UNIWERSYTET PRZYRODNICZY W LUBLINIE
WYDZIAŁ NAUK O ŻYWNOŚCI I BIOTECHNOLOGII

mgr inż. Iwona Niedźwiedź

Wykorzystanie zimnej plazmy w konserwacji win czerwonych

The application of cold plasma in the preservation of red wines

Rozprawa doktorska

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Lublin 2022

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WYKAZ PUBLIKACJI NAUKOWYCH STANOWIĄCYCH PRZEDMIOT ROZPRAWY DOKTORSKIEJ

PUBLIKACJA P1

Niedźwiedź I., Waśko A., Pawłat J., Polak-Berecka M. 2019. The state of research on antimicrobial activity of cold plasma. Polish Journal of Microbiology, 68(2), 153.

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PUBLIKACJA P2

Niedźwiedź I., Juzwa W., Skrzypiec K., Skrzypek T., Waśko A., Kwiatkowski M., Pawłat J., Polak-Berecka M. 2020. Morphological and physiological changes in *Lentilactobacillus hilgardii* cells after cold plasma treatment. Scientific Reports, 10(1), 1-14.

MNiSW	IF	UDZIAŁ PROCENTOWY	ILOŚĆ CYTOWAŃ
140	4,379	60%	3

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PUBLIKACJA P3

Niedźwiedź I., Płotka-Wasylka J., Kapusta I., Simeonov V., Stój A., Waśko A., Pawłat J., Polak-Berecka M. 2022. The impact of cold plasma on the phenolic composition and biogenic amine content of red wine. Food Chemistry, 381, 132257.

MNiSW	IF	UDZIAŁ PROCENTOWY	ILOŚĆ CYTOWAŃ
200	9,231	60%	2

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PUBLIKACJA P4

Niedźwiedź I., Simeonov V., Waśko A., Polak-Berecka M. 2022. Comparison of the effect of cold plasma with conventional preservation methods on red wine quality using chemometrics analysis. Molecules, 27(20), 7048.

MNiSW	IF	UDZIAŁ PROCENTOWY	ILOŚĆ CYTOWAŃ
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STRESZCZENIE

Świadomość społeczeństwa na temat zdrowej żywności wzrasta. Powoduje to duże oczekiwania względem producentów żywności, którzy muszą zapewnić na rynku produkty bezpieczne dla zdrowia oraz w jak najmniejszym stopniu przetworzone. W tym celu prowadzone są procesy utrwalania żywności, których głównym zadaniem jest ochrona przed zepsuciem i skażeniem mikrobiologicznym, ograniczenie niepożądanych zmian sensorycznych oraz zachowanie wysokiej wartości odżywczej. Chociaż klasyczne termiczne metody konserwacji żywności wciąż są najczęściej wykorzystywane, to wysoka temperatura procesu ogranicza ich stosowanie w niektórych gałęziach przemysłu.

Wino to tradycyjny napój alkoholowy, którego kultura produkcji i konsumpcji widoczna jest na całym świecie od wieków. Ze względu na zawartość związków bioaktywnych należących głównie do grupy polifenoli, przypisuje się mu liczne właściwości prozdrowotne. Aby zapewnić trwałość i bezpieczeństwo mikrobiologiczne wina do jego konserwacji powszechnie wykorzystuje się siarczyny. Związki te jednak wywierają negatywny wpływ na zdrowie konsumenta, co potwierdzają badania naukowe. Przyczyniło się to do wzmożonych poszukiwań alternatywnych metod konserwacji wina. Szczególną uwagę poświęca się nietermicznym metodom, które oprócz eliminacji niepożądanych mikroorganizmów, nie wpływałyby znacząco na stabilność końcową produktu. Zimna plazma (CP) ze względu na swoje zalety, do których zaliczyć możemy szerokie działanie przeciwdrobnoustrojowe, niską temperaturę prowadzenia procesu, krótki czas sterylizacji, a także stosunkowo niski koszt staje się obiecującym rozwiązaniem w aspekcie konserwacji napojów alkoholowych.

Głównym celem badań przeprowadzonych w ramach niniejszej rozprawy doktorskiej była ocena możliwości wykorzystania zimnej plazmy w konserwacji czerwonego wina zrealizowana poprzez (i) ustalenie mechanizmów działania CP na komórki drobnoustrojów zanieczyszczających wina, uwzględniając charakterystykę zachodzących zmian fizjologicznych i morfologicznych komórek *L. hilgardii* (ii) poznanie oddziaływań CP na właściwości fizykochemiczne i aktywność biologiczną czerwonego wina. (iii) porównanie efektów działania CP z metodami konserwacji

wykorzystującymi dodatek pirosiarczynu potasu na jakość czerwonego wina z uwzględnieniem okresu przechowywania.

Stan fizjologiczny bakterii *L. hilgardii* po działaniu CP oceniono poprzez badanie przeżywalności komórek za pomocą klasycznej metody hodowlanej, pomiarów gęstości optycznej OD_{600} oraz z wykorzystaniem cytometrii przepływowej. Do określenia związku między aktywnością metaboliczną a morfologią komórki zastosowano skaningową mikroskopię elektronową (SEM), mikroskopię sił atomowych (AFM), oraz spektroskopię w podczerwieni z transformacją Fouriera (FTIR). Połączenie metody AFM z cytometrią przepływową pozwoliło na analizę zmian po działaniu CP w poszczególnych subpopulacjach komórek, co jest podejściem innowacyjnym, dotychczas nie opisanym w literaturze międzynarodowej. Po raz pierwszy także, wpływ CP na właściwości fizykochemiczne i aktywność biologiczną czerwonego wina porównano z metodą tradycyjną i łączoną uwzględniając trzymiesięczny okres przechowywania. Przeprowadzono również jakościowe i ilościowe oznaczenie profilu związków fenolowych oraz amin biogennych metodą UPLC-PDA-MS/MS oraz GC-MS. Analiza chemometryczna została wykorzystana w celu opisania zależności pomiędzy zastosowaną metodą konserwacji a poszczególnymi badanymi parametrami świadczącymi o jakości wina.

W pracy wykazano, że CP opóźnia wzrost i podziały bakterii *L. hilgardii*, a wydłużenie czasu jej działania prowadzi do pełnej eliminacji ich ze środowiska. Ważnym wynikiem badań podjętych w dysertacji było stwierdzenie odzyskiwania żywotności przez komórki bakterii znajdujące się w stanie anabiozy. Ponowna hodowla wydzielonej subpopulacji potwierdziła, że przynajmniej część komórek znajdowało się w stanie VBNC (ang. viable but nonculturable). Ponadto, z wykorzystaniem techniki AFM potwierdzono zmianę morfologii komórek subpopulacji pośredniej, która może wskazywać na adaptację bakterii do niekorzystnych warunków środowiska. Odnotowano także liczne zmiany struktury powierzchni komórek martwych, charakteryzujące się wzrostem chropowatości i elastyczności, mogące wynikać z działania na ścianę komórkową bakterii reaktywnych związków tlenu zawartych w strumieniu plazmy.

Wykazano także, że wpływ CP na zawartość związków fenolowych uzależniony był od warunków prowadzenia procesu oraz budowy chemicznej konkretnego związku

reagującego ze składnikami plazmy. W badanych próbkach wina po 3–miesięcznym okresie przechowywania stwierdzono redukcję ogólnej zawartości związków fenolowych oraz obniżenie aktywności przeciwutleniającej niezależnie od zastosowanej metody konserwacji. Efekt ten był najłagodniejszy dla konserwacji metodą tradycyjną oraz CP (5 min; He/N_2). W przeprowadzonych badaniach po raz pierwszy wskazano możliwość wykorzystania zimnej plazmy do redukcji amin biogennych w czerwonym winie. Pozytywnie należy ocenić fakt, że konserwacja wina CP nie wpłynęła na pH oraz barwę w sposób, który mógłby być dostrzegalny dla konsumenta. Z wykorzystaniem niehierarchicznej analizy skupień wykazano przyporządkowanie konserwacji metodą CP do klastra z próbkami kontrolnym. Skupienie to charakteryzowało się pośrednimi wartościami poszczególnych parametrów jakościowych wina. Wykorzystując analizę chemometryczną w pracy po raz pierwszy wykazano, że istnieją specyficzne zależności między zastosowaną metodą konserwacji a poszczególnymi parametrami świadczącymi o jakości wina tj. barwa, właściwości przeciwutleniające, zawartość związków fenolowych i amin biogennych oraz czystość mikrobiologiczna.

Wyniki przeprowadzonych analiz dowodzą, że dobór odpowiednich warunków plazmowania może w przyszłości spowodować, iż CP stanie się alternatywną metodą konserwacji wina.

SUMMARY

Public awareness of healthy food is increasing. This creates high expectations for food producers, who must provide the market with products that are safe for health and as little processed as possible. To this end, food preservation processes are carried out, the main aim of which is to protect against spoilage and microbiological contamination, to reduce undesirable sensory changes and to preserve high nutritional value. Although classical thermal food preservation methods are still the most widely used, the high process temperature limits their use in some industries.

Wine is a traditional alcoholic beverage whose culture of production and consumption has been evident throughout the world for centuries. Due to its content of bioactive compounds belonging mainly to the polyphenol group, it is attributed with numerous health-promoting properties. In order to ensure the shelf-life and microbiological safety of wine, sulphites are commonly used for its preservation. However, these compounds have a negative impact on consumer health, as confirmed by scientific studies. This has contributed to an increased search for alternative wine preservation methods. Particular attention is being paid to non-thermal methods that, in addition to eliminating unwanted microorganisms, would not significantly affect the final stability of the product. Cold plasma (CP) is becoming a promising solution for the preservation of alcoholic beverages due to its advantages, which include a broad antimicrobial effect, low process temperature, short sterilisation time and relatively low cost.

The main objective of the research carried out within the framework of this dissertation was to evaluate the possibility of using cold plasma in the preservation of red wine realised by (i) determining the mechanisms of action of CP on the cells of wine-contaminating microorganisms, taking into account the characteristics of the physiological and morphological changes occurring in *L. hilgardii* cells (ii) finding out the effects of CP on the physicochemical properties and biological activity of red wine. (iii) to compare the effects of CP with preservation methods using the addition of potassium metabisulfite on the quality of red wine, taking into account the storage period.

The physiological state of the bacterium *L. hilgardii* after CP treatment was assessed by examining cell survival using the classical culture method, OD_{600} optical

density measurements and using flow cytometry. Scanning electron microscopy (SEM), atomic force microscopy (AFM), and Fourier transform infrared spectroscopy (FTIR) were used to determine the relationship between metabolic activity and cell morphology. The combination of AFM and flow cytometry allowed the analysis of post-CP treatment changes in individual cell subpopulations, an innovative approach not previously reported in the international literature. Also, for the first time, the effect of CP on the physicochemical properties and biological activity of red wine was compared with the traditional and combined method, taking into account a three-month storage period. Qualitative and quantitative determination of the profile of phenolic compounds and biogenic amines by UPLC-PDA-MS/MS and GC-MS was also carried out. A chemometric analysis was used to describe the relationship between the preservation method used and the individual wine quality parameters studied.

In this study, CP was shown to retard the growth and division of *L. hilgardii*, while prolonging its duration of action leads to their complete elimination from the environment. An important result of the research undertaken in the dissertation was the finding of recovery of viability by bacterial cells in a state of anabiosis. Re-culture of the isolated subpopulation confirmed that at least some of the cells were in the VBNC (viable but nonculturable) state. In addition, a change in cell morphology of the intermediate subpopulation was confirmed using AFM, which may indicate adaptation of the bacteria to adverse environmental conditions. Numerous changes in the surface structure of the dead cells, characterised by an increase in roughness and elasticity, were also noted, possibly due to the action of reactive oxygen compounds contained in the plasma stream on the bacterial cell wall.

It was also shown that the effect of CP on the content of phenolic compounds depended on the process conditions and the chemical structure of the specific compound reacting with the plasma components. In the wine samples studied, a reduction in the total phenolic compound content and a decrease in antioxidant activity were found after a 3-month storage period, regardless of the preservation method used. This effect was mildest for preservation by the traditional method and CP (5 min; He/N_2). The study indicated for the first time the possibility of using cold plasma to reduce biogenic amines in red wine. On a positive note, CP wine preservation did not affect pH and colour in a way that could be perceived by the consumer. Using non-hierarchical cluster analysis, the allocation of CP preservation to a cluster with control samples was shown.

This cluster was characterised by intermediate values of individual wine quality parameters. Using chemometric analysis, the study showed for the first time that there are specific relationships between the preservation method used and individual parameters indicative of wine quality, i.e., colour, antioxidant properties, content of phenolic compounds and biogenic amines, and microbiological purity. The results of the analyses indicate that the selection of appropriate conditions for the plasma process could make CP an alternative method of wine preservation in the future.

1. WPROWADZENIE

Pierwsza opublikowana praca **(P1)** wchodząca w skład mojej dysertacji jest przeglądem piśmiennictwa opisującym mechanizmy działania zimnej plazmy na komórki drobnoustrojów oraz cząstki wirusów. W publikacjach badawczych **P2, P3** oraz **P4** scharakteryzowano zimną plazmę jako nową nietermiczną metodę utrwalania żywności w kontekście konserwacji czerwonego wina. Dodatkowo w monografii nie będącej częścią cyklu publikacyjnego wchodzącego do rozprawy doktorskiej pt. „**Innovative technologies in sustainable food production: cold plasma processing. [AUT.] MAGDALENA POLAK-BERECKA, IWONA NIEDŹWIEDŹ. W: Sustainable production technology in food / edited by José M. Lorenzo, Paulo E.S. Munekata, Francisco J. Barba Amsterdam 2021, Academic Press, s. 165-177,** omówiono rolę i znaczenie zimnej plazmy w zrównoważonej produkcji żywności. Niniejszy rozdział jest podsumowaniem zebranych informacji, które stanowiły podstawę do podjęcia tematyki badawczej, będącej przedmiotem mojej rozprawy doktorskiej.

Wino jest napojem alkoholowym, którego tradycja wytwarzania i spożywania znana jest na świecie od wieków (Gajek i in. 2021). Postępujące w ostatnich latach zmiany klimatyczne przyczyniły się do zwiększenia uprawy winorośli i produkcji wina również w chłodniejszych regionach Europy Środkowo-Wschodniej, w tym także w Polsce (Koźmiński i in. 2020). Dostępna na rynku bogata oferta tych produktów sprawia, że klienci przywiązują coraz większą wagę do jakości wypijanego trunku, zwłaszcza jego cech fizyko-chemicznych i organoleptycznych (Dumitriu i in. 2021). Coraz większą uwagę przywiązuje się także do prozdrowotnych właściwości czerwonego wina, wynikających z bogatej zawartości polifenoli, które charakteryzują się aktywnością przeciwutleniającą (Borges i in. 2013; Gris i in. 2013). Wiele badań epidemiologicznych wskazuje, że umiarkowane spożycie czerwonego wina korzystnie wpływa na układ nerwowy i sercowo-naczyniowy (Snopek i in. 2018), chroni przed cukrzycą (Rasines-Perea i in. 2017) oraz ma działanie prewencyjne w stosunku do niektórych nowotworów (Haunschild i Marx, 2022).

Ze względu na niskie pH, obecność alkoholu oraz substancji o aktywności przeciwbakteryjnej, wino uważane jest za środowisko niekorzystne dla rozwoju mikroflory. Jednak niektóre drobnoustroje wykształciły szereg mechanizmów

adaptacyjnych, pozwalających im przetrwać w tych warunkach. Do tej grupy należą przede wszystkim drożdże *Brettanomyces*, bakterie kwasu mlekowego (LAB – lactic acid bacteria) oraz bakterie kwasu octowego (Niu i in. 2019; Puligundla i in. 2018). LAB stanowią dominującą grupę mikroorganizmów występującą w moszczu winogronowym oraz winie. Szczegółowe badania przeprowadzone przez Rodas i wsp. (2005) wykazały, że większość izolatów należy do gatunku *Lentilactobacillus hilgardii* (*L. hilgardii*). Jednym z głównych zagrożeń wynikających z metabolizmu bakterii mlekowych jest synteza karbaminianu etylu, który ma właściwości rakotwórcze. Ponadto LAB są głównymi producentami amin biogennych (BAs – biogenic amins), takich jak histamina (HIS), tyramina (TYR), fenyloetyloamina (2-PE) i spermidyna (SPD). Aminy biogenne są odpowiedzialne za obniżenie jakości sensorycznej wina, ale także ich wysokie stężenie może wywoływać niepożądane efekty fizjologiczne u konsumenta objawiające się bólem głowy, nudnościami czy tachykardią (Puligundla i in. 2018).

Aby zapobiec rozwojowi niepożądanej mikroflory i przedłużyć okres przydatności produktu do spożycia przeprowadza się proces konserwacji. Chociaż klasyczne termiczne metody utrwalania produktów spożywczych nadal odgrywają bardzo ważną rolę w technologii żywności, to nie są one odpowiednie dla procesów winifikacji (Niu i wsp. 2019). Dlatego w produkcji wina powszechnie stosowanym konserwantem jest dwutlenek siarki (SO_2), dodawany zazwyczaj w postaci pirosiarczynu potasu ($K_2S_2O_5$), który wykazuje właściwości przeciwbakteryjne i przeciwutleniające oraz zapobiega nieenzymatycznemu utlenianiu win (Casquete i in. 2021). Z drugiej strony, związek ten może mieć negatywny wpływ na jakość wina, powodując pogorszenie właściwości sensorycznych produktu, a także negatywnie oddziaływać na zdrowie konsumentów, wywołując u nich bóle głowy, zapalenie skóry czy biegunkę (Pozo-Bayón i in. 2012). Dlatego Światowa Organizacja Zdrowia (WHO) wprowadziła przepisy określające dopuszczalną dawkę SO_2 ograniczając tym samym jego stosowanie. Przyczyniło się to do wzmożonych poszukiwań nowych strategii, które pozwoliłyby na zmniejszenie stężenia lub nawet zastąpienie siarczynów w procesie konserwacji wina (Cordero-Bueso i in. 2019). Mając to na uwadze, badacze są obecnie szczególnie zainteresowani nietermicznymi metodami konserwacji żywności, które pozwolą na usunięcie niepożądanych mikroorganizmów z matrycy żywności bez znaczącego wpływu na końcową stabilność produktu (Puligundla i in. 2018). Do takich

metod należy zimna plazma (CP – cold plasma), której popularność stale wzrasta, o czym świadczy liczba opracowań naukowych w tej tematyce z ostatniej dekady.

1.1 Charakterystyka zimnej plazmy

Termin „plazma" został wprowadzony do nauki w 1927 roku przez amerykańskiego fizykochemika Irvinga Langmuira, który użył tej nazwy do opisu zjonizowanego gazu o unikatowych właściwościach. Zimna plazma uważana za czwarty stan materii jest rodzajem zjonizowanego bądź częściowo zjonizowanego gazu, który zawiera wiele naładowanych cząstek, atomów w stanie wzbudzonym i podstawowym, reaktywnych związków oraz fotonów UV (Brisset i in. 2016). Ze względu na udowodnione działanie sterylizujące CP znalazła zastosowanie w wielu branżach m.in. w medycynie (Metelmann i in. 2018), przemyśle spożywczym (Hojnik i in. 2017), rolnictwie oraz ochronie środowiska (Polčic i in. 2018). Szerokie spektrum wykorzystania zimnej plazmy wiąże się z jej zdolnością do inaktywacji czynników biologicznych, takich jak: wirusy, bakterie, spory, drożdże czy grzyby (Bourke i in. 2017). W zależności od warunków generowania plazmy, można ją sklasyfikować ze względu na: ciśnienie atmosferyczne (plazma niskociśnieniowa, wysokociśnieniowa), temperaturę (plazma niskotemperaturowa, wysokotemperaturowa) oraz skład gazu plazmotwórczego (plazma jednoskładnikowa, wieloskładnikowa) (Bourke i in. 2017; Liao i in. 2017). W inaktywacji czynników biologicznych wykorzystuje się plazmę nietermiczną, która charakteryzuję się temperaturą elektronów (T_e) = 10^4~10^5 K, oraz brakiem równowagi termodynamicznej. Oznacza to, że elektrony posiadają wyższą temperaturę niż temperatura cząstek neutralnych ($T_e \geq T_n$), co powoduje, że temperatura samego procesu wzrasta nieznacznie (Laskowska i in. 2016). Temperatura plazmy jest niezwykle istotna przy obróbce materiałów termolabilnych - nieodpornych na działanie wysokich temperatur. Plazmę nierównowagową otrzymuje się poprzez zastosowanie różnorodnych wyładowań elektrycznych tj.; koronowe, mikrofalowe, jarzeniowe oraz wyładowania barierowe. Typ zastosowanego źródła plazmy bezpośrednio wpływa na skład i liczebność generowanych przez nią komponentów, a co za tym idzie determinuje jej późniejsze wykorzystanie technologiczne (Wang i in. 2019). W zależności od rodzaju użytego gazu składniki plazmy będą odmienne. W plazmie, której gazem roboczym jest tlen lub azot bądź mieszanina tych gazów, powstają reaktywne formy tlenu (ROS – reactive oxygen species) i reaktywne formy azotu (RNS – reactive nitrogen species). W przypadku wykorzystania CP w rolnictwie, przemyśle

spożywczym oraz medycynie, wyładowania barierowe i dżety plazmowe są najczęściej wykorzystywane do generowania zimnej plazmy. Wynika to z ich prostej konstrukcji oraz łatwości modyfikacji (Wiktor i in. 2013).

1.2. Mechanizm działania zimnej plazmy

Pomimo iż w literaturze międzynarodowej jest wiele doniesień naukowych wskazujących na wysoką skuteczność zimnej plazmy w eliminacji niepożądanych mikroorganizmów, to sam mechanizm tego procesu wciąż nie jest w pełni poznany (Liao i in. 2017). Kluczowe w inaktywacji biologicznej jest działanie reaktywnych związków, wysokoenergetycznych elektronów, zjonizowanych atomów i cząstek oraz promieniowania UV (Bourke i in. 2017). W kontakcie z materiałem biologicznym wysoką reaktywnością charakteryzują sią związki pochodzące z tlenu bądź azotu; O, O_2, O_3, OH, NO, i NO_2. Ich działanie powoduje utlenianie lipidów i białek błony, zakłócając tym samym jej prawidłowe funkcjonowanie, a powstałe uszkodzenia prowadzą do przerwania ciągłości błony komórkowej (Afshari i Hosseini, 2014). Na integralność błon, znaczący wpływ wywierają również siły elektrostatyczne. Wytworzone przez plazmę naładowane cząstki, gromadzą się po zewnętrznej stronie membrany doprowadzając do jej dezintegracji (Maciejewska i in. 2016). Przerwanie ciągłości struktur powierzchniowych komórek jest również efektem procesu elektroporacji. Zjawisko to polega na wzroście istniejących oraz powstaniu nowych mikroporów komórkowych pod wpływem pulsacyjnego pola elektryczego (Moreau i in. 2008). Pierwsze badania sugerowały, że za inaktywację mikroorganizmów odpowiada degradacja DNA wywołana działaniem promieniowania UV. Obecne w plazmie fotony mogą powodować zmiany w strukturze materiału genetycznego, prowadząc do powstania dimerów zasad azotowych i uszkadzając zdolność do replikacji DNA (Maciejewska i in. 2016). Udział każdego z wyżej wymienionych mechanizmów oraz ich skuteczność w inaktywacji biologicznej jest różny i zależy przede wszystkim od parametrów procesu generowania plazmy, czynników środowiskowych oraz rodzaju i właściwości drobnoustrojów. Nieliczne doniesienia naukowe wskazują także na możliwość indukowania przez zimną plazmę stanu VBNC (ang. viable but nonculturable) niektórych bakterii (Dolezalova i Lukes, 2015). Komórki w tym stanie charakteryzują się niską aktywnością metaboliczną, nie rosną na standardowych stałych podłożach hodowlanych i nie są w stanie się replikować, a tym samym są bardzo trudne

do wykrycia z zastosowaniem standardowych metod oceny czystości mikrobiologicznej produktów spożywczych (Schottroff i in. 2018).

1.3. Zastosowanie zimnej plazmy w produkcji żywności

Zimna plazma ze względu na szeroko udowodnione działanie inaktywacyjne w stosunku do różnych grup mikroorganizmów jest interesującą metodą w kontekście produkcji żywności (Pan i in. 2019). W literaturze przedmiotu dobrze opisano i potwierdzono sterylizujące działanie CP w stosunku do niepożądanych mikroorganizmów zasiedlających odmienne matryce żywnościowe takie jak powierzchnia warzyw (Jahid i in. 2014), soki owocowe (Shi i in. 2011) czy produkty pochodzenia zwierzęcego (Jayasena i in.2015). Poziom redukcji drobnoustrojów zależny jest od specyficznych właściwości produktu poddawanego procesowi plazmowania, ale także od warunków prowadzenia procesu (Jahid i in. 2014). Poza eliminacją niepożądanej mikroflory istotne jest również by wykorzystywane metody utrwalania nie wywierały negatywnego wpływu na właściwości fizykochemiczne i odżywcze produktu spożywczego (Ekezie i in. 2017). Reaktywne związki obecne w strumieniu plazmy oprócz efektu, jaki wywierają na komórki drobnoustrojów, mogą również wpływać na samą naturę produktu, zmieniając niektóre jego cechy t.j. barwę, teksturę czy zawartość związków bioaktywnych.

Dostępne dane literaturowe wskazują, że technologia zimnej plazmy w produkcji żywności może zostać wykorzystana w sektorze rolnictwa zrównoważonego. Udowodniono, że jej zastosowanie zwiększa oporność eksponowanych roślin na patogeny grzybicze, redukując tym samym wykorzystanie chemicznych środków ochrony roślin, które wywierają negatywny wpływ zarówno na zdrowie konsumentów jak również na środowisko. Dodatkowo CP powoduję redukcję niepożądanych mikroorganizmów i owadów w materiale siewnym (El-Aziz i in. 2014) oraz przyczynia się do poprawy kiełkowania nasion zwiększając szybkość i wydajność tego procesu (Tong i in. 2014; Ling i in. 2014). Podniesienie wydajności kiełkowania jest korzystne w sektorze przemysłu słodowniczego czy piwowarskiego, ponieważ skutkuje zdecydowanym zmniejszeniem zużycia energii. Zimna plazma może zostać także wykorzystana do degradacji mykotoksyn grzybiczych, które jak wskazują dane FAO zanieczyszczają aż ¼ światowych upraw. Mykotoksyny toksyczne dla zwierząt

i ludzi, charakteryzują się dużą odpornością na działanie wysokich temperatur tym samym ich eliminacja jest trudna (Hojnik i in. 2017).

Technologia zimnej plazmy staje się obiecującym rozwiązaniem, które w przyszłości może zastąpić konwencjonalne metody dekontaminacji produktów spożywczych, materiałów medycznych jak również powietrza. Technika ta posiada wiele zalet, do których zaliczyć możemy wysoką skuteczność redukcji miana cząstek wirusów oraz liczby mikroorganizmów, tworzenie nietoksycznych produktów ubocznych, jak również stosunkowo niski koszt procesu. Ważnym aspektem, dotychczas nie opisanym w literaturze przedmiotu są reakcje ochronne mikroorganizmów indukowane działaniem plazmy. Doniesienia naukowe na temat możliwości wprowadzania komórek niektórych bakterii w stan VBNC wzbudzają wątpliwości co do pełnego bezpieczeństwa tej metody sterylizacji. Dodatkowo kluczowe jest również poznanie efektów, jakie może wywierać ta technika na właściwości fizykochemiczne, a także zawartość i przemiany związków aktywnych biologicznie w produkcie.

Dotychczas w literaturze przedmiotu brak jest doniesień badających działanie CP na komórki w układzie modelowym bakterii zanieczyszczających wina oraz oceniających przydatność tej metody w konserwacji czerwonego wina w aspekcie cech fizykochemicznych, technologicznych i wartości odżywczych w porównaniu z metodami konwencjonalnymi. W oparciu o powyższe informacje, celowe wydaje się podjęcie badań nad wykorzystaniem zimnej plazmy w konserwacji win czerwonych.

2. HIPOTEZY BADAWCZE I CEL PRACY

HIPOTEZA GŁÓWNA

Zimna plazma może być wykorzystywana jako alternatywna metoda konserwacji czerwonego wina.

HIPOTEZY SZCZEGÓŁOWE

1. Zimna plazma działa antybakteryjnie względem *Lentilactobacillus hilgardii* oraz może wpływać na morfologię i fizjologię komórek indukując u nich stan VBNC.
2. Zimna plazma może wpływać w sposób jakościowy i ilościowy na zawartość związków bioaktywnych w czerwonym winie oraz na jego właściwości fizykochemiczne.
3. Efektem zastosowania zimnej plazmy jako metody konserwacji czerwonego wina może być poprawa jakości produktu w porównaniu z zastosowaniem metody tradycyjnej wykorzystującej pirosiarczyn potasu.

CEL PRACY

Celem pracy była ocena możliwości zastosowania zimnej plazmy jako alternatywnej metody konserwacji czerwonego wina.

W celu weryfikacji hipotez założono szczegółowe cele badawcze:

1. Charakterystyka przeżywalności oraz zmian fizjologicznych i morfologicznych komórek *L. hilgardii* poddanych działaniu zimnej plazmy.
2. Określenie wpływu zimnej plazmy na profil związków fenolowych oraz zawartość amin biogennych.
3. Poznanie działania CP na aktywność biologiczną i właściwości fizykochemiczne czerwonego wina.
4. Zbadanie efektu działania CP na jakość czerwonego wina.
5. Porównanie cech technologicznych i fizykochemicznych próbek wina poddanych konserwacji CP oraz metodą tradycyjną z pirosiarczynem potasu i metodą łączoną (zimna plazma + niskie stężenia $K_2S_2O_5$), z uwzględnieniem okresu przechowywania.

3. MATERIAŁY I METODY BADAWCZE

3.1. Materiały i metody badawcze zastosowane w publikacji P2

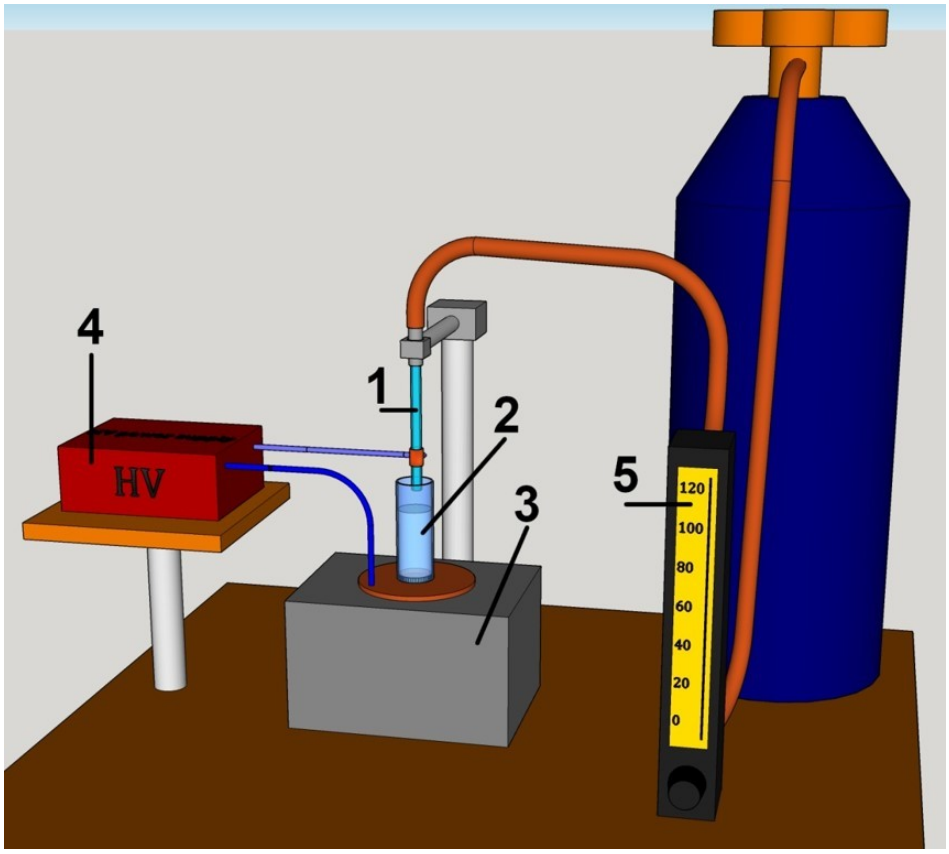
MATERIAŁ BADAWCZY

Materiał badawczy stanowił szczep kolekcyjny *Lentilactobacillus hilgardii* (*L. hilgardii*; NRRL B-1843, Agricultural Research Service Culture Collection, USA) wyizolowany z wina. Wybór powyższego modelu eksperymentalnego podyktowany był tym, iż jak wskazuje literatura, bakterie te charakteryzują się wysoką odpornością na niekorzystne warunki środowiska w jakim bytują np. mogą przetrwać w środowisku zawierającym nawet do 20% alkoholu i są dominującymi mikroorganizmami stanowiącymi zanieczyszczenia w winie.

SYSTEM PLAZMOWANIA

Doświadczenie rozpoczęto od badań wstępnych, które miały na celu dobór najskuteczniejszych warunków prowadzenia procesu. Wpływ typu zastosowanego wyładowania elektrycznego, rodzaj gazu plazmotwórczego oraz czas trwania procesu na efektywność sterylizującą oceniono poprzez badanie stopnia przeżywalności komórek metodą płytkową. Na podstawie uzyskanych wyników do oceny stanu fizjologicznego i zmian morfologicznych komórek *L. hilgardii* pod wpływem zimnej plazmy został wybrany system plazmowy, wykorzystujący generator wyładowań dielektrycznych (DBD - dielectric barrier discharge) oraz gaz roboczy będący mieszaniną helu i tlenu. Z kolei czas trwania procesu ustalono na 5, 10 i 15 min. a wyniki odnoszono do próbek kontrolnych nie poddanych działaniu CP. Generowana w reaktorze plazma pod ciśnieniem atmosferycznym miała postać strumienia wychodzącego z ceramicznej rury o średnicy wewnętrznej i zewnętrznej odpowiednio 1,4 mm i 3,4 mm. W odległości 10 mm od końca rury znajdowała się wysokonapięciowa elektroda pierścieniowa. Uziemiona elektroda płaska wykonana z miedzianego laminatu PCB umieszczona była na mieszadle magnetycznym. Ciekłą próbkę o objętości 6 mL umieszczono w szklanej probówce o średnicy wewnętrznej 20 mm, bezpośrednio w obszarze wyładowań pomiędzy dwiema elektrodami. Odległość między końcem reaktora a powierzchnią cieczy wynosiła 2 mm. Próbki były mieszane podczas ekspozycji na zimną plazmę za pomocą mieszadła z PTFE Natężenie przepływu mieszaniny gazów 96 L/h helu z 1,8 L/h tlenu było regulowane przez regulatory przepływu gazów. Reaktor był

zasilany napięciem RMS 5,1 kV o częstotliwości 21,43 kHz i średniej mocy 6 W. Schemat systemu plazmowego przedstawiono na **Rycinie 1**.



Rycina 1. Układ doświadczalny: 1- reaktor strumienia plazmy; 2 - próbka w szklanym pojemniku; 3 - mieszadło magnetyczne; 4 - zasilacz wysokiego napięcia; 5 - regulator przepływu gazu (Publikacja **P2**).

Poniżej wymieniono skrócony opis wykorzystanych metod badawczych w publikacji P2:

1. Badanie przeżywalności komórek przeprowadzono z wykorzystaniem klasycznej metody hodowlanej na płytkach Petriego oraz pomiaru spektrofotometrycznego gęstości optycznej OD_{600} mikrohodowli z wykorzystaniem systemu Bioscreen C (Labsystem, Helsinki, Finlandia).
2. Aktywność metaboliczną i żywotność komórek *L. hilgardii* badano przy użyciu zestawu BacLight Redox Sensor Green Kit (Life Technologies, Carlsbad, CA, USA) oraz cytometru przepływowego BD FACS Aria III (Becton Dickinson, San Jose, CA, USA).

3. Resuscytację komórek przeprowadzono według procedury Liu i wsp. (2018) z niewielkimi modyfikacjami. Krzywą wzrostu populacji wykonano poprzez spektrofotometryczny pomiar gęstości optycznej OD_{600}, z wykorzystaniem systemu Bioscreen C (Labsystem, Helsinki, Finlandia).
4. Ocenę zmian morfologicznych na powierzchni komórek dokonano z wykorzystaniem skaningowej mikroskopii elektronowej (SEM – scanning electron microscopy) oraz mikroskopii sił atomowych (AFM – atomic force microscopy).
5. Widma FTIR otrzymano przez zastosowanie spektrometru FT-IR Nicolet 8700 (Thermo Scientific, Waltham, MA, USA) wyposażonego w rozdzielacz wiązki KBr i detektor MCT/A. Widma FTIR mierzono i analizowano przy użyciu oprogramowania Atlus Microscopy Software for OMNIC-8.

3.2. Materiały i metody badawcze zastosowane w publikacji P3 i P4

MATERIAŁ BADAWCZY

Materiał badawczy stanowiło czerwone wino wyprodukowane w winnicy „Dom Bliskowice" (Polska, woj. lubelskie) z winogron odmiany Regent i Rondo (1:1), zebranych w październiku 2019 roku. Po zbiorze, winogrona odszypułkowano i zmiażdżono. Miazgę przeniesiono do kadzi fermentacyjnych w celu maceracji i wstępnej fermentacji. Procesy te prowadzono przez 10 dni w temperaturze powietrza, przy udziale naturalnych drożdży. Następnie wina zostały oddzielone od frakcji stałej poprzez tłoczenie za pomocą prasy pneumatycznej i przeniesione do zbiornika fermentacyjnego o pojemności 1000L. Fermentacja trwała 30 dni na osadzie w temperaturze powietrza. Następnie wino przelano do kolejnego zbiornika, celem dokończenia fermentacji i przeprowadzenia spontanicznej fermentacji malolaktycznej. Do czasu pobrania badanych prób wino nie było poddane żadnym zabiegom konserwującym. Uzyskane wino podzielono na trzy partie i poddano trzem metodom konserwacji:

- Metodą tradycyjną - dodatek 30 mg/L lub 100 mg/L pirosiarczynu potasu;
- metodą łączoną – zimna plazma i dodatek 30 mg/L pirosiarczynu potasu;
- zimną plazmą.

Następnie badania próbek wina przeprowadzono bezpośrednio po procesie konserwacji oraz po trzech miesiącach przechowywania (15°C, ograniczony dostęp światła). W **Tabeli 1**. przedstawiono charakterystykę badanych prób.

Tabela 1. Sposób kodowania próbek i ich charakterystyka

Kod próbki	Metoda konserwacji	Czas trwania procesu	Gaz roboczy
PRZED PRZECHOWYWANIEM			
1.	kontrola	0	-
2.	zimna plazma	2	He /O_2
3.	zimna plazma	5	He /O2
4.	zimna plazma	10	He /O2
5.	zimna plazma	2	He / N_2
6.	zimna plazma	5	He / N_2
7.	zimna plazma	10	He / N_2
8.	metoda tradycyjna - 30mg/L pirosiarczynu potasu	0	-
9.	metoda łączona	2	He / O_2
10.	metoda łączona	5	He / O_2
11.	metoda łączona	10	He / O_2
12.	metoda łączona	2	He / N_2
13.	metoda łączona	5	He / N_2
14.	metoda łączona	10	He / N_2
15.	metoda tradycyjna - 100 mg/L pirosiarczynu potasu	0	-
PO PRZECHOWYWANIU			
16.	kontrola	0	-
17.	zimna plazma	2	He / O_2
18.	zimna plazma	5	He / O_2
19.	zimna plazma	10	He / O_2
20.	zimna plazma	2	He / N_2
21.	zimna plazma	5	He / N_2
22.	zimna plazma	10	He / N_2

23.	metoda tradycyjna - 30mg/L pirosiarczynu potasu	0	-
24.	metoda łączona	2	He / O_2
25.	metoda łączona	5	He / O_2
26.	metoda łączona	10	He / O_2
27.	metoda łączona	2	He / N_2
28.	metoda łączona	5	He / N_2
29.	metoda łączona	10	He / N_2
30.	metoda tradycyjna - 100 mg/L pirosiarczynu potasu	0	-

SYSTEM PLAZMOWANIA

Próbki wina poddawano działaniu zimnej plazmy przez 2; 5 lub 10 min. Jako gaz roboczy stosowano mieszaninę helu i azotu lub helu i tlenu. Próbki eksponowano na działanie CP przy użyciu reaktora strumienia plazmy DBD. Objętość 50 mL czerwonego wina wlewano do sterylnego szklanego pojemnika i umieszczano na mieszadle magnetycznym. Reaktor DBD składał się z ceramicznej rury gazowej o średnicy wewnętrznej 1,4 mm. Dwie metalowe elektrody były rozmieszczone w następujący sposób: pierścieniowa elektroda wysokiego napięcia była umieszczona 10 mm od końca dyszy, a płaska, laminowana miedzią elektroda PCB służyła jako uziemienie. Elektroda miedziana była umieszczona na mieszadle magnetycznym, tuż pod pojemnikiem na próbki. Odległość między końcem rurki reaktora a powierzchnią cieczy wynosiła 2 mm. Natężenia przepływu mieszanin gazów substratowych wynosiły 96 L/h helu z domieszkami tlenu lub azotu w ilości 1,8 L/h. Natężenia przepływu były regulowane przez regulatory przepływu gazu (Zakład Automatyki "ROTAMETR", Gliwice, Polska).

Poniżej wymieniono skrócony opis wykorzystanych metod badawczych w publikacji P3 i P4:

1. Jakościowe i ilościowe oznaczenie profilu związków fenolowych wykonano przy użyciu ultrawydajnej chromatografii cieczowej z odwróconą fazą (UPLC-PDA-MS/MS). Zastosowano system UPLC-PDA-MS/MS Waters ACQUITY (Walters, Milford, MA, USA).
2. Jakościowe i ilościowe oznaczenie amin biogennych oznaczono przy użyciu chromatografii gazowej połączonej ze spektrometrią mas (GC-MS).

3. Specyficzne korelacje pomiędzy różnymi metodami konserwacji, a zawartością amin biogennych i wybranych związków fenolowych określono z wykorzystaniem wieloczynnikowej statystycznej eksploracji danych. Do analizy danych wykorzystano następujące metody chemometryczne: analizę skupień (hierarchiczną i niehierarchiczną lub klasteryzację K-means), dwukierunkową analizę łączenia, analizę składowych głównych oraz analizę czynnikową. Analizy przeprowadzono przy użyciu oprogramowania STATISTICA 8.0.
4. Wartość pH próbek czerwonego wina mierzono potencjometrycznie przy użyciu pH-metru Hanna HI 221 (Hanna Instruments, Woonsocket, RI, USA).
5. Barwę próbek czerwonego wina określono za pomocą kolorymetru X-Rite 8200 (X-Rite, Inc., Michigan, USA) z wykorzystaniem przestrzeni barw CIELab (Metoda OIV-MA-AS2-11, 2006).
6. Całkowitą zawartość związków fenolowych (TC – total phenolic content) oraz całkowitą zawartość antocyjanów (TA – total anthocyanin content) w próbkach wina czerwonego oznaczono metodą spektrofotometryczną.
7. Potencjał antyoksydacyjny oznaczono spektrofotometrycznie poprzez ocenę zdolności do neutralizowania wolnych rodników generowanych z ABTS i DPPH oraz jonów Fe^{3+} do Fe^{2+} (FRAP).
8. Ogólną zawartość bakterii mezofilnych w próbkach czerwonego wina oceniono z wykorzystaniem klasycznej metody hodowlanej.
9. Wieloczynnikowa eksploracja danych statystycznych została wykorzystana do opisania specyficznych korelacji pomiędzy zastosowanymi różnymi metodami konserwacji a właściwościami fizykochemicznymi i biologicznymi próbek wina. Zastosowane metody chemometryczne obejmowały analizę skupień (hierarchiczną i niehierarchiczną), analizę czynnikową oraz składowych głównych. Różnice między średnimi wartościami danych (pH, barwa, TC, TC, ABTS, DPPH, logN), testowano na istotność statystyczną przy $p < 0,05$ za pomocą analizy wariancji i testu Tuckey'a.

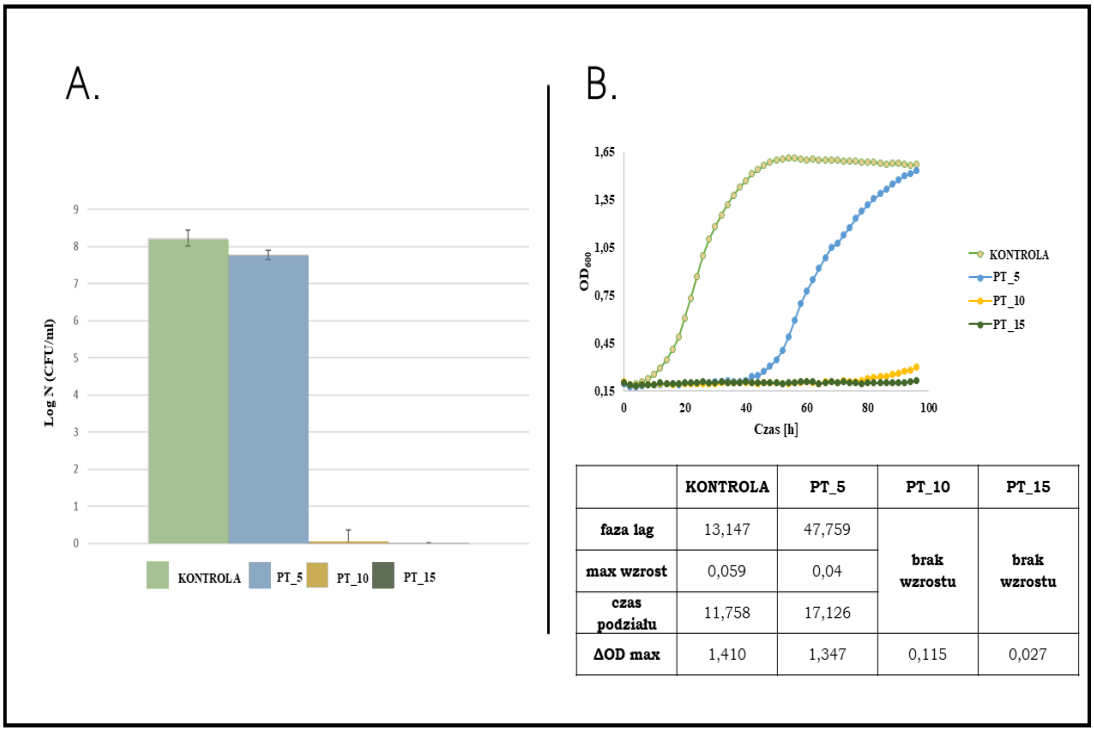
Szczegółowy opis metodyki znajduje się w poszczególnych publikacjach wchodzących w skład rozprawy doktorskiej.

4. PREZENTACJA WYBRANYCH WYNIKÓW BADAŃ ORAZ ICH DYSKUSJA

Postawiony w niniejszej rozprawie doktorskiej cel badawczy zrealizowano w dwóch etapach prac eksperymentalnych, których wyniki opisano w publikacjach **P2** oraz **P3** i **P4**. W pierwszym etapie badań podjęto próbę ustalenia mechanizmów działania zimnej plazmy na komórki *L. hilgardii* (Publikacja **P2**). Z kolei w drugim oceniono wpływ CP na właściwości fizykochemiczne i zawartość związków fenolowych w czerwonym winie. (Publikacja **P3** i **P4**). Poniżej przedstawiono opis uzyskanych wyników.

4.1 Ocena stanu fizjologicznego i zmian morfologicznych komórek *Lentilactobacillus hilgardii* po ekspozycji na zimną plazmę (P2)

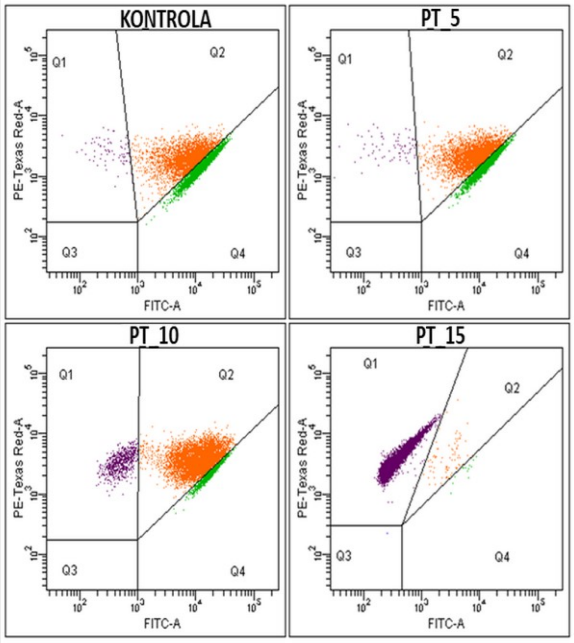
W pierwszej części eksperymentu oceny stanu fizjologicznego komórek *L. hilgardii* dokonano z wykorzystaniem klasycznej metody hodowlanej, a krzywą wzrostu populacji uzyskano dzięki wykonaniu pomiarów spektrofotometrycznych gęstości optycznej OD_{600}, z wykorzystaniem systemu Bioscreen C (**Rycina 2.**).



	KONTROLA	PT_5	PT_10	PT_15
faza lag	13,147	47,759	brak wzrostu	brak wzrostu
max wzrost	0,059	0,04		
czas podziału	11,758	17,126		
ΔOD max	1,410	1,347	0,115	0,027

Rycina 2. A. - Redukcja ilości komórek *L. hilgardii* po ekspozycji na zimną plazmę (PT_5 - 5 min ekspozycji, PT_10 -10 min., PT_15 -15 min). **B.** - Parametry wzrostu *L. hilgardii* po ekspozycji na zimną plazmę (Publikacja **P2**).

Całkowite zahamowanie wzrostu bakterii zaobserwowano po 10-minutowej ekspozycji na zimną plazmę. Natomiast już 5-minutowa sterylizacja spowodowała wydłużenie fazy lag oraz czasu podziału odpowiednio o 263% i 46% w stosunku do kontroli. Wydłużenie fazy lag sugeruje, że komórki dłużej przystosowują się do warunków panujących w środowisku. Prawdopodobną przyczyną tego zjawiska może być fakt, że w strumieniu plazmy, generowanej z wykorzystaniem tlenu, znajdują się liczne reaktywne związki takie jak: tlen singletowy, ozon, nadtlenek wodoru lub aniony ponadtlenkowe, które w bezpośredniej interakcji z drobnoustrojami mogą wywoływać u nich stres oksydacyjny tym samym opóźniając ich podziały i wzrost w podłożu mikrobiologicznym (Liu i in. 2018). Przedstawione powyżej metody hodowlane opierają się jednak jedynie na ocenie zdolności komórek do podziałów i wzrostu w określonych warunkach. Na tej podstawie możliwa jest ich klasyfikacja na frakcje martwe i żywe, co stanowi tylko skrajne stany żywotności, bowiem drobnoustroje mogą występować w tzw. stanach pośrednich. Z tego powodu stan fizjologiczny komórek kontrolnych oraz poddanych działaniu CP oceniono również z wykorzystaniem cytometrii przepływowej (**Rycina 3**).



SUBPOPULACJE

	KOMÓRKI AKTYWNE (Q4)	KOMÓRKI POŚREDNIE (Q2)	KOMÓRKI MARTWE (Q1)
KONTROLA	56,1 %	42,9 %	0,8 %
PT_5	55,6 %	43,5 %	0,9 %
PT_10	14,4 %	**77,5 %**	8,1 %
PT_15	0,2 %	0,9 %	**98,8 %**

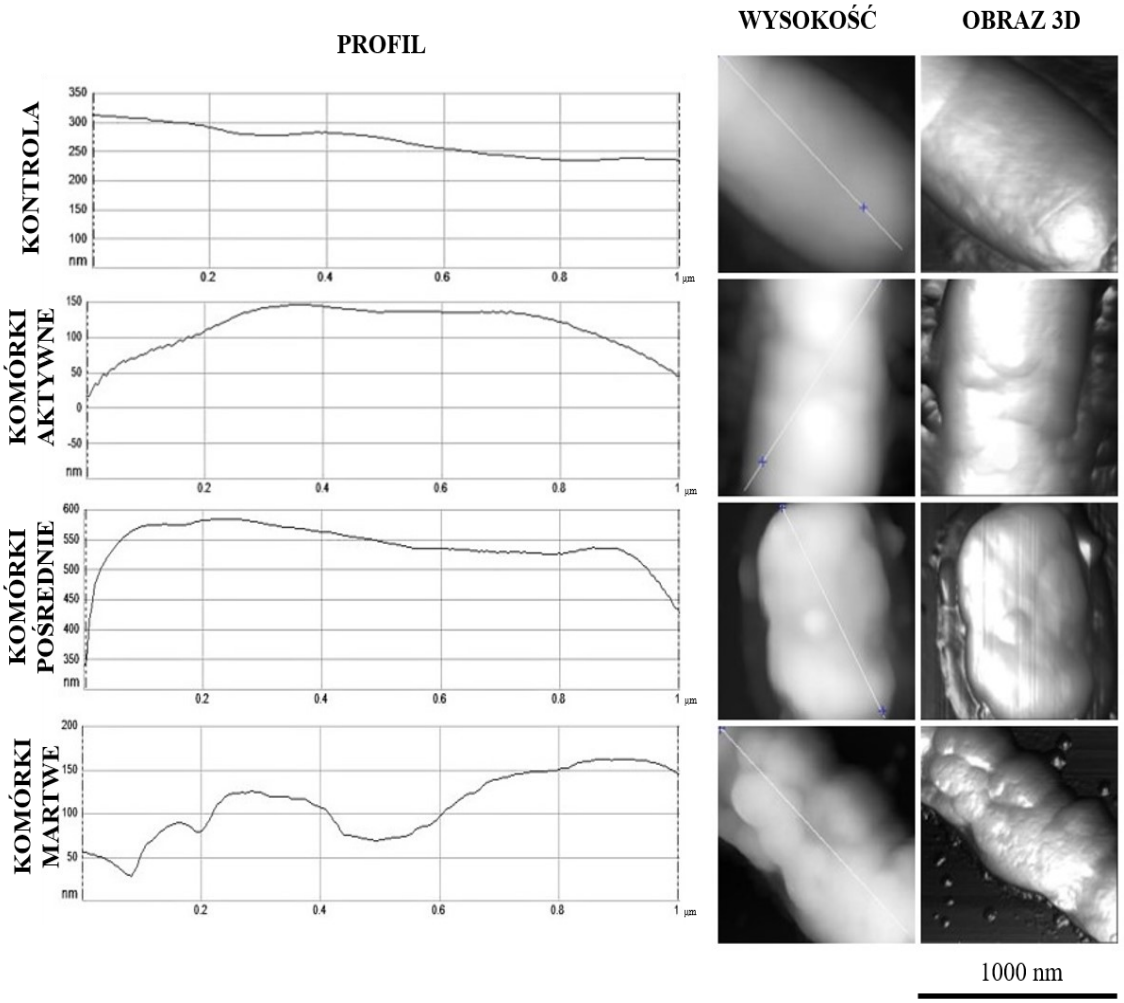
Rycina 3. Analiza stanu fizjologicznego komórek *L. hilgardii* metodą cytometrii przepływowej (Publikacja **P2**).

