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WYDZIAŁ NAUK O ZWIERZĘTACH I BIOGOSPODARKI

mgr inż. Kamil Cezary Drabik

Dynamika zmian jakości zachodzących w jajach konsumpcyjnych w różnych warunkach przechowywania

Dynamics of quality changes occurring in table eggs under various storage conditions

Praca doktorska

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J. Batkowska

Promotor:

dr hab. Justyna Batkowska, prof. uczelni

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Spis treści

Wykaz publikacji wchodzących w skład pracy doktorskiej

Drabik K., Próchniak T., Kasperek K., Batkowska J. (2021) The use of the dynamics of changes in table eggs during storage to predict the age of eggs based on selected quality traits. *Animals*, 11(11):3192. DOI: 10.3390/ani11113192

Liczba punktów w roku publikacji: ***MEiN = 100; IF=2,752***

Indywidualny wkład pracy w publikację ***(75%)****: zaplanowanie i przeprowadzenie doświadczenia, gromadzenie danych, redagowanie manuskryptu, korekta po recenzjach.*

Drabik K., Próchniak T., Spustek D., Wengerska K., Batkowska J. (2021) The impact of package type and temperature on the changes in quality and fatty acids profile of table eggs during their storage. *Foods*; 10(9): 2047. DOI: 10.3390/foods10092047.

Liczba punktów w roku publikacji: ***MEiN = 70; IF=4,350***

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Drabik K., Batkowska J., Próchniak T., Horecka B. (2021) Citric acid as a factor limiting changes in the quality of table eggs during their storage. *Poultry Science*, 100(4), 100995.DOI: 10.1016/j.psj.2021.01.018.

Liczba punktów w roku publikacji: ***MEiN = 140; IF=3.352***

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Streszczenie

Jakość jaj oraz ich stabilność w czasie przechowywania należy analizować zgodnie z przyjętym prawodawstwem, które reguluje zarówno parametry jakości, jak również maksymalny czas obrotu jajami konsumpcyjnymi. Co istotne, nie określona ono konkretnych warunków przechowywania surowca, zastrzegając tylko, że przechowywanie jaj w warunkach chłodniczych jest zarezerwowane wyłącznie dla konsumenta finalnego. Jednak w jajach, jak we wszystkich produktach żywnościowych, wraz z czasem przechowywania dochodzi do pogorszenia jakości. Konieczne jest zatem zapewnienie właściwych metod przechowywania, tak aby ograniczyć wpływ czasu na jakość surowca jajczarskiego.

Celem pracy była analiza dynamiki zmian jakości jaj konsumpcyjnych w czasie przechowywania w kontekście możliwości ich predykcji oraz opracowanie metod hamowania procesów wpływających na obniżenie jakości jaj konsumpcyjnych. Postawiono hipotezy badawcze w brzmieniu: analiza zmienności dziennej cech jakości jaj może przyczynić się do stworzenie modeli matematycznych pozwalających na prognozowanie tych zmian, jak również czasu, jaki upłynął od momentu zniesienia jaja, jak również, że hamowanie procesu starzenia jaj konsumpcyjnych jest możliwe na drodze modyfikacji warunków przechowywania i/lub z wykorzystaniem substancji ograniczających przepuszczalność porów skorupowych.

Przeprowadzono 3 niezależne doświadczenia. W pierwszym z nich ocenę cech jakości jaj wykonywano codziennie w czasie jego wydłużonego do 35 dni przechowywania, a uzyskane w ten sposób dane pozwoliły na wytworzenie wielkocechowego modelu matematycznego, który po dalszej obróbce statystycznej pozwala finalnie na określenie z dużą pewnością (pow. 95%) wieku jaja na podstawie jego mierzalnych cech zarówno niedestrukcyjnych, jak i destrukcyjnych. Wykorzystano metody statystyczne oparte o równania regresji liniowej (dla cech charakteryzujących się zmiennością liniową) oraz wielomianowej dla cech o zależności nieliniowej.

W doświadczeniu 2 czynnikami doświadczalnymi była rodzaj opakowania jaj (wytłaczanki tekturowe i plastikowe) oraz temperatura magazynowania (chłodnicza i pokojowa). Taki układ doświadczalny pozwolił na przeprowadzenie symulacji zmian jakości surowca pozostającego w obrocie handlowym w warunkach zbliżonych do konsumenckich. Dodatkowym elementem było także określenie rodzaju opakowania detalicznego, które ~~daje~~ umożliwia zachowanie jakości jaj w dłuższym czasie przechowywania i obrotu handlowego. Pozaoceną jakości jaj konsumpcyjnych w pracy analizowano również zmienność profilu

kwasów tłuszczowych żółtka oraz zmiany wartości ich indeksów w zależności od czynników doświadczalnych oraz czasu przechowywania.

Drugą metodą pozwalającą na ograniczenie tempa negatywnych zmian jakościowych jaj w czasie ich przechowywania było pokrywanie skorup wodnym roztworem kwasu cytrynowego o różnym stężeniu (doświadczenie 3). Z uwagi na znaczną ilość wapnia w skorupie jaja, pod wpływem działania słabego kwasu organicznego dochodzi do reakcji, w wyniku której powstają sole tego kwasu- cytryniany. W efekcie gromadzą się one nie tylko na powierzchni skorupy, ale również w porach skorupowych ograniczając ich przepuszczalność, co prowadzi do ograniczenia wymiany gazowej między treścią jaja a środowiskiem zewnętrznym, a tym samym przyczynia się do obniżenia intensywności negatywnych zmian jakości. Obserwacje te potwierdzono dodatkowo wykonując zdjęcia przekrojów i powierzchni skorup poddanych działaniu kwasu cytrynowego z wykorzystaniem techniki skaningowej mikroskopii elektronowej (SEM).

Przeprowadzone badania pozwoliły na potwierdzenie postawionych uprzednio hipotez badawczych. Wykazano, że w przeciwieństwie do dostępnego piśmiennictwa, nie wszystkie cechy jakości jaj charakteryzują się liniowymi zmianami w czasie przechowywania, zatem wskazane jest wykorzystanie regresji wielomianowej. Dodatkowo analiza regresji wielokrotnej pozwala na wyznaczenie modelu predykcyjnego umożliwiającego wnioskowanie o „wieku jaja" na podstawie dokonanych pomiarów wybranych cech. Opakowanie ma wpływ na ograniczenie tempa zachodzących zmian, w jajach przechowywanych w wytłaczankach z tworzywa sztucznego, pogarszanie jakości zachodzi wolniej niż w przypadku jaj przechowywanych w wytłaczankach tekturowych. Intensywność tych zmian jest zbliżona do tej obserwowanej w warunkach chłodniczych dla opakowań tekturowych, zatem wytłaczanki z tworzyw sztucznych mogą być alternatywą dla przechowalnictwa chłodniczego. Zależności te potwierdzono ograniczeniem intensywności zmian profilu kwasów tłuszczowych, zwłaszcza w kontekście kwasów wielonienasyconych. Zastosowanie kwasu cytrynowego jako substancji pokrywającej skorupy jaj, również pozwala na zahamowanie procesów prowadzących do pogorszenia jakości jaj konsumpcyjnych (ograniczenie ubytku masy, dłuższe zachowanie struktury białka, spowolnienie wzrostu odczynu treści) zmieniając dynamikę tych zmian.

Słowa kluczowe: jaja kurze, przechowywanie jaj, modele statystyczne, zmiany jakości, uszczelnianie porów skorupowych

Summary

The quality of eggs and their stability during the storage must be analysed in the legislation adopted, which regulates both the quality parameters and the maximum period for table eggs trading. Importantly, it does not lay down specific storage conditions for this raw material, stipulating only that the refrigerated storage of eggs is exclusively reserved for the final consumer. However, in eggs, as in all food products, quality deteriorates with storage time. Therefore, it is necessary to provide appropriate methods to reduce the impact of time on the quality of raw egg material.

The aim of the thesis was to analyse the dynamics of changes in egg quality during storage in the context of their prediction and to develop methods to inhibit processes affecting the deterioration of table eggs. Research hypotheses were formulated that analysis of the daily variation of egg quality traits may contribute to the development of mathematical models to predict these changes as well as the time elapsed since laying, and that inhibition of the ageing process in table eggs is possible by modification of storage conditions and/or with the use of substances limiting shell pore permeability.

Three independent experiments were conducted. In the 1st one, evaluation of egg quality traits was performed daily during its extended storage up to 35 days, and the data thus obtained made it possible to create a multivariate mathematical model which, after further statistical processing, makes it possible to determine with high certainty (above 95%) the age of an egg based on its measurable traits, both non-destructive and destructive. Statistical methods based on linear regression equations (for traits characterised by linear variation) and polynomial regression equations for traits with non-linear dependence were used.

In 2nd experiment, the experimental factors were the type of eggs packaging (cardboard and plastic egg boxes) and the storage temperature (refrigerated and room temperature). Such an experimental arrangement made it possible to simulate changes in the quality of commercially traded eggs under similar conditions to those of consumers. An additional element was the determination of the type of egg box, which allows for longer preservation of egg quality during storage and trading. Apart from the evaluation of the quality of the eggs, the study also analysed the variation in the yolk fatty acid profile and changes in their index values depending on experimental factors and storage time.

