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Opracowanie metody modyfikacji właściwości mąki pszennej z przeznaczeniem do zastosowań w piekarnictwie

Development of a method for modifying the properties of wheat flour for use in bakery industry

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P1. Piotr Lewko, Agnieszka Wójtowicz, Marek Gancarz: Distribution of arabinoxylans and their relationship with physiochemical and rheological properties in wheat flour mill streams as an effective way to predict flour functionality. Applied Science, 2023, 13, (9), 5458. https://doi.org/10.3390/app13095458 (**IF 2,50, 100 pkt. MNiSW**)

P2. Piotr Lewko, Agnieszka Wójtowicz, Monika Różańska-Boczula: Effect of extruder configuration and extrusion-cooking processing parameters on selected characteristics of NSP-rich wheat flour as a hybrid treatment with xylanase addition. Processes, 2024, 12, 1159. https://doi.org/10.3390/pr12061159 (**IF 2,80, 70 pkt. MNiSW**)

P3. Piotr Lewko, Agnieszka Wójtowicz, Michał Rudaś: Effect of processing conditions of enzymatic, extrusion and hybrid treatment methods on composition and selected techno-functional properties of developed wheat flour. International Journal of Food Science (**w recenzji; IF 2,70, 100 pkt. MNiSW**)

P4. Piotr Lewko, Agnieszka Wójtowicz, Daniel M. Kamiński: The influence of processing using conventional and hybrid methods on the composition, polysaccharide profiles and selected properties of wheat flour enriched with baking enzymes. Foods, 2024, 13, 2957. https://doi.org/10.3390/foods13182957 (**IF 4,70, 140 pkt. MNiSW**)

P5. Piotr Lewko, Agnieszka Wójtowicz, Marek Gancarz: Application of conventional and hybrid thermal-enzymatic modified wheat flours as clean label bread improvers. Applied Science, 2024, 14, 7659. https://doi.org/10.3390/app14177659 (**IF 2,50, 100 pkt. MNiSW**)

Artykuły stanowiące osiągnięcie naukowe przedstawiono w kolejności odpowiadającej głównym tezom pracy. Punktację podano według listy czasopism punktowanych Ministerstwa Nauki i Szkolnictwa Wyższego (MNiSW), a Impact Factor (IF) według Journal Citation Reports za rok 2024.

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Spis treści

STRESZCZENIE

Opracowanie metody modyfikacji właściwości mąki pszennej z przeznaczeniem do zastosowań w piekarnictwie

Celem pracy było opracowanie technologii produkcji mąki funkcjonalnej o zwiększonej wodochłonności i zdefiniowanej charakterystyce reologicznej, która stosowana samodzielnie lub w mieszankach piekarniczych pozwoli na zwiększenie wydajności pieczywa. W ramach badań opracowano mąkę bazową (F) z wybranych frakcji mąk będących ubocznymi pasażami przemiałowymi, uzyskiwanymi podczas produkcji mąk niskowyciągowych. Mąka ta, składająca się z pasaży o wysokiej wodochłonności i zwiększonej zawartości nieskrobiowych polisacharydów oraz arabinoksylanów, została poddana analizie parametrów fizykochemicznych i reologicznych. Uzyskaną mąkę zmodyfikowano enzymatycznie za pomocą celulazy, ksylanazy i/lub ich mieszanin, a następnie poddano różnym metodom obróbki fizycznej. Zastosowano obróbkę termiczną na sucho (T), hydrotermiczną (H) oraz ekstruzyjną (E) w różnych warunkach procesowych. Następnie przeprowadzono badania techno-funkcjonalnych cech zmodyfikowanych mąk oraz przetestowano ich dodatek w recepturach pieczywa. Modyfikacje mąki bazowej (F) miały zróżnicowany wpływ na jej skład, reologię oraz strukturę, w zależności od zastosowanych warunków obróbki i użytych enzymów. Konwencjonalne i hybrydowe metody obróbki z użyciem enzymów celulazy lub kompleksu celulaza-ksylanaza spowodowały zmiany w składzie frakcji polisacharydowych (szczególnie arabinoksylanów) oraz w reologii zmodyfikowanej mąki, co znacząco wpłynęło na jej właściwości. Podczas wypieku chleba z dodatkiem modyfikowanych mąk największą efektywność w zwiększeniu wodochłonności ciasta osiągnięto przy użyciu mąk o zachowanych funkcjach białka glutenowego, czyli modyfikowanych termicznie oraz z użyciem ekstruzji dwuślimakowej. Modyfikowane mąki mogą być dodawane do pieczywa w ilości do 20% po obróbce termicznej (z użyciem lub bez enzymów) oraz do 10% po obróbce ekstruzyjnej dwuślimakowej i hybrydowej. Wprowadzenie tych mąk do receptur chleba spowodowało wzrost wodochłonności, poprawę wydajności i objętości pieczywa, bez negatywnego wpływu na strukturę, teksturę i barwę miękiszu. Przeprowadzone prace badawcze potwierdziły, że zintegrowanie obróbki enzymatycznej z obróbką termiczną lub ekstruzyjną, prowadzoną w określonych warunkach procesowych na skomponowanej z wybranych pasaży mące pszennej, pozwoliło na otrzymanie mąki funkcjonalnej o zwiększonej wodochłonności i zdefiniowanej charakterystyce reologicznej. Uzyskane mąki mogą być wykorzystywane jako dodatki do specjalistycznych mąk dedykowanych dla branży piekarniczej, wpływając na poprawę cech reologicznych mieszanek oraz zwiększając wodochłonność i wydajność pieczywa.

Słowa kluczowe: mąka pszenna modyfikowana, arabinoksylany, eznymy, modyfikacja termiczna, ekstruzja

SUMMARY

Development of a method for modifying the properties of wheat flour for use in bakery industry

The objective of the research was to develop a production technology of functional flour with enhanced water absorption capacity and defined rheological characteristics, which, when used alone or in baking mixes, would improve bread yield. As part of the research, a base flour (F) was developed from selected useless fractions of milling streams, obtained during the production of low-extraction flours. This flour, consisting of passages with high water absorption and increased content of non-starch polysaccharides and arabinoxylans, was subjected to analysis of physicochemical and rheological parameters. The obtained flour was enzymatically modified using cellulase, xylanase and/or their mixtures, and then subjected to various methods of physical treatment. Dry heat treatment (T), hydrothermal (H) and extrusion (E) processing were used under various process conditions. Then, studies were carried out on the techno-functional features of the modified flours and their addition to bread recipes was tested. Modifications of the base flour (F) showed a varied effect on its composition, rheology and structure, depending on the processing conditions and enzymes used. Conventional and hybrid methods of processing using cellulase enzyme or cellulase-xylanase complex caused changes in the composition of polysaccharide fractions (especially arabinoxylans) and in the rheology of the modified flour, which significantly affected its properties. During baking bread with the addition of modified flours, the highest effectiveness in increasing the water absorption of the dough was achieved using flours with preserved gluten protein functions, especially modified by thermal treatment and twin-screw extrusion. Modified flours can be added to bread recipes in amounts of up to 20% after dry heat treatment (with or without enzymes) and up to 10% after twin-screw and hybrid enzyme-assisted extrusion processing. The introduction of these flours to bread recipes resulted in increased water absorption, improved yield and bread volume, without negative effects on the structure, texture and colour of bread crumb. The conducted research confirmed that the integration of enzymatic action with thermal or extrusion treatment, carried out under specific processing conditions on wheat flour composed of selected passages, allowed obtaining functional flours with increased water absorption and defined rheological characteristics. The obtained flours can be used as additives to specialist flours dedicated to the bakery industry, improving the rheological properties of the mixtures and increasing the water absorption and yield of bread.

Keywords: modified wheat flour, arabinoxylans, enzymes, thermal modification, extrusion

1. WPROWADZENIE

Pszenica zwyczajna (*Triticum aestivum L.*) jest zbożem szeroko wykorzystywanym w produkcji żywności, począwszy od chleba, ciastek, herbatników i wafli, po produkcję makaronów i płatków śniadaniowych [Marti i in. 2015]. Cel technologiczny użycia mąki zależy od jej właściwości (jako efekt jej składu chemicznego), jak również od interakcji między jej poszczególnymi składnikami [Zawieja i in. 2020]. Dlatego tylko niektóre odmiany pszenicy nadają się do konkretnych rodzajów produktów, nadając im określone cechy funkcjonalne. Na przykład, „mocne" mąki pszenne są preferowane do wypieku chleba, pizzy czy produkcji makaronu, gdzie pożądana jest wysoka zawartość białka i mocna sieć glutenowa. Z kolei mąka „słaba" jest preferowana do produkcji ciastek i wafli, gdzie wymagana jest niska zawartość białka i słaba matryca glutenowa. Technologiczna jakość mąki nie jest związana tylko z zawartością białka i charakterystyką skrobi, ale jest również wynikiem skomplikowanych interakcji między makrocząsteczkami, które są odpowiedzialne za jakość ciasta i wydajność pieczywa [Marti i in. 2015].

Mąka pszenna jest podstawowym surowcem do produkcji wyrobów piekarniczych. Chleb, jako jeden z najpowszechniejszych produktów spożywczych na świecie, jest uważany za kluczowy w żywieniu człowieka ze względu na swoją dostępność i wartość odżywczą, gdyż jest bardzo dobrym źródłem węglowodanów, białka, błonnika pokarmowego, witamin i minerałów [Cauvain i in. 2012]. Postęp w dziedzinie młynarstwa i piekarstwa zaowocował rozwojem technologii produkcji wyrobów piekarniczych. Są one stale udoskonalane, co pozwala przemysłowi wprowadzać na rynek spożywczy produkty na bazie pszenicy o szczególnych walorach prozdrowotnych i funkcjonalnych. Obecne trendy badawcze koncentrują się w szczególności na udoskonaleniach żywieniowych i technologicznych w produktach zbożowych przy użyciu przede wszystkim różnych dodatków mogących stanowić składnik „czystej etykiety" oraz procesów produkcyjnych [Capelli i in. 2020, Vargas i in. 2021]. Efekty prac badawczych, takich jak użycie dodatku alternatywnych składników do standardowej mąki pszennej (np. owadów, roślin strączkowych, owoców, warzyw, ziół, mikroalg lub produktów ubocznych przemysłu rolno-spożywczego) [Campbell i in. 2016, Zhang i in. 2021, Wójcik i in. 2023, Jurkaninová i in. 2024, Mahmoud i in. 2024, Zarzycki i in. 2024], a także zastosowanie nowoczesnych technologii przetwarzania zbóż [Lee i in. 2021, Tayefe i in. 2020], pozwala znacząco udoskonalać produkty piekarnicze, zwłaszcza mąki i pieczywo pełnoziarniste [Tebben i in. 2018].

Aby zapewnić pieczywu odpowiednią wydajność, stosowana do jego produkcji mąka powinna charakteryzować się zestawem cech decydujących o jej jakości i przydatności technologicznej, które ogólnie można nazwać wartością wypiekową mąki. W uzyskaniu odpowiedniej jakości pieczywa bardzo ważna jest również zdolność mąki do odpowiedniej absorbcji wody i wytworzenia ciasta zdolnego produkować gaz - dwutlenek węgla w procesie fermentacji, oraz zatrzymać go w postaci porów w trakcie wypieku, co w efekcie nadaje pieczywu odpowiednią objętość.

Kwestia wodochłonności mąki jest jednym z kluczowych aspektów finansowych przemysłu piekarskiego, czyli zwiększania wydajności produkcji, poprzez uzyskanie z mniejszej ilości mąki większej ilości pieczywa [Martinez i in. 2013]. Wydajność pieczywa ma ogromne znaczenie dla komercyjnych producentów chleba, a jednym ze sposobów na jej poprawę jest właśnie dodawanie większej ilości wody do receptur wypiekowych. Może to jednak powodować trudności technologiczne, gdyż ciasto z nadmiarem wody staje się lepkie, a to może wpływać negatywnie na teksturę końcową pieczywa [Jiang i in. 2022]. Zdolność pochłaniania wody przez mąkę związana jest z ilością poszczególnych składników, takich jak białko, pentozany czy uszkodzona skrobia [Martinez i in. 2013]. Ilość tych składników w mące pszennej jest bezpośrednio związana z procesem przemiału pszenicy w młynach przemysłowych.

Mielenie pszenicy to mechaniczny, wieloetapowy i złożony proces stopniowego rozdrabniania ziarna, w którym bielmo najpierw oddziela się od łuski otrębowej, a następnie, poprzez serię przejść przez maszyny rozdrabniające i przesiewające, tworzy tzw. pasaże przemiałowe [Prabhasnakar i in. 2000, Banu i in. 2010, Vukić i in. 2020]. Tak więc mielenie pszenicy polega na oddzieleniu mączystego bielma od otrąb i redukcji cząstek bielma do mąki. Główną komercyjną i przemysłową metodą przemiału ziarna, ze względu na uzyskiwane wysokie wydajności, jest mielenie walcowe. Odbywa się ono w kilku etapach i przeprowadzane jest na walcach różnego typu, w tym walcach rozdrabniających, które wyposażone w rowkowania zaprojektowane są do rozcierania ziarna i usuwania bielma oraz zarodków z powłoki otrąb, a także stopniowego mielenia bielma na mąkę, walcach rozczynowych generujących kaszki o różnych rozmiarach cząstek oraz walcach wymiałowych, które redukują kaszki do mąki.

Każdy młyn wytwarza od kilku do nawet kilkudziesięciu pasaży przemiałowych, z których każda mąka różni się właściwościami fizykochemicznymi i reologicznymi. Na końcu procesu przemiału są one mieszane, aby uzyskać tzw. mąki gatunkowe. W celu wyprodukowania mąki handlowej, często konieczne jest połączenie różnych mąk gatunkowych

i ich staranne wymieszanie. Tak przygotowane produkty trafiają następnie do sprzedaży, głównie do restauracji i piekarni, gdzie są wykorzystywane do produkcji chleba, ciast i pizzy, lub do różnych zakładów spożywczych, które używają ich do produkcji makaronów, produktów ekstrudowanych czy pieczywa cukierniczego [Ahmed i in. 2017].

Produkcja szerokiej gamy typów mąki pszennej jest wynikiem odpowiedniego doboru pasaży przemiałowych pod kątem charakterystyki finalnego produktu. Nie każdy pasaż jest jednakowo przydatny w tworzeniu specjalistycznych mąk pszennych, co stanowi wyzwanie przy optymalizacji produkcji. Dlatego kluczowe jest dokładne testowanie i przewidywanie jakości poszczególnych pasaży przemiałowych, niezależnie od planowanego zastosowania końcowego produktu. Określenie rozmieszczenia składników, które pozytywnie lub negatywnie wpływają na jakość mąki w poszczególnych frakcjach, jest istotne dla oceny efektywności przemiału [Ramseyer i in. 2011], a optymalna kombinacja pasaży ma kluczowe znaczenie dla uzyskania najlepszych właściwości technologicznych produktu finalnego [Vukić i in. 2020].

Badania nad różnicami między poszczególnymi pasażami są szeroko publikowane w literaturze tematu. Różnice te obejmują cechy takie jak właściwości reologiczne [Banu i in. 2010, Vukić i in. 2020, Indrania i in. 2003, Gómez i in. 2010, Liu i in. 2011, Pojić i in. 2013, Pojić i in. 2014], a także właściwości fizykochemiczne, w tym zawartość i rozmieszczenie białka, skład poszczególnych białek, zawartość składników mineralnych [Prabhasnakar i in. 2000, Banu i in. 2010, Gómez i in. 2010, Liu i in. 2011, Pojić i in. 2014, Every i in. 2002, Iqbal i in. 2015, Sutton i in. 2006, Wang i in. 2007], aktywność enzymów endogennych [Dornez i in. 2006, Every i in. 2006, Gebruers i in. 2002, Rani i in. 2001], zawartość tłuszczu [Prabhasnakar i in. 2000, Indrania i in. 2003, Abdel-Haleem i in. 2019], stopień uszkodzenia skrobi [Banu i in. 2010, Pojić i in. 2014, Sutton i in. 2006], zawartość pentozanów (arabinoksylanów) i ich frakcji [Ramseyer i in. 2011, Wang i in. 2006] czy zawartość przeciwutleniaczy [Engelsen i in. 2009].

Oprócz białka i skrobi, to właśnie arabinoksylany (AX) są istotnymi składnikami ziarna pszenicy, które znacząco wpływają na właściwości mąki. Arabinoksylany to nieskrobiowe polisacharydy obecne w bielmie (3–5% całkowitego bielma), warstwie aluronowej i ścianach komórkowych otrąb (około 60–70% całkowitej zawartości ściany komórkowej). Arabinoksylany są zbudowane z pojedynczego głównego łańcucha składającego się z reszt ksylozy połączonych wiązaniem β→1,4, do których w pozycjach C-3 i jednocześnie C-2 i C-3 dołączone są pojedyncze reszty arabinozy [Izydorczyk i in. 1991]. W przypadku otrąb pszennych AX stanowią od 10,9 do 26,0% wszystkich ich frakcji [Zannini i in. 2022]. Podobnie

jak białko i składniki mineralne, arabinoksylany nie są równomiernie rozmieszczone w ziarnie pszenicy – ich stężenie w środkowym bielmie jest znacznie niższe niż w zewnętrznych warstwach ziarna [Ramseyer i in. 2011].

Pomimo niskiej zawartości arabinoksylanów w mąkach handlowych, mają one istotny wpływ na interakcje między białkami, formowanie glutenu oraz konsystencję ciasta, co szczególnie oddziałuje na jakość końcową wyrobów piekarniczych, takich jak chleb [Delcour i in. 1991, Michniewicz i in. 1992, Wang i in. 2003]. Arabinoksylany wyróżniają się zdolnością do wiązania dużych ilości wody, odgrywając kluczową rolę w gospodarce wodnej podczas tworzenia ciasta [Labat i in. 2000].

Całkowitą zawartość arabinoksylanów (T-AX) można empirycznie podzielić na frakcje arabinoksylanów rozpuszczalnych w wodzie WEAX (ang. water extractable arabinoxylans) lub arabinoksylanów nierozpuszczalnych w wodzie WUAX (ang. water unextractable arabinoxylans). WUAX i WEAX mają różne właściwości fizykochemiczne [Ramseyer i in. 2011]. WUAX wpływa na mobilność molekularną wody [Izydorczyk i in. 1991] i negatywnie oddziałuje na jakość chleba przez wiązanie dużych ilości wody, co uniemożliwia właściwe nawodnienie skrobi i glutenu. WUEX wpływa także na właściwe formowanie pęcherzyków gazu podczas fermentacji w cieście chlebowym [Gebruers i in. 2002]. WEAX mają unikalne właściwości fizyczne, takie jak zdolność do wiązania 10 razy więcej wody niż ich własna masa [Jelaca i in. 1971, Patil i in. 1975], tworząc bardzo lepkie roztwory i żele dzięki ich kowalencyjnemu sieciowaniu [Hoseney i in. 1981, Izydorczyk i in. 1990]. Wszystkie te właściwości mają bezpośredni funkcjonalny wpływ na formowanie glutenu i właściwości ciasta. Ogólnie uważa się, że WEAX mają pozytywny wpływ na jakość chleba [Rouau i in. 1994], a WUAX oddziaływują negatywnie [Jelaca i in. 1972, Kim i in. 1977]. We frakcji WEAX reszty kwasu ferulowego są dostępne do oksydacyjnego sieciowania indukowanego przez wolne rodniki i są częściowo odpowiedzialne za zmiany lepkości ciasta [Ramseyer i in. 2011].

Wcześniejsze badania nad rozmieszczeniem arabinoksylanów w strumieniach przemiałowych scharakteryzowały w ograniczonym zakresie zależność między zawartością AX w poszczególnych frakcjach mąki a przydatnością technologiczną poszczególnych pasaży mącznych [Delcour i in. 1999, Dornez i in. 2006, Every i in. 2006, Wang i in. 2006, Ramseyer i in. 2011, Pojić i in. 2014, Vukić i in. 2020]. Badania te często skupiały się bardziej na charakterystyce strukturalnej AX z różnych pasaży mącznych, a w mniejszym stopniu na porównaniu ich zawartości z wynikami najczęściej stosowanych metod oceny jakości i przydatności technologicznej. Zrozumienie różnic między cechami funkcjonalnymi różnych

pasaży mącznych umożliwiłoby efektywne kształtowanie ich składu i jakości końcowej mieszanki dla różnych zastosowań [Ramseyer i in. 2011]. Dlatego interesujące jest określenie rozmieszczenia arabinoksylanów w pasażach przemiałowych w celu skomponowania odpowiednich mieszanek, aby uzyskać funkcjonalną mąkę o specyficznych właściwościach, którą można przeznaczyć do różnych zastosowań technologicznych.

Pasaże przemiałowe najbogatsze we frakcje włókniste pochodzą z zewnętrznych części ziarna. Jak opisali Lewko i in. [2023], różnice w składzie poszczególnych mąk pasażowych wynikają bezpośrednio z pochodzenia konkretnych frakcji z anatomicznych części ziarna oraz wpływu procesów mielenia, takich jak mechaniczne uszkodzenie skrobi. Frakcje zawierające fragmenty otrąb, często o różnych rozmiarach, są uważane za niepożądane w standardowych mąkach chlebowych ze względu na obniżenie jakości ciasta, zmniejszenie jego elastyczności oraz redukcję objętości chleba [Schmiele i in. 2012, Bucsella i in. 2016, Noort i in. 2010]. Z tego powodu frakcje te są sprzedawane jako produkty bogate w błonnik, w postaci suplementów lub jako składnik pasz dla zwierząt [Kaur i in. 2019].

Niewykorzystane frakcje mogą stanowić nawet około 10% całkowitej produkcji w przedsiębiorstwie młynarskim, a ich ilość jest jeszcze większa w młynach skoncentrowanych na produkcji mąk dla branży makaronowej, gdzie surowiec musi charakteryzować się niską zawartością składników mineralnych i wysokim wyciągiem, bez zanieczyszczeń z łuski ziarniaka. Frakcje te, pozbawione najbardziej wartościowych składników technologicznych, nie mogą być stosowane jako pełnowartościowa mąka pełnoziarnista ani mąka chlebowa bez dodatkowego połączenia z jakościową mąką handlową. Dlatego kluczowe jest opracowanie dodatkowych rozwiązań technologicznych, które pozwolą na efektywniejsze wykorzystanie i istotne zmniejszenie ilości takich niewykorzystanych frakcji podczas przemiału.

Rosnące zastrzeżenia konsumentów dotyczące składników żywności i czystego etykietowania również mają wpływ na przemysłową produkcję chleba ze zwiększoną wydajnością i nowymi recepturami [Vargas i in. 2021]. Konsumenci poszukują produktów z czystą etykietą, bez dodatków oznaczonych znakiem E, ale o odpowiedniej jakości [Li i in. 2023]. Niestety, niektóre polepszacze pieczywa są postrzegane jako nieznane lub szkodliwe substancje chemiczne, które mogą powodować problemy zdrowotne [Vargas i in. 2021]. Niektóre cechy mąki, ulepszone za pomocą metod obróbki termicznej, mogą mieć pozytywny wpływ na końcową jakość produktów piekarniczych z oznaczeniem czystej etykiety.

Aby poprawić funkcjonalność mąki pszennej poprzez modyfikację fizyczną, można zastosować różne technologie obróbki mąki. Do najpopularniejszych należą procesy termiczne oraz hydrotermiczne, wykorzystujące podwyższona temperaturę oraz wodę i parę [Ma i in.

2021, Keppler i in. 2018, Bucsella i in. 2016, Hu i in. 2017, Delatte i in. 2019]. Dodatkowo, modyfikacje mogą być wspomagane przez wybrane enzymy, takie jak celulaza czy ksylanaza [de Souza i in. 2021, Melim Migel i in. 2013]. Współczesne technologie, takie jak wtrysk pary SE (ang. steam explosion), wysokie ciśnienie hydrostatyczne HHP (ang. high pressure processing), homogenizacja wysokociśnieniowa HPH (ang. high-pressure homogenization), pulsacyjne pole elektryczne PEF (ang. pulsed electric field) czy obróbka plazmowa, również mogą być stosowane w celu zmiany lub poprawy właściwości ziarna pszenicy, mąk pełnoziarnistych czy frakcji otrąb, co ma istotny wpływ na ich cechy chemiczne, reologiczne i hydratacyjne [Li i in. 2023]. Odpowiednio przeprowadzone modyfikacje fizyczne mogą znacząco ograniczyć negatywny wpływ dodatku mąki pełnoziarnistej lub niezmodyfikowanych frakcji zawierających otręby na jakość wypieku, zwłaszcza w odniesieniu do reologii ciasta i jakości pieczywa [Gómez i in. 2011, Martinez i in. 2013, Jiang i in. 2022]. Intensywnie rozwijają się również technologie ekstrakcji wybranych składników z ziaren, takich jak błonnik pokarmowy (rozpuszczalny i nierozpuszczalny), β-glukany, witaminy i przeciwutleniacze [Wójcik i in. 2023, Cingöz i in. 2023, Li i in. 2023].

Procesy termiczne, obróbka hydrotermiczna lub ciśnieniowo-termiczna są skuteczne w modyfikowaniu właściwości fizycznych, reologicznych, technologicznych i funkcjonalnych oraz w stabilizacji trwałości mąki pszennej i innych produktów zbożowych [Cai i in. 2015, Arcila i in. 2015, Long i in. 2014]. Obróbka termiczna zmniejsza aktywność naturalnych enzymów, ogranicza zawartość wody i zmienia frakcje lipidowe obecne głównie w mące bogatej w otręby, wydłużając w ten sposób okres przydatności do spożycia mąk i produktów zbożowych [Bucsella i in. 2016]. W temperaturze powyżej 50°C białka glutenowe ulegają rozfałdowaniu lub tworzą agregaty, co modyfikuje wytrzymałość ciasta [Wang i in. 2017]. Obróbka termiczna na sucho jest mniej intensywna i mniej degradująca niż ogrzewanie z użyciem pary, głównie z powodu ograniczonego dostępu do wody dostarczonej w procesie hydrotermicznym i mniejszej ruchliwości cząsteczek [Mann i in. 2013]. Intensywność zmian w białku, skrobi i błonniku zachodzących podczas obróbki hydrotermicznej zależy od zawartości wody, profilu czasowo-temperaturowego oraz rodzaju obróbki, takiego jak np. zastosowanie podgrzewanych walców, autoklawowanie, gotowanie parą, atomizacja lub ekstruzja [Chiu i Solarek 2009]. Podczas modyfikacji hydrotermicznych dochodzi do wstępnego skleikowania skrobi i częściowej denaturacji białek, co sprawia, że mąka pełnoziarnista lub otręby charakteryzują się po obróbce zwiększoną lepkością. Zaś hydrotermicznie modyfikowane mąki mogą być wykorzystywane do produkcji funkcjonalnych

składników, takich jak zagęszczacze do zup, sosów, polew czy w żywności dla niemowląt i dzieci [Bucsella i in. 2016].

Bardziej intensywne przetwarzanie uzyskuje się dzięki technice ekstruzji, która łączy działanie podwyższonej temperatury, ciśnienia, sił ścinających i czasu przebywania z różną dostępnością wody w trakcie przetwarzania. Składniki pszenicy, zwłaszcza gluten i skrobia, odgrywają kluczową rolę w kształtowaniu struktury, reologii i tekstury ekstrudatów. Główny efekt fragmentacji lub agregacji białek podczas ekstruzji wynika z międzycząsteczkowych zmian w wiązaniach disiarczkowych [Wu i in. 2024]. Skrobia może być częściowo lub całkowicie skleikowana, w zależności od zawartości wody w surowcu, temperatury, intensywności ścinania i konfiguracji ekstrudera. Ekstruzja jest uznawana za skuteczną metodę przekształcania nierozpuszczalnych frakcji błonnika w jego rozpuszczalne formy [Kong i in. 2023, Zhang i in. 2022].

Inną, powszechnie stosowaną metodą modyfikacji skrobi, mąki lub otrąb jest stosowanie enzymów. Dzięki tej metodzie można efektywnie zmieniać skład frakcji włóknistych, zwiększać czas rozwoju ciasta, poprawiać jego stabilność oraz wzmacniać właściwości absorpcyjne mąki [Melim Miguel i in. 2013]. W piekarnictwie kluczową rolę odgrywają takie enzymy jak: amylazy, które przekształcają skrobię w cukry proste i dekstryny, oksydazy wzmacniające i wybielające ciasto, hemicelulazy poprawiające wytrzymałość glutenu, proteazy redukujące jego elastyczność, oraz lipazy, które wydłużają okres przydatności do spożycia. Enzymy te mają także istotny wpływ na formowanie ciasta, objętość chleba, teksturę produktów, reakcje brązowienia i zmiany barwy podczas pieczenia oraz zmniejszenie retrogradacji i czerstwienia pieczywa [Whitehurst i Van Oort 2016]. Badania potwierdzają również pozytywny wpływ celulazy i ksylanazy na funkcjonalność polisacharydów nieskrobiowych, które, jak wspomniano, występują głównie w zewnętrznych warstwach ziaren zbóż [Bender i in. 2017]. Amylaza, celulaza i proteaza stosowane są również w ekstruzji enzymatycznej, odgrywając rolę w przemianach skrobi, poprawie jakości ciasta czy przy produkcji bioetanolu [Deng i in. 2023].

Celulazy są niezwykle ważnymi enzymami zarówno w przemyśle, jak i w świecie przyrody, ponieważ odgrywają główną rolę w globalnym cyklu węglowym, degradując nierozpuszczalną celulozę do rozpuszczalnych cukrów [Deng i in. 2023]. Ksylanazy, stosowane w przemyśle spożywczym, szczególnie w sektorze piekarniczym, wpływają na zwiększenie stabilności ciasta, uzyskanie bardziej miękkiej i jednolitej struktury miękiszu oraz poprawienie objętości chleba. Działanie ksylanaz powoduje redystrybucję wody z fazy pentozanowej do fazy glutenowej, co zwiększa objętość pieczywa poprzez poprawę elastyczności glutenu.

Dodatkowo, ksylanazy opóźniają proces czerstwienia, poprawiają teksturę chleba o wysokiej zawartości błonnika oraz stabilizują jakość mąki używanej do wypieku chleba pszennego. Ze względu na złożoną i heterogeniczną strukturę ksylanów, ksylanazy muszą działać grupowo, aby skutecznie hydrolizować takie polisacharydy jak arabinoksylany i glukuronoksylany [Chen i in. 2019, Zhou i in. 2010].

Kilku badaczy połączyło enzymatyczną i termiczną/ekstruzyjną obróbkę produktów zbożowych, zwłaszcza frakcji otrąb. Na przykład Kong i współautorzy [2023] przetestowali koenzymatyczną i ekstruzyjną obróbkę otrąb pszennych, stosując celulazę, ksylanazę, wysokotemperaturową α-amylazę i kwaśną proteazę, zarówno pojedynczo, jak i w połączeniu, aby zbadać wpływ na rozpuszczalne w wodzie arabinoksylany (WEAX). Wyniki pokazały istotny wzrost zawartości WEAX, zdolności wiązania wody i tłuszczu oraz zdolności adsorpcji cholesterolu, co prawdopodobnie wynika z utworzenia luźniejszej i bardziej porowatej mikrostruktury w tak modyfikowanych otrębach.

Ekstruzja enzymatyczna to nowa metoda, w której ekstruder pełni funkcję ciągłego bioreaktora lub reaktora enzymatycznego, przyspieszając reakcje enzymatyczne [Zeng i in. 2017]. Obróbka przy użyciu ekstruzji wspomaganej enzymatycznie może skutecznie oddziaływać na złożone biopolimery o wysokim stopniu polimeryzacji, krystaliczności i wytrzymałości strukturalnej, tworząc porowatą mikrostrukturę i odsłaniając miejsca reaktywne dla enzymów [Deng i in. 2023]. Badacze stosujący tę technikę do modyfikacji otrąb zbożowych i frakcji bogatych w błonnik [Román i in. 2017, Dang i Vasanthan 2018] wykazali, że połączenie obróbki enzymatycznej z ekstruzją poprawiło rozpuszczalność błonnika pokarmowego w otrębach ryżowych i innych składnikach rozpuszczalnych, przy czym sekwencyjna obróbka ekstruzyjno-enzymatyczna znacząco zwiększyła całkowitą zawartość rozpuszczalnych pentozanów w porównaniu z obróbką indywidualną lub równoczesną. Kong i współpracownicy [2023] badali wpływ współdziałania ekstruzji i hydrolizy enzymatycznej (z użyciem celulazy, ksylanazy, α-amylazy wysokotemperaturowej i kwaśnej proteazy) na ekstrahowalne wodą arabinoksylany oraz właściwości fizykochemiczne otrąb pszennych z ziarna o czarnej barwie. W zależności od rodzaju, poziomu i aktywności enzymów oraz dodatkowego przetwarzania i kolejności obróbki, funkcjonalne polisacharydy w mące pszennej ulegały różnorodnym modyfikacjom.

W ramach prac badawczych zaplanowanych w niniejszej pracy, postanowiono zbadać możliwość zagospodarowania ubocznych pasaży przemiałowych, powstających w trakcie wytwarzania mąki stosowanej w produkcji makaronu. Skomponowana z nich mąka,

charakteryzująca się zwiększoną zawartością polisacharydów nieskrobiowych, mogłaby stanowić dodatek poprawiający jakość mąk stosowanych w przemyśle piekarniczym.

W trakcie badań wybrano pasaże przemiałowe o najkorzystniejszym składzie, aby zagospodarować niewykorzystane frakcje i stworzyć nową kompozycję mąki. Do modyfikacji jej właściwości zastosowano różne metody, takie jak modyfikacja fizyczna, enzymatyczna oraz hybrydowa. Zbadano wpływ tych metod na cechy opracowanej mąki oraz przetestowano jej zastosowanie w produkcji pieczywa. W efekcie zaproponowano gotowe do wdrożenia rozwiązanie, polegające na opracowaniu metody modyfikacji właściwości mąki pszennej w warunkach przemysłowych.

2. PROBLEMY BADAWCZE I CELE NAUKOWE

Zaplanowane badania miały na celu rozwiązanie następującego problemu badawczego:

- Opracowanie parametrów procesowych umożliwiających otrzymanie mąki funkcjonalnej ze skomponowanych w odpowiednich proporcjach pasaży przemiałowych o zdefiniowanym składzie i charakterystyce reologicznej, która, samodzielnie lub w mieszankach stosowanych w piekarnictwie, umożliwi zwiększenie wodochłonności mąki i wydajności pieczywa bez pogorszenia jego cech jakościowych oraz będzie stanowić możliwość ograniczenia ilości frakcji ubocznych powstających podczas przemiały ziarna pszenicy.

Do rozwiązania problemu badawczego ustalono następują cele naukowe:

- Analiza odmian pszenicy zwyczajnej pod kątem zwiększonej zawartości nieskrobiowych polisacharydów w tym arabinoksylanów, wybór odmiany do dalszych badań.
- Pełna charakterystyka fizykochemiczna i reologiczna pasaży przemiałowych pszenicy zwyczajnej i opracowanie kompozycji nowej mąki o zwiększonej zawartości arabinoksylanów przy zagospodarowaniu frakcji ubocznych.
- Weryfikacja wpływu parametrów procesowych na poszczególne wyróżniki mąki podczas obróbki skomponowanej mąki z zastosowaniem zróżnicowanych warunków obróbki termicznej, hydrotermicznej, procesu ekstruzji, prowadzonych samodzielnie lub wspieranych enzymatycznie.
- Ocena przydatności skomponowanej mąki poddanej zintegrowanej obróbce enzymatycznej i termicznej/hydrotermicznej/ekstruzyjnej do wprowadzenia jako dodatek w recepturach wybranych wyrobów piekarniczych.

Zakres badań obejmował charakterystykę i przygotowanie surowca w postaci wyselekcjonowanych pasaży przemiałowych a następnie przeprowadzenie różnych modyfikacji procesowych na skomponowanej mące z dodatkiem enzymów piekarniczych. Zakres prac składał się z następujących etapów:

Badania wstępne:

- ocena wybranych 15 odmian pszenic zwyczajnych pod kątem przede wszystkim zróżnicowania w składzie nieskrobiowych polisacharydów, takich jak arabinoksylany, ocena zastosowania szybkich metod analitycznych dostępnych w elewatorach zbożowych do szybkościowej analizy zawartości nieskrobiowych polisacharydów, wybór odmiany pszenicy zwyczajnej do etapu prac badawczych.

Prace badawcze:

- przemiał wybranej odmiany pszenicy i ocena fizykochemiczna oraz reologiczna pasaży przemiałowych,
- opracowanie mąki składającej się z wyselekcjonowanych pasaży i ocena jej parametrów fizykochemicznych i reologicznych,
- modyfikacja enzymatyczna i obróbka termiczna, hydrotermiczna oraz ekstruzyjna opracowanej mąki,
- ocena parametrów techno-funkcjonalnych i reologicznych mąki modyfikowanej w różnych warunkach,
- analiza porównawcza ciasta i pieczywa uzyskanego z dodatkiem mąk modyfikowanych w różnych warunkach procesowych.

Prace wdrożeniowe:

- opracowanie zaleceń technologicznych i procesowych do wytwarzania i selekcji pasaży przemiałowych powstających w trakcie przemiału mąki na surowiec do produkcji makaronu,
- opracowanie zaleceń technologicznych do prowadzenia procesu modyfikacji skomponowanej z wybranych pasaży przemiałowych mąki pszennej,
- przygotowanie dokumentacji produkcyjnej w postaci opisów technicznych procesów i kart specyfikacji produktów.

3. MATERIAŁY I PROCEDURY BADAWCZE

Badania przeprowadzano w laboratoriach Katedry Techniki Cieplnej i Inżynierii Procesowej Uniwersytetu Przyrodniczego w Lublinie oraz laboratoriach firmy PZZ Lubella GMW Sp. z o.o. W trakcie badań korzystano także z aparatury Centralnego Laboratorium Badawczego UP w Lublinie, Instytutu Hodowli i Aklimatyzacji Roślin w Radzikowie oraz w Katedrze Chemii Organicznej i Krystalochemii UMCS w Lublinie.

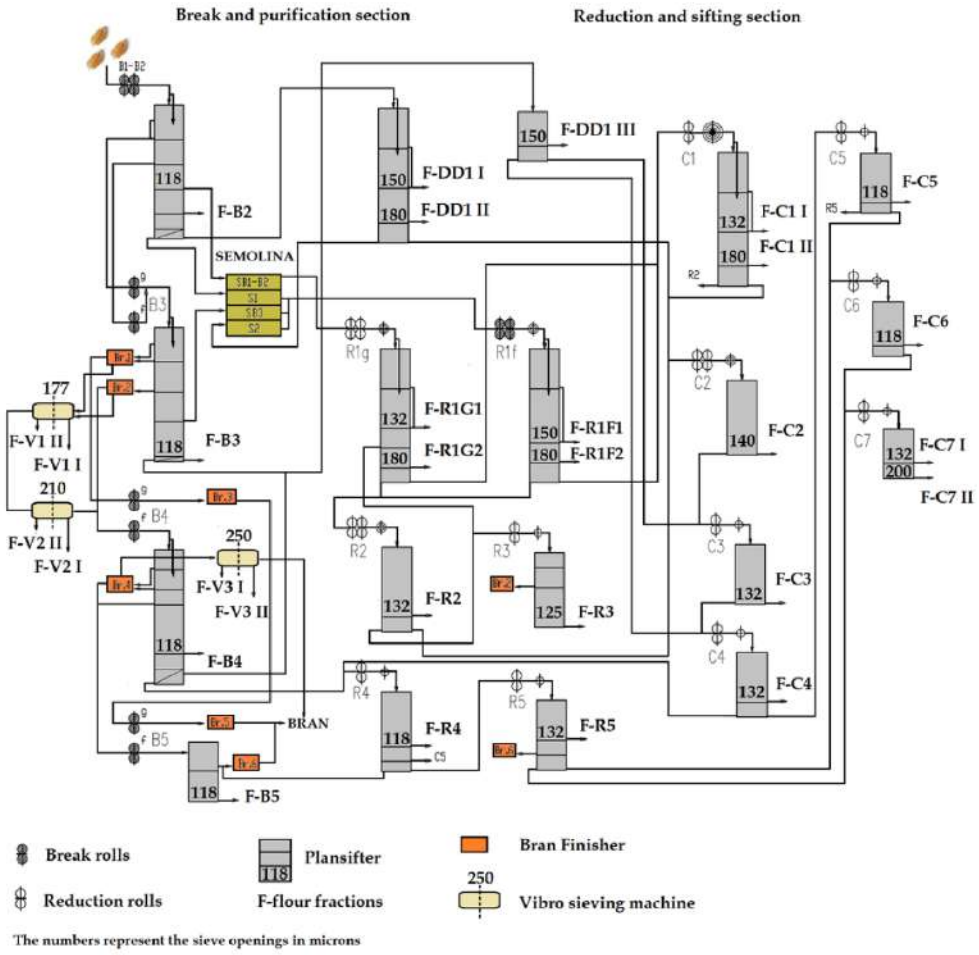
3.1. Materiały badawcze

a) Odmiany pszenicy zwyczajnej

Surowcem doświadczalnym w ramach badań wstępnych było 15 odmian komercyjnych ziaren pszenicy ozimej uzyskanych z firmy PZZ Lubella (Lublin, Polska). Do testów wybrano następujące odmiany najczęściej dostarczane w roku 2020: Bonavita, Hondia, Expo, Kilimanjaro, Danubius, Patinas, Komandor, Pananonikus, Bertold i Laudis; a także do porównania z odmianmi uprawianymi w Polsce wybrano odmiany uprawiane na terenie Europy: Summit, Patwin, Hartline, Pueblo White i Ruta. Wszystkie ziarna spełniały wymagania jakościowe surowca do produkcji mąki makaronowej. Parametry jakościowe ziaren zostały przetestowane przy użyciu standardowych testów ziaren i potencjalnych szybkich testów reologicznych dla śruty pszennej, które można zastosować podczas procedur zakupu pszenicy.

b) Pasaże przemiałowe

Po testach laboratoryjnych poszczególnych odmian do przemiału wykorzystano odmianę pszenicy IS Laudis, charakteryzującą się wysoką zawartością polisacharydów nieskrobiowych [**P1**]. Pszenicę oczyszczono i kondycjonowano do 16% zawartości wilgoci, a następnie zmielono w młynie walcowym o skali przemysłowej (w PZZ LUBELLA GMW Sp. z o.o., Lublin, Polska) o przepustowości 11 800 kg/h przy wyciągu mąki 78%. Proces mielenia obejmował etapy rozdrabniania (pasaże śrutowe), redukcję (pasaże rozczynowe i wymiałowe), przesiewanie oraz sortowanie (odsiewacze płaskie i szczotkarki wibracyjne) i klasyfikację (odsiewacze kwadratowe). Schematyczny diagram etapów przemysłowego mielenia przedstawiono na rysunku 1 [**P1-Fig. 1**].



Rys. 1. Schematyczny diagram zastosowanej stacji mielenia przemysłowego [**P1-Fig. 1**]: Break Rolls - walce śrutowe, Reduction Rolls - walce rozczynowe i wymiałowe, Plansifter - odsiewacz płaski, F - flour fractions - mąki pasażowe, Bran Finisher - rzutnik otrębowy, Vibro sieving machine - szczotkarka wibracyjna.

Indeksy I i II w oznaczeniach pasaży na rys. 1 odnoszą się do mąk pasażowych uzyskanych w tej samej sekcji mielenia i odsiewania, różniących się granulacją. Indeks I odnosi się do mąki o drobniejszej granulacji (≤150 µm), natomiast indeks II – do mąki o większej granulacji (150–280 µm). W przypadku pasaży V1–V3, z każdej szczotkarki wibracyjnej pobrano dwie oddzielne frakcje (I i II) o podobnej wielkości cząstek, ale różnych parametrach fizykochemicznych, pochodzących z dwóch części sita szczotkarki. Po zakończeniu mielenia uzyskano 30 różnych pasaży mącznych, oznaczonych na rys. 1 jako „F - mąki pasażowe". W skład tych pasaży wchodziły: 4 pasaże śrutowe/rozdrabniające B (B2, B3, B4, B5), 17 pasaży wymiałowych C i rozczynowych R (C1I, C1II, C2, C3, C4, C5, C6, C7I, C7II, R1F1, R1F2, R1G1, R1G2, R2, R3, R4, R5), 3 pasaże sortujące DD (DD1I, DD1II, DD1III) oraz 6 pasaży mąk filtracyjnych V (V1I, V1II, V2I, V2II, V3I, V3II), jak przedstawiono na schemacie (rys.

1). Wszystkie próbki mąk pasażowych były pobierane oddzielnie i przechowywane w szczelnych plastikowych workach przed dalszymi analizami.

c) Nowoopracowana mąka (F - mąka bazowa)

Aby ocenić możliwości zagospodarowania dotychczas niewykorzystywanych frakcji w młynach produkujących głównie mąkę makaronową, opracowano mąkę pszenną o zwiększonej zawartości polisacharydów nieskrobiowych i arabinoksylanów, pozyskaną z wybranych pasaży rozczynowych, wymiałowych oraz filtracyjnych [**P1**]. Skład opracowanej mąki pszennej był następujący (%): białko 14,62 ± 0,06, tłuszcz 1,31 ± 0,01, popiół 0,72 ± 0,02, nierozpuszczalny błonnik pokarmowy (IDF) 3,94 ± 0,04, rozpuszczalny błonnik pokarmowy (SDF) 2,86 ± 0,02 i całkowity błonnik pokarmowy (TDF) 6,80 ± 0,03. Ponadto, skład polisacharydów opracowanej mąki był następujący: całkowita zawartość arabinoksylanów (T-AX) 1,91 ± 0,06, w tym 1,31 ± 0,04 frakcji nierozpuszczalnej (I-AX) i 0,60 ± 0,02 frakcji rozpuszczalnej (S-AX), oraz całkowita zawartość polisacharydów nieskrobiowych (T-NSP) 3,40 ± 0,00, w tym 2,06 ± 0,01 frakcji nierozpuszczalnej (I-NSP) i 1,34 ± 0,00 frakcji rozpuszczalnej (S-NSP).

d) Enzymy piekarnicze

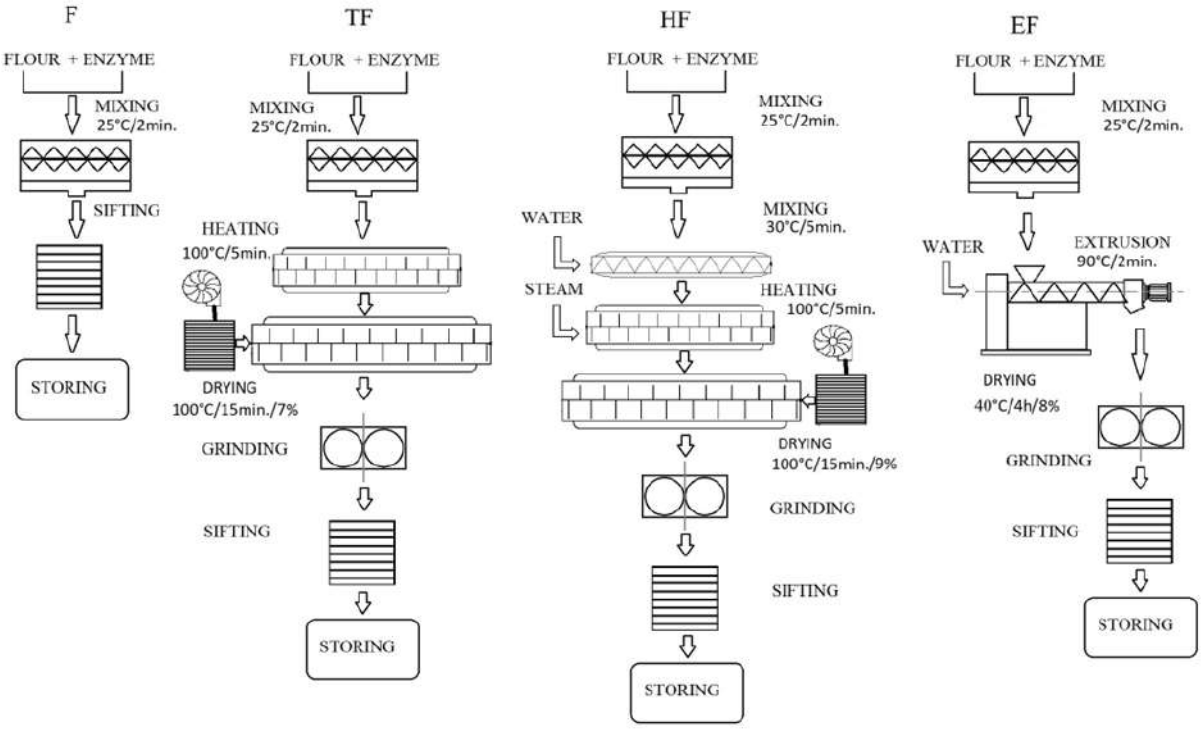
Do mąki w ramach modyfikacji enzymatycznej i hybrydowej dodawano komercyjne enzymy piekarnicze: C-Bakezyme® WholeGain - celulaza z *Trichoderma reesei* (DSM Food Specialities B.V., Holandia) o deklarowanej aktywności enzymu 1475 EGU/g (± 5%) i X-VERON 292 - ksylanaza z *Aspergillus niger* (AB Enzymes GmbH, Niemcy) o deklarowanej aktywności enzymu min. 1701 XylH/g.

3.2. Przeprowadzone procesy modyfikacji

3.2.1. Modyfikacja enzymatyczna

Mąkę pszenną (F) o wilgotności 14% przygotowano z zastosowaniem dodatku 120 ppm sproszkowanej celulazy (FC) lub z mieszaniną 50+60 ppm kompleksu celulazy-ksylanazy (FCX). Składniki mieszano przez 2 minuty w temperaturze pokojowej przy użyciu laboratoryjnego mieszalnika wstęgowego (Konstal - Zakład Mechaniczny CNC Zbigniew Własiuk, Lublin, Polska), pozostawiono na 2 godziny w temp. pokojowej przed pomiarami do

zainicjowania aktywności enzymów i przesiano do uzyskania jednolitej wielkości cząstek (rys. 2).



Rys. 2. Schemat zastosowanych modyfikacji mąki [**P4-Fig. 1**].

3.2.2. Modyfikacja termiczna

Obróbkę termiczną bez dodatku wody (T) przeprowadzono dla mąki pszennej (TF) oraz mąki z dodatkiem enzymów (TFC i TFCX) przy użyciu prototypowej instalacji do obróbki mąki należącej do PZZ LUBELLA GMW Sp. z o.o. w Lublinie. Schemat i warunki procesu przedstawiono na rysunku 2. W trakcie 5-minutowej obróbki termicznej, próbki o wilgotności 14% podgrzewano wstępnie w układzie ślimakowym prekondycjonera w temperaturze 30°C w celu aktywacji enzymów piekarniczych. Następnie mąkę transportowano do kondycjonera (bębna z obracającym się wirnikiem łopatkowym) przy temperaturze płaszcza grzewczego ustawionej na 100°C. Temperatura produktu podczas testów nie przekraczała 50°C. Wydajność procesu wynosiła 650 kg produktu na godzinę. Po obróbce termicznej lub hybrydowej obróbce termiczno-enzymatycznej mąkę suszono w suszarni z olejowym płaszczem grzewczym, przy wykorzystaniu gorącego powietrza o temperaturze 100°C, aby zatrzymać aktywność enzymów i osiągnąć końcową wilgotność mąki 7%. Następnie mąkę przesiewano za pomocą odsiewacza kwadratowego (Toruńskie Zakłady Urządzeń Młyńskich Spomasz S.A., Toruń, Polska), a 200 kg produktu pobierano do dalszych badań.

3.2.3. Modyfikacja hydrotermiczna

Podczas modyfikacji hydrotermicznej (H) opracowana mąka bazowa (HF) i mąka z dodatkiem enzymów (HFC i HFCX), przygotowana zgodnie z procedurą przedstawioną na rys. 2, była przetwarzana w prototypowej instalacji (należącej do PZZ LUBELLA GMW Sp. z o.o. w Lublinie). Porcje mąki wymieszane przez 2 minuty z enzymami przeniesiono do jednoślimakowego prekondycjonera o temp. 30°C w celu aktywacji enzymów piekarniczych. Dodawano 20 l/h wody i przetransportowano do kondycjonera (bębna z obracającym się wirnikiem łopatkowym) dodatkowo ogrzewając wtryskiem pary wodnej przez 5 minut przy ustawionej temperaturze płaszcza grzewczego 100°C, aby osiągnąć temperaturę produktu mierzoną podczas testów nieprzekraczającą 65°C. Następnie produkt suszono w suszarni z olejowym płaszczem grzewczym, z użyciem gorącego powietrza o temperaturze 100°C, aby zatrzymać aktywność enzymów i osiągnąć końcową wilgotność 9%. Wydajność prototypowej instalacji z wtryskiem pary wynosiła 650 kg/h. Próbki zmielono i przesiano za pomocą odsiewacza kwadratowego (Toruńskie Zakłady Urządzeń Młyńskich Spomasz S.A., Toruń, Polska) w celu dokładnego wymieszania materiału i usunięcia agregatów. Do kolejnych testów zebrano 200 kg mąki modyfikowanej hydrotermicznie lub hybrydowo.

3.2.4. Modyfikacja ekstruzyjna w niskiej temperaturze - ekstruder jednoślimakowy

Testy ekstruzji i hybrydowej ekstruzji wspomaganej enzymami przeprowadzono przy użyciu prototypowego jednoślimakowego ekstrudera EXP-45-32 (Zamak Mercator, Skawina, Polska) z matrycą formującą 3 mm. Zastosowano dwie wersje ekstrudera w różnej konfiguracji układu plastyfikującego o L/D = 16:1 i L/D = 20:1. Zastosowano konwencjonalny ślimak o ciągłym uzwojeniu w wersji krótszej oraz zaprojektowany i wykonany ślimak z elementami mieszającymi (zwój z nacięciami na ślimacznicy rozmieszczonymi naprzemiennie z ciągłym zwojem) umieszczonym na trzech czwartych długości ślimaka w wersji dłuższej (L/D = 20:1). Podczas badania zastosowano trzy zmienne: poziom dowilżenia mąki, prędkość obrotową ślimaka i dawkę enzymu. Nowoopracowaną mąkę pszenną (F) wymieszano na sucho z dodatkiem enzymu ksylanazy w ilościach 50 i 100 ppm. Następnie mieszanki dowilżono, aby uzyskać zawartość wody w mieszance wynoszącą 23, 25 i 27%, pozostawiono na 2 godziny w temp. pokojowej w celu aktywacji enzymu i przesiano, aby zapewnić jej ujednorodnienie i odpowiednie uwodnienie. Dowilżone próbki poddano następnie ekstruzji niskotemperaturowej przy prędkościach ślimaka 40, 60 i 80 obr./min w temperaturach od 40 do 80°C w poszczególnych strefach ekstrudera. W wersji L/D = 16 użyto czterech sekcji,

przy czym ustawione temperatury zaczynające się od strefy podawania wynosiły 30, 40, 60 i 80°C, podczas gdy w wersji L/D = 20 użyto pięciu sekcji, przy czym nastawy temperatury zaczynające się od strefy podawania wynosiły 30, 40, 60, 80 i 80°C. Temperatura matrycy formującej wynosiła 80°C. Temperatury i prędkości ślimaka utrzymywano na stałym poziomie podczas poszczególnych eksperymentów przy każdym zmiennym ustawieniu. Ekstruder jednoślimakowy EXP-45-32 jest wyposażony w precyzyjny system ogrzewania/chłodzenia każdej sekcji cylindra, dzięki czemu kontrola temperatury była bardzo stabilna. Próbki pobierano po ustabilizowaniu procesu, co najmniej 30 minut po zmianie parametrów. Uzyskane ekstrudaty cięto za pomocą układu tnącego podłączonego do ekstrudera, suszono w laboratoryjnej suszarce półkowej w temperaturze 40°C do końcowej zawartości wilgoci poniżej 9% i mielono na młynie nożowym LMN-100 (TestChem, Radlin, Polska) na proszek o wielkości cząstek poniżej 300 µm. Końcową wilgotność badano metodą suszarkową.

Rys. 3. Ekstruder jednoślimakowy z modułowym układem plastyfikującym (L/D = 16 lub L/D = 20) (*opracowanie własne*).

3.2.5. Modyfikacja ekstruzyjna w niskiej temperaturze - ekstruder dwuślimakowy

Modyfikację mąki metodą ekstruzji bez (EF) lub z różnymi kombinacjami enzymów (EFC i EFCX) przeprowadzono przy użyciu współbieżnego dwuślimakowego ekstrudera Evolum25 (Clextral, Firminy, Francja) o konfiguracji L/D = 24 ze ślimakami o średnicy 25 mm i matrycą o średnicy 3 mm (rys. 4). Konfiguracja ślimaków składała się z 32 modułów umieszczonych na rowkowanym wale o łącznej długości 24 D, z modułami zasilającymi 3,75D,

modułami transportowymi 12,5D, elementami mieszającymi 1,5D, elementami mieszającymi z rowkowaniem 2D, modułami kompresyjnymi 4D i pierścieniem dystansowym 0,25D, złożonymi w określonej kolejności. Prędkość podawania utrzymywano na poziomie 10 kg/h za pomocą wolumetrycznego podajnika grawitacyjnego Brabender (Duisburg, Niemcy), podczas gdy prędkość ślimaka ustawiono na 400 obr./min przez cały czas trwania prób. Poziom dowilżenia wsadu ustalono na 23, 25 i 27%, obliczony na podstawie początkowej wilgotności mąki, podczas gdy woda w odpowiednich ilościach (odpowiednio 1,2, 1,5 i 1,8 l/h) była pompowana przez pompę wodną bezpośrednio do drugiej strefy cylindra ekstrudera. Podczas procesu ekstruzji monitorowano temperatury w poszczególnych sekcjach ekstrudera, temperaturę produktu i ciśnienie robocze wewnątrz cylindra. Ekstruder wykorzystuje elektryczne grzałki oporowe oraz układ wężownic do grzania/chłodzenia sześciu stref monitorowanych przez termopary. Temperaturę przetwarzania ustawiono następująco: 40/50/60/65/70/80°C, temperaturę matrycy formującej ustawiono na 85°C, mierzona temperatura produktu wynosiła 82 - 95°C (wyższa przy niższej wilgotności wsadu), mierzone ciśnienie na matrycy wynosiło 80 - 102 bar (malejące przy wyższej wilgotności wsadu). Ekstrudaty cięto nożem dwuostrzowym pracującym z prędkością 300 obr./min. Próbki pobierano, gdy ekstruder pracował stabilnie. Ekstrudaty uzyskane po ekstruzji suszono w temperaturze 40°C w laboratoryjnej suszarce półkowej, aby zapewnić bezpieczne przechowywanie poniżej 8% wilgotności, a następnie mielono w młynku laboratoryjnym (TestChem, Radlin, Polska) do wielkości cząstek poniżej 300 µm i przechowywano do dalszych analiz.

Rys. 4. Ekstruder dwuślimakowy EVOLUM25 firmy Clextral (*opracowanie własne*).

3.3.Metody badawcze

3.3.1. Wilgotność metodą suszarkową

Wilgotność mąki bazowej i mąk modyfikowanych [**P1, P3, P4**] określono wg PN-ISO 712:2002 za pomocą referencyjnej metody suszarkowej z wykorzystaniem suszarki laboratoryjnej typu KBC-G-100/250 (POL-EKO-APARATURA Sp. J., Wodzisław Śląski). Próbki o masie 5 g umieszczono w naczynkach wagowych i suszono przez 1 godzinę w temperaturze 130°C. Wilgotność obliczono za pomocą odpowiedniego wzoru:

$$W = \frac{a-b}{a-c}\ x\ 100\ [\%] \quad (1)$$

gdzie: W – wilgotność materiału [%],

a – masa naczynka z próbką przed suszeniem [g],

b – masa naczynka z próbką po suszeniu [g],

c – masa pustego naczynka [g].

Jako wynik końcowy przyjęto średnią arytmetyczną z uzyskanych wyników. Oznaczenia przeprowadzono w trzech powtórzeniach dla każdej z mąk przed modyfikacją oraz po procesach modyfikacyjnych. W przypadku rozdrobnionych ekstrudatów, pomiary wykonano 24 godziny po ekstruzji i suszeniu, po osiągnięciu temperatury otoczenia.

Aby osiągnąć zakładaną wilgotność materiału w procesie dowilżania przed ekstruzją, obliczenia przeprowadzono zgodnie ze wzorem podanym przez Jurgę [1985]:

$$x = \frac{Mx(W_c - W_m)}{100 - W_c}\ [\text{kg}] \quad (2)$$

gdzie: x- ilość wody, jaką należy użyć do dowilżenia materiału [kg],

M – masa materiału [kg],

W_c – zakładana wilgotność receptury [%],

W_m – faktyczna wilgotność surowców [%].

Obliczoną ilość wody mieszano z suchymi mąkami w mieszarce wstęgowej i pozostawiano w celu ujednorodnienia rozprowadzenia wody w całej masie próby, lub dozowano bezpośrednio do ekstrudera podczas ekstruzji dwuślimakowej.

3.3.2. Ocena twardości ziarniaków ziaren różnych odmian pszenic zwyczajnych

Urządzenie SKCS 4100 (SKCS 4100, Perten Instruments, Stockholm, Sweden) analizuje poszczególne ziarna, waży je, a następnie miażdży między zębatym wirnikiem a stopniowo zwężającą się szczeliną półksiężycową. Średnia twardość jest obliczana na podstawie danych uzyskanych z próbki 300 ziaren każdej odmiany [Cetiner i in. 2020]. System

SKCS 4100 klasyfikuje pszenicę zgodnie ze wskaźnikiem twardości (HI) określonym zgodnie z metodą AACC 55.31 [2010]. Dane podano jako średnie z trzech niezależnych analiz.

3.3.3. Zawartości popiołu całkowitego

Zawartość popiołu ogółem [**P1, P2, P3, P4, P5**] oznaczano wg PN-EN ISO 2171:2010/ AACC 08–01 [2010] przez mineralizację próbki w piecu muflowym w temp. 900°C przez 2 godziny. Dane podano jako średnie z trzech niezależnych analiz.

3.3.4. Zawartość mokrego glutenu

Zawartość glutenu i indeks glutenu [**P1**] oznaczono wg ICC 155 [2018] za pomocą urządzenia do mechanicznego wymywania glutenu Glutomatic 2200 (PerkinElmer Inc., Waltham, MA, USA). Indeks glutenu (GI) jest miarą proporcji mokrego glutenu, który nie przechodzi przez sito podczas wirowania. Dane podano jako średnie z trzech niezależnych analiz.

3.3.5. Stopień uszkodzenia skrobi

Oznaczenie uszkodzonej skrobi [**P1**] przeprowadzono wg PN-EN ISO 17715:2015-0) z użyciem analizatora SDmatic (Chopin Technologies, Francja), który mierzy absorpcję jodu z uszkodzonej mechanicznie skrobi w rozcieńczonej zawiesinie mąki (Ai%). Dane podano jako średnie z trzech niezależnych analiz.

3.3.6. Liczba opadania

Aktywność α-amylazy [**P1**] określono metodą Hagberga-Pertena wg ICC 107/1 [2018] za pomocą aparatu do liczby opadania Falling Number 1305 (PerkinElmer Inc., Waltham, MA, USA). Dane podano jako średnie z trzech niezależnych analiz.

3.3.7. Zawartość pentozanów ogółem, frakcji rozpuszczalnych i nierozpuszczalnych

Zawartość polisacharydów nieskrobiowych (NSP) [**P1, P3, P4**] oceniono metodą chromatografii gazowej według Englysta i Cummingsa [1984], zgodnie z AOAC 994.13 [2010]. Całkowita zawartość NSP (T-NSP) to ilość cukrów: arabinozy, ksylozy, mannozy, galaktozy i glukozy [AACC 2010, Herlich 1990]. Analiza ta pozwoliła na rozdzielenie polisacharydów nieskrobiowych na frakcje rozpuszczalne (S-NSP) i nierozpuszczalne (I-NSP)

oraz określenie składu jakościowego i ilościowego polisacharydów w obu frakcjach. Całkowitą zawartość arabinoksylanów (T-AX) oraz frakcji nierozpuszczalnych (I-AX) i rozpuszczalnych (S-AX) obliczono na podstawie zawartości każdej frakcji. Po kwasowej hydrolizie frakcji rozpuszczalnej i nierozpuszczalnej wykryto monosacharydy w każdej frakcji. Otrzymane hydrolizaty przekształcono w lotne octany alditolu. Do każdej próbki (1 ml) dodano 2 krople 2-oktanolu, 0,26–0,28 ml 12M roztworu amoniaku i 0,1 ml roztworu borowodorku sodu w amoniaku (100 mg BH_4 w 1 ml 3M NH_4OH). Po 40 minutach inkubacji w temperaturze 40°C do hydrolizatu dodano 0,1 ml lodowatego kwasu octowego, wymieszano, a następnie do 0,2 ml pobranej próbki dodano 0,2 ml 1-metyloimidazolu i 2 ml bezwodnika octowego. Przygotowany roztwór chłodzono przez 30 minut, następnie dodano 4 ml wody destylowanej i 1,15 ml dichlorometanu i wytrząsano przez 1 minutę. Fazę wodną usunięto, a fazę organiczną przeanalizowano na chromatografie gazowym Autosystem XL firmy Perkin Elmer (Shelton, CT, USA), wyposażonym w autosampler, rozdzielacz, detektor z płomieniową jonizacją (FID) i kapilarną kolumnę kwarcową Rtx-225 (0,53 mm × 30 m). Parametry pracy chromatografu: gaz nośny hel, przepływ 2 ml/min, temperatura wtryskiwacza 275°C, temperatura detektora 275°C. Program temperatury kolumny: temperatura początkowa 185°C, 1 minuta; wzrost 5°C/min do 215°C; izoterma 215°C, 10 minut [Fraś 2011]. Dane podano jako średnie z trzech powtórzeń przeprowadzonej analizy.

3.3.8. Ocena zdolności pochłaniania wybranych roztworów

Zdolność pochłaniania wybranych roztworów metodą Solvent Retention Capacity (SRC) [**P1, P3, P4**] AACC 56-11.02 [2010]. SRC to masa rozpuszczalnika zatrzymana przez spęczniały osad mąki po wirowaniu i jest wyrażana jako procent pierwotnej masy mąki (skorygowanej do 14% wilgotności). Rozpuszczalnikami były: woda demineralizowana, 50% wag. roztwór sacharozy w wodzie, 5% wag. roztwór kwasu mlekowego w wodzie, 5% wag. roztwór węglanu sodu w wodzie. Wyznaczono SRCWa jako pojemność retencji wody, SRCSu jako pojemność retencji rozpuszczalnika sacharozy, SRCLa jako pojemność retencji rozpuszczalnika kwasu mlekowego, SRCSc jako pojemność retencji rozpuszczalnika węglanu sodu). Próbkę mąki (5 ± 0,050 g) przeniesiono do 50 ml probówki wirówkowej i zmieszano z 25 g rozpuszczalnika [Guzmán i in. 2015]. Próbkę pozostawiono do solwatacji na 20 min, wstrząsając co 5 min przez 5 s. Następnie probówki wirowano przy 2500 obr./min przez 15 min. Supernatant zlano, a probówki pozostawiono do wyschnięcia na 10 min. Następnie próbkę zważono i obliczono SRC [Kweon i in. 2011]. Dodatkowo obliczono (wskaźnik wydajności glutenu) GPI zgodnie z opisem Vukić i in. [2020] w oparciu o metodę ICC 186 [2018], dzieląc

wartość SRCLa przez łączne wartości SRCSu i SRCSc. Dane podano jako średnie z trzech powtórzeń analizy.

3.3.9. Ocena farinograficzna

Właściwości reologiczne poszczególnych mąk [**P1, P4, P5**] określono przy użyciu standardowej procedury Farinograph wg ICC method 115/1, [Wang i in. 2017]. Analizowano absorpcję wody (WA) (% wody potrzebnej do uzyskania konsystencji ciasta 500 BU), czas rozwoju ciasta (DT) (czas do osiągnięcia konsystencji 500 BU), DoS — stopień rozmiękczenia ciasta, liczbę jakości (QN) i stabilność ciasta (S). Dane podano jako średnie z trzech niezależnych analiz.

3.3.10. Ocena alweograficzna

Do oceny parametrów reologicznych [**P1, P3, P4, P5**] zastosowano standardową metodę wg ICC 121 [2018] z użyciem Alweografu (AlveoLab, Chopin Technologies, Francja). W trakcie analizy oceniano wartość wypiekową (W) jako powierzchnię pod krzywą równą pracy potrzebnej do odkształcenia 1 g ciasta wyrażoną w 10^{-4}J, sprężystość ciasta (P) wyrażoną w mm jako maksymalna wytrzymałość ciasta na odkształcenie, rozciągliwości ciasta (L) wyrażoną w mm jako długość wykresu, będącą punktem pękania pęcherzyka, indeks elastyczności (Ie) wyrażony w % jako stosunku ciśnienia w pęcherzyku ciasta po wprowadzenia 200 ml powietrza do sprężystości ciasta P [Codină i in. 2010] oraz wskaźnika utwardzenia odkształceniowego (SH) [Jødal i in. 2021]. Dane podano jako średnie z trzech niezależnych analiz.

3.3.11. Ocena właściwości reologicznych przy zmiennej temperaturze

Właściwości reologiczne ciasta [**P1, P2, P3, P4, P5**] badano przy użyciu urządzenia Mixolab 2 (Chopin Technologies, Francja) opartego na protokole Chopin+ (wg ISO 17718-1:2013) z następującymi ustawieniami: prędkość mieszania 80 obr./min, całkowity czas analizy 45 min, masa ciasta 75 g, temperatura wody do analizy 30°C. Mąkę i wodę dodawano odpowiednio, aby uzyskać ciasto o maksymalnej konsystencji 1,10 Nm (± 0,05) podczas pierwszej fazy testu. Test Mixolab przeprowadzono przy użyciu standardowego protokołu: 8 min w 30°C, ogrzewanie przez 15 min z szybkością 4°C/min, utrzymywanie w 90°C przez 7 min, chłodzenie przez 10 min do 50°C z szybkością 4°C/min i utrzymywanie w 50°C przez 5 min [Szafrańska i in. 2015]. Następujące cechy reologiczne zostały wyznaczone: absorpcja wody (WA), osłabienie białka (C2), kleikowanie skrobi (C3), aktywność amylazy (C4),

retrogradacja skrobi (C5), nachylenie α - między końcem okresu ogrzewania w 30°C a C2 jako szybkość osłabiania białka pod wpływem temperatury, nachylenie β - między punktami C2 a C3 jako wskaźnik szybkości kleikowania, nachylenie γ - między C3 i C4 jako szybkość degradacji enzymatycznej (α - amylazy) [Dubat 2010]; cechy dodatkowe: C2–C1 - poziom osłabienia białka glutenowego, C3–C2 - zakres kleikowania skrobi, C4–C3 - poziom stabilności w temperaturze 90°C i C5–C4 - poziom żelowania skrobi w trakcie chłodzenia [Dubat i in. 2013]. Dane podano jako średnie z trzech niezależnych analiz.

3.3.12. Badanie wydajności procesu ekstruzji

Wydajność procesu ekstruzji [**P2**] została oceniona poprzez zmierzenie masy ekstrudatu wytworzonego w określonym czasie dla każdej z badanych mąk i parametrów procesu [Wójtowicz i in. 2023]. Wydajność została obliczona według następującego wzoru:

$$Q = \frac{m}{t} \text{ [kg/h]} \quad (3)$$

gdzie: Q – wydajność ekstrudera [kg/h],

m – masa ekstrudatu, który uzyskano w trakcie pomiaru [kg],

t – czas pomiaru [h].

Badania przeprowadzono w 5 powtórzeniach dla każdej serii, a jako wynik przyjęto średnią wartość z uzyskanych pomiarów.

3.3.13. Wyznaczanie energochłonności procesu ekstruzji

Energochłonność procesu ekstruzji [**P2**] poszczególnych mąk została określona poprzez obliczenie wskaźnika jednostkowego zapotrzebowania na energię mechaniczną (SME - ang. specific mechanical energy). Pobór mocy rejestrowano za pomocą wbudowanego oprogramowania, uwzględniając parametry silnika ekstrudera, takie jak obciążenie i wydajność procesu ekstruzji w poszczególnych próbach i wyznaczono wg wzoru [Stojceska i in. 2008, Lisiecka i in. 2020]:

$$SME = \frac{n}{n_m} x \frac{O}{100} x \frac{P}{Q} \text{ [kWh/kg]} \quad (4)$$

gdzie: SME- wskaźnik specyficznego zużycia energii mechanicznej [kWh/kg],

n – obroty ślimaka [obr./min],

n_m – obroty znamionowe ślimaka [obr./min],

O – obciążenie silnika w stosunku do maksymalnego [%],

P – moc znamionowa [kW],

Q – wydajność procesu [kg/h].

Energochłonność procesu obliczono na podstawie trzech powtórzeń dla różnych uzyskanych ekstrudatów przy założonych warunkach obróbki ciśnieniowo-termicznej, a za wynik końcowy przyjęto średnią z tych pomiarów.

3.3.14. Pomiar zawartości białka

Analizę zawartości białka [**P3, P4, P5**] wykonano zgodnie z metodą Kiejdahla wg AACC 46-10 [2010], stosując przelicznik azotu na białko ×6,25. Dane podano jako średnie z trzech niezależnych analiz.

3.3.15. Pomiar zawartości tłuszczu

Analizę zawartości tłuszczu [**P3, P4, P5**] przeprowadzono metoda Soxhleta zgodnie z AACC 30–10 [2010], polegającej na ekstrakcji tłuszczu z próby z użyciem eteru naftowego. Dane podano jako średnie z trzech niezależnych analiz.

3.3.16. Pomiar zawartości rozpuszczalnych (SDF) i nierozpuszczalnych (IDF) frakcji oraz całkowitej ilości błonnika (TDF)

Całkowitą zawartość błonnika pokarmowego (TDF) i jego frakcje (rozpuszczalny SDF i nierozpuszczalny IDF) [**P3, P4, P5**] oznaczono metodą enzymatyczno-wagową zgodnie z normą AACC 32-07 [2010].

3.3.17. Pomiar właściwości amylograficznych

Właściwości kleikowania [**P3, P4**] zgodnie z procedurą ICC169 [2018] oceniono na urządzeniu Brabender Viscograph-E (Brabender GmbH & Co., Niemcy) pracującym z prędkością 75 obr./min i momentem obrotowym 700 cmg. Wymieszano 80 g mąki (obliczonej do zawartości wilgoci 14%) i 450 ml wody destylowanej, przygotowaną zawiesinę umieszczono w komorze grzewczej i przymocowano wrzeciono. Profil grzania/chłodzenia był następujący: grzanie od 30°C do 93°C z szybkością 1,5°C/min, utrzymywanie w 93°C przez 15 min, chłodzenie do 50°C z szybkością 3°C/min i końcowe utrzymywanie w 55°C przez 15 min. Lepkość rejestrowano jako opór przy mieszaniu. Następujące charakterystyki kleikowania uzyskano za pomocą oprogramowania Viscograph-E: lepkość maksymalna (ang. maximum viscosity), lepkość na końcu procesu termostatowania (ang. through viscosity), lepkość końcowa (ang. final viscosity), zmniejszenie lepkości w czasie kleikowania (ang. breakdown), wzrost lepkości podczas chłodzenia (ang. setback), a także temperatury początku (ang.

beginning of gelatinization) i końca kleikowania (ang. end of gelatinization). Dane podano jako średnie z trzech niezależnych analiz.

3.3.18. Analiza struktury za pomocą dyfrakcji rentgenowskiej

Próbki w proszku kondycjonowano przez 3 dni w kontrolowanej temperaturze (19°C) i stałej wilgotności względnej powietrza (28%), a następnie umieszczono w autosamplerze. Do analizy struktury [**P3, P4**] zastosowano metodę dyfrakcji rentgenowskiej XRD (X-ray diffraction) wykorzystującą wysokorozdzielczy dyfraktometr proszkowy Empyrean (PANalytical, Almelo, Holandia) z promieniowaniem Cu Kα1 (λ = 1,54178 Å). Próbki mierzono w zakresie od 5 do 70° przy geometrii kąta dyfrakcji θ–2θ, z czasem zliczania 400 s na punkt danych i rozmiarem kroku 0,01° [Tomaszewska i in. 2021]. Uzyskano wartości bazowe, a różnice w strukturze analizowano przy określonych kątach pików. Krystaliczność określono przy użyciu oprogramowania WAXFIT [Combrzyński i in. 2021]. Wszystkie dane znormalizowano, a tło aproksymowano funkcją hiperboliczną. Dane dotyczące mąk natywnych dopasowano do modelu Gaussa–Cauchy'ego zawierającego 15 funkcji opisujących fazę krystaliczną i 2 funkcje opisujące fazę amorficzną. Dane dotyczące mąk ekstrudowanych dopasowano do modelu Gaussa–Cauchy'ego zawierającego 13 funkcji opisujących fazę krystaliczną i 2 funkcje opisujące fazę amorficzną. Stopień krystaliczności (%) obliczono za pomocą oprogramowania WAXFIT po dopasowaniu modeli i skorygowaniu tła jako stosunku powierzchni pod krzywymi fazy krystalicznej do sumy faz krystalicznej i amorficznej [Rabiej 2017, Yoo i in. 2002].

3.3.19. Analiza mikrostruktury z użyciem skaningowego mikroskopu elektronowego

Mikrostrukturę mąk natywnych i modyfikowanych [**P3, P4**] obserwowano za pomocą skaningowego mikroskopu elektronowego Vega Tescan LMU (Tescan, Czechy). Próbki sproszkowane montowano na aluminiowych stolikach za pomocą dwustronnej taśmy srebrowej i natryskiwano złotem za pomocą Sputter Coater Emitech K550X (Emitech, Wielka Brytania). Napięcie przyspieszające SEM wynosiło 20 keV, a współczynnik absorpcji 9 pA. Zdjęcia SME wykonano przy powiększeniach ×600 i ×2000 [Bouasla i in. 2016].

3.3.20. Pomiar współrzędnych barwy

Aby ocenić charakterystykę barwy miękiszu i skórki chleba 24 h po upieczeniu [**P5**], użyto kolorymetru NH310 (3NH TECHNOLOGY CO Ltd., Chiny). Współrzędne barwy

badano zgodnie z systemem CIE-Lab, gdzie L^* opisuje jasność w zakresie od 0 (czarny) do 100 (biały), współrzędna chromatyczna a^* jako balans między czerwienią (wartości dodatnie) i zielenią (wartości ujemne), a współrzędna chromatyczna b^* jako balans między żółcią (jeśli dodatnia) i błękitem (jeśli ujemna) [Jurkaninová i in. 2024]. Końcowe wartości współrzędnych L^*, a^* i b^* miękiszu chleba i skórki wyrażono jako średnie z co najmniej 5 pomiarów każdego wyznacznika barwy z 3 indywidualnych bochenków chleba. ΔE obliczono jako całkowitą różnicę barwy [Jurkaninová i in. 2024]. Przed każdym pomiarem kolorymetr kalibrowano przy użyciu białej płytki kalibracyjnej dostarczonej z urządzeniem.

3.3.21. Określenie cech jakościowych pieczywa

Objętość chleba (ml) [**P5**] badano metodą wypierania nasion rzepaku zgodnie z normą AACC 10-05 [2010], stosując znaną objętość/masę nasion rzepaku zastąpionych bochenkiem chleba i obliczaną jako objętość chleba do masy chleba [Cingöz i in. 2024].

Gęstość chleba (g/cm^3) [**P5**] obliczano jako stosunek masy do objętości pojedynczego bochenka [Cacak-Pietrzak i in. 2021].

Stratę wypiekową (%) [**P5**] oceniano jako różnicę masy ciasta i bochenka bezpośrednio po upieczeniu do właściwej masy ciasta [Zhang i in. 2021].

Utratę masy (%) [**P5**] sprawdzano jako różnicę masy gorącego chleba tuż po upieczeniu i po 24 godzinach przechowywania [Cacak-Pietrzak i in. 2021].

Wydajność chleba (%) [**P5**] obliczano jako stosunek masy ciasta pomnożonej przez wydajność ciasta do masy zimnego chleba po upieczeniu [Ambrosewicz-Walacik i in. 2016]. Dane podano jako średnie z trzech niezależnych eksperymentów.

3.3.22. Wypiek laboratoryjny metodą jednofazową

Próbka kontrolna chleba (K) została przygotowana bez dodatku modyfikowanych mąk. Przygotowanie chleba kontrolnego wyglądało następująco: mąkę pszenną chlebową typu 750 zmieszano z 2% soli i 3% drożdży, a następnie dodano wodę, aby uzyskać konsystencję ciasta 400 BU [**P5**]. Ciasto chlebowe przygotowano metodą bezpośrednią jednoetapową [Jurkaninová i in. 2024] z niewielkimi modyfikacjami [**P5**]. Aby przygotować testowane chleby, zwykłą mąkę pszenną chlebową zastąpiono opracowaną mąką (F), mąkami modyfikowanymi enzymatycznie (FC, FCX), a także mąkami modyfikowanymi termicznie, hydrotermicznie i hybrydowo - wspomaganymi enzymatycznie (TF, TFC, TFCX i HF, HFC, HFCX, odpowiednio) w ilościach 10 i 20% (w/w). Wszystkie składniki mieszano w mikserze laboratoryjnym przez 6 min (JMP12, Fimar Food Processing Equipment, Vericchio, Włochy),

przygotowane ciasto podzielono na kawałki o masie 300 g umieszczono w foremkach na chleb (ok. 10×10×10 cm) i poddano fermentacji w temperaturze 30°C przy 75% wilgotności względnej (RH) przez 50 minut w komorze garwoniczej (MIWE US 2.0, Arnstein, Niemcy) kontrolowanej przez automatyczny system kontroli temperatury i wilgotności z dokładnością 1°C i 1% RH dostarczony z urządzeniem. Po fermentacji chleb wypiekano w temperaturze 210/200/190/210°C przez 30 s/2 min/20 min/3,5 min – łącznie 26 minut w piecu MIWE AERO backcombi (Arnstein, Niemcy). Po umieszczeniu bochenków w piecu, wprowadzano parę przez 30 s w ilości 0,08 l. Temperaturę wewnątrz pieca do wypieku kontrolowano za pomocą automatycznego systemu kontroli temperatury z dokładnością do 1°C dostarczonego przez producenta. Po upieczeniu bochenki wyjmowano z foremek i ważono. Następnie chleb schładzano przez 1 godzinę i ponownie ważono, pakowano w torby polietylenowe i przechowywano w temperaturze 21°C przed testami. Wszystkie procedury powtarzano trzykrotnie dla każdej receptury.

3.3.23. Wyznaczenie wskaźnika absorpcji wody WAI i wskaźnika rozpuszczalności wody WSI

Wskaźnik absorpcji wody WAI (ang. water absorption index) i wskaźnik rozpuszczalności w wodzie WSI (ang. water solubility index) w pieczywie [**P5**] badano zgodnie z Soja i in. [2023] metodą wirówkową. WAI wyrażono jako g wody wchłoniętej przez g chleba. WSI wyrażono jako % składników rozpuszczalnych w wodzie po badaniu WAI. Pomiary wykonano w 3 powtórzeniach, za wynik przyjęto średnią arytmetyczną z pomiarów.

3.3.24. Ocena cech tekstury pieczywa

Właściwości teksturalne chleba kontrolnego i pieczywa przygotowanego z dodatkiem mąk modyfikowanych [**P5**] określono metodą TPA w teście podwójnego ściskania. Test przeprowadzono trzykrotnie przy użyciu urządzenia ZwickRoell BDO-FB0.5TH (Zwick GmbH & Co., Ulm, Niemcy) zgodnie z protokołem TPA z oprogramowaniem testXpert®13.3. Próbki chleba wycinano ze środkowej części miękiszu (3×3×1 cm). Do testów wykorzystano komorę Ottawa, przy prędkości roboczej głowicy 100 mm/min w przeprowadzonym teście podwójnego ściskania do 50% wysokości próbki i 10-sekundowym odstępie między cyklami. Przeanalizowano krzywe TPA, a właściwości teksturalne oceniono jako wartości średnie z 5 powtórzeń. Określono następujące cechy: jędrność jako najwyższy pik w pierwszym cyklu ściskania, adhezję jako pracę potrzebną do oddzielenia miękiszu od tłoka, sprężystość jako odległość wykrytej wysokości podczas drugiego cyklu ściskania podzieloną przez pierwotną

odległość ściskania, gumiastość i żujność obliczone na podstawie jędrności, spójności i sprężystości oraz spójność jako obszar pod wykresem podczas drugiego cyklu ściskania podzielony przez obszar podczas pierwszego ściskania [Nishinari i in. 2018].

3.4. Analiza statystyczna wyników

3.4.1. Jednoczynnikowa ANOVA - test post hoc - test fishera

Dane z wielokrotnych pomiarów poddano jednokierunkowej analizie wariancji (ANOVA) [**P1**] przy użyciu oprogramowania Statistica 13.3 (StatSoft, Inc., Tulsa, OK, USA), a następnie testowi post hoc najmniejszej istotnej różnicy Fishera (LSD) w celu porównania średnich na poziomie istotności 0,05

3.4.2. Jednoczynnikowa ANOVA - test post hoc - test Tukeya

Uzyskane dane poddano jednokierunkowej analizie wariancji (ANOVA) [**P2, P3, P4, P5**] za pomocą oprogramowania Statistica 13.3 (StatSoft, Inc., Tulsa, OK, USA), a następnie testowi post hoc Tukeya w celu porównania średnich na poziomie istotności 0,05.

3.4.3. Macierz korelacji Pearsona

Współczynniki korelacji Pearsona [**P1, P2, P3, P4, P5**] zostały zastosowane do oceny korelacji między badanymi właściwościami przy użyciu oprogramowania Statistica 13.3 (StatSoft, Inc., Tulsa, USA) w 95% przedziale ufności.

3.4.4. Metoda powierzchni odpowiedzi RSM

Uzyskane wyniki z procesu ekstruzji i właściwości reologicznych modyfikowanych mąk pszennych [**P2**] poddano analizie metodą powierzchni odpowiedzi RSM (ang. Response Surface Methodology) przy użyciu zmiennych wejściowych. Wyniki testów analizowano za pomocą RSM, wybierając niezależne czynniki poziomu dowilżenia, prędkości obrotowej ślimaka i dozy enzymu oraz ich interakcje, oddzielnie dla konfiguracji ekstrudera L/D = 16 i L/D = 20. Zastosowano RSM z dopasowaniem kwadratowym i utworzono modele dla każdej kombinacji niezależnych czynników. Dla każdej konfiguracji ekstrudera równania regresji drugiego rzędu zastosowano niezależnie, przy użyciu programu Statistica 13.3 (Statsoft, Tulsa, USA):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11}(X_1)_2 + \beta_{22}(X_2)_2 + \beta_{33}(X_3)_2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (5)$$

gdzie: Y jest wskazanym współczynnikiem odpowiedzi, *β_0* reprezentuje wartość stałą, *β_i* reprezentuje współczynnik liniowy, *β_ij* gdzie i = j reprezentuje współczynnik kwadratowy, a *β_ij* gdzie i ≠ j reprezentuje współczynnik interakcyjny. X_1, X_2 i X_3 reprezentują zmienne wejściowe poziomu dowilżenia (M), prędkości ślimaka (S) i dawki enzymu (E) i zostały zakodowane na poziomach −1, 0 i 1 dla każdego czynnika, odpowiednio. Wszystkie współczynniki zostały scharakteryzowane pod kątem istotności jako nieznacznie istotne ($p < 0,10$), istotne ($p < 0,05$) lub bardzo istotne ($p < 0,01$).

3.4.5. Analiza głównych składowych (PCA)

Oprogramowanie Statistica (wersja 12.0, StatSoft Inc., Tulsa, OK, USA) zastosowano do analizy głównych składowych PCA (ang. principal component analysis) [**P1, P5**] i określenia współczynników korelacji na poziomie istotności 0,05. Analiza głównych składowych została wykorzystana do określenia związku między poszczególnymi cechami mąki. Macierz wejściowa została automatycznie przeskalowana. Optymalną liczbę głównych składowych uzyskanych w analizie dla każdej macierzy określono na podstawie kryterium Cattela.

4. OMÓWIENIE NAJWAŻNIEJSZYCH REZULTATÓW

4.1. Badania wstępne

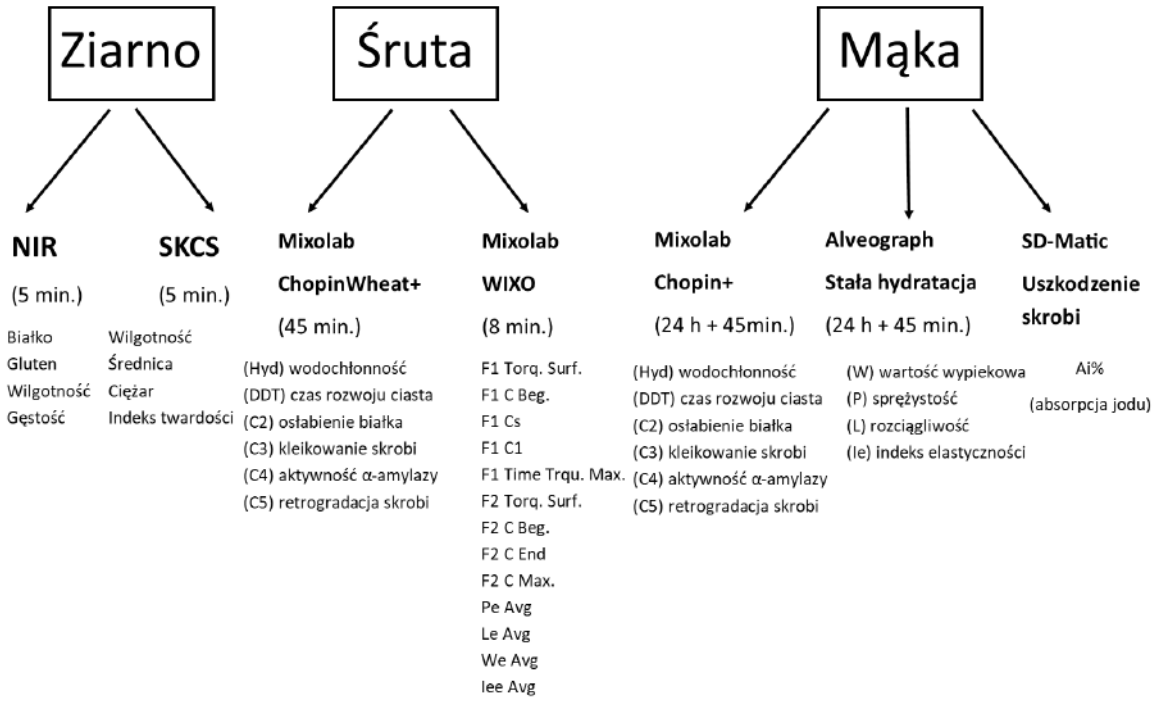
Najbardziej interesująca z punktu widzenia producenta w przemyśle zbożowo-młynarskim jest ocena cech jakościowych ziarna, które mogą charakteryzować się dużym poziomem zmienności w poszczególnych odmianach i w ten sposób negatywnie wpływać na oczekiwaną jakość uzyskiwanych produktów. W punktach skupu analizowanych jest tylko kilka kluczowych parametrów fizykochemicznych ziarna, które laboratorium przyzakładowe może wykonać w ciągu maksymalnie kilkunastu minut. Analizy te skupiają się głównie na ocenie kompleksu białkowo-skrobiowego ziarna. Technologiczna jakość mąki nie zależy jedynie od zawartości białka i właściwości skrobi, lecz wynika także z kompleksowych interakcji między makrocząsteczkami, które odpowiadają za jakość i wydajność produktów wytwarzanych z mąki pszennej [Marti i in. 2015]. Takimi składnikami są m.in. obecne w pszenicy nieskrobiowe polisacharydy, a szczególnie arabinoksylany. W warunkach skupu nie ma możliwości kontroli poziomu tych istotnych technologicznie składników.

W ramach badań wstępnych skoncentrowano się na analizie właściwości fizykochemicznych i reologicznych ziaren różnych odmian pszenicy zwyczajnej w odniesieniu do zawartości poszczególnych frakcji nieskrobiowych polisacharydów. Przebadano dziesięć odmian pszenicy krajowej oraz pięć odmian pszenicy uprawianej w Europie, uwzględniając ich skład chemiczny, kompozycję polisacharydów i ich frakcji oraz właściwości fizykochemiczne i reologiczne. Badania prowadzono zarówno dla ziarna, śruty pszennej, jak i mąki. Wykorzystano testy szybkościowe, jak i długotrwałe testy reologiczne, przeprowadzane na otrzymanych w przemiale laboratoryjnym mąkach z poszczególnych odmian (rys. 5).

Badania obejmowały określenie całkowitej zawartości arabinoksylanów (polisacharydów nieskrobiowych) oraz poziomów ich frakcji rozpuszczalnych i nierozpuszczalnych za pomocą chromatografii gazowej. Ta metoda, choć precyzyjna, jest kosztowna, czasochłonna i pracochłonna. W celu porównania skuteczności szybszych metod, możliwych do zastosowania w skupach ziarna do szacowania zawartości polisacharydów, zastosowano różne techniki statystyczne, w tym analizę korelacji i głównych składowych (PCA).

Pomimo zaobserwowanych różnic, znalezienie szybkiej metody oceny zawartości arabinoksylanów okazało się trudnym zadaniem. Nie stwierdzono zależności między

zawartością poszczególnych frakcji arabinoksylanów a wynikami standardowych oraz dodatkowych testów fizykochemicznych i reologicznych.



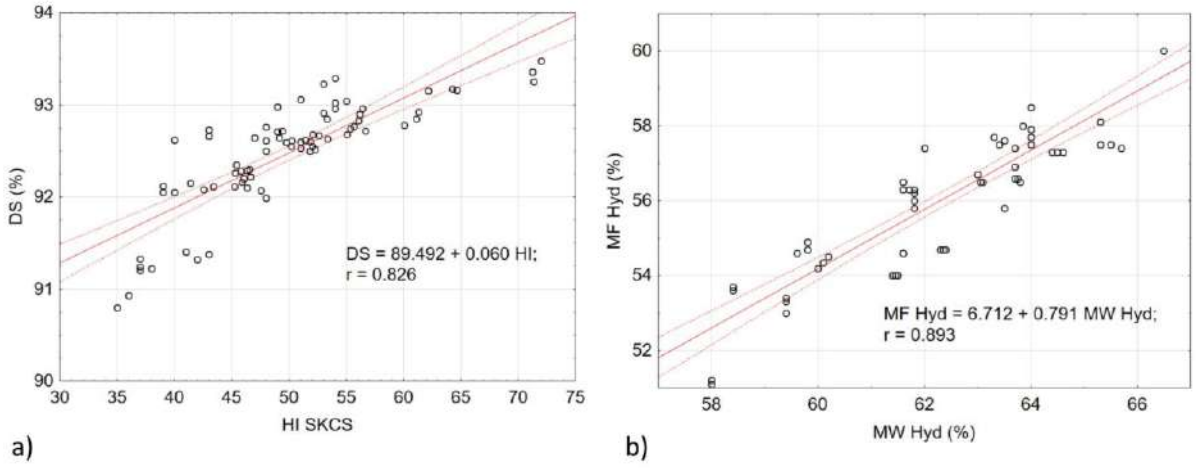
Rys. 5. Porównanie czasu analiz i możliwości wyznaczania poszczególnych cech jakościowych dla urządzeń wykorzystywanych w ocenie jakościowej różnych form pszenicy zwyczajnej w przedsiębiorstwach zbożowych (*opracowanie własne*).

Szereg przeprowadzonych testów pozwolił jednak udoskonalić procedurę oceny ziarna w warunkach skupu. Ocena ziarna lub zmielonych ziaren pszenicy znacznie skraca czas oczekiwania na wyniki długotrwałych testów reologicznych, zwłaszcza w odniesieniu do wielogodzinnych procedur analitycznych mąki otrzymywanej z poszczególnych partii ziarna, i może być przeprowadzona niemal natychmiast po dostarczeniu ziaren do magazynu lub młyna z użyciem szybkich testów. W oparciu o szybkie testy ziarna i śruty pszennej oraz z wykorzystaniem wyznaczonych współczynników korelacji i równań regresji można efektywniej zarządzać kierowaniem partii ziarna z przeznaczeniem na specjalistyczne cele technologiczne.

Najważniejsze rezultaty badań, jakie udało się otrzymać w trakcie badań wstępnych, to uzyskanie wysokiego współczynnika korelacji między indeksem twardości a uszkodzeniem skrobi. W przypadku urządzeń SKCS i SD-Matic analiza statystyczna wykazała istotną dodatnią korelację między twardością ziarna (HI) a uszkodzoną skrobią (DS) ($r = 0,83$ przy $p < 0,05$). Testy wykazały, iż wraz ze spadkiem twardości ziarna uzyskiwano mniejsze wartości

uszkodzenia skrobi w mąkach wytworzonych z tego ziarna (rys. 6a). Tak więc analiza ziarna na urządzeniu SKCS może być pomocna w skutecznym oszacowywaniu parametru uszkodzenia skrobi w wytwarzanej z tego ziarna mące, a tym samym w ocenie jakości ziaren pszenicy.