Analiza z wykorzystaniem cytometrii przepływowej w połączeniu ze specyficznym barwieniem fluorescencyjnym i sortowaniem komórek umożliwiła dokonanie pełnej charakterystyki stanów fizjologicznych *L. hilgardii* w analizowanych próbkach. Zestaw BacLight Redox Sensor Green Vitality Kit (Thermo Scientific) został wykorzystany do scharakteryzowania i rozróżnienia poszczególnych stanów fizjologicznych badanych bakterii na podstawie pomiarów aktywności metabolicznej. Interpretacja wyników doświadczenia polegała na określeniu procentowego udziału zdefiniowanych subpopulacji komórek martwych (Q1), średnio aktywnych (Q2) oraz aktywnych (Q4). Oceniono rozkład poszczególnych subpopulacji w całej populacji mikroorganizmów w badanych próbach. Procentowy udział zdefiniowanych subpopulacji drobnoustrojów wykazał znaczne różnice w rozkładzie poszczególnych frakcji (Q4, Q2, Q1) pomiędzy próbką kontrolą a próbkami poddanymi działaniu CP (**Rycina 3**). W próbce PT_10, w której klasyczne metody hodowlane wskazywały na całkowite zahamowanie wzrostu bakterii, odnotowano aż 77,5% komórek w stanie pośrednim, a tylko 8,1% komórek martwych. Dopiero zastosowanie 15-minutowej ekspozycji na CP powodowało dominację subpopulacji Q1, która stanowiła 98,8% całej populacji. Wysoki udział drobnoustrojów, pozostających w stanie pośrednim, może skutkować fałszywie negatywnymi wynikami oznaczeń liczby mikroorganizmów z zastosowaniem klasycznych metod hodowlanych, ponieważ część komórek z tej grupy, w sprzyjających warunkach, może podlegać procesom regeneracji i odzyskać żywotność. Bakterie tworzące subpopulację komórek średnio aktywnych (Q2) mogą reprezentować populację bakterii uszkodzonych lub znajdujących się w stadium VBNC. Stan VBNC jest strategią bakterii mającą na celu przetrwanie niekorzystnych warunków środowiska tj. ekstremalne temperatury, podwyższone ciśnienie osmotyczne, ekspozycja na światło lub zawartość tlenu (Schottroff i in. 2018). Jak omówiono wcześniej, w strumieniu plazmy, którego gazem roboczym jest tlen, stężenie ROS jest wysokie, co może być czynnikiem indykującym wejście bakterii w omawiane stadium. W celu sprawdzenia czy bakterie należące do frakcji średnio aktywnej (PT_10; Q2) odzyskają żywotność oraz by zweryfikować hipotezę, że zimna plazma indukuje stan VBNC, dokonano izolacji specyficznych subpopulacji na drodze sortowania komórek. Otrzymane izolaty poddano procesowi resuscytacji na odpowiednio przygotowanym podłożu mikrobiologicznym. W przeprowadzonym doświadczeniu zaobserwowano wzrost komórek z frakcji Q4 i Q2 (Publikacja **P2 – Rycina 6**), co potwierdziło hipotezę, że ekspozycja *L.hilgardii* na CP wprowadza część komórek w stan VBNC i że niektóre

średnio aktywne komórki w odpowiednich warunkach środowiska zdolne są do ponownego namnażania.

Do określenia zależności między stanem fizjologicznym a morfologią komórki po ekspozycji na zimną plazmę zastosowano skaningową mikroskopię elektronową oraz mikroskopię sił atomowych. W niniejszym opracowaniu omówiono wyniki uzyskane z wykorzystaniem metody AFM.

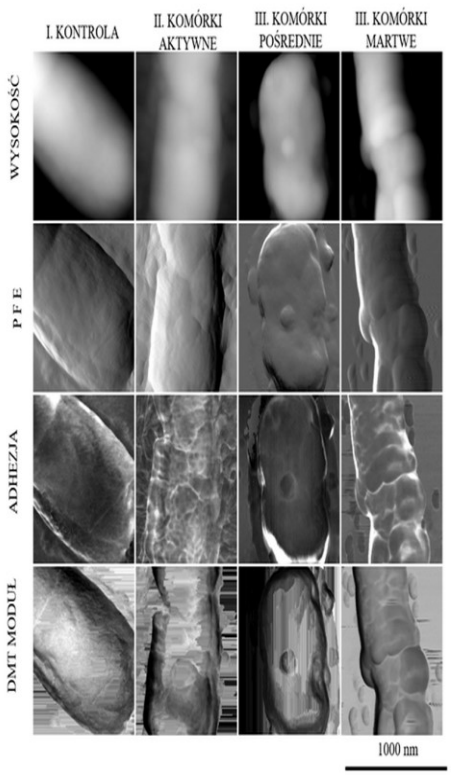
Technika AFM została wykorzystana do skorelowania obserwacji w skali nanometrycznej z właściwościami nanomechanicznymi i fizycznymi powierzchni komórek (**Rycina 4 i 5**). Analiza ta pozwoliła określić rozbieżności w topografii i właściwościach powierzchniowych komórek (PT_10) w trzech różnych stanach fizjologicznych (aktywny, średnio aktywny, martwy) oraz komórek kontrolnych. Należy podkreślić, iż połączenie metody cytometrii przepływowej z analizą morfologii bakterii potraktowanych CP, a następnie posortowanych na subpopulacje jest podejściem nowatorskim nie odnotowanym wcześniej w literaturze międzynarodowej.



Rycina 4. Profil przekroju powierzchni komórek *L. hilgardii* w trzech różnych stanach fizjologicznych po 10 – minutowej eskpozycji na zimną plazmę.

Na uzyskanych obrazach 3D widoczne są komórki kontrolne charakteryzujące się typowym dla nich kształtem i gładką powierzchnią. Z kolei, morfologia komórek badanych znajdujących się w poszczególnych stanach fizjologicznych różniła się znacząco. Wartym odnotowania są zmiany morfologiczne, jakie zaszły we frakcji Q2. Wielkość komórek uległa zmniejszeniu, a profil średnicy przekroju wzrósł dwukrotnie co świadczy o zmianie kształtu komórek z typowego wydłużonego dla *L.hilgardii* na kulisty. Obserwacje te znajdują potwierdzenie w dostępnej literaturze naukowej omawiającej zmiany zachodzące podczas przejścia bakterii w stan VBNC (Pienaar i in. 2016). Miniaturyzacja komórek oraz przyjęcie formy kulistej stanowią strategię przetrwania niekorzystnych warunków. Zmiana kształtu prawdopodobnie pozytywnie wpływa na pobieranie składników odżywczych przez komórkę ze względu na zwiększoną powierzchnię wchłaniania (Yoon i in. 2017). Widoczne różnice w stosunku do kontroli zaobserwowano również w subpopulacji Q1, w której powierzchnia

komórek odznaczała się głębokimi, nieregularnymi zagłębieniami. Pomimo to, integralność struktur zewnętrznych nie została przerwana. Jest to zgodne z wcześniejszymi doniesieniami naukowymi wskazującymi na wyższą oporność na działanie CP bakterii Gram-dodatnich, których gruba ściana komórkowa chroni przed uszkodzeniami zewnętrznymi, a ich uszkodzenie i eliminacja najprawdopodobniej następuje na skutek interakcji ROS z komponentami wewnętrznymi komórki (Laroussi i in. 2003). Dzięki zastosowaniu techniki AFM, oprócz obrazów ukazujących różnice w powierzchni komórek, uzyskaliśmy dane liczbowe, które pozwoliły na obliczenie wartości odpowiadających chropowatości powierzchni, adhezji i sprężystości (moduł DMT - model Derjaguina-Mullera-Toropova) komórek (**Rycina 5**).



	KONTROLA	KOMÓRKI AKTYWNE (Q4)	KOMÓRKI POŚREDNIE (Q2)	KOMÓRKI MARTWE (Q1)
DMT MODUL	1,81 ± 0.24 GPa	1,97 ± 0.19 GPa	2,19 ± 0.17 GPa	1,45 ± 0.28 GPa
CHROPOWATOŚĆ	2,04 ± 0.55 nm	2,11 ± 0.57 nm	2,70 ± 0.75 nm	7,07 ± 3.1 nm
ADHEZJA	0,32 ± 0.08 nN	0,29 ± 0.09 nN	0,43 ± 0.09 nN	0,24 ± 0.02 nN

Rycina 5. Obrazowanie AFM komórek posortowanych w subpopulacje po 10-minutowym traktowaniu plazmą z wartościami modułu DMT, chropowatości oraz adhezji ich powierzchni.

Analiza danych wykazała, iż subpopulacja Q1 charakteryzowała się najniższą wartością modułu DMT 1,45 ± 0,28 GPa, co wskazuje na wzrost elastyczności

powierzchni komórek badanych bakterii po działaniu CP. Dla tej subpopulacji oznaczono również najwyższą wartość chropowatości 7,07 ± 3,1 nm. Zwiększona szorstkość sugeruje głębokie zmiany struktur zewnętrznych, co koreluje ze zmianami zaobserwowanymi na obrazach uzyskanych metodą SEM i AFM. W przypadku komórek średnio aktywnych, które zdolne są do ponownego wzrostu odnotowano zbliżone wartości wszystkich wyznaczanych parametrów w porównaniu z frakcją żywą. Natomiast biorąc pod uwagę widoczną zmianę kształtu bakterii występujących w tej subpopulacji możemy przypuszczać, iż wzrost sztywności do wartości 2,19 ± 0,17 GPa wynikał z przejścia komórki w formę bardziej kulistą.

Ostatnim przeprowadzonym doświadczeniem na tym etapie badań była analiza chemicznych modyfikacji zewnętrznej struktury komórek *L. hilgardii* poddanych działaniu zimnej plazmy (PT_10) przeprowadzona przy użyciu techniki FT-IR (Publikacja **P2, Rycina 10**). W badaniach zaobserwowano znaczną liczbę zmian widmowych związanych z wibracjami grup funkcyjnych. Odnotowano spadek intensywności widma przy długości fali 2920 cm^{-1}, związany z grupą kwasów tłuszczowych CH_3 i CH_2. Spadek ten prawdopodobnie był wynikiem zaburzeń struktury błony komórkowej (Khan i in. 2016). Dodatkowo zaobserwowano intensywne pasma pochodzące od jonu karboksylanowego, a także wzrost intensywności pasma 1720 cm^{-1} wskazujący na tworzenie się nowych grup karbonylowych, takich jak aldehydy ketony lub kwasy. Wystąpienie tych dwóch sygnałów może być związane z degradacją ściany i błony komórkowej (Kiwi i in. 2005). Występujący również wzrost intensywności pasma przy długości fali 1045 cm^{-1}. związany z symetrycznymi drganiami rozciągającymi S = O sugeruje, że CP może powodować utlenianie cząsteczek zawierających siarkę, takich jak aminokwasy (metionina, cysteina) czy białka (Lackmann i in. 2013; Ravi i in. 2011).

4.2. Ocena wpływu zimnej plazmy na jakość wina czerwonego z uwzględnieniem okresu przechowywania (P3, P4)

W drugim etapie badań określono wpływ plazmy niskotemperaturowej nierównowagowej na właściwości fizykochemiczne oraz aktywność biologiczną czerwonego wina (**P3**, **P4**). Na podstawie danych literaturowych oraz wyników uzyskanych w publikacji **P2,** do dalszych badań wybrano dwa systemy plazmowania różniące się zastosowanym gazem roboczym – mieszanina He/O_2 lub He/N_2 . Czas trwania procesu ustalono na 2; 5; lub 10 min. W publikacji **P3** przeanalizowano profil

związków fenolowych oraz zawartość amin biogennych w próbkach wina czerwonego poddanych działaniu zimnej plazmy. Z kolei, w publikacji **P4** oceniono wpływ zimnej plazmy na właściwości technologiczne i funkcjonalne badanych próbek czerwonego wina. Dodatkowo, w celu zweryfikowania hipotezy, że zimna plazma może być alternatywną metodą konserwacji wina, w obydwu pracach porównano wpływ działania CP z metodą łączoną (CP + 30 mg/L $K_2S_2O_5$) oraz metodą konwencjonalną (30 lub 100 mg/L $K_2S_2O_5$) na końcową jakość produktu z uwzględnieniem trzymiesięcznego okresu przechowywania.

Polifenole to jedna z najliczniejszych i najważniejszych grup związków występujących w czerwonym winie. Odpowiadają one za barwę (antocyjany), smak (taniny) i aromat produktu. Dodatkowo wykazują aktywność antyoksydacyjną, co sprawia, że korzystnie wpływają na profilaktykę chorób układu krążenia i innych przewlekłych stanów chorobowych (Snopek i in.2018). Zawartość polifenoli w winie zależy m.in. od szczepu winorośli, technologii produkcji wina, procesów starzenia oraz stosowanych metod konserwacji. Przeprowadzone w pracy analizy umożliwiły określenie różnic jakościowych i ilościowych w profilu związków fenolowych w badanych próbkach wina, które poddano różnym procesom konserwacji oraz trzymiesięcznemu przechowywaniu (Publikacja **P3)**. Z wykorzystaniem ultrasprawnej chromatografii cieczowej oznaczono 54 związki, w tym 24 z grupy antocyjanów, 7 flawonoli, 12 katechin, 7 kwasów fenolowych oraz 4 związki należące do stilbenów (Publikacja **P3** – Materiały Dodatkowe). Najliczniej oznaczoną grupę stanowiły antocyjany, które są odpowiedzialne za odcień i stabilność barwy wina oraz świadczą o jego końcowej jakości. Antocyjany są związkami nietrwałymi, które w środowisku wodnym ulegają odwracalnym przemianom wywołując tym samym zmianę barwy zależną od pH środowiska. Ponadto ulegają one degradacji w trakcie procesów technologicznych na skutek działania różnych czynników tj. temperatura, tlen czy światło (He et al. 2012; Yue et al. 2021). Powyższe właściwości antocyjanów znajdują potwierdzenie w uzyskanych przez nas wynikach, które wskazują, iż zarówno proces przechowywania, jak również zastosowana metoda konserwacji wpłynęły na końcową zawartość antocyjanów. Proces przechowywania spowodował redukcję tych związków w każdej z badanych prób (od 8,23 do 47,51%). Natomiast wpływ procesu konserwacji uzależniony był od rodzaju zastosowanej metody. Bezpośrednio po dodaniu pirosiarczynu potasu do czerwonego wina (próbka nr. 8 i 15) odnotowano niewielki

wzrost sumarycznej zawartości antocyjanów w porównaniu z próbką kontrolną (nr 1). Ponadto próbka z dodatkiem 100 mg/L $K_2S_2O_5$ charakteryzowała się ich najwyższą zawartością (836,32 mg/L). Również po trzymiesięcznym okresie przechowywania (próbka nr 23 i 30) odnotowaliśmy zbliżone stężenie antocyjanów w porównaniu do wina nie konserwowanego (próbka nr 16), jednakże dodatek pirosiarczynu potasu w stężeniu 100 mg/L spowodował obniżenie zawartości tych związków o 5,77%. Uzyskane wyniki wskazują, iż zastosowana metoda tradycyjna wywiera minimalny wpływ na obniżenie zawartości antocyjanów. W badaniach nad wpływem CP na profil związków fenolowych odnotowano, że zarówno czas trwania procesu, jak również rodzaj użytego gazu przyczynił się do zmiany zawartości poszczególnych związków. Badanie zawartości antocyjanów w próbkach nieprzechowywanych wskazuje, iż zastosowanie CP przez 10 min. (He/O_2) spowodowało najwyższą redukcję sumarycznej zawartości antocyjanów w stosunku do kontroli (580,36 mg/L). Natomiast obserwując działanie CP generowanej z wykorzystaniem mieszaniny gazów hel/azot odnotowaliśmy wzrost ich zawartości wraz ze wzrostem czasu trwania procesu. Sumaryczna zawartość po czasie 2, 5 oraz 10 min wyniosła kolejno: 707,23; 747,74; 755,25 mg/L (**Tabela 2**). Różnice w działaniu poszczególnych gazów na stabilność antocyjanów w badanych próbkach wina prawdopodobnie wynikają z powstających w trakcie generowania plazmy odmiennych reaktywnych związków. Interesujące są wyniki analizy próbek po trzymiesięcznym przechowywaniu. W próbkach poddanych 5-minutowej konserwacji CP oraz metodą łączoną odnotowano wyższą zawartość niektórych antocyjanów w porównaniu z próbkami, które były plazmowane tylko przez 2 min. Dodatkowo ten sam czas działania CP generowanej z wykorzystaniem mieszaniny gazów He/N_2 spowodował, że sumaryczna zawartość antocyjanów była porównywalna do próbki kontrolnej oraz z dodatkiem 30 mg/L $K_2S_2O_5$, a wyższa o 4,34% od próbki z dodatkiem 100 mg/L pirosiarczynu potasu.

Tabela 2. Sumaryczna zawartość związków fenolowych i amin biogennych w badanych próbkach czerwonego wina

Kod Próbki*	Antocyjany [mg/L]	Kwasy fenolowe [mg/L]	Flawanole [mg/L]	Flawan-3-ole [mg/L]	Stilbeny [mg/L]	Związki fenolowe ogółem [mg/L]
1.	731,78 ± 30,97	22,65 ± 1,38	3,74 ± 0,25	50,52 ± 3,68	1,87 ± 0,18	810,55 ± 36,46
2.	633,34 ± 31,01	23,26 ± 1,41	3,40 ± 0,31	51,99 ± 3,74	1,77 ± 0,16	713,77 ± 36,63
3.	682,58 ± 33,31	24,17 ± 1,47	3,41 ± 0,15	47,88 ± 3,44	1,73 ± 0,04	759,77 ± 38,41
4.	580,36 ± 27,83	23,19 ± 1,41	3,02 ± 0,14	42,78 ± 3,06	1,65 ± 0,15	651,00 ± 32,59
5.	707,23 ± 34,97	24,68 ± 1,48	3,28 ± 0,15	53,61 ± 3,86	1,86 ± 0,18	790,65 ± 40,64
6.	747,74 ± 36,77	23,91 ± 1,43	3,17 ± 0,13	50,31 ± 3,60	1,79 ± 0,17	826,92 ± 42,10
7.	755,25 ± 37,09	23,56 ± 1,40	3,17 ± 0,14	47,21 ± 3,38	1,78 ± 0,17	830,97 ± 42,18
8.	811,73 ± 40,18	23,52 ± 1,42	3,25 ± 0,14	53,06 ± 3,83	1,87 ± 0,18	893,43 ± 45,75
9.	797,03 ± 39,62	23,39 ± 1,41	3,19 ± 0,14	51,55 ± 3,70	1,85 ± 0,18	877,00 ± 45,05
10.	768,28 ± 37,77	23,74 ± 1,42	3,17 ± 0,14	47,04 ± 3,39	1,79 ± 0,17	844,03 ± 42,89
11.	710,95 ± 41,17	24,13 ± 1,44	2,93 ± 0,22	42,46 ± 2,65	1,65 ± 0,15	782,12 ± 45,63
12.	818,52 ± 49,80	24,61 ± 1,46	3,11 ± 0,24	53,52 ± 3,49	1,82 ± 0,22	901,57 ± 55,21
13.	777,79 ± 47,80	24,31 ± 1,44	3,12 ± 0,25	50,95 ± 3,28	1,84 ± 0,22	858,01 ± 52,99
14.	778,08 ± 47,66	23,95 ± 1,41	3,12 ± 0,26	47,76 ± 3,06	1,75 ± 0,22	854,66 ± 52,61

15.	836,32 ± 52,29	22,95 ± 1,35	3,15 ± 0,32	52,93 ± 3,50	1,85 ± 0,22	917,19 ± 57,68
16.	671,28 ± 40,63	23,88 ± 1,41	2,87 ± 0,24	44,21 ± 3,01	1,68 ± 0,21	743,92 ± 45,50
17.	513,69 ± 25,18	24,52 ± 1,44	2,92 ± 0,24	34,86 ± 2,25	1,43 ± 0,12	577,42 ± 29,23
18.	578,80 ± 32,07	24,34 ± 1,44	2,67 ± 0,22	35,76 ± 2,33	1,47 ± 0,13	643,02 ± 36,19
19.	426,16 ± 18,32	24,13 ± 1,41	2,62 ± 0,21	21,19 ± 1,31	1,34 ± 0,12	475,45 ± 21,37
20.	493,18 ± 23,61	24,66 ± 1,46	2,64 ± 0,22	34,67 ± 2,20	1,66 ± 0,19	556,81 ± 27,68
21.	661,23 ± 35,66	23,91 ± 1,84	3,19 ± 0,14	33,57 ± 1,92	1,38 ± 0,11	732,28 ± 39,67
22.	476,48 ± 23,95	24,01 ± 1,76	3,02 ± 0,11	25,04 ± 1,42	1,19 ± 0,10	529,74 ± 27,34
23.	675,11 ± 38,12	22,71 ± 1,76	2,98 ± 0,13	40,79 ± 2,36	1,45 ± 0,07	743,04 ± 42,44
24.	473,92 ± 23,22	23,70 ± 1,76	2,74 ± 0,11	28,71 ± 1,71	1,13 ± 0,06	530,19 ± 28,86
25.	489,98 ± 24,25	24,18 ± 1,78	2,73 ± 0,10	27,71 ± 1,59	1,15 ± 0,06	545,75 ± 27,78
26.	443,38 ± 23,18	23,90 ± 1,79	2,72 ± 0,14	24,06 ± 1,37	1,16 ± 0,06	495,23 ± 26,54
27.	429,64 ± 21,25	24,50 ± 1,79	2,70 ± 0,10	30,80 ± 0,84	1,15 ± 0,06	488,79 ± 24,04
28.	495,68 ± 25,88	24,52 ± 1,80	2,87 ± 0,12	30,83 ± 1,78	1,23 ± 0,06	555,13 ± 29,64
29.	502,65 ± 27,27	23,82 ± 1,76	2,52 ± 0,12	30,50 ± 1,67	1,20 ± 0,05	560,70 ± 30,87
30.	632,53 ± 35,77	22,48 ± 1,77	3,00 ± 0,11	42,21 ± 2,44	1,32 ± 0,06	701,53 ± 40,16

Dane przedstawiono jako średnie ±SD (odchylenie standardowe). * sposób kodowania próbek przedstawiono w **Tabeli 1**.

W badanych próbkach czerwonego wina oznaczono również kwasy fenolowe: kwas galusowy, protokatechowy, kaftarowy, kutarowy, kawowy, kumarowy oraz ferulowy. Stwierdzono, że kwas galusowy występował w najwyższym stężeniu (9,52 – 11,86 mg/L). W próbkach przed przechowywaniem najwyższą sumaryczną zawartość tych substancji 24,68 mg/L odnotowano po 2 minutowej ekspozycji na zimą plazmę (He/N_2), zawartość ta była wyższa o 8,25% w stosunku do kontroli. Obserwacja ta znajduje potwierdzenie w literaturze przedmiotu, korzystne działanie CP na zawartość kawasów hydroksycynamonowych zostało opisane przez Herceg i wsp. (2016). Związki należące do tej grupy charakteryzują się wyższą stabilnością, co prawdopodobnie wpływa na ich mniejszą reaktywność z rodnikami powstającymi w trakcie generowania zimnej plazmy. W przeciwieństwie do antocyjanów zawartość kwasów fenolowych po przechowywaniu w większości próbek wzrosła. Interesujące wyniki uzyskaliśmy przy ocenie zawartości kwasu protokatechowego (Publikacja **P3 - Tabela 2**). Po przechowywaniu jego stężenie było wyższe w stosunku do próbek analizowanych bezpośrednio po konserwacji, a w przypadku próbek poddanych działaniu CP (10 min., He/N_2) odnotowano nawet 4-krotny wzrost (0,84 mg/L). Z kolei najniższą zawartość kwasu protokatechowego oznaczono w próbkach z dodatkiem 100 mg/L $K_2S_2O_5$ (0,19 mg/L). Podobną tendencję zaobserwowano w przypadku zawartości pozostałych kwasów. Na podstawie dostępnych danych literaturowych oraz uzyskanych przez nas wyników zawartości antocyjanów w badanych próbach czerwonego wina, możemy przypuszczać, iż wzrost stężenia kwasów fenolowych w próbkach konserwowanych CP związany był ze spadkiem zawartości antocyjanów. Związki te bowiem mogą być degradowane do kwasów fenolowych, a głównymi produktami ich rozpadu są kwasy protokatechowe, kwasy wanilinowe, kwasy syringowe oraz p-kumarowy (Yang i in. 2018). Prace innych autorów sugerują także, że krótki czas działania plazmy na matrycę żywnościową powoduje dysocjację aglomeratów lub cząstek, co prowadzi do wzrostu zawartości związków fenolowych (Garofuli´c i in. 2015). Z wykorzystaniem UPLC-PDA-MS/MS oznaczono również flawanole, flawan-3-ole oraz stilbeny. Sumaryczna zawartość flawanoli w badanych próbkach wina była niewielka i wahała się w przedziale od 2,52 – 3,74 mg/L. Najwyższe stężenie odnotowano w próbce kontrolnej nie przechowywanej, natomiast najniższe w próbce po przechowywaniu, konserwowanej metodą łączoną (10 min., He/N_2). Spośród flawan-3-oli najwyższe stężenie w badanych próbkach odnotowano dla (+)-katechiny 25,67 mg/L (próbka nr 5) oraz procyjanidyny B1 10,46 mg/L (próbka nr 12). W badanych próbkach wina

oznaczano także cis– oraz trans-resweratrol. Zawartość cis-resweratrolu w próbkach przed i po przechowywaniu pozostała na niezmienionym poziomie. Niewielki wzrost jego zawartości odnotowano w próbkach poddanych działaniu CP (10 min., He/O_2), a następnie przechowywanych przez 3 miesiące. Odwrotną zależność zaobserwowano dla trans-resweratrolu. Otrzymane przez nas wyniki wskazują, że wpływ procesu przechowywania jak również zastosowanej metody konserwacji na profil związków fenolowych wina nie jest jednoznaczny i zależy w głównej mierze od właściwości chemicznych poszczególnych związków. Do tej pory w literaturze nie ma wielu doniesień wyjaśniających mechanizm działania zimnej plazmy na cechy produktów spożywczych. Jednakże w trakcie generowania CP obserwowana jest emisja światła, procesy kawitacyjne, wytwarzanie fal uderzeniowych a także generowanie wolnych rodników, co bezpośrednio przyczynia się do degradacji wielu związków organicznych, także związków fenolowych (He i in. 2012).

Zawartość związków fenolowych w czerwonym winie bezpośrednio wpływa na jego aktywność przeciwutleniającą, a tym samym determinuje jego właściwości prozdrowotne. Wpływ różnych metod konserwacji na pojemność antyoksydacyjną badanych próbek określono poprzez oznaczenie zdolności do neutralizowania wolnych rodników generowanych z ABTS i DPPH oraz do redukcji jonów Fe^{3+} do Fe^{2+} (FRAP) (Publikacja **P4**). Najwyższą aktywność przeciwutleniającą odnotowano w próbce nieprzechowywanej, konserwowanej metodą tradycyjną (100 mg/L $K_2S_2O_5$), z wartościami DPPH, ABTS i FRAP wynoszącymi odpowiednio 77,31%, 95,56% i 12,41 mmTE/L. Co ciekawe, jak omówiono powyżej, próbka ta charakteryzowała się również najwyższą sumaryczną zawartością związków fenolowych (917,19 mg/L). Najniższy potencjał przeciwutleniający zaobserwowano natomiast po zastosowaniu CP (10 min; He/O_2) i procesu przechowywania. Wyniki DPPH, ABTS i FRAP były niższe odpowiednio o 67,03%, 2,83% i 30,82% w porównaniu do próbki kontrolnej. Podobnie jak w przypadku zawartości poszczególnych związków fenolowych, wpływ czasu trwania procesu konserwacji na aktywność antyoksydacyjną nie jest jednoznaczny. Brak jest również danych literaturowych wyjaśniających oddziaływanie CP na właściwości antyoksydacyjne czerwonego wina. Część autorów sugeruje spadek potencjału oksydacyjnego wraz ze wzrostem czasu plazmowania (Hou i in. 2019). Jednakże, w naszych badaniach po 5-minutowej ekspozycji na CP (He/N_2) wykazano o 19% silniejszą zdolność do zmiatania wolnych rodników DPPH w porównaniu do

efektów uzyskanych po 2-minutowej sterylizacji. Ponownie, wynik ten jest zbieżny z obserwacjami zmian zawartości związków fenolowych. Przedstawione wyniki wskazują, że wpływ CP na aktywność antyoksydacyjną czerwonego wina jest silnie skorelowany z zawartością związków fenolowych. Podobne wnioski znajdujemy również w pracach innych autorów, którzy badali właściwości antyoksydacyjne wina (Rodríguez-Vaquero i in. 2020; Yue i in. 2021). W kontekście rozważań nad potencjalnym zastosowaniem zimnej plazmy jako alternatywnej metody utrwalania czerwonego wina, należy podkreślić, iż różnica między uzyskanymi wynikami z analizy prób poddanych konserwacji CP (5 min., He/N_2) i metodą tradycyjną (100 mg/L $K_2S_2O_5$), a następnie przechowywanych była nieistotna statystycznie.

Oprócz prozdrowotnych związków fenolowych, w winie obecne są również aminy biogenne, których zawartość uzależniona jest od stężenia prekursorów aminokwasów w produkcie, pH, stężenia alkoholu oraz dwutlenku siarki. BAs powstają głównie na drodze dekarboksylacji aminokwasów będącej wynikiem działalności mikroorganizmów głównie należących do LAB. Wysoka zawartość tych substancji może mieć negatywny wpływ na zdrowie człowieka wywołując m.in. nudności czy biegunkę. Z powyższych względów w publikacji **P3**, po raz pierwszy w literaturze przedmiotu, podjęto próbę określenia wpływu zimnej plazmy na zawartość amin biogennych w próbkach czerwonego wina z wykorzystaniem metody GC-MS. Wyniki przedstawiono w **Tabeli 3**. Zidentyfikowano sześć amin biogennych: TRP, PUT, HIS, TYR, CAD i 2-PE, spośród których najwyższe wartości odnotowano dla HIS. Koresponduje to z wynikami innych badaczy, którzy wskazują, że jest ona dominującą aminą biogenną w winach (Płotka i in. 2018). Najwyższą zawartość HIS stwierdzono w próbce kontrolnej nie konserwowanej (przed przechowywaniem: 818 ± 34 µg/L; po przechowywaniu: 821 ± 30 µg/L). Znaczną redukcję zawartości tego związku (584 ± 34 µg/L) zaobserwowano po zastosowaniu konserwacji metodą łączoną (10 min, He/O_2). Również po trzech miesiącach przechowywania zawartość HIS w tej próbce nie uległa istotnej zmianie (586 ± 33 µg/L). Analizując wpływ sposobu konserwacji próbek wina na zawartość pozostałych amin biogennych we wszystkich przypadkach omawiana wyżej metoda łączona powodowała najwyższą redukcję tych związków. Co więcej, uzyskany efekt utrzymywał się po 3-miesięcznym okresie przechowywania. Uzyskane wyniki wskazują, że na skuteczność CP w redukcji amin biogennych w badanych próbkach wina wpływa czas trwania procesu oraz rodzaj zastosowanego gazu

roboczego. Wydłużenie procesu do 10 min. oraz zastosowanie jako gazu roboczego mieszaniny helu i tlenu korzystnie wpłynęło na eliminację tych związków z matrycy żywnościowej. Do tej pory w literaturze brakuje informacji dotyczących analizy wpływu CP na zawartość amin biogennych w winie. Mając jednak na uwadze, że za powstawanie tych związków w głównej mierze odpowiedzialne są mikroorganizmy obecne w produkcie, możemy przypuszczać, iż CP, która ma szeroko udowodnione działanie biobójcze, przyczynia się tym samym do redukcji poziomu amin biogennych w czerwonym winie. Stwierdzenie to mogą potwierdzać wyniki oznaczeń całkowitej liczby bakterii mezofilnych omówione w publikacji **P4**. Zaobserwowano, iż, każda z zastosowanych metod konserwacji spowodowała redukcję ogólnej liczby drobnoustrojów, a w przypadku zastosowania metody łączonej (10 min., He/O_2) nie wykryto wzrostu mikroorganizmów w podłożu mikrobiologicznym. Wyraźnie widoczne jest także, że skuteczność inaktywacyjna CP wzrastała wraz z wydłużeniem czasu trwania procesu. Podobna zależność została odnotowana przez wielu autorów (Lacombe i in. 2015; Pankaj i in. 2017). Analiza danych uzyskanych po trzech miesiącach przechowywania wskazywała na wzrost liczby mikroorganizmów w każdej z badanych prób, jednakże ponownie najniższą ilość mikroorganizmów odnotowano po zastosowaniu metody łączonej (10 min., He/O_2). Stopień redukcji całkowitej ilości bakterii w tej próbce wynosił 4,21 log CFU/mL względem próbki kontrolnej. Należy również zauważyć, że powyższa metoda wykazała wyższą skuteczność eliminacji mikroorganizmów w porównaniu z tradycyjną metodą konserwacji wina (100 mg/L $K_2S_2O_5$). Inaktywacyjne działanie CP na mikroorganizmy zostało dobrze udokumentowane przez wielu autorów. Badano zarówno jej bezpośredni efekt na komórki mikroorganizmów (Khan i in. 2016), jak również jej wpływ na bezpieczeństwo mikrobiologiczne produktów spożywczych (Choi i in. 2016; Tappi i in. 2016). Wyniki badań opisane w literaturze przedmiotu są zbieżne z naszymi i wskazują, że skuteczność obróbki zimną plazmą zależy od wielu parametrów procesu, jak również od matrycy żywnościowej, która jest poddawana procesowi konserwacji.

Tabela 3. Zawartość amin biogennych oznaczonych w próbkach czerwonego wina

Kod Próbki*	TRP (µg/L)	PUT (µg/L)	HIS (µg/L)	TYR (µg/L)	CAD (µg/L)	2-PE (µg/L)
1.	4,089 ± 0,012	489 ± 25	818 ± 34	27,74 ± 0,16	58,73 ± 0,15	18,70 ± 0,054
2.	3,670 ± 0,011	475 ± 24	799 ± 31	27,58 ± 0,17	54,15 ± 0,12	18,68 ± 0,049
3.	3,578 ± 0,008	455 ± 25	734 ± 37	27,34 ± 0,17	52,21 ± 0,12	18,73 ± 0,047
4.	3,551 ± 0,009	449 ± 23	732 ± 36	26,43 ± 0,13	52,01 ± 0,14	18,63 ± 0,050
5.	3,662 ± 0,010	471 ± 22	784 ± 29	27,51 ± 0,18	53,94 ± 0,13	18,71 ± 0,048
6.	3,589 ± 0,008	466 ± 27	741 ± 33	27,44 ± 0,16	52,27 ± 0,11	18,75 ± 0,044
7.	3,540 ± 0,010	457 ± 22	742 ± 34	26,78 ± 0,17	52,22 ± 0,13	18,66 ± 0,051
8.	2,918 ± 0,008	344 ± 25	654 ±34	<LOD	48,29 ± 0,16	23,74± 0,044
9.	2,705 ± 0,011	324 ± 23	627 ± 38	<LOD	44,54 ± 0,14	23,77± 0,047
10.	2,678 ± 0,013	299 ± 20	622 ± 33	<LOD	39,79 ± 0,12	23,68± 0,050
11.	1,972 ± 0,006	278 ± 19	584 ± 34	<LOD	38,09 ± 0,14	23,76± 0,048
12.	2,802 ± 0,014	348 ± 24	654 ± 38	<LOD	43,87 ± 0,16	23,72± 0,051
13.	2,732 ± 0,016	320 ± 21	641 ± 32	<LOD	40,17 ± 0,13	23,63± 0,047
14.	2,052 ± 0,008	291 ± 19	601 ± 36	<LOD	37,89 ± 0,11	23,69± 0,052
15.	3,878 ± 0,013	466 ± 23	773 ± 30	<LOD	52,42 ± 0,17	25,88± 0,054
16.	4,086 ± 0,011	490 ± 24	821 ± 30	27,71 ± 0,15	58,66 ± 0,18	18,78 ± 0,044
17.	3,674 ± 0,010	479 ± 22	794 ± 29	27,66 ± 0,16	54,05 ± 0,14	18,75 ± 0,043

18.	3,581 ± 0,012	457 ± 24	739 ± 35	27,91 ± 0,14	52,18 ± 0,13	18,79 ± 0,051
19.	3,560 ± 0,010	449 ± 21	732 ± 33	26,38 ± 0,12	52,09 ± 0,18	18,60 ± 0,044
20.	3,669 ± 0,010	476 ± 26	789 ± 31	27,79 ± 0,21	53,99 ± 0,15	18,77 ± 0,044
21.	3,593 ± 0,013	471 ± 23	748 ± 27	27,49 ± 0,18	52,30 ± 0,17	18,70 ± 0,038
22.	3,547 ± 0,011	457 ± 20	739 ± 31	26,85 ± 0,15	52,28 ± 0,10	18,71 ± 0,047
23.	2,915 ± 0,009	349 ± 24	658 ± 33	<LOD	48,33 ± 0,16	23,81± 0,056
24.	2,711 ± 0,013	332 ± 21	629 ± 38	<LOD	44,50 ± 0,19	23,84± 0,031
25.	2,684 ± 0,012	309 ± 24	617 ± 31	<LOD	39,83 ± 0,10	23,77± 0,062
26.	1,979 ± 0,011	279 ± 19	586 ± 33	<LOD	38,04 ± 0,14	23,85± 0,045
27.	2,811 ± 0,017	353 ± 22	659 ± 36	<LOD	43,95 ± 0,11	23,77± 0,044
28.	2,729 ± 0,014	320 ± 19	646 ± 31	<LOD	40,20 ± 0,17	23,56± 0,039
29.	2,058 ± 0,009	289 ± 21	613 ± 35	<LOD	37,84 ± 0,15	23,71± 0,057
30.	3,874 ± 0,012	469 ± 25	773 ± 34	<LOD	52,47 ± 0,20	25,93± 0,061

TRP - tryptamina, PUT - putrescyna, HIS - histamina, TYR - tyramina, CAD - kadaweryna,
2-PE – 2-fenyloetyloamina. Dane przedstawiono jako średnie ±SD (odchylenie standardowe).
*sposób kodowania próbek przedstawiono w **Tabeli 1**.

W dalszym etapie badań przeprowadzono ocenę wpływu różnych procesów konserwacji na pH oraz barwę czerwonego wina (**Tabela 4**). W badanych próbkach nie zaobserwowano znaczących zmian w kwasowości, a pH było na poziomie ~3,52 przed przechowywaniem i ~3,49 po przechowywaniu. Wartości te były zbliżone do pH próbki kontrolnej i nie odbiegały od kwasowości komercyjnie dostępnych win czerwonych (Sáenz-Navajas i in. 2011).

Dla konsumentów istotnym parametrem świadczącym o jakości wina jest barwa. Wpływ poszczególnych metod konserwacji i czasu przechowywania na barwę czerwonego wina oceniono za pomocą przestrzeni CIE L* a* b*. Analiza jasności barwy (parametr L*) wykazała, że bezpośrednio po zastosowaniu różnych metod konserwacji następuje wzrost jasności produktu. Najwyższe wartości L* odnotowano dla metody łączonej 15,13 (5 min, He/N_2), oraz tradycyjnej 14,94. W odniesieniu do czerwoności (a*) obserwowano tą samą zależność. Zaobserwowano, że próbki konserwowane CP (He/N_2) bądź poprzez dodatek pirosiarczynu potasu charakteryzowały się wyższymi wartościami tego parametru. Za czerwoną barwę wina odpowiadają głównie antocyjany, które z SO_2 mogą tworzyć wiązania, równocześnie przyczyniając się do zwiększenia jasności produktu (Silva i in. 2021). Po trzech miesiącach przechowywania odnotowano ciemniejszy kolor produktu w stosunku do kontroli w próbkach 17, 19, 24, 26 i 29. (odpowiednio około 9,35; 13,44; 41,23; 20,39; 15,94%). Do konserwacji czterech z nich zastosowano zimną plazmę (gaz roboczy: He/O_2), co może wskazywać na udział tlenu i jego reaktywnych związków w procesie ciemnienia wina. Mechanizm tego zjawiska może być związany z obecnością ozonu i rodników hydroksylowych powstających w procesie wytwarzania CP. Związki te mogą powodować oksydacyjne rozszczepienie chromoforów, prowadząc do rozpadu antocyjanów i utraty barwy produktu (Sarangapani i in. 2017). Wartym odnotowania jest także fakt, że zastosowanie CP lub metody łączonej (5 min., He/N_2) praktycznie nie spowodowało zmiany wartości parametru L* w stosunku do próby kontrolnej. Koreluje to z uzyskanymi przez nas wynikami dotyczącymi zawartości antocyjanów w badanych próbkach czerwonego wina. Analizując pozostałe parametry składające się na model CIELab (a*; b*), stwierdzono takie same zależności jak w przypadku parametru jasności. Wzrost wartości parametru b* wskazujący na większy udział barwy żółtej prawdopodobnie związany był z utlenianiem flawanoli. Z kolei, wzrost C* (*chroma*)

świadczy o zwiększonej klarowności produktu, co mogło być spowodowane sedymentacją koloidów w winie podczas przechowywania (De Souza i in. 2018).

W celu określenia, czy obserwowane zmiany mogą wpływać na ocenę jakości wina przez konsumentów, obliczono parametr ΔE*, który wskazuje, czy różnice w barwie pomiędzy próbką kontrolną a badaną mogą być dostrzeżone przez ludzkie oko (ΔE* ≥ 3) (Tkacz i in. 2020). W próbkach badanych bezpośrednio po ekspozycji na czynnik sterylizujący, zastosowanie CP przez 2, 5 lub 10 min (gaz roboczy He/O_2) nie wpłynęło na barwę wina w sposób zauważalny. Jednak wydłużenie czasu trwania procesu spowodowało wzrost wartości tego parametru z 0,19 do 3,04. Po przechowywaniu, najniższe wartości ΔE* odnotowano dla próbek wina konserwowanych CP lub metodą łączoną (He/N_2; 5 min), ΔE* odpowiednio 1,12 i 1,01. Ponadto zmiana barwy po zastosowaniu większości zabiegów była niezauważalna (ΔE* ≤ 3,0) lub prawie niezauważalna (ΔE* ≤6,0) dla ludzkiego oka, z wyjątkiem metody łączonej (2 min., He/O2) i konwencjonalnej (100 mg/L $K_2S_2O_5$): ΔE* odpowiednio 11,81 i 10,98.

Tabela 4. Wpływ metody konserwowania oraz procesu przechowywania na pH i barwę wina czerwonego

Kod Próbki*	pH	L*	a*	b*	C*	H*	ΔE*
1.	3,54± 0,01^{de}	11,23 ± 0,02^{e}	41,68 ± 0,08^{ef}	19,35 ± 0,07^{g}	45,96 ± 0,13^{ef}	24,91 ± 0,03^{e}	-
2.	3,55 ± 0,02^{e}	11,28 ± 0,03^{ef}	41,84 ± 0,22^{ef}	19,45 ± 0,11^{g}	46,14 ± 0,05^{fg}	24,93 ± 0,07^{e}	0,20 ± 0,01
3.	3,52 ± 0,01^{bcde}	11,81 ± 0,07^{g}	42,43 ± 0,07^{fghij}	20,36 ± 0,02^{h}	47,06 ± 0,07^{i}	25,64 ± 0,12^{f}	1,39 ± 0,09
4.	3,52 ± 0,01^{bcde}	12,56 ± 0,06^{ij}	43,16 ± 0,17^{jk}	21,65 ± 0,22^{l}	48,29 ± 0,06^{jk}	26,64 ± 0,09^{ij}	3,04± 0,12
5.	3,48 ± 0,01^{abcd}	11,02 ± 0,05^{e}	41,51 ± 0,16^{e}	19,01 ± 0,03^{f}	45,65 ± 0,0^{e}	24,61 ± 0,10^{e}	0,43 ± 0,02
6.	3,49 ± 0,01^{abcde}	13,26 ± 0,22^{k}	44,01 ± 0,12^{lmn}	22,87 ± 0,10^{m}	49,60 ± 0,23^{l}	27,46 ± 0,06^{m}	4,68 ± 0,16
7.	3,55 ± 0,02^{e}	12,55 ± 0,52^{ij}	43,29 ± 0,37^{kl}	21,70 ± 0,10^{l}	48,41 ± 0,19^{jk}	26,62 ± 0,03^{ij}	3,14 ± 0,09
8.	3,54 ± 0,01^{de}	14,79 ± 012^{n}	45,89 ± 0,04^{qr}	25,24 ± 0,10^{q}	52,37 ± 0,33^{p}	28,82 ± 0,20^{pq}	8,07 ± 0,22
9.	3,54 ± 0,01^{de}	12,31 ± 0,10^{hij}	43,05 ± 0,25^{ijk}	21,23 ± 0,14^{jk}	48,00 ± 0,14^{j}	26,24 ± 0,15^{h}	2,56 ± 0,18
10.	3,52 ± 0,00^{bcde}	12,69 ± 0,14^{j}	43,44 ± 1,27^{klm}	21,87 ± 0,05^{l}	48,64 ± 0,05^{k}	26,73 ± 0,17^{jk}	3,40 ± 0,10
11.	3,52 ± 0,01^{bcde}	14,10 ± 0,06^{m}	44,88 ± 0,12^{op}	23,98 ± 0,14^{o}	51,04 ± 0,14^{n}	28,51 ± 0,12^{op}	6,32 ± 0,13
12.	3,52 ± 0,02^{bcde}	14,33 ± 0,08^{m}	45,27 ± 0,11^{pq}	24,62 ± 0,10^{p}	51,54 ± 0,16^{o}	28,54 ± 0,18^{op}	7,09 ± 0,09
13.	3,51 ± 0,01^{bcde}	15,13 ± 0,14^{n}	46,08 ± 0,22^{qr}	25,80 ± 0,16^{r}	52,81 ± 0,12^{q}	29,24 ± 0,13^{rs}	8,73 ± 0,18
14.	3,48 ± 0,01^{abcd}	12,13 ± 0,15^{ghi}	42,75 ± 0,15^{ghijk}	20,91 ± 0,07^{i}	46,92 ± 0,11^{i}	26,06 ± 0,13^{gh}	2,09 ± 0,03
15.	3,52 ± 0,02^{bcde}	14,94 ± 0,06^{n}	46,16 ± 0,09^{r}	25,44 ± 0,12^{q}	52,79 ± 0,13^{pq}	29,03 ± 0,12^{qr}	8,42 ± 0,01

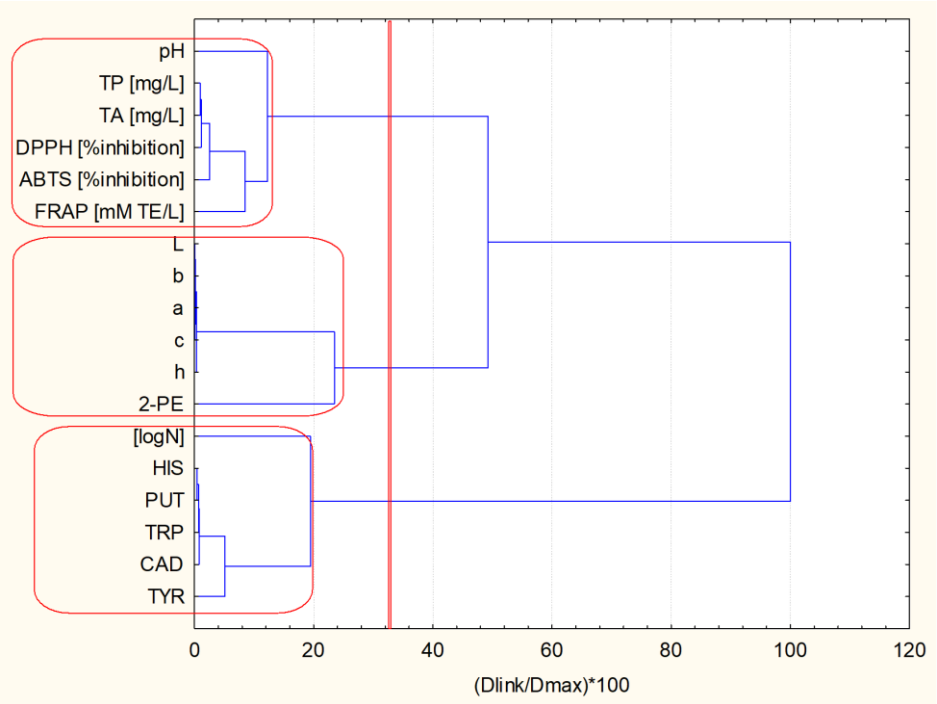
16.	3,53 ± 0,01^{cde}	11,71 ± 0,15^{fg}	42,22 ± 0,04^{efghi}	20,19 ± 0,14^{h}	46,80 ± 0,08^{hi}	25,55 ± 0,10^{f}	1,11 ± 0,12
17.	3,50 ± 0,02^{abcde}	10,18 ± 0,15^{d}	39,55 ± 0,05^{cd}	17,56 ± 0,11^{e}	43,27 ± 0,14^{d}	23,93 ± 0,12^{d}	2,97 ± 0,09
18.	3,48 ± 0,01^{abcd}	13,62 ± 0,09^{kl}	44,21 ± 0,07^{mno}	23,48 ± 0,09^{n}	50,05 ± 0,08^{m}	27,97 ± 0,10^{n}	5,40 ± 0,08
19.	3,47 ± 0,01^{abc}	9,72 ± 0,09^{c}	39,89 ± 0,12^{d}	16,75 ± 0,09^{d}	43,26 ± 0,12^{d}	22,80 ± 0,09^{c}	3,50 ± 0,10
20.	3,53 ± 0,02^{cde}	12,74 ± 0,08^{j}	42,99 ± 0,11^{ijk}	21,96 ± 0,09^{l}	48,28 ± 0,10^{jk}	27,05 ± 0,10^{kl}	3,29 ± 0,10
21.	3,50 ± 0,01^{abcde}	11,74 ± 0,10^{g}	42,13 ± 0,15^{efgh}	20,24 ± 0,14^{h}	46,74 ± 0,11^{hi}	25,67 ± 0,10^{f}	1,12 ± 0,12
22.	3,48 ± 0,01^{abcd}	13,91 ± 0,12^{lm}	44,34 ± 0,13^{no}	23,98 ± 0,12^{o}	50,41 ± 0,06^{m}	22,41 ± 0,09^{o}	5,97 ± 0,08
23.	3,53 ± 0,01^{cde}	12,35 ± 0,06^{hij}	43,05 ± 0,11^{jk}	21,30 ± 0,11^{k}	48,03 ± 0,14^{j}	26,32 ± 0,08^{hi}	2,63 ± 0,09
24.	3,53 ± 0,02^{cde}	6,6 ± 0,08^{a}	34,28 ± 0,14^{a}	11,39 ± 0,03^{a}	36,13 ± 0,04^{a}	18,37 ± 0,04^{a}	11,81 ± 0,07
25.	3,49 ± 0,01^{abcde}	12,73 ± 0,05^{j}	42,76 ± 0,06^{hijk}	21,97 ± 0,06^{l}	48,07 ± 0,07^{j}	27,18 ± 0,04^{lm}	3,21 ± 0,06
26.	3,47 ± 0,01^{abc}	8,94 ± 0,05^{b}	38,39 ± 0,07^{b}	15,41 ± 0,11^{b}	41,37 ± 0,04^{b}	21,87 ± 0,07^{b}	5,62 ± 0,09
27.	3,48 ± 0,00^{abcd}	12,03 ± 0,03^{gh}	41,92 ± 0,06^{efg}	20,73 ± 0,12^{i}	46,77 ± 0,09^{hi}	26,32 ± 0,04^{hi}	1,61 ± 0,09
28.	3,46 ± 0,01^{ab}	11,73 ± 0,08^{g}	41,79 ± 0,05^{ef}	20,22 ± 0,06^{h}	46,43 ± 0,09^{gh}	25,82 ± 0,05^{fg}	1,01 ± 0,07
29.	3,44 ± 0,03^{a}	9,44 ± 0,06^{c}	38,94 ± 0,06^{bc}	16,28 ± 0,06^{c}	42,21 ± 0,07^{c}	22,69 ± 0,07^{c}	4,49 ± 0,06
30.	3,47 ± 0,01^{abc}	16,52 ± 0,12^{o}	47,60 ± 0,09^{s}	26,94 ± 0,03^{s}	54,70 ± 0,05^{r}	29,51 ± 0,07^{s}	10,98 ± 0,05

Wartości oznaczone różnymi literami (a-s) w obrębie jednej kolumny różnią się istotnie ($P < 0,05$). Dane przedstawiono jako średnie ±SD (odchylenie standardowe). *sposób kodowania próbek przedstawiono w **Tabeli 1**.

W niniejszej pracy doktorskiej analiza chemometryczna została wykorzystana w celu opisania specyficznych zależności między zastosowaną metodą konserwacji i poszczególnymi parametrami świadczącymi o jakości wina (Publikacja **P4**).