A second method to reduce the progress of negative quality changes of eggs during storage was to coat the shells with an aqueous solution of citric acid at different concentrations (3rd experiment). Due to the high amount of calcium in the egg shell, a weak

organic acid causes a reaction that results in the formation of salts of this acid - citrates. As a consequence, they accumulate not only on the surface of the shell but also in the shell pores limiting their permeability, which leads to a limitation of gas exchange between the egg content and the external environment and thus contributes to a decrease in the intensity of negative quality changes. These observations were further confirmed by taking photographs of cross-sections and surfaces of shells treated with citric acid using the scanning electron microscopy (SEM) technique.

The research carried out allowed us to confirm the research hypotheses set out previously. It was shown, contrary to the available literature, that not all egg quality traits are characterised by linear changes during the storage time, so it is advisable to use polynomial regression. In addition, multiple regression analysis allows the determination of a predictive model allowing the inference of "egg age" based on measurements of selected traits. The packaging has an impact on limiting the rate of changes occurring, in eggs stored in plastic boxes, deterioration occurs more slowly than in eggs stored in cardboard ones. The intensity of these changes is similar to that observed under refrigerated conditions for cardboard boxes, so plastic boxes may be an alternative to refrigerated storage. These relationships were confirmed by reducing the intensity of changes in the fatty acid profile, especially in the context of polyunsaturated acids. The use of citric acid as a shell coating substance also allows inhibiting the processes leading to a deterioration of table eggs quality (reduction of weight loss, longer preservation of egg albumen structure, slowing down the increase of egg content pH) by changing the dynamics of these changes.

Keywords: hen eggs, egg storage, statistical models, quality changes, shell pore sealing

Wstęp

Jaja kurze, ze względu na swoje wartości odżywcze oraz relatywnie niską cenę stanowią jedno z podstawowych źródeł białka zwierzęcego w diecie człowieka. Na ich jakość oddziałuje szereg czynników związanych zarówno z prowadzeniem stada produkcyjnego, wiekiem i genotypem ptaków, czy też zastosowanymi dodatkami żywieniowymi. Jednocześnie, niezależnie od powyższych, na jaja pozostające w obrocie handlowym oddziałuje przede wszystkim czas, jaki upłynął od ich zniesienia. Wraz z wydłużeniem tego okresu dochodzi do zmian o podłożu biologicznym, chemicznym i fizycznym, które wpływają negatywnie na jakość surowca jajczarskiego.

W Unii Europejskiej, obrót jajami konsumpcyjnymi został uregulowany przez Rozporządzenie Komisji (WE) nr 589/2008 z dnia 23 czerwca 2008 r. ustanawiającym szczegółowe zasady wykonywania rozporządzenia Rady (WE) nr 1234/2007 w sprawie norm handlowych w odniesieniu do jaj. Akt ten wprowadza ograniczenia zarówno w czasie pozostawania jaj konsumpcyjnych w obrocie handlowym (28 dni), jak również określa cechy jakościowe, jakie muszą spełniać jaja sklasyfikowane jako klasa A. Niestety podczas tworzenia wspomnianego aktu prawnego pominięto znaczną większość zaleceń ujętych w poprzednio obowiązujących normach (PN-86 A-86504:1986 oraz PN-A-86503:1998) odnośnie warunków związanych z przechowywaniem jaj na etapie magazynowania, pakowania i dystrybucji. Określono jedynie konieczność ochrony surowca przed nagłymi zmianami temperatury oraz ekspozycją na warunki środowiskowe. Ograniczenie w prawodawstwie regulacji w zakresie warunków przechowywania jaj konsumpcyjnych pozostających w obrocie handlowym sprawiło, że przechowalnictwo chłodnicze (poniżej 6 °C) zostało zarezerwowane wyłącznie dla konsumentów końcowych, mimo, że liczne prace badawcze wskazują na pozytywny wpływ obniżenia temperatury na hamowanie niekorzystnych zmian jakości surowca jajczarskiego (Jin i wsp., 2011; Brodacki i wsp., 2019). Należy jednak zauważyć, ze niezależnie od warunków środowiskowych czy zastosowania metod ochronnych, zmiany jakości jaj konsumpcyjnych w czasie ich

przechowywania mają charakter naturalny i ciągły, a całkowita ich eliminacja nie jest możliwa.

Zmiany zachodzące w jajach w czasie przechowywania

Bezpośrednio po zniesieniu jaja, rozpoczyna się oddziaływanie na nie warunków zewnętrznych. Postępują także procesy biochemiczne wpływające w sposób pośredni lub bezpośredni na jakość surowca. Ogólnie rzecz biorąc zmiany związane ze „starzeniem się" surowca można podzielić na te, które dotyczą cech całego jaja, jak również poszczególnych jego elementów.

Należy zwrócić uwagę, że w jajach, jak niemal wszystkich układach biologicznych, zmiany pojedynczej cechy pociągają ze sobą przemiany rzutujące na pozostałe charakterystyki jakościowe surowca. W przypadku jaj podstawową zmianą w czasie jest ubytek jego masy. Zmienność ta ma charakter ciągły i liniowy (Ragni i wsp., 2006), a co ważniejsze, została dotychczas odnotowana niezależnie od zastosowanych zmian w zakresie temperatury przechowywania jaj (Jones i wsp., 2018; Brodacki i wsp., 2019) czy zastosowanych substancji o charakterze protekcyjnym (Oliveira i wsp., 2020). Ze względu na warstwową budowę jaja, proces parowania wody możliwy jest z uwagi na obecność porów skorupowych, które dodatkowo zabezpieczone są warstwą mucynową. Jej ochronne działanie utrzymuje się jednak wyłącznie w początkowym okresie przechowywania (Rodriguz-Navarro i wsp., 2013). Ubytek masy jaja na drodze parowania, wiąże się również z pogłębianiem komory powietrznej, która, według wspomnianego już Rozporządzenia Komisji (WE) 589/2008, stanowi jeden z elementów podlegających ocenie w zakresie jakości jaj konsumpcyjnych.

Zmiany jakości dotyczą także poszczególnych elementów treści jaja. Wraz z czasem dochodzi do ubytku masy białka na drodze zarówno parowania, jak i poprzez występującą dyfuzję z białka do żółtka (Menezes i wsp., 2012). Te zmiany pociągają za sobą kolejne, jak obniżenia wysokości białka czy spadek liczby związanych z nią jednostek Haugh'a. Wcześniejsze badania (Heath, 1977) wskazywały na to, że to właśnie ubytek masy białka jest jednym z najważniejszych czynników wpływających na zmianę jego struktury. W chwili obecnej równie istotna rolę przypisuje się

alkalizacji białka na drodze uwalniania dwutlenku węgla w wyniku dysocjacji kwasu węglowego (Monira i wsp., 2003), co z kolei prowadzi do rozluźnienia wiązań w obrębie kompleksu owomucyna-lizozym, który jest jednym z czynników odpowiedzialnych za utrzymanie właściwej struktury białka gęstego. Badania są zgodne co do roli czasu w zachodzeniu tych zmian, jak również dowodzą, że obniżenie temperatury przechowalnictwa może skutecznie zmniejszać ich intensywność (Jin i wsp., 2011; Akter i wsp., 2014).

Wraz z czasem zmianom ulegają również cechy jakości żółtka. Przede wszystkim dochodzi do wzrostu jego masy, ze względu na opisany już ruch wody między białkiem i żółtkiem. Zmiany te pociągają za sobą następne, a więc obniżenie wartości indeksu kształtu (Li i wsp., 2017). Finalnie, w efekcie rosnącej objętości kuli żółtkowej może dochodzić do przerwania błony witelinowej i wymieszania elementów morfologicznych treści, pozbawiając w ten sposób jaja przydatności technologicznej.

Poza zamianami cech wpływających na wartość technologiczną jaj, zmianom w czasie przechowywania może ulegać także ich skład chemiczny. Z uwagi na fakt, że żółtko jaja stanowi cenne źródło wielonienasyconych kwasów tłuszczowych (UFA), szczególnie istotnym w tym zakresie zagadnieniem jest peroksydacja lipidów w czasie przechowywania. Wysoka zawartość UFA w żółtku sprawia, że jest ono tym bardziej narażone na utlenianie (Hayat i wsp. 2010).

Metody ograniczania zmian jakości jaj konsumpcyjnych

Jako, że obniżenie temperatury przechowywania jaj konsumpcyjnych zostało mocno ograniczone obowiązującym ustawodawstwem poszukuje się metod alternatywnych, które umożliwiłyby jak najdłuższe zachowanie właściwych cech jakościowych oraz przydatności technologicznej surowca. Zasadniczo można podzielić je na dwie grupy obejmujące modyfikację atmosfery oraz sposób pakowania, a także pokrywanie skorup jaj substancjami pozwalającymi na obniżenie intensywności parowania wody przez pory skorupowe.

Prowadzone dotychczas prace badawcze w tym zakresie wykazały skuteczność takich rozwiązań jak pakowanie próżniowe (Aygun i Sert, 2013), czy wykorzystanie atmosfery modyfikowanej (Rocculi i wsp., 2009; Jia i wsp. 2019). Pakowanie

żywności w warunkach atmosfery modyfikowanej jest szeroko rozpowszechnione w przemyśle spożywczym, jednak w odniesieniu do jaj konsumpcyjnych pozostaje wyłącznie w sferze badań naukowych. Niemniej jednak doniesienia na ten temat wskazują, że wzbogacenie atmosfery w dwutlenek węgla (50% CO_2: 50%N_2) pozwala na ograniczenie ubytku masy jaj w czasie ich przechowywania, jak również zapewnia utrzymanie właściwej struktury białka gęstego. Warto wspomnieć również, że badania Pasquali i wsp. (2012) wykazały, że pakowanie jaj w atmosferze zawierającej 100% dwutlenku węgla przyczyniło się do redukcji liczebności bakterii tlenowych na powierzchni skorupy, przy jednoczesnym braku rozwoju mikroorganizmów innych grup, co może przekładać się na zwiększone bezpieczeństwo konsumentów finalnych.