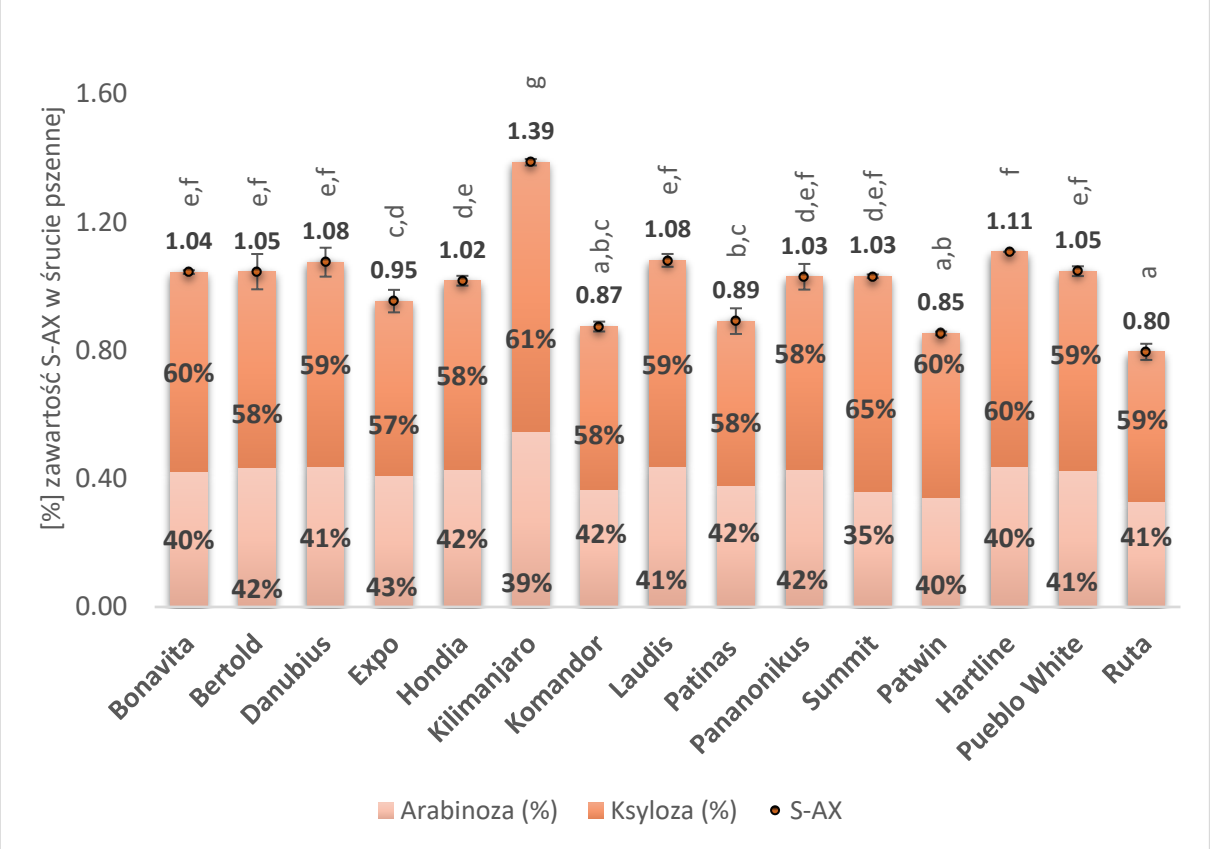
Zależność absorpcji wody śruty pełnoziarnistej w odniesieniu do mąki uzyskanej z tych samych próbek wykazała, iż w tym przypadku odnotowano wysoką istotną dodatnią korelację między wodochłonnością (Hyd) śruty pszennej (MW) i mąki (MF) ($r = 0,89$, przy $p < 0,05$) (rys. 6b). Ta szybka metoda może zatem wyeliminować potrzebę wykonywania przemiału laboratoryjnego dla partii ziarna w celu oceny wodochłonności mąki, parametru istotnego w wielu kierunkach technologicznego zastosowania.



Rys. 6. Wyniki korelacji pomiędzy: a) wskaźnikiem twardości (HI) ziaren pszenicy mierzonym urządzeniem SKCS i zawartością uszkodzonej skrobi (DS) w mące mierzoną urządzeniem SD-Matic; b) wodochłonnością (Hyd) śruty pszennej MW i mąki pszennej MF mierzoną urządzeniem Mixolab (*opracowanie własne*).

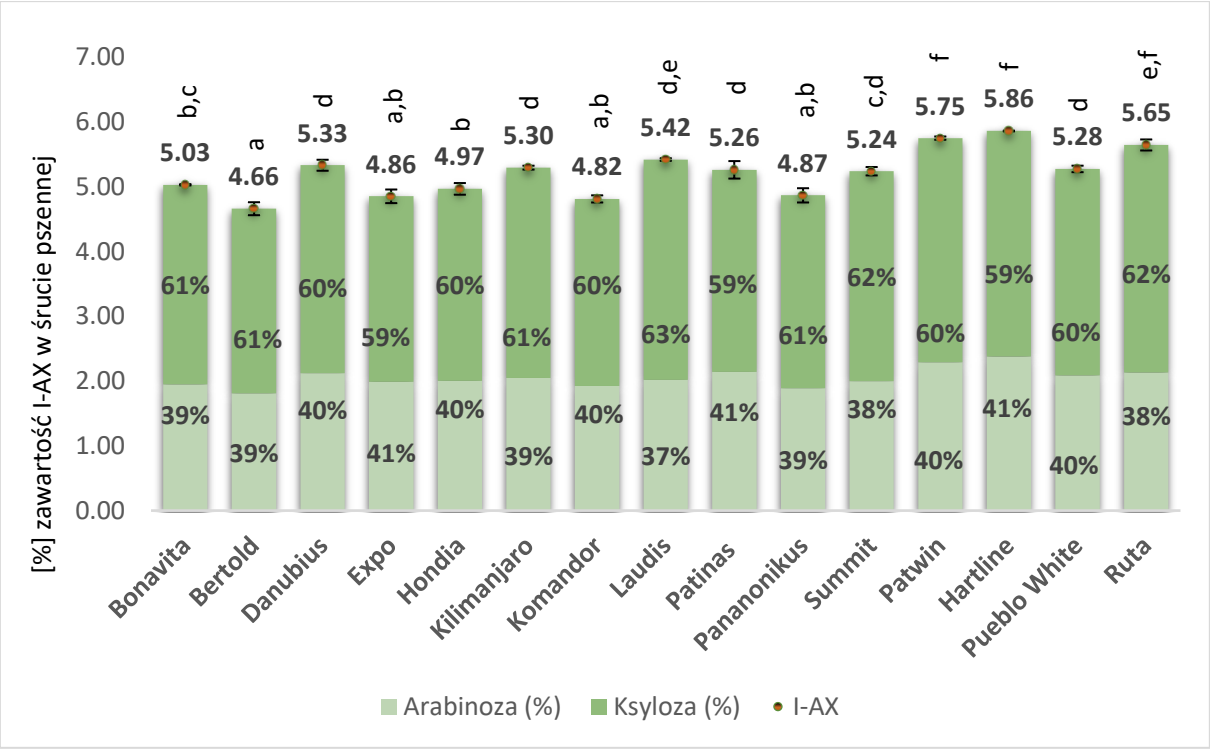
Badania wstępne pozwoliły również oszacować różnorodność frakcji polisacharydów w odmianach krajowych w porównaniu z analizowanymi odmianami pszenicy uprawianymi poza Polską, w powiązaniu z danymi literaturowymi [Török i in. 2019, Kiszonas i in. 2013] opisującymi zawartość tych frakcji w różnych odmianych pszenicy zwyczajnej. Na wykresach 7 i 8 przedstawiono zawartość rozpuszczalnych (S-AX) i nierozpuszczalnych (I-AX) frakcji arabinoksylanów wraz z procentowym udziałem frakcji arabinozy i ksylozy. Wyznaczone w pracach badawczych zawartości S-AX i I-AX mieściły się w przedziale od 0,80 do 1,39 % oraz od 4,66 do 5,86 %, co jest zgodne z danymi literaturowymi dotyczącymi mąk pełnoziarnistych otrzymanych z pszenicy zwyczajnej [Török i in. 2019]. W przeciwieństwie jednak do wyników przedstawionych przez Töröka i in. [2019], zawartości S-AX i I-AX w badanych próbkach

były bardzo zbliżone do siebie, przy czym jedynie odmiana Komandor wykazywała podwyższoną zawartość S-AX. Większą zmiennością składu tych frakcji charakteryzowało się bielmo ziarniaków [Török i in. 2019]. Zawartość I-AX również utrzymywała się na podobnym poziomie w badanych próbkach. Nie zaobserwowano istotnych różnic w zawartości poszczególnych frakcji między badanymi odmianami uprawianymi w Polsce a odmianami uprawianymi poza Polską.



Rys. 7. Frakcje rozpuszczalnych arabinoksylanów w śrucie pszennej różnych odmian pszenicy, z procentowym udziałem cukrów arabinozy i ksylozy (*opracowanie własne*).

Na właściwości technologiczne i odżywcze AX duży wpływ ma zastąpienie szkieletu ksylozy resztami arabinozy [Török i in. 2019]. Na rysunkach 7 i 8 przedstawiono zatem stosunek A/X jako wskaźnika substytucji w odniesieniu do zawartości S-AX i I-AX. Stosunek arabinozy do ksylozy w S-AX był zróżnicowany, a zawartość arabinozy wahała się od 35% dla odmiany Summit do 43% dla odmiany Expo. Podobne wartości uzyskano w I-AX, gdzie procentowa zawartość arabinozy kształtowała się na poziomie od 37% dla odmiany Laudis do 41% dla odmian Expo, Patinas i Hartline.



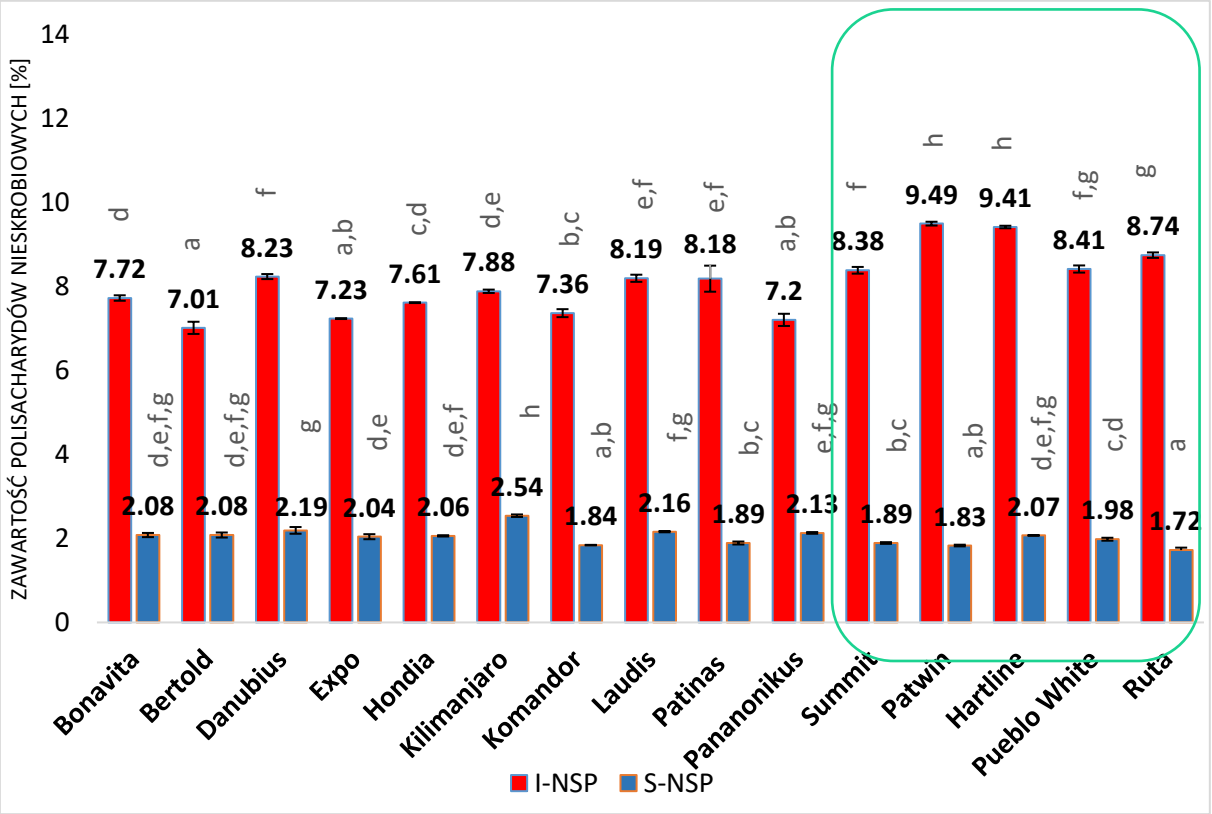
Rys. 8. Frakcje nierozpuszczalnych arabinoksylanów w śrucie pszennej z procentowym udziałem cukrów arabinozy i ksylozy (*opracowanie własne*).

Wyniki w tabeli 1 ukazują różnorodność zmielonego w formie śruty ziarna ze zbiorów 2020 r. w postaci zawartości polisacharydów nieskrobiowych (T-NSP) i arabinoksylanów (T-AX) oraz ich frakcji ekstrahowalnych wodą (S-NSP, S-AX) i nieekstrahowalnych wodą (I-NSP, I-AX). Całościowe wyniki wykazały, że zawartość S-NSP i I-NSP wahała się od 1,72 do 2,54% i od 9,09 do 11,78%, odpowiednio. Zawartości S-AX i I-AX wynosiły odpowiednio od 0,80 do 1,39% i od 4,66 do 5,86%.

Tabela 1. Zróżnicowanie zawartości NSP oraz AX w badanych odmianach (*opracowanie własne*).

	S-AX	**I-AX**	**T-AX**	**S-NSP**	**I-NSP**	**T-NSP**
Min-Max (% masy śruty)	0,80-1,39	4,66-5,86	5,69-6,97	1,72-2,54	7,01-9,49	9,09-11,48
Średnia (% masy śruty)	1,02	5,22	6,24	2,03	8,07	10,10
Współczynnik zmienności	13%	7%	6%	9%	9%	7%
Średni rozkład procentowy w śrucie	16%	84%	100%	20%	80%	100%

Wyniki badań wykazały, że zawartość rozpuszczalnych i nierozpuszczalnych frakcji NSP i AX, w zależności od odmiany pszenicy, była na zbliżonym poziomie, szczególnie w przypadku odmian krajowych (Rys. 9).



Rys. 9. Skład nieskrobiowych polisacharydów w śrucie pszennej (*opracowanie własne*).

Wyższe wartości uzyskano w odmianach uprawianych poza terytorium Polski. Może to wynikać z warunków środowiskowych, w jakich uprawiane były te odmiany. Jak podaje Kiszonas [2013] wysokie temperatury powietrza i susza, a także efektywność wykorzystania wody przez odmiany pszenicy zwyczajnej to najczęstsze czynniki środowiskowe wpływające na zawartość i skład arabinoksylanów.

Wiedza pozyskana w trakcie badań wstępnych pozwoliła na wytypowanie i wybór odmiany IS Laudis, jako przedstawiciela odmian pszenicy zwyczajnej dedykowanych do wykorzystania na surowiec makaronowy, która w kolejnym etapie badań posłużyła jako surowiec przemiałowy. Odmiana ta charakteryzowała się odpowiednimi parametrami jakościowymi, a także wysoką zawartością AX i NSP (Tab. 2. i Rys. 7, 8)

Tabela 2. Najważniejsze cechy odmiany pszenicy IS LAUDIS wybranej do prac badawczych (*opracowanie własne*).

Szklistość ziarniaka	79%
Twardość SKCS (HI)	61
Wodochłonność śruty -Mixolab	65,4%
Poziom retrogradacji skrobi Mixolab (C5)	1,76 Nm
Szacowana wartość siły wypiekowej Mixolab	252-332 $\times 10^{-4}$ J
Współczynniki pochłaniania (SRC) Sacharoza i woda	106,9% i 77,1%

4.2.Prace badawcze

Celem pracy **P1** była ocena poszczególnych frakcji ziarna pszenicy oraz zbadanie zależności między właściwościami reologicznymi pasaży przemiałowych a zawartością arabinoksylanów, w celu przewidywania oczekiwanej funkcjonalności mąki. Testowaną odmianą pszenicy była odmiana IS Laudis, a do przemiału wykorzystano młyn walcowy o skali przemysłowej (w PZZ LUBELLA GMW Sp. z o.o., Lublin, Polska) z kilkoma sekcjami walców, jak przedstawiono na rysunku 1 w rozdziale 3.1. W trakcie przemiału wytworzono 30 pasaży przemiałowych. Każda otrzymana frakcja mąki została przeanalizowana indywidualnie. Właściwości fizykochemiczne, w tym wilgotność, zawartość popiołu, liczba opadania, zawartość glutenu mokrego, indeks glutenu, zawartość uszkodzonej skrobi i cechy reologiczne zostały wyznaczone za pomocą metod opisanych w rozdziale 3.3, oraz przedstawionych w pracy **P1**. Zbadano całkowitą zawartość polisacharydów nieskrobiowych i arabinoksylanów, a także frakcji rozpuszczalnych i nierozpuszczalnych w poszczególnych pasażach przemiałowych. Wyniki wykazały istotne różnice między pasażami przemiałowymi pod względem parametrów fizykochemicznych i właściwości reologicznych, a także zawartości frakcji rozpuszczalnych i nierozpuszczalnych polisacharydów nieskrobiowych i arabinoksylanów.

Zawartość polisacharydów, arabinoksylanów i ich frakcji w uzyskanych pasażach mącznych podano w tabeli [**P1-Tab. 7**]. Całkowitą zawartość polisacharydów nieskrobiowych

(T-NSP) oznaczono metodą chromatografii gazowej i stanowi ona sumę cukrów: arabinozy, ksylozy, mannozy, galaktozy i glukozy. Analiza ta pozwala na rozdzielenie polisacharydów nieskrobiowych na dwie frakcje: rozpuszczalną i nierozpuszczalną oraz określenie składu polisacharydów i poszczególnych cukrów w obu frakcjach. W badanych frakcjach mąki całkowita ilość polisacharydów nieskrobiowych wahała się od 2,70 do 24,70% i była najwyższa głównie w końcowych pasażach rozczynowych, wymiałowych oraz w pasażach mąk filtracyjnych. Stwierdzono, że zawartość T-NSP była istotnie ($r=0,85$; $p < 0,05$) skorelowana z zawartością składników mineralnych w badanych mąkach. Zaobserwowano wysoką zawartość T-NSP dla pasażu R1F1 (4,39%), którego mąka pochodziła ze środkowego bielma ziarniaka pszenicy.

Podobnie wysoką zawartość T-NSP stwierdzono we frakcjach DD1I i C1II, odpowiednio 4,66% i 4,11%, w których ilość frakcji I-NSP i S-NSP była również wysoka. Najwyższą ilość T-NSP stwierdzono w pasażu C7II (24,70%), który zawierał największą ilość frakcji pochodzących z najbardziej zewnętrznych partii bielma i z warstwy aluronowej ziarniaka, a zatem zawierał najwyższą zawartość składników mineralnych ze wszystkich badanych frakcji. Zawartość I-NSP w tej części wynosiła prawie 90% całkowitej ilości T-NSP. Zawartość frakcji nierozpuszczalnej (I-NSP) w poszczególnych pasażach była podobna jak dla frakcji T-NSP i przyjmowała najwyższe wartości dla mąk z pasaży o wyższym poziomie zawartości frakcji zewnętrznych ziarniaka, a zatem zawartości popiołu ($r=0,86$; $p < 0,05$). Frakcje S-NSP charakteryzowały się bardziej równomiernym rozkładem we wszystkich badanych mąkach pasażowych i wykazywały jedynie niewielki związek z zawartością popiołu ($r=0,43$; $p < 0,05$).

Tabela [**P1-Tab. 7**] przedstawia również nierozpuszczalne (I-AX) i rozpuszczalne (S-AX) frakcje arabinoksylanów. Stanowią one część sumy polisacharydów nieskrobiowych, które odgrywają dużą rolę w produkcji wyrobów piekarniczych [Delcour i in. 1991, Michniewicz i in. 1992, Labat i in. 2000]. Wartości dla I-AX wynosiły od 0,93% dla pasażu B3 do 15,07% dla pasażu C7II. Natomiast, dla S-AX wartości wahały się od 0,55% w pasażu B3 do 1,37% w C7II. Ogólnie rzecz biorąc, suma obu frakcji wahała się od 1,48 do 16,45%. Stopniowy przyrost wartości dla poszczególnych frakcji był ogólnie podobny do przyrostu I-NSP i S-NSP. Zawartość I-AX i T-AX wzrastała znacząco wraz ze wzrostem składników mineralnych w poszczególnych frakcjach ($r=0,85$; $p < 0,05$), z wyjątkiem mąk z pasaży R1F1 i DD1I. Stwierdzono również, że rozkład S-AX był bardziej równomierny i mniej związany z zawartością popiołu ($r=0,45$; $p < 0,05$) i częścią ziarna, z której pochodził dany pasaż. Podobne obserwacje zgłosili Delcour i in. [1999], gdzie wartości frakcji NSP wzrastały wraz

ze wzrostem zawartości składników mineralnych. Wyraźny przyrost związany z zawartością popiołu zaobserwowano dla T-AX, natomiast nierównomierny dla S-AX. Ponadto wyjaśniono, że S-AX może wzrastać tylko wtedy, gdy mąka jest zmieszana z większą ilością bardzo drobnej frakcji łuski ziarniaka (otrąb), co zaobserwowano w tym badaniu dla pasaży C7II, C7I i R5. Jak opisali Li i in. [2022], intensywny przemiał w końcowych pasażach rozczynowych i wymiałowych, prowadzący do uzyskania drobno rozdrobnionych fragmentów tych frakcji, może przyczynić się do zwiększenia zawartości błonnika rozpuszczalnego, również poprzez rozbijanie wiązań glikozydowych w celulozie i nierozpuszczalnej hemicelulozie, szczególnie w S-AX.

Jak przedstawiono również w pracy **P1**, zawartość T-AX, I-AX i I-NSP, potwierdzona analizą PCA, była silnie dodatnio skorelowana z zawartością popiołu (składników mineralnych) w pasażach przemiałowych oraz z absorpcją wody [**P1-Fig. 3**]. Zaobserwowano, że wysoka zawartość tych składników skutecznie zapobiegała tworzeniu się sieci glutenowej i odpowiedniej konsystencji ciasta w analizach reologicznych. Podobnie, jak w badaniach Ramseyera i in. [2011], zauważono zwiększającą się ilość S-AX, I-AX i T-AX wraz z kolejnymi pasażami mącznymi w trakcie przemiału. Jednocześnie zaobserwowano, że zwiększenie zawartości S-AX w poszczególnych pasażach nie było powiązane z wynikami zawartości popiołu oraz I-AX i T-AX.

Zawartość popiołu była najbardziej różnicującym parametrem poszczególne pasaże. Niska zawartość popiołu (około 0,6% lub mniej) wskazuje na brak frakcji zewnętrznych ziarniaka w uzyskanej frakcji mąki. Jest to cecha pożądana przez przemysł i konsumentów, a mąki handlowe skomponowane z pasaży o niskiej zawartości popiołu i wysokiej zawartości białka glutenowego są szeroko wykorzystywane przez producentów wyrobów cukierniczych czy makaronowych. Wysoka zawartość popiołu (1,6% i więcej) świadczy natomiast o dużej zawartość frakcji zewnętrznych ziarniaka, a tym samym charakteryzuje się wysoką zawartością błonnika pokarmowego, przeciwutleniaczy i minerałów [Engelsen i in. 2009]. Najniższą zawartość popiołu stwierdzono w pasażach B2–B3, C1–C5, DD1 i R1–R3, pasaże te zawierały najwięcej części ziarniaka pochodzących ze środkowej części bielma. Natomiast najwyższą zawartość popiołu zaobserwowano w pasażach B5, C6–C7, R4–R5 i V1–V3. Były to więc pasaże najbogatsze w różne frakcje błonnika [**P1-Tab. 1]**.

W badanych frakcjach mąk pasażowych, w pracy **P1**, skupiono się również na parametrze wodochłonności mąki. Parametr ten charakteryzowano trzema różnymi metodami. Wykonano analizę farinograficzną, test na urządzeniu Mixolab, a także ocenę zdolności pochłaniania wybranych roztworów metodą SRC. W przeprowadzonych analizach wyższą

absorpcję wody (WA) zaobserwowano w szczególności w końcowych pasażach śrutowych (B4, B5), wymiałowych (C4–C7) i rozczynowych (R4, R5), a także w pasażach mąk filtracyjnych (V1–V3) [**P1-Tab. 3, 4 i 6**]. Wysoka WA w tych frakcjach jest najprawdopodobniej spowodowana wyższą zawartością popiołu [Banu i in. 2010] i uszkodzonej skrobi [Di Stasio i in. 2007] przy wysokich współczynnikach korelacji wynoszących odpowiednio 0,86 i 0,72 dla tych cech ($p < 0,05$). Końcowe pasaże w poszczególnych sekcjach charakteryzowały się również zwiększającą się zawartością polisacharydów nieskrobiowych, takich jak arabinoksylany, oraz zwiększonymi uszkodzeniami w granulkach skrobi, które są w stanie zatrzymywać większe ilości wody w tych mąkach, niż w mąkach o niskiej zawartości popiołu.

Po przeprowadzeniu testów zgodnie z procedurą farinograficzną i skorygowaniu do stałej zawartości wilgotności (14%) mąki, oceniono, że pasaże o wyższej zawartości uszkodzonej skrobi SD nie zostały całkowicie uwodnione, co spowodowało większą sztywność ciasta [**P1-Tab. 3**]. Wysoka absorpcja wody (WA) wpływała na wyższą wydajność przygotowanego ciasta. Z kolei długi czas rozwoju (DT) i niski wskaźnik rozmiękczenia ciasta (DoS) charakteryzowały odporność tych mąk pasażowych na zmniejszenie konsystencji ciasta, przy większej tolerancji na obróbkę mechaniczną. Oceniono, że czas rozwoju ciasta (DT) osiągał najwyższe wartości dla mąk śrutowych, ale także dla mąk filtracyjnych i końcowych pasaży rozczynowych i wymiałowych o wyższej zawartości popiołu. Badania wykazały również, że granulacja frakcji wpływała na czas rozwoju ciasta. Dlatego mąki pasażowe o większej granulacji (powyżej 180 μm), takie jak R1F2, R1F2, C1II, miały skrócony czas rozwoju (2,1–2,3 min). Najwyższą stabilność ciasta osiągnięto w pierwszych pasażach śrutowych (B2, B3) i pasażach sortujących (DD1) wspomagających rozsortowywanie najwydajniejszych frakcji. W kolejnych pasażach w poszczególnych sekcjach stabilność ciasta (S) znacznie obniżała się z 17,9 do 2,7 min. Oceniono, iż ten spadek stabilności wynika z pogarszającej się jakości glutenu i zwiększającej się ilości frakcji błonnikowych, które zaczynają znacznie wpływać na układ ciasta [**P1-Tab. 3**]. Destabilizacja ciasta pszennego następować może, gdy narasta ilość mechanicznie uszkodzonej skrobi znajdującej się w sieci glutenowej, rozrywając wiązania disiarczkowe i zmiękczając ciasto.

Z uzyskanych w pracy **P1** danych wyznaczono, iż SRCWa wahało się od 61,867 do 159,775%, SRCSu od 92,951 do 178,502%, SRCLa od 96,788 do 180,035%, a SRCSc od 74,394 do 193,406% [**P1-Tab. 6**]. Porównując wyniki SRCWa z absorpcją wody mierzoną za pomocą analizy na urządzeniach Farinograf i Mixolab, współczynniki korelacji wynosiły odpowiednio 0,93 i 0,92 przy $p < 0,05$. Analiza ta dodatkowo pozwoliła skutecznie ocenić,

które z komponentów w poszczególnych pasażach odgrywały najważniejszą rolę w ogólnej wodochłonności danej frakcji, a szczególnie, w których mąkach arabinoksylany odpowiadały głównie za wysoką wodochłonność. W analizie SRC to pojemność retencji rozpuszczalnika sacharozy SRCSu wskazuje na ilość arabinoksylanów w analizowanej próbce mąki. W przeciwieństwie do badań Vukić i in. [2020], w pracy **P1** wykazano wyższe wartości SRCSu w mąkach z pasaży śrutowych. Prawdopodobnie było to spowodowane obecnością bielma sąsiadującego z warstwą aleuronową, która występuje w dużych ilościach w tych frakcjach, jeśli proces mielenia, w tej sekcji, jest prowadzony z dużą intensywnością. Wysokie wartości SRCSu zaobserwowano również w pasażach mąk filtracyjnych (V2–V3).

W celu oceny wpływu absorbcji wody przez poszczególne składniki mąki na ogólną jakość mąk pasażowych w badaniach w pracy **P1-Tab. 6**, wyznaczono współczynnik GPI (ang. Gluten Performance Index). Został on uznany przez Kweon i in. [2011] za lepszy wskaźnik przewidywalności funkcjonalności glutenu niż sam SRCLa. Opisuje on ogólną zdolność gluteniny (białka glutenu) do funkcjonowania wśród innych sieci modulujących, takich jak uszkodzona skrobia lub pentozany/arabinoksylany. Lindgren i Simsek [2016] potwierdzili w swoich badaniach istnienie dodatniej korelacji między GPI a wybranymi parametrami reologicznymi mąki. Podobne zależności odnotowano w tym badaniu. GPI korelował negatywnie z WA i rozmiękczeniem ciasta po 12 minutach (DoS12) z farinografu (odpowiednio -0,77 i -0,62 przy $p < 0,05$) i pozytywnie z parametrami wyznaczonymi w analizie alweograficznej L, W, Ie i SH (odpowiednio 0,73, 0,62, 0,83, 0,83 przy $p < 0,05$). GPI w badanych pasażach wahało się od 0,42 do 0,91 (średnio 0,66) [**P1-Tab. 6**]. Podobnie, jak w przypadku wyników SRCLa, mąki z bielma centralnego, a także te o zwiększonej granulacji (R1F2, R1G2) wykazały najwyższe wartości. Zgodnie z wcześniejszymi badaniami Lindgren i Simsek [2016] oraz Kweon i in. [2016], oceniono iż wartość GPI może być także wykorzystywana do określenia jakości poszczególnych mąk pasażowych, dobrze charakteryzując ich przydatność technologiczną.

Wszystkie otrzymane wyniki w pracy **P1**, w celu uzyskania informacji o wzajemnych powiązaniach poszczególnych cech jakościowych pasaży, poddano analizie statystycznej PCA. Wykazała ona, że pierwsze dwa główne komponenty PC1 i PC2 opisują zmienność układu w 70,47%, gdzie parametry zawarte między dwoma czerwonymi okręgami mają największy wpływ na zmienność układu [**P1-Fig. 3**]. W pracy **P1** analiza PCA wykazała, że A (zawartość składników mineralnych), M-WA (wodochłonność w analizie Mixolab), SRCWa, SRCSu, SRCSc, I-AX, T-AX, I-NSP i T-NSP są silnie i dodatnio skorelowane ze sobą. Stąd wyniki uzyskane z pomiarów instrumentalnych, zwłaszcza zawartość popiołu, absorpcja wody

mierzona za pomocą procedury Mixolab, parametry analizy SRC, takie jak SRCSu (50% roztworach sacharozy) i SRCSc (5% roztworach węglanu sodu), mogą być przydatne do przewidywania zawartości całkowitej ilości polisacharydów i arabinoksylanów, a także ich nierozpuszczalnych frakcji. Wyznaczono również dodatnią i silną korelację pomiędzy parametrami GPI, nachyleniem M-β i pewnymi cechami mierzonymi za pomocą analizy Mixolab oraz parametrami wyznaczonymi w analizie Mixolab M-C3, M-C4, M-C5, M-C3−C2, M-C5−C4. Wykazano silną i dodatnią korelację pomiędzy parametrami M-C2−C1 i M-C2. Na podstawie wyników analizy PCA zaobserwowano również ujemną i silną korelację pomiędzy A, M-WA, SRCSc, SRCWa, SRCSu, I-AX, T-AX, I-NSP, T-NSP oraz FN, GPI, M-β, M-C3, M-C4, M-C5, M-C3−C2, M-C5−C4.

Przedstawione w pracy **P1** porównanie pasaży przemiałowych z młyna pszennego, a także inne porównania z danych literaturowych [Brütsch i in. 2017, Prabhasnakar i in. 2000, Ramseyer i in. 2011, Pojić i in. 2014, Wang i in. 2006], wskazują możliwości wykorzystania tych wyników do komponowania specjalistycznych mąk gatunkowych. Stąd opracowywanie kompozycji mąki w celu uzyskania jej określonej funkcjonalności może się także opierać na metodach instrumentalnych, a nie na długich i kosztownych analizach chemicznych. Badanie mąk pochodzących z różnych pasaży przy użyciu szybkich analiz reologicznych, takich jak analiza Mixolab, w porównaniu do czasochłonnych analiz chemicznych izolacji frakcji polisacharydów pozwala (w pewnym zakresie) na szacowanie zawartości tych frakcji w mące. Jest to aspekt bardzo istotny z punktu widzenia tworzenia mąk specjalistycznych o zwiększonej zawartości arabinoksylanów, o funkcjach wysokiej absorpcji wody i cechach jakościowych odpowiednich do produkcji wyrobów piekarniczych, szczególnie w warunkach produkcyjnych.

Badacze, tacy jak Michniewicz i in. [1992], badając te cechy określali na przykład wpływ rozpuszczalnych i nierozpuszczalnych w wodzie pentozanów pszennych oraz rozpuszczalnych w wodzie pentozanów żytnich na niektóre cechy wypiekowe mąki pszennej. W swojej pracy wykazali, że preparaty pentozanów wyraźnie zwiększyły wodochłonność farinograficzną, natomiast dodatek rozpuszczalnych w wodzie pentozanów (w ilości 2%) zwiększał właściwą objętość bochenka. Natomiast frakcje nierozpuszczalne nie wpłynęły znacząco na poprawę tego parametru. Według Michniewicz i in. [1992], przy stałej konsystencji ciasta, chleby wzbogacone pentozanami miały wyższą wilgotność i wyższą aktywność wody. Co więcej, wykazali wyższe wskaźniki retrogradacji amylopektyny mierzone metodą DSC (różnicowej kalorymetrii skaningowej) w przypadku chlebów wzbogaconych pentozanami, prawdopodobnie ze względu na ich wyższą zawartość wody. Wywnioskowano, że rozpuszczalne w wodzie pentozany opóźniały proces agregacji między cząsteczkami

amylozy, o czym świadczy ilość i rodzaj węglowodanów ekstrahowanych wodą z cząsteczek chleba.

Jak wykazano w ramach pracy **P1**, parametry najbardziej różnicujące poszczególne mąki, takie jak zawartość popiołu, białka glutenowe, enzymy amylolitycznye lub polisacharydy nieskrobiowe i arabinoksylany, miały bezpośredni wpływ na oczekiwane parametry reologiczne służące do komponowania frakcji zgodnie z założoną przydatnością technologiczną. Chociaż wyniki pracy **P1** odnosiły się tylko do konkretnej odmiany pszenicy zwyczajnej IS Laudis i konkretnego schematu mielenia, można założyć, że wyniki będą również spójne dla innych odmian, szczególnie, tak jak zaobserwowano w badaniach wstępnych, dla różnych odmian stanowiących surowiec do produkcji wyrobów makaronowych.

Z perspektywy wykorzystania poszczególnych pasaży, istotne jest zrozumienie subtelnych różnic w zawartości tych składników szczególnie w końcowych frakcjach schematu mielenia. Pozwala to na lepsze wykorzystanie dodatkowych cech jakościowych, które zazwyczaj są tracone z powodu wykluczenia tych pasaży przemiałowych podczas przemiału ziarna na surowiec do produkcji wyrobów makaronowych. Testy frakcji mącznych z wykorzystaniem szybkich analiz reologicznych, takich jak analiza Mixolab i połączenie analizy głównych składowych ze współczynnikami korelacji Pearsona do analizy tych zależności, pozwalają na identyfikację istotnych zależności między testowanymi parametrami.

Lepsze zrozumienie pochodzenia różnych frakcji oraz roli arabinoksylanów i ich frakcji w procesie mielenia daje możliwość opracowania mieszanek mąk handlowych o pożądanej funkcjonalności.

Bazując na informacjach pozyskanych w pracy **P1**, w toku kolejnego etapu prac badawczych, opracowana została mąka pszenna (F) składająca się z wybranych pasaży przemiałowych zestawionych głównie z frakcji końcowych pasaży wymiałowych i rozczynowych, charakteryzująca się zwiększona zawartością polisacharydów nieskorobiowych, w tym arabinoksylanów, ale również posiadająca cechy fizykochemiczne i reologiczne pozwalające na zastosowanie jej w produkcji wyrobów piekarniczych. Opracowana mąka F posłużyła jako mąka bazowa do dalszych prac badawczych.

W następnym etapie badań, zaprezentowanych w publikacji **P2,** badano wpływ konfiguracji ekstrudera jednoślimakowego i zmiennych parametrów przetwarzania, takich jak konwencjonalna ekstruzja lub hybrydowa obróbka z enzymem ksylanazą, na wydajność i energochłonność ekstruzji i wybrane cechy opracowanej mąki pszennej o podwyższonej zawartości nieskrobiowych polisacharydów (F). Zastosowano dwie konfiguracje układu

plastyfikującego ekstrudera L/D = 16 i L/D = 20 z różnymi profilami ślimaków. Oceniono interakcje między zmiennymi przetwarzania (poziom dowilżenia 23, 25, 27%; prędkość ślimaka 40, 60, 80 obr./min; poziom ksylanazy 0, 50, 100 ppm), aby wskazać jednostkowe zapotrzebowanie energetyczne procesu ekstruzji i właściwości reologiczne modyfikowanej mąki. Podczas ekstruzji w niskiej temperaturze monitorowano i rejestrowano kilka cech: ciśnienie ekstruzji (bar), moment obrotowy (Nm), obciążenie silnika (%), moc czynną (kW), wydajność przetwarzania (kg/h) i specyficzne zapotrzebowanie jednostkowe energii mechanicznej (SME). Obliczenia wykonano oddzielnie dla obu konfiguracji ekstrudera, L/D = 16 i L/D = 20, użytych w eksperymencie.

Mąki pszenne komponowane jako mieszanka wybranych pasaży końcowych sekcji śrutowania, redukcji i wymielania mogą zawierać wysoką zawartość arabinoksylanów. Wynika to z włączenia frakcji odpadowych uzyskanych podczas mielenia ziarna [Miller i in. 2008] i może przynosić istotne korzyści jakościowe, gdy proces komponowania mieszanki oparty jest o kompleksową analizę poszczególnych frakcji, dzięki czemu możliwe jest zastosowanie odpowiednich proporcji określonych pasaży. Mąki bogatsze we frakcje włókniste mogą charakteryzować się nieco gorszymi właściwościami reologicznymi niż standardowa mąka chlebowa. Wynika to bezpośrednio ze zmniejszonej ilości wysokojakościowego białka glutenowego, znajdującego się głównie w środkowej części ziarna pszenicy, ale jednocześnie pozwala na włączenie dużych ilości frakcji mąk pasażowych, zwykle usuwanych podczas mielenia niskopopiołowych mąk handlowych. W pracy **P2** wodochłonność opracowanej mąki wynosiła 60,5 ± 0,1%, czas rozwoju wynosił 1,92 ± 0,19 min, a stabilność ciasta wynosiła 9,73 ± 0,12 min. Na podstawie uzyskanych wyników przedstawionych w pracy [**P2-Tab. 5 i 6**] stwierdzono, że absorpcja wody przez ekstrudowaną mąkę pszenną z enzymem ksylanazą zależy od zmiennych przetwarzania i konfiguracji układu plastyfikującego L/D. W pracy **P2** hydratacja wody ekstrudowanych próbek wahała się od 61,0 do 68,3%, gdy do wstępnej obróbki mąki pszennej zastosowano L/D = 16, i od 63,9 do 69,3%, gdy składniki przetwarzano w konfiguracji L/D = 20.

Wykazano, że ekstruzja przyczynia się do zwiększenia właściwości hydratacyjnych różnych produktów [Mosibo i in. 2022]. Intensywne ścinanie przy niskiej wilgotności i wysokiej prędkości ślimaka powoduje degradację skrobi z krystalicznym topnieniem cząsteczek amylopektyny, a także dekstrynizacją, co ma wpływ na właściwości hydratacyjne. Ponadto, hydroliza białka, która jest możliwa podczas ekstruzji, może wpływać na absorpcję wody, szczególnie w surowcach o wysokiej zawartości białka [Alam i in. 2014]. Ekstrudowana mąka uzyskana po przetworzeniu przy wysokiej wilgotności i niskiej prędkości obrotowej

ślimaka może być cenna dla poprawy stabilności ciasta ze względu na nieniszczący wpływ takiej obróbki na hydrolizę lub aktywność enzymów [Mosibo i in. 2022].

Dodatkowo jedną z metod, która może wpływać na przemiany surowców zbożowych po procesie ekstruzji jest obróbka enzymatyczno-ekstruzyjna [Kong i in. 2023]. Kombinację tych procesów można stosować do ziaren zbóż i mąki w warunkach wysokiego stężenia substratu dla działania enzymów oraz podwyższonej temperatury, ciśnienia i naprężenia ścinającego przy różnych poziomach dowilżenia materiału. W mieszankach surowców zbożowych z dodatkiem enzymów znajduje się dużo składników termolabilnych, takich jak białka (także enzymatyczne) lub tłuszcz w mące, co wymaga stosowania niskich temperatur przetwarzania i skutkuje brakiem ekspandowania produktu gotowego oraz ograniczonymi przemianami zależnymi od temperatury w materiałach ekstrudowanych [Santala i in. 2013]. Można tego również uniknąć, stosując enzymy termostabilne, które mogą poprawić teksturę produktu [Alam i in. 2014]. W ekstruderze podczas przetwarzania zachodzi szereg interakcji między zmiennymi przetwarzania a aktywnością enzymatyczną, które wpływają na zachowanie reologiczne powstałego ciasta. Martínez i in. [2014] zastosowali ekstruzję do modyfikacji mąki pszennej, a przetwarzanie znacznie zwiększyło właściwości hydratacyjne; konkretnie 5-krotnie zdolność wiązania wody i 9-krotnie pęcznienie w porównaniu z nieobrobioną mąką pszenną.

Należy zauważyć, że ekstrudowane mąki pszenne mogą zwiększać wydajność chleba w procesach piekarniczych [Martínez i in. 2013]. Ich dodatek do receptur wypiekowych może również mieć wpływ na różne właściwości reologiczne ciasta. Fizykochemiczna modyfikacja włókien i bogatych w błonnik frakcji mąki, poprzez zastosowanie wysokich temperatur i warunków ścinania w procesie ekstruzji, jest możliwa w celu poprawy ich właściwości funkcjonalnych.

Zwykle mąki pszenne używane do wypieku chleba, zawierające wysokie poziomy dobrej jakości glutenu, mają dobrą odporność na mechaniczną obróbkę (np. miesienie, kształtowanie) w porównaniu do mąk z końcowych pasaży rozczynowych i wymiałowych zawierających więcej zewnętrznych warstw, które są bogatsze w błonnik i polisacharydy nieskrobiowe oraz arabinoksylany [Lewko i in. 2023]. Dlatego też w pracy **P2** istotne było określenie wpływu zastosowanego procesu ekstruzji jednoślimakowej z różną konfiguracją układu plastyfikujacego i parametrów procesu na jakość kompleksu białkowego w obecności dodatku enzymów piekarniczych.

Odpowiednim wskaźnikiem charakteryzującym te właściwości jest parametr C2 (osłabienie białka - wpływ temperatury na zachowanie białek glutenowych, determinujących jakość mąki) w analizie reologicznej na urządzeniu Mixolab. Przy zastosowaniu obu

konfiguracji układu plastyfikującego w trakcie ekstruzji zaobserwowano niewielki wzrost wartości parametru C2, gdy stosowano wyższy poziom dowilżenia [**P2-Fig. 5**]. Zwiększona dawka enzymu miała również niewielki wpływ na wartość osłabienia białka: niższe wartości C2 uzyskano, gdy ekstruzję przeprowadzono z wyższymi dawkami ksylanazy w obu konfiguracjach ekstrudera. Ogólnie rzecz biorąc, wydłużona konfiguracja ekstrudera L/D = 20 pozwoliła uzyskać niższe wartości C2, co może być spowodowane zastosowaniem dodatkowego elementu mieszającego w tej konfiguracji ślimaka, który także rozluźnił upłynnione ciasto, a tym samym spowodował mniej intensywną obróbkę składników białkowych (zwłaszcza glutenu) w testowanej mące o wyższej zawartości NSP. Ekstruzja jednoślimakowa spowodowała intensywne osłabienia białka, co można zaobserwować jako wyższe wartości C2, zwłaszcza przy wyższej wilgotności wsadu. Jednak zastosowanie enzymu ograniczyło rozrywanie sieci białkowej, dając niższe wartości C2. Jeśli wartość C2 jest wysoka, ciasto uzyskane z przetworzonej mąki jest mniej elastyczne i wykazuje ograniczony rozwój. Sama mąka ekstrudowana nie nadaje się do stosowania w wypieku chleba, ale częściowe zastąpienie mąki chlebowej o niskiej zawartości C2 mąką ekstrudowaną może być pomocne w spowolnieniu tworzenia się pęcherzyków gazu podczas fermentacji i wyrastania ciasta, oraz może pozytywnie utrzymać wewnętrzną strukturę chleba.

Podobne badania przeprowadzili Moreno-Rivas i in. [2014]. Zastosowali oni jednoślimakowy ekstruder laboratoryjny o L/D = 25:1, o nominalnym współczynniku sprężania 2:1 i otworze matrycy 3 mm, który pracował z prędkością 45 obr./min w zakresie temperatur 60, 70, 80 i 90°C, aby przetworzyć mąkę kukurydzianą nixtamalizowaną z ksylanazą i bez jej udziału. Donieśli, że ekstrudowana mąka kukurydziana, z ksylanazą i bez niej, miała zwiększoną rozpuszczalność białka, a efekt ten był mniejszy, gdy przy ekstruzji użyto enzymu. Co więcej, dodatek ksylanazy zmniejszył wpływ procesu ekstruzji na rozpuszczalność białek ekstrudowanej mąki kukurydzianej. Ponadto, w produktach ekstrudowanych bez i z enzymem ksylanazą, znacznie spadła zawartość tłuszczu, co było spowodowane rozpadem lipidów lub tworzeniem kompleksów między amylozą i kwasami tłuszczowymi. W rezultacie proces ten umożliwił wydłużenie okresu przydatności do spożycia, ekstrudowanych produktów mącznych [Hebeda i in. 1991]. Z kolei Bucsella i in. [2016] zauważyli znaczące zmiany po zastosowaniu metod termicznych i hydrotermicznych w obróbce mąki do ciast i chleba. Stwierdzili niewielki wzrost wartości C2 po 5-minutowej obróbce hydrotermicznej, ale dłuższe czasy obróbki (10 i 20 minut) obniżyły wartości C2 zarówno dla mąki do ciastek, jak i do chleba, prawdopodobnie z powodu zmian w konformacji białek pod wpływem ogrzewania.

W odniesieniu do zmian w strukturze białka powstających podczas ekstruzji przy niskiej wilgotności stwierdzono, że niektóre wiązania białkowe zawierały mniej wiązań disiarczkowych niż te tworzone przy wyższych poziomach dowilżenia [Fisher 2004]. Oceniono, że sieć białkowa utworzona podczas ekstruzji przy niskiej wilgotności mogła zawierać więcej podjednostek białkowych, co wyjaśniałoby niższą rozpuszczalność białka. Tak więc, oprócz wpływu energii cieplnej i mechanicznej, poziom dowilżenia jest ważny dla charakteru sieciowania disiarczkowego podczas ekstruzji.

W badaniach w pracy **P2** zauważono ponadto, iż kleikowanie skrobi (C3) było niższe, gdy dodano enzym przed ekstruzją, a aktywność amylazy (C4) zmniejszyła się wraz ze zwiększonym dodatkiem ksylanazy. Dodatek enzymu na poziomie 50 i 100 ppm w ekstrudowanej mące pszennej ograniczył również tendencję do retrogradacji (C5) (o 8–24% dla L/D = 16 i 8–23% dla L/D = 20) w porównaniu do ekstrudowanej mąki pszennej bez dodatków; dlatego wywnioskowano, że mąka ekstrudowana wspomagana enzymatycznie stosowana jako dodatek do produkcji wyrobów piekarniczych może wydłużyć ich okres przydatności do spożycia.

Zgodnie z wynikami pracy **P2**, wymagania dotyczące zapotrzebowania specyficznej energii mechanicznej (SME) podczas obróbki ekstruzyjnej mąki pszennej z dodatkiem enzymu ksylanazy zależały od konfiguracji L/D i warunków procesowych [**P2-Fig. 5**]. SME jest dobrym ilościowym wskaźnikiem intensywności procesu ekstruzji, stopnia zachodzących przekształceń makrocząsteczkowych i oddziaływań, tj. konwersji skrobi, a w konsekwencji właściwości reologicznych powstałego produktu [Hebeda i in. 1991]. Wyższe SME zwykle skutkuje większym stopniem skleikowania skrobi oraz większym stopniem redukcji wielkości jej cząsteczek i ekspandowania ekstrudatu [Oliveira i in. 2017].

W pracy **P2** statystycznie istotny model regresji dla SME (z umiarkowaną wartością współczynnika determinacji i istotnym wynikiem testu F) uzyskano tylko dla konfiguracji układu plastyfikującego ekstrudera L/D = 20, co wskazuje na dodatnią liniową zależność z M (poziomem dowilżenia), E (dawką enzymu) i efektem interakcji M × E [**P2-Tab. 2**]. Zaobserwowano zintegrowany wpływ poziomu dowilżenia i dawki enzymu na SME. Oba czynniki niezależnie przyczyniły się do zwiększenia SME (L/D = 20). Co więcej, SME wzrastało wraz ze wzrostem M i E dla L/D = 20. Współczynniki korelacji nie były istotne zarówno dla L/D = 16, jak i L/D = 20. Zmiany M, S lub E nie miały znaczącego wpływu na SME w ekstruzji przy zastosowaniu L/D = 16. Jednak wyższe wartości SME odnotowano dla konfiguracji L/D = 20, szczególnie przy wysokim poziomie dowilżenia i maksymalnej dawce ksylanazy w obrabianej mące. Do takiego efektu mogły przyczynić się różnice w strukturze

ciasta, wynikające ze stosowania elementu mieszającego w konstrukcji ślimaka oraz z obecności częściowo zhydrolizowanych polisacharydów, na które działa ksylanaza podczas ekstruzji przy wysokiej wilgotności i w niskich temperaturach (poniżej 80°C).

Ksylanaza może zmieniać nierozpuszczalne frakcje polisacharydów (szczególnie arabinoksylany) w rozpuszczalne i bardziej reaktywne struktury, które pochłaniają więcej wody i sprawiają, że ciasto poddawane obróbce jest gęstsze, a zatem wymaga większego nakładu energii przetwórczej. Badacze, tacy jak Deng i in. [2023], testowali otręby pszenne przetwarzane przez ekstruzję enzymatyczną przy poziomach dowilżenia 30 i 40% i stwierdzili wyższe nakłady energii mechanicznej podczas ekstruzji przy przetwarzaniu materiału o niższej wilgotności. Wysokie SME może sprzyjać tworzeniu luźnej i porowatej struktury ułatwiającej przenikanie roztworu zawierającego ksylanazę. Niższa prędkość ślimaka skutkuje dłuższym czasem przebywania i mniejszą intensywną obróbką mechaniczną wytwarzaną przez ekstruder [Deng i in. 2023].

Ponadto, zwiększenie prędkości obrotowej ślimaka prowadzi do wyższych wartości SME, co skutkuje depolimeryzacją lignocelulozy i przekształceniem łańcuchów arabinoksylanowych w rozpuszczalne małe cząsteczki. W efekcie tworzą się kompleksy z białkami, a tym samym obserwowane są zmiany w reologicznych cechach zależnych od funkcji białka ciasta pszennego. Dlatego też wywnioskowano, że ekstruzja przy wyższej prędkości ślimaka wytwarza więcej rozpuszczalnych arabinoksylanów (pentozanów), nawet jeśli nie dodaje się żadnych enzymów [Deng i in. 2023].

Jako parametr systemowy, SME reprezentuje ilość energii mechanicznej przenoszonej na materiał wsadowy podczas procesu ekstruzji i może być używane do oceny intensywności tego procesu. Stwierdzono, że na SME mają wpływ wilgotność wsadu, szybkość podawania, prędkość ślimaka oraz temperatura cylindra [Kowalski i in. 2020]. Obecność błonnika oraz zwiększona wilgotność mogą zmniejszać lepkość przetwarzanego materiału w ekstruderze, co wpływa na rozkład sił ścinających, mieszanie, obróbkę mechaniczną, a tym samym na moment obrotowy silnika i wartość SME [Oliveira i in. 2017]. Wyższa wilgotność wsadu i temperatura prowadzą zazwyczaj do obniżenia SME, a wyższa prędkość ślimaka powoduje zwiększone zapotrzebowanie energetyczne [Ma i in. 2018, Kharat i in. 2019, Allai i in. 2022, Kesre i in. 2022, Fischer i in. 2004, Bouasla i in. 2016, Feng i in. 2014]. Zmniejszenie SME obniża zawartość skrobi szybkostrawnej, a zwiększa poziom skrobi wolno trawionej (opornej), co pozytywnie wpływa na jakość ekstrudatów [Feng i in. 2014]. Największy wpływ na SME przypisuje się wilgotności wsadu, przy czym większa ilość białka i tłuszczu w recepturze wpływa na wyższą lepkość ciasta i tym samym na wzrost SME.

Przedstawiona w publikacji **P2** analiza statystyczna potwierdziła, że poziom dowilżenia i prędkość ślimaka były zmiennymi o najistotniejszym wpływie na cechy mąki pszennej modyfikowanej ekstruzją jednoślimakową. Najważniejszymi czynnikami z punktu widzenia jakości ekstrudowanej mąki pszennej o podwyższonej zawartości NSP i jej dalszego wykorzystania w mieszankach mąki chlebowej powinny być niska energochłonność ekstruzji SME, zwiększona wodochłonność i ograniczona tendencja do retrogradacji.

Wyniki badań wykazały, że zastosowanie różnych konfiguracji ekstrudera i profili ślimaka miało znaczący wpływ zarówno na parametry procesowe, jak i na właściwości reologiczne mąki pszennej. Dłuższa konfiguracja ekstrudera L/D = 20 z profilem ślimaka z elementami mieszającymi pozwoliła uzyskać modyfikowaną mąkę przy niższym ciśnieniu ekstruzji i zapotrzebowaniu na energię niż w wersji L/D = 16. Wyniki uzyskane dla ekstrudowanych mąk modyfikowanych wykazały różnice w absorpcji wody, a także we właściwościach reologicznych. Ekstrudowana mąka pszenna charakteryzowała się wyższymi właściwościami hydratacyjnymi (o 12,9% dla L/D = 16 i 14,4% dla L/D = 20 w porównaniu z mąką niemodyfikowaną) i ograniczoną tendencją do retrogradacji (o 28,6% dla L/D = 16 i 24,6% dla L/D = 20 w porównaniu z mąką niemodyfikowaną). Ponadto, niższy początkowy poziom dowilżenia (23%) wpływał na wyższą zdolność pochłaniania wody przez mąkę. Najbardziej znaczące różnice zaobserwowano w parametrze C2 z analizy Mixolab (osłabienie białka), jeśli zastosowano różne konfiguracje układu plastyfikującego L/D = 16 lub L/D = 20. Zwiększona ilość zastosowanego enzymu (100 ppm) ograniczyła negatywny wpływ obróbki ekstruzyjnej na ten parametr.

Zgodnie z wynikami badań z publikacji **P2**, zalecane warunki ekstruzji jednoślimakowej to 23% wilgotności wsadu, prędkość ślimaka 40 obr./min i dodatek enzymu 100 ppm do opracowanej mąki z wykorzystaniem wydłużonej konfiguracji ekstrudera L/D = 20 wykorzystującej ślimak z dodatkowym elementem mieszającym. Mąka przetworzona przy takich zaleceniach mogłaby być zastosowana jako polepszacz w postaci dodatku do handlowych mąk piekarniczych.

Ze względu na zaobserwowany w pracy **P2** tylko niewielki wzrost właściwości hydratacyjnych mąki, a jednocześnie widoczny wpływ zastosowanych enzymów piekarniczych na poprawę parametrów wytworzonej mąki w trakcie procesu, w kolejnym etapie badań mąkę (F) poddano procesowi niskotemperaturowej ekstruzji z wykorzystaniem ekstrudera dwuślimakowego.

W badaniach opisanych w publikacji **P3**, mąka pszenna (F) charakteryzująca się zwiększoną zawartością polisacharydów nieskrobiowych, została poddana modyfikacji z użyciem enzymów, których ilość określono na podstawie wstępnych testów i sugestii producenta enzymu. Należały do nich celulaza oraz ksylanaza, które zastosowano w dwóch kombinacjach podczas modyfikacji enzymatycznych: samodzielnie enzym celulazę użyto w ilości 120 ppm (próbki oznaczone literą C), a mieszaninę celulazy i ksylanazy użyto w ilościach odpowiednio 60 ppm i 50 ppm (próbki oznaczone literą CX). Następnie poszczególne mąki: bazową (F) oraz mąki z enzymami (FC i FCX) poddano obróbce ekstruzyjnej w niskiej temperaturze (do 85°C). Konwencjonalna hydroliza enzymatyczna z użyciem celulazy i mieszanki celulaza-ksylanaza, a także ekstruzja i hybrydowe metody ekstruzji w obecności enzymów zostały przetestowane w zmiennych warunkach procesowych. Ekstruzję mąki pszennej prowadzono przy poziomie dowilżenia 23-27% w zakresie temperatur 40-80°C.

W pracy **P3** otrzymane mąki natywne i modyfikowane poddano szeregowi testów reologicznych w celu oceny parametrów jakościowych wpływających na poprawę funkcjonalności wyselekcjonowanej mąki bazowej (F) i możliwość wykorzystania zmodyfikowanych mąk w przemyśle piekarniczym.

Cechy reologiczne mąki bazowej (F), ekstrudowanej i poddanej obróbce enzymatyczno-ekstruzyjnej, były testowane za pomocą urządzenia Mixolab i przedstawione w tabeli [**P3- Tab. 6**]. Oceniono, iż wodochłonność (Hyd) była zbliżona (około 60%) dla mąki bazowej (F), nawet jeśli zastosowano obróbkę enzymatyczną (FC i FCX). Znaczący wzrost Hyd zaobserwowano we wszystkich ekstrudowanych mąkach, który wahał się od 94,3% dla próbki EF27 do 117,7% dla EF23 i obniżał przy wyższym dowilżeniu surowców. Mniejsze różnice odnotowano między mąkami poddanymi hybrydowej obróbce enzymatyczno-ekstruzyjnej, niezależnie od użytego enzymu. Czas rozwoju ciasta (DT) we wszystkich testowanych modyfikowanych mąkach był dłuższy niż dla mąki bazowej (F), a najbardziej znaczące różnice zaobserwowano, jeśli do mąki pszennej dodano celulazę (FC) i kompleks celulaza-ksylanaza (FCX), w tych przypadkach obserwowano odpowiednio o 28% i 40% wydłużenie czasu rozwoju ciasta. Powiązanym parametrem jest stabilność ciasta, ze znaczącą ujemną korelacją do właściwości hydratacyjnych (-0,973; $p < 0,05$) dla wszystkich próbek. Zastosowanie hydrolizy enzymatycznej do modyfikacji mąki pszennej obniżyło stabilność ciasta tylko nieznacznie, podczas gdy ekstruzja i hybrydowa obróbka enzymatyczno-ekstruzyjna obniżyły stabilność ciasta ponad dwukrotnie, a w przypadku próbki EF23 nawet trzykrotnie. W mąkach poddanych obróbce EF i EFC stabilność nieznacznie wydłużyła się wraz ze zwiększaniem

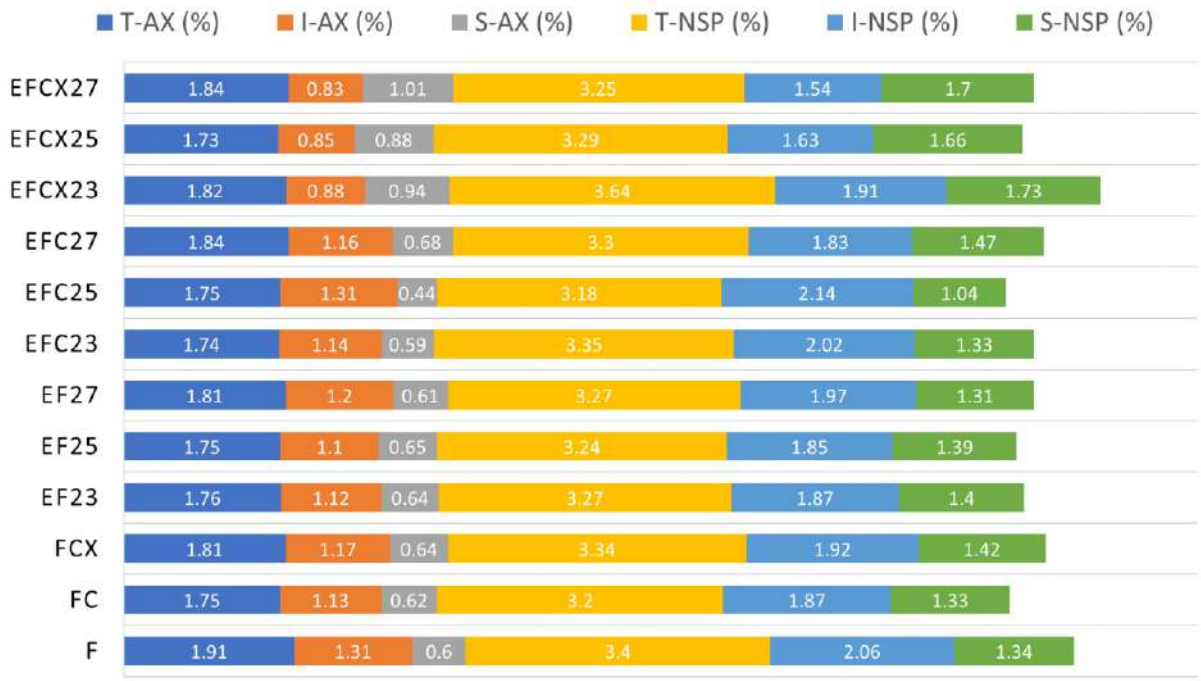
wilgotności wsadu, ale jeśli zastosowano EFCX, zaobserwowano odwrotną tendencję. Zmniejszenie stabilności ciasta może być zatem związane z degradacją matrycy glutenowej zachodzącą podczas procesu ekstruzji z powodu podwyższonej temperatury, ponieważ obróbka w wysokiej temperaturze modyfikuje właściwości składników matrycy glutenowej [Demuth i in. 2020]. Zdolność absorpcji wody przez ekstrudowaną mąkę pszenną wzrasta wraz ze wzrostem intensywności obróbki, ale stabilność ciasta ma tendencję do zmniejszania się [Martinez i in. 2013]. Zdolność absorpcji wody przez ciasto zależy głównie od składu mąki i wzrasta wraz ze wzrostem zawartości białka, pentozanów i uszkodzonej skrobi.

Przeprowadzone w pracy **P3** badania wykazały również specyficzny wpływ hybrydowej obróbki enzymatyczno-ekstruzyjnej mąki pszennej na frakcje polisacharydów, co skutkowało znacznym zmniejszeniem zawartości nierozpuszczalnych arabinoksylanów (rys. 10) oraz zwiększeniem zawartości rozpuszczalnych arabinoksylanów i polisacharydów nieskrobiowych, zwłaszcza, gdy podczas ekstruzji dwuślimakowej zastosowano mieszankę enzymów celulaza-ksylanaza (próbki EFCX).

Zaobserwowano, że wszystkie zastosowane zabiegi obniżały zawartość T-AX poprzez zmniejszanie nierozpuszczalnych i zwiększanie rozpuszczalnych frakcji AX, z bardziej widoczną zmianą, gdy użyto jako dodatek celulazy niż przez połączone działanie kompleksu celulaza-ksylanaza na arabinoksylany. Podobną tendencję zaobserwowano w odniesieniu do zawartości T-NSP. W obu frakcjach zaobserwowano znaczący wzrost rozpuszczalnych AX i NSP, przy czym najbardziej efektywnym działaniem wpływającym na zwiększenie ilości rozpuszczalnych składników w modyfikowanej mące pszennej było zastosowanie połączenia kompleksu enzymów i procesu ekstruzji (EFCX). Arabinoksylany (AX) są głównymi polimerami w ścianie komórkowej ziarna pszenicy, a zatem głównym składnikiem błonnika pokarmowego [Gartaula i in. 2018]. Frakcje arabinoksylanów AX można scharakteryzować stosunkiem arabinozy do ksylozy (A/X). Stosunek frakcji rozpuszczalnych wynoszący około 0,6 sugeruje większą zawartość bielma w mące, podczas gdy stosunek bliższy 1,0 wskazuje, że polisacharydy pochodzą z warstw zewnętrznych.

W opracowanej mące bazowej (F) wyznaczono stosunek I-A/X na poziomie 0,723 i stosunek S-A/X na poziomie 0,842 - wskazujący na obecność frakcji otrąb w badanej mące. Skład S-AX i I-AX (rys. 10) w opracowanej mące F użytej w badaniach, z większą ilością nierozpuszczalnych frakcji arabinoksylanów i polisacharydów nieskrobiowych, wyraźnie pokazał, że większość nierozpuszczalnych frakcji pochodzi z warstw zewnętrznych niż z bielma (I-AX 1,31% i S-AX 0,60% w mące pszennej F). Zarówno obróbka enzymatyczna, ekstruzja, jak i hybrydowe zabiegi enzymatyczno-ekstruzyjne obniżyły ilość

nierozpuszczalnych frakcji - głównie z powodu obniżenia całkowitej zawartości arabinoksylanów. Najbardziej znaczące obniżenie I-AX i zwiększenie ilości S-AX było widoczne po ekstruzji mąki pszennej z dodanymi enzymami, ale bez zmiany ilości T-AX [**P3-Tab. 3**]. Wyniki te sugerują, że metoda obróbki EFCX miała największy wpływ na rozpuszczalność AX, w porównaniu do niewielkiego wpływu spowodowanego tylko zastosowaniem procesu ekstruzji, nawet przy zmiennych parametrach.



Rys. 10. Kompozycja NSP i ich frakcji w modyfikowanej ekstruzyjnie i hybrydowo opracowanej mące pszennej (*opracowanie własne*).

W literaturze tematu dla kilku surowców stosowano połączone działanie dodatku enzymów i procesu ekstruzji. Zhou i in. [2010] zastosowali obróbkę ksylanazą i alkaliami do ekstrakcji arabinoksylanów z otrąb pszennych i stwierdzili, że stosunek arabinozy do ksylozy wynosił odpowiednio 0,56 i 0,83, pomimo, że masa cząsteczkowa AX poddanych obróbce alkaliami była około 10 razy większa od masy cząsteczkowej AX poddanych tylko obróbce ksylanazą. Również Chen i in. [2019] przetestowali różne metody ekstrakcji AX z pszenżyta i stwierdzili, że metoda ekstrakcji, w pierwszym etapie kompleksem celulazy-ksylanazy, a następnie ekstrakcja alkaliczna z pozostałego po ekstrakcji enzymatycznej surowca, jest najbardziej odpowiednia do uzyskania najwyższej wydajności arabinoksylanów (ze wskaźnikiem A/X wynoszącym 1,52, przy czym stosunek ten wyniósł tylko 0,25 A/X, jeśli zastosowano czystą ekstrakcję enzymatyczną). Z kolei Ma i in. [2020] stwierdzili, że metody

enzymatyczne z endoksylanazami są wydajne w ekstrakcji nieekstrahowalnych wodą WUAX i że połączona ekstrakcja celulazą i endoksylanazą daje wyższe efekty uzysku AX niż zastosowanie tylko ksylanazy. Według przedstawionych wyników badań hybrydowa ekstrakcja enzymatyczna AX z surowego włókna kukurydzianego była wydajniejsza niż metody chemiczne podawane przez innych autorów. Chen i in. [2019] zaobserwowali w otrębach pszennych zawierających wodę i poddanych procesowi ekstruzji, że wiązania nierozpuszczalnych polisacharydów, na przykład celulozy i hemicelulozy, oraz ciągła matryca włókien zostały częściowo rozerwane i uwolniły rozpuszczalne sacharydy po ekstruzji ze względu na połączone efekty intensywnego ścinania, oraz innych wynikających z tego procesu zjawisk wewnątrz ekstrudera pod wpływem wysokiej temperatury. Podobne spostrzeżenia zgłosili Arcila i in. [2015] opisując, iż ekstruzja z niższą wilgotnością wsadu (15%) była skuteczniejsza w przekształcaniu nieekstrahowanych frakcji hemicelulozowych w ekstrahowane polisacharydy i w zwiększaniu ilości rozpuszczalnych składników błonnika pokarmowego w ekstrudowanych otrębach pszennych ze względu na większe ścinanie i rozrywanie mechaniczne. Efekt ten był odwrotny do stosowania wysokiej wilgotności (30%), gdzie woda działała jako plastyfikator w cylindrze ekstrudera.

Co więcej, zastosowanie ekstrudera dwuślimakowego w temperaturze 50°C zwiększyło rozpuszczalność arabinoksylanów (AX) przy niskiej zawartości wody (poniżej 54%) w porównaniu do mieszania otrąb z ksylanazą za pomocą łopatek miksera. W związku z tym, ekstruzja umożliwiła wydajne działanie enzymu przy niskiej zawartości wody ze względu na zwiększoną dyfuzję enzymu ksylanazy i przez tworzenie ciągłej masy w ekstruderze [Santala i in. 2013]. Jak stwierdzono w pracy Anderssona i in. [2017], ekstrahowalność AX w otrębach pszennych i żytnich wzrastała w zależności od parametrów ekstruzji. Opisano, że zwiększona ekstrahowalność błonnika pokarmowego i arabinoksylanów, w połączeniu z utrzymywaną zawartością β-glukanów w otrębach pszennych i żytnich sprawia, że ekstrudowane otręby są bardziej wartościowe jako dodatek do zastosowania w przemyśle spożywczym. Również Fadel i in. [2018], opisali proces ekstruzji jako innowacyjną obróbkę wstępną, przydatną do zwiększenia rozpuszczalności frakcji AX.

W toku kolejnych analiz w pracy **P3** oceniono, iż wszystkie testy obejmujące ekstruzję w niskiej temperaturze (bez enzymów lub z enzymami) powodowały obniżenie zawartości tłuszczu w modyfikowanych mąkach z powodu tworzenia kompleksów amylozowo-lipidowych [**P3-Tab. 2**], co zostało również potwierdzone przeprowadzoną z użyciem dyfrakcji rentgenowskiej analizą krystaliczności.

W analizie krystaliczności badanych próbek oceniono, iż różnice w strukturze mąk natywnych i ekstrudowanych były niewielkie, prawdopodobnie dlatego, że krystaliczność typu A uległa przegrupowaniu, a krystaliczność typu V utworzyła się w ekstrudowanych próbkach zawierających skrobię ze względu na jej kleikowanie podczas obróbki metodą ekstruzji [Saiah i in. 2007, Leblanc i in. 2008, Oliveira i in. 2017]. Krystaliczność w ekstrudatach jest również efektem tworzenia kompleksów między amylozą i endogennymi lipidami (kompleks amylozowo-lipidowy). Kompleksy te są na ogół wytwarzane po kleikowaniu skrobi w obecności ciepła i wody ze względu na przemiany skrobi podczas procesu ekstruzji i rozpad podwójnych helis amylopektyny, podczas gdy część wolnych lipidów może tworzyć kompleksy inkluzyjne z cząsteczkami amylozy [Jafari i in. 2017]. Potwierdza to również niską zawartość tłuszczu w próbkach ekstrudowanej mąki pszennej opisanych w pracy **P3** [**P3-Tab. 2**], oraz niewielkie różnice w krystaliczności ze względu na metodę obliczeniową opartą na stosunku powierzchni pod krzywą struktur krystalicznych i amorficznych. Największy spadek krystaliczności (13,39%) odnotowano w mące ekstrudowanej [**P3-Fig. 2c**], przy najniższej wilgotności wsadu oraz przy użyciu celulazy lub kompleksu celulaza-ksylanaza (krystaliczność odpowiednio 13,43% i 14,48%) przy dowilżeniu 25% [**P3-Fig. 3**]. Zwiększenie poziomu dowilżenia wsadu do 27% zwiększyło krystaliczność do 15,54%, co przypisano intensywniejszemu skleikowaniu skrobi i zmianie struktury krystalicznej. Oliveira i in. [2017] wykazali, że utrata krystaliczności podczas ekstruzji wynika z mechanicznego rozerwania wiązań molekularnych spowodowanego intensywnymi siłami ścinającymi. Przy niskiej wilgotności ekstrudaty zawierają mieszankę skleikowanych i stopionych ziaren skrobi oraz są pofragmentowane. W badaniach w pracy **P3** najwyższą krystaliczność (17,27% i 16,43%) zaobserwowano przy 23% i 27% poziomie dowilżenia mąki ekstrudowanej z dodatkiem kompleksu celulaza-ksylanaza, co ograniczało tworzenie fazy krystalicznej.

Podsumowując wyniki pracy **P3** wykazano, iż obróbka ekstruzyjna przy niższym dowilżeniu mieszanki, zarówno bez enzymów, jak i z enzymami, zwiększyła lepkość maksymalną, zmniejszyła lepkość w czasie kleikowania (breakdown), wodochłonność, C2 (stopień osłabienia białka) i zdolność retencji wszystkich rozpuszczalników (w analizie SRC), ale jednocześnie zmniejszyła przyrost lepkości podczas chłodzenia (setback) oraz stabilność ciasta, C3 (skleikowanie skrobi), C4 (aktywność amylazy), C5 (retrogradację skrobi) w analizie Mixolab, a także wskaźnik wydajności glutenu GPI (analiza SRC). Dodatkowo, hydroliza kompleksem celulaza-ksylanaza wykazała silniejszy wpływ na modyfikację mąki pszennej niż samo traktowanie celulazą, szczególnie poprzez zwiększenie zawartości

rozpuszczalnych AX i NSP, wodochłonności oraz czasu rozwoju ciasta DDT (analiza Mixolab) i GPI w modyfikowanej mące.

Zaprezentowane w pracy **P3** wyniki badań potwierdziły, że hybrydowa modyfikacja enzymatyczno-ekstruzyjna ma większy wpływ na skład polisacharydów oraz techno-funkcjonalne właściwości mąki pszennej niż indywidualna obróbka enzymatyczna lub ekstruzyjna.

Modyfikowana hybrydowo enzymatyczno-ekstruzyjnie mąka pszenna, w odpowiednich warunkach przetwarzania, może być wykorzystywana w przemyśle piekarniczym, jako źródło rozpuszczalnych arabinoksylanów, jako środek zagęszczający lub jako składnik o wysokiej absorpcji wody i zdolności zatrzymywania rozpuszczalników oraz wpływać na zmniejszenie retrogradacji skrobi w gotowych produktach.

Kolejnym etapem prac badawczych było porównanie wpływu różnych procesów termicznych celem wyselekcjonowania najbardziej korzystnych warunków modyfikacji fizycznych i zmiennych procesowych umożliwiających otrzymanie mąki modyfikowanej o zwiększonej zdolności pochłaniania wody i korzystnych właściwościach reologicznych.

W pracy doświadczalnej przedstawionej w publikacji **P4**, opracowana mąka pszenna (F) charakteryzująca się zwiększoną zawartością polisacharydów nieskrobiowych, z dodatkiem celulazy (C) oraz kompleksu celulaza-ksylanaza (CX), została poddana konwencjonalnym i hybrydowym metodom obróbki. Przeprowadzono suchą obróbkę termiczną (T), obróbkę hydrotermiczną (H) oraz ekstruzję dwuślimakową (E) bez lub z dodatkiem enzymów jako metody hybrydowe. Analizowano skład chemiczny i profil polisacharydów, wybrane parametry fizykochemiczne i reologiczne oraz cechy strukturalne zmodyfikowanych mąk pszennych.

Modyfikacje opracowanej mąki pszennej bazowej (F) miały zróżnicowany wpływ na skład, reologię i strukturę, w zależności od warunków obróbki oraz zastosowania enzymów. Konwencjonalne i hybrydowe metody obróbki, obejmujące włączenie enzymów celulazy i/lub kompleksu enzymów celulaza-ksylanaza, spowodowały zmiany w składzie frakcji polisacharydowych (zwłaszcza arabinoksylanów) oraz w reologii zmodyfikowanej mąki, zmieniając znacząco właściwości techno-funkcjonalne otrzymanych mąk.

Zastosowanie ogrzewania na sucho, obróbki hydrotermicznej i ekstruzji dwuślimakowej, zarówno jako metod obróbki indywidualnej, jak i hybrydowej z dodatkiem enzymów, w zróżnicowany sposób wpłynęło na charkterystykę mąki. Obserwowano znaczące

obniżenie zawartości tłuszczu w próbkach poddawanych modyfikacji ekstruzyjnej zarówno bez, jak i z dodatkiem enzymów (próbki EF, EFC i EFCX). Alam i in. [2016] stwierdzili, że tłuszcz jest w stanie tworzyć kompleksy ze skrobią lub białkiem podczas procesu ekstruzji, a uzyskane wyniki potwierdzają tę obserwację. Zawartość tłuszczu była co najmniej 5-krotnie niższa niż w mące natywnej i w próbkach poddanych obróbce termicznej. Dodanie enzymu miało nieznaczny wpływ na zawartość tłuszczu.

Proces obróbki termicznej na sucho (T) okazał się mieć najmniej destrukcyjny wpływ na jakość białka, zwłaszcza, jeśli zastosowano kompleks enzymów celulaza-ksylanaza. Testowana mąka bazowa F charakteryzowała się dobrymi właściwościami wypiekowymi, z siłą wypiekową W wynoszącą 273×10^{-4}J. Dodanie celulazy (FC) lub kompleksu celulaza-ksylanaza (FCX) nieznacznie zmniejszyło elastyczność i zwiększyło rozciągliwość ciasta, utrzymując wskaźnik elastyczności Ie i wskaźnik SH na podobnym poziomie [**P4-Tab. 9**]. Zastosowanie obróbki cieplnej znacznie zmniejszyło wartość wypiekową mąki TF, głównie poprzez zmniejszenie sprężystości ciasta (P); pogorszyło również parametry Ie i SH, odpowiednio z 49,27% (F) do 44,83% (TF) i z 1,66 (F) do 1,48 (TF). Zastosowanie modyfikacji hybrydowej poprzez włączenie do mieszanki enzymów, zwłaszcza w mące TFCX, pozwoliło uzyskać jakość zbliżoną do jakości próbek F, FC i FCX, szczególnie w zakresie sprężystości ciasta (P). Sucha obróbka termiczna i hybrydowa z enzymami zwiększyły rozciągliwość ciasta L, ale nieznacznie obniżyły wytrzymałość wypiekową W, wskaźnik elastyczności Ie i wskaźnik SH, przy mniejszych różnicach, jeśli testowano próbki TFC i TFCX.

Intensywna obróbka metodą hydrotermiczną lub ekstruzyjną miały negatywny wpływ na jakość frakcji białkowych, ale znacząco zmieniały właściwości żelujące, zarówno bez, jak i z dodatkiem enzymów. Brak prawidłowego formowania matrycy glutenowej został spowodowany albo wysoką temperaturą pary, albo zintegrowanym termiczno-enzymatycznym zniszczeniem białek glutenowych, które zmieniły swoją strukturę. W wyniku przetwarzania procesowego w podwyższonych temperaturach (powyżej 50°C) zachodzą zmiany w konformacji cząsteczkowej białka, takie jak rozwijanie się białek glutenowych, tworzenie agregatów glutenu ze zmniejszoną ekstrahowalnością, zmiany między reakcjami wymiany wiązań sulfhydrylowych/disulfidowych prowadzące do polimeryzacji gluteniny, modyfikacja rozkładu masy cząsteczkowej, co może mieć wpływ na końcową zawartość białka i możliwość tworzenia nieekstrahowalnych białek polimerowych UPP (ang. Unextrectable Polymeric Proteins), zwłaszcza w obecności skrobi, co potwierdzili Guerrieri i Cerletti [1996] na podstawie wewnętrznych zmian konformacyjnych fluorescencji w białkach, Hu i in. [2017] na obrazach CSLM, lub Schofield i in. [1983] za pomocą analizy chromatograficznej.

W rezultacie, otrzymane w analizie alweograficznej ciasto z mąki po obróbce hydrotermicznej charakteryzowało się bardzo słabą rozciągliwością L (ponad dwukrotnie niższą niż w przypadku mąki natywnej F) i zwiększoną wartością wskaźnika SH, szczególnie w próbkach HF i HFC. Wskaźnik P/L próbek z dodatkiem enzymu FC, FCX był podobny jak dla mąki bazowej F, nieznacznie niższe wartości odnotowano w mące poddanej obróbce termicznej bez oraz z enzymami. Większe różnice stwierdzono w wartościach współczynnika P/L, z ponad trzykrotnie wyższymi wartościami, w testach ciasta wykonanego z mąk HF, HFC i HFCX. Nie można było wyznaczyć wskaźnika elastyczności Ie w próbkach modyfikowanych hydrotermicznie. Chociaż współczynnik SH zwiększył się do wyższego poziomu niż w mące bazowej, było to spowodowane wzrostem sztywności ciasta, a nie rzeczywistą poprawą wydajności mąki. W przypadku obróbki ekstruzyjnej i częściowo obróbki hydrotermicznej nie udało się określenie właściwości reologicznych matrycy glutenowej z powodu częściowego skleikowania skrobi i denaturacji białka glutenowego. Żadna z ekstrudowanych mąk nie była w stanie uformować ciasta. W cieście wytworzonym z mąki pszennej, wzbogaconym o ekstrudowane otręby Gómez i in. [2011] zaobserwowali zmiany w parametrach analizy alweograficznej P, L i W z powodu przerwanej matrycy glutenowo skrobiowej i negatywnego wpływu na zatrzymywanie gazu w trakcie fermentacji, co skutkowało zmniejszeniem rozciągliwości ciasta i wzrostem sprężystości związanym ze słabymi właściwościami utrzymania prawidłowej konsystencji ciasta. Jødal i Larsen [2021] wskazali wysokie wartości P/L jako odporne i nierozciągliwe ciasto, podczas gdy niskie wartości współczynnika P/L określały ciasto słabe i rozciągliwe.

W pracy **P4** parametry reologiczne modyfikowanych mąk analizowane były również w matrycy ciasta o konsystencji wyznaczanej w oparciu o metody przywołane w punktach 3.3.8 i 3.3.10 metodyki. W przeprowadzonych analizach wodochłonność mąki, badana za pomocą pomiaru hydratacji, różniła się nieznacznie w zależności od zastosowanej metody. Hydratacja badana za pomocą urządzenia Mixolab była bardzo dobrze skorelowana ($r=0,90$ przy $p < 0,05$) z absorpcją wody wyznaczaną przez Farinograf, skorygowaną do wilgotności 14% dla wszystkich testowanych mąk. Jednak, w przeciwieństwie do Farinografu, Mixolab pozwolił ocenić absorpcję wody w mące modyfikowanej procesem ekstruzji dwuślimakowej [**P4-Tab. 7 i 8**]. Analizując wyniki badania z użyciem Farinografu, oceniono, iż proces modyfikacji termicznej mąki (TF) wykazał nieistotny wpływ na absorpcję wody, zwłaszcza jeśli wilgotność została skorygowana do 14%. Podobne obserwacje poczynili Hu i in. [2017] opisujący modyfikację termiczną ziaren pszenicy przed mieleniem oraz Bucsella i in. [2016], którzy analizowali obróbkę termiczną i hydrotermiczną mąk pszennych przeznaczonych do

wypieku ciasta i chleba. W badaniach w pracy **P4**, obróbka hydrotermiczna wspomagana parą zmniejszyła skorygowaną do 14% wilgotności wodochłonność mąki HF w porównaniu z mąką bazową F [**P4-Tab. 8**]. We wszystkich modyfikacjach dodatek enzymów spowodował niewielki wzrost absorpcji wody, szczególnie w przypadku zastosowania kompleksu CX, najwyższą wartość uzyskano dla TFCX. Obróbka termiczna mąki może stworzyć bardziej wzmocnioną strukturę ciasta bez zmiany jego właściwości hydratacyjnych [Bucsella i in. 2016]. Proces obróbki termicznej z enzymami spowodował również wzrost DT (czas rozwoju ciasta), najniższe wartości wyznaczono dla TF, zaś najwyższy dla TFCX [**P4-Tab. 8**].

Z kolei, w analizie z wykorzystaniem urządzenia Mixolab, mąka bazowa F wykazała absorpcję wody na poziomie 60,5%, podczas gdy zarówno obróbka termiczna, jak i hydrotermiczna w próbkach TF i HF, zmniejszyła absorpcję wody. Dodanie enzymów C i CX we wszystkich poddanych obróbce mąkach spowodowało niewielki wzrost absorpcji wody. W przypadku modyfikacji ekstruzyjnej, podobnie jak w badaniu SRC [**P4-Tab. 5**], zaobserwowano ponad dwukrotny wzrost absorbcji wody w porównaniu z innymi testowanymi procesami przetwarzania, prawdopodobnie w wyniku znacznego pęcznienia skrobi lub już częściowemu skleikowaniu podczas obróbki w ekstruderze dwuślimakowym.

Urządzenie Mixolab mierzy parametr C2 jako utratę konsystencji ciasta podczas narażenia na stres fizyczno-mechaniczny i termiczny, po tym etapie testu (denaturacji białka pod wpływem ciepła) dominują zależne od jakości węglowodanów etapy kleikowania skrobi (C3), aktywności α-amylazy (C4) i retrogradacji skrobi (C5). Wyniki charakterystyk reologicznych badanych za pomocą procedury Mixolab przedstawiono w tabeli [**P4-Tab. 7**].

Parametr osłabienia białka C2 dla próbek TF nie różnił się od mąki bazowej F, ale zaobserwowano znaczący wzrost tego parametru w mąkach poddanych obróbce hydrotermicznej i procesowi ekstruzji. Najwyższy C2 zaobserwowano w próbce HFC, a efekt ten był najprawdopodobnie związany z najwyższą zawartością błonnika, zwłaszcza frakcji nierozpuszczalnych, co skutkowało pogorszeniem właściwości wypiekowych mąki, potwierdzonych wynikami analizy alweograficznej. Testowane ciasto wykonane z mąki HF poddanej obróbce hydrotermicznej wspomaganej parą charakteryzowało się wysoką sprężystością i niską elastycznością spowodowaną znacznymi zmianami w konformacji białek glutenowych, które uniemożliwiły prawidłowy rozwój ciasta lub białka glutenowe zostały całkowicie zdegradowane.

Włączenie kompleksu enzymów celulaza-ksylanaza spowodowało znaczący wzrost frakcji rozpuszczalnych arabinoksylanów, które pełnią funkcję strukturotwórczą w matrycy ciasta i uczestniczą w zarządzaniu wodą. Jeśli chodzi o skład frakcji rozpuszczalnych NSP

i AX [**P4-Tab. 3**], dodatek celulazy i kompleksu celulaza-ksylanaza zwiększył zawartość frakcji rozpuszczalnych niemal wszystkich cukrów, co sugeruje powstawanie bardziej rozpuszczalnych frakcji dzięki aktywności enzymów. Sucha obróbka termiczna obniżyła zawartość rozpuszczalnej mannozy i ksylozy, a dodatek enzymów wykazał zróżnicowany wpływ na próbki TFC i TFCX. Obróbka HF bez enzymów powodowała powstawanie zwiększonej zawartość rozpuszczalnych cukrów w porównaniu z mąką bazowa F. Dodatek kompleksu C lub CX do mąki bazowej F obniżył ilość rozpuszczalnej mannozy, ale zawartość innych cukrów, takich jak S-glukoza i S-arabinoza, wzrosła lub pozostała na podobnym do mąki bazowej F poziomie.

Najbardziej znaczące różnice zaobserwowano, jeśli zastosowano ekstruzję lub hybrydową obróbkę enzymatyczno-ekstruzyjną. W ich wyniku odnotowano szczególnie silny wzrost zawartości rozpuszczalnej arabinozy i ksylozy, podczas gdy stosunek S-A/X był najniższy. Mąka poddana obróbce HFC charakteryzowała się dużą ilością T-NSP i wysoką zawartością frakcji I-AX i I-NSP spośród badanych mąk modyfikowanych [**P4-Tab. 4**]. Po dodaniu tylko enzymu celulazy, ilość rozpuszczalnych frakcji polisacharydów nieskrobiowych zmniejszyła się, a ilość nierozpuszczalnych frakcji wzrosła, podczas gdy po dodaniu kompleksu enzymów CX zauważono wzrost ilości rozpuszczalnych i obniżenie zawartości nierozpuszczalnych frakcji NSP. Może to być efekt interakcji obróbki hydrotermicznej w obecności łatwo dostępnej wody z wtrysku pary podczas tego procesu oraz działania enzymów. Różne aktywności celulazy, takie jak celobiohydrolaza i endoglukanaza, mogą hydrolizować włókna celulozy. Celulaza użyta w tym badaniu wykazywała zarówno wysoką aktywność celobiohydrolazy, jak i aktywność endoglukanazy, i była odpowiedzialna za rozkład polimerów celulozy. Główną aktywnością celobiohydrolazy jest otwieranie fibryli ułatwiając działanie ksylanazy, która rozkłada składniki ścian komórkowych, szczególnie we frakcjach mąk z składnikami zewnętrznej części ziarniaka pszenicy. Końcowym efektem jest zwiększenie ilości rozpuszczalnych frakcji i ułatwienie działania innych enzymów. Tak więc połączenie celulazy i ksylanazy może poprawić jakość matrycy glutenowej, ponieważ rozbija włókna celulozy, wpływając na poprawę stabilności glutenu i zatrzymywanie gazu, bez zakłócania działania aktywności ksylanazy, która hydrolizuje arabinoksylany do ich rozpuszczalnej formy [Tebben i in. 2018]. W mąkach ekstrudowanych bez i z enzymami w wyniku obróbki technologicznej również zaobserwowano znaczne obniżenie zawartości nierozpuszczalnych i jednoczesny wzrost rozpuszczalnych arabinoksylanów [**P4-Tab. 4**]. W prawie wszystkich próbkach poddanych modyfikacji konwencjonalnej i hybrydowej zawartość T-NSP oniżyła się, z wyjątkiem TF i wszystkich próbek poddanych obróbce

hydrotermicznej (HF, HFC, HFCX), w porównaniu z mąką bazową F. Natomiast frakcje rozpuszczalnych S-NSP były na wyższym poziomie w próbkach FCX, a także w HF i HFCX, jak również w mące poddanej obróbce ekstruzyjnej EFC i EFCX.

Stwierdzono, że metody modyfikacji, z wyjątkiem TF, nieznacznie obniżyły ilość całkowitą arabinoksylanów w porównaniu do mąki bazowej F, prawdopodobnie z powodu częściowej ich hydrolizy przez enzymy lub tworzenia kompleksów. W mąkach modyfikowanych odnotowano dodatnie korelacje pomiędzy zawartością I-arabinozy (0,610), I-ksylozy (0,626), I-AX (0,639), I-NSP (0,682), T-NSP (0,634) a ilością nierozpuszczalnego błonnika IDF. Frakcje rozpuszczalnego błonnika obecne w modyfikowanej mące były również dodatnio skorelowane z T-NSP na poziomie 0,633, a całkowita zawartość błonnika była skorelowana z I-AX (0,606), I-NSP (0,661), T-NSP (0,698). Korelacje te nie były ekstremalnie wysokie, ale istotne przy założonym $p < 0,05$.

Stwierdzono również ujemne korelacje pomiędzy I-ksylozą a SRCWa (–0,607) i SRCLa (–0,620). Tak więc, wraz ze wzrostem ilości nierozpuszczalnych frakcji I-NSP wartości parametrów SRC zmniejszyły się dla wszystkich użytych rozpuszczalników i odnotowano istotne ujemne współczynniki korelacji pomiędzy I-NSP a SRCWa (–0,659), SRCSu (–0,670), SRCLa (–0,696) i SRCSc (–0,642), odpowiednio. Ponadto, stwierdzono istotną korelację pomiędzy T-NSP a SRCSu (–0,613). Spośród niektórych rozpuszczalnych frakcji pojedynczych cukrów stwierdzono jedynie dodatnie korelacje jako istotne pomiędzy S-ksylozą a SRCWa (0,619), SRCLa (0,610) i SRCSc (0,601), co oznacza, że im wyższy był poziom S-ksylozy, tym lepsza była zdolność absorpcji mąki, przy czym najbardziej widoczne działanie na wzrost wodochłonności było związane z wysokimi wartościami SRCLa. Stwierdzono również pewne istotne korelacje pomiędzy parametrami analiz reologicznych, sprężystością ciasta P a I-arabinozą (–0,627), I-ksylozą (–0,709) oraz I-AX (–0,711), co może wyjaśniać wpływ nierozpuszczalnych frakcji polisacharydów nieskrobiowych na właściwości ciasta. Potwierdzono również, że rozpuszczalne frakcje polisacharydów są powiązane z P, a istotne korelacje potwierdzono S-ksylozą (0,828), S-NSP (0,856) i S-AX (0,792). W swoich badaniach Andersson i in. [2017] opisali zwiększenie zawartości błonnika pokarmowego, w tym AX i jego rozpuszczalnych frakcji, poprzez proces ekstruzji. Zastosowane intensywne siły ścinające podczas ekstruzji powodują redukcję długości włókien, dlatego prawdopodobnie zwiększa to dostępną powierzchnię dla hydrolizy enzymatycznej. Według Yağcı i in. [2022], modyfikacje ekstruzyjne przeprowadzone przy maksymalnych temperaturach cylindra 40, 75 i 110°C, umożliwiły minimalną degradację hemicelulozy w otrębach z kaszy bulgur. Zaobserwowali

również znaczną redukcję zawartości glukozy po wstępnej obróbce ekstruzyjnej, ale wzrost zawartości hemicelulozy, ksylozy i arabinozy po hybrydowej ekstruzji alkalicznej otrąb.

W pracy **P4** wykonano obrazy makroskopowe z wykorzystaniem techniki SEM (ang. scanning electron microscope) w celu zobrazowania zmian, jakie zostały spowodowane przeprowadzonymi procesami modyfikacji konwencjonalnych i hybrydowych. Zaobserwowano znaczące zmiany w strukturze i mikrostrukturze zmodyfikowanych mąk, szczególnie, gdy zastosowano ekstruzję dwuślimakową. Mikrostrukturę mąki pszennej natywnej i poddanej obróbce przedstawiono na rysunku [**P4-Fig. 3**]. Zdjęcia SEM wykonano przy powiększeniach ×600 i ×2000. Mąka bazowa F [**P4-Fig. 3a**] wykazała obecność różnych frakcji widocznych jako duże i małe granulki skrobi, części składników włóknistych pochodzących z otrąb oraz wydłużone struktury pochodzące z białka. Różnorodne rozmiary granulek skrobi pszennej potwierdziły obecność obu typów granulek: typu A (średnica powyżej 9,9 μm) i typu B (średnica poniżej 9,9 μm). W mące pszennej granulki typu A zajmują do 70% objętości i 10% całkowitej liczby granulek skrobi, a granulki typu B zawierają około 30% objętości i 90% całkowitej liczby granulek [Lu i in. 2014]. Donoszono również o bardzo drobnych (<2,0 μm) granulkach skrobi typu C, chociaż ten typ może również reprezentować granulki typu B [Liu i in. 2023]. Granulki skrobi typu A i typu B wykazują różną morfologię, przy czym granulki typu A mają kształt dysku z możliwymi rowkami lub wgłębieniami, a granulki typu B mają kształty kuliste, elipsoidalne, kanciaste i nieregularne oraz są ściśle upakowane w bielmie [Shevkani i in. 2017].

W mące natywnej po dodaniu enzymów można zaobserwować niewielką aglomerację w mące FC i FCX (odpowiednio **P4-Fig. 3 b i 3c**]. Może to być wynikiem aktywności enzymów i rozpoczęcia hydrolizy liniowych frakcji polisacharydów, zwłaszcza celulozy i hemicelulozy, widocznych jako grupy sklejonych cząstek mąki, przy czym skrobia typu A i B są umieszczone blisko siebie. Dodatek enzymów do mąki o niskiej zawartości wilgoci powoduje niepełną hydrolizę, ale struktura mąki pszennej może się zmienić, np. zwiększyć zawartość S-NSP, zwłaszcza S-AX, a tym samym zwiększyć możliwości poprawy wodochłonności i DT mąki.

Po suchej obróbce termicznej zauważalne są bardziej pojedyncze granulki skrobi o większych wymiarach, co sugeruje obecność podgrzanych i spęczniałych granulek skrobi umieszczonych luźno w mące TF [**P4-Fig. 3d**]. W mące TF odnotowano zwiększoną zawartość I-AX, I-NSP i T-AX, T-NSP, niższą lepkość i hydratację w porównaniu z F, ale dodanie enzymów wywołało przeciwny efekt w próbkach TFC i TFCX, szczególnie, gdy włączono kompleks celulaza-ksylanaza. W tych mąkach zauważono drobniejsze upakowanie cząstek

[**P4-Fig. 3e i 3f**] z pustymi przestrzenimi między granulkami skrobi, co pozwoliło na większą absorpcję rozpuszczalników (analiza SRC), zwiększone DT i rozciągliwość ciasta. Niższa wilgotność mąki TF może jednak również przyczyniać się do tego efektu.