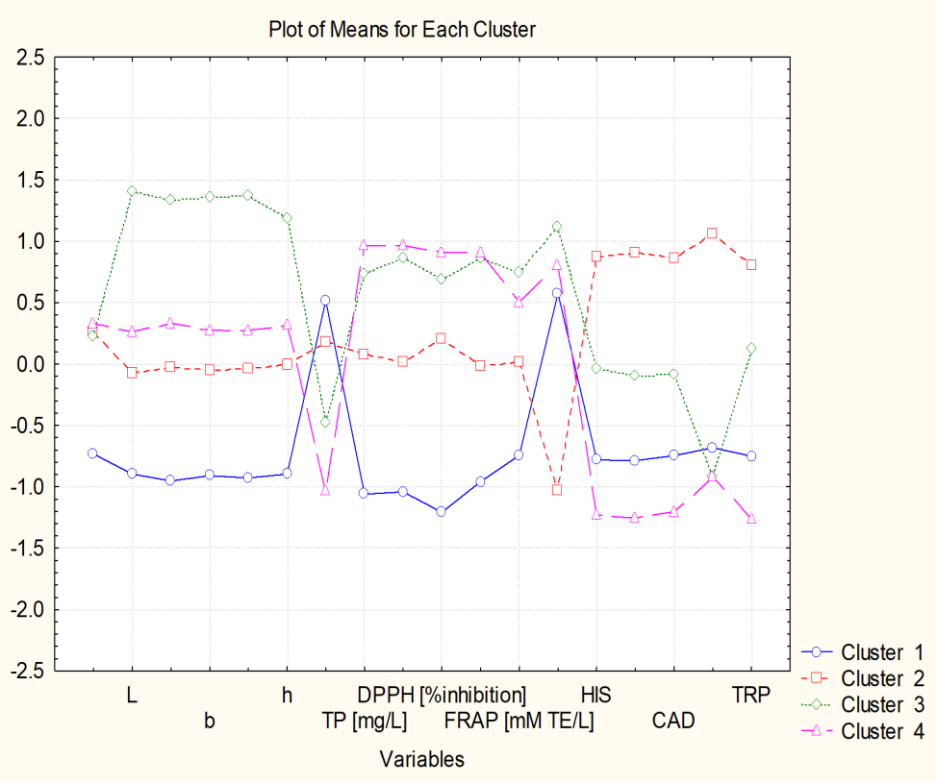
W wyniku hierarchicznej analizy skupień otrzymano dendrogram grupujący 18 zmiennych (**Rycina 6**). Wyodrębniono trzy główne skupienia (poziom istotności 1/3Dmax), które przedstawiają się następująco:

- pH, TP, TA, DPPH, ABTS, FRAP - klaster wskazuje na wpływ kwasowości, składu fenolowego i aktywności antyoksydacyjnej próbek (czynnik utleniania);
- L, a, b, c, h, 2-PE - klaster składający się w całości ze składowych barwy; i świadczy o wpływie barwy jako ważnego deskryptora badanego wina;
- LogN, HIS, PUT, TRP, CAD, TYR - skupisko trzecie jest reprezentacją powiązania pomiędzy parametrem mikrobiologicznym a zawartością amin biogennych (czynnik biologiczny).



Rycina 6. Hierarchiczny dendrogram dla powiązania 18 zmiennych. TP – ogólna zawartość fenoli; TA – ogólna zawartość antocyjanów; TRP – tryptamina; PUT – putrescyna; HIS – histamina; TYR – tyramina; CAD – kadaweryna; 2-PE – 2-fenyloetyloamina (Publikacja **P4**).

Niehierarchiczna analiza skupień została wykorzystana w celu sprawdzenia, które zmienne (deskryptory) są specyficzne dla podziału grupy obiektów (badane próby) na 4 skupienia. **Rycina 7** przedstawia średnie wartości poszczególnych zmiennych dla każdego ze zidentyfikowanych klastrów.



Rycina 7. Wykres średnich wartości każdej zmiennej dla każdego zidentyfikowanego skupienia. *Sekwencja zmiennych jest następująca: pH, L, a, b, c, h, logN, TP, TA, DDPH, ABTS, FRAP, 2-PE, HIS, PUT, CAD, TYR, TRP. ** TP – ogólna zawartość fenoli; TA – ogólna zawartość antocyjanów; TRP – tryptamina; PUT – putrescyna; HIS – histamina; TYR – tyramina; CAD – kadaweryna; 2-PE – 2-fenyloetyloamina (Publikacja **P4**).

Do skupienia pierwszego przypisano próbki nr: 19; 23; 24; 25; 26; 27; 28; 29 – wszystkie obiekty należące do tego skupienia były przechowywane przez 3 miesiące. Większość z nich konserwowano metodą łączoną z wyjątkiem próbki nr 19 (CP: 10 min., He/O_2) oraz nr 23 (metoda tradycyjna: 30 mg/L). Klaster ten charakteryzował się najniższymi poziomami parametrów barwy. Kolejną cechą tej grupy próbek był najniższy poziom parametrów antyoksydacyjnych oraz umiarkowany poziom amin biogennych. Zaobserwowano także najwyższy poziom log N i relatywnie wysoki poziom 2-PE.

Skupienie drugie obejmowało próbki nr: 1; 2; 3; 4; 5; 6; 7; 10; 16; 17; 18; 20; 21; 22, w którym, co warte podkreślenia, przyporządkowano zarówno próbki przed i po przechowywaniu konserwowane CP oraz próbki kontrolne. Charakteryzuje się ono umiarkowanymi poziomami prawie wszystkich zmiennych.

Pozostałe dwa skupienia są stosunkowo niewielkie. Skupienie trzecie (próbka nr 8; 12; 13; 15; 30), o najwyższym poziomie deskryptorów barwy, zawiera głównie próbki konserwowane metodą tradycyjną. Do klastra czwartego przypisano próbki konserwowane CP (He/O_2) - 9; 10, 11, 14. Charakteryzował się on niskim poziomem amin biogennych. Potwierdziło to nasze wcześniejsze obserwacje, że wydłużenie czasu działania zimnej plazmy oraz zastosowanie tlenu do generowania plazmy korzystnie wpływa na redukcję amin biogennych.

5. WNIOSKI I STWIERDZENIA

1. Na podstawie wykonanych analiz można stwierdzić, że skuteczność dekontaminacyjna zimnej plazmy zależy zarówno od rodzaju użytego gazu roboczego, jak również czasu trwania procesu.
2. Zastosowanie cytometrii przepływowej pozwoliło na wykrycie (po procesie plazmowania) subpopulacji komórek *L. hilgardii* średnio aktywnych metabolicznie, które w sprzyjających warunkach środowiska odzyskały zdolność do wzrostu w podłożu mikrobiologicznym.
3. Dziesięciominutowe działanie zimnej plazmy na komórki *L. hilgardii* doprowadziło do indukcji stanu VBNC.
4. Po raz pierwszy w literaturze przedmiotu, z wykorzystaniem techniki AFM, oceniono zmiany morfologiczne bakterii *L.hilgardii* poddanych dziesięciominutowemu działaniu CP, a następnie posortowano je na subpopulacje komórek żywych, średnio aktywnych oraz martwych.
5. Na skutek działania zimnej plazmy, subpopulacja komórek martwych *L. hilgardii* charakteryzowały się dużą chropowatością struktur zewnętrznych, mogącą wynikać z działania na ścianę komórkową bakterii zawartych w strumieniu plazmy reaktywnych związków tlenu.
6. Kształt komórek *L. hilgardii* w stanie pośrednim różnił się od kształtu komórek żywych i martwych. Zmiany morfologii komórek mogły być związane z adaptacją do niekorzystnych warunków środowiska.
7. Wpływ zimnej plazmy na zawartość związków fenolowych w próbkach czerwonego wina uzależniony był od warunków prowadzenia procesu oraz budowy chemicznej konkretnego związku reagującego ze składnikami plazmy.
8. Pięciominutowe działanie na próbki czerwonego wina zimną plazmą z gazem roboczym hel/azot wpływało w mniejszym stopniu na profil związków fenolowych, aktywność biologiczną oraz właściwości fizykochemiczne badanych próbek.
9. Niezależnie od zastosowanej metody konserwacji próbek czerwonego wina proces przechowywania spowodował redukcję ogólnej zawartości związków fenolowych oraz obniżenie aktywności przeciwutleniającej.

10. W przeprowadzonych badaniach po raz pierwszy wskazano możliwość wykorzystania zimnej plazmy do redukcji amin biogennych w czerwonym winie.
11. Zastosowanie zimnej plazmy nie wpłynęło na kwasowość oraz barwę badanych próbek czerwonego wina w stopniu zauważalnym dla konsumenta.
12. Przeprowadzone analizy wykazały, że po uprzednim doborze i optymalizacji warunków plazmowania, zimna plazma może stanowić alternatywną metodę konserwacji czerwonego wina.
13. Na podstawie analizy chemometrycznej wykazano po raz pierwszy, że istnieją specyficzne zależności między zastosowaną metodą konserwacji a poszczególnymi parametrami świadczącymi o jakości wina tj. barwa, właściwości przeciwutleniające, zawartość związków fenolowych i amin biogennych oraz czystość mikrobiologiczna.

6. LITERATURA

Afshari, R., & Hosseini, H. (2014). Non-thermal plasma as a new food preservation method, its present and future prospect. *Archives of Advances in Biosciences*, *5*(1).

Borges, A., Ferreira, C., Saavedra, M. J., & Simões, M. (2013). Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial Drug Resistance*, *19*(4), 256-265. https://doi.org/10.1089/mdr.2012.0244

Bourke, P., Ziuzina, D., Han, L., Cullen, P. J., & Gilmore, B. F. (2017). Microbiological interactions with cold plasma. *Journal of Applied Microbiology*, *123*(2), 308-324. https://doi.org/10.1111/jam.13429

Brisset, J. L., & Pawlat, J. (2016). Chemical effects of air plasma species on aqueous solutes in direct and delayed exposure modes: discharge, post-discharge and plasma activated water. *Plasma Chemistry and Plasma Processing*, *36*(2), 355-381. https://doi.org/10.1007/s11090-015-9653-6

Casquete, R., Benito, M. J., Pérez-Nevado, F., Martínez, A., Martín, A., & de Guía Córdoba, M. (2021). Addition of grape skin and stems extracts in wines during the storage to reduce the sulfur dioxide: Impact on red wine quality. *International Journal of Environmental Research and Public Health*, *18*(5), 2783. https://doi.org/10.3390/ijerph18052783

Choi, S., Puligundla, P., & Mok, C. (2016). Corona discharge plasma jet for inactivation of *Escherichia coli* O157: H7 and Listeria monocytogenes on inoculated pork and its impact on meat quality attributes. *Annals of Microbiology*, *66*(2), 685-694. https://doi.org/10.1007/s13213-015-1147-5

Cordero-Bueso, G., Moraga, J., Ríos-Carrasco, M., Ruiz-Muñoz, M., Cantoral, J. M. (2019). Bacteriophages as an Up-and-Coming Alternative to the Use of Sulfur Dioxide in Winemaking. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.02931

De Souza, J. F., de Souza Nascimento, A. M., Linhares, M. D. S. S., Dutra, M. D. C. P., dos Santos Lima, M., & Pereira, G. E. (2018). Evolution of phenolic compound profiles and antioxidant activity of Syrah red and sparkling Moscatel wines stored in bottles of different colors. *Beverages*, *4*(4), 89. https://doi.org/10.3390/beverages4040089

Dolezalova, E., & Lukes, P. (2015). Membrane damage and active but nonculturable state in liquid cultures of *Escherichia coli* treated with an atmospheric pressure

plasma jet. *Bioelectrochemistry*, *103*, 7-14. https://doi.org/10.1016/j.bioelechem.2014.08.018

Dumitriu, G. D., Teodosiu, C., Gabur, I., Cotea, V. V., Peinado, R. A., & López de Lerma, N. (2021). Alternative Winemaking Techniques to Improve the Content of Phenolic and Aromatic Compounds in Wines. *Agriculture*, *11*(3), 233. https://doi.org/10.3390/agriculture11030233

Ekezie, F. G. C., Sun, D. W., & Cheng, J. H. (2017). A review on recent advances in cold plasma technology for the food industry: Current applications and future trends. *Trends in Food Science & Technology*, 69, 46-58. https://doi.org/10.1016/j.tifs.2017.08.007

El-Aziz, M. F., Mahmoud, E. A., & Elaragi, G. M. (2014). Non thermal plasma for control of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae). *Journal of Stored Products Research*, *59*, 215-221. https://doi.org/10.1016/j.jspr.2014.03.002

Gajek, M., Pawlaczyk, A., & Szynkowska-Jozwik, M. I. (2021). Multi-Elemental Analysis of Wine Samples in Relation to Their Type, Origin, and Grape Variety. *Molecules, 26*(1), 214. https://doi.org/10.3390/molecules26010214

Garofuli´c, I. E., Jambrak, A. R., Milo˘sevi´c, S., Dragovi´c-Uzelac, V., Zori´c, Z., & Herceg, Z. (2015). The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca (*Prunus cerasus* var. *Marasca*) juice. *LWT-Food.Science and Technology*, *62*(1), 894–900. https://doi.org/10.1016/j.lwt.2014.08.036

Gris, E. F., Mattivi, F., Ferreira, E. A., Vrhovsek, U., Pedrosa, R. C., & Bordignon-Luiz, M. T. (2013). Phenolic profile and effect of regular consumption of Brazilian red wines on in vivo antioxidant activity. *Journal of Food Composition and Analysis*, *31*(1), 31-40. https://doi.org/10.1016/j.jfca.2013.03.002

Haunschild, R., & Marx, W. (2022). On Health Effects of Resveratrol in Wine. International *Journal of Environmental Research and Public Health*, *19*(5), 3110. https://doi.org/10.3390/ijerph19053110

He, F., Liang, N. N., Mu, L., Pan, Q. H., Wang, J., Reeves, M. J., & Duan, C. Q. (2012). Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their color expression. *Molecules*, *17*(2), 1571–1601. https://doi.org/10.3390/molecules17021571

Herceg, Z., Kovaˇcevi´c, D. B., Kljusuri´c, J. G., Jambrak, A. R., Zori´c, Z., & Dragovi´c-Uzelac, V. (2016). Gas phase plasma impact on phenolic compounds in pomegranate juice. *Food Chemistry*, *190*, 665–672. https://doi.org/10.1016/j.foodchem.2015.05.135

Hojnik, N., Cvelbar, U., Tavčar-Kalcher, G., Walsh, J. L., & Križaj, I. (2017). Mycotoxin decontamination of food: Cold atmospheric pressure plasma versus "classic" decontamination. *Toxins*, *9*(5), 151. https://doi.org/10.3390/toxins9050151

Hou, Y., Wang, R., Gan, Z., Shao, T., Zhang, X., He, M., & Sun, A. (2019). Effect of cold plasma on blueberry juice quality. *Food Chemistry*, *290*, 79-86. https://doi.org/10.1016/j.foodchem.2019.03.123

Jahid, I. K., Han, N., & Ha, S. D. (2014). Inactivation kinetics of cold oxygen plasma depend on incubation conditions of *Aeromonas hydrophila* biofilm on lettuce. *Food Research International*, *55*, 181-189. https://doi.org/10.1016/j.foodres.2013.11.005

Khan, M. S. I., Lee, E. J., & Kim, Y. J. (2016). A submerged dielectric barrier discharge plasma inactivation mechanism of biofilms produced by *Escherichia coli* O157: H7, *Cronobacter sakazakii*, and *Staphylococcus aureus*. *Scientific Reports*, *6*(1), 1-11. https://doi.org/10.1038/srep37072

Kiwi, J., & Nadtochenko, V. (2005). Evidence for the mechanism of photocatalytic degradation of the bacterial wall membrane at the TiO2 interface by ATR-FTIR and laser kinetic spectroscopy. *Langmuir*, *21*(10), 4631-4641. https://doi.org/10.1021/la0469831

Koźmiński, C., Mąkosza, A., Michalska, B., & Nidzgorska-Lencewicz, J. (2020). Thermal Conditions for Viticulture in Poland. *Sustainability*, *12*(14), 5665. https://doi.org/10.3390/su12145665

Lackmann, J. W., Schneider, S., Edengeiser, E., Jarzina, F., Brinckmann, S., Steinborn, E., Havenith, M., Benedikt J. & Bandow, J. E. (2013). Photons and particles emitted from cold atmospheric-pressure plasma inactivate bacteria and biomolecules independently and synergistically. *Journal of The Royal Society Interface*, *10*(89), 20130591. https://doi.org/10.1098/rsif.2013.0591

Lacombe, A., Niemira, B. A., Gurtler, J. B., Fan, X., Sites, J., Boyd, G., & Chen, H. (2015). Atmospheric cold plasma inactivation of aerobic microorganisms on blueberries and effects on quality attributes. *Food Microbiology*, *46*, 479-484. https://doi.org/10.1016/j.fm.2014.09.010

Laroussi, M., Mendis, D. A., & Rosenberg, M. (2003). Plasma interaction with microbes. *New Journal of Physics*, *5*(1), 41. https://doi.org/10.1088/1367-2630/5/1/341

Laskowska, M., Boguslawska-Was, E., Kowal, P., Holub, M., & Dabrowski, W. (2016). Skuteczność wykorzystania niskotemperaturowej plazmy w mikrobiologii i medycynie. Postępy Mikrobiologii, 55(2).

Liao, X., Liu, D., Xiang, Q., Ahn, J., Chen, S., Ye, X., & Ding, T. (2017). Inactivation mechanisms of non-thermal plasma on microbes: A review. *Food Control*, *75*, 83-91. https://doi.org/10.1016/j.foodcont.2016.12.021

Ling, L., Jiafeng, J., Jiangang, L., Minchong, S., Xin, H., Hanliang, S., & Yuanhua, D. (2014). Effects of cold plasma treatment on seed germination and seedling growth of soybean. *Scientific Reports*, *4*(1), 1-7. https://doi.org/10.1038/srep05859

Liu, J., Deng, Y., Li, L., Li, B., Li, Y., Zhou, S., ... & Peters, B. M. (2018). Discovery and control of culturable and viable but non-culturable cells of a distinctive Lactobacillus harbinensis strain from spoiled beer. *Scientific Reports*, *8*(1), 1-10. https://doi.org/10.1038/s41598-018-28949-y

Maciejewska, M., Bauer, M., & Dawgul, M. (2016). Nowoczesne metody zwalczania biofilmu bakteryjnego. *Postępy Mikrobiologii*, *55*(1).

Metelmann, H. R., Seebauer, C., Miller, V., Fridman, A., Bauer, G., Graves, D. B., ... & Von Woedtke, T. (2018). Clinical experience with cold plasma in the treatment of locally advanced head and neck cancer. *Clinical Plasma Medicine*, *9*, 6-13. https://doi.org/10.1016/j.cpme.2017.09.001

Moreau, M., Orange, N., & Feuilloley, M. G. J. (2008). Non-thermal plasma technologies: new tools for bio-decontamination. *Biotechnology Advances*, *26*(6), 610-617. https://doi.org/10.1016/j.biotechadv.2008.08.001

Niu, D., Wang, L. H., Zeng, X. A., Wen, Q. H., Brennan, C. S., Tang, Z. S., & Wang, M. S. (2019). Effect of ethanol adaption on the inactivation of *Acetobacter* sp. by pulsed electric fields. *Innovative Food Science & Emerging Technologies*, *52*, 25-33. https://doi.org/10.1016/j.ifset.2018.11.009

Pan, Y., Cheng, J. H., & Sun, D. W. (2019). Cold plasma-mediated treatments for shelf life extension of fresh produce: A review of recent research developments. *Comprehensive Reviews in Food Science and Food Safety*, *18*(5), 1312-1326. https://doi.org/10.1111/1541-4337.12474

Pankaj, S. K., Wan, Z., Colonna, W., & Keener, K. M. (2017). Effect of high voltage atmospheric cold plasma on white grape juice quality. *Journal of the Science of Food and Agriculture*, *97*(12), 4016-4021. https://doi.org/10.1002/jsfa.8268

Pienaar, J. A., Singh, A., & Barnard, T. G. (2016). The viable but non-culturable state in pathogenic *Escherichia coli*: a general review. *African Journal of Laboratory Medicine*, *5*(1), 1-9. https://doi.org//10.4102/ajlm.v5i1.368

Polčic, P., Pakosová, L., Chovančíková, P., & Machala, Z. (2018). Reactive cold plasma particles generate oxidative stress in yeast but do not trigger apoptosis. *Canadian Journal of Microbiology*, *64*(6), 367-375. https://doi.org/10.1139/cjm-2017-0753.

Pozo-Bayón, M. Á., Monagas, M., Bartolome, B., & Moreno-Arribas, M. V. (2012). Wine features related to safety and consumer health: an integrated perspective. *Critical Reviews In Food Science and Nutrition*, *52*(1), 31-54. https://doi.org/10.1080/10408398.2010.489398

Puligundla, P., Pyun, Y. R., & Mok, C. (2018). Pulsed electric field (PEF) technology for microbial inactivation in low-alcohol red wine. *Food Science and Biotechnology*, *27*(6), 1691-1696. https://doi.org/10.1007/s10068-018-0422-1

Rasines-Perea, Z., & Teissedre, P. L. (2017). Grape polyphenols' effects in human cardiovascular diseases and diabetes. *Molecules*, *22*(1), 68. https://doi.org/10.3390/molecules22010068

Ravi, J., Hills, A. E., Cerasoli, E., Rakowska, P. D., & Ryadnov, M. G. (2011). FTIR markers of methionine oxidation for early detection of oxidized protein therapeutics. European *Biophysics Journal*, *40*(3), 339-345. https://doi.org/10.1007/s00249-010-0656-1

Rodas, A. M., Ferrer, S., & Pardo, I. (2005). Polyphasic study of wine *Lactobacillus* strains: taxonomic implications. International *Journal of Systematic and Evolutionary Microbiology*, *55*(1), 197-207. https://doi.org/10.1099/ijs.0.63249-0

Rodríguez-Vaquero, M. J., Vallejo, C. V., & Aredes-Fernández, P. A. (2020). Antibacterial, antioxidant and antihypertensive properties of polyphenols from argentinean red wines varieties. *Open Journal of Pharmacology and Pharmacotherapeutics*, *5*(1), 001-006. https://doi.org/10.17352/ojpp.000010

Sáenz-Navajas, M. P., Echavarri, F., Ferreira, V., & Fernández-Zurbano, P. (2011). Pigment composition and color parameters of commercial Spanish red wine samples: Linkage to quality perception. *European Food Research and Technology*, *232*(5), 877-887. https://doi.org/10.1007/s00217-011-1456-2

Sarangapani, C., O'Toole, G., Cullen, P. J., & Bourke, P. (2017). Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. *Innovative Food Science & Emerging Technologies*, *44*, 235-241. https://doi.org/10.1016/j.ifset.2017.02.012

Schottroff, F., Fröhling, A., Zunabovic-Pichler, M., Krottenthaler, A., Schlüter, O., & Jäger, H. (2018). Sublethal injury and viable but non-culturable (VBNC) state in microorganisms during preservation of food and biological materials by non-thermal processes. *Frontiers in Microbiology*, 2773. https://doi.org/10.3389/fmicb.2018.02773

Shi, X. M., Zhang, G. J., Wu, X. L., Li, Y. X., Ma, Y., & Shao, X. J. (2011). Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. *IEEE Transactions on Plasma Science*, *39*(7), 1591-1597. https://doi.org/10.1109/TPS.2011.2142012

Silva, F. V., & Van Wyk, S. (2021). Emerging non-thermal technologies as alternative to SO_2 for the production of wine. *Foods*, *10*(9), 2175. https://doi.org/10.3390/foods10092175

Snopek, L., Mlcek, J., Sochorova, L., Baron, M., Hlavacova, I., Jurikova, T., ... & Sochor, J. (2018). Contribution of red wine consumption to human health protection. *Molecules*, *23*(7). https://doi.org/10.3390/molecules23071684

Tappi, S., Gozzi, G., Vannini, L., Berardinelli, A., Romani, S., Ragni, L., & Rocculi, P. (2016). Cold plasma treatment for fresh-cut melon stabilization. *Innovative Food Science & Emerging Technologies*, *33*, 225-233. https://doi.org/10.1016/j.ifset.2015.12.022

Tkacz, K., Modzelewska-Kapituła, M., Więk, A., & Nogalski, Z. (2020). The applicability of total color difference ΔE for determining the blooming time in longissimus lumborum and semimembranosus muscles from Holstein-Friesian bulls at different ageing times. *Applied Sciences*, *10*(22), 8215. https://doi.org/10.3390/app10228215

Tong, J., He, R., Zhang, X., Zhan, R., Chen, W., & Yang, S. (2014). Effects of atmospheric pressure air plasma pretreatment on the seed germination and early growth of *Andrographis paniculata*. *Plasma Science and Technology*, *16*(3), 260. https://doi.org/10.1088/1009-0630/16/3/16

Wang, R. X., Nian, W. F., Wu, H. Y., Feng, H. Q., Zhang, K., Zhang, J., ... & Fang, J. (2012). Atmospheric-pressure cold plasma treatment of contaminated fresh fruit

and vegetable slices: inactivation and physiochemical properties evaluation. *The European Physical Journal D*, *66*(10), 1-7.https://doi.org/10.1140/epjd/e2012-30053-1

Wiktor, A., Sledz, M., Nowacka, M., & Witrowa-Rajchert, D. (2013). Możliwości zastosowania niskotemperaturowej plazmy w technologii żywności. *Żywność Nauka Technologia Jakość*, *20*(5).

Yang, P., Yuan, C., Wang, H., Han, F., Liu, Y., Wang, L., & Liu, Y. (2018). Stability of anthocyanins and their degradation products from cabernet sauvignon red wine under gastrointestinal pH and temperature conditions. *Molecules*, *23*(2), 354. https://doi.org/10.3390/molecules23020354

Yoon, J. H., Bae, Y. M., & Lee, S. Y. (2017). Effects of varying concentrations of sodium chloride and acidic conditions on the behavior of *Vibrio parahaemolyticus* and *Vibrio vulnificus* cold-starved in artificial sea water microcosms. *Food Science and Biotechnology*, *26*(3), 829-839. https://doi.org/10.1007/s10068-017-0105-3

Yue, X. F., Jing, S. S., Ni, X. F., Zhang, K. K., Fang, Y. L., Zhang, Z. W., & Ju, Y. L. (2021). Anthocyanin and phenolic acids contents influence the color stability and antioxidant capacity of wine treated with mannoprotein. *Frontiers in Nutrition*, *8*, 691784. https://doi.org/10.3389/fnut.2021.691784

7. ZESTAWIENIE DOROBKU NAUKOWEGO

RB-XV-68/2022

21.10.2022, Lublin

Biblioteka Główna UP w Lublinie
Bibliografia Publikacji Pracowników Uniwersytetu Przyrodniczego w Lublinie

Raport autora: mgr inż. Iwona Niedźwiedź

1. Publikacje w czasopismach naukowych

1.1. Publikacje w czasopiśmie naukowym posiadającym Impact Factor IF

Lp	Opis bibliograficzny	IF	Pkt. ministerialne
1.	**Biological activity of an *Epilobium angustifolium* L. (fireweed) infusion after in vitro digestion.** [AUT.] KLAUDIA KOWALIK, [AUT. KORESP.] MAGDALENA POLAK-BERECKA, [AUT.] MONIKA PRENDECKA-WRÓBEL, DOMINIKA PIGOŃ-ZAJĄC, IWONA NIEDŹWIEDŹ, DOMINIK SZWAJGIER, EWA BARANOWSKA-WÓJCIK, ADAM WAŚKO. *Molecules (Basel, Online)* 2022 Vol. 27 Issue 3 Article number 1006, il., bibliogr., sum. DOI: 10.3390/molecules27031006	4,927	140,00
2.	**Comparison of the effect of cold plasma with conventional preservation methods on red wine quality using chemometrics analysis.** [AUT. KORESP.] IWONA NIEDŹWIEDŹ, [AUT.] VASIL SIMEONOV, ADAM WAŚKO, MAGDALENA POLAK-BERECKA. *Molecules (Basel, Online)* 2022 Vol. 27 Issue 20 Article number 7048, il., bibliogr., sum. DOI: 10.3390/molecules27207048	4,927	140,00
3.	**The impact of cold plasma on the phenolic composition and biogenic amine content of red wine.** [AUT.] IWONA NIEDŹWIEDŹ, JUSTYNA PŁOTKA-WASYLKA, IRENEUSZ KAPUSTA, VASIL SIMEONOV, ANNA STÓJ, ADAM WAŚKO, JOANNA PAWŁAT, [AUT. KORESP.] MAGDALENA POLAK-BERECKA. *Food Chem.* 2022 Vol. 381 Article number 132257, il., bibliogr., sum. DOI: 10.1016/j.foodchem.2022.132257	9,231	200,00
4.	**New insight into bacterial interaction with the matrix of plant-based fermented foods.** [AUT.] KLAUDIA GUSTAW, IWONA NIEDŹWIEDŹ, [AUT. KORESP.] KAMILA RACHWAŁ, [AUT.] MAGDALENA POLAK-BERECKA. *Foods* 2021 Vol. 10(7) Article number 1603, il., bibliogr., sum. DOI: 10.3390/foods10071603	5,561	100,00
5.	**Morphological and physiological changes in *Lentilactobacillus hilgardii* cells after cold plasma treatment.** [AUT.] IWONA NIEDŹWIEDŹ, WOJCIECH JUZWA, KRZYSZTOF SKRZYPIEC, TOMASZ SKRZYPEK, ADAM WAŚKO, MICHAŁ KWIATKOWSKI, JOANNA PAWŁAT, [AUT. KORESP.] MAGDALENA POLAK-BERECKA. *Sci. Rep. (Nat. Publ. Group)* 2020 Vol.10 Article number: 18882, il., bibliogr. sum. DOI: 10.1038/s41598-020-76053-x	4,380	140,00

6.	**The state of research on antimicrobial activity of cold plasma.** [AUT.] IWONA NIEDŹWIEDŹ, ADAM WAŚKO, JOANNA PAWŁAT, [AUT. KORESP.] MAGDALENA POLAK-BERECKA. *Pol. J. Microbiol.* 2019 Vol. 68 No. 2 153-164, il., bibliogr., sum. DOI: 10.33073/pjm-2019-028	0,897	40,00
	Suma:	29,923	760,00

2. Monografie naukowe

2.1 Autorstwo rozdziału w monografii naukowej

Lp	Opis bibliograficzny	Pkt. ministerialne
1.	**Modele *in vitro* w badaniach nad biodostępnością składników odżywczych.** [AUT.] KLAUDIA KOWALIK, IWONA NIEDŹWIEDŹ, ADAM WAŚKO, MAGDALENA POLAK-BERECKA. W: Substancje bioaktywne w surowcach i produktach spożywczych. Systemy produkcji i pakowania żywności zapewniające ich zachowanie w łańcuchu żywnościowym pod redakcją Joanny Stadnik Lublin 2022, Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie, s. 165-186, 978-83-7259-353-5.	20,00
2.	**Innovative technologies in sustainable food production: cold plasma processing.** [AUT.] MAGDALENA POLAK-BERECKA, IWONA NIEDŹWIEDŹ. W: Sustainable production technology in food / edited by José M. Lorenzo, Paulo E.S. Munekata, Francisco J. Barba Amsterdam 2021, Academic Press, s. 165-177, il, bibliogr., sum, 978-0-12-821233-2. DOI: 10.1016/B978-0-12-821233-2.00007-1	50,00
3.	**Nietermiczne metody utrwalania żywności (Non-thermal methods of food preservation).** [AUT. KORESP.] NIEDŹWIEDŹ IWONA, [AUT.] POLAK-BERECKA MAGDALENA. W: Badania i rozwój młodych naukowców w Polsce. Nauki przyrodnicze. Część III: Żywienie i żywność Poznań 2019, Młodzi Naukowcy, s. 120-125, ilu. bibliogr., streszcz, 978-83-66139-81-7.	5,00
	Suma:	75,00

3. Materiały konferencyjne

Lp	Opis bibliograficzny
1.	**Cytometria przepływowa jako metoda do sortowania mikroorganizmów zanieczyszczających wina w oparciu o stan fizjologiczny komórki.** [AUT. KORESP.] IWONA NIEDŹWIEDŹ, [AUT.] MAGDALENA POLAK-BERECKA. W: Badania i rozwój młodych naukowców w Polsce 2020. Materiały konferencyjne- wiosna. Część III. Redakcja naukowa dr. Jędrzej Nyćkowiak i dr. hab Jacek Leśny s. 69. Poznań 2020, Młodzi Naukowcy, 978-83-66392-65-6.
2.	**Induction of the viable but non-culturable state in food contaminating microorganisms by non thermal processes of preservation.** [AUT. KORESP.] IWONA NIEDŹWIEDŹ, [AUT.] MAGDALENA POLAK-BERECKA. W: Badania i rozwój młodych naukowców w Polsce 2020. Materiały konferencyjne-

wiosna. Część III. Redakcja naukowa dr. Jędrzej Nyćkowiak i dr. hab Jacek Leśny s. 68. Poznań 2020, Młodzi Naukowcy, 978-83-66392-65-6.

3. **Mechanizmy tolerancji mikroorganizmów na alkohol (Mechanisms of alcohol tolerance of microorganisms).** [AUT.] IWONA NIEDŹWIEDŹ, MAGDALENA POLAK-BERECKA. W: Przegląd badań prowadzonych przez doktorantów s. 36. Lublin 2020, Uniwersytet Przyrodniczy w Lublinie, 978-83-7259-318-4. DOI: 10.24326/srzp.2020.1

4. **High hydrostatic pressure technology for microbial inactivation in low-alcohol beverages.** [AUT.] IWONA NIEDŹWIEDŹ, M[AGDALENA] POLAK-BERECKA. W: Rolnictwo - żywność - zdrowie : VI Forum Młodych Przyrodników, Lublin 25.05.2019 : program i streszczenia prac / redakcja: Paulina Gil-Kulik, Jolanta Karwat s. 57. Lublin 2019, Stowarzyszenie Młodych Naukowców, 978-83-939764-5-4.

5. **Impact of critical proces parameters on the anibacterial effectiveness of low-temperature plasma.** [AUT.] IWONA NIEDŹWIEDŹ, MAGDALENA POLAK-BERECKA. W: Badania i Rozwój Młodych Naukowców w Polsce 2019 : materiały konferencyjne - jesień. Część 3 - Lublin / Redakcja naukowa Jędrzej Nyćkowiak, Jacek Leśny s. 56. Poznań 2019, Młodzi Naukowcy, 978-83-66392-58-8.

6. **Novel techniques for microbial decontamination in the food industry.** [AUT.] IWONA NIEDŹWIEDŹ, MAGDALENA POLAK-BERECKA. W: Badania i rozwój młodych naukowców w Polsce 2019. Materiały konferencyjne - wiosna. Część pierwsza - Lublin / Red. nauk. Jędrzej Nyćkowiak, Jacek Leśny s. 101. Poznań, Młodzi Naukowcy, 978-83-66139-83-1.

7. **Peptydy przeciwdrobnoustrojowe w zwalczaniu biofilmu.** [AUT.] ANTONINA KRAWCZYK, AGNIESZKA VOGT, IWONA NIEDŹWIEDŹ, MAGDALENA POLAK-BERECKA. W: XVI Międzynarodowe Seminarium Studenckich Kół Naukowych nt. "Środowisko - Zwierzę - Produkt" : VI Konferencja Doktorantów, Lublin, 8 kwietnia 2019 r. / Uniwersytet Przyrodniczy w Lublinie s. 97. Lublin 2019, Wydaw. Uniwersytetu Przyrodniczego.

8. **Probiotyki jako składnik żywności funkcjonalnej.** [AUT.] KLAUDIA KNIEĆ, AGATA KUSIO, IWONA NIEDŹWIEDŹ, MAGDALENA POLAK-BERECKA. W: XVI Międzynarodowe Seminarium Studenckich Kół Naukowych nt. "Środowisko - Zwierzę - Produkt" : VI Konferencja Doktorantów, Lublin, 8 kwietnia 2019 r. / Uniwersytet Przyrodniczy w Lublinie s. 96. Lublin 2019, Wydaw. Uniwersytetu Przyrodniczego.

9. **Skuteczność antybakteryjna niskotemperaturowej plazmy.** [AUT.] IWONA NIEDŹWIEDŹ, MAGDALENA POLAK-BERECKA. W: XVI Międzynarodowe Seminarium Studenckich Kół Naukowych nt. "Środowisko - Zwierzę - Produkt" : VI Konferencja Doktorantów, Lublin, 8 kwietnia 2019 r. / Uniwersytet Przyrodniczy w Lublinie s. 176. Lublin 2019, Wydaw. Uniwersytetu Przyrodniczego.

10. **Drożdże *Saccharomyces cerevisiae* jako organizmy modelowe.** [AUT.] PATRYK OSTANEK, IWONA NIEDŹWIEDŹ, MAGDALENA MICHALAK. W: XV Międzynarodowe Seminarium Studenckich Kół Naukowych nt. "Środowisko - Zwierzę - Produkt" : V Konferencja Doktorantów, Lublin, 17 kwietnia 2018 r. / Uniwersytet Przyrodniczy w Lublinie s. 87, Tytuł równolegle w języku angielskim. Lublin 2018, Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie.

11. **Potencjał antyoksydacyjny bakterii probiotycznych.** [AUT.] MAGDALENA MICHALAK, IWONA NIEDŹWIEDŹ, MAGDALENA POLAK-BERECKA. W: XV Międzynarodowe Seminarium Studenckich Kół Naukowych nt. "Środowisko - Zwierzę - Produkt" : V Konferencja Doktorantów, Lublin, 17 kwietnia 2018 r. / Uniwersytet Przyrodniczy w Lublinie s. 126, Tytuł równolegle w języku angielskim. Lublin 2018, Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie.

12. **Potencjał antyoksydacyjny bakterii probiotycznych.** [AUT.] M[AGDALENA] MICHALAK, IWONA NIEDŹWIEDŹ, MAGDALENA POLAK-BERECKA. W: XV Międzynarodowe Seminarium Studenckich Kół Naukowych nt. "Środowisko - Zwierzę - Produkt" : V Konferencja Doktorantów, Lublin, 17 kwietnia 2018 r. / Uniwersytet Przyrodniczy w Lublinie s. 126, Tytuł równolegle w języku angielskim.

Lublin 2018, Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie.

13. **Właściwości przeciwzapalne flawonoidów.** [AUT.] IWONA NIEDŹWIEDŹ, URSZULA SZYMANOWSKA. W: XV Międzynarodowe Seminarium Studenckich Kół Naukowych nt. "Środowisko - Zwierzę - Produkt" : V Konferencja Doktorantów, Lublin, 17 kwietnia 2018 r. / Uniwersytet Przyrodniczy w Lublinie s. 69, Tytuł równolegle w języku angielskim. Lublin 2018, Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie.

14. **Wpływ polifenoli na mikrobiotę przewodu pokarmowego.** [AUT.] MAGDALENA MICHALAK, IWONA NIEDŹWIEDŹ, PATRYK OSTANEK, MAGDALENA POLAK-BERECKA. W: XV Międzynarodowe Seminarium Studenckich Kół Naukowych nt. "Środowisko - Zwierzę - Produkt" : V Konferencja Doktorantów, Lublin, 17 kwietnia 2018 r. / Uniwersytet Przyrodniczy w Lublinie s. 133, Tytuł równolegle w języku angielskim. Lublin 2018, Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie.

15. **Wykorzystanie metody PCR – DGGE w analizie mikroorganizmów różnych środowisk.** [AUT.] MAGDALENA MICHALAK, KAROLINA BARYŁA, ILONA DRĄG, IWONA NIEDŹWIEDŹ. W: XV Międzynarodowe Seminarium Studenckich Kół Naukowych nt. "Środowisko - Zwierzę - Produkt" : V Konferencja Doktorantów, Lublin, 17 kwietnia 2018 r. / Uniwersytet Przyrodniczy w Lublinie s. 157, Tytuł równolegle w języku angielskim. Lublin 2018, Wydawnictwo Uniwersytetu Przyrodniczego w Lublinie.

Sumarycznie: IF 29,923; 835,00 pkt. MNiSW/MEiN

Szczegółowe wyjaśnienia:

- Wskaźnik Impact Factor został podany na podstawie bazy Journal Citation Reports (JCR) dla roku wydania publikacji z wyjątkiem publikacji z roku 2022, gdzie jego wartość została podana na podstawie ostatniej edycji JCR ed. 2021
- Punktacja została podana na podstawie Wykazu Czasopism Punktowanych MNiSW/MEiN obowiązujących dla roku wydania publikacji z wyjątkiem:
  • publikacji z 2017, którym punkty zostały przypisane na podstawie "Wykazu czasopism naukowych zawierający historię czasopisma z publikowanych wykazów za lata 2013-2016";
  • publikacji z roku 2019, 2020, 2021, 2022, którym punkty zostały przypisane na podstawie: „Komunikatu Ministra Edukacji i Nauki z dnia 21 grudnia 2021 r. o zmianie i sprostowaniu komunikatu w sprawie wykazu czasopism naukowych i recenzowanych materiałów z konferencji międzynarodowych".
- Punktacja za monografie została przyznana na podstawie rozporządzeń obowiązujących w konkretnych latach opublikowania prac

Wykaz przygotowała:
Katarzyna Brzezińska
/mgr Katarzyna Brzezińska/

8. ZAŁĄCZNIKI

- kopie publikacji naukowych wchodzących w skład rozprawy doktorskiej.

Polish Journal of Microbiology
2019, Vol. 68, No 2, 153–164
https://doi.org/10.33073/pjm-2019-028

MINIREVIEW

The State of Research on Antimicrobial Activity of Cold Plasma

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Submitted 17 January 2019, revised 15 April 2019, accepted 17 April 2019

Abstract

Microbiological contamination is a big challenge to the food industry, medicine, agriculture, and environmental protection. For this reason, scientists are constantly looking for alternative methods of decontamination, which ensure the effective elimination of unwanted biological agents. Cold plasma is a new technology, which due to its unique physical and chemical properties becomes a point of interest to a growing group of researchers. The previously conducted experiments confirm its effective action, e.g. in the disinfection of skin wounds, air, and sewage treatment, as well as in food preservation and decontamination. The reactive compounds present in the plasma: high-energy electrons, ionized atoms and molecules, and UV photons are the key factors that cause an effective reduction in the number of microorganisms. The mechanism and effectiveness of the cold plasma are complex and depend on the process parameters, environmental factors and the type and properties of the microorganisms that are to be killed. This review describes the current state of knowledge regarding the effectiveness of the cold plasma and characterizes its interaction with various groups of microorganisms based on the available literature data.

Key words: biofilm, cellular response, cold plasma, mycotoxin, viruses

Introduction

Cold plasma (CP) or nonthermal plasma (NTP) and, in particular, cold atmospheric plasma (CAP) is gaining increasing scientists' interest, given the possibility of application thereof in medicine (Boudam and Moisan 2010; Isbary et al. 2010; Vandamme et al. 2010; Metelmann et al. 2018), food industry (Afshari and Hosseini 2014; Hojnik et al. 2017, Pignata et al. 2017), agriculture, and environmental protection (Bogaerts and Neyts 2018; Pawłat et al. 2018a; 2018b; Siddique et al. 2018). Many investigations have confirmed the positive effect of the plasma on the anticancer therapy (Kim et al. 2009; Vandamme et al. 2010), disinfection of skin wounds (Isbary et al. 2010; Lademann et al. 2011), surgical instruments and materials in contact with food (Boudam and Moisan 2010; Dasan et al. 2017b), purification of air, water, wastewater, and sewage, as well as preservation and decontamination of food (Gallagher et al. 2007; Korachi et al. 2009; Pawłat 2013; Chizoba Ekezie et al. 2017; Wolny-Koładka et al. 2017). Such a broad spectrum of the cold plasma applications is associated with its ability to inactivate biological factors as viruses (Terrier et al. 2009; Su et al. 2018), bacteria (Isbary et al. 2010; Samoń et al. 2014; Kartaschew et al. 2016), spores (Deng et al. 2006; Boudam and Moisan 2010), yeasts (Korachi et al. 2009; Metelmann et al. 2018), or fungi (Bayliss et al. 2012; Panngom et al. 2014; Siddique et al. 2018).

William Crookes discovered plasma in 1879 while the concept of the plasma was first used in the article by Irving Langmuir's entitled "Oscillations in ionized gases" in 1928. Since then, plasma physics has become an important field of research. The plasma processing is used from the seventies when it was used to etch semiconductor materials. Then, in the 1980s, it was used in the computer industry, especially in the production of miniaturized circuits. In the last decade of the twentieth century, there has been a development of the plasma generation technology at an atmospheric pressure, which allowed to eliminate the expensive vacuum chambers. This has resulted in wider applications of

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cold plasma in medicine, environmental protection and food preservation (Misra et al. 2016).

Plasma, which is regarded as the fourth state of matter is a type of ionized gas containing many charged particles (OH^-, H_2O^+, electrons), reactive compounds (reactive oxygen species – ROS, which include hydroxyl radical, superoxide anion hydrogen peroxide, and reactive nitrogen species – RNS, i.e., peroxynitrite), the molecules in the excited and basic states, and UV photons (UVB, UVC) (Brisset and Pawłat 2016; Bruggeman et al. 2016).

Plasma can be classified according to the generation conditions, i.e. atmospheric pressure (low-pressure plasma, high-pressure plasma), temperature (low-temperature plasma, high-temperature plasma), and the composition of plasma-generating gas (one-component plasma, multi-component plasma) (Dzimitrowicz et al. 2015; Bourke et al. 2017). The division of the plasma due to the temperature depends on the temperature of the electrons (T_e). The high-temperature plasma is characterized by $T_e = 10^6 \sim 10^8$ K, whereas for the low-temperature plasma the T_e value is in the range from 10^4 to 10^5 K (Fridman et al. 2005). In addition, the low-temperature plasma is divided into thermal and non-thermal plasma due to the thermodynamic equilibrium. Inactivation of biological factors uses a non-thermal plasma that is characterized by a thermodynamic imbalance. Therefore, the electrons have a higher temperature than the temperature of the neutral particles (T_n), and the temperature of the gas (T_g), and thus, the temperature of the process increases slightly (Laskowska et al. 2016; Liao et al. 2017).

Non-equilibrium plasma (with temperatures of electrons substantially exceeding the temperatures of the other gas components) can be generated with the use of various electric discharges, e.g. corona, microwave, gliding arc, and dielectric barrier discharges as plasma sources. As a result of these discharges, the energy from the electric field is collected by electrons as a result of their collision, and only part of the energy is transferred to neutral molecules which results in the formation of the $T_e \geq T_n$ state, characterized by non-thermal plasma. The type of the plasma source has directed the effect on the composition and the number of components generated by the plasma and determines the technological application of the plasma. The temperature of the plasma is extremely important in the processing of the materials that are sensitive to high temperatures. The optimization of the power supply system, an appropriate geometry of the discharge system, and the type of gas are essential when active agents generated by cold plasma (e.g. reactive compounds), whose temperature does not exceed several tens of degrees Celsius, have to be standardized. For the applications in agriculture, food industry, and medicine, the dielectric barrier discharges (DBD) and plasma jets are used most frequently for the generation of cold plasma due their simple structure and ease of modification (Pawłat et al. 2016; Bourke et al. 2017).

The mechanism involved in the cold plasma sterilization process has not yet been elucidated. The numerous attempts made by scientists confirm the inactivation of virus particles and microbial cells by the plasma, but the relationships between them have not been explored in details (Liao et al. 2017). The reactive compounds, high-energy electrons, ionized atoms and particles, and UV radiation are involved in the process of biological inactivation (Bourke et al. 2017). When in contact with biological material, the compounds derived from oxygen or nitrogen, i.e., O, O_2, O_3, OH·, NO, and NO_2 are characterized by high reactivity. Their effects include oxidation of membrane lipids and proteins, which results in the disturbances of the proper membrane function and, finally, the disruption of the cell membrane (Afshari and Hosseini 2014). Membrane integrity is highly influenced by electrostatic forces. The charged particles generated by the plasma accumulate on the outer side of the membrane, thereby leading to its disintegration (Liao et al. 2017). The discontinuity of the cell surface structures can also be an effect of the electroporation process. This phenomenon involves an increase in the number of the existing cell micropores and the emergence of new ones induced by the pulsed electric field (Wiktor et al. 2013). The degradation of DNA caused by UV radiation could also be involved in the inactivation of microorganisms. Photons present in the plasma can alter the structure of the nucleic acids, leading to the formation of nitrogen base dimers and impairment of DNA replication capacity (Beggs 2002; Liao et al. 2017). The contribution of each of the mechanisms and their effectiveness in biological inactivation vary and depend primarily on the parameters of the plasma generation process, environmental factors, and the type and properties of the microorganisms (Fig. 1) (Bayliss et al. 2012).

The aim of this review is to present the current knowledge on the antimicrobial activity of the plasma and to discuss the molecular mechanism of interactions between cold atmospheric plasma and various groups of microorganisms.

Effect of cold plasma on viruses

The cold plasma technology becomes a promising solution for inactivation of pathogenic viruses that cause infections in humans, animals, and plants (Bourke et al. 2017). The specific mechanisms of CP inactivation of viruses have not been elucidated yet. The studies carried out so far demonstrated that exposure to cold plasma can lead to the modification and/or degradation of proteins, nucleic acids, and lipids of viral envelopes (Pradeep and Chulkyoon 2016). The

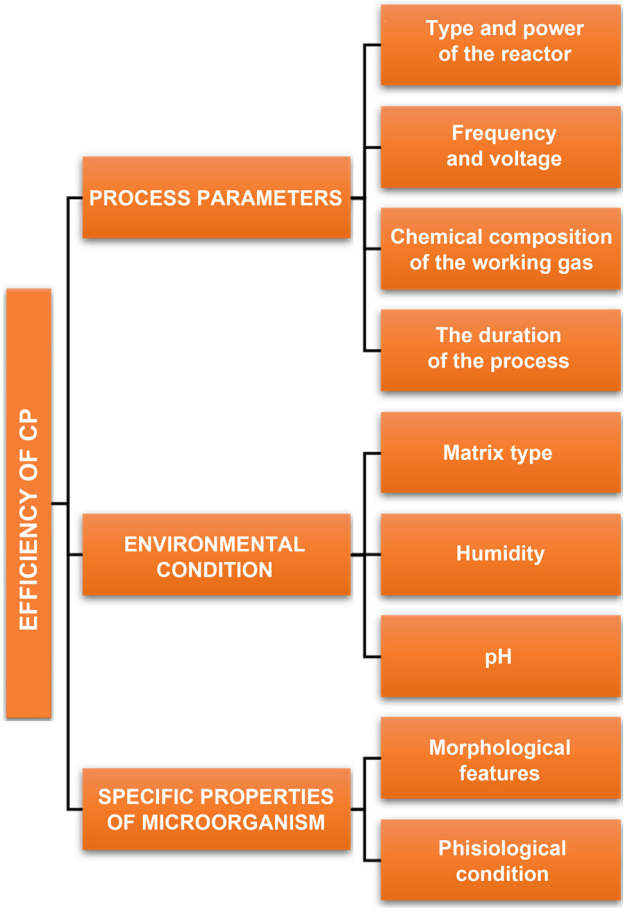

Fig. 1. Factors influencing the effectiveness of decontamination using cold plasma. Based on Liao et al. 2017.

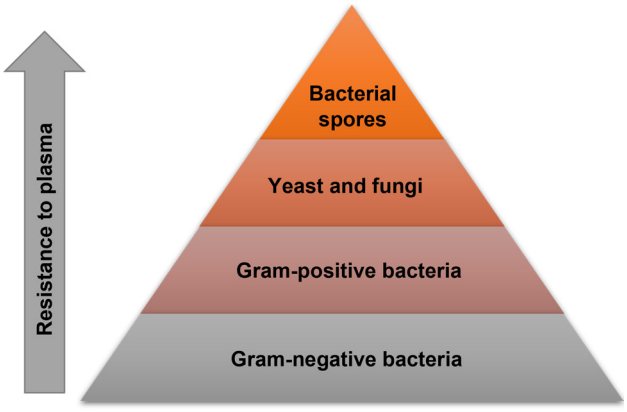

Fig. 2. Pyramid of the sensitivity of microorganisms to plasma*. Based on Klämpfl T.G. et al. 2012; and Liao et al. 2017.

* Sensitivity of individual groups of microorganisms can vary depending on the conditions of the process.

researches on the impact of the plasma on bacteriophages (a λ phage model system) suggested that the damage of capsid proteins is directly involved in the inactivation of viruses (Yasuda et al. 2010).

Cold plasma has been demonstrated to inactivate animal viruses. Terrier et al. (2009) investigated three types of viruses with the considerable clinical importance, which cause respiratory infections, e.g. respiratory syncytial virus (RSV), human parainfluenza virus type 3 (HPIV-3), and influenza A virus subtype H5N2. The plasma generated with air as the working gas contributed to a significant decrease in the titer of all viruses tested. Researchers indirectly associated this finding with the presence of ozone in the generated plasma that induces the protein damage and lipid peroxidation. The effect of the plasma on adenoviruses was analyzed by Zimmermann et al. (2011), who used 4.7-kV micro-discharges for the generation of the plasma. These viruses have double-stranded DNA (dsDNA) and exhibit low sensitivity to physical and chemical factors. They are causative agents of ophthalmological, respiratory, and gastrointestinal diseases. Using a green fluorescent protein (GFP) and a firefly luciferase, the researchers demonstrated that both viral infectivity and replication were inhibited. The activity of reactive nitrogen species (RNS) and their intermediates was proposed as a mechanism of the plasma effect on the DNA structure and immunogenicity of the virus. Some investigations were focused on the susceptibility of noroviruses (NoV), which cause gastrointestinal infections and pose a major problem in the food industry. Ahlfeld et al. (2015) studied the effect of CP on the inactivation of NoV from fecal samples. They treated the samples with a plasma stream and evaluated the effectiveness of inactivation at a variable time of the process. They achieved 1.23 log and 1.69 log reduction in the viral titer after 10 and 15 minutes, respectively (Bourke et al. 2017). The recent research also suggests that plasma-activated solutions (PASs) can contribute to effective decontamination. The effectiveness of inactivation of the Newcastle disease virus (ND) by the plasma-activated solutions (H_2O, 0.9% NaCl, 0.3% H_2O_2) was assessed. The scanning electron microscope's (SEM) images revealed morphological changes in virus particles, and the reduction of their titer and RNA degradation have also been shown (Su et al. 2018).

Effect of cold plasma on microbial cells

Microorganisms are a key target in the investigation of the plasma efficiency, as susceptibility to the sterilization process may vary between microorganisms, even within species and strains (Fig. 2). It largely depends on the structure of cellular envelopes and the microbial growth phase (Liao et al. 2017).

Bacteria

The exact mechanism of inactivation of bacterial cells by cold plasma is still unknown, although the issue has been extensively studied. Permeabilisation of

the cell membrane or cell wall leading to leakage of intracellular components, i.e. potassium, nucleic acids, and proteins, is regarded as one of the mechanisms of inactivation of this group of microorganisms. Furthermore, inactivation of bacteria occurs via the oxidative damage to intracellular proteins and DNA related to the effect of the plasma compounds (Mai-Prochnow et al. 2014). The overall mechanisms of microbial inactivation with plasma reactive species are presented in Fig. 3. The impact of the plasma stream on the external bacterial structures was investigated by Laroussi et al., who use the plasma generated with dielectric barrier discharge (DBD) against *Escherichia coli* and *Bacillus subtilis* cells. For Gram-negative *E. coli* with a cell wall composed of an outer membrane and thin peptidoglycan (murein), there was substantial damage to the membrane resulting in the cytoplasm leakage. The authors suggest that the visible changes resulted from the electrostatic rupture of the outer membrane. In turn, the microscopic image of Gram-positive *B. subtilis* cells with a thick cell wall did not show the significant morphological modifications and the decontamination was most probably due to interactions of reactive compounds with the intracellular components. The differences in the effect of the plasma on this microbial group are related to a different structure of cell walls in these microorganisms (Laroussi et al. 2003). A comprehensive analysis of chemical modifications induced by CAP in the structure of Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*) bacteria was performed by Kartaschew et al. (2016) with use of a Fourier Transform Infrared Spectroscopy (FTIR) technique. The spectral images revealed that the plasma caused an increase in the number of symmetric stretching vibrations that reveal the formation of carboxylic groups (COO^-). The investigators also noted the spectra in the absorbance range of 1720 cm^{-1} that indicate the formation of carbonyl groups (C = O) related to the presence of aldehydes, ketones, or acids. The resulting signals were associated with the destruction of the cell wall and cellular membrane by ROS. Another phenomenon induced by the activity of the plasma is the change of the cell membrane potential. This leads to disturbances in the function of the protonomotoric force and, consequently, abnormal ATP synthesis and the impairment of cell metabolism and division (Brun et al. 2018).