Poza zmianą składu atmosfery w otoczeniu jaj, inną metodą jest pokrywanie skorup jaj substancjami, mającymi na celu uszczelnienie porów skorupowych. Metoda ta pozwala w znacznym stopniu ograniczyć ubytek wody z jaj, a w szerszej perspektywie, również hamować zmiany pozostałych elementów morfologicznych jaja na które wspominany ubytek wody wpływa w sposób istotny. Dotychczas wykorzystywano szereg substancji (Tabela 1), które mogą obniżać tempo negatywnych zmian jakości jaj konsumpcyjnych w czasie ich przechowywania.

Mimo że wykorzystywane dotychczas substancje mają zupełnie różną budowę i właściwości chemiczne, ich zastosowanie sprowadza się do ograniczenia przepuszczalności porów skorupowych poprzez ich uszczelnienie. Co oczywiste, w efekcie finalnym obserwuje się ograniczenie ubytku masy oraz zmniejszone tempo pogłębiania się komory powietrznej (Nongtaodum i wsp., 2013, Drabik i wsp., 2018b). Dodatkowo, brak możliwości uwalniania CO_2 z treści jaja hamuje proces alkalizacji, obserwowany jako niższe wartości pH białka (Torrico i wsp., 2011) i żółtka (Pires i wsp., 2019). Co równie istotne w większość prac, w których wykorzystano substancje powlekające skorupę, wskazuje się na zahamowanie obniżania się wartości indeksu żółtka. Efekt ten sugeruje nie tylko ograniczenie ubytku wody z jaj, ale również minimalizację jej ruchów między białkiem i żółtkiem.

Tabela 1. Wykaz substancji wykorzystywanych w pokrywaniu skorup jaj

Pochodzenie	Substancja pokrywająca	Piśmiennictwo
Odzwierzęce	chitosan	Caner, 2005; Kim i wsp., 2009, Jo i wsp., 2011
	propolis	Copur i wsp., 2008; Akpinar i wsp., 2015
	białka serwatkowe	Caner 2005
	żelatyna	Al-Hajo i wsp., 2012
Roślinne	oleje roślinne	Eke i wsp.,2013; Nongtaodum i wsp., 2013;Ryu i wsp., 2011
	izolaty białkowe np. z ryżu	Pires i wsp., 2019
	soki i wyciągi roślinne	Drabik i wsp., 2018a
Inne	glicerol	Nongtaodum i wsp., 2013, Drabik i wsp., 2018b
	oleje mineralne	Jirangrat i wsp., 2010; Ryu i wsp., 2011
	emulsje z chitozanem	Torrico i wsp., 2011, Wardy i wsp., 2011,

Hipoteza badawcza i cel pracy

Hipotezy badawcze

- Analiza zmienności dziennej cech jakości surowca jajczarskiego daje możliwość tworzenia modeli matematycznych pozwalających na prognozowanie tych zmian, jak również czasu, jaki upłynął od momentu zniesienia jaja
- Możliwe jest hamowanie procesu starzenia surowca na drodze modyfikacji warunków przechowywania i/lub z wykorzystaniem substancji ograniczających przepuszczalność porów skorupowych

Cel pracy

Celem pracy była analiza dynamiki zmian jakości jaj konsumpcyjnych w czasie przechowywania w kontekście możliwości ich predykcji oraz opracowanie metod hamowania procesów wpływających na obniżenie jakości jaj konsumpcyjnych.

Materiał i metody

Dla przejrzystości zastosowano umowny podział wyników zgodnie z pracami, w których zostały opublikowane (Tabela 2).

Tabela 2. Prace, w których opublikowano prezentowane wyniki badań

	Dane bibliograficzne
Praca 1	**Drabik K.,** Próchniak T., Kasperek K., Batkowska J. (2021) The use of the dynamics of changes in table eggs during storage to predict the age of eggs based on selected quality traits. *Animals*, 11(11):3192. DOI: 10.3390/ani11113192
Praca 2	**Drabik K.**, Próchniak T., Spustek D., Wengerska K., Batkowska J. (2021) The impact of package type and temperature on the changes in quality and fatty acids profile of table eggs during their storage. *Foods*; 10(9): 2047. DOI: 10.3390/foods10092047
Praca 3	**Drabik K.**, Batkowska J., Próchniak T., Horecka B. (2021). Citric acid as a factor limiting changes in the quality of table eggs during their storage. *Poultry Science*, 100(4), 100995, DOI: 10.1016/j.psj.2021.01.018

Materiał do badań stanowiło w sumie 2470 konsumpcyjnych jaj kurzych, pochodzących od ptaków utrzymywanych w systemie klatkowym. Jaja zakupiono bezpośrednio z fermy produkcyjnej w dniu ich zniesienia, z zachowaniem pochodzenia od jednego stada ptaków (z jednego budynku), a następnie oznakowano indywidualnie, zważono i podzielono na odpowiednie grupy doświadczalne (Tabela 3).

Tabela 3. Ogólna charakterystyka układów doświadczalnych w dysertacji

	Praca 1		**Praca 2**				**Praca 3**		
Czynnik*	-		Rodzaj opakowania i temperatura przechowywania				brak (CA0) ***n=260***	roztwór kwasu cytrynowego 10% (CA10) ***n=260***	roztwór kwasu cytrynowego 15% (CA15) ***n=260***
Klasa wagowa	M ***n=365***	L ***n=365***	L				M		
Rodzaj wytłaczanki	tekturowa		tekturowa (PT) ***n=240***	z tworzywa sztucznego (PP) ***n=240***	tekturowa (CT) ***n=240***	z tworzywa sztucznego (CP) ***n=240***	tekturowa		
Temperatura przechowywania	14 °C		24 °C		5 °C		14 °C		
Wilgotność	70%		-		-		70%		
Czas przechowywania	35 dni		42 dni				28 dni		
Częstotliwość pomiarów	Codziennie		Co 14 dni				Co 7 dni		
Analizy dodatkowe	-		profil kwasów tłuszczowych, indeksy kwasów tłuszczowych				analiza ultrastruktury skorupy z wykorzystaniem skaningowej mikroskopii elektronowej (SEM)		
Razem (***N***)	**730**		**960**				**780**		

* Czynnik czasu, który występował we wszystkich pracach został pominięty w tabeli

Dla wszystkich jaj objętych badaniami ujednolicono sprzęt oraz metody analityczne. Do badań jakości wykorzystano aparat obciążeniowy Instron Mini 55 oraz zestaw analityczny EQM (Egg Quality Measurement, TSS®). Analizie poddano następujące cechy całego jaja:

- indeks kształtu jaja (**IJ**, jako stosunek jego szerokości i długości, przy użyciu suwmiarki elektronicznej),
- głębokość komory powietrznej (**GKP**, po prześwietleniu owoskopem, według skali),
- masa jaja (**MJ**, przy użyciu wagi laboratoryjnej o dokładności 0,01 g),
- ciężar właściwy jaja (**MWJ**, na podstawie pomiaru masy jaja w powietrzu i w wodzie, zgodnie z zasadą Archimedesa),
- proporcje poszczególnych elementów jaja (jako stosunek ich masy do masy całego jaja).

W przypadku jakości skorupy jaj oceniano następujące cechy tych elementów:

- kolor (**KS**, jako procent odbitego światła),
- masa (**MS**, przy użyciu wagi laboratoryjnej o dokładności 0,01 g),
- grubość (**GS**, za pomocą śruby mikrometrycznej, na "równiku"),
- gęstość (**GSS**, obliczona na podstawie powierzchni skorupy i objętości, wg Shafey, 2002),
- przepuszczalność (**SP**, według wzoru zaproponowanego przez Ar i wsp., 1974).

W przypadku treści jaja mierzono:

- wysokość białka gęstego (**WB**),
- na podstawie której obliczano jednostki Haugha (**HU**),
- masę żółtka (**MŻ**, przy użyciu wagi laboratoryjnej o dokładności 0,01 g),
- barwę żółtka (**KŻ**, przy użyciu 16-punktowej skali Roche, DSM®) oraz indeks żółtka (**IŻ**, jako stosunek wysokości i średnicy).

Pomiar pH białka (**pHB**) i pH żółtka (**pHŻ**) wykonano przy użyciu pH-metru z kombinowaną elektrodą szklaną.

Analiza profilu kwasów tłuszczowych

W **pracy 2** po 28 dniach przechowywania pobrano, a następnie zliofilizowano po 10 prób żółtek z każdej badanej grupy.

Profil kwasów tłuszczowych w żółtkach jaj analizowano metodą chromatografii gazowej zgodnie z normami PN-EN ISO 5508: 1996 i PN-EN ISO 5509: 2001. Do analizy użyto chromatografu gazowego Varian 450-GC z detektorem płomieniowo-jonizacyjnym (FID). Na podstawie udziału poszczególnych kwasów tłuszczowych i ich grup obliczono następujące wskaźniki:

- PI - indeks peroksydacyjności (Arakawa i Sagai, 1986),
- AI - indeks patogenności (Ulbricht i Southgate, 1991)
- TI - indeks trombogenności (Ulbricht i Southgate, 1991),
- DFA - pożądane kwasy tłuszczowe (Medeiros i wsp., 2014),
- HFSA - hipercholesterolemiczne nasycone kwasy tłuszczowe (Renna i wsp., 2012)
- h/H - stosunek hipocholesterolemiczny/ hipercholesterolemiczny (Domaradzki i wsp., 2019).