W mące poddanej obróbce parą (próbka HF) zaobserwowano widoczną częściową aglomerację [**P4-Fig. 3g**], która była bardziej intensywna przy włączeniu enzymów [**P4-Fig. 3h i 3i**]. Tworzenie się tych aglomeratów ze spęczniałymi i częściowo skleikowanymi granulkami skrobi oraz przy obecności drobniejszych granulek o mniejszych wymiarach zmniejszyło zdolności hydratacyjne tej maki, skróciło rozciągliwość ciasta i obniżyło wartość wypiekową wraz ze wzrostem maksymalnej lepkości. Małe granulki typu B charakteryzują się większą odpornością na hydrolizę i wykazują niższą temperaturę kleikowania niż struktura typu A, którą zaobserwowano po obróbce HF i EF.

Najbardziej znaczące zmiany zaobserwowano w mące ekstrudowanej bez [**P4-Fig. 3j**] i z dodatkiem enzymów [**P4-Fig. 3k i 3l**]. Według Bouasla i in. [2016] surowa mąka jest upłynniana wewnątrz ekstrudera przy dostępie wody i kleikowana w zależności od temperatury procesu. Otrzymane obrazy potwierdzają te obserwacje, przedstawiając stopioną i zwartą strukturę wewnętrzną matrycy skrobia-białko-lipid z dużymi skupiskami utworzonymi z powodu asocjacji po obróbce w obecności wody (27%) i braku wolnych granulek skrobi, szczególnie w próbkach EFC i EFCX [**P4-Fig. 3k i 3l**]. Do podobnych wniosków doszli Wu i in. [2024], którzy wykazali, że działanie ekstrudera spowodowało rozkład skrobi. W ekstrudowanych mąkach obniżony I-NSP i zwiększony S-AX w stopionej strukturze wewnętrznej znacznie poprawiły właściwości hydratacyjne mąki, ale zmniejszyły stabilność ciasta, GPI, C3, C4 i C5 – i tym samym uniemożliwiły utworzenie struktury ciasta. Cervantes-Ramírez i in. [2020] zaobserwowali zintegrowaną amorficzną matrycę skrobi kukurydzianej utworzoną jako efekt obróbki ekstruzyjnej. Jednakże niektóre granulki pozostały widoczne, ponieważ dodane kwasy tłuszczowe działały jak powłoka ochronna (smarująca) podczas ekstruzji i znacznie redukowały uszkodzenia fizyczne granulek skrobi.

Właściwości techno-funkcjonalne zmodyfikowanych mąk, zwłaszcza ich właściwości hydratacyjne i poziom skleikowania, jak również parametry reologiczne ciasta, mogą być poprawiane przez odpowiednią metodę obróbki, aby umożliwić ich zastosowanie w różnych aplikacjach. Mąki ekstrudowane o wysokiej absorpcji wody mogą stanowić interesującą alternatywę dla podgotowanych skrobi lub hydrokoloidów w procesie produkcji pieczywa, przy niewielkim dozowaniu wykazują znaczący wpływ na zwiększenie wydajności pieczywa. Co więcej, tego typu mąki nie muszą być oznaczane jako dodatki sygnowane literą E, ale jako

mąka pszenna, co ułatwić może wykazanie tego dodatku na „czystej etykiecie” jako obecnie preferowanego trendu w przemyśle spożywczym.

Kolejne badania zaprezentowane w pracy **P5** obejmowały wykorzystanie uzyskanych zmodyfikowanych mąk jako składników "czystej etykiety" w pieczywie pszennym w celu zweryfikowania cech technologicznych i jakościowych uzyskanych podczas ich zastosowania jako dodatków w produktach piekarniczych.

W pracy doświadczalnej **P5** mąkę F oraz zmodyfikowane mąki FC, FCX, TF, TFC, TFCX, HF, HFC, HFCX dodano w ilości 10 i 20% do komercyjnej (handlowy typ 750) mąki pszennej chlebowej (PZZ Lubella), a następnie przygotowano chleb foremkowy (rys. 11), aby sprawdzić przydatność dodatku mąk modyfikowanych do poprawy właściwości wypiekowych. Przeanalizowano właściwości zarówno mieszanek mąki, ciasta, jak i chleba, m. in. skład chemiczny, parametry reologiczne, czy cechy jakościowe i teksturę pieczywa.

Rys. 11. Wypiek chleba foremkowego z dodatkiem mąk modyfikowanych (*opracowanie własne*).

Po przeanalizowaniu badanych mieszanek za pomocą analizy farinograficznej [**P5-Tab. 1**], zaobserwowano, że zwiększona tendencja do wchłaniania wody widoczna w modyfikowanych mąkach przełożyła się także na wzrost absorpcji wody (WA) w mieszankach chlebowych. Było to szczególnie zauważalne w ciastach wytworzonych z receptur zawierających mąki poddane obróbce termicznej przy zawartościach zarówno 10, jak i 20%. Tak więc przy dodatku mąki TFCX10 WA wzrosło o 3,5%, przy TFCX20 o 5,4%, a przy TFC20 o 5,7% w porównaniu z kontrolną recepturą K. Zwiększenie absorpcji wody analizowanych mieszanek mącznych stwierdzono również w przypadku próbek zawierających mąki poddane obróbce hydrotermicznej, ale bez istotnych różnic, gdy podczas tej obróbki zastosowano enzymy piekarnicze. Wyniki pozwoliły na dobór odpowiedniej ilości wody do przygotowania ciasta chlebowego metodą foremkową o stałej konsystencji 400 BU,

umożliwiając porównanie wpływu dodatku modyfikowanych mąk na jakość i wydajność chleba.

Jak potwierdzono w pracy **P1**, zwiększony poziom polisacharydów nieskrobiowych w opracowanej mące bazowej F (zwłaszcza frakcji nierozpuszczalnych, które pochodziły głównie z końcowych pasaży rozczynowych i wymiałowych, a także z mąk pasaży filtracyjnych) był dodatnio skorelowany z absorpcją wody. Zatem prezentowana w pracy **P5** zwiększona zdolność absorpcji wody w mieszankach chlebowych przy dodatku mąki modyfikowanej na poziomie 10 i 20% może być również związana z obecnością polisacharydów nieskrobiowych, które zostały nieznacznie zmodyfikowane za pomocą obróbki termicznej, hydrotermicznej i hybrydowej.

W celu oceny właściwości reologicznych mieszanek mąki kontrolnej z dodatkiem mąk modyfikowanych i scharakteryzowania wpływu poszczególnych modyfikacji na końcową jakość ciasta chlebowego dla wszystkich próbek testowych przeprowadzono analizę alweograficzną. Wyniki badania właściwości ciasta, przy wykorzystania urządzenia Alweograf przedstawiono w tabeli [**P5-Tab.1**]. Testowana komercyjna mąka chlebowa (K) charakteryzowała się odpowiednimi parametrami do wypieku chleba, przy czym siła wypiekowa W wynosiła 212×10^{-4}J, a współczynnik sprężystości i rozciągliwości ciasta P/L wynosił 1,26. Po dodaniu mąki o zwiększonej zawartości polisacharydów nieskrobiowych (F) w ilościach 10 i 20% zaobserwowano niewielkie zwiększenie rozciągliwości ciasta (L), a tym samym poprawę wartości wypiekowej (W). Dodatek mąk modyfikowanych enzymatycznie FC i FCX nie wpłynął na zmianę cech alweograficznych, a uzyskane parametry były zbliżone do mąki kontrolnej K. Gdy zmodyfikowane mąki poddane obróbce cieplnej (TF) zostały użyte jako dodatki do mąki kontrolnej, stwierdzono pogorszenie wartości wypiekowej (W), głównie z powodu zmniejszenia sprężystości ciasta (P). Również parametry elastyczności ciasta Ie i SH uległy pogorszeniu. Należy zauważyć, że gdy jako dodatek zastosowano mąki modyfikowane termicznie z dodatkiem enzymów piekarniczych, parametr sprężystości (P) powrócił do wartości obserwowanej dla mąki kontrolnej K. Stwierdzono również poprawę rozciagliwości ciasta (L), wartości wypiekowej (W) i wskaźnika elastyczności (Ie); poprawa tych parametrów była bardziej widoczna w próbach z dodatkiem mąki modyfikowanej z kompleksem enzymatycznym (TFCX). W badaniach Buscella i in. [2016] analizowano mąkę do wyrobu ciast i mąkę chlebową, które znacząco różniły się jakością i wartością wypiekową. Mąki te zostały poddane obróbce termicznej bez dodatku wody oraz obróbce hydrotermicznej. Przeprowadzono analizę zarówno zawiesiny mąki, jak i matrycy ciasta i zaobserwowano, że obróbka termiczna poprawiła stabilność ciasta, choć bardziej intensywnie w przypadku mąki

o słabszej wartości wypiekowej, stwierdzono również, że zmieniły się właściwości skrobi mąki chlebowej w testach Mixolab [Bucsella i in. 2016]. Analiza wartości wskaźnika sedymentacji Zeleny'ego, oceniająca jakość białka glutenowego, wykazała znaczące obniżenie dla mąki chlebowej poddanej suchej obróbce termicznej w porównaniu z mąką chlebową niepoddaną obróbce [Bucsella i in. 2016]. Rezultat ten przypisano zmianom w strukturze glutenu spowodowanym przegrupowaniom wiązań disiarczkowych.

W pracy **P5** również zaobserwowano, że dodatek mąki bazowej F poddanej wyłącznie termicznej obróbce, pogorszył parametry jakościowe określone za pomocą analizy alweograficznej. Jednocześnie, zastosowanie metody modyfikacji hybrydowej, poprzez udział w mieszance enzymów piekarniczych, szczególnie TFCX z kompleksem celulaza-ksylanaza, pozwoliło na synergistyczną modyfikację, która poprawiła ogólną jakość białek glutenowych, co uwidoczniło się w poprawie elastyczności ciasta, a także w zauważalnej poprawie właściwości hydratacyjnych mąki (zwiększona absorpcja wody) [**P5-Tab. 1**]. W przypadku zastosowania dodatku mąk modyfikowanych obróbką hydrotermiczną stwierdzono zmniejszenie rozciągliwości ciasta (L) i znaczny wzrost jego sprężystości (P) we wszystkich badanych mieszankach. Spowodowało to istotną zmianę w konfiguracji wykresu P/L, którego wartość wzrosła o 26% dla ciasta z dodatkiem 10% mąki przetworzonej hydrotermicznie (HF10) w porównaniu do mąki kontrolnej, oraz o 49%, jeśli do mieszanki dodano 20% mąki z hybrydową enzymatyczno-hydrotermiczną modyfikacją (HFCX20). Obróbka hydrotermiczna mąki bazowej F spowodowała istotne zmiany w konformacji białek glutenowych, co wpłynęło przede wszystkim na zmniejszenie elastyczności ciasta. Jednocześnie, nie obserwowano istotnych różnic między dodatkiem mąk poddanych tylko obróbce hydrotermicznej, a mąkami obrabianymi tą metodą wspomaganą enzymatycznie. Chociaż, zarówno wartość wypiekowa W, jak i współczynnik SH, wzrosły do wyższego poziomu niż w mące kontrolnej (K), było to spowodowane zwiększoną sztywnością ciasta, a nie rzeczywistą poprawą jakości mąki, ponieważ nie zaobserwowano zwiększenie absorpcji wody w tej mieszance. Podobne wyniki uzyskano w pracy Martineza i in. [2013], którzy stosowali ekstrudowane mąki jako dodatki do chleba. Tutaj dodatek ekstrudowanych mąk znacznie zwiększył elastyczność ciasta i zmniejszył jego rozciągliwość [Martinez i in. 2013]. Należy również zauważyć, że mąki zmodyfikowane metodą hydrotermiczną lub hybrydową wspomaganą enzymami, będąc składnikiem receptury, nie tworzyły pożądanej struktury ciasta. Ich dodatek powodował pogorszenie formowania się skórki chleba, mimo, że zwiększały one objętość i wydajność wypieku, w porównaniu do chleba kontrolnego z mąki K.

Jakość i wygląd chleba są ważnymi czynnikami zarówno dla producentów, jak i konsumentów [Cauvain i in. 2012, Vargas i in. 2021, Campbell i in. 2016, Zhang i in. 2021, Wójcik i in. 2023, Jurkaninová i in. 2024, Mahmoud i in. 2024, Zarzycki i in. 2024]. Chleby z mąki pełnoziarnistej lub uzupełnione o dodatek niemodyfikowanych otrąb, ze względu na zmniejszoną zdolność ciasta do zatrzymywania gazów, charakteryzują się mniejszą objętością bochenka, a tym samym gorszą jakością wypieku [Schmiele i in. 2023, Bucsella i in. 2016]. Producenci preferują wysoką wydajność chleba o dużej objętości bochenka i zwiększonej zdolności absorpcji wody podczas wyrobu ciasta chlebowego, natomiast konsumenci wolą regularną strukturę skórki i jednorodny rozkład porów w miękiszu chleba. Dodawanie większej ilości wody do receptur ciasta jest powszechnym podejściem do zwiększenia efektywności produkcji chleba. Jednak zwiększenie ilości wody w cieście może spowodować pogorszenie zdolności ciasta do wyrabiania, ponieważ staje się ono zbyt mokre i lepkie, a to wpływa na końcową objętość i teksturę chleba [Gomez i in. 2011]. Ponadto, wyższa zawartość wody w cieście może skrócić okres przydatności chleba do spożycia ze względu na zagrożenia mikrobiologiczne. Wykazano, że dodatek mąk modyfikowanych fizycznie, bogatych we frakcje włókniste, powoduje zmiany w jakości pieczywa [Li i in. 2023].

Wyniki wybranych cech jakościowych wypiekanego pieczywa przygotowanego z dodatkiem mąk modyfikowanych przedstawiono w tabeli [**P5-Tab. 3**]. Analizując jakość i charakterystykę wydajności wypiekanego chleba uzyskanego z receptur z dodatkiem mąk modyfikowanych można zauważyć, że dodatek mąk modyfikowanych enzymatycznie i procesowo miał znaczący wpływ na objętość testowanego chleba. Efekt ten był również widoczny w parametrach objętości poszczególnych bochenków. Objętość chleba znacznie wzrosła, gdy do składu chleba dodano mąki poddane obróbce, w większości przypadków, gdy poziom dodatku wynosił 20% mąk modyfikowanych.

Bardzo dobre wyniki w zwiększaniu objętości bochenka uzyskano, gdy jako dodatki zastosowano wyłącznie mąki z dodatkiem enzymu (FC10, FC20 i FCX20). Hilhorst i in. [1999] podali, że dodanie ksylanaz może poprawić właściwości użytkowe ciasta pszennego, powodować lepsze wyrastanie i objętość chleba. Dodanie do mieszanek chlebowych mąki modyfikowanej procesem termicznym lub mąki poddanej obróbce hybrydowymi metodami wspomaganymi enzymami również poprawiło objętość chleba, ale bez istotnych różnic pomiędzy zastosowanymi metodami przetwarzania. Wykazano istotny wzrost objętości chleba, gdy do receptury chleba dodano mąki TFC10 i HF20 poddane obróbce termicznej i hydrotermicznej w ilości odpowiednio 10 i 20%. Należy zauważyć, że większa objętość bochenka chleba przygotowanego z dodatkiem mąki obrabianej hydrotermicznie z enzymami

była również wynikiem nierównomiernego rozłożenia pęcherzyków gazu, które w dużej liczbie znajdowały się pod skórką chleba, powodując jej odchylanie się na bok i zapadanie się bochenka w końcowej fazie wypieku, co widać na uzyskanych zdjęciach chleba przedstawionych na rysunku [**P5-Fig. 2**]. Sugeruje to, że konsystencja ciasta mogła być zbyt luźna i że ilość dodawanej do receptur wody powinna zostać zmniejszona w przypadku dodawania mąk modyfikowanych hydrotermicznie [Bucsella i in. 2017].

Gdy mąki modyfikowane hydrotermicznie były używane jako dodatek do chleba, widoczny był negatywny wpływ na końcową jakość wytworzonego pieczywa. Problemy z zatrzymywaniem gazu w chlebie były prawdopodobnie spowodowane utratą przez mąki w ten sposób modyfikowane właściwości tworzenia siatki glutenowej. Według Hong i in. [2023], modyfikacja mąki pszennej poprzez obróbkę parą przegrzaną powoduje denaturację białka i początkowe kleikowanie zawartych w nim granulek skrobi, zmniejszając w ten sposób dostęp wody do fazy białkowej ze względu na jej większą absorpcję. Wynikający z tego problem z tworzeniem ciągłej matrycy ciasta wpływa również na osłabienie jakości glutenu i zmniejszenie jego elastyczności. Efekt ten zauważono także w analizach alweograficznych [**P5-Tab.1**]. Obecność dodatków mąki HF, HFC i HFCX w cieście chlebowym spowodowała obniżenie wytrzymałości ciasta i problem z zatrzymywaniem pęcherzyków gazu w matrycy ciasta. Pomimo zastosowania enzymów piekarniczych w trakcie modyfikacji, które zwiększyły ilość frakcji rozpuszczalnego błonnika, nie wyeliminowano negatywnego wpływu procesu modyfikacji hydrotermicznej na jakość białka glutenowego.

W pracy **P5** wyznaczono, że objętość właściwa chleba znacznie zwiększała się po dodaniu mąki poddanej obróbce termicznej, hydrotermicznej i hybrydowej lub po włączeniu FC10 i FC20 do receptury chleba [**P5-Tab. 3**]. W tych próbkach zaobserwowano również istotne zmniejszenie gęstości chleba (0,30–0,33 g/cm^3) w porównaniu z próbkami kontrolnymi (0,35 g/cm^3), ze względu na większą liczbę porów w miękiszu niż w chlebie kontrolnym.

Stwierdzono również, że objętość chleba i objętość właściwa silnie ujemnie korelowały z gęstością chleba (odpowiednio –0,997 i –0,987; przy $p < 0,05$). W swoich badaniach Tao i in. [2021] opisali zwiększoną objętość właściwą chleba (z 1,63 do 2,15 g/cm^3), gdy do modyfikacji skrobi pszennej dodanej do receptury chleba zastosowano ekstruzję niskotemperaturową. W badaniach w pracy **P5** wykazano, iż dodatek mąk modyfikowanych nie spowodował zwiększenia strat podczas wypieku w porównaniu do chleba kontrolnego, podczas gdy zaobserwowano zwiększenie utraty wagi po 24 godzinach od wypieku. Większe straty wypiekowe po 24 godzinach były odnotowane, gdy porównano receptury z dodatkiem mąki modyfikowanej i kontrolną, jednak nieistotne statystycznie. Największą ususzkę podczas

wypieku zaobserwowano w chlebach kontrolnych. Natomiast w chlebach z dodatkiem mąk modyfikowanych ususzka była nieco niższa, co prawdopodobnie wskazuje na zwiększoną zdolność do zatrzymywania wody podczas wypieku mieszanek z udziałem mąk modyfikowanych. Nieznacznie wyższa utrata masy bochenków chleba po schłodzeniu i po 24 h od wypieku była prawdopodobnie efektem retrogradacji skrobi, którą poddano obróbce metodami T i H. Wyniki te potwierdzają dane z analizy WAI, w której wyniki były również niższe w chlebie kontrolnym i wyższe w tym wytworzonym z modyfikowanymi dodatkami [**P5-Tab. 5**]. Dodanie mąki TF do receptury zmniejszyło retrogradację skrobi w cieście, co ilustrują wyniki pomiaru C5, podczas gdy dodanie mąki poddanej obróbce hydrotermicznej zwiększyło poziom retrogradacji [**P5-Fig. 1**]. Dane te zostały również potwierdzone analizą tekstury chleba, gdzie po schłodzeniu, wewnętrzna konsystencja miękiszu była mniej sprężysta [**P5-Tab. 6**].

W podobnej pracy Kurek i in. [2017] stwierdzili, że wartości objętości właściwej chleba pszennego zależą od rodzaju użytej mąki, przy czym najniższą objętość właściwą zaobserwowali w chlebie pełnoziarnistym (0,82 cm^3/g), a najwyższą w kontrolnych próbkach chleba z mąki pszennej chlebowej (1,60 cm^3/g). Ma i in. [2021] zbadali wpływ obróbki parą przegrzaną na właściwości fizykochemiczne mąki do wypieków. Zauważyli, że obróbka parą może poprawić pewne cechy jakościowe ciasta, takie jak objętość i jakość miękiszu. W swoich badaniach odkryli, że obróbka parą przegrzaną zwiększyła poziom skleikowania skrobi i osłabiała wytrzymałość glutenu z powodu denaturacji, a zmiany tych właściwości fizykochemicznych mąki wykazały wpływ na ogólną jakość ciasta [Ma i in. 2021, Hu i in. 2017]. W przygotowanych w pracy **P5** badaniach obróbka enzymami w pewnym stopniu wyeliminowała negatywne aspekty modyfikacji termicznych. Natomiast obróbka hydrotermiczna, mimo zastosowania kompleksu enzymatycznego, negatywnie wpłynęła na jakość zmodyfikowanej mąki, której dodatek spowodował widoczne w uzyskanym pieczywie problemy z zatrzymywaniem pęcherzyków gazu w cieście podczas wyrastania i wypieku.

Z punktu widzenia ekonomicznego i jakościowego najbardziej pożądanym rezultatem modyfikacji receptury wypiekowej jest wygenerowanie najwyższej objętości właściwej pieczywa. Badacze, tacy jak Alamri i in. [2022] w swojej pracy podali zakres objętości właściwej między 2,55 a 3,14 cm^3/g dla chleba z dodatkiem 1 i 2% gum roślinnych, przy czym najniższą wartość uzyskano dla chleba kontrolnego z mąki z pszenicy zwyczajnej. W przeprowadzonych w pracy **P5** badaniach, jako jeden z istotnych parametrów, obliczono zmianę wydajności uzyskanego chleba w stosunku do kontroli dla poszczególnych wypiekanych bochenków. Istotny wzrost zaobserwowano w przypadku chlebów wypieczonych

z receptur zawierających dodatek mąk HFC20, HFCX20, HFCX10, TFCX 20 i TFC20. Jednakże, jak wspomniano wcześniej, chleby upieczone z dodatkiem mąk HF charakteryzowały się niższą zdolnością do zatrzymywania gazów podczas fermentacji, szczególnie, gdy dodano 10% mąk HFC i HFCX, a pęcherzyki przemieszczały się pod skórką, powodując jej odstawanie od miękiszu [**P5-Fig. 2**].

Takich zmian nie zaobserwowano, gdy jako dodatek zastosowano mąkę poddaną obróbce termicznej T. Chleby z dodatkami TF, TFC i TFCX charakteryzowały się zwiększoną lub podobną liczbą porów, jak w chlebie kontrolnym K; były one równomiernie rozmieszczone w miękiszu, zwiększając całkowitą objętość bochenków. Ambrosewicz-Walacik i in. [2016] w pokrewnej pracy testowali wydajność chleba z drożdżami lub chleba fermentowanego na zakwasie w oparciu o różne składy ciast. Podali, że wydajność chleba wahała się od 131,1%, jeśli drożdże były używane w jasnym chlebie pszennym do 134,8% w chlebie pszennym pełnoziarnistym, podczas gdy wydajność chleba wahała się od 152,8% do 162,4% odpowiednio, gdy zastosowano naturalny zakwas. Znacznie wyższą wydajność chleba uzyskano, jeśli użyto mąki żytniej, zwłaszcza pełnoziarnistej (188,3%), a do przygotowania chleba użyto naturalnego zakwasu. W pracy **P5** oceniono, iż zastosowanie termicznie zmodyfikowanych mąk w standardowej recepturze chleba pszennego dało najwyższą wydajność chleba wynoszącą 150,02% przy zwiększeniu absorpcji wody przez ciasto do 66%, zwłaszcza, gdy zastosowano metodę wspomaganą enzymami do modyfikacji opracowanej mąki, bez negatywnego wpływu na teksturę lub barwę otrzymanych chlebów.

Ze względu na dużą ilość przeprowadzonych analiz i uzyskanych czynników wpływających na końcową jakość poszczególnych prób testowych, w pracy **P5** wykonano analizę głównych składowych PCA w celu określenia parametrów, które mają największy wpływ na zmienność całego układu. W toku wstępnej analizy uzyskano osiemnaście nowych zmiennych, z których pierwsze trzy główne składowe wyjaśniały ponad 50% zmienności układu. Pierwsze dwie główne składowe wyjaśniały 40,16% zmienności układu (PC1 w 24,95% i PC2 w 15,21%), a parametry zawarte między dwoma okręgami miały największy wpływ na zmienność układu [**P5-Fig. 3**]. W wyniku tej analizy określono jako istotne czternaście parametrów z czterdziestu badanych parametrów: C2, C3, C4, C5, L, IDF, TDF, wydajność pieczywa (bread yield), W, le, SH, P, P/L i zawartość węglowodanów (carbohydrates) [**P5-Fig. 3a**]. Pozostałe parametry miały niewielki wpływ na zmienność układu. Ponadto C2, C3, C4, C5, W, le, SH, P i P/L były silnie i dodatnio skorelowane. Tę samą zależność zaobserwowano pomiędzy IDF, TDF i wydajnością pieczywa. Silna, ale ujemna korelacja występowała między parametrami C2, C3, C4, C5, W, Ie, SH, P, P/L i L, podczas

gdy silna i ujemna korelacja została również określona pomiędzy IDF, TDF, wydajnością chleba i zawartością węglowodanów. Nie wyznaczono korelacji pomiędzy C2, C3, C4, C5, W, Ie, SH, P, P/L a zawartością IDF, TDF, wydajnością chleba i poziomem węglowodanów. Nie zaobserwowano również korelacji pomiędzy L i IDF, TDF i wydajnością chleba oraz między L i zawartością węglowodanów. Analiza PCA wykazała również [**P5-Fig. 3a i 3b**], że HF, HFC i HFCX były silnie i dodatnio skorelowane z C2, C3, C4, C5, W, Ie, SH, P i P/L oraz silnie i ujemnie z L. Mąki F10, F20, TFC i TFCX były silnie i dodatnio skorelowane z L. Z kolei K, FC, TF i FCX były silnie i dodatnio skorelowane z węglowodanami. Analiza PCA wykazała, że pierwsza główna składowa PC1 odróżniała mąkę modyfikowaną hybrydowo termiczno-enzymatycznie od mąki kontrolnej i mąki obrabianej termiczno-enzymatycznie w 24,95% [**P5-Fig. 3b**]. Dodatnie wartości głównej składowej PC1 opisywały wyniki dla mąk F i T oraz kontroli, a ujemne wartości głównej składowej PC1 opisywały wyniki dla H. Druga główna składowa PC2 charakteryzowała mąki F, TF i TFCX (dodatnie wartości PC2) oraz K, FC i FCX (ujemne wartości PC2) w 15,21%.

Pierwsza i trzecia główna składowa wyjaśniały 36,99% zmienności układu (PC1 w 24,95% i PC3 w 12,04%). Parametry zawarte między dwoma okręgami miały największy wpływ na zmienność układu [**P5-Fig. 4**]. W wyniku analizy określono dwanaście parametrów z czterdziestu badanych parametrów: C2, C3, C4, C5, wydajność chleba, W, Ie, SH, P, P/L, objętość właściwa (specific volume) i objętość pieczywa (bread volume) [**P5-Fig. 4a**]. Pozostałe parametry miały niewielki wpływ na zmienność układu. C2, C3, C4, C5, W, Ie, SH, P i P/L były silnie i dodatnio skorelowane. Tę samą zależność zaobserwowano dla objętości właściwej i objętości pieczywa. Silna, ale ujemna korelacja występowała pomiędzy parametrami: objętość właściwa, objętość pieczywa i wydajność pieczywa. Nie zaobserwowano korelacji pomiędzy C2, C3, C4, C5, W, Ie, SH, P, P/L a wydajnością pieczywa, objętością właściwą i objętością pieczywa.Analiza PCA wykazała również [**P5-Fig. 4a i 4b**], że HF, HFC i HFCX są silnie i dodatnio skorelowane z C2, C3, C4, C5, W, Ie, SH, P i P/L. Z kolei F10, F20, TFC i TFCX są silnie i dodatnio skorelowane z objętością właściwą i objętością pieczywa. Próby K, FC, TF i FCX były silnie i dodatnio skorelowane z wydajnością pieczywa. Analiza PCA wykazała, że pierwsza główna składowa PC1 odróżniała mąkę modyfikowaną hybrydowo enzymatyczno- hydrotermicznie od mąki kontrolnej i mąki obrabianej termiczno-enzymatycznie w 24,95% [**P5-Fig. 4b**]. Dodatnie wartości głównej składowej PC1 opisywały wyniki dla mąk F i T oraz kontrolnej, a ujemne wartości głównej składowej PC1 opisywały wyniki dla H. Trzecia główna składowa PC3 charakteryzowała mąki

F20, FC, FCX i TFCX (ujemne wartości PC3) oraz K, F, TF i TFC (dodatnie wartości PC3) w 12,04%.

Podsumowując, wyniki pracy **P5** potwierdziły możliwość zastąpienia standardowych mąk pszennych mąkami poddanymi obróbce termicznej, hydrotermicznej lub hybrydowej wspomaganej enzymami w recepturach na chleb w celu poprawy wydajności pieczywa. Zastosowane procesy modyfikacji spowodowały pewne istotne zmiany w reologii ciasta, gdy w kompozycji kontrolna handlowa mąka chlebowa została zastąpiona w 10 lub 20% mąkami modyfikowanymi. Zgodnie z wynikami badań, właściwości ciasta chlebowego różniły się w zależności od zastosowanego procesu modyfikacji i ilości dodanej modyfikowanej mąki. Obróbka hybrydowa przy obecności enzymów pozwoliła w pewnym stopniu wyeliminować negatywne aspekty procesów fizycznych w trakcie procesu modyfikacji opracowanej mąki.

Obróbka hydrotermiczna, pomimo zastosowania kompleksu enzymatycznego, negatywnie wpłynęła na jakość modyfikowanej mąki, której dodanie spowodowało problemy z zatrzymywaniem pęcherzyków gazu w cieście chlebowym podczas wyrastania i wypieku. Właściwości chleba pod względem składu chemicznego, cech jakościowych, tekstury i barwy były nieznacznie różne, jeśli dodano zmodyfikowane mąki. Zastosowanie mąki modyfikowanej poddanej obróbce termicznej jako dodatku do handlowej mąki chlebowej wpłynęło na uzyskanie najwyższej wydajności chleba wynoszącej 150,02% i zwiększyło absorpcję wody przez ciasto o 6%, zwłaszcza, gdy do opracowanej mąki zastosowano metodę modyfikacji wspomaganą dodatkiem enzymów; osiągnięto to bez negatywnego wpływu na teksturę lub barwę otrzymanych chlebów. Wytworzone ciasta z dodatkiem mąki modyfikowanej termicznie mogą być wykorzystywane do przetwarzania, mieszania i fermentacji bez pogorszenia jakości końcowej pieczywa, jednocześnie ze zwiększoną objętością chleba (16%).

4.3. Prace wdrożeniowe

Prace wdrożeniowe prowadzono z wykorzystaniem prototypowej instalacji do termicznej obróbki mąki będącej na wyposażeniu firmy PZZ Lubella w Lublinie. Serię testów obróbki ekstruzyjnej i enzymatyczno-ekstruzyjnej przeprowadzono w Centrum Badawczo Rozwojowym przy firmie PZZ Lubella w Lublinie z wykorzystaniem ekstrudera dwuślimakowego oraz w warunkach przemysłowych w obietach firmy. Badania cech jakościowych prowadzono w przyzakładowym laboratorium i studium wypiekowym.

Proces uzyskiwania określonych frakcji mąki bazowej F opierał się o charakterystykę przemiałową mąki oraz kaszy do produkcji wyrobów makaronowych, które stanowią jeden z głównych asortymentów produktów wytwarzanych przez młyn przemysłowy PZZ Lubella. Frakcja pozostała po odseparowaniu mąki oczyszczonej jest częściowo wykorzystywana do produkcji mąk pełnoziarnistych, jako składnik wyrobów galanterii śniadaniowej wytwarzanych przez PZZ Lubella, mąk piekarniczych lub sprzedawana jest jako składnik do mieszanek paszowych (analogicznie jak frakcja otrąb). Proces mielenia obejmował etapy rozdrabniania (pasaże śrutowe), redukcję (pasaże rozczynowe i wymiałowe), przesiewanie oraz sortowanie i klasyfikację. Schematyczny diagram etapów przemysłowego mielenia przedstawiono w pracy **P1** [**P1-Fig. 1**].

Określono procentowy udział w mące bazowej F i wydajność poszczególnych pasaży w trakcie produkcji jasnej mąki niskowyciągowej (informacja niejawna objęta tajemnicą prawnie chronioną). Pomiar wydajności i skład procentowy ustalono w trakcie standardowego przemiału i wykonano poprzez separcję poszczególnych frakcji do oddzielnych pojemników przez czas 10 minut i wyliczenie wydajności w kg/godz., zaś wydajność poszczególnych frakcji przy kolejnych przemiałach może się nieznacznie różnić w poszczególnych szarżach produkcyjnych.

Podczas wyboru poszczególnych pasaży jako składowych mąki bazowej F, wykorzystano wyniki pracy **P1** oraz wieloletnie doświadczenie technologiczne inż. Krzysztofa Gaczkowskiego, kierownika Młyna Pszennego, który pełnił również funkcję opiekuna naukowego z ramienia firmy podczas relazacji prac wdrożeniowych w ramach rozprawy doktorskiej. Przede wszystkim wzięto pod uwagę wysoką całkowitą zawartość arabinoksylanów T-AX, także ich frakcji rozpuszczalnych S-AX, przy jednoczesnym spełnieniu wymagań parametrów reologicznych i fizykochemicznych, z tolerancją uwzględniającą procentowy udział określonej frakcji w całej mieszance mąki. Mąki pasażowe musiały charakteryzować się odpowiednią do wypieku wartością wypiekową W, której wartość wynikła z odpowiedniego stosunku parametrów sprężystości P i rozciągliwości ciasta L. Analizowano również wodochłonność poszczególnych frakcji różnymi metodami opisanymi w rozdziale 3.2 rozprawy (WA, M-WA, SRCWa), stabilność (S i M-S), stopień rozmiękczenia (DoS) i parametry jakościowe białka glutenowego (SRCLa i M-C2).

W celu ujednorodnienia i homogenizacji uzyskanej mieszanki, w kolejnym kroku mąka rozdzielana była na trzy oddzielne silosy, a następnie mieszana w mieszarce okresowej. W czasie mieszania pobierana była okresowo próba mąki, która stanowiła reprezentatywną próbę średnią, dla której laboratorium przyzakładowe wykonywało każdorazowo oznaczenia

parametrów zgodnie z dokumentami normatywnymi obowiązującymi w przedsiębiorstwie. Po spełnieniu wymagań jakościowych zawartych w dokumentacji, mąka mieszana była poprzez zadozowanie do każdej tony mąki bazowej F określonej ilości enzymu ksylanazy i celulazy, dokładne proporcje enzymów zastosowanych w pracach wdrożeniowych stanowią informację niejawną.

Podczas obróbki termicznej bądź hybrydowej mąka z dodatkiem enzymów transportowana była pneumatycznie do komory przyjęciowej prototypowej instalacji do termicznej obróbki mąki, gdzie zgodnie z wytycznymi zawartymi w dokumencie wewnętrznym firmy „Wskazówki do prowadzenia procesu" prowadzony był proces termicznej obróbki mąki. Po całościowej ocenie laboratoryjnej, zmodyfikowana w zaproponowanej technologii mąka może zostać skierowana do mieszalni młyna pszennego i zadozowana jako składnik specjalistycznych mąk piekarniczych. Z technologicznego oraz ekonomicznego punktu widzenia, za maksymalną ilość dodatku uznano 20% mąki modyfikowanej.

Na podstawie przeprowadzonych prac badawczych opisanych w publikacjach **P3** i **P4** jako element prac wdrożeniowych przeprowadzono także obróbkę technologiczną opracowanej mąki F z zastosowaniem procesu ekstruzji dwuślimakowej w podniesionej skali technologicznej z wykorzystanie ekstrudera dwuślimakowego będącego na wyposażeniu Centrum Badawczo-Rozwojowego firmy PZZ Lubella. Jako surowiec do testów wykorzystano opracowaną mąkę bazową F, mąkę bazową z dodatkiem enzymu celulazy (FC) oraz mąkę bazową z dodatkiem kompleksu celulazy i ksylanazy (FCX), przygotowane w sposób analogiczny jak opisano w punkcie 3.2.5 rozprawy. Przeprowadzono serie testów produkcyjnych otrzymywania modyfikowanych mąk, które następnie poddawano analizom laboratoryjnym oraz testom wypiekowym.

Wytworzone w procesie ekstruzyjnej modyfikacji mąki zostały dodane w ilości do 20% do komercyjnej mąki chlebowej i przetestowane pod kątem przydatności technologicznej jako dodatek do specjalistycznych mąk piekarniczych. Mieszanki mąki handlowej z mąkami modyfikowanymi zostały przetestowane w zakresie właściwości reologicznych z wykorzystaniem analizy alweograficznej, farinograficznej oraz w zmiennej temperaturze na urządzeniu Mixolab, zgodnie z metodyką przedstawioną w rozdziale 3.3. Następnie przygotowano chleb foremkowy, aby przetestować ich przydatność do poprawy właściwości wypiekowych pieczywa. Próby wypiekowe prowadzono z wykorzystaniem pieca MIWE AERO backcombi będącego na wyposażeniu studia wypiekowego w PZZ Lubella. Równocześnie oceniano właściwości ciasta i chleba.

Analizując jakość i charakterystykę wydajności wypiekanego chleba uzyskanego z receptur z dodatkiem mąk modyfikowanych ekstruzyjnie można zauważyć, że dodatek mąk modyfikowanych ekstruzyjnie i wspomaganych enzymami miał znaczący wpływ na wydajność testowanego chleba. Efekt ten był również widoczny w wartościach objętości poszczególnych bochenków. Generalnie, objętość zwiększała się lub pozostała na poziomie zbliżonym do chleba z mąki bez dodatków. Również objętość właściwa chleba z dodatkiem mąk modyfikowanych była większa lub na podobnym poziomie do pieczywa kontrolnego. Gęstość chleba związana z ilością i rozmieszczeniem porów w miękiszu, była na zbliżonym poziomie do pieczywa z mąki kontrolnej. Chleby z dodatkiem mąk modyfikowanych do 10% charakteryzowały się zwiększoną lub podobną liczbą porów, jak w chlebie kontrolnym, które były równomiernie rozmieszczone w miękiszu, zwiększając całkowitą objętość bochenków, co jest bardzo korzystnym efektem zastosowanej mąki modyfikowanej ekstruzyjnie. Taki dodatek mąk modyfikowanych ekstruzyjnie zwiększał wydajność pieczywa, a istotny wzrost zaobserwowano w przypadku chlebów wytworzonych z receptur zawierających dodatek mąk z kompleksem enzymów, który był proporcjonalny do ilości zastosowanego dodatku modyfikowanej mąki w recepturze pieczywa. Testy laboratoryjne pozwoliły opracować zalecane parametry procesowe konieczne do uzyskania podwyższonej wydajności wypiekowej oraz założonych funkcjonalności nowopracowanej modyfikowanej mąki poddanej obróbce ekstruzyjnej i hybrydowej z enzymami piekarniczymi.

Szczegółowy zakres obróbki technologicznej, warunki procesowe oraz charakterystyki opracowanych modyfikowanych mąk z przeznaczeniem na dodatki do mieszanek specjalistycznych dla branży piekarniczej zostały opracowane w ramach prac wdrożeniowych jako noty technologiczne gotowe do wdrożenia w firmie PZZ Lubella i są traktowane jako informacja niejawna objęta tajemnicą prawnie chronioną.

5. WNIOSKI

1. Przeprowadzone prace badawcze pozwoliły na potwierdzenie hipotezy, że zintegrowanie obróbki enzymatycznej z obróbką termiczną lub ekstruzyjną prowadzoną w różnych warunkach procesowych na skomponowanej z wybranych pasaży mące pszennej pozwoli na otrzymanie mąki funkcjonalnej o zwiększonej wodochłonności i zdefiniowanej charakterystyce reologicznej, która samodzielnie lub w mieszankach stosowanych w piekarnictwie umożliwi zwiększenie wydajności pieczywa.
2. Skomponowana z wybranych pasaży przemiałowych mąka pszenna (F) charakteryzowała się podwyższoną zawartością nieskrobiowych polisacharydów i arabinoksylanów. Wykorzystanie skomponowanej mąki w praktyce produkcyjnej pozwoli na ograniczenie ilości niewykorzystanych dotychczas frakcji ubocznych powstających podczas przemiału ziarna pszenicy.
3. Modyfikacje opracowanej mąki pszennej bazowej (F) miały zróżnicowany wpływ na skład, reologię i strukturę, w zależności od warunków obróbki oraz zastosowania enzymów. Konwencjonalne i hybrydowe metody obróbki, obejmujące włączenie enzymów celulazy i/lub kompleksu celulaza-ksylanaza, spowodowały zmiany w składzie frakcji polisacharydowych (zwłaszcza arabinoksylanów) oraz w reologii zmodyfikowanej mąki, zmieniając znacząco właściwości techno-funkcjonalne otrzymanych mąk.
4. Podczas wypieku chleba z dodatkiem mąk obrabianych termicznie i hydrotermicznie najbardziej efektywnym w zwiększaniu wodochłonności ciasta okazał się dodatek mąk o zachowanych funkcjach białka glutenowego, czyli modyfikowanych termicznie.
5. Zaobserwowano poprawę wydajności pieczywa przy zastosowaniu dodatku opracowanej mąki (F) modyfikowanej z udziałem enzymów piekarniczych: celulazy i ksylanazy.
6. Modyfikowane mąki mogą stanowić dodatek do pieczywa w ilości do 20% po obróbce termicznej bez i z enzymami oraz w mniejszej ilości do 10% po obróbce ekstruzyjnej dwuślimakowej i hybrydowej, powodując zwiększenie wodochłonności mąki, poprawę wydajności i objętości pieczywa, bez ujemnego wpływu na strukturę, teksturę i barwę miękiszu chleba z dodatkiem opracowanych mąk funkcjonalnych.
7. W efekcie prac wdrożeniowych w podniesionej skali jako produkt gotowy do wdrożenia do praktyki produkcyjnej w firmie PZZ Lubella GMW Sp. z o.o. została wybrana mieszanka mąki bazowej F z dodatkiem kompleksu enzymów celulazy i ksylanazy modyfikowana metodą obróbki termicznej oraz z użyciem ekstruzji dwuślimakowej. Procesy te został

przeskalowane do produkcji w skali przemysłowej i uzyskane mąki mogą być stosowane jako dodatki do piekarniczych mąk specjalistycznych dedykowanych dla branży piekarniczej wpływające na cechy reologiczne mieszanek, w tym podwyższające wodochłonność i wydajność pieczywa.

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7. OŚWIADCZENIA WSPÓŁAUTORÓW PUBLIKACJI

Lublin, data 24.09.2024

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1. Piotr Lewko, Agnieszka Wójtowicz, Marek Gancarz: Distribution of arabinoxylans and their relationship with physiochemical and rheological properties in wheat flour mill streams as an effective way to predict flour functionality. Applied Science, 2023, 13, (9), 5458. https://doi.org/10.3390/app13095458

mój udział polegał na opracowaniu koncepcji oraz metodologii badań, prowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników, a także na przeglądzie literatury oraz tworzeniu, recenzji i edycji manuskryptu. Wkład w artykuł wynosił: 75%.

2. Piotr Lewko, Agnieszka Wójtowicz, Monika Różańska-Boczula: Effect of extruder configuration and extrusion-cooking processing parameters on selected characteristics of NSP-rich wheat flour as a hybrid treatment with xylanase addition. Processes, 2024, 12, 1159. https://doi.org/10.3390/pr12061159

mój udział polegał na współopracowaniu koncepcji oraz metodologii badań, prowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników, a także na tworzeniu, recenzji i edycji manuskryptu. Wkład w artykuł wynosił: 60%.

3. Piotr Lewko, Agnieszka Wójtowicz, Michał Rudaś: Effect of processing conditions of enzymatic, extrusion and hybrid treatment methods on composition and selected techno-functional properties of developed wheat flour. International Journal of Food Science (w recenzji)

mój udział polegał na współopracowaniu koncepcji oraz metodologii badań, prowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników, a także na tworzeniu, recenzji i edycji manuskryptu. Wkład w artykuł wynosił: 70%.

4. Piotr Lewko, Agnieszka Wójtowicz, Daniel M. Kamiński: The influence of processing using conventional and hybrid methods on the composition, polysaccharide profiles and selected properties of wheat flour enriched with baking enzymes. Foods 2024, 13, 2957. https://doi.org/10.3390/foods13182957

mój udział polegał na opracowaniu koncepcji oraz metodologii badań, prowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników, a także na tworzeniu, recenzji i edycji manuskryptu. Wkład w artykuł wynosił: 70%.

5. Piotr Lewko, Agnieszka Wójtowicz, Marek Gancarz: Application of conventional and hybrid thermal-enzymatic modified wheat flours as clean label bread improvers. Applied Science, 2024, 14, 7659. https://doi.org/10.3390/app14177659

mój udział polegał na opracowaniu koncepcji oraz metodologii badań, prowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników, a także na tworzeniu, recenzji i edycji manuskryptu. Wkład w artykuł wynosił: 75%.

Piotr Lewko

Podpis

Lublin, data 24.09.2024

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracach:

1. Piotr Lewko, Agnieszka Wójtowicz, Marek Gancarz: Distribution of arabinoxylans and their relationship with physiochemical and rheological properties in wheat flour mill streams as an effective way to predict flour functionality. Applied Science, 2023, 13, (9), 5458. https://doi.org/10.3390/app13095458

mój udział polegał na nadzorowaniu i administrowaniu projektem, przeglądzie literatury oraz udziale w przygotowaniu, edycji i recenzji manuskryptu. Wkład w artykuł wynosił: 20%.

2. Piotr Lewko, Agnieszka Wójtowicz, Monika Różańska-Boczula: Effect of extruder configuration and extrusion-cooking processing parameters on selected characteristics of NSP-rich wheat flour as a hybrid treatment with xylanase addition. Processes, 2024, 12, 1159. https://doi.org/10.3390/pr12061159

mój udział polegał na współopracowaniu koncepcji doświadczeń, nadzorze prowadzonych prac laboratoryjnych, interpretacji wyników oraz przygotowania manuskryptu, recenzji i jego ostatecznej wersji. Wkład w artykuł wynosił: 30%.

3. Piotr Lewko, Agnieszka Wójtowicz, Michał Rudaś: Effect of processing conditions of enzymatic, extrusion and hybrid treatment methods on composition and selected techno-functional properties of developed wheat flour. International Journal of Food Science (w recenzji)

mój udział polegał na zaprojektowaniu doświadczeń, nadzorze prac laboratoryjnych, analizie i interpretacji wyników oraz przygotowaniu szkicu manuskryptu, recenzji i jego statecznej wersji. Wkład w artykuł wynosił: 20%.

4. Piotr Lewko, Agnieszka Wójtowicz, Daniel M. Kamiński: The influence of processing using conventional and hybrid methods on the composition, polysaccharide profiles and selected properties of wheat flour enriched with baking enzymes. Foods 2024, 13, 2957. https://doi.org/10.3390/foods13182957

mój udział polegał na opracowaniu koncepcji oraz metodologii badań, interpretacji wyników oraz przygotowaniu manuskryptu, recenzji i jego ostatecznej wersji. Wkład w artykuł wynosił: 20%.

5. Piotr Lewko, Agnieszka Wójtowicz, Marek Gancarz: Application of conventional and hybrid thermal-enzymatic modified wheat flours as clean label bread improvers. Applied Science, 2024, 14, 7659. https://doi.org/10.3390/app14177659

mój udział polegał na współpracowaniu koncepcji doświadczeń, interpretacji wyników oraz przygotowania manuskryptu, recenzji i jego ostatecznej wersji. Wkład w artykuł wynosił: 20%.

Wójtowicz Agnieszka

.....

Podpis

Lublin, data 24.09.2024

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracach:

1. Piotr Lewko, Agnieszka Wójtowicz, Marek Gancarz: Distribution of arabinoxylans and their relationship with physiochemical and rheological properties in wheat flour mill streams as an effective way to predict flour functionality. Applied Science, 2023, 13, (9), 5458. https://doi.org/10.3390/app13095458

mój udział polegał na wykonaniu części analizy statystycznej. Wkład w artykuł wynosił: 5%.

2. Piotr Lewko, Agnieszka Wójtowicz, Marek Gancarz: Application of conventional and hybrid thermal-enzymatic modified wheat flours as clean label bread improvers. Applied Science, 2024, 14, 7659. https://doi.org/10.3390/app14177659

mój udział polegał na wykonaniu części analizy statystycznej. Wkład w artykuł wynosił: 5%.

.....
Podpis

Lublin, data 24.09.2024

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1. Piotr Lewko, Agnieszka Wójtowicz, Monika Różańska-Boczula: Effect of extruder configuration and extrusion-cooking processing parameters on selected characteristics of NSP-rich wheat flour as a hybrid treatment with xylanase addition. Processes, 2024, 12, 1159. https://doi.org/10.3390/pr12061159

mój udział polegał na wykonaniu pogłębionej analizy statystycznej otrzymanych rezultatów.
Wkład w artykuł wynosił: 10%.

M. Różańska-Boczula

Podpis

Lublin, data 24.09.2024

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1. Piotr Lewko, Agnieszka Wójtowicz, Michał Rudaś: Effect of processing conditions of enzymatic, extrusion and hybrid treatment methods on composition and selected techno-functional properties of developed wheat flour. International Journal of Food Science (w recenzji)

mój udział polegał na obsłudze urządzeń i archiwizacji obrazów przy analizie makroskopowej.
Wkład w artykuł wynosił: 10%.

Podpis

Lublin, data 24.09.2024

dr hab. Daniel M. Kamiński
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Oświadczenie o współautorstwie

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1. Piotr Lewko, Agnieszka Wójtowicz, Daniel M. Kamiński: The influence of processing using conventional and hybrid methods on the composition, polysaccharide profiles and selected properties of wheat flour enriched with baking enzymes. Foods 2024, 13, 2957. https://doi.org/10.3390/foods13182957

mój udział polegał na wykonaniu pomiarów struktury krystalicznej prób oraz archiwizacji wyników. Wkład w artykuł wynosił: 10%.

Podpis

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Distribution of Arabinoxylans and Their Relationship with Physiochemical and Rheological Properties in Wheat Flour Mill Streams as an Effective Way to Predict Flour Functionality

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Abstract: The aim of the study was to evaluate wheat flour fractions and the relationships between the rheological properties of wheat flour mill streams with arabinoxylans content to predict flour functionality. The tested wheat variety was IS Laudis and an industrial milling station with several roll sections was employed to reach 30 passages of flour streams. Each mill stream fraction was analyzed separately. Several physiochemical (moisture, ash content, falling number, wet gluten content, gluten index, and damaged starch content) and rheological properties were evaluated by means of utilizing various test apparatus and techniques. The total content of non-starch polysaccharides and arabinoxylans, as well as soluble and insoluble fractions were investigated. Results showed significant differences between the mill streams in terms of the content of physicochemical parameters and rheological properties, as well as in soluble and insoluble fractions of non-starch polysaccharides and arabinoxylans. The relationships between the tested parameters and PCA analysis can be useful for millers who can then select and blend several flour streams to obtain a maximum amount of flour with specified characteristics. A better understanding of the origin and function of different fractions and the role of arabinoxylans and their fractions in the milling process will allow the development of wheat flour blends with the desired functionality. Flours from late reduction and sizing passages (C and R) as well as from sorting filter (V) streams showed high ash content as well as T-NSP and T-AX levels, so the final content of NSP in flour blends may be balanced by the application of the proper amount of C6–C7 and R5 stream flours.

Keywords: wheat; mill stream; rheological properties; arabinoxylans; functionality

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1. Introduction

Wheat plays a very important role in human nutrition, as it is the main ingredient of many food products produced around the world. The main benefits of the intake of these products are due to their main components, i.e., starch and protein. The functionality and mutual interactions between starch and protein enable the formation of a specific viscoelastic network of links called 'gluten'. Gluten makes it possible to produce a wide range of products based on wheat flour [1].

Wheat milling is a mechanical, multi-stage, and complex process of gradual grinding, in which the endosperm is first separated from the bran layers and then, through a series of grinding operations, creates mill streams of wheat flour [2–4]. Wheat milling induces the separation of the floury endosperm from the bran and the reduction of the endosperm particles into flours. Roller milling is the principal commercial milling method as it has

a very high capacity. Roller milling is conducted on break rolls (which are designed to break and remove the endosperm and germ from the bran coat, and gradually grind endosperm into flour), sizing rolls (so as to generate 'middling' with various particle sizes), and reduction rolls (which reduce the middling to flour).

Each mill produces from a few to even dozens of flour streams, with large differences in the physicochemical properties of individual flours. These are mixed at the end of the process to produce so-called 'composite flours'. These stream mixes are dedicated to restaurants and bakeries for producing breads, cakes, and pizzas, or to various food plants for producing pasta, extruded products, or biscuits [5].

A wide range of wheat flour is produced, as a result of various combinations of flour stream mixing possibilities. Indeed, not every flour stream is equally useful for generating a composition of specialized wheat flour. Significant differences in the parameters of flour streams affect the difficulty in optimizing flour production. Therefore, it becomes very important to precisely test and predict the quality of flour streams, regardless of the ultimate applications of the final composed flour. Determining the distribution of beneficial and harmful components in mill streams is important for aspects such as assessing the quality of wheat milling [6], and the optimal combination of flour streams is of great importance to obtain the best baking quality [4].

Research on the differences between individual flour streams has been continuous. These differences concern such features as rheological properties [3,4,7–11], or physicochemical characteristics such as content and distribution of protein, protein composition or ash content [2,3,8,9,11–15], distribution, amount of enzymes [16–19], fat content [2,7,20], starch damage degree [3,11,14], pentosans and its fractions [6,21], or antioxidants content [22].

Apart from protein and starch, arabinoxylans (AX) are important components of wheat grain. These significantly affect the properties of flour. Arabinoxylans are non-starch polysaccharides that are present in the endosperm (3–5% of the total endosperm), aleurone, and bran cell walls (about 60–70% of the total cell wall). Arabinoxylans are built of a single main chain consisting of xylose residues linked by a β-1,4 chain, to which single arabinose residues are attached in positions C-3 and simultaneously C-2 and C-3 [23]. In the case of wheat bran, AX constitutes 10.9 to 26% of all bran fractions [24]. As with protein and ash, arabinoxylans are not evenly distributed in the wheat grain. The concentration of AX in the middle endosperm is much lower compared to the outer layers of the wheat grains [6].

Despite the low content of AX in flour, they have an important impact on the quality of gluten and dough, affecting especially bread quality [25,26]. A special feature of AX is the binding of very large amounts of water. Hence, it plays an important role in water management during bread production [27]. The interactions of proteins and AX affect the properties of gluten and dough [28]. WEAX have unique physical properties such as the ability to bind 10 times their own weight of water [29,30], forming highly viscous solutions and gels due to their covalent cross-linking [31,32]. All these properties have a direct functional impact on the formation of gluten and the properties of the dough. In general, it is believed that WEAX have a positive effect on bread quality [33] and WUAX have a negative effect [34,35].

The total arabinoxylan (TAX) content can be empirically divided into water extractable arabinoxylans (WEAX) or water non-water extractable arabinoxylans (WUAX) fractions. WUAX and WEAX have different physicochemical properties [6]. WUAX impinges upon the molecular mobility of water [23] and negatively influences the quality of bread by binding large amounts of water, which prevents proper hydration of starch and gluten. WUEX also affects the proper formation of air bubbles during fermentation in bread dough [18]. In the WEAX fraction, ferulic acid residues are available for oxidative crosslinking induced by free radicals and are partly responsible for changes in dough viscosity [6]. It is therefore of interest to determine the distribution of arabinoxylans in the flour streams in order to compose proper flour stream blends to obtain a functional flour with specific properties without the use of additives or further treatments.

Earlier studies on the distribution of arabinoxylans in mill streams characterized the relationship between the content of AX in individual flour fractions and the technological suitability of specific flour passages to a limited extent [4,11,16,17,21,36]. These studies have often been more focused on the structural characterization of AX from various mill streams and less on their comparison with the most commonly used methods of quality and technological suitability assessment. Understanding the differences between the functional characteristics of different mill streams would improve the efficiency of their composition and the quality of the final blend for various applications [6].

The aim of the study was to evaluate several physiochemical and rheological properties of wheat flour mill streams, including arabinoxylans content and fractions structure, to analyze its distribution so as to optimize flour passage composition.

2. Materials and Methods

IS Laudis wheat variety, characterized by a high content of non-starch polysaccharides, was used for the milling test. The wheat was cleaned and conditioned to 16% of moisture content and milled in an industrial scale Roller Mill (in PZZ LUBELLA GMW Sp. z o.o., Lublin, Poland) with a throughput of 11,800 kg/h and an extraction rate of 78%. The milling process consisted of breaking, reduction, sizing, sifting, and sorting. A schematic diagram of the industrial milling stages is shown in Figure 1.

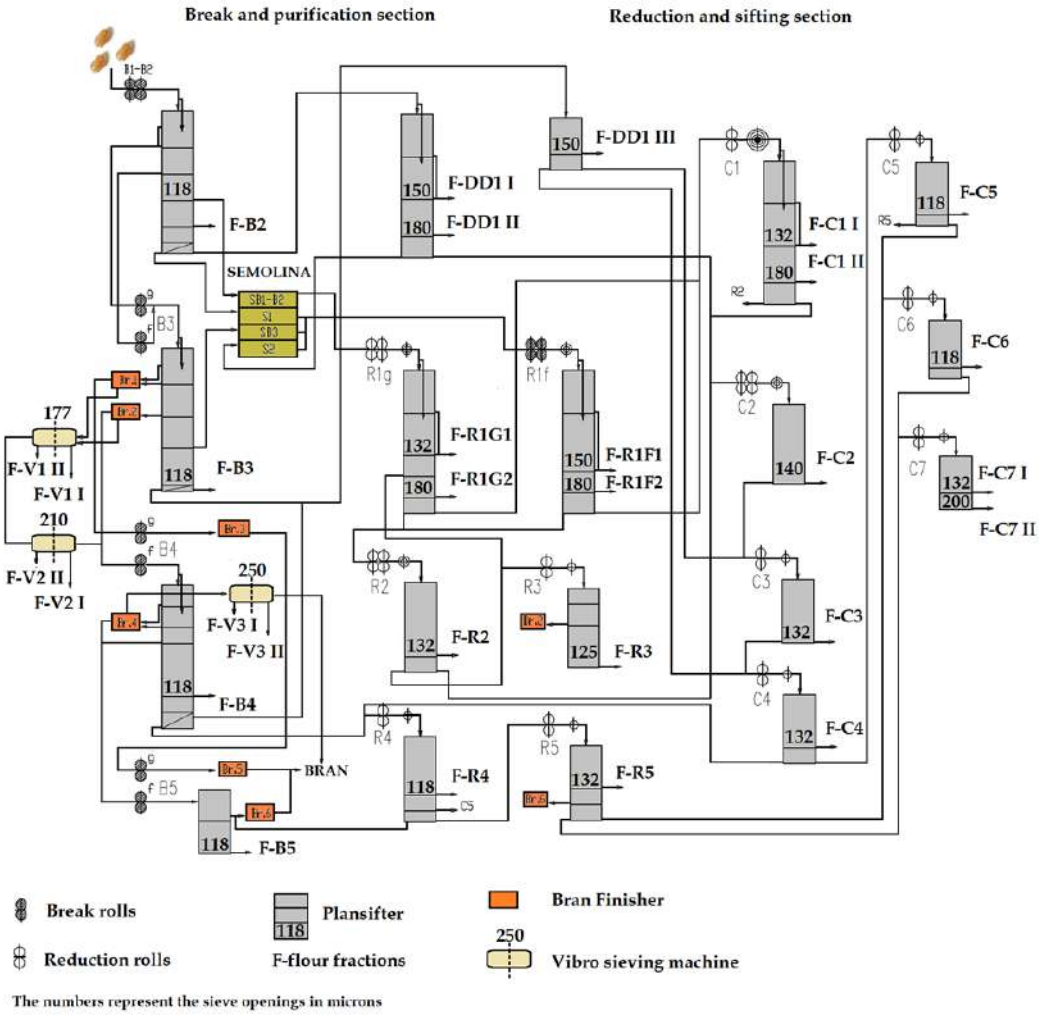

Figure 1. Schematic diagram of industrial milling station applied.

Indexes I and II refer to passages produced in the same grinding section that differ in granulation, wherein Index I refers to a fine flour and Index II to a coarse one. For V1–V3

streams in each vibro-sifter, two separate fractions (I and II) differing in quality, but with similar particle size, were collected in half of the sifter length. After milling, 30 different flour streams were obtained, marked as "F—flour" on the scheme, consisting of 4 breaking flour streams (B2, B3, B4, B5), 17 reduction and sizing mill flour streams (C1I, C1II, C2, C3, C4, C5, C6, C7I, C7II, R1F1, R1F2, R1G1, R1G2, R2, R3, R4, R5), 3 sifting flour streams (DD1I, DD1II, DD1III) and 6 sorting filter streams flour (V1I, V1II, V2I, V2II, V3I, V3II) as shown in the Scheme (Figure 1) for individual streams. All samples were collected separately and were kept in tight plastic bags prior to further analyzes.

The selected physicochemical properties of the flour mill streams were determined as follows according to ICC Standard Methods [37]: moisture content (MC) according to PN-ISO 712:2002 (ICC 110/1), ash content (A) according to PN-EN ISO 2171:2010 (ICC 104/1), falling number (FN) determined according to PN-EN ISO 3093:2010 (ICC 107/1) standard method, as well as wet gluten content (G) and gluten index (GI) according to PN-EN ISO 21415-2:2008 (ICC 155) by using a Perten Glutomatic 2200 (PerkinElmer Inc., Waltham, MA, USA). Damaged starch (SD) content of the flour mill streams samples was determined by means of an SD-Matic (Chopin Technologies, Villeneuve la Garenne, France) which provides results in AACC units [38] (PN-EN ISO 17715:2015-01).

Rheological tests were performed with the following devices: Alveograph (Chopin Technologies, Villeneuve-la-Garenne, France) according to PN-EN ISO 27971:2015-07 (ICC 121), Brabender Farinograph-E apparatus (Duisburg, Germany) according to PN-EN ISO 5530-1:2015-01 (ICC 115/1), and Mixolab (Chopin Technologies, Villeneuve-la-Garenne, France) according to PN-EN ISO 17718-1:2015-01 (ICC 173) [37].

Standard Alveograph procedure was applied to evaluate the baking strength (W) as the surface area under the curve, dough strength (P) as the maximum pressure needed to blow the dough bubble expressing dough resistance, extensibility (L) as the length of the curve expressing dough extensibility, elasticity index (Ie) [39] and strain hardening index (SH) [40].

The rheological properties of the dough prepared from each mill stream were determined using Farinograph standard procedure. Water absorption (WA) (% of the water needed to obtain a dough consistency of 500 BU), dough development time (DT) (time to reach a consistency of 500 BU), DoS—degree of softening, Farinograph quality number (QN), and dough stability (S) were recorded.

Rheological properties of dough prepared from each mill stream were also studied using the Chopin Mixolab based on the Chopin+ flour protocol with the following settings: mixing speed—80 rpm, total analysis time—45 min, dough weight—75 g, hydration water temperature 30 °C. Flour and water were added accordingly to obtain a dough with a maximum consistency of 1.10 Nm (±0.05) during the first test phase. The Mixolab test was performed using a standard protocol: 8 min at 30 °C, heating for 15 min at a rate of 4 °C/min, holding at 90 °C for 7 min, cooling for 10 min to 50 °C at a rate of 4 °C/min and holding at 50 °C for 5 min [41]. The following rheological features were tested with Mixolab: (i) primary readings: water absorption (M-WA), protein weakening (M-C2), starch gelatinization (M-C3), amylase activity (M-C4), starch retrogradation (M-C5), slope M-α—between the end of 30 °C period and M-C2 as the speed of the protein weakening under heating effect, slope M-β—between M-C2 and as an indicator of pasting (gelatinization) speed, slope M-γ—between M-C3 and M-C4 as enzymatic (α-amylase) degradation speed [42]; (ii) secondary parameters: M-C2–C1—protein weakening range, M-C3–C2—starch gelatinization range (pasting), M-C4–C3—cooking stability range, and M-C5–C4—cooling setback (gelling) [43].

Solvent Retention Capacity (SRC) tests were performed according to an approved AACC 56-11.02 method [38]. SRC is the weight of solvent retained by the swollen flour deposit after centrifugation and is expressed as a percentage of the original flour weight (adjusted to 14% moisture). Solvents were: deionized water, 50 wt% sucrose in water, 5 wt% lactic acids in the water, 5 wt% sodium carbonate in water (WaSRC—water retention capacity; SuSRC—sucrose solvent retention capacity; LaSRC—lactic acid solvent retention

capacity; ScSRC—sodium carbonate solvent retention capacity). Herein, a flour sample (5 ± 0.050 g) was transferred to a 50 mL centrifuge tube and mixed with 25 g of solvent [44]. In the next step, the sample was left to solvate for 20 min with shaking every 5 min for 5 s. The tubes were then centrifuged at 2500 rpm for 15 min. The supernatant was poured off and the tubes were allowed to dry for 10 min. The sample was subsequently weighed and the SRC was calculated [45]. Additionally, GPI (gluten performance index) was calculated as described by Vukić et al. [4] based on the ICC method [37] by dividing the LaSRC value by the combined values of SuSRC and ScSRC.

The content of non-starch polysaccharides (NSP) was determined by gas chromatography according to Englyst and Cummings [46] and AOAC 994.13 [47]. The total NSP (T-NSP) content is the amount of sugars: arabinose, xylose, mannose, galactose, and glucose [47]. This analysis allows us to separate the non-starch polysaccharides into two fractions: soluble (S-NSP) and insoluble (I-NSP), and to determine the composition of polysaccharides in both fractions. Total arabinoxylans content (T-AX) and soluble (S-AX) and insoluble (I-AX) fractions were assessed.

All analyzes were performed in triplicate. Data were subjected to one-way analysis of variance (ANOVA) using Statistica 13.3 software (StatSoft, Inc., Tulsa, OK, USA) followed by the Fisher's least significant difference (LSD) post hoc test to compare means at the 0.05 significance level. Statistica software (version 12.0, StatSoft Inc., Tulsa, OK, USA) was applied for Principle Component Analysis (PCA) and determination of correlation coefficients at the level of significance 0.05. The analysis of the main components was employed to determine the relationship between physiochemical and rheological features and arabinoxylans in the individual flour streams. PCA input data matrix was scaled automatically. The optimal number of principal components obtained in the analysis was determined on the basis of the Cattel criterion.

3. Results

The obtained physiochemical and rheological properties of the individual mill streams are presented in Tables 1–4. The ash content in the individual mill streams was one of the most differentiating components that demonstrated dependence upon the obtained fraction. Low ash content (around 0.6 or less) of flour fractions indicates the absence of bran in the flour (i.e., more white flour), which is often desired by industry and consumers. In contrast, high ash content (1.6 and above) reveals high content of bran, dietary fiber, antioxidants, and minerals in the flour [22]. Various passages of the tested flour showed significant differences between the obtained mill streams. The lowest content of ash was found in flour streams B2–B3, C1–C5, DD1, and R1–R3 (Table 1) which is evident that from these passages, the flour was white and came from the endosperm. In contrast, the highest ash content was observed in B5, C6–C7, R4–R5, and V1–V3 flour streams. This demonstrates that the feed was rich in the outer layer and bran fractions of wheat grains. Although B2 flour often has the lowest ash content among broken flour, B2 flour was characterized by a higher content of ash compared to the flour stream B3, probably due to the release of accumulated surface dust from the wheat [48] and the presence of some bran fraction in first break streams.

Table 1. Selected physiochemical properties of IS Laudis wheat flour streams.

Flour Stream	A (%)	G (%)	GI (-)	FN (s)	SD (%)
B2	0.671 ± 0.026^{g}	$35.99 \pm 1.67^{j,k}$	$94.8 \pm 2.2^{e,f}$	$388 \pm 14^{d,e}$	93.01 ± 0.39^{i}
B3	0.620 ± 0.009^{f}	38.78 ± 0.59^{l}	$96.3 \pm 0.9^{f,g,h,i}$	$423 \pm 26^{h,j,k,l}$	92.90 ± 0.23^{i}
B4	0.936 ± 0.002^{i}	36.62 ± 0.27^{k}	$95.5 \pm 0.5^{e,f,g,h}$	$410 \pm 4^{e,f,g,h,i,j,k}$	94.00 ± 0.16^{j}
B5	1.282 ± 0.002^{m}	$43.56 \pm 0.16^{m,n}$	86.5 ± 0.5^{b}	$446 \pm 4^{l,m}$	95.84 ± 0.02^{m}

Table 1. *Cont.*

Flour Stream	A (%)	G (%)	GI (-)	FN (s)	SD (%)
C1I	$0.440 \pm 0.009^{a,b}$	$30.86 \pm 0.04^{f,g}$	$97.5 \pm 0.5^{g,h,i}$	$430 \pm 2^{j,k,l}$	92.30 ± 0.10^{h}
C1II	$0.465 \pm 0.003^{b,c}$	$29.53 \pm 0.12^{c,d,e,f}$	99.0 ± 0.0^{i}	$446 \pm 2^{l,m}$	85.09 ± 0.03^{b}
C2	$0.466 \pm 0.015^{b,c}$	$28.65 \pm 0.25^{b,c,d,e}$	$98.5 \pm 0.5^{h,i}$	359 ± 1^{c}	89.32 ± 0.07^{h}
C3	0.489 ± 0.012^{c}	$32.10 \pm 0.09^{g,h}$	99.0 ± 0.0^{i}	$410 \pm 5^{e,f,g,h,i,j,k}$	90.65 ± 0.03^{f}
C4	0.733 ± 0.006^{h}	$29.59 \pm 0.15^{c,d,e,f}$	$94.0 \pm 1.0^{d,e,f}$	$419 \pm 5^{g,h,i,j,k,l}$	$96.00 \pm 0.01^{m,n}$
C5	0.755 ± 0.001^{h}	$27.59 \pm 0.07^{b,c}$	$98.5 \pm 0.5^{h,i}$	$436 \pm 5^{k,l,m}$	$95.91 \pm 0.06^{m,n}$
C6	1.461 ± 0.003^{o}	23.84 ± 0.06^{a}	$93.0 \pm 0.0^{d,e}$	$429 \pm 4^{j,k,l}$	$95.69 \pm 0.05^{l,m}$
C7I	2.352 ± 0.002^{p}	ND	ND	$410 \pm 4^{e,f,g,h,i,j,k}$	97.20 ± 0.03^{p}
C7II	3.931 ± 0.002^{s}	ND	ND	74 ± 4^{a}	87.60 ± 0.12^{d}
DD1I	$0.584 \pm 0.007^{d,e}$	$32.96 \pm 0.26^{h,i}$	99.0 ± 0.0^{i}	$444 \pm 2^{l,m}$	90.73 ± 0.11^{f}
DD1II	$0.579 \pm 0.005^{d,e}$	$30.66 \pm 0.17^{e,f,g}$	$96.5 \pm 2.5^{f,g,h,i}$	$394 \pm 1^{d,e,f,g}$	86.83 ± 0.03^{c}
DD1III	0.565 ± 0.001^{d}	$37.54 \pm 0.12^{k,l}$	$96.0 \pm 0.0^{e,f,g,h,i}$	463 ± 3^{m}	90.33 ± 0.05^{f}
R1F1	$0.471 \pm 0.007^{b,c}$	$30.26 \pm 0.10^{d,e,f,g}$	$97.0 \pm 0.0^{f,g,h,i}$	$385 \pm 1^{c,d,e,f}$	93.84 ± 0.15^{j}
R1G1	0.486 ± 0.010^{c}	$28.54 \pm 0.25^{b,c,d}$	$91.5 \pm 0.5^{c,d}$	$428 \pm 5^{j,k,l}$	96.71 ± 0.05^{o}
R1G2	$0.428 \pm 0.001^{a,c}$	27.05 ± 0.07^{b}	$98.0 \pm 0.0^{g,h,i}$	$388 \pm 4^{c,d,e,f}$	91.51 ± 0.06^{g}
R1F2	0.418 ± 0.003^{a}	$29.69 \pm 0.17^{d,e,f}$	$97.5 \pm 0.5^{g,h,i}$	$405 \pm 5^{c,d,e,f}$	81.97 ± 0.04^{a}
R2	$0.589 \pm 0.002^{d,e}$	$29.57 \pm 0.42^{c,d,e,f}$	$98.0 \pm 0.0^{g,h,i}$	$424 \pm 4^{h,i,j,k,l}$	92.12 ± 0.13^{h}
R3	$0.609 \pm 0.004^{e,f}$	$28.67 \pm 0.21^{b,c,d,e}$	$95.5 \pm 0.5^{e,f,g,h}$	$421 \pm 5^{g,h,i,j,k,l}$	$95.89 \pm 0.06^{m,n}$
R4	1.063 ± 0.001^{k}	$29.33 \pm 0.18^{c,d,e,f}$	$96.0 \pm 0.0^{e,f,g,h,i}$	$412 \pm 2^{e,f,g,h,i,j,k}$	$96.38 \pm 0.06^{n,o}$
R5	2.633 ± 0.006^{r}	ND	ND	301 ± 3^{b}	98.09 ± 0.07^{r}
V1I	1.008 ± 0.014^{j}	$36.39 \pm 0.04^{j,k}$	$94.0 \pm 0.0^{d,e,f}$	$377 \pm 3^{c,d}$	$95.21 \pm 0.04^{k,l}$
V1II	1.012 ± 0.003^{j}	$37.12 \pm 0.06^{k,l}$	$88.5 \pm 0.5^{b,c}$	362 ± 6^{c}	97.20 ± 0.06^{p}
V2I	$1.041 \pm 0.01^{j,k}$	$34.40 \pm 0.24^{i,j}$	$95.0 \pm 0.0^{e,f,g}$	$403 \pm 5^{d,e,f,g,h,i,j}$	90.39 ± 0.04^{f}
V2II	1.284 ± 0.008^{m}	$28.64 \pm 0.16^{b,c,d}$	59.0 ± 0.0^{a}	$398 \pm 4^{d,e,f,g,h,i}$	91.56 ± 0.06^{g}
V3I	1.397 ± 0.007^{n}	41.79 ± 0.18^{m}	87.0 ± 0.0^{b}	$414 \pm 4^{f,g,h,i,j,k}$	96.61 ± 0.01^{r}
V3II	1.218 ± 0.006^{l}	44.62 ± 0.17^{n}	87.0 ± 1.0^{b}	$395 \pm 4^{d,e,f,g,i}$	94.96 ± 0.07^{k}

A—ash content; G—gluten content; GI—gluten index; FN—falling number; SD—damaged starch; ND—no data; $^{a–r}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Table 2. Alveograph features of IS Laudis wheat flour streams.

Flour Stream	P (mm)	L (mm)	W (J 10^{-4})	P/L	Ie (%)	SH Index
B2	66.4 ± 0.9^{b}	146.7 ± 11.1^{m}	$289.4 \pm 12.5^{k,m}$	$0.46 \pm 0.04^{a,b}$	$58.53 \pm 0.96^{m,n}$	1.81 ± 0.02^{n}
B3	62.3 ± 1.5^{a}	173.8 ± 19.8^{n}	$309.5 \pm 21.5^{m,n}$	0.36 ± 0.04^{a}	60.23 ± 0.63^{n}	$1.82 \pm 0.03^{m,n}$
B4	$83.3 \pm 1.2^{d,e,f}$	$107.7 \pm 12.0^{j,k,l}$	$210.3 \pm 14.5^{d,e}$	$0.78 \pm 0.09^{b,c,d}$	$41.93 \pm 0.93^{f,g}$	$1.33 \pm 0.04^{b,c,d}$
B5	75.7 ± 1.2^{c}	119.0 ± 6.2^{l}	$191.0 \pm 7.8^{b,c,d}$	$0.64 \pm 0.03^{a,b,c,d}$	38.83 ± 0.15^{e}	$1.30 \pm 0.04^{b,c}$
C1I	$81.0 \pm 0.0^{c,d,e}$	$113.0 \pm 2.0^{k,l}$	$283.3 \pm 6.1^{j,k,l,m}$	$0.72 \pm 0.02^{a,b,c,d}$	$55.93 \pm 0.21^{l,m}$	$1.79 \pm 0.01^{l,m,n}$
C1II	$90.0 \pm 1.7^{g,h,i}$	$88.3 \pm 4.0^{g,h,i,j,k}$	$256.0 \pm 10.1^{g,h,i,j}$	$1.02 \pm 0.04^{d,e,f}$	$52.97 \pm 0.23^{j,k}$	$1.74 \pm 0.02^{j,k,l,m,n}$
C2	$82.7 \pm 1.5^{d,e,f}$	$94.3 \pm 4.2^{h,i,j,k,l}$	$261.3 \pm 8.5^{g,h,i,j,l}$	$0.88 \pm 0.04^{c,d,e}$	$57.20 \pm 0.36^{m,n}$	$1.82 \pm 0.00^{m,n}$
C3	$89.7 \pm 2.1^{g,h}$	$106.3 \pm 1.2^{j,k,l}$	$290.7 \pm 7.0^{k,l,m}$	$0.84 \pm 0.02^{c,d,e}$	$54.20 \pm 0.17^{k,l}$	$1.74 \pm 0.01^{j,k,l,m,n}$
C4	$103.7 \pm 0.6^{l,m}$	$66.0 \pm 2.0^{d,e,f,g}$	$211.0 \pm 2.6^{d,e,f}$	$1.57 \pm 0.06^{h,i,j}$	$40.93 \pm 0.47^{e,f}$	$1.51 \pm 0.03^{e,f,g,h}$
C5	$95.7 \pm 1.5^{i,j,k}$	$60.7 \pm 1.5^{c,d,e,f}$	$171.7 \pm 6.0^{b,c}$	$1.57 \pm 0.02^{h,i,j}$	34.67 ± 0.74^{d}	$1.38 \pm 0.02^{b,c,d,e}$
C6	$111.7 \pm 2.5^{n,o}$	$40.0 \pm 1.0^{a,b,c}$	106.3 ± 10.5^{a}	2.79 ± 0.12^{l}	6.45 ± 0.50^{a}	ND
C7I	$93.3 \pm 2.3^{h,i,j}$	$31.0 \pm 6.6^{a,b}$	80.3 ± 4.9^{a}	3.11 ± 0.71^{l}	ND	ND
C7II	ND	ND	ND	ND	ND	ND
DD1I	$80.3 \pm 2.1^{c,d}$	$104.0 \pm 1.7^{i,j,k,l}$	$270.0 \pm 4.6^{h,i,j,k,l}$	$0.78 \pm 0.03^{b,c,d}$	$57.77 \pm 0.21^{m,n}$	$1.79 \pm 0.01^{l,m,n}$
DD1II	$86.7 \pm 1.5^{e,f,g}$	$88.3 \pm 3.1^{g,h,i,j,k}$	$245.0 \pm 6.9^{f,g,h,i}$	$0.98 \pm 0.02^{d,e,f}$	$52.90 \pm 0.20^{j,k}$	$1.72 \pm 0.03^{j,k,l,m,n}$
DD1III	68.7 ± 1.5^{b}	148.3 ± 9.5^{m}	$293.0 \pm 4.4^{k,l,m}$	$0.46 \pm 0.04^{a,b,c}$	$57.27 \pm 0.84^{m,n}$	$1.79 \pm 0.02^{l,m,n}$
R1F1	$109.3 \pm 2.1^{m,n}$	$87.3 \pm 4.0^{g,h,i,j}$	330.0 ± 14.4^{n}	$1.26 \pm 0.06^{e,f,g,h}$	$57.57 \pm 0.06^{m,n}$	$1.83 \pm 0.01^{m,n}$
R1G1	121.3 ± 0.6^{p}	$68.3 \pm 6.1^{d,e,f,g}$	$296.7 \pm 16.1^{g,i,j,k,l,m}$	$1.79 \pm 0.17^{i,j,k}$	$53.73 \pm 0.21^{k,l}$	$1.81 \pm 0.02^{m,n}$
R1G2	$107.7 \pm 3.1^{m,n}$	$72.0 \pm 2.6^{d,e,f,g,h}$	$272.3 \pm 3.5^{k,m,n}$	$1.50 \pm 0.10^{g,h,i}$	$54.17 \pm 0.67^{k,l}$	$1.78 \pm 0.03^{k,l,m,n}$
R1F2	$86.3 \pm 0.6^{e,f,g}$	$81.3 \pm 4.2^{e,f,g,h,i}$	$235.7 \pm 8.3^{e,f,g}$	$1.06 \pm 0.06^{d,e,f,g}$	$54.30 \pm 0.44^{k,l}$	$1.76 \pm 0.02^{k,l,m,n}$
R2	$101.3 \pm 1.2^{k,l}$	$75.3 \pm 1.5^{d,e,f,g,h}$	$238.0 \pm 2.0^{e,f,g,h}$	$1.35 \pm 0.04^{f,g,h,i}$	46.23 ± 0.06^{h}	$1.63 \pm 0.01^{h,i,j,k}$
R3	$87.3 \pm 0.6^{f,g}$	$108.3 \pm 3.8^{j,k,l}$	$265.0 \pm 6.1^{g,h,i,j,k,l}$	$0.81 \pm 0.04^{b,c,d,e}$	49.93 ± 0.51^{i}	$1.64 \pm 0.02^{h,i,j,k,l}$
R4	$98.7 \pm 0.6^{j,k,l}$	$56.7 \pm 2.5^{c,d,e}$	160.7 ± 1.5^{b}	$1.74 \pm 0.09^{i,j}$	29.20 ± 0.20^{b}	1.23 ± 0.07^{b}
R5	$90.0 \pm 1.0^{g,h,i}$	25.0 ± 1.0^{a}	85.7 ± 8.1^{a}	3.60 ± 0.13^{m}	ND	1.05 ± 0.34^{a}

Table 2. *Cont.*

Flour Stream	P (mm)	L (mm)	W (J 10^{-4})	P/L	Ie (%)	SH Index
V1I	143.7 ± 6.0^{r}	$80.0 \pm 5.0^{e,f,g,h,i}$	365.3 ± 22.0^{o}	$1.79 \pm 0.10^{i,j,k}$	50.30 ± 0.27^{i}	$1.67 \pm 0.01^{i,j,k,l,m}$
V1II	151.3 ± 1.2^{s}	$77.0 \pm 4.6^{e,f,g,h}$	382.3 ± 9.5^{o}	$1.97 \pm 0.13^{j,k}$	$51.27 \pm 0.25^{i,j}$	$1.72 \pm 0.03^{j,k,l,m,n}$
V2I	$112.7 \pm 2.3^{n,o}$	$50.7 \pm 2.1^{b,c,d}$	$184.3 \pm 4.0^{b,c,d}$	2.23 ± 0.13^{k}	31.57 ± 2.45^{c}	$1.42 \pm 0.04^{c,d,e,f}$
V2II	$79.3 \pm 2.5^{c,d}$	$83.7 \pm 0.6^{f,g,h,i,j}$	$198.7 \pm 7.0^{c,d}$	$0.95 \pm 0.03^{d,e,f}$	47.23 ± 0.21^{h}	$1.59 \pm 0.03^{g,h,i,j}$
V3I	$115.7 \pm 2.3^{o,p}$	$78.3 \pm 5.1^{e,f,g,h}$	$259.3 \pm 3.8^{g,h,i,j,l}$	$1.48 \pm 0.12^{g,h,i}$	43.23 ± 0.60^{g}	$1.47 \pm 0.02^{d,e,f,g}$
V3II	$103.7 \pm 1.5^{l,m}$	$75.7 \pm 3.5^{e,f,g,h}$	$238.0 \pm 3.5^{e,f,g,h}$	$1.37 \pm 0.08^{f,g,h,i}$	46.10 ± 0.00^{h}	$1.57 \pm 0.03^{f,g,h,i}$

P—dough tenacity; L—extensibility; W—baking strength; P/L—dough configuration index; Ie—elasticity index; SH—strain hardening index; ND—no data; [a–r]—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Table 3. Farinograph features of IS Laudis wheat flour streams.

Flour Stream	WA (%)	DT (min)	S (min)	DoS 10 (BU)	DoS 12 (BU)	QN (-)
B2	$56.68 \pm 0.63^{d,e}$	7.4 ± 1.0^{g}	16.2 ± 0.9^{j}	11.67 ± 3.43^{a}	$48.17 \pm 3.37^{a,b,c}$	$136.89 \pm 6.35^{l,m}$
B3	$57.43 \pm 0.16^{f,g}$	$7.0 \pm 1.1^{e,f,g}$	17.9 ± 0.3^{k}	8.33 ± 3.50^{a}	$42.40 \pm 7.86^{a,b}$	155.67 ± 8.14^{n}
B4	62.90 ± 0.00^{k}	$5.5 \pm 0.4^{d,e}$	6.3 ± 0.2^{d}	$38.67 \pm 1.53^{e,f,g}$	$77.33 \pm 2.08^{i,j,k}$	$88.33 \pm 3.79^{e,f,g}$
B5	$66.27 \pm 0.06^{m,n}$	$6.2 \pm 0.5^{d,e,f,g}$	$5.9 \pm 0.3^{c,d}$	$36.00 \pm 6.08^{d,e,f}$	$69.00 \pm 6.00^{g,h,i,j}$	$92.33 \pm 5.51^{f,g,h}$
C1I	$56.47 \pm 0.21^{c,d,e}$	$3.2 \pm 0.3^{a,b,c}$	$10.5 \pm 0.9^{g,h}$	$35.33 \pm 7.57^{d,e,f}$	$53.33 \pm 5.13^{b,c,d,e}$	$87.67 \pm 18.04^{e,f,g}$
C1II	55.53 ± 0.06^{b}	2.4 ± 0.4^{a}	9.7 ± 0.6^{g}	$39.67 \pm 2.52^{e,f,g}$	$54.00 \pm 3.00^{b,c,d,e,f}$	$75.67 \pm 3.22^{d,e,f}$
C2	$55.67 \pm 0.06^{b,c}$	$2.9 \pm 0.5^{a,b}$	$12.2 \pm 0.8^{h,i}$	$34.67 \pm 4.04^{d,e,f}$	$48.00 \pm 2.65^{a,b,c,d}$	$86.67 \pm 4.16^{e,f,g}$
C3	$57.20 \pm 0.10^{e,f,g}$	2.7 ± 0.2^{a}	$9.2 \pm 0.4^{f,g}$	$42.67 \pm 1.53^{f,g}$	$58.67 \pm 1.53^{c,d,e,f,g,h}$	$72.67 \pm 2.52^{d,e,f}$
C4	61.70 ± 0.00^{j}	$4.8 \pm 0.9^{b,c,d}$	$7.5 \pm 0.1^{d,e,f}$	$40.33 \pm 2.08^{e,f,g}$	$77.00 \pm 4.00^{i,j,k}$	$88.33 \pm 2.52^{e,f,g}$
C5	61.83 ± 0.06^{j}	$4.9 \pm 0.3^{c,d}$	6.2 ± 0.1^{d}	$43.33 \pm 2.08^{f,g}$	$81.67 \pm 0.58^{j,k}$	$83.67 \pm 2.31^{d,e,f}$
C6	69.13 ± 0.06^{o}	$5.6 \pm 0.1^{d,e,f}$	$4.2 \pm 0.5^{a,b,c}$	$61.67 \pm 4.16^{h,i}$	87.33 ± 5.51^{k}	$76.00 \pm 2.00^{d,e,f}$
C7I	69.83 ± 0.06^{o}	$5.7 \pm 0.3^{d,e,f}$	2.7 ± 0.4^{a}	116.67 ± 6.35^{j}	182.33 ± 5.86^{l}	$74.00 \pm 1.73^{d,e,f}$
C7II	ND	ND	ND	ND	ND	ND
DD1I	$56.23 \pm 0.06^{b,c,d}$	$4.7 \pm 0.7^{b,c,d}$	15.2 ± 1.2^{j}	$18.33 \pm 5.03^{a,b,c}$	40.00 ± 0.00^{a}	$129.33 \pm 10.26^{k,l,m}$
DD1II	$55.67 \pm 0.06^{b,c}$	2.2 ± 0.3^{a}	$9.3 \pm 0.5^{f,g}$	$45.67 \pm 2.08^{f,g}$	$56.33 \pm 2.08^{c,d,e,f,g}$	$69.00 \pm 3.00^{c,d,e}$
DD1III	$57.07 \pm 0.06^{e,f}$	7.8 ± 0.3^{g}	$16.5 \pm 0.6^{j,k}$	8.67 ± 2.52^{a}	$50.00 \pm 0.00^{a,b,c,d,e}$	$144.33 \pm 7.77^{m,n}$
R1F1	$57.90 \pm 0.10^{g,h}$	2.7 ± 0.3^{a}	$8.8 \pm 0.3^{f,g}$	$44.00 \pm 2.65^{f,g}$	$55.00 \pm 3.46^{c,d,e,f}$	$64.00 \pm 2.65^{b,c,d}$
R1G1	61.47 ± 0.06^{j}	2.1 ± 0.2^{a}	$3.9 \pm 0.8^{a,b}$	66.00 ± 4.58^{i}	$81.33 \pm 6.03^{j,k}$	43.33 ± 7.10^{a}
R1G2	$57.00 \pm 0.20^{d,e,f}$	2.3 ± 0.2^{a}	$6.0 \pm 0.7^{c,d}$	$50.00 \pm 5.57^{g,h}$	$63.67 \pm 3.22^{e,f,g,h}$	$50.67 \pm 4.62^{a,b,c}$
R1F2	54.30 ± 0.17^{a}	2.2 ± 0.2^{a}	$7.1 \pm 0.9^{d,e}$	$50.67 \pm 3.79^{g,h}$	$61.00 \pm 2.65^{d,e,f,g,h}$	$49.00 \pm 6.56^{a,b}$
R2	59.03 ± 0.06^{i}	2.5 ± 0.2^{a}	$7.4 \pm 0.7^{d,e,f}$	49.00 ± 6.56^{g}	$69.00 \pm 6.25^{g,h,i,j}$	$71.00 \pm 7.00^{d,e}$
R3	$58.67 \pm 0.06^{h,i}$	2.5 ± 0.2^{a}	$8.8 \pm 0.1^{e,f,g}$	$42.33 \pm 1.53^{f,g}$	$61.33 \pm 1.53^{e,f,g,h}$	$81.67 \pm 3.06^{d,e}$
R4	64.50 ± 0.10^{l}	$5.1 \pm 0.4^{c,d}$	$5.9 \pm 0.2^{b,c,d}$	$36.67 \pm 3.06^{e,f}$	$71.00 \pm 1.73^{h,i,j}$	$92.33 \pm 3.79^{f,g,h}$
R5	71.40 ± 0.10^{p}	$6.1 \pm 0.2^{d,e,f,g}$	2.8 ± 0.1^{b}	109.67 ± 4.16^{j}	208.67 ± 1.53^{m}	$80.33 \pm 1.53^{d,e,f}$
V1I	66.23 ± 0.35^{m}	5.2 ± 0.9^{d}	$8.9 \pm 0.8^{e,f,g}$	$24.33 \pm 3.51^{b,c,d}$	$71.33 \pm 3.51^{h,i,j}$	$108.33 \pm 6.51^{h,i,j}$
V1II	67.07 ± 0.15^{n}	$6.2 \pm 0.4^{d,e,f,g}$	10.0 ± 0.5^{g}	$18.00 \pm 2.00^{a,b,c}$	$66.67 \pm 7.02^{f,g,h,i}$	$119.00 \pm 7.94^{i,j,k}$
V2I	59.17 ± 0.15^{i}	$4.8 \pm 0.4^{b,c,d}$	$12.1 \pm 0.3^{h,i}$	$18.00 \pm 2.65^{a,b,c}$	$55.33 \pm 1.53^{c,d,e,f}$	$122.00 \pm 3.61^{i,j,k,l}$
V2II	$62.17 \pm 0.38^{j,k}$	$5.1 \pm 0.2^{c,d}$	$9.3 \pm 0.1^{f,g}$	$28.33 \pm 2.08^{c,d,e}$	$64.67 \pm 3.79^{e,f,g,h,i}$	$104.00 \pm 2.65^{g,h,i}$
V3I	65.77 ± 0.29^{m}	$6.3 \pm 0.9^{d,e,f,g}$	13.2 ± 0.8^{i}	$15.33 \pm 1.53^{a,b}$	$55.67 \pm 2.52^{c,d,e,f}$	$127.67 \pm 2.52^{j,k,l,m}$
V3II	65.70 ± 0.10^{m}	$7.4 \pm 0.3^{f,g}$	13.2 ± 0.8^{i}	11.67 ± 1.16^{a}	$57.67 \pm 2.52^{c,d,e,f,g}$	$133.33 \pm 6.66^{k,l,m}$

WA—water absorption; DT—development time; S—stability, DoS10,12—dough softening in time; QN—quality number; ND—no data; [a–p]—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Table 4. Mixolab® primary features of IS Laudis wheat flour streams.