The changes in the membrane integrity may have a direct effect on DNA, mainly through the discontinuation of interactions with membrane proteins and formation of pores in the cell membrane by which nucleic acids can be released from the cell (Coutinho et al. 2018). Plasma-induced release of DNA is possible after the prior DNA fragmentation, and the effectiveness of this process depends on the conformation of the nucleic acid. In comparison with linear DNA, its supercoiled circular form is regarded as the resistant form of the nucleic acids (Moreau et al. 2008; Alkawareek et al. 2014). In general, it is believed that the plasma effect on DNA is a result of the activity of both reactive compounds and UV photons (Coutinho et al. 2018). In the cell, the DNA-protein crosslinks (DPCs) can be formed, which lead to the formation of hard-to-repair damage. Reactive oxygen and nitrogen species produced by the plasma oxidize proteins by the generation of hydroperoxide groups in their structure, which can form strong intramolecular crosslinks with the nucleic acids. In the experiment conducted by Guo et al. (2018), *E. coli* cells after treatment with the plasma stream with an air and 1% He were subjected to two versions of a Comet assay (with and without proteinase K) to assess the formation of DPC in DNA of the analyzed bacterium. In the fluorescent DNA image of the cells treated with the plasma,

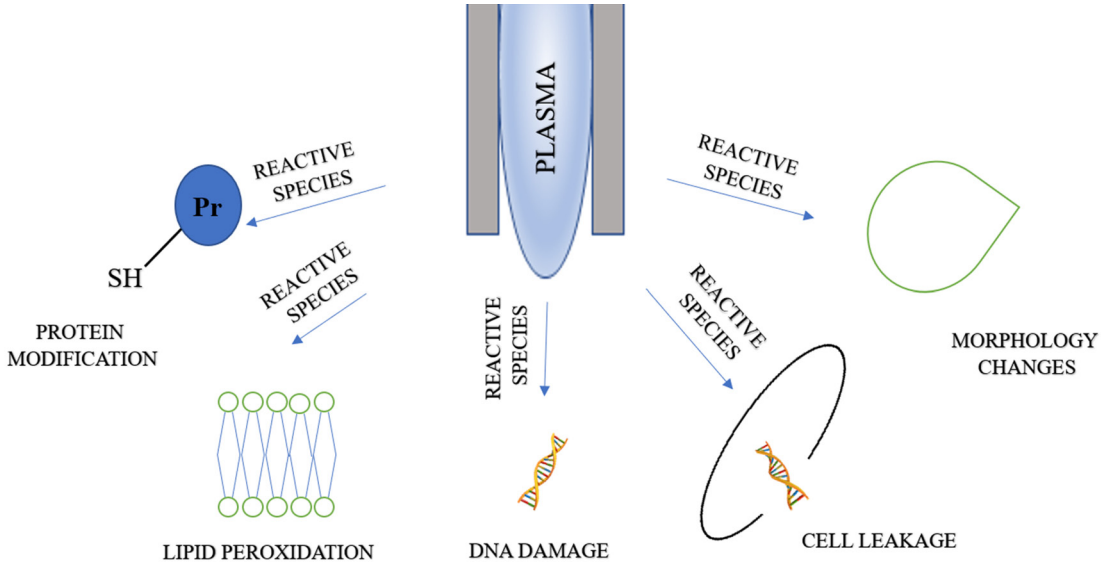

Fig. 3. Mechanisms of microbial inactivation with plasma reactive species. Based on Bourke et al. 2017.

the researchers observed short "tails" indicating the damaged fragments without proteinase treatment, and much longer tails after the treatment with proteinase. This suggested the presence of DPC crosslinks, which were destroyed by the enzyme digesting the proteins. Then, the proteins were by the CsCl density gradient ultracentrifugation, treated with a nuclease, and their analysis revealed a higher concentration and diversity of the proteins than in the samples that were not treated with the plasma. This may be another piece of evidence supporting the hypothesis that the plasma-induced damage to nucleic acids is a result of the protein-DNA covalent bonding. An important issue is the CP effect on the genes encoding antibiotic resistance. The resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with the *mecA* gene, which encodes protein *PBP2a* characterized by low affinity to β-lactam antibiotics. The most recent investigations of the CP applications for inactivation of *S. aureus* MRSA demonstrated that the cumulative energy delivered to the surface of the plasma-treated sample was 0.12 kJ/cm^2 and 0.35 kJ/cm^2, respectively, when air was used as the working gas. Five-log reduction in the number of *S. aureus* MRSA was observed even at the lower energy values, whereas higher values (0.35 kJ/cm^2) were required for more effective degradation of the resistance gene. The isolated *mecA* gene exhibited higher sensitivity to the plasma activity compared to the intracellular gene. This is closely related to the protective effect of cell constituents, which are the first to interact with the reactive plasma components (Liao et al. 2018). The investigations also highlighted the changes in the membrane potential and integrity induced by the various intensities of the plasma activity. After application of the plasma treatment with a cumulative energy level of 0.12 kJ/cm^2, the authors observed that a membrane potential coefficient was close to the depolarization value (Liao et al. 2018).

The use of cold plasma leads to the degradation of cellular proteins. Large proteins (50–90 kDa) are destroyed as the first. It was revealed by analysis of the samples where the concentration of the proteins with a molecular mass below 25 kDa increased after the plasma treatment (Hosseinzadeh Colagar et al. 2013). The probable mechanism of the degradation consists of the destruction of hydrogen, sulfide, and peptide bonds by reactive compounds present in the plasma. This results in the changes of the primary, secondary, and tertiary protein structure that lead to a decline in the enzymatic activity of the cell (Mai-Prochnow et al. 2014). Recently, researchers have investigated the effect of CP on proteins in thermophilic bacteria that pose a serious problem in the food industry due to their high resistance to the classical decontamination methods, i.e. high temperature or chemical denaturing agents. Attri et al. (2018) treated a highly stable MTH 1880 protein from the thermophilic bacterium *Methanobacterium thermoautotrophicum* with CP generated by DBD and analyzed it with the circular dichroism (CD), fluorescence, and nuclear magnetic resonance (NMR) spectroscopy techniques. Additionally, molecular dynamics (MD) simulation was carried out for both the native and plasma-treated protein. The investigators achieved a partial destabilization of MTH protein only after a long time of exposure to CP (20 min), which resulted in a decrease of 5°C in its melting point and an increase in its susceptibility to thermal denaturation. To elucidate the mechanism of the plasma effect on cellular proteins, the proteomic profile of *Salmonella enteritidis* was determined and as the result, 1096 proteins were identified with 249 of them present only in the plasma-treated samples and nine only in control samples. Under the impact of CP, the proteins that were overexpressed came mainly from the carbohydrate and nucleotide metabolism pathways and were associated with RNA transcription. It indicates an increased energy metabolism in cells as a defense response of bacteria (Ritter et al. 2018). The plasma-induced changes in bacterial metabolism were reported by Laroussi et al. (2003), who demonstrated the reduced utilization of L-lactic acid and increased consumption of D-sorbitol (Moreau et al. 2008). Interestingly, the effect of plasma on microorganisms does not always lead to cell death but can also reduce their metabolism, therefore, the cells do not undergo division and become viable but not culturable (VBNC). Dolezalova et al. (2015) treated a suspension of *E. coli* cells with the plasma and measured bacterial viability with a conventional culture method and by assessment of the fluorescent images of the cells stained with the LIVE/DEAD kits. The result of the former method suggested a 7.0-log reduction of the number of cells, whereas a reduction of only 1.0 log was indicated with the latter technique. These findings suggest that the plasma may induce the VBNC state of the cells.

Spores. Through evolutionary adaptation, bacterial spores have acquired the possibility to survive in adverse environmental conditions. They are characterized by resistance to disinfectants, chemical sterilants, drying processes, and thermal inactivation. This poses a serious threat to food and pharmaceutical industries as well as medicine, where spores are a permanent source of contamination (Liao et al. 2017). The major cause of such high resistance of spores is their structure, which differs substantially from that of vegetative forms of bacteria. Spores are composed of impermeable outer layers creating a specific barrier for the external factors. Additionally, they are characterized by low water content, which accounts for approximately 15% of the entire cell (Olesiak and Stępniak 2012). The interior of the spore contains a rigid structure, i.e. an inner membrane permeable only to small molecules (< 200 Daltons). When

the membrane is damaged, dipicolinic acid (DPA) is released and endospore germination is not induced. DPA ensures considerably higher (up to 50-fold) resistance to UV radiation in spores (Olesiak and Stępniak 2012). In the very core of the spore, the DNA protection function against chemical and physical agents is fulfilled by small acid-soluble SASP proteins, which are closely related to the nucleic acids (Kądzielska et al. 2012). The precise mechanism of the plasma effect on spores has not been clarified to date. Some researchers suggest that spores are inactivated mainly via interactions between the reactive plasma compounds and the external spore structures (Hong et al. 2009). The morphological changes in *B. subtillis* endospores induced by various CF-related factors were presented by Deng et al. (2006) on scanning electron microscopy (SEM) images. The changes in the spore size, leakage of the cytoplasm content, and final membrane disruption were observed. These results suggest that oxidation by reactive oxygen species is the main factor contributing to the reduction of the number of viable *B. subtilis* cells, whereas the electric field, UV photons, and charged particles play a minor role in this process. Other investigators demonstrated that inactivation of endospores was primarily caused by damage to their outer layers resulting in leakage of DPA and hydration of the core (Tseng et al. 2012). The importance of the external structures for resistance to cold plasma was analyzed by Raguse et al. (2016), who investigated wild-type *B. subtilis* spores and mutants deficient in some surface structures. Additionally, the investigators examined the effect of the gas applied on the effectiveness of sterilization. Their results indicated a substantial impact of surface structures and the type of gas on the resistance of spores. A mixture of oxygen and argon was the most effective in inactivation of bacterial spores, as it caused significant damage to the external layers. In contrast, the mutants deficient in surface structures exhibited the highest sensitivity to plasma. The recent studies conducted by Connor et al. (2017) also have emphasized the impact of environmental conditions on plasma efficiency. The studies on the resistance of *Clostridium difficile* spores to plasma were conducted in three different environments (the spores suspended in water and dry spores with or without 0.03% BSA). The greatest reduction in the spore number in the shortest time was observed in a dry environment. In contrast, organic matter and moist environment extended the time of the spore inactivation by plasma (Klampf et al. 2014).

Another aspect that should be explored more deeply is the mechanism of CP effect on sporal DNA. There is a contradictory data on the effect of UV photons generated in the sterilization process on nucleic acids. The results of investigations conducted by Tseng et al. (2012) did not show DNA degradation in *B. subtilis* spores after a 20-min exposure to the plasma. This finding can be supported by the conclusion reported by Fiebrandt et al. (2016), who suggested that cell layers absorb UV radiation, thereby protecting DNA from damage. This mechanism is plausible given the structure of spores. Surface structures of spores are characterized by a high content of proteins with amino acid side chains forming endogenous chromophores. These compounds can be the main target of photooxidation, thereby protecting the cell interior from harmful radiation.

Some investigations have indicated that the plasma inactivates spores by the impact on key metabolic proteins. In their study, Dobrynin et al. (2010) suggested that reactive oxygen species penetrating the cell interior could cause oxidation of proteins involved in germination or inactivate germination receptors located in the inner membrane of spores. In turn, Wang et al. (2011) compared the kinetics of germination of the cold plasma-treated *B. subtilis* spores and untreated spores. They conducted the experiments in an environment enriched with nutrient germinants (L-valine) and non-nutrient germinants (dodecylamine, Ca^{2+}DPA). Their results indicated the potential inactivation effects of the plasma on germination receptors but the germination induced by L-valine was inhibited.

Bacterial biofilm. Many microorganisms live in the environment as biofilms rather than free-living organisms. Biofilm was defined as the cells adhering to a solid surface and surrounded by an extracellular matrix produced by them (Czapka et al. 2018). Such populations exhibit higher resistance to adverse external factors (antibiotics, temperature, and pH); therefore, they pose a serious challenge in both medicine and food industry (Maciejewska et al. 2016). There are numerous reports demonstrating the sensitivity of biofilms to CF; however, the time required for full inactivation thereof is longer than for planktonic cells (Mai-Prochnow et al. 2014; Flynn et al. 2015). This was confirmed by Jahid et al. (2014), who compared the effect of CP on planktonic *Aeromonas hydrophila* cells and bacterial biofilm on the surface of lettuce. The experiment showed that a 15-s plasma treatment was sufficient to reduce the number of planktonic populations by > 5 logs. In contrast, the cell population on the lettuce surface was substantially reduced after a 5-min process of the plasma treatment (Jahid et al. 2014; Bourke et al. 2017). The extracellular matrix constitutes approx. 90% of biofilm and its presence largely determines the effectiveness of sterilization processes (Czyzewska-Dors et al. 2018). The matrix composition varies and depends on species of microorganisms forming the biofilm. The basic components of the extracellular matrix are polysaccharides, lipids, proteins, and nucleic acids. These compounds constitute a protective barrier against antibiotics or temperature as well as photons, reactive

compounds, and charged particles in the plasma. Since these agents have to penetrate the protective layer, the biofilm inactivation time is prolonged (Mai-Prochnow et al. 2014). The results of investigations conducted by Ermolaeva et al. (2011) demonstrated the differences in the survival of bacteria in different biofilm layers, suggesting that the effectiveness of CP depends on the biofilm thickness. Microscopic evaluation of the viability of *P. aeruginosa* biofilm showed a greater number of bacterial cells in its deeper layers. This probably explains the proportional decline of the sterilization effectiveness with the increasing biofilm thickness. Another determinant of the sterilization effectiveness is the species of biofilm-forming microorganisms. This issue was investigated by Mai-Prochnow et al. (2016) who compared the effects of CP on bacterial biofilm formed by Gram-positive *B. subtillis* and Gram-negative *P. aeruginosa*. Almost complete reduction of the bacteria in Gram-negative biofilm was observed in contrast to Gram-positive biofilm where a 10-min plasma treatment resulted in <1 log reduction. The presence of reactive oxygen compounds contributing to the cell wall disintegration in the emission spectra suggests that the cell wall thickness may be correlated with the duration of the cold-plasma inactivation. In conclusion, the sterilants present in the plasma inactivate biofilms through damage to the extracellular matrix, cell wall, cellular membrane, and internal cell structures. They can also induce the VBNC state in the cells (Ziuzina et al. 2015; Bourke et al. 2017).

Bacterial cell response to cold-plasma treatment. Elucidation of mechanisms of cell response to the cold plasma treatment is an important issue and requires further exploration. Hitherto, the changes in gene transcription and expression induced by exposure of cells to plasma have been already analyzed. The available scientific publications demonstrated a potential increase in the expression of SOS regulon, oxidation-related genes, and the genes encoding DNA repair processes. In turn, the expression of housekeeping genes was reduced (Sharma et al. 2009; Roth et al. 2010). Sharma et al. investigated the effect of plasma on genomic DNA in *E. coli* and performed a microarray analysis of the samples after 2-min plasma exposure. The increased expression of the gene involved in superoxide radical scavenging (*katG*) and the *recA* gene responsible for DNA recombination and repair was noted (Sharma et al. 2009). Similar results were reported by Roth et al. (2010) who analyzed the samples of highly radiation-resistant bacteria (*Deinococcus radiodurans*) and found an increase in the expression of the genes involved in DNA repair, oxidative stress responses, and cell wall synthesis. The process of the nucleic acids repair may result in the emergence of mutations that will determine increased resistance to CP. UV radiation, which induces the formation of nitrogen base dimers in nucleic acids is the main mutagenic factor. On the other hand, ROS and RNS present in the plasma exert a destructive effect on cellular components; thus, contribute to bacterial death and minimize the conservation of mutation effects (Boxhammer et al. 2013).

Yeast

Yeasts, which are a valuable source of many enzymes, are widely used in food biotechnology and microbiology in both fermentation and food-enrichment processes (Krzyczkowska et al. 2008). With their low pathogenicity as well as a unique structure and properties, some yeast species, e.g. *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* have become an inseparable element of molecular biology, serving as a model for an eukaryotic organism. Furthermore, a simple and cost-efficient culture of yeasts has contributed to the increasing interest in these organisms as objects for the elucidation of the effect of CP on cells (Wawrzycka 2011). The experiments were performed by Nishime and coworkers (2017) on the effectiveness of the plasma generated by DBD discharges with helium addition against various microorganisms. The researchers studied *Enterococcus faecalis* (Gram-positive) and *P. aeruginosa* (Gram-negative) bacteria as well as *Candida albicans* yeasts. They reported an inactivating effect of the plasma on all of the microorganisms investigated. Nevertheless, in comparison with the bacterial cells, *C. albicans* exhibited higher resistance, which can be explained mainly by the differences in their cell structure. In turn, the investigations conducted by Colonna et al. (2017) were focused on the effect of the plasma generated with dry air and a gas mixture (65% O_2, 30% CO_2, 5% N_2) on *S. cerevisiae* cells at a different density of cellular suspension and duration of the exposure. The results confirmed a correlation between the effectiveness of sterilization and the process parameters. For the samples with higher cellular density, a longer timer of exposure of the suspension to the plasma was required for a complete cell degradation. Moreover, the reduction effect monitored at various time points indicated higher efficiency of a plasma stream with a gas mixture. The effect of environmental conditions on the effectiveness of inactivation was examined by Ryu et al. (2013), who observed a decrease of the number of *S. cerevisiae* cells suspended in different media (water, saline, and YPD). The most serious CP-induced damage to membrane lipids and genomic DNA was observed in yeast cells suspended in water. This was associated with the highest content of hydroxyl radicals generated in the water medium during the process.

Polcic et al. (2018), who used genetic mutants of yeasts to identify the role of oxidative stress and apoptosis

in the decontamination process made an attempt at the elucidation of the mechanisms of CP effect on *S. cerevisiae*. The results of the experiments indicated higher susceptibility of strains with superoxide dismutase deficiency than those deprived of the key components of the apoptotic pathway, as their sensitivity to the plasma activity did not change. This proves that reactive oxygen species are one of the most important factors involved in the inactivation of yeast cells, and the apoptosis process itself does not play a key role in this case. An important issue requiring comprehensive investigations is the impact of CP on intracellular proteins. The treatment of *S. cerevisiae* with argon plasma was found to lead to protein ubiquitination and formation of the insoluble protein aggregates in the yeast cytoplasm. These researchers also underlined the potential of the CP-induced generation of endoplasmic reticulum (ER) stress, which is characteristic of eukaryotic cells. This was confirmed by an increase in the activity of the endoplasmic reticulum transmembrane protein Ire1p induced by accumulation of unfolded proteins in ER (Itooka et al. 2018). As demonstrated in many research reports, the cold-plasma technique can be used for inactivation of enzymes (Li et al. 2011; Surowsky et al. 2013; Tolouie et al 2017; Tolouie et al. 2018). The authors suggest that the ability of plasma to inhibit enzymes is associated with loss of the protein secondary structure. It is an effect of interactions with reactive compounds generated in the gas used in the process (Misra et al. 2016). Colonna et al. (2017) investigated the impact of CP on enzymes produced by yeasts in a study on the plasma generated with dry air. Their analyses were focused on *S. cerevisiae* yeast invertase, which lost its activity at a level of > 96% after 75 s of the treatment.

Fungi

Plant diseases caused by fungal pathogens pose a serious crisis in agriculture, as they cause huge economic losses worldwide. As demonstrated in the recent research reports, the cold-plasma technology can become an alternative plant protection method either by inactivating fungal cells or improving the resistance of infected hosts (Dasan et al. 2017a; Siddique et al. 2018). The investigations that confirmed the susceptibility of this microbial group to the CP activity have been conducted by many researchers (Suhem et al. 2013; Sohbatzadeh et al. 2016; Nikmaram et al. 2018). Some of them suggested that the inactivation of fungal cells using CP mainly involves the production of reactive compounds which seems to have their destructive effect on the cell wall and inner membrane of fungi (Ye et al. 2012; Lu et al. 2014; Dasan et al. 2016; Dasan et al. 2017a). In their study, Ye et al. (2012) assessed the effect of the plasma (working gas: air) generated by corona discharges on the cells of *Penicillium expansum*, i.e. one of the most important pathogens causing spoilage of the stored fruit. SEM images revealed disruption of the external structures of *P. expansum*, which resulted in cytoplasmic leakage. In turn, an analysis of transmission electron microscopy (TEM) images indicated the plasma-induced alterations in the cell, i.e. an increased volume of the protoplasm, stretching of vacuoles, and disintegration of the membrane, which contributed to cell lysis.

Besides their impact on external structures and intracellular organelles, the reactive compounds present in the plasma and the generated UV radiation cause damage to nucleic acids and oxidation of proteins and lipids. Lu et al. (2014) have investigated the effect of CP on *Cladosporium fulvum* by determination of the concentration of malondialdehyde (MDA), i.e. the basic product of lipid peroxidation. The findings reported confirmed the hypothesis that the activity of sterilizing agents in fungal cells triggers the peroxidation process. The similar observations were reported by another research team investigating the effect of CP generated at atmospheric pressure on *Aspergillus flavus* cells. The investigators determined the activity of thiobarbituric acid (TBA) in the samples, which showed a linkage between the membrane damage and lipid oxidation process (Simoncicova et al. 2018).

The other mechanism that may reduce the number of fungal cells is apoptosis or necrosis, as suggested by Panngom et al. (2014) to identify the mechanism of inactivation of *Fusarium oxysporum* cells, the authors stained the cells with Annexin V and propidium iodide and treated them with argon plasma for 1, 5, and 10 min. The numbers of viable, necrotic, and apoptotic cells were analyzed with flow cytometry. The majority of the cells were propidium iodide-stained after 5- and 10-min of the treatment with CP. This indicates that the necrosis process was the main mechanism of inactivation of the fungal cells.

An important aspect that requires further research is the increase in resistance to fungal pathogens in the plasma-treated plants. The experiments conducted by Filatova et al. (2016) demonstrated a substantially reduced incidence of diseases caused by *Fusarium* spp. and *Ustilago maydis* in wheat, lupine, and maize after exposure of the seeds to CP. The stimulating and fungicidal effect of the plasma resulted in an increase in the yield of the spring wheat, maize, and lupine seeds by 4–6%, 1.5–2%, and 20–40%, respectively, in comparison with the control. After additional assays that should be carried out in the future, CP may become an alternative to chemical plant protection agents, which may effectively minimize the negative impact of fungicides on the environment (Siddique et al. 2018).

Cold-plasma inactivation of mycotoxins. The UN Food and Agriculture data indicate that 25% of world

crops are contaminated with mycotoxins produced by fungi during plant growth or crop storage. *Aspergillus*, *Fusarium*, and *Penicillium* molds are the major producers of toxic secondary metabolites, including aflatoxin, fumonisin, zearalenone, ochratoxin, and deoxynivalenol, which are most toxic to mammals. The elimination of these compounds from food products is problematic due to their high thermostability; for instance, the temperature of aflatoxin degradation ranges from 237 to 306°C (Pankaj et al. 2017). The latest physicochemical methods that can potentially be used for elimination of mycotoxins include the cold-plasma sterilization (Ouf et al. 2015; Hojnik et al. 2017; Pankaj et al. 2017; Shi et al.2017). There is a possibility of the complete degradation and reduction of aflatoxin B1 (AFB_1), deoxy nivalenol (DON), and nivalenol (NIV) cytotoxicity with the use of argon plasma generated by microwave discharges (Hojnik et al. 2017). The reduction of the toxicity of these compounds may result from structural changes induced by the sterilization process. Wang et al. (2015) applied low-temperature radio-frequency plasma and reported 88.3% reduction in AFB_1 concentration after a 10-minute process. The analysis of degradation products revealed five different compounds characterized by loss of the double bond between C8 and C9 in the furan ring. Besides its effect on the standard mycotoxin solutions, the plasma has been found to exert a reductive effect on the compounds contained in food products (Ouf et al. 2015; Siciliano et al. 2016; Shi et al. 2017). Aflatoxins present in hazelnuts were decontaminated using CP (1000 W, 12 min), which resulted in an over 70% decline in the AFB_1 concentration (Siciliano et al. 2016). In turn, the concentration of this maize contaminant was reduced by 62% and 82% after the 1- and 10-min plasma treatments, respectively (Shi et al. 2017). However, the atmospheric plasma treatment of contaminated nuts did not allow complete removal of the mycotoxin. The best results were obtained with the highest power (1150 W) and the longest operating time (12 min), which enabled the reduction of AFB1 approx. by 70%. Given the high efficiency of degradation of standard mycotoxin solutions and mycotoxins contained in food, the cold plasma method is becoming a promising solution that may replace conventional techniques in the future. In addition to the high efficiency of compound degradation, the relatively low cost of the process, as well as environmental safety, also favor the use of CP (Hojnik et al. 2017).

Summary

The cold plasma technology is becoming a promising solution with the potential to replace conventional techniques of decontamination of food products, medical materials, and air in the future. This technique has many advantages, e.g. high efficiency in reducing the viral particles load and the number of microorganisms, formation of non-toxic by-products, and a relatively low cost of the process (Liao et al. 2016). Although many studies focused on this issue, the precise molecular mechanism of the plasma effect on cells of different microbial groups has not been clarified yet. The available reports on the possibility of induction of VBNC state in some bacteria raise doubts about the safety of this sterilization method (Dolezalova and Lukes 2015). In addition, this technology has some disadvantages, i.e. a small working surface and poor permeability. In food products, this technique may cause increased lipid oxidation, increased acidity of the product, reduced color intensity and decrease in firmness of fruits (Misra et al. 2016; Chizoba Ekezie et al. 2017). Therefore, it is necessary to conduct further research that will allow for the optimization of process parameters, explain the doubts concerning the biting mechanisms of plasma operation and promote the application of cold plasma in the industry with no negative effects on human health or the environment.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Afshari R, Hosseini H. Non-thermal plasma as a new food preservation method, its present and future prospect. J Paramed Sci. 2014;5:116–120.

Ahlfeld B, Li Y, Boulaaba A, Binder A, Schotte U, Zimmermann JL, Morfill G, Klein G. Inactivation of a foodborne norovirus outbreak strain with nonthermal atmospheric pressure plasma. MBio. 2015 Feb 27;6(1):e02300–e02314. doi:10.1128/mBio.02300-14 Medline

Alkawareek MY, Alshraiedeh NH, Higginbotham S, Flynn PB, Algwari QT, Gorman SP, Graham WG, Gilmore BF. Plasmid DNA damage following exposure to atmospheric pressure nonthermal plasma: kinetics and influence of oxygen admixture. Plasma Med. 2014;4(1-4):211–219. doi:10.1615/PlasmaMed.2015011977

Attri P, Han J, Choi S, Choi EH, Bogaerts A, Lee W. CAP modifies the structure of a model protein from thermophilic bacteria: mechanisms of CAP-mediated inactivation. Sci Rep. 2018 Dec; 8(1):10218. doi:10.1038/s41598-018-28600-w Medline

Bayliss DL, Walsh JL, Iza F, Shama G, Holah J, Kong MG. Complex responses of microorganisms as a community to a flowing atmospheric plasma. Plasma Process Polym. 2012 Jun;9(6):597–611. doi:10.1002/ppap.201100104

Beggs CB. A quantitative method for evaluating the photoreactivation of ultraviolet damaged microorganisms. Photochem Photobiol Sci. 2002 Jun 7;1(6):431–437. doi:10.1039/b202801h Medline

Bogaerts A, Neyts EC. Plasma technology: an emerging technology for energy storage. ACS Energy Lett. 2018 Apr 13;3(4):1013–1027. doi:10.1021/acsenergylett.8b00184

Boudam MK, Moisan M. Synergy effect of heat and UV photons on bacterial-spore inactivation in an N_2 –O_2 plasma-afterglow sterilizer. J Phys D Appl Phys. 2010 Jul 28;43(29):295202. doi:10.1088/0022-3727/43/29/295202

Bourke P, Ziuzina D, Han L, Cullen PJ, Gilmore BF. Microbiological interactions with cold plasma. J Appl Microbiol. 2017 Aug;123(2):308–324. doi:10.1111/jam.13429 Medline

Boxhammer V, Li YF, Köritzer J, Shimizu T, Maisch T, Thomas HM, Schlegel J, Morfill GE, Zimmermann JL. Investigation of the mutagenic potential of cold atmospheric plasma at bactericidal dosages. Mutat Res-Gen Tox En. 2013;753(1):23–28.

Brisset JL, Pawłat J. Chemical effects of air plasma species on aqueous solutes in direct and delayed exposure modes: discharge, post-discharge and plasma activated water. Plasma Chem Plasma Process. 2016 Mar;36(2):355–381. doi:10.1007/s11090-015-9653-6

Bruggeman PJ, Kushner MJ, Locke BR, Gardeniers JGE, Graham WG, Graves DB, Hofman-Caris RCHM, Maric D, Reid JP, Ceriani E, et al. Plasma–liquid interactions: a review and roadmap. Plasma Sources Sci Technol. 2016 Sep 30;25(5):053002. doi:10.1088/0963-0252/25/5/053002

Brun P, Bernabè G, Marchiori C, Scarpa M, Zuin M, Cavazzana R, Zaniol B, Martines E. Antibacterial efficacy and mechanisms of action of low power atmospheric pressure cold plasma: membrane permeability, biofilm penetration and antimicrobial sensitization. J Appl Microbiol. 2018 Aug;125(2):398–408. doi:10.1111/jam.13780 Medline

Chizoba Ekezie F-G, Sun DW, Cheng JH. A review on recent advances in cold plasma technology for the food industry: current applications and future trends. Trends Food Sci Technol. 2017 Nov; 69:46–58. doi:10.1016/j.tifs.2017.08.007

Colonna W, Wan Z, Pankaj SK, Keener KM. High-voltage atmospheric cold plasma treatment of yeast for spoilage prevention. Plasma Med. 2017;7(2):97–107. doi:10.1615/PlasmaMed.2017019201

Connor M, Flynn PB, Fairley DJ, Marks N, Manesiotis P, Graham WG, Gilmore BF, McGrath JW. Evolutionary clade affects resistance of *Clostridium difficile* spores to Cold Atmospheric Plasma. Sci Rep. 2017 Dec;7(1):41814. doi:10.1038/srep41814 Medline

Coutinho NM, Silveira MR, Rocha RS, Moraes J, Ferreira MVS, Pimentel TC, Freitas MQ, Silva MC, Raices RSL, Ranadheera CS, et al. Cold plasma processing of milk and dairy products. Trends Food Sci Technol. 2018 Apr;74:56–68. doi:10.1016/j.tifs.2018.02.008

Czapka T, Maliszewska I, Olesiak-Bańska J. Influence of atmospheric pressure non-thermal plasma on inactivation of biofilm cells. Plasma Chem Plasma Process. 2018 Nov;38(6):1181–1197. doi:10.1007/s11090-018-9925-z

Czyzewska-Dors E, Dors A, Pomorska-Mól M. Właściwości biofilmu bakteryjnego warunkujące oporność na antybiotyki oraz metody jego zwalczania. Zycie Wet. 2018;93(11):765–771.

Dasan BG, Boyaci IH, Mutlu M. Nonthermal plasma treatment of *Aspergillus* spp. spores on hazelnuts in an atmospheric pressure fluidized bed plasma system: impact of process parameters and surveillance of the residual viability of spores. J Food Eng. 2017a Mar;196:139–149. doi:10.1016/j.jfoodeng.2016.09.028

Dasan BG, Mutlu M, Boyaci IH. Decontamination of Aspergillus flavus and *Aspergillus parasiticus* spores on hazelnuts via atmospheric pressure fluidized bed plasma reactor. Int J Food Microbiol. 2016 Jan;216:50–59. doi:10.1016/j.ijfoodmicro.2015.09.006 Medline

Dasan BG, Onal-Ulusoy B, Pawłat J, Diatczyk J, Sen Y, Mutlu M. A new and simple approach for decontamination of food contact surfaces with gliding arc discharge atmospheric non-thermal plasma. Food Bioprocess Technol. 2017b Apr;10(4):650–661. doi:10.1007/s11947-016-1847-2

Deng X, Shi J, Kong MG. Physical mechanisms of inactivation of *Bacillus subtilis* spores using cold atmospheric plasmas. IEEE Trans Plasma Sci. 2006 Aug;34(4):1310–1316. doi:10.1109/TPS.2006.877739

Dobrynin D, Fridman G, Mukhin YV, Wynosky-Dolfi MA, Rieger J, Rest RF, Gutsol AF, Fridman A. Cold plasma inactivation of *Bacillus cereus* and *Bacillus anthracis* (anthrax) spores. IEEE Trans Plasma Sci. 2010 Aug;38(8):1878–1884. doi:10.1109/TPS.2010.2041938

Dolezalova E, Lukes P. Membrane damage and active but nonculturable state in liquid cultures of *Escherichia coli* treated with an atmospheric pressure plasma jet. Bioelectrochemistry. 2015 Jun;103:7–14. doi:10.1016/j.bioelechem.2014.08.018 Medline

Dzimitrowicz A, Jamróz P, Nowak P. Sterylizacja za pomocą niskotemperaturowej plazmy, generowanej w warunkach ciśnienia atmosferycznego. Postepy Mikrobiol. 2015;54(2):195–200.

Ermolaeva SA, Varfolomeev AF, Chernukha MY, Yurov DS, Vasiliev MM, Kaminskaya AA, Moisenovich MM, Romanova JM, Murashev AN, Selezneva II, et al. Bactericidal effects of non-thermal argon plasma *in vitro*, in biofilms and in the animal model of infected wounds. J Med Microbiol. 2011 Jan 01;60(1):75–83. doi:10.1099/jmm.0.020263-0 Medline

Fiebrandt M, Lackmann JW, Raguse M, Moeller R, Awakowicz P, Stapelmann K. VUV absorption spectroscopy of bacterial spores and DNA components. Plasma Phys Contr Fusion. 2017 Jan 01; 59(1):014010. doi:10.1088/0741-3335/59/1/014010

Filatova I, Azharonok V, Lyushkevich V, Zhukovsky A, Mildažienė V, Pauzaite G, Zukiene RAM. 2016. The effect of pre-sowing plasma seeds treatment on germination, plants resistance to pathogens and crop capacity. Paper presented at: 1st International Workshop on Plasma Agriculture; Camden, NJ.

Flynn PB, Higginbotham S, Alshraiedeh NH, Gorman SP, Graham WG, Gilmore BF. Bactericidal efficacy of atmospheric pressure non-thermal plasma (APNTP) against the ESKAPE pathogens. Int J Antimicrob Agents. 2015 Jul;46(1):101–107. doi:10.1016/j.ijantimicag.2015.02.026 Medline

Fridman A, Chirokov A, Gutsol A. Non-thermal atmospheric pressure discharges. J Phys D Appl Phys. 2005 Jan 21;38(2):R1–R24. doi:10.1088/0022-3727/38/2/R01

Gallagher MJ, Vaze N, Gangoli S, Vasilets VN, Gutsol AF, Milovanova TN, Anandan S, Murasko DM, Fridman AA. Rapid inactivation of airborne bacteria using atmospheric pressure dielectric barrier grating discharge. IEEE Trans Plasma Sci. 2007 Oct; 35(5):1501–1510. doi:10.1109/TPS.2007.905209

Guo L, Zhao Y, Liu D, Liu Z, Chen C, Xu R, Tian M, Wang X, Chen H, Kong MG. Cold atmospheric-pressure plasma induces DNA – protein crosslinks through protein oxidation. Free Radic Res. 2018 Jul 03;52(7):783–798. doi:10.1080/10715762.2018.1471476 Medline

Hojnik N, Cvelbar U, Tavčar-Kalcher G, Walsh J, Križaj I. Cold atmospheric pressure plasma versus "classic" decontamination. Toxins (Basel). 2017 Apr 28;9(5):151. doi:10.3390/toxins9050151 Medline

Hong YF, Kang JG, Lee HY, Uhm HS, Moon E, Park YH. Sterilization effect of atmospheric plasma on *Escherichia coli* and *Bacillus subtilis* endospores. Lett Appl Microbiol. 2009 Jan;48(1):33–37. doi:10.1111/j.1472-765X.2008.02480.x Medline

Hosseinzadeh Colagar A, Memariani H, Sohbatzadeh F, Valinataj Omran A. Nonthermal atmospheric argon plasma jet effects on *Escherichia coli* biomacromolecules. Appl Biochem Biotechnol. 2013 Dec;171(7):1617–1629. doi:10.1007/s12010-013-0430-9 Medline

Isbary G, Morfill G, Schmidt HU, Georgi M, Ramrath K, Heinlin J, Karrer S, Landthaler M, Shimizu T, Steffes B, et al. A first prospective randomized controlled trial to decrease bacterial

load using cold atmospheric argon plasma on chronic wounds in patients. Br J Dermatol. 2010 May;163(1):78–82. doi:10.1111/j.1365-2133.2010.09744.x Medline

Itooka K, Takahashi K, Kimata Y, Izawa S. Cold atmospheric pressure plasma causes protein denaturation and endoplasmic reticulum stress in *Saccharomyces cerevisiae.* Appl Microbiol Biotechnol. 2018 Mar;102(5):2279–2288. doi:10.1007/s00253-018-8758-2 Medline

Jahid IK, Han N, Ha SD. Inactivation kinetics of cold oxygen plasma depend on incubation conditions of *Aeromonas hydrophila* biofilm on lettuce. Food Res Int. 2014 Jan;55:181–189. doi:10.1016/j.foodres.2013.11.005

Kądzielska J, Obuch-Woszczatyński P, Pituch H, Młynarczyk G. *Clostridium perfringens* jako czynnik etiologiczny biegunki poantybiotykowej. Postepy Mikrobiol. 2012;51(1):17–25.

Kartaschew K, Baldus S, Mischo M, Bründermann E, Awakowicz P, Havenith M. Cold atmospheric-pressure plasma and bacteria: understanding the mode of action using vibrational microspectroscopy. J Phys D Appl Phys. 2016 Sep 21;49(37):374003. doi:10.1088/0022-3727/49/37/374003

Kim GC, Kim GJ, Park SR, Jeon SM, Seo HJ, Iza F, Lee JK. Air plasma coupled with antibody-conjugated nanoparticles: a new weapon against cancer. J Phys D Appl Phys. 2009 Feb 07;42(3):032005. doi:10.1088/0022-3727/42/3/032005

Klämpfl TG, Isbary G, Shimizu T, Li YF, Zimmermann JL, Stolz W, Schlegel J, Morfill GE, Schmidt HU. Cold atmospheric air plasma sterilization against spores and other microorganisms of clinical interest. Appl Environ Microbiol. 2012 Aug 01;78(15):5077–5082. doi:10.1128/AEM.00583-12 Medline

Klämpfl TG, Shimizu T, Koch S, Balden M, Gemein S, Li YF, Mitra A, Zimmermann JL, Gebel J, Morfill GE, et al. Decontamination of nosocomial bacteria including *Clostridium difficile* spores on dry inanimate surface by cold atmospheric plasma. Plasma Process Polym. 2014 Oct;11(10):974–984. doi:10.1002/ppap.201400080

Korachi M, Turan Z, Şentürk K, Şahin F, Aslan N. An investigation into the biocidal effect of high voltage AC/DC atmospheric corona discharges on bacteria, yeasts, fungi and algae. J Electrost. 2009 Jul;67(4):678–685. doi:10.1016/j.elstat.2009.03.002

Krzyczkowska J, Stolarzewicz I, Bellok D, Bellok M, Białecka-Florjańczyk E. Wpływ modyfikacji pożywki na biokatalityczne właściwości drożdży. Żywn Nauka Technol Jakość. 2008;15(5):299–306.

Lademann O, Kramer A, Richter H, Patzelt A, Meinke MC, Roewert-Huber J, Czaika V, Weltmann K-D, Hartmann B, Koch S. Antisepsis of the follicular reservoir by treatment with tissue-tolerable plasma (TTP). Laser Phys Lett. 2011 Apr;8(4):313–317. doi:10.1002/lapl.201010123

Laroussi M, Mendis DA, Rosenberg M. Plasma interaction with microbes. New J Phys. 2003;5(1):41.1–41.10.

Laskowska M, Bogusławska-Wąs E, Kowal P, Hołub M, Dąbrowski W. Skuteczność wykorzystania niskotemperaturowej plazmy w mikrobiologii i medycynie. Postepy Mikrobiol. 2016;55(2):172–181.

Li HP, Wang LY, Li G, Jin LH, Le PS, Zhao HX, Xing XH, Bao CY. Manipulation of lipase activity by the helium radio-frequency, atmospheric-pressure glow discharge plasma jet. Plasma Process Polym. 2011 Mar 22;8(3):224–229. doi:10.1002/ppap.201000035

Liao X, Cullen PJ, Liu D, Muhammad AI, Chen S, Ye X, Wang J, Ding T. Combating *Staphylococcus aureus* and its methicillin resistance gene (*mecA*) with cold plasma. Sci Total Environ. 2018 Dec;645:1287–1295. doi:10.1016/j.scitotenv.2018.07.190 Medline

Liao X, Liu D, Xiang Q, Ahn J, Chen S, Ye X, Ding T. Inactivation mechanisms of non-thermal plasma on microbes: A review. Food Control. 2017 May;75:83–91. doi:10.1016/j.foodcont.2016.12.021

Lu Q, Liu D, Song Y, Zhou R, Niu J. Inactivation of the tomato pathogen *Cladosporium fulvum* by an atmospheric-pressure cold plasma jet. Plasma Process Polym. 2014 Nov;11(11):1028–1036. doi:10.1002/ppap.201400070

Maciejewska M, Bauer M, Dawgul M. Nowoczesne metody zwalczania biofilmu bakteryjnego. Postepy Mikrobiol. 2016;55(1):3–11.

Mai-Prochnow A, Clauson M, Hong J, Murphy AB. Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. Sci Rep. 2016 Dec;6(1):38610. doi:10.1038/srep38610 Medline

Mai-Prochnow A, Murphy AB, McLean KM, Kong MG, Ostrikov KK. Atmospheric pressure plasmas: infection control and bacterial responses. Int J Antimicrob Agents. 2014 Jun;43(6):508–517. doi:10.1016/j.ijantimicag.2014.01.025 Medline

Metelmann HR, Seebauer C, Miller V, Fridman A, Bauer G, Graves DB, Pouvesle J-M, Rutkowski R, Schuster M, Bekeschus S, et al. Clinical experience with cold plasma in the treatment of locally advanced head and neck cancer. Clin Plasma Med. 2018 Mar;9:6–13. doi:10.1016/j.cpme.2017.09.001

Misra NN, Pankaj SK, Segat A, Ishikawa K. Cold plasma interactions with enzymes in foods and model systems. Trends Food Sci Technol. 2016 Sep;55:39–47. doi:10.1016/j.tifs.2016.07.001

Misra NN, Schlüter O, Cullen PJ. Plasma in food and agriculture. Amsterdam (Netherlands): Academic Press. 2016. p. 1–16.

Moreau M, Orange N, Feuilloley MGJ. Non-thermal plasma technologies: new tools for bio-decontamination. Biotechnol Adv. 2008 Nov;26(6):610–617. doi:10.1016/j.biotechadv.2008.08.001 Medline

Nikmaram H, Rezaei Kanavi M, Ghoranneviss M, Balagholi S, Ahmadieh H, Roshandel D, Amini M. Cold atmospheric pressure plasma jet for the treatment of *Aspergillus keratitis.* Clin Plasma Med. 2018 Mar;9:14–18. doi:10.1016/j.cpme.2017.12.075

Nishime TMC, Borges AC, Koga-Ito CY, Machida M, Hein LRO, Kostov KG. Non-thermal atmospheric pressure plasma jet applied to inactivation of different microorganisms. Surf Coat Tech. 2017 Feb;312:19–24. doi:10.1016/j.surfcoat.2016.07.076

Olesiak P, Stępniak L. Skuteczność wybranych związków dezynfekcyjnych wobec przetrwalników *Bacillus.* Inżynieria i Ochrona Środowiska. 2012;15:41–50.

Ouf SA, Basher AH, Mohamed AAH. Inhibitory effect of double atmospheric pressure argon cold plasma on spores and mycotoxin production of *Aspergillus niger* contaminating date palm fruits. J Sci Food Agric. 2015 Dec;95(15):3204–3210. doi:10.1002/jsfa.7060 Medline

Pankaj SK, Shi H, Keener KM. A review of novel physical and chemical decontamination technologies for aflatoxin in food. Trends Food Sci Technol. 2018 Jan;71:73–83. doi:10.1016/j.tifs.2017.11.007

Panngom K, Lee SH, Park DH, Sim GB, Kim YH, Uhm HS, Park G, Choi EH. Non-thermal plasma treatment diminishes fungal viability and up-regulates resistance genes in a plant host. PLoS One. 2014 Jun 9;9(6):e99300. doi:10.1371/journal.pone.0099300 Medline

Pawłat J, Kwiatkowski M, Terebun P, Murakami T. RF-powered atmospheric-pressure plasma jet in surface treatment of high-impact polystyrene. IEEE Trans Plasma Sci. 2016 Mar;44(3):314–320. doi:10.1109/TPS.2015.2436061

Pawłat J, Starek A, Sujak A, Kwiatkowski M, Terebun P, Budzeń M. Effects of atmospheric pressure plasma generated in GlidArc reactor on *Lavatera thuringiaca* L. seeds' germination. Plasma Process Polym. 2018a Feb;15(2):1700064. doi:10.1002/ppap.201700064

Pawłat J, Starek A, Sujak A, Terebun P, Kwiatkowski M, Budzeń M, Andrejko D. Effects of atmospheric pressure plasma jet operating with DBD on *Lavatera thuringiaca* L. seeds' germination. PLoS One. 2018b Apr 9;13(4):e0194349. doi:10.1371/journal.pone.0194349 Medline

Pawłat J. Atmospheric pressure plasma jet for decontamination purposes. Eur Phys J Appl Phys. 2013;61:1–11.

Pignata C, D'Angelo D, Fea E, Gilli G. A review on microbiological decontamination of fresh produce with nonthermal plasma. J Appl Microbiol. 2017 Jun;122(6):1438–1455. doi:10.1111/jam.13412 Medline

Polčic P, Pakosová L, Chovančíková P, Machala Z. Reactive cold plasma particles generate oxidative stress in yeast but do not trigger apoptosis. Can J Microbiol. 2018 Jun;64(6):367–375. doi:10.1139/cjm-2017-0753 Medline

Pradeep P, Chulkyoon M. Non-thermal plasmas (NTPs) for inactivation of viruses in abiotic environment. Res J Biotechnol. 2016;11:91–96.

Raguse M, Fiebrandt M, Denis B, Stapelmann K, Eichenberger P, Driks A, Eaton P, Awakowicz P, Moeller R. Understanding of the importance of the spore coat structure and pigmentation in the *Bacillus subtilis* spore resistance to low-pressure plasma sterilization. J Phys D Appl Phys. 2016 Jul 20;49(28):285401. doi:10.1088/0022-3727/49/28/285401

Ritter AC, Santi L, Vannini L, Beys-da-Silva WO, Gozzi G, Yates J 3rd, Ragni L, Brandelli A. Comparative proteomic analysis of foodborne *Salmonella* Enteritidis SE86 subjected to cold plasma treatment. Food Microbiol. 2018 Dec;76:310–318. doi:10.1016/j.fm.2018.06.012 Medline

Roth S, Feichtinger J, Hertel C. Response of *Deinococcus radiodurans* to low-pressure low-temperature plasma sterilization processes. J Appl Microbiol. 2010 Jun;109(5):1521–1530. doi:10.1111/j.1365-2672.2010.04771.x Medline

Ryu YH, Kim YH, Lee JY, Shim GB, Uhm HS, Park G, Choi EH. Effects of background fluid on the efficiency of inactivating yeast with non-thermal atmospheric pressure plasma. PLoS One. 2013 Jun 14;8(6):e66231. doi:10.1371/journal.pone.0066231 Medline

Samoń R, Czapiński J, Grządziel J, Płonka M, Pawłat J, Diatczyk J. Ocena działania bakteriobójczego niskotemperaturowej plazmy nierównowagowej generowanej w reaktorze RF. Eur J Med Technol. 2014;2:17–26.

Sharma A, Collins G, Pruden A. Differential gene expression in *Escherichia coli* following exposure to nonthermal atmospheric pressure plasma. J Appl Microbiol. 2009 Nov;107(5):1440–1449. doi:10.1111/j.1365-2672.2009.04323.x Medline

Shi H, Ileleji K, Stroshine RL, Keener K, Jensen JL. Reduction of aflatoxin in corn by high voltage atmospheric cold plasma. Food Bioprocess Technol. 2017 Jun;10(6):1042–1052. doi:10.1007/s11947-017-1873-8

Siciliano I, Spadaro D, Prelle A, Vallauri D, Cavallero M, Garibaldi A, Gullino M. Use of cold atmospheric plasma to detoxify hazelnuts from aflatoxins. Toxins (Basel). 2016 Apr 26;8(5):125. doi:10.3390/toxins8050125 Medline

Siddique SS, Hardy GESJ, Bayliss KL. Cold plasma: a potential new method to manage postharvest diseases caused by fungal plant pathogens. Plant Pathol. 2018 Jun;67(5):1011–1021. doi:10.1111/ppa.12825

Šimončicová J, Kaliňáková B, Kováčik D, Medvecká V, Lakatoš B, Kryštofová S, Hoppanová L, Palušková V, Hudecová D, Ďurina P, et al. Cold plasma treatment triggers antioxidative defense system and induces changes in hyphal surface and subcellular structures of *Aspergillus flavus.* Appl Microbiol Biotechnol. 2018 Aug;102(15): 6647–6658. doi:10.1007/s00253-018-9118-y Medline

Sohbatzadeh F, Mirzanejhad S, Shokri H, Nikpour M. Inactivation of *Aspergillus flavus* spores in a sealed package by cold plasma streamers. J Theor Appl Phys. 2016 Jun;10(2):99–106. doi:10.1007/s40094-016-0206-z

Su X, Tian Y, Zhou H, Li Y, Zhang Z, Jiang B, Yang B, Zhang J, Fang J. Inactivation efficacy of non-thermal plasma activated solutions against Newcastle disease virus. Appl Environ Microbiol. 2018 Feb 23;84(9):e02836-17. doi:10.1128/AEM.02836-17

Suhem K, Matan N, Nisoa M, Matan N. Inhibition of *Aspergillus flavus* on agar media and brown rice cereal bars using cold atmospheric plasma treatment. Int J Food Microbiol. 2013 Feb;161(2): 107–111. doi:10.1016/j.ijfoodmicro.2012.12.002 Medline

Surowsky B, Fischer A, Schlueter O, Knorr D. Cold plasma effects on enzyme activity in a model food system. Innov Food Sci Emerg Technol. 2013 Jul;19:146–152. doi:10.1016/j.ifset.2013.04.002

Terrier O, Essere B, Yver M, Barthélémy M, Bouscambert-Duchamp M, Kurtz P, VanMechelen D, Morfin F, Billaud G, Ferraris O, et al. Cold oxygen plasma technology efficiency against different airborne respiratory viruses. J Clin Virol. 2009 Jun;45(2): 119–124. doi:10.1016/j.jcv.2009.03.017 Medline

Tolouie H, Mohammadifar MA, Ghomi H, Hashemi M. Cold atmospheric plasma manipulation of proteins in food systems. Crit Rev Food Sci Nutr. 2018;58(15):2583–2597. doi:10.1080/10408398.2017.1335689 Medline

Tolouie H, Mohammadifar MA, Ghomi H, Yaghoubi AS, Hashemi M. The impact of atmospheric cold plasma treatment on inactivation of lipase and lipoxygenase of wheat germs. Innov Food Sci Emerg Technol. 2018 Jun;47:346–352. doi:10.1016/j.ifset.2018.03.002

Tseng S, Abramzon N, Jackson JO, Lin WJ. Gas discharge plasmas are effective in inactivating *Bacillus* and *Clostridium* spores. Appl Microbiol Biotechnol. 2012 Mar;93(6):2563–2570. doi:10.1007/s00253-011-3661-0 Medline

Vandamme M, Robert E, Pesnel S, Barbosa E, Dozias S, Sobilo J, Lerondel S, Le Pape A, Pouvesle JM. Antitumor effect of plasma treatment on U87 glioma xenografts: preliminary results. Plasma Process Polym. 2010 Mar 22;7(3-4):264–273. doi:10.1002/ppap.200900080

Wang G, Zhang P, Setlow P, Li Y. Kinetics of germination of wet-heat-treated individual spores of *Bacillus* species, monitored by Raman spectroscopy and differential interference contrast microscopy. Appl Environ Microbiol. 2011 May 15;77(10):3368–3379. doi:10.1128/AEM.00046-11 Medline

Wang SQ, Huang GQ, Li YP, Xiao JX, Zhang Y, Jiang WL. Degradation of aflatoxin B1 by low-temperature radio frequency plasma and degradation product elucidation. Eur Food Res Technol. 2015 Jul;241(1):103–113. doi:10.1007/s00217-015-2439-5

Wawrzycka D. Drożdże jako model w badaniach chorób neurodegeneracyjnych. Postepy Hig Med Dosw. 2011 Jun 2;65:328–337. doi:10.5604/17322693.945767

Wiktor A, Śledź M, Nowacka M, Witrowa-Rajchert D. Możliwości zastosowania niskotemperaturowej plazmy w technologii żywności. Żywn Nauka Technol Jakość. 2013. 5:5–14.