Skaningowa mikroskopia elektronowa

W **pracy 3** po 28 dniach przechowywania, z 4 jaj z każdej grupy pobrano próby skorup w celu określenia wpływu zastosowanego kwasu cytrynowego na możliwe zmiany ich ultrastruktury. Zostały one poddane analizie mikroskopowej przy użyciu skaningowego mikroskopu elektronowego (SEM). Zdjęcia przekrojów poprzecznych wykonano przy użyciu skaningowego mikroskopu elektronowego FEI QUANTA 200 SEM (Hillsboro, OR).

Analizy statystyczne

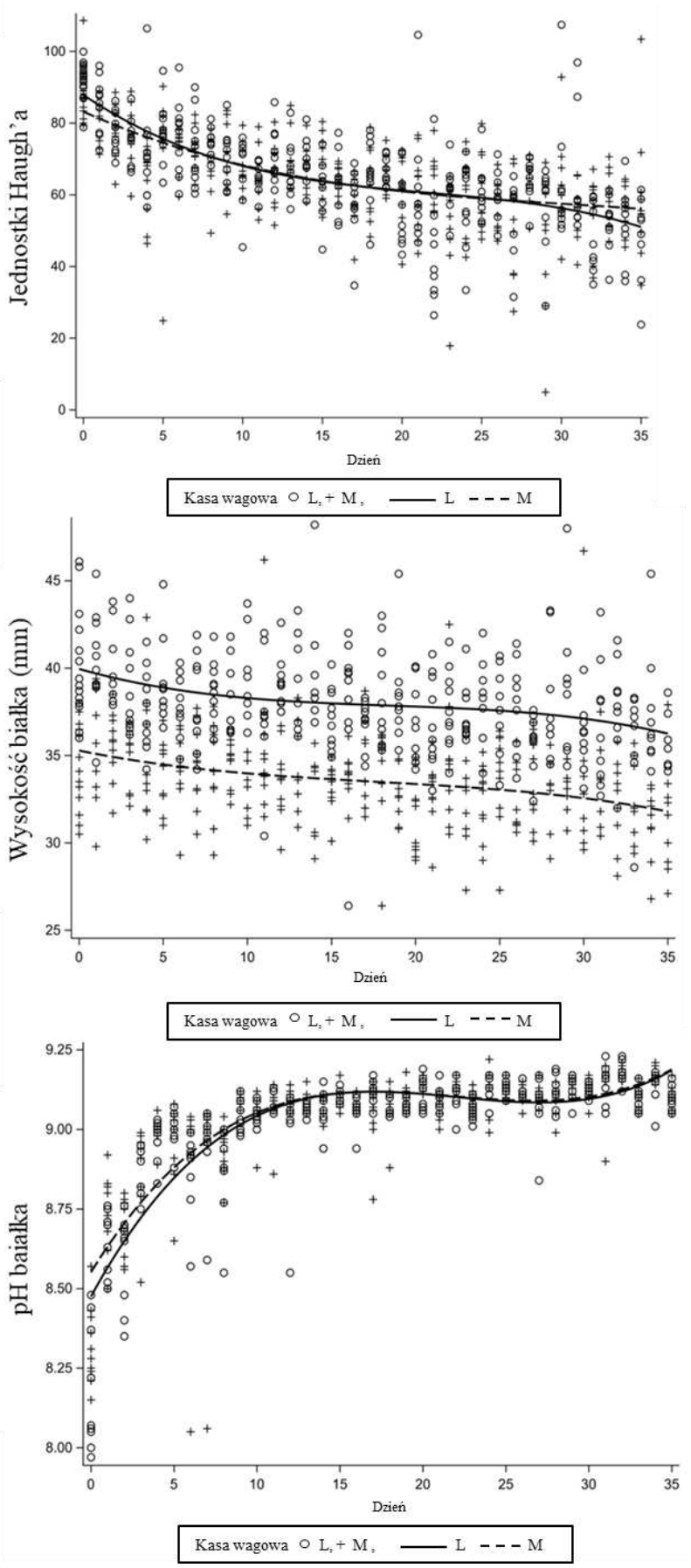
W **pracy 1** zastosowano następujące procedury: CORR, REG, TTEST, GLM w oprogramowaniu SAS (Statistical Analysis System ver. 9.4, 2013). Wpływ czasu przechowywania na wartości cech przedstawiono w postaci korelacji rang Spearmana oraz równań regresji. Zależność pomiędzy czasem przechowywania, masą jaja a masą właściwą jaja analizowano modelami liniowymi regresji, natomiast w przypadku

jednostek Haugh'a, masy białka, wielkości komory powietrznej, indeksu żółtka oraz pH białka i żółtka zastosowano wielomiany zwyczajne trzeciego stopnia. Do prognozowania dnia przechowywania jaja użyto wartości masy właściwej jaja, pH żółtka i białka, indeksu żółtka, jednostek Haugh'a oraz głębokości komory powietrznej w liniowym modelu regresji. Dopasowanie modelu, przy kolejno dodawanych cechach, przedstawiono za pomocą współczynnika R^2 oraz statystyki Mallow'a C(p). Weryfikację modeli regresji przeprowadzono poprzez oszacowanie wartości cech, a następnie porównanie oszacowanych wartości z wartościami oczekiwanymi za pomocą testu t-Studenta dla zmiennych łączonych.

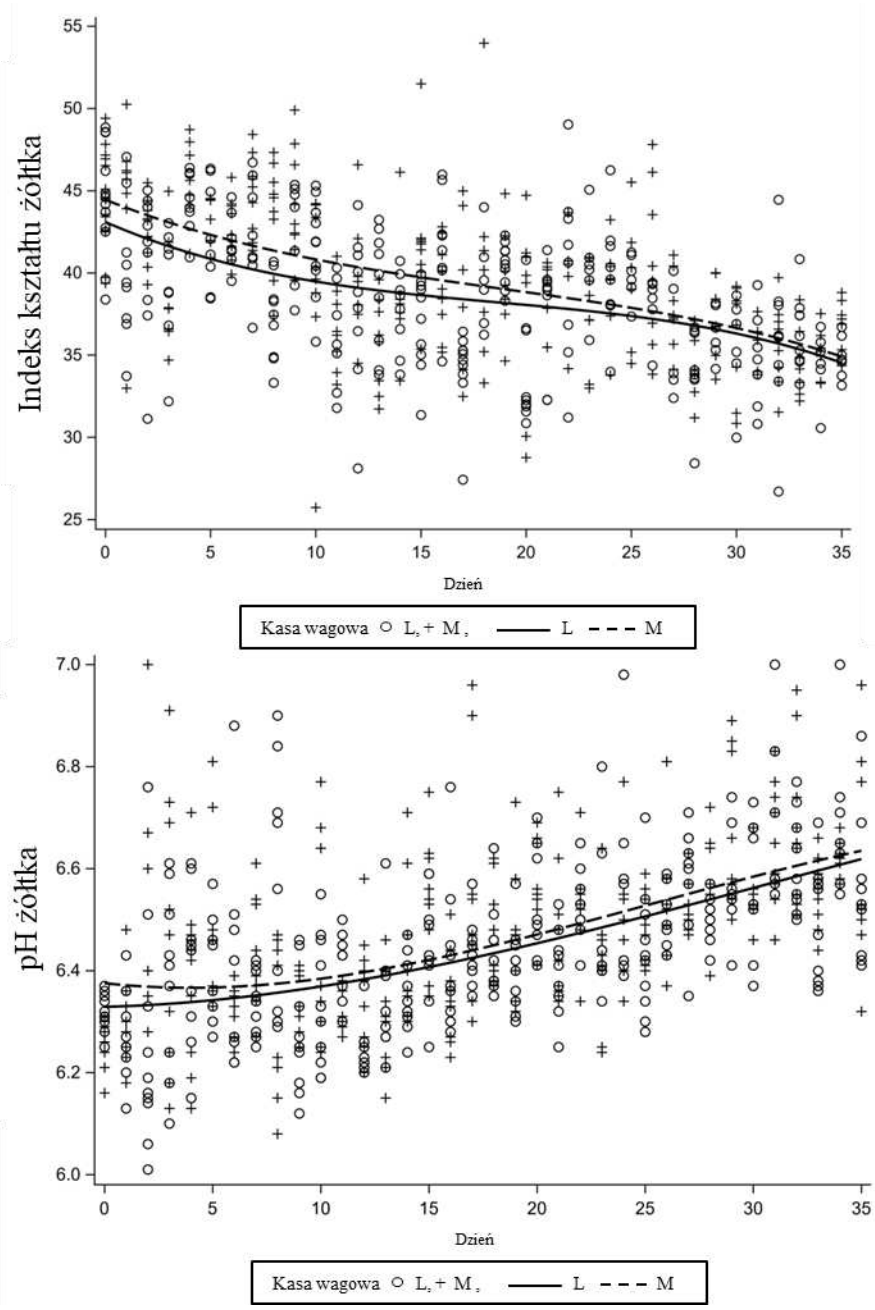
Dane z **prac 2** i **3** poddano analizie statystycznej z wykorzystaniem pakietu statystycznego SPSS 24.0. Do oceny normalności rozkładu cech wykorzystano test Shapiro-Wilka. Następnie zastosowano dwuczynnikowy model analizy wariancji, uwzględniający pojedyncze zmienne oraz interakcję między nimi. Porównania grup wykonano przy użyciu testu porównań wielokrotnych Tukey'a. W **pracy 2** czynnikami wpływającymi na parametry jakościowe jaj były rodzaj opakowania i temperatura przechowywania. Natomiast w **pracy 3** czynnikami modelu był czas przechowywania i powlekanie jaj kwasem cytrynowym.