Flour Stream	M-WA (%)	M-C2 (Nm)	M-C3 (Nm)	M-C4 (Nm)	M-C5 (Nm)	M-α	M-β	M-γ
B2	56.97 ± 0.70 ^{d,e}	0.463 ± 0.007 ^{j}	2.542 ± 1.992 ^{m}	1.689 ± 0.031 ^{m}	2.810 ± 0.033 ^{h,i}	−0.099 ± 0.016 ^{a}	0.576 ± 0.167 ^{h,i,j,k,l,m}	−0.050 ± 0.030 ^{f,g}
B3	57.80 ± 0.11 ^{f}	0.471 ± 0.009 ^{j,k,l}	1.839 ± 0.017 ^{l}	1.627 ± 0.045 ^{k,l}	2.853 ± 0.052 ^{i}	−0.084 ± 0.008 ^{a,b}	0.576 ± 0.136 ^{g,h,i,j,k,l,m}	−0.056 ± 0.028 ^{f,g}
B4	62.07 ± 0.15 ^{j,k}	0.392 ± 0.006 ^{c}	1.590 ± 0.009 ^{g}	1.373 ± 0.005 ^{e,f}	2.273 ± 0.017 ^{f}	−0.117 ± 0.011 ^{a}	0.510 ± 0.018 ^{d,e,f,g,h,i,j,k,l}	−0.030 ± 0043 ^{f,g}
B5	65.77 ± 0.50 ^{m,n}	0.365 ± 0.017 ^{b}	1.303 ± 0.013 ^{b}	1.073 ± 0.023 ^{b,c}	1.850 ± 0.045 ^{c}	−0.127 ± 0.002 ^{a}	0.368 ± 0.040 ^{a,b,c,d,e,f}	−0.042 ± 0.022 ^{f,g}
C1I	56.53 ± 0.06 ^{c,d}	0.490 ± 0.003 ^{m,n,o,p,r}	2.038 ± 0.020 ^{o}	1.505 ± 0.046 ^{g,h,i}	3.131 ± 0.048 ^{k,l}	−0.102 ± 0.007 ^{a,b}	0.719 ± 0.054 ^{l,m,n,o}	−0.207 ± 0.064 ^{a,b,c}
C1II	55.90 ± 0.10 ^{a,b,c}	0.507 ± 0.003 ^{r}	2.203 ± 0.013 ^{r}	1.682 ± 0.015 ^{l,m}	3.262 ± 0.014 ^{m,n}	−0.095 ± 0.001 ^{a,b}	0.829 ± 0.019 ^{o}	−0.177 ± 0.032 ^{a,b,c,d}
C2	56.20 ± 0.10 ^{b,c}	0.491 ± 0.002 ^{n,o,p,r}	2.149 ± 0.014 ^{p}	1.634 ± 0.014 ^{k,l,m}	3.322 ± 0.020 ^{n}	−0.082 ± 0.003 ^{a,b}	0.788 ± 0.003 ^{n,o}	−0.215 ± 0.090 ^{a,b}
C3	57.50 ± 0.10 ^{e,f}	0.487 ± 0.002 ^{l,m,n,o,p}	2.013 ± 0.008 ^{o}	1.527 ± 0.012 ^{h,i,j}	3.051 ± 0.018 ^{j,k}	−0.103 ± 0.005 ^{a,b}	0.693 ± 0.031 ^{k,l,m,n,o}	−0.115 ± 0.008 ^{b,c,d,e,f,g}
C4	61.30 ± 0.00 ^{i,j}	0.457 ± 0.005 ^{i,j}	1.693 ± 0.009 ^{h}	1.435 ± 0.025 ^{f,g}	2.489 ± 0.039 ^{g}	−0.106 ± 0.006 ^{a}	0.465 ± 0.039 ^{c,d,e,f,g,h,i,j,k}	−0.063 ± 0.010 ^{e,f,g}
C5	61.50 ± 0.00 ^{i,j,k}	0.438 ± 0,004 ^{h,i}	1.666 ± 0.004 ^{h}	1.152 ± 0.005 ^{c,d}	2.462 ± 0.035 ^{g}	−0.101 ± 0.007 ^{a,b}	0.431 ± 0.006 ^{b,c,d,e,f,g,h,i}	−0.101 ± 0.049 ^{c,d,e,f,g}
C6	66.10 ± 0.10 ^{n}	0.435 ± 0.004 ^{g,h}	1.475 ± 0.015 ^{e}	1.137 ± 0.015 ^{c,d}	1.968 ± 0.028 ^{d}	−0.085 ± 0.003 ^{a,b}	0.337 ± 0.041 ^{a,b,c,d,e}	−0.061 ± 0.011 ^{e,f,g}
C7I	67.13 ± 0.06 ^{o,p}	0.355 ± 0.003 ^{b}	1.315 ± 0.012 ^{b}	1.049 ± 0.002 ^{b}	1.785 ± 0.021 ^{c}	−0.078 ± 0.005 ^{a,b}	0.250 ± 0.019 ^{a,b,c}	−0.053 ± 0.022 ^{f,g}
C7II	100.10 ± 0.0 ^{r}	0.697 ± 0.013 ^{s}	1.177 ± 0.029 ^{a}	0.647 ± 0.033 ^{a}	0.880 ± 0.028 ^{a}	−0.070 ± 0.039 ^{a,b}	0.202 ± 0.012 ^{a,b}	−0.028 ± 0,005 ^{f,g}
DD1I	56.50 ± 0.00 ^{c,d}	0.495 ± 0.004 ^{n,o,p,r}	2.013 ± 0.007 ^{o}	1.865 ± 0.023 ^{n}	3.174 ± 0.033 ^{l,m}	−0.101 ± 0.004 ^{a,b}	0.720 ± 0.018 ^{l,m,n,o}	−0.057 ± 0.026 ^{f,g}
DD1II	55.60 ± 0.00 ^{a,b}	0.502 ± 0.005 ^{o,p,r}	2.150 ± 0.003 ^{p}	2.000 ± 0.025 ^{o}	3.349 ± 0.041 ^{n,o}	−0.099 ± 0.011 ^{a,b}	0.750 ± 0.060 ^{m,n,o}	−0.054 ± 0.030 ^{f,g}
DD1III	57.50 ± 0.00 ^{e,f}	0.471 ± 0.003 ^{j,k,l,m}	1.958 ± 0.008 ^{n}	1.425 ± 0.024 ^{f,g}	3.140 ± 0.007 ^{k,l}	−0.088 ± 0.009 ^{a,b}	0.671 ± 0.062 ^{j,k,l,m,n,o}	−0.170 ± 0.031 ^{a,b,c,d,e}
R1F1	58.20 ± 0.00 ^{f,g}	0.480 ± 0.003 ^{k,l,m,n}	1.968 ± 0.003 ^{n}	1.566 ± 0.060 ^{i,j,k}	2.781 ± 0.032 ^{h,i}	−0.030 ± 0.122 ^{b}	0.632 ± 0.025 ^{h,i,j,k,l,m,n,o}	−0.079 ± 0.038 ^{d,e,f,g}
R1G1	61.10 ± 0.17 ^{i}	0.465 ± 0.005 ^{j,k}	1.801 ± 0.010 ^{j,k}	1.529 ± 0.017 ^{h,i,j}	2.341 ± 0.021 ^{f}	−0.109 ± 0.007 ^{a}	0.473 ± 0.055 ^{c,d,e,f,g,h,i,j,k}	−0.070 ± 0011 ^{d,e,f,g}
R1G2	57.50 ± 0.00 ^{e,f}	0.505 ± 0.002 ^{p,r}	2.053 ± 0.007 ^{o}	1.480 ± 0.019 ^{g,h}	3.158 ± 0.015 ^{l}	−0.105 ± 0.005 ^{a,b}	0.661 ± 0.008 ^{i,j,k,l,m,n,o}	−0.191 ± 0.065 ^{a,b,c}
R1F2	55.07 ± 0.06 ^{a}	0.483 ± 0.009 ^{k,l,m,n,o}	2.253 ± 0.011 ^{s}	1.714 ± 0.039 ^{m}	3.432 ± 0.034 ^{o}	−0.100 ± 0.007 ^{a,b}	0.858 ± 0.026 ^{o}	−0.277 ± 0.031 ^{a}
R2	58.10 ± 0.20 ^{f,g}	0.482 ± 0.005 ^{k,l,m,n}	1.941 ± 0.014 ^{n}	1.439 ± 0.006 ^{f,g}	2.813 ± 0.047 ^{h,i}	−0.111 ± 0.011 ^{a}	0.653 ± 0.005 ^{i,j,k,l,m,n,o}	−0.131 ± 0.038 ^{b,c,d,e,f}
R3	58.60 ± 0.00 ^{g}	0.465 ± 0.003 ^{j,k}	1.834 ± 0.012 ^{k,l}	1.332 ± 0.029 ^{e}	2.748 ± 0.046 ^{h}	−0.089 ± 0.002 ^{a,b}	0.568 ± 0.027 ^{e,f,g,h,i,j,k,l,m,n}	−0.222 ± 0.029 ^{a,b}
R4	63.13 ± 0.00 ^{g}	0.425 ± 0.007 ^{e,f,g,h}	1.541 ± 0.010 ^{f}	1.338 ± 0.010 ^{e}	2.300 ± 0.027 ^{f}	−0.103 ± 0.008 ^{a,b}	0.369 ± 0.006 ^{a,b,c,d,e,f}	−0.047 ± 0.050 ^{f,g}
R5	67.97 ± 0.21 ^{p}	0.319 ± 0.003 ^{a}	1.159 ± 0.008 ^{a}	0.992 ± 0.003 ^{b}	1.676 ± 0.022 ^{b}	−0.069 ± 0.008 ^{a,b}	0.164 ± 0.016 ^{a}	−0.025 ± 0.010 ^{f,g}
V1I	66.27 ± 0.15 ^{n,o}	0.414 ± 0.004 ^{d,e,f}	1.421 ± 0.002 ^{d}	1.220 ± 0.006 ^{d}	2.120 ± 0.014 ^{e}	−0.127 ± 0.013 ^{a}	0.375 ± 0.014 ^{a,b,c,d,e,f,g}	−0.027 ± 0.020 ^{f,g}
V1II	67.10 ± 0.10 ^{o,p}	0.397 ± 0.005 ^{c,d}	1.377 ± 0.005 ^{c}	1.173 ± 0.006 ^{d}	2.023 ± 0.020 ^{d,e}	−0.123 ± 0.005 ^{a}	0.354 ± 0.034 ^{a,b,c,d,e,f}	−0.034 ± 0.011 ^{f,g}
V2I	59.90 ± 0.10 ^{h}	0.433 ± 0.004 ^{f,g,h}	1.771 ± 0.009 ^{i,j}	1.700 ± 0.012 ^{m}	3.173 ± 0.025 ^{l,m}	−0.100 ± 0.012 ^{a,b}	0.277 ± 0.005 ^{a,b,c,d}	−0.014 ± 0.016 ^{g}
V2II	62.20 ± 0.10 ^{k}	0.418 ± 0.004 ^{e,f,g}	1.739 ± 0.006 ^{i}	1.609 ± 0.014 ^{j,k,l}	2.954 ± 0.043 ^{j}	−0.108 ± 0.005 ^{a}	0.571 ± 0.025 ^{f,g,h,i,j,k,l,m,n}	−0.035 ± 0.043 ^{f,g}
V3I	65.70 ± 0.20 ^{m,n}	0.411 ± 0.005 ^{c,d,e}	1.456 ± 0.006 ^{d,e}	1.333 ± 0.016 ^{e}	2.350 ± 0.020 ^{f}	−0.122 ± 0.005 ^{a}	0.401 ± 0.014 ^{b,c,d,e,f,g,h}	−0.057 ± 0.024 ^{f,g}
V3II	64.90 ± 0.10 ^{m}	0.427 ± 0.003 ^{e,f,g,h}	1.471 ± 0.002 ^{e}	1.385 ± 0.016 ^{e,f}	2.480 ± 0.018 ^{g}	−0.113 ± 0.014 ^{a}	0.441 ± 0.020 ^{c,d,e,f,g,h,i,j}	−0.042 ± 0.008 ^{f,g}

M-WA—water hydration capacity; M-C2—protein weakening; M-C3—starch gelatinization; M-C4—amylase activity; M-C5—starch retrogradation; M-α slope—speed of the protein weakening under heating effect; M-β slope—an indicator of pasting (gelatinization) speed; M-γ slope—the enzymatic (α-amylase) degradation speed; $^{a–r}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

In agreement with Pojić et al. [11], we determined that the ash content in the wheat flour streams ranged from 0.440 to 0.755%, with a noticeable increase from B2 to B4 due to the gradual reduction of the gap between the rollers, which resulted in the release of the aleurone layer, fine bran, and germ particles together with the endosperm. In the IS Laudis variety flour, we noted that the ash content tends to increase gradually in successive breaking streams B1–B5 (from 0.671 to 1.282%, respectively), followed by a decrease in fraction streams R1–R3 and early reducing flour streams (C1–C5) with a further increase in later reduction streams C6 (2.352%) and C7 (3.931%), which is consistent with the findings reported by others [2,14]. The increase in the ash content also revealed an increase in contamination with non-endosperm tissue in a later break and reduction streams. Ash content may be related to flour yield, as reported by Banu et al. [3], as we saw around 70% yield, with a mean of 0.44% ash content from the first reduction passages, followed by a final extraction with yield over 76% with 0.51% of ash. As suggested by Every et al. [12,17], flour streams with high ash content may be characterized by a high content of lipoxygenase and dehydroascorbate reductase, and these components may negatively affect the bread quality [9]. When the ash content was higher than 2%, we found it not possible to evaluate the gluten content and gluten index due to the inability of equipment settings.

The IS Laudis wheat variety is a high-gluten wheat, so each of the passages obtained during milling was characterized by a high content of wet gluten in the range of 23.84–44.62%. Wet gluten content is often used as a parameter of protein quality to determine bread dough fermentation tolerance [11]. The high content of this ingredient in the obtained passages is considered an indicator of high quality within the obtained flours.

In the breaking passages B2–B5, which are suited to a gradual grinding of wheat grain and then separation of endosperm from bran, the content of wet gluten increased with progressing milling from 35.99 to 43.56% (Table 1). In the flour stream from the B3 break passage, the gluten content was at a higher level than in the B2 and B4 passages, which was probably due to the fact that the flour from this passage was characterized by a lower content of minerals. A similar dependence was observed in sorting systems DD1I–DD1II and DD1III, these being an extension of wheat meal screening from passages B2 and B3, respectively (Figure 1). Similar observations were made by Banu et al. [3] and Pojić et al. [11].

The increase in the protein content in various flour streams is affected by the increase in the presence of peripheral endosperm and protein-rich bran particles [15]. Flour streams of sorting passages coming from plansifters R1 to R4 contained comparable wet gluten content. Those with an ash content over 1%, such as R5, did not contain enough gluten proteins to perform the test. Streams of reducing passages were characterized by a lower content of wet gluten in comparison to breaking passages, fluctuating in the range of 27.05–30.26%, respectively. Lower values were achieved by flours separated from R1F2 and R1G2, which were characterized by a higher (coarse) particle size. Generally, due to the lower ash content and the lack of peripheral parts of the endosperm, they can be defined as originating from the central parts of the endosperm. The reducing passages C1–C7 are used to break up the endosperm to the largest possible amount of flour. Thus, flour streams from C1–C6 demonstrated wheat gluten that ranged from 23.84–32.10% and, similarly to the sorting passages, at a higher level of ash, they either did not form gluten (passages C7I and C7II), or the amount of wet gluten was very low (passages C5 and C6). The correlation coefficient calculated between A and G was only 0.34 at $p < 0.05$.

As reported by Pojić et al. [11], the differences in wet gluten content are due to the protein content and the sourcing of the bran and fat. As the grinding progresses, the protein content increases. In the endosperm, the gluten-forming protein predominates over the non-gluten-forming protein. An increase in protein content is therefore correlated with an increase in wet gluten content [49]. The inclusion of bran and aleurone, rich in non-gluten protein, increases the protein content, but not the wet gluten content. Flours with a high proportion of these outer layers tend to have a low wet gluten content. In addition, fat and bran particles can interfere with the gluten network, weakening the network and making

precise determination difficult or impossible [11]. Such relationships are shown by the gluten index (GI) determined in this study, which is related to the content of wet gluten and shows the ratio of glutenin and gliadin in wheat flour. If GI shows a higher value, this means that the wheat quality is better.

This outcome is not always correlated with gluten content in several streams. We found the correlation coefficient between A and GI to be -0.61 at $p < 0.05$. As shown in Table 1, flour streams B5 and V3II were characterized by high gluten content (G), but the gluten index (GI) was low, as the gluten quality was poor. Other relationships were found in V2II, where gluten content, as well as GI, were low (28.64 and 59.0, respectively). This relationship can be explained by the fact that the flour from this passage is another screening of the same filter flour and may be characterized by a reduced quality of gluten proteins. In addition, similar to passages C7I, C7II, and R5, it contains a high content of insoluble arabinoxylans, which may hinder the formation of the gluten network. As observed by Curić et al. [50], flours for baking, in which the GI was above 90%, contained gluten too strong for baking, the resulting bread is characterized by reduced volume, similarly, when the GI was below 75%, the bread is of poor quality. Flours with a GI in the range of 75 to 90% give the best bread with good sensory properties and volumes [50]. Therefore, it seems important to create mixtures of baking flour from passages with different GI in order to obtain flour with the above-mentioned optimal parameters GI.

Differences in protein content and composition have been known for many years, with sub-aleurone cells being richer in protein and having fewer regular starch granules than other starch endosperm cells [49]. The most detailed study to explain protein and gluten relationships in wheat grains was conducted using sequential pearling to remove six outside fractions, each representing on average about 8% of grain weight [51]. A comparison of these fractions and the milled core (corresponding to about 50% of the grain weight) showed that although the total protein content decreased from the outer layers to the center of the grain, the proportion of gluten proteins increased from about 50–55% to about 75% of the total protein in the grain [52].

A characteristic feature of the tested IS Laudis wheat is a high falling number (FN) characterized by a low activity of amylolytic enzymes. In passage flours derived from the central, ventral, and dorsal endosperm, the variability of the falling number was small, 380–460 s, and resulted rather from different content of damaged starch, as explained by Banu et al. [3] and Rani et al. [19]. In our work, only flour streams containing a high content of minerals from the outer layers, such as flour streams R5 and C7II, were distinguished by increased α-amylase activity and the FN results were very low (301 and 74 s, respectively). According to results presented by Rani et al. [19], Every et al. [12], and Dornez et al. [16], amylolytic enzymes are located mainly in the peripheral parts of the grain and the ash content and α-amylase are similarly distributed in the flour passages [3]. In our study, we calculated the correlation coefficient to be -0.75 at $p < 0.05$ between A and FN.

A measure of the flour's functionality is the amount of damaged starch (SD) that can be attributed to the mechanical impact acting on grain particles during grinding. Progressive milling causes an increase in the degree of starch damage, which was also confirmed by Banu et al. [53] and Pojić et al. [11]. In the research of Tian et al. [54] on the grinding of wheat endosperm (hard red winter wheat) in a ball mill, an upward trend in the content of damaged starch was shown with the progress of flour fractions extraction, but it slowed down with an increase in the grinding time. In our research, the flour streams coming from breaking and sorting passages showed a lower degree of starch damage than from the reducing passages. According to the results presented in Table 1, SD content increased with the subsequent passage of individual sections. Lower SD values were observed for the initial sorting and reducing passages with lower roller pressure, especially those for which the particle size was coarse (C1II, C2, R1G2, R1F2). The grain is broken down by grooved break rollers which exert less pressure on the endosperm than smooth reduction rollers. In the case of reduction passes, the size of the gap decreases and the pressure of the rolls increases, increasing the starch damage [11].

Table 2 shows the selected properties of wheat flour streams as measured by Alveograph. Several properties were tested in most flour streams: baking strength (W), dough tenacity (P), extensibility (L), elasticity index (Ie), and strain hardening index (SH). High values of dough tenacity (P) (90–151 mm) were noted mainly for the flour streams from the first fractions of the sorting passages (R1–R2), the final sorting and reducing passages (R4–R5, C4–C7I) and the filtration passages from vibro-sifter (V1I–V2I, V3). The lowest *p* values were obtained by flours of breaking passages and passage-supporting sieving (B2–B5, DD1). These differences result from the origin of the wheat flour stream fraction from the grain zone: the amount of gluten from the middle endosperm is lower than that from the outer aleurone layers [7,9].

The different rheological behavior of the breaking and reducing flour streams can be explained by the higher ratio of polymeric to monomeric proteins in the breaking streams than in the reducing streams [15]. Breaking passages, especially early ones, release relatively clean endosperm particles, while final passages tend to scrape residual endosperm particles from the peripheral endosperm layer, along with fine bran and germ particles [48]. In addition, the final reducing passages (C4–C6) contain more damaged starch and have higher water absorption, which improves the elasticity of the dough. When performing the alveographic test, which is carried out at constant dough moisture, flour containing a higher content of damaged starch would not be fully hydrated, resulting in higher dough tenacity [11]. Conversely, higher extensibility (L) values were obtained for the flour fraction of breaking passages (B2–B5) and intermediate flours of reducing and sorting passages (C1–C3, R3).

Resistance to extension was found to be negatively correlated with damaged starch content [10]. Hence, lower L values were obtained for the flour streams from the final passages. The above characteristic determines the value of the dough configuration index P/L. High P/L indicates a resistant and inextensible dough, while low P/L indicates a weak and extensible dough [40]. The lowest were found for flour streams coming from breaking passages (B2–B5), initial reducing (C1I, C2, C3), and then sorting (DD1, R3) passages. According to the observations of Banu et al. [3], streams fractions derived from dorsal and ventral endosperm were characterized by a higher baking strength (W) and elasticity index (Ie), while lower values were recorded for fractions from the sub-aleurone zone. The higher W will be, the stronger the flour will be during kneading, but the elasticity index has to be considered. The relationships between water absorption and parameters P, W, and P/L can be achieved during measurement, and relationships between P and absorption capacity are very often due to starch damage and lowering of the strength of the flour. The higher W values would suggest more stable flour during kneading [55].

Strain Hardening Index (SH) is related to the properties of the gluten network, especially large glutenin molecules, which are responsible for the branching and entanglement of the gluten polymers and thus the strength and development of the gluten network [40]. Van Vliet [56] found that a high rate of strain hardening is crucial for bread dough development and yield by it facilitating the inflation of dough bubbles into larger volumes and thinner cell walls. Many studies have shown a correlation between bread volume and SH [56–58]. The results presented in Table 2 show that the highest SH values and, at the same time, suitability for bread baking, are shown by the first breaking (B2, B3), sorting (R1F1–R1F2), and reducing (C1–C3) passage flour streams. Interestingly, the V1I and V1II filtration passages flour streams obtained from the vibro-sifter are also characterized by high SH values. The R4 and R5 flour streams showed the lowest values of the SH index, so the application of these streams is not recommended for bread flour composition. Samples containing high ash content (C5–C7II) did not show the ability to form gluten networks, so results are missing in these flour streams. These results were not included in PCA analysis due to the absence of alveographic results for some flour streams, i.e., P, L, W, and P/L could not be evaluated in the C7II stream, Ie was not found in C7I and C7II stream flours, and SH index was not available for C6, C7I, and C7II streams flour. These flour streams included very high amounts of ash content (Table 1) because of the presence of external

grain layers which excluded gluten from the obtained flour. Thus, it was not possible to test selected properties using the Alveograph procedure.

The results of selected features measured with the Farinograph are presented in Table 3. From the individual numerical values of farinographic parameters read from the chart (Figure 2a), conclusions can be drawn about the baking quality of individual flours and the direction of their use. The farinographic assessment of flour allows for testing the dough in conditions similar to the production conditions, thanks to which it enables a more complete determination of flour quality and suitability for mechanical processing than basic methods, such as protein content or gluten amount. The differences in farinographic results indices of passage flours depend on the grain fraction from which it is extracted in the technological scheme of the mill.

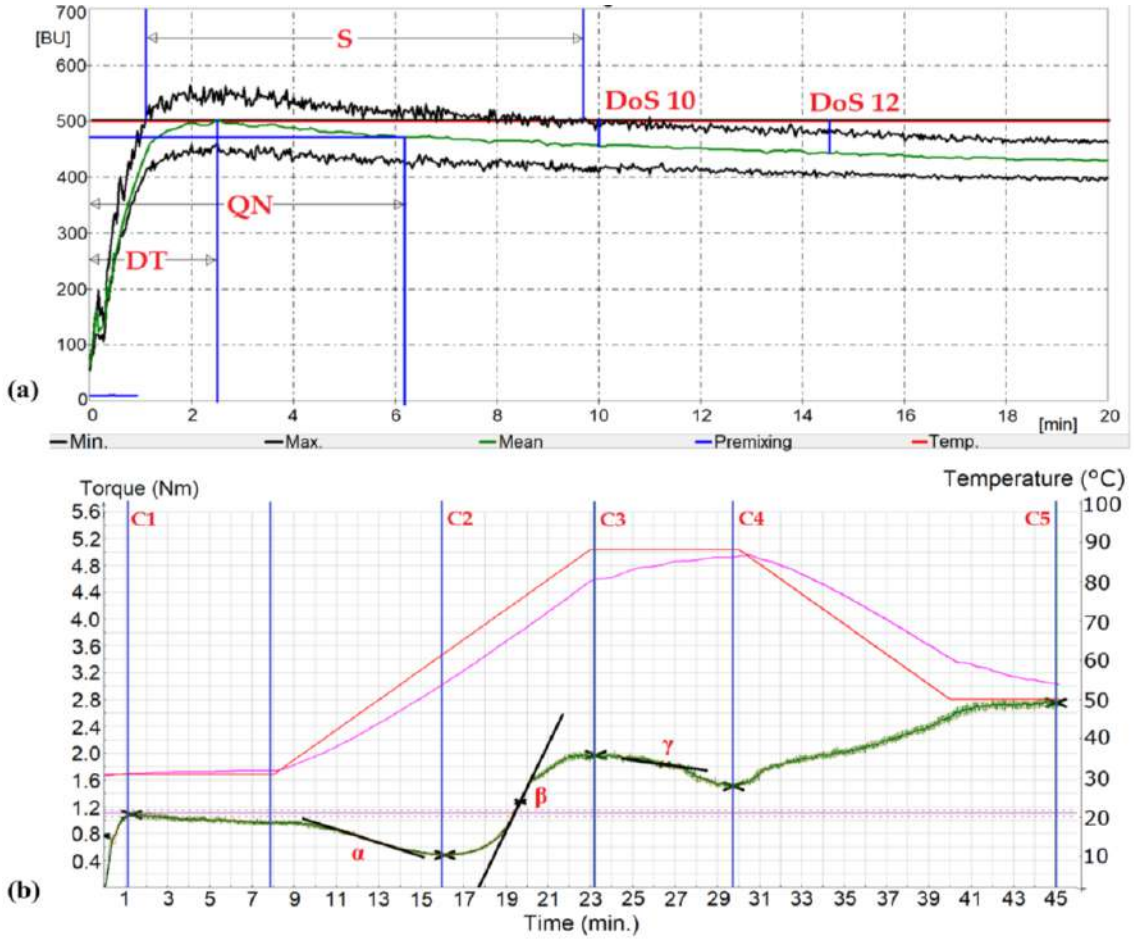

Figure 2. Example graphs of Farinograph (**a**) and Mixolab® (**b**) obtained for R1F1 flour mill stream: DT—development time; QN—quality number; S—stability, DoS10,12—dough softening in time 10 and 12 min; C2—protein weakening; C3—starch gelatinization; C4—amylase activity; C5—starch retrogradation; α slope—speed of the protein weakening under heating effect; β slope—an indicator of pasting (gelatinization) speed; γ slope—enzymatic (α-amylase) degradation speed.

In the tested flour streams from various passages, higher water absorption (WA) was observed in particular in the final breaking (B4, B5), reducing (C4–C7), and sorting (R4, R5) passages, as well as filter flour streams (V1–V3). High WA is most likely due to an increase in the content of ash [3] and damaged starch [59] with high correlation coefficients of 0.86 and 0.72, respectively ($p < 0.05$). Consecutive passages were characterized by increasing content of non-starch polysaccharides, such as arabinoxylans, which can be found in larger amounts in bran, and the increasing damage in starch granules, which are able to retain larger amounts of water in these flours than in those with low ash. Upon performing tests

following the Farinograph procedure, and correcting to a constant moisture content (14%) of flour, we concluded that streams containing higher SD content would not be completely hydrated resulting in higher dough strength. Here, the higher the water absorption (WA), the higher the yield of the prepared dough. In turn, long development time (DT) and low dough softening (DoS) characterize resistance to weakening of the dough with greater tolerance to mechanical processing.

We noted that dough development time (DT) had the highest values for breaking stream flours, but also for filter flours and final sorting and reducing passages with higher ash content. Our work also indicated that the granularity of the fraction also affects the development time of the dough. Therefore, very thick (above 180 µm) fractions (R1F2, R1F2, C1II) had a shortened development time (2.1–2.3 min). In our research, dough stability (S) decreased significantly from 17.9 to 2.7 min with successive flour streams for breaking, sorting, and reducing passages. The highest stability was achieved by the initial breaking flour streams (B2, B3) and the sieving passages (DD1) supporting fraction separation. Thus, the decrease in stability results from low-quality gluten or when other ingredients of the flour start to affect the dough system.

Destabilization occurs when mechanically damaged starch is in the gluten network, breaking the disulfide bond and softening the dough. The lowest values of DoS, both in the 10th and 12th minute of the test, were characterized by breaking passages, especially the initial (B2–B3) and, as in the case of dough stability, additional sorting passages (DD1). Interestingly, low dough softening was also found in flour streams from passages of filter flours (V1–V3). The quality number (QN) was related to the results of other farinographic results as the softening and stability parameters described above [1]. Higher S was positively correlated with higher QN with a correlation coefficient of 0.86 at $p < 0.05$ but negatively correlated with DoS results obtained both for 10 and 12 min (−0.88 and −0.84 at $p < 0.05$, respectively). Flour streams B2 and B3 as well as DD1 or V1–V3 flours were characterized by high QN results.

Tables 4 and 5 show Mixolab® primary and secondary features of IS Laudis wheat flour streams. Several properties can be evaluated and calculated via the Mixolab® procedure. As in the farinographic analysis, fractions from the middle endosperm showed low dough water hydration (M-WA), measured by obtaining a consistency of 1.1 Nm (M-C1), in direct correlation with the water absorption (WA) parameter determined in the farinographic analysis (0.98 at $p < 0.05$). This proves the possibility of using Mixolab® as an alternative instrument for the analysis of flour water absorption parameters, with less sample use needed and greater possibilities of analysis.

Table 5. Mixolab® secondary features of IS Laudis wheat flour streams.

Flour Stream	M-C2−C1 (Nm)	M-C3−C2(Nm)	M-C4−C3 (Nm)	M-C5−C4 (Nm)
B2	−0.635 ± 0.013 ^{e,f,g,h}	1.411 ± 0.019 ^{m}	−0.186 ± 0.017 ^{f,g,h,i}	1.120 ± 0.035 ^{i}
B3	−0.622 ± 0.006 ^{f,i}	1.368 ± 0.017 ^{l}	−0.212 ± 0.045 ^{e,f,g}	1.225 ± 0.060 ^{j,k}
B4	−0.709 ± 0.013 ^{b,c}	1.198 ± 0.008 ^{i}	−0,216 ± 0.005 ^{e,f,g,h}	0.900 ± 0.020 ^{e,f}
B5	−0.758 ± 0.018 ^{a}	0.938 ± 0.005 ^{c}	−0,229 ± 0.015 ^{e,f}	0.777 ± 0.022 ^{b,c,d}
C1I	−0.604 ± 0.017 ^{i,j}	1.549 ± 0.018 ^{p}	−0.533 ± 0.027 ^{a,b}	1.626 ± 0.036 ^{r,s}
C1II	−0.615 ± 0.011 ^{f,i,j}	1.696 ± 0.013 ^{s}	−0.520 ± 0.028 ^{a,b}	1.580 ± 0.029 ^{p,r}
C2	−0.589 ± 0.004 ^{j}	1.657 ± 0.012 ^{r}	−0.514 ± 0.024 ^{a,b}	1.687 ± 0.026 ^{s}
C3	−0.598 ± 0.005 ^{i,j}	1.527 ± 0.008 ^{p}	−0.486 ± 0.018 ^{b}	1.524 ± 0.008 ^{n,p}
C4	−0.657 ± 0.018 ^{d,e,g}	1.236 ± 0.004 ^{j}	−0.258 ± 0.026 ^{e}	1.054 ± 0.036 ^{g,h,i}
C5	−0.646 ± 0.012 ^{e,f,g,h}	1.229 ± 0.006 ^{i,j}	−0.514 ± 0.007 ^{a,b}	1.310 ± 0.030 ^{k,l}
C6	−0.688 ± 0.005 ^{c,d}	1.040 ± 0.011 ^{f,g}	−0.338 ± 0.002 ^{c,d}	0.830 ± 0,014 ^{c,d,e}
C7I	−0.736 ± 0.010 ^{a,b}	0.960 ± 0.010 ^{c,d}	−0.266 ± 0.010 ^{d,e}	0.736 ± 0.019 ^{b,c}
C7II	−0.394 ± 0.007 ^{k}	0.480 ± 0.021 ^{a}	−0.530 ± 0.010 ^{a,b}	0.233 ± 0.023 ^{a}
DD1I	−0.595 ± 0.010 ^{i,j}	1,518 ± 0.005 ^{o,p}	−0.148 ± 0.016 ^{h,i,j,k}	1.309 ± 0.014 ^{k,l}
DD1II	−0.602 ± 0.011 ^{i,j}	1,648 ± 0.006 ^{r}	−0.150 ± 0.028 ^{g,h,i,j,k}	1.349 ± 0.042 ^{l,m}
DD1III	−0.618 ± 0.019 ^{f,h,i,j}	1.487 ± 0.009 ^{m,o}	−0.533 ± 0.030 ^{a,b}	1.715 ± 0.019 ^{s}

Table 5. *Cont.*

Flour Stream	M-C2−C1 (Nm)	M-C3−C2(Nm)	M-C4−C3 (Nm)	M-C5−C4 (Nm)
R1F1	−0.604 ± 0.004 ^{i,j}	1.488 ± 0.002 ^{m,o}	−0.402 ± 0.061 ^{c}	1.214 ± 0.028 ^{j,k}
R1G1	−0.655 ± 0.015 ^{d,e,g}	1.337 ± 0.012 ^{k,l}	−0.273 ± 0.007 ^{d,e}	0.813 ± 0.005 ^{c,d,e}
R1G2	−0.622 ± 0.012 ^{f,g,h,i,j}	1.548 ± 0.005 ^{p}	−0.573 ± 0.014 ^{a}	1.678 ± 0.032 ^{s}
R1F2	−0.612 ± 0.015 ^{f,i,j}	1.769 ± 0.006 ^{t}	−0.538 ± 0.030 ^{a,b}	1.718 ± 0.006 ^{s}
R2	−0.654 ± 0.005 ^{d,e,g,h}	1.459 ± 0.012 ^{n}	−0.502 ± 0.013 ^{a,b}	1.374 ± 0.042 ^{l,m}
R3	−0.642 ± 0.005 ^{e,f,g,h}	1.369 ± 0.009 ^{l}	−0.501 ± 0.021 ^{a,b}	1.415 ± 0.027 ^{m,n}
R4	−0.699 ± 0.013 ^{c}	1.116 ± 0.003 ^{h}	−0.204 ± 0.008 ^{e,f,g,h}	0.962 ± 0.018 ^{f,g}
R5	−0.757 ± 0.013 ^{b}	0.840 ± 0.006 ^{b}	−0.166 ± 0.006 ^{f,g,h,i,j}	0.684 ± 0.019 ^{b}
V1I	−0.713 ± 0.005 ^{b,c}	1.007 ± 0.002 ^{e,f}	−0.201 ± 0.008 ^{e,f,g,h,i}	0.900 ± 0.020 ^{e,f}
V1II	−0.689 ± 0.009 ^{c,d}	0.980 ± 0.009 ^{d,e}	−0.204 ± 0.008 ^{e,f,g,h}	0.849 ± 0.020 ^{d,e}
V2I	−0.656 ± 0.019 ^{d,e,g}	1.338 ± 0.007 ^{k,l}	−0.070 ± 0.004 ^{l}	1.473 ± 0.014 ^{n,o}
v2II	−0.703 ± 0.009 ^{b,c}	1.321 ± 0.005 ^{k}	−0.130 ± 0.009 ^{i,j,k,l}	1.345 ± 0.042 ^{l,m}
V3I	−0.656 ± 0.008 ^{d,e,g}	1.045 ± 0.001 ^{g}	−0.123 ± 0.016 ^{j,k,l}	1.017 ± 0.028 ^{g,h}
V3II	−0.659 ± 0.006 ^{d,e}	1.044 ± 0.004 ^{g}	−0.086 ± 0.015 ^{k,l}	1.095 ± 0.002 ^{h,i}

M-C2−C1—protein weakening range, M-C3−C2—starch gelatinization range (pasting); M-C4−C3—cooking stability range; M-C5−C4—cooling setback (gelling); $^{a–r}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Mixolab® allows to evaluate the rheological properties of the dough (testing of the protein-starch complex) at a variable temperature. Using Mixolab®, the flour's water absorption is determined, and the characteristics characterizing the susceptibility of the dough to proteolytic enzymes (C2), the activity of amylolytic enzymes (C3), and starch retrogradation (C5) are read from the graph. Testing the rheological properties of the dough using the Mixolab® apparatus is carried out in two stages. In the first stage, the water absorption of the flour is determined, corresponding to the dough consistency of 1.1 ± 0.05 Nm. In the second stage, changes in the characteristics of the dough during its formation and further mixing under changing temperature conditions for 45 min are examined [42]. The graph (Figure 2b), which can be divided into five phases, records the changes in the resistance of the dough to the stirrers when mixing the dough. In the first phase, lasting 8 min at a constant dough temperature (30 °C), the properties of the dough during its formation are determined. In the second phase, during further mixing and at the same time increasing the temperature at a rate of 4 °C/min, the consistency of the dough decreases. When the temperature reaches the initial gelatinization temperature (phase 3), gelatinization of the starch begins, which is manifested by an increase in the consistency of the dough. In the fourth phase, a further increase in temperature to 90 °C causes the liquefaction of the starch paste and thus the reduction of the resistance of the dough to the stirrers. Lowering the temperature to 50 °C in phase 5 causes recrystallization of amylose, which in the graph is manifested by an increase in the consistency of the dough, referred to as retrogradation. In the third, fourth, and fifth phases of the graph, the properties of the starch complex are examined [43]. The graph also reads indicators describing the rate of dough consistency reduction during the initial temperature increase in the second phase (α), dough consistency increase due to starch gelatinization (β), and consistency reduction due to enzymatic hydrolysis (γ).

The lowest values of the M-C2 (protein weakening) parameter, excluding passage C7II (with extremely high bran content), were found in flour streams with increasing ash content (−0.86 at $p < 0.05$). Fractions from the periphery of the endosperm were found to be characterized by proteins from these zones of the grain, which may result in greater proteolytic activity [3]. These M-C2 results corresponded to the M-C2−C1 parameter (protein weakening range) (0.89 at $p < 0.05$) and were not related to the M-α slope (protein breakdown rate) (Figure 2b).

Flours from the breaking and initial sorting and reduction passages, derived from the central endosperm, were characterized by high values of the M-β slope (Table 4) found on the curve (Figure 2b), which characterize the starch gelatinization rate [3,60,61]. These fractions have reduced enzymatic activity. Passages originating from the outer zone have

a lower M-β slope due to the higher content of amylolytic enzymes. We noted that the increased presence of peripheral kernel particles rich in protein, minerals, and amylolytic enzymes affected the decrease in viscosity. The fact that α-amylase is mostly located in the peripheral parts of a wheat kernel indicated the similar distribution of α-amylase and ash content among flour mill streams, where final reduction flours are characterized by higher α-amylase activity [12,16,19].

Accordingly, more intense shear stress of flour streams during reduction passages induced stronger mechanical damage of starch granules than during breaking stages. Since the recorded peak viscosity is an indirect measure of the present α-amylase status along with the starch granule quality, the higher α-amylase activity and higher quantity of mechanically damaged starch in the final reduction passages resulted in lower peak viscosity [62].

High values of the M-C3 point are associated with reduced M-WA (-0.95 at $p < 0.05$) and less SD (-0.82 at $p < 0.05$) and are characteristic of the middle endosperm flour fraction. For parameter M-C4 (amylase activity), the final passages from each section showed the lowest parameters (Table 4), while flour streams from the middle endosperm and filter flour (V2) revealed the highest M-C4 values. Low values of M-γ slope, characterized by a small difference for M-C4−C3 (cooking stability range), were found in V1–V3 filter flours, but also in the DD1 sorting flour streams. M-C5 values indicate starch retrogradation [60,61,63] and were lower for breaking passages (B2–B5) and sorting passages flours (R4–R5) and, as in the studies of Banu et al. [3], in the reduction passages (C5–C6) of flour streams from peripheral parts of the kernel. This relationship was associated with the cooling setback M-C5−C4 index (Table 5) at the correlation level of 0.93 ($p < 0.05$).

There is a strong correlation between the results obtained through the application of the different procedures assessed in this study. In the Mixolab® analysis, a decrease in the M-C4 torque and an increase in the M-C4−C3 differential is generally seen as a decrease in the falling number in the tested flour [55]. Herein, FN and viscosity assessments of M-C4−C3 from Mixolab®, as well as WA and SD allowed analysis of some properties tested by means of the Alveograph procedure. Of note, the alveographic procedure and selected values provide little predictive information about dough behavior, unlike the Mixolab® "hot" stage (M-C2, M-C3, M-C4, and M-C5). This Mixolab® data is therefore extremely useful. Moreover, Mixolab® data shows good differentiation between the mill streams, allowing inferences on the properties of both protein and starch complex, and the amylolysis/retrogradation phase. A further advantage of the test device is that it gives researchers the ability to analyze flours with a high content of bran fraction and ash, which is difficult or impossible when utilizing other devices evaluating such rheological properties. Hence, using Mixolab® gives the possibility to assess the similarities of various mill streams of flours so as to study the complete milling process and produce reproducible flour blends [55].

Table 6 shows the results of wheat flour stream SRC as determined utilizing a variety of liquids. The Solvent Retention Capacity (SRC) method indicates the hydration capacity of wheat flour, and the flour's ability to absorb various solutions is measured depending on the chemical composition, i.e., the amount and quality of gluten, starch, and pentosans. This attribute is assessed utilizing distilled water (Wa), 50% sucrose (Su), 5% lactic acid (La), and 5% sodium carbonate (Sc). Water absorption depends on all of the flour components listed above, thus providing an overall picture of the water-holding capacity of the dough system. The absorbed sucrose solution determines the properties of pentoses, which are formed as a result of pentosan hydrolysis. The volume of the absorbed lactic acid solution characterizes the hydration properties of gluten depending on the quality and quantity of gluten proteins, while the absorbed Na_2CO_3 solution provides information on the degree of starch damage [45]. Large differences in SRC values were found in the tested flour streams.

Table 6. SRC values of IS Laudis wheat flour streams.

Flour Stream	WaSRC (%)	SuSRC (%)	LaSRC (%)	ScSRC (%)	GPI (-)
B2	62.642 ± 0.593 ^{a,b}	115.695 ± 1.534 ^{e,f,g,h,i}	161.558 ± 1.010 ^{n}	79.567 ± 0.704 ^{d}	0.827 ± 0.007 ^{m}
B3	63.017 ± 0.58 ^{a,b}	120.274 ± 1,153 ^{i,j,k,l}	180.035 ± 1.701 ^{s}	78.150 ± 0.692 ^{c}	0.907 ± 0.007 ^{o}
B4	71.675 ± 0.574 ^{g}	119.569 ± 0.640 ^{h,i,j,k,l}	134.362 ± 0.501 ^{h}	94.163 ± 0.304 ^{j}	0.629 ± 0.005 ^{e}
B5	75.508 ± 0.302 ^{h}	124.692 ± 1370 ^{k,l,m}	129.109 ± 0.412 ^{g}	97.265 ± 0.412 ^{k}	0.582 ± 0.003 ^{d}
C1I	65.164 ± 0.523 ^{c,d}	114.885 ± 0.570 ^{d,e,f,g,h,i,j}	162.432 ± 0.114 ^{n,o}	86.106 ± 0.198 ^{e,f,g}	0.808 ± 0.002 ^{l}
C1II	63.932 ± 0.228 ^{a,b,c}	94.796 ± 0.747 ^{a}	128.096 ± 0.228 ^{g}	78.541 ± 0.228 ^{c,d}	0.739 ± 0.004 ^{i}
C2	63.009 ± 0.302 ^{a,b}	104.997 ± 1.951 ^{b}	147.579 ± 0.685 ^{k}	80.345 ± 0.198 ^{d}	0.796 ± 0.011 ^{k,l}
C3	64.177 ± 0.302 ^{b,c}	114.794 ± 2.205 ^{d,e,f,g,h,i,j}	154.734 ± 0.457 ^{m}	84.806 ± 0.498 ^{e}	0.775 ± 0.008 ^{j,k}
C4	75.233 ± 0.115 ^{h}	121.145 ± 0,574 ^{h,i,j,k,l}	143.074 ± 1.033 ^{j}	100.673 ± 0.500 ^{l}	0.645 ± 0.005 ^{e,f}
C5	73.052 ± 0.496 ^{g}	114.671 ± 0.911 ^{d,e,f,g,h,i}	138.012 ± 1.532 ^{i}	102.573 ± 0.711 ^{m}	0.635 ± 0.006 ^{e}
C6	86.747 ± 0.856 ^{j}	113.323 ± 1.262 ^{c,d,e,f,g,h}	100.100 ± 0.340 ^{b}	125.105 ± 0.631 ^{p}	0.420 ± 0.003 ^{a}
C7I	87.798 ± 0.490 ^{j}	107.871 ± 1.369 ^{b,c,d}	114.951 ± 0.585 ^{d,e}	136.063 ± 0.981 ^{r}	0.471 ± 0.002 ^{b}
C7II	159.775 ± 2.833 ^{k}	178.502 ± 12.648 ^{n}	163.080 ± 1.323 ^{n,o}	193.406 ± 0.683 ^{t}	0.439 ± 0.014 ^{a}
DD1I	62.096 ± 0.307 ^{a,b}	110.142 ± 1.686 ^{b,c,d,e,f,g}	149.610 ± 0.307 ^{k,l}	77.642 ± 0.580 ^{b,c}	0.797 ± 0.008 ^{l}
DD1II	61.867 ± 0.464 ^{a}	105.465 ± 1.508 ^{b,c}	128.637 ± 0.876 ^{g}	76.065 ± 0.307 ^{a,b}	0.709 ± 0.002 ^{h}
DD1III	62.331 ± 0.463 ^{a,b}	115.751 ± 1.219 ^{d,e,f,g,h,i,j}	166.634 ± 0.530 ^{p,r}	77.422 ± 0.116 ^{b,c}	0.863 ± 0.008 ^{n}
R1F1	69.124 ± 0.115 ^{f}	120.538 ± 1.966 ^{h,i,j,k,l}	164.314 ± 1.852 ^{o,p}	95.562 ± 0.230 ^{j,k}	0.760 ± 0.013 ^{j}
R1G1	78.190 ± 0.576 ^{i}	117.884 ± 0.984 ^{g,h,i,j,k,l}	169.014 ± 1.282 ^{r}	113.562 ± 0.461 ^{o}	0.730 ± 0.004 ^{i}
R1G2	66.829 ± 0.230 ^{d,e}	109.832 ± 0.305 ^{b,c,d,e,f}	152.437 ± 1.055 ^{l,m}	87.699 ± 0.115 ^{f,g,h}	0.772 ± 0.006 ^{j}
R1F2	62.023 ± 0.691 ^{a}	92.951 ± 0.399 ^{a}	121.951 ± 0.610 ^{f}	74.394 ± 0.399 ^{a}	0.729 ± 0.004 ^{h,i}
R2	68.044 ± 0.115 ^{e,f}	109.922 ± 1.490 ^{b,c,d,e,f}	131.159 ± 0.413 ^{g,h}	87.958 ± 0.198 ^{g,h}	0.663 ± 0.005 ^{f,g}
R3	69.252 ± 0.527 ^{f}	121.621 ± 0.996 ^{i,j,k,l}	154.675 ± 0.690 ^{m}	90.160 ± 0.527 ^{g,h}	0.730 ± 0.006 ^{i}
R4	77917 ± 0.198 ^{i}	122.578 ± 0.457 ^{j,k,l,m}	118.158 ± 0.749 ^{e}	103.645 ± 0.198 ^{m}	0.522 ± 0.004 ^{c}
R5	90.464 ± 0.297 ^{k}	129.955 ± 0.971 ^{m}	117.460 ± 0.624 ^{e}	148.082 ± 1.866 ^{s}	0.422 ± 0.002 ^{a}
V1I	86.505 ± 0.606 ^{j}	109.513 ± 0.499 ^{b,c,d,e}	146.734 ± 1.301 ^{k}	110.835 ± 0.525 ^{n}	0.666 ± 0.006 ^{f,g}
V1II	87.365 ± 0.458 ^{i}	110.637 ± 0.595 ^{b,c,d,e,f,g}	151.362 ± 1.196 ^{l}	114.008 ± 0.344 ^{o}	0.674 ± 0.004 ^{g}
V2I	66.753 ± 0.231 ^{d,e}	117.492 ± 0.924 ^{f,g,h,i,j,k}	113.692 ± 1.677 ^{d}	79.888 ± 0.115 ^{d}	0.576 ± 0.011 ^{d}
V2II	72.040 ± 0.528 ^{g}	115.615 ± 0.502 ^{d,e,f,g,h,i,j}	96.788 ± 0.599 ^{a}	86.011 ± 0.200 ^{e,f}	0.480 ± 0.005 ^{b}
V3I	79.754 ± 0.499 ^{i}	125.271 ± 0.794 ^{k,l,m}	106.614 ± 0.198 ^{c}	99.271 ± 0.397 ^{l}	0.475 ± 0.002 ^{b}
V3II	73.711 ± 0.303 ^{g,h}	126.770 ± 1.952 ^{l,m}	116.065 ± 1.300 ^{d,e}	88.975 ± 0.499 ^{h,i}	0.538 ± 0.004 ^{c}

SRC—Solvent Retention Capacity; Wa—distilled water; Su—50% sucrose; La—5% lactic acid; Sc—5% sodium carbonate; GPI—Gluten Performance Index; $^{a–p}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

In the data we derived, WaSRC ranged from 61.867 to 159.775%, SuSRC from 92.951 to 178.502%, LaSRC from 96.788 to 180.035%, and ScSRC from 74.394 to 193.406%. According to the results listed in Table 6, the lowest water absorption (WaSRC) was found in the DD1 sorting passages, the breaking passages, and the initial sorting and reduction passages. Comparing the WaSRC results with water absorption measured via the application of farinograph and Mixolab® procedures, the correlation coefficients were 0.93 and 0.92, respectively, at $p < 0.05$.

The SuSRC values indicate the amount of arabinoxylans in the analyzed flour sample. Unlike in the studies of Vukić et al. [4], we noted higher SuSRC values in breaking stream flours. These were probably brought about by the presence of endosperm adjacent to the aleurone layer. High values of SuSRC were also obtained in the reduction and filter flour streams (V2–V3). Our work indicates that the highest ScSRC values (indicating damaged starch) are in the flour streams from the final reducing passages (C4–C7II) and sorting passages (R3–R5), as well as in the filter and sorting flour streams (V1I, V1II, and R1G1), for which also the starch damage DS measured by the amperometric method was high (Table 1).

LaSRC value is an indicator of gluten quality and functionality and reveals the amount of glutenin protein in passage flours [45]. The high values of this index are related mainly to the content of middle endosperm fractions derived in reducing C1–C5, initial sorting with fine granulation (R1F1 and R1G1), breaking passages (B2, B3) and supporting sorting flour streams (DD1I, DD1III). The C7II flour is also characterized by a high level of LaSRC, but this outcome was probably due to the very high content of the bran hydrophilic fraction,

but not the quality and quantity of glutenin. This notion is confirmed by the low value of the GPI parameter for this passage flour stream (Table 6).

Gluten Performance Index (GPI) was considered by Kweon et al. [45] as being a better indicator of the predictability of gluten functionality than LaSRC alone. It describes the general ability of glutenin to function among other modulating networks such as damaged starch or pentosans/arabinoxylans. Lindgren and Simsek [64] confirmed in their research the existence of a positive correlation between GPI and selected passage flour rheological parameters. Similar relationships were noted in this study. GPI was negatively correlated with WA and dough softening at 12 min (DoS12) of the farinograph (−0.77 and −0.62 at $p < 0.05$, respectively), and strongly positively correlated with L, W, Ie, and alveograph SH (0.73, 0.62, 0.83, 0.83 at $p < 0.05$, respectively). The GPI in the tested flour streams ranged from 0.42 to 0.91 (average 0.66). As with the LaSRC results, central endosperm flours, also those with increased granulation (R1F2, R1G2) demonstrated the highest values. In line with earlier research by Lindgren and Simsek [64] and Kweon et al. [45], the GPI value can be used to determine the baking quality of flour, in particular, bread.

Polysaccharides content, arabinoxylans content, and fractions in the obtained flour streams are listed in Table 7. The total content of non-starch polysaccharides (T-NSP) was determined by gas chromatography and is the sum of the sugars: arabinose, xylose, mannose, galactose, and glucose. This analysis allows separating non-starch polysaccharides into two fractions: soluble and insoluble, and determining the composition of polysaccharides in both fractions. In the tested flour streams, the total amount of non-starch polysaccharides ranged from 2.70 to 24.70% and was the highest mainly in the final fractions of reduction and sorting passages and in filtration streams flours. We saw that the T-NSP content was strongly related to the ash content (A) in the tested flours, and the calculated correlation coefficient was at the level of 0.85 (at $p < 0.05$). We also observed a high content of T-NSP for the R1F1 passage (4.39%), the flour of which comes from the middle endosperm and, however, contains a small amount of ash.

Table 7. Non-starch polysaccharides and arabinoxylans content in IS Laudis wheat flour streams.

Flour Stream	T-NSP (%)	S-NSP (%)	I-NSP (%)	T-AX (%)	I-AX (%)	S-AX (%)
B2	$3.80 \pm 0.00^{i,j,k}$	$1.72 \pm 0.05^{g,h,i}$	$2.08 \pm 0.04^{g,h}$	$2.05 \pm 0.01^{g,h,i,j}$	$1.30 \pm 0.02^{f,g}$	$0.75 \pm 0.03^{e,f,g,h,i}$
B3	2.70 ± 0.00^{a}	1.29 ± 0.04^{a}	1.41 ± 0.04^{a}	1.48 ± 0.00^{a}	0.93 ± 0.04^{a}	0.55 ± 0.04^{a}
B4	$4.17 \pm 0.12^{n,o}$	$1.85 \pm 0.05^{i,j}$	2.31 ± 0.07^{i}	$2.42 \pm 0.05^{m,n}$	$1.61 \pm 0.04^{j,k}$	$0.81 \pm 0.01^{h,i,j}$
B5	$3.89 \pm 0.04^{j,k,l,m}$	$1.57 \pm 0.03^{d,e,f}$	2.32 ± 0.07^{i}	$2.29 \pm 0.07^{l,m,n}$	$1.61 \pm 0.07^{i,j,k}$	$0.68 \pm 0.00^{c,d,e,f}$
C1I	$3.09 \pm 0.02^{b,c}$	$1.57 \pm 0.01^{d,e,f}$	$1.52 \pm 0.03^{a,b}$	$1.71 \pm 0.08^{b,c}$	$1.01 \pm 0.08^{a,b,c}$	$0.71 \pm 0.01^{d,e,f,g}$
C1II	$4.12 \pm 0.08^{m,n}$	$1.41 \pm 0.04^{a,b,c}$	$2.70 \pm 0.11^{j,k}$	$2.23 \pm 0.03^{k,l,m}$	$1.58 \pm 0.05^{i,j}$	$0.65 \pm 0.02^{b,c,d,e}$
C2	$3.35 \pm 0.03^{d,e,f}$	$1.55 \pm 0.02^{d,e,f}$	$1.80 \pm 0.01^{c,d,e}$	$1.86 \pm 0.04^{b,c,d,e}$	$1.13 \pm 0.01^{c,d,e}$	$0.74 \pm 0.05^{e,f,g,h,i}$
C3	$3.29 \pm 0.08^{c,d,e}$	$1.63 \pm 0.04^{e,f,g}$	$1.66 \pm 0.04^{b,c}$	$1.79 \pm 0.01^{b,c,d}$	$1.08 \pm 0.02^{b,c,d}$	$0.71 \pm 0.01^{d,e,f,g}$
C4	$3.64 \pm 0.09^{g,h,i}$	$1.71 \pm 0.03^{g,h}$	$1.93 \pm 0.06^{e,f,g}$	$1.95 \pm 0.05^{d,e,f,g,h,i}$	$1.25 \pm 0.08^{e,f,g}$	$0.70 \pm 0.03^{d,e,f,g}$
C5	$3.66 \pm 0.02^{g,h,i,j}$	$1.71 \pm 0.03^{g,h}$	$1.95 \pm 0.02^{e,f,g}$	$2.03 \pm 0.05^{f,g,h,i,j}$	$1.27 \pm 0.08^{e,f,g}$	$0.77 \pm 0.03^{f,g,h,i,j}$
C6	6.47 ± 0.14^{t}	2.07 ± 0.07^{k}	4.40 ± 0.07^{m}	3.75 ± 0.11^{r}	2.83 ± 0.09^{n}	$0.91 \pm 0.02^{k,l}$
C7I	7.98 ± 0.04^{v}	2.15 ± 0.05^{k}	5.83 ± 0.09^{o}	4.63 ± 0.06^{t}	3.68 ± 0.03^{p}	$0.94 \pm 0.03^{k,l}$
C7II	24.70 ± 0.16^{w}	2.48 ± 0.03^{l}	22.22 ± 0.19^{p}	16.45 ± 0.10^{u}	15.07 ± 0.09^{r}	1.37 ± 0.01^{n}
DD1I	4.66 ± 0.12^{r}	$1.82 \pm 0.06^{h,i,j}$	2.84 ± 0.06^{k}	2.61 ± 0.00^{o}	1.84 ± 0.04^{l}	$0.77 \pm 0.03^{f,g,h,i,j}$
DD1II	$3.74 \pm 0.06^{h,i,j,k}$	$1.65 \pm 0.01^{e,f,g}$	$2.08 \pm 0.05^{g,h}$	$2.19 \pm 0.04^{j,k,l}$	$1.34 \pm 0.01^{g,h}$	$0.85 \pm 0.03^{j,k}$
DD1III	$3.28 \pm 0.12^{c,d,e}$	1.876 ± 0.066^{j}	1.41 ± 0.05^{a}	$1.90 \pm 0.10^{d,e,f,g}$	$0.95 \pm 0.05^{a,b}$	0.95 ± 0.05^{l}
R1F1	$4.39 \pm 0.06^{o,p}$	2.59 ± 0.02^{l}	$1.807 \pm 0.035^{c,d,e}$	$2.37 \pm 0.07^{m,n}$	$1.17 \pm 0.04^{d,e,f}$	1.20 ± 0.04^{m}
R1G1	2.95 ± 0.01^{b}	$1.40 \pm 0.05^{a,b,c}$	$1.55 \pm 0.03^{a,b}$	1.51 ± 0.01^{a}	$0.94 \pm 0.03^{a,b}$	$0.56 \pm 0.02^{a,b}$
R1G2	$3.18 \pm 0.13^{b,c,d}$	$1.60 \pm 0.06^{e,f,g}$	$1.58 \pm 0.06^{a,b}$	1.69 ± 0.05^{b}	$1.00 \pm 0.03^{a,b,c}$	$0.69 \pm 0.02^{c,d,e,f,g}$
R1F2	$3.67 \pm 0.01^{g,h,i,j}$	$1.46 \pm 0.01^{b,c,d}$	$2.21 \pm 0.00^{h,i}$	$1.92 \pm 0.04^{d,e,f,g,h}$	$1.30 \pm 0.01^{f,g}$	$0.61 \pm 0.03^{a,b,c,d}$
R2	$4.04 \pm 0.07^{l,m,n}$	$1.68 \pm 0.03^{f,g,h}$	2.36 ± 0.04^{i}	$2.28 \pm 0.01^{l,m,n}$	$1.47 \pm 0.03^{h,i}$	$0.81 \pm 0.04^{i,j}$
R3	$3.26 \pm 0.06^{c,d,e}$	$1.59 \pm 0.04^{d,e,f,g}$	$1.68 \pm 0.02^{b,c,d}$	$1.87 \pm 0.06^{c,d,e,f}$	$1.09 \pm 0.01^{b,c,d}$	$0.78 \pm 0.05^{g,h,i,j}$
R4	$3.96 \pm 0.00^{k,l,m,n}$	$1.33 \pm 0.03^{a,b}$	2.63 ± 0.03^{j}	$2.32 \pm 0.03^{l,m,n}$	$1.74 \pm 0.04^{k,l}$	$0.59 \pm 0.01^{a,b}$
R5	6.96 ± 0.06^{u}	$1.52 \pm 0.02^{c,d,e}$	5.44 ± 0.04^{n}	4.09 ± 0.09^{s}	3.48 ± 0.03^{o}	$0.61 \pm 0.05^{a,b,c,d}$

Table 7. *Cont.*

Flour Stream	T-NSP (%)	S-NSP (%)	I-NSP (%)	T-AX (%)	I-AX (%)	S-AX (%)
V1I	$3.82 \pm 0.02^{i,j,k,l}$	1.93 ± 0.00^{j}	$1.89 \pm 0.02^{d,e,f,g}$	$2.08 \pm 0.03^{h,i,j,k}$	$1.30 \pm 0.04^{f,g}$	$0.78 \pm 0.01^{g,h,i,j}$
V1II	$3.452 \pm 0.081^{e,f,g}$	$1.59 \pm 0.05^{e,f,g}$	$1.86 \pm 0.03^{c,d,e,f}$	$1.99 \pm 0.03^{e,f,g,h,i}$	$1.28 \pm 0.01^{f,g}$	$0.71 \pm 0.04^{e,f,g,h}$
V2I	$3.56 \pm 0.06^{f,g,h}$	$1.52 \pm 0.01^{c,d,e}$	$2.04 \pm 0.08^{f,g,h}$	$1.89 \pm 0.01^{d,e,f,g}$	$1.29 \pm 0.02^{f,g}$	$0.60 \pm 0.01^{a,b,c}$
V2II	5.53 ± 0.01^{s}	$1.72 \pm 0.07^{g,h,i}$	3.81 ± 0.07^{l}	3.23 ± 0.03^{p}	2.49 ± 0.00^{m}	$0.74 \pm 0.03^{e,f,g,h,i}$
V3I	4.43 ± 0.01^{s}	$1.72 \pm 0.07^{g,h,i}$	$2.71 \pm 0.15^{j,k}$	$2.44 \pm 0.00^{n,o}$	$1.74 \pm 0.04^{k,l}$	$0.70 \pm 0.04^{d,e,f,g}$
V3II	$3.69 \pm 0.01^{h,i,j}$	$1.36 \pm 0.00^{a,b}$	2.33 ± 0.01^{i}	$2.11 \pm 0.00^{i,j,k}$	$1.53 \pm 0.00^{i,j}$	$0.57 \pm 0.00^{a,b}$

T-NSP—total non-starch polysaccharides; S-NSP—soluble non-starch polysaccharides; I-NS—insoluble non-starch polysaccharides; T-AX—total arabinoxylans; I-AX—insoluble arabinoxylans; S-AX—soluble arabinoxylans; $^{a–u}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Upon analyzing the insoluble (I) and soluble (S) NSP fractions, S-NSP stood at 2.58%. A similarly high content of T-NSP was found in the DD1I and C1II fractions, respectively, 4.66% and 4.11%, in which the amount of both I-NSP and S-NSP fractions was also high. The highest amount of T-NSP was found in flour stream C7II (24.70%), which contains the highest amount of bran fraction, and thus ash, of all the tested fractions. The content of I-NSP in this passage is almost 90%. In the insoluble fraction (I-NSP), the growth gradient is similar to that of T-NSP and generally takes the highest values for flour streams from passages with a higher degree of extraction and thus ash content (0.86 at $p < 0.05$) from the outer parts of the kernel. The S-NSP fractions were characterized by a more even distribution in all the tested passage flours and were only slightly related to the ash content (0.43 at $p < 0.05$).

Table 7 also shows the insoluble (I-AX) and soluble (S-AX) fractions of arabinoxylans. These are part of the sum of non-starch polysaccharides that contribute to better bread production [25–27]. The values for I-AX were from 0.93% for flour stream B3, to 15.07% for flour stream C7II. Similarly, for S-AX, values ranged from 0.55% in the B3 flour stream, to 1.37% in C7II. Over all, the sum of both fractions ranged from 1.48 to 16.45%. The gradient of the value increase for the individual fractions was generally similar to that of the I-NSP and S-NSP fractions. For I-AX and T-AX, the values increased significantly with the amount of ash (0.85 at $p < 0.05$), except for flours from the R1F1 and DD1I streams. We also found that the S-AX distribution was more even and less related to ash content (0.45 at $p < 0.05$) and extraction level. Similar observations were reported by Delcour et al. [36] where the values of the NSP fraction increased with the increase of ash. A clear gradient was observed for T-AX while a fuzzy one was noted for S-AX. Moreover, we discovered that S-AX can increase only when the flour is enriched with a greater amount of very fine bran fraction, which is observed in this study for flour streams C7II, C7I, and R5. As reported by Li et al. [65] intensive grinding of the bran layer leading to obtaining finely divided fragments of these fractions may contribute to increasing the content of soluble fiber, also by breaking glycosidic bonds in cellulose and insoluble hemicellulose, especially in S-AX.

As presented in previous studies, the content of T-AX, I-AX, and I-NSP, as confirmed by the PCA analysis (Figure 3), was strongly positively correlated with the flour stream ash content and water absorption. We observed that the high content of these components effectively prevented the formation of a gluten network and the appropriate consistency of the dough in rheological analyses. As in the studies of Ramseyer et al. [6], we noted an increasing amount of S-AX, I-AX, and T-AX, along with the progressing flour extraction. At the same time, we saw that the increase in the content of S-AX in individual passages was different than the results of ash content, I-AX, and T-AX.

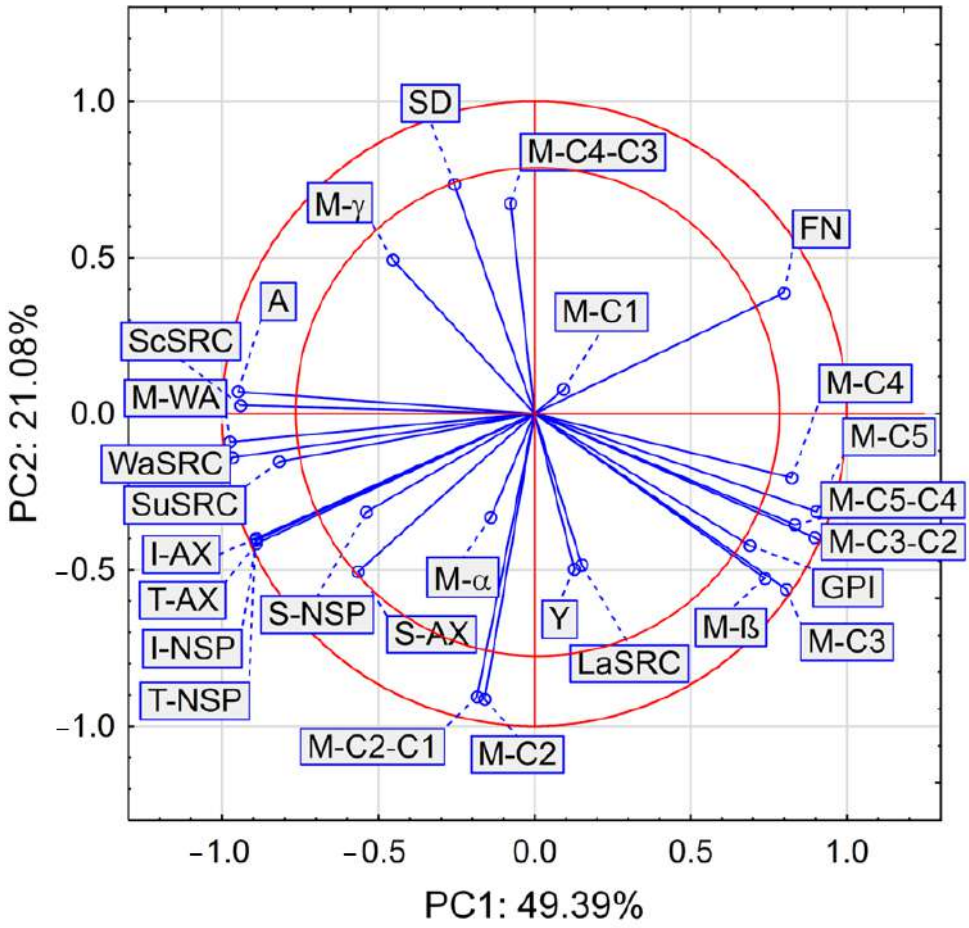

Figure 3. PCA analysis of selected properties of wheat flour streams.

We therefore conclude that the testing of flour mill streams coming from various passages using rapid rheological analyses, such as the Mixolab® analysis, compared to time-consuming chemical analyzes of the isolation of polysaccharides fractions, allows (to some extent) the possibility of indicating the content of these fractions in flours. Michniewicz et al. [26] demonstrated, for example, the effects of water-soluble and water-insoluble wheat pentosans and water-soluble rye pentosans on certain baking characteristics of wheat flour. In their work, all three pentosan preparations markedly increased the farinograph water absorption, while the addition of water-soluble pentosans (at 2%, w/w) increased the specific loaf volume. However, insoluble fractions did not significantly affect this parameter. According to Michniewicz et al. [26], at a constant dough consistency, pentosan-supplemented breads had higher moisture contents and water activity values. Moreover, in their study, higher retrogradation rates of the amylopectin, as measured by DSC, were shown for breads supplemented with pentosans, presumably due to their higher moisture content. The conclusion was that water-soluble pentosans retarded the aggregation process between amylose molecules, as evidenced by the amount and type of water-extractable carbohydrates from bread crumbs.

Figure 3 presents the results of the PCA analysis of selected flour streams. Here, certain physiochemical and rheological properties, as well as polysaccharides and arabinoxylans were taken into account.

The PCA analysis shows that the first two main components PC1 and PC2 describe the variability of the system to a level of 70.47%, however, the parameters that are contained between the two red circles in Figure 3 have the greatest impact on the variability of the system. In our study, PCA showed that A, M-WA, WaSRC, SuSRC, ScSRC, I-AX, T-AX, I-NSP, and T-NSP are strongly and positively correlated with each other. Hence, the

results obtained from instrumental measurements, especially ash content, water absorption measured with the Mixolab® procedure, and solvent retention capacity in water, 50% sucrose, and 5% sodium carbonate solutions may be useful for the prediction of the content of total polysaccharides and arabinoxylans, as well as their insoluble fractions.

We also found a positive and strong correlation between the parameters GPI, the slope of M-β, and certain characteristics measured by means of Mixolab® and labeled as M-C3, M-C4, M-C5, M-C3−C2, M-C5−C4. Figure 3 reveals a strong and positive correlation between the parameters M-C2−C1 and M-C2. Based on the results of PCA analysis we observed a negative and strong correlation between A, M-WA, ScSRC, WaSRC, SuSRC, I-AX, T-AX, I-NSP, T-NSP and FN, GPI, M-β, M-C3, M-C4, M-C5, M-C3−C2, M-C5−C4. We also noted a strong negative correlation between M-C2, M-C2−C1, and SD values. That parameter SD is indicative of the amount of damaged starch best describes the passages B4, B5, C4, C6, C7I, R4, R5, R1G1, V1I, V1II, V3I, V3II which are the final flour fractions from the specific milling scheme used in the experiment, and give flour fractions after intensive treatment by breaking, reducing and sifting passages. The presented comparison of flour mill stream passages, as well as other comparisons [1,2,6,11,21], shows the possibilities of using such data to compose specialized flour mixtures. Hence, flour mixing to achieve a particular flour functionality can be based on instrumental methods instead of long and costly chemical analyzes. As was shown in previous studies, the parameters most differentiating individual flours, such as the content of ash, gluten proteins, amylolytic enzymes, or non-starch polysaccharides and arabinoxylans, had a direct impact on the characterized rheological parameters used to direct the fraction in accordance with the assumed technological usefulness. In our work, we observed that the high content of T-AX, I-AX, and I-NSP effectively prevented the formation of the gluten network and the appropriate consistency of the dough in rheological analyses. Although the results of this study apply only to a specific variety of IS Laudis common wheat and a specific milling scheme, it can be assumed that the results will also be consistent for other varieties. A better understanding of the origin of different fractions and the role of arabinoxylans and their fractions in the milling process will allow the development of wheat flour blends with the desired functionality.

4. Conclusions

In conclusion, there are large differences between the mill streams in terms of the content of physicochemical parameters and rheological properties, as well as soluble and insoluble fractions of non-starch polysaccharides and arabinoxylans. These differences result directly from the origin of specific fractions from the anatomical parts of the kernel and the impact of grinding processes, mechanical damage to starch, and sieving during grain milling. All these operations greatly affect the overall quality of the flours. From the point of view of using the passages, it seems important to know about the subtle differences in the content of these components in the final fractions of the milling scheme. Flour passage tests using rapid rheological analyses, such as the Mixolab® analysis and the combination of principal component analysis with Pearson's correlation coefficients for the analysis of these relationships, allows for the identification of strict relationships between the tested parameters. Based on this information, millers can select and blend several flour streams for the maximum amount of flour at specified characteristics, especially ash content and non-starch polysaccharides.

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processes

MDPI

Article

Effect of Extruder Configuration and Extrusion Cooking Processing Parameters on Selected Characteristics of Non-Starch Polysaccharide-Rich Wheat Flour as Hybrid Treatment with Xylanase Addition

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Abstract: The effects of a single-screw extruder configuration and processing variables such as conventional extrusion or hybrid treatments with xylanase were tested on the extrusion performance and selected characteristics of the developed non-starch polysaccharide-rich (NSP-rich) wheat flour. L/D 16 and 20 extruder configurations with various screw profiles were used. The interactions between processing variables (moisture content 23, 25, 27%; screw speed 40, 60, 80 rpm; xylanase level 0, 50, 100 ppm) were assessed to indicate energy consumption and the rheological properties of flour. The results showed that the possibility of obtaining enzyme-assisted extruded flour products derived from flours of varying characteristics depended on the processing conditions. The application of various extruder configurations and screw profiles showed significant effects on both processing behavior and rheological characteristics. The longer L/D 20 extruder configuration using a screw profile with mixing elements allowed us to obtain products with lower extrusion pressure (max. 20.8 bar) and energy requirements (max. SME = 33.1 kWh/kg) and better rheological properties (max. Hyd = 69.2%, less intensive starch gelatinization with max. C3 = 1.47 Nm) than the L/D 16 version. The extruded wheat flour was characterized by improved hydration properties and limited retrogradation tendency, especially when hybrid extrusion with xylanase was applied. This may lead to favorable results, as the newly developed enzymatic extrusion modification method produces NSP-rich wheat flour with specific techno-functional and rheological characteristics that can be seen as a potential "clean label" enhancer in bakery products. Our statistical analysis confirmed feed moisture and screw speed to be the variables with the most significant effect on wheat flour features.

Keywords: extrusion; screw configuration; processing variables; screw speed; energy consumption; wheat flour; rheological properties; hydration; RSM analysis

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1. Introduction

Extrusion cooking is an economical processing method that allows the rapid direct or indirect transformation of proteins, starch and cellulose polymers to be achieved [1]. The extrusion of cereal-based products has advantages over other processing methods because of its low cost, short processing time, high productivity, versatility, unique product shapes, and energy savings [2]. In order to produce extruded goods, it is crucial to understand that the physicochemical changes that occur during extrusion cooking processing depend on the conditions and equipment applied [3]. Extrusion is used for the production of ready-to-eat cereals, snacks and food additives with different swelling properties. Most changes during extrusion cooking (swelling, viscosity or water absorption) depend on

the initial starch content. The thermo-mechanical treatment of cereal materials causes starch modification, especially partial or full gelatinization, depending on the feed moisture content, temperature range, shearing intensity, extruder configuration, treatment intensity and processing conditions. Several authors reported various levels of starch damage and gelatinization depending on the processing variables used, especially water content, screw speed and energy input during processing [4–6]. Wheat flour also has a protein content of more than 10%, and a major part of wheat protein is gluten. This component is responsible for the unique structures of bakery products.

In extrusion cooking processing, the contained food components may have greater or lesser involvement in the formation of the specific textural and microstructural properties of the extrudates [7,8]. The main process connected with the extrusion of plant materials or cereals containing proteins is protein aggregation, or the simultaneous fragmentation and aggregation of wheat proteins due to intermolecular disulfide bonding. This can change the rheological behavior of dough [9–11]. In the work of Wu et al. [12], wheat flour was extruded by single- and a double-screw extruders under various parameters, followed by quantifying the protein content, analyzing the free sulfhydryl and disulfide bonds and lysinoalanine, and reducing the total sugar in the extruded flour. The extrusion of wheat and rye bran at variable conditions was found by Anderson et al. [13] to be an effective method for increasing the extractability of dietary fibers, especially AX, and the extruded bran showed improved nutritional properties, such as fermentability. Aktas-Akyildiz et al. [14] showed that extrusion treatment can be used to disrupt the microstructure of wheat bran and thus to increase its soluble fiber content. Demuth et al. [15] found significant changes in the structure of water-soluble wheat bran arabinoxylans processed through extrusion. Singkhornart et al. [16] noted a reduction in sugar and soluble arabinoxylan content in corn bran due to changes in feed moisture content and screw speed with/without chemical pretreatment. The combined enzymatic and thermal/extrusion treatments of cereal products may increase the changes in cereals after treatments. Kong et al. [17], for example, tested co-enzymatic and extrusion treatment of black wheat bran, using cellulase, xylanase, high-temperature α-amylase and acid protease individually or in combination. They noted significant increases in water extractable arabinoxylan content, water and oil holding capacity, and cholesterol adsorption capacity, probably due to the creation of a looser and more porous microstructure.

Enzymatic extrusion can be applied to cereal grains and flour under the conditions of high substrate concentration and under elevated temperature, pressure and shear stress at various moisture levels. However, the presence of thermolabile ingredients as enzymes or fat in flour requires the use of low processing temperatures with a consequent lack of product expansion and limited temperature-dependent changes in the extruded materials [18]. This can also be avoided by using thermostable enzymes that can improve product texture [19]. Bakery enzymes play important roles in dough formation and bread volume; product crispness, color and browning reactions during baking; and in reducing retrogradation and staling [20]. Research confirms the positive effect of cellulase or xylanase on the functionality of non-starch polysaccharides, found mainly in the outer layers of cereal grains [21]. Xylanase is responsible for water distribution from the pentosan phase to the gluten phase [22]. This is the cause of xylanase's impact on bread volume improvement via increasing the extensibility of gluten due to a rise in gluten volume fraction [23,24]. Additionally, xylanases have been shown to delay staling, enhance the texture of high-fiber bread and balance out the variable quality of flour used in baking wheat bread.

There are some reports about improving the properties of cereal products by extrusion or enzymatic extrusion treatments. Moreno-Rivas et al. [25] reported single-screw extrusion in the temperature range of 60, 70, 80 and 90 °C suitable for limiting fat extractability and increasing protein solubility in nixtamalized corn flour, without or with xylanase, which lowered this effect. Of note, limited fat content may extend the shelf life of extruded flour products [26]. Martinez et al. [27,28] tested the single-screw extrusion of common wheat flour with 4–16% initial water content and in the temperature range of 60–140 °C. These

extruded flours treated at variable conditions were added in concentrations of 5% and were used as additives in bread preparation. The authors found that the addition of extruded wheat flour caused an increase in dough hydration of about 9% when extreme conditions were applied, due to the higher starch gelatinization degree and damage. The stability of dough with added extruded flour decreased if more intensive treatment conditions were applied during extrusion. When alveographic analysis was performed on the supplemented flour, the results showed that a 5% addition had no negative effect on the overall strength values of the dough. The extrusion of flour decreased extensibility but improved dough tenacity, most visibly when extreme extrusion conditions were applied. Also, the addition of extruded flour may be suggested as a replacement for pregelatinized starch [28].

Bread improvers may be chemical agents with oxidation-reduction properties or directly added enzymes that may affect the gluten network of wheat flour. Industrial bread production and recipes have been changing in recent years due to growing consumer concerns about food ingredients. Some bread improvers are perceived as unknown and harmful chemicals, and some of them may cause health problems [29]. Extruded wheat flour may be an interesting additive as a replacement for chemically modified starches, pregelatinized (hydroxypropylated or cross-linked) starches [30] or hydrocolloids, and may change the properties of bread flour, especially in the context of increasing baking efficiency or bread texture [28,31]; however, the techno-functional properties of extruded flours may vary depending on the processing conditions. Moreover, there is a lack of knowledge about the impact of the single-screw extruder configuration or a multivariate analysis of the impact of processing conditions and enzyme levels on the extrusion behavior, technical–functional and rheological properties of wheat flour, especially those containing high levels of non-starch polysaccharides. So, the aim of this work was to apply variable conditions to the low-temperature single-screw extrusion process using two different extruder configurations (L/D 16 and L/D 20) and screw designs to modify the developed NSP-rich wheat flour in the absence/presence of xylanase and to evaluate the influence of variables on the process performance, techno-functional and rheological characteristics of the extruded flour.

2. Materials and Methods

2.1. Raw Materials and Proximate Composition

A new wheat flour blend (type 750 from common wheat of the Laudis variety), containing selected breaking, milling, reducing and sifting passages developed according to Lewko et al. [32] and characterized by a high content of non-starch polysaccharides, was used for tests. The proximate composition of NSP-rich wheat flour was tested according to the following methods: protein (Nx6.25) with the AACC 46–10 method, fat with the AACC 30–10 method, ash with the AACC 08–01 method [33] and total dietary fiber (TDF) and its soluble (SDF) and insoluble (IDF) fractions with the enzymatic–gravimetric 991.43 method [34]. Additionally, polysaccharide composition was tested by gas chromatography according to the method described by Lewko et al. [32]. All analyses were performed in triplicate. The enzyme VERON 292—Xylanase from *Aspergillus niger* with the declared enzyme activity of min. 1701 XylH/g was supplied by Barentz Sp. z o.o. (AB Enzymes, Darmstadt, Germany).

2.2. Extrusion Processing

The tests of conventional extrusion and hybrid enzyme-assisted extrusion treatments were carried out with the use of a prototype single-screw extruder, EXP-45-32, with a forming die of 3 mm (built by Zamak Mercator, Skawina, Poland). Two versions of the extruder with L/D = 16 and L/D = 20 were used: a conventional screw with a continuous spiral pattern in the shorter version, and a newly designed screw with mixing elements (helices with indentations on a spiral pattern located alternately with a continuous spiral pattern) placed on three-quarters of the screw length in the longer version. Three variables were applied in the experiment: feed moisture, screw speed and enzyme dose. The newly developed

wheat flour blend [32] was fortified with the addition of powdered xylanase in amounts of 50 and 100 ppm and dry-mixed. In conventional extrusion, this step was avoided. Next, the mixtures were moistened to obtain the initial blend moisture contents of 23, 25 and 27%, left for 2 h for enzyme activation and sieved to ensure the homogeneity of composition and moisture distribution. Moistened samples were subsequently subjected to low-temperature extrusion cooking at the screw speeds of 40, 60 and 80 rpm at temperatures ranging from 40 to 80 °C in the individual zones of the extruder. In the L/D 16 version, four sections were used, with the temperature settings starting from the feeding zone being 30, 40, 60 and 80 °C, while in the L/D 20 version, five sections were used with the temperature settings starting from the feeding zone being 30, 40, 60, 80 and 80 °C. The forming die temperature was 80 °C. Temperature and screw speeds (rpm) were kept constant throughout individual experiments at each variable setting. The EXP-45-32 single-screw extruder is well equipped with a precise barrel heating/cooling system combined with individual chillers for each section, so the temperature control was very stable. Samples were collected after process stabilization at least 30 min after changing variables. The obtained extrudates were cut into small pieces by a cutting device connected to the extruder and dried in a laboratory shelf dryer at 40 °C to a final moisture content below 9% and ground on an LMN-100 knife mill (TestChem, Radlin, Poland) to powder with a particle size below 300 µm. The final moisture content was tested with the moisture analyzer MA.50.R.WH (Radwag, Radom, Poland).

2.3. Extrusion Performance

During the single-screw extrusion (with two different extruder L/D configurations being applied), the processing conditions were monitored and the following data shown on the LCD screen of the extruder control cabinet were archived: the extrusion pressure (bar), torque (Nm), engine load (%) and active power (kW). The processing efficiency (kg/h) was calculated as the amount of flour obtained at a certain time according to the following equation [35,36]:

$$Q = \frac{m}{t} \text{ (kg/h)} \quad (1)$$

where: Q—processing efficiency (kg/h), m—mass of the obtained extrudate (kg), and t—measurement time (h).

Specific mechanical energy (SME) was calculated based on the extruder characteristics and the obtained process output according to the following equation [35,36]:

$$SME = \frac{n \times L \times P}{n_{max} \times 100 \times Q} \text{ (kWh/kg)} \quad (2)$$

where: SME—specific mechanical energy (kWh/kg), n—screw rotations (rpm), P—electric power (kW), L—engine load (%), n_{max}—maximum screw rotations (rpm), and Q—process efficiency (kg/h).

All parameters were registered in triplicate.

2.4. Rheological Properties of Modified Flour

The selected techno-functional and rheological characteristics were tested in conventional extrusion-treated and hybrid enzymatic-extrusion-treated flours without and with the addition of xylanase enzyme and subjected to diverse extrusion procedures. The rheological properties of modified flours were examined by using Mixolab® (Chopin Technologies, Villeneuve-la-Garenne, France) according to ISO 17718-1:2013 [37]. In brief, the Chopin+ flour protocol with the following settings was used: mixing speed—80 rpm, total analysis time—45 min, dough weight—75 g, hydration water temperature—30 °C. Modified flour and water were added accordingly to obtain a dough with a maximum consistency of 1.10 Nm (±0.05) during the first test phase. The Mixolab test was performed using a standard protocol: 8 min at 30 °C, heating for 15 min at a rate of 4 °C/min, holding at 90 °C for 7 min, cooling for 10 min to 50 °C at a rate of 4 °C/min and holding at 50 °C for

5 min [38]. The following techno-functional and rheological features were tested with the Mixolab procedure: water absorption (Hyd), based on the amount of water necessary to reach maximum torque during mixing (1.10 Nm); dough stability (Stab.); protein weakening (C2), based on the mechanical work and temperature increase; maximum torque during the heating stage (C3), expressing the rate of starch gelatinization; minimum torque during the heating period (C4), indicating the stability of the hot gel formed and the amylase activity; C5, the torque after cooling at 50 °C, representing starch retrogradation during the cooling stage; slope α—between the end of the 30 °C period and C2, showing the speed of the protein weakening under a heating effect; slope β—between C2 and C3, acting as an indicator of pasting (gelatinization) speed; and slope γ—between C3 and C4, showing enzymatic (α-amylase) degradation speed [36,39,40]. All the measurements were performed in triplicate.

2.5. Statistical Analysis

Table 1 presents the experimental design along with the coded value representations.

Table 1. Experimental design trials with coded value representations.

Trial Number	M = Feed Moisture (%)	M Code	S = Screw Speed (rpm)	S Code	E = Enzyme Dose (ppm)	E Code
1	23	−1	40	−1	0	−1
2	23	−1	40	−1	50	0
3	23	−1	40	−1	100	1
4	23	−1	60	0	0	−1
5	23	−1	60	0	50	0
6	23	−1	60	0	100	1
7	23	−1	80	1	0	−1
8	23	−1	80	1	50	0
9	23	−1	80	1	100	1
10	25	0	40	−1	0	−1
11	25	0	40	−1	50	0
12	25	0	40	−1	100	1
13	25	0	60	0	0	−1
14	25	0	60	0	50	0
15	25	0	60	0	100	1
16	25	0	80	1	0	−1
17	25	0	80	1	50	0
18	25	0	80	1	100	1
19	27	1	40	−1	0	−1
20	27	1	40	−1	50	0
21	27	1	40	−1	100	1
22	27	1	60	0	0	−1
23	27	1	60	0	50	0
24	27	1	60	0	100	1
25	27	1	80	1	0	−1
26	27	1	80	1	50	0
27	27	1	80	1	100	1

All responses from extrusion processing and the rheological properties of the modified wheat flours were subjected to response surface analysis using RSM (Response Surface Methodology) using the input variables [41]. The test results were analyzed with RSM by selecting the independent factors of moisture content, screw speed, enzyme dose and its interactions, for the L/D 16 and L/D 20 extruder configurations separately. RSM with quadratic fit was applied and models were created for each combination of independent factors. For each extruder configuration, the second-order regression equations were independently applied using Statistica 13.3 (Statsoft, Tulsa, OK, USA) and the open-source software R, version 4.3.1, package: RSM, version 2.10.4 [42]:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11}(X_1)^2 + \beta_{22}(X_2)^2 + \beta_{33}(X_3)^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (3)$$

where Y is the response factor being indicated, β_0 represents a constant value, β_i for $i = 1, 2, 3$ represents a linear coefficient, β_{ij} where $i = j$ represents a quadratic coefficient, and β_{ij} where $i \neq j$ represents an interactive coefficient when $i, j = 1, 2, 3$. X_i and X_j represent the input variables of moisture (M), screw speed (S) and enzyme dose (E) and were coded at levels of −1, 0 and 1 for each factor, respectively. All coefficients were characterized for significance as either slightly significant ($p < 0.10$), significant ($p < 0.05$) or very significant ($p < 0.01$).

The coefficient of determination (R^2) expressed the model's validation and a F-test ascertained its statistical significance. The significance of differences was assessed by the analysis of variance test (ANOVA) followed by the Tukey post hoc test at the 0.05 significance level. Homogenous groups were indicated with similar letters at the significance level of 0.05.

3. Results and Discussion

3.1. Effect of Variables on Extrusion Performance

NSP-rich wheat flour from selected breaking, milling, reducing and sifting passages was developed [32] to determine the possibility of using previously unused passages produced in the milling factory with increased content of polysaccharides and arabinoxylans. The proximate composition of NSP-rich wheat flour was as follows (g/100 g): protein 14.62 ± 0.06, fat 1.31 ± 0.01, ash 0.72 ± 0.02, insoluble dietary fiber 3.94 ± 0.04, soluble dietary fiber 2.86 ± 0.02 and total dietary fiber 6.80 ± 0.03. Additionally, the polysaccharide composition of the flour used in the experiment was as follows: total arabinoxylans 1.91 ± 0.06, including 1.31 ± 0.04 of insoluble fraction and 0.60 ± 0.02 of soluble fraction, and total non-starch polysaccharides 3.40 ± 0.00, including 2.06 ± 0.01 of insoluble fraction and 1.34 ± 0.00 of soluble fraction. Wheat flour may vary depending on the content and composition of the milling fractions. Previously, an aleurone-rich wheat milling fraction was developed at the industrial scale [43]. Bucsella et al. [44] found and characterized an aleurone-rich flour composed from bran-rich milling fractions that lacked the outer layers of the grain. This aleurone-rich flour showed a different composition (20% protein, 15% dietary fiber) to that of commercial fiber-rich wheat fractions (9–13% protein, 9% dietary fiber). The higher amount of inner layers on the seed coat than in white or wholegrain flour resulted in a higher fat content (4%), which may decrease the shelf life of bran-rich wheat flour products. Thermal treatments, such as dry thermal heating, hydrothermal treatment or extrusion, may increase the storage stability of wholegrain flour or bran-rich products due to the formation of lipid–amylose complexes [3]. Additionally, extrusion seems to be a processing method that increases the proportion of extractable dietary fiber, including arabinoxylans, and makes cereal bran and bran-rich flours more sensorially acceptable [45].

Extrusion processing was tested using either a conventional treatment or a hybrid treatment with the addition of xylanase to check the possibility of obtaining extruded flour with certain characteristics that depended on processing conditions (two different extruder configurations and screw profiles). During the low-temperature extrusion processing of the developed NSP-rich wheat flour without or with the addition of xylanase, several features were monitored and registered—the extrusion pressure (bar), torque (Nm), engine load (%), active power (kW), processing efficiency (kg/h) and specific mechanical energy (SME)—separately for both extruder configurations, L/D 16 and L/D 20, used in the experiment.

Figure 1 shows the results of extrusion pressure affected by L/D configuration and the interaction of each of the two input variables if L/D 16 and L/D 20 were applied for processing, respectively: E × M (Figure 1a,d), E × S (Figure 1b,e) and M × S (Figure 1c,f).

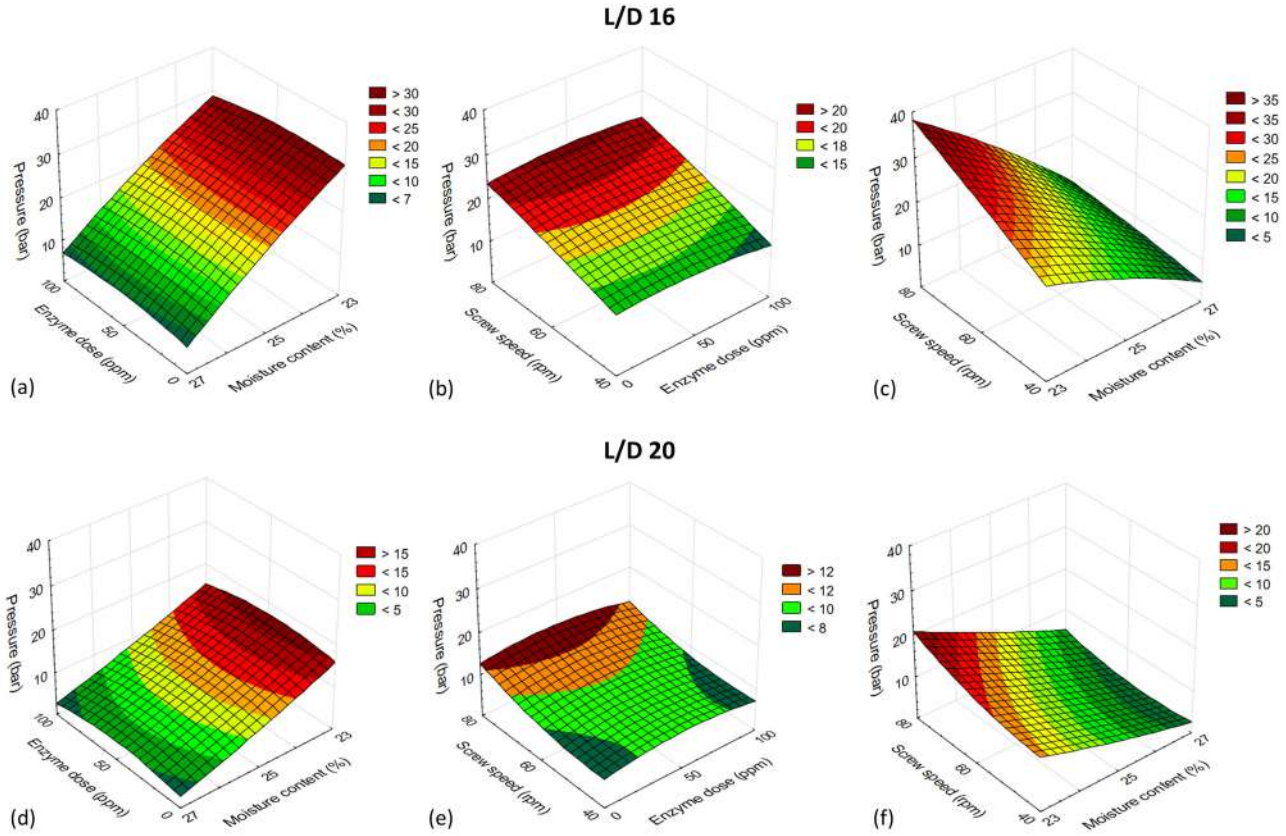

Figure 1. Processing pressure during the extrusion cooking of wheat flour with xylanase enzyme. L/D 16: (**a**) E × M, (**b**) E × S, (**c**) M × S; L/D 20: (**d**) E × M, (**e**) E × S, (**f**) M × S. M—moisture; S—screw speed; E—enzyme dose.

Pressure results varied from 3.5 to 41.7 bar for the short extruder and from 2.5 to 21.3 bar for the elongated configuration. The lowest pressure was noted at the highest (27%) moisture content and increased to max values when extrusion was carried out at 23%—the lowest feed moisture. The most significant parameters affecting the changes in extrusion pressure were the feed moisture and screw speed applied in both versions of the extruder. In the shorter version of the extruder, much higher pressure was generated at a similar flour feed rate due to the smaller internal space. This may have affected the filling degree of the interzonal elements of the screw helix or flights and, consequently, much higher pressure was required to transfer the hot, high-viscosity molten mass in through the forming die. The application of a newly designed screw profile with mixing elements (a helix with indentations on a spiral pattern alternating with a continuous spiral pattern) in the L/D 20 extruder configuration induced better mixing and disengagement of the molten dough. This resulted in much lower pressure results and less intensive compression of the material between the external surfaces of the screw and the internal grooved surfaces of the barrel. Such an outcome can also modify the residence time distribution during processing and thus may have effect on wheat flour components transformation intensity. NSP-rich wheat flour was found to have significant negative linear relationships between pressure and M but significant positive linear relationships between pressure and S for L/D 16 and L/D 20 (Table 2).

Table 2. Regression coefficients for response surface models of processing conditions of wheat flour, using coded inputs.

	Pressure (bar)	Torque (Nm)	Load (%)	Active Power (kW)	Process Efficiency (kg/h)	SME (kWh/kg)
			L/D 16			
Const.	20.640 ***	69.181 ***	19.542 ***	0.881 ***	25.991 ***	0.214
M	−11.557 ***	16.581 ***	4.684 ***	−0.090	−0.720	−0.006
S	5.150 ***	−1.966	−0.556	−0.010	8.827 ***	−0.019
E	−0.350	0.120	0.034	0.005	−0.800	0.005
M × M	−1.160	26.089 ***	7.370 ***	−0.303 ***	−0.773	−0.039
S × S	−0.248	−7.076	−1.999	0.015	0.507	−0.001
E × E	−1.203	3.323	0.939	−0.289 **	0.107	−0.087
M × E	0.601	−4.346	−1.228	0.005	−0.040	−0.005
S × E	0.509	1.987	0.562	−0.091	0.080	−0.023
M × S	−3.123 ***	1.181	0.333	0.012	0.240	−0.005
p-value of F test	<0.0001 ***	0.0431 **	0.0431 **	0.0736 *	<0.0001 ***	0.2325
R^2	0.913	0.356	0.356	0.299	0.885	0.142
			L/D 20			
Const.	9.4098 ***	112.420	31.754	0.272 ***	27.004 ***	0.099 ***
M	−6.435 ***	14.803	4.180	0.047	0.787	0.035 **
S	2.608 ***	−0.799	−0.223	−0.057	7.347 ***	−0.025
E	−0.227	6.265	1.772	0.068	−0.147	0.040 **
M × M	0.467	−5.042	−1.422	−0.019	−6.093 ***	0.025
S × S	1.576 **	−20.803	−5.873	−0.012	−0.093	−0.036
E × E	−1.837 **	18.051	5.100	0.106	1.587	0.044
M × E	0.154	6.494	1.831	0.092	−0.460	0.048 **
S × E	−0.361	7.511	2.126	−0.131 **	−1.020	−0.037 **
M × S	−1.426 **	4.815	1.356	−0.080	0.860	−0.031
p-value of F test	<0.0001 ***	0.3593	0.3596	0.0412 **	0.0007 ***	0.0035 ***
R^2	0.915	0.062	0.062	0.357	0.700	0.551

SME—specific mechanical energy; M—moisture; S—screw speed; E—enzyme dose. * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$.

The pressure was also found to have a significant positive quadratic relationship with S and a significant negative quadratic relationship with E if the L/D 20 extruder was used. A significant minor effect of the M × S interaction was found in both extruder configurations (L/D 16, L/D 20). The interaction between moisture and screw speed significantly influenced the extrusion pressure. An antagonistic effect indicates that higher moisture levels combined with an increased screw speed resulted in a lower extrusion pressure (L/D 16). The high coefficient of determination R^2 and the significant result of the F test proved that the obtained model can adequately describe pressure. Upon comparing the constant values of pressure models as prepared for both configurations of the extruder, in the L/D 20 configuration, a significantly lower pressure was generated. Moreover, in each configuration, the pressure decreased significantly with an increase in the initial moisture content M (r = −0.866 for L/D 16 and r = −0.869 for L/D 20). Because many of the regression coefficients deviated further from zero for the L/D 16 configuration, this means that pressure-related changes are more important for L/D 16 than for L/D 20. The longer barrel length and mixing element of the screw resulted in a longer residence time and a lower compression of the extruded material within the internal spaces between barrel and screw, so the pressure observed during the extrusion of wheat flour was twice as low in the L/D 20 configuration than in the L/D 16 version. Increasing the initial moisture of the flour during processing in the L/D 16 configuration also lowered the pressure inside the barrel. Here, only a slight effect of enzyme dose was evident (if 100 ppm was used). The decrease in pressure was noted at the lowest initial moisture content, suggesting the partial hydrolysis of fibrous fractions by xylanase and thus a loosening of the structure of the treated material.

Pilli et al. [19] found significant effects of independent variables on the die pressure during twin-screw extrusion of wheat flour with enzymes added. They reported this parameter as negatively influenced by barrel temperature and dough moisture, i.e., pressure decreased at high barrel temperature and dough moisture. Die pressure decreases when water content increases because of the reduction in molten/gelatinized starch viscosity inside the extruder [46,47]. Also, Kantrong et al. [48] found the feed moisture of the material to be the most important process parameter affecting pressure inside the extruder barrel, the strain applied to the extrudates and the SME, resulting in differences in a product's characteristics. The interaction between dough moisture and xylanase shows a positive effect on the pressure, which, in our experiment, increased with the increase in dough moisture and xylanase content, and this could be attributed to the decrease in enzymatic activity caused by the high moisture content [46]. Water is required to maintain the catalytically active conformation of an enzymatic system. On the other hand, during the denaturation process, it acts as a plasticizer, which allows the enzyme molecules to unfold, resulting in the loss of native conformation [49].