Wolny-Koładka K, Pawłat J, Terebun P, Kwiatkowski M, Diatczyk J. Ocena możliwości zastosowania plazmy niskotemperaturowej w celu higienizacji zmieszanych odpadów komunalnych służących do produkcji paliwa alternatywnego. Przegl Elektrotechn. 2017 Nov 5;1(11):211–215. doi:10.15199/48.2017.11.43

Yasuda H, Miura T, Kurita H, Takashima K, Mizuno A. Biological evaluation of DNA damage in bacteriophages inactivated by atmospheric pressure cold plasma. Plasma Process Polym. 2010 Mar 22;7(3-4):301–308. doi:10.1002/ppap.200900088

Ye S, Song X, Liang JL, Zheng S, Lin Y. Disinfection of airborne spores of *Penicillium expansum* in cold storage using continuous direct current corona discharge. Biosyst Eng. 2012 Oct;113(2): 112–119. doi:10.1016/j.biosystemseng.2012.06.013

Zimmermann JL, Dumler K, Shimizu T, Morfill GE, Wolf A, Boxhammer V, Schlegel J, Gansbacher B, Anton M. Effects of cold atmospheric plasmas on adenoviruses in solution. J Phys D Appl Phys. 2011 Dec 21;44(50):505201. doi:10.1088/0022-3727/44/50/505201

Ziuzina D, Boehm D, Patil S, Cullen PJ, Bourke P. Cold plasma inactivation of bacterial biofilms and reduction of quorum sensing regulated virulence factors. PLoS One. 2015 Sep 21;10(9):e0138209. doi:10.1371/journal.pone.0138209 Medline

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Morphological and physiological changes in *Lentilactobacillus hilgardii* cells after cold plasma treatment

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Atmospheric cold plasma (ACP) inactivation of *Lentilactobacillus hilgardii* was investigated. Bacteria were exposed to ACP dielectric barrier discharge with helium and oxygen as working gases for 5, 10, and 15 min. The innovative approach in our work for evaluation of bacterial survival was the use in addition to the classical plate culture method also flow cytometry which allowed the cells to be sorted and revealed different physiological states after the plasma treatment. Results showed total inhibition of bacterial growth after 10-min of ACP exposure. However, the analysis of flow cytometry demonstrated the presence of 14.4% of active cells 77.5% of cells in the mid-active state and 8.1% of dead cells after 10 min. In addition, some of the cells in the mid-active state showed the ability to grow again on culture medium, thus confirming the hypothesis of induction of VBNC state in *L .hilgardii* cells by cold plasma. In turn, atomic force microscopy (AFM) which was used to study morphological changes in *L. hilgardii* after plasma treatment at particular physiological states (active, mid-active, dead), showed that the surface roughness of the mid-active cell (2.70 ± 0.75 nm) was similar to that of the control sample (2.04 ± 0.55 nm). The lack of considerable changes on the cell surface additionally explains the effective cell resuscitation. To the best of our knowledge, AFM was used for the first time in this work to analyze cells which have been sorted into subpopulations after cold plasma treatment and this is the first work indicating the induction of VBNC state in *L. hilgardii* cells after exposure to cold plasma.

Wine is one of the most frequently consumed alcoholic beverages in the world[1]. Due to the presence of alcohol as well as the low pH and compounds with antibacterial activity, wine is considered an unfavourable environment for the development of microflora. However, some microorganisms have developed a number of adaptive mechanisms that allow them to survive the unfavourable conditions prevailing in wine at particular stages of production, thus adversely affecting its physicochemical properties. This group includes mainly *Brettanomyces* yeasts, lactic acid bacteria (LAB), and acetic acid bacteria[2,3]. LAB are the most numerous group of bacteria in grape must and wine. In wine production, one of the main risks arising from the metabolism of lactic bacteria is the synthesis of ethyl carbamate. This compound has carcinogenic properties. Moreover, LABs are the main producers of biogenic amines (BA) such as histamine, tyramine, phenylethylamine, and spermidine. Biogenic amines are responsible for lowering the sensory quality of wine, but also their presence can be dangerous to consumer health. An example is histamine, which can cause ailments such as vomiting, headaches, and rash[4,5]. Extensive research conducted by Rodas et al. (2005) showed that the majority of isolates of LAB in wine belongs to *Lentilactobacillus hilgardii* species[6,7]. The strains of this species were found in wine with 20% ethanol content[6–8]. The aforementioned high resistance of *L. hilgardii* strains to high alcohol concentrations and other conditions in wine is a huge problem in winemaking because elimination thereof from the product is difficult. This is related

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to the metabolic characteristics and structure of the bacterial cell membrane. The presence of S-layer proteins on the cell surface and long unsaturated fatty acids (C19) in the direct membrane increases the resistance of the bacteria to alcohol. In the context of protection against adverse conditions, the S-surface layer plays an important role due to its stability at various pH and resistance to temperature, radiation, and proteolysis[9]. In addition, as shown by recent studies, *L. hilgardii* is likely to produce bacterial cellulose, which can considerably increase resistance to the sterilizing agent[10]. In comparison with other wine bacteria such as *Pediococcus pentosaceus* and *Oenococcus oeni*, *L. hilgardii* exhibits higher resistance to flavonoids and phenolic acids[11]. High resistance of *L. hilgardii* to stress conditions is a huge problem in winemaking because elimination thereof from the product is difficult. Although the classic thermal methods of food preservation are still very important in food technology, heat processes can negatively affect the unique properties of product such as taste, colour, and flavour[2]. Scientists around the world are paying special attention to non-thermal methods that will eliminate undesirable microorganisms but will not significantly affect the final stability of the product[3].

Non-thermal plasma (NTP), also known as cold plasma (CP), is one of the technologies for non-thermal food preservation with great potential as a new decontamination technique. The term cold plasma was first used by Irving Langmuir in 1928 and refers to a partially or fully ionized gas, which consists of molecules in the excited and basic state, reactive compounds, charged particles and UV photons[12]. A number of available publications indicate effective elimination of undesirable microorganisms from food products[13–15]. This wide range of cold plasma applications is related to its ability to inactivate viruses[16,17], bacteria[18], spores[19], yeasts[20] and fungi[21]. Nevertheless, the exact mechanisms of cold plasma antimicrobial activity are still unknown. Numerous publications indicate that reactive nitrogen and oxygen compounds, high-energy electrons, ionized atoms and particles as well as UV photons present in the plasma stream damage the external structures of microorganisms, have a negative effect on internal cellular components and cause DNA degradation[15,22]. The integrity and proper functioning of the cell membrane of microorganisms is mainly affected by oxidative stress and probably also as some scientists indicate, by generated electrostatic forces. Reactive compounds, mainly oxygen, cause the oxidation of lipids and proteins contained in the membrane, while charged particles accumulating on the outer side of the membrane cause the interruption of its continuity[14,22]. In addition, under the influence of an electric field, an electroporation process may occur, which manifests itself in the formation of micropores[15]. The resulting damage to external cell structures may lead to the leakage of intracellular components. On the other hand, the action of UV photons may cause the formation of nitrogen base dimers, thus disturbing the DNA replication process[14,22]. However, it is known from the available studies that the effectiveness of cold plasma depends on many factors, e.g. operating condition (reactor type, flow, gas pressure, power of plasma excitation, gas type, process duration), exposure mode (direct or indirect), environmental conditions (pH, humidity, matrix type) and the individual morphological and physiological characteristics of the cell[23,24]. Thus, it is necessary to optimize the conditions of plasma treatment in order to make the sterilization with this method effective. Nevertheless, CP has many advantages over conventional techniques, such as the process temperature, which is close to the ambient temperature and therefore does not adversely affect the properties of the product. Furthermore, compared to conventional methods, it is environmentally friendly and cost efficient due to water saving[25]. However, due to the generation of oxidative stress in the cell and the action of an electric field, cold plasma may induce some bacteria to enter the viable but non-culturable (VBNC) stage. Bacteria in this state are characterized by an inability to grow on microbiological media despite their physiological and metabolic activity. The classical plate culture method is most frequently used to determine the microbiological quality of the product. However, this technique does not detect microorganisms that have entered the VBNC state. This is a critical problem in food technology causing adulterated results. Therefore, it is necessary to apply methods that will allow determination of a reliable influence of the new non-thermal decontamination technique on microbial cells[26].

In this study, inactivation of *L. hilgardii* by cold atmospheric plasma was investigated in a model experimental system. To ensure that the effectiveness of the method was not misinterpreted and damaged cells could be detected, their survival was compared by classical culture, optical density measurement and flow cytometry. Morphological and physiological changes of plasma treated cells were analysed using scanning electron microscopy, atomic force microscopy and Fourier transform-infrared spectroscopy.

Materials and methods

Bacterial strains and growth. The *L. hilgardii* strain (NRRL B-1843) was grown overnight (18 h) in MRS (OXOID LTD., Hampshire, England) at 30 °C. Cells were harvested by centrifugation at 8000×*g* for 15 min. The cell pellet was washed once with sterile phosphate-buffered saline (PBS pH 7.4) and twice with sterile water. The pellet was resuspended in sterile water, the bacterial density was measured with a spectrophotometer (SmartSpec Plus Spectrophotometr, USA) at 600 nm, and the number of cells was estimated at 10^8 CFU mL^{-1} according to the previously established growth curve.

Experimental design. In order to establish the experimental conditions, preliminary studies were carried out to assess the efficacy of cold plasma inactivation of *L. hilgardii* cells using various critical plasma parameters such as the type of plasma generator (with different configurations and power supplies) and the type of gas used. For the verification tests, two electrode gliding arc (GAD) reactor[27,28], radio-frequency (RF) plasma jet[29,30], dielectric barrier discharge (DBD) plasma jet with two ring electrodes on the ceramic tube[31,32] and DBD plasma jet with one ring electrode on the ceramic tube and grounded electrode below the container were used. After the exposure for 2.5 min, the most effective gas mixture was selected and the operating time was extended to 5 min only for this single gas mixture and the selected reactor. The impact of process parameters was assessed by determining the survival of cold plasma-treated cells were determined by standard counting method on MRS plates. Colonies on plates were enumerated after 6 days incubation at 30 °C, and viable counts were expressed

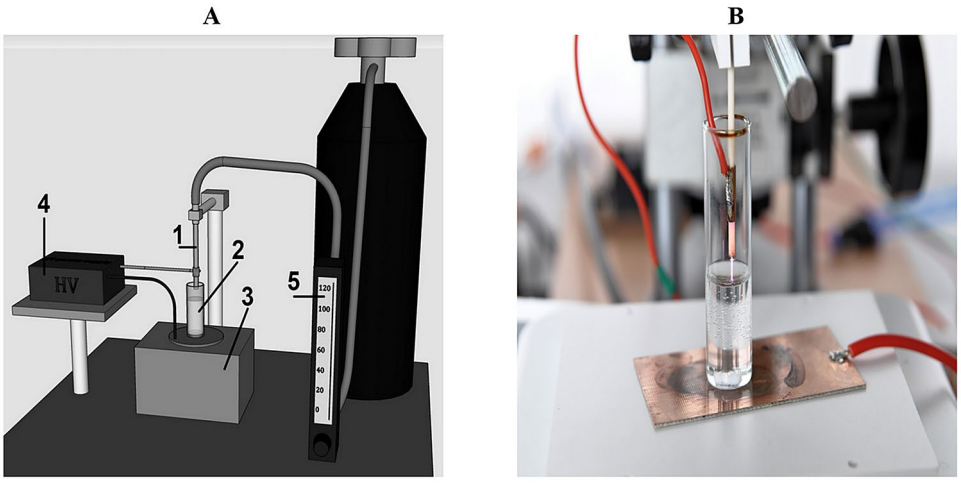


Figure 1. Experimental set-up for plasma treatment: 1—plasma jet reactor; 2—sample in a glass test tube; 3—magnetic stirrer; 4—high voltage power supply; 5—gas flow controller (**A** scheme of experimental set-up; **B** photo of reactor).

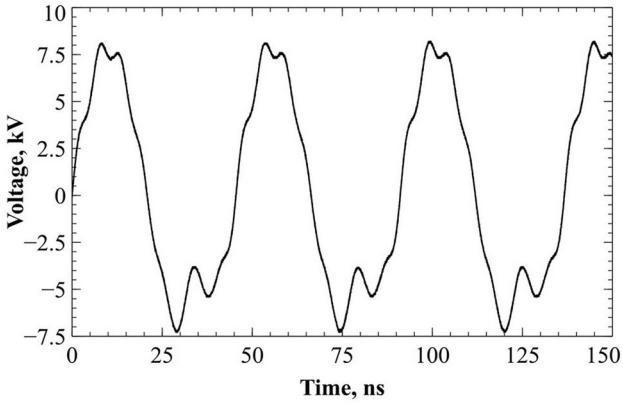


Figure 2. Voltage signal between electrodes.

as CFU/mL, as averaged values. Each experiment was performed in triplicate. Finally, process parameters were selected from the obtained data.

Cold plasma treatment of bacteria. *Exposure of microorganisms to cold plasma.* Four sterile glass tubes containing 6 mL of *L. hilgardii* suspension (10^8 CFU mL^{-1}) in sterile water were prepared. One of them was a control sample not treated with cold plasma. The others were subjected to non-thermal plasma exposure for 5, 10, and 15 min respectively. After that, the samples were transferred to sterile falcons. Part of the research material was immediately used for bacterial growth analysis and SEM imaging. The rest of the material was frozen in dry ice with 20% glycerol and stored at − 80 °C for further experiments.

Plasma treatment system. The cold plasma treatment was performed using a dielectric barrier discharge (DBD) reactor with the power supply of push–pull topology. Helium and oxygen were used as working gases. The atmospheric pressure plasma generated in the reactor had a form of a jet emerging from a ceramic tube with internal and external diameters of 1.4 mm and 3.4 mm, respectively. A high-voltage ring electrode was located 10 mm from the end of the tube. A grounded flat electrode made of copper PCB laminate was placed on a magnetic stirrer. 6 mL of a liquid sample were placed in a glass test tube with a 20-mm internal diameter, directly in the discharge area between the two electrodes. The distance between the end of the reactor and the surface of the liquid was 2 mm. The samples were mixed during the exposure to cold plasma with a PTFE stir bar (Fig. 1). The flow rate of the gas mixture of 96 L/h of helium with 1.8 L/h of oxygen was adjusted by gas flow controllers. The reactor was supplied by RMS voltage of 5.1 kV with frequency of 21.43 kHz and mean power of 6 W. The sine-like voltage signal is shown in Fig. 2.

Bacterial growth parameters. Growth parameters of *L. hilgardii* before and after the plasma treatment were determined by plate counting method and measurements of optical density (OD_{600}) in microcultures with a Bioscreen C system (Labsystem, Helsinki, Finland). Briefly, bacterial cultures were centrifuged and suspended in physiological saline and set to the same optical density of 0.2 at 600 nm. Bacteria were grown in 250 µL of MRS medium, which was transferred into 100-well Bioscreen plates in triplicate, and inoculated with 50 µL of the bacterial suspension. The experiment was performed in aerobic conditions by measuring the OD_{600} every 2 h for 96 h. The following growth curve parameters (i.e. lag time, doubling time, max specific growth rate) were determined using the PYTHON script according to Hoeflinger et al.[33].

Flow cytometry. Cellular metabolic activity and viability of microbial cells from the treated and non-treated samples (10^8 CFU mL^{-1}) were examined using a BacLight Redox Sensor Green Kit (Life Technologies, Carlsbad, CA, USA) and a flow cytometer (cell sorter) BD FACS Aria III (Becton Dickinson, San Jose, CA, USA). Preparation of samples together with configuration and settings of the instrument were based on the protocol described in Duber et al.[34]. The BacLight Redox Sensor Green Kit contains RedoxSensor Green reagent and propidium iodide. The RedoxSensor Green reagent is a fluorogenic redox indicator dye[35]. This compound is subjected to conversion by microbial reductases involved in the electron transport chain. Following excitation (maximum = 490 nm), the converted dye emits green fluorescence (maximum = 520 nm). The intensity of green fluorescence emission is directly proportional to the cellular redox potential (CRP), indicating the levels of metabolic activity of microbial cells[36]. Propidium iodide (PI) is an indicator of cellular integrity facilitating discrimination of live and dead microbial cells. The microbial cells were characterized by two non-fluorescent parameters: forward scatter (FSC) and side scatter (SSC) and two fluorescent parameters: green fluorescence (FITC) from the RedoxSensor Green reagent collected using a 530/30 band pass filter and red fluorescence (PE-TexasRed) from the propidium iodide (PI) reagent collected using a 616/23 band pass filter. For excitation of both fluorescent reagents, 488-nm blue laser was employed. Data were acquired in a four-decade logarithmic scale as area signals (FSC-A, SSC-A, FITC-A, and PE-TexasRed -A) and analysed with FACS DIVA software (Becton Dickinson).

The analysis facilitated detection of dead, mid-active, and active microbial cells. Sub-populations Q1, Q2, and Q4, i.e. dead, mid-active, and active microbial cells, respectively, were selected based on differences in the level of metabolic activity measured as the cellular redox potential (CRP)[37,38]. The definition of the discrete sub-populations was supported by gating in the dot plots of green fluorescence (FITC-A) versus side scatter (PE-TexasRed-A) using quartile gates. The calculation of CRP values was performed using medians of green fluorescence (FITC-A) signals of gated sub-populations.

Microbial cells from the defined sub-populations (Q1, Q2, and Q4) were isolated using a BD FACS Aria III cell sorter. Cell sorting was preceded by the doublet discrimination procedure with the use of height versus width scatter signal measurement (SSC-H vs. SSC-W and FSC-H vs. FSC-W) in order to discriminate single cells from conglomerates allowing high purity sorting. AccuDrop beads (Becton Dickinson, San Jose, CA, USA) were used to setup the drop delay for the best sorting performance. The FACS Aria III cell sorter settings were also established so as to achieve the highest purity level. The cells were sorted to 5 mL cytometry tubes.

Recovery of the subpopulation cells. The recovery method of Liu et al. (2018) was used with minor modifications[39]. Cells, which had previously been sorted using flow cytometry into subpopulations in different physiological states (active, mid-active, and dead cells) and stored at − 80 °C with cryoprotectant, were resuspended in fresh sterilized MRS broth supplemented with Tween-20 and amino acids was performed for recovery of potential VBNC *L. hilgardii* cells. Bacteria were grown in 250 µL of MRS medium, which was transferred into 100-well Bioscreen plates in triplicate, and inoculated with 50 µL of the bacterial suspension. The experiment was performed in aerobic conditions by measuring the OD_{600} every 4 h for 168 h. Next one hundred microliters of *L. hilgardii* cells were plated onto supplemented MRS agar. After incubation at 32 °C, microbial growth and colony formation on agar plate was observed.

Detection of morphological changes. *Scanning electron microscopy (SEM).* *L. hilgardii* cells (10^8 CFU mL^{-1}) before and after the cold plasma treatment were subjected to SEM analysis in order to investigate morphological changes in the cell surface due to potential cell damage. Bacteria were transferred directly from the culture onto microscopic stubs, frozen in liquid nitrogen, and transferred to the cryo system (Polaron Range, PP 7480). The samples were sublimed (15 min, − 85 °C), sputter-coated with platinum, and transferred into the microscope chamber. The bacteria were observed with the use of an in-lens detector, EHT 3 kV, SEM (Zeiss, ULTRA PLUS).

Atomic force microscopy (AFM). Control samples and samples treated with cold plasma for 10 min, which had previously been sorted using flow cytometry into subpopulations in different physiological states (active, mid-active, and dead cells), were analysed using AFM. After sorting concentration of cells in each samples were respectively; 10^4 CFU mL^{-1} (active cells), 10^5 CFU mL^{-1} (mid-active cells) and 10^5 CFU mL^{-1} (dead cells). Samples were prepared according to Zdybicka-Barabas et al.[40] and Rachwał et al.[41]. After three rounds of centrifugation (8000*g*, 10 min, 4 °C) of the cells in sterile water, the pellets were resuspended in sterile water at $OD_{600} = 0.1$. Next, 5 µL of the bacterial suspension were applied to the centre of mica discs and allowed to dry overnight at room temperature. NanoScope Analysis vet. 1.40 software V AFM (Veeco Oyster Bay, NY, USA) was used for imaging the bacterial cell surface (Analytical Laboratory, Faculty of Chemistry, UMCS, Lublin, Poland). The measurements were performed in the 'Peak Force QNM' operation mode using a silicon tip with a spring constant of 24 N m^{-1} (NSG30, NT-MDT, Russia) and resonance frequency of 300 kHz. The resolution of

Option	Plasma generator	Gas used	Log N (CFU/mL) 2.5 min	Log N (CFU/mL) 5 min
A	Glide arc	Air	8.86 ± 0.21	Nt
		Nitrogen	8.83 ± 0.15	Nt
		Helium/oxygen	8.83 ± 0.09	7.78 ± 0.07
B	Plasma jet	Air	8.83 ± 0.17	Nt
		Nitrogen	8.81 ± 0.25	Nt
		Helium/oxygen	8.72 ± 0.18	7.28 ± 0.12
C	DBD	Air	8.45 ± 0.09	Nt
		Nitrogen	8.40 ± 0.12	Nt
		Helium/oxygen	7.89 ± 0.05	6.04 ± 0.09
D	RF	air	8.57 ± 0.07	Nt
		nitrogen	8.58 ± 0.13	Nt
		helium/oxygen	7.93 ± 0.21	6.93 ± 0.11

Table 1. Number of live cells of *Lentilactobacillus hilgardii* after cold plasma treatment for 2.5 min and 5 min using various plasma generators (*nt* not tested). Cell concentrations for control samples at each stage of the experiment were 8.89 ± 0.11 (2.5 min) and 7.81 ± 0.19 (5 min) log CFU/mL respectively. *DBD* dielectric barrier discharge, *RF* radio frequency discharge.

the scans was 256 × 256. Three bacterial cells from each mica disc were analysed. The topographies of the tested samples were determined on the basis of height and peak force error images, the DMT module, and adhesion forces between the cell surface and the tip. The data were analysed with Nanoscope Analysis ver. 1.40 software (Veeco, Oyster Bay, NY, USA). The values of average root-mean-square (RMS) roughness and adhesion forces of the cell surface were calculated using 30 fields (120 × 120 nm) in 1 × 1 µm images of the bacterial cell surface. The Student's *t* test was used to establish the differences between the control samples and bacterial cells in the active, mid-active, and dead states after the 10-min plasma treatment, and P values of < 0.05 were considered significant. The three-dimensional images and section profiles of the *L. hilgardii* cells were generated using Nanoscope Analysis.

Fourier transform-infrared spectroscopy (FT-IR). FT-IR spectra were collected on a Nicolet 8700 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA) equipped with a KBr beam-splitter and a Mercury Cadmium Telluride MCT/A detector in the transreflection mode. The spectra were recorded over the range of 4000–650 cm^{-1}. Each spectrum represented an average of 120 scans and was apodized with the Happ–Genzel function; the number of scan points was 8480. The spectral resolution was 8 cm^{-1}. Each sample was measured five times. Each spectrum was baseline corrected and then the spectra were normalized. Assignment of the functional groups of the FT-IR spectra was carried out according to Naumann et al.[42]. To prepare the samples, bacteria (10^8 CFU mL^{-1}) were ground on an aluminium-coated microscope glass slide. The background spectrum was recorded for every sample. FTIR spectra were measured and analysed using Atlus Microscopy Software for OMNIC-8.

Results and discussion

Effect of non-thermal plasma on inactivation of *L. hilgardii* cells. The inactivation mechanisms of cold plasma on microbial cells has been confirmed by numerous scientific reports[14,43]. However, there are no studies describing the effects of this sterilizing agent on cells of wine spoilage bacteria such as *L. hilgardii*. Previous reports indicate that the effectiveness of cold plasma inactivation is influenced not only by the environmental conditions (matrix type, pH) and the process parameters (type of reactor, duration of the process) but also by the specific properties of microorganisms[14,15,44]. For this reason, the first step of our experiment was to carry out initial optimization of the process conditions, which included the selection of the reactor, working gas and duration of the plasma treatment of *L. hilgardii* cells. Based on the survival of bacterial cells exposed to cold plasma in various conditions, we decided to use a dielectric barrier discharging cold atmospheric plasma with a mixture of helium and oxygen as working gases in the next stages of the experiment (Table 1).

An important point in understanding the exact mechanism of the cold plasma action on bacterial cells is to determine the time required for complete growth inhibition[45]. For this purpose, the present study was to evaluated the differences between the application of traditional cells counting techniques and FCM analysis. Experimental data for the *L. hilgardii* growth parameters after cold plasma exposure were determined in comparison with the control sample using the classical plate culture method, optical density measurement and flow cytometry techniques. The inhibition of *L. hilgardii* growth after non-thermal plasma treatment for 5, 10, and 15 min is presented in Figs. 3 and 4. Complete inhibition of bacterial growth was observed after the 10-min cold plasma exposure. However, the 5-min plasma treatment resulted in 263% lag time extension and 46% doubling time extension, compared to the control samples. The distinct lag phase suggested adaption to the stress growth conditions and the onset of repair mechanisms. Since the working gas used was a mixture of helium and oxygen, a high concentration of reactive oxygen species, i.e. singlet oxygen, ozone, hydrogen peroxide, or superoxide anion,

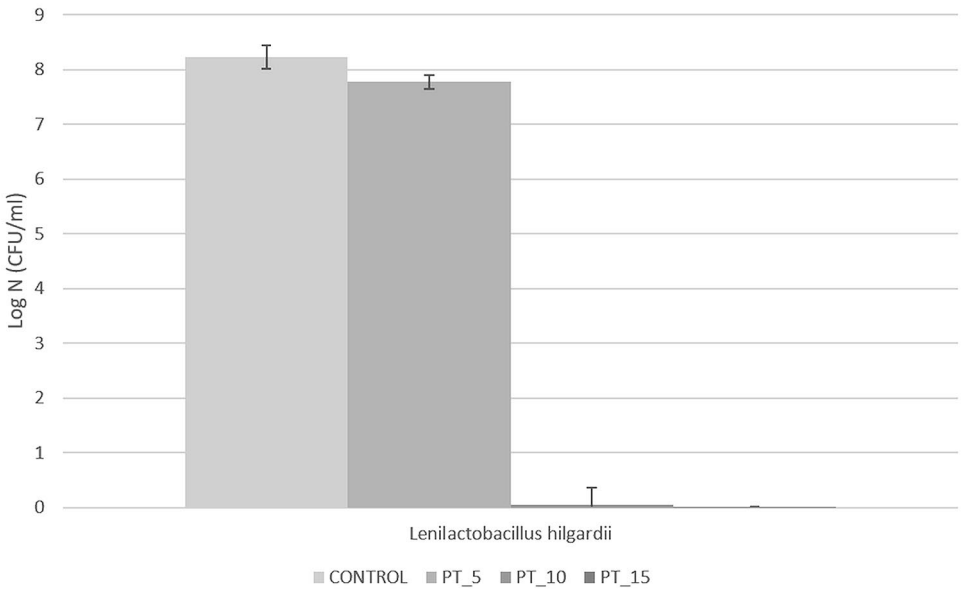
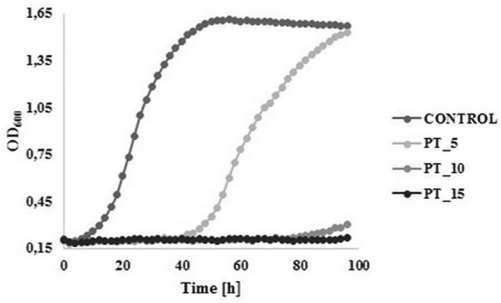


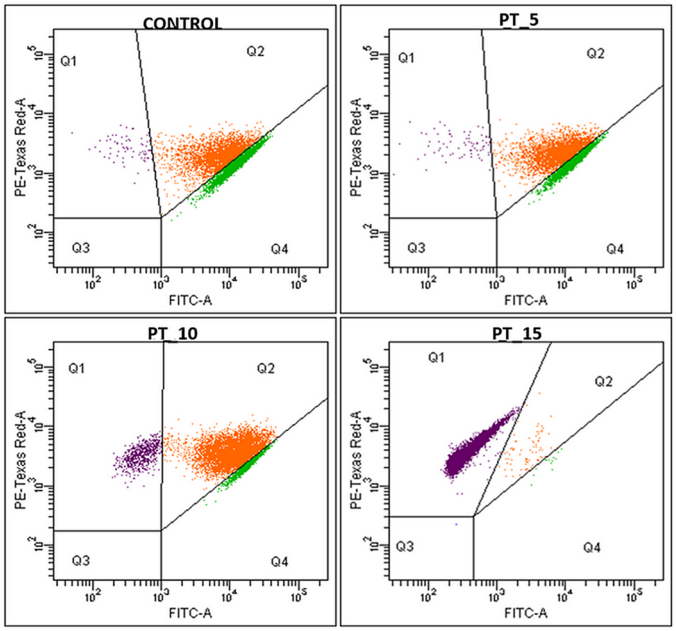
Figure 3. *L. hilgardii* strains population reduction under treatment of DBD. Bars represent standard deviation.



	CONTROL	PT_5	PT_10	PT_15
lag_time	13.147	47.759	No growth	No growth
max growth	0.059	0.04		
doubling time	11.758	17.126		
ΔOD max	1.410	1.347	0.115	0.027

Figure 4. *L. hilgardii* growth parameters after plasma treatment (*PT_5* 5-min plasma treatment, *PT_10* 10-min plasma treatment, *PT_15* 15-min plasma treatment).

was probably present in the plasma stream. For this reason, we suspect that ROS induced oxidative stress in the cell[46]. Nevertheless, lactic acid bacteria have developed a number of defensive mechanisms against ROS. These mainly include excessive production of antioxidant enzymes such as peroxide dismutase (peroxide degradation), which is encoded by the sodA gene and its expression occurs in the presence of oxygen[47]. However, other studies, in which DBD was also the plasma source and the working gas was an argon/oxygen mixture, showed that the time needed to fully inactivate Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Vibrio parahaemolyticus*) and Gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus*) was 5 min. Nevertheless, these differences may result from the fact that the number of living cells was determined only by plate counting method[48]. Flow cytometry in combination with specific fluorescent staining facilitated characterization of the physiological states of bacterial cells in the tested samples. The BacLight Redox Sensor Green Vitality Kit (Thermo Scientific) was used to characterize and distinguish the different physiological states of the treated microbial strains based on measurements of cellular metabolic activity. The flow cytometry analysis facilitated



	ACTIVE (Q4)	MID-ACTIVE (Q2)	DEAD (Q1)
CONTROL	56.1 %	42.9 %	0.8 %
PT_5	55.6 %	43.5 %	0.9 %
PT_10	14.4 %	77.5 %	8.1 %
PT_15	0.2 %	0.9 %	98.8 %

Figure 5. Flow cytometry analysis of the physiological state of *L. hilgardii* cells (active, mid-active, dead) after exposure to cold plasma (*PT_5* 5-min plasma treatment, *PT_10* 10-min plasma treatment, *PT_15* 15-min plasma treatment).

discrimination of the discrete sub-populations of active (Q4), mid-active (Q2), and dead (Q1) microbial cells. The interpretation of the results of the experiment consisted in determining the percentages of the defined sub-populations of dead, mid-active, and active cells (assessment of their distribution in the entire population of microorganisms in the tested samples. The percentages of the defined microbial sub-populations demonstrated considerable differences in the distribution of individual sub-populations of active, mid-active, and dead microbial cells (sub-population Q4, Q2, and Q1, respectively) between the untreated (control) and cold plasma-treated samples (Fig. 5). The microbial cells from mid-active sub-population (Q2) may represent the dormant and injured cells (including VBNC), which may undergo a resuscitation process[36] and regain viability[49] which leads to the recovery of culturability. The substantial resolution of the applied assay helped to characterize and distinguish the different physiological states to monitor the germicidal effect of the tested anti-microbial procedure. The first indication of the bactericidal effect was demonstrated in the sample subjected to the 10-min plasma exposure (PT_10) as an 8.1% increase in the percentage of dead bacterial cells. This was accompanied by a considerable increase in the percentage of mid-active cells (77.5% vs. 42.9% for the untreated control). The *L. hilgardii* sample subjected to the 15-min cold plasma treatment (PT_15) demonstrated a virtually detrimental effect with the dominance of dead bacterial cells (98.8%). The flow cytometry evaluation of the metabolic activity of the microbial cells helped to discriminate between different physiological states of microbial cells in the plasma-treated samples. This facilitated monitoring of the microbial heterogeneity to evaluate the impact of the tested anti-microbial procedure on the viability and vitality of the microbial community at the single-cell level. As shown by the bacterial growth curve in Fig. 4, *L. hilgardii* subjected to the 10-min plasma treatment exhibited cell growth on the fourth day of culture. Our results demonstrate that cold plasma treatment may have constituted an environmental stress that initiated response strategies in the *L. hilgardii* cells. Pavlov and Ehrenberg have described a model in which bacteria manipulate their own gene expression to adapt quickly to changes in the environment[50]. Microbial response provides bacteria with an ability to concentrate on growth metabolism instead of multiplying under environmental stress[51]. In our experiment, we can assume that the *L. hilgardii* response includes the viable but non-culturable (VBNC) state, in which the bacteria cannot form a colony on solid media but still possess the capability of renewed metabolic activity, namely, to be still alive in favourable conditions. These strategies inevitably pose a potential hazard to the food processing industry. The VBNC state is a bacterial strategy to survive adverse conditions causing cellular stress, i.e. extreme temperatures, elevated osmotic pressure, exposure to light or oxygen content. As mentioned earlier in the plasma stream, whose working gas is oxygen, the ROS concentration is high, which can potentially induce cell entry into the VBNC state[46]. In order to confirm our hypothesis that cold plasma induces VBNC state in *L. hilgardii* cells, we have performed resuscitation of sub-population cells on supplemented MRS broth and MRS agar. Figure 6 shows the growth of the recovery cells. There was observed the growth of active and mid-active cells and the lack of growth of dead cells. This may confirm our assumption that some of the mid-active cells of *L. hilgardii* are able to be cultured under appropriate conditions. The resuscitation experiment was carried out after storing the samples at − 80 °C (with cryoprotectant), therefore we observe a longer stationary phase. It is worth noting the faster growth of cells in the VBNC state in relation to active cells. At this stage of the experiment we can assume that the faster and more intensive cell divisions may be influenced by defense mechanisms activated under environmental stress, such as cold plasma. Although our study did not include investigation of changes in gene expression under the influence of

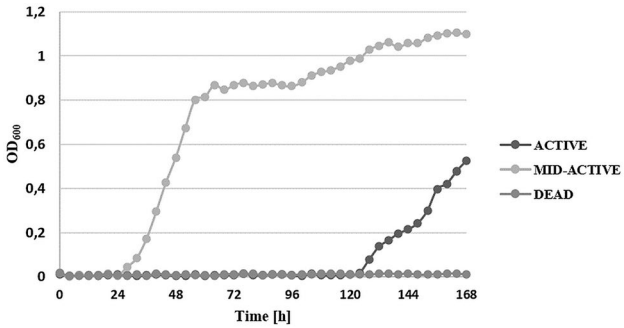


Figure 6. Recovery of the subpopulation *L. hilgardii* cells after 10-min plasma treatment.

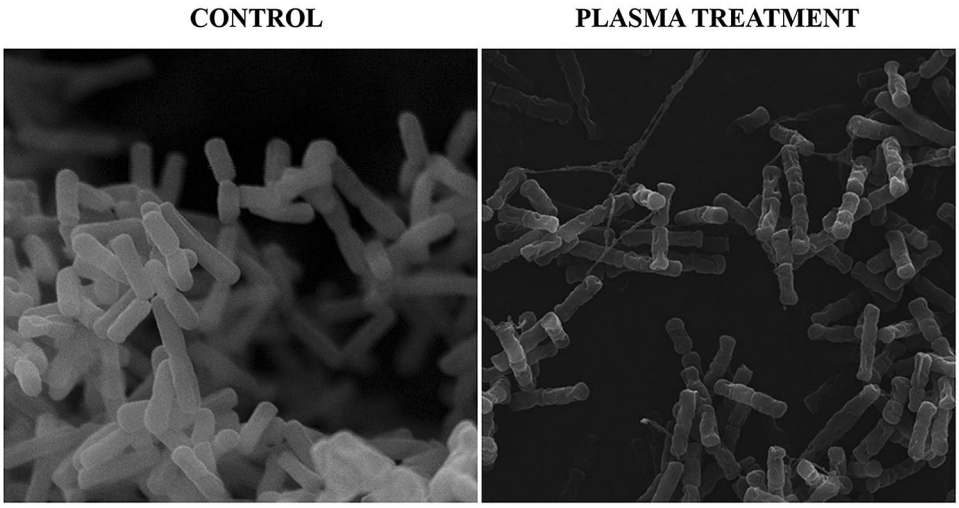


Figure 7. Scanning electron micrographs of *L. hilgardii cells*: untreated (control) and after 10-min cold plasma treatment.

cold plasma, however, on the basis of studies carried out by other authors we can assume that the potential entry into VBNC was related to the loss of catalase activity[52]. Recent studies indicate that *Schleiferilactobacillus harbinensis* (previously *Lactobacillus herbinesis*)[7] strains from rotten beer can enter the VBNC state and, what is important, the cells recover their ability to grow on media with catalase[46]. Furthermore, other reports confirmed that new non-thermal decontamination techniques such as high hydrostatic pressure (HHP) induced cell entry into the VBNC state[26,53]. Unfortunately, in most literature reports on the efficacy of cold plasma, only a few authors use detection methods that are more sensitive than the plate culture methods[26]. A possibility of induction the VBNC state by cold plasma was reported by Dolezalova and Lukas[54] who received a 7 log reduction in *E. coli* growth assessed by the plate count as a result of treatment with an atmospheric pressure plasma jet. In turn, the LIVE/DEAD technique showed only 1 log reduction of *E. coli*. These results show that the classical culture methods are insufficient to determine the effectiveness of new sterilization methods and may cause falsification of the results. In addition, cold plasma may induce the VBNC state in *L. hilgardii* cells.

Effect of cold plasma on bacterial cell morphology. Cold plasma inactivation is primarily based on the interaction of its components with the cell surface. The oxygen and nitrogen compounds in the plasma cause oxidation of lipids and proteins of the membrane and, consequently, interruption of its continuity. Additionally, the plasma contains charged particles, which accumulate on the outer side of the membrane leading to its disintegration[15]. In order to determine the relationship between the physiological state and morphology of the cell after exposure to cold plasma, we used various microscopic techniques to characterize cell wall damage at the nanoscale. Scanning electron microscopy (SEM) was used to examine the cell surface. Atomic force microscopy (AFM) was used to correlate the nanometric scale observations with the nanomechanical and physical properties. The SEM analysis was carried out to observe the morphological changes in plasma-untreated bacterial cells in comparison to the cells treated with the plasma for 10 min (Fig. 7).

The untreated *L. hilgardii* cells are visible as short or long rod-shaped cells with a smooth surface. In contrast, most of the treated cells have an irregular shape with deep cavities on the surface. In some cells, the outer layer looks as if it has been etched. This effect is probably caused by radicals (OH^- or NO^-) that cause erosion and

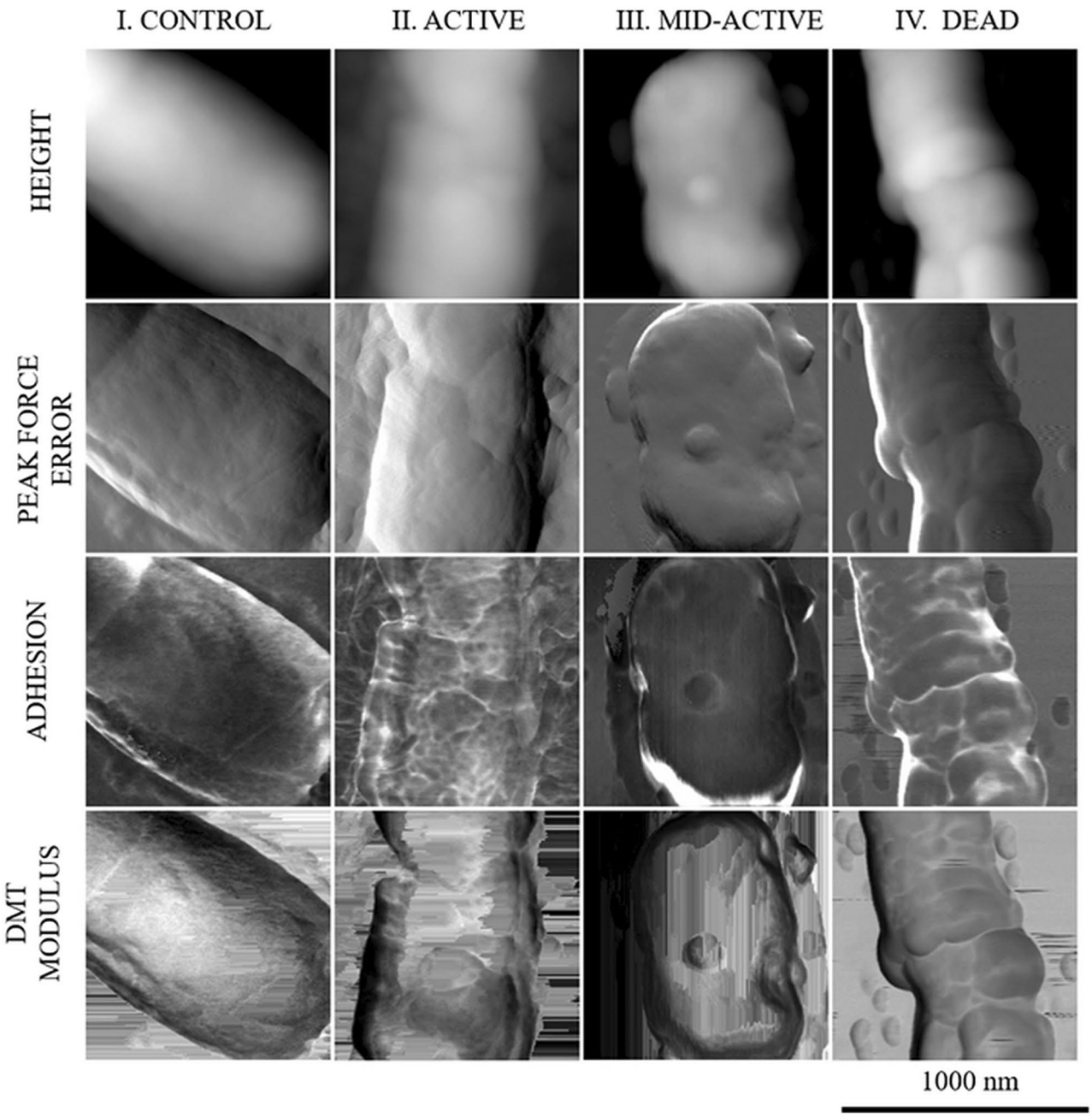


Figure 8. AFM imaging of cells sorted into subpopulations after 10-min plasma treatment.

digestion of the cell membrane[26]. However, the substantial number of cells in the intermediate state indicates that not all cells have suffered sufficiently serious external damage that would lead to their death. Previous studies have pointed out differences in the action of cold plasma on bacterial cells due to the different structure of external cellular covers. In general, Gram-positive bacteria are considered to be more resistant to CP than Gram-negative species, and the mechanism of their inactivation based on reactions of ROS (reactive oxygen species) will cause severe damage to intracellular components (e.g., DNA) but not cell leakage[55]. In our study, atomic force microscopy (AFM) images were used for further investigations of the changes in *L. hilgardii* before and after the CP treatment. The analysis allowed determination of the discrepancies in the topography and surface properties of cells after the 10-min exposure to cold plasma in three different physiological states (active, mid-active, dead) compared with cells that were not exposed to the sterilizing agent. To the best of our knowledge, AFM was used for the first time in this work to evaluate cells sorted into subpopulations after cold plasma treatment. Cells in the control sample had a typical rod shape and smooth surface, whereas cells subjected to the 10-min plasma treatment (active, mid-active) had a more irregular shape, which can be seen as the height and peak force error images (Fig. 8). The cells in the intermediate state were shorter and smaller. Many authors point out a marked reduction in the cell size in the VBNC state (shorter length), which is a starvation survival strategy[56,57]. Interestingly, the section profile (Fig. 9) also indicates that these cells were almost twice the height

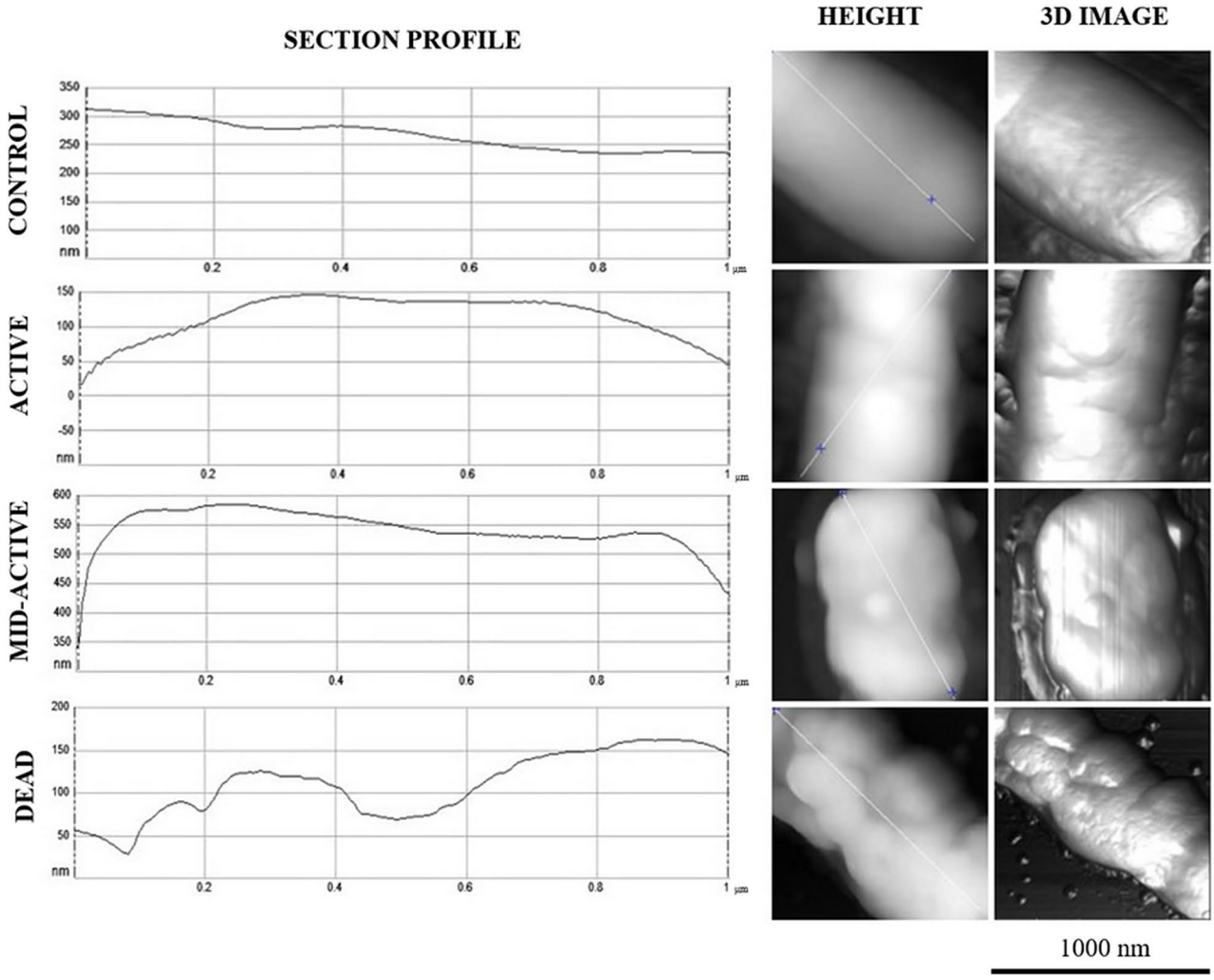


Figure 9. Section profile of the cell surface of *L. hilgardii* cells in three different physiological states after plasma treatment.

	Control	Active	Mid-active	Dead
DMT modules	1.81 ± 0.24 GPa	1.97 ± 0.19 GPa	2.19 ± 0.17 GPa	1.45 ± 0.28 GPa
Roughness	2.04 ± 0.55 nm	2.11 ± 0.57 nm	2.70 ± 0.75 nm	7.07 ± 3.1 nm
Adhesion	0.32 ± 0.08 nN	0.29 ± 0.09 nN	0.43 ± 0.09 nN	0.24 ± 0.02 nN

Table 2. Values of DMT Modules, roughness and adhesion of cells surface sorted into subpopulations after 10-min plasma treatment.

of the control cells. Similar results were obtained by Hung et al.[58], who observed *Vibrio parahaemolyticus* in the VBNC state as bulged cells. The transition from rod-shaped bacterial morphology into coccoid morphology has been characterized for some bacteria in the literature[59,60]. A change in the shape is likely to contribute positively to the uptake of nutrients by the cell. This is related to the large cell surface area compared to the cellular volume[61]. However, considerable differences in cell topography can be observed in cells whose physiological condition indicates that they are dead. The section profile (Fig. 9) indicated that these cells had deep, irregular depressions on their surface but it seemed that the integrity of the outer layers was not interrupted. This is consistent with previous reports demonstrating that inactivation of Gram-positive bacteria is the result of ROS action on the internal components of cells. Moreover, the nanomechanical properties of cells in the examined physiological states in comparison with the control are shown in the peak force error, DMT module, and adhesion images. Thanks to the application of AFM technique, in addition to images showing the differences in the cell surface, we obtained figures that allowed us to calculate values corresponding to cell surface RMS roughness, adhesion, elasticity, and stiffness (Table 2).

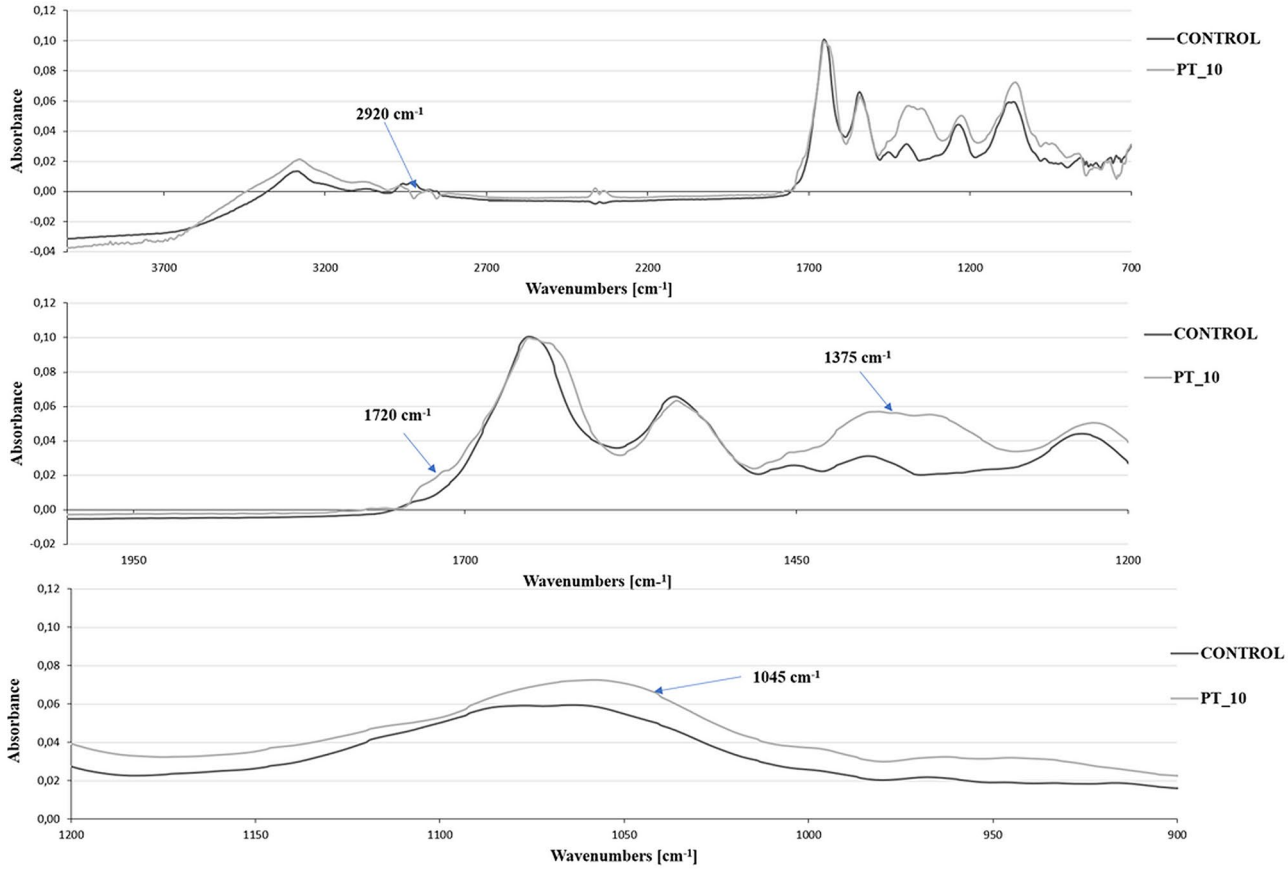


Figure 10. FT-IR spectra of *L. hilgardii* cells: untreated (control) and after 10-min cold plasma treatment.

The DMT Module in the dead cells was 1.45 ± 0.28 GPa, which indicated that the surface was more flexible than surface of control cells (1.81 ± 0.24 GPa) after the plasma treatment. In turn, the highest stiffness (2.19 ± 0.17 GPa) was typical for cells in the intermediate state; however, the results were similar to those of the control and living cells. An increase in stiffness is probably associated with a change in the cell shape. Research conducted by Vattakaven et al.[62] showed that *Vibrio tasmaniensis* in the VBNC state changed into coccal forms with a thicker cell wall after 150-day cold- starvation[62]. In addition, the roughness of the dead cells (7.07 ± 3.1 nm) was statistical significantly higher in relation to the control (2.04 ± 0.55 nm) ($p < 0.05$). The enhanced roughness suggests disorganization of the cell wall surface and correlates with the changes observed by SEM. The roughness of the living cells should be underlined. Despite the exposure to cold plasma, the roughness of their outer structures was almost the same as that of the cells in the control sample. These unchanged cell surface properties in the viable and intermediate states suggest that some of mid-active bacteria can resume growth and divisions in favourable environmental conditions.