Wyniki i dyskusja

Analiza dynamiki zmian jakości surowca jajczarskiego potwierdziła opisywane dotychczas w literaturze zmiany jaj w czasie ich przechowywania (Brodacki i wsp., 2019, Pires i wsp., 2019) takie jak ubytek masy i masy właściwej, zmniejszenie liczby jednostek Haugh'a, wzrost pH białka i żółtka etc. Istotnym w tym zakresie wynikiem jest natomiast fakt, że wśród wszystkich badanych cech zaledwie dwie (masa i masa właściwa) charakteryzują się zmiennością liniową. Wyznaczone równania regresji wielomianowej zilustrowane na rycinach 1. i 2. wskazują, że zmienność cech białka (WB, HU, pHB) i żółtka (IŻ, pHŻ) ma charakter nieliniowy. Stwierdzenie braku zmienności liniowej ma w tym zakresie kluczowe znaczenie, gdyż dotychczasowe wyniki badań (Ragni i wsp., 2006; Soltani i wsp., 2015), mimo stosowania zaawansowanych narzędzi, upraszczały podobne modele do regresji liniowej, co finalnie mogło prowadzić do błędów w prognozowaniu zmienności cech jakości jaj w czasie ich przechowywania.



Ryc. 1. Zmiany cech jakości białka wraz z czasem przechowywania jaj



Ryc. 2. Zmiany cech jakości żółtka wraz z czasem przechowywania

Najważniejszym wynikiem w tym zakresie jest stworzenie modelu statystycznego opartego o podstawowe cechy jakości jaj, które nie wymagają wykorzystania specjalistycznych narzędzi diagnostycznych, w celu predykcji czasu jaki upłynął od zniesienia jaja. Z uwagi na wykorzystanie obróbki danych analizy regresji krokowej (ang. *Stepwise analyses*), możliwe stało się dobranie cech mających

najwyższe znaczenie predykcyjne, przy jednoczesnym ograniczeniu ryzyka błędu przeszacowania modelu (Tabela 4).

Tabela 4. Model statystyczny stosowany do przewidywania wieku jaj (w dniach)

Etap	Zmienna	Częściowy R^2	R^2 modelu	C(p)	β	Pr > |t|
Intercept (β_0)					17,90251	0,5232
1	MW	0,5176	0,5176	382,072	-152,42621	<,0001
2	pHB	0,1096	0,6272	175,732	10,63105	<,0001
3	pHŻ	0,0447	0,6719	92,7676	12,82286	<,0001
4	IŻ	0,0206	0,6926	55,5100	-0,33684	<,0001
5	HU	0,0182	0,7108	22,8532	-0,12071	<,0001
6	GKP	0,0094	0,7202	7,00	1,11087	<,0001
Test t-studenta (dla zmiennych połączonych) dla dzień vs. oszacowany dzień: średnia różnica = -0,00031; SD= 5,49; Pr. > |t| = 0,999						

Zdając sobie sprawę z istotnego miejsca jaj w diecie człowieka, zbadano również możliwości hamowania zmian jakości jaj konsumpcyjnych na drodze doboru właściwego opakowania detalicznego oraz temperatury przechowywania (**Praca 2**). Stwierdzono najlepsze efekty w przypadku przechowalnictwa w temperaturze chłodniczej, przy czym, zaobserwowane różnice w przypadku wydłużonego czasu przechowywania w wytłaczankach z tworzyw sztucznych pozostają na zbliżonym poziomie do tych, które odnotowano dla klasycznych tekturowych opakowań i obniżonej temperatury (Tabela 5).

Stwierdzono również zmniejszenie tempa pogłębiania komory powietrznej, co więcej, w przypadku tego czynnika stwierdzono istotność zarówno czasu, rodzaju opakowania, jak i interakcji wskazanych czynników. Należy w tym miejscu zauważyć, że nawet przy znacznie wydłużonym czasie przechowywania (42 dni), głębokość komory powietrznej przekroczyła wartości graniczne dla jaj konsumpcyjnych klasy A określone przez Rozporządzenie Komisji (WE) 589/2008 (6 mm).

Z pobranych w czasie analiz prób żółtek, wyznaczono skład kwasów tłuszczowych (Tabela 6) oraz określono ich indeksy (Tabela 7). Wśród PUFA, stwierdzono, że wraz z czasem przechowywania zmianie uległa zawartość 3

wielonienasyconych kwasów tłuszczowych (PUFA, *polyunsaturated fatty acids)*. Wśród nich kwas arachidonowy (C20:4 *n6*) oraz kwas dokozaheksaenowy (DHA, C22:6 *n3*) pozostawały na istotnie najwyższym poziomie w żółtkach jaja przechowywanych w warunkach chłodniczych w opakowaniach plastikowych w porównaniu z pozostałymi grupami doświadczalnymi.

W zakresie indeksów kwasów tłuszczowych istotne różnice zaobserwowano jedynie w przypadku grupy kwasów *n3*. Najwyższą ich zawartość odnotowano w żółtkach jaj przechowywanych chłodniczo (CT and CP). Uzyskane dla tych grup wartości nie różniły się istotnie od tych uzyskanych dla jaj świeżych (czas 0).

Z uwagi na fakt, że żółtko jaja stanowi cenne źródło wielonienasyconych kwasów tłuszczowych, szczególnie istotnym w tym zakresie zagadnieniem jest peroksydacja lipidów w czasie przechowywania. Wysoka zawartość UFA w żółtku sprawia, ze jest ono tym bardziej narażone na utlenianie (Hayat i wsp. 2010). Mając na uwadze powyższe konieczne jest zapewnienie możliwie jak najwyższej stabilności oksydacyjnej pozyskiwanych jaj. Wiele prac skupia się w tym zakresie na stosowaniu dodatków paszowych o charakterze antyoksydacyjnym jak: aldehyd cynamonowy (Cimrin i wsp., 2020) czy wyciągów roślinnych z tymianku czy oregano (Cimrin i wsp., 2019). W badaniach własnych nie wykorzystywano podobnych dodatków, a materiał badawczy został ujednolicony, zatem obserwowana zmienność wynikała wyłącznie z zastosowanych metod przechowalnictwa.

Tabela 5. Zmiany cech całego jaja w zależności od sposobu pakowania oraz temperatury przechowywania

Cecha	Czas (dni)	grupa				Razem	*SEM*	Czynnik		
		PT	PP	CT	CP			W	T	W×T
Masa jaja (g)	0	63,29^{a}	63,36^{a}	63,30^{a}	64,83^{b}	63,66	*0,159*			
	14	62,36^{a}	64,10^{ab}	64,11^{bc}	64,49^{a}	63,76	*0,268*	*	*	-
	28	61,56^{a}	61,85^{a}	62,34^{a}	64,31^{b}	62,51	*0,263*	*	*	-
	42	59,95^{ab}	59,16^{a}	60,71^{a}	64,08^{a}	60,92	*0,297*	*	*	*
masa właściwa (g/cm^{3})	0	1,079					*0,002*			
	14	1,059^{a}	1,072^{b}	1,081^{c}	1,081^{c}	1,073	*0,001*	*	*	*
	28	1,078	1,054	1,072	1,076	1,070	*0,011*	*	-	-
	42	1,019^{a}	1,043^{b}	1,067^{c}	1,070^{c}	1,049	*0,003*	*	*	*
ubytek masy jaja (%)	14	4,37^{c}	3,27^{b}	2,61^{a}	2,22^{a}	3,15	*0,112*	*	*	*
	28	6,56^{c}	4,33^{b}	3,00^{a}	2,87^{a}	4,15	*0,215*	*	*	*
	42	8,47^{c}	5,39^{b}	3,25^{a}	3,41^{a}	5,22	*0,279*	*	*	*
Przewodność wodna skorupy (mg/d/torr)	14	2,28^{c}	1,73^{b}	1,37^{a}	1,17^{a}	1,64	*0,057*	*	*	*
	28	1,73^{c}	1,08^{b}	0,77^{a}	0,75^{a}	1,07	*0,056*	*	*	*
	42	1,48^{c}	0,91^{b}	0,54^{a}	0,60^{a}	0,90	*0,049*	*	*	*
Głębokość komory powietrznej (mm)	14	3,83^{c}	2,80^{b}	2,10^{a}	2,23^{ab}	2,74	*0,109*	*	*	*
	28	5,20^{c}	3,98^{b}	2,80^{a}	2,55^{a}	3,63	*0,159*	*	*	*
	42	6,33^{b}	4,52^{a}	4,20^{a}	3,40^{a}	4,63	*0,206*	*	*	-

$^{a, b, c}$ – średnie oznaczone różnymi literami różnią się istotnie przy p≤0,05, *- istotność wpływu czynnika

PT – opakowanie tekturowe, temp. pokojowa, PP - opakowanie plastikowe, temp. pokojowa, CT - opakowanie tekturowe, temp. chłodnicza, CP - - opakowanie plastikowe, temp. chłodnicza; W – rodzaj wytłaczanki, T – temperatura

Tabela 6. Zmiany wybranych wielonienasyconych kwasów tłuszczowych (PUFA) w czasie przechowywania w zależności od temperatury i rodzaju opakowania