The screw construction used in the experiment in both configurations had a compression ratio of 3:1, so along with the extruder length, the flow of the melted material was limited by a shallow channel impeding the flow of material inside the barrel as a result of the non-Newtonian behavior of the raw plant materials during extrusion. This effect is opposite to that of plastic or rubber [2,3]. The application of a screw profile with a mixing element in L/D 20 caused a loosening of the compaction of the molten material, which was once again agglomerated just before the forming die. This is the effect of viscosity changes within the transferred material due to it being heated, melted and compressed, as well as its change from a powder form to a viscous liquid due to the addition of water inside the barrel sections towards the exit of the forming die [2].

In Tables 3 and 4, torque, load and active power data are listed for both extruder configurations, L/D 16 and L/D 20, as dependent on processing variables. As the viscosity is measured through torque determination in the RVA system, the higher viscosity may also be interpreted by the torque obtained in the extrusion process. Because the torque value is measured in the extrusion process, the extruder can also be treated as a torque rheometer [50]. Here, it is evident that torque (Nm) values were in general higher in the L/D 20 version of the extruder (with its additional mixing zone). The torque reported for L/D 16 varied between 56.6 and 130.6 Nm, while that of the L/D 20 version was between 59.0 and 178.3 Nm (Table 3). This difference is due to the presence of an additional mixing element within the screw configuration in the L/D 20 version, and hence a longer material residence time, and also some disturbance of the material flow inside the barrel. In the case of engine torque, a significant positive linear and quadratic relationship with M was found, but only for L/D 16 (Table 2). This means that the torque values during the extrusion of NSP-rich wheat flour with this extruder configuration increased with the increase in M. For the L/D 20 configuration, no statistically significant relationships between torque and the tested factors were demonstrated (Table 2, p-value > 0.05). The obtained data weakly fit the applied model due to the low R^2 values.

Increasing torque, when the initial moisture content was increased, may be the effect of a higher dough viscosity inside the barrel due to the initiation of the gelatinization of wheat starch under processing temperatures, as well as the greater addition of water to the treated mass. This observation and conclusion were confirmed by the positive linear and quadratic relationship with M. Similar observations were reported by Kowalski et al. [47], who tested the extrusion of a waxy wheat variety through a co-rotating twin-screw extruder. In their experiment, they found a significant positive quadratic feed moisture effect and positive interactive effect of feed moisture and screw speed with respect to motor torque. They also noted that a non-waxy wheat flour produced much higher pressure and torque as compared to waxy varieties during extrusion. However, there were insignificant and ambiguous dependencies observed between the processing variables and the levels of enzyme added.

Table 3. Torque, load and active power registered during wheat flour extrusion for the L/D 16 extruder configuration (mean values, n = 3).

Processing Variables			L/D 16		
M	**S**	**E**	**Torque (Nm)**	**Load (%)**	**Active Power (kW)**
23	40	0	$60.4 \pm 7.6^{a,b}$	17.1 ± 2.5^{a}	$0.477 \pm 0.093^{e,f,g}$
		50	$106.3 \pm 11.1^{d,e,f,g}$	$30.0 \pm 4.4^{b,c,d,e,f}$	$0.863 \pm 0.080^{i,j}$
		100	$76.9 \pm 7.4^{a,b,c,d}$	$21.7 \pm 3.0^{a,b,c,d}$	$0.473 \pm 0.057^{e,f,g}$
	60	0	$77.3 \pm 8.8^{a,b,c,d}$	$21.8 \pm 3.1^{a,b,c,d}$	$0.301 \pm 0.065^{a,b,c,d,e}$
		50	$59.5 \pm 6.3^{a,b}$	16.8 ± 2.7^{a}	$0.898 \pm 0.080^{i,j}$
		100	$82.3 \pm 8.6^{a,b,c,d,e}$	$23.3 \pm 3.0^{a,b,c,d,e}$	$0.435 \pm 0.061^{c,d,e,f,g}$
	80	0	$73.9 \pm 7.5^{a,b,c,d}$	$20.9 \pm 2.9^{a,b,c}$	$0.412 \pm 0.080^{c,d,e,f,g}$
		50	$75.1 \pm 7.7^{a,b,c,d}$	$21.2 \pm 2.0^{a,b,c,d}$	$0.199 \pm 0.092^{a,b,c}$
		100	$74.1 \pm 6.3^{a,b,c,d}$	$20.9 \pm 2.9^{a,b,c}$	$0.307 \pm 0.074^{a,b,c,d,e}$
25	40	0	$64.8 \pm 5.7^{a,b}$	$18.3 \pm 1.7^{a,b}$	$0.335 \pm 0.070^{a,b,c,d,e,f}$
		50	$65.2 \pm 6.4^{a,b}$	$18.4 \pm 1.6^{a,b}$	$0.421 \pm 0.025^{c,d,e,f,g}$
		100	$65.0 \pm 6.5^{a,b}$	$18.4 \pm 2.1^{a,b}$	$0.591 \pm 0.063^{g,h}$
	60	0	$59.7 \pm 5.4^{a,b}$	16.9 ± 1.3^{a}	$0.407 \pm 0.051^{b,c,d,e,f,g}$
		50	$90.7 \pm 10.3^{b,c,d,e,f}$	$25.6 \pm 3.2^{a,b,c,d,e,f,g}$	1.155 ± 0.100^{k}
		100	$68.3 \pm 7.1^{a,b}$	$19.3 \pm 1.9^{a,b}$	$0.561 \pm 0.089^{f,g,h}$
	80	0	56.6 ± 4.7^{a}	16.0 ± 1.6^{a}	$1.024 \pm 0.095^{j,k}$
		50	$71.2 \pm 8.1^{a,b,c,d,e}$	$20.1 \pm 5.0^{a,b,c}$	1.194 ± 0.152^{k}
		100	$58.6 \pm 4.8^{a,b}$	16.6 ± 5.3^{a}	$0.594 \pm 0.072^{g,h}$
27	40	0	$117.8 \pm 12.4^{f,g}$	$33.3 \pm 4.1^{d,e,f,g}$	$0.229 \pm 0.029^{a,b,c,d}$
		50	$112.6 \pm 11.2^{e,f,g}$	$31.8 \pm 5.2^{c,d,e,f}$	$0.746 \pm 0.096^{h,i}$
		100	$84.2 \pm 9.9^{a,b,c,d,e}$	$23.8 \pm 3.3^{a,b,c,d,e,f}$	$0.467 \pm 0.071^{d,e,f,g}$
	60	0	129.2 ± 14.6^{g}	36.5 ± 4.5^{g}	$0.197 \pm 0.025^{a,b,c}$
		50	$104.5 \pm 12.5^{c,d,e,f,g}$	$29.5 \pm 3.2^{b,c,d,e,f}$	$0.304 \pm 0.065^{a,b,c,d,e}$
		100	127.6 ± 16.8^{g}	$36.1 \pm 6.4^{f,g}$	0.119 ± 0.018^{a}
	80	0	126.0 ± 17.9^{g}	$35.6 \pm 5.6^{e,f,g}$	$0.168 \pm 0.075^{a,b}$
		50	$121.7 \pm 16.5^{f,g}$	$34.6 \pm 6.1^{e,f,g}$	$0.420 \pm 0.087^{c,d,e,f,g}$
		100	130.6 ± 18.9^{g}	36.9 ± 7.0^{g}	0.099 ± 0.017^{a}

M—moisture; S—screw speed; E—enzyme dose. $^{a–k}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

The results of the engine load (%) during the extrusion cooking of wheat flour with xylanase enzyme, dependent upon L/D configuration and processing variables, are presented in Table 3. The results varied from 16.0 to 36.9% if L/D 16 was employed (Table 3) and from 16.7 to 47.3% if L/D 20 was used for processing (Table 4). When analyzing the engine load, significant positive linear and quadratic relationships with M were obtained, but only if the L/D 16 configuration was used for treatment (Table 2). This implies that the load for this extruder configuration increased with the increase in the initial moisture of the treated wheat flour. Higher values were observed because the higher initial moisture contents brought about greater gelatinization and increased dough viscosity during flour extrusion. In contrast, with the L/D 20 configuration, no statistically significant relationships were evident for the load in relation to the tested factors (Table 2, *p*-values > 0.05).

The active power (kW) results for wheat flour without/with enzymes as extruded under variable processing conditions are summarized in Tables 3 and 4. Different factors determining changes in the active power depending on the extruder configuration were indicated. Accordingly, the L/D 16 configuration showed a significant negative quadratic relationship with M and E (Table 2), while the long L/D 20 version of the extruder displayed a significant negative relationship with the S × E interaction effect. On comparing the constant values of the obtained models in both configurations, it can be concluded that the active power was significantly higher for L/D 16, especially when low initial moisture and low screw speeds were applied during processing (Table 3). This may be connected

with the effect of pressure increase due to the formation of the melted starch–protein matrix (which demonstrates high viscosity due to being partly gelatinized because of access to heat and water). The lowest values were observed for both extruder configurations if the initial moisture and enzyme level were the highest. Strong correlation coefficients were found between active power and SME (r values were 0.883 for L/D 16 and 0.865 for L/D 20 extruder configurations). Due to the low R^2, the obtained models for active power (L/D 16, L/D 20) are not recommended to describe this factor. Further research is necessary to obtain more adequate models describing the variability of active power depending on important experimental factors.

Table 4. Torque, load and active power registered during wheat flour extrusion for the L/D 20 extruder configuration (mean values, n = 3).

Processing Variables			L/D 20		
M	**S**	**E**	**Torque (Nm)**	**Load (%)**	**Active Power (kW)**
23	40	0	$90.6 \pm 9.3^{a,b,c,d,e,f}$	$25.6 \pm 3.8^{a,b,c,d,e,f,g}$	$0.294 \pm 0.096^{a,b,c,d,e}$
		50	$87.6 \pm 8.1^{a,b,c,d,e,f}$	$24.7 \pm 3.6^{a,b,c,d,e,f,g}$	$0.133 \pm 0.085^{a,b}$
		100	$91.5 \pm 9.5^{a,b,c,d,e,f}$	$25.8 \pm 3.5^{a,b,c,d,e,f,g}$	$0.270 \pm 0.096^{a,b,c,d}$
	60	0	$150.0 \pm 18.5^{h,i,j,k}$	$42.4 \pm 4.3^{i,j,k,l}$	$0.176 \pm 0.032^{a,b,c}$
		50	$94.3 \pm 10.7^{a,b,c,d,e,f}$	$26.6 \pm 5.1^{a,b,c,d,e,f,g}$	$0.296 \pm 0.066^{a,b,c,d,e}$
		100	$80.9 \pm 8.6^{a,b,c,d,e}$	$22.8 \pm 3.9^{a,b,c,d,e}$	$0.512 \pm 0.098^{e,f,g}$
	80	0	59.0 ± 5.9^{a}	16.7 ± 2.5^{a}	$0.429 \pm 0.075^{d,e,f}$
		50	$78.6 \pm 7.8^{a,b,c,d,e}$	$22.2 \pm 3.6^{a,b,c,d,e}$	$0.195 \pm 0.092^{a,b,c}$
		100	$84.2 \pm 8.1^{a,b,c,d,e}$	$23.8 \pm 2.8^{a,b,c,d,e,f}$	0.103 ± 0.069^{a}
25	40	0	$64.4 \pm 6.2^{a,b}$	$18.2 \pm 3.4^{a,b}$	$0.313 \pm 0.093^{a,b,c,d,e}$
		50	$109.5 \pm 16.6^{c,d,e,f,g,h}$	$30.9 \pm 2.7^{b,c,d,e,f,g,h,i,j}$	$0.237 \pm 0.045^{a,b,c,d}$
		100	$112.4 \pm 15.9^{d,e,f,g,h,i}$	$31.8 \pm 3.8^{d,e,f,g,h,i,j}$	$0.692 \pm 0.073^{g,h,i}$
	60	0	178.3 ± 16.8^{k}	50.4 ± 6.2^{l}	$0.134 \pm 0.099^{a,b}$
		50	$66.6 \pm 5.3^{a,b,c}$	$18.8 \pm 2.6^{a,b,c}$	$0.251 \pm 0.032^{a,b,c,d}$
		100	$154.5 \pm 19.4^{i,j,k}$	$43.6 \pm 5.5^{j,k,l}$	$0.254 \pm 0.041^{a,b,c,d}$
	80	0	$70.7 \pm 7.9^{a,b,c,d}$	$20.0 \pm 2.9^{a,b,c,d}$	$0.555 \pm 0.086^{f,g,h}$
		50	$71.5 \pm 8.8^{a,b,c,d}$	$20.2 \pm 3.1^{a,b,c,d}$	$0.372 \pm 0.065^{c,d,e,f}$
		100	$167.4 \pm 19.9^{j,k}$	$47.3 \pm 5.3^{k,l}$	$0.197 \pm 0.084^{a,b,c,d}$
27	40	0	$102.7 \pm 15.5^{a,b,c,d,e,f,g}$	$29.0 \pm 3.8^{a,b,c,d,e,f,g,h}$	$0.340 \pm 0.076^{b,c,d,e,f}$
		50	$141.0 \pm 16.7^{g,h,i,j,k}$	$39.8 \pm 4.6^{h,i,j,k,l}$	$0.191 \pm 0.019^{a,b,c}$
		100	$110.2 \pm 12.7^{c,d,e,f,g,h}$	$31.1 \pm 3.1^{c,d,e,f,g,h,i,j}$	0.893 ± 0.098^{i}
	60	0	$128.6 \pm 17.5^{f,g,h,i,j}$	$36.3 \pm 4.5^{f,g,h,i,j,k}$	$0.270 \pm 0.061^{a,b,c,d}$
		50	$105.5 \pm 14.9^{b,c,d,e,f,g}$	$29.8 \pm 3.5^{b,c,d,e,f,g,h,i}$	$0.288 \pm 0.042^{a,b,c,d,e}$
		100	$131.2 \pm 17.1^{f,g,h,i,j}$	$37.1 \pm 3.7^{g,h,i,j,k}$	$0.782 \pm 0.069^{h,i}$
	80	0	$118.5 \pm 16.3^{e,f,g,h,i}$	$33.5 \pm 4.8^{e,f,g,h,i,j}$	0.086 ± 0.064^{a}
		50	$102.1 \pm 15.4^{a,b,c,d,e,f,g}$	$28.9 \pm 4.2^{a,b,c,d,e,f,g,h}$	$0.292 \pm 0.087^{a,b,c,d,e}$
		100	$143.3 \pm 18.8^{g,h,i,j,k}$	$40.5 \pm 5.6^{h,i,j,k,l}$	$0.112 \pm 0.032^{a,b}$

M—moisture; S—screw speed; E—enzyme dose; $^{a–l}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

The extrusion cooking processing efficiencies of wheat flour with xylanase enzyme that are dependent on L/D configuration and processing variables are presented in Figure 2.

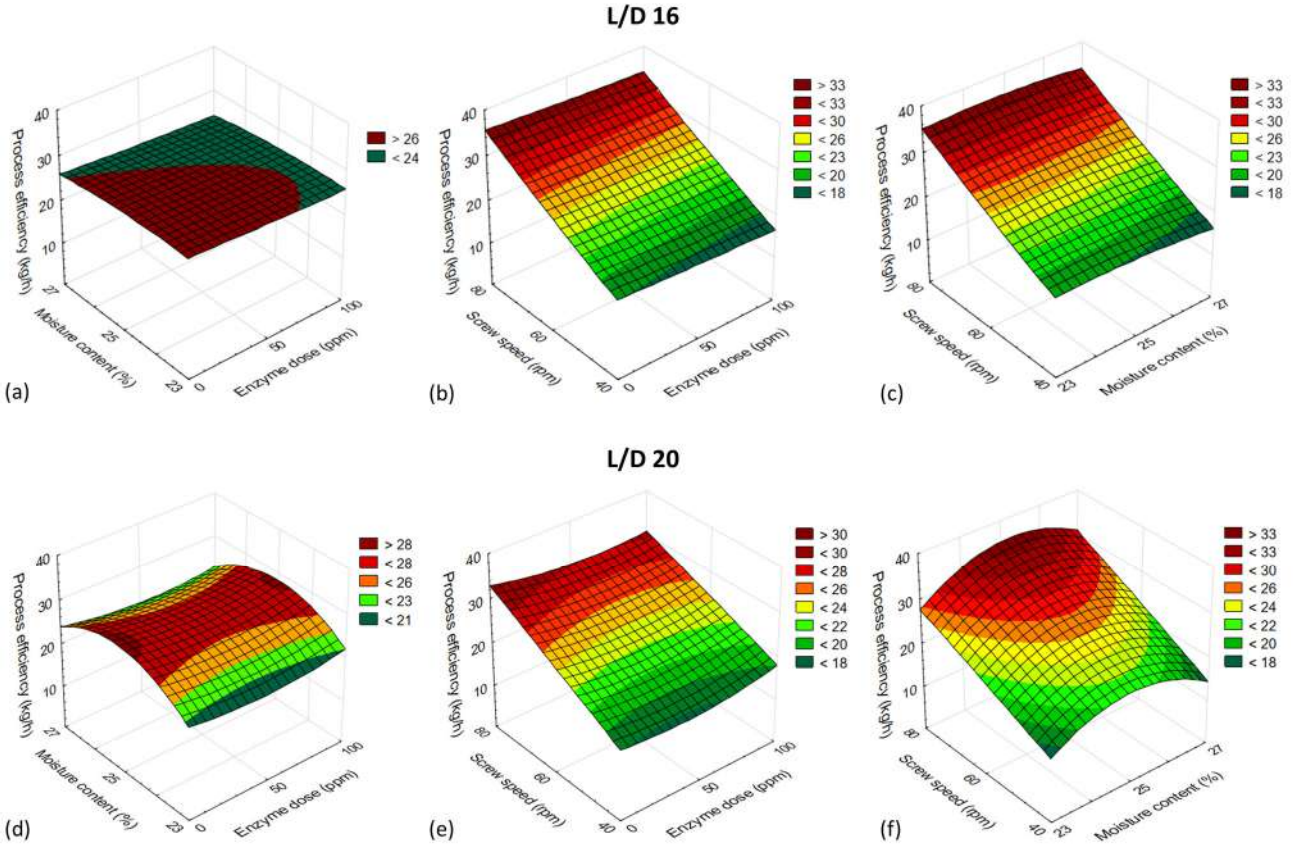

Figure 2. Processing efficiency during the extrusion cooking of wheat flour with xylanase enzyme. L/D 16: (**a**) E × M, (**b**) E × S, (**c**) M × S; L/D 20: (**d**) E × M, (**e**) E × S, (**f**) M × S. E—enzyme dose; M—moisture; S—screw speed.

Generally, processing with a longer extruder configuration (L/D 20) resulted in lower output due to the extended residence time of the treated flour inside the barrel. Accordingly, efficiency varied between 14.88 and 39.36 kg/h if L/D 16 was employed and between 13.92 and 36.96 kg/h if L/D 20 was used. More visible differences between the output results were observed with regard to the variables of the moisture content and enzyme addition. Here, the highest efficiency was evident at 25% initial moisture. A further increase in moisture slightly lowered processing output, probably due to more intense starch gelatinization and increased dough viscosity, and thus lowered the flow intensity of the dense and sticky material. In analyzing the extrusion processing efficiency of wheat flour with the addition of xylanase, a significant positive linear relationship with S was demonstrated for both configurations. This effect indicates that processing efficiency increased with the increase in S for both extruder configurations. High correlation coefficient values (r = 0.953 for L/D 16 and r = 0.792 for L/D 20, respectively) were indicated. The values of process efficiency were not susceptible to changes in the initial moisture content M of wheat flour and the enzyme addition E. When comparing the coefficients of both models, it can be concluded that the differences between the considered configurations with regard to processing efficiency were small. In the case of the longer configuration (L/D 20), a significant negative quadratic relationship with M was additionally demonstrated. This outcome was similar to that of previous studies. In the work of Wójtowicz et al. [35], the processing efficiency and SME of cereal-based snack pellets fortified with edible cricket flour processed with a similar extruder configuration to L/D 20 was noted for varying from 12.08 to 37.20 kg/h, with efficiency lowering with increasing initial water content, especially when a low screw speed was applied.

According to the outcome of our experiment, the specific mechanical energy (SME) requirements during the extrusion cooking of wheat flour amended with the xylanase

enzyme depend on the L/D configuration and processing values (Figure 3). The SME is a good quantitative descriptor of the extrusion processes of the extent of macromolecular transformations and interactions that take place, i.e., starch conversion, and consequently, the rheological properties of the melt [26]. A higher SME usually results in a greater degree of starch gelatinization and greater extents of starch molecular size reduction and extrudate expansion [41].

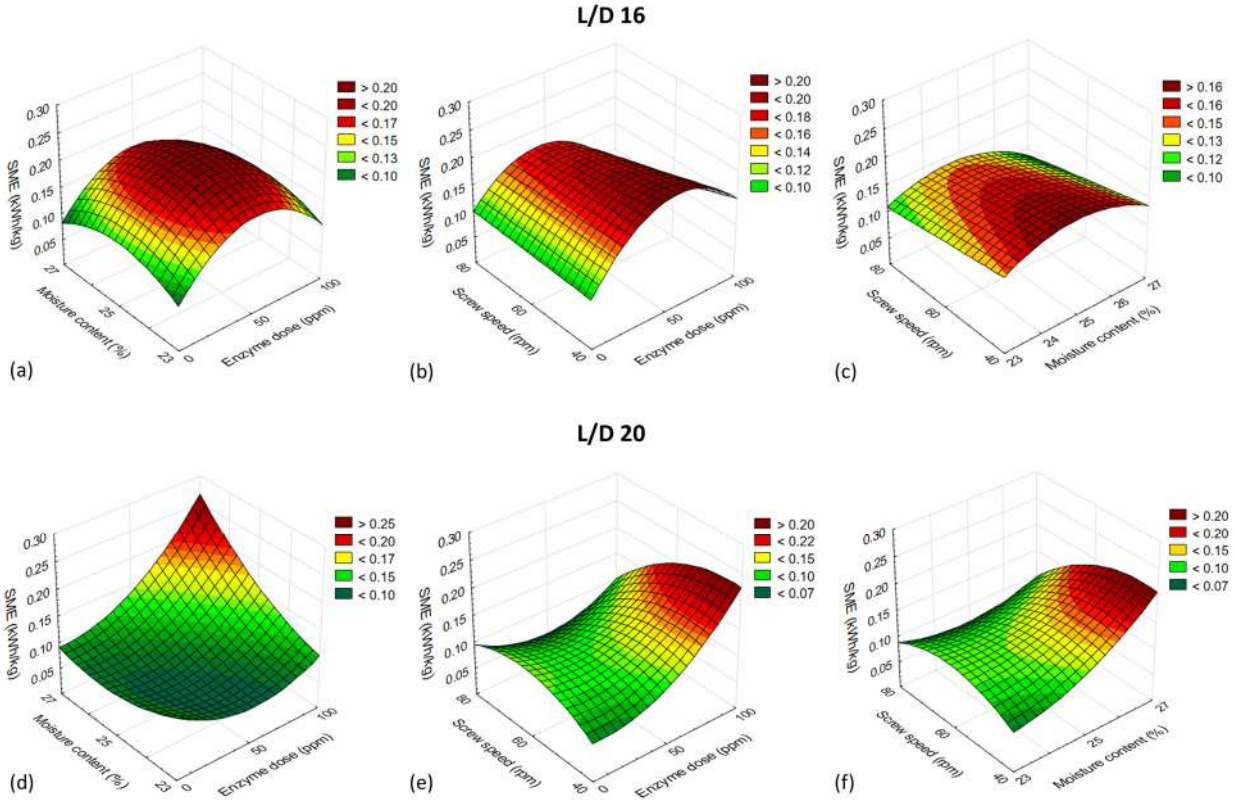

Figure 3. SME during the extrusion cooking of wheat flour with xylanase enzyme: L/D 16: (**a**) E × M, (**b**) E × S, (**c**) M × S; L/D 20: (**d**) E × M, (**e**) E × S, (**f**) M × S. SME—specific mechanical energy; E—enzyme dose; M—moisture; S—screw speed.

A statistically significant regression model for the SME (with a moderate value of the coefficient of determination and a significant F test result) was obtained only for the L/D 20 extruder configuration, indicating a positive linear relationship with M, E and the M × E interaction effect (Table 2). The combined effect of moisture and enzyme dose on the SME was observed. Both factors independently contributed to improving the SME (L/D 20). Moreover, the SME increased with increasing M and E for L/D 20. Still, the correlation coefficients were not significant for both L/D 16 and L/D 20. In L/D 16 extrusion, the SME was not significantly affected by changes in M, S or E (Table 2). Higher energy values were, however, noted for the L/D 20 configuration, especially at high moisture content and the highest dose of xylanase in the extruded blend. This consequence may have come about as a result of differences in dough structure, due to using the mixing element in screw construction and due to the presence of partly hydrolyzed polysaccharides being acted upon by xylanase under high-moisture extrusion at low temperatures (below 80 °C).

As is known, xylanase can change insoluble fractions of polysaccharides (especially xylans) into soluble and more reactive structures that absorb more water and make the treated dough denser, and hence require greater processing energy input. Deng et al. [50], for example, tested wheat bran processed by enzymatic extrusion at moisture levels of 30 and 40% and found higher specific mechanical energy input from the extruder at lower moisture, which softened the fiber. High mechanical energy input might be conducive

to forming a loose and porous structure that facilitates the penetration of the xylanase-containing solution. The lower screw speed is a result of longer residence time and lower mechanical force produced from the extruder [51]. Furthermore, an increase in the screw speed leads to higher specific mechanical energy SME input, which results in depolymerization of lignocellulose and conversion of arabinoxylan chains into soluble small molecules. This effect may create complexes with proteins and thus changes in the rheological characteristics of the wheat dough's protein-dependent functions. Therefore, it was inferred that extrusion at a higher screw speed produces more soluble pentose, even if no enzymes are added [51].

SME input is responsible for the intensity of changes during extrusion cooking, especially when HTST (high-temperature short-time) treatment is applied [1]. As a system parameter, SME represents the amount of mechanical energy transferred to the feed material during extrusion, and it can be used to indicate extrusion intensity. SME was found to be dependent on feed moisture, feed rate, screw speed and barrel temperature [47]. The presence of fiber and increased moisture may have provided a reduction in the viscosity of the melt in the extruder by changing the distributions of shear, mixing, mechanical heat and convective heat and thus affecting motor torque and SME [41]. At higher screw speed and temperature, a greater increase in SME was noticed by Kharat et al. [52] during the extrusion of select major millets. This can be attributed to the changes in the material viscosity in the barrel due to the increased shear. Ma et al. [50] extruded wheat flour with various co-rotating twin-screw extruders and they found decreased SME when the water content increased from 18 to 24% at both die temperatures of 95 °C and 139 °C. Allai et al. [53] also reported upon the effect of processing conditions on SME during the extrusion of wholegrain breakfast cereals. Here, feed moisture content and barrel temperature were found to be inversely proportional to SME. Wójtowicz et al. [35] stated that increased quantity of insect flour (20 and 30%) in wheat–corn-based snack pellets resulted in better SME stability at variable screw speeds and applied moisture content, and that SME results differed slightly between these extrudates because of the increased amount of fat from the cricket flour in the recipes. This fat played a lubrication role during processing (single-screw extruder), enhancing the slippage of the material. Kesre and Masatcioglu [54], in turn, noted a decrease in torque and SME values with increasing barrel temperature because of a reduction in dough viscosity. Fischer [55] concluded that the mechanical energy input during extrusion decreased with increasing extrusion temperature (140 to 180 °C) (wheat flour). In addition, higher moisture (24%) resulted in lower SME values and the difference between moisture levels decreased with increasing extrusion temperature. Robin et al. [56] extruded wholewheat flour in a co-rotating twin-screw extruder under water levels of 18 or 22%, screw speeds of 400 or 800 rpm and barrel temperatures of 140 or 180 °C, and noted that SME lowered when temperature and water content increased, and that SME was higher at a higher screw speed. Adding to the aforementioned, Lisiecka and Wójtowicz [36] found the lowest SME for extrudates supplemented with fresh onion and processed at the lowest screw speed by using a single-screw extruder. Moreover, Bouasla and Wójtowicz [57] reported that SME is significantly affected by feed moisture and screw speed, where increased feed moisture appeared to cause a slight increase in SME, but increased screw speed from 60 rpm to 100 rpm caused the SME to increase sharply when a single-screw extruder was used in pre-cooked rice pasta processing. This could be due to the higher viscosity of the dough inside the extruder, an effect caused by the more intensive gelatinization of the dough during processing when high moisture content (over 30%) and high screw speed (100 rpm) are applied. Kantrong et al. [48] used RSM to test the effect of processing conditions on several characteristics of twin-screw-extruded snacks and found that feed moisture had the most influence on SME—more than barrel temperature and applied screw speed. Moreover, they noted that higher levels of protein in the formula may contribute to higher dough viscosity, and thus higher SME is required. Feng and Lee [58], in turn, concluded that a decrease in SME significantly decreases rapidly digestible starch content and significantly increases slowly digestible starch (SDS) levels in rice-based snacks.

This outcome can have nutritionally positive effects in food products. In general, feed moisture content was reported as the most significant factor affecting the SME [41,47].

3.2. Rheological Characteristics of Extruded Flours

Wheat flours developed as a blend of selected breaking, milling, reducing and sifting passages can possess high arabinoxylan content. This results from the inclusion of waste fractions obtained during grain grinding [30], and can be valuable when selected and mixed in well-defined proportions. A flour richer in fibrous fractions, however, demonstrates different rheological characteristic than a common bread flour. In our work, the hydration ability of the flour we developed was 60.5 ± 0.1%, the development time was 1.92 ± 0.19 min. and dough stability was 9.73 ± 0.12 min. These characteristics are a bit lower than that of commercial bread flour, but allow the incorporation of large amounts of fractions usually wasted during grinding. This flour was processed either without or with xylanase enzyme and underwent diverse extrusion conditions (variations in initial moisture content, screw speed and enzyme levels), whereupon its rheological characteristics were analyzed. Schmiele et al. [40] reported 57.80% of water absorption, 11.25 min development time and 18.80 min dough stability for commercial wheat flour. Bucsella et al. [59] noted a water absorption 61.5%, 3.6 min DDT and around 7 min of dough stability for wheat flour when measured with Mixolab. Commercial bread and cake showed 58 and 60% water absorption, 6.6 and 7.9 min dough stability and 1.2 and 4.2 min of DDT, respectively [60].

Based on the obtained results presented in Tables 5 and 6, the water absorption of the extruded wheat flour with xylanase enzyme was found to depend upon processing variables and L/D configuration. Here, the water hydration of the extruded samples varied from 61.0 to 68.3% when L/D 16 was employed to pretreat the wheat flour, and from 63.9 to 69.3% when the components were processed in the L/D 20 configuration. Although in the case of the shorter L/D 16 extruded configuration, the F test result was statistically significant, due to the moderate R^2 result, the estimated model cannot be recommended for optimization purposes. It is probably necessary to fit a higher-order model, which requires conducting extended experimental studies. We found in the analyzed relationships of variables that when subject to extrusion through the L/D 20 apparatus, the hydration results were not susceptible to changes in initial moisture (M), screw speed (S) or enzyme dose (E) factors (Table 7). Butt et al. [61] reported bacterial xylanase as having a higher impact on water absorption capacity than fungal xylanase, but this application is more suitable for modifying bran fiber or high-fibrous fractions of flour. In our research, due to the application of bread flour, fungal xylanase was added due to being more reactive for the insoluble fractions of xylans present in the developed flour [62]. Xylanase can change the insoluble fractions of polysaccharides (especially xylans) into soluble and more reactive structures that absorb more water and make the treated dough more dense, and hence a requirement for more processing energy input is observed, as mentioned in Section 3.1. When 50 or 100 ppm was added to NSP-rich flour, the effect of the enzyme level was insignificant, as well as the other variables used in the experiment. Nevertheless, all treatments increased the extruded flour's hydration properties as compared to native flour (hydration of 60.5%), with a negligible effect of processing variables or xylanase enzyme level. Similar observations have been reported by Medina-Rendon et al. [8] when comparing the water absorption of non-extruded flour with the values of the extrudates obtained with a single-screw extruder. Herein, the extrudates showed equivalent or higher water absorption values. Extrusion has been demonstrated to contribute to the increase in the hydration properties of different products [63]. High shear at low moisture and high screw speed causes the degradation of starch with the crystal melting of amylopectin molecules as well as dextrinization, which have an effect on hydration properties. Also, protein hydrolysis, which is possible during extrusion, may affect water absorption, especially in raw materials with high protein content. The extruded flour obtained after processing with high moisture and low screw speed may be valuable for improving dough stability because of its nondestructive effects on hydrolysis or enzyme activity [63]. Higher feed moisture was found to

limit the mechanical disruption and fragmentation of starch granules because water acts as a plasticizer in the extruder [64]. Some research reported an association between the quantity of damaged starch and the water absorption of the extruded flour [4,5,65]. Studies performed with the application of extrusion to obtain pregelatinized starches indicated starch granule breakdown and damage during this process and increased water absorption due to the partial gelatinization of starch in the presence of water and heat. Pasqualone et al. [65] reported increased water absorption after industrial-scale extrusion cooking of lentil flour with two temperature/screw profiles, and they observed a significant increase in water absorption (90.8–94.7%) after processing at lower temperatures and screw speeds as compared to native lentil flour (41.1%). Similarly, Tao et al. [5] reported the increased water absorption of dough with added extruded wheat starch into bread formulation because of the crystallinity loss in the extruded starch that was beneficial for the quality of bread with the addition of extruded flour. Liu et al. [4] recommended single-screw extrusion at lower-than-conventional temperatures (50–150 °C) as an easy and flexible process to modify rice starch at the initial moisture from 30 to 70%, significantly improving the degree of gelatinization. Low temperatures are required for processing when enzymes are directly added to the extruder. In the extruder, during processing, a number of interactions occur between processing variables and enzymatic activity [19], and these affect the rheological behavior of the resulting dough. Martínez et al. [27] used extrusion to modify wheat flour, and the processing significantly increased hydration properties; specifically, 5-fold water binding capacity and 9-fold swelling compared with untreated wheat flour.

Of note, extruded wheat flours have been reported to increase the bread yield in bakery processes [28]. The composition of the blend may also have an effect on rheological properties. The physicochemical modification of fiber and fiber-rich fractions by using high temperatures and shear conditions of extrusion processing is possible for enhancing their functional properties. The dough-mixing and pasting properties of blends of wholewheat flour and different types (commercial yellow pea flour, yellow pea flour, green pea flour, red lentil flour, and chickpea flour) and amounts (5, 15 and 25%, based on total composite flour weight) of pulse flours were analyzed by Zhang et al. [66] using Mixolab. They found a higher water absorption value as the amount of pulse flour increased in the blend, compared to basic wholewheat flour. The rheological parameters of wheat flour provide a measure of dough water absorption, viscoelastic strength and stability for the tolerance of over-mixing dough. Usually, the wheat flours used in bread making containing high levels of good-quality gluten have good machinability properties and good tolerance to over-mixing in comparison to flours of poorer quality containing more outer layers from reducing and sifting passages, and which are richer in fiber and non-starch polysaccharides and arabinoxylans [32]. Lee et al. [67] found that extrusion was the most effective treatment approach for improving the dough-mixing properties of wholewheat meal containing bran, e.g., the resistance of a composed dough with puffed or HTHP-cooked wheat bran rapidly decreased after reaching a Mixograph peak, showing a significant increase in breakdown resistance. In contrast, the addition of extruded bran significantly increased the midline peak time by 0.5 min, as compared to untreated wheat bran.

Gómez et al. [68] found that extrusion increased the dough development time when bran was added to bread flour. However, the Mixograph results presented by Gajula et al. [31] indicate that high-temperature and high-shear-extrusion processing deteriorated protein quality and caused poor water absorption and viscoelastic strength in pre-cooked bran-supplemented flour as compared to control wheat flour. The high thermal and mechanical energy input during extrusion was found to have caused the denaturation of proteins, thus negatively affecting dough development, but this effect was especially noticeable when HTST treatment was applied with low feed moisture. In contrast, low temperatures and the low shear extrusion of flour caused a lower peak viscosity than under HTST conditions and resulted in improvements in the dough properties due to some kind of synergistic effect of the pre-cooked fiber and starch on water binding and swelling at low bran levels [31].

Table 5. Rheological properties of wheat flour extruded using L/D 16 extruder configuration (mean values, n = 3).

Processing Variables			L/D 16				
M	**S**	**E**	**Hyd (%)**	**Stab. (min)**	***α***	***β***	***γ***
23	40	0	67.0 ± 0.3 ^{g,h,i}	5.1 ± 0.12 ^{a,b,c}	−0.042 ± 0.002 ^{a,b,c,d}	0.236 ± 0.019 ^{a,b,c}	−0.042 ± 0.001 ^{a,b,c,d,e}
		50	68.0 ± 0.4 ^{h,i}	5.6 ± 0.15 ^{b,c,d}	−0.046 ± 0.017 ^{a,b,c,d}	0.158 ± 0.015 ^{a,b}	−0.046 ± 0.022 ^{a,b,c,d,e}
		100	67.2 ± 0.2 ^{g,h,i}	5.6 ± 0.2 ^{b,c,d}	−0.052 ± 0.022 ^{a,b,c,d}	0.240 ± 0.03 ^{a,b,c}	−0.080 ± 0.008 ^{a,b,c,d}
	60	0	67.3 ± 0.2 ^{g,h,i}	5.0 ± 0.16 ^{a,b}	−0.022 ± 0.015 ^{d}	0.192 ± 0.007 ^{a,b,c}	−0.028 ± 0.002 ^{b,c,d,e}
		50	63.8 ± 0.1 ^{d,e,f}	9.8 ± 0.12 ^{l}	−0.082 ± 0.02 ^{a,b}	0.240 ± 0.001 ^{a,b,c}	−0.080 ± 0.01 ^{a,b,c,d}
		100	67.5 ± 0.2 ^{h,i}	4.4 ± 0.1 ^{a}	−0.026 ± 0.015 ^{c,d}	0.204 ± 0.08 ^{a,b,c}	−0.098 ± 0.08 ^{a,b,c}
	80	0	68.3 ± 0.2 ^{i}	6.2 ± 0.5 ^{c,d,e,f}	−0.034 ± 0.02 ^{b,c,d}	0.204 ± 0.053 ^{a,b,c}	−0.028 ± 0.003 ^{b,c,d,e}
		50	65.9 ± 0.2 ^{g}	7.1 ± 0.2 ^{f,g,h,i,j}	−0.056 ± 0.008 ^{a,b,c,d}	0.142 ± 0.002 ^{a}	−0.072 ± 0.013 ^{a,b,c,d,e}
		100	68 ± 0.3 ^{h,i}	6.7 ± 0.2 ^{d,e,f,g}	−0.046 ± 0.002 ^{a,b,c,d}	0.288 ± 0.004 ^{c}	−0.084 ± 0.01 ^{a,b,c,d}
25	40	0	60.6 ± 0.2 ^{a}	7 ± 0.2 ^{f,g,h,i}	−0.090 ± 0.015 ^{a}	0.260 ± 0.025 ^{a,b,c}	−0.070 ± 0.015 ^{a,b,c,d,e}
		50	62.8 ± 0.1 ^{b,c,d,e}	5.6 ± 0.2 ^{b,c,d}	−0.070 ± 0.005 ^{a,b,c,d}	0.260 ± 0.03 ^{a,b,c}	−0.120 ± 0.010 ^{a}
		100	61.5 ± 0.2 ^{a,b,c}	10.2 ± 0.6 ^{l}	−0.078 ± 0.02 ^{a,b,c}	0.286 ± 0.025 ^{c}	0.002 ± 0.006 ^{e}
	60	0	61 ± 0 ^{a}	7.7 ± 0.3 ^{g,h,i,j,k}	−0.048 ± 0.001 ^{a,b,c,d}	0.270 ± 0.002 ^{b,c}	−0.064 ± 0.008 ^{a,b,c,d,e}
		50	63.9 ± 0.25 ^{d,e,f}	5.6 ± 0.05 ^{b,c,d}	−0.064 ± 0.002 ^{a,b,c,d}	0.202 ± 0.04 ^{a,b,c}	−0.088 ± 0.02 ^{a,b,c}
		100	62.5 ± 0.3 ^{b,c,d}	6.6 ± 0.2 ^{d,e,f,g}	−0.058 ± 0.04 ^{a,b,c,d}	0.278 ± 0.02 ^{b,c}	−0.066 ± 0.02 ^{a,b,c,d,e}
	80	0	61.5 ± 0.15 ^{a,b,c}	6.9 ± 0.2 ^{e,f,g,h}	−0.038 ± 0.005 ^{a,b,c,d}	0.300 ± 0.015 ^{c}	−0.030 ± 0.052 ^{b,c,d,e}
		50	64.5 ± 0.2 ^{g,h}	5.3 ± 0.23 ^{e,f,g,h}	−0.048 ± 0.02 ^{a,b,c,d}	0.242 ± 0.025 ^{a,b,c}	−0.110 ± 0.008 ^{a,b,c,d}
		100	61.4 ± 0.2 ^{a,b}	4.3 ± 0.08 ^{a}	−0.028 ± 0.012 ^{c,d}	0.262 ± 0.002 ^{a,b,c}	−0.108 ± 0.003 ^{a,b}
27	40	0	64.0 ± 0.2 ^{e,f}	5.6 ± 0.15 ^{b,c,d}	−0.074 ± 0.009 ^{a,b,c,d}	0.246 ± 0.030 ^{a,b,c}	−0.006 ± 0.005 ^{d,e}
		50	64.3 ± 0.3 ^{f}	8.5 ± 0.1 ^{k}	−0.066 ± 0.020 ^{a,b,c,d}	0.24 ± 0.020 ^{a,b,c}	−0.046 ± 0.00 ^{a,b,c,d,e}
		100	63.5 ± 0.2 ^{d,e,f}	8.1 ± 0.6 ^{i,j,k}	−0.064 ± 0.03 ^{a,b,c,d}	0.236 ± 0.030 ^{a,b,c}	−0.084 ± 0.003 ^{a,b,c,d}
	60	0	66.0 ± 0.3 ^{g}	7.9 ± 0.2 ^{h,i,j,k}	−0.064 ± 0.001 ^{a,b,c,d}	0.234 ± 0.090 ^{a,b,c}	−0.100 ± 0.025 ^{a,b,c}
		50	62.9 ± 0.2 ^{c,d,e,f}	5.8 ± 0.2 ^{b,c,d,e}	−0.058 ± 0.022 ^{a,b,c,d}	0.256 ± 0.019 ^{a,b,c}	−0.030 ± 0.035 ^{b,c,d,e}
		100	64.0 ± 0.1 ^{e,f}	7.4 ± 0.2 ^{g,h,i,j,k}	−0.062 ± 0.015 ^{a,b,c,d}	0.240 ± 0.004 ^{a,b,c}	0.004 ± 0.022 ^{e}
	80	0	68.0 ± 0.2 ^{h,i}	8.2 ± 0.2 ^{j,k}	−0.044 ± 0.02 ^{a,b,c,d}	0.198 ± 0.070 ^{a,b,c}	−0.044 ± 0.005 ^{a,b,c,d,e}
		50	67.3 ± 0.2 ^{g,h,i}	8.3 ± 0.3 ^{k}	−0.030 ± 0.023 ^{b,c,d}	0.208 ± 0.031 ^{a,b,c}	−0.024 ± 0.028 ^{c,d,e}
		100	63.5 ± 0.1 ^{d,e,f}	7.1 ± 0.2 ^{f,g,h,i,j}	−0.070 ± 0.005 ^{a,b,c,d}	0.212 ± 0.045 ^{a,b,c}	−0.090 ± 0.015 ^{a,b,c}

M—moisture; S—screw speed; E—enzyme dose; Hyd—hydration; Stab.—dough stability; *α*—slope of angle *α*; *β*—slope of angle *β*; *γ*—slope of angle *γ*; $^{a–l}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

The stability time of the dough indicates the flour's strength. The dough stability results of enzymatic-extrusion-modified wheat flour, presented in Tables 5 and 6, demonstrate increased values with an increasing level of initial feed moisture, especially when the elongated extruder L/D 20 was employed for processing. The mixing elements incorporated into the longer extruder screw profile created a less compact and dense dough structure due to greater mixing, and this resulted in greater dough stability. No regression model was obtained in any extruder settings that could adequately describe the variability of the dough stability depending on the experimental factors tested (Table 7). We think that further research is necessary, with the application of a central composite design with levels based on the presented results to find an optimization method, either to develop a higher-level model (so far this is not possible in this research, because to develop a third-level model, research is needed that takes five levels of each factor into account) or take into account other factors that may affect dough stability. Dough stability was highly correlated with C2 values, indicating protein weakening (r values were 0.604 for L/D 16 and 0.829 for L/D 20), and with C5 values when the elongated extruder configuration was applied (r = 0.793).

Zhang et al. [69] reported decreasing dough strength, in some cases, almost double, when pulse flours were incorporated in blends with wholewheat-based dough, probably due to the dilution of gluten when grain flour was replaced by pulse flour. Pasqualone et al. [64] reported significantly increased stability time of extruded lentil flour after pro-

cessing with an industrial-scale line at two temperature/screw profiles (2.1–2.4 min) as compared to the native lentil flour (1.5 min). Martinez et al. [28] noted a decrease in protein stability in bread flours with the addition of extruded wheat flour in the amount of 5%; farinographic examination showed a connection with the degradation of the gluten matrix during extrusion as a result of an increase in temperature, even up to 140 °C.

Table 6. Rheological properties of wheat flour extruded using L/D 16 and L/D 20 extruder configurations (mean values, n = 3).

Processing Variables			L/D 20				
M	**S**	**E**	**Hyd (%)**	**Stab. (min)**	***α***	***β***	***γ***
23	40	0	68.0 ± 0.2 ^{h,i}	7.6 ± 0.2 ^{j,k}	−0.036 ± 0.005 ^{c,d,e}	0.262 ± 0.09 ^{a,b,c,d}	−0.070 ± 0.052 ^{a,b,c,d}
		50	67.0 ± 0.3 ^{e,f,g}	5.5 ± 0.2 ^{d,e,f}	−0.066 ± 0.02 ^{a,b,c,d,e}	0.224 ± 0.019 ^{a,b,c}	−0.090 ± 0.008 ^{a,b,c}
		100	66.5 ± 0.4 ^{d,e}	6.0 ± 0.2 ^{e,f,g}	−0.038 ± 0.012 ^{c,d,e}	0.222 ± 0.004 ^{a,b}	−0.108 ± 0.003 ^{a,b}
	60	0	69.2 ± 0.1 ^{k}	6.3 ± 0.2 ^{g,h}	−0.040 ± 0.009 ^{b,c,d,e}	0.224 ± 0.019 ^{a,b,c}	−0.088 ± 0.005 ^{a,b,c,d}
		50	66.8 ± 0.3 ^{e,f}	4.6 ± 0.3 ^{a,b,c}	−0.040 ± 0.02 ^{b,c,d,e}	0.234 ± 0.015 ^{a,b,c,d}	−0.060 ± 0.001 ^{a,b,c,d}
		100	67.2 ± 0.3 ^{f,g}	4.6 ± 0.2 ^{a,b,c}	−0.056 ± 0.03 ^{a,b,c,d,e}	0.268 ± 0.03 ^{a,b,c,d}	−0.066 ± 0.003 ^{a,b,c,d}
	80	0	66.8 ± 0.2 ^{e,f}	4.7 ± 0.1 ^{a,b,c}	−0.042 ± 0.015 ^{b,c,d,e}	0.276 ± 0.007 ^{a,b,c,d}	−0.062 ± 0.025 ^{a,b,c,d}
		50	66.5 ± 0.2 ^{d,e}	5.3 ± 0.2 ^{c,d,e}	−0.036 ± 0.005 ^{c,d,e}	0.240 ± 0.001 ^{c,d,e}	−0.034 ± 0.001 ^{b,c,d,e}
		100	67.0 ± 0.2 ^{e,f,g}	4.4 ± 0.2 ^{a}	−0.054 ± 0.002 ^{a,b,c,d,e}	0.246 ± 0.080 ^{a,b,c,d}	−0.084 ± 0.008 ^{a,b,c,d}
25	40	0	65.5 ± 0.1 ^{b,c}	6.2 ± 0.2 ^{f,g,h}	−0.066 ± 0.001 ^{a,b,c,d,e}	0.312 ± 0.700 ^{a,b,c,d}	−0.064 ± 0.02 ^{a,b,c,d}
		50	68.2 ± 0.0 ^{i}	8.4 ± 0.1 ^{l,m,n}	−0.034 ± 0.002 ^{c,d,e}	0.268 ± 0.031 ^{a,b,c,d}	−0.064 ± 0.02 ^{a,b,c,d}
		100	65.3 ± 0.1 ^{b}	7.6 ± 0.1 ^{j,k}	−0.098 ± 0.040 ^{a}	0.242 ± 0.045 ^{a}	−0.114 ± 0.035 ^{a}
	60	0	67.0 ± 0.3 ^{e,f,g}	8.8 ± 0.2 ^{n}	−0.078 ± 0.02 $^{a,b,c,d\ e}$	0.228 ± 0.053 ^{a,b,c,d}	−0.084 ± 0.022 ^{a,b,c,d}
		50	65.6 ± 0.2 ^{b,c}	5.3 ± 0.2 ^{c,d,e}	−0.017 ± 0.015 ^{e}	0.202 ± 0.002 ^{a,b,c,d}	−0.060 ± 0.005 ^{a,b,c,d}
		100	68.9 ± 0.1 ^{j,k}	7.8 ± 0.1 ^{k,l}	−0.056 ± 0.020 ^{a,b,c,d,e}	0.164 ± 0.004 ^{a,b,c}	−0.094 ± 0.028 ^{a,b,c}
	80	0	67.2 ± 0.2 ^{f,g}	6.0 ± 0.2 ^{e,f,g}	−0.026 ± 0.008 ^{d,e}	0.288 ± 0.025 ^{a,b,c,d}	−0.070 ± 0.015 ^{a,b,c,d}
		50	63.9 ± 0.2 ^{a}	4.5 ± 0.1 ^{a,b}	−0.040 ± 0.002 ^{b,c,d,e}	0.250 ± 0.030 ^{e}	0.028 ± 0.001 ^{e}
		100	67.0 ± 0.3 ^{e,f,g}	5.2 ± 0.6 ^{b,c,d}	−0.062 ± 0.001 ^{a,b,c,d,e}	0.228 ± 0.025 ^{a,b,c,d}	−0.056 ± 0.022 ^{a,b,c,d}
27	40	0	66.0 ± 0.2 ^{c,d}	8.6 ± 0.5 ^{m,n}	−0.078 ± 0.022 ^{a,b,c,d}	0.192 ± 0.002 ^{a,b,c,d}	−0.068 ± 0.008 ^{a,b,c,d}
		50	63.9 ± 0.1 ^{a}	8.8 ± 0.2 ^{n}	−0.082 ± 0.015 ^{a,b,c}	0.172 ± 0.030 ^{c,d,e}	−0.028 ± 0.003 ^{c,d,e}
		100	63.7 ± 0.2 ^{a}	6.8 ± 0.2 ^{h,i}	−0.070 ± 0.020 ^{a,b,c,d,e}	0.186 ± 0.020 ^{c,d,e}	−0.034 ± 0.013 ^{b,c,d,e}
	60	0	68.4 ± 0.3 ^{i,j}	8.5 ± 0.2 ^{l,m,n}	−0.040 ± 0.023 ^{b,c,d,e}	0.128 ± 0.030 ^{d,e}	−0.014 ± 0.010 ^{d,e}
		50	66.0 ± 0.3 ^{c,d}	7.4 ± 0.2 ^{i,j,k}	−0.068 ± 0.005 ^{a,b,c,d,e}	0.224 ± 0.040 ^{a,b,c,d}	−0.050 ± 0.015 ^{a,b,c,d}
		100	64.0 ± 0.2 ^{a}	6.9 ± 0.3 ^{h,i,j}	−0.080 ± 0.002 ^{a,b,c}	0.118 ± 0.020 ^{a,b,c,d}	−0.050 ± 0.010 ^{a,b,c,d}
	80	0	67.5 ± 0.3 ^{g,h}	8.0 ± 0.3 ^{k,l,m}	−0.092 ± 0.017 ^{a,b}	0.234 ± 0.015 ^{c,d,e}	−0.032 ± 0.002 ^{c,d,e}
		50	65.1 ± 0.1 ^{b}	4.5 ± 0.1 ^{a,b}	−0.046 ± 0.022 ^{a,b,c,d,e}	0.250 ± 0.025 ^{e}	0.028 ± 0.010 ^{e}
		100	68.1 ± 0.2 ^{h,i}	8.1 ± 0.2 ^{k,l,m,n}	−0.058 ± 0.015 ^{a,b,c,d,e}	0.186 ± 0.002 ^{b,c,d,e}	−0.038 ± 0.080 ^{b,c,d,e}

M—moisture; S—screw speed; E—enzyme dose; Hyd—hydration; Stab.—dough stability; *α*—slope of angle *α*; *β*—slope of angle *β*; *γ*—slope of angle *γ*; $^{a–n}$—means indicated with similar letters in columns do not differ significantly at *α* = 0.05.

Table 7. Regression coefficients for response surface model of rheological properties of wheat flour extruded via L/D 16 and L/D 20 using coded inputs.

	Hyd (%)	**Stab. (min)**	**C2 (Nm)**	**C3 (Nm)**	**C4 (Nm)**	**C5 (Nm)**	***α***	***β***	***γ***
					L/D 16				
Const.	61.985 ***	6.659	0.634	1.381 ***	0.972 ***	1.410 ***	−0.061	0.245	−0.081
M	−1.083 ***	0.633	0.013	0.029 **	0.050 ***	0.161 ***	−0.007	0.009	0.008
S	0.528	−0.067	0.000	−0.023 **	−0.029 **	−0.034 **	0.010	−0.006	−0.005
E	−0.256	0.044	−0.015	−0.016	−0.070 ***	−0.138 ***	−0.002	0.006	−0.011
M × M	3.728 ***	0.222	0.019	−0.082 ***	−0.083 ***	0.002	0.006	−0.041	0.018
S × S	0.528	0.056	0.006	0.007	0.027	0.070 **	0.000	−0.001	0.001
E × E	−0.222	−0.178	−0.016	0.032	0.048 **	0.149 ***	0.006	0.027	0.012
M × E	−0.592	0.042	−0.013	−0.016	−0.006	−0.033	0.001	−0.008	0.012
S × E	−0.458	−0.783	−0.008	0.006	0.007	0.006	−0.003	0.003	−0.011
M × S	0.583	−0.192	0.001	−0.014	0.003	0.003	0.005	−0.009	0.000
p-value of F test	0.001 ***	0.728	0.142	0.002 ***	0.0002 ***	<0.0001 ***	0.219	0.105	0.606
R^2	0.646	0.021	0.217	0.582	0.691	0.886	0.152	0.256	0.020

Table 7. *Cont.*

	Hyd (%)	Stab. (min)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)	α	β	γ
				L/D 20					
Const.	66.352	6.300 ***	0.639	1.354 ***	0.942 ***	1.573 ***	−0.042	0.221 ***	−0.051 ***
M	−0.683	1.033 ***	0.017	−0.030 ***	0.018	0.135 ***	−0.011	−0.028 ***	0.021 ***
S	0.278	−0.822 ***	−0.015	−0.004	−0.028 **	−0.117 ***	0.006	0.007	0.018 ***
E	−0.439	−0.406	−0.018	−0.063 ***	−0.055 ***	−0.123 ***	−0.004	−0.016	−0.005
M × M	0.028	−0.167	−0.010	−0.029 **	−0.004	−0.097 **	−0.004	−0.027	0.012
S × S	−0.722	−0.233	−0.016	0.021	0.015	0.022	−0.004	0.039 **	0.010
E × E	0.961	0.750	0.018	−0.016	0.033	0.164 ***	−0.012	−0.007	−0.030 ***
M × E	−0.233	0.025	0.002	−0.031***	−0.020	0.016	0.003	−0.003	0.003
S × E	0.383	0.083	0.006	−0.002	−0.019	−0.034	0.001	−0.002	0.003
M × S	0.692	0.092	0.014	0.016	−0.002	0.006	0.002	0.006	0.000
p-value of F test	0.131	0.021 **	0.196	<0.0001 ***	0.004 ***	<0.0001 ***	0.314	0.020 **	0.006 ***
R^2	0.227	0.424	0.170	0.783	0.551	0.810	0.089	0.427	0.512

M—moisture; S—screw speed; E—enzyme dose; Hyd—hydration; Stab.—dough stability; C2—protein weakening; C3—starch gelatinization; C4—amylase activity; C5—starch retrogradation; α—slope of angle α; β—slope of angle β; γ—slope of angle γ; ** $p < 0.05$, *** $p < 0.01$.

Figure 4 presents the example curves obtained via the Mixolab® Chopin+ procedure for a native flour and for extruded flours amended without/with xylanase. In Figure 4, the measure points for the main dough properties prepared from the treated flours are indicated as protein weakening (C2), starch gelatinization (C3), amylase activity (C4) and starch retrogradation (C5). The developed native NSP-rich wheat flour tested with Mixolab® showed C2 at 0.477 ± 0.01 Nm, C3 at 1.709 ± 0.01 Nm, C4 at 1.479 ± 0.01 Nm and C5 at 2.519 ± 0.00 Nm.

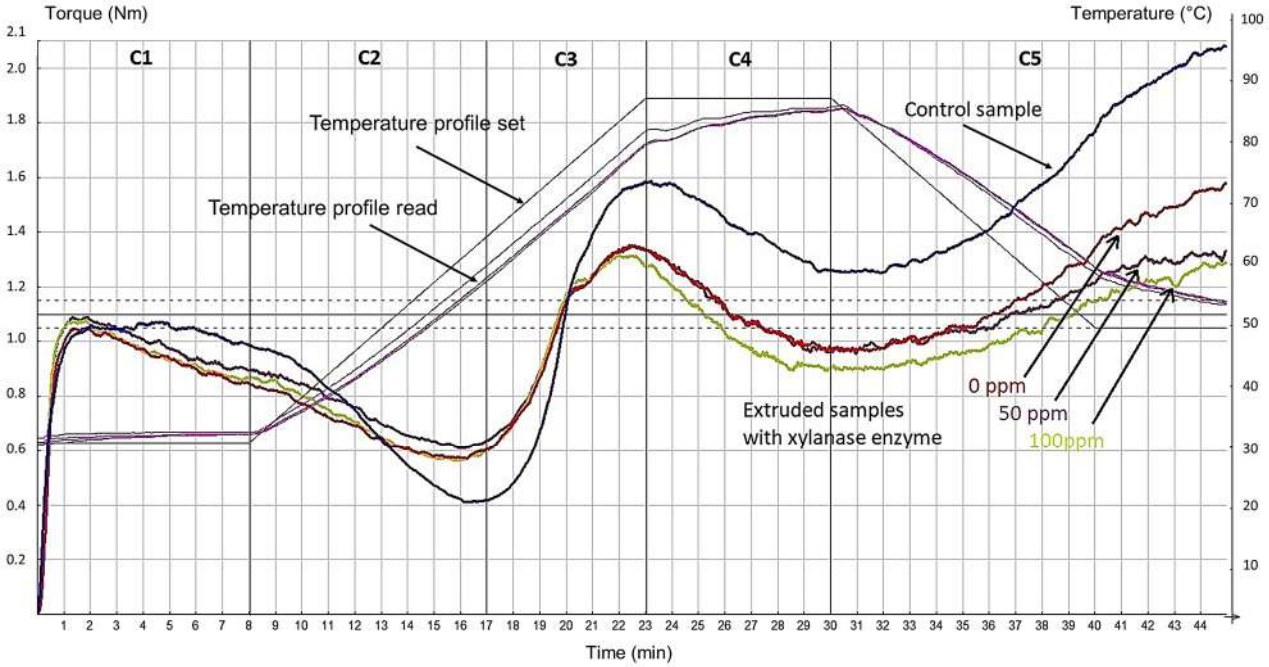

Figure 4. Mixolab® apparatus curve profile with identified tested points referring to rheological properties: results of control and hybrid enzymatic-extrusion-treated wheat flour extruded at 80 rpm and 23% of initial moisture. C1—beginning heating; C2—protein weakening; C3—starch gelatinization; C4—amylase activity; C5—starch retrogradation.

The rheological properties of the dough prepared from the extruded flours are presented in Figures 5–8 and in Tables 5 and 6. Our work demonstrates that the low-temperature extrusion process significantly affects some of the rheological properties of the NSP-rich wheat flour, and the scale of this depends on the L/D extruder configuration and

the processing variables applied. We noted that the single-screw extrusion treatment of the NSP-rich wheat flour without or with xylanase addition using two different extruder configurations slightly influenced C2 values (Figure 5), probably due to treatment at similar conditions. C2 for native wheat flour was 0.47 Nm. For extruded wheat flour, C2 was 0.61–0.67 Nm for L/D 16 and 0.59–0.65 Nm for L/D 20, so the extrusion treatment significantly increased the protein weakening of the treated flour. C2, as a protein weakening indicator, was measured at a temperature that may have denatured the protein, and protein weakening values were not differentiated significantly as extrusion-dependent variables. C2 parameters, registered in the first stage of dough heating, indicated that the modified flours were not susceptible to changes in the factors of initial moisture (M), screw speed (S) or enzyme dose (E) in any of the settings or extruder configurations: L/D 16 and L/D 20 (Table 7).

With regard to the changes in protein structure formed during extrusion at low moisture, some protein linkages were found to involve fewer disulfide bonds than those built up at higher moisture levels. We think that the protein network formed under low moisture extrusion could have incorporated more protein subunits, which would explain the lower protein solubility. Thus, besides the influence of thermal and mechanical energy input, moisture content is important for the nature of disulfide cross-linking during extrusion [55]. In both extruder configurations, we observed a slight increase in C2 parameter values when the initial feed moisture content increased (Figure 5a,c,d,f). Increased enzyme dose also had a slight effect on protein weakening values: lower C2 values were obtained when extrusion was undertaken with higher xylanase doses in both extruder configurations (Figure 5b,d,e). In general, the elongated L/D 20 extruder configuration allowed us to obtain lower C2 values, and this may be due to the application of a mixing element to the screw configuration mixing element, which loosened the melted dough and thus caused a less intensive treatment of protein components (especially gluten) in the tested NSP-rich flour.

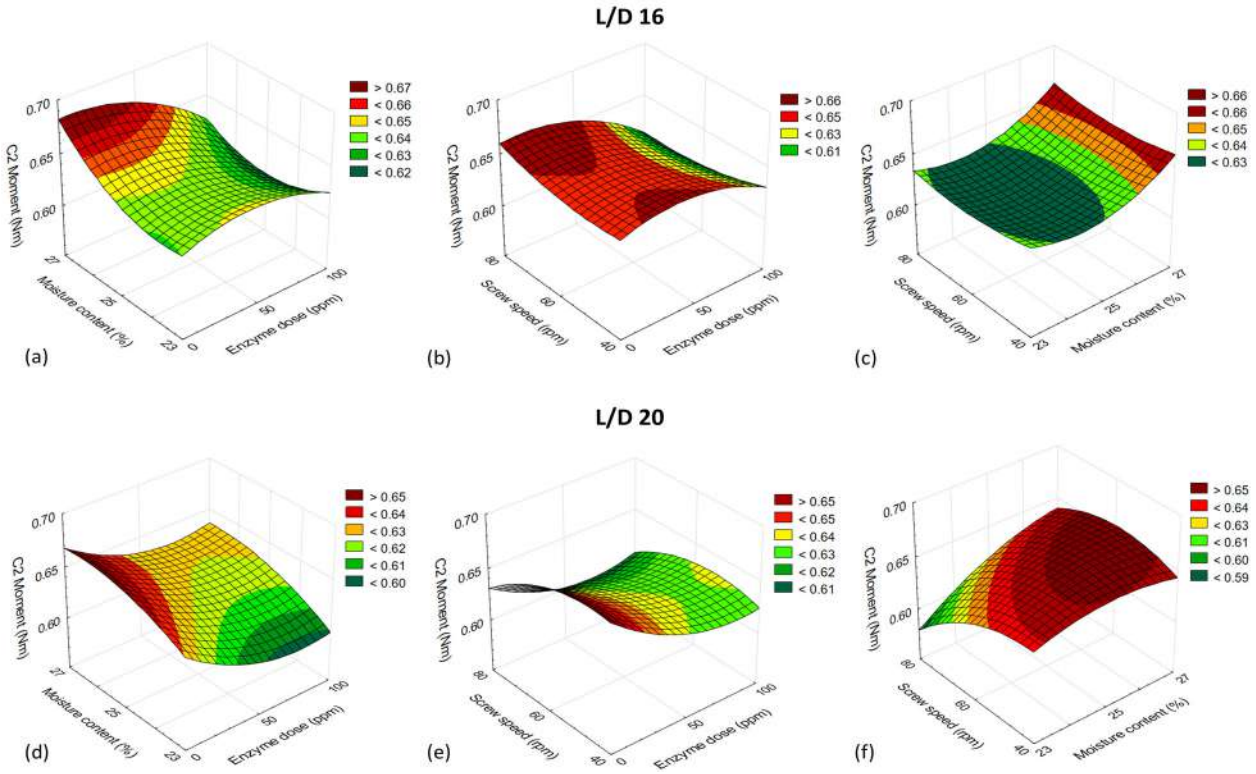

Figure 5. Processing variables and L/D configuration effects on the C2 of extruded wheat flour with xylanase enzyme: L/D 16: (**a**) E × M, (**b**) E × S, (**c**) M × S; L/D 20: (**d**) E × M, (**e**) E × S, (**f**) M × S. C2—protein weakening; E—enzyme dose; M—moisture; S—screw speed.

In general, extrusion processing caused an increase in protein weakening that can be observed as higher C2 values, especially at higher feed moisture. But the application of the enzyme limited the disruption of the protein network, giving lower C2 values. If C2 is high in value, the dough obtained with the treated flour is less elastic and exhibits limited development. Extruded flour itself is impossible to apply in bread making, but the partial replacement of bread flour with a low C2 characteristic with extruded flour can be helpful to slow down the formation of pores during fermentation and ovenspring and can positively maintain the internal structure of bread. Moreno−Rivas et al. [25] applied a single-screw laboratory extruder with L/D = 25:1, a nominal compression ratio of 2:1 and a die opening of 3 mm, working at 45 rpm and in the temperature ranges of 60, 70, 80, and 90 °C to treat nixtamalized corn flour with and without xylanase. They reported that the extruded nixtamalized corn flour, with and without xylanase, had increased protein solubility, and this effect was lower when extruded with xylanase. What is more, the addition of xylanase reduced the effect that the extrusion process had on the solubility proteins of the extruded nixtamalized corn flour. Additionally, fat content decreased significantly in the extruded products without and with xylanase enzyme, due to lipid breakdown or the formation of complexes between amylose and fatty acids, making it possible to extend the shelf life of extruded flour products [26]. Schmiele et al. [40] reported C2 values ranging from 0.46 to 0.58 Nm for wheat flour filled with soy protein and fructooligosaccharides depend on the content of additives. Bucsella et al. [60] reported significant changes after the treatment of cake and bread flour with thermal and hydrothermal methods. They found a slight increase in C2 values after hydrothermal treatment for 5 min, but longer treatment times (10 and 20 min) decreased the C2 values of both cake and bread wheat flour due to changes in protein conformation by heating.

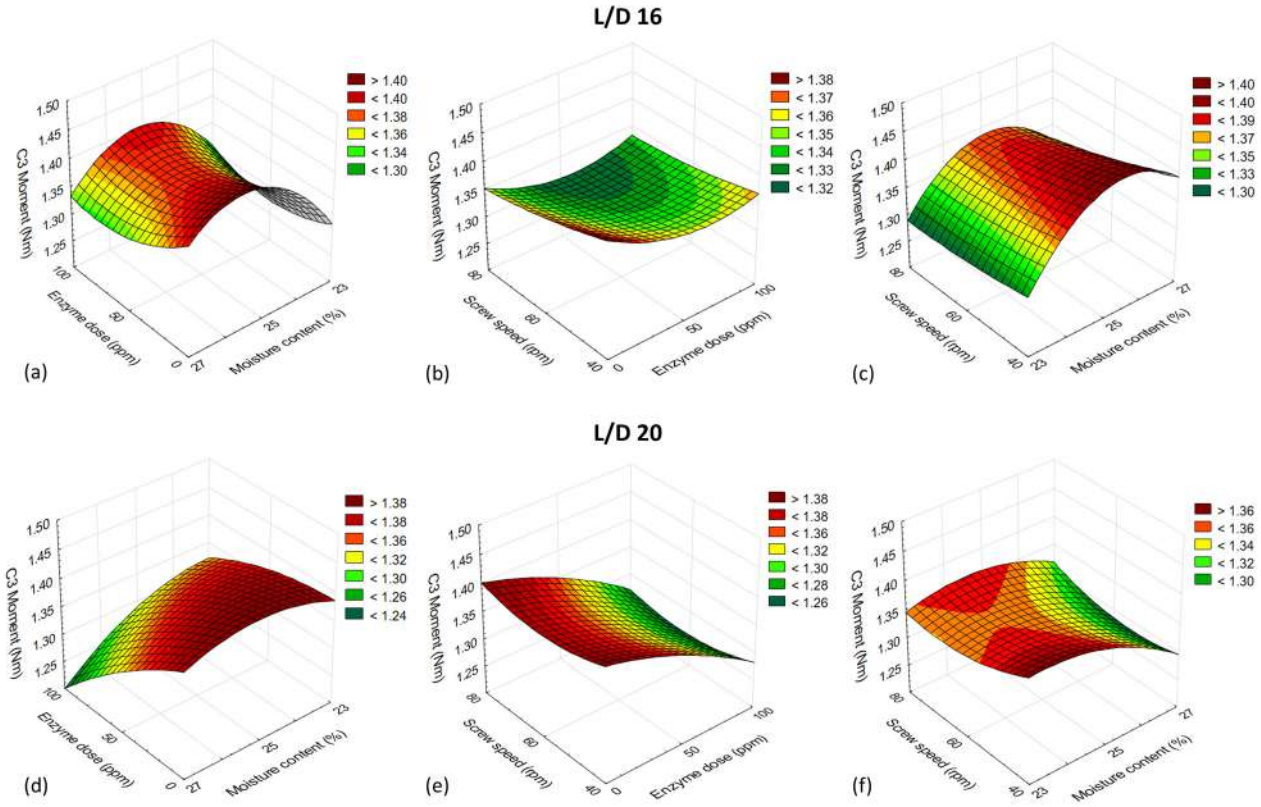

Figure 6. Processing variables and L/D configuration effects on the C3 of extruded wheat flour with xylanase enzyme: L/D 16: (**a**) E × M, (**b**) E × S, (**c**) M × S; L/D 20: (**d**) E × M, (**e**) E × S, (**f**) M × S. C3—starch gelatinization; E—enzyme dose; M—moisture; S—screw speed.

Figure 6 presents the C3 results (starch gelatinization torque data) of NSP-rich wheat flour exposed to variable conditions and either the L/D 16 or L/D 20 extruder configurations. The C3 for native flour was 1.71 Nm, and after processing, values ranged from 1.244 to 1.491 Nm when wheat flour was extruded with the short L/D 16 extruder configuration, and from 1.181 to 1.470 Nm if processed via the elongated extruder L/D 20. Treatment via the short L/D 16 version of the extruder engendered higher starch gelatinization levels due to more intensive treatment, in contrast to processing via the L/D 20 apparatus with a screw profile equipped with a mixing zone. In this case, the C3 results showed a strong negative correlation with enzyme dose ($r = -0.743$). This mixing zone allowed for a longer residence time of the treated wheat flour and the activation of the xylanase enzyme. Additionally, the screw mixing element loosened the internal structure of the melted treated flour, which could worsen the gelatinization levels of the starch present in wheat flour. For both extruder settings (L/D 16, L/D 20), regression models explaining the variability of C3 in relation to the experimental factors tested were estimated.

The significant results of the F test and the high values of the coefficient of determination confirmed the adequacy of the obtained relationships. Statistical analysis of the C3 values showed a positive linear C3 relationship with respect to M and a negative quadratic relationship with M, as well as a negative linear relationship with S (Table 7). The changes in the C3 values of the treated flour were more dominated by a parabolic (quadratic) relationship with initial feed moisture if the L/D 16 configuration was used for treatment. Regarding the C3 results obtained for the L/D 20 version, there was a small negative M × E interaction effect, indicating that C3 increases as M and E decrease for this device configuration. Both factors (M, E) independently contributed to the decrease in C3, indicating an additive effect of this interaction. It can be seen that in comparing the regression coefficients, the influence of enzyme dose is slightly stronger than other factors (Table 7). This could be due to the partial hydrolysis of polysaccharides by xylanase (probably either starch), the effect of which lowered C3 values in the obtained enzymatic-extruded flour. More important changes in modified flour caused by xylanase level were visible when the elongated L/D 20 extruder configuration was used for processing (Figure 6d,e), due to less intensive mechanical treatment via the mixing element and the possibly more intensive effect of the enzyme on flour structure. This resulted in lower gelatinization levels in the treated flour.

The amylographic profile of the dry-heated test wheat flour showed a drastic change, i.e., an earlier onset time and higher peak viscosity than that of the control, suggesting easier gelatinization after treatment [70]. Gujala et al. [31] used two extrusion conditions: low-temperature−low-shear (LTLS), where the barrel temperature was set at 30, 32, 34, 36, 38 and 40 °C in individual zones and a 200 rpm screw speed was used, and high-temperature−high-shear (HTHS), with temperature settings of 30, 40, 50, 60, 70, and 80 °C and a screw speed of 250 rpm was applied in a twin-screw extruder for pre-cooking wheat flours substituted with 0, 10, 20 and 30% wheat bran, and with a moisture content maintained at 30%. They found that the application of extrusion pre-cooking of fibers in bran-enriched flour led to synergistic interactions between the bran and other components, and increased its functionality for use in baked products. The dough rheological properties, measured using a Mixograph, showed dough behavior when heated above the characteristic temperature in the presence of water, where native starch granules undergo gelatinization, which essentially involves the disruption of the molecular order in the granules, causing the starch granules to swell and amylose to leach out.

Amylase activity (C4) is another rheological parameter possible to identify when the Chopin+ protocol is applied to wheat flour (1.48 Nm for native flour). We noted that the results of C4 obtained for the enzymatic-extruded wheat flour depended on the extruder configuration and processing variables (Figure 7). Values of amylase activity represented by C4 were slightly higher if L/D 20 was employed for processing, especially when the interactions of M and S were analyzed (Figure 7c and Figure 7f, for L/D 16 and L/D 20, respectively).

Increasing feed moisture content brought about higher values of C4, especially when the enzyme was not used in the processed blends (Figure 7a,c). However, the strongest changes in the C4 feature were obtained when the shorter L/D 16 configuration was employed for wheat flour processing. This generated a quadratic negative dependence on M (Table 7). Here, enzyme addition induced lower results that were independent of the extruder configuration used. For both extruder configurations, a significant negative linear relationship was obtained between C4 and S and E, which demonstrated that amylase activity decreases as S and E increase, with the effect of xylanase enzyme level being slightly stronger than the applied screw speed. For both configurations, the adequacy of the models describing C4 was demonstrated by moderate R^2 results and statistically significant F test results. Moreover, if the L/D 16 version was used for treatment, significant positive linear effects of M and quadratic E on the C4 values were demonstrated. Significant negative correlation coefficients were found between C4 and enzyme dose for both extruder configurations used (r values were −0.582 for L/D 16 and −0.649 for L/D 20). The effect of xylanase of various origins may have a variable impact on dough rheological properties tested with Mixolab, but, in general, xylanase supplementation decreases protein resistance to mixing that is connected with lowered protein weakening (C2). Moreover, wheat flour gelatinization decreases (C3–C2) and the stability of the hot-formed gel increases (C3–C4) [61].

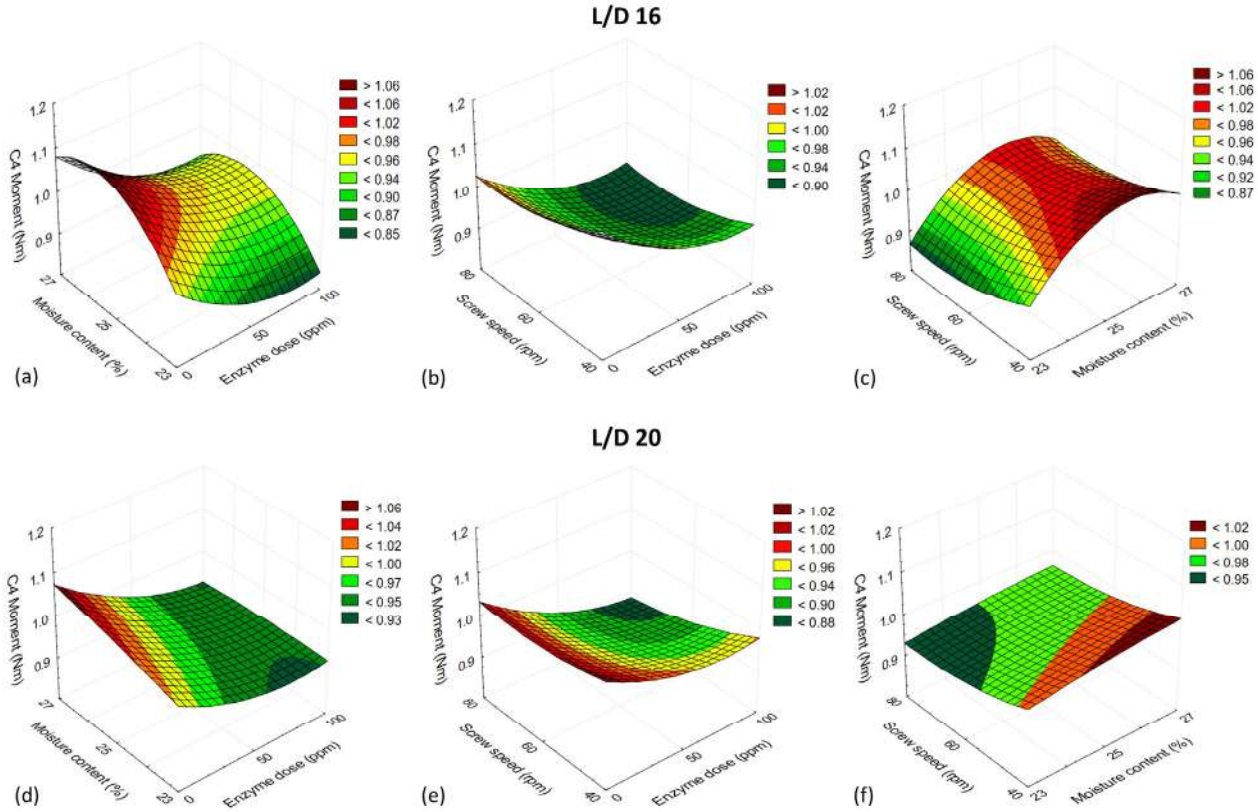

Figure 7. Processing variables and L/D configuration effects on the C4 of extruded wheat flour amended with xylanase enzyme: L/D 16: (**a**) E × M, (**b**) E × S, (**c**) M × S; L/D 20: (**d**) E × M, (**e**) E × S, (**f**) M × S. C4—amylase activity; E—enzyme dose; M—moisture; S—screw speed.

The C5 value indicates retrogradation tendency (as a starch gelling torque) and it was evident that the extrusion treatment lowered retrogradation tendency after cooling as compared to native flour (2.52 Nm). This outcome can have a positive effect on bread stability if extruded flour is added as a "clean label" water hydration improver, and limitation of bread staling with this additive is expected. In the cereal industry, hydrothermal and dry heat treatment processes are applied mainly for extending the shelf life of flours

or products (normally 3–9 months for wholegrain wheat flour and 9–15 months for white wheat flour—this may be extended by at least 3 months after treatment) and modifying the techno-functional properties of the flours during food production. The C5 values of the treated NSP-rich wheat flour are presented in Figure 8. As shown in Figure 8, increased levels of added xylanase decreased C5 values and lowered retrogradation tendencies in the hybrid enzymatic-extruded modified wheat flour. The C5 values presented in Figure 8a,b indicate a lowered retrogradation tendency with increasing enzyme level after NSP-rich wheat flour extrusion, and that this effect is not dependent on the extruder configuration. Slightly lower values were obtained after extrusion via the shorter L/D 16 configuration. This is especially noticeable when analyzing the combined effect of screw speed and feed moisture content (Figure 8c,f). For both configurations, the adequacy of the models describing C5 was demonstrated by high R^2 results and statistically significant F test results. On average, the C5 values were slightly higher for the L/D 20 extruder configuration (Figure 8). Strong correlation coefficients were found between C5 and C4 values for both extruder configurations applied (r values were 0.814 for L/D 16 and 0.767 for L/D 20). Additionally, C5 values were negatively correlated with extrusion pressure, with similar r values obtained for both L/D configurations (r = −0.649 and −0.642, respectively).

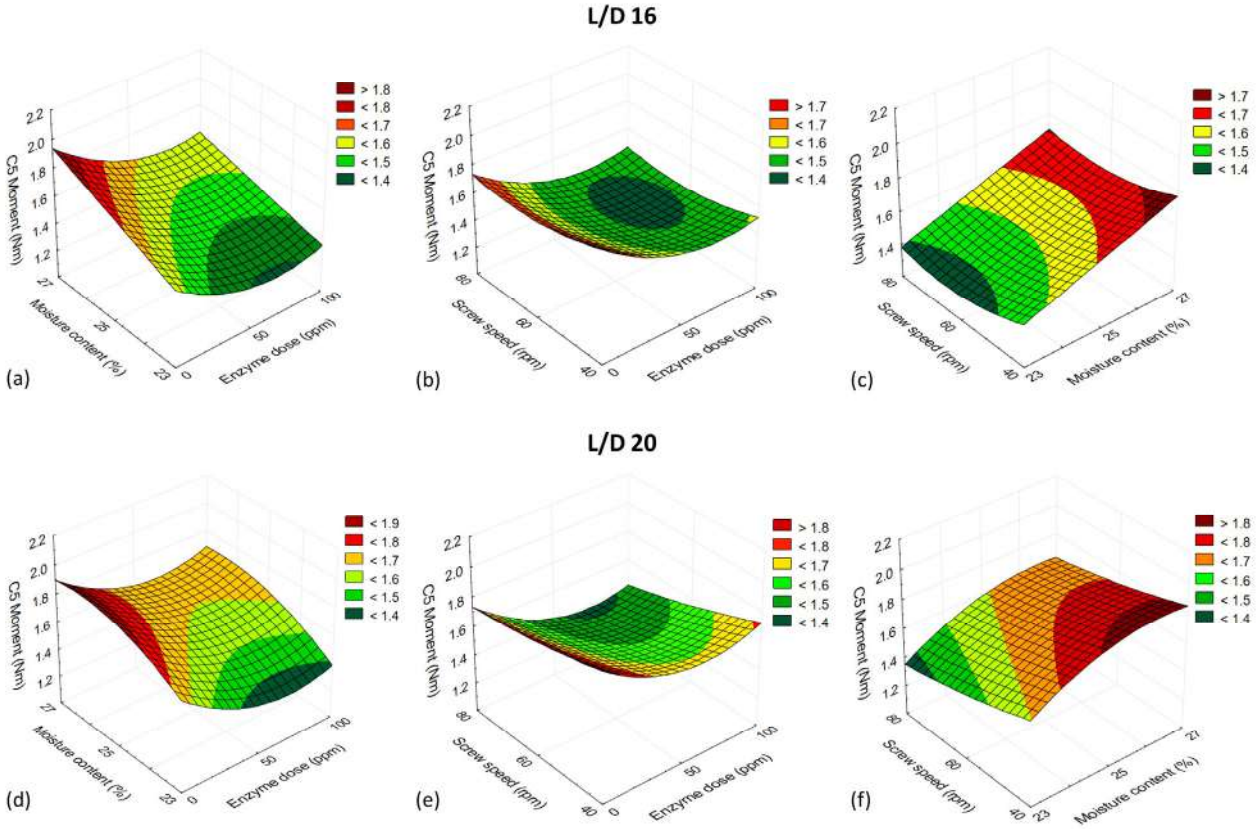

Figure 8. Processing variables and L/D configuration effects on the C5 of extruded wheat flour with xylanase enzyme: L/D 16: (**a**) E × M, (**b**) E × S, (**c**) M × S; L/D 20: (**d**) E × M, (**e**) E × S, (**f**) M × S. C5—starch retrogradation; E—enzyme dose; M—moisture; S—screw speed.

Slope α, between the end of the 30 °C period and C2, can be designated as the speed of the protein weakening (protein breakdown) (α, °) under the heating effect. Moreover, slope β, between C2 and C3, serves as an indicator of pasting (gelatinization) speed (β, °), and slope γ, between C3 and C4, represents the enzymatic (α-amylase) degradation speed (cooking stability rate) (γ, °) [32,70]. The greater angle of a slope indicates more intensive changes [41]. Results of measured values of slopes are presented in Tables 5 and 6, respectively, if L/D 16 and 20 was applied for wheat flour processing. The α slope of the

modified flour was not susceptible to changes in the tested factors, M, S or E, in either extruder configuration (L/D 16, L/D 20) (Table 7). In the case of the β angle values, a significant negative linear relationship with feed moisture M was obtained for the L/D 20 configuration, which indicates that the β slope decreases with the increase in M, and a positive relationship with the square of screw speed S were noted (Table 7). However, when the short extruder version L/D 16 was used for wheat flour processing, the β angle was not susceptible to initial moisture (M), screw speed (S) or enzyme dose (E) factor changes (Table 7). The values of this slope (Tables 5 and 6) were correlated with the C3 results of modified flour with r values of 0.684 and 0.644 for L/D 16 and 20, respectively. Similarly, the γ angle values, established between C3 and C4, obtained during the testing of the enzymatic-extruded wheat flour, were not susceptible to changes in the initial moisture (M), screw speed (S) or enzyme dose (E) processing variables when the L/D 16 extruder configuration was utilized (Table 7). If the elongated L/D 20 configuration was employed, significant positive linear dependences between the γ angle and M and S were obtained, which indicates that the γ slope increased with the increases in feed moisture M and screw speed S. A significant negative quadratic trend between the γ angle and enzyme dose E was also established. This parabolic relationship seems to have a stronger impact on the variability of γ in relation to the other variables (Table 7, coefficients further from zero).

The addition of xylanase enzyme may have effects on the handling properties of dough, the ovenspring and the bread volume. Hilhorst et al. [22] reported that the addition of xylanase to the dough increased loaf volume and improved the crumb structure of the baked product, but made the dough more sticky and less firm. Furthermore, staling was retarded. This effect has been ascribed to the redistribution of water from hemicellulose to gluten, which would render the gluten more extensible. Andersson et al. [13], in turn, reported the significant effects of screw speed, temperature and water content on oligosaccharide content, with the highest extractability found at high screw speed, high temperature and low water content. They also noted the significant effect of the interaction between screw speed and water content, with a more pronounced effect of screw speed at the lowest water content. The yield of oligosaccharides was significantly improved by traditional extrusion and enzymatic extrusion, which was mainly ascribed to extrusion-induced damage to the cell walls in the fiber-rich raw materials.

4. Conclusions

Low-temperature enzyme-assisted extrusion of NSP-rich wheat flour is possible with various extruder configurations and processing variables. The results show that the obtained extruded NSP-rich flour properties were significantly affected by processing conditions, especially the initial feed moisture and the screw speed applied during processing. The application of various extruder configurations and screw profiles demonstrated significant effects on both the wheat flour's processing behavior and rheological characteristics. The longer L/D 20 extruder configuration with a screw profile with mixing elements obtained a modified flour with lower extrusion pressure and energy requirements than the L/D 16 version. The results obtained for the extruded flours showed differences in water absorption, as well as in rheological characteristics. The extruded wheat flour was characterized by improved hydration properties (by 12.9% for L/D 16 and 14.4% for L/D 20 as compared to native flour) and limited retrogradation tendency (by 28.6% for L/D 16 and 24.6% for L/D 20 as compared to native flour). Moreover, the lower initial moisture level (23%) induced a higher hydration ability. The most significant differences were observed in C2 (protein weakening) if different configurations of the plasticizing unit L/D 16 or L/D 20 were used. The increased enzyme amount limited the negative effects of extrusion treatment on C2. Furthermore, starch gelatinization (C3) was lower when the enzyme was added prior to extrusion, and amylase activity (C4) decreased with increased xylanase addition. The addition of xylanase enzyme at the levels of 50 and 100 ppm in extruded wheat flour also limited C5—retrogradation tendency (by 8–24% for L/D 16 and 8–23% for L/D 20)—as compared to extruded wheat flour; hence, enzymatic-extruded flour used

as an additive in flour bread can elongate bread shelf life. Statistical analysis confirmed that feed moisture and screw speed were variables with the most significant effects on extrusion-modified wheat flour features. The most important factors from the point of view of the quality of the extruded NSP-rich wheat flour and its further use in bread flour mixtures should be low extrusion energy requirements, improved hydration properties and limited retrogradation tendency. So, the recommended single-screw extrusion conditions are 23% feed moisture, 40 rpm screw speed and 100 ppm enzyme addition before processing with an elongated L/D 20 extruder configuration utilizing a screw with mixing elements. Flour processed in these conditions may have a positive impact on the possibility of using modified wheat flour rich in NPS as a "clean label" improver in bakery products.

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P3. Piotr Lewko, Agnieszka Wójtowicz, Michał Rudaś: Effect of processing conditions of enzymatic, extrusion and hybrid treatment methods on composition and selected techno-functional properties of developed wheat flour. International Journal of Food Science (**w recenzji; IF 2,70, 100 pkt. MNiSW**)

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Effect of processing conditions of enzymatic, extrusion and hybrid treatment methods on composition and selected techno-functional properties of developed wheat flour

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Abstract

In this study, wheat flour characterized by a high content of non-starch polysaccharides was fortified with enzymes and then subjected to low temperature (up to 85 °C) extrusion-cooking treatment. Conventional enzymatic hydrolysis with cellulase and cellulase-xylanase blend, as well extrusion and hybrid enzymatic-extrusion treatments were tested under variable conditions. Extrusion of wheat flour was applied at 23-27% initial moisture at the temperature range of 40-80°C. Proximate composition, polysaccharides content and its fractions, as well rheological and techno-functional properties, were tested. Extruded and hybrid-modified wheat flour showed significant decrease in fat, ash, insoluble fibre content, gelatinization beginning temperature, dough stability, starch gelatinization, amylase activity, starch retrogradation and gluten performance index, whereas increased hydration capacity, max viscosity, setback, protein weakening and solvent retention capacity was evidenced in the presence of all tested solvents. Soluble and insoluble fractions of non-starch polysaccharides were, however, significantly different, especially if the hybrid cellulase-xylanase-extrusion method was applied to wheat flour. Moreover, crystalline structure of wheat flour changed significantly after extrusion and hybrid treatments. In addition, microstructure showed significant agglomeration of the extruded flours due to starch gelatinization and formation of melted phase in all extruded and hybrid treated flours, with visible fibrous particles coming from outer layers of wheat grains as polysaccharides fractions. Extruded wheat flour, characterized by increased viscosity, hydration and solvent retention ability, can be used as a "clean label" improver in mixtures for various bakery products, especially bread.

1. Introduction

The extrusion-cooking process is increasingly used in many fields of food, feed or biopolymers processing [1,2]. Controlling the effect of the extrusion-cooking conditions on the changes in basic and functional components, as well as physical properties and texture, is still a big challenge [3,4]. The inclusion of health-promoting and functional components and valuable

by-products may easy improve the nutritional potential of the extruded products [5,6]. The extrusion process has been studied for decades for its potential in the processing various cereals or plant-based products. Extrusion processing brings about protein and starch structure changes and gelatinization of starch granules [7,8], and is widely used to develop infant foods, snack foods, ready-to-eat breakfast cereals, modified starches, meat analogues, pet foods, among other products [9-15]. During extrusion, the thermomechanical action may cause denaturation of proteins, and alteration of the protein structure and solubility because of the combining effect of heat, shear force, pressure and oxygen [16, 17]. Extrusion of flours produces gelatinized, melted or fragmented starch with increased damaged starch content, together with a reduction in lipid oxidation due to inactivation of enzymes, an increase in soluble fibre, protein fragmentation, and a possible reduction in thermolabile vitamins, antinutritional factors and microbial load [1]. The disruption of the starch granules by gelatinization occurring during extrusion also makes starch more accessible and susceptible towards enzymatic hydrolysis afterwards - leading to a more intensive solubility [18]. These changes allow for the adjustment of its rheological and hydration properties in response to arising needs demanded by new food trends. Extruded wheat flours may therefore be an interesting alternative to chemically pregelatinized (hydroxypropylated or cross-linked) starches or hydrocolloids in the bakery industry. Extrusion-cooking have been widely reported in the processing of bran to change the rheological and chemical characteristics of fibrous fractions e.g. in wheat bran [19,20], corn bran [21], rice bran [2,22]. Blasting extrusion processing (BEP) has also been developed so as to modify wheat bran by enabling partial disruption of its cellulose and hemicellulose, as well as to bring about the release of its soluble saccharides from its original continuous fibre matrix [23].

Some researchers have attempted to apply combined or hybrid methods together with extrusion-cooking to modify selected components by the addition, among others, of specific chemicals [18,21,24,25], microwaving [26], ultrasound treatment [27] or enzymatic digestion [22]. Zhou et al. [18] investigated ethanolic-assisted extrusion of corn starch and reported destruction and reorganization crystalline structure during extrusion, with significant positive effect of ethanolic extrusion to obtain cold water-swelling starch. Enzymatic extrusion is a quite new method, in which the extruder is used as a continuous bioreactor or enzyme reactor to accelerate the enzymatic reaction [28]. Enzymatic-supported extrusion treatment can effectively act on complex biopolymers with a high degree of polymerization, crystallinity and structural strength, and can impose a porous microstructure and expose the reactive site of the enzyme [2]. Some researchers have employed enzymatic-extrusion to modify cereal bran or fibre rich fractions [29]. Dang & Vasanthan [22], for example, found that application of both enzyme treatment and extrusion improved the solubility of rice bran dietary fibre and other soluble components, especially as the sequential extrusion-enzyme treatment significantly increased the total soluble pentosan content, compared to individual or simultaneous treatments. Kong et al. [30], in turn, tested the effect of co-modification by extrusion and enzymatic hydrolysis (with cellulase, xylanase, high-temperature α-amylase, and acid protease) on water extractable arabinoxylan and physicochemical properties of black wheat bran. Depending on the type of enzymes, its level and activity or additional processing and its treatment order, with regard to wheat flour, its functional polysaccharides may be diversely modified.

The use of enzymes in baking technology is a very common practice. They are responsible for the improvement of dough machinability and bakery product quality. Significant improvements can be obtained by adding enzymes to flour due to slowing down of the crumb firming. Among others, these include increase in bread volume, improvement in crispness and

colour of the crumb crust, better sensory characteristics and increased shelf-life of products [31]. Different types of enzymes can be applied to change the selected fractions of cereals and plants, i.e. as proteases to hydrolyse rice proteins [32], amylases to enzymatic saccharification of various starch origins [33], or cellulase and hemicellulases to fibrous fractions modification [34]. At present, amylase, cellulase and protease are used in enzymatic extrusion playing role in liquefying starch, producing bioethanol and improving dough quality [2]. Cellulases are extremely important enzymes both industrially and in the natural world, because they play a major role in the global carbon cycle by degrading insoluble cellulose to soluble sugars. Xylanase, for example, are cellulase enzymes are used in the food industry in the bakery sector to enhance the stability of dough, to generate a softer and uniform crumb structure, and to increase the specific volume of breads. Through the action of xylanase, the contained water becomes distributed from the pentosan phase to the gluten phase, thus amplifying volume via increased extensibility of the gluten due to the rise in gluten volume fraction. Additionally, xylanases have been discovered to delay staling, improve the texture of high fibre bread, and balance out the variable quality of flour used in baking wheat bread. Xylanases are required to act collectively to hydrolyse xylans (e.g., arabinoxylans and glucuronoxylans) due to the structural heterogeneity and complexity of xylans and the specificities and modes of catalytic action of xylanases [35,36].

Non-starch polysaccharides (NSP) and arabinoxylans (AX) are interesting cereal components. These are cereal fibre compounds found predominantly in the cell walls of the endosperm and aleurone layer in cereal grains. Among the cereal grains (and grinding processes), arabinoxylan content differs significantly at 7.6–12.1% in rye, 4.8–7.6% in wheat, 4.4–8.1% in barley and 1.7–2.7% in oat [37]. Arabinoxylans are an important dietary fibre source due to their contained functionalities and health benefits, including cholesterol lowering activity, faecal bulking effect, prebiotic effects, high antioxidant activity, vitamins, minerals, and the presence of other bioactive components [36,38,39]. Kaur et al. [40] reported two main types of arabinoxylans: water extractable arabinoxylans WE-AX (one-third) and water unextractable WU-AX (two-thirds). These are characterized by different composition, as well as action to water and functionality, e.g. WE-AX are semi-flexible and highly soluble in water due to high glucose and arabinose substitution, they stabilize gas pores, slow down bread staling and improve dough quality by increasing water absorption and bread volume; but WU-AX are more rigid and insoluble due to the presence of cellulose and hemicellulose fractions [27]. The AXs are the major polymers present in the cell wall of wheat grain and exhibit different physicochemical properties (hydration properties, water solubility, viscosity). Moreover, these properties or its structure may change through technological treatments.

The objective of our study was to evaluate the effects of enzyme addition, low-temperature extrusion-cooking and hybrid enzymatic-extrusion treatment on particular properties of developed wheat flour consisting of selected breaking, milling, reducing and sifting passages. Treatments were selected as individual or hybrid, with various types and levels of enzymes (cellulase and xylanase) under variable extrusion conditions. Non-treated native flour (F), enzyme fortified flour (FC and FCX) and extruded samples treated without (EF) or with the presence of enzymes (EFC and EFCX) were tested for proximate chemical composition, non-starch polysaccharides and arabinoxylans contents and composition, pasting properties, rheological and techno-functional characteristics, as well solvents retention capacity and gluten performance. Structural changes were observed via X-ray diffraction analysis and on the basis of microscopic pictures taken via scanning electron microscopy.