FT-IR. The analysis of the chemical modifications in the outer structure of plasma-treated *L. hilgardii* bacteria was performed using the FT-IR technique. A comparison of the control sample with the sample subjected to 10-min cold plasma exposure demonstrates a substantial number of spectral changes associated with vibrations of functional groups such as carbohydrates, fatty acids, proteins, or nucleic acids (Fig. 10). The 3100–2800 cm^{-1} spectral region is the fatty acid region, 1800–1500 cm^{-1} represents the amide region, 1500–1200 cm^{-1} is a mixed region containing signals from proteins, fatty acids, and phosphate-carrying compounds, 1200–900 cm^{-1} is the polysaccharide region, and 900–700 cm^{-1} is the "fingerprint region"[63]. In first region, a decrease around the bands ~2960 cm^{-1} and 2920 cm^{-1} was observed after the plasma treatment, which is related to destruction of antisymmetric stretching in the CH_3 and CH_2 groups of fatty acids. Similar results were presented by Khan et al.[64], who examined the effect of DBD on the mechanism of bacterial biofilm inactivation. The authors suggested that the decrease in the intensity of the spectra corresponded to cell membrane disturbances[64]. The FT-IR investigations show very prominent increase around 1375 cm^{-1} which may be related to symmetric stretching vibration of C=O indicating a formation of the carboxylate groups (COO^-). Figure 10 shows a rising shoulder at 1720 cm^{-1}, indicating the formation of new carbonyl groups like aldehydes, ketones, or acids. Probably, the occurrence of these two signals is associated with the degradation of the cell wall and membrane caused by ROS (e.g. OH^- and O^{2-})[65,66]. There is also an increase in the band at 1045 cm^{-1}. As described in other reports, protein treatment with CAP is probably associated with symmetrical tensile vibrations of S=O and radical oxidation of sulphur-containing amino acid methionine[67–69]. This demonstrates that plasma can cause oxidation of sulphur-

containing molecules such as amino acids (methionine, cysteine), proteins, or compounds with antioxidant effects, e.g. glutathione (GSH).

Conclusion

In this work a model experimental system was used to assess the effect of cold plasma on *L. hilgardii* cells. Differences between cell viability results obtained by plate count method, optical density measurement and flow cytometry are presented. In the classical plate culture method and optical density measurement complete inhibition of bacterial growth was observed after the 10 min cold plasma exposure. On the other hand, flow cytometry analysis indicating the levels of metabolic activity of microbial cells revealed the presence of three physiological states: active (14.4%), mid-active (77.5%) and dead (98.8%) of *L. hilgardii* population after 10 min plasma treatment. Re-cultivation of the sorted subpopulation seems to confirm that at least some of the mid active cells were in the VBNC state. Moreover, application of AFM technique indicated that morphological changes in external cell structures in the intermediate state did not show any considerable damage compared to control cells. This also suggests that cells can grow again in favorable conditions. However, entry of the food spoilage bacteria in VBNC state is a potential threat to the food industry. The classical plate culture method, which is not able to detect the mid-active bacteria, is commonly used to determine the microbiological purity of a food product. For new non-thermal food preservation methods such us cold plasma, whose exact mechanism of microbial inactivation is not yet known, it is necessary to use more sensitive detection methods that will give a reliable result on the microbiological purity of the product. In summary, the flow cytometry used in this work, combined with cell sorting that allows the analysis of different subpopulations, is a valuable new approach in explaining the bactericidal effect of cold plasma and requires further research will help explain the exact mechanism of microbiological inactivation in the future.

Received: 15 June 2020; Accepted: 16 October 2020
Published online: 03 November 2020

References

1. Dimopoulou, M., Hatzikamari, M., Masneuf-Pomarede, I. & Albertin, W. Sulfur dioxide response of *Brettanomyces bruxellensis* strains isolated from Greek wine. *Food Microbiol.* **78**, 155–163 (2019).
2. Niu, D. *et al.* Effect of ethanol adaption on the inactivation of *Acetobacter* sp. by pulsed electric fields. *Innov. Food Sci. Emerg.* **52**, 25–33 (2019).
3. Puligundla, P., Pyun, Y. R. & Mok, C. Pulsed electric field (PEF) technology for microbial inactivation in low-alcohol red wine. *Food Sci. Biotechnol.* **27**(6), 1691–1696 (2018).
4. Henríquez-Aedo, K., Durán, D., Garcia, A., Hengst, M. B. & Aranda, M. Identification of biogenic amines-producing lactic acid bacteria isolated from spontaneous malolactic fermentation of Chilean red wines. *LWT* **68**, 183–189 (2016).
5. Košmerl, T., Šućur, S. & Prosen, H. Biogenic amines in red wine: the impact of technological processing of grape and wine. *Acta Agric. Slov.* **101**(2), 249–261 (2013).
6. Rodas, A. M., Ferrer, S. & Pardo, I. Polyphasic study of wine *Lactobacillus* strains: taxonomic implications. *Int. J. Syst. Evol. Microbiol.* **55**(1), 197–207 (2005).
7. Zheng, J. *et al.* A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int. J. Syst. Evol. Microbiol.* **70**(4), 2782–2858 (2020).
8. König, H., & Fröhlich, J. Lactic acid bacteria. In *Biology of Microorganisms on Grapes, in Must and in Wine* 3–41 (Springer, 2017).
9. Hynönen, U. & Palva, A. *Lactobacillus* surface layer proteins: structure, function and applications. *Appl. Microbiol. Biotechnol.* **97**(12), 5225–5243 (2013).
10. Khan, H., Kadam, A. & Dutt, D. Studies on bacterial cellulose produced by a novel strain of *Lactobacillus* genus. *Carbohydr. Polym.* **229**, 115513. https://doi.org/10.1016/j.carbpol.2019.115513 (2020).
11. Alberto, M. R., Farías, M. E. & Manca de Nadra, M. C. Effect of gallic acid and catechin on *Lactobacillus hilgardii* 5w growth and metabolism of organic compounds. *J. Agric. Food Chem.* **49**(9), 4359–4363 (2001).
12. Bruggeman, P. J. *et al.* Plasma–liquid interactions: a review and roadmap. *Plasma Sources Sci. Technol.* **25**(5), 053002. https://doi.org/10.1088/0963-0252/25/5/053002 (2016).
13. Ekezie, F. G. C., Sun, D. W. & Cheng, J. H. A review on recent advances in cold plasma technology for the food industry: current applications and future trends. *Trends Food Sci. Technol.* **69**, 46–58 (2017).
14. Liao, X. *et al.* Inactivation mechanisms of non-thermal plasma on microbes: a review. *Food Control* **75**, 83–91 (2017).
15. Niedźwiedź, I., Waśko, A., Pawłat, J. & Polak-Berecka, M. The state of research on antimicrobial activity of cold plasma. *Pol. J. Microbiol.* **68**(2), 153–164 (2019).
16. Aboubakr, H. A., Parra, F. S., Collins, J., Bruggeman, P. & Goyal, S. M. Ìn situ inactivation of human norovirus GII. 4 by cold plasma: ethidium monoazide (EMA)-coupled RT-qPCR underestimates virus reduction and fecal material suppresses inactivation. *Food Microbiol.* **85**, 103307. https://doi.org/10.1016/j.fm.2019.103307 (2020).
17. Bermudez-Aguirre, D. Advances in the inactivation of microorganisms and viruses in food and model systems using cold plasma. In *Advances in Cold Plasma Applications for Food Safety and Preservation* 49–91 (Academic Press, 2020).
18. Gök, V., Aktop, S., Özkan, M. & Tomar, O. The effects of atmospheric cold plasma on inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* and some quality characteristics of pastırma—a dry-cured beef product. *Innov. Food Sci. Emerg.* **56**, 102188. https://doi.org/10.1016/j.ifset.2019.102188 (2019).
19. Liao, X. *et al.* Bacterial spore inactivation induced by cold plasma. *Crit. Rev. Food Sci. Nutr.* **59**(16), 2562–2572 (2019).
20. Wang, Y., Wang, Z., Yang, H., & Zhu, X. Gas phase surface discharge plasma model for yeast inactivation in water. *J. F**ood Eng.* 110117; https://doi.org/10.1016/j.jfoodeng.2020.110117 (2020).
21. Misra, N. N., Yadav, B., Roopesh, M. S. & Jo, C. Cold plasma for effective fungal and mycotoxin control in foods: mechanisms, inactivation effects, and applications. *Compr. Rev. Food Sci. Food Saf.* **18**(1), 106–120 (2019).
22. Bourke, P., Ziuzina, D., Han, L., Cullen, P. J. & Gilmore, B. F. Microbiological interactions with cold plasma. *J. Appl. Microbiol.* **123**(2), 308–324 (2017).
23. Jayasena, D. D. *et al.* Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: effects on pathogen inactivation and meat-quality attributes. *Food Microbiol.* **46**, 51–57 (2015).
24. Ziuzina, D., Patil, S., Cullen, P. J., Keener, K. M. & Bourke, P. Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* inoculated on fresh produce. *Food Microbiol.* **42**, 109–116 (2014).

25. Pankaj, S. K., Wan, Z. & Keener, K. M. Effects of cold plasma on food quality: a review. *Foods* **7**(1), 4 (2018).
26. Schottroff, F. *et al.* Sublethal injury and viable but non-culturable (VBNC) state in microorganisms during preservation of food and biological materials by non-thermal processes. *Front. Microbiol.* **9**, 2773. https://doi.org/10.3389/fmicb.2018.02773 (2018).
27. Starek, A. *et al.* Evaluation of selected microbial and physicochemical parameters of fresh tomato juice after cold atmospheric pressure plasma treatment during refrigerated storage. *Sci. Rep.* **9**, 8407. https://doi.org/10.1038/s41598-019-44946-1 (2019).
28. Pawłat, J. *et al.* Evaluation of oxidative species in gaseous and liquid phase generated by mini-gliding arc discharge. *Plasma Chem. Plasma P.* **39**(3), 627–642 (2019).
29. Pawlat, J., Terebun, P., Kwiatkowski, M. & Diatczyk, J. RF atmospheric plasma jet surface treatment of paper. *J. Phys. D Appl. Phys.* **49**(37), 1–12 (2016).
30. Pawłat, J., Kwiatkowski, M., Terebun, P. & Murakami, T. RF-powered atmospheric-pressure plasma jet in surface treatment of high-impact polystyrene. *IEEE Trans. Plasma Sci.* **44**(3), 314–320 (2015).
31. Pawłat, J. *et al.* Effects of atmospheric pressure plasma jet operating with DBD on *Lavaterathuringiaca* L. seeds' germination. *PLoS ONE* **13**(4), e0194349. https://doi.org/10.1371/journal.pone.0194349 (2018).
32. Kwiatkowski, M., Terebun, P., Mazurek, P. A. & Pawłat, J. Wettability of polymeric materials after dielectric barrier discharge atmospheric-pressure plasma jet treatment. *Sensors Mater.* **30**(5), 1207–1212 (2018).
33. Hoeflinger, J. L., Hoeflinger, D. E. & Miller, M. J. A dynamic regression analysis tool for quantitative assessment of bacterial growth written in Python. *J. Microbiol. Methods* **132**, 83–85 (2017).
34. Duber, A. *et al.* Exploiting the real wastewater potential for resource recovery—n-caproate production from acid whey. *Green Chem.* **20**(16), 3790–3803 (2018).
35. Gray, D., Yue, R. S., Chueng, C. Y. & Godfrey, W. Bacterial vitality detected by a novel fluorogenic redox dye using flow cytometry. Abstr. 105th Meet. Am. Soc. Microbiol. (2005).
36. Díaz, M., Herrero, M., García, L. A. & Quirós, C. Application of flow cytometry to industrial microbial bioprocesses. *Biochem. Eng. J.* **48**(3), 385–407 (2010).
37. Juzwa, W., Duber, A., Myszka, K., Białas, W. & Czaczyk, K. Identification of microbes from the surfaces of food-processing lines based on the flow cytometric evaluation of cellular metabolic activity combined with cell sorting. *Biofouling* **32**(8), 841–851 (2016).
38. Juzwa, W., Myszka, K. & Białas, W. Investigation of the population dynamics within a *Pseudomonas aeruginosa* biofilm using a flow based biofilm model system and flow cytometric evaluation of cellular physiology. *Biofouling* **34**(8), 835–850 (2018).
39. Liu, J. *et al.* Induction and recovery of the viable but nonculturable state of hop-resistance *Lactobacillus brevis*. *Front. Microbiol.* **9**, 2076. https://doi.org/10.3389/fmicb.2018.02076 (2018).
40. Zdybicka-Barabas, A., Januszanis, B., Mak, P. & Cytryńska, M. An atomic force microscopy study of *Galleria mellonella* apolipophorin III effect on bacteria. *BBA-Biomembranes* **1808**(7), 1896–1906 (2011).
41. Rachwał, K. *et al.* The regulatory protein RosR affects *Rhizobiumleguminosarum* bv. trifolii protein profiles, cell surface properties, and symbiosis with clover. *Front. Microbiol.* **7**, 1302. https://doi.org/10.3389/fmicb.2016.01302 (2016).
42. Naumann, D., Helm, D. & Labischinski, H. Microbiological characterizations by FT-IR spectroscopy. *Nature* **351**(6321), 81–82 (1991).
43. Kartaschew, K. *et al.* Cold atmospheric-pressure plasma and bacteria: understanding the mode of action using vibrational micro-spectroscopy. *J. Phys. D Appl. Phys.* **49**(37), 374003. https://doi.org/10.1088/0022-3727/49/37/374003 (2016).
44. Brun, P. *et al.* Antibacterial efficacy and mechanisms of action of low power atmospheric pressure cold plasma: membrane permeability, biofilm penetration and antimicrobial sensitization. *J. Appl. Microbiol.* **125**(2), 398–408 (2018).
45. Han, L. *et al.* Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for *Escherichia coli* and *Staphylococcus aureus*. *Appl. Environ. Microbiol.* **82**(2), 450–458 (2016).
46. Liu, J. *et al.* Discovery and control of culturable and viable but non-culturable cells of a distinctive *Lactobacillus harbinensis* strain from spoiled beer. *Sci. Rep.* **8**(1), 1–10 (2018).
47. Papadimitriou, K. *et al.* Stress physiology of lactic acid bacteria. *Microbiol. Mol. Biol. Rev.* **80**(3), 837–890 (2016).
48. Olatunde, O. O., Benjakul, S. & Vongkamjan, K. Dielectric barrier discharge cold atmospheric plasma: bacterial inactivation mechanism. *J. Food Saf.* **39**(6), e12705. https://doi.org/10.1111/jfs.12705 (2019).
49. Al-Qadiri, H. M., Lin, M., Al-Holy, M. A., Cavinato, A. G. & Rasco, B. A. Detection of sublethal thermal injury in *Salmonella enterica* serotype *Typhimurium* and *Listeria monocytogenes* using fourier transform infrared (FT-IR) spectroscopy (4000 to 600 cm^{-1}). *J. Food Sci.* **73**(2), 54–61 (2008).
50. Pavlov, M. Y. & Ehrenberg, M. Optimal control of gene expression for fast proteome adaptation to environmental change. *Proc. Natl. Acad. Sci. U.S.A.* **110**(51), 20527–20532 (2013).
51. Wu, D. *et al.* Microbial response to some nonthermal physical technologies. *Trends Food Sci. Technol.* **95**, 107–117 (2019).
52. Kong, I. S. *et al.* Role of catalase and oxyR in the viable but nonculturable state of *Vibrio vulnificus*. *FEMS Microbiol. Ecol.* **50**(3), 133–142 (2004).
53. Kimura, K., Morimatsu, K., Inaoka, T. & Yamamoto, K. Injury and recovery of *Escherichia coli* ATCC25922 cells treated by high hydrostatic pressure at 400–600 MPa. *J. Biosci. Bioeng.* **123**(6), 698–706 (2017).
54. Dolezalova, E. & Lukes, P. Membrane damage and active but nonculturable state in liquid cultures of *Escherichia coli* treated with an atmospheric pressure plasma jet. *Bioelectrochemistry* **103**, 7–14 (2015).
55. Laroussi, M., Mendis, D. A. & Rosenberg, M. Plasma interaction with microbes. *New J. Phys.* **5**(1), 41 (2003).
56. Pienaar, J. A., Singh, A. & Barnard, T. G. The viable but non-culturable state in pathogenic *Escherichia coli*: a general review. *Afr. J. Lab. Med.* **5**(1), 1–9 (2016).
57. Yoon, J. H., Bae, Y. M. & Lee, S. Y. Effects of varying concentrations of sodium chloride and acidic conditions on the behavior of *Vibrio parahaemolyticus* and *Vibrio vulnificus* cold-starved in artificial sea water microcosms. *Food Sci. Biotechnol.* **26**(3), 829–839 (2017).
58. Hung, W. C., Jane, W. N. & Wong, H. C. Association of a D-alanyl-D-alanine carboxypeptidase gene with the formation of aberrantly shaped cells during the induction of viable but nonculturable *Vibrio parahaemolyticus*. *Appl. Environ. Microbiol.* **79**(23), 7305–7312 (2013).
59. Brenzinger, S. *et al.* Structural and proteomic changes in viable but non-culturable *Vibrio cholerae*. *Front. Microbiol.* **10**, 793 (2019).
60. Pianetti, A. *et al.* Morphological changes of *Aeromonas hydrophila* in response to osmotic stress. *Micron* **40**(4), 426–433 (2009).
61. Krebs, S. J. & Taylor, R. K. Nutrient-dependent, rapid transition of *Vibrio cholerae* to coccoid morphology and expression of the toxin co-regulated pilus in this form. *Microbiology* **157**(Pt 10), 2942 (2011).
62. Vattakaven, T., Bond, P., Bradley, G. & Munn, C. B. Differential effects of temperature and starvation on induction of the viable-but-nonculturable state in the coral pathogens *Vibrio shiloi* and *Vibrio tasmaniensis*. *Appl. Environ. Microbiol.* **72**(10), 6508–6513 (2006).
63. Dziuba, B., Babuchowski, A., Nałęcz, D. & Niklewicz, M. Identification of lactic acid bacteria using FTIR spectroscopy and cluster analysis. *Int. Dairy J.* **17**(3), 183–189 (2007).
64. Khan, M. S. I., Lee, E. J. & Kim, Y. J. A submerged dielectric barrier discharge plasma inactivation mechanism of biofilms produced by *Escherichia coli* O157: H7, *Cronobacter sakazakii*, and *Staphylococcus aureus*. *Sci. Rep.* **6**, 37072. https://doi.org/10.1038/srep37072 (2016).
65. Kiwi, J. & Nadtochenko, V. Evidence for the mechanism of photocatalytic degradation of the bacterial wall membrane at the TiO_2 interface by ATR-FTIR and laser kinetic spectroscopy. *Langmuir* **21**(10), 4631–4641 (2005).

66. Hou, Y., Li, X., Zhao, Q., Chen, G. & Raston, C. L. Role of hydroxyl radicals and mechanism of *Escherichia coli* inactivation on Ag/AgBr/TiO_2 nanotube array electrode under visible light irradiation. *Environ. Sci. Technol.* **46**(7), 4042–4050 (2012).
67. Lackmann, J. W. *et al.* Effects of the effluent of a microscale atmospheric pressure plasma-jet operated with He/O_2 gas on bovine serum albumin. *Plasma Med.* **3**(1–2), 115–124 (2013).
68. Lackmann, J. W. *et al.* Photons and particles emitted from cold atmospheric-pressure plasma inactivate bacteria and biomolecules independently and synergistically. *J. R. Soc. Interface* **10**(89), 20130591. https://doi.org/10.1098/rsif.2013.0591 (2013).
69. Ravi, J., Hills, A. E., Cerasoli, E., Rakowska, P. D. & Ryadnov, M. G. FTIR markers of methionine oxidation for early detection of oxidized protein therapeutics. *Eur. Biophys. J.* **40**(3), 339–345 (2011).

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Competing interests

The authors declare no competing interests.

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Food Chemistry 381 (2022) 132257

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The impact of cold plasma on the phenolic composition and biogenic amine content of red wine

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ARTICLE INFO

Keywords:
Cold plasma
Phenolic compounds
Biogenic amines
Chemometric analysis
Wine preservation
Red wine

ABSTRACT

The effect of cold plasma (CP) on phenolic compound (PC) and biogenic amine (BA) contents of red wine was investigated for the first time. The influence of CP was compared with the effects of a wine preservation using potassium metabisulfite and a combined method. The PC profile was determined by UPLC-PDA-MS/MS while BAs using DLLME-GC–MS. Chemometric analysis also was used. The content of PCs was 3.1% higher in the sample preserved by CP treatment (5 min, helium/nitrogen) compared to a sample preserved by the addition of potassium metabisulfite (100 mg/L). On a positive note, CP treatment reduced the concentration of BAs in the wine samples. The lowest BA contents were recorded after 10 min of cold plasma (helium/oxygen) treatment with the addition of potassium metabisulfite (1120.85 μg/L). The results may promote interest in CP as a potential alternative method for the preservation of wine and other alcoholic beverages.

1. Introduction

Wine is an alcoholic beverage, the tradition of production and consumption of which has been known around the world for centuries (Gajek et al., 2021). The recent climatic changes have brought about alterations in the geographical distribution of areas used for viticulture. As a consequence of global warming, a significant increase in the area of vineyards and wine production has been observed across Central and Eastern Europe, including Poland (Koźmiński et al., 2020). Wine is produced by fermentation of sugars contained in fruit using naturally occurring microorganisms or starter cultures. This product is a complex matrix consisting of water, alcohol, carbohydrates, organic acids, polyphenols, minerals, and aromatic substances (Robles et al., 2019).

One of the most abundant and important groups of compounds found in wines are polyphenols. They are responsible for the color (anthocyanins), taste (tannins), and aroma of wines. Additionally, they show antioxidant activity, which makes them beneficial in the prevention of cardiovascular diseases and other chronic medical conditions (Snopek et al., 2018). The polyphenol content of wine depends on the grapevine strain and the grape variety, the winemaking technology, the aging processes, and the wine preservation methods used. Red wines are characterized by a higher content of polyphenolic compounds (PCs) compared to white wines, and thus show a higher antioxidant activity (Robles et al., 2019). Due to the growing consumer awareness of the health benefits associated with the consumption of polyphenol-rich products as well as the knowledge of the impact of these compounds on the final quality of a product, wine producers are looking for solutions that would minimize the loss of polyphenols during the entire winemaking process.

Besides health-promoting phenolic compounds, wines also contain biogenic amines (BAs), which may have a negative impact on human health. They are nitrogenous compounds that are mainly formed by the decarboxylation of amino acids, which in wine is the result of the activity of microbes such as yeast or lactic acid bacteria (LAB) (Smit et al., 2012). The content of biogenic amines depends mainly on the concentration of amino acid precursors in a product's matrix, but also on pH as well as alcohol and sulfur dioxide contents, which directly affect the growth of microorganisms (Papageorgiou et al., 2018). In addition, the presence of amino acid precursors is influenced by the grape variety, the geographical region, vinification methods, and the aging process

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https://doi.org/10.1016/j.foodchem.2022.132257
Received 29 October 2021; Received in revised form 10 January 2022; Accepted 24 January 2022
Available online 29 January 2022

(Płotka-Wasylka et al., 2018a). The biogenic amines most commonly found in wines include histamine (HIS), cadaverine (CAD), tyramine (TYR), 2-phenylethylamine (2-PE), putrescine (PUT), and tryptamine (TRP) (Esposito et al., 2019). High concentrations of biogenic amines in the final product may cause undesirable physiological effects in the consumer, such as headaches, nausea, or tachycardia (Naila et al., 2010).

In order to prevent the negative effects of microorganisms on the quality of wine, methods of eliminating the undesirable microbes have been developed. Although classic thermal methods of food preservation still play a very important role in food technology, they are not suitable for vinification processes as they can negatively affect the unique taste, color and flavor of wine (Niu et al., 2019). Instead, sulfur dioxide, which has decontaminating and antioxidant properties, is commonly added to wine to remove unwanted microorganisms (Christofi et al., 2020). Furthermore, the addition of sulfur dioxide is thought to prevent enzymatic and non-enzymatic oxidation of wines (Esparza et al., 2020). However, despite its positive effects, it can cause allergic reactions in some consumers, which is why the World Health Organization (WHO) has introduced restrictions on its use. This has contributed to an increased search for new strategies to minimize or even replace SO_2 (Cordero-Bueso et al., 2019), but the problem of biogenic amines still remains unsolved. Therefore, scientists are looking for effective non-thermal preservation methods which allow to remove undesirable microorganisms without significantly affecting the final stability of the product (Puligundla et al., 2018).

Cold plasma is one of the most recent non-thermal methods used in sterilization processes. Numerous scientific publications confirm its effective antimicrobial activity, which is connected with the influence of reactive compounds, atoms in the excited and basic state, and UV photons on microbial cells (Bourke et al., 2017). Reactive compounds are produced by subjecting a working gas to various electrical discharges such as barrier discharge and corona discharge. Importantly, in the context of cold plasma applications in the food industry, the temperature of the free electrons in the working gas is lower than that of the other particles, which directly results in a slight increase in process temperature (Niedźwiedź et al., 2019). To date, there are few reports in the literature explaining the mechanism of action of cold plasma on food products (Alves Filho et al., 2020; Gavahian et al., 2018). During CP generation, light emission, cavitation processes, shock wave generation, and free radical generation are accompanied, which can directly contribute to the degradation of many organic compounds (He et al., 2012). However, there is limited information in the literature regarding the impact of cold plasma on the final quality of alcoholic beverages, which means this problem is worth delving into.

The objective of the present study was to investigate the effect of a new wine preservation method using cold plasma on the phenolic composition and biogenic amine content of red wine. An additional objective was to compare the effect of preserving wine samples using the traditional method (addition of potassium metabisulfite at 30 mg/L or 100 mg/L) and a combined method (cold plasma and the addition of potassium metabisulfite at 30 mg/L) with the effect of cold plasma alone. Wine sample storage was also considered in the study. In addition, chemometric analysis was conducted to discover specific relationships between the different wine preservation methods and the content of bioamines and selected phenolic compounds. To the best of our knowledge, there is no research so far regarding the effect of cold plasma treatment on the content of phenolic compounds and biogenic amines in red wine samples in comparison with the effect of this method combined with potassium metabisulfite.

2. Materials and methods

2.1. Chemicals and materials

All reference materials used in the determination of the biogenic amines such as tryptamine hydrochloride, putrescine dihydrochloride, histamine dihydrochloride, tyramine hydrochloride, cadaverine hydrochloride and 2-phenylethylamine hydrochloride, as well as hexylamine (internal standard, IS), were purchased from Sigma-Aldrich (St. Louis, MO, USA). The derivatization reagent (isobutyl chloroformate) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Stock solutions of BAs and IS (both at 1 mg/mL) were prepared daily in ultrapure water and stored at + 4 °C in silanized screw-capped vials with solid PTFE-lined caps (Supelco, Bellefonte, PA, USA). Methanol, used as a dispersive solvent, was a high purity grade solvent purchased from Fluka (Seelze, Germany). High purity grade chloroform, applied as an extractive solvent, was obtained from Sigma. 0.1 M HCl was supplied by Fluka. Other chemicals were of an analytical grade.

Analytical standards for phenolic profile determination such as cyanidin-3-O-glucoside, delphicin-3-O-glucoside, isorhamnetin-3-O-glucoside, kaempferol-3-O-glucoside, malvidin-3-O-glucoside, myricetin-3-O-glucoside, peonidin-3-O-glucoside, petunidin-3-O-glucoside, quercetin-3-O-glucoside, quercetin-4′-O-glucoside, quercetin-3-O-rutinoside, (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-gallate, procyanidin A1 and A2, *trans*-resveratrol, and *trans*-piceid were purchased from Extrasynthese (Lyon, France). Caftaric acid, caffeic acid, coutaric acid, gallic acid, ferulic acid, protocatechuic acid, and p-coumaric acid were purchased from PhytoLab (Vestenbergsgreuth, Germany). Formic acid (LC-MS grade) was purchased from Fischer Scientific (Schwerte, Germany). Acetonitrile was purchased from POCH (Gliwice, Poland).

2.2. Wine samples

The red wine used in this study was produced at Dom Bliskowice Winery (Lublin Province, Poland) from grapes of Regent and Rondo (1:1) varieties harvested in October 2019. The wine was subjected to different preservation processes (control sample – not preserved; cold plasma treatment; addition of 30 mg/L potassium metabisulfite; addition of 30 mg/L potassium metabisulfite combined with cold plasma treatment; and addition of 100 mg/L potassium metabisulfite). The samples were then analyzed immediately after preservation and also after three months of storage (15 °C, limited light) (Table 1).

2.3. Cold plasma treatment of wine

Wine samples were exposed to cold plasma for 2-, 5- and 10-min. Mixtures of helium and nitrogen or helium and oxygen were used as the working gas. The samples were treated using a DBD (Dielectric Barrier Discharge) plasma jet reactor. The volume of 50 ml of wine was poured to a sterilized glass container and placed on a magnetic stirrer. To ensure homogenous exposure to plasma treatment, samples were stirred with a PTFE stir bar placed inside the sample. The DBD reactor consisted of a 1.4 mm internal diameter ceramic gas tube. Two metal electrodes were located as follows: a ring-shaped high voltage electrode was positioned 10 mm from the end of the jet and A flat, copper PCB laminated electrode was used as the ground. The latter electrode was placed on the magnetic stirrer, just beneath the sample container. The distance between the end of the reactor's tube and the surface of the liquid was 2 mm. The flow rates of the substrate gas mixtures were 96 L/h of helium with 1.8 L/h of oxygen or nitrogen admixtures. The flow rates were adjusted by gas flow controllers (Automatic Works "ROTAMETR," Gliwice, Poland). A schematic view of the experimental set-up is presented in Fig. 1. The mean power of the power supply was 6 W. For both gas mixtures, the sine-like voltage signals were quite similar, with a subtle difference in the maximum voltage, which was slightly higher in the case of the helium and oxygen mixture and ranged 8.3 kV.

A K-type thermocouple connected to a DT-847U meter was used to measure the temperature of the sample after plasma treatment. In the course of the experiment, the maximum registered temperature of the sample did not exceed 32 °C, so the treatment can be considered a cold

Table 1
Characterization of samples and sample coding.

Sample	Preservation method	Cold plasma exposure time	Gas used for preservation	Storage
1	no preservation	0	No	No
2	cold plasma	2	He /O_2	No
3	cold plasma	5	He /O2	No
4	cold plasma	10	He /O2	No
5	cold plasma	2	He / N_2	No
6	cold plasma	5	He / N_2	No
7	cold plasma	10	He / N_2	No
8	30 mg/L potassium metabisulfite	0	No	No
9	cold plasma and 30 mg/L potassium metabisulfite	2	He / O_2	No
10	cold plasma and 30 mg/L potassium metabisulfite	5	He / O_2	No
11	cold plasma and 30 mg/L potassium metabisulfite	10	He / O_2	No
12	cold plasma and 30 mg/L potassium metabisulfite	2	He / N_2	No
13	cold plasma and 30 mg/L potassium metabisulfite	5	He / N_2	No
14	cold plasma and 30 mg/L potassium metabisulfite	10	He / N_2	No
15	100 mg/L potassium metabisulfite	0	No	No
16	no preservation	0	No	Yes
17	cold plasma	2	He / O_2	Yes
18	cold plasma	5	He / O_2	Yes
19	cold plasma	10	He / O_2	Yes
20	cold plasma	2	He / N_2	Yes
21	cold plasma	5	He / N_2	Yes
22	cold plasma	10	He / N_2	Yes
23	30 mg/L potassium metabisulfite	0	No	Yes
24	cold plasma and 30 mg/L potassium metabisulfite	2	He / O_2	Yes
25	cold plasma and 30 mg/L potassium metabisulfite	5	He / O_2	Yes
26	cold plasma and 30 mg/L potassium metabisulfite	10	He / O_2	Yes
27	cold plasma and 30 mg/L potassium metabisulfite	2	He / N_2	Yes
28	cold plasma and 30 mg/L potassium metabisulfite	5	He / N_2	Yes
29	cold plasma and 30 mg/L potassium metabisulfite	10	He / N_2	Yes
30	100 mg/L potassium metabisulfite	0	No	Yes

one.

2.4. Determination of polyphenolic compounds

The protocol reported by Kapusta et al. (2018) was used to determine polyphenolic compounds in the wine samples. The qualitative and quantitative determination of the phenolic compound profile was performer using ultra-performance reverse-phase liquid chromatography (UPLC-PDA-MS/MS). The UPLC-PDA-MS/MS Waters ACQUITY system (Walters, Milford, MA, USA) used consisted of a sample manager, a binary pump manager, a column manager, a photodiode array (PDA) detector, and a tandem quadrupole mass spectrometer (TQD) with electrospray ionization (ESI). A BEH C18 column (100 mm × 2.1 mm i.d., 1.7 μm, Waters) was used to separate the compounds. Wine samples were filtered before the analysis through a 0.45-μm Millipore filter and then injected onto the chromatographic column. The injected sample volume was 5 μL. The experiment was conducted in duplicate. Waters MassLynx software v.4.1 was used to collect and analyze the results. The results obtained are expressed in mg/L.

The method was validated for parameters such as linearity, accuracy (relative error, RE), limit of detection (LOD), limit of quantification (LOQ), and precision (relative standard deviation, RSD). Quantification was determined by external standard calibration. Stock standard solutions of the polyphenols were prepared with methanol. Six calibrators of each standard were prepared by dilution of stock solutions, and the calibration curve was generated by plotting the peak area ratio of the polyphenol versus the nominal concentration ranging from 0.05 to 5 mg ml^{-1} ($R2 \leq 0.999$). The regression equation was obtained by weighted (1/c2) least-squares linear regression. The LOD was determined as a signal-to-noise ratio (S/N) of 3:1, and the LOQ was determined as a S/N of > 10. An acceptable RE within ± 20% and an RSD not exceeding 20% should be obtained.

2.5. GC–MS determination of biogenic amine content

The protocol reported by Płotka-Wasylka et al. (2018b) was used to determine biogenic amines (BAs) in the wine samples. Isolation of analytes was carried out simultaneously with their derivatization. The selected analytes were determined qualitatively and quantitatively using gas chromatography combined with mass spectrometry (GC–MS). A gas chromatography (GC) 7890A (Agilent Technologies, Santa Clara, CA, USA) system was interfaced with an inert mass selective detector 5975C (Agilent Technologies, Santa Clara, CA, USA) with an electron impact ionization chamber (EI). A ZB-5MS capillary column (30 m × 0.25 mm I.D., 0.25 μm) supplied by Zebron Phenomenex was used for chromatographic separation. The injection was performed in the splitless mode at 230 °C. The interface was set at 250 °C. The injected sample volume was 2 μL. Helium was the carrier gas with a constant pressure of 30 psi. The oven temperature program was as follows: 50 °C held for 1 min, ramped to 280 °C at 15 °C /min and held for 9 min (total run time was 25.3 min). The analysis was carried out in the selected ion monitoring (SIM) mode. The MS parameters were set as follows: EI ionization with 70 eV energy; ion source temperature, 250 °C. All the ion fragments with their relative intensities at the specific retention times were considered as a valid confirmation criterion and were used to identify the selected BAs. An Agilent ChemStation was used for data collection and GC–MS control.

The optimized method was evaluated using the following validation parameters: linearity, precision, sensitivity and accuracy in accordance to quality assurance protocol. Linearity was examined by application of 10 different concentrations. Hexylamine was used as internal standard. Limits of detection (LODs) and limits of quantification (LOQs) were calculated to estimate the sensitivity of the methodology. Both LODs and LOQs were calculated from spiked samples ($n = 3$) and the minimum detectable analyte amount with a signal-to-noise ratio of 3 and 10, respectively, was established. The intra-day (RSD_r) and inter-day (RSD_R) precision were determined by the application of five replicates of wine samples spiked at two levels (0.10 and 0.25 mg/L). In addition to validation parameters, recovery rates were estimated using the ratio of the peak areas of the spiked samples of known concentration of biogenic amines to those of spiked water solution ($n = 3$). The matrix effect (ME) of the optimized method was also evaluated by application the procedure described by Matuszewski et al. (2003). The ME was examined at a concentration level of 0.25 mg/L and calculated by comparing the mean peak area of the analyte standards in the water solution (a, $n = 3$) with the mean peak area of an analyte spiked post-extraction (b, $n = 3$). The following Equation was used:

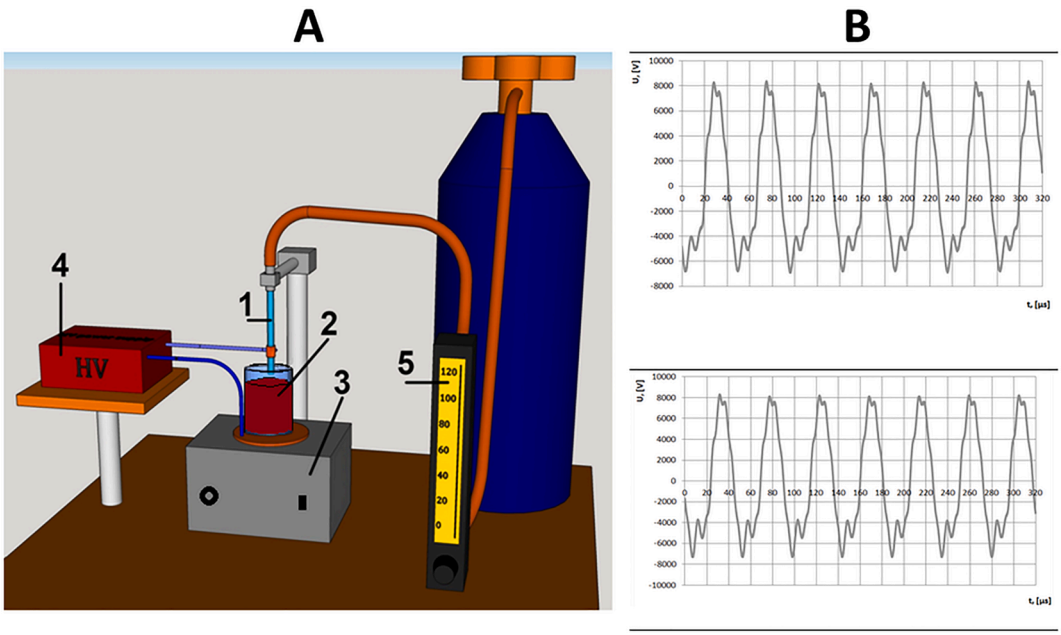

Fig. 1. A. Experimental set-up for plasma treatment of wine: 1– plasma jet reactor; 2 – sample in a glass container; 3 – magnetic stirrer; 4 – high voltage power supply; 5 – gas flow controller. B. Voltage signal between electrodes for the selected gas mixtures.

$$ME[\%] = \frac{b}{a} \times 100\% \quad (1)$$

The MEs, were ranged from 79% and 99%. In general, ME has no impact on the qualitative and quantitative results of this method and can be omitted. Additionally, it was proven that it is justified to use an internal standard (IS) for calibration. Information on determined validation parameters and average recoveries (%) obtained with the optimized method in spiked wine samples are given in Table S.5 (Supplementary Material).

2.6. Chemometric analysis

In the present study, multivariate statistical data mining was used to discover the specific correlations between the different wine preservation methods and determine the content of bioamines and selected phenolic compounds. The following chemometric methods were used for intelligent data analysis: cluster analysis (hierarchical and non-hierarchical or K-means clustering), two-way joining analysis, principal component analysis, and factor analysis. The analysis was performed using STATISTICA 8.0 software.

3. Results and discussion

3.1. Polyphenolic content

Red wine is a rich source of phenolic compounds that exert beneficial effects on the human health due to their antioxidant properties. Many studies have been conducted which indicate that the profile of phenolic compounds in a wine depends on the geographical location of the vineyard, the type of grapes, the method of production and preservation, and storage time (Manns et al., 2013; Stój et al., 2020). To evaluate the effect of the preservation method and storage time on the phenolic compound content of red wine samples, UPLC-PDA-MS/MS was used. A total of 54 compounds were determined in the studied samples by UPLC: 24 anthocyanins, 7 flavonols, 12 flavon-3-ols, 7 phenolic acids, and 4 stilbenes (Supplementary Material – Table S.1). The Retention times, molecular ion masses and the basic MS2 fragments of the individual phenolic compounds are presented in Supplementary Material - Table S.2. The contents of selected phenolic compounds detected in our red wine samples are shown in Table 2.

3.1.1. Anthocyanins

The most abundant group were anthocyanins, especially malvidin 3-O-glucoside-5-O-glucoside, malvidin 3-O-glucoside and delphinidin 3-O-glucoside, a profile that is characteristic of Rondo variety wines (Stój et al., 2020; Kapusta et al., 2018). The basic structure of anthocyanins is their aglycone part (Khoo et al., 2017). In the examined wine, derivatives of five aglycones were determined: delphinic, malvidin, petunidin, peonidin and cyanidin. Anthocyanins are unstable compounds that can undergo reversible transformations in aqueous environments due to pH changes, thus affecting the color of the product. In addition, these compounds may degrade during processing when exposed to various factors, such as temperature, oxygen, or light (He et al., 2012; Yue et al., 2021). This is consistent with our results, which indicated that the storage process as well as the preservation method used affected the final anthocyanin concentration. Analyzing the influence of the storage process, we noted an 8.23 to 47.51 % reduction in the subtotal levels of these compounds in each of the tested samples compared to samples which had not been stored. Additionally, a lower decrease in the content of diglycoside anthocyanins was observed, which indicates that they exhibit a higher stability than monoglycoside anthocyanins (Table S.1). This observation is confirmed by numerous scientific reports (He et al., 2012; Kim et al., 2010). The most stable molecule with the lowest level of reduction in all samples was cyanidin-3-O-glucoside-5-O-glucoside. By contrast, cyanidin-3-O-glucoside was the most susceptible to degradation, which was directly related to its structure. Malvidin-3-O-glucoside and peonidin-3-O-glucoside do not have hydroxyl groups in the ortho position, which makes them relatively more resistant to oxidation than cyanidin-3-O-glucoside (He et al., 2012). Our results indicate that the content of each anthocyanin in both non-stored and stored samples was also dependent on the preservation method applied. In the present study, three preservation methods were used: cold plasma (variable process conditions), addition of potassium metabisulfite (30 mg/L or 100 mg/L) and a method combining the use of cold plasma with the addition of potassium metabisulfite at 30 mg/L. In the wine samples analyzed immediately after the addition of potassium metabisulfite (Table S.1 – samples no. 8 and 15), we observed a slight increase in the total content of anthocyanins compared to the control

Table 2
Contents of selected phenolic compounds in red wine samples determined by UPLC-PDA-MS/MS (n = 2).

Sample no.*	3gM (mg/L)	3gD (mg/L)	3gC (mg/L)	3kGM (mg/L)	3kGPet (mg/L)	3kGPeo (mg/L)	PCA (mg/L)
1	191.76 ± 3.02	82.38 ± 5.62	2.96 ± 0.01	45.67 ± 1.13	18.99 ± 1.84	4.94 ± 0.26	0.16 ± 0.00
2	142.6 ± 3.02	49.26 ± 5.60	1.91 ± 0.01	27.87 ± 1.07	13.35 ± 1.75	2.92 ± 0.26	0.2 ± 0.00
3	153.32 ± 3.24	50.93 ± 5.79	2.11 ± 0.01	31.25 ± 1.19	15.38 ± 2.02	3.22 ± 0.28	0.22 ± 0.00
4	126.35 ± 2.67	39.49 ± 4.49	1.67 ± 0.01	23.74 ± 0.91	11.36 ± 1.49	2.48 ± 0.22	0.2 ± 0.00
5	157.68 ± 3.34	55.64 ± 6.33	2.03 ± 0.01	33.75 ± 1.29	16.33 ± 2.15	3.32 ± 0.29	0.2 ± 0.00
6	166.42 ± 3.52	57.24 ± 6.51	2.07 ± 0.01	34.97 ± 1.34	16.4 ± 2.15	3.61 ± 0.32	0.21 ± 0.00
7	170.08 ± 3.60	58.03 ± 6.60	2.07 ± 0.01	35.98 ± 1.38	17.17 ± 2.26	3.63 ± 0.32	0.21 ± 0.00
8	182.88 ± 3.87	64.70 ± 7.36	2.44 ± 0.01	39.71 ± 1.52	19.23 ± 2.53	4.03 ± 0.35	0.17 ± 0.00
9	177.2 ± 3.75	64.19 ± 7.30	2.25 ± 0.01	37.73 ± 1.44	18.94 ± 2.49	3.89 ± 0.34	0.2 ± 0.00
10	170.57 ± 3.61	58.29 ± 6.63	2.2 ± 0.01	37.34 ± 1.43	17.98 ± 2.36	3.70 ± 0.33	0.22 ± 0.00
11	150.23 ± 9.16	57.69 ± 9.27	1.91 ± 0.05	30.75 ± 3.14	11.63 ± 0.89	2.99 ± 0.24	0.17 ± 0.01
12	178.48 ± 10.88	75.63 ± 12.16	2.41 ± 0.06	39.46 ± 4.03	15.64 ± 1.19	3.80 ± 0.31	0.18 ± 0.01
13	166.59 ± 10.16	72.04 ± 11.58	2.23 ± 0.05	38.68 ± 3.95	15.28 ± 1.16	3.63 ± 0.30	0.18 ± 0.01
14	171.68 ± 10.47	69.42 ± 11.16	2.08 ± 0.05	38.43 ± 3.93	14.93 ± 1.14	3.66 ± 0.30	0.19 ± 0.01
15	190.82 ± 11.63	64.7 ± 13.17	2.43 ± 0.06	43.86 ± 4.48	17.36 ± 1.32	3.96 ± 0.32	0.13 ± 0.01
16	149.42 ± 9.11	61.47 ± 9.88	2.12 ± 0.05	30.64 ± 3.13	12.09 ± 0.92	2.94 ± 0.24	0.32 ± 0.02
17	106.81 ± 6.51	15.13 ± 2.43	0.41 ± 0.01	18.55 ± 1.90	7.43 ± 0.57	2.03 ± 0.17	0.79 ± 0.06
18	121.59 ± 7.41	40.16 ± 6.46	1.45 ± 0.04	22.42 ± 2.29	8.69 ± 0.66	2.27 ± 0.19	0.7 ± 0.05
19	54.59 ± 3.33	14.78 ± 2.38	0.52 ± 0.01	6.96 ± 0.71	3.05 ± 0.23	1.07 ± 0.09	0.8 ± 0.06
20	101.6 ± 6.19	11.50 ± 1.85	0.34 ± 0.01	17.1 ± 1.75	6.32 ± 0.48	1.88 ± 0.15	0.78 ± 0.06
21	138.68 ± 2.00	53.03 ± 6.97	1.67 ± 0.10	26.58 ± 0.43	13.18 ± 2.12	2.59 ± 0.01	0.73 ± 0.02
22	79.88 ± 1.15	12.89 ± 1.69	0.39 ± 0.04	11.36 ± 0.18	5.78 ± 0.93	1.44 ± 0.01	0.84 ± 0.02
23	143.55 ± 2.07	61.70 ± 8.11	2.28 ± 0.14	28.27 ± 0.46	14.36 ± 2.31	2.83 ± 0.01	0.25 ± 0.01
24	92.75 ± 1.34	7.81 ± 1.03		14.19 ± 0.23	7.29 ± 1.17	1.63 ± 0.01	
			0.25 ± 0.02				0.81 ± 0.02
25	97.01 ± 1.40	11.90 ± 1.56	0.33 ± 0.02	15.12 ± 0.25	7.74 ± 1.24	1.71 ± 0.01	0.79 ± 0.02
26	76.38 ± 1.10	19.42 ± 2.55	0.67 ± 0.04	11.26 ± 0.18	5.9 ± 0.95	1.4 ± 0.01	0.80 ± 0.02
27	84.70 ± 1.22	9.37 ± 1.23	0.28 ± 0.02	12.73 ± 0.21	6.49 ± 1.04	1.52 ± 0.01	0.84 ± 0.02
28	100.49 ± 1.45	23.42 ± 3.08	0.67 ± 0.04	16.68 ± 0.27	8.91 ± 1.43	1.83 ± 0.01	0.83 ± 0.02
29	97.29 ± 1.40	34.25 ± 4.50	1.18 ± 0.07	17.31 ± 0.28	8.86 ± 1.42	1.92 ± 0.01	0.74 ± 0.02
30	132.67 ± 1.91	57.73 ± 7.58	2.07 ± 0.13	26.45 ± 0.43	13.35 ± 2.15	2.7 ± 0.01	0.19 ± 0.00

3gM – malvidin 3-O-glucoside; 3gD – delphinidin 3-O-glucoside; 3gC – cyanidin 3-O-glucoside; 3kGM – malvidin 3-O-(600-O-coumaryl) - glucoside; 3kGPet – petunidin 3-O-(600-O-coumaryl) -glucoside; 3kGPeo – peonidin 3-O-(60 0-O-coumaryl) - glucoside; PCA – protocatechuic acid.
* the coding of the samples is shown in Table 1.

sample (no. 1). Moreover, the sample with the addition of 100 mg/L potassium metabisulfite had the highest content of anthocyanins (836.32 mg/L) compared to the other samples (580.36–811.73 mg/L). In our study, however, the total content of anthocyanins after the three-month storage period in samples subjected to sulfurization (Table S.1–samples 23 and 30) was similar to the control sample (sample 16); only the addition of 100 mg/L potassium metabisulfite caused a decrease in the content of these compounds by 5.77%. These results indicate that the application of potassium metabisulfite has a minimal effect on the reduction of the anthocyanin content. In our experiment, we evaluated the impact of cold plasma treatment time (2, 5, 10 min) and the type of working gas used (helium/oxygen and helium/nitrogen) on the profile of phenolic compounds in red wine samples. Additionally, we tested the effect of cold plasma treatment combined with potassium metabisulfite (30 mg/L) treatment. Our results showed that both the duration of the process and the type of gas used contributed to a change in the content of individual compounds. The analysis of the level of anthocyanins in unstored samples indicated that the application of cold plasma for 10 min with the mixture of helium/oxygen as the working gas resulted in the highest reduction in the total anthocyanin content compared to the control (Table S.1). On the other hand, in wine samples exposed to cold plasma with helium/nitrogen as the working gas, an increase in anthocyanin concentration was observed, which was the larger the longer the samples were exposed to treatment. The total anthocyanin content after 2, 5, and 10 min was, respectively, 707.23, 747.74, and 755.25 mg/L. Also, higher anthocyanin concentrations were recorded in the samples exposed to cold plasma with the addition of potassium metabisulfite compared to the same samples exposed to cold plasma alone (Table S.1). This was probably related to the protective effect of sulfate on anthocyanins. We also observed a similar relationship related to the working gas used. Again, wine samples exposed to cold plasma generated using a mixture of helium and oxygen showed a higher reduction in the anthocyanin content compared to samples using a mixture of helium and nitrogen as the working gas. The disparities in the effect of the individual gases on anthocyanin stability were probably due to the fact that the different gases produced different reactive compounds during plasma generation. When oxygen is used in the working gas mixture, the plasma stream may contain hydrogen peroxide,

hydroxyl radical, peroxyl anion or singlet oxygen, all of which can cause significant degradation of anthocyanins (Arjunan et al., 2015). Since red wine is a complex matrix and undergoes various chemical processes, the effects of different preservation methods on phenolic compounds after three months of storage were also analyzed. Interesting results were observed in most of the samples exposed to 5 min of cold plasma. A higher content of some anthocyanins was noted compared to samples that were plasma-treated for only 2 min (Table 2). In addition, the decrease in the anthocyanin content compared to the sample preserved by the same method but not stored was also lower than after a 2-min exposure to cold plasma. For example, for a 5-min cold plasma treatment with helium/nitrogen as the working gas, the total anthocyanin content before storage was 747.74 mg/L and dropped after storage by 11.57 % to 661.23 mg/L. By contrast, a 2-min cold plasma treatment resulted in a 30.27 % reduction in anthocyanins compared to the non-stored sample (Table S.1). Moreover, higher anthocyanin contents were observed in the samples subjected to 5 min of cold plasma treatment without potassium metabisulfite, which was an inverse relationship to that observed in the samples before storage. This may indicate that cold plasma, despite the initial degradation of anthocyanins, produces a better overall preservation effect than the mixed method. In addition, when samples with the same exposition time were compared, the anthocyanin content in samples exposed to cold plasma generated using the helium/nitrogen gas mixture was similar to that of the control sample and the sample with 30 mg/L potassium metabisulfite, and 4.34 % higher than that of the sample with 100 mg/L potassium metabisulfite.

3.1.2. Phenolic acids

The contents of phenolic acids such as gallic acid, protocatechuic acid, caftaric acid, cutaric acid, caffeic acid, coumaric acid and ferulic acid were also determined in the studied wine. Gallic acid was the most abundant of those compounds at concentrations from 9.52 mg/L to 11.86 mg/L. In the samples before storage, the highest total content of phenolic acids was noted after a 2-min exposure to cold plasma – 24.68 mg/L (helium/nitrogen), a value that was 8.25% higher compared to the control sample (Table S.1). A study conducted on white wine by Lukić et al. (2019) also reported a slight increase in the content of some phenolic acids as a result of cold plasma exposure. Cold plasma also had a beneficial effect on the content of hydroxycinnamic acids in pomegranate juice (Herceg et al., 2016). Acids belonging to this group are characterized by a higher stability, which probably translates into their lower reactivity with the radicals formed during cold plasma generation.

In contrast to anthocyanins, the content of phenolic acids increased after storage in most samples (Table S.1). Interesting results were observed for the content of protocatechuic acid. In each sample after storage, the content of this acid increased compared to the non-stored samples. However, cold plasma treatment (10 min, helium/nitrogen) resulted in a substantial, up-to-4-fold increase in the content of this compound compared to non-stored samples (0.84 mg/L). The lowest content of this compound was observed in samples with 100 mg/L potassium metabisulfite (0.19 mg/L) (Table 2). The contents of other acids showed a similar trend. Based on the literature data and our own results on the anthocyanin content, we can assume that such a large increase in protocatechuic acid in samples exposed to cold plasma was related to a decrease in the anthocyanin content. Under cold plasma treatment, anthocyanins degrade to phenolic acids, and the main products of their decomposition are protocatechuic, vanillic, syringic, and p-coumaric acids (Yang et al., 2018). Garofulić et al. (2015), who evaluated the effect of cold plasma treatment on the contents of anthocyanins and phenolic acids in cherry juice, suggested that plasma acting on the food matrix for a short time caused the dissociation of agglomerates or particles, leading to an increase in the content of phenolic compounds.

3.1.3. Other phenolic compounds

In another experiment, we used ultraperformance chromatography to determine flavanols, flavan-3-ols, and stilbenes in the examined red wine samples. The content of flavanols in the wine was low and their total content ranged from 3.74 mg/L to 2.52 mg/L. The highest concentration was recorded in the non-stored control sample, while the lowest concentration was recorded after storage in the sample preserved by cold plasma (10 min, helium/nitrogen working gas) with the addition of potassium metabisulfite. The most abundant flavan-3-olswere (+) - catechin at 25.67 mg/L (sample no. 5) and procyanidin B1 at 10.46 mg/L (sample no. 12). *Cis* - and *trans*-resveratrol were also determined in the studied wine samples. The content of *cis*-resveratrol in the samples before and after storage was practically the same. A slight increase in its content was observed after storage in the sample exposed to cold plasma (10 min, helium/oxygen). An inverse correlation was noted for *trans*-resveratrol (Table S.1).