Czas (dni)	0	28				Razem	*SEM*	Czynnik		
Grupa		PT	PP	CT	CP			W	T	W×T
C18:2 *n6c* + C18:2 *n6t*	20,608	24,066	17,280	17,166	20,016	19,827	*1,005*	-	-	*
C18:3 *n6*γ	0,078	4,250	0,092	0,078	0,090	0,918	*0,832*	-	-	-
C18:3 *n6* α	0,610^{ab}	0,360^{a}	0,804^{b}	0,728^{ab}	0,596^{ab}	0,620	*0,049*	-	-	*
C20:2 *n6*	0,232	0,150	0,146	0,144	0,194	0,173	*0,012*	-	-	-
C20:3 *n6*	0,102	0,064	0,106	0,094	0,112	0,096	*0,008*	*		-
c20:4 *n6*	1,412^{ab}	0,938^{a}	1,258^{ab}	1,292^{ab}	1,494^{b}	1,279	*0,064*	*	*	-
C20:3 *n3*	0,008	0,004	0,012	0,008	0,004	0,007	*0,002*	-	-	-
C22:2 *n6*	0,040	0,046	0,054	0,060	0,058	0,052	*0,004*	-	-	-
C22:6 *n3*	0,000^{a}	0,024^{ab}	0,164^{ab}	0,182^{ab}	0,350^{b}	0,144	*0,041*	-	-	-

$^{a, b, c}$ – średnie oznaczone różnymi literami różnią się istotnie przy p≤0,05, *- istotność wpływu czynnika

PT – opakowanie tekturowe, temp. pokojowa, PP - opakowanie plastikowe, temp. pokojowa, CT - opakowanie tekturowe, temp. chłodnicza, CP - - opakowanie plastikowe, temp. chłodnicza; W – rodzaj wytłaczanki, T - temperatura

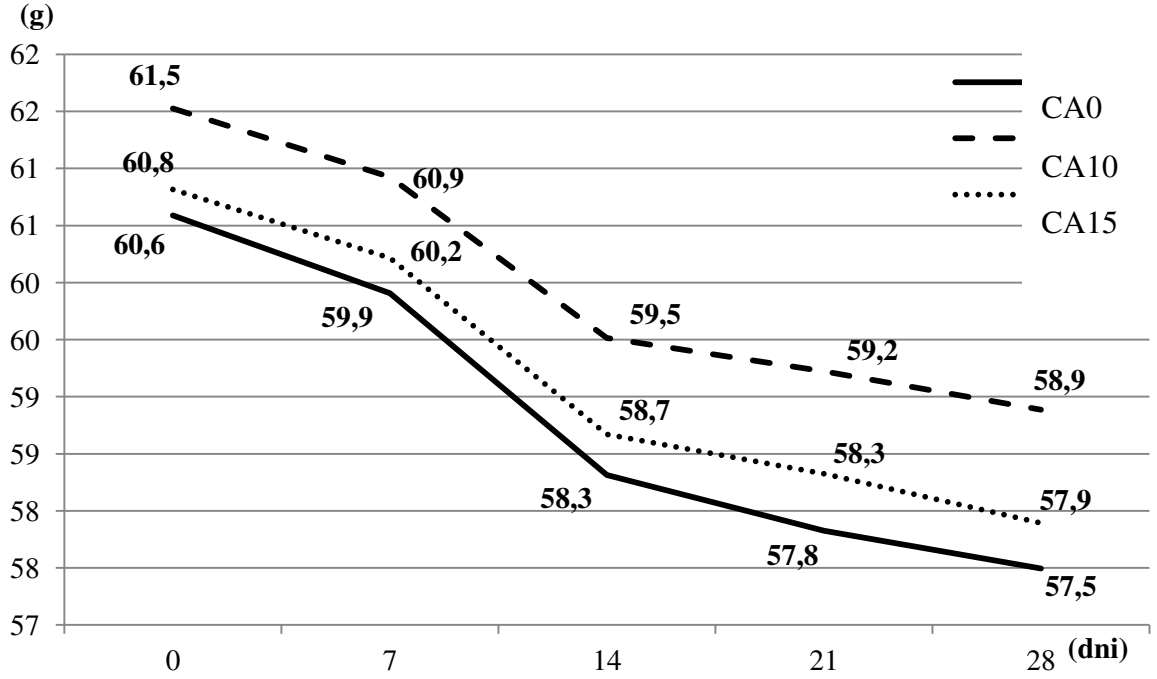
Tabela 7. Indeksy kwasów tłuszczowych w żółtku jaja w zależności od sposobu pakowania oraz temperatury przechowywania

Czas (dni)	0	28				Razem	*SEM*	Czynnik		
Grupa		PT	PP	CT	CP			W	T	W×T
SFA	30,680	31,386	30,630	29,588	29,762	30,409	*0,287*	-	-	-
MUFA	42,154	42,662	44,584	45,942	43,118	43,692	*0,530*	-	-	-
PUFA	23,090	22,114	19,916	19,752	22,914	21,557	*0,594*	-	-	-
n3	0,618^{ab}	0,514^{a}	0,980^{b}	0,918^{ab}	0,950^{b}	0,796	*0,056*	*	-	-
n6	22,472	21,600	18,936	18,834	21,964	20,761	*0,590*	-	-	-
n9	39,182	39,690	40,652	42,592	40,404	40,504	*0,497*	-	-	-
PI	29,178	38,570	26,967	26,959	31,726	30,680	*2,496*	-	-	-
AI	0,374	0,319	0,387	0,360	0,358	0,359	*0,016*	-	*	-
TI	0,880	0,747	0,864	0,824	0,823	0,827	*0,037*	-	*	-
DFA	71,968	70,184	71,024	72,394	72,838	71,682	*0,344*	-	*	-
HSFA	23,626	19,646	23,860	22,642	22,692	22,493	*0,968*	-	*	-
H/h	2,623	2,495	2,533	2,732	2,761	2,634	*0,040*	-	*	-

$^{a, b, c}$ – średnie oznaczone różnymi literami różnią się istotnie przy p≤0,05, *- istotność wpływu czynnika

PT – opakowanie tekturowe, temp. pokojowa, PP - opakowanie plastikowe, temp. pokojowa, CT - opakowanie tekturowe, temp. chłodnicza, CP - - opakowanie plastikowe, temp. chłodnicza; W – rodzaj wytłaczanki, T - temperatura

Mając na uwadze, że podstawową i najbardziej widoczną zmianą surowca jajczarskiego w czasie jego przechowywania jest ubytek masy, stwierdzono, że wykorzystanie kwasu cytrynowego jako protektanta pozwala na zahamowanie tego procesu (Wykres 1).



Wykres 1 Zmiany masy jaj w czasie w zależności od zastosowanego stężenia kwasu

Jako że zmniejszenie masy jaja pociąga za sobą kolejne zmiany w ogólnej charakterystyce jakości jaja, stwierdzono także istotny wpływ kwasu cytrynowego na masę właściwą i głębokość komory powietrznej w czasie (Tabela 8). Podobne obserwacje można znaleźć w dostępnej literaturze, jednak dotyczą one innych substancji uszczelniających pory skorupowe (Drabik i wsp., 2018b; Pires i wsp.; 2019; Pires i wsp., 2020). Zasadnicza różnica w tym zakresie polega na samym mechanizmie zachodzącej reakcji. W badaniach własnych uszczelnienie porów skorupowych nastąpiło na skutek reakcji między budującym skorupę wapniem, a zastosowanym kwasem. W efekcie uzyskane sole kwasu cytrynowego dostając się do porów skorupowych znacząco ograniczyły możliwość wymiany gazowej między treścią jaja a środowiskiem zewnętrznym. Jednocześnie wyniki badań w zakresie jakości treści jaja nie wskazały bezpośredniego oddziaływania zastosowanego kwasu cytrynowego, co potwierdza brak zmian pH białka i żółtka. Można zatem przypuszczać, że działanie

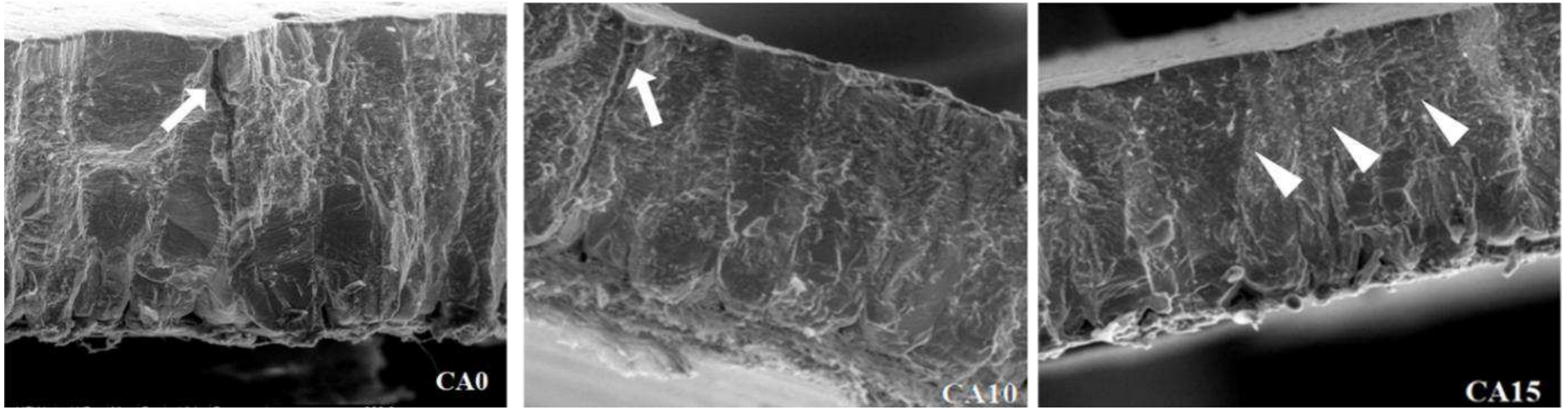
kwasu skupiło się wyłącznie na powierzchni skorupy, nie przenikając do wnętrza jaja. Obserwacje te zostały potwierdzone za pomocą zdjęć mikroskopowych (Ryc. 3), na których zaznaczono obszary zmienione na skutek działania czynnika doświadczalnego. W przypadku skorup jaja z grupy CA0 zaobserwowano otwarte pory skorupowe, które pod wpływem działania 10% kwasu cytrynowego uległy zasklepieniu (CA10). Zastosowanie wyższej dawki kwasu spowodowało silniejsze oddziaływania na skorupę jaj, co wykazały badania mikroskopowe, które wskazały na wzrost integralności skorupy.