2. Materials and Methods

2.1. Raw materials and enzymes

New flour blend (F) from common wheat of the Laudis variety was employed for the tests. This consisted of blends subject to selected breaking, milling, reducing and sifting passages, composed in accordance with our previous research [41] as a raw material suitable for the production of wheat bread, characterized by an average gluten content of 31%, ash 0.72%, descent time 340 s, and average water absorption 60%. Commercially available baking enzymes were used to fortify the flour (the amount of enzyme being determined based on preliminary tests and suggestions of the enzyme manufacturer). These included: Bakezyme® WholeGain - cellulase from *Trichoderma reesei* (DSM Food Specialities B.V., Delft, The Netherlands) with declared enzyme activity 1,475 EGU/g, and VERON 292 - xylanase from *Aspergillus niger* (AB Enzymes GmbH, Darmstadt, Germany) with declared enzyme activity min 1701 XylH/g. Two combinations of enzymatic modification were investigated: the Bakezyme® WholeGain cellulase enzyme was used in an amount of 120 ppm (samples marked C), and the mixture of Bakezyme® WholeGain cellulase and VERON 292 xylanase were employed in amounts of 60 ppm and 50 ppm, respectively (samples marked CX). Wheat flour was fortified with these enzymes by gentle mixing of flour with dry enzymes in a powdered form and left for 0.5 h in a room temperature to initiate the enzymes activity. The fortified flours were then tested (FC and FCX) or subjected to extrusion processing under variable conditions. Experimental design with selected variables and coded samples is presented in Table 1.

Table 1. Experiment design and variables coded.

Sample	Treatment	Enzyme type	Enzyme content (ppm)	Moistening level (%)
F	native	-	-	-
FC	native	Cellulase	120	-
FCX	native	Cellulase+Xylanase	60+50	-
EF23	extruded	-	-	23
EF25	extruded	-	-	25
EF27	extruded	-	-	27
EFC23	extruded	Cellulase	120	23
EFC25	extruded	Cellulase	120	25
EFC27	extruded	Cellulase	120	27
EFCX23	extruded	Cellulase+Xylanase	60+50	23
EFCX25	extruded	Cellulase+Xylanase	60+50	25
EFCX27	extruded	Cellulase+Xylanase	60+50	27

F - flour, E - extrusion treatment, C - cellulase enzyme, X - xylanase enzyme;

2.2. Extrusion process

The extrusion-cooking processing of flour without (EF) or with different enzyme combinations (EFC and EFCX) was carried out using an Evolum25 co-rotating twin-screw extruder (Clextral, Firminy, France) of L/D=24 configuration with screws of 25 mm in diameter and with single open die with a diameter of 3 mm. Twin screw configuration consisted 32 modules on one grooved shaft, with the total length of 24 D, with 3.75D supply modules, 12.5D transport modules, 1.5D mixing elements, 2D mixing with groove elements, 4D compression modules and 0.25D interface ring, set in a certain order. Feeding rate was maintained at 10 kg/h by means of a Brabender volumetric gravity feeder, while screw speed was set at 400 rpm throughout the experiments. The level of feed moisture was set to 23, 25 and 27% as calculated based on the initial moisture of flour, while water in proper amounts (1.2, 1.5 and 1.8 l/h,

respectively) was pumped by a water pump directly to the second zone of the extruder barrel. During the extrusion process, the temperatures in individual sections of the extruder, the product temperature and the working pressure inside the barrel were monitored. The extruder utilizes electrical resistance heaters to create six heating/cooling zones with electrical resistance heaters monitored by thermocouple sensors. Processing temperature was set as follows: 40/50/60/65/70/80 °C, forming die temperature set at 85 °C, product temperature measured 82 - 95 °C (increasing with lower feed moisture), pressure on the die measured 80 – 102 bar (decreasing with higher feed moisture). Extrudates were cut with a two-blade knife working with speed of 300 rpm. Samples were collected when the extruder was working stably. The samples obtained after extrusion were dried at 40 °C in a laboratory shelf dryer to ensure safe storage below 12% moisture content, and were ground in a laboratory grinder (TestChem, Radlin, Poland) to particle size below 500 μm and stored for further analyses. The effects of treatment conditions on selected characteristics were tested on dry flour, diluted flour suspension or on the dough matrix to compare the physiochemical characteristics of modified flours.

2.3. Proximate chemical composition

Proximate chemical characteristics were determined for protein (Nx6.25; AACC 46–10 method), fat (AACC 30–10 method), and ash (AACC 08–01 method) contents [42]. The 991.43 enzymatic-gravimetric method [43] was applied to evaluate the content of dietary fibre (TDF) and its soluble (SDF) and insoluble (IDF) fractions.

2.4. Non-starch polysaccharides and arabinoxylans profile

The content of non-starch polysaccharides (NSP) was assessed by gas chromatography according to Englyst and Cummings [44]. The total NSP (T-NSP) content is the amount of sugars: arabinose, xylose, mannose, galactose and glucose according to AACC standard procedure 32-25 and AOAC 994.13 [42,45]. This analysis allowed to separate non-starch polysaccharides for soluble (S-NSP) and insoluble (I-NSP) fractions, and to determine the qualitative and quantitative composition of polysaccharides in both fractions. Total arabinoxylans content (T-AX) and insoluble (I-AX) and soluble (S-AX) fractions were calculated based on the content of each fraction. After acid hydrolysis of soluble and insoluble fractions monosaccharides were detected in each fraction. The obtained hydrolysates were converted into volatile alditol acetates. To each sample (1 ml) 2 drops of 2-octanol, 0.26–0.28 ml of 12M ammonia solution and 0.1 ml of sodium borohydride solution in ammonia (100 mg BH_4 in 1 ml of 3M NH_4OH) were added. After 40 minutes of incubation at 40 °C, 0.1 ml of glacial acetic acid was added to the hydrolysate, mixed, and then 0.2 ml of 1-methylimidazole and 2 ml of acetic anhydride were added to 0.2 ml of the collected sample. The prepared solution was cooled for 30 minutes, then 4 ml of distilled water and 1.15 ml of dichloromethane were added and shaken for 1 minute. The aqueous phase was removed and the organic phase was analyzed on an Autosystem XL gas chromatograph from Perkin Elmer (Shelton, CT, U.S.), equipped with an autosampler, a split injector, a flame ionization detector (FID) and an Rtx-225 capillary quartz column (0.53 mm × 30 m). Chromatograph operating parameters: carrier gas helium, flow 2 ml/min, injector temperature 275 °C, detector temperature 275 °C. Column temperature program: initial temperature 185 °C, 1 minute; increase 5°C/min to 215 °C; isotherm 215 °C, 10 minutes [46].

2.5. Rheological properties of dough

Rheological properties of dough prepared from the tested native and modified flours were studied using a Chopin Mixolab (Chopin Technologies, Villeneuve-la-Garenne, France) based on the Chopin+ flour protocol with the following settings: mixing speed - 80 rpm, total analysis time - 45 min, dough weight - 75 g, hydration water temperature 30 °C. Flour and water were added accordingly to obtain a dough with a maximum consistency of 1.10 Nm (± 0.05) during the first test phase. Mixolab test was performed using a standard protocol: 8 min at 30 °C, heating for 15 min at a rate of 4 °C/min, holding at 90 °C for 7 min, cooling for 10 min to 50 °C at a rate of 4 °C/min and holding at 50 °C for 5 min. The following rheological features were tested via Mixolab: water absorption (Hyd), protein weakening (C2), starch gelatinization (C3), amylase activity (C4), starch retrogradation (C5) [47].

2.6. Pasting properties

Pasting properties were measured by employing a Brabender Viscograph-E (Brabender GmbH & Co. KG, Duisburg, Germany) at 75 rpm and 700 cmg torque according to ICC 169 procedure [48]. Slurries made up of 80 g flour (adjusted to 14% moisture content) and 450 ml distilled water were first added in the Viscograph-E canister. The canister was then placed in the heating chamber and spindles attached. The slurry was heated from 30 °C to 93 °C at a rate of 1.5 °C/min; held at 93 °C for 15 min; cooled to 50 °C at a rate of 3 °C/min; and finally held at 55 °C for 15 min. Resistance to stirring was recorded as viscosity in Brabender Units (BU). The beginning and end of gelatinization temperature (°C), maximum viscosity (BU), trough viscosity (BU), final viscosity (BU), breakdown (max viscosity minus trough viscosity, BU), setback (final viscosity minus trough viscosity, BU) were determined using the Viscograph-E software.

2.7. Hydration properties

Solvent Retention Capacity (SRC) tests were performed according to the AACC approved method 56-11 [42,49]. SRC is the weight of solvent retained by the swollen flour deposit after centrifugation and is expressed as a percentage of the original flour weight (adjusted to 14% moisture). Solvents were: deionized water (Wa), 50 wt% sucrose in water (Su), 5 wt% lactic acid in water (La), 5 wt% sodium carbonate in water (Sc). In this test, a flour sample (5 ± 0.05 g) was transferred to a 50 mL centrifuge tube and mixed with 25 g of solvent. In the next step, the sample was left to solvate for 20 minutes with shaking every 5 minutes for 5 seconds. The tubes were then centrifuged at 2500 rpm (646 g force) for 15 min in an Eppendorf 5702 centrifuge (Eppendorf AG, Hamburg, Germany).The supernatant was poured off and the tubes were allowed to dry for 10 minutes. Sample was weighed and the water retention capacity (WaSRC), sucrose solvent retention capacity (SuSRC), lactic acid solvent retention capacity (LaSRC), sodium carbonate solvent retention capacity (ScSRC) were calculated [50]. Additionally, gluten performance index (GPI) was determined as described by Rani et al. [49] based on the ICC method by dividing the LaSRC value by the combined values of SuSRC and ScSRC.

2.8. Structure analysis with X-ray diffractometer

Powdered samples were conditioned by 3 days at controlled temperature (19 °C) and at a constant relative humidity (28%) and placed in an autosampler. The X-ray diffraction (XRD) method was applied utilizing a high-resolution Empyrean powder diffractometer (PANalytical,

Almelo, The Netherlands) with Cu Kα1 radiation (λ = 1.54178 Å). Samples were measured over a range from 5 to 70° at diffraction angle geometry θ–2θ, with a counting time of 400 s per data point and a step size of 0.01° [51]. Obtained results were baselined and differences in the structure were analysed at specific peak angles. Crystallinity was determined using WAXFIT software [52]. All data were normalized and background was approximated by hyperbolic function. Data for native flours were fitted to a Gauss–Cauchy model containing 15 functions to describe crystalline phase and 2 functions to describe amorphous phase. Extruded flours data were fitted to a Gauss–Cauchy model containing 13 functions to describe crystalline phase and 2 functions to describe amorphous phase. The degree of crystallinity (%) was calculated via WAXFIT software after fitting models and correcting backgrounds as a ratio between surface areas under the curves of the crystalline phase to the sum of the crystalline and amorphous phases [53,54].

2.9. Microstructure characteristic by SEM

The microstructure of native and modified flours was observed with a scanning electron microscope Vega Tescan LMU (Tescan, Czech Republic). Powdered samples were mounted on aluminium specimen stubs with double-sided adhesive silver tape and sprayed with gold using Sputter Coater Emitech K550X (Emitech, United Kingdom). The accelerating voltage of the SEM was 20 keV, with absorption currency 9 pA. SME pictures were taken with magnifications x600 and x2000 [9].

2.9. Statistical analysis

All analyses were performed in triplicate and results are expressed as average values ± standard deviation. Data were subjected to one-way analysis of variance (ANOVA) using Statistica 13.3 software (StatSoft, Inc., Tulsa, OK, USA) followed by the Tukey's post hoc test to compare means at the 0.05 significance level. Homogenous groups were indicated with similar letters at significance level 0.05. The correlation matrix with Pearsons' correlation was assessed between all the tested properties, and significant coefficients were found using Statistica 13.3 software (StatSoft, Inc., Tulsa, USA) within the 95% confidence interval.

3. Results and Discussion

3.1. Proximate composition

Proximate chemical composition of untreated, extruded, enzyme fortified and hybrid treated wheat flours is presented in Table 2. Protein content of native wheat flour F was at 14.65% and similar results were noted if fortification with enzymes was applied (14.52-14.64%). Extrusion and hybrid enzymatic-extrusion treatment with combined CX blend increased or remain unchanged protein content in the modified flours (except of EFC25 sample) as compared to native wheat flour F. As opposed to our results, Zhou et al. (2010) found lowering the content of protein (9.85%) in xylanase-extracted de-starched wheat bran and with alkali treatment (4.10%) against of 18.4% in native bran. Moreno-Rivas et al. [55] also found that xylanase addition had a reduction effect on the proteins solubility in extruded nixtamalized corn flour, probably by the interaction with arabinoxylans.

Table 2. Proximate chemical composition of untreated, extruded and hybrid treated wheat flours.

Sample	Protein (%)	Fat (%)	Ash (%)	IDF (%)	SDF (%)	TDF (%)
F	14.62 ± 0.06 ^{e}	1.31 ± 0.01 ^{b}	0.72 ± 0.02 ^{f,g}	3.94 ± 0.04 ^{b}	2.86 ± 0.02 ^{b}	6.80 ± 0.03 ^{c}
FC	14.52 ± 0.03 ^{e}	1.39 ± 0.02 ^{a}	0.74 ± 0.02 ^{d,e,f}	3.81 ± 0.03 ^{c}	2.33 ± 0.02 ^{e}	6.14 ± 0.05 ^{d}
FCX	14.64 ± 0.01 ^{e}	1.30 ± 0.01 ^{b}	0.73 ± 0.01 ^{e,f,g}	4.10 ± 0.03 ^{a}	2.56 ± 0.03 ^{c}	6.66 ± 0.06 ^{c}
EF23	14.66 ± 0.03 ^{d,e}	0.16 ± 0.02 ^{e}	0.76 ± 0.01 ^{d,e,f}	3.08 ± 0.03 ^{f}	2.42 ± 0.02 ^{d}	5.50 ± 0.03 ^{g}
EF25	14.80 ± 0.03 ^{b}	0.29 ± 0.02 ^{c}	0.82 ± 0.02 ^{a,b,c}	3.79 ± 0.03 ^{c}	1.83 ± 0.02 ^{h}	5.53 ± 0.15 ^{g}
EF27	14.76 ± 0.02 ^{b}	0.21 ± 0.01 ^{d}	0.77 ± 0.02 ^{d,e}	3.06 ± 0.03 ^{f}	2.24 ± 0.02 ^{f}	5.30 ± 0.02 ^{h}
EFC23	14.67 ± 0.02 ^{c,d,e}	0.23 ± 0.01 ^{d}	0.78 ± 0.02 ^{b,c,d}	3.37 ± 0.03 ^{e}	2.57 ± 0.02 ^{c}	5.94 ± 0.01 ^{e}
EFC25	14.17 ± 0.03 ^{g}	0.20 ± 0.02 ^{d,e}	0.85 ± 0.02 ^{a}	3.43 ± 0.02 ^{e}	2.08 ± 0.02 ^{g}	5.50 ± 0.03 ^{g}
EFC27	14.66 ± 0.03 ^{d,e}	0.16 ± 0.02 ^{e}	0.77 ± 0.02 ^{d,e}	4.18 ± 0.03 ^{a}	2.93 ± 0.03 ^{b}	7.12 ± 0.02 ^{b}
EFCX23	14.73 ± 0.03 ^{b,c,d}	0.29 ± 0.03 ^{c}	0.78 ± 0.02 ^{c,d}	3.62 ± 0.03 ^{d}	4.12 ± 0.03 ^{a}	7.74 ± 0.04 ^{a}
EFCX25	14.76 ± 0.03 ^{b,c}	0.22 ± 0.02 ^{d}	0.70 ± 0.02 ^{g}	2.91 ± 0.04 ^{g}	2.61 ± 0.03 ^{c}	5.52 ± 0.01 ^{g}
EFCX27	14.90 ± 0.02 ^{a}	0.30 ± 0.03 ^{c}	0.82 ± 0.02 ^{a,b}	3.13 ± 0.03 ^{f}	2.56 ± 0.05 ^{c}	5.70 ± 0.02 ^{f}

F - flour, E - extrusion treatment, C - cellulase enzyme, X - xylanase enzyme; TDF – total dietary fibre; SDF – soluble dietary fibre; IDF insoluble dietary fibre; n=3

$^{a-g}$ – means indicated with similar letters in columns do not differ significantly at α=0.05

We noted that fat content decreased significantly after extrusion treatment of flour, both without or with enzymes added. This is due to formation of lipid-starch complexes under extrusion conditions - as reported by many authors [1,4,12,24]. Ash content in the tested samples do not differ significantly if flour was fortified with both enzymes (FC and FCX) and was lower than in samples after extrusion. Moreover, cellulase addition significantly lowered TDF (6.14%) in the untreated flour (FC), especially the SDF fraction (2.33%) as compared with native flour F (6.80% and 2.86%, respectively). Extrusion without enzymes at variable feed moisture lowered TDF content (5.30-5.53%), in both IDF (3.06-3.79%) and SDF (1.83-2.42%) fractions. When the hybrid enzymatic-extrusion method was applied, the fibre content in treated flour varied depending on processing conditions. Cellulase addition and the highest moisture (27%) increased TDF content in the treated flour (7.12%), both soluble (2.93%) and insoluble (4.18%) fractions. The opposite effect was noted if enzymes blend CX was added to flour and then subjected to extrusion – here, the lowest feed moisture (23%) in sample EFCX23 significantly increased the formation of fibre (7.74%), especially the SDF fraction (4.12%) as compared to native flour (sample F). The lowest TDF was noted in EF samples extruded without enzymes addition without a strong effect of feed moisture. In almost all samples, content of IDF was lower after modification than in native flour, this may suggest SDF is more sensitive to enzymatic or extrusion treatment [30]. The conversion of IDF to SDF might be the effect of high shear and high temperature during extrusion treatment which possibly breakdown the larger molecules, leading to a decrease in TDF and IDF contents, but at the same time an increase in solubility of fibres [19]. Additionally, enzymes, like cellulase or xylanase, can modify SDF content and enzyme assisted extrusion was found as an effective treatment to increase oligosaccharides soluble fractions, for example in rice bran [2]. Ye et al. [56] tested various methods of wheat bran treatment and they reported fermentation and extrusion effects on increasing the soluble dietary fibre content in processed bran. Accordingly, extrusion treatment may increase fibre solubility as we noted that the water-binding capacities of extruded brans were lower than that of non-extruded wheat bran [57]. The results presented by Aktas-Akyildiz et al. [19] confirmed increased SDF content in bran samples subjected to enzymatic hydrolysis. Extrusion treatment conditions, especially the screw speed as the most effective parameter, showed that extrusion can disrupt the wheat bran microstructure and thus increase the soluble fibre content. Similar observations in improvement of dietary fibre solubility during extrusion of wheat bran were noted by other authors [57-59]. In our study, not bran - but developed wheat flour [41] was used in the experiment, so the results may be different due to the higher presence of starch and proteins in the flour. As stated by Alam et al.

[1], these are the components responsible for formation of complexes and starch gelatinization under extrusion, even if low temperature processing is applied.

3.2. Insoluble and soluble polysaccharides characteristics

The content and fractions of polysaccharides are very interesting with regard to their nutritional and technological roles. Cereal bran is rich in non-starch polysaccharides, mainly hemicellulose, including arabinoxylan (AX) and β-glucan, as well as cellulose and lignin [36,40]. Among these non-starch polysaccharides, AXs are important dietary fibre components. Wheat AXs are present in endosperm (3–5% of total endosperm), aleurone and bran cell walls (approximately 60–70% of the entire cell wall) [39]. If flour is not completely refined to white flour, the presence of polysaccharides may have positive impact on hydration, rheological and health-promoting properties. The tested flour used in the experiment was composed by Lewko et al. [41] from selected passages and is characterized by the high content of total AX (1.91%) and total NSP (3.40%) (Table 3).

Table 3. Non-starch polysaccharides and arabinoxylans content in untreated, extruded and hybrid treated wheat flours.

Sample	T-AX (%)	I-AX (%)	S-AX (%)	T-NSP (%)	I-NSP (%)	S-NSP (%)
F	1.91 ± 0.06^{a}	$1.31 \pm 0.04^{a,b}$	0.60 ± 0.02^{e}	3.40 ± 0.00^{b}	$2.06 \pm 0.01^{a,b}$	$1.34 \pm 0.00^{c,d}$
FC	$1.75 \pm 0.02^{a,b}$	1.13 ± 0.02^{c}	0.62 ± 0.01^{e}	$3.20 \pm 0.04^{c,d}$	$1.87 \pm 0.03^{d,e}$	$1.33 \pm 0.01^{c,d}$
FCX	$1.81 \pm 0.00^{a,b}$	$1.17 \pm 0.01^{a,b,c}$	$0.64 \pm 0.01^{e,d}$	$3.34 \pm 0.01^{b,c,d}$	$1.92 \pm 0.01^{c,d,e}$	$1.42 \pm 0.00^{b,c}$
EF23	$1.76 \pm 0.06^{a,b}$	1.12 ± 0.05^{c}	$0.64 \pm 0.01^{e,d}$	$3.27 \pm 0.05^{b,c,d}$	$1.87 \pm 0.00^{d,e}$	$1.40 \pm 0.05^{b,c}$
EF25	$1.75 \pm 0.07^{a,b}$	1.10 ± 0.02^{c}	$0.65 \pm 0.05^{e,d}$	$3.24 \pm 0.04^{c,d}$	1.85 ± 0.05^{e}	$1.39 \pm 0.01^{b,c,d}$
EF27	$1.81 \pm 0.04^{a,b}$	$1.20 \pm 0.02^{a,b,c}$	0.61 ± 0.02^{e}	$3.27 \pm 0.03^{b,c,d}$	$1.97 \pm 0.01^{b,c,d}$	1.31 ± 0.02^{d}
EFC23	$1.74 \pm 0.06^{a,b}$	1.14 ± 0.02^{c}	0.59 ± 0.03^{e}	$3.35 \pm 0.07^{b,c}$	$2.02 \pm 0.02^{a,b,c}$	$1.33 \pm 0.06^{c,d}$
EFC25	$1.75 \pm 0.06^{a,b}$	1.31 ± 0.06^{a}	0.44 ± 0.00^{f}	3.18 ± 0.04^{d}	2.14 ± 0.03^{a}	1.04 ± 0.01^{e}
EFC27	$1.84 \pm 0.13^{a,b}$	$1.16 \pm 0.12^{b,c}$	0.68 ± 0.01^{d}	$3.30 \pm 0.06^{b,c,d}$	1.83 ± 0.04^{e}	1.47 ± 0.02^{b}
EFCX23	$1.82 \pm 0.00^{a,b}$	0.88 ± 0.02^{d}	0.94 ± 0.01^{b}	3.64 ± 0.06^{a}	$1.91 \pm 0.01^{c,d,e}$	1.73 ± 0.05^{a}
EFCX25	1.73 ± 0.04^{b}	0.85 ± 0.04^{d}	0.88 ± 0.02^{c}	$3.29 \pm 0.04^{b,c,d}$	1.63 ± 0.02^{f}	1.66 ± 0.06^{a}
EFCX27	$1.84 \pm 0.05^{a,b}$	0.83 ± 0.07^{d}	1.01 ± 0.02^{a}	$3.25 \pm 0.12^{b,c,d}$	1.54 ± 0.11^{f}	1.70 ± 0.01^{a}

F - flour, E - extrusion treatment, C - cellulase enzyme, X - xylanase enzyme; T-AX – total arabinoxylans; I-AX – insoluble arabinoxylans; S-AX – soluble arabinoxylans; T-NSP – total non-starch polysaccharides; S-NSP– soluble non-starch polysaccharides; I-NS– insoluble non-starch polysaccharides; n=3

$^{a-e}$ – means indicated with similar letters in columns do not differ significantly at $\alpha=0.05$.

We observed that all the applied treatments lowered T-AX content by decreasing insoluble and increasing soluble fractions of AX with more significant effect of cellulase addition than by combined cellulase-xylanase hydrolysis on arabinoxylans. A similar tendency was observed regarding T-NSP content. In both fractions, significant increase was seen for soluble AX and NSP, with the most significant effect being that of hybrid EFCX treatment on the increase of soluble components in the modified wheat flour. Arabinoxylans (AX) are the major polymers in the cell wall of wheat grain, and therefore a major component of dietary fibre. In wheat, both soluble and insoluble fractions of polysaccharides are present, coming from the endosperm and outer layers [60]. AX fractions may be characterized by an arabinose to xylose ratio (A/X). Soluble fractions ratio of about 0.6 suggest more endosperm content in flour, whereas a ratio closer to 1 indicates that the polysaccharides come from the outer layers. We found in our F sample an I-A/X ratio at 0.723 and an S-A/X ratio at 0.842 - indicating the presence of bran fractions in the tested flour. The composition of S-AX and I-AX (Table 3) in the developed flour F used in our study, with more insoluble fractions of arabinoxylans and non-starch polysaccharides [41], clearly showed that most insoluble fractions come from the outer layers than from endosperm (I-AX 1.31% and S-AX 0.60% in wheat flour F). Both enzymatic hydrolysis, extrusion and hybrid enzymatic-extrusion treatments lowered the amounts of

insoluble fractions - mostly due to lowering of total arabinoxylans content. The most significant decrease of I-AX and increase in S-AX amount was visible after hybrid extrusion of wheat flour with combined enzymes, but without changing T-AX. This outcome suggests that the EFCX treatment method has the greatest effect on the solubility of AX, in comparison to a slight effect brought about due to extrusion conditions.

Zhou et al. [36] used xylanase and alkali treatment to extract arabinoxylans from wheat bran and they found a ratio of arabinose to xylose of 0.56 and 0.83, respectively, despite the molecular weight of alkali-treated AX being about 10 times that of xylanase-treated. Chen et al. [35] tested various methods of extraction of AX from triticale and they found that the cellulase-xylanase extraction method followed by alkaline extraction from the residues of complex enzyme extraction to be the most suitable for obtaining the highest yield of arabinoxylans (with a 1.52 index of A/X, however, this ratio is only 0.25 A/X if pure enzymatic extraction was applied). Ma et al. [61] state that enzymatic methods with endoxylanases are efficient for extracting water-unextractable AXs and that a combined cellulase-endoxylanase extraction gives higher AX yields than xylanase, while the double-enzymatic extraction of AX from fresh corn fibre was more efficient than the chemical methods reported by other authors. Chen et al. [20] observed in wheat bran containing water and fed into the extrusion unit, that the bonds of insoluble polysaccharides, for instance, cellulose and hemicellulose, and the continuous fibre matrix were partially disrupted and released soluble saccharides after extrusion due to the combination effects of high shear, turbulence and cavitation, along with temperature. A similar observation was reported by Arcila et al. [57] as the extrusion with lower feed moisture (15%) was more effective in converting unextractable hemicellulosic fractions to extractable polysaccharides and increasing the amount of soluble dietary fibre components in extruded wheat bran due to more abrasion and mechanical disruption. This effect is opposite to the use of high moisture (30%) where water acts as a plasticizer in the extruder barrel. Moreover, the use of a co-rotating twin screw extruder at 50 °C enhanced arabinoxylan (AX) solubilization at low water content (below 54 %), as compared to blade-mixing. Accordingly, extrusion enabled efficient enzyme action at low water content due to the enhanced diffusion of xylanase enzyme by the formation of a continuous mass in the extruder [62]. As found in Andersson et al. [63], the extractability of AX in wheat and rye bran increased depending on the extrusion parameters. They noted that the enhanced extractability of dietary fibre and arabinoxylan, in combination with maintained content of β-glucan in wheat and rye bran, makes the extruded bran more valuable as additive in the food industry. Beyond the aforementioned, Fadel et al. [58] reported extrusion cooking to be an innovative pre-treatment useful to increase the solubility of AXs.

In Table 4 and Table 5, detailed composition of insoluble and soluble non-starch polysaccharides in native and modified wheat flour is presented. As seen in the tables, higher content of soluble fractions of non-starch polysaccharides was observed for mannose and galactose, but glucose, arabinose and xylose higher content of insoluble fractions was observed, both in native and modified flour. The application of the hybrid method with combined enzymes (EFCX) showed strong decrease of I-Arabinose and I-Xylose (Table 4) and strong increase of soluble fractions of these polysaccharides (Table 5). When extrusion treatment was applied to wheat flour, all the results of S-A/X were lower than for unmodified F flour (Table 5), albeit, less important differences were noted for I-A/X ratio (Table 4). What is interesting, the addition of enzymes to wheat flour (FC and FCX samples) without other treatments increased the level of soluble fractions of all soluble polysaccharides (Table 5) as well as I-Mannose, while other insoluble components were also lower after enzymatic action (Table 4). In our study, T-NSP content was significantly correlated with SDF and TDF, with

correlation coefficients of 0.851 and 0.730, respectively. S-AX was also highly correlated with S-NSP (0.958) and I-NSP (-0.794) and with I-AX (-0.900), whereas for I-AX, slightly lower correlations were noted with S-NSP (-0.867) and I-NSP (0.827). Moreover, individual polysaccharide fractions showed correlations with protein content, for example, S-Arabinose (0.706), S-Xylose (0.678), S-AX (0.698), I-NSP (-0.662) and S-NSP (0.752). In addition, strong negative correlations were found between insoluble and soluble fractions of polysaccharides (Arabinose, Xylose and AX with values from -0.801 to -0.910).

Table 4. Insoluble non-starch polysaccharides and arabinoxylans content in untreated, extruded and hybrid treated wheat flours.

Sample	I-Mannose (%)	I-Galactose (%)	I-Glucose (%)	I-Arabinose (%)	I-Xylose (%)	I-A/X (-)
F	0.131 ± 0.002^{a,b}	0.078 ± 0.003^{a,b}	0.548 ± 0.024^{a,b}	0.549 ± 0.019^{a,b}	0.759 ± 0.019^{a}	0.723 ± 0.007^{a,b}
FC	0.133 ± 0.005^{a,b}	0.074 ± 0.002^{b}	0.535 ± 0.005^{a,b}	0.458 ± 0.027^{c,d}	0.674 ± 0.004^{a,b}	0.680 ± 0.044^{a,b}
FCX	0.142 ± 0.017^{a,b}	0.081 ± 0.007^{a,b}	0.528 ± 0.003^{a,b}	0.518 ± 0.020^{a,b,c}	0.651 ± 0.033^{b}	0.798 ± 0.072^{a}
EF23	0.129 ± 0.007^{a,b}	0.078 ± 0.003^{a,b}	0.541 ± 0.052^{a,b}	0.474 ± 0.020^{a,b,c}	0.647 ± 0.033^{b}	0.733 ± 0.007^{a,b}
EF25	0.144 ± 0.023^{a,b}	0.080 ± 0.005^{a,b}	0.522 ± 0.045^{a,b}	0.468 ± 0.009^{b,c,d}	0.634 ± 0.015^{b}	0.739 ± 0.003^{a,b}
EF27	0.120 ± 0.005^{b}	0.074 ± 0.001^{b}	0.573 ± 0.003^{c}	0.507 ± 0.007^{a,b,c}	0.694 ± 0.014^{a,b}	0.731 ± 0.005^{a,b}
EFC23	0.124 ± 0.005^{b}	0.077 ± 0.002^{a,b}	0.676 ± 0.004^{b}	0.459 ± 0.011^{c,d}	0.685 ± 0.035^{a,b}	0.671 ± 0.050^{b}
EFC25	0.162 ± 0.010^{a}	0.098 ± 0.011^{a}	0.565 ± 0.010^{c}	0.550 ± 0.022^{a}	0.760 ± 0.037^{a}	0.724 ± 0.007^{a,b}
EFC27	0.131 ± 0.014^{a,b}	0.069 ± 0.014^{b}	0.469 ± 0.052^{d}	0.516 ± 0.078^{a,b,c}	0.642 ± 0.043^{b}	0.800 ± 0.068^{a}
EFCX23	0.128 ± 0.001^{b}	0.077 ± 0.004^{a,b}	0.828 ± 0.018^{a}	0.388 ± 0.000^{d,e}	0.490 ± 0.015^{c}	0.793 ± 0.023^{a,b}
EFCX25	0.131 ± 0.014^{a,b}	0.079 ± 0.002^{a,b}	0.565 ± 0.038^{c}	0.371 ± 0.003^{e}	0.481 ± 0.035^{c}	0.774 ± 0.050^{a,b}
EFCX27	0.129 ± 0.003^{b}	0.091 ± 0.016^{a,b}	0.496 ± 0.028^{a,b}	0.353 ± 0.013^{e}	0.474 ± 0.057^{c}	0.749 ± 0.063^{a,b}

F - flour, E - extrusion treatment, C - cellulase enzyme, X - xylanase enzyme; I-A/X – insoluble arabinose to xylose ratio; n=3

$^{a-e}$ – means indicated with similar letters in columns do not differ significantly at α=0.05.

A strong correlation (0.98) was observed by Barron et al. [38] between the AX and TDF contents, indicating that AX can be used to estimate TDF content in wheat fractions and wheat-based food products. What is more, Kaur et al. [40] reported A/X ratios to be considerably lower than 1 for wheat brans of four different wheat varieties (between 0.33 and 0.62). These authors also reported different A/X ratios for bran fractions rich in AXs. They found A/X ratios between 0.09 and 1.37 (for water-extractable fractions), 0.33 and 1.82 (for alkali-extractable fractions), and 0.38 and 0.7 (for cellulosic arabinoxylans). Hell et al. [64] used individual or enzyme mixes composed of cellulase from *Trichoderma reesei*, xylanase Pentopan Mono BG and GH 43 α-L-arabinofuranosidase from *Bifidobacterium adolescentis* to modify wheat bran after extrusion as one of the modification methods.

Demuth et al. [65] reported that application of twin screw extrusion at a maximum temperature of 133 °C increased accessibility of cellulose, released more glucose and observed high solubilization of xylose than in untreated wheat bran. Similarly to our study, they reported a slight decrease in arabinose and xylose content after extrusion as compared to untreated wheat

bran, which implies selectivity of this treatment towards arabinoxylan. Demuth et al. [65] also speculated that the extrudate from enzymatic extrusion might be more accessible to xylanase for preparation of feruloyl oligosaccharides (FOs). In addition, compared with traditional hydrolysis to remove starch and protein, enzymatic extrusion might greatly shorten the time required for these steps [2].

Table 5. Soluble non-starch polysaccharides and arabinoxylans content in untreated, extruded and hybrid treated wheat flours.

Sample	S-Mannose (%)	S-Galactose (%)	S-Glucose (%)	S-Arabinose (%)	S-Xylose (%)	S-A/X (-)
F	0.265 ± 0.008^{b,c,d}	0.118 ± 0.000^{a,b}	0.352 ± 0.017^{a,b}	0.276 ± 0.008^{b}	0.328 ± 0.012^{e}	0.842 ± 0.008^{a}
FC	0.275 ± 0.001^{a,b,c}	0.123 ± 0.003^{a,b}	0.313 ± 0.012^{a,b}	0.281 ± 0.002^{b}	0.335 ± 0.003^{e}	0.839 ± 0.000^{a,b}
FCX	0.274 ± 0.006^{a,b,c}	0.124 ± 0.002^{a,b}	0.381 ± 0.005^{a,b}	0.287 ± 0.001^{b}	0.351 ± 0.007^{e}	0.819 ± 0.013^{a,b}
EF23	0.257 ± 0.010^{c,d}	0.123 ± 0.003^{a,b}	0.387 ± 0.066^{a,b}	0.289 ± 0.000^{b}	0.347 ± 0.005^{e}	0.834 ± 0.012^{a,b}
EF25	0.236 ± 0.025^{d}	0.103 ± 0.008^{b}	0.401 ± 0.069^{a}	0.285 ± 0.007^{b}	0.363 ± 0.038^{d,e}	0.787 ± 0.062^{a,b}
EF27	0.257 ± 0.001^{c,d}	0.119 ± 0.000^{a,b}	0.322 ± 0.000^{a,b}	0.268 ± 0.008^{b}	0.341 ± 0.007^{e}	0.788 ± 0.005^{a,b}
EFC23	0.257 ± 0.003^{c,d}	0.152 ± 0.010^{a}	0.328 ± 0.036^{a,b}	0.256 ± 0.020^{b}	0.338 ± 0.016^{e}	0.758 ± 0.023^{a,b}
EFC25	0.190 ± 0.010^{e}	0.101 ± 0.015^{b}	0.307 ± 0.003^{b}	0.190 ± 0.009^{c}	0.252 ± 0.010^{f}	0.758 ± 0.066^{a,b}
EFC27	0.300 ± 0.020^{a}	0.121 ± 0.013^{a,b}	0.369 ± 0.025^{a,b}	0.283 ± 0.025^{b}	0.401 ± 0.013^{d}	0.708 ± 0.087^{b}
EFCX23	0.291 ± 0.007^{a,b}	0.139 ± 0.016^{a}	0.360 ± 0.016^{a,b}	0.402 ± 0.011^{a}	0.541 ± 0.003^{b}	0.742 ± 0.017^{a,b}
EFCX25	0.245 ± 0.006^{c,d}	0.105 ± 0.011^{b}	0.382 ± 0.013^{a,b}	0.387 ± 0.025^{a}	0.489 ± 0.023^{c}	0.793 ± 0.089^{a,b}
EFCX27	0.262 ± 0.006^{b,c,d}	0.104 ± 0.025^{b}	0.328 ± 0.008^{a,b}	0.418 ± 0.005^{a}	0.590 ± 0.010^{a}	0.709 ± 0.002^{a,b}

F - flour, E - extrusion treatment, C - cellulase enzyme, X - xylanase enzyme; S-A/X – soluble arabinose to xylose ratio; n=3

$^{a-u}$ – means indicated with similar letters in columns do not differ significantly at α=0.05.

3.3. Rheological characteristic of native and modified wheat flour

Rheological features, as tested with Mixolab device for untreated, extruded and hybrid treated wheat flours are presented in Table 6. According to the results of this work, hydration capacity was similar (around 60%) for native flours (F), even if enzymatic treatments (FC and FCX) were applied. Significant increase of Hyd was observed in all the extruded flours, and this varied from 94.3% for the EF27 sample, to 117.7% for EF23 and decreased significantly with higher feed moisture. Smaller differences were noted between hybrid enzymatic-extrusion treated samples regardless of the enzyme used. Dough development time (DT) of in all tested modified flours (Table 6) was longer than indicated for native flour (F) and the most significant differences were observed if cellulase (FC) and combined cellulase-xylanase (FCX) were added to wheat flour, in these cases, by 28% and 40% of time elongation, respectively. A connected parameter is a dough stability, with significant negative correlation to hydration capacity (-0.973) for all the samples. Application of enzymatic hydrolysis to wheat flour lowered only slightly the dough stability, whereas extrusion and hybrid enzymatic-extrusion treatments lowered dough stability by more than double, and for EF23 sample, even triple (Table 6). In EF and EFC treated flours, stability slightly elongated with increase of feed

moisture, but if EFCX was applied, an opposite tendency was observed. The reduction in dough stability may therefore be related to the degradation of the gluten matrix occurring during the extrusion process due to the increase in temperature, as high-temperature treatments will modify the characteristics of the components of the gluten matrix [65].

Table 6. Mixolab features of untreated, extruded and hybrid treated wheat flours.

Sample	Hyd (%)	DT (min)	S (min)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
F	60.5 ± 0.1^{g}	1.92 ± 0.19^{d}	9.73 ± 0.12^{a}	0.477 ± 0.006^{d}	1.709 ± 0.010^{a}	1.479 ± 0.006^{a}	2.519 ± 0.001^{a}
FC	60.6 ± 0.1^{g}	2.66 ± 0.76^{c,d}	9.60 ± 0.36^{a}	0.432 ± 0.003^{e,f}	1.673 ± 0.006^{e}	1.433 ± 0.034^{b}	2.414 ± 0.025^{b}
FCX	61.2 ± 0.2^{g}	3.22 ± 0.34^{c}	9.37 ± 0.15^{a}	0.423 ± 0.003^{f}	1.674 ± 0.009^{a}	1.351 ± 0.010^{c}	2.173 ± 0.037^{c}
EF23	117.7 ± 1.0^{a}	1.91 ± 0.04^{d}	2.60 ± 0.05^{d}	0.452 ± 0.007^{e}	0.474 ± 0.006^{e}	0.326 ± 0.006^{h}	0.543 ± 0.027^{h}
EF25	106.6 ± 0.5^{b}	2.38 ± 0.10^{d}	3.76 ± 0.16^{b,c}	0.526 ± 0.003^{b}	0.664 ± 0.012^{b,c,d}	0.497 ± 0.001^{d}	0.801 ± 0.007^{d,e}
EF27	94.3 ± 1.5^{f}	2.59 ± 0.01^{c,d}	3.83 ± 0.06^{b}	0.524 ± 0.019^{b}	0.745 ± 0.021^{b}	0.491 ± 0.020^{d,e}	0.844 ± 0.026^{d}
EFC23	103.5 ± 0.4^{c}	2.18 ± 0.29^{d}	3.47 ± 0.06^{b,c}	0.498 ± 0.009^{c,d}	0.663 ± 0.009^{b,c,d}	0.466 ± 0.005^{d,e,f}	0.740 ± 0.034^{f}
EFC25	99.3 ± 0.1^{e}	2.49 ± 0.14^{c,d}	3.71 ± 0.04^{b,c}	0.521 ± 0.004^{b}	0.656 ± 0.015^{b,c,d}	0.459 ± 0.003^{e,f}	0.753 ± 0.010^{e,f}
EFC27	95.2 ± 0.1^{f}	2.13 ± 0.20^{d}	3.87 ± 0.06^{b}	0.529 ± 0.003^{b}	0.639 ± 0.099^{c,d}	0.443 ± 0.002^{f}	0.777 ± 0.006^{e,f}
EFCX23	102.7 ± 0.3^{c,d}	2.33 ± 0.15^{d}	3.80 ± 0.10^{b,c}	0.509 ± 0.003^{b,c}	0.663 ± 0.006^{b,c,d}	0.458 ± 0.002^{e,f}	0.801 ± 0.006^{d,e}
EFCX25	96.0 ± 0.5^{f}	2.66 ± 0.04^{c,d}	3.63 ± 0.12^{b,c}	0.524 ± 0.010^{b}	0.711 ± 0.009^{b,c}	0.467 ± 0.005^{d,e,f}	0.813 ± 0.012^{d,e}
EFCX27	101.2 ± 0.3^{d}	2.66 ± 0.01^{c,d}	3.42 ± 0.03^{c}	0.566 ± 0004^{a}	0.576 ± 0.002^{d}	0.366 ± 0.003^{g}	0.657 ± 0.012^{g}

F – flour, E - extrusion treatment, C – cellulase enzyme, X – xylanase enzyme; Hyd – hydration capacity; DT – dough development time; S – stability; C2 – protein weakening; C3 – starch gelatinization; C4 – amylase activity; C5 – starch retrogradation; n=3

$^{a-h}$ – means indicated with similar letters in columns do not differ significantly at α=0.05.

Water absorption capacity of extruded wheat flour rises with increasing treatment intensity, but dough stability tends to decrease [66]. The water absorption capacity of dough depends mainly on the flour composition, and increases with increasing protein, pentosan and damaged starch content. Indeed, the association between the quantity of damaged starch and the water absorption capacity of flour has been long established. Studies have also been performed with pregelatinized starches, which show a similar behaviour to damaged starch because the starch granules break down during this process. Pasqualone et al. [67] reported increased water absorption after industrial scale extrusion-cooking of lentil flour with two temperature/screw profiles (EF1: temp. 80-90-100°C, rpm 220, and EF2: temp. 100-105-115°C, rpm 230), they found a significantly higher water absorption for sample EF1 (94.7 g/100 g) extruded at lower temperature and screw speed than EF2 (90.8 g/100 g) and native flour (41.1 g/100 g).

Results of protein weakening (C2), starch gelatinization (C3), amylase activity (C4) and starch retrogradation (C5) of native wheat flour, enzyme hydrolysed, extruded and hybrid method treated are presented in Table 6. Significant differences were noted between samples that had passed through non-thermal and thermal modifications. Application of cellulase (FC) and combined cellulase-xylanase (FCX) hydrolysis in environmental conditions caused only little effects on all rheological properties evaluated via Mixolab procedure. Increasing water addition to the extruded samples, however, brought about increasing values of C2, indicating more

intensive protein weakening in the extruded samples without or with enzymes addition. This outcome can be an indicator of protein destruction after treatment. Starch gelatinization indicator (C3) showed the most significant differences in EF samples without enzymes extruded with various feed moisture: as water content increased the C3 values raised. In addition, lower differences and opposite tendencies were found if enzymes were added during the extrusion. This outcome seems to indicate the effect of combined enzymatic-extrusion effect on starch gelatinization. The lowest values of C2, C3, C4 and C5 indicators were also found in EF23 samples characterized by the highest hydration, which might be the result of the most intensive treatment of wheat flour with limited water amount causing thermomechanical damage of both proteins and starchy components under twin-screw extrusion treatment (Table 6). The greatest differences (more than 2.5-3.0 times lower values) were noted for C4 and C5, indicating significant differences in amylase activity and starch retrogradation of the extruded flour, as compared to native and enzyme that were only hydrolysed. The effect of hybrid cellulase-extruded and combined cellulase-xylanase extrusion was negligible without specific effects of enzymes addition or water dosing on rheological characteristics measured with Mixolab. Statistical analysis showed that C3, C4, and C5 results were in strong negative correlation with Hydration of the tested flours (-0.974, -0.963, -0.965, respectively). Moreover, high and significant correlations were found between Stability of dough and C3 (0.994), C4 (0.996) and C5 (0.995) of tested modified wheat flour. Unlike the total arabinoxylan, which contributes towards good viscoelastic properties of wheat flour dough, the A/X ratio does not have any remarkable effect on the rheological properties of wheat flour dough, as reported by Kaur et al. [40]. Rheological properties of raw materials and pasting or hydration characteristics explain the techno-functional properties of wheat flour and dough. Some authors reported that the addition of AX influenced rheological and mixing properties of dough and water absorption due to high-water binding capacity [37]. During the bread-making process, addition of AXs can be advantageous due to their high-water binding capacity [40].

3.4. Pasting properties of native and modified wheat flour

Pasting properties indicate the thermal sensitivity and ability of starches or flours to create a viscous structure under heating and cooling and can support to identify the retrogradation tendency by breakdown and setback results. The results of pasting properties of native, enzyme treated, extruded and hybrid treated wheat flours are presented in Table 7. Native wheat flour tested under Viscograph procedure showed max viscosity of 745 BU (which corresponds to 1564.5 mPa·s), application of cellulase FC as well as combined cellulase-xylanase FCX lowered max viscosity significantly to 625 and 573 BU, respectively. This suggest the partial hydrolysis of flour due to applied enzymes activity by degrading insoluble cellulose to soluble sugars by cellulase and hydrolysing xylans (e.g., arabinoxylans and glucuronoxylans) by xylanase.

Table 7. Pasting properties of untreated, extruded and hybrid treated wheat flours.

Sample	Maximum viscosity (BU)	Trough viscosity (BU)	Final viscosity (BU)	Breakdown (BU)	Setback (BU)	Beginning of gelatinization (°C)	End of gelatinization (°C)
F	745 ± 4^{e}	208 ± 2^{f}	583 ± 1^{g}	537 ± 3^{c}	376 ± 2^{g}	60.2 ± 0.0^{a}	86.6 ± 0.1^{d}
FC	625 ± 2^{h}	154 ± 1^{i}	445 ± 1^{i}	471 ± 2^{d}	291 ± 1^{i}	60.5 ± 0.0^{a}	85.5 ± 0.0^{f}
FCX	573 ± 1^{i}	134 ± 1^{j}	373 ± 4^{j}	439 ± 2^{e}	239 ± 3^{j}	60.2 ± 0.1^{a}	85.2 ± 0.1^{g}
EF23	1057 ± 6^{b}	366 ± 3^{b}	765 ± 2^{d}	692 ± 4^{a}	399 ± 1^{e}	31.1 ± 0.1^{e}	86.2 ± 0.1^{e}
EF25	1220 ± 9^{a}	526 ± 5^{a}	1073 ± 4^{a}	694 ± 4^{a}	547 ± 1^{a}	42.4 ± 1.2^{b}	88.0 ± 0.0^{a}
EF27	649 ± 2^{g}	229 ± 1^{e}	649 ± 2^{f}	421 ± 2^{f}	420 ± 3^{d}	$38.2 \pm 0.9^{c,d}$	86.7 ± 0.2^{d}
EFC23	941 ± 16^{c}	362 ± 5^{b}	847 ± 10^{b}	579 ± 11^{b}	485 ± 5^{b}	$36.6 \pm 2.0^{c,d}$	87.6 ± 0.1^{b}
EFC25	689 ± 1^{f}	246 ± 1^{d}	670 ± 4^{e}	443 ± 2^{e}	424 ± 4^{d}	35.4 ± 2.1^{d}	86.9 ± 0.1^{c}
EFC27	578 ± 8^{i}	193 ± 2^{g}	581 ± 5^{g}	386 ± 6^{g}	389 ± 3^{f}	$37.2 \pm 0.8^{c,d}$	86.3 ± 0.1^{e}
EFCX23	915 ± 4^{d}	340 ± 11^{c}	830 ± 8^{c}	575 ± 7^{b}	490 ± 3^{b}	38.6 ± 1.1^{c}	87.7 ± 0.2^{b}
EFCX25	689 ± 5^{f}	243 ± 9^{d}	679 ± 3^{e}	446 ± 4^{e}	436 ± 6^{c}	38.9 ± 0.8^{c}	87.0 ± 0.1^{c}
EFCX27	546 ± 8^{j}	174 ± 2^{h}	520 ± 4^{h}	372 ± 6^{g}	346 ± 2^{h}	35.2 ± 0.6^{d}	86.2 ± 0.1^{e}

F - flour, E - extrusion treatment, C - cellulase enzyme, X - xylanase enzyme; n=3

$^{a-j}$ – means indicated with similar letters in columns do not differ significantly at α=0.05.

The combined effect of both enzymes acting together demonstrated more significance on pasting properties, both in native flour and in hybrid enzymatic-extrusion treatment. Differences in pasting behaviour are presented in Figure 1 depending on treatment methods. Application of low temperature extrusion-cooking of native flour (EF) under various feed moistening caused increase of max viscosity if 23 and 25% feed moisture was applied. At the highest moisture (27%), applied max viscosity was even lower even that for native F, but higher than observed for flour modified by enzymes only (FC and FCX). Hybrid enzyme-extrusion treatment showed slightly higher viscosity if extruded at 23% moisture due to the more intensive mechanical treatment at low moisture, other conditions, however, did not significantly affect wheat flour viscosity, the results obtained were similar to that for pure enzyme hydrolysis [17]. Trough viscosity or holding strength means the trough at the minimum hot paste viscosity and could be connected with water holding capacity of swollen starch after heating and cooling stages [68]. Trough viscosity decreased after enzymes addition but was higher than in native flour when extrusion treatment was applied, especially at 23 and 25% of feed moisture. Significant tendency to decrease the final viscosity with increasing flour feed moisture was noted for both enzyme type applied, but the results for hybrid enzymatic-extrusion treatment were higher by around 30% than that for only enzymatically hydrolysed wheat flour (FC and FCX). Application of individual enzymes significantly lowered breakdown and setback values due to changes in various fractions of polysaccharides via partial hydrolysis of carbohydrates by cellulose and combined cellulase-xylanase action. We also noted that Breakdown and Setback values were significantly correlated with max viscosity (0.961 and 0.733, respectively). Temperature of beginning gelatinization was similar (60.2 - 60.5 °C) for all tested native flours without (F) or with enzymes (FC and FCX), so any effect of enzymes on gelatinization tendency was notable. In the extruded samples treated by thermal and mechanical treatment, as well as by hybrid treatment at temperatures up to 80 °C, partial gelatinization occurred inside the extruder, hence, the temperature registered for the beginning of gelatinization were from 35.2 to 42.4 °C when 25 and 27% feed moisture was applied. Lower gelatinization temperature was reported in the EF23 sample. This effect was due to more intensive treatment under low moisture content, which results in greater starch degradation due to intensive mechanical shearing. Moreover, great correlation was found between Setback and fat content (0.974), the extruded samples were much lower in fat, probably due to formation of lipid-starch or lipid-protein complexes through the extrusion processing [1] and this affected higher Setback in these samples. Robin et al. [17] reported a peak viscosity of the raw whole wheat flour at 1800 mPa s via RVA pasting profile, and after extrusion, peak viscosity lowered

significantly (from 117 to 292 mPa s), suggesting that extrusion disrupted the crystalline organization of starch, generating an amorphous state. However, full gelatinization of the starch granules did not occurred because of limited water content during extrusion (ranging between 18 and 22%), and increasing the water content led to a decreased degree of starch transformation as indicated by the highest viscosity being at maximum water content and minimum screw speed.

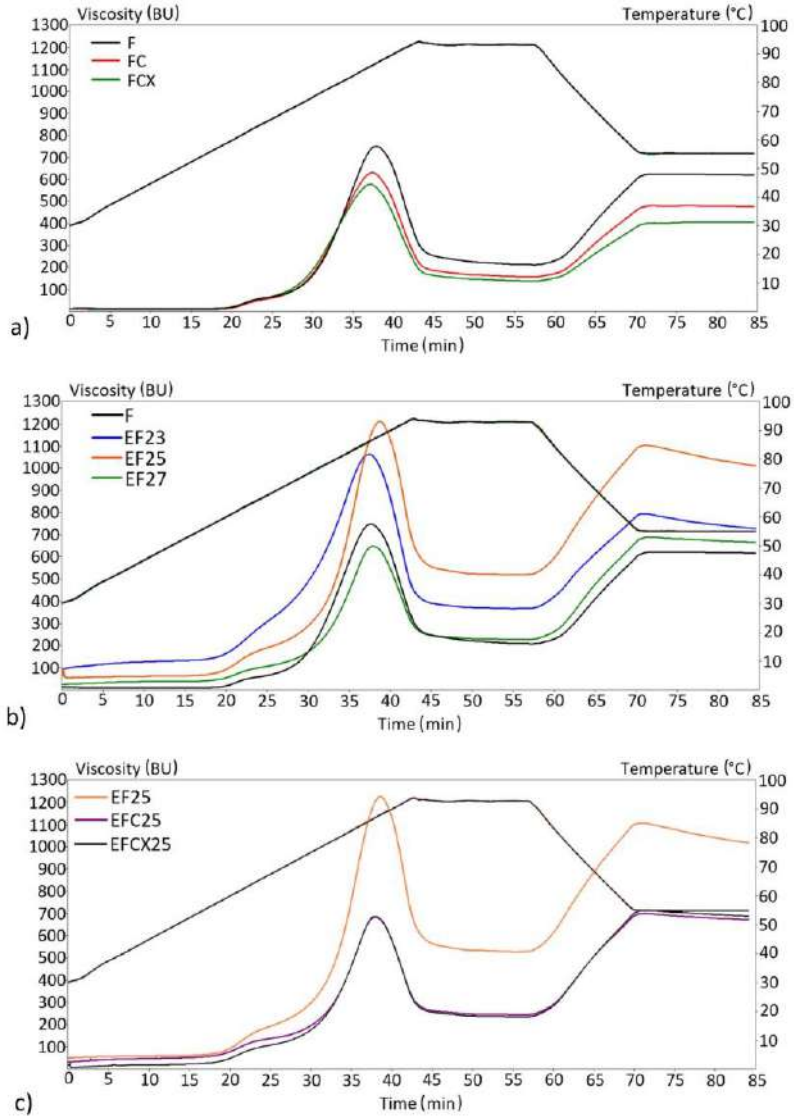

Figure 1: Pasting curves of wheat flour modified under various conditions: (a) native wheat flour, with cellulase (FC) and cellulase-xylanase (FCX), (b) extruded at various feed moisture without enzymes, and (c) hybrid extruded at 25% moisture with cellulase (EFC25) and cellulase-xylanase (EFCX25).

De Pilli et al. [31] studied the effects of two commercially available enzymes (protease and amylase) on selected properties of products obtained from wheat and almond flour extruded at low temperature (54 °C) via twin-screw extruder. They reported a decreased dough viscosity during the extrusion at increased moisture content due to the pressure decreasing at high dough moisture. This trend could be attributed to the decrease of enzymatic activity caused by high

moisture content. Water is required to maintain the catalytically active conformation of enzymatic system. However, during the denaturation process, it acts as a plasticizer, which allows the enzyme molecules to unfold, resulting in the loss of native conformation. Mitrus et al. [12] observed that the addition of increased amounts of water raised the peak viscosity of extruded bean flour, and the results showed that the twin-screw extrusion-cooking process reduced the retrogradation tendency of bean paste due to starch degradation occurring during processing because of values breakdown decrease and setback increase with increased water feeding. Pasqualone et al. [67] tested extruded lentil flour and reported increase of viscoamylograph initial viscosity compared with native flour, with significantly higher value when lower temperature and lower screws speed were applied. Additionally, they noted lower degree of starch retrogradation in extruded lentil flours than in native one. Pasting profile of wheat flours studied by Román et al. [29] showed lower viscosity profiles of not-enzymatically treated and extruded flours, confirming that gelatinization occurred during thermal treatments, similarly as in our research. Breakdown and setback values were also reduced. Both extruded and native samples with additional enzymatic treatment showed very low viscosity and flat pasting profile with no peak viscosity as a result of the hydrolytic activity of the enzyme on the starch [29]. Uthumporn et al. [33] found in heat-treated corn and potato starch significant increase in setback viscosity compared to control starches, indicating great tendency to retrogradation. After enzymatic hydrolysis, where amylose was degraded into shorter chain oligosaccharides that are easier to re-associate and solubilize, the setback values increased, showing positive correlation with the solubility. Deng et al. [2], in turn, tested rice bran processed via the enzymatic-extrusion method and found higher viscosity of bran when the screw rate was low at low moisture content due to the higher specific mechanical energy input from the extruder at lower moisture, which softened the fibre. Higher mechanical energy input might, therefore, be conducive to forming a loose and porous structure that facilitates penetration of the xylanase-containing solution.

3.5. Hydration characteristics of native and modified wheat flour

SRC may be a suitable indicator of the true hydration ability of flour. In contrast, flour is subjected to mechanical forces or kinetic effect during the measuring of water absorption by Mixolab or RVA, and the true value may be effected by increased starch or protein mechanical damage [50]. The SRC method also enables identifying separate functional contributions from damaged starch (Sc solvent), pentosans (Su solvent), and gluten (La solvent) at the same time - mostly identifying differences during flour milling intensity. Flour dedicated to various applications are characterized by holding specific properties e.g. for bread production, high water absorption, good gluten strength and relatively high damaged starch and arabinoxylans is required, but for cookies, minimal gluten strength, low damaged starch and arabinoxylans and low water absorption is recommended. Table 8 shows the SRC values of untreated, extruded and hybrid treated wheat flours tested via various media. In our work, native wheat flour (F) (non-treated) was characterized by low solvent retention capacity to all tested solvents as compared to the extruded. Moreover, cellulase and combined cellulase-xylanase hydrolysis of native flour only slightly increased SRC values, in many cases without significant differences between F and FC or FCX samples. Application of extrusion increased more than triple SCRWa, more than double SRCSu and SRCLa, and almost four times SRCSc of the extruded flour at the lowest feed moisture. With increasing water addition during simple extrusion of wheat flour without enzymes, a significant and constant lowering of all SRC results was observed. In hybrid enzymatic-extrusion treated samples the range of SRC values showed similar tendency if cellulase was used individually (EFC samples), whereas in EFCX hybrid treated samples, the results were not linearly dependent on water addition and the

highest SRC values were observed if 27% of moisture was applied in relation to SRC of Wa, La and Sc solvents.

Table 8. Solvent retention capacity (SRC) values of untreated, extruded and hybrid treated wheat flours.

Sample	SRCWa (%)	SRCSu (%)	SRCLa (%)	SRCSc (%)	GPI (-)
F	70.005 ± 0.062^{j}	114.973 ± 0.770^{k}	118.294 ± 0.595^{j}	87.839 ± 0.256^{g}	0.583 ± 0.005^{b}
FC	70.427 ± 0.049^{j}	116.916 ± 1.228^{j}	118.289 ± 0.624^{j}	86.971 ± 0.080^{g}	0.580 ± 0.001^{c}
FCX	75.496 ± 0.278^{i}	119.827 ± 0.876^{i}	126.504 ± 0.950^{i}	88.702 ± 0.153^{g}	0.607 ± 0.003^{a}
EF23	236.652 ± 0.530^{a}	216.253 ± 0.725^{a}	236.923 ± 0.276^{a}	344.999 ± 2.037^{a}	0.422 ± 0.002^{f}
EF25	196.950 ± 0.530^{d}	198.022 ± 0.477^{b}	$209.170 \pm 0.385^{b,c}$	295.467 ± 2.609^{b}	$0.424 \pm 0.002^{e,f}$
EF27	167.158 ± 0.227^{h}	161.398 ± 0.224^{h}	172.324 ± 0.952^{h}	235.073 ± 1.585^{f}	$0.435 \pm 0.001^{c,d,e}$
EFC23	193.667 ± 0.479^{e}	189.778 ± 0.471^{d}	202.480 ± 0.609^{d}	276.106 ± 4.925^{c}	$0.435 \pm 0.006^{c,d,e}$
EFC25	191.321 ± 0.869^{f}	$183.074 \pm 0.305^{e,f}$	192.849 ± 2.034^{f}	$272.395 \pm 6.960^{c,d}$	$0.424 \pm 0.011^{e,f}$
EFC27	177.957 ± 0.609^{g}	166.261 ± 0.363^{g}	183.706 ± 1.429^{g}	248.603 ± 2.871^{e}	0.443 ± 0.001^{c}
EFCX23	203.601 ± 0.980^{c}	191.850 ± 0.433^{c}	206.880 ± 1.042^{c}	291.260 ± 1.326^{b}	$0.428 \pm 0.001^{d,e,f}$
EFCX25	195.729 ± 0.382^{d}	182.101 ± 0.202^{f}	198.613 ± 0.284^{e}	264.583 ± 0.268^{d}	0.445 ± 0.001^{c}
EFCX27	216.286 ± 0.156^{b}	184.618 ± 0.087^{e}	210.365 ± 0.588^{b}	295.415 ± 0.440^{b}	$0.438 \pm 0.001^{c,d}$

F - flour, E - extrusion treatment, C - cellulase enzyme, X - xylanase enzyme; SRC - Solvent Retention Capacity; Wa - distilled water; Su - 50% sucrose; La - 5% lactic acid; Sc - 5% sodium carbonate; GPI - Gluten Performance Index; n=3

$^{a-k}$ – means indicated with similar letters in columns do not differ significantly at $\alpha=0.05$

Water-soluble pentosans (also known as water-extractable arabinoxylans) are considered to have great water holding capacity and this property may explain increased hydration of solvents due to increasing content of soluble fractions of arabinoxylans (Table 2) in the tested flours modified with extrusion (EF) or hybrid treatment (EFC and EFCX), as well as because of damaged starch or gluten by extrusion treatment - as confirmed by significantly lower gelatinization temperatures obtained in pasting tests. Gluten performance index (GPI) can calculated based on the SRC results. GPI has been found to be an even better predictor of the overall performance of flour glutenin in the environment of other modulating networks of flour polymers, e.g., in bread [50]. Results presented in Table 8 for GPI of native, enzymatic treated and hybrid treated flour clearly indicated significant decrease of GPI in extruded samples without significant effects of the enzymes used in hybrid treatments. GPI values were strongly negatively correlated with SRC Wa, Su, La, Sc (-0.957, -0.928, -0.923, -0.964, respectively). All SRC and GPI results were, surprisingly, significantly positively correlated with fat content in the tested flours (correlation coefficients from 0.903 to 0.981) and with Hyd (from 0.989 to 0.994), whereas a strong negative correlation was found between Hyd and GPI (-0.961). Moreover, significant positive relations of Setback (from 0.650 to 0.711) and negative of gelatinization beginning temperature (from -0.916 to -0.965) were found with all SRC results. The results of the work of Moreno-Rivas et al. [55] showed that extruded nixtamalized corn flour, with and without xylanase, had increased protein solubility, compared to non-treated and non-extruded, and this effect was lower in extruded nixtamalized corn flour with xylanase. Moreover, insoluble protein diminished in corn flours either with or without xylanase enzyme. The addition of xylanase reduces the effect that the extrusion process has on the solubility proteins of extruded nixtamalized corn flour.

3.7. Structure diffraction analysis of modified flours

To analyse the internal structure of native and extruded wheat flour without or with enzyme addition processed at variable moisture levels, we applied the X-ray diffraction technique. The X-ray diffraction patterns identified in native and extruded and hybrid treated wheat flour samples are collected in Figure 2a. Two main profiles were identified in wheat flour: for native and enzyme hydrolysed flour (Fig. 2b) and for extruded and hybrid enzymatic-extruded flour

(Fig. 2c and 2d). The native flours, rich in starch, with present A (large) and B (small) granules of wheat starch displayed typical A-type X-ray diffraction patterns at 2θ with sharp peaks: a first peak around 15°, a strong doublet near 17 and 18°, and a third main reflection around 23°. These are characteristic for the crystalline structures of cereal starches. In native samples (F, FC and FCX), only a small peak at 20° was noted. Such effect (2θ ≈ 20°) represents the amorphous peak of amylose and lipids, and these are in low amounts in native wheat flour, and, indeed, were found to decrease slightly more after enzymes addition (Fig. 2b). Other enzymes effects were visible in FC and FCX samples at 23° as compared to native flour F. Such results indicate that native wheat kernel starch had typical A-type crystal characteristics [69,70]. After the extrusion, much higher peak intensity at 2θ ≈ 20° was observed, both without (Fig. 2c) or with enzymes addition (Fig. 2d) - indicating higher content of amylose and lipids complexes in the extruded flour. This outcome is confirmed by the lower extractable fat content in this (Table 2). Moreover, changes in peak intensity at 2θ ≈ 23° were observed. In the extruded samples, these peaks were lower in intensity than that for the native flours. Lower relative intensity of peaks at 2θ ≈ 15° were also seen, especially when low feed moisture 23% was used during extrusion (Fig. 2c). With regard to the effect of moisture content, a diminished peak at 18° was found with limited water amount (EF23), with absence of a 17° angle peak (almost absent in EF25, but visible in EF27). If high feed moisture was applied during processing, a larger surface area under the curve was observed in the region between 25° and 50°, suggesting higher amounts of structure with amorphous characteristics after extrusion. Some changes in this region were also observed with enzyme addition: if CX complex was used, the intensity of 15° peak was higher (Fig. 2d) and the surface area under the curve between 25° and 50° was greater when complex CX was added (as seen in the EFCX25 and EFCX27 samples) (Fig. 2a and 2d). The strong doublet peaks around 2θ ≈ 17° and 18° were not observed in the extruded wheat flour, suggesting a transformation of starch crystalline structure in this region. After the extrusion treatment (without or with enzymes), significant changes were observed at the 20° diffraction angle. In this region, the native flour showed a low intensity peak (Fig. 2b), whereas after extrusion, in all extruded samples, this peak was very intensive with the altitude similar in intensity as for 18° (Fig. 2c and 2d). As stated previously, Liu et al. [69] identified the peak at 2θ ≈ 20° as the amorphous peak of amylose and the lipids. A higher peak intensity indicates a higher content of amylose and lipid complexes formed after extrusion. This effect was confirmed by the notable lowering of extractable fat content, as compared to native (non-extruded) flour. Additionally, microstructure analysis confirmed the formation of an amorphous melted structure after extrusion, with singular starch granules embedded in a continuous starch-protein matrix, as confirmed by SEM. Zeng et al. [70] found X-ray diffraction patterns to be true “fingerprints” of the crystal structure within starch grains. Accordingly, the crystal structure of starch can be identified as one of four types (A, B, C and V-type) due to the presence of characteristic X-ray diffraction lines at proper diffraction angles. A, B and C-type indicates the crystalline structures commonly present in natural cereal starches, and, while V-type is crystalline, it is typical of the complexes formed by amylose and lipids. In our study, the A-granules with larger particle size show sharper X-ray diffraction patterns than the other starches, and this may be the explanation for the flattening of the curve of diffraction due to the structure differences in native and extruded samples as observed on SEM microscopic pictures, where after extrusion, only a few swollen granules are present.

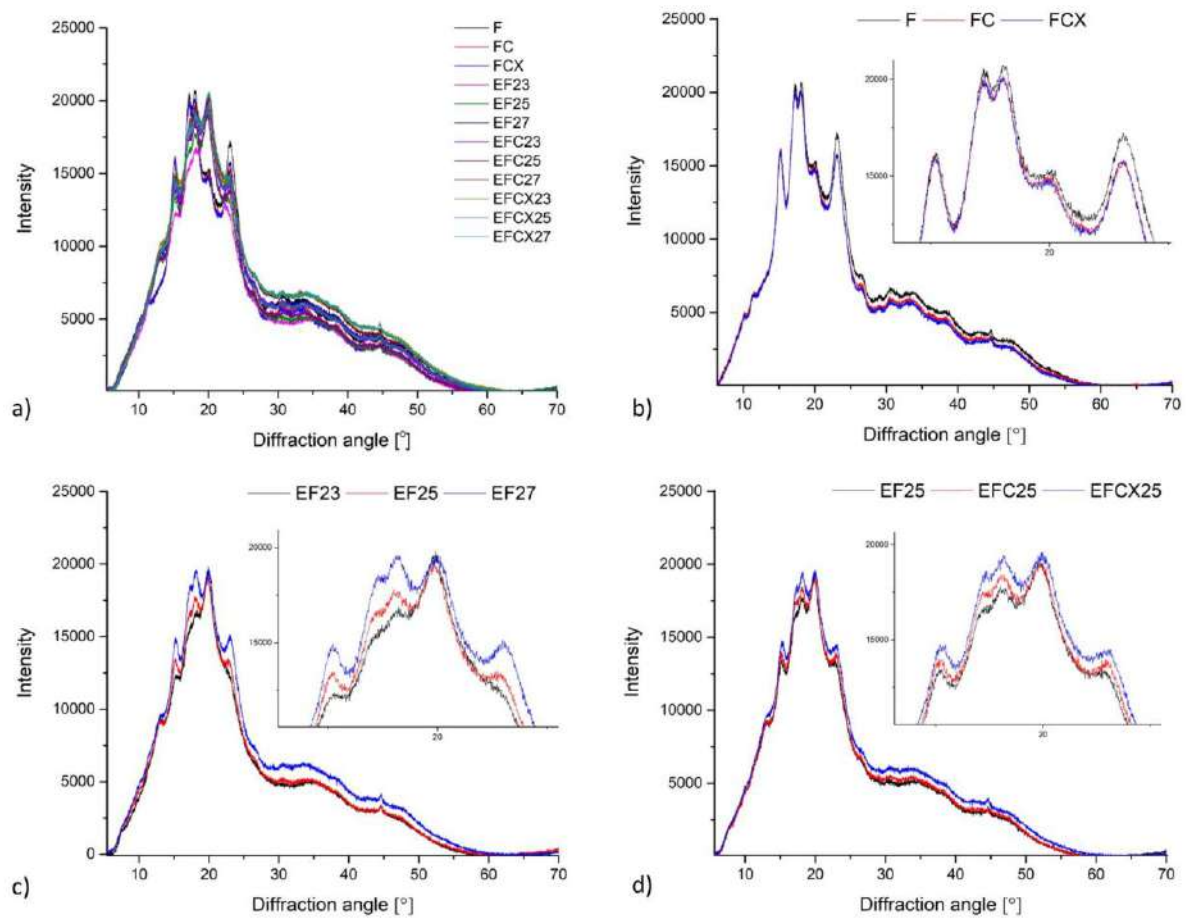

Fig. 2: X-ray diffraction patterns of native, extruded and hybrid enzymatic-extrusion treated wheat flour: (a) comparison of patterns of native (F) and extruded (EF) samples without or with enzymes; (b) effect of enzymes addition to native wheat flour; (c) effect of feed moisture of extruded wheat flour without enzymes; (d) effect of enzymes addition extruded samples at 25% feed moisture; C – cellulase; CX – cellulase-xylanase complex.

Crystallinity of the tested samples as native flours without or with enzymes as well as extruded and hybrid enzymatic-extruded flours was obtained on the base of X-ray diffraction results obtained at the range of θ–2θ from 5 to 70° (4922 points) fitted to a Gauss–Cauchy model. Two models were used for calculations due to significant changes between native and extruded X-ray diffraction curves and peak number and intensity. Native flours without and with addition of enzymes were analysed as a function of 15 peaks. In the extruded flours the curves obtained were less sharp with 13 significant peaks taken for fitting the model. The results are presented in Figure 3. Native wheat flour was characterized 15.11% of crystallinity and addition of enzymes did not affected significantly on crystallinity changes in non-processed flours. For a native wheat Saiah et al. [71] and Leblanc et al. [72] confirmed the presence of an A-type structure, characteristic to cereal starches. They noted after measurement at θ–2θ from 5 to 35° a degree of crystallinity at 40% w/w for wheat starch and at 30% w/w for the wheat flour because of the presence of highly amorphous multipolymer gluten in wheat. Yoo and Jane [54] tested structural properties at the range of θ–2θ from 4 to 40° of starches isolated from waxy wheat, amylose-reduced Kanto 107 variety, normal hard red winter wheat Centura, and commercial wheat starch and they noted 18.0, 14.5, 12.0 and 13.0% percentage crystallinity calculated based on X-ray diffractograms. Waxy wheat starch had significantly greater crystallinity than others.

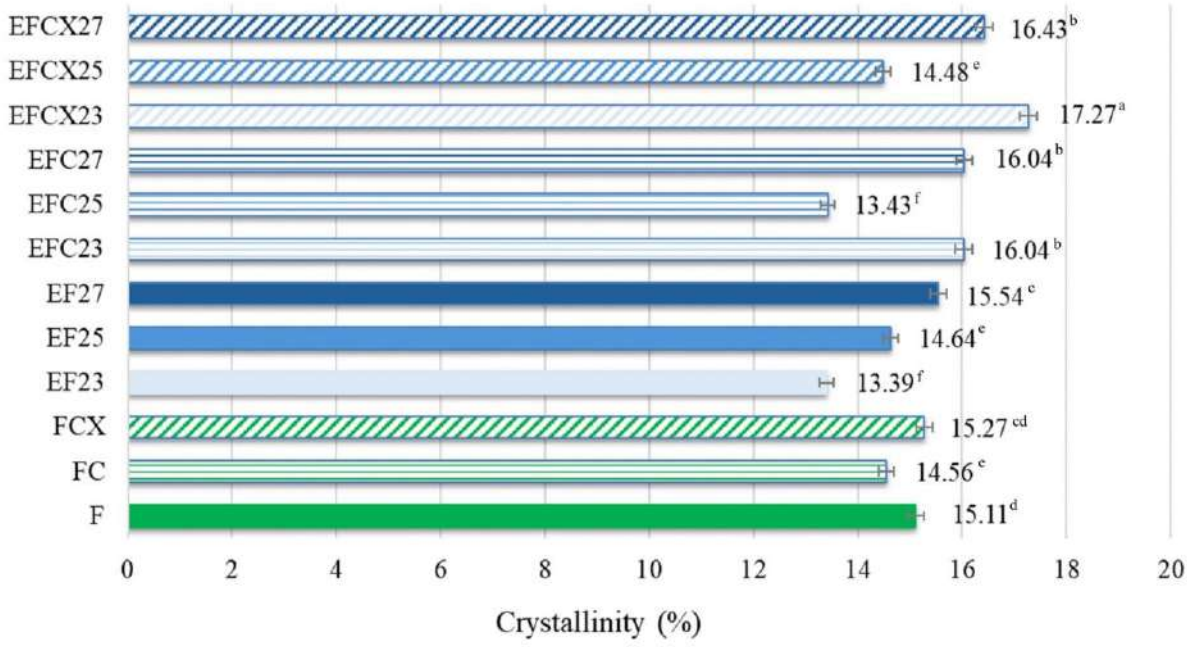

Fig. 3: Crystallinity results of untreated, extruded and hybrid treated wheat flours.

Differences in crystallinity of native and extruded samples were small, probably because the A-type crystallinity became rearranged and V-type is formed in the extruded samples containing starch due to its melting under treatment via extrusion [71-73]. The crystallinity in extrudates is also the effect of formation of complexes between amylose and endogenous lipids (amylose– lipid complex). Amylose–lipid complexes are generally produced after gelatinization of starch in a presence of heat and water due to starch melting during extrusion cooking and destruction of amylopectin double helices, whereas part of the free lipids can form a helical inclusion complexes with the amylose molecules [74]. This confirms also the low fat content in our extruded wheat flour samples, as presented in Table 1, and small differences in crystallinity due to the calculation method based on the ratio of area under the curve of crystalline and amorphous structures. The most significant effect of lowering crystallinity (13.39%) was observed in wheat samples extruded alone at the lowest feed moisture, as visible in Fig 2c, and extruded with cellulase or xylanase-cellulase complex at 25% of feed moisture (13.43 and 14.48%, respectively). Increase in initial moisture when wheat flour was extruded caused increase of crystallinity to 15.54% when 27% feed moisture was applied. This may be the effect of greater starch gelatinization at increased access to water under 80-85°C extrusion temperature and more intensive changes from A-type structure into V-type (both crystalline) what was observed also on diffraction curve (Fig. 2c) as a highest peak at 23°. Oliveira et al. [73] reported that the loss of crystallinity during extrusion is caused by mechanical disruption of the molecular bonds due to the intense shear fields within the extruder. Therefore, under extrusion at low moisture content (expanded products), a mixture of small amounts of gelatinized and melted states of starch, as well as fragmentation exist simultaneously. They found values of crystallinity of corn flour and wholegrain wheat flour in the range of 16.97–23.81% for the extrudates treated in L/D 29 twin screw extruder at 75 and 100°C and 16-22% feed moisture against 25.20–34.00% for unprocessed flours and flour blends, but samples were tested within a narrower range of θ–2θ from 3 to 35° at a scanning rate of 1°/min. They noted no significant effect of any variable studied on extrudate crystallinity despite differences in crystallinity values. In our research the highest crystallinity (17.27 and 16.43%) was observed in flour with xylanase-cellulase complex extruded both at 23 and 27% of initial moisture,

respectively, and similar tendency was noted if the only cellulase was added before extrusion. Application of 25% feed moisture in both cases (EFC25 and EFCX25) limited formation of crystalline phase when wheat flour was supported by enzymes before extrusion. Jafari et al. [74] tested native sorghum flour, exhibiting A-type X-ray diffraction patterns with crystallinity of 30.84%, as well as extruded sorghum flour. Extrusion cooking lowered crystallinity in the range of 17.59-29.94% depend on processing conditions when sorghum flour was extruded at 110 and 160°C with 10, 14 and 18% of food moisture in a co-rotating twin screw extruder with L/D 10. A-type and V-type crystalline peaks became narrower when feed moisture and die temperature decreased, what suggest reorganization of helices into packed structures but they tested only range of 4-40° so any amorphous structures over this range were not taken into account, as in our case. Increasing shear forces intensity at low moisture content could disrupt molecular bonds increasing the availability of amylose chains while the high temperature could promote molecular movement and more amylose-lipid complexes could be formed at low moisture content and high temperature during extrusion process. So, decreasing of feed moisture and increasing of die temperature showed a negative effect on starch crystallinity in extruded samples [74]. Similar effect was observed in our extruded wheat flour (samples EF23, EF25 and EF27, Figure 3). After a single screw extrusion at 120 °C and 40 rpm Saiah et al. [71] and Leblanc et al. [72] found peak located at 22.6° characteristic for a V-type crystalline structure, but also the peak located at 17.3° of very small magnitude indicating the existence of a residual A-type crystalline structure in the extruded materials with the degree of crystallinity of 14% in all extruded samples. Similar peaks ($2\theta \approx 17°$ and 23°) were observed in our native and extruded wheat flour samples with various intensity (Fig. 2). A very small fraction of granules (observed on microscopic pictures) may be not transformed in the extrusion process due to incomplete restructuring under presented conditions.

3.7. Microstructure observations

Microstructure observations with SEM presented in Fig. 4 showed that the addition of enzymes had only a slight effect upon the internal structures of FC and FCX flours (Fig. 4b, 4c), as compared to native (Fig. 4a). Moreover, in samples with both enzymes, the partly swollen starch granules were visible as larger amounts of finer granules (Fig. 4b and 4c) when compared with native flour (Fig. 4a). The A-starch granules also displayed a disk shape with diameters of 12–28 μm, and the isolated B- starch granules appeared as a spherical shape with diameters of about 2.5–8.5 μm, while very minute (<2.0 μm) C-type starch granules were indicated - although this type of granule may represent a B-type granule [70]. We also observed some fibrous fractions coming from the outer layer of the grains used for the developed flour composition. This suggests the presence of polysaccharides in native wheat flours. Similar observations were made by Zeng et al. [70] for white and wholegrain wheat flour. Here, they observed two types of starch granule distribution: large granules measuring about 35 μm and small granules about 2-10 μm. Application of low-temperature extrusion processing caused significant changes in the structure of the modified flours. In all extruded (EF) and hybrid-extruded (EFC and EFCX) samples, melting and agglomeration of components were observed, with only singular starch and fibre particles visible (under high magnifications). In Figs. 4d-4l, for example, swollen starch granules embedded in the amorphous structure of gelatinized starch-protein matrix are visible in a molten mass under various magnifications (x600 and x2000). The extruded wheat flour also presented almost null presence of native starch granules with a defined structure. The effect of increasing feed moisture on more compact and homogenous internal structure was observed especially in samples extruded at 27% of moisture both without (Fig. 4f) and with enzymes addition (Fig. 4i and 4l), where a more uniform melted phase was noted than in samples with lower moisture during processing without or with

enzymes (Fig. 4e, 4h and 4k, respectively). In samples processed at 23% moisture, starch ungelatinized granules were observed (Fig. 4d, 4g and 4j). This suggests that insufficient water was available for starch to complete gelatinization at the temperature range used in the experiment. The application of cellulase and xylanase-cellulase complex created a more loose structure of melted fractions with visible particles inside agglomerates formed by the extrusion process, especially at low feed moisture (Fig. 4g and 4j).

Other authors observed changes in the microstructure of modified flours or starch after extrusion processing. Moreno-Rivas et al. [55] reported remarkable damage and surface erosion of starch granules after extrusion, although such structure changes were also observed in conventional nixtamalization as an effect of lime presence and soaking time. Liu et al. [75] reported significant changes in microstructure of rice starch after extrusion at 30-70% of moisture, with almost complete gelatinization and shape destruction of starch treated with the highest moisture. Enríquez-Castro et al. [76] observed significant reduction in the number of granules and an increase in the irregular shape and pore surface in the microstructures of extruded nixtamalized corn flour. This effect probably came about due to higher enzymatic hydrolysis and starch digestibility. Extrusion-cooking carried out with low moisture contents causes a high amount of starch granules to be fragmented and embedded in the endosperm matrix, as well as for some granules to be dispersed out of it. Under thermal treatment, especially extrusion, agglomeration of starch granules results in amorphous structures, and dextrinization is possible. Alam et al. [77], for example, applied twin screw extruder at temperature up to 130°C, but with limited feed moisture (17 and 19%) to obtain crispy products with high content of fibre coming from rice bran. Jafari et al. [74] observed microstructure of native and extruded sorghum flour and they found significant variation in starch granule shapes. Moreover, for mildest extrusion condition (10% feed moisture at 110°C) some nongelatinized starch granules were observed. However, with more intensive extrusion condition most of starch granules were completely melted and visible as agglomerated particles, similarly to our observations.

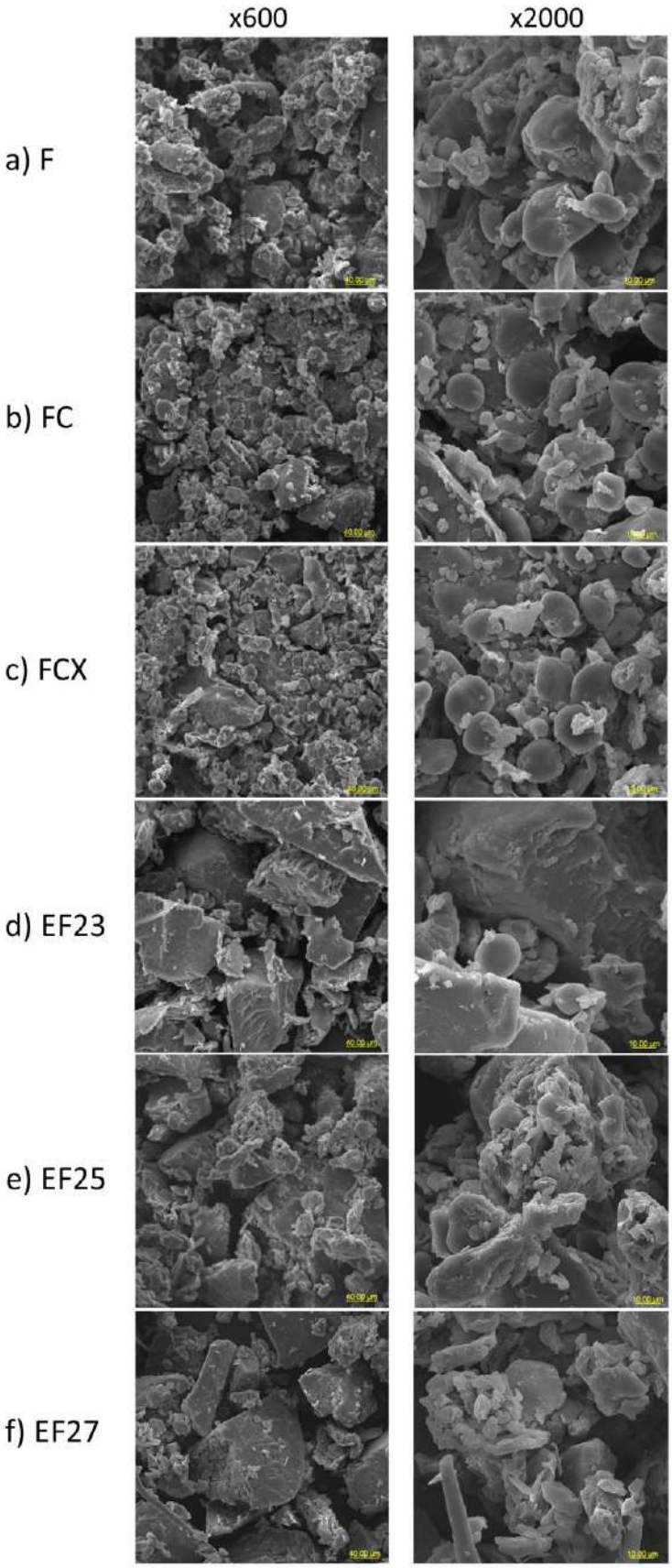

Fig.3: continued

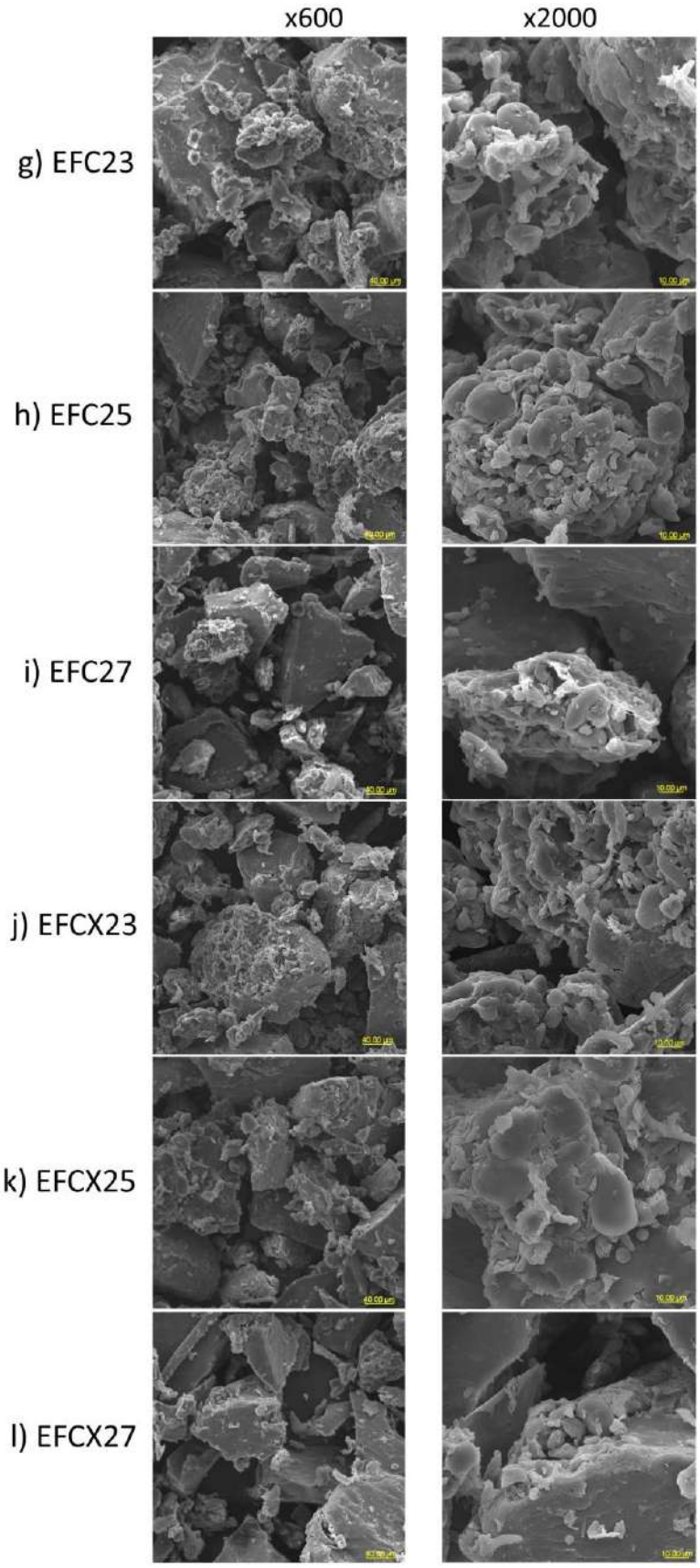

Fig. 4: SEM micrographs of flours at low (x600) and high (x2000) magnifications. Non-treated (a), enzymatically treated with C and CX (b, c), extrusion treated (d, e, f), hybrid enzymatic-extrusion treated with C (g, h, i) and CX (j, k, l) flours processed at 23, 25 and 27% of feed moisture, respectively.

4. Conclusions

The obtained results confirmed that hybrid enzymatic-extrusion modification has a greater affect upon polysaccharides composition and the techno-functional properties of wheat flour than individual enzymatic or extrusion treatment. In our work, a specific effect of hybrid enzymatic-extrusion treatment of wheat flour was observed on polysaccharides fractions, the outcome of which significantly decreased insoluble arabinoxylans content and increased soluble arabinoxylans and non-starch polysaccharides content if a combined cellulase-xylanase blend was used during extrusion (EFCX samples). Moreover, all treatments involving low-temperature extrusion (without or with enzymes) caused lowering of fat content in the modified flours due to the formation of amylose-lipids complexes confirmed by crystallinity analysis. In addition, extrusion treatments at lower feed moisture without or with enzymes increased viscosity, breakdown, hydration, C2 and all solvents retention capacity, but decreased setback, stability, C3, C4, C5, and gluten performance index of the modified wheat flour. In addition, combined cellulase-xylanase hydrolysis showed a stronger effect on wheat flour modification than did cellulase treatment alone, especially by lowering viscosity and pasting properties and SCR values, and by increasing soluble AX and NSP, hydration, dough development time and GPI in the modified flour. Hybrid enzymatic-extrusion modified wheat flour treated at proper processing conditions may be used in the bakery industry as a source of soluble arabinoxylans, as a thickening agent, or as a binder with high water absorption and solvents retention capacity and lower setback (which indicates the decreasing retrogradation tendency of the modified flour). The presented results can be helpful in the processing of wheat flour with specific techno-functional properties. Moreover, the practices enable this flour to be listed as a “clean label” additive. Application of modified flours as bread improvers will be tested in the next research step.

Data Availability

All data are included in this paper.

Conflicts of Interest

The authors declare no personal conflict of interest regarding the publication of this paper.

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The Influence of Processing Using Conventional and Hybrid Methods on the Composition, Polysaccharide Profiles and Selected Properties of Wheat Flour Enriched with Baking Enzymes

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Abstract: In this study, a developed wheat flour blend (F), consisting of a high content of non-starch polysaccharides, was fortified with cellulase (C) and a cellulase–xylanase complex (CX) and then processed via conventional and hybrid treatment methods. Dry heating (T), hydrothermal treatment (H) and extrusion processing (E) were applied without or with enzyme addition as hybrid treatments. Proximate composition and polysaccharide profiles selected techno-functional and structural properties of modified wheat flours, were analyzed. Conventional and hybrid treatments induced changes in polysaccharide fraction compositions (especially the arabinoxylans) and the rheology of modified flour. Dry heating caused an inconsiderable effect on flour composition but reduced its baking value, mainly by reducing the elasticity of the dough and worsening the strain hardening index, from 49.27% (F) to 44.83% (TF) and from 1.66 (F) to 1.48 (TF), respectively. The enzymes added improved the rheological properties and baking strength, enhancing the quality of gluten proteins. Hydrothermal enzyme-assisted treatment increased flour viscosity by 14–26% and improved the dough stability by 12–21%; however, the use of steam negatively affected the protein structure, weakening dough stretchiness and elasticity. Extrusion, especially enzyme-assisted, significantly increased the hydration properties by 55–67% but lowered dough stability, fat content and initial gelatinization temperature due to the changes in the starch, mostly induced by the hybrid enzymatic–extrusion treatment. The structure of extruded flours was different from that obtained for other treatments where the peak intensity at 20° was the highest, suggesting the presence of amorphous phases of amylose and lipids. The results can be helpful in the selection of processing conditions so as to obtain flour products with specific techno-functional properties.

Keywords: dry heating; hydrothermal treatment; extrusion cooking; wheat flour; bakery enzyme; arabinoxylans; rheological properties; structure

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1. Introduction

Dry heating, hydrothermal or pressure–thermal treatments are useful in modifying the physical, rheological, technological and functional features or storage stability of wheat flour and other cereal products [1–3]. Thermal treatment reduces the natural enzyme activity, limits the moisture content and changes lipid fractions present mostly in bran-rich flour, thus extending the shelf life of cereal flours and products [4]. Dry heating mostly affects gluten proteins and starch molecules. At temperatures above 50 °C, gluten proteins are straightened or form gluten aggregates, thus modifying dough strength. As reported in other research, the changes during heating between 55 °C and 95 °C are connected with

variable conformational changes, particularly in the glutenin proteins, and compositions of protein fractions. Data presented by Wang et al. [5] indicate that there are heat-induced alterations in gluten proteins at temperatures above 55 °C, which appear to be involved in the loss of functionality (baking performance) on heating. Between 55 and 75 °C, chemical changes occur in the protein component, new disulfide bond formation is observed, and the glutenin fraction is affected predominantly, as reported by Schofield et al. [6]. Glutenin changes that occurred in the temperature range of 65–70 °C were observed because of cross-linking temperature. The glutenin proteins are unfolded upon heating up to 75 °C, and this facilitates sulfhydryl/disulfide bond rearrangement [5,7]. At temperatures above 75 °C, the gliadin proteins are also affected, but the most important changes were observed around 80 °C due to protein polymer cross-linking. Within the temperature limit of 95 °C, gliadin binds with glutenin through non-covalent interactions and thus forms a viscoelastic gluten protein network [6]. Treatment above 95 °C is far more drastic, as in HTST processing, which causes thermal denaturation and induces permanent modifications in conformation and association, affecting the protein structure and producing interchain disulfide binding [8].

In comparison to hydrothermal treatment, dry heat treatment does not require external water and can be stored directly without drying, making it convenient for industrial production operations [9]. Moreover, dry thermal treatment is less drastic than heating in the presence of steam, mostly by limiting access to additional water. The changing intensity in protein, starch and fiber taking place during hydrothermal treatment depends on the water content, the time–temperature profile and the type of treatment: heated rolls, autoclaving, steaming, atomization or extrusion [10]. In hydrothermal modifications, the pregelatinization of starch and the partial denaturation of proteins occur; hence, cereal flour or bran is characterized by increased viscosity post-treatment. These hydrothermally modified flours can be used to produce functional flours used as thickeners or binders in soups, sauces, coatings or baby food [4].

More intensive treatment is obtained by the extrusion-cooking technique, which combines the effect of temperature, pressure, shearing forces and residence time with varied water accessibility in processing. Wheat components, especially gluten, are significantly involved in the structural, rheological and textural formation of the extrudates, and the main effect of protein fragmentation or aggregation in the extrusion process is generated through intermolecular changes in the disulfide bonds [11]. Moreover, starch can be partly or completely gelatinized as influenced by the initial moisture in raw materials and moisturizing level, range of treatment temperature, intensity of shearing and configuration of the device. Extrusion cooking is considered an effective treatment for converting insoluble fibers into soluble fractions [12,13].

Enzyme addition is a common method for starch, flour or bran modification, as enzyme addition may modify fiber fraction compositions, dough development ability and stability or increase absorption properties of flours. The main enzymes useful in bakery are amylases to change starch into simple sugars and dextrins, oxidases to enhance dough strengthening and whitening, hemicellulases for improving the gluten strength, proteases for reducing its elasticity and lipases for elongating the shelf life. All enzymes execute significant roles in the formation of bread dough and pan volume, texture, color and browning reactions throughout the time of baking or retrogradation and staling reduction [14]. The addition of cellulase or xylanase has been reported to improve the functionality of non-starch polysaccharides, which are mainly found in the external layers of cereal grains [15].