3.2. Biogenic amine content

DLLME-GC–MS was applied to determine the concentrations of biogenic amines in the red wine samples analyzed. The results are presented in Table 3. Six biogenic amines were identified: TRP, PUT, HIS, TYR, CAD, and 2-PE, with histamine having the highest concentrations in all samples. This finding corresponds with the results reported by other researchers who indicate that histamine is the most abundant biogenic amine in wines (Płotka-Wasylka et al., 2018). High concentrations of histamine in a product can cause negative health effects in the consumer, so it is important to use methods that will reduce the content of this compound in the food matrix (Esposito et al., 2019). In our experiment, the highest HIS content was found in the unpreserved control sample (before storage: 818 ± 34 μg/L; after storage: 821 ± 30 μg/L). A significantly lower content of this compound was observed in the sample that had been exposed to cold plasma for 10 min using a helium/oxygen mixture as the working gas (584 ± 34 μg/L) in combination with the addition of 30 mg/L potassium metabisulfite. Also, after three months of storage, the HIS content of this sample did not change significantly (586 ± 33 μg/L). When the effect of the wine preservation method on the content of other biogenic amines was analyzed, in all cases the 10-min application of cold plasma (helium/oxygen as working gas) with 30 mg/L potassium metabisulfite resulted in the highest reduction in the level of these compounds. Moreover, this effect persisted after storage. To date, the literature provides no information on or explanation of the effect of cold plasma on the content of biogenic amines in wine. However, because the formation of these compounds depends mainly on the microorganisms present in the food matrix (Restuccia et al., 2018), it can be assumed that cold plasma, which has a well-proven biocidal activity against unwanted microorganisms, indirectly contributes to the reduction of biogenic amines in food products (Bourke et al., 2017; Lu et al., 2014). Our results also showed that the efficiency of cold plasma in reducing biogenic amines in wine samples was affected by the duration of treatment and the type of working gas used. Increasing the duration of the process to 10 min and the use of a mixture of helium and oxygen as the working gas favourably affected the elimination of these compounds from the product matrix. The influence of the duration of the process as well as the type of gases used on the sterilizing efficiency of cold plasma has also been demonstrated by other authors. Hou et al. (2019), who sterilized blueberry juice using cold plasma for 2, 4 and 6 min, recorded the highest reduction in *Bacillus* spp. populations after the time of 6 min. Also, our previous study on the effects of cold plasma on *Lentilactobacillus hilgardii* cells showed that increasing the duration of the process as well as using a mixture of helium and oxygen as the working gas resulted in higher cell reduction than using a mixture of helium and nitrogen (Niedźwiedź et al., 2020).

3.3. Chemometric analysis

The major goal of multivariate statistical data mining was to reveal hidden specific relations between differently treated (different

Table 3
Concentrations of selected biogenic amines determined in wine samples by DLLME-GC–MS; $n = 3$.

Sample no.*	TRP (μg/L)	PUT (μg/L)	HIS (μg/L)	TYR (μg/L)	CAD (μg/L)	2-PE (μg/L)
1	4.089 ± 0.012	489 ± 25	818 ± 34	27.74 ± 0.16	58.73 ± 0.15	18.70 ± 0.054
2	3.670 ± 0.011	475 ± 24	799 ± 31	27.58 ± 0.17	54.15 ± 0.12	18.68 ± 0.049
3	3.578 ± 0.008	455 ± 25	734 ± 37	27.34 ± 0.17	52.21 ± 0.12	18.73 ± 0.047
4	3.551 ± 0.009	449 ± 23	732 ± 36	26.43 ± 0.13	52.01 ± 0.14	18.63 ± 0.050
5	3.662 ± 0.010	471 ± 22	784 ± 29	27.51 ± 0.18	53.94 ± 0.13	18.71 ± 0.048
6	3.589 ± 0.008	466 ± 27	741 ± 33	27.44 ± 0.16	52.27 ± 0.11	18.75 ± 0.044
7	3.540 ± 0.010	457 ± 22	742 ± 34	26.78 ± 0.17	52.22 ± 0.13	18.66 ± 0.051
8	2.918 ± 0.008	344 ± 25	654 ± 34	<LOD	48.29 ± 0.16	23.74 ± 0.044
9	2.705 ± 0.011	324 ± 23	627 ± 38	<LOD	44.54 ± 0.14	23.77 ± 0.047
10	2.678 ± 0.013	299 ± 20	622 ± 33	<LOD	39.79 ± 0.12	23.68 ± 0.050
11	1.972 ± 0.006	278 ± 19	584 ± 34	<LOD	38.09 ± 0.14	23.76 ± 0.048
12	2.802 ± 0.014	348 ± 24	654 ± 38	<LOD	43.87 ± 0.16	23.72 ± 0.051
13	2.732 ± 0.016	320 ± 21	641 ± 32	<LOD	40.17 ± 0.13	23.63 ± 0.047
14	2.052 ± 0.008	291 ± 19	601 ± 36	<LOD	37.89 ± 0.11	23.69 ± 0.052
15	3.878 ± 0.013	466 ± 23	773 ± 30	<LOD	52.42 ± 0.17	25.88 ± 0.054
16	4.086 ± 0.011	490 ± 24	821 ± 30	27.71 ± 0.15	58.66 ± 0.18	18.78 ± 0.044
17	3.674 ± 0.010	479 ± 22	794 ± 29	27.66 ± 0.16	54.05 ± 0.14	18.75 ± 0.043
18	3.581 ± 0.012	457 ± 24	739 ± 35	27.91 ± 0.14	52.18 ± 0.13	18.79 ± 0.051
19	3.560 ± 0.010	449 ± 21	732 ± 33	26.38 ± 0.12	52.09 ± 0.18	18.60 ± 0.044
20	3.669 ± 0.010	476 ± 26	789 ± 31	27.79 ± 0.21	53.99 ± 0.15	18.77 ± 0.044
21	3.593 ± 0.013	471 ± 23	748 ± 27	27.49 ± 0.18	52.30 ± 0.17	18.70 ± 0.038
22	3.547 ± 0.011	457 ± 20	739 ± 31	26.85 ± 0.15	52.28 ± 0.10	18.71 ± 0.047
23	2.915 ± 0.009	349 ± 24	658 ± 33	<LOD	48.33 ± 0.16	23.81 ± 0.056
24	2.711 ± 0.013	332 ± 21	629 ± 38	<LOD	44.50 ± 0.19	23.84 ± 0.031
25	2.684 ± 0.012	309 ± 24	617 ± 31	<LOD	39.83 ± 0.10	23.77 ± 0.062
26	1.979 ± 0.011	279 ± 19	586 ± 33	<LOD	38.04 ± 0.14	23.85 ± 0.045
27	2.811 ± 0.017	353 ± 22	659 ± 36	<LOD	43.95 ± 0.11	23.77 ± 0.044
28	2.729 ± 0.014	320 ± 19	646 ± 31	<LOD	40.20 ± 0.17	23.56 ± 0.039
29	2.058 ± 0.009	289 ± 21	613 ± 35	<LOD	37.84 ± 0.15	23.71 ± 0.057
30	3.874 ± 0.012	469 ± 25	773 ± 34	<LOD	52.47 ± 0.20	25.93 ± 0.061

TRP – tryptamine, PUT – putrescine, HIS – histamine, TYR – tyramine, CAD – cadaverine, 2-PE – 2-phenylethylamine
* the coding of the samples is shown in Table 1.

preservation conditions) wine samples (a total of 30 cases) characterized by 13 chemical variables (bioamines and phenolic compounds). Another important task was to find similarity patterns depending on the storage conditions and, beyond that, to identify specific chemical descriptors responsible for the classification of the different wine samples.

The following chemometric methods were used in the intelligent data analysis:

- Cluster analysis (hierarchical and non-hierarchical or K-means clustering).
- Two-way joining.
- Principal components analysis and factor analysis.

Hierarchical clustering was performed on standardized input data (z-normalization), with squared Euclidean distance as a similarity measure, using Ward's method of linkage and Sneath's significance test. Fig. 2 A1 shows a hierarchical clustering dendrogram of the 13 chemical variables. Three major clusters were identified at Sneath's significance level of 1/3Dmax:

C1: 3gM, 3kGM, 3kGPeo, 3kGPet, 3gD, 3gC – phenolic cluster.

*C*2: HIS, PUT, TRP, CAD, TYR – amine cluster.

C3: 2PE, PCA – mixed cluster.

The hierarchical clustering of the chemical variables identified three patterns of similarity which could be conditionally determined as phenolic, amine and mixed clusters. There was a good separation between the phenolic and the amine variables, which indicated that both groups of variables had a separate impact on the quality of the different wine samples which was unrelated to the preservation or storage conditions. Fig. 2 A2 shows a hierarchical dendrogram linking 30 wine samples (with different preservation and storage conditions). Three major clusters of cases were formed (under the same clustering conditions):

C1: 17, 19, 20, 22, 24, 25, 26, 27, 28, 29 – samples after storage and preservation by plasma and by plasma in combination with potassium metabisulfite.

*C*2: 8, 9, 10, 11, 12, 13, 14, 23 – samples before storage with preservation by plasma and potassium metabisulfite.

C3: 1, 2, 3, 4, 5, 6, 7, 15, 16, 18, 21, 30 – samples before storage with plasma preservation.

Cluster 1 mainly included samples after storage preserved by plasma and plasma plus potassium metabisulfite. Cluster 2 chiefly consisted of samples before storage but preserved by plasma or by potassium metabisulfite. Cluster 3 aggregated 12 plasma-preserved samples before storage. The clustering of the wine samples showed separation into patterns which differed in the treatment and storage conditions.

K-means clustering is a non-supervised clustering method in which clusters are not formed spontaneously but according to a preliminary hypothesis regarding the possible number of clusters. This a priori segmentation is based on an algorithm which selects centroids in the dataset under a predefined distance measure. The results of K-means clustering for the formation of 3 clusters of variables and 3 clusters of cases were identical to those obtained by hierarchical clustering. The members of the non-hierarchical clusters were the same. This is illustrated in Table 3 in Supplementary Materials (S.3) which shows cluster membership data for cases and variables along with the respective distances between the members in each identified cluster. It was important to reveal the role of the chemical variables as specific descriptors for each of the identified clusters. Fig. 3A presents the average values of each chemical variable for each cluster. The cluster which included plasma-preserved samples before storage (C1 in the plot below) was characterized by the highest levels of amines, moderate (rather high) levels of phenolic compounds and low levels of 2-PE and PCA. The cluster with samples before storage, preserved by plasma and potassium metabisulfite (*C*2 in the plot below) was characterized by the lowest levels of amines, the highest levels of phenolic compounds, the highest level of 2-PE, and the lowest level of PCA. The cluster with samples stored after preservation by plasma and by plasma in combination with potassium metabisulfite (C3 in the plot below) was characterized by moderate levels of amines, the lowest levels of phenolic compounds, moderate levels of 2-PE and the highest levels of PCA. It is readily seen that the storage conditions led to changes in the levels of all the chemical variables, which additionally depended on the preservation treatment used. In general, the levels of phenolic compounds fell after storage, whereas levels of amines were high before storage and plasma

preservation but decreased substantially following preservation with potassium metabisulfite or after storage.

The relationship between the chemical variables and the wine samples is shown additionally in the plot of the results of two-way joining cluster analysis, in which variables and cases are in respective correspondence (Fig. 3B).

The plot confirms the conclusions above about the determination of specific chemical descriptors for the wine sample clusters.

Both chemometric methods are very similar and their basic task is to find hidden factors (principal components or factors) responsible for the structure of the data matrix. Additionally, they are typical projection methods and, as such, lead to a dimensionality reduction of the system under consideration. In the working algorithm, the data matrix is decomposed into a factor loading matrix and a factor score matrix, the former presenting the newly defined special directions in the variables space, and the latter – the new coordinates of the objects. Both of these matrices need to be correctly interpreted in order to find specific relationships between objects and variables. In our dataset, two latent factors were responsible for the data structure. The first of them, which explained 51.3 % of the total variance of the system, could be tentatively named the "phenolic factor," and the second factor, with 40.5 % of explained variance could be called the "amine factor." This is largely consistent with the results of cluster analysis. Table 4 (Supplementary Materials S.4), in which statistically significant loadings are given in bold, shows that the variables 2-PE and PCA are reversely correlated to the rest of the significant factor loadings with regard to factor 1 and factor 2, and this specificity corresponds to the formation of the mixed cluster in cluster analysis. An interpretation of the data in the loadings table leads to the conclusion that the data structure is dependent on two latent relationships between the variables – a relationship between phenolic compounds as a similarity group and a relationship between biogenic amines as another similarity pattern. The graphical plot of the factor loadings in Fig. 2B clearly illustrates these relationships. Both clusters of variables are well-defined, and the more specific role of 2-PE as opposite to the amine group and PCA as opposite to the phenolic group is indicated. The factor scores plot illustrates the formation of three patterns of similarity between the wine samples. It matches the hierarchical and K-means clusters of wine samples almost perfectly.

4. Conclusion

In this study, for the first time, the effect of cold plasma on the content of phenolic compounds and biogenic amines in red wine was evaluated with respect to storage time. In addition, the effect of cold plasma was compared with the traditional method of preservation (addition of 30 mg/L and 100 mg/L of potassium metabisulfite) and a combined method (cold plasma with 30 mg/L of potassium metabisulfite). In general, cold plasma treatment caused a decrease in the total content of phenolic compounds in the wine samples subjected to three months of storage. However, the application of cold plasma for 5 min with helium/nitrogen as the working gas reduced the content of these compounds by only 2.85 % compared to the control. Moreover, the content of phenolic compounds was 3.1% higher in the sample preserved by this method compared to the sample preserved by the addition of potassium metabisulfite at a dose of 100 mg/L.). The highest degree of degradation in wine samples was observed in anthocyanin content. On the basis of the observed changes in their amount it can be observed that a higher degree of reduction of these compounds was observed after exposure to cold plasma with the use of helium/oxygen gas mixture in comparison with the mixture of oxygen and nitrogen. Also, the increase in process time resulted in their greater loss in the wine sample. Additionally, cold plasma increased the content of phenolic acids in the studied samples. Protocatechuic acid content increased up to fourfold in wine samples preserved by cold plasma (10 min, helium/nitrogen). Importantly, the use of cold plasma resulted in a reduction of biogenic amines, which can cause adverse health reactions in the consumer. The highest degree of reduction was observed in the samples exposed to 10 min of cold plasma (helium/oxygen). Our results indicate that the

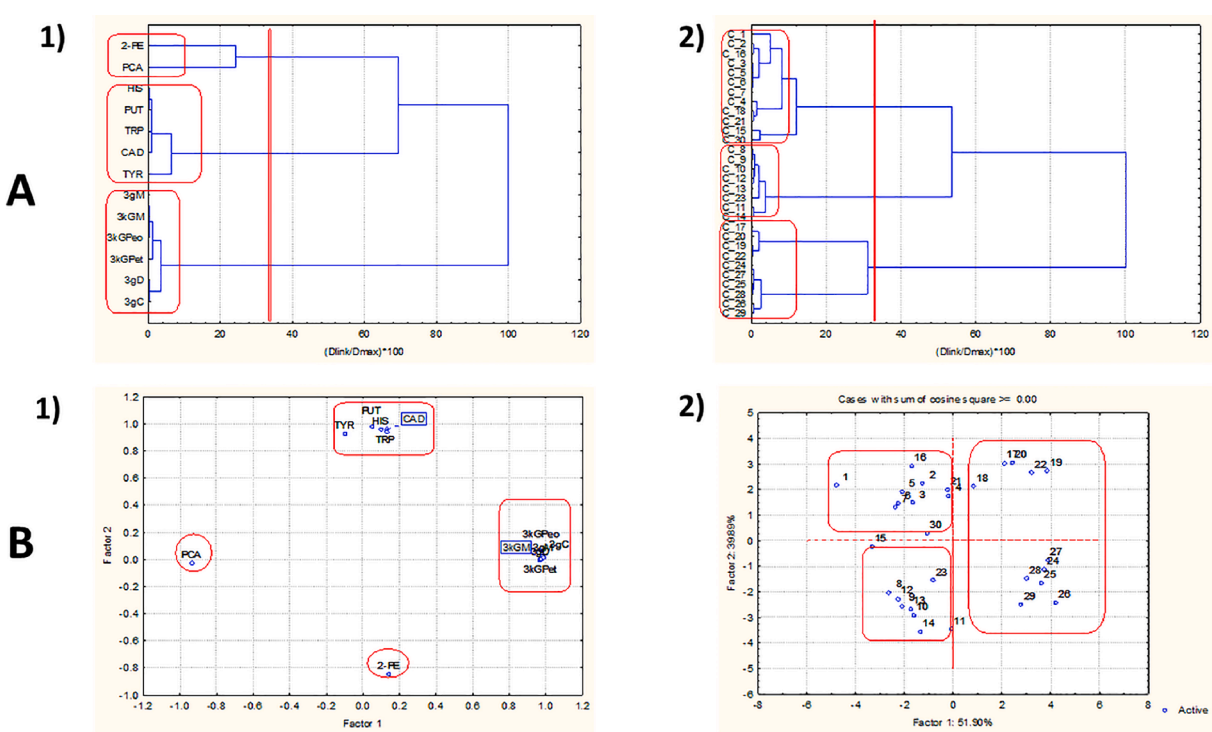

Fig. 2. A. Hierarchical dendrogram. 1) clustering of 13 chemical variables 2) – clustering of wine samples. B. 1) Plot of factor loadings. 2) Plot of factor scores. 3gM – malvidin 3-O-glucoside; 3gD – delphinidin 3-O-glucoside; 3gC – cyanidin 3-O-glucoside; 3kGM – malvidin 3-O-(600-O-coumaryl)-glucoside; 3kGPet – petunidin 3-O-(600-O-coumaryl)-glucoside; 3kGPeo – peonidin 3-O-(60 0-O-coumaryl)-glucoside; PCA – protocatechuic acid; TRP – tryptamine, PUT – putrescine, HIS – histamine, TYR – tyramine, CAD – cadaverine, 2-PE – 2-phenylethylamine.

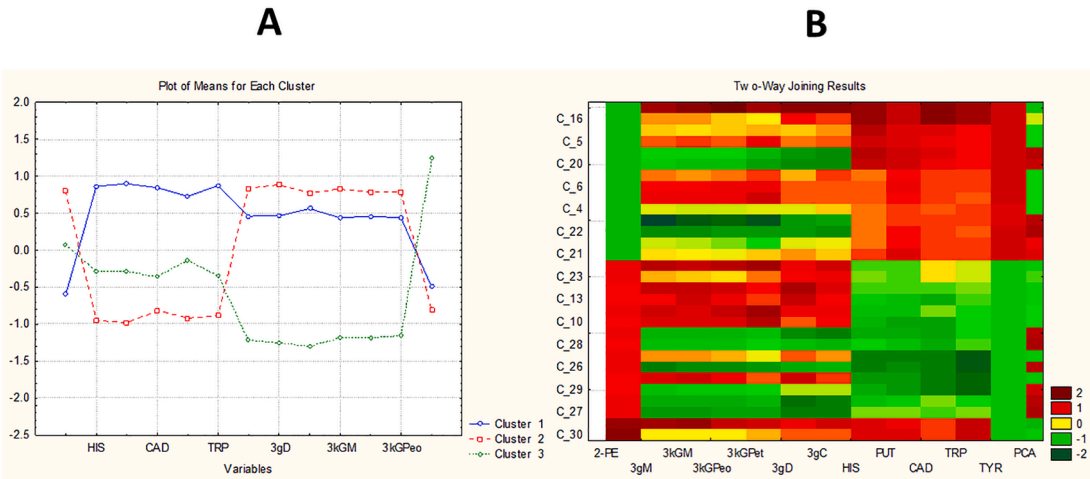

Fig. 3. A. Plot of means for each variable for each identified cluster B. Correspondence between wine samples and chemical variables. 3gM – malvidin 3-O-glucoside; 3gD – delphinidin 3-O-glucoside; 3gC – cyanidin 3-O-glucoside; 3kGM – malvidin 3-O-(600-O-coumaryl)-glucoside; 3kGPet – petunidin 3-O-(600-O-coumaryl)-glucoside; 3kGPeo – peonidin 3-O-(60 0-O-coumaryl)-glucoside; PCA – protocatechuic acid; TRP – tryptamine, PUT – putrescine, HIS – histamine, TYR – tyramine, CAD – cadaverine, 2-PE – 2-phenylethylamine.

influence of the storage process as well as the preservation method on the phenolic profile and the content of biogenic amines is not unambiguous and depends mainly on the chemical properties of the individual compounds. However, the reported effects of cold plasma and cold plasma combined with the addition of potassium metabisulfite on the analyzed compounds allow us to assume that in the future these methods can be successfully used to reduce the use of SO_2 in winemaking.

To conclude, cold plasma may become an alternative method for the preservation of wine or other alcoholic beverages in the future, ensuring adequate product safety and preserving the pro-health values of these products. However, it is necessary to investigate the possible risks resulting from the formation of toxic substances or free radicals that may be hazardous to human health. Also, further research is needed to optimize the process conditions of cold plasma treatment.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Iwona Niedźwiedź: Conceptualization, Project administration, Investigation, Methodology, Validation, Writing – original draft, Visualization. **Justyna Płotka-Wasylka:** Methodology, Software, Validation, Resources, Writing – original draft, Writing – review & editing. **Ireneusz Kapusta:** Methodology, Software. **Vasil Simeonov:** Methodology, Software, Writing – original draft, Writing – review & editing. **Anna Stój:** Resources, Validation. **Adam Waśko:** Conceptualization, Writing – review & editing. **Joanna Pawłat:** Resources, Writing – original draft, Visualization. **Magdalena Polak-Berecka:** Supervision, Conceptualization, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132257.

References

Alves Filho, E. G., de Brito, E. S., & Rodrigues, S. (2020). Effects of cold plasma processing in food components. In *Advances in Cold Plasma Applications for Food Safety and Preservation* (pp. 253–268). Academic Press. https://doi.org/10.1016/B978-0-12-814921-8.00008-6.

Arjunan, K. P., Sharma, V. K., & Ptasinska, S. (2015). Effects of atmospheric pressure plasmas on isolated and cellular DNA—a review. *International Journal of Molecular Sciences, 16*(2), 2971–3016. https://doi.org/10.3390/ijms16022971

Bourke, P., Ziuzina, D., Han, L., Cullen, P. J., & Gilmore, B. F. (2017). Microbiological interactions with cold plasma. *Journal of Applied Microbiology, 123*(2), 308–324. https://doi.org/10.1111/jam.2017.123.issue-210.1111/jam.13429

Christofi, S., Malliaris, D., Katsaros, G., Panagou, E., & Kallithraka, S. (2020). Limit SO2 content of wines by applying High Hydrostatic Pressure. *Innovative Food Science & Emerging Technologies, 62*, 102342. https://doi.org/10.1016/j.ifset.2020.102342

Cordero-Bueso, G., Moraga, J., Ríos-Carrasco, M., Ruiz-Muñoz, M., & Cantoral, J. M. (2019). Bacteriophages as an Up-and-Coming Alternative to the Use of Sulfur Dioxide in Winemaking. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.02931

Esparza, I., Martínez-Inda, B., Cimminelli, M. J., Jimeno-Mendoza, M. C., Moler, J. A., Jiménez-Moreno, N., & Ancín-Azpilicueta, C. (2020). Reducing SO2 doses in red wines by using grape stem extracts as antioxidants. *Biomolecules, 10*(10), 1369. https://doi.org/10.3390/biom10101369

Esposito, F., Montuori, P., Schettino, M., Velotto, S., Stasi, T., Romano, R., & Cirillo, T. (2019). Level of biogenic amines in red and white wines, dietary exposure, and histamine-mediated symptoms upon wine ingestion. *Molecules, 24*(19), 3629. https://doi.org/10.3390/molecules24193629

Gajek, M., Pawlaczyk, A., & Szynkowska-Jozwik, M. I. (2021). Multi-Elemental Analysis of Wine Samples in Relation to Their Type, Origin, and Grape Variety. *Molecules, 26*(1), 214. https://doi.org/10.3390/molecules26010214

Garofulić, I. E., Jambrak, A. R., Milošević, S., Dragović-Uzelac, V., Zorić, Z., & Herceg, Z. (2015). The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca *(Prunus cerasus* var. *Marasca*) juice. *LWT-Food. Science and Technology, 62*(1), 894–900. https://doi.org/10.1016/j.lwt.2014.08.036

Gavahian, M., Chu, Y. H., Khaneghah, A. M., Barba, F. J., & Misra, N. N. (2018). A critical analysis of the cold plasma induced lipid oxidation in foods. *Trends in Food Science & Technology, 77*, 32–41. https://doi.org/10.1016/j.tifs.2018.04.009

He, F., Liang, N. N., Mu, L., Pan, Q. H., Wang, J., Reeves, M. J., & Duan, C. Q. (2012). Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their color expression. *Molecules, 17*(2), 1571–1601. https://doi.org/10.3390/molecules17021571

Herceg, Z., Kovačević, D. B., Kljusurić, J. G., Jambrak, A. R., Zorić, Z., & Dragović-Uzelac, V. (2016). Gas phase plasma impact on phenolic compounds in pomegranate juice. *Food Chemistry, 190*, 665–672. https://doi.org/10.1016/j.foodchem.2015.05.135

Hou, Y., Wang, R., Gan, Z., Shao, T., Zhang, X., He, M., & Sun, A. (2019). Effect of cold plasma on blueberry juice quality. *Food Chemistry, 290*, 79–86. https://doi.org/10.1016/j.foodchem.2019.03.123

Kapusta, I., Cebulak, T., & Oszmiański, J. (2018). Characterization of polish wines produced from the interspecific hybrid grapes grown in south-east Poland. *European Food Research and Technology, 244*(3), 441–455. https://doi.org/10.1007/s00217-017-2972-5

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research, 61*(1), 1361779. https://doi.org/10.1080/16546628.2017.1361779

Kim, M., Yoon, S. H., Jung, M., & Choe, E. (2010). Stability of meoru *(Vitis coignetiea)* anthocyanins under photochemically produced singlet oxygen by riboflavin. *New Biotechnology, 27*(4), 435–439. https://doi.org/10.1016/j.nbt.2010.01.003

Koźmiński, C., Mąkosza, A., Michalska, B., & Nidzgorska-Lencewicz, J. (2020). Thermal Conditions for Viticulture in Poland. *Sustainability, 12*(14), 5665. https://doi.org/10.3390/su12145665

Lu, H., Patil, S., Keener, K. M., Cullen, P. J., & Bourke, P. (2014). Bacterial inactivation by high-voltage atmospheric cold plasma: Influence of process parameters and effects on cell leakage and DNA. *Journal of Applied Microbiology, 116*(4), 784–794. https://doi.org/10.1111/jam.12426

Lukić, K., Vukušić, T., Tomašević, M., Ćurko, N., Gracin, L., & Ganić, K. K. (2019). The impact of high voltage electrical discharge plasma on the chromatic characteristics and phenolic composition of red and white wines. *Innovative Food Science & Emerging Technologies, 53*, 70–77. https://doi.org/10.1016/j.ifset.2017.11.004

Manns, D. C., Coquard Lenerz, C. T., & Mansfield, A. K. (2013). Impact of processing parameters on the phenolic profile of wines produced from hybrid red grapes Maréchal Foch, Corot noir, and Marquette. *Journal of Food Science, 78*(5), C696–C702. https://doi.org/10.1111/1750-3841.12108

Matuszewski, B. K., Constanzer, M. L., & Chavez-Eng, C. M. (2003). Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC−MS/MS. *Analytical Chemistry, 75*(13), 3019–3030. https://doi.org/10.1021/AC020361S

Naila, A., Flint, S., Fletcher, G., Bremer, P., & Meerdink, G. (2010). Control of biogenic amines in food—existing and emerging approaches. *Journal of Food Science, 75*(7), R139–R150. https://doi.org/10.1111/j.1750-3841.2010.01774.x

Niedźwiedź, I., Juzwa, W., Skrzypiec, K., Skrzypek, T., Waśko, A., Kwiatkowski, M., & Polak-Berecka, M. (2020). Morphological and physiological changes in *Lentilactobacillus hilgardii* cells after cold plasma treatment. *Scientific Reports, 10*(1), 1–14. https://doi.org/10.1038/s41598-020-76053-x

Niedźwiedź, I., Waśko, A., Pawłat, J., & Polak-Berecka, M. (2019). The state of research on antimicrobial activity of cold plasma. *Polish Journal of Microbiology, 68*(2), 153–164. https://doi.org/10.33073/pjm-2019-028

Niu, D., Wang, L. H., Zeng, X. A., Wen, Q. H., Brennan, C. S., Tang, Z. S., & Wang, M. S. (2019). Effect of ethanol adaption on the inactivation of Acetobacter sp. by pulsed electric fields. *Innovative Food Science & Emerging Technologies, 52*, 25–33. https://doi.org/10.1016/j.ifset.2018.11.009

Papageorgiou, M., Lambropoulou, D., Morrison, C., Kłodzińska, E., Namieśnik, J., & Płotka-Wasylka, J. (2018). Literature update of analytical methods for biogenic amines determination in food and beverages. *TrAC Trends in Analytical Chemistry, 98*, 128–142. https://doi.org/10.1016/j.trac.2017.11.001

Płotka-Wasylka, J., Simeonov, V., Morrison, C., & Namieśnik, J. (2018). Impact of selected parameters of the fermentation process of wine and wine itself on the biogenic amines content: Evaluation by application of chemometric tools. *Microchemical Journal, 142*, 187–194. https://doi.org/10.1016/j.microc.2018.06.038

Płotka-Wasylka, J., Simeonov, V., & Namieśnik, J. (2018). Evaluation of the impact of storage conditions on the biogenic amines profile in opened wine bottles. *Molecules, 23*(5), 1130. https://doi.org/10.1016/j.jfca.2017.07.042

Puligundla, P., Pyun, Y. R., & Mok, C. (2018). Pulsed electric field (PEF) technology for microbial inactivation in low-alcohol red wine. *Food Science and Biotechnology, 27*(6), 1691–1696. https://doi.org/10.1007/s10068-018-0422-1

Restuccia, D., Loizzo, M. R., & Spizzirri, U. G. (2018). Accumulation of biogenic amines in wine: Role of alcoholic and malolactic fermentation. *Fermentation, 4*(1), 6. https://doi.org/10.3390/fermentation4010006

Robles, A. D., Fabjanowicz, M., Płotka-Wasylka, J., & Konieczka, P. (2019). Organic acids and polyphenols determination in Polish wines by ultrasound-assisted solvent extraction of porous membrane-packed liquid samples. *Molecules, 24*(23), 4376. https://doi.org/10.3390/molecules24234376

Smit, A. Y., Engelbrecht, L., & Du Toit, M. (2012). Managing your wine fermentation to reduce the risk of biogenic amine formation. *Frontiers in Microbiology, 3*, 76. https://doi.org/10.3389/fmicb.2012.00076

Snopek, L., Mlcek, J., Sochorova, L., Baron, M., Hlavacova, I., Jurikova, T., … Sochor, J. (2018). Contribution of red wine consumption to human health protection. *Molecules, 23*(7), 1684. https://doi.org/10.3390/molecules23071684

Stój, A., Kapusta, I., & Domagała, D. (2020). Classification of red wines produced from Zweigelt and Rondo grape varieties based on the analysis of phenolic compounds by UPLC-PDA-MS/MS. *Molecules, 25*(6), 1342. https://doi.org/10.3390/molecules25061342

Yang, P., Yuan, C., Wang, H., Han, F., Liu, Y., Wang, L., & Liu, Y. (2018). Stability of anthocyanins and their degradation products from cabernet sauvignon red wine under gastrointestinal pH and temperature conditions. *Molecules, 23*(2), 354. https://doi.org/10.3390/molecules23020354

Yue, X. F., Jing, S. S., Ni, X. F., Zhang, K. K., Fang, Y. L., Zhang, Z. W., & Ju, Y. L. (2021). Anthocyanin and Phenolic Acids Contents Influence the Color Stability and Antioxidant Capacity of Wine Treated With Mannoprotein. *Frontiers in Nutrition, 8*, 335. https://doi.org/10.3389/fnut.2021.691784

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Comparison of the Effect of Cold Plasma with Conventional Preservation Methods on Red Wine Quality Using Chemometrics Analysis

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Abstract: In this study, the effect of cold plasma (CP) on the physicochemical and biological properties of red wine was investigated in comparison with the effects of the conventional preservation method and the combined method. In addition, the effect of storage time after the application of each of the analyzed methods was evaluated. The study examined the effects of the different preservation methods on the pH, color, phenolic content, antioxidant activity and microbiological purity of the red wine. Chemometric analysis was used to discover the relationship between the preservation method used and wine quality. In the wine samples tested, a reduction in phenolic compounds and a decrease in antioxidant activity were noted after storage. This effect was mildest for preservation methods with the addition of potassium metabisulphite and those in which a mixture of helium and nitrogen was used as the working gas. On a positive note, the CP treatment did not affect the color of the wine in a way perceptible to the consumer: ΔE^*—1.12 (He/N_2; 5 min). In addition, the lowest growth of microorganisms was detected in the CP-treated samples. This indicates the potential of cold plasma as an alternative method to the use of potassium metabisulfite in wine production, which may contribute to its wider use in the alcohol industry in the future.

Keywords: cold plasma; phenolic compounds; antioxidant activity; chemometric analysis; wine preservation; red wine; shelf life

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Citation: Niedźwiedź, I.; Simeonov, V.; Waśko, A.; Polak-Berecka, M. Comparison of the Effect of Cold Plasma with Conventional Preservation Methods on Red Wine Quality Using Chemometrics Analysis. *Molecules* **2022**, *27*, 7048. https://doi.org/10.3390/molecules27207048

Academic Editor: Carmen González-Barreiro

Received: 7 September 2022
Accepted: 17 October 2022
Published: 19 October 2022

1. Introduction

Wine is a traditional alcoholic beverage, which has been present in the cultures of the whole world for thousands of years. The first references to winemaking can be found in records from Georgia dating back to 6000 BC. For centuries, the culture of wine production and consumption has been developing in the hot-climate areas today occupied by Greece, Italy, Spain and Portugal, which are some of the most important winemaking countries in Europe [1]. In recent years, climatic changes have contributed to increased grapevine cultivation and wine production also in the hitherto cold Western regions of the continent [2]. Large-scale production of wine all over the world has resulted in a wide range of wines being available on the market.

On the consumption end of the wine business, growing consumer awareness makes customers attach increasing importance to the quality of the wine they drink, especially its physical, chemical and organoleptic properties [3]. The main parameters consumers assess are alcohol content, composition, acidity, color, aroma and taste. In addition, more and more importance is given to the health-promoting properties of wine [4], in particular red wine, which is considered to have some potent health benefits. The salutary effects of red wine are due to its complex chemical composition, which, in addition to water, sugars and alcohol, includes bioactive compounds. Among the most important bioactive compounds are polyphenols, which are characterized by antioxidant [5] and antibacterial

properties [6], and have a beneficial effect on the nervous and cardiovascular systems [7]. Many epidemiological studies indicate that moderate red wine consumption contributes to a reduction in mortality, protects individuals against diabetes [8] and has a preventive effect on certain cancers [9].

The final quality of wine depends on the numerous chemical reactions occurring in the complex food matrix during the many stages of the winemaking process that include fermentation, preservation and aging. The fermentation of grapes involves a large number of microorganisms, whose uncontrolled growth can lead to the deterioration of the product or even its complete spoilage. To avoid this, different preservation methods are used, which prevent the development of undesirable microflora and extend the shelf life of the product [10]. A common preservative used for this purpose is sulfur dioxide (SO_2), which exhibits antimicrobial and antioxidant properties and prevents the non-enzymatic oxidation of wines [11]. However, this compound can have a negative impact on the quality of wine by causing sensory changes and it can also be potentially harmful to consumers' health as it can lead to headaches, dermatitis or diarrhea [12]. For this reason, regulations have been introduced to determine the acceptable dose of SO_2 in wine production. However, wines without the addition of sulphates are considered healthier, hence the growing interest in new preservation methods, which would minimize or eliminate the use of sulphur compounds. The use of thermal technologies in wine preservation is not appropriate, as they can adversely affect the taste, color and aroma of wine through the loss of bioactive compounds when exposed to the temperature used. With this in mind, researchers are currently particularly interested in new non-thermal methods of food preservation, such as high hydrostatic pressure (HHP) or pulsed electric field (PEF), which, in addition to providing microbiological safety, due to the low temperature of the process, have less impact on product quality, e.g., by not contributing to high losses of thermolabile compounds [13].

Such methods also include cold plasma, which is considered to be the fourth state of matter [14]. The concept of plasma was introduced in 1928 by the American physical chemist Irving Langmuir to describe a low-pressure electrical discharge [15]. Food technology makes use of low-temperature non-equilibrium plasma, which is characterized by a thermal imbalance between electrons and other gas molecules such as ions, inert particles or radicals. The lower temperature of the electrons relative to the heavy gas molecules allows the food preservation process to be carried out at temperatures close to ambient [16]. Plasma components, whose amount and type depend on the working gas used, enable, as indicated by numerous scientific reports, the effective elimination of microorganisms from the food matrix [2,17]. The mechanism of microbial inactivation using cold plasma is still not fully understood despite many studies [18,19]. However, it is believed that the reactive compounds, UV photons, and excited and ground state atoms produced during cold plasma generation can disintegrate microbial surface structures or degrade genetic material [20,21]. In the context of the use of cold plasma in the food industry, it is worth noting its advantages, such as the short duration of the process, low temperature, minimal water consumption and effective decontamination, which allow this method to be viewed as a sustainable food production technology [22].

Due to increasing consumer expectations of product quality and microbiological safety, it is necessary to search for alternative preservation techniques. This is particularly important in the alcoholic beverage industry, where the application of conventional methods using high temperatures is undesirable. The present work is a continuation of our previous research [2], in which we qualitatively and quantitatively determined phenolic compounds and biogenic amines in wine samples subjected to different preservation treatments. The methods studied were the conventional preservation method (addition of potassium metabisulfite at 30 mg/L or 100 mg/L), cold plasma and a combined method (cold plasma and addition of potassium metabisulfite at 30 mg/L). In the present study, the scope of the research was extended to analyze the effects of the above-mentioned preservation methods and storage time on the physicochemical properties (pH, color, total

polyphenol content, total anthocyanin content, antioxidant properties) and microbiological safety of red wine.

2. Results and Discussion

2.1. Physicochemical Properties

Physicochemical properties such as pH, color, total phenolic compounds, total anthocyanin content and antioxidant properties play a crucial role in wine quality assessment. Therefore, in this study, we investigated how these parameters were affected by three different preservation processes (cold plasma, a method combining cold plasma with the addition of 30 mg/L potassium metabisulfite, and the traditional method involving the addition of 100 mg/L potassium metabisulfite) and storage time (Tables 1 and 2).

2.1.1. Determination of pH and Color Measurement

Analyzing the influence of different preservation processes on the pH value of wine samples, we did not observe major changes in acidity (Table 1). The pH was ~3.52 before storage and ~3.49 after storage. These values were similar to the pH of the control sample and did not differ from the acidity of commercially available red wines [23].

The effect of the different preservation methods and storage time on wine color was evaluated using the CIELab model. An analysis of color lightness (parameter L*) showed that, in non-stored samples, the value of this parameter was higher than in the control. The highest L* values were recorded in the samples treated with the combined method—15.13 (cold plasma: 5 min, He/N_2), and the traditional method—14.94. When analyzing the a* parameter indicating the redness of the product, we observed the same dependencies. Higher values of this parameter were recorded in samples where the addition of potassium metabisulfite was used (in a sample with addition of potassium metabisulfite at a dose of 30 mg/L–45.89 and 100 mg/L–46.14). In general, samples preserved with cold plasma using helium/nitrogen as the working gas and samples with the addition of potassium metabisulfite were characterized by higher values of this parameter. The red color of wine is mainly due to anthocyanins. SO_2 can form bonds with them, which directly contributes to the increased brightness of the product. From the point of view of commercial wine quality assessment, this is an undesirable effect [13].

After three months, samples 17, 19, 24, 26 and 29 were darker in color compared to the control (about 9.35; 13.44; 41.23; 20.39; and 15.94%, respectively). Cold plasma (working gas: He/O_2) was used to preserve four of them, which may indicate the involvement of oxygen and its reactive compounds in the process of wine darkening. The mechanism of this phenomenon may be related to ozone and hydroxyl radicals resulting from cold plasma generation. These compounds can cause oxidative cleavage of chromophores, leading to the breakdown of anthocyanins and loss of product color [24]. However, it should be noted that the 5 min treatment with cold plasma alone (He/N_2) as well as with the addition of potassium metabisulfite (30 mg/L) did not cause any change in the value of the L* parameter compared to the control sample. A slight darkening of wine under cold plasma was also observed by Lukić et al. (2019) [25], who studied the effect of cold plasma generated by high voltage pulsed power supply (working gas: argon, treatment time: 2, 5 or 10 min.) on white and red wine. Those authors observed a decrease in the value of the L* parameter with increasing time of exposure to cold plasma. Analyzing the other parameters that comprise the CIELab model (a*; b*), we found the same relationships as in the case of the brightness parameter. The highest value of the parameter indicating the redness of the product (a*) was recorded in the sample with the addition of potassium metabisulfite at a dose of 100 mg/L and was 47.60. An increase in the parameter b* indicates a greater proportion of yellow color in the wine, which may be due to the oxidation of flavanols. Conversely, an increase in C* indicates an increase in the clarity of the product, which may be due to sedimentation of colloids in the wine during storage [26]. Nevertheless, data explaining the effect of cold plasma on wine color are still lacking, and the results are inconclusive.

Table 1. Effect of different preservation processes and storage time on pH and color of red wine.

Sample	Preservation Methods	pH	L*	a*	b*	C*	H*	ΔE*
			BEFORE STORAGE					
1.	no preservation	3.54 ± 0.01 ^{de}	11.23 ± 0.02 ^{e}	41.68 ± 0.08 ^{ef}	19.35 ± 0.07 ^{g}	45.96 ± 0.13 ^{ef}	24.91 ± 0.03 ^{e}	-
2.	cold plasma (2 min *; He/O_2 **)	3.55 ± 0.02 ^{e}	11.28 ± 0.03 ^{ef}	41.84 ± 0.22 ^{ef}	19.45 ± 0.11 ^{g}	46.14 ± 0.05 ^{fg}	24.93 ± 0.07 ^{e}	0.20 ± 0.01
3.	cold plasma (5 min; He/O_2)	3.52 ± 0.01 ^{bcde}	11.81 ± 0.07 ^{g}	42.43 ± 0.07 ^{fghij}	20.36 ± 0.02 ^{h}	47.06 ± 0.07 ^{i}	25.64 ± 0.12 ^{f}	1.39 ± 0.09
4.	cold plasma (10 min; He/O_2)	3.52 ± 0.01 ^{bcde}	12.56 ± 0.06 ^{ij}	43.16 ± 0.17 ^{jk}	21.65 ± 0.22 ^{l}	48.29 ± 0.06 ^{jk}	26.64 ± 0.09 ^{ij}	3.04 ± 0.12
5.	cold plasma (2 min; He/N_2)	3.48 ± 0.01 ^{abcd}	11.02 ± 0.05 ^{e}	41.51 ± 0.16 ^{e}	19.01 ± 0.03 ^{f}	45.65 ± 0.0 ^{e}	24.61 ± 0.10 ^{e}	0.43 ± 0.02
6.	cold plasma (5 min; He/N_2)	3.49 ± 0.01 ^{abcde}	13.26 ± 0.22 ^{k}	44.01 ± 0.12 ^{lmn}	22.87 ± 0.10 ^{m}	49.60 ± 0.23 ^{l}	27.46 ± 0.06 ^{m}	4.68 ± 0.16
7.	cold plasma (10 min; He/N_2)	3.55 ± 0.02 ^{e}	12.55 ± 0.52 ^{ij}	43.29 ± 0.37 ^{kl}	21.70 ± 0.10 ^{l}	48.41 ± 0.19 ^{jk}	26.62 ± 0.03 ^{ij}	3.14 ± 0.09
8.	30 mg/L potassium metabisulfite	3.54 ± 0.01 ^{de}	14.79 ± 012 ^{n}	45.89 ± 0.04 ^{qr}	25.24 ± 0.10 ^{r}	52.37 ± 0.33 ^{p}	28.82 ± 0.20 ^{pq}	8.07 ± 0.22
9.	cold plasma (2 min; He/O_2) and 30 mg/L potassium metabisulfite	3.54 ± 0.01 ^{de}	12.31 ± 0.10 ^{hij}	43.05 ± 0.25 ^{ijk}	21.23 ± 0.14 ^{jk}	48.00 ± 0.14 ^{j}	26.24 ± 0.15 ^{h}	2.56 ± 0.18
10.	cold plasma (5 min; He/O_2) and 30 mg/L potassium metabisulfite	3.52 ± 0.00 ^{bcde}	12.69 ± 0.14 ^{j}	43.44 ± 1.27 ^{klm}	21.87 ± 0.05 ^{l}	48.64 ± 0.05 ^{k}	26.73 ± 0.17 ^{jk}	3.40 ± 0.10
11.	cold plasma (10 min; He/O_2) and 30 mg/L potassium metabisulfite	3.52 ± 0.01 ^{bcde}	14.10 ± 0.06 ^{m}	44.88 ± 0.12 ^{op}	23.98 ± 0.14 ^{o}	51.04 ± 0.14 ^{n}	28.51 ± 0.12 ^{op}	6.32 ± 0.13
12.	cold plasma (2 min; He/N_2) and 30 mg/L potassium metabisulfite	3.52 ± 0.02 ^{bcde}	14.33 ± 0.08 ^{m}	45.27 ± 0.11 ^{pq}	24.62 ± 0.10 ^{p}	51.54 ± 0.16 ^{o}	28.54 ± 0.18 ^{op}	7.09 ± 0.09
13.	cold plasma (5 min; He/N_2) and 30 mg/L potassium metabisulfite	3.51 ± 0.01 ^{bcde}	15.13 ± 0.14 ^{n}	46.08 ± 0.22 ^{qr}	25.80 ± 0.16 ^{r}	52.81 ± 0.12 ^{q}	29.24 ± 0.13 ^{rs}	8.73 ± 0.18
14.	cold plasma (10 min; He/N_2) and 30 mg/L potassium metabisulfite	3.48 ± 0.01 ^{abcd}	12.13 ± 0.15 ^{ghi}	42.75 ± 0.15 ^{ghijk}	20.91 ± 0.07 ^{i}	46.92 ± 0.11 ^{i}	26.06 ± 0.13 ^{gh}	2.09 ± 0.03
15.	100 mg/L potassium metabisulfite	3.52 ± 0.02 ^{bcde}	14.94 ± 0.06 ^{n}	46.16 ± 0.09 ^{r}	25.44 ± 0.12 ^{q}	52.79 ± 0.13 ^{pq}	29.03 ± 0.12 ^{qr}	8.42 ± 0.01
			AFTER STORAGE					
16.	no preservation	3.53 ± 0.01 ^{cde}	11.71 ± 0.15 ^{fg}	42.22 ± 0.04 ^{efghi}	20.19 ± 0.14 ^{h}	46.80 ± 0.08 ^{hi}	25.55 ± 0.10 ^{f}	1.11 ± 0.12

Table 1. *Cont.*

Sample	Preservation Methods	pH	L*	a*	b*	C*	H*	ΔE*
17.	cold plasma (2 min; He/O_2)	3.50 ± 0.02^{abcde}	10.18 ± 0.15^{d}	39.55 ± 0.05^{cd}	17.56 ± 0.11^{e}	43.27 ± 0.14^{d}	23.93 ± 0.12^{d}	2.97 ± 0.09
18.	cold plasma (5 min; He/O_2)	3.48 ± 0.01^{abcd}	13.62 ± 0.09^{kl}	44.21 ± 0.07^{mno}	23.48 ± 0.09^{n}	50.05 ± 0.08^{m}	27.97 ± 0.10^{n}	5.40 ± 0.08
19.	cold plasma (10 min; He/O_2)	3.47 ± 0.01^{abc}	9.72 ± 0.09^{c}	39.89 ± 0.12^{d}	16.75 ± 0.09^{d}	43.26 ± 0.12^{d}	22.80 ± 0.09^{c}	3.50 ± 0.10
20.	cold plasma (2 min; He/N_2)	3.53 ± 0.02^{cde}	12.74 ± 0.08^{j}	42.99 ± 0.11^{ijk}	21.96 ± 0.09^{l}	48.28 ± 0.10^{jk}	27.05 ± 0.10^{kl}	3.29 ± 0.10
21.	cold plasma (5 min; He/N_2)	3.50 ± 0.01^{abcde}	11.74 ± 0.10^{g}	42.13 ± 0.15^{efgh}	20.24 ± 0.14^{h}	46.74 ± 0.11^{hi}	25.67 ± 0.10^{f}	1.12 ± 0.12
22.	cold plasma (10 min; He/N_2)	3.48 ± 0.01^{abcd}	13.91 ± 0.12^{lm}	44.34 ± 0.13^{no}	23.98 ± 0.12^{o}	50.41 ± 0.06^{m}	22.41 ± 0.09^{o}	5.97 ± 0.08
23.	30 mg/L potassium metabisulfite	3.53 ± 0.01^{cde}	12.35 ± 0.06^{hij}	43.05 ± 0.11^{jk}	21.30 ± 0.11^{k}	48.03 ± 0.14^{j}	26.32 ± 0.08^{hi}	2.63 ± 0.09
24.	cold plasma (2 min; He/O_2) and 30 mg/L potassium metabisulfite	3.53 ± 0.02^{cde}	6.6 ± 0.08^{a}	34.28 ± 0.14^{a}	11.39 ± 0.03^{a}	36.13 ± 0.04^{a}	18.37 ± 0.04^{a}	11.81 ± 0.07
25.	cold plasma (5 min; He/O_2) and 30 mg/L potassium metabisulfite	3.49 ± 0.01^{abcde}	12.73 ± 0.05^{j}	42.76 ± 0.06^{hijk}	21.97 ± 0.06^{l}	48.07 ± 0.07^{j}	27.18 ± 0.04^{lm}	3.21 ± 0.06
26.	cold plasma (10 min; He/O_2) and 30 mg/L potassium metabisulfite	3.47 ± 0.01^{abc}	8.94 ± 0.05^{b}	38.39 ± 0.07^{b}	15.41 ± 0.11^{b}	41.37 ± 0.04^{b}	21.87 ± 0.07^{b}	5.62 ± 0.09
27.	cold plasma (2 min; He/N_2) and 30 mg/L potassium metabisulfite	3.48 ± 0.00^{abcd}	12.03 ± 0.03^{gh}	41.92 ± 0.06^{efg}	20.73 ± 0.12^{i}	46.77 ± 0.09^{hi}	26.32 ± 0.04^{hi}	1.61 ± 0.09
28.	cold plasma (5 min; He/N_2) and 30 mg/L potassium metabisulfite	3.46 ± 0.01^{ab}	11.73 ± 0.08^{g}	41.79 ± 0.05^{ef}	20.22 ± 0.06^{h}	46.43 ± 0.09^{gh}	25.82 ± 0.05^{fg}	1.01 ± 0.07
29.	cold plasma (10 min; He/N_2) and 30 mg/L potassium metabisulfite	3.44 ± 0.03^{a}	9.44 ± 0.06^{c}	38.94 ± 0.06^{bc}	16.28 ± 0.06^{c}	42.21 ± 0.07^{c}	22.69 ± 0.07^{c}	4.49 ± 0.06
30.	100 mg/L potassium metabisulfite	3.47 ± 0.01^{abc}	16.52 ± 0.12^{o}	47.60 ± 0.09^{s}	26.94 ± 0.03^{s}	54.70 ± 0.05^{r}	29.51 ± 0.07^{s}	10.98 ± 0.05

$^{a–s}$ Values with the different superscript letters within one column are significantly different ($p < 0.05$). * Cold plasma exposure time—2, 5, or 10 min. ** Working gas (a mixture of He/O_2 or He/N_2).

Table 2. Effect of different preservation processes and storage time on polyphenolic content and antioxidant activity of red wine.