Tabela 8. Najważniejsze zmiany jakości cech całego jaja w zależności od zastosowanego stężenia kwasu cytrynowego

Cecha	Czas (dni)	Grupa			*SEM*	Czynnik		
		CA0	CA10	CA15		CA	T	CA×T
Masa właściwa (g/cm^3)	0	1.088			*0.004*	*	*	-
	7	1.071^{a}	1.079^{b}	1.079^{b}	*0.001*			
	14	1.069	1.073	1.074	*0.001*			
	21	1.058^{a}	1.066^{b}	1.070^{b}	*0.001*			
	28	1.054	1.055	1.052	*0.001*			
Ubytek masy (%)	0-7	1.085	1.019	0.991	*0.026*	*	*	*
	7-14	2.774^{b}	2.247^{a}	2.559^{ab}	*0.075*			
	14-21	1.012^{b}	0.512^{a}	0.477^{a}	*0.069*			
	21-28	0.952^{b}	0.558^{a}	0.625^{a}	*0.033*			
	0-28	5.703^{b}	4.278^{a}	4.551^{a}	*0.126*			
Głębokość komory powietrznej (mm)	7	2.683^{c}	1.450^{a}	1.733^{b}	*0.086*	*	*	*
	14	4.093^{b}	2.383^{a}	2.517^{a}	*0.115*			
	21	4.567^{b}	3.650^{a}	3.383^{a}	*0.102*			
	28	4.700	4.400	4.550	*0.082*			

a, b – średnie różnią się istotnie przy $p \leq 0.05$, *- istotność wpływu czynnika

CA0- grupa kontrolna, CA10- kwas cytrynowy 10%, CA 15- kwasy cytrynowy 15%



Ryc. 3. Obraz mikroskopowy (SEM) przekroju poprzecznego skorup poddanych działaniu kwasu cytrynowego. CA0- grupa kontrolna, CA10- kwas cytrynowy 10%, CA 15- kwasy cytrynowy 15%

Wnioski

1. W przeciwieństwie do dostępnych w piśmiennictwie prac badawczych, badania własne wykazały, że tylko dwie z analizowanych cech jakości jaj konsumpcyjnych (masa i masa właściwa) charakteryzują się zmiennością liniową w czasie przechowywania, dla pozostałych konieczne jest wykorzystanie regresji wielomianowej.
2. Na podstawie masy właściwej jaja, głębokości komory powietrznej, liczby jednostek Haugh'a, indeksu żółtka a także pH białka i żółtka przy zastosowaniu równań regresji (prostej i wielomianowej) można z dużą dokładnością oszacować liczbę dni od momentu jego zniesienia.
3. Na ograniczenie tempa zachodzących zmian ma wpływ opakowanie detaliczne. Stwierdzono, że dla jaj przechowywanych w wytłaczankach z tworzywa sztucznego, pogarszanie jakości surowca zachodzi wolniej niż w przypadku tych przechowywanych w wytłaczankach tekturowych. Intensywność tych zmian jest zbliżona do tej obserwowanej w warunkach chłodniczych dla opakowań tekturowych, zatem wytłaczanki z tworzyw sztucznych mogą być alternatywą dla przechowalnictwa chłodniczego.
4. Przechowalnictwo w opakowaniach z tworzyw sztucznych wpływa również na zmniejszenie intensywności zmian profilu kwasów tłuszczowych, zwłaszcza w kontekście wielonienasyconych kwasów tłuszczowych (PUFA).
5. Zastosowanie kwasu cytrynowego jako substancji pokrywającej skorupy jaj, wpływa na zahamowanie procesów prowadzących do pogorszenia jakości jaj konsumpcyjnych (ograniczenie ubytku masy, dłuższe zachowanie struktury białka frakcji gęstej, spowolnienie zmian odczynu treści) zmieniając dynamikę tych zmian.

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The Use of the Dynamics of Changes in Table Eggs during Storage to Predict the Age of Eggs Based on Selected Quality Traits

Kamil Drabik, Tomasz Próchniak *, Kornel Kasperek and Justyna Batkowska

Institute of Biological Basis of Animal Production, University of Life Sciences in Lublin, 13 Akademicka St., 20-950 Lublin, Poland; kamil.drabik@up.lublin.pl (K.D.); kornel.kasperek@up.lublin.pl (K.K.); justyna.batkowska@up.lublin.pl (J.B.)

* Correspondence: tomasz.prochniak@up.lublin.pl

Simple Summary: The freshness is the most important characteristic of table eggs. EU legislation does not provide clear guidelines how to store table eggs or how to elongate their shelf life. Changes occurring in eggs after laying are a natural consequence of the passage of time, and there is no method for precise determination of "age" in a randomly chosen egg. The dynamics of changes of individual quality features of the raw material during its extended storage period of up to 35 days were determined. For this purpose, the evaluation of quality traits was performed daily, and the data thus obtained made it possible to create a multivariate mathematical model which, after further statistical processing, makes it possible to determine with high certainty (above 95%) the age of an egg on the basis of its measurable traits, both non-destructive and destructive. The study allowed us to select easily measurable egg quality traits, whose values clearly change in time. The detailed data of daily variability and methods of data statistical analysis are not only of scientific importance, but are also a useful diagnostic tool in assessing the freshness of table eggs on the basis of their quality characteristics.

Abstract: The aim of the study was to determine daily changes in some egg quality parameters, indirectly reflecting egg freshness, and to assess the possibility of predicting time from laying using mathematical methods. The study material consisted of 365 table eggs of medium (M, ≥53 g and <63 g) and large (L, ≥63 g and <73 g) weight classes (commercial stock, cage system, brown-shelled eggs) collected on the same day. Eggs were numbered individually and placed on transport trays and stored (14 °C, 70% RH). Every day, for 35 days, egg quality characteristics were analyzed (10 eggs per group). The change of traits in time was analyzed on the basis of linear and polynomial regression equations, depending on the trait. Based on model fitting, eight traits were selected as those most affected by storage time: egg weight and specific weight, Haugh units, albumen weight, air cell depth, yolk index, albumen and yolk pH. These traits, excluding those related to the weight, were then used in a multiple linear regression model to predict egg age. All regression models presented in this study were characterized by high predictive efficiency, which was confirmed by comparison of the observed and estimated values.

Keywords: egg storage; time; egg weight class; statistical models; regression

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1. Introduction

Quality of table eggs is often studied. It is influenced by a number of factors related to bird origin, feeding, flock age or rearing system. However, regardless of them, soon after laying, biophysical and chemical changes take place in eggs, which have negative influence on egg quality.

The storage of table eggs in the EU is regulated by the Commission Regulation (EC) No 589/2008 [1]. The important element described in the Regulation is minimal shelf life

date defined at 28 days and quality parameters to be met by eggs classified as Class A. In terms of storage, the most important of them is the air cell depth, for which the limit has been adopted as 6 mm for eggs classified as an Class A.

At the same time, the air cell is not the only feature that changes with egg storage time. The primary and most easily observable trait is egg weight loss [2,3]. The change in egg mass occurs primarily through evaporation of water from the egg contents via gas exchange that occurs between the egg contents and the external environment [4]. Due to the fact that weight loss during storage occurs regardless of weight grade or storage conditions, it may cause a number of problems for distributors and producers of table eggs. In the case of eggs with weights close to the lower limits of weight classes specified in the Regulation, the weight may decrease during the trade, and this change, despite its natural causes, is considered an adulteration (mislabeling).

Specific morphological elements of the egg are also affected by the quality changes. There is a thinning of the albumen dense fraction, and a change in its mass and pH [5,6]. Similar changes also occur in the yolk, which, due to the diffusion of water from the albumen [7], increases its mass while its shape index value decreases. Similar to the albumen, the yolk pH also tends to increase with the storage time.

In the aspect of storage, it is necessary to discuss the factors that can affect the intensity of changes in raw material. One of the most important is temperature, while its reduction has an inhibitory effect on the intensity of the occurring changes [8,9]. No less relevant in this respect is relative humidity, as providing it at high levels reduces water evaporation from the egg content, inhibiting other changes in egg quality [10].

Recently, the alternative methods of egg protection against negative changes in their quality during the storage have also gained popularity. Among them, it is possible to separate two main groups: those using modification of the atmosphere in the egg environment [11,12] or those using shell pore sealers [13–15].

The application of protection treatments or lowering the temperature affects the inhibition of the intensity of changes, but does not inhibit them completely. Moreover, although the bird rearing system or the flock age determine the quality of the egg material, the general trends occurring irrespective of the egg origin are evident for storage [16,17].

Therefore, these observations indicate a natural origin of the mentioned variability. Unfortunately, the majority of works dealing with changes in the quality of table eggs during storage focus on specific periods, usually 7 days long. This allows the analysis of the influence of time on external and internal characteristics of eggs, but makes it impossible to evaluate daily changes of their quality. Therefore, it seems reasonable to analyze their dynamics, which would enable bidirectional analysis, i.e., changes in the egg material quality on particular days, or vice versa, i.e., it would allow determination of the age of an egg on the basis of its quality characteristics. Determination of daily variability will not only allow for detailed observations, which may contribute to better understanding of the process of egg material quality deterioration, but also be used as a diagnostic tool in its assessment. In the case of eggs remaining on the market, for example, quality control mainly consists of comparing the weight declared by the producer with the actual situation. Therefore, non-compliance is possible. The use of the model can therefore verify whether the indicated inconsistencies are due to incorrect classification of the raw material or are related to its natural variability during storage. At present, the characteristics laid down in the Regulation remain the basis for this, while others are omitted. The identification of traits whose variability over time shows a stable tendency to change may resolve the problem of the hitherto inadequate assessment of material and eliminate erroneous conclusions regarding potential adulteration of the marketed eggs.

The aim of the study was to determine daily changes in some egg quality parameters, indirectly reflecting freshness, and to assess the possibility of predicting time from laying using mathematical methods.

2. Materials and Methods

2.1. Eggs

A total of 730 table hen eggs collected on the same day, 365 eggs each of medium (M, ≥53 g and <63 g) and large (L, ≥63 g and <73 g) weight classes. The eggs were purchased from a farm keeping a commercial flock of caged brown-shelled egg layers. Birds were kept in accordance with the requirements of Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. The stocking density was 750 cm^2/bird (5–6 birds per cage). Additionally, cages were equipped with a group nest, litter, perches (15 cm per laying hen) and claw-shortening devices. Birds were maintained according to zoo-hygienic and welfare requirements. All birds were fed with the same complete feed for laying hens, suitable for the age of the birds and laying phase (ME 11.6 MJ, protein 17.0%, fiber 3.9%, Ca 3.8%, P 0.55%).

The choice of material was determined by the facts that the typical consumer usually chooses eggs from these two classes, as the most common on the market, they generally come from cage farming and the brown eggshell is preferred.

2.2. Egg Quality Analyses

Eggs were individually numbered and placed blunt end up on transport carton trays, then stored at 14 °C and 70% humidity (typical storage conditions). On the day of starting the experiment (day 0), egg quality characteristics were assessed (15 eggs each), then the same testing procedures were repeated by the same laboratory team for the next 35 days (10 eggs per group daily). Additionally, every 7 days all the eggs included in the experiment were weighed. The Instron Mini 55 (Instron®, Norwood, MA, USA) compression apparatus and the TSS® Egg Quality Measurement (EQM, TSS®, York, UK) analytical kit were used. The characteristics of the whole egg were analyzed as follows:

- egg shape index (EI, as a ratio of its width and length, using electronic caliper),
- air cell depth (ACD, by candling, according to scale),
- egg weight (EW, using laboratory balance with 0.01 g accuracy),
- egg specific gravity (SG, based on egg weight measurement in the air and in the water, according to Archimedes principle),
- proportions of particular egg elements (as the ratio of their weight to the weight of whole egg).

In the case of eggshell quality, the following traits of this element were evaluated:

- color (SC, as a percentage of reflected light),
- weight (SW, using laboratory balance with 0.01 g accuracy),
- thickness (ST, by micrometer screw, at the "equator"),
- density (SD, calculated based on shell area and volume, according to Shafey [18].

In the case of egg content, the height of dense albumen (AH) was measured, and based on it the Haugh units (HU) were calculated [19]. As far as the yolk traits are concerned, its weight (YW, using laboratory balance with 0.01 g accuracy), color (YC, using 16-point Roche scale, DSM®) and index (YI, as a ratio of height and diameter) were estimated. The pH of albumen (ApH) and yolk (YpH) were measured using a pH meter with a combined glass electrode.

2.3. Statistical Analyses

The following statistical analyses were used: CORR, REG, TTEST, GLM procedures of SAS software (Statistical Analysis System, 9.4, Cary, NC, USA, 2013). The effect of storage time on trait values is presented as Spearman's rank correlations and regression equations. Regression models depending on the trait analyzed were selected on the basis of graphical analysis of residuals and the value of the R^2 coefficient. Consequently, egg weight and specific weight were analyzed by linear regression models (Model 1), while for Haugh

units, albumen weight, air cell depth, yolk index and albumen and yolk pH, a polynomial of the third degree regression was used (Model 2).

$$\text{Model 1}: \ Y = \beta_0 + \beta_1 * \text{day} + \varepsilon_i$$

$$\text{Model 2}: \ Y = \beta_0 + \beta_1 * \text{day} + \beta_2 * \text{day}^2 + \beta_3 * \text{day}^3 + \varepsilon_i$$

where:

Y—estimated value of egg trait
β_0—intercept
β_1, β_2, β_3—coefficient of the polynomial term

A multivariate linear regression model (model 3) was used to predict egg storage day. Weight-related traits were dropped from the model to eliminate the influence of egg weight class. Consequently, estimation was based on SG, ApH, YpH, YI, HU and ACD. No high correlations (0.8 and above) were found between the traits used in the storage day prediction model. The lack of multicollinearity between traits was also confirmed through the variance inflation factor and tolerance. Model fit, with successively added traits, was presented using R^2 and Mallows' statistic C(p).

$$\text{Model 3}: \ Y = \beta_0 + \beta_1 * \text{SG} + \beta_2 * \text{ApH} + \beta_3 * \text{YpH} + \beta_4 * \text{YI} + \beta_5 * \text{HU} + \beta_6 * \text{ACD} + \varepsilon_i$$

where:

Y—estimated value of egg trait
β_0—intercept
β_1–β_6—coefficient of the polynomial term

The verification of the presented models (Model 1, 2, 3) was performed by estimating the trait values and then comparing the estimated values with the expected ones using Student's *t*-test for combined variables. For Models 1 and 2, trait values were predicted based on the day of storage. For Model 3, the day of storage was predicted from the trait values.

The Supplementary Material presents basic statistics of the analyzed traits including the significance of differences verified by two-factor analysis of variance with Tukey's test (proc GLM). Egg weight classes (2 levels) and day of egg storage in a 7-day period (6 levels) were used as factors.

3. Results

3.1. Dynamics of Egg Quality Changes during Storage

Only those traits for which the regression models had an $R^2 > 0.1$ (EW, SG, ACD, HU, AW, ApH, YI, YpH) are presented in the results. The complete data are included as Supplementary Materials (Table S1).

Independently of the weight class, Spearman correlation analysis showed a significant effect of storage time on the value of traits, except for YW (Table 1). It was found that the value of characteristics such as egg weight, specific weight, albumen (HU, AH, AW) and yolk features (YI, YC) decreased significantly with time. On the other hand, the air cell depth as well as the albumen and yolk pH were positively correlated with the storage time.

In terms of egg weight and its specific weight, a linear relationship with the egg material storage time was found (Figure 1A,B), while for the other egg quality traits non-linear relationships were found, so their analysis was based on polynomial regression models. For SG, a small scatter of results from the simple regression was found, characterized by a high fit ($R^2 = 0.53$). The occurrence of outliers in this range is due to the deepening of the air cell, which in some cases caused the inability to analyze the wet egg weight, which resulted in a value of ≈1.0. When comparing the increase in air cell depth, an identical relationship was noted within both weight classes up to day 10 of the experiment. After this time, the tendency to a higher rate of ACD deepening was characterized by L class eggs.

Table 1. Spearman's correlation coefficients between particular quality traits of eggs and days of storage.

Class	M		L	
Trait	**ρ**	***p*-Value**	**ρ**	***p*-Value**
EW	−0.20287	0.0001	−0.20407	0.0001
SG	−0.82275	<0.0001	−0.79993	<0.0001
ACD	0.76409	<0.0001	0.75573	<0.0001
HU	−0.62356	<0.0001	−0.69372	<0.0001
AW	−0.35078	<0.0001	−0.32698	<0.0001
ApH	0.73197	<0.0001	0.77513	<0.0001
YI	−0.59984	<0.0001	−0.50617	<0.0001
YpH	0.38091	<0.0001	0.38466	<0.0001

EW—egg weight, SG—egg specific gravity, ACD—air cell depth, HU—Haugh units, AW—albumen weight, ApH—albumen pH, YI—yolk index, YpH—yolk pH.

Analysis of albumen quality traits (Figure 2) showed a small difference according to weight class for HU (Figure 2A). After 5 days of the experiment, these differences were imperceptible. An interesting observation concerns albumen pH (Figure 2C). This trait was characterized by fluctuating variability during storage. An increase in pH was observed up to about the 15th day of the experiment, with the highest intensity of changes observed in the initial period (10th day) of the experiment. In later stages of the study (up to the 30th day), a slight decrease in the trait value was observed. It is worth mentioning that ApH was the trait with the highest fitting index to the regression equation.

In the case of the yolk quality characteristics (Figure 3), a decrease in the yolk index value was observed with storage time regardless of egg weight class. In the yolk pH (Figure 3B), in contrast to egg albumen, initially a slight increase in the value of the trait (up to the 10th day of the experiment) was found, after which the increase continued more intensively