Soluble and insoluble arabinoxylans (AXs) and other non-starch polysaccharides (NSPs) as fractions of total dietary fiber are found in wheat flour and bran [16]. Most arabinoxylans intertwined with other macromolecules or embedded in cell walls are difficult to extract with water, but selected processing conditions can increase or change the structure and solubility or extractability of AX and other NSP fractions [17,18]. Singh et al. [19] found extrusion suitable for increasing the proportion of extractable dietary fiber, including AXs. AXs and NSPs are dietary fibers with variable properties depending on cereal variety, treatment method and internal composition. They are quite resistant to

high-temperature treatments, due to contained hemicelluloses of varied degradation temperatures (243–279 °C for xylan and 332 °C for arabinoxylan, xyloglucan and β-glucan) [20]. Demuth et al. [21] reported significant structure changes in wheat bran water-soluble fractions of arabinoxylans after extrusion processing. Treatment of corn bran, as reported by Singkhornart et al. [22], reduced the content of sugars and soluble arabinoxylans after extrusion depending on the application or not of some chemical pretreatment or the application of various moisture levels or screw speed during modification. Several researchers have modified cereal products using coupled thermal/extrusion processing with enzyme applications, especially in bran fractions. Kong et al. [12], for example, tested coupled enzymatic–extrusion modification of bran from black wheat, by application of cellulase, high-temperature α-amylase, acid protease and xylanase alone or in combination to determine the effect upon water extractable arabinoxylan (WEAX). WEAX content and cholesterol adsorption capacity as well as oil and water retention ability increased dramatically, most likely as a result of the effect of loose porous structures after treatment.

The aim of this study was to assess the application of conventional dry thermal heating, hydrothermal treatment and low-temperature extrusion treatment in modifying developed wheat flour properties whether as individual or hybrid treatments with cellulase or cellulase–xylanase enzyme additions. In the work, chemical, rheological and structural properties were determined, with the hypothesis being that hybrid treatment more greatly affects the polysaccharide compositions, hydration capacity and techno-functional properties of modified developed wheat flour than does individual enzymatic, thermal, hydrothermal or extrusion treatment.

2. Materials and Methods

2.1. Materials

A newly developed flour blend (F) from common wheat of the Laudis variety was used for the tests. This consisted of flours of high arabinoxylan amounts from selected breaking, milling, reducing, sifting and sorting passages developed and composed by Lewko et al. [23], characterized by the wet gluten content of 31% (tested according to the ICC 155 method [24] by using a Perten Glutomatic 2200) (PerkinElmer Inc., Waltham, MA, USA) and a falling number of 340 s (tested according to ICC 107/1 standard method) [24].

Commercial baking enzymes were added to the flour as powders: Bakezyme® WholeGain—cellulase from *Trichoderma reesei* (DSM Food Specialties B.V., Delft, The Netherlands) with declared enzyme activity 1475 EGU/g (±5%); and VERON 292—xylanase from *Aspergillus niger* (AB Enzymes GmbH, Darmstadt, Germany) with declared enzyme activity min 1701 XylH/g. Cellulase enzymes were employed for wheat flour fortification in an amount of 120 ppm (samples marked with C), and a complex of cellulase and xylanase enzymes was used for flour fortification in amounts of 60 ppm and 50 ppm, respectively (samples marked CX). The amount of the enzymes was determined based on preliminary tests and on the recommendations of the enzyme manufacturers.

2.2. Enzymatic Hydrolysis

Wheat flour (F) at 14% moisture content was prepared with 120 ppm of powdered cellulase (FC) and a combined 50 + 60 ppm of cellulase–xylanase complex (FCX). Components were mixed for 2 min at room temperature by using a laboratory ribbon mixer (Konstal—Zakład Mechaniczny CNC Zbigniew Własiuk, Lublin, Poland), left for resting for 2 h before measurements and sifted to uniform particle size below 300 μm (Figure 1).

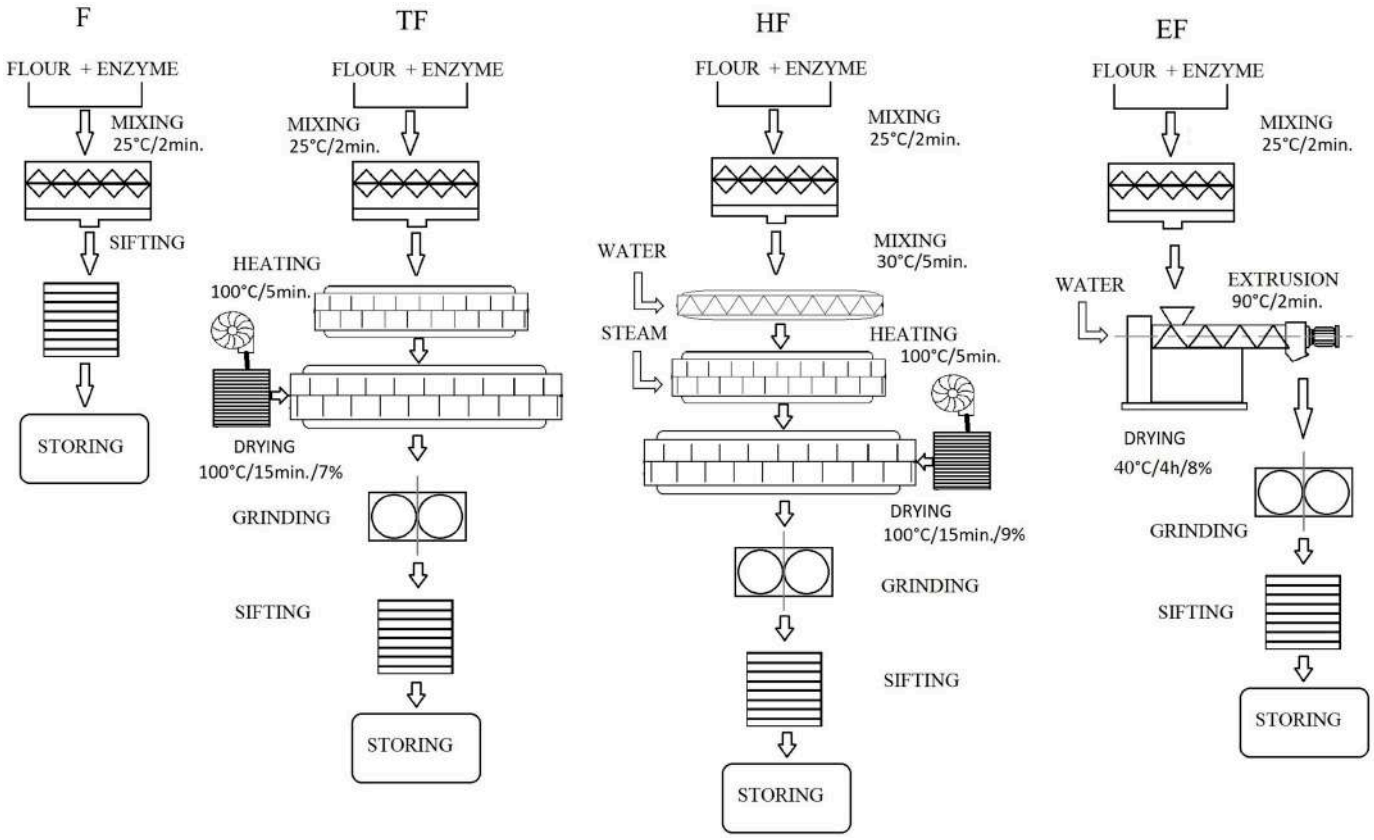

Figure 1. Schematic diagram of treatment methods.

2.3. Dry Heat Treatment

Dry thermal treatment (T) was carried out for wheat flour (TF) and flour fortified with enzymes (TFC and TFCX) by using an installation for thermal treatment owned by PZZ Lubella, Lublin, Poland (prototype installation). A schematic diagram of treatment runs is presented in Figure 1 with applied conditions. During the 5 min dry heat treatment, mixed samples at 14% moisture content were heated inside the barrel with a rotating screw, where the heating jacket temperature was set to 100 °C, with the product temperature measured during the tests to not exceed 50 °C. The prototype installation used for the tests was equipped with sensors located inside the barrel with a rotating screw. The temperature in the barrel was regulated by the temperature of the oil in the heating jacket of the device and the efficiency of the supplied amount of flour. After reaching the required processing temperature, the heating jacket was stopped and the flour was removed immediately by a two-way distributor; if the parameters were exceeded or were too low, the flour was directed to a separate tank, and the flour processed in the assumed conditions was collected as a test sample for further tests and sent for drying. The efficiency of this procedure is 650 kg of product/h. After dry heating or hybrid heat–enzymatic treatment, the flour was dried in an air dryer at 100 °C with an oil heating jacket to stop enzyme activity and to obtain a final moisture of 7%. To check the final moisture content, a fast method was employed using NIR Perten DA7250 (PerkinElmer Inc., Waltham, MA, USA). The samples were ground and sieved using a square sifter (Toruńskie Zakłady Urządzeń Młyńskich Spomasz S.A., Toruń, Poland) to homogenize the material, remove aggregates and obtain particle sizes below 300 µm, and 200 kg was collected for further research.

2.4. Hydrothermal Treatment

During the hydrothermal modification (H), the base wheat flour (HF) and flour fortified with enzymes (HFC and HFCX), prepared according to the procedure shown in Figure 1, were processed in a prototype installation (owned by PZZ Lubella). Flour samples

mixed for 2 min with enzymes were transferred to a single-screw pre-conditioner at 30 °C with 20 L/h of water and additionally heated with steam injection for 5 min at a set heating jacket temperature of 100 °C, to reach a product temperature measured during the tests not exceeding 65 °C. The prototype installation used for the tests was equipped with sensors located inside the barrel with a rotating screw. The temperature in the barrel was regulated by the temperature of the oil in the heating jacket of the device and the efficiency of the supplied amount of flour. After reaching the required processing temperature, the heating jacket was stopped and the flour was removed immediately by a two-way distributor; if the parameters were exceeded or were too low, the flour was directed to a separate tank, and the flour processed in the assumed conditions was collected as a test sample for further tests and sent for drying. The obtained flour was then dried in an air dryer at 100 °C with an oil heating jacket to stop enzyme activity and to obtain a final moisture of 9%. To check the final moisture content, a fast method was employed using NIR Perten DA7250 (PerkinElmer Inc., Waltham, MA, USA). The efficiency of prototype installation with steam injection is 650 kg/h. The samples were ground and sieved using a square sifter (Toruńskie Zakłady Urządzeń Młyńskich Spomasz S.A., Toruń, Poland) to homogenize the material, remove aggregates and obtain particle sizes below 300 µm. A total of 200 kg of hydrothermally or hybrid-modified flour was gathered for subsequent tests.

2.5. Low-Temperature Extrusion-Cooking Treatment

Tests on the extrusion processing (E) of flour without (EF) and with various enzyme additions (EFC and EFCX) were carried out using an Evolum25 co-rotating twin-screw extruder (Clextral, Firminy, France) with an L/D = 24 configuration, with screws of 25 mm in diameter and with a single open die with a diameter of 3 mm. The feeding rate of dry components was set at 10 kg/h by using a volumetric gravity feeder (Brabender® GmbH & Co. KG, Duisburg, Germany). The tests were performed at the feed moisture level of 27% by adding the water via a water pump directly into the second section of the extruder working at 400 rpm rotational screws speed. During the monitored low-temperature extrusion process, the temperatures in individual sections of the extruder were set from 40 °C in the dosing section and 50, 60, 65, 70 and 80 °C in the subsequent sections, up to 85 °C at the die, and were stabilized by a heating/cooling jacket system. The total residence time of treatment inside the extruder did not exceed 2 min (Figure 1). Obtained samples were shredded via a cutting knife and dried at 40 °C in a laboratory shelf dryer to ensure storage moisture below 8%, which was checked by a rapid moisture analyzer MA50R.WH (Radwag Wagi Elektroniczne, Radom, Poland). Extrudates were ground in a TestChem laboratory grinder (Radlin, Poland) and sifted to obtain a similar particle size below 300 µm, and samples were taken for further tests.

2.6. Proximate Composition Analysis

The selected chemical characteristics of native and modified flours were determined according to standard methods: AACC 46–10 method for protein (Nx6.25), AACC 30–10 method for fat and AACC 08–01 method for ash [25]. The 991.43 method was used to evaluate soluble (SDF) and insoluble (IDF) fractions and the content of total dietary fiber (TDF) [26,27]. Moisture content was evaluated in accordance with the ICC 110/1 method [24].

2.7. Content and Fractions of Non-Starch Polysaccharides and Arabinoxylan Analysis

The content of non-starch polysaccharides (NSPs) was determined by gas chromatography according to Englyst and Cummings [28]. The total NSP (T-NSP) content, soluble (S-NSP) and insoluble (I-NSP) fractions, as well as the content of total arabinoxylans (T-AX), insoluble (I-AX) and soluble (S-AX) fractions, were ascertained according to the procedure described by Fraś [29]. After acid hydrolysis of soluble and insoluble fractions, monosaccharides were detected in each fraction. The obtained hydrolysates were converted into volatile alditol acetates. To each sample (1 mL), 2 drops of 2-octanol, 0.26–0.28 mL of 12 M ammonia

solution and 0.1 mL of sodium borohydride solution in ammonia (100 mg BH_4 in 1 mL of 3M NH_4OH) were added. After 40 min of incubation at 40 °C, 0.1 mL of glacial acetic acid was added to the hydrolysate and mixed, and then 0.2 mL of 1-methylimidazole and 2 mL of acetic anhydride were added to 0.2 mL of the collected sample. The prepared solution was cooled for 30 min and then 4 mL of distilled water and 1.15 mL of dichloromethane were added and shaken for 1 min. The aqueous phase was removed, and the organic phase was analyzed on an Autosystem XL gas chromatograph from Perkin Elmer (Shelton, CT, USA), equipped with an autosampler, a split injector, a flame ionization detector (FID) and an Rtx-225 capillary quartz column (0.53 mm × 30 m). Chromatograph operating parameters included the following: carrier gas helium, flow 2 mL/min, injector temperature 275 °C and detector temperature 275 °C. For the column temperature program, parameters included the following: initial temperature 185 °C, 1 min; increase 5 °C/min to 215 °C; and isotherm 215 °C, 10 min [29]. Gas chromatography allowed for the identification of the soluble and insoluble fractions of individual sugars: arabinose, xylose, mannose, galactose and glucose.

2.8. Hydration and Retention Properties

Solvent Retention Capacity (SRC) tests of native and modified flours were carried out in accordance with the AACC 56-11.02 approved procedure [25]. SRC was calculated as the retained solvent mass after centrifugation of the swollen flour and expressed as a percentage of the dry flour mass (amended to 14% moisture). Several solvent types were used: deionized water (WaSRC), 50 wt% sucrose-in-water solution (SuSRC), 5 wt% lactic acid-in-water solution (LaSRC) and 5 wt% sodium carbonate-in-water solution (ScSRC). A total of 5 g of samples placed in a 50 mL centrifuge tube was mingled with 25 g of solvents. Samples were then allowed to stand for 20 min and stirred every 5 min for 5 s to solvate. Subsequently, samples underwent centrifugation at 2500 rpm for 15 min, and the filtrate was poured off and allowed to stand for 10 min. SRC was calculated after weighting the samples. Calculation of the gluten performance index (GPI) included a division of LaSRC results by consolidating the results of SuSRC and ScSRC [30].

2.9. Pasting Properties

Pasting properties according to the ICC 169 procedure [24] were evaluated on a Brabender Viscograph-E (Brabender GmbH & Co., Duisburg, Germany) working with 75 rpm and 700 cmg torque. In total, 80 g of flour (adjusted to 14% moisture content) and 450 mL of distilled water were mixed, the prepared slurry was placed in the heating chamber, and the spindle was attached. The heating/cooling profile was as follows: heating from 30 °C to 93 °C at a rate of 1.5 °C/min, holding at 93 °C for 15 min, cooling to 50 °C at a rate of 3 °C/min and finally, holding at 55 °C for 15 min. Viscosity (mPas) was recorded as a resistance to stirring. The following pasting characteristics were obtained via Viscograph-E software (version 4.1.1): maximum viscosity, trough viscosity, final viscosity, breakdown (max viscosity minus trough viscosity) and setback (final viscosity minus trough viscosity), as well as the beginning and end of gelatinization temperatures.

2.10. Rheological Tests

Rheological properties were evaluated using the listed instruments: Mixolab (Chopin Technologies, Villeneuve-La-Garenne, France) in accordance with the ICC 173 method, Brabender Farinograph-E apparatus (Brabender, Duisburg, Germany) in accordance with the ICC 115/1 method and Alveograph (Chopin Technologies, Villeneuve-La-Garenne, France) in accordance with the ICC 121 method [24].

Rheological properties by Mixolab were determined based on the Chopin+ flour protocol with the following settings: mixing speed—80 rpm; total analysis time—45 min; dough weight—75 g; and hydration water temperature—30 °C. Flour and water were added accordingly to obtain a dough maximum consistency of 1.10 Nm (±0.05). The test was performed using a standard protocol: 8 min at 30 °C, heating for 15 min at a rate

of 4 °C/min, holding for 7 min at 90 °C, cooling down to 50 °C for 10 min at a speed of 4 °C/min and finally, holding for 5 min at 50 °C [31]. Several properties have been assessed: water absorption (Hyd), protein weakening (C2), starch gelatinization (C3), amylase activity (C4) and starch retrogradation (C5) [32].

Rheological properties were tested by the Farinograph working with a standardized protocol [33]. Water absorption (WA) was expressed as the % of the water required to obtain a dough with a consistency of 500 BU or corrected at 14%, and dough development time (DT) was observed as the time to attain a consistency of 500 BU); dough stability (S), degree of softening (DoS and DoS12 after 12 min) and quality number (QN) were recorded.

Alveograph working with a standard procedure was used to determine the following characteristics: baking strength (W) calculated from the surface area under the curve, dough strength (P) calculated as the maximum pressure required to form the dough bubble expressing dough resistance, extensibility (L) of the dough as the length of the curve, elasticity index (Ie) [34], strain hardening index (SH) and P/L as a configuration ratio [35].

2.11. X-ray Diffraction Analysis

The ground flour samples were subjected to X-ray diffraction (XRD) using a high-resolution Empyrean powder diffractometer (PANalytical, The Netherlands) with Cu Kα1 radiation (λ = 1.54178 Å). Samples were measured in θ–2θ geometry, over an angle range from 10 to 70°, with a step size of 0.01° and a counting time of 400 s per data point. All measurements were carried out at room temperature and an RH of 28% [36].

2.12. Microstructure Observations

Flour microstructure was observed with a scanning electron microscope Vega Tescan LMU (Tescan, Brno, Czech Republic) at an accelerating voltage of 20 keV. Powdered samples were mounted on aluminum specimen stubs with double-sided adhesive silver tape and sprayed with gold using Sputter Coater Emitech K550X (Emitech, Essex, UK). SME pictures were taken with magnifications of 600× and 2000× [37].

2.13. Statistical Analysis

All analyses were performed in triplicate. One-way ANOVA was performed using Statistica 13.3 software (StatSoft, Inc., Tulsa, OK, USA), followed by Tukey's least significant difference (LSD) post hoc test to compare means at the 0.05 significance level. Pearson's correlation coefficients were determined to find the correlations between variables using Statistica 13.3 software (StatSoft, Inc., Tulsa, OK, USA) within the 95% confidence interval.

3. Results and Discussion

3.1. Effect of Treatment Method on Proximate Composition

Basic chemical components in the developed wheat flour varied depending on treatment methods. The moisture content of native flour without or with enzymes was similar around 14%. Processed flours exhibited lower moisture content due to the drying after treatment and values were from 7.01% to 7.23% for dry thermal treatments, from 8.98% to 9.28% for hydrothermal treatments and from 8.06% to 8.26% for extruded flours, with the unessential effect of enzymes added (Table 1). These moisture values were also used during testing rheological properties as a base for specific calculations. The results of chemical components are presented as is and were slightly dependent on the flour moisture content. The content of protein in native wheat flour was 14.62%, and the application of enzymes did not significantly affect protein content (Table 1). Application of dry heating, hydrothermal treatment and extrusion, both as individual or hybrid treatments with the addition of enzymes, resulted in lower moisture of flour, which had an effect on the protein content results presented in Table 1 as expressed in wet mass. These results showed increased protein content in treated samples, but, as expressed in dry mass, the protein content decreased in all treated samples. As a result of processing at high temperatures (above 50 °C), changes occur in the molecular conformation of the protein, such as the unfolding

of gluten proteins, the formation of gluten aggregates, with reduced extractability, changes between sulfhydryl/disulfide bond exchange reactions leading to glutenin polymerization and modified molecular mass distribution, which may affect the final protein content and the possibility of forming unextractable polymeric proteins (UPPs), especially in the presence of starch, as confirmed by Guerrieri and Cerletti [8] based on internal fluorescence conformational changes in proteins, Hu et al. [33] on CSLM images and Schofield et al. [6] by chromatographic analysis. Differences between samples were very small in proximate composition, but it was worth noting that when hybrid treatment with CX complex addition occurred, irrespective of the treatment method, the protein content was higher in all modified flours with CX, both as expressed in wet and dry mass. The probable mechanism of the CX enzyme complex action, which was used to improve gluten quality, is the destruction of cellulose fibers by cellulase and the limitation of water absorption by water-unextractable arabinoxylans by xylanases, thus improving gluten hydration [38]. A significant lowering of extractable fat content was observed in samples undergoing extrusion modification both without/with enzyme addition (EF, EFC and EFCX samples). Alam et al. [39] stated that fat is able to form complexes with starch or protein during extrusion cooking, and the obtained results confirm this observation. The fat content was at least 5-fold lower than in native flour and thermally treated samples. Enzyme addition had an indistinct effect on fat content, especially in F, FC and FCX, as well as in TF-, TFC- and TFCX-modified flours. In samples processed with hydrothermal treatment, both C and CX enzyme additions decreased fat content from 1.37% in HF to 1.30% in HFC and HFCX samples. Application of modification methods slightly increased ash content in all samples, as compared to untreated and enzymatic fortified flour.

Table 1. Selected chemical properties of untreated and hybrid-treated flours.

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	IDF (%)	SDF (%)	TDF (%)	IDF/SDF (-)
F	13.93 0.08 ^{e}	14.62 ± 0.06 ^{a,b}	1.31 ± 0.01 ^{d}	0.72 ± 0.02 ^{a}	3.94 ± 0.04 ^{d}	2.86 ± 0.01 ^{e}	6.80 ± 0.03 ^{e}	1.38
FC	13.89 ± 0.09 ^{e}	14.52 ± 0.03 ^{a}	1.39 ± 0.02 ^{f}	0.74 ± 0.02 ^{a,b,c,d}	3.81 ± 0.03 ^{c}	2.33 ± 0.03 ^{b}	6.14 ± 0.05 ^{c}	1.63
FCX	13.76 ± 0.07 ^{e}	14.64 ± 0.01 ^{a,b,c}	1.30 ± 0.01 ^{d}	0.73 ± 0.01 ^{a,b}	4.10 ± 0.03 ^{e}	2.56 ± 0.01 ^{c}	6.66 ± 006 ^{d}	1.61
TF	7.01 ± 0.06 ^{a}	14.75 ± 0.05 ^{c,d}	1.34 ± 0.03 ^{d,e}	074 ± 0.01 ^{a,b,c}	4.70 ± 0.02 ^{h}	2.87 ± 0.02 ^{e}	7.57 ± 0.01 ^{h}	1.64
TFC	7.17 ± 0.13 ^{a}	14.92 ± 0.04 ^{e,f}	1.38 ± 0.01 ^{e,f}	0.76 ± 0.01 ^{b,c,d,e}	4.25 ± 0.03 ^{f}	2.79 ± 0.01 ^{d}	7.04 ± 0.04 ^{f,g}	1.52
TFCX	7.23 ± 0.11 ^{a}	15.11 ± 0.05 ^{g}	1.36 ± 0.02 ^{e,f}	0.82 ± 0.02 ^{f}	4.22 ± 0.01 ^{f}	2.74 ± 0.03 ^{d}	6.96 ± 0.03 ^{f}	1.54
HF	8.98 ± 0.09 ^{c}	14.85 ± 0.03 ^{d,e}	1.37 ± 0.02 ^{e,f}	0.76 ± 0.02 ^{b,c,d,e}	3.91 ± 0.04 ^{d}	3.12 ± 0.01 ^{g}	7.02 ± 0.07 ^{f,g}	1.25
HFC	9.17 ± 0.12 ^{c,d}	14.81 ± 0.04 ^{d,e}	1.30 ± 0.01 ^{d}	0.78 ± 0.01 ^{d,e}	4.60 ± 0.03 ^{g}	2.89 ± 0.01 ^{e,f}	7.49 ± 0.03 ^{h}	1.59
HFCX	9.28 ± 0.06 ^{d}	15.02 ± 0.06 ^{f,g}	1.30 ± 0.02 ^{d}	0.73 ± 0.02 ^{a,b}	4.05 ± 0.04 ^{e}	2.55 ± 0.03 ^{c}	6.60 ± 0.06 ^{d}	1.58
EF	8.18 ± 0.06 ^{b}	14.76 ± 0.04 ^{c,d}	0.22 ± 0.02 ^{b}	0.77 ± 0.01 ^{c,d,e}	3.05 ± 0.03 ^{a}	2.24 ± 0.02 ^{a}	5.29 ± 0.05 ^{a}	1.37
EFC	8.06 ± 0.08 ^{b}	14.67 ± 0.05 ^{b,c}	0.16 ± 0.02 ^{a}	0.78 ± 0.01 ^{e}	4.20 ± 0.03 ^{f}	2.95 ± 0.03 ^{f}	7.15 ± 0.06 ^{g}	1.42
EFCX	8.26 ± 0.13 ^{b}	14.89 ± 0.05 ^{e,f}	0.29 ± 0.02 ^{c}	0.83 ± 0.01 ^{f}	3.15 ± 0.02 ^{b}	2.57 ± 0.02 ^{c}	5.73 ± 0.03 ^{b}	1.23

F—flour; T—dry thermal treatment; H—hydrothermal treatment; E—extrusion treatment; C—cellulase enzyme; X—xylanase enzyme; TDF—total dietary fiber; IDF—insoluble dietary fiber; SDF—soluble dietary fiber; $^{a–h}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

The greatest differences were observed in dietary fiber content and its fractions. Application of C and a CX complex under environmental conditions lowered TDF content in FC and FCX samples, with an 18–19% increased ratio of insoluble/soluble fractions of dietary fiber (Table 1). TDF increased significantly after dry thermal treatment without (TF) and with added enzymes (TFC and TFCX), but the IDF/SDF ratio was lower after the hybrid method application. Individual hydrothermal treatments slightly increased the TDF content, with increasing soluble fraction content in the HF samples, as compared to the native sample. Hybrid HFC and EFC treatments showed increased content of TDF due to cellulase action on fibrous components present in flour under pressure–thermal conditions, hence the release of more insoluble fiber fractions. In contrast, CX addition significantly lowered TDF content in HFCX and EFCX samples. The synergic effect of both enzymes also significantly lowered the detection possibility of fibrous fractions—probably due to their enzymatic hydrolysis being supported by the integrated water temperature effect. A

significant increase in SDF was observed in HF and in extruded samples which generated similar or lower values of the IDF/SDF ratio than in native F wheat flour (Table 1).

The impact of extrusion conditions as extruder barrel temperature or screw speed, on different dietary fiber components, may vary depending on processing conditions. Alam et al. [39], for example, reported a significant increase in SDF and TDF fractions in extruded rye bran when both in-barrel water feeding and pre-conditioning were applied during twin-screw extrusion at 130 °C. A decrease in TDF may also be observed due to the degradation of insoluble parts into smaller-molecular-weight compounds by extrusion. Lee et al. [40] tested the effect of various cooking methods on wheat bran and reported extrusion to be the most effective in increasing the TPC, the SDF content, the bulk density of bran and the midline peak time.

3.2. Influence of Modification Conditions on Polysaccharide Fractions

Consumption of AX, the dominant dietary fiber component in bran, helps to reduce glucose and insulin levels in food. Tables 2 and 3 present the effect of the modification method on NSP and AX compositions of insoluble and soluble fractions in treated wheat flour without/with enzyme addition. Table 4 reveals summarized NSP and AX total insoluble and soluble fractions depending on the treatment method.

Table 2. Insoluble non-starch polysaccharide and arabinoxylan content in untreated and hybrid-treated flours.

Sample	Insoluble Polysaccharides					
	I-Mannose (%)	**I-Galactose (%)**	**I-Glucose (%)**	**I-Arabinose (%)**	**I-Xylose (%)**	**I-A/X (-)**
F	0.131 ± 0.002 ^{a,b}	0.078 ± 0.003 ^{a,b,c}	0.548 ± 0.024 ^{b,c}	0.549 ± 0.019 ^{d}	0.759 ± 0.019 ^{e,f}	0.723 ± 0.007 ^{a}
FC	0.133 ± 0.005 ^{a,b}	0.074 ± 0.002 ^{a,b}	0.535 ± 0.005 ^{b,c}	0.458 ± 0.027 ^{b,c}	0.674 ± 0.004 ^{c,d}	0.680 ± 0.044 ^{a}
FCX	0.142 ± 0.017 ^{a,b}	0.081 ± 0.007 ^{a,b,c}	0.528 ± 0.003 ^{a,b,c}	0.518 ± 0.020 ^{b,c,d}	0.651 ± 0.033 ^{b,c}	0.798 ± 0.072 ^{a}
TF	0.137 ± 0.016 ^{a,b}	0.097 ± 0.008 ^{b,c}	0.662 ± 0.018 ^{e}	0.582 ± 0.009 ^{d}	0.842 ± 0.024 ^{g}	0.692 ± 0.009 ^{a}
TFC	0.129 ± 0.009 ^{a,b}	0.081 ± 0.005 ^{a,b,c}	0.538 ± 0.012 ^{b,c}	0.536 ± 0.013 ^{c,d}	0.735 ± 0.010 ^{d,e,f}	0.729 ± 0.027 ^{a}
TFCX	0.168 ± 0.013 ^{b}	0.072 ± 0.013 ^{a,b}	0.630 ± 0.015 ^{d,e}	0.498 ± 0.031 ^{b,c,d}	0.691 ± 0.019 ^{c,d,e}	0.721 ± 0.025 ^{a}
HF	0.154 ± 0.015 ^{a,b}	0.087 ± 0.009 ^{a,b,c}	0.654 ± 0.001 ^{e}	0.503 ± 0.004 ^{b,c,d}	0.695 ± 0.007 ^{c,d,e}	0.723 ± 0.013 ^{a}
HFC	0.231 ± 0.031 ^{c}	0.100 ± 0.001 ^{c}	0.657 ± 0.001 ^{e}	0.539 ± 0.040 ^{c,d}	0.787 ± 0.002 ^{f,g}	0.685 ± 0.052 ^{a}
HFCX	0.153 ± 0.006 ^{a,b}	0.081 ± 0.004 ^{a,b,c}	0.569 ± 0.012 ^{c}	0.446 ± 0.009 ^{b}	0.589 ± 0.022 ^{b}	0.758 ± 0.013 ^{a}
EF	0.120 ± 0.005 ^{a}	0.074 ± 0.001 ^{a,b}	0.573 ± 0.003 ^{c,d}	0.507 ± 0.007 ^{b,c,d}	0.694 ± 0.014 ^{c,d,e}	0.731 ± 0.005 ^{a}
EFC	0.131 ± 0.014 ^{a,b}	0.069 ± 0.014 ^{a}	0.469 ± 0.052 ^{a}	0.516 ± 0.078 ^{b,c,d}	0.642 ± 0.043 ^{b,c}	0.800 ± 0.068 ^{a}
EFCX	0.129 ± 0.003 ^{a,b}	0.091 ± 0.016 ^{a,b,c}	0.496 ± 0.028 ^{a,b}	0.353 ± 0.013 ^{a}	0.474 ± 0.057 ^{a}	0.749 ± 0.063 ^{a}

F—flour; T—dry thermal treatment; H—hydrothermal treatment; E—extrusion treatment; C—cellulase enzyme; X—xylanase enzyme; I-A/X—insoluble arabinose-to-xylose ratio; $^{a–g}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Table 3. Soluble non-starch polysaccharide and arabinoxylan content in untreated and hybrid-treated flours.

Sample	Soluble Polysaccharides					
	S-Mannose (%)	**S-Galactose (%)**	**S-Glucose (%)**	**S-Arabinose (%)**	**S-Xylose (%)**	**S-A/X (-)**
F	0.265 ± 0.008 ^{b,c}	0.118 ± 0.000 ^{a}	0.352 ± 0.017 ^{a,b,c,d}	0.276 ± 0.008 ^{a,b}	0.328 ± 0.012 ^{b}	0.842 ± 0.008 ^{c,d}
FC	0.275 ± 0.001 ^{c,d}	0.123 ± 0.003 ^{a}	0.313 ± 0.012 ^{a}	0.281 ± 0.002 ^{a,b}	0.335 ± 0.003 ^{b}	0.839 ± 0.000 ^{c,d}
FCX	0.274 ± 0.006 ^{c,d}	0.124 ± 0.002 ^{a}	0.381 ± 0.005 ^{d,e}	0.287 ± 0.001 ^{a,b}	0.351 ± 0.007 ^{b}	0.819 ± 0.013 ^{b,c,d}
TF	0.239 ± 0.014 ^{a,b}	0.121 ± 0.00 ^{a}	0.356 ± 0.004 ^{b,c,d}	0.296 ± 0.002 ^{b}	0.324 ± 0.022 ^{b}	0.916 ± 0.069 ^{d}
TFC	0.242 ± 0.012 ^{a,b}	0.131 ± 0.005 ^{a}	0.382 ± 0.002 ^{d,e}	0.263 ± 0.006 ^{a}	0.322 ± 0.009 ^{b}	0.818 ± 0.002 ^{a,b,c,d}
TFCX	0.249 ± 0.013 ^{a,b,c}	0.115 ± 0.004 ^{a}	0.324 ± 0.025 ^{a,b}	0.271 ± 0.004 ^{a,b}	0.342 ± 0.004 ^{b}	0.791 ± 0.002 ^{a,b,c}
HF	0.295 ± 0.005 ^{d}	0.131 ± 0.002 ^{a}	0.392 ± 0.008 ^{d,e}	0.275 ± 0.017 ^{a,b}	0.396 ± 0.024 ^{c}	0.698 ± 0.085 ^{a}
HFC	0.227 ± 0.007 ^{a}	0.118 ± 0.013 ^{a}	0.420 ± 0.024 ^{e}	0.281 ± 0.012 ^{a,b}	0.268 ± 0.011 ^{a}	1.048 ± 0.00 ^{e}
HFCX	0.241 ± 0.008 ^{a,b}	0.121 ± 0.011 ^{a}	0.415 ± 0.004 ^{e}	0.360 ± 0.001 ^{c}	0.468 ± 0.009 ^{d}	0.769 ± 0.018 ^{a,b,c}
EF	0.257 ± 0.001 ^{b,c}	0.119 ± 0.000 ^{a}	0.322 ± 0.000 ^{a,b}	0.268 ± 0.008 ^{a,b}	0.341 ± 0.007 ^{b}	0.788 ± 0.005 ^{a,b,c}
EFC	0.300 ± 0.020 ^{d}	0.121 ± 0.013 ^{a}	0.369 ± 0.025 ^{c,d}	0.283 ± 0.025 ^{a,b}	0.401 ± 0.013 ^{c}	0.708 ± 0.087 ^{a,b}
EFCX	0.262 ± 0.006 ^{b,c}	0.104 ± 0.025 ^{a}	0.328 ± 0.008 ^{a,b}	0.418 ± 0.005 ^{d}	0.590 ± 0.010 ^{e}	0.709 ± 0.002 ^{a,b}

F—flour; T—dry thermal treatment; H—hydrothermal treatment; E—extrusion treatment; C—cellulase enzyme; X—xylanase enzyme; S-A/X—soluble arabinose-to-xylose ratio; $^{a–e}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Table 4. Non-starch polysaccharide and arabinoxylan content in untreated and hybrid-treated flours.

Sample	Polysaccharide Fractions					
	I-AX (%)	S-AX (%)	T-AX (%)	I-NSP (%)	S-NSP (%)	T-NSP (%)
F	1.31 ± 0.04 ^{d,e,f}	0.604 ± 0.020 ^{b,c}	1.912 ± 0.058 ^{b,c}	2.064 ± 0.009 ^{d,e}	1.340 ± 0.004 ^{a}	3.404 ± 0.004 ^{b,c}
FC	1.13 ± 0.02 ^{b,c}	0.616 ± 0.005 ^{b,c}	1.749 ± 0.018 ^{a}	1.875 ± 0.025 ^{b,c}	1.327 ± 0.012 ^{a}	3.202 ± 0.037 ^{a}
FCX	1.17 ± 0.01 ^{b,c,d}	0.638 ± 0.009 ^{c,d}	1.807 ± 0.004 ^{a,b}	1.921 ± 0.008 ^{b,c,d}	1.416 ± 0.001 ^{b}	3.337 ± 0.007 ^{a,b,c}
TF	1.42 ± 0.03 ^{f}	0.620 ± 0.020 ^{b,c}	2.044 ± 0.013 ^{c}	2.319 ± 0.074 ^{f}	1.335 ± 0.037 ^{a}	3.654 ± 0.037 ^{e}
TFC	1.27 ± 0.00 ^{c,d,e}	0.585 ± 0.015 ^{a,b}	1.856 ± 0.012 ^{a,b}	2.020 ± 0011 ^{c,d,e}	1.340 ± 0.004 ^{a}	3.360 ± 0.015 ^{a,b,c}
TFCX	1.19 ± 0.05 ^{c,d,e}	0.613 ± 0.007 ^{b,c}	1.802 ± 0.042 ^{a,b}	2.059 ± 0.064 ^{d,e}	1.301 ± 0.035 ^{a}	3.361 ± 0.099 ^{a,b,c}
HF	1.20 ± 0.00 ^{c,d,e}	0.671 ± 0.007 ^{d,e}	1.869 ± 0.010 ^{a,b}	2.093 ± 0.027 ^{e}	1.489 ± 0.018 ^{c}	3.581 ± 0.045 ^{d,e}
HFC	1.33 ± 0.04 ^{e,f}	0.548 ± 0.023 ^{a}	1.875 ± 0.016 ^{a,b}	2.314 ± 0.069 ^{f}	1.313 ± 0.027 ^{a}	3.627 ± 0.042 ^{e}
HFCX	1.04 ± 0.03 ^{b}	0.828 ± 0.008 ^{f}	1.863 ± 0.023 ^{a,b}	1.838 ± 0.018 ^{b}	1.605 ± 0.015 ^{d}	3.443 ± 0.003 ^{d}
EF	1.20 ± 0.02 ^{c,d,e}	0.609 ± 0.015 ^{b,c}	1.810 ± 0.036 ^{a,b}	1.967 ± 0.014 ^{b,c,d,e}	1.307 ± 0.016 ^{a}	3.274 ± 0.030 ^{a,b}
EFC	1.16 ± 0.12 ^{b,c}	0.684 ± 0.012 ^{e}	1.842 ± 0.134 ^{a,b}	1.828 ± 0.041 ^{b}	1.473 ± 0.021 ^{b,c}	3.302 ± 0.062 ^{a,b,c}
EFCX	0.83 ± 0.07 ^{a}	1.009 ± 0.015 ^{g}	1.836 ± 0.055 ^{a,b}	1.543 ± 0.111 ^{a}	1.703 ± 0.012 ^{e}	3.246 ± 0.123 ^{a,b}

F—flour; T—dry thermal treatment; H—hydrothermal treatment; E—extrusion treatment; C—cellulase enzyme; X—xylanase enzyme; I-AX—insoluble arabinoxylans; S-AX—soluble arabinoxylans; T-AX—total arabinoxylans; I-NSPs—insoluble non-starch polysaccharides; S-NSPs—soluble non-starch polysaccharides; T-NSPs—total non-starch polysaccharides; $^{a–g}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Regarding the insoluble sugars present in native and modified flour, after applying dry thermal treatment TF, the samples demonstrated increasing content of insoluble fractions of mannose, galactose, glucose, arabinose and xylose, as compared to native F flour (Table 2). However, enzyme treatment significantly lowered insoluble glucose, arabinose and xylose fractions in FC and FCX samples, as compared to native samples. Moreover, enzyme addition in TFC and TFCX hybrid treatment, with the exception of I-mannose, lowered insoluble sugar content, while HF and hybrid HFC and HFCX methods formed more insoluble fractions of mannose, galactose and glucose, whereas I-arabinose levels lowered significantly. After extrusion and hybrid treatments, insoluble arabinose and xylose were lowered most significantly. Similar to the composition of soluble fractions of NSP and AX (Table 3), cellulase and cellulase–xylanase complex incorporation increased the soluble fraction contents of almost all sugars, suggesting the formation of more soluble fractions after enzymatic activity.

Dry thermal treatment lowered soluble mannose and xylose content, and enzyme additions showed differential effects on TFC and TFCX samples. HF treatment without enzymes demonstrated increased soluble sugar content compared to native F flour. C or CX complex addition lowered soluble mannose, but other sugar contents increased (such as S-glucose and S-arabinose) or seemed similar to native flour. The most significant differences were observed if extrusion or hybrid enzymatic–extrusion treatment was applied. Accordingly, an especially strong increase in soluble arabinose and xylose was noted, while the S-A/X ratio was the lowest. In general, modification methods, except for TF, slightly lowered the amount of total arabinoxylan content compared to native F flour, probably due to partial hydrolysis by enzymes or the formation of complexes.

A significant decrease in insoluble AX and a simultaneous increase in soluble AX were observed in extruded samples, without/with enzymes (Table 4). In almost all treated and hybrid-treated samples, the content of T-NSP decreased except for TF and all hydrothermally treated samples (HF, HFC and HFCX), as compared to native F. In contrast, soluble NSP fractions increased in FCX samples, and in HF and HFCX, as well as in EFC- and EFCX-treated flours.

In general, modification methods, except for TF, slightly lowered the amount of total arabinoxylan content compared to native F flour, probably due to partial hydrolysis by enzymes or the formation of complexes. Positive correlations (significant at $p < 0.05$) were noted in treated flours between I-arabinose (0.610), I-xylose (0.626), I-AX (0.639), I-NSP (0.682) and T-NSP (0.634) with insoluble fiber content. Soluble fiber fractions present in modified flour were also correlated positively with T-NSP at 0.633, and total fiber content

was correlated with I-AX (0.606), I-NSP (0.661) and T-NSP (0.698). These correlations were not extremely high but were significant at $p < 0.05$. Andersson et al. [17] reported an increase in extractable dietary fiber, including AX and its soluble fractions, through extrusion processing. The applied high shearing forces cause fiber length reduction; therefore, it probably increases the accessible surface area for enzymatic hydrolysis. According to Yağcı et al. [41], extrusion experiments conducted at maximum barrel temperatures of 40, 75 and 110 °C enabled minimal degradation of bulgur bran hemicellulose. They also observed a significant reduction in glucose content after extrusion pretreatment, but an increase in hemicellulose, xylan and arabinose contents after combined alkali–extrusion treatments of bran. Corn fiber pretreated in a twin-screw extruder with different chemicals (NaOH and H_2SO_4 solutions) showed that increasing screw speed improved reducing sugar, soluble arabinoxylan content and the yield of corn fiber gum [22].

3.3. Effect of Treatment on Hydration and Retention Properties

Preferred techno-functional properties depend on flour designation in the bakery industry. For example, bread flour requires high water absorption, good gluten strength and relatively high damaged starch and arabinoxylan content, while cookie flour requires low water absorption, minimal gluten strength and low damaged starch and arabinoxylan content [42]. The absorption and retention capacity of flour or dough components may indicate allusive main components responsible for flour quality. The SRC method with no additional shearing and heating of components with La is useful for indicating the protein quality; Sc reveals starch quality and Su uncovers polysaccharide contents (especially pentosane structures). The results of SRC measurements of untreated, individual and hybrid method treatments for wheat flour are presented in Table 5.

Table 5. SRC values of untreated and hybrid-treated flours.

Sample	SRCWa (%)	SRCSu (%)	SRCLa (%)	SRCSc (%)	GPI (-)
F	70.005 ± 0.062 ^{d}	114.973 ± 0.770 ^{c,d}	118.294 ± 0.595 ^{c,d}	87.839 ± 0.256 ^{b,c}	0.583 ± 0.005 ^{c}
FC	70.427 ± 0.049 ^{d}	116.916 ± 1.228 ^{d}	118.289 ± 0.624 ^{c,d}	86.971 ± 0.080 ^{b,c}	0.580 ± 0.001 ^{b,c}
FCX	75.496 ± 0.278 ^{e}	119.827 ± 0.876 ^{e}	126.504 ± 0.950 ^{e}	88.702 ± 0.153 ^{c}	0.607 ± 0.003 ^{d}
TF	66.757 ± 0.171 ^{b}	113.042 ± 0.921 ^{c}	112.155 ± 0.959 ^{b}	85.038 ± 0.233 ^{b}	0.566 ± 0.002 ^{b}
TFC	68.594 ± 0.097 ^{c}	115.545 ± 0.407 ^{d}	115.279 ± 1.645 ^{b,c}	87.596 ± 0.073 ^{b,c}	0.567 ± 0.007 ^{b,c}
TFCX	70.049 ± 0.820 ^{d}	119.550 ± 1.698 ^{e}	120.105 ± 1.256 ^{d}	87.912 ± 0.360 ^{b,c}	0.579 ± 0.011 ^{b,c}
HF	64.765 ± 0.292 ^{a}	100.706 ± 0.493 ^{b}	102.786 ± 1.578 ^{a}	81.098 ± 0.066 ^{a}	0.565 ± 0.008 ^{b}
HFC	66.656 ± 0.169 ^{b}	96.238 ± 0.761 ^{a}	102.717 ± 0.601 ^{a}	81.218 ± 0.424 ^{a}	0.579 ± 0.001 ^{b,c}
HFCX	66.324 ± 0.146 ^{b}	99.793 ± 0.774 ^{b}	105.804 ± 1.943 ^{a}	81.667 ± 0.422 ^{a}	0.583 ± 0.010 ^{c}
EF	167.158 ± 0.227 ^{f}	161.398 ± 0.224 ^{f}	172.324 ± 0.952 ^{f}	235.073 ± 1.585 ^{d}	0.435 ± 0.001 ^{a}
EFC	177.957 ± 0.609 ^{g}	166.261 ± 0.363 ^{g}	183.706 ± 1.429 ^{g}	248.603 ± 2.871 ^{e}	0.443 ± 0.001 ^{a}
EFCX	216.286 ± 0.156 ^{h}	184.618 ± 0.087 ^{h}	210.365 ± 0.588 ^{h}	295.415 ± 0.440 ^{f}	0.438 ± 0.001 ^{a}

F—flour; T—dry thermal treatment; H—hydrothermal treatment; E—extrusion treatment; C—cellulase enzyme; X—xylanase enzyme; SRC—Solvent Retention Capacity; Wa—distilled water; Su—50% sucrose-in-water solution; La—5% lactic acid-in-water solution; Sc—5% sodium carbonate-in-water solution; GPI—gluten performance index; $^{a–h}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Native F flour demonstrated 70.005% SRCWa, 114.973% SRCSu, 118.294% SRCLa and 87.839% SRCSc. Enzymatic action increased the Wa, Su, La and Sc retention capacity of FC and FCX flours, whereas TF treatment without enzymes slightly decreased the absorption and retention capacity, and when cellulase was used in the hybrid TFC flour, water hydration decreased. Moreover, La and Sc solutions in TFCX samples brought about opposite trends, and a slight increase in Su absorption was noted in both TFC and TFCX samples, indicating increased activity of fibrous fractions under dry heating. Furthermore, hydrothermal treatments without/with enzymes lowered SRC values, with the most significant decrease in SRCLa, suggesting limited absorption by proteins present in HF-, HFC- and HFCX-modified flours due to the possible formation of unextractable polymeric proteins (UPPs), especially in the presence of starch, after treatment above

50 °C, as reported by Guerrieri and Cerletti [8], Hu et al. [33] and Schofield et al. [6]. Van Steertegem et al. [43] reported decreased SRCLa values of commercial wheat flour subjected to either 2 or 5 h heating at 80 or 100 °C, indicating that heat treatment restricted the swelling ability of the protein network, which was related to protein cross-linking within the flour particles. Longer and more severe heat treatments indicated more cross-linking, leading to lower LaSRC, but when heat treatment was relatively mild, increases in WaSRC and SuSRC were observed. Moreover, they found decreases in both SDSEP (proteins extractable in sodium dodecyl sulfate (SDS)-containing medium) and free SH groups as a result of heating, indicating that the gluten proteins formed covalent disulfide (SS) cross-links and hence polymerized after treatment, thereby increasing gluten protein polymer size values and thus preventing the gluten proteins from swelling in the La-containing media [43]. Similar results were noted in our results for HF samples and this effect was limited after treatments with enzymes added.

Extrusion and hybrid enzymatic–extrusion treatments generated increased absorption ability and retention capacity in modified EF, EFC and EFCX flour, probably due to significant changes in proteins, starch and fiber because of the treatment intensity. Combined EFCX treatment strongly affected SRC due to having the highest values of retention capacity of all applied solvents (Table 5). This again reveals the intensity of extrusion and hybrid enzymatic–extrusion conditions on the tested wheat flour. Additionally, after any form of extrusion treatment, compared to other treatments, GPI values were the lowest. GPI, considering the overall effect of protein, starch and fibrous fractions, indicates the overall performance of glutenin capability in simulating dough behavior. Other treatments showed negligible or slight decreasing effects on GPI values, and only the FCX method increased GPI. GPI results were significantly (at $p < 0.05$) correlated in a positive trend with insoluble fiber content in modified flours with a coefficient of 0.628, with dough stability S (0.925), with rheological features C3 (0.955), C4 (0.928) and C5 (0.921), but negatively with Hyd (−0.968).

A negative correlation was found between I-xylose with SRCWa (−0.607) and SRCLa (−0.620). So, with an increased level of insoluble fractions, the I-NSP values of SRC decreased for all solvents used, and significant ($p < 0.05$) negative correlation coefficients were noted between I-NSP and SRCWa (−0.659), SRCSu (−0.670), SRCLa (−0.696) and SRCSc (−0.642). Moreover, a significant (at $p < 0.05$) correlation was found between T-NSP and SRCSu (−0.613). For certain soluble fractions of simple sugars, only significant positive correlations at $p < 0.05$ were found as significant between S-xylose and SRCWa (0.619), SRCLa (0.610) and SRCSc (0.601), which means that if S-xylose was higher, the flour absorption ability would be improved with the most visible action of gluten explained by SRCLa values. Also, hydration (Hyd) properties of modified flours were significantly (at $p < 0.05$) positively correlated with SRCWa values (0.992), SCRSu (0.970), SRC La (0.978) and SRCSc (0.995). Strong significant negative correlations at $p < 0.05$ were found between S, C3, C4, C5 and all SRC results for all solvents used, and correlation coefficients varied from −0.947 to −0.983. So, it can be stated that the hydration properties of modified flours are strongly related to rheological features related to starch changes after treatments.

Keppler et al. [44] tested the dry heat treatment of soft wheat flour by heating a thin layer at temperatures between 110 °C and 200 °C for between 1 and 30 min. They noted improved swelling ability and increased interactions of flour polymers (in particular arabinoxylans) of heat-treated flour at ambient conditions as tested by SRC tests. An increase in individual solvent (La, SC and Su) retention capacity was observed when flour was heated at an elongated time, and a decrease in GPI was significant at prolonged heating time. They connected the SRC profile with the impact of thermal treatment on the arabinoxylan fraction of flour, showing increased swelling behavior in the SRC test mostly upon heat.

Ma et al. [9] investigated the effects of wheat bran pretreatments by autoclaving, roasting, jet cooking, extrusion, puffing and high-temperature high-pressure cooking on steamed bread and pancake properties. All the pretreatments of bran imparted negative influences

on the gluten index of whole wheat flour. These treatments significantly increased the water absorption index, water retention capacity and SDF content, but differently affected the microstructure, the median particle size, the bulk density of wheat bran and the dough mixing properties. Extrusion of bran may improve the crumb structure quality, stress relaxation score and springiness in steam bread, and autoclaving of bran improved the moistness of pancakes [40].

3.4. Impact of Heat Treatment Processes on Pasting Properties

Pasting properties indicate changes in starchy components, e.g., partial or complete gelatinization by thermal or mechanical treatments. Peak and hot viscosity are the maximum and the lowest viscosity of the starch paste for heating, respectively, and they reveal binding water effects and starch granule swelling. The final viscosity reflects the stability of cooled starch paste. Breakdown and setback indicate the paste's resistance to heat and shear and the paste's retrogradation as a result of cooling [45]. The results of pasting characteristics of native, enzyme-supported and individual or hybrid-treated wheat flour are presented in Table 6.

Table 6. Pasting properties of untreated and hybrid-treated flours.

Sample	Maximum Viscosity (mPas)	Through Viscosity (mPas)	Final Viscosity (mPas)	Breakdown (mPas)	Setback (mPas)	Beginning of Gelatinization (°C)	End of Gelatinization (°C)
F	1564 ± 9 ^{h}	436 ± 3 ^{i}	1225 ± 2 ^{g}	1128 ± 6 ^{g}	789 ± 4 ^{h}	60.2 ± 0.0 ^{c}	86.6 ± 0.1 ^{i}
FC	1313 ± 4 ^{e}	322 ± 1 ^{e}	935 ± 3 ^{e}	990 ± 3 ^{e}	612 ± 2 ^{e}	60.5 ± 0.0 ^{c}	85.5 ± 0.0 ^{f}
FCX	1204 ± 3 ^{d}	281 ± 3 ^{d}	783 ± 9 ^{c}	923 ± 4 ^{d}	502 ± 7 ^{c}	60.2 ± 0.1 ^{c}	85.2 ± 0.1 ^{e}
TF	1014 ± 15 ^{a}	194 ± 3 ^{a}	496 ± 12 ^{a}	820 ± 12 ^{b}	302 ± 9 ^{a}	60.3 ± 0.1 ^{c}	83.2 ± 0.1 ^{a}
TFC	1108 ± 17 ^{b}	240 ± 2 ^{b}	643 ± 2 ^{b}	867 ± 16 ^{c}	402 ± 1 ^{b}	60.3 ± 0.1 ^{c}	83.9 ± 0.1 ^{b}
TFCX	1148 ± 10 ^{c}	256 ± 4 ^{c}	661 ± 7 ^{b}	901 ± 2 ^{c,d}	404 ± 2 ^{b}	60.0 ± 0.1 ^{c}	84.3 ± 0.1 ^{c}
HF	1432 ± 26 ^{g}	351 ± 3 ^{f}	892 ± 9 ^{d}	1073 ± 29 ^{f}	540 ± 9 ^{d}	60.2 ± 0.1 ^{c}	84.6 ± 0.1 ^{d}
HFC	1975 ± 7 ^{j}	623 ± 1 ^{l}	1519 ± 6 ^{j}	1353 ± 7 ^{i}	896 ± 4 ^{j}	60.3 ± 0.1 ^{c}	85.8 ± 0.1 ^{g}
HFCX	1785 ± 6 ^{i}	533 ± 5 ^{k}	1290 ± 12 ^{h}	1253 ± 2 ^{h}	757 ± 7 ^{g}	60.1 ± 0.1 ^{c}	85.3 ± 0.1 ^{e,f}
EF	1363 ± 4 ^{f}	480 ± 1 ^{j}	1362 ± 4 ^{i}	883 ± 3 ^{c}	882 ± 5 ^{j}	38.2 ± 0.9 ^{b}	86.7 ± 0.2 ^{i}
EFC	1215 ± 16 ^{d}	405 ± 5 ^{h}	1221 ± 10 ^{g}	810 ± 12 ^{a,b}	816 ± 5 ^{i}	37.2 ± 0.8 ^{b}	86.3 ± 0.1 ^{h}
EFCX	1150 ± 16 ^{c}	366 ± 4 ^{g}	1092 ± 7 ^{f}	781 ± 13 ^{a}	726 ± 5 ^{f}	35.2 ± 0.6 ^{a}	86.2 ± 0.1 ^{h}

F—flour; T—dry thermal treatment; H—hydrothermal treatment; E—extrusion treatment; C—cellulase enzyme; X—xylanase enzyme; $^{a–l}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

The maximum viscosity of native wheat flour was 1564 mPas, and the application of enzymes lowered the maximum, trough and final viscosity of enzyme-fortified samples. In dry-thermal-modified samples, viscosity was significantly lower as compared with native flour, and the application of enzymes induced increased viscosity in the TFC and TFCX samples. Hydrothermal treatment was the most influencing method for increasing viscosity, and, in this case, hybrid HFC treatment produced the highest viscosity values. Moreover, breakdown and setback were the highest, with the same beginning gelatinization temperature. Here, in TF, HF and hybrid treatments, the effect of enzymes was opposite to that in the F and E methods. Extruded EF and hybrid EFC- and EFCX-treated flours showed lower maximum viscosity than native F, FC or FCX samples, but the trough and final viscosities were higher than in native or dry-thermal-modified flours. A significantly lower beginning gelatinization temperature was noted in all extruded samples, indicating that starch was partly melted and gelatinized. Applied treatments resulted in increased setback values in HFC, EF and EFC samples (Table 6), suggesting increased starch paste stability and gel hardness. Other treatments, especially when enzymes were applied as individual C, a CX complex or in hybrid dry thermal treatment, lowered setback values.

Bucsella et al. [4] confirm that the hydrothermal process increased peak viscosity and setback values, compared to untreated flours, while treated bread flour showed higher peak viscosity than treated cake flour. Similar observations were reported for increased

temperature and retention time [46]. Therefore, hydrothermally treated starch granules are more rigid and resistant to quick heating due to altered swelling behavior, as stated by McCann et al. [47]. The presence of protein and starch in wheat flour differentiates pasting properties by competition of protein and starch for water during hydration. Pasting properties were measured with greater access to water oppositely to dough tested in Mixolab because of the altered diffusion of water into starch granules through the formation of a starch–protein matrix and starch gelatinization during the pasting procedure [48]. This behavior was not observed in dough because of less accessible water [4]. In our research, significant (at $p < 0.05$) correlations were observed between breakdown values and C3 (0.677), C4 (0.745) and C5 (0.734), as indicated mostly by starch transformations during heating. Additionally, a significant correlation at $p < 0.05$ between pasting properties and dough properties was similar to breakdown with dough stability (0.746) and C2 (0.714), as a result of protein weakening. Where the highest protein destruction occurred, the highest C2 level showed the highest max viscosity with a correlation coefficient of 0.826 at $p < 0.05$. For samples HFC and HFCX, the highest gel formation properties were observed as confirmed by the highest viscosity because of the high stiffness of dough as confirmed by high C2 values, which correlated with setback values (0.626). These observations may be the effect of the hybrid action of enzymes used with the high level of the insoluble fraction of I-NSP in HFC and the high level of S-NSP in HFCX-modified flour (Table 4). Breakdown was also significantly (at $p < 0.05$) negatively correlated (−0.693) with SRCSu responsible for arabinoxylan absorption. But these pasting analysis results were less connected with other modified flour properties than results obtained in the dough matrix tested by Mixolab. Hu et al. [33] reported that the application of superheated steam into wheat grains resulted in lower gelatinization temperature and higher peak viscosity with increased time and temperature of treatments up to 200 °C, upon which starch damage was observed. Peak and final viscosities, as well as breakdown and setback, decreased gradually with increased content of damaged starch due to better hydration, and weakening and breakdown of the gelatinized granules were observed [49]. Ma et al. [50] reported that superheated steam processing resulted in a rise in peak viscosity compared with native wheat flour; similar to our research, these changes were correlated with the denaturation protein barriers surrounding starch granules. Deng et al. [51] found higher viscosity of enzymatic–extrusion-treated rice bran when the screw rate was low at low moisture content, due to the higher specific mechanical energy input, which softened the fiber and created a loose and porous structure. Higher water content in raw material, higher gelatinization and a more rapid increase in viscosity at low temperatures were reported by Liu et al. [52] if rice starch was extruded with a single-screw extruder. The pasting profile of wheat flours studied by Román et al. [53] showed lower viscosity profiles of non-enzymatically treated and extruded flours, confirming that gelatinization occurred during thermal treatments, similarly shown in our research. Breakdown and setback values were also reduced. Both extruded and native samples with additional enzymatic treatment showed very low viscosity and flat pasting profiles with no peak viscosity as a result of the hydrolytic activity of the enzyme on the starch.

3.5. Heat Treatment Impact on Rheological Dough Properties

In our study, water absorption tested by hydration measurement varied depending on the testing method. Hydration as tested via Mixolab corresponded very well (r = 0.900 at $p < 0.05$) to the water absorption determined by the Farinograph when corrected to 14% initial moisture. However, unlike the Farinograph, the Mixolab allowed for the assessment of the water absorption of modified flour subjected to the extrusion process. Native flour F demonstrated water absorption of 60.5%, while both thermal and hydrothermal treatment in the TF and HF samples reduced water absorption (Table 7). The addition of C and CX enzymes in all treated samples resulted in a slight increase in water absorption. For extrusion processing, as in the SRC study, a more than twofold increase in hydration was observed compared to other tested methods as a result of starch swelling or partial

gelatinization during processing. The analyzed development time (DT) of dough from native F flour was short (1.92 min), but the addition of enzymes in the FC and FCX samples significantly enhanced this parameter. Dry thermal treatment resulted in a 2.5-fold increase in the DT without significant impact of enzyme use. Hydrothermal treatment elongated DT, especially in the HFC sample, to 7.40 min; this flour was characterized by high S-A/X (1.048) and the highest total fiber content (7.49%). In samples subjected to the extrusion treatment, DT did not differ significantly compared to unprocessed samples. Moreover, the stability of flour treated by dry heating was similar to F, while hydrothermal steam-assisted treatment resulted in increased stability, and low-temperature extrusion reduced stability by almost triple as compared to native flour with no significant effect of enzyme addition.

Mixolab measures the C2 parameter as a dough consistency loss during the exposure to physical–mechanical and thermal stress, and after heat-induced protein denaturation carbohydrate-dependent starch gelatinization (C3), amylase activity (C4) and starch gelling (C5) dominated. The results of rheological characteristics tested with the Mixolab procedure are presented in Table 7.

Table 7. Mixolab features of untreated and hybrid-treated flours.

Sample	Hyd (%)	DT (min)	S (min)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
F	60.5 ± 0.1 ^{b}	1.92 ± 0.19 ^{a}	9.73 ± 0.12 ^{e}	0.477 ± 0.01 ^{c}	1.709 ± 0.01 ^{c,d}	1.479 ± 0.01 ^{e}	2.519 ± 0.00 ^{e,f}
FC	60.6 ± 0.1 ^{b}	2.66 ± 0.76 ^{a,b}	9.60 ± 0.36 ^{d,e}	0.432 ± 0.00 ^{a,b}	1.673 ± 0.006 ^{c}	1.433 ± 0.03 ^{d,e}	2.414 ± 0.03 ^{d,e}
FCX	61.2 ± 0.2 ^{b}	3.22 ± 0.34 ^{b}	9.37 ± 0.15 ^{c,d,e}	0.423 ± 0.00 ^{a}	1.674 ± 0.01 ^{c}	1.351 ± 0.01 ^{c}	2.173 ± 0.04 ^{c}
TF	57.6 ± 0.3 ^{a}	4.94 ± 0.39 ^{c}	9.23 ± 0.06 ^{b,c,d}	0.454 ± 0.01 ^{b,c}	1.665 ± 0.01 ^{c}	1.352 ± 0.02 ^{c}	2.343 ± 0.07 ^{d}
TFC	60.8 ± 0.2 ^{b}	4.81 ± 0.20 ^{c}	9.03 ± 0.15 ^{b,c}	0.448 ± 0.01 ^{a,b}	1.666 ± 0.01 ^{c}	1.393 ± 0.01 ^{c,d}	2.402 ± 0.06 ^{d,e}
TFCX	61.6 ± 0.2 ^{b}	4.68 ± 0.07 ^{c}	8.83 ± 0.23 ^{b}	0.441 ± 0.01 ^{a,b}	1.657 ± 0.01 ^{c}	1.382 ± 0.02 ^{c,d}	2.330 ± 0.05 ^{c,d}
HF	58.1 ± 0.4 ^{a}	2.37 ± 0.38 ^{a,b}	10.87 ± 0.23 ^{f}	0.572 ± 0.01 ^{e}	1.781 ± 0.01 ^{d}	1.571 ± 0.05 ^{f}	2.675 ± 0.13 ^{f}
HFC	58.3 ± 0.2 ^{a}	7.40 ± 0.94 ^{d}	11.90 ± 0.10 ^{g}	0.770 ± 0.00 ^{g}	1.985 ± 0.00 ^{e}	1.832 ± 0.01 ^{g}	3.072 ± 0.02 ^{g}
HFCX	58.7 ± 1.1 ^{a}	3.14 ± 0.54 ^{a,b}	11.77 ± 0.15 ^{g}	0.699 ± 0.01 ^{f}	1.939 ± 0.02 ^{e}	1.801 ± 0.01 ^{g}	2.952 ± 0.07 ^{g}
EF	94.3 ± 1.5 ^{c}	2.59 ± 0.01 ^{a,b}	3.83 ± 0.06 ^{a}	0.524 ± 0.02 ^{d}	0.745 ± 0.02 ^{b}	0.491 ± 0.02 ^{b}	0.844 ± 0.03 ^{b}
EFC	95.2 ± 0.1 ^{c}	2.13 ± 0.20 ^{a,b}	3.87 ± 0.06 ^{a}	0.529 ± 0.00 ^{d}	0.639 ± 0.10 ^{a}	0.443 ± 0.00 ^{b}	0.777 ± 0.01 ^{a,b}
EFCX	101.2 ± 0.3 ^{d}	2.66 ± 0.01 ^{a,b}	3.42 ± 0.03 ^{a}	0.566 ± 000 ^{e}	0.576 ± 0.00 ^{a}	0.366 ± 0.00 ^{a}	0.657 ± 0.01 ^{a,b}

F—flour; T—dry thermal treatment; H—hydrothermal treatment; E—extrusion treatment; C—cellulase enzyme; X–xylanase enzyme; Hyd—hydration capacity; DT—development time; S—stability C2—protein weakening; C3—starch gelatinization; C4—amylase activity; C5—starch retrogradation; $^{a–g}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

The C2 protein weakening parameter for TF samples did not differ from F, but a significant increase was observed for flours subjected to hydrothermal and extrusion processing. The highest C2 was observed in the HFC sample, and the effect was probably related to the highest fiber content, especially insoluble fractions, which resulted in the deterioration of the flour's baking properties, as confirmed by alveographic analysis results presented later (in Table 9). Tested dough made with steam-assisted hydrothermally treated flour HF was characterized by high springiness and low elasticity caused by significant changes in the conformation of gluten proteins, preventing proper development of the gluten network or destruction of gluten. As described by Hong et al. [48], modification of wheat flour using superheated steam treatment causes the denaturation of proteins and the initial gelatinization of starch granules contained in wheat flour, thus reducing the access of water to the protein phase due to greater absorption by modified starch. The effects of these changes may be visible by the problem with the dough formation ability caused by the weakening of the gluten quality and a reduction in its elasticity. An increase in the C2 parameter compared to F was also observed for samples subjected to low-temperature extrusion. These changes were induced by the loss of the gluten–protein matrix properties due to high mechanical shearing. For C2, after heating to 60 °C, if the gluten is damaged, the C2 in HF is high, similar to what was reported by Lewko et al. [54] for low-temperature single-screw extrusion. For these tests, high elasticity P and low extensibility L were also noted (Table 9). The C3 and C4 parameters did not differ significantly in native flours without/with enzyme incorporation. The addition of enzymes to native flour reduced

starch retrogradation level (C5) in FCX samples. Similarly, the thermal process did not affect the C3 value but slightly reduced the amylase activity of C4 and starch retrogradation (C5), without the significant influence of the enzymes. The highest differences in the impact of the processing on starch-related parameters after hydrothermal treatment were observed, wherein significant increases in C3, C4 and C5 (especially in the presence of C and the CX complex) were indicated. Some significant correlations were found between dough features tested with Mixolab and pasting properties, as reported in Section 3.4.

The extrusion process, in turn, significantly reduced the C3, C4 and C5 values, probably due to the high water absorption of flours obtained after this treatment. The above parameters had values inversely proportional to the flour's water absorption (Hyd) with significant correlation coefficients at $p < 0.05$ of −0.980 with C3, −0.958 with C4, and −0.961 with C5, the lowest being for EFCX flour. Reduction in the gelling ability (C3) may be altered by the protein–pentosan–lipid complexes formed in extrusion processing. Bucsella et al. [4] reported that the C3 parameter at the heating phase is higher in cookie flour than in bread flour. In contrast, the gelling ability of both types of flour during the cooling phase (C5) is similar. Moreover, thermally treated flour shows higher C2 and lower weakening with similar DT of cake flour, but with prolonged stability. Minor differences between untreated and dry-treated flours, especially in C3 and C4 values, may be because of the insignificant effect of dry heating on starch structure and gelling properties. Bucsela et al. [4], comparing RVA and Mixolab, found that starch behaves differently in the dough matrix and differently in the suspension (difference in water content to flour), and flours were more degraded by the high process temperature of 96 °C in hydrothermal treatment. These changes were more similar to our results for flour samples treated using low-temperature extrusion—then, the described dependencies of rheological features can be confirmed which are consistent with the cited work [4]. Moreover, in our research, the applied parameters of hydrothermal treatment used in the prototype installation were less aggressive in order to better protect the enzyme used as additives from the effect of high temperature.

Farinographic assessment allows for predicting the baking quality and may indicate directions for the technological use of the tested products [33]. Table 8 shows the results of the Farinograph tests. Flours subjected to extrusion and hybrid enzymatic–extrusion processing could not be analyzed on a Farinograph due to the probable complete loss of wheat gluten functionality and the very high water absorption obtained.

Table 8. Farinograph properties of untreated and hybrid-treated flours.

Sample	WA500 (%)	WA 14% (%)	DT (min)	S (min)	DoS (BU)	DoS12 (BU)	QN (-)
F	60.7 ± 0.3 ^{a}	59.5 ± 0.3 ^{c}	3.1 ± 0.6 ^{a}	14.1 ± 0.7 ^{c}	21.7 ± 3.2 ^{d,e,f}	38.0 ± 2.6 ^{c}	118.3 ± 10.3 ^{a}
FC	61.0 ± 0.1 ^{a,b}	60.2 ± 0.1 ^{d}	2.7 ± 0.3 ^{a}	12.1 ± 0.9 ^{a,b}	29.3 ± 5.0 ^{f}	46.3 ± 3.1 ^{d,e}	96.3 ± 9.9 ^{a}
FCX	61.3 ± 0.0 ^{b}	60.5 ± 0.0 ^{d,e}	3.8 ± 0.1 ^{a,b}	13.1 ± 0.1 ^{b,c}	19.7 ± 1.2 ^{c,d,e}	42.0 ± 0.0 ^{c,d}	128.0 ± 1.7 ^{a,b}
TF	66.0 ± 0.2 ^{e}	58.5 ± 0.2 ^{b}	5.9 ± 0.8 ^{b,c}	11.5 ± 0.4 ^{a}	17.0 ± 1.0 ^{c,d,e}	58.7 ± 2.5 ^{f}	126.0 ± 2.6 ^{a,b}
TFC	67.9 ± 0.1 ^{f}	60.8 ± 0.1 ^{e}	6.1 ± 0.9 ^{c}	11.5 ± 0.3 ^{a}	15.3 ± 3.1 ^{b,c,d}	57.7 ± 3.5 ^{f}	126.7 ± 5.5 ^{a,b}
TFCX	68.2 ± 0.1 ^{f}	61.8 ± 0.1 ^{f}	6.5 ± 0.9 ^{c}	11.5 ± 0.6 ^{a}	12.0 ± 2.0 ^{a,b,c}	53.3 ± 2.5 ^{e,f}	132.7 ± 2.5 ^{a,b}
HF	64.6 ± 0.1 ^{d}	57.3 ± 0.1 ^{a}	2.9 ± 0.1 ^{a}	18.4 ± 0.1 ^{d}	24.3 ± 4.7 ^{e,f}	27.7 ± 3.8 ^{b}	161.3 ± 36.3 ^{b}
HFC	63.5 ± 0.1 ^{c}	58.3 ± 0.1 ^{b}	18.5 ± 1.6 ^{d}	17.3 ± 0.6 ^{d}	7.7 ± 2.1 ^{a,b}	ND	200.0 ± 0.0 ^{c}
HFCX	63.7 ± 0.1 ^{c}	58.5 ± 0.2 ^{b}	6.8 ± 0.2 ^{c}	18.4 ± 0.1 ^{d}	5.7 ± 0.6 ^{a}	7.3 ± 1.2 ^{a}	200.0 ± 0.0 ^{c}

F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; X—xylanase enzyme; WA500—water absorption at 500 BU; WA 14%—water absorption corrected for 14%; DT—dough development time; S—stability; DoS—dough softening in time; DoS12—dough softening after 12 min; QN—quality number; ND—no data; $^{a–f}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

The process of thermal heating of flour showed an insignificant impact on water absorption, especially if moisture was adjusted to 14%. Similar observations were made by Hu et al. [33] embracing thermal modification of wheat grains before milling, and Bucsella et al. [4] encompassing thermal and hydrothermal treatment on wheat flour for cakes and bread. In our study, steam-assisted hydrothermal treatment reduced the corrected water

absorption of flour compared to F flour. In all treatments, the addition of enzymes resulted in a slight increase in water absorption, especially with CX, with the highest value being obtained for TFCX. Thermal processing of flour may create a more strengthened dough structure without changing its hydration properties [4]. Thermal treatment of flour also resulted in dough DT increase (the lowest for TF and the highest for TFCX). Hydrothermal treatment HF did not change the dough DT, as compared to native flour F. In the HFCX samples, DT was similar to samples subjected only to thermal treatment and was twice as long as in the HF test. The longest dough development time was observed for the HFC sample (18.5 min). These findings are in agreement with research presented by Hu et al. [33], who tested the superheated steam (SS) effect of flour and dough behavior. Dough development time and stability all showed an increasing trend with the extension of superheated steam processing time. After 4 min of processing, the development and stability times increased from NF of 1.2 min and 1.1 min to 8.5 min and 7.1 min for modified flour with superheated steam. The dough exhibited longer stability time and always showed a less weakening index. Dough development time reflected the resistance of the dough against the blades. Dough stability and the degree of softening gave an indication of dough strength and tenacity. The increase in development time and stability showed an enhanced resistance to successive mixing and an improved capacity to sustain shear stress. Combined with the lower degree of softening, it suggested that dough made of SS-treated flours was much stronger and tenacious than that made of NF and the effect of SS treatment on protein aggregation as well as protein–starch interaction [33]. HFC-treated flour was characterized by a high amount of T-NSP and a high content of I-AX and I-NSP fractions among the modified flours tested (Table 4). After adding only cellulase, the amount of soluble fractions of non-starch polysaccharides decreased and the amount of insoluble ones increased, while with the addition of a CX complex, an increase in the amount of soluble and a decrease in the amount of insoluble NSP fractions was noted. This may be the effect of the interaction of hydrothermal treatment in the presence of easily accessible water from steam injection combined with enzyme activity. Various cellulase activities, such as cellobiohydrolase and endoglucanase, can hydrolyze cellulose. The cellulase used in this study contained both a high-activity cellobiohydrolase polymer and an endoglucanase and was responsible for the breakdown of cellulose polymers. The main activity of cellobiohydrolase is opening the fibrils to xylanase action, which breaks down cell wall components, especially in the whole grain fraction. The final effect is increasing the amount of insoluble fractions and facilitating the action of other enzymes. So, the combination of cellulase and xylanase may improve the quality of the crumb because it breaks up cellulose fibers responsible for improved gluten stability and gas retention, without interfering with the action of xylanase, which hydrolyzes arabinoxylans to their soluble form [38].

P result elasticity from Alveograph tests decreased from 140 mm for HF to 97 mm in HFC, thereby decreasing W from 227 $\times$ 10^{-4} J in HF to 134 $\times$ 10^{-4} J in HFC (Table 9), which indicates that a large amount of insoluble NSP fractions influenced the quality of gluten proteins. These insoluble fractions influenced the result of long dough development time (DT) (18.5 min for HFC) and a small DoS of 7.7 BU for HFC because after using the enzyme cellulase and hydrothermal treatment processing of flour with a large amount of insoluble NSP, the dough made from this flour absorbed water very slowly and developed slowly, while the stiff dough was indicated by high C2 and the highest viscosity due to starch gelatinization, as confirmed by Viscograph tests. During Farinographic analysis, after obtaining optimal consistency, partially heated and gelatinized starch and insoluble fractions of polysaccharides competed for water, and the formed structure did not give the possibility of gluten development. In HFCX, a higher amount of soluble fractions improved gluten development ability (DT was 6.8 min), but still, in dough, the C2 value was high (0.699 Nm), with high springiness (P = 147 mm) and low extensibility (L = 31 mm), because denatured proteins after hydrothermal treatment were stiff and not elastic anymore.

Flour stability (S) was found to be dependent on the processing method used. When dry heated, stability decreased slightly to 11.5 min compared to F, but so did the degree of softening (DoS). The most significant differences were observed between HF and hybrid-treated HFC and HFCX flours; a significant decrease in DoS was observed from 24.3 BU to 7.7 BU and 5.7 BU, respectively. DoS12 values were higher by more than double if measured at 12 min of the test for F and with enzymes added (FC or FCX), but 3.5–4 times higher in TF, TFC and TFCX samples, compared to DoS values. Hydrothermal treatment resulted in a significant increase in flour stability to 18.4 min, without significant variability when enzymes were used, but as a result of extended development time. The presence of cellulase or cellulase–xylanase enzymes in TFC and TFCX, as well as in HFC and HFCX samples, increased QN as compared to native and treated flour without enzymes.

Gómez et al. [55] reported that extruded wheat bran addition (2.5 to 20%) increased. DT. Tayefe et al. [56] added hydrothermally treated rice bran to wheat dough which changed the starch present in the bran into a pregelatinized form, hence improving water molecule retention and, when added to the wheat dough, increasing its water absorption capacity, elongating dough development time but lowering dough stability by lowering the gluten content. Tao et al. [57] found increased water absorption of wheat starch extruded at a temperature of 50–70 °C; the addition of 15% of extruded starch decreased DT and strengthened wheat dough consistency.

The modified flours were tested via the Alveograph procedure, which involves measuring the resistance of a dough sample prepared from flour and sodium chloride solution while blowing it evenly. The results of the Alveograph tests are presented in Table 9.

Table 9. Alveograph properties of untreated and hybrid-treated flours.

Sample	P (mm)	L (mm)	W (10^{-4} J)	P/L (-)	Ie (%)	SH (-)
F	111 ± 3^{b}	$77 \pm 6^{c,d}$	273 ± 18^{d}	1.45 ± 0.10^{a}	49.27 ± 0.81^{c}	1.66 ± 0.02^{c}
FC	$105 \pm 1^{b,c,d}$	74 ± 6^{c}	$253 \pm 12^{c,d}$	1.42 ± 0.12^{a}	48.87 ± 0.46^{c}	1.67 ± 0.03^{c}
FCX	$107 \pm 1^{c,d}$	$78 \pm 3^{c,d}$	$266 \pm 8^{c,d}$	1.37 ± 0.03^{a}	49.60 ± 0.61^{c}	1.66 ± 0.02^{c}
TF	82 ± 3^{a}	87 ± 1^{d}	199 ± 6^{b}	0.95 ± 0.04^{a}	44.83 ± 0.29^{a}	1.48 ± 0.05^{a}
TFC	$98 \pm 2^{b,c}$	86 ± 3^{d}	$243 \pm 10^{c,d}$	1.12 ± 0.02^{a}	46.50 ± 0.70^{b}	$1.54 \pm 0.02^{a,b}$
TFCX	$101 \pm 5^{b,c}$	$84 \pm 3^{c,d}$	$249 \pm 16^{c,d}$	1.21 ± 0.03^{a}	46.47 ± 0.35^{b}	$1.55 \pm 0.01^{a,b}$
HF	140 ± 6^{e}	38 ± 1^{b}	227 ± 13^{c}	3.71 ± 0.21^{b}	ND	2.08 ± 0.02^{e}
HFC	97 ± 3^{b}	26 ± 7^{a}	134 ± 31^{a}	$3.97 \pm 0.96^{b,c}$	ND	1.84 ± 0.03^{d}
HFCX	147 ± 4^{e}	$31 \pm 1^{a,b}$	194 ± 7^{b}	4.70 ± 0.19^{c}	ND	$1.61 \pm 0.06^{b,c}$

F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; X—xylanase enzyme; P—dough tenacity; L—extensibility; W—baking strength; P/L—configuration ratio; Ie—elasticity index; SH—strain hardening index; ND—no data; $^{a–e}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

The tested base flour F was characterized by good baking properties, with a baking strength value W of 273×10^{-4} J. The addition of the cellulase FC or cellulase–xylanase complex FCX slightly reduced the elasticity and increased the extensibility of the dough, maintaining the elasticity index Ie and the strain hardening index SH at a similar level. The use of thermal treatment significantly reduced the baking value of TF flour, mainly by reducing the elasticity of the dough (P); it also worsened the Ie and SH parameters, from 49.27% (F) to 44.83% (TF) and from 1.66 (F) to 1.48 (TF), respectively. The use of hybrid modification through the incorporation of baking enzymes in the mixture, especially TFCX, allowed for obtaining quality close to that of the F, FC and FCX samples, especially in dough elasticity (P). Dry thermal and hybrid treatment increased the dough extensibility L, but slightly lowered baking strength W, elasticity index Ie and strain hardening SH, with a lesser difference if TFC and TFCX samples were tested (Table 9).

We noted that the applied steam-assisted hydrothermal treatment caused significant changes in the conformation of gluten proteins, preventing proper development of the gluten network or destruction of gluten. This was due either to the steam's high temperature or by the integrated thermal–enzymatic destruction of the gluten network, which

became brittle and short. As a result, the dough showed very poor dough extensibility L (more than a double lowering than that of native F) and increased SH, especially in HF and HFC samples. The P/L index was similar for native F and enzyme-added samples FC and FCX, and slightly lower values were noted in thermally treated flours without/with enzymes. The greater differences were found in P/L with more than triple higher results in the testing of dough made of HF, HFC and HFCX flours. Elasticity index Ie was not possible to obtain in the hydrothermally modified samples. Although the SH coefficient increased to higher levels than in the base flour, this was due to an increase in dough stiffness rather than an actual improvement in flour performance.

Some important correlations were found between rheological properties tested with various methods. Alveograph features were correlated with dough quality from Farinograph tests, with a significant positive correlation at $p < 0.05$ of p values with dough stability (0.760) and negatively with DoS12 (−0.928). L results were negatively significantly correlated at $p < 0.05$ with S (−0.946), but positively with DoS12 (0.920). Moreover, baking strength W was negatively correlated with DT (−0.830). P/L was strongly (at $p < 0.05$) correlated with dough stability (0.935) and with DoS12 (−0.923). There were also some significant (at $p < 0.05$) correlations found between dough tenacity P and I-arabinose (−0.627), I-xylose (−0.709) and I-AX (−0.711), which can explain the effect of insoluble fractions of non-starch polysaccharides on dough properties. Soluble fractions of polysaccharides were also found to be related to P, and significant correlations were found for S-xylose (0.828), S-NSP (0.856) and S-AX (0.792) at $p < 0.05$.

For the extrusion treatment and partly for the hydrothermal treatment, it was not possible to assess the rheological properties of the gluten matrix (Ie values) due to the starch gelatinization and denaturation of the gluten protein. All the extruded flours were unable to successfully form a dough when used alone. In wheat dough fortified with extruded bran, Gómez et al. [55] observed changes in P, L and W because of the interrupted gluten–starch matrix and a negative effect on gas retention, resulting in a dough extensibility reduction and tenacity increase associated with poor handling characteristics of doughs. Jødal and Larsen [35] indicated high P/L as a resistant and non-stretchable dough, while low P/L revealed a weak and stretchy dough.

3.6. Treatment Effect on X-ray-Detected Structure

The treated flour X-ray diffractometry profiles are shown in Figure 2. Zeng et al. [58] consider the X-ray diffraction pattern as the "fingerprint" of plant starch crystallinity. Regarding X-ray diffraction lines, the crystal structure of starch can be divided into four types, where A, B and C types are the natural crystal structures of starch, and the structure V type is typical for complexes created by amylose and lipids.

The wheat starches present standard A-type crystallinity diffraction patterns at 2θ with peaks near 15°, a strong doublet around 2θ ≈ 17° and 18° and a third main reflection around 23° [45]. The peak at 2θ ≈ 20° is the amorphous peak of amylose and lipids, and a higher peak intensity suggests its higher content [59]. In wheat, depending on the variety, if the relative intensity of the peak at 23° is higher, then the 15° peak intensity is generally lower. The presence of peaks at certain angles differs depending on wheat flour treatment conditions and enzyme presence. In native, thermal and hydrothermal modified samples, all characteristic patterns were found to be at similar diffraction angles: at 15°, with a strong doublet at 17 and 18°, and at 23°. In wheat flour treated via steam (HF), however, X-ray patterns showed sharper peaks in comparison to that of the other treatments (Figure 2a), suggesting the prevalence of A-type starch [58]. Dry thermal treatment in TF samples induced relocation of the highest peak point to 20°, indicating that these samples contain the lowest amount of amorphous structures. Regarding the extruded flour, the peak intensity at 20° was the highest, suggesting the presence of amorphous phases of amylose and lipids; however, the 17° peak was the lowest. In TF, the peak intensity was the lowest at 20° and 22°, and in TF and EF, significant decreases in peak intensity at 15 and 17° were observed.

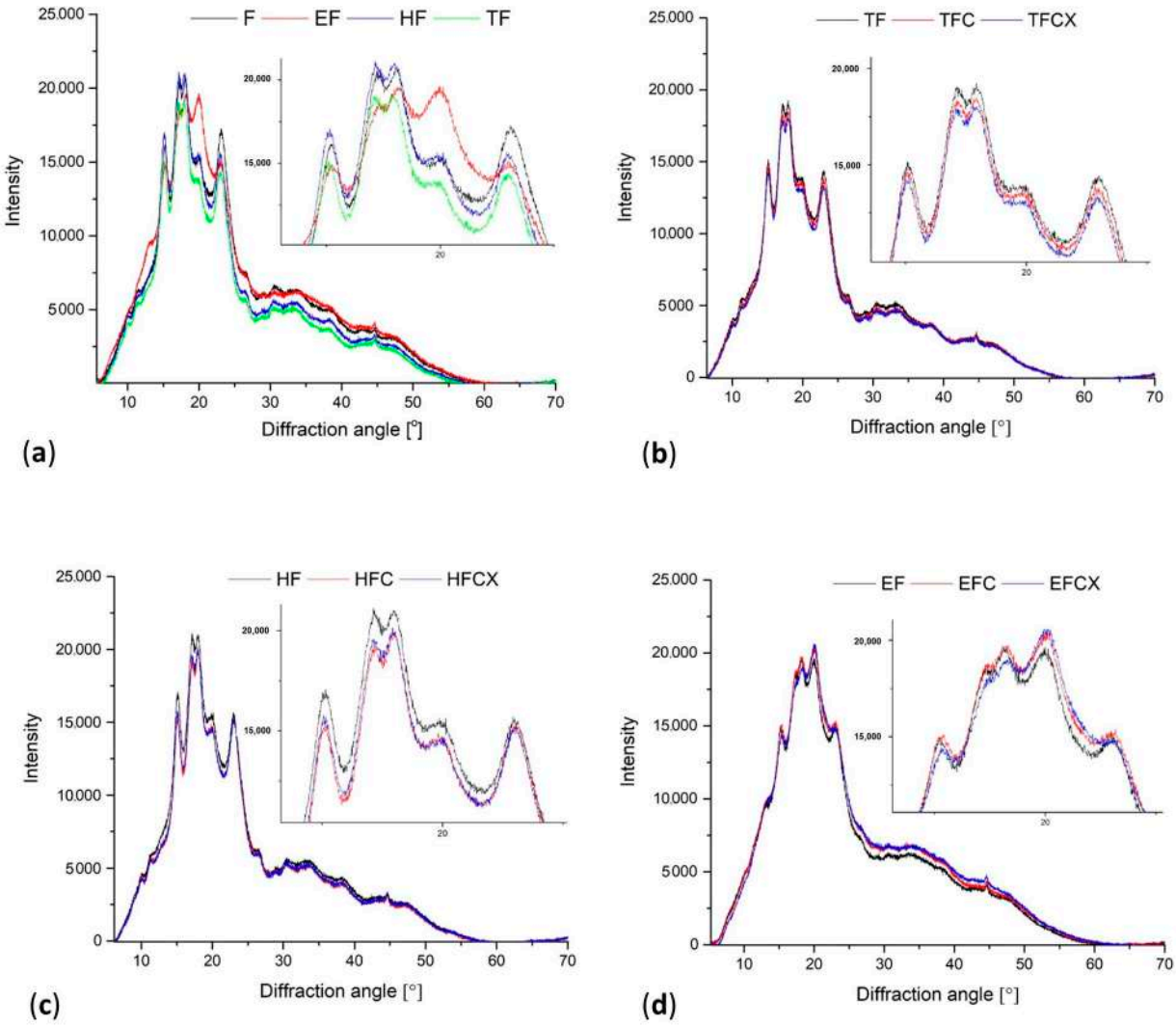

Figure 2. X-ray diffraction patterns of modified wheat flour: (**a**) comparison of various treatments without enzymes, (**b**) effect of enzymes in dry thermal treatment, (**c**) effect of enzymes in hydrothermal and (**d**) effect of enzymes in extrusion treatment.

Figure 2b–d reveal the pattern differences in modified flours without/with added enzymes. Differences were slight in dry-thermal-treated samples (Figure 2b), but in TFC and TFCX, the heights of all peak intensities were lower. A similar but stronger trend was seen in HFC and HFCX, with a more significant decrease in peak intensities, as compared to enzyme-free HF (Figure 2c). The extruded samples EFC and EFCX, which contain enzymes (Figure 2d), showed higher peak positions at 20°, as compared to EF samples. CX complex addition, however, lowered the intensity of the peaks at 15° and at 17° and 18°, suggesting a reorganization of A-type crystallinity. Indeed, Li et al. [45] found that amylose content was significantly positively correlated with the intensity of the diffraction peak at 23° and the crystallinity of diffraction peaks at 17–18°. The introduction of enzymes into hybrid enzymatic–extrusion treatments also changed diffraction intensity at the range of 25 to 45° by increasing the surface area under the baselined curves, as compared to EF, suggesting more amorphous structures in the EFC and EFCX samples, as compared to other treatment methods (Figure 2a). Tao et al. [57] identified in native wheat starch the presence of all four A-type patterns, and after extrusion, the crystalline peaks were less pronounced, especially at 17, 18 and 23°. The loss of crystallinity was brought about because high extrusion temperature caused thermal degradation of the starch amylopectin fraction at its branching points. Liu et al. [52] used a single-screw extrusion treatment to modify rice starch and

reported a gradual weakening of all peaks associated with A-type crystallinity patterns after extrusion. These became more amorphous as the initial moisture content increased in the extruded starch. They found a clear crystallinity peak at 20° for extruded starch (not present in native rice starch), indicating V-type crystallinity that can be associated with the formation of the amylose–lipid complex. Merayo et al. [60] reported changes in a peak located at 20° (2θ scale), indicating the formation of amylose–lipid complexes in their investigation of the extrusion of red and yellow corn flours during spaghetti production. He et al. [61] tested cellulose nanofiber/polyaniline film composites and found two peaks at 2θ = 15.24° and 22.4° for cellulose-based film (this is the classic cellulose I structure). These peaks were superimposed on broad scatterings placed between 15° and 25°, which were ascribed to the periodicity parallel and perpendicular to the polymer chains of polymerized composite film, respectively.

3.7. Microstructure of Native and Processed Flours

The microstructure of native and treated wheat flours is presented in Figure 3. SEM pictures were taken with low (600×) and high (2000×) magnifications. Native flour (Figure 3a) displayed the presence of various fractions visible as large and small starch granules, parts of fibrous components coming from bran and elongated structures coming from protein. The diverse wheat starch granule sizes confirmed the presence of both granule types: A-type (diameter over 9.9 μm) and B-type (diameter below 9.9 μm). In native wheat flour, A-type granules contain up to 70% of the volume and 10% of the total number of starch granules, and B-type granules contain approximately 30% of the volume and 90% of the total number of granules [62]. Very fine (<2.0 μm) C-type starch granules have also been reported, although this type of granule may also represent a B-type granule [59]. A-type and B-type starch granules show different morphologies, wherein the A-type has a disk-like shape with possible grooves or indentations, and the B-type exhibits spherical, ellipsoidal, angular and irregular shapes, and is tightly packed within the endosperm [49].

In a native flour, after the enzyme addition, slight agglomeration may be observed in FC and FCX flours (Figure 3b,c, respectively). This may be a result of enzyme activity and the initiation of hydrolysis of the linear fractions of polysaccharides, especially cellulose and hemicellulose, visible as groups of glued flour particles with both A- and B-type starch placed close to each other. Enzyme addition to low-moisture flour results in incomplete hydrolysis, but the structure of wheat flour can change, e.g., an increase in S-NSP content, especially S-AX, can thus increase hydration possibilities and DT, but lower viscosity, breakdown and setback.

After dry thermal treatment, more singular starch granules of larger dimensions are noticeable, suggesting the presence of heated and swollen starch granules placed loosely in the TF flour (Figure 3d). In TF, increased content of I-AX and I-NSP and of T-AX and T-NSP, lower viscosity and hydration were noted, as compared to F, but the addition of enzymes generated an opposite effect in TFC and TFCX samples, especially when the cellulase–xylanase complex was incorporated. In these samples, we noted finer particle packing (Figure 3e,f) with empty space between starch granules, which allowed for more solvent absorption and increased DT and dough extensibility. The lower moisture of dry heated flours might, however, contribute to this effect.

In steam-treated flours (the HF sample), we observed a visible partial agglomeration (Figure 3g) that was more intensive with enzyme incorporation (Figure 3h,i). Formation of these agglomerates with swollen and partly gelatinized starch granules and with the presence of more finer granules of lower dimensions sufficiently decreased hydration ability, shortened dough extensibility and lowered baking strength with increasing maximum viscosity.

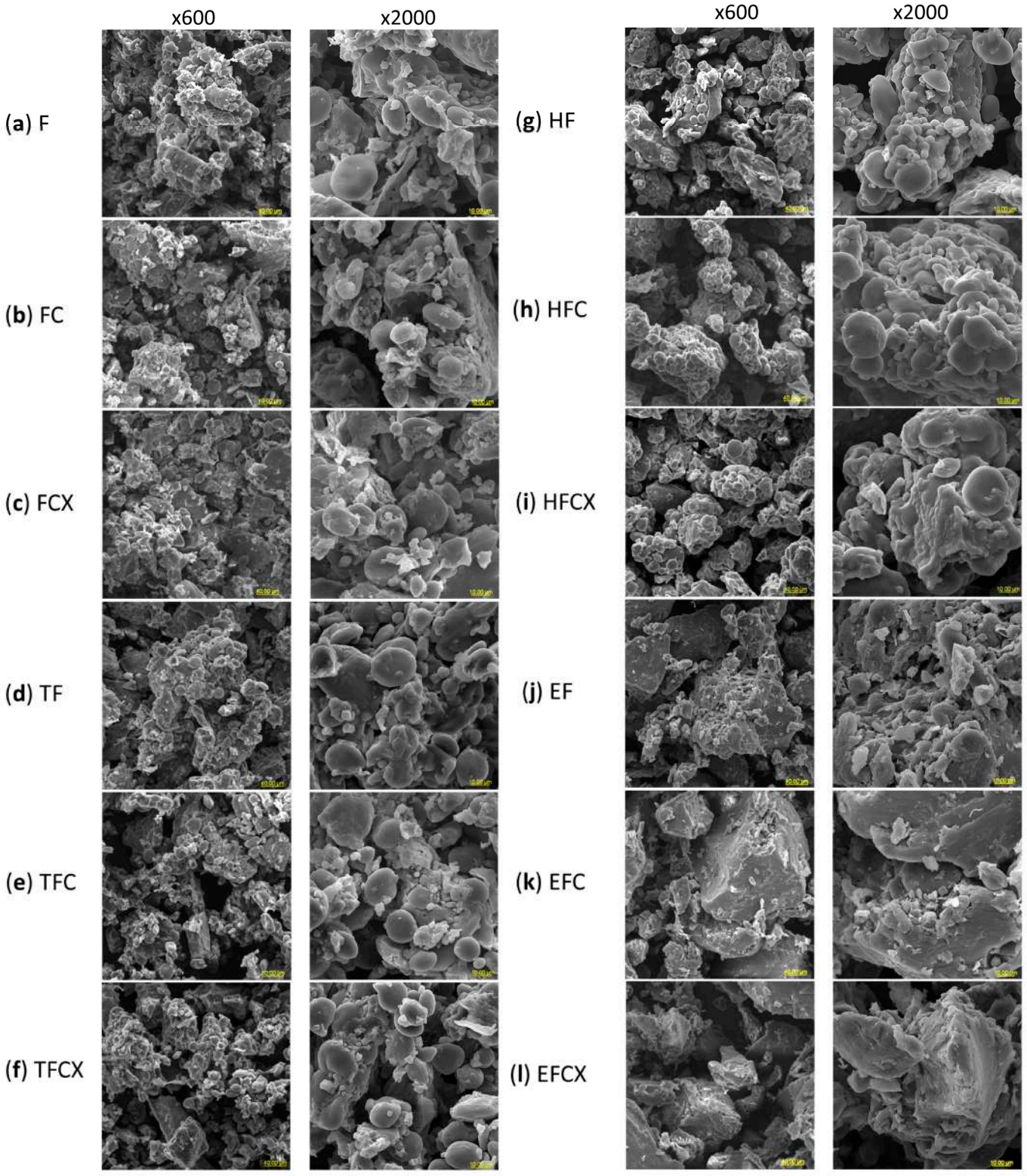

Figure 3. SEM structure of modified wheat flour at various magnifications (600× and 2000×): (**a**) native flour, (**b**) native flour with cellulase, (**c**) native flour with cellulase–xylanase complex, (**d**) dry-treated flour, (**e**) dry-treated flour with cellulase, (**f**) dry-treated flour with cellulase–xylanase complex, (**g**) hydrothermally treated flour, (**h**) hydrothermally treated flour with cellulase, (**i**) hydrothermally treated flour with cellulase–xylanase complex, (**j**) extruded flour, (**k**) extruded flour with cellulase and (**l**) extruded flour with cellulase–xylanase complex.