Sample Number	Preservation Methods	TPC [mg/L]	TAC [mg/L]	DPPH [% Inhibition]	ABTS [% Inhibition]	FRAP [mM TE/L]
		BEFORE STORAGE				
1.	no preservation	2442.75 ± 12.30 ^{mn}	690.92 ± 3.00 ^{lmno}	72.22 ± 0.87 ^{hij}	95.24 ± 1.73 ^{a}	10.35 ± 0.53 ^{fghijk}
2.	cold plasma (2 min *; He/O_2 **)	2300.25 ± 10.15 ^{j}	647.92 ± 3.64 ^{ijk}	67.82 ± 1.27 ^{h}	95.13 ± 2.54 ^{a}	10.16 ± 0.59 ^{fghij}
3.	cold plasma (5 min; He/O_2)	2497.03 ± 11.26 ^{pq}	607.00 ± 17.82 ^{h}	74.14 ± 1.46 ^{jk}	95.39 ± 0.95 ^{a}	10.72 ± 0.11 ^{hijkl}
4.	cold plasma (10 min; He/O_2)	2449.53 ± 4.02 ^{n}	634.56 ± 3.08 ^{hi}	72.22 ± 0.88 ^{hij}	94.81 ± 1.40 ^{a}	9.81 ± 0.19 ^{defghi}
5.	cold plasma (2 min; He/N_2)	2483.46 ± 5.26 ^{op}	693.84 ± 4.98 ^{mno}	70.50 ± 1.09 ^{hij}	95.22 ± 2.33 ^{a}	12.06 ± 0.46 ^{lm}
6.	cold plasma (5 min; He/N_2)	2388.46 ± 3.59 ^{k}	642.35 ± 10.51 ^{ij}	72.61 ± 4.31 ^{ijk}	94.26 ± 1.68 ^{a}	8.24 ± 0.27 ^{abcd}
7.	cold plasma (10 min; He/N_2)	2517.39 ± 4.07 ^{q}	649.17 ± 3.01 ^{ijk}	72.03 ± 0.99 ^{hij}	95.39 ± 1.56 ^{a}	11.64 ± 0.61 ^{jklm}
8.	30 mg/L potassium metabisulfite	2422.39 ± 3.03 ^{lm}	702.19 ± 8.22 ^{nop}	73.75 ± 1.84 ^{ijk}	95.27 ± 1.12 ^{a}	11.42 ± 0.65 ^{jklm}
9.	cold plasma (2 min; He/O_2) and 30 mg/L potassium metabisulfite	2381.68 ± 4.80 ^{k}	658.35 ± 5.74 ^{ijk}	72.61 ± 2.52 ^{ijk}	95.42 ± 0.62 ^{a}	11.45 ± 0.43 ^{jklm}
10.	cold plasma (5 min; He/O_2) and 30 mg/L potassium metabisulfite	2483.46 ± 5.71 ^{op}	670.46 ± 5.75 ^{jklm}	69.73 ± 1.51 ^{hij}	95.30 ± 1.25 ^{a}	10.17 ± 0.24 ^{fghij}
11.	cold plasma (10 min; He/O_2) and 30 mg/L potassium metabisulfite	2463.10 ± 8.96 ^{no}	662.67 ± 22.00 ^{ijkl}	72.22 ± 2.35 ^{hij}	95.04 ± 1.03 ^{a}	9.81 ± 0.25 ^{defghi}
12.	cold plasma (2 min; He/N_2) and 30 mg/L potassium metabisulfite	2578.46 ± 9.04 ^{s}	673.80 ± 6.31 ^{klmn}	70.11 ± 0.97 ^{hij}	95.51 ± 0.93 ^{a}	12.09 ± 0.76 ^{lm}
13.	cold plasma (5 min; He/N_2) and 30 mg/L potassium metabisulfite	2415.61 ± 4.34 ^{l}	658.56 ± 14.13 ^{ijk}	69.16 ± 1.08 ^{hi}	95.24 ± 0.37 ^{a}	11.45 ± 0.69 ^{jklm}
14.	cold plasma (10 min; He/N_2) and 30 mg/L potassium metabisulfite	2551.31 ± 3.46 ^{r}	707.20 ± 5.21 ^{op}	73.95 ± 0.95 ^{jk}	95.42 ± 1.59 ^{a}	11.86 ± 0.77 ^{klm}
15.	100 mg/L potassium metabisulfite	2598.81 ± 5.18 ^{s}	730.16 ± 14.61 ^{p}	77.31 ± 1.34 ^{k}	95.56 ± 1.55 ^{a}	12.41 ± 0.59 ^{m}
		AFTER STORAGE				
16.	no preservation	1954.20 ± 5.37 ^{i}	571.10 ± 1.67 ^{g}	57.21 ± 0.89 ^{g}	94.80 ± 0.93 ^{a}	11.32 ± 0.61 ^{ijklm}
17.	cold plasma (2 min; He/O_2)	1750.64 ± 5.38 ^{e}	485.10 ± 7.23 ^{cd}	46.66 ± 0.65 ^{ef}	93.36 ± 0.56 ^{a}	8.17 ± 0.14 ^{abc}
18.	cold plasma (5 min; He/O_2)	1791.35 ± 4.18 ^{f}	476.47 ± 12.11 ^{cd}	36.13 ± 0.57 ^{d}	93.86 ± 1.16 ^{a}	8.15 ± 0.46 ^{abc}
19.	cold plasma (10 min; He/O_2)	1587.79 ± 6.28 ^{a}	425.40 ± 3.26 ^{a}	23.81 ± 0.34 ^{a}	92.54 ± 0.51 ^{a}	7.16 ± 0.17 ^{a}

Table 2. *Cont.*

Sample Number	Preservation Methods	TPC [mg/L]	TAC [mg/L]	DPPH [% Inhibition]	ABTS [% Inhibition]	FRAP [mM TE/L]
20.	cold plasma (2 min; He/N_2)	1832.06 ± 7.03 ^{g}	436.95 ± 11.12 ^{ab}	31.44 ± 1.70 ^{cd}	94.14 ± 1.88 ^{a}	10.41 ± 0.56 ^{ghijk}
21.	cold plasma (5 min; He/N_2)	1944.20 ± 5.09 ^{i}	529.91 ± 15.49 ^{ef}	50.18 ± 1.30 ^{f}	94.20 ± 1.44 ^{a}	11.46 ± 0.77 ^{jklm}
22.	cold plasma (10 min; He/N_2)	1791.35 ± 7.91 ^{f}	436.12 ± 4.89 ^{ab}	46.07 ± 1.03 ^{ef}	93.20 ± 0.88 ^{a}	9.05 ± 0.30 ^{bcdefg}
23.	30 mg/L potassium metabisulfite	1832.06 ± 5.95 ^{g}	543.83 ± 9.96 ^{fg}	31.99 ± 0.67 ^{cd}	94.51 ± 0.73 ^{a}	10.07 ± 0.42 ^{efghij}
24.	cold plasma (2 min; He/O_2) and 30 mg/L potassium metabisulfite	1750.64 ± 8.50 ^{e}	467.57 ± 10.85 ^{c}	30.85 ± 0.25 ^{bc}	94.20 ± 1.44 ^{a}	9.73 ± 0.16 ^{cdefghi}
25.	cold plasma (5 min; He/O_2) and 30 mg/L potassium metabisulfite	1628.50 ± 7.48 ^{b}	437.23 ± 5.03 ^{ab}	26.16 ± 0.71 ^{ab}	93.46 ± 1.74 ^{a}	7.82 ± 0.16 ^{ab}
26.	cold plasma (10 min; He/O_2) and 30 mg/L potassium metabisulfite	1709.92 ± 4.34 ^{d}	433.54 ± 2.55 ^{ab}	32.02 ± 0.98 ^{cd}	93.30 ± 1.66 ^{a}	8.52 ± 0.36 ^{abcde}
27.	cold plasma (2 min; He/N_2) and 30 mg/L potassium metabisulfite	1669.21 ± 5.93 ^{c}	458.38 ± 7.07 ^{bc}	35.51 ± 0.52 ^{cd}	93.17 ± 1.84 ^{a}	9.12 ± 0.42 ^{bcdefg}
28.	cold plasma (5 min; He/N_2) and 30 mg/L potassium metabisulfite	1709.92 ± 2.37 ^{d}	482.32 ± 6.27 ^{cd}	43.72 ± 0.90 ^{e}	93.70 ± 0.66 ^{a}	9.19 ± 0.60 ^{bcdefgh}
29.	cold plasma (10 min; He/N_2) and 30 mg/L potassium metabisulfite	1832.06 ± 6.93 ^{g}	501.24 ± 4.12 ^{de}	44.90 ± 1.65 ^{e}	94.20 ± 1.45 ^{a}	8.81 ± 0.31 ^{bcdef}
30.	100 mg/L potassium metabisulfite	1913.49 ± 11.40 ^{h}	553.57 ± 9.45 ^{fg}	50.18 ± 2.61 ^{f}	94.72 ± 0.56 ^{a}	10.31 ± 0.57 ^{fghijk}

$^{a–s}$ Values with the different superscript letters within one column are significantly different ($p < 0.05$); * Cold plasma exposure time—2, 5, or 10 min. ** Working gas (a mixture of He/O_2 or He/N_2).

In order to determine whether the changes we observed could affect the assessment of wine quality by the consumer, the parameter ΔE* was calculated, which indicates whether the differences in color between the control sample and the test sample can be perceived by the human eye (ΔE* $\geq$ 3) [27]. In samples that were tested immediately after exposure to a sterilizing agent, the application of cold plasma for 2, 5 or 10 min (working gas He/O_2) did not visibly affect the coloring of wine. However, when the duration of the process was prolonged, the value of this parameter increased from 0.19 to 3.04. The highest values of ΔE* were recorded for wine samples preserved using the combined method (5 min, He/N_2)—8.72, and the traditional method (addition of 100 mg/L potassium metabisulfite)—8.42. After storage, the lowest ΔE* values were recorded for the wine samples preserved with the combined method (helium/nitrogen; 5 min), ΔE* of 1.01, and cold plasma treatment (helium/nitrogen; 5 min), ΔE* of 1.12. However, the change in color, for most treatments, was imperceptible (ΔE* $\leq$ 3.0) or almost imperceptible (ΔE* $\leq$ 6.0) to the human eye, with the exception of the combined method (2 min, He/O_2) and the conventional method: ΔE* of 11.81 and 10.98, respectively.

2.1.2. Polyphenolic Content

The total contents of phenolic compounds and anthocyanins were determined using the spectrophotometric method (Table 2). Measurement of TPC immediately after the application of the different preservation processes did not show considerable differences among the methods used. Most samples showed a slight increase in the content of these compounds compared to the control. The highest concentration of TPC was recorded in the sample with the addition of 100 mg/L potassium metabisulfite and was 6.39% higher than in the unpreserved sample. These results contrast with the data obtained by Lukić et al. (2019), who treated a Cabernet Sauvignon red wine with cold plasma and reported a reduction in phenolic compounds from 1816.06 mg GAE/L to 1606.57 mg GAE/L immediately after 10 min exposure to CP. However, those authors used different process parameters in their study (a different type of plasma generator and working gas), which may have led to differences in the results obtained. Numerous literature data on the effects of cold plasma on the physicochemical properties of food products highlight differences in CP efficiency depending on the process parameters used [28]. The results we obtained after three months of storage showed that the content of TPC in each of the samples was reduced in comparison with the control sample (from 20 to 33% degradation). There are limited reports in the literature explaining the mechanism of action of cold plasma on food products [29,30]. In the generation of CP, emission of light occurs, a shock wave is generated, cavitation processes can develop and, most importantly, free radicals are generated. All this can contribute directly to the degradation of many organic compounds [31]. The highest degree of TPC degradation was obtained in the sample subjected to 10 min of cold plasma treatment (He/O_2), which is consistent with our previous research findings [2]. Cold plasma generated using a mixture of helium and oxygen as the working gas contains many reactive compounds formed from oxygen, such as hydroxyl radical, peroxyl anion or singlet oxygen, which directly interact with the chemical compounds in the food matrix and can cause their degradation. This topic has been discussed more extensively in recent studies [2,32]. However, comparing the content of phenolic compounds after 3-month storage, we noted in the cold-plasma-preserved sample (He/O_2; 5 min) a lower content of these compounds by 0.51% compared to the unpreserved stored sample. Furthermore, the concentration of TPC was 1.62% higher compared to the conventionally preserved sample.

Similar relationships were noted when we investigated the total anthocyanin content. However, in contrast to the TPC results, the number of anthocyanins decreased immediately after application of the preservation agent and degraded even more after time. An analysis of the effect of process duration on the content of anthocyanins after 3-month storage showed no clear relationship between increasing process duration and a decrease in the content of these compounds in the samples. Such a trend was observed only for methods in which cold plasma with the working gas He/O_2 was used as the sterilizing agent. In

the case of cold plasma (He/N_2) or combined treatments, the content of these compounds was higher after 5 min of application of the sterilizing agent compared to 2 min. Similar correlations were also noted in our previous publication, in which we qualitatively and quantitatively determined the content of individual phenolic compounds in the same wine samples using UPLC/MS/MS [2]. Furthermore, when analyzing the anthocyanin content of the stored samples, we clearly observed their higher content in the samples with potassium metabisulfite. In the conventionally preserved samples (addition of potassium metabisulfite at 30 mg/L or 100 mg/L), the anthocyanin content was lower by 4.77% and 3.07%, respectively, compared to the non-preserved stored sample. This is probably related to the protective effect of sulphur dioxide on the enzymatic and non-enzymatic oxidation of wines [33].

2.1.3. Antioxidant Activity

Wine has health-promoting properties due to a high content of phenolic compounds, which gives it a strong antioxidant capacity. In the present study, we used DPPH, ABTS and FRAP methods to determine the effect of the different preservation processes studied and three-month storage on the antioxidant capacity of wine.

The results of these assays are presented in Table 2. The findings obtained using the different analytical methods did not always have the same correlations associated with the preservation method used, which may be due to the distinct interactions of the bioactive compounds with the reagents used in the assay. Nevertheless, across all assays, the highest antioxidant activity was recorded in the unstored sample with the addition of 100 mg/L potassium metabisulfite, with DPPH, ABTS and FRAP values of 77.31%, 95.56% and 12.41 mmTE/L, respectively. Interestingly, as discussed above, this sample also had the highest TPC and TAC contents. However, the sample preserved by cold plasma (10 min; He/O_2) and then stored had the lowest antioxidant capacity. DPPH, ABTS and FRAP values for this sample were 67.03%, 2.83% and 30.82% lower, respectively, compared to the control sample. As in the case of the total content of phenolic compounds and anthocyanins, the effect of the duration of the preservation process on antioxidant activity is not clear. There are no literature data on the direct effect of cold plasma on the antioxidant properties of wine. However, it was shown that exposure of blueberry juice to CP resulted in a decrease in its oxidative potential with increasing treatment time [28]. In contrast, in our study, the radical scavenging activity determined by DPPH was 19% higher after 5 min CP treatment (He/N_2) compared to 2 min cold plasma exposure. The present results indicate that antioxidant activity is strongly related to the content of phenolic compounds. Similar conclusions have also been reached by other authors who have studied the antioxidant properties of wine [34,35].

2.2. Microbial Contamination

Microbiological contamination of food products not only causes earlier spoilage of food, but can also be a threat to consumers' health. In addition, a shortened shelf life of products generates large amounts of food waste, which is a serious problem in the face of world hunger. Therefore, scientists are still looking for effective food preservation methods. This paper presents a quantitative analysis of the microbiological contamination of wine samples subjected to different preservation methods immediately after the application of a sterilizing agent, as well as after a three-month storage period. In Figure 1A, showing the degree of reduction in microorganisms in the samples that were not subjected to storage, we can see that each of the preservation methods resulted in a reduction of the total number of mesophilic bacteria, and when the combined method (10 min, He/O_2) was used, no microorganisms were detected in the sample. In addition, an analysis of the graph indicates that the inactivation efficiency of cold plasma increased with increasing process duration. A similar relationship has been reported by numerous authors [18,36–38]. For example, Pankaj et al. (2017), who studied the effect of cold plasma (DBD; working gas: air; treatment time: 1–4 min.) on white grape juice reported a reduction in *Saccharomyces cerevisiae* by

7.4 log CFU/mL after 4 min of CP treatment. When we analyzed our samples after 3 months of storage (Figure 1B), we noted a growth in the number of microorganisms in each sample; however, the number of microorganisms was also the lowest in the sample treated for 10 min by the combined method using He/O_2 as the working gas. The microorganism reduction rate in this sample was 4.21 log number of cfu compared to a stored sample that had not been subjected to any preservation treatment. It should also be noted that this method showed a higher microbial elimination efficiency than the addition of 100 mg/L potassium metabisulfite. The inactivating effect of cold plasma on microorganisms has already been well-documented by numerous authors. Both its direct effect on microbial cells [39,40] and its contribution to the microbiological safety of food products have been analyzed [41–43]. The findings available in the literature correspond with ours and indicate that the effectiveness of cold plasma treatment depends on the numerous parameters of the process as well as the food matrix that is subjected to the preservation process.

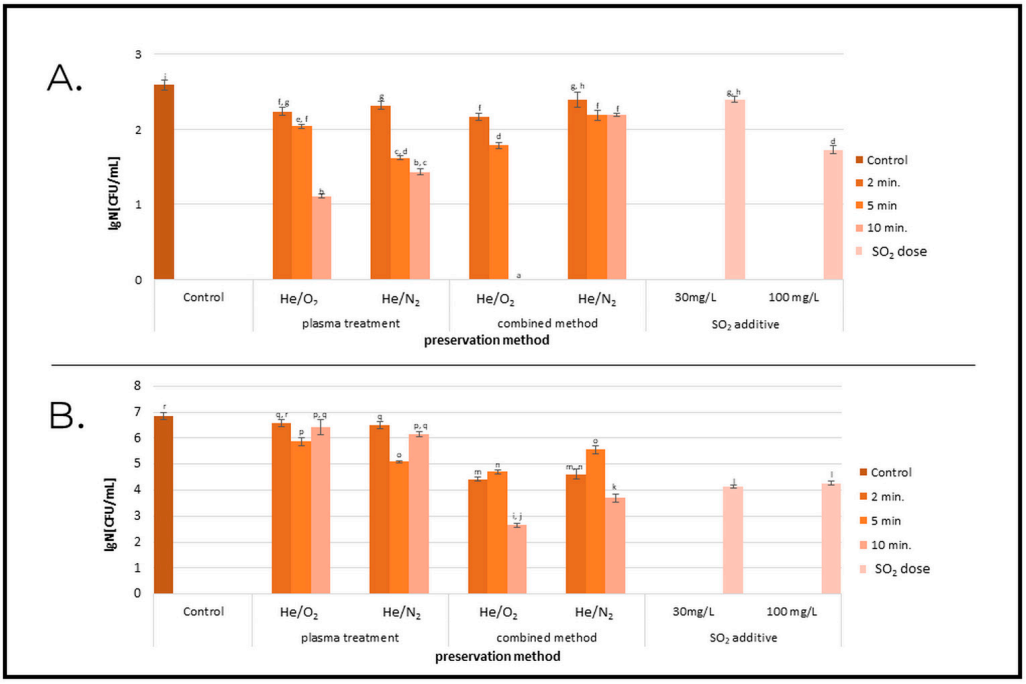

Figure 1. Effect of inactivation of bacterial growth at different preservation methods. (**A**)—before storage. (**B**)—after storage. Cold plasma exposure time—2, 5, or 10 min. Working gas was a mixture of He/O_2 or He/N_2; $^{a–r}$ Values with the different superscript letters are significantly different ($p < 0.05$).

Biological contamination of wines poses a serious problem not only because it can cause spoilage and reduce the shelf life of these products, but also because the microorganisms present in wine may increase the content of biogenic amines [44]. In our previous publication, we determined the biogenic amine content in the same samples we used in the present experiments [2]. A comparison of those results with the number of microorganisms determined in this study suggests that the content of microorganisms may correlate with the concentration of biogenic amines in wine. For example, the lowest total contents of biogenic amines were recorded in samples 11 and 14 (925.82 µg/L and 955.63 µg/L, respectively), in which the total number of mesophilic bacteria was the lowest in this study.

2.3. Chemometric Analysis

The major goal of the multivariate statistical analysis of the experimental data was to reveal patterns of similarity between the objects or between the variables; to identify specific descriptors responsible for the partitioning of the wine samples; and to elucidate

the dataset structure by finding the optimal number of latent variables (factors) able to explain the maximal amount of explained variance of the system.

The following chemometric methods were used in the intelligent data analysis:

- Cluster analysis.
- Factor analysis and principal components analysis.

2.3.1. Cluster Analysis

Figure 2 shows a hierarchical dendrogram for the clustering of the 18 variables studied (input data standardized by z-transform, squared Euclidean distances as a similarity measure and Ward's method of linkage). Three major clusters (significance level 1/3Dmax) were identified as follows:

C1: pH, TP, TA, DPPH, ABTS, FRAP—cluster 1 indicates the impact of the acidity, phenolic composition and antioxidant activity of the samples (oxidation factor);

C2: L*, a*, b*, C*, H*, 2-PE—cluster 2 is almost entirely composed of color characteristics; the linkage of 2-PE to the color estimates is surprising to some extent but, in general, cluster 2 reflects color impact as an important descriptor of the investigated wine;

C3: LogN, HIS, PUT, TRP, CAD, TYR—cluster 3 is a representation of the linkage between the microbiological parameter and the biogenic amines (biological factor).

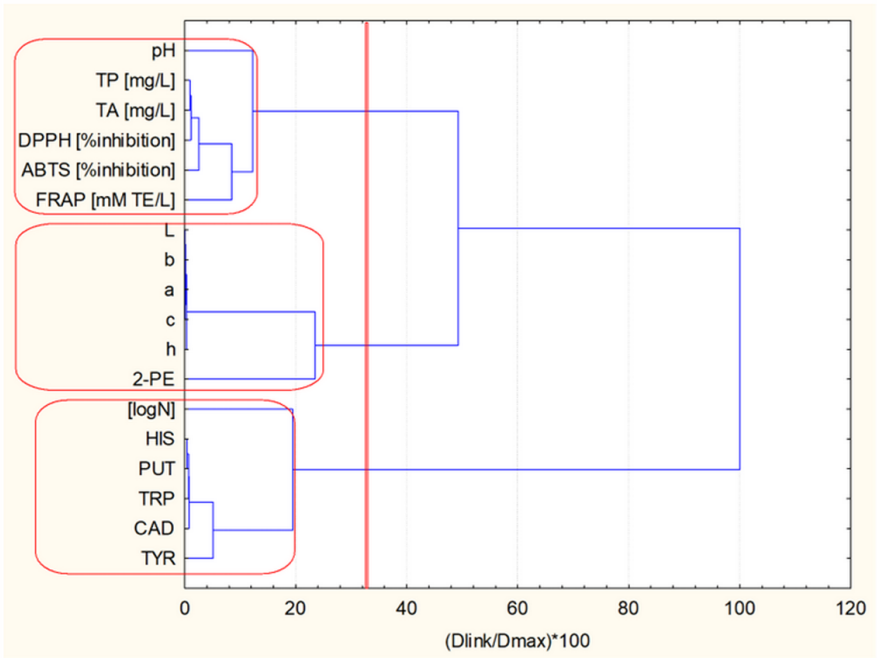

Figure 2. Hierarchical dendrogram for linkage of 18 variables. TRP—tryptamine; PUT—putrescine; HIS—histamine; TYR—tyramine; CAD—cadaverine; 2-PE—2-phenylethylamine.

The application of non-hierarchical clustering (K-means) for the a priori selected number of three clusters confirmed the partitioning obtained by hierarchical cluster analysis. Table S1 (Supplementary Material) shows the members of each cluster identified using the K-means clustering approach. The only (insignificant) difference between the clusters obtained using the K-means algorithm versus hierarchical clustering was their numbering (clusters 1 and 2 were formed in reverse order).

In Figure 3A, the average values for each identified cluster of variables are presented for each of the 30 wine samples. Four different groups of objects are visible in Figure 3A.

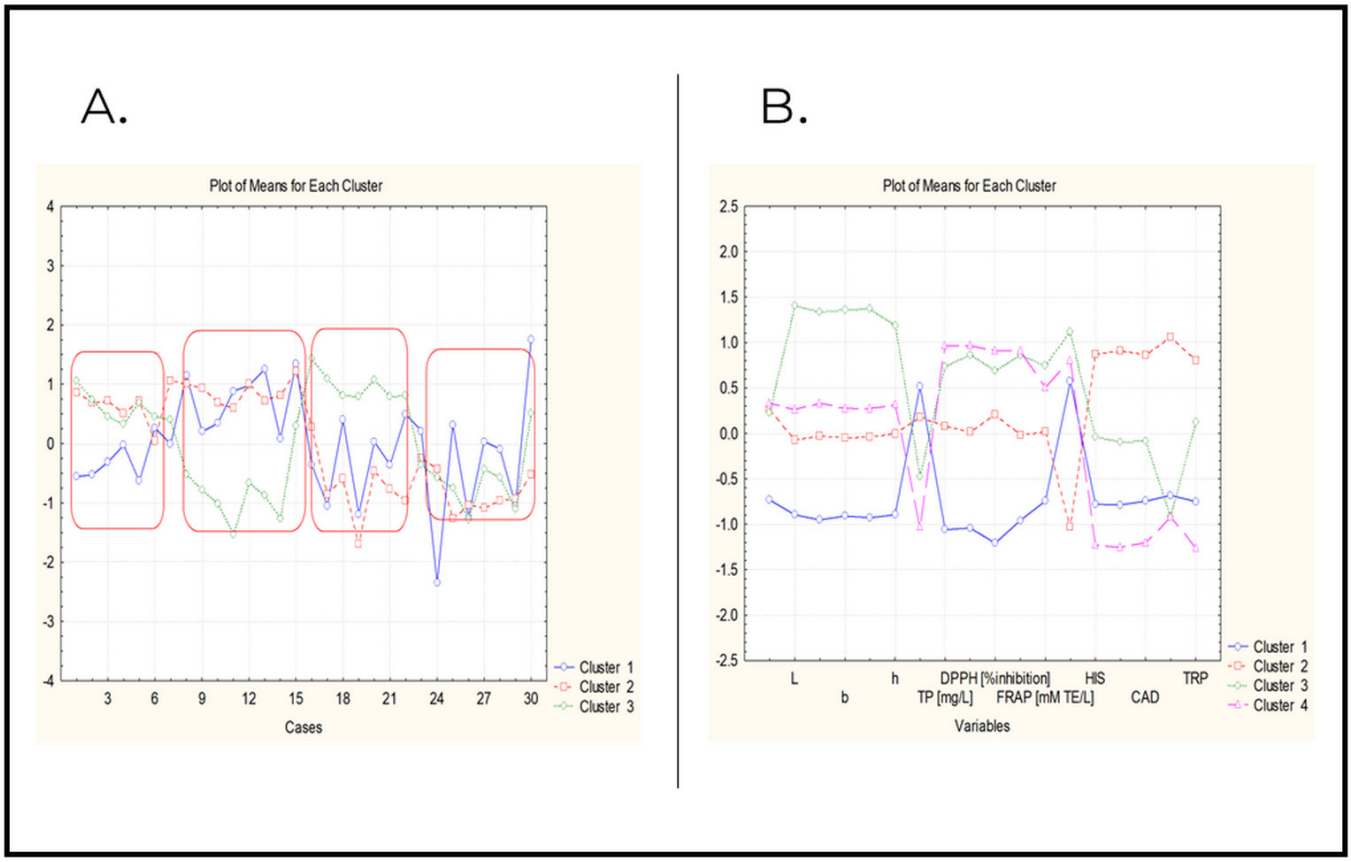

Figure 3. (**A**) Plot of average values for each cluster of variables for each object wine sample. (**B**) Plot of average values of each variable for each identified cluster. The sequence of variables is as follows: pH, L*, a*, b*, C*, H*, logN, TP, TA, DDPH, ABTS, FRAP, 2-PE, HIS, PUT, CAD, TYR, TRP. TRP—tryptamine; PUT—putrescine; HIS—histamine; TYR—tyramine; CAD—cadaverine; 2-PE—2-phenylethylamine.

The first group includes samples with numbers 1–7, the second 8–15, the third 16–22 and the last, the fourth group—samples 23–30. In general, the first group of samples is characterized by low levels of the "color" factor, the second one by low levels of bioamines (biological impact), the third one by low levels of oxidation impact and the fourth one by high levels of the "color" factor. Additionally, it can be stated that the first group are plasma-preserved samples; the second group are samples preserved with plasma and metabisulfite; the third group are samples treated with plasma after storage; and the fourth group are samples treated with plasma and metabisulfite after storage. It could be assumed from this part of the statistical analysis that the time of treatment and the concentration of the metabisulfite added were not significant factors.

Table S2 (Supplementary Materials) shows the non-hierarchical partitioning of the 30 wine samples (objects), which reveals patterns of similarity between the objects described by all the variables. A preliminary hypothesis assumed the existence of four clusters depending on the conditions of preservation (plasma or plasma plus metabisulfite) and storage (without storage and after storage).

It is of substantial interest to see which variables (descriptors) are specific for the partitioning of the group of objects into four clusters.

Figure 3B shows the average values of each variable for each of the identified clusters.

Cluster 1 is characterized by the lowest levels of color parameters (all samples preserved by plasma and metabisulfite and stored afterwards). Another feature of this group of samples is the lowest levels of antioxidant parameters and moderate levels of biogenic

amines. Very specific for this cluster is the highest level of log N and the relatively high level of 2-PE.

Cluster 2 is the largest one (it mainly contains samples preserved with plasma, with and without storage). It is characterized by moderate levels of almost all the variables and the highest levels of biogenic amines.

The other two clusters are relatively small (cluster 3, with the highest levels of color descriptors, chiefly contains non-preserved control samples and cluster 4 mainly consists of samples preserved using helium/oxygen), characterized by high levels of antioxidants and low levels of biogenic amines.

The graphical relation between the objects and the variables is illustrated by the two-way clustering diagram in Figure 4A.

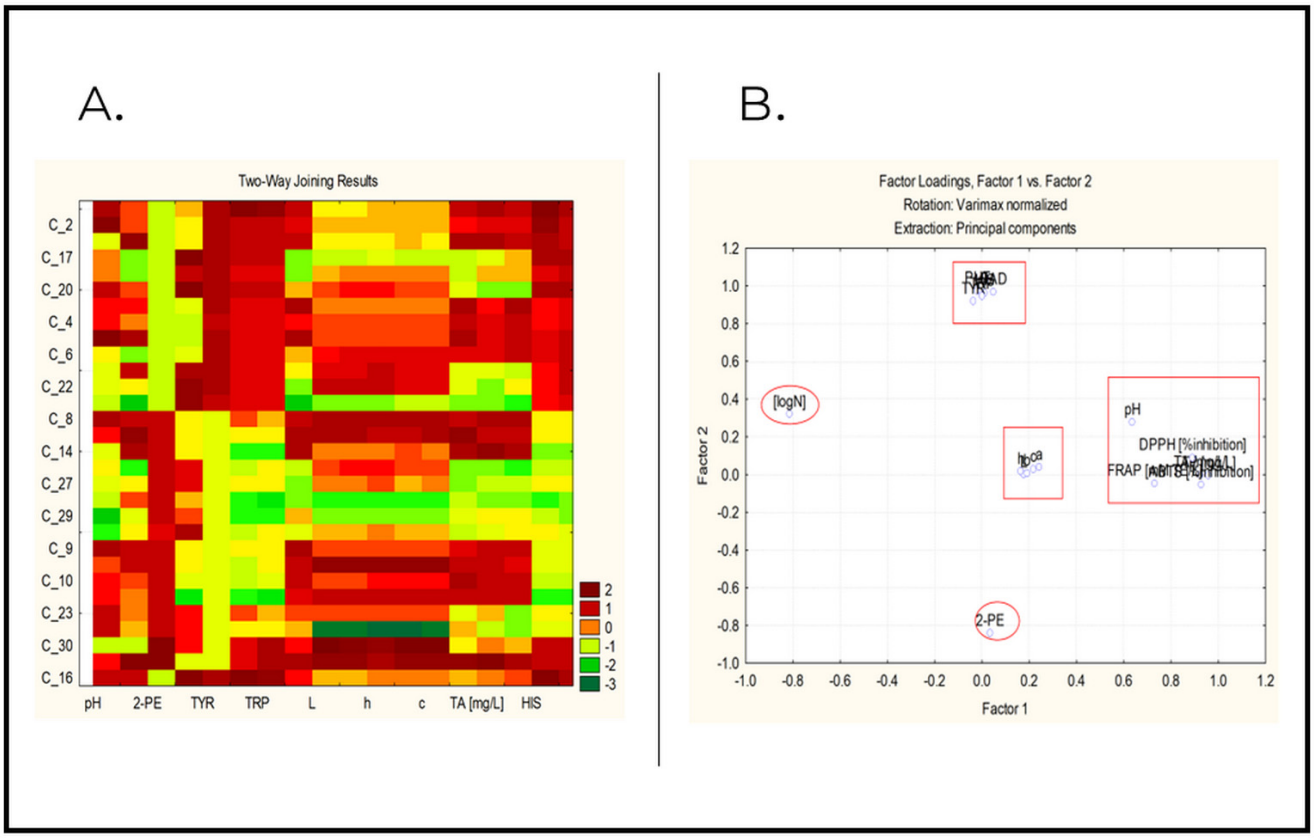

Figure 4. (**A**)—Two-way joining plot. (**B**)—2D plot of factor loadings. TRP—tryptamine; PUT—putrescine; HIS—histamine; TYR—tyramine; CAD—cadaverine; 2-PE—2-phenylethylamine.

The dark brown regions represent strong relations (e.g., biogenic amines are strongly associated with objects 2, 17, 20, 4, 6 and 22), and green regions indicate a lack of a strong relation (e.g., TA are weakly associated with objects 17, 22, 14, 27, 29 and 23).

2.3.2. Factor Analysis and Principal Component Analysis

Table S3 (Supplementary Material) shows the factor loadings for the 18 variables studied.

Three latent factors explain over 80% of the total variance of the system. In general, the grouping by high factor loadings in the different latent components corresponds to the results obtained by cluster analysis. The first latent factor, which explains over 30% of the total variance, could be provisionally named an "antioxidant" factor due to the significant loadings of the phenolic compounds, pH and antioxidants. The negative sign of logN indicates another level of the relationship.

The second latent factor explains nearly 30% of the total variance and includes high loadings for biogenic amines, which is why it can be tentatively referred to as a "biogenic

amines factor". The only exception is 2-PE (negative sign of the loading), but this could be explained by the low variability of the variable (it takes only two values for all objects).

The third latent factor could be conditionally called a "color" factor, as it includes all the color indicators (it explains nearly 30% of the total variance).

Figure 4B shows a 2D plot of the factor loadings. It very clearly illustrates the formation of the three latent factors and the special positions of logN and 2-PE.

3. Materials and Methods

3.1. Wine

In this study, we investigated a red wine produced at the Dom Bliskowice winery located in Poland's Lubelskie Province. Two grape varieties, Rondo and Regent (1:1), obtained from the October 2019 harvest, were used to produce the wine. The test samples included wine subjected to different preservation processes: cold plasma treatment, preservation with the addition of 30 mg/L or 100 mg/L potassium metabisulfite and a method combining cold plasma with the addition of 30 mg/L potassium metabisulfite. The control sample was wine not subjected to any preservation process. Samples were assayed immediately after preservation and again after three months of storage.

3.2. Cold Plasma Treatment

50 mL of wine contained in a sterile glass container was placed on a magnetic stirrer to ensure that the plasma generated in the Dielectric Barrier Discharge (DBD) reactor was uniformly applied to the samples. A mixture of helium and nitrogen or helium and oxygen was used as the working gas. The preservation process was carried out for 2, 5 or 10 min. The exact methodology for cold plasma treatment was described in an earlier publication by Niedźwiedź et al. (2022).

3.3. Determination of pH and Color Measurement

The pH value of the red wine samples was measured potentiometrically using a Hanna HI 221 pH meter (Hanna Instruments, Woonsocket, RI, USA). The electrode tip was thoroughly rinsed each time with distilled water both before and after measurement.

Color parameters were determined with an X-Rite 8200 colorimeter (X-Rite, Inc., Grand Rapids, MI, USA) using the CIELab color space (Method OIV-MA-AS2-11, 2006). Black slides and white plates were used to calibrate the colorimeter. The spectra were registered directly on the wine, using a 10 mm optical path glass cell. Color was expressed by the CIE coordinates: L*—clarity, lightness; a*—red/green color components; and b*—blue/yellow color components, and by its derived magnitudes: C*—chroma and H*—hue angle. In order to determine the degree of color change between the study samples and the control, delta E (ΔE*) was calculated according to the following formula:

$$\Delta E^* = \sqrt{(\Delta \boldsymbol{L}^2 + \Delta \boldsymbol{a}^2 + \Delta \boldsymbol{b}^2)} \quad (1)$$

where: $\Delta \boldsymbol{L}$; $\Delta \boldsymbol{a}$; and $\Delta \boldsymbol{b}$ are the difference between the value of a given parameter of the test sample and the control sample.

3.4. Determination of Polyphenolic Compounds

3.4.1. Total Phenolic Content

The total phenolic content (TPC) of red wine was determined by the Folin–Ciocalteu method with a minor modification. Wine samples were diluted 1:9 with distilled water. Fifty microliters (50 μL) of diluted sample was mixed with 750 μL of Folin–Ciocalteu reagent. After 5 min, 750 μL of sodium hydrogen carbonate ($NaHCO_3$) was added to the mixture followed by incubation for 2 h at room temperature. The absorbance was measured at a wavelength of 760 nm against the blank sample. Results were expressed as mg/L gallic acid equivalents (mg GAE/L).

3.4.2. Total Anthocyanin Content

For the determination of the total anthocyanin content (TAC), two reaction solutions were prepared by mixing (1) 50 µL of diluted wine sample (10×) with 200 µL of KCl buffer (pH 1.0) and (2) 50 µL of diluted wine sample (10×) with 200 µL of $CH_3COONa \times 3\ H_2O$ buffer (pH 4.5). Subsequently, the samples were incubated for 15 min. After that time, the absorbance was measured at two wavelengths: 520 nm and 700 nm. TAC was calculated using the formulas below, and the results were expressed as cyanidin-3-glucoside equivalents.

$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5} \quad (2)$$

$$C = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)} \quad (3)$$

where A—absorbance of sample; C—anthocyanin concentration; MW—molar mass of cyanidin-3-glucoside; DF—sample dilution; ε—cyanidin-3 molar extinction coefficient of glucoside; and 1—length of light path.

3.5. Determination of Antioxidant Activity

3.5.1. DPPH Inhibition

Antioxidant activity was determined using the DPPH radical according to a slightly modified method proposed by Brand-Williams et al. (1995) [45] and Szwajgier et al. (2021) [46]. A 10-fold diluted test sample in a volume of 20 µL was mixed with 255 µL of methanol and 30 µL of a DPPH solution with a fixed absorbance (1.5 at 515 nm). A blank sample was prepared in an identical manner by replacing the diluted wine sample with 20 µL of deionized water. Then, after 4 min of incubation, the absorbance of the samples was measured at 515 nm using a microplate reader. The experiment was performed in three replicates, and the results were expressed as % inhibition of DPPH including standard deviations. The calculation was performed according to the following equation:

$$\%DPPHinhibition = (A_B - A_S)/A_B\ \times 100, \quad (4)$$

where A_B—absorbance of the blank sample, A_S—absorbance of the test sample.

3.5.2. ABTS

Antioxidant activity was assayed using the radical cation ABTS according to Miller et al. (1993) [47] with modifications. For analysis, the test sample diluted 10-fold in a volume of 20 µL was mixed with 180 µL of distilled water and 185 µL of ABTS solution (absorbance = 1.5 at 734 nm). After 4 min, absorbance was measured. The negative blank sample contained 0.2 mL of distilled water and 0.185 µL of ABTS solution. Sample background was subtracted during calculations. All samples were analyzed in three replicates, and the results were expressed as % inhibition of ABTS including standard deviations

3.5.3. FRAP

Antioxidant activity was determined based on the degree of ferric ion reduction using the FRAP method according to the procedure described by Szwajgier et al. (2021). A FRAP solution was prepared for the analysis by mixing 2.5 mL of a 5 mM 2,4,6-tris(2-pyridyl)-(S)-triazine (TPTZ) solution (in a 40 mM HCl solution), 2.5 mL of a 5 mM FeCl3 solution and 25 mL of acetate buffer (0.3 M pH 3.6). The mixture was then heated in a water bath at 37 °C for 20 min. After reagent preparation, 20 ul of a wine sample was mixed with 1.9 mL of the FRAP solution and shaken for 30 min at room temperature. Absorbance was measured at 593 nm after shaking. Results were expressed as Trolox equivalents (mg Trolox/mL).

3.6. Microbiological Analysis

The microbiological purity of the wine samples was evaluated using a pour plate method. For this purpose, a series of ten-fold dilutions was first prepared by mixing 1 mL

of wine with 9 mL of saline solution. Then, 1 mL of the appropriately diluted solution was applied to the center of a sterile Petri dish, and 15 mL of sterile, cooled nutrient agar medium (BTL, Łódź, Poland) was poured over it. The medium with the test sample was mixed well and incubated for 72 h at 30 °C. After incubation, colonies were counted, and the number of viable cells was determined as the mean of log colony forming units (cfu) per mL of sample ± standard deviation.

3.7. Chemometric Analysis

Multivariate statistical data mining was used to discover specific correlations between the different wine preservation methods and their effects on the physicochemical and biological properties of wine. The chemometric methods used included cluster analysis (hierarchical and non-hierarchical) and factor analysis. The input dataset consisted of 30 objects (wine samples subjected to different preservation methods) described by 18 variables that could be divided into 6 categories: pH, color, microbiological analysis, polyphenolic compounds, antioxidant activity—which were determined in this study—and biogenic amines, which had been determined in an earlier work (Niedźwiedź et al., 2022). Statistical analysis was conducted using STATISTICA 8.0 software (New York, NY, USA).

In addition, all data obtained were expressed as mean ± standard deviation ($n \geq 3$). Differences between mean data values were tested for statistical significance at $p < 0.05$ using analysis of variance and Tuckey's test.

4. Conclusions

The physicochemical and biological properties of wine play a key role in the consumer's assessment of its quality. In the present study, we investigated the effect of the use of cold plasma as a preservation method on the physicochemical and biological properties of red wine and compared it with the effects of using a conventional preservation method (addition of 30 or 100 mg/L of potassium metabisulfite) and a combined method (cold plasma with 30 mg/L of potassium metabisulfite). In addition, the effect of storage time after the application of each of the analyzed methods was assessed to determine the potential of the respective techniques to extend the shelf life of the product.

More specifically, we analyzed the effects of the different preservation methods on the pH; color; total content of phenolic compounds and anthocyanins; and antioxidant activity, as well as the biological safety of wine. Color is a parameter that plays a decisive role in the evaluation of wine quality. We examined changes in wine color under the influence of preservative factors after three-month storage using the CIELab space. The least prominent color changes were observed in samples treated with the combined method (helium/nitrogen; 5 min), ΔE*—1.01, and cold plasma (helium/nitrogen; 5 min), ΔE*—1.12. By contrast, the most perceptible changes were noted in samples preserved with the traditional method: ΔE*—11.81. In addition, a reduction in the content of phenolic compounds, and thus a decrease in antioxidant activity, was observed in stored samples. This effect was the mildest for preservation methods involving the addition of potassium metabisulfite and those that used a mixture of helium and nitrogen as the working gas. These results correspond with the data we obtained in a previous publication. When analyzing the effect of the selected preservation methods on the biological purity of the wine, we observed a lower number of microorganisms in the methods where cold plasma was used. After 3 months of storage, the total content of microorganisms in the samples was lower by 4.21 and 3.17 log(N), for the sample treated for 10 min with the combined method using He/O_2 and He/N_2, respectively, compared to the sample stored unpreserved. The above results suggest that the action of cold plasma using a mixture of helium and nitrogen as the working gas has a smaller impact on the final quality of the wine. However, the impact of the process duration on individual parameters is ambiguous and requires further detailed studies.

Obtained data allow us to assume that, in the future, cold plasma may contribute to the reduction or elimination of the use of SO_2 in the wine industry. However, since still

relatively little is known about the influence of cold plasma on wine, it is necessary to conduct further research in order to be able to fully exploit the potential of this technology on an industrial scale in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27207048/s1, Table S1: Members of clusters obtained by K-means clustering of 18 variables; Table S2: Members of each identified cluster by K-means clustering; and Table S3: Factor loadings.

Author Contributions: Conceptualization, I.N., M.P.-B. and A.W.; methodology, I.N. and V.S.; software, V.S.; validation, I.N. and V.S.; formal analysis, I.N.; investigation, I.N.; writing—original draft preparation, I.N. and V.S.; writing—review and editing, I.N., M.P.-B. and A.W.; visualization, I.N. and V.S.; supervision, M.P.-B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Griffith, M.P. Ancient wine: The search for the origins of viniculture. *Econ. Bot.* **2004**, *58*, 488. [CrossRef]
2. Niedźwiedź, I.; Płotka-Wasylka, J.; Kapusta, I.; Simeonov, V.; Stój, A.; Waśko, A.; Pawłat, J.; Polak-Berecka, M. The impact of cold plasma on the phenolic composition and biogenic amine content of red wine. *Food Chem.* **2022**, *381*, 132257. [CrossRef] [PubMed]
3. Dumitriu, G.D.; Teodosiu, C.; Gabur, I.; Cotea, V.V.; Peinado, R.A.; López de Lerma, N. Alternative Winemaking Techniques to Improve the Content of Phenolic and Aromatic Compounds in Wines. *Agriculture* **2021**, *11*, 233. [CrossRef]
4. Robles, A.D.; Fabjanowicz, M.; Płotka-Wasylka, J.; Konieczka, P. Organic acids and polyphenols determination in Polish wines by ultrasound-assisted solvent extraction of porous membrane-packed liquid samples. *Molecules* **2019**, *24*, 4376. [CrossRef]
5. Gris, E.F.; Mattivi, F.; Ferreira, E.A.; Vrhovsek, U.; Filho, D.W.; Pedrosav, R.C.; Bordignon-Luiz, M.T. Phenolic profile and effect of regular consumption of Brazilian redwines on in vivo antioxidant activity. *J. Food Compos. Anal.* **2013**, *31*, 31–40. [CrossRef]
6. Borges, A.; Ferreira, C.; Saavedra, M.J.; Simoes, M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb. Drug Resist.* **2013**, *19*, 256–265. [CrossRef]
7. Snopek, L.; Mlcek, J.; Sochorova, L.; Baron, M.; Hlavacova, I.; Jurikova, T.; Sochor, J. Contribution of red wine consumption to human health protection. *Molecules* **2018**, *23*, 1684. [CrossRef] [PubMed]
8. Rasines-Perea, Z.; Teissedre, P.L. Grape Polyphenols' Effects in Human Cardiovascular Diseases and Diabetes. *Molecules* **2017**, *22*, 68. [CrossRef] [PubMed]
9. Haunschild, R.; Marx, W. On Health Effects of Resveratrol in Wine. *Int. J. Environ. Res. Public Health* **2022**, *19*, 3110. [CrossRef]
10. Vaquero, C.; Loira, I.; Raso, J.; Álvarez, I.; Delso, C.; Morata, A. Pulsed Electric Fields to improve the use of non-Saccharomyces starters in red wines. *Foods* **2021**, *10*, 1472. [CrossRef]
11. Casquete, R.; Benito, M.J.; Pérez-Nevado, F.; Martínez, A.; Martín, A.; de Guía Córdoba, M. Addition of Grape Skin and Stems Extracts in Wines during the Storage to Reduce the Sulfur Dioxide: Impact on Red Wine Quality. *Int. J. Environ. Res. Public Health* **2021**, *18*, 2783. [CrossRef] [PubMed]
12. Pozo-Bayón, M.Á.; Monagas, M.; Bartolomé, B.; Moreno-Arribas, M.V. Wine features related to safety and consumer health: An integrated perspective. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 31–54. [CrossRef] [PubMed]
13. Silva, F.V.M.; van Wyk, S. Emerging Non-Thermal Technologies as Alternative to SO_2 for the Production of Wine. *Foods* **2021**, *10*, 2175. [CrossRef]
14. Rathod, N.B.; Kahar, S.P.; Ranveer, R.C.; Annapure, U.S. Cold plasma an emerging nonthermal technology for milk and milk products: A review. *Int. J. Dairy Technol.* **2021**, *74*, 615–626. [CrossRef]
15. Niedźwiedź, I.; Waśko, A.; Pawłat, J.; Polak-Berecka, M. The state of research on antimicrobial activity of cold plasma. *Polish J. Microbiol.* **2019**, *68*, 153. [CrossRef]
16. Misra, N.N.; Pankaj, S.K.; Segat, A.; Ishikawa, K. Cold plasma interactions with enzymes in foods and model systems. *Trends Food Sci. Technol.* **2016**, *55*, 39–47. [CrossRef]
17. Starek, A.; Pawłat, J.; Chudzik, B.; Kwiatkowski, M.; Terebun, P.; Sagan, A.; Andrejko, D. Evaluation of selected microbial and physicochemical parameters of fresh tomato juice after cold atmospheric pressure plasma treatment during refrigerated storage. *Sci. Rep.* **2019**, *9*, 8407. [CrossRef] [PubMed]

18. Lacombe, A.; Niemira, B.A.; Gurtler, J.B.; Fan, X.; Sites, J.; Boyd, G.; Chen, H. Atmospheric cold plasma inactivation of aerobic microorganisms on blueberries and effects on quality attributes. *Food Microbiol.* **2015**, *46*, 479–484. [CrossRef]
19. Mandal, R.; Singh, A.; Singh, A.P. Recent developments in cold plasma decontamination technology in the food industry. *Trends Food Sci. Technol.* **2018**, *80*, 93–103. [CrossRef]
20. Bourke, P.; Ziuzina, D.; Han, L.; Cullen, P.J.; Gilmore, B.F. Microbiological interactions with cold plasma. *J. Appl. Microbiol.* **2017**, *123*, 308–324. [CrossRef]
21. Mai-Prochnow, A.; Clauson, M.; Hong, J.; Murphy, A.B. Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. *Sci. Rep.* **2016**, *6*, 38610. [CrossRef] [PubMed]
22. Bourke, P.; Ziuzina, D.; Boehm, D.; Cullen, P.J.; Keener, K. The potential of cold plasma for safe and sustainable food production. *Trends Biotechnol.* **2018**, *36*, 615–626. [CrossRef] [PubMed]
23. Sáenz-Navajas, M.P.; Echavarri, F.; Ferreira, V.; Fernández-Zurbano, P. Pigment composition and color parameters of commercial Spanish red wine samples: Linkage to quality perception. *Eur. Food Res. Technol.* **2011**, *232*, 877–887. [CrossRef]
24. Sarangapani, C.K.; O'Toole, G.; Cullen, P.; Bourke, P. Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. *Innov. Food Sci. Emerg. Technol.* **2017**, *44*, 235–241. [CrossRef]
25. Lukić, K.; Vukušić, T.; Tomašević, M.; Ćurko, N.; Gracin, L.; Ganić, K.K. The impact of high voltage electrical discharge plasma on the chromatic characteristics and phenolic composition of red and white wines. *Innov. Food Sci. Emerg. Technol.* **2019**, *53*, 70–77. [CrossRef]
26. De Souza, J.F.; De Souza Nascimento, A.M.; Linhares, M.d.S.S.; Dutra, M.d.C.P.; Dos Santos Lima, M.; Pereira, G.E. Evolution of Phenolic Compound Profiles and Antioxidant Activity of Syrah Red and Sparkling Moscatel Wines Stored in Bottles of Different Colors. *Beverages* **2018**, *4*, 89. [CrossRef]
27. Tkacz, K.; Modzelewska-Kapituła, M.; Więk, A.; Nogalski, Z. The Applicability of Total Color Difference ΔE for Determining the Blooming Time in *Longissimus lumborum* and *Semimembranosus* Muscles from Holstein-Friesian Bulls at Different Ageing Times. *Appl. Sci.* **2020**, *10*, 8215. [CrossRef]
28. Hou, Y.; Wang, R.; Gan, Z.; Shao, T.; Zhang, X.; He, M.; Sun, A. Effect of cold plasma on blueberry juice quality. *Food Chem.* **2019**, *290*, 79–86. [CrossRef]
29. Alves Filho, E.G.; de Brito, E.S.; Rodrigues, S. Effects of cold plasma processing in food components. In *Advances in Cold Plasma Applications for Food Safety and Preservation*; Academic Press: Cambridge, MA, USA, 2020; pp. 253–268. [CrossRef]
30. Gavahian, M.; Chu, Y.H.; Khaneghah, A.M.; Barba, F.J.; Misra, N.N. A critical analysis of the cold plasma induced lipid oxidation in foods. *Trends Food Sci. Technol.* **2018**, *77*, 32–41. [CrossRef]
31. He, F.; Liang, N.N.; Mu, L.; Pan, Q.H.; Wang, J.; Reeves, M.J.; Duan, C.Q. Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their color expression. *Molecules* **2012**, *17*, 1571–1601. [CrossRef]
32. Arjunan, K.P.; Sharma, V.K.; Ptasinska, S. Effects of atmospheric pressure plasmas on isolated and cellular DNA—A review. *Int. J. Mol. Sci.* **2015**, *16*, 2971–3016. [CrossRef] [PubMed]
33. Esparza, I.; Martínez-Inda, B.; Cimminelli, M.J.; Jimeno-Mendoza, M.C.; Moler, J.A.; Jiménez-Moreno, N.; Ancín-Azpilicueta, C. Reducing SO_2 doses in red wines by using grape stem extracts as antioxidants. *Biomolecules* **2020**, *10*, 1369. [CrossRef]
34. Rodríguez-Vaquero, M.J.; Vallejo, C.V.; Aredes-Fernández, P.A. Antibacterial, antioxidant and antihypertensive properties of polyphenols from argentinean red wines varieties. *J. Pharmacol. Pharmacother.* **2020**, *5*, 001–006. [CrossRef]
35. Yue, X.F.; Jing, S.S.; Ni, X.F.; Zhang, K.K.; Fang, Y.L.; Zhang, Z.W.; Ju, Y.L. Anthocyanin and phenolic acids contents influence the color stability and antioxidant capacity of wine treated with mannoprotein. *Front. Nutr.* **2021**, *8*, 691784. [CrossRef] [PubMed]
36. Kim, J.S.; Lee, E.J.; Choi, E.H.; Kim, Y.J. Inactivation of *Staphylococcus aureus* on the beef jerky by radio-frequency atmospheric pressure plasma discharge treatment. *Innov. Food Sci. Emerg. Technol.* **2014**, *22*, 124–130. [CrossRef]
37. Pankaj, S.K.; Wan, Z.; Colonna, W.; Keener, K.M. Effect of high voltage atmospheric cold plasma on white grape juice quality. *J. Sci. Food and Agric.* **2017**, *97*, 4016–4021. [CrossRef]
38. Selcuk, M.; Oksuz, L.; Basaran, P. Decontamination of grains and legumes infected with *Aspergillus* spp. and *Penicillum* spp. by cold plasma treatment. *Bioresour. Technol.* **2008**, *99*, 5104–5109. [CrossRef]
39. Khan, M.S.I.; Lee, E.J.; Kim, Y.J. A submerged dielectric barrier discharge plasma inactivation mechanism of biofilms produced by *Escherichia coli* O157: H7, *Cronobacter sakazakii*, and *Staphylococcus aureus*. *Sci. Rep.* **2016**, *6*, 37072. [CrossRef]
40. Niedźwiedź, I.; Juzwa, W.; Skrzypiec, K.; Skrzypek, T.; Waśko, A.; Kwiatkowski, M.; Pawłat, J.; Polak-Berecka, M. Morphological and physiological changes in *Lentilactobacillus hilgardii* cells after cold plasma treatment. *Sci. Rep.* **2020**, *10*, 18882. [CrossRef]
41. Choi, S.; Puligundla, P.; Mok, C. Corona discharge plasma jet for inactivation of Escherichia coli O157: H7 and *Listeria monocytogenes* on inoculated pork and its impact on meat quality attributes. *Ann. Microbiol.* **2016**, *66*, 685–694. [CrossRef]
42. Tappi, S.; Gozzi, G.; Vannini, L.; Berardinelli, A.; Romani, S.; Ragni, L.; Rocculi, P. Cold plasma treatment for fresh-cut melon stabilization. *Innov. Food Sci. Emerg. Technol.* **2016**, *33*, 225–233. [CrossRef]
43. Wang, R.X.; Nian, W.F.; Wu, H.Y.; Feng, H.Q.; Zhang, K.; Zhang, J.; Zhu, W.D.; Becker, K.H.; Fang, J. Atmospheric-pressure cold plasma treatment of contaminated fresh fruit and vegetable slices: Inactivation and physiochemical properties evaluation. *Eur. Phys. J. D* **2012**, *66*, 276. [CrossRef]
44. Restuccia, D.; Loizzo, M.R.; Spizzirri, U.G. Accumulation of biogenic amines in wine: Role of alcoholic and malolactic fermentation. *Fermentation* **2018**, *4*, 6. [CrossRef]

45. Brand-Williams, W.; Cuvelier, M.E.; Berset, C.L.W.T. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
46. Szwajgier, D.; Baranowska-Wójcik, E.; Kukula-Koch, W.; Kowalik, K.; Polak-Berecka, M.; Waśko, A. Evolution of the anti-cholinesterase, antioxidant, and anti-inflammatory activity of *Epilobium angustifolium* L. infusion during in vitro digestion. *J. Funct. Foods* **2021**, *85*, 104645. [CrossRef]
47. Miller, N.J.; Rice-Evans, C.; Davies, M.J.; Gopinathan, V.; Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* **1993**, *84*, 407–412. [CrossRef]