Small B-type granules are characterized by greater resistance to hydrolysis and exhibit lower gelatinization temperatures than the A-type structure that was observed after HF and EF treatments. The most significant changes were observed in the extruded flour, without (Figure 3j) and with enzyme addition (Figure 3k,l). According to Bouasla et al. [37], raw flour is molten inside an extruder and gelatinized in the extended range. We confirmed this by seeing the melted and compact internal structure of the starch–protein–lipid matrix with large clusters formed due to association after treatment in the presence of water (27%) and the absence of free starch granules, especially in the EFC and EFCX samples (Figure 3k,l). A similar conclusion was found by Wu et al. [11]; they showed that the extruder action caused the starch to decompose. In the extruded flours, the lowered I-NSP and increased S-AX in this melted internal structure significantly enhanced the flour's hydration properties, but decreased dough stability, GPI, C3, C4 and C5—and made dough creation impossible. Cervantes-Ramírez et al. [63] observed an integrated amorphous matrix of corn starch formed as the effect of extrusion treatment. However, some granules remained visible due to the fatty acids acting as a protective (lubricating) coating during extrusion and significantly reducing physical damage of starch granules.

4. Conclusions

Wheat flour blend modification had variable effects on composition, rheology and structure depending on treatment conditions and enzyme applications. The results confirmed that hybrid treatments incorporating cellulase and/or cellulase–xylanase complex enzymes modified polysaccharide compositions and techno-functional properties. The thermal treatment turned out to have the least destructive effect on the protein quality, especially if the cellulase–xylanase enzyme complex was incorporated. The dough matrix, however, became more resistant to mixing because of protein structure improvement. Intensive treatment in hydrothermal and extrusion methods had a negative impact on the quality of protein fractions but significantly changed gelling properties whether performed without/with enzymes. Incorporating the cellulase–xylanase enzyme complex resulted in a significant increase in the soluble fractions of arabinoxylans, which have a structure-forming function in the dough matrix and participate in water management. Significant changes were observed in the structure and microstructure of the modified flours, especially when extrusion was applied. The techno-functional properties of modified flours, especially their hydration and pasting properties, as well as dough rheology, may be developed by proper treatment methods for their use in various applications. Extruded flours with high water absorption may be an interesting alternative to pregelatinized/modified starch or hydrocolloids in the bakery production process with low dosing and a significant effect in increasing the bread yield. Furthermore, these types of flours do not need to be labeled as additives but as wheat flour, which will facilitate clear labeling—currently the preferred trend in the food industry. Further investigation will include using the obtained modified flours as clean-label ingredients in wheat bread in order to verify the technological and quality features obtained during processing in the final bakery products.

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Application of Conventional and Hybrid Thermal-Enzymatic Modified Wheat Flours as Clean Label Bread Improvers

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Abstract: A new flour blend (F) composed of selected milling and leaving passages with a high content of non-starch polysaccharides underwent thermal (T), hydrothermal (H) or hybrid processing and was used along with cellulase (C) and cellulase-xylanase complex (CX) to produce bread. This modified flour can be considered a clean label product. In this study, blends of common and treated flours were tested for dough properties and rheology. The modified flours were added at 10 and 20% to the base wheat flour. A pan bread was then prepared to test their suitability for bread baking. Dough and bread properties were subsequently assessed. Accordingly, dough with added thermally, hydrothermally, and hybrid modified flours revealed differences in rheology. Addition of hybrid enzymatic-hydrothermal treated flour increased dough tenacity by 23% and baking strength by 26%, but decreased dough extensibility by 19%, whereas hybrid enzymatic-thermal modification increased water absorption by 6% and bread yield from 146.77% to 150.02% when modified flour was added at 20%. Breads with added modified flours demonstrated a 16% increase in bread volume, 8% lower baking loss, and 14% greater density, with no negative effect on color and texture. Thus, hybrid thermal-enzymatic treatment of the developed flours can be recommended as a suitable method for enhancing the utilization of waste flour fractions and increasing their value by enabling them to be considered as clean label bread improvers.

Keywords: thermal treatment; enzymatic treatment; hydrothermal; wheat flour; dough rheology; bread quality

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1. Introduction

Bread, as one of the most common food products in the world, is considered crucial in human nutrition due to its accessibility and nutritional value. It is a very good source of carbohydrates, protein, dietary fiber, vitamins, and minerals [1]. Progress in the milling and bakery industries has resulted in the development of technology for the production of bakery products. These products are constantly improved, which allows industry to introduce wheat-based products with special health-promoting and functional values to the food market. Current research trends are focused on nutritional and technological improvements in cereal-based products using various additives and production processes that, ideally, allow these products to be recognized as clean label products [2,3]. The outcome of such work is that the addition of alternative components to standard wheat flour (insects, legumes, fruits, vegetables, herbs, microalgae, or by-products from the agri-food industry) [4–9], as well as the use of modern grain processing technologies [10,11] has

enabled improvement of bakery products, especially of whole grain flours and breads [12].

To improve the functional properties of wheat flour through physical modification, various flour treatment technologies can be used. The most popular are dry heating or hydrothermal treatment with steam [13–17]. Additional modifications may be supported by utilizing selected enzymes such as cellulase or xylanase [18,19]. Modern technologies that can be applied to change or improve cereals' properties include steam explosion (SE), high-hydrostatic-pressure (HHP), high-pressure homogenization (HPH), pulsed electric field (PEF), and plasma processing. The use of these technologies has significant effects on the resulting flour's chemical, rheological, and hydration properties, as reported by Li and Niu [20]. Introducing specific physical modifications to grains or flours could reduce the negative effects of supplementation with wholegrain flour or unmodified bran-rich fractions on bread quality, especially with regard to dough rheology and bread quality [21–23]. Processes for extracting selected ingredients and components with desired properties from grains, mostly based on soluble and insoluble dietary fiber or beta-glucans, vitamins, and antioxidants, are under intense development [6,24,25].

Heating, as a physical treatment, via various methods (dry heating, hydrothermal, extrusion) may effectively modify the techno-functional properties of wheat grain and flour without the introduction of undesirable chemical additives. Hence, the developed products can be considered clean label additives [7]. Even if enzymes are used in such modification processes, after drying, the enzymes are inactivated, so enzymatically assisted modification can also be considered a clean label approach in bread production [12,13]. Each of the aforementioned modifications are intended to improve the various attributes of bakery products, especially their nutritional attributes, but, unfortunately, they often also have a negative impact on the technological and production properties of dough and, consequently, bakery products, e.g., bread [2].

Mill streams richest in fiber fractions come from the outer parts of the grain. As we described in our previous studies [26], differences in the composition of individual main milling streams of wheat result directly from the origin of specific fractions from the anatomical parts of the grain and the influence of grinding processes such as the mechanical damage of starch. Fractions containing bran parts, also of various sizes, are considered undesirable as a component of standard bread flours due to lower overall quality of dough and a decrease in dough elasticity and bread volume [26–29]; as such, they are sold as bran-rich products and supplements or animal feed [30]. Such items bring in less profit to the mill owners. These underutilized fractions amount to about 10% of the total production in a milling company, so it is economically important to find technological solutions for reducing the quantity of these underutilized fractions by increasing their use in higher quality products.

Economics and growing consumer concerns about food ingredients and clean labelling have had an impact on industrial bread production, resulting in enhanced efficiency and new recipes [3]. Consumers are looking for clean label products, without E-marked additives, but also with proper quality [20]. Unfortunately, some bread improvers are perceived as being unknown and harmful chemicals that may be unhealthy to consume [3]. In contrast, thermal processing methods may have a positive impact on the final quality of bakery products while enabling these to be considered "clean label". In this present study, we investigate the possibility of using a developed wheat flour with an increased content of non-starch polysaccharides [26], fortified with baking enzymes, and additionally treated via various physical methods for bread production. Such modifications may offset the negative effects of treatments on the final quality of flour and help in the utilization of unused flour fractions as clean label additives to commercial bread flour blends.

The aim of the study was to investigate the impact of the addition of flours subjected to enzymatic, thermal, hydrothermal, and enzyme-assisted hybrid modifications on the quality of bread dough during mixing and fermentation, and on the characteristics of bread, as compared to the use of conventional flour.

2. Materials and Methods

2.1. Materials

Common wheat flour type 750 produced in PZZ Lubella was used as the base raw material suitable for the production of wheat bread. This was characterized by a moisture content of 14.3%, a gluten content of 28.2%, an ash content of 0.77%, and a falling number of 280 s. The developed flour before modifications was characterized as having a moisture content of 13.7%, a gluten content of 31.0%, an ash content of 0.78%, and a falling number of 340 s. The functional flours were prepared by undergoing thermal and hydrothermal treatments as described in Section 2.2 and were added in amounts of 10 and 20% of the total common flour base. The quantities of the incorporated modified flours were selected based on a preliminary study and on economic profitability. In the preliminary study, at over 20% of modified flours, the tested bread showed a tendency to collapse during fermentation and baking, thus 20% was considered the upper limit in the full study (any greater amount would lower the final product quality, hence lowering the profitability of production).

Selected batches of the functional flours underwent hybrid enzyme-assisted treatments. Commercial baking enzymes were employed to fortify the flour (the amount of the enzyme was determined based on preliminary tests and on the suggestions of the enzyme manufacturer). The following baking enzymes were used in the experiment: Bakezyme® WholeGrain-cellulase from *Trichoderma reesei* (DSM Food Specialities B.V., Delft, The Netherlands) with declared enzyme activity 1475 EGU/g (+/− 5%); VERON 292-xylanase from *Aspergillus niger*, (AB Enzymes GmbH, Darmstadt, Germany) with declared enzyme activity min 1701 XylH/g. The cellulase enzyme was added in the amount of 120 ppm (samples marked as C), and the complex of cellulase and xylanase enzyme was incorporated in amounts of 60 ppm and 50 ppm, respectively (samples marked CX). Salt (Ciech S.A., Warszawa, Poland) and commercial bakery yeast (Lallemand, Lublin, Poland) were also used in the recipe.

2.2. Flour Modification Procedure

Enzymatic modification of the developed wheat flour (F) [31] was performed through the addition of 120 ppm of powdered cellulase (FC) and a combined 50 + 60 ppm of cellulase-xylanase complex (FCX). Components were mixed for 5 min at room temperature using a laboratory ribbon mixer (Konstal-Zakład Mechaniczny CNC Zbigniew Własiuk, Lublin, Poland) and left for 2 h to start enzymatic action.

A prototype installation (owned by PZZ Lubella) with an efficiency of 650 kg/h was used to obtain the modified flours. This consisted of cylindrical barrels with heating jackets that incorporated screw transporting-mixing elements.

Dry thermal treatment (T) was carried out for the tested wheat flour (TF) and for the flours incorporating the enzymes (TFC and TFCX) after mixing in a continuous ribbon mixer for 2 min at 25 °C. The processed flours underwent 5 min of dry heating at 15% moisture content inside the barrel, wherein the heating jacket temperature was set to 100 °C; the product temperature was measured during the tests so as not to exceed 50 °C.

Hydrothermal modification (H) of base wheat flour (HF) and the flours incorporating the enzymes (HFC and HFCX) was performed after mixing in a continuous ribbon mixer for 2 min at 25 °C using a prototype installation equipped with an additional steam-assisted preconditioner. Herein, flour samples without/with enzymes were mixed at 30 °C for 2 min and transferred to a single-screw preconditioner with 20 L/h of water, with a set jacket temperature of 100 °C, and were subjected to steam injection for 5 min. The product temperature measured during the tests did not exceed 65 °C.

The heated or hybrid enzymatic-assisted treated samples were subsequently dried in an air dryer at 100 °C for at least 15 min to end enzyme activity. A final moisture content of below 9% was achieved.

All samples were ground and sieved using a square sifter (Toruńskie Zakłady Urządzeń Młyńskich Spomasz S.A., Toruń, Poland) to homogenize the material and remove aggregates to obtain particle sizes below 300 μm. The samples were then stored at room temperature in closed plastic bags before tests.

2.3. Rheological Properties of Flours and Dough with Added Modified Flours

Rheological tests were performed with the following devices: Mixolab (Chopin Tech-nologies, Villeneuve-la-Garenne, France) according to ICC method 173, Brabender Farinograph-E apparatus (Duisburg, Germany) according to ICC method 115/1, and Alveograph (Chopin Technologies, Villeneuve-la-Garenne, France) according to ICC method 121 [32].

Rheological properties of blends prepared with additions of the modified flours were studied using a Chopin Mixolab based on the Chopin+ flour protocol with some modifications. The device was equipped with an additional attachment (the set includes a dough feeder and a special nozzle for this application) to control the quality of the prepared dough. For this purpose, 75 g of dough prepared during the bread preparation procedure described in Section 2.4 was transferred directly to the mixer and the test was begun according to the standard protocol at the following settings: dough weight—75 g, total analysis time—45 min, mixing speed—80 rpm, hydration water temperature 30 °C [33]. The Mixolab test was performed using a standard protocol: 8 min at 30 °C, heating for 15 min at a rate of 4 °C/min, holding at 90 °C for 7 min, cooling for 10 min to 50 °C at a rate of 4 °C/min, and holding at 50 °C for 5 min [14]. The following rheological features were evaluated via the Mixolab procedure: protein weakening (C2), starch gelatinization (C3), amylase activity (C4), starch retrogradation (C5) [34].

The rheological properties of the dough prepared without and with modified flours were determined using the Farinograph procedure [16] with some modification according to preparation of bread by way of the pan method. Water absorption (WA) was tested at the consistency of 400 BU, as recommended for this type of bread as prepared with pans (% of water needed to obtain a dough consistency of 400 BU or corrected at 14%).

Standard testing procedure was applied using Alveograph (Chopin Technologies, Villeneuve-la-Garenne, France) to investigate the blends with the addition of modified flours. The following features were assessed: the baking strength (W) as the surface area under the curve obtained, dough strength (P) as the maximum pressure needed to blow the dough bubble expressing dough resistance, dough extensibility (L) as the length of the curve expressing dough extensibility, elasticity index (Ie) [35], strain hardening index (SH), and P/L as configuration ratio [36]. All rheological tests were performed in triplicate.

2.4. Bread Preparation

The control bread sample (K) was prepared without the addition of modified flours. The control bread preparation was as follows: common wheat bread flour 750 type was mixed with 2% of salt and 3% of yeast, and water was added to obtain a dough consistency of 400 BU. The bread dough was prepared by way of the direct one-step method [7] with slight modifications. To prepare the tested breads, common bread wheat flour was replaced with developed flour (F), enzymatically modified flours (FC, FCX), as well as with thermal, hydrothermal, and hybrid enzymatic-assisted modified flours (TF, TFC, TFCX and HF, HFC, HFCX, respectively) in amounts of 10 and 20% (*w*/*w*). All ingredients were mixed in a laboratory mixer for 6 min (JMP12, Fimar Food Processing Equipment, Vericchio, Italy). The prepared dough was then divided into 300 g pieces and placed in loaf pans (approx. 10 × 10 × 10 cm) and fermented at 30 °C and 75% relative humidity (RH) for 50 min in a climatic chamber (MIWE US 2.0, Arnstein, Germany) controlled by an incorporated automatic temperature and humidity control system with an accuracy of 1 °C and 1% RH, respectively. After fermentation, the bread was then baked at 210/200/190/210 °C for 30 s/2 min/20 min/3.5 min—for a total of 26 min in a MIWE AERO backcombi oven (Arnstein, Germany). After loaf placement, steam was introduced for 30 s in an amount of

0.08 L. The temperature inside the baking oven was controlled by an incorporated automatic temperature control system with an accuracy of 1 °C. Post-baking, the loaves were removed from the tins and weighed. The breads were then cooled down for 1 h and weighed again, packed in polyethylene bags, and stored at 21 °C before the tests. All procedures were repeated in triplicate for each flour sample.

2.5. Proximate Composition Analysis of Bread

The chemical composition of ground dried bread samples was determined according to standard methods: AACC 46-10 method for protein (Nx6.25), AACC 30-10 method for fat, and AACC 08-01 method for ash [37]. The 991.43 method was applied to evaluate soluble (SDF) and insoluble (IDF) fractions and the content of total dietary fiber (TDF) [38]. Total carbohydrates and caloric values with Atwater energy equivalents were calculated for the tested breads [39]. All tests were performed in triplicate.

2.6. Bread Quality Tests

Specific bread volume (mL) was tested by way of the rapeseed displacement method according to AACC 10-05 standard [37] by using a known volume/mass of rapeseeds replaced by bread loaf and calculated as bread volume to bread weight [40]. Bread density (g/cm^3) was calculated as the weight to volume ratio of single loaf. Baking loss (%) was evaluated as the difference of dough and loaf mass directly after baking to proper dough mass [6]. Weight loss (%) was checked as the difference between mass of the hot bread just after baking and after 24 h of storage. Bread yield (%) was calculated as the ratio of dough mass multiplied by dough yield to the mass of cold bread after baking [41]. Data were given as the averages of three independent experiments.

2.7. Water Absorption Index and Water Solubility Index Assessment

Water absorption index (WAI) and water solubility index (WSI) in breads were determined according to Soja et al. [42]. WAI was expressed as g of water absorbed by g of bread. WSI was expressed as % of components soluble in water after WAI testing. Measurements were conducted in triplicate.

2.8. Color Profile of Bread

To evaluate the color characteristics of bread crumb and crust 24 h after baking, the NH310 colorimeter was used (3NH TECHNOLOGY Co., Ltd., Guangzhou, China). Color assessment followed the CIE-Lab system, where L^* describes the lightness and ranged from 0 (black) to 100 (white), the a^* chromatic coordinate is determined as the balance between red (positive values) and green (negative values), and the b^* chromatic coordinate is ascertained as the balance between yellow (if positive) and blue (if negative) [7]. The final values of the L^*, a^*, and b^* coordinates of bread crumb and crust were expressed as the means of at least five measurements of each color determinant from three individual bread loaves. ΔE was calculated as the total color difference [7]. Before each measurement, the colorimeter was calibrated using a supplied white calibration plate.

2.9. Bread Crumb Texture Analysis

The textural properties of control bread and samples prepared with modified flours were determined in triplicate using a ZwickRoell BDO-FB0.5TH (Zwick GmbH and Co., Ulm, Germany) instrument, according to the TPA protocol, and testXpert®13.3 software. Bread samples were cut from the middle part of the crumb (3 × 3 × 1 cm). An Ottawa cell was employed for the testing, and had a working head speed of 100 mm/min in the double compression test to 50% of sample height and 10 s distance between cycles. TPA curves were analyzed, and textural properties were evaluated as mean values of five replications. The following features were determined: firmness as the highest peak during the first compression run, adhesion as the work needed to separate crumb and piston, springiness

as the distance of the detected height during the second compression cycle divided by the original compression distance, gumminess and chewiness calculated on the base of firmness, cohesiveness, and springiness, and cohesiveness as the area of work during the second compression cycle divided by the area of work during the first compression [43].

2.10. Statistical Analysis

The obtained data were subjected to one-way analysis of variance (ANOVA) via the Statistica 13.3 software (StatSoft, Inc., Tulsa, OK, USA) application, followed by Tukey post hoc test to compare means at the 0.05 significance level. Pearson's correlation coefficients were found to evaluate the correlations between the tested properties using Statistica 13.3 software (StatSoft, Inc., Tulsa, OK, USA) within the 95% confidence interval.

Statistica software (version 12.0, StatSoft Inc., Tulsa, OK, USA) was used for statistical analyses. Principal component analysis (PCA), analysis of variance, and determination of correlations were performed at the significance level of $\alpha = 0.05$. Principal component analysis was applied to determine the relationship between conventional and hybrid thermal-enzymatic modified wheat flours and the studied parameters. The PCA data matrix for statistical analysis of the research results consisted of 40 columns (parameters) and 20 rows (Type of material). The input matrix was automatically rescaled. The optimal number of principal components obtained in the analysis for each matrix was determined based on the Cattel criterion.

3. Results

3.1. Flour and Dough Features Analysis

The developed flour was characterized as having an increased content of proteins, polysaccharides, and arabinoxylans due to it containing mostly the outer fractions of the wheat grain [14]. The composition of the NSP-rich developed wheat flour before modifications was as follows (%): protein—14.62 ± 0.06, fat—1.31 ± 0.01, ash—0.78 ± 0.02, insoluble dietary fiber—3.94 ± 0.04, soluble dietary fiber—2.86 ± 0.02, and total dietary fiber—6.80 ± 0.03. The composition of polysaccharides in the developed flour before modifications was as follows (%): total arabinoxylans—1.91 ± 0.06, which consisted of 1.31 ± 0.04 of insoluble fraction and 0.60 ± 0.02 of soluble fraction, and total non-starch polysaccharides—3.40 ± 0.00, which consisted of 2.06 ± 0.01 of insoluble fraction and 1.34 ± 0.00 of soluble fraction [31].

The following rheological features were evaluated through the Mixolab procedure: protein weakening (C2), starch gelatinization (C3), amylase activity (C4), and starch retrogradation (C5) [34]. In order to qualitatively assess individual samples, using the Mixolab device, an analysis of the dough taken from the mixer was carried out just before forming the dough in pans. This allowed us to check whether the dough had proper consistency and the flour was properly hydrated. This is made evident by reading the dough resistance at point C1 of the graph [5]. The properties of the finished dough, prepared for shaping, were tested at specific analysis points in accordance with the adopted methodology for the Chopin + protocol, i.e., C2, C3, C4, C5. Additional analysis allowed for effective control of the dough consistency, as the calculated average consistency for all dough samples at point C1 was 0.763 Nm ± 0.01 Nm. Figure 1 presents the rheological properties of the tested doughs with added modified flours in amounts of 10 and 20%.

The results of the analyses confirmed that the addition of flours modified by the addition of FC and FCX enzymes did not significantly affect the tested features, such as the level of C2 protein weakening, C3 starch gelatinization, C4 amylase activity, or the level of C5 starch retrogradation (Figure 1).

Figure 1. Rheological features of raw materials composition with added modified flours as compared to control common bread flour: (**a**) protein weakening (C2); (**b**) starch gelatinization (C3); (**c**) amylase activity (C4); (**d**) starch retrogradation (C5); K—control; F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; CX—cellulase-xylanase enzyme complex; 10 and 20—% of modified flour in bread recipe; dash line—level for control sample; $^{a-l}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

In tests with the addition of flour modified by thermal treatment, a slight increase in the C2 point value was observed for flours without the use of enzymes TF10 and TF20, indicating greater stiffness of the dough (Figure 1a). Incorporating hybrid enzymatic-thermal modified flours TFC10-TFCX20 resulted in a reduction of the C2 parameter values to the level of the control flour K. Mixtures with the addition of TF, TFC, and TFCX flours after thermal treatment were characterized by a reduction in C4 amylase activity without a significant effect of enzyme activity (Figure 1c), and the level of retrogradation of C5 starch was similar to the value for the control flour (Figure 1d).

The greatest differences in the values indicated via the Mixolab procedure were observed for mixtures with the addition of the hydrothermally processed flours–HF, HFC, and HFCX–where the values of all tested parameters–C2, C3, C4, C5–increased, as compared to the control flour, which indicates the large impact of flours modified with this method on the dough's protein and starch complexes. These flours were characterized by a higher level of protein weakening, starch gelatinization, reduced activity of amylolytic enzymes, and a higher level of starch retrogradation than the control flour K. High-temperature treatment with steam and water had a negative effect on the protein-starch complex of the tested HF, HFC, and HFCX flours. Partial denaturation of gluten proteins occurred, thus bringing about difficulties in creating a gluten network matrix (unpublished data) and affecting an increase in the C2 parameter of protein weakening. The hydrothermal process induced initial starch gelatinization, hence, generating, through inactivation of amylolytic enzymes, a significant increase in starch gelatinization (C3), amylase activity (C4), and starch retrogradation (C5). These observations are supported by the PCA analysis presented in Section 3.5, which showed a commonality of effect in all the hydrothermally treated flours.

The addition of developed flour treated with enzymes in the hybrid processing increased this effect. Of note, the parameters from this analysis were well correlated with the parameters obtained in the alveographic analysis of bread composition mixtures consistency features. We found significant correlations between C2, C3, C4, C5 and dough tenacity (P) values with coefficients of 0.842, 0.825, 0.773, and 0.785, respectively, as well as dough configuration index (P/L) with r values of 0.845, 0.850, 0.812, and 0.794, respectively. Slightly lower but still significant negative correlations were noted between C2–C5 and dough extensibility L (range of r coefficients form −0.601 to −0.687). Starch gelatinization (C3), amylase activity (C4), and starch retrogradation (C5) were also significantly negatively correlated with water absorption (WA), with values of r ranging from −0.706 to −0.723.

Jurkaninová et al. [7] tested unfermented bread dough using the Mixolab procedure and they reported a negligible effect of herb extract addition on dough rheological characteristics, whereas their C2-C5 results were higher than that of our tested bread dough. Mahmoud et al. [8] assessed bread products with the addition of microalgae, and the results of their Mixolab rheological tests were similar, the C2 range being 0.23–0.44 Nm and the C3 being 2.12–2.74 Nm, while the C4 varied from 2.00 to 2.41 Nm, and, finally, C5 ranged between 3.06–4.27 Nm.

Upon analyzing the tested flour mixtures via farinographic analysis (Table 1), we observed that the improved tendency to absorb water seen in the modified developed flours had the greatest impact on the increase in water absorption (WA) within the bread mixtures. This was especially noticeable in the doughs created from flours incorporating thermally treated flours at both 10 and 20% values. Thus, for TFCX10 flour, WA increased by 3.5%, for TFCX20 by 5.4% and for TFC20 by 5.7%, as compared to the control K recipe. An increase in water absorption of the analyzed flour blends was also found for samples incorporating hydrothermally processed flours, but without significant differences when baking enzymes were used during this treatment. The results allowed the selection of the appropriate amount of water to prepare pan method bread dough with a constant consistency of 400 BU, enabling comparison of the impact of the addition of modified flours on the quality and bread yield.

Cacak-Pietrzak et al. [44] tested the addition of dried crushed roots of *Taraxacum officinale* to wheat flour and observed decreased WA from 57.5% (control sample) to 55.5% (sample with 6% TO), but significant differences were found even if 1% TO rich in inulin content was applied. Inulin, as a dietary fiber fraction rich in soluble components (such as inulin-type fructans), has a limited ability to absorb water [45]. In our samples, the increased level of non-starch polysaccharides in the developed flour rich in arabinoxylans (especially the insoluble fractions that came mostly from the final fractions of the reduction and sorting passages, as well as from filtration stream flours) was positively correlated with the water absorption [26]. Hence, the increased water absorption ability in blends with 10 and 20% of developed modified flour content may be connected with the presence of non-starch polysaccharides that were slightly modified by thermal or hydrothermal and hybrid treatments.

Tayefe et al. [11] reported increased WA in dough with the addition of hydrothermally treated bran. Their explanation for this outcome is that hydrothermal treatment causes interaction between water molecules and structural fibers through hydrogen bonding and thus increases the WA. Moreover, this hydrothermal processing may convert the starch present in developed flour to pregelatinized starch, which is characterized by improved retention of water molecules. They also reported an increase in dough development time and a decrease in dough stability when 6 and 9% of HT treated bran were added to the component bread flour. Similarly, when extruded at 50, 60, and 70 °C, wheat starch added to bread flour caused increased water absorption, as tested with Farinograph by Tao et al. [46].

The results of dough property testing utilizing an Alveograph are presented in Table 1 for control common bread flour and for blends with enzymatic, thermally, hydrothermally, and hybrid enzyme-assisted modified methods. The tested base common bread flour (K) was characterized by appropriate parameters for baking bread, wherein the baking force W was 212 J 10^{-4}, with the dough elasticity and extensibility coefficient P/L being 1.26. After the addition of flour with increased content of non-starch polysaccharides (F), in amounts of both 10 and 20%, a slight increase in dough extensibility (L) was observed, and thus an improvement in baking value (W). However, with the addition of enzymatically modified flours, the flours had these values at a level similar to the control flour. When modified heat-treated raw materials (TF) were employed as additions to the control flour, a deterioration in the baking value (W) was found, mainly due to a decrease in dough elasticity (P). Here, the dough elasticity parameters (Ie) and (SH) also deteriorated. Of note, when thermally modified flours with enzymatic fortification were used as an additive, the elasticity parameters (P) increased to those observed for the control flour.

Improvement in dough elasticity (L), baking value (W), and elasticity index (Ie) was also observed; the improvement in these properties was more visible in the tests with the addition of the enzyme complex (TFCX). Buscella et al. [15] analyzed wheat cakes and bread flours that differed in quality and baking value and were subjected to heat treatment without water and to hydrothermal treatment. They conducted analyses of both the suspension and the dough matrix and observed that heat treatment improved the stability of the dough, albeit more intensively for the flour with weaker baking value, and that the viscosity properties of bread flour also changed [15]. The sedimentation value indicating the quality of gluten protein of bread flour subjected to dry heat treatment also showed a significant decrease compared to untreated bread flour [15]. This outcome was attributed to changes in the gluten structure due to the rearrangement of disulfide bonds [15]. We also observed that the addition of bread flour subjected only to dry heating worsened the quality parameters determined by alveographic analysis. The use of the hybrid modification method through the participation of bakery enzymes in the mixture, especially TFCX with the cellulase-xylanase complex, allowed us to obtain a level of quality that improved the overall quality of gluten proteins, evident in the improvement of dough elasticity, as well as in the notable improvement of the hydration properties of the flour (increased water absorption) (Table 1). In the case of the addition of flours modified by hydrothermal

treatment, a decrease in dough extensibility and a significant increase in its elasticity were found in all tested blends. This resulted in a significant change in the configuration of the P/L chart, the value of which increased by 26% compared to the control flour and for dough with the addition of 10% of hydrothermally processed (HF10) flour, and to 49% if 20% of hybrid enzymatic-hydrothermal flour (HFCX20) was added to the blend.

Table 1. Consistency characteristics of tested bread dough compositions with 10 and 20% *w*/*w* added modified flours treated through various methods.

Bread Sample	WA (%)	P (mm)	L (mm)	W (J10^{-4})	P/L (-)	Ie (%)	SH (-)
K	62.5 ± 0.2 ^{a}	92 ± 2 ^{d,e}	73 ± 3 ^{d,e,f,g}	212 ± 3 ^{c,d}	1.26 ± 0.06 ^{f,g}	47.67 ± 0.55 ^{e,f}	1.60 ± 0.04 ^{e}
F10	62.8 ± 0.1 ^{a,b}	93 ± 1 ^{e,f}	89 ± 3 ^{j,k}	235 ± 3 ^{g}	1.05 ± 0.04 ^{a,b,c}	46.97 ± 0.35 ^{d,e}	1.52 ± 0.01 ^{b,c}
F20	64.1 ± 0.1 ^{e,f}	97 ± 2 ^{g}	87 ± 1 ^{j,k}	239 ± 4 ^{g,h}	1.10 ± 0.01 ^{b,c,d,e}	45.77 ± 0.12 ^{b,c}	1.52 ± 0.02 ^{b,c}
FC10	63.5 ± 0.2 ^{c,d}	88 ± 0 ^{b,c}	78 ± 1 ^{g,h,i}	212 ± 2 ^{c,d}	1.13 ± 0.02 ^{c,d,e,f}	48.13 ± 0.15 ^{f,g}	1.61 ± 0.01 ^{e,f}
FC20	63.0 ± 0.1 ^{b}	95 ± 1 ^{f,g}	72 ± 2 ^{d,e,f,g}	223 ± 2 ^{e,f}	1.32 ± 0.05 ^{g}	48.83 ± 0.06 ^{g}	1.64 ± 0.02 ^{f}
FCX10	63.2 ± 0.2 ^{b,c}	92 ± 1 ^{d,e}	73 ± 2 ^{e,f,g}	212 ± 6 ^{c,d}	1.26 ± 0.03 ^{f,g}	47.23 ± 0.42 ^{d,e,f}	1.59 ± 0.01 ^{d,e}
FCX20	64.0 ± 0.1 ^{e,f}	92 ± 1 ^{d,e}	70 ± 1 ^{d,e,f}	205 ± 6 ^{b,c}	1.32 ± 0.01 ^{g}	47.00 ± 0.46 ^{d,e}	1.60 ± 0.01 ^{e}
TF10	64.1 ± 0.2 ^{e,f}	91 ± 1 ^{d,e}	74 ± 2 ^{f,g}	201 ± 2 ^{b}	1.23 ± 0.03 ^{e,f,g}	43.93 ± 0.15 ^{a}	1.47 ± 0.00 ^{a}
TF20	65.3 ± 0.2 ^{i}	86 ± 1 ^{b}	68 ± 4 ^{c,d,e}	184 ± 3 ^{a}	1.26 ± 0.09 ^{f,g}	45.50 ± 0.10 ^{b}	1.55 ± 0.03 ^{c,d}
TFC10	64.3 ± 0.2 ^{f,g}	80 ± 0 ^{a}	81 ± 2 ^{h,i}	186 ± 2 ^{a}	0.99 ± 0.03 ^{a,b}	44.33 ± 0.15 ^{a}	1.49 ± 0.01 ^{a,b}
TFC20	66.0 ± 0.1 ^{j}	86 ± 0 ^{b}	91 ± 1 ^{k}	221 ± 2 ^{d,e,f}	0.94 ± 0.01 ^{a}	47.33 ± 0.21 ^{d,e,f}	1.53 ± 0.01 ^{b,c}
TFCX10	64.7 ± 0.1 ^{g,h}	90 ± 0 ^{c,d}	83 ± 1 ^{i,j}	225 ± 2 ^{f}	1.08 ± 0.02 ^{a,b,c,d}	47.43 ± 0.06 ^{d,e,f}	1.58 ± 0.01 ^{d,e}
TFCX20	65.9 ± 0.2 ^{j}	92 ± 1 ^{d,e}	77 ± 3 ^{g,h}	213 ± 3 ^{c,d,e}	1.19 ± 0.05 ^{d,e,f,g}	46.63 ± 0.23 ^{c,d}	1.55 ± 0.01 ^{c,d}
HF10	64.1 ± 0.1 ^{e,f}	107 ± 1 ^{h}	67 ± 1 ^{b,c,d}	244 ± 1 ^{g,h}	1.59 ± 0.03 ^{h}	50.37 ± 0.15 ^{h}	1.70 ± 0.01 ^{g}
HF20	65.1 ± 0.2 ^{h,i}	113 ± 1 ^{j}	68 ± 1 ^{c,d,e}	268 ± 3 ^{j}	1.66 ± 0.01 ^{h,i}	51.97 ± 0.12 ^{i}	1.74 ± 0.00 ^{g,h}
HFC10	63.9 ± 0.1 ^{d,e}	110 ± 1 ^{i}	62 ± 1 ^{a,b}	246 ± 3 ^{h}	1.78 ± 0.01 ^{i,j}	52.03 ± 0.12 ^{i}	1.77 ± 0.01 ^{h,i}
HFC20	64.3 ± 0.2 ^{f,g}	114 ± 2 ^{j}	62 ± 2 ^{a,b}	257 ± 5 ^{i}	1.83 ± 0.08 ^{j}	53.10 ± 0.61 ^{j}	1.79 ± 0.01 ^{i}
HFCX10	64.0 ± 0.1 ^{e,f}	106 ± 1 ^{h}	63 ± 3 ^{a,b,c}	237 ± 6 ^{g,h}	1.68 ± 0.07 ^{h,i}	50.63 ± 0.68 ^{h}	1.74 ± 0.01 ^{g,h}
HFCX20	64.3 ± 0.2 ^{f,g}	111 ± 2 ^{i,j}	59 ± 2 ^{a}	242 ± 1 ^{g,h}	1.88 ± 0.08 ^{j}	52.13 ± 0.40 ^{i,j}	1.79 ± 0.00 ^{i}

K—control; F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; CX—cellulase-xylanase enzyme complex; 10 and 20—% of modified flour in bread recipe; WA—water absorption; P—dough tenacity; L—extensibility; W—baking strength; P/L—dough configuration index; Ie —elasticity index; SH—strain hardening index; $^{a–j}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Hydrothermal treatment carried out in the developed flour also brought about significant changes in the conformation of gluten proteins by reducing their elasticity. We did not observe significant differences between the addition of hydrothermally treated flours or those treated with enzyme assisted modification. Although both the baking value W and the SH coefficient increased to a higher level than in the control flour (K), this was due to increased dough stiffness and not due to improvement in flour blend quality, as we did not observe an increase in flour water absorption. Similar results were obtained in the work of Martinez et al. [22], who used extruded flours as additives to bread. Here, the addition of extruded flours significantly increased the elasticity of the dough and reduced its extensibility [22].

3.2. Bread Proximate Composition

Table 2 summarizes the main chemical components present in bread made with a common bread flour and when 10 and 20% *w*/*w* was replaced by modified flours without or with enzymatic assistance. The level of protein in common bread flour was 13.22% and all breads with added modified flours were higher in protein content than the control bread. This is the effect of the composition of the developed flour (F), which was characterized by higher protein content (14.62%) due to selection of appropriate milling

passages, as well as to the contribution of selected fractions [31]. The bread formed from the developed flour, whether modified enzymatically, thermally, or hydrothermally without or with enzyme assistance, when replacing common flour at 10 and 20%, had increased total protein content, with higher values when 20% was added to the basic bread recipe.

Moreover, fat content in the developed flour was 1.31 ± 0.01%, and, after thermal modification or enzymatic modifications, was similarly ranged—at 1.30–1.38. Bucsella et al. [47], when testing aleurone-rich flour, found a different composition (20% protein, 15% dietary fiber) to that of commercial fiber-rich wheat fractions (9–13% protein, 9% dietary fiber). They noted that the presence of a higher content of inner layers of the seed coat than seen in white or wholegrain flour also resulted in a high fat content (4%). In our work, we saw that the fortified bread was lower in fat if modified flours were added, especially following HFC and HFCX application at 20%. Accordingly, significantly lowered fat content was found to be extractable during bread analysis. This limitation may be the effect of the formation complexes with amylose that usually come about during hydrothermal or extrusion treatment at increased temperatures [48]. The final temperature of these flours was around 65 °C, hence the temperature effect was more intense than that which occurs under dry thermal treatment (TF). Additionally, during baking temperatures, formation of fat-induced complexes may take place.

Table 2. Proximate composition of bread obtained with addition of modified flours.

Bread Sample	Protein [%]	Fat [%]	Ash [%]	IDF [%]	SDF [%]	TDF [%]	Carbohydrates [%]	Caloric Value [kcal/100 g]
K	13.22 ± 0.06 ^{a}	0.30 ± 0.01 ^{g,h}	1.02 ± 0.02 ^{a,b,c}	5.43 ± 0.02 ^{c}	2.79 ± 0.01 ^{f,g}	8.21 ± 0.02 ^{c}	77.25 ± 0.05 ^{m}	397.40 ± 0.13 ^{g,h}
F10	13.46 ± 0.06 ^{b,c}	0.27 ± 0.01 ^{f,g}	1.04 ± 0.02 ^{a,b,c,d}	7.10 ± 0.02 ^{l}	3.08 ± 0.01 ^{h,i}	10.17 ± 0.02 ^{l}	75.06 ± 0.03 ^{d}	397.19 ± 0.13 ^{f,g,h}
F20	13.54 ± 0.02 ^{c,d}	0.22 ± 0.01 ^{e}	0.98 ± 0.02 ^{a}	7.19 ± 0.02 ^{m}	3.38 ± 0.02 ^{j}	10.57 ± 0.02 ^{o}	74.69 ± 0.02 ^{c}	397.20 ± 0.09 ^{f,g,h}
FC10	13.28 ± 0.02 ^{a}	0.13 ± 0.02 ^{b,c}	1.05 ± 0.02 ^{a,b,c,d}	5.28 ± 0.01 ^{b}	2.64 ± 0.02 ^{d,e,f}	7.93 ± 0.02 ^{b}	75.76 ± 0.02 ^{g}	396.47 ± 0.16 ^{b,c,d}
FC20	13.86 ± 0.04 ^{g,h}	0.23 ± 0.01 ^{e,f}	1.02 ± 0.02 ^{a,b,c}	6.76 ± 0.01 ^{j}	3.01 ± 0.02 ^{h}	9.78 ± 0.02 ^{j}	76.96 ± 0.07 ^{k}	397.09 ± 0.11 ^{e,f,g}
FCX10	13.25 ± 0.05 ^{a}	0.22 ± 0.02 ^{e}	1.15 ± 0.01 ^{e}	6.51 ± 0.02 ^{i}	2.40 ± 0.02 ^{b,c}	8.91 ± 0.01 ^{e}	76.40 ± 0.05 ^{j}	396.28 ± 0.21 ^{a,b}
FCX20	13.72 ± 0.04 ^{e,f}	0.20 ± 0.01 ^{d,e}	1.08 ± 0.02 ^{c,d}	5.50 ± 0.02 ^{d}	3.43 ± 0.01 ^{j}	8.94 ± 0.01 ^{e}	76.05 ± 0.05 ^{h,i}	396.68 ± 0.13 ^{b,c,d,e}
TF10	13.42 ± 0.04 ^{b}	0.27 ± 0.02 ^{f,g}	1.03 ± 0.01 ^{a,b,c}	5.28 ± 0.01 ^{a}	2.60 ± 0.02 ^{d,e}	7.88 ± 0.02 ^{a}	77.47 ± 0.00 ^{n}	397.25 ± 0.04 ^{g,h}
TF20	13.43 ± 0.03 ^{b}	0.29 ± 0.01 ^{g,h}	1.03 ± 0.02 ^{a,b,c}	6.73 ± 0.02 ^{j}	2.53 ± 0.02 ^{c,d}	9.27 ± 0.01 ^{h}	76.14 ± 0.01 ^{i}	397.55 ± 0.04 ^{h}
TFC10	13.43 ± 0.01 ^{b}	0.20 ± 0.02 ^{d,e}	1.01 ± 0.02 ^{a,b,c}	5.91 ± 0.03 ^{f}	2.44 ± 0.01 ^{b,c}	8.35 ± 0.02 ^{d}	76.93 ± 0.02 ^{k}	397.36 ± 0.18 ^{g,h}
TFC20	13.56 ± 0.03 ^{d}	0.28 ± 0.02 ^{g}	1.07 ± 0.02 ^{b,c,d}	7.72 ± 0.02 ^{o}	3.21 ± 0.02 ^{i}	10.92 ± 0.02 ^{p}	74.24 ± 0.03 ^{a}	396.74 ± 0.16 ^{c,d,e}
TFCX10	13.81 ± 0.04 ^{f,g}	0.09 ± 0.02 ^{a,b}	1.11 ± 0.03 ^{d}	6.18 ± 0.01 ^{g}	2.81 ± 0.01 ^{g}	9.02 ± 0.02 ^{f}	75.97 ± 0.05 ^{h}	396.01 ± 0.22 ^{a}
TFCX20	13.92 ± 0.02 ^{h}	0.12 ± 0.01 ^{a,b}	1.06 ± 0.02 ^{b,c,d}	7.34 ± 0.02 ^{n}	3.07 ± 0.01 ^{h,i}	10.40 ± 0.02 ^{n}	74.50 ± 0.02 ^{b}	396.34 ± 0.09 ^{a,b,c,d}
HF10	13.44 ± 0.02 ^{b,c}	0.20 ± 0.02 ^{d,e}	1.05 ± 0.03 ^{a,b,c,d}	6.94 ± 0.01 ^{k}	2.33 ± 0.03 ^{b}	9.27 ± 0.01 ^{h}	76.04 ± 0.04 ^{h,i}	396.77 ± 0.08 ^{d,e,f}
HF20	13.64 ± 0.04 ^{d,e}	0.28 ± 0.01 ^{g}	1.01 ± 0.06 ^{a,b,c}	6.46 ± 0.02 ^{h}	2.72 ± 0.20 ^{e,f,g}	9.07 ± 0.01 ^{g}	76.00 ± 0.06 ^{h}	397.38 ± 0.20 ^{g,h}
HFC10	13.43 ± 0.03 ^{b}	0.08 ± 0.02 ^{a}	1.00 ± 0.02 ^{a,b}	7.09 ± 0.02 ^{l}	3.19 ± 0.01 ^{i}	10.26 ± 0.02 ^{m}	75.23 ± 0.04 ^{e}	396.38 ± 0.16 ^{a,b,c,d}
HFC20	13.70 ± 0.02 ^{e}	0.13 ± 0.02 ^{b,c}	1.01 ± 0.02 ^{a,b,c}	5.81 ± 0.01 ^{e}	2.09 ± 0.02 ^{a}	7.89 ± 0.02 ^{b}	77.07 ± 0.04 ^{l}	397.63 ± 0.18 ^{h}
HFCX10	13.70 ± 0.01 ^{e}	0.07 ± 0.02 ^{a}	1.02 ± 0.02 ^{a,b,c}	7.16 ± 0.02 ^{m}	2.80 ± 0.01 ^{g}	9.95 ± 0.01 ^{k}	75.26 ± 0.02 ^{e}	396.29 ± 0.16 ^{a,b,c}
HFCX20	13.82 ± 0.02 ^{f,g,h}	0.18 ± 0.02 ^{c,d}	1.05 ± 0.03 ^{a,b,c,d}	6.55 ± 0.01 ^{i}	2.96 ± 0.01 ^{h}	9.52 ± 0.02 ^{i}	75.43 ± 0.02 ^{f}	396.67 ± 0.17 ^{b,c,d,e}

K—control; F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; CX—cellulase-xylanase enzyme complex; 10 and 20—% of modified flour in bread recipe; TDF—total dietary fiber; IDF—insoluble dietary fiber; SDF—soluble dietary fiber; $^{a–n}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Our work saw that ash content in common bread was 1.02%, but that in the developed flour, ash content was lower (0.78%) [31]. Thus, the substitution of common flour with 10 and 20% of the developed F flour decreased ash content in the prepared bread. The ash content in treated flours varied from 1.00 to 1.15%, with a slight decreasing effect of C and CX enzymes addition on ash content in the produced bread (Table 2). In our work, dietary fiber and its fractions were tested in bread composed according to control (K) and modified recipes. The content of fibrous fractions in the developed flour showed IDF 3.94%, SDF 2.86%, and TDF 6.80% [31]. In contrast, the TDF in control bread was 8.21%

and demonstrated a predominance of insoluble fraction IDF. In breads fortified with untreated developer flour (F10, F20), the content of TDF was significantly higher due to the incorporation of passages rich in non-starch polysaccharides derived from the outer layer of the original wheat grains [26].

As mentioned previously, increased content of fibrous fractions, especially insoluble, may have an effect on dough properties through an increase in water binding ability [49]. We also found high correlation between insoluble fractions and the TDF content ($r = 0.933$ at $p < 0.05$). In all breads with added hydrothermally treated flour at 20% content, both without and with enzymes, a lower level of TDF was noted than that for 10% of the additive, in addition to significantly lower amounts of insoluble fiber fractions. This may be the effect of enhanced enzymes activity being improved upon by temperature and steam action, because cellulases and xylanases act mostly on fibrous fractions of polysaccharides, causing partial hydrolysis of pentosanes [19,48,49]. In contrast, replacement of 20% of bread composition by modified flours resulted in higher content of TDF in bread than if 10% was applied—no matter the treatment or supplementation. In all cases, the effect of the addition of enzymes was ambiguous or similar in all dietary fiber fractions analysis. Analysis of carbohydrates content showed a slight decrease if modified flours were added to the bread mixture, and thus some slight changes in caloric values were noted (Table 2).

3.3. Bread Quality and Appearance

Bread quality and appearance are important factors for both producers and consumers [1,3–9]. Breads made from whole grain flour or supplemented with unmodified bran addition, due to the reduced ability of the dough to retain gases, are characterized by having smaller loaf volume and, hence, deteriorated baking quality [27,28]. Producers prefer a high yield of bread with high loaf volume and increased water absorption ability during bread dough making, but consumers prefer a regular crust structure and homogenous pores distribution in the bread crumb. Adding more water to dough recipes is a common approach to increasing bread production. However, increasing the amount of water in the dough can result in a deterioration of the dough's kneading ability, as it becomes too wet and sticky, and this affects the final volume and texture of the bread [21]. Additionally, a higher water content in the dough can reduce the shelf life of the bread due to microbiological hazards. The addition of physically modified flours rich in fibrous fractions have been demonstrated to bring about changes in the bread quality [20]. The results of selected quality characteristics of baked bread prepared with the addition of modified flours are presented in Table 3.

In analyzing the quality and performance characteristics of the baked bread obtained from recipes with the addition of modified flours, it can be noticed that the addition of enzymatically and process-modified flours had a significant impact on the volume of the tested bread. This effect was also evident in the specific loaf volume values. Bread volume increased significantly when treated flours were added to the bread composition, in most cases, when 20% of modified flours were added.

Very good results in increasing the loaf volume were obtained when only flours subjected to enzymatic fortification (FC10, FC20, and FCX20) were used as additives. Hilhorst et al. [50] reported that the addition of xylanases may enhance the handling properties of wheat dough, the ovenspring, and the bread volume. The addition of process-modified flours or flours treated using hybrid enzyme-assisted methods to the bread mixtures also improved the bread volume, but without significant differences among the applied processing methods. Significant increase in bread volume was also demonstrated if thermally and hydrothermally treated TFC10 and HF20 flours were added to the bread recipe.

Table 3. Selected quality characteristics of baked bread prepared with the addition of modified flours.

Bread Sample	Bread Volume (mL)	Specific Volume (cm^3/g)	Bread Density (g/cm^3)	Baking Loss (%)	Weight Loss (%)	Bread Yield (%)
K	755 ± 10 ^{a,b}	2.84 ± 0.02 ^{a,b}	0.35 ± 0.00 ^{g,h}	9.79 ± 0.49 ^{a}	2.80 ± 0.21 ^{a,b}	146.77 ± 0.64 ^{b,c,d,e}
F10	778 ± 8 ^{b,c}	2.95 ± 0.03 ^{b,c}	0.34 ± 0.00 ^{f,g}	9.33 ± 0.33 ^{a}	3.06 ± 0.20 ^{a,b,c}	147.21 ± 0.32 ^{b,c,d,e,f}
F20	832 ± 13 ^{d,e,f,g}	3.12 ± 0.05 ^{d,e}	0.32 ± 0.01 ^{c,d,e}	9.34 ± 0.47 ^{a}	3.13 ± 0.19 ^{b,c,d,e}	147.72 ± 0.70 ^{b,c,d,e,f,g}
FC10	860 ± 9 ^{g,h,i}	3.28 ± 0.04 ^{g,h,i}	0.31 ± 0.00 ^{a,b}	9.48 ± 0.09 ^{a}	3.63 ± 0.10 ^{e}	146.30 ± 0.02 ^{b}
FC20	880 ± 10 ^{i}	3.34 ± 0.05 ^{i}	0.30 ± 0.00 ^{a}	9.48 ± 0.19 ^{a}	2.98 ± 0.12 ^{a,b,c}	146.93 ± 0.31 ^{b,c,d,e,f}
FCX10	750 ± 17 ^{a}	2.77 ± 0.08 ^{a}	0.35 ± 0.01 ^{g,h}	9.31 ± 0.34 ^{a}	3.46 ± 0.30 ^{c,d,e}	147.28 ± 0.41 ^{b,c,d,e,f,g}
FCX20	847 ± 12 ^{f,g,h}	3.21 ± 0.03 ^{e,f,g,h}	0.31 ± 0.00 ^{a,b,c,d}	9.64 ± 0.43 ^{a}	3.22 ± 0.10 ^{b,c,d,e}	146.48 ± 0.55 ^{b,c}
TF10	810 ± 10 ^{d,e}	3.13 ± 0.05 ^{d,e,f}	0.32 ± 0.01 ^{c,d,e}	9.39 ± 0.26 ^{a}	3.64 ± 0.27 ^{f}	146.77 ± 0.04 ^{a}
TF20	817 ± 3 ^{d,e,f}	3.11 ± 0.01 ^{d,e}	0.32 ± 0.00 ^{d,e}	9.61 ± 0.56 ^{a}	3.58 ± 0.25 ^{d,e}	147.46 ± 0.76 ^{b,c,d,e,f,g}
TFC10	873 ± 6 ^{h,i}	3.32 ± 0.03 ^{h,i}	0.30 ± 0.00 ^{a}	9.42 ± 0.06 ^{a}	3.61 ± 0.08 ^{e}	146.59 ± 0.17 ^{b,c,d}
TFC20	813 ± 6 ^{d,e}	3.07 ± 0.01 ^{c,d}	0.33 ± 0.00 ^{e,f}	9.40 ± 0.39 ^{a}	2.80 ± 0.24 ^{a,b}	150.02 ± 0.28 ^{i}
TFCX10	828 ± 3 ^{d,e,f}	3.12 ± 0.01 ^{d,e}	0.32 ± 0.00 ^{c,d,e}	9.44 ± 0.62 ^{a}	2.79 ± 0.11 ^{a,b}	148.13 ± 0.96 ^{e,f,g,h}
TFCX20	837 ± 6 ^{e,f,g}	3.16 ± 0.03 ^{d,e,f,g}	0.32 ± 0.00 ^{b,c,d,e}	9.30 ± 0.38 ^{a}	2.92 ± 0.15 ^{a,b}	149.60 ± 0.45 ^{h,i}
HF10	833 ± 15 ^{d,e,f,g}	3.14 ± 0.07 ^{d,e,f}	0.32 ± 0.01 ^{b,c,d,e}	9.08 ± 0.26 ^{a}	3.18 ± 0.18 ^{b,c,d,e}	147.90 ± 0.34 ^{c,d,e,f,g}
HF20	860 ± 17 ^{g,h,i}	3.25 ± 0.06 ^{f,g,h,i}	0.31 ± 0.01 ^{a,b,c}	9.11 ± 0.22 ^{a}	3.47 ± 0.04 ^{c,d,e}	147.58 ± 0.30 ^{b,c,d,e,f,g}
HFC10	803 ± 6 ^{c,d}	3.03 ± 0.03 ^{c,d}	0.33 ± 0.00 ^{e,f}	9.58 ± 0.18 ^{a}	2.60 ± 0.17 ^{a}	148.05 ± 0.12 ^{d,e,f,g}
HFC20	810 ± 1 ^{d,e}	3.05 ± 0.02 ^{c,d}	0.33 ± 0.00 ^{e,f}	9.53 ± 0.17 ^{a}	2.89 ± 0.05 ^{a,b}	148.40 ± 0.23 ^{f,g,h}
HFCX10	833 ± 15 ^{d,e,f,g}	3.13 ± 0.05 ^{d,e,f}	0.32 ± 0.00 ^{c,d,e}	9.27 ± 0.47 ^{a}	3.10 ± 0.10 ^{a,b,c,d}	148.76 ± 0.77 ^{g,h,i}
HFCX20	823 ± 6 ^{d,e,f}	3.11 ± 0.00 ^{d,e}	0.32 ± 0.00 ^{c,d,e}	9.17 ± 0.23 ^{a}	3.08 ± 0.11 ^{a,b,c}	148.43 ± 0.41 ^{f,g,h}

K—control; F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; CX—cellulase-xylanase enzyme complex; 10 and 20—% of modified flour in bread recipe; $^{a–i}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

It should be noted that the higher loaf volume of breads prepared with the addition of hydrothermally treated flour with enzymes was also the result of uneven distribution of gas bubbles, which were located in large numbers under the bread crust, causing it to stand aside and bringing about the collapse of the loaf in the final stage of baking, as visible in the obtained bread pictures presented in Figure 2. This suggests that the consistency of the dough may have been too loose, and that the amount of water added to the recipes should be reduced when adding hydrothermally modified flours [47].

When flours modified hydrothermally were used as an additive for baking, a negative impact on the final quality of the produced bread was evident. The problems with gas retention in bread were probably due to flours modified in this way losing their gluten network formation properties. According to Hong et al. [51], modification of wheat flour via superheated steam treatment causes protein denaturation and brings about the initial gelatinization of its contained starch granules, thus reducing the access of water to the protein phase due to its greater absorption. The resulting problem with the formation of a continuous network by starch is that it results in a weakening of the gluten quality and a reduction in its elasticity. This effect was noted in the alveographic analyses. The presence of HF, HFC, and HFCX flour components in the bread dough induced a lowering of dough strength and a problem with gas bubble containment in the dough matrix. Despite the use of bakery enzymes for processing, which increased the soluble fiber fraction, this did not eliminate the negative impact of the process on gluten protein quality.

In our work, specific bread volume increased significantly with the addition of thermal, hydrothermal, and hybrid treated developed flour, or if FC10 and FC20 was incorporated within the bread recipe (Table 3). A significant decrease in bread density (0.30–0.33

g/cm^3) was also observed in these samples, as compared to control (0.35 g/cm^3), due to the larger number of pores in the crumb than in the control bread.

We also found that bread volume and specific volume were highly negatively correlated with bread density (r = −0.997 and 0.987, respectively, at $p < 0.05$). Accordingly, Tao et al. [46] reported increased specific bread volume (from 1.63 to 2.15 cm^3/g) when low temperature extrusion was applied to process wheat starch added to a bread recipe. Moreover, in our work, the addition of modified flours did not cause an increase in baking loss compared to the control bread, while an increase in weight loss after 24 h was observed. The increase in baking loss after 24 h was, however, slightly greater, albeit statistically insignificant, when modified and control flour recipes were compared. The greatest baking loss was observed in the control breads. The loss during baking of bread incorporating modified flours was lower, which indicates a heightened ability to retain water during baking; however, after 24 h, the weight loss was slightly higher, which may be the effect of retrogradation of starch treated via T and H methods after baking and cooling. The WAI values were also lower in the control bread and higher in that made with the modified additives. The addition of dry thermal heating reduced the retrogradation of the dough, as illustrated by the results of the C5 measurement, while the addition of hydrothermally treated flour increased the retrogradation, which is confirmed by the C5 results. Here, after cooling, the internal consistency was less springy, as confirmed by texture measurements.

In related work, Kurek et al. [52] found that values of a specific volume of wheat bread depend on the flour type used, with the lowest specific volume observed in wholegrain bread (0.82 cm^3/g) and the highest in control white bread samples (1.60 cm^3/g). Ma et al. [13] investigated the effect of superheated steam treatment on enhancing the physicochemical properties of flour for baked products. They noted that steam treatment could improve certain dough quality characteristics, such as volume and crumb quality. In their research, they found that the superheated steam treatment increased the starch gelatinization level and weakened the gluten strength due to denaturation, and that changes in these physicochemical properties of flour showed an effect on dough quality [13,16]. In our research, treatment with enzymes eliminated the negative aspects of the physical processes. In contrast, Hydrothermal treatment, despite the use of an enzyme complex, negatively affected the quality of the modified flour, the addition of which caused problems with bread dough gas bubble retention during rising and baking.

From an economic and quality point of view, the most desired outcome of flour/recipe modification is generating the highest specific volume. Alamri et al. [53] reported a specific volume between 2.55 and 3.14 cm^3/g for bread with addition of 1 and 2% of plant gums, with the lowest value obtained for common wheat flour control bread. Wholegrain or fiber enriched dough generally has more phytic acid, which reduces alpha-amylase activity, which, in turn, causes a decrease in bread specific volume. A much higher specific bread volume was obtained by Zhan et al. [5] for whole wheat bread (3.82 cm^3/g), and replacement of wheat flour by pulses decreased the specific volume of supplemented bread because of the lower water absorption of pulse flour.

In our study, change in the yield of the obtained bread was calculated for individual baked loaves. A significant increase was observed in breads made with recipes containing the addition of HFC20, HFCX20, HFCX10, TFCX 20, and TFC20 flours; however, as mentioned earlier, breads baked with the addition of HF flours were characterized by a lower ability to retain gases during fermentation, especially when 10% HFC and HFCX flours were added, and the bubbles moved under the crust, causing it to stand apart after baking (Figure 2). Such changes were not observed when dry heat-treated flour was used as an additive.

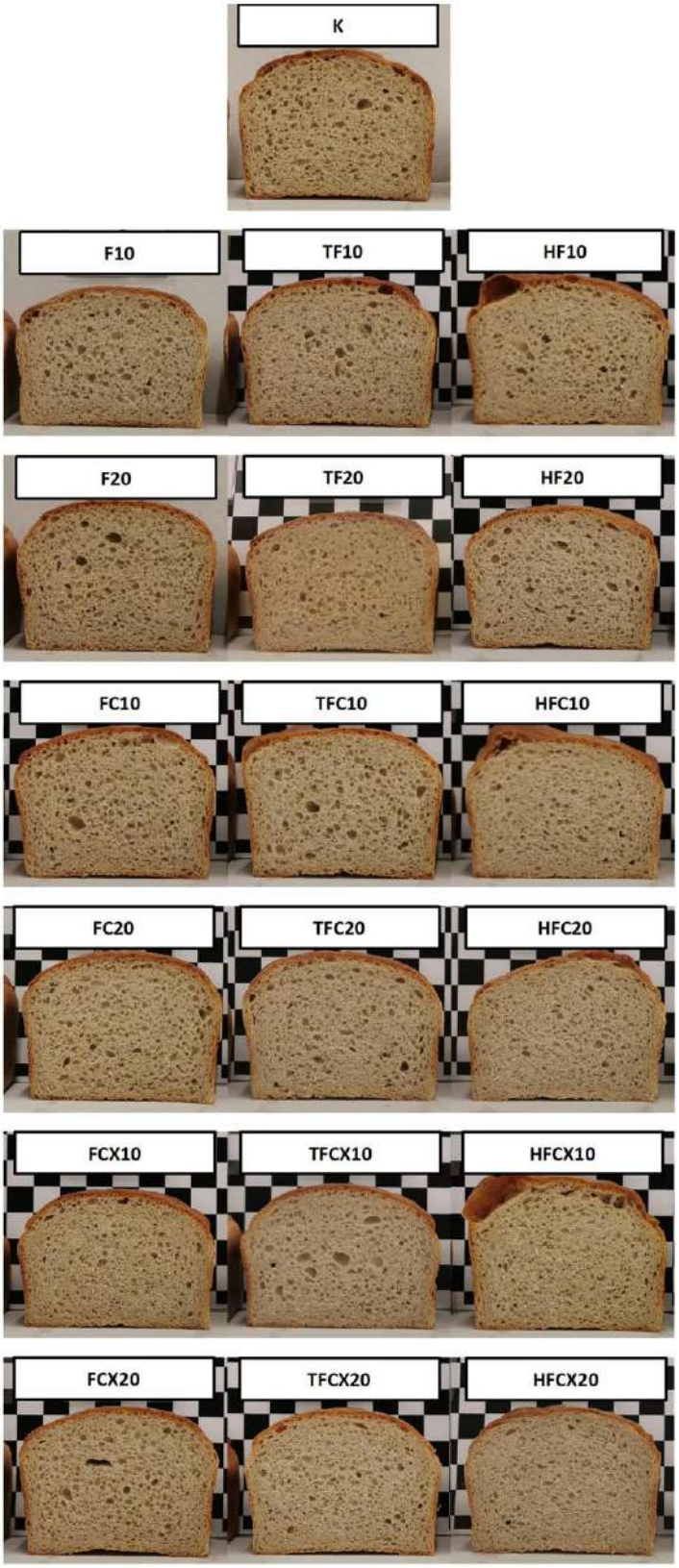

Figure 2. Bread samples with the addition of modified flours: K—control; F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; CX—cellulase-xylanase enzyme complex; 10 and 20—% of modified flour in bread recipe.

As shown in Figure 2, breads with TF, TFC, and TFCX additions were characterized by an increased or similar number of pores as in the control bread K; these were located

evenly in the crumb, increasing the overall volume of the loaves. Ambrosewicz-Walacik et al. [41], in a related work, tested bread yield of yeast or sourdough fermented bread based on various compositions of doughs. They reported that bread yield varied from 131.1% if yeast were used in white wheat bread to 134.8% in wholemeal wheat bread, whereas bread yield ranged from 152.8% to 162.4%, respectively, when natural sourdough was applied. A much higher bread yield was obtained if rye flour was used, especially wholegrain (188.3%), with natural sourdough being used for bread preparation.

3.4. Bread Physical Properties

The color profile of bread crumb and crust are quality parameters mostly associated with the attractiveness of bakery products to the consumer. Development of non-enzymatic browning, as the effect of sugars caramelization or the Maillard reaction, on the surface of baked goods is important because of the formation of a marketable beige-brown color and specific flavor [7]. Table 4 presents the results of color profile evaluation of bread crumb and crust depending on the addition of the developed flour (F) as modified via the researched methods.

Table 4. Color profile evaluation of bread crumb and crust.

Bread	Bread Crumb				Bread crust			
Sample	*L**	*a**	*b**	ΔE	*L**	*a**	*b**	ΔE
K	65.29 ± 0.74 ^{a,b}	1.97 ± 0.13 ^{a}	10.48 ± 0.34 ^{a,b,c}	ref	45.60 ± 1.66 ^{c,d}	13.45 ± 0.20 ^{a,b,c}	22.69 ± 0.90 ^{c,d,e}	ref
F10	65.02 ± 2.59 ^{a,b}	1.95 ± 0.17 ^{a}	10.61 ± 0.38 ^{a,b,c}	0.30	45.33 ± 1.55 ^{c,d}	13.54 ± 0.27 ^{b,c,d}	23.36 ± 1.17 ^{c,d,e}	0.73
F20	63.75 ± 1.37 ^{a,b}	2.13 ± 0.20 ^{a}	10.48 ± 0.79 ^{a,b,c}	1.55	45.75 ± 2.51 ^{c,d}	13.07 ± 0.85 ^{a,b,c}	22.50 ± 2.47 ^{c,d,e}	0.46
FC10	62.51 ± 1.99 ^{a}	1.85 ± 0.23 ^{a}	9.80 ± 0.72 ^{a,b}	2.86	43.20 ± 1.16 ^{a,b,c}	13.07 ± 0.42 ^{a,b,c}	20.84 ± 1.47 ^{a,b,c,d}	3.05
FC20	64.06 ± 2.95 ^{a,b}	2.01 ± 0.22 ^{a}	10.44 ± 0.19 ^{a,b,c}	1.23	48.46 ± 1.92 ^{d}	12.87 ± 0.30 ^{a,b}	23.84 ± 0.85 ^{d,e}	3.14
FCX10	62.36 ± 1.86 ^{a}	1.83 ± 0.20 ^{a}	10.16 ± 0.40 ^{a,b,c}	2.95	41.13 ± 2.02 ^{a,b}	13.10 ± 0.33 ^{a,b,c}	19.48 ± 1.56 ^{a,b}	5.52
FCX20	61.95 ± 1.11 ^{a}	2.17 ± 0.26 ^{a}	10.26 ± 0.71 ^{a,b,c}	3.35	44.05 ± 1.48 ^{a,b,c}	13.72 ± 0.25 ^{b,c,d}	22.42 ± 0.31 ^{b,c,d,e}	1.59
TF10	62.52 ± 1.92 ^{a}	2.08 ± 0.20 ^{a}	10.47 ± 0.43 ^{a,b,c}	2.77	46.61 ± 1.90 ^{c,d}	13.90 ± 0.49 ^{c,d}	24.14 ± 0.80 ^{e}	1.82
TF20	64.97 ± 0.66 ^{a,b}	2.04 ± 0.07 ^{a}	10.70 ± 0.42 ^{b,c}	0.39	44.62 ± 1.68 ^{b,c}	12.98 ± 0.45 ^{a,b,c}	21.90 ± 1.88 ^{a,b,c,d,e}	1.34
TFC10	64.46 ± 0.90 ^{a,b}	1.98 ± 0.15 ^{a}	10.48 ± 0.54 ^{a,b,c}	0.83	43.39 ± 1.68 ^{a,b,c}	13.07 ± 0.62 ^{a,b,c}	20.68 ± 2.01 ^{a,b,c}	3.01
TFC20	62.42 ± 1.08 ^{a}	1.91 ± 0.24 ^{a}	10.04 ± 0.64 ^{a,b,c}	2.90	44.95 ± 1.82 ^{c,d}	13.69 ± 0.51 ^{b,c,d}	22.51 ± 1.42 ^{c,d,e}	0.71
TFCX10	65.53 ± 1.91 ^{b}	2.08 ± 0.16 ^{a}	10.82 ± 0.35 ^{c}	1.33	43.76 ± 1.90 ^{a,b,c}	13.38 ± 0.43 ^{a,b,c}	21.50 ± 1.50 ^{a,b,c,d,e}	2.19
TFCX20	63.50 ± 1.77 ^{a,b}	2.03 ± 0.24 ^{a}	10.88 ± 0.55 ^{c}	1.83	41.90 ± 1.15 ^{a,b}	12.82 ± 0.30 ^{a,b}	19.93 ± 0.52 ^{a,b}	6.09
HF10	63.14 ± 2.37 ^{a,b}	1.91 ± 0.22 ^{a}	10.75 ± 0.32 ^{b,c}	2.17	44.86 ± 1.85 ^{c,d}	12.93 ± 0.54 ^{a,b}	21.78 ± 1.59 ^{a,b,c,d,e}	1.29
HF20	64.28 ± 2.42 ^{a,b}	1.81 ± 0.27 ^{a}	10.06 ± 0.60 ^{a,b,c}	1.10	45.63 ± 1.92 ^{c,d}	12.81 ± 0.31 ^{a,b}	21.49 ± 1.04 ^{a,b,c,d,e}	1.37
HFC10	62.77 ± 1.66 ^{a}	2.00 ± 0.13 ^{a}	9.95 ± 0.40 ^{a,b,c}	2.57	45.81 ± 1.43 ^{c,d}	13.53 ± 0.36 ^{b,c}	22.87 ± 1.65 ^{c,d,e}	0.28
HFC20	62.31 ± 1.90 ^{a}	1.88 ± 0.14 ^{a}	9.64 ± 0.36 ^{a}	3.10	44.19 ± 1.25 ^{a,b,c}	12.76 ± 0.44 ^{a,b}	20.86 ± 1.15 ^{a,b,c,d}	2.41
HFCX10	63.58 ± 0.42 ^{a,b}	1.86 ± 0.14 ^{a}	10.45 ± 0.41 ^{a,b,c}	1.71	45.38 ± 1.31 ^{c,d}	13.50 ± 0.36 ^{b,c}	22.99 ± 1.76 ^{c,d,e}	0.37
HFCX20	63.64 ± 0.42 ^{a,b}	1.96 ± 0.08 ^{a}	10.10 ± 0.23 ^{a,b,c}	1.69	45.13 ± 2.68 ^{c,d}	14.49 ± 0.74 ^{d}	23.45 ± 1.48 ^{c,d,e}	1.37

K—control; F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; CX—cellulase-xylanase enzyme complex; 10 and 20—% of modified flour in bread recipe; *L**—lightness (0–100); *a**—greenest(−)—redness(+) balance; *b**—blueness(−)—yellowness(+) balance; ΔE—total color change; $^{a–e}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

In our work, the control bread crumb and crust had *L** values 65.26 and 45.60, respectively. Breads with the addition of TH20 and HF20 were lighter than other samples with added modified flours, and the color profile was similar to the control. The use of modified flours with enzymes significantly decreased bread crumb lightness, and breads with the addition of FC10, FCX10, and FCX20 and HFC10 and HFC20 showed *L** values ranging between 61.92 and 62.77. Other results were comparable with negligible effects of processing methods and enzymes used.

Bread crust color profiles were similar in lightness and *a** values; however, a wider range of results were noted if the crust yellowness *b** was evaluated (19.93–24.14). The more intensive redness or yellowness and darker crust color seen in some resulting breads may be the effect of nonenzymatic browning reactions or surface caramelization occurring under high temperature baking, especially when an increased content of reducing sugars is present in the bread composition [21]. In our work, ΔE was calculated separately for bread crumb and crust, and the results were, in almost all tested breads, below a value of 5, which is an easily recognized color difference.

Some differences were found between the tested breads and control sample if CX enzyme complex was added to the developed flour modifications. Especially in FCX10 and TFCX20, bread crust total color change values notably differed from the control. This could have come about due to the action of the enzyme complex upon the contained polysaccharides (which were hydrolyzed into simple sugars). This effect was not observed in the hydrothermally treated samples, probably due to the intensiveness of the treatment, which resulted in the complete deactivation of the enzymes. In the tested breads, however, no clear trend was observed, and the obtained breads were visually very similar. This confirms the appropriate quality of the resulting breads and that the additives did not have a deteriorating effect.

In a related work, Zhang et al. [5] tested the color of bread crumb prepared with whole what and supplemented with various pulse flours up to 25%. Here, the addition of pulse flour limited *L** and *b** coordinates, but, similarly, no significant trend was observed. Jurkaninová et al. [7] observed *L** values from 59.0 to 69.8 for bread crust of breads fortified with herb extracts, which is within the standard range of lightness. They determined that *a** ranged from 8.79 to 15.70 and *b** from 32.98 to 38.33, which, while indicating a red–yellow area of crust color that is favorable for baked goods, demonstrated that the color profile of the crumb was much less intensive in redness and more yellow. Mahmoud et al. [8], in turn, tested the addition of microalgae to bread and they reported twice as much a decrease of crust and crumb lightness and a more intensive green and more yellow tint of crumb when various algaes were added. In our bread with the developed treated flour, the lightness and yellowness of crust and crumb were lower, but redness was more intensive than in the control.

Testing of WAI and WSI is helpful in identifying the integration of components in products that undergo variable heat-related treatments, for example, thermal treatment, baking, or extrusion [17,42]. In all the tested breads fortified with the modified developed flours, an increased WAI was obtained as compared to control bread K (Table 5). This confirms the ability of the dough to absorb more water when the developed modified flour was added to breads. When starch granules are partly gelatinized, they have tendency to absorb and hold water, but after exceeding the gelatinization point temperature, the starch granules become broken, and they do not have the ability to absorb water [54]. Of additional interest, thermal and hydrothermal treatment may be responsible for increasing swelling capacity; in our work, the addition of treated flours showed the tendency to increase WAI. During the steam process, wheat flour components are restructured and may present different affinities with water, because strong water binding capacity can be related to interactions between water and the carbohydrates and proteins present inside the flour. Delatte et al. [17] noted some differences in the WAI and WSI of untreated and modified flours when steam treated. They reported WAI of 0.929 to 2.089 g/g in the former, and up to 4.169 to 7.450 g/g in the latter, depending on the amount of steam added during treatment. In our study, increased WAI was noted if thermally, hydrothermally, and enzyme-assisted modified flours were incorporated into the basic bread recipe, as compared to control common wheat bread (Table 5), but differences were not strictly dependent on treatment method. WAI varied from 2.668 g/g in control bread K to 3.754 g/g in the TFC10 fortified bread.

Table 5. WAI and WSI of breads.

Bread Sample	WAI (g/g)	WSI (%)
K	2.668 ± 0.044 ^{a}	6.847 ± 0.276 ^{d,e}
F10	2.930 ± 0.019 ^{b}	7.199 ± 0.087 ^{e,f}
F20	2.692 ± 0.046 ^{a}	7.840 ± 0.148 ^{g}
FC10	3.093 ± 0.078 ^{c,d}	6.700 ± 0.290 ^{c,d,e}
FC20	3.362 ± 0.010 ^{g}	6.710 ± 0.148 ^{c,d,e}
FCX10	2.691 ± 0.046 ^{a}	7.133 ± 0.143 ^{d,e,f}
FCX20	3.266 ± 0.065 ^{e,f,g}	6.985 ± 0.148 ^{d,e,f}
TF10	3.290 ± 0.035 ^{f,g}	7.173 ± 0.355 ^{e,f}
TF20	2.694 ± 0.071 ^{a}	7.270 ± 0.137 ^{e,f,g}
TFC10	3.754 ± 0.031 ^{i}	6.548 ± 0.289 ^{b,c,d}
TFC20	3.086 ± 0.062 ^{c,d}	7.555 ± 0.005 ^{f,g}
TFCX10	3.209 ± 0.024 ^{d,e,f}	6.143 ± 0.286 ^{a,b,c}
TFCX20	3.144 ± 0.048 ^{d,e,f}	7.046 ± 0.209 ^{d,e,f}
HF10	3.125 ± 0.039 ^{c,d,e}	6.981 ± 0.162 ^{d,e,f}
HF20	2.673 ± 0.061 ^{a}	6.904 ± 0.228 ^{d,e}
HFC10	3.521 ± 0.081 ^{h}	5.832 ± 0.276 ^{a}
HFC20	3.118 ± 0.016 ^{c,d}	6.847 ± 0.143 ^{d,e}
HFCX10	2.978 ± 0.018 ^{b,c}	7.539 ± 0.011 ^{f,g}
HFCX20	3.147 ± 0.016 ^{d,e,f}	5.974 ± 0.008 ^{a,b}

K—control; F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; CX—cellulase-xylanase enzyme complex; 10 and 20—% of modified flour in bread recipe; WAI—water absorption index; WSI—water solubility index; $^{a–i}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

WSI, as an indicator of degradation of molecular components, is usually employed to measure the degree of starch conversion during baking and may be an indicator of the amount of soluble polysaccharide released from the starch component after processing [55]. The decrease in WSI may be because of the reduction of carbohydrates, especially starch, in the recipe as replaced by the developed modified flour (Table 2), and thus limited gelatinization is one of the important effects of utilized baking procedure. Since WSI indicates the water solubility of non-bounded food components into water after WAI measurements, a tendency towards WSI increase with lowering of WAI was observed for most of the obtained results. Soluble components evaluated after bread testing indicated enhanced solubility if F developed flour was added to bread at 10 and 20%; here, the increase was associated with higher TDF in bread (Table 2) produced from fibrous fractions of selected passages in this new flour [26]. The highest WSI results were noted in bread with the addition of FC20 (Table 5). This outcome confirms the cellulase enzyme action on polysaccharides fractions in enzyme modified flour. Quite low WSI values were noted in breads with added enzyme-hydrothermal hybrid treated flour, both with cellulase and cellulase-xylanase complex application. All WSI values were below 8%. This confirms that the breads have both an appropriate internal structure and combination of most components within the protein-starch matrix of the bread dough after baking.

Texture measurements of the bread crumb showed some differences in the main textural properties of the tested breads, with the results from double-compression tests being presented in Table 6. Accordingly, control bread firmness was 15.47 N, springiness was 0.81, chewiness was 5.00 N, and cohesiveness was 0.40. Substitution of bread wheat flour with thermal, hydrothermal, or enzyme-assisted hybrid treated developed flour induced slight changes in the textural properties of the bread crumb. Most of the tested breads showed higher firmness values when thermal and hydrothermal modification of the added flour was applied. Moreover, the addition of untreated F flours increased bread hardness. Furthermore, lower compression forces were noted if enzymes were added to

the developed flour. This outcome may be the effect of enzyme action on the fibrous flour fractions and subsequently on loosening the internal structure of bread crumb fortified with FC and FCX flours. Adhesion, as the work needed to separate the measuring element from the crumb surface, was very low in all the tested breads and differences were insignificant. Similarly, low values and insignificant differences were found for bread springiness.

Table 6. Texture of bread with the addition of modified flours.

Bread Sample	Firmness (N)	Adhesion (mJ)	Springiness (–)	Gumminess (N)	Chewiness (N)	Cohesiveness (-)
K	15.47 ± 1.22 ^{a,b,c,d}	0.09 ± 0.06 ^{a,b}	0.81 ± 0.07 ^{a}	6.19 ± 0.28 ^{a,b,c,d}	5.00 ± 0.61 ^{a}	0.40 ± 0.02 ^{a,b}
F10	20.20 ± 1.59 ^{f,g}	0.04 ± 0.06 ^{a}	0.86 ± 0.01 ^{a}	7.70 ± 0.51 ^{c,d,e}	6.65 ± 0.51 ^{a,b}	0.38 ± 0.01 ^{a,b}
F20	17.47 ± 3.31 ^{c,d,e,f,g}	0.07 ± 0.06 ^{a,b}	0.72 ± 0.14 ^{a}	7.25 ± 1.51 ^{b,c,d,e}	5.18 ± 1.25 ^{a,b}	0.41 ± 0.03 ^{a,b}
FC10	13.67 ± 0.80 ^{a,b,c}	0.08 ± 0.02 ^{a,b}	0.82 ± 0.01 ^{a}	5.44 ± 0.18 ^{a,b}	4.44 ± 0.17 ^{a}	0.40 ± 0.02 ^{a,b}
FC20	13.77 ± 1.67 ^{a,b,c}	0.07 ± 0.01 ^{a,b}	0.86 ± 0.02 ^{a}	5.89 ± 0.30 ^{a,b,c}	5.04 ± 0.35 ^{a}	0.43 ± 0.03 ^{b}
FCX10	14.67 ± 0.15 ^{a,b,c,d,e}	0.13 ± 0.00 ^{a,b}	0.78 ± 0.07 ^{a}	6.15 ± 0.15 ^{a,b,c,d}	4.77 ± 0.49 ^{a}	0.39 ± 0.02 ^{a,b}
FCX20	12.93 ± 1.02 ^{a}	0.08 ± 0.03 ^{a,b}	0.85 ± 0.03 ^{a}	5.25 ± 0.17 ^{a}	4.46 ± 0.14 ^{a}	0.41 ± 0.03 ^{a,b}
TF10	20.97 ± 1.29 ^{g,h}	0.06 ± 0.06 ^{a,b}	0.67 ± 0.10 ^{a}	7.94 ± 0.36 ^{d,e}	5.36 ± 0.97 ^{a,b}	0.38 ± 0.01 ^{a,b}
TF20	17.43 ± 0.86 ^{b,c,d,e,f,g}	0.08 ± 0.05 ^{a,b}	0.69 ± 0.21 ^{a}	6.63 ± 0.48 ^{a,b,c,d}	4.67 ± 1.66 ^{a}	0.38 ± 0.04 ^{a,b}
TFC10	15.53 ± 0.38 ^{a,b}	0.09 ± 0.02 ^{a,b}	0.86 ± 0.01 ^{a}	5.45 ± 0.14 ^{a,b}	4.69 ± 0.11 ^{a}	0.40 ± 0.00 ^{a,b}
TFC20	24.83 ± 0.99 ^{h}	0.09 ± 0.01 ^{a,b}	0.87 ± 0.01 ^{a}	9.02 ± 1.47 ^{e}	7.79 ± 1.26 ^{b}	0.36 ± 0.06 ^{a,b}
TFCX10	19.53 ± 0.46 ^{e,f,g}	0.03 ± 0.02 ^{a}	0.72 ± 0.13 ^{a}	7.04 ± 0.70 ^{a,b,c,d}	5.04 ± 0.76 ^{a}	0.36 ± 0.03 ^{a,b}
TFCX20	15.63 ± 1.70 ^{a,b,c,d,e}	0.20 ± 0.05 ^{b}	0.74 ± 0.06 ^{a}	5.55 ± 0.30 ^{a,b}	4.06 ± 0.31 ^{a}	0.36 ± 0.03 ^{a,b}
HF10	18.07 ± 0.31 ^{d,e,f,g}	0.11 ± 0.03 ^{a,b}	0.70 ± 0.12 ^{a}	6.95 ± 0.48 ^{a,b,c,d}	4.89 ± 1.13 ^{a}	0.39 ± 0.04 ^{a,b}
HF20	16.13 ± 0.12 ^{a,b,c,d,e}	0.09 ± 0.04 ^{a,b}	0.72 ± 0.10 ^{a}	6.44 ± 0.27 ^{a,b,c,d}	4.66 ± 0.82 ^{a}	0.40 ± 0.01 ^{a,b}
HFC10	17.20 ± 0.20 ^{b,c,d,e,f,g}	0.06 ± 0.06 ^{a,b}	0.74 ± 0.17 ^{a}	6.63 ± 0.33 ^{a,b,c,d}	4.94 ± 1.31 ^{a}	0.39 ± 0.03 ^{a,b}
HFC20	18.17 ± 1.40 ^{d,e,f,g}	0.16 ± 0.14 ^{a,b}	0.76 ± 0.07 ^{a}	6.27 ± 0.26 ^{a,b,c,d}	4.78 ± 0.63 ^{a}	0.34 ± 0.02 ^{a}
HFCX10	16.07 ± 0.72 ^{a,b,c,d,e}	0.08 ± 0.05 ^{a,b}	0.75 ± 0.08 ^{a}	6.13 ± 0.32 ^{a,b,c,d}	4.60 ± 0.73 ^{a}	0.38 ± 0.01 ^{a,b}
HFCX20	16.30 ± 1.35 ^{a,b,c,d,e,f}	0.06 ± 0.02 ^{a,b}	0.67 ± 0.08 ^{a}	6.41 ± 0.47 ^{a,b,c,d}	4.29 ± 0.77 ^{a}	0.39 ± 0.02 ^{a,b}

K—control; F—flour; T—dry thermal treatment; H—hydrothermal treatment; C—cellulase enzyme; CX—cellulase-xylanase enzyme complex; 10 and 20—% of modified flour in bread recipe; $^{a–h}$—means indicated with similar letters in columns do not differ significantly at $\alpha = 0.05$.

Chewiness of breads supplemented with F10 and F20, and those which contained unchanged fibrous fractions in the developed flour, was slightly higher than that of the control bread K. For other bread samples, the results did not differ significantly, except for the addition of TFC20. This increased bread chewiness. All baked breads were similar in cohesiveness, which confirms the visual observations of bread crumb presence of a compact internal structure (Figure 2). In general, the addition of modified flours did not significantly deteriorate the texture of the bread, while bread yield, bread volume, and specific volume of prepared breads with the modified developed flour were improved significantly. Zhang et al. [5] found that increasing the substitution level of pulse flours significantly enhanced the starch retrogradation value and resulted in a harder bread texture, as both polysaccharides and protein in the pulse flours have an effect on bread hardness. Tao et al. [46], in turn, found that the hardness of the bread crumb is limited if extruded flour was added; this is because bread containing extruded starch had a softer texture and a more porous crumb structure. The addition of plant gums to bread recipes also significantly reduces the firmness of bread via a clear effect of crumb-softening, as reported by Alamri et al. [53].

3.5. PCA Analysis

From the PCA analysis performed to determine the parameters that have the greatest influence on the variability of the system, eighteen new variables were obtained, of which the first three principal components explain more than 50% of the variability of the system. Indeed, the first two principal components explain 40.16% of the variability of the system (PC1 in 24.95% and PC2 in 15.21%), and the parameters that are contained between the two circles have the greatest influence on the variability of the system. As a result of this procedure, fourteen parameters were determined from the forty parameters examined: C2, C3, C4, C5, L, IDF, TDF, bread yield, W, le, SH, P, P/L, and carbohydrates (Figure 3a). The remaining parameters have a weak influence on the variability of the system. Moreover, C2, C3, C4, C5, W, le, SH, P, and P/L are strongly and positively correlated. The same relationship was observed for IDF, TDF, and bread yield. A strong but negative correlation occurs between parameters C2, C3, C4, C5, W, Ie, SH, P, P/L, and L, while a strong and negative correlation was also determined for IDF, TDF, bread yield, and carbohydrates. There is no correlation between C2, C3, C4, C5, W, Ie, SH, P, P/L, and IDF, TDF, bread yield, and carbohydrates. There is also no correlation between L and IDF, TDF and bread yield, and between L and carbohydrates.

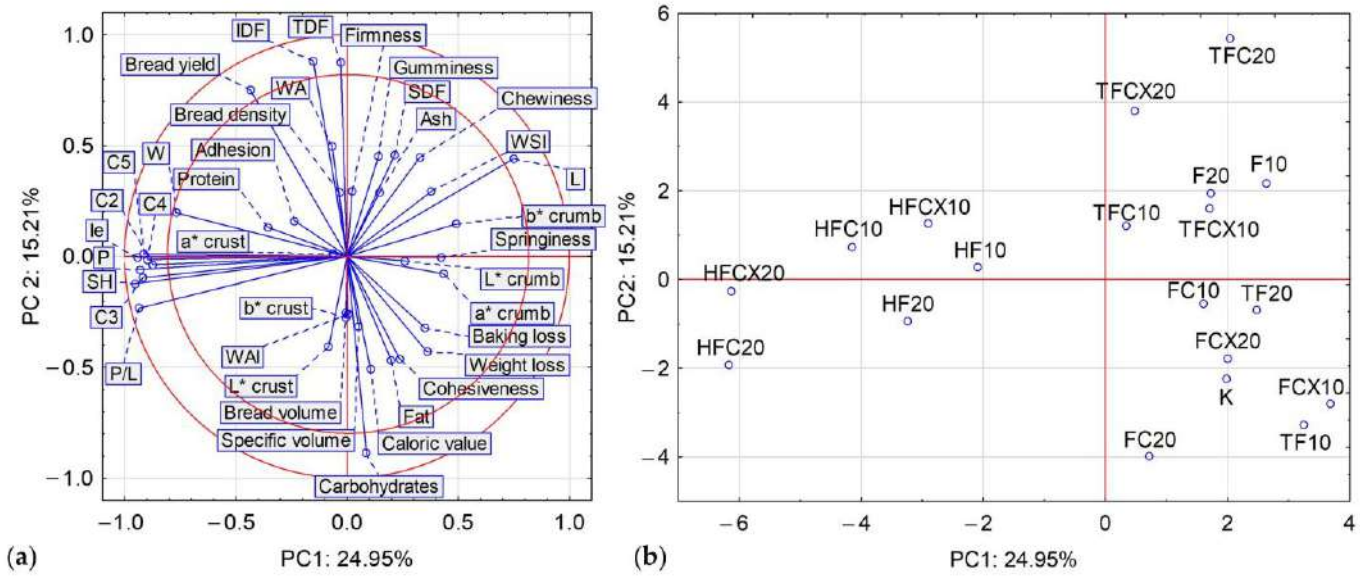

Figure 3. PCA analysis: (**a**) projection of variables all parameters on the PC1 and PC2 loadings plot; (**b**) projection of modified wheat flours on the PC1 and PC2 scores plot.

The PCA analysis also shows (Figure 3a,b) that HF, HFC, and HFCX are strongly and positively correlated with C2, C3, C4, C5, W, Ie, SH, P, and P/L, and strongly and negatively with L. In turn, F10, F20, TFC, and TFCX are strongly and positively correlated with L. In turn, K, FC, TF, and FCX are strongly and positively correlated with carbohydrates. The PCA analysis shows that the first principal component PC1 distinguishes hybrid thermal-enzymatic flour from control flour and thermal-enzymatic flour by 24.95% (Figure 3b). Positive values of the principal component PC1 describe the results of F and T and the control, and negative values of the principal component PC1 describe the results for H. The second principal component PC2 characterizes F, TF, and TFCX (positive values of PC2) and K, FC, and FCX (negative values of PC2) to 15.21%.

The first and third principal components explain 36.99% of the variability of the system (PC1 to 24.95% and PC2 to 15.21%). The parameters that are contained between the two circles have the greatest influence on the variability of the system. As a result of the analysis, twelve parameters were determined from the forty parameters studied: C2, C3, C4, C5, bread yield, W, Ie, SH, P, P/L, specific volume, and bread volume (Figure 4a). The

remaining parameters have a weak influence on the variability of the system. C2, C3, C4, C5, W, Ie, SH, P, and P/L are strongly and positively correlated. The same relationship was observed for specific volume and bread volume. A strong but negative correlation occurs between the parameters: specific volume, bread volume, and bread yield. There is no correlation between C2, C3, C4, C5, W, Ie, SH, P, P/L, and bread yield, specific volume, and bread volume.

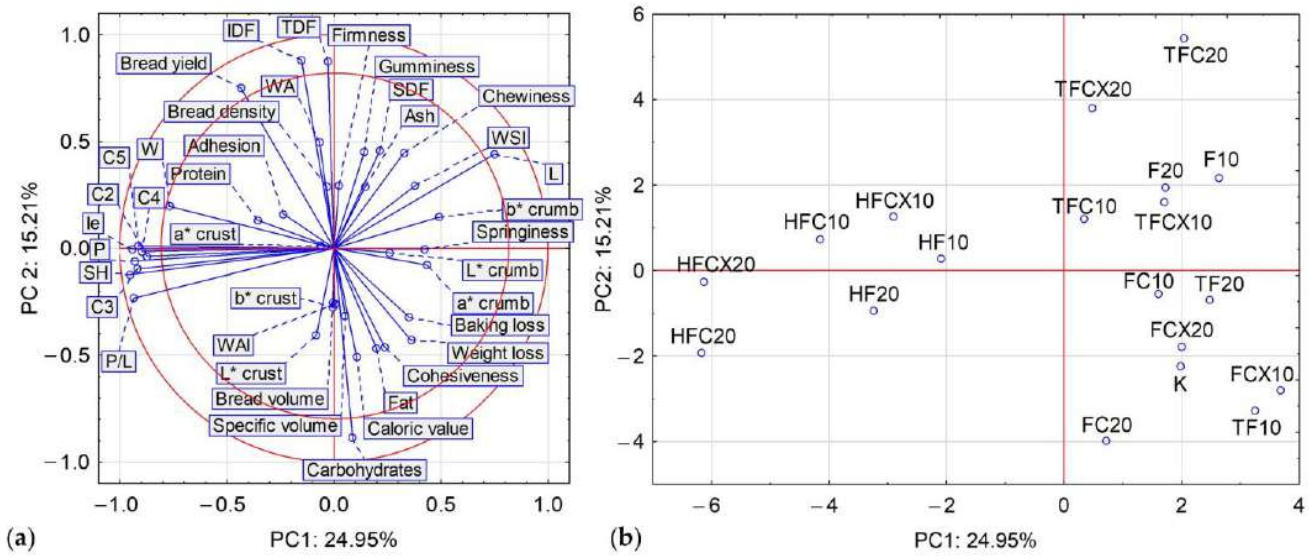

Figure 4. PCA analysis: (**a**) projection of variables parameters on the PC1 and PC3 loadings plot; (**b**) projection of modified wheat flours on the PC1 and PC3 scores plot.

The PCA analysis also shows (Figure 4a,b) that HF, HFC, and HFCX are strongly and positively correlated with C2, C3, C4, C5, W, Ie, SH, P, and P/L. In turn, F10, F20, TFC, and TFCX are strongly and positively correlated with specific volume and bread volume. In turn, K, FC, TF, and FCX are strongly and positively correlated with bread yield.

The PCA analysis shows that the first principal component PC1 distinguishes hybrid thermal-enzymatic flour from flour and thermal-enzymatic flour to 24.95% (Figure 4b). Positive values of the PC1 principal component describe the results of F and T and the control, and negative values of the PC1 principal component describe the results for H. The third principal component PC3 characterizes F20, FC, FCX, and TFCX (negative PC2 values) and K F, TF, and TFC (positive PC2 values) to 12.04%.

4. Conclusions

The presented research has both scientific and practical impact due to it providing the possibility to find technological solutions for reducing the amount of underutilized wheat flour fractions (10% of production in a milling company) by incorporating it into blends that may be applicable for bread making with appropriate properties. Moreover, new knowledge acquired about the characteristics of developed flour modified with thermal, hydrothermal, and enzyme-assisted hybrid treatment and its application in bread is valuable for the baking industry and in providing food for a fast-growing population.

The presented research results confirmed the possibility of substituting standard wheat flours with flours treated via thermal, hydrothermal, or enzyme-assisted hybrid methods in bread recipes for the purpose of improving bread yield in a clean label manner. The investigated modification processes brought about some significant changes in dough rheology when 10 or 20% of modified flours replaced common wheat flour in bread composition. According to our research results, bread dough properties varied depending on modification process and level of added modified flour. Treatment with enzymes allowed us to eliminate the negative aspects of the physical processes. The hydrothermal

treatment, despite the use of an enzyme complex, negatively affected the quality of modified flour, the addition of which caused problems with gas bubble retention in the bread dough during rising and baking. When modified flours were introduced, the protein weakening values increased by 19%. This indicates that the resulting bread product showed enhanced bread dough stiffness. However, dry thermal heated flours without or with enzymes showed significantly decreased amylase activity and starch retrogradation results by 2–11% and 2–13%, respectively, as compared to the control sample or to the hydrothermally modified flours. This indicates that thermal treatment deteriorated the flour parameters, but the addition of enzymes resulted in a dough quality similar to control, with increased bread efficiency related to the higher water absorption of the flour.

Bread properties, in terms of chemical composition, quality features, texture, and color, were slightly different if modified flours were added. Application of thermal treated modified flours to a common wheat bread recipe gave the highest bread yield of 150.02% and increased water absorption of the dough by 6%, especially when the enzyme-assisted method was applied to the developed flour; this was achieved without a negative impact on the texture or color of the obtained breads. It should be noted that the developed flours modified via hydrothermal method or hybrid enzyme-assisted method, when added to bread composition, did not produce a desired dough structure, and the addition resulted in a deteriorated bread crust formation, despite the increasing of bread volume and yield, as compared to control common wheat flour bread. However, all types of additives lowered baking loss and weight loss of the produced bread by 8% as compared to control sample. The resulting doughs with added thermally modified flour may be suitable for processing, mixing, and fermentation without deterioration of the quality of the bread and with improved bread volume (16%).

The presented results can be readily implemented in practice by milling companies as a solution for reducing the amount of underutilized fractions. Moreover, the composition of the new flour blends with the added developed modified flour (with its specific properties) may be offered as a ready-to-use bakery blend with a clean label as a bread improver. As a limitation in the use of the obtained modified flours, it should be noted that the addition of more than 20% of the total blend would not be profitable for bread producers as such amounts lower the final product quality. Future research, however, will be focused on production optimization, as well as exploring the possibilities of incorporating other flours into bread-making and evaluating the effects of the aforementioned additions on dough and bread quality. Moreover, additional tests will be carried out using other baking enzymes (e.g., bacterial xylanase) that could modify the processed flours more effectively.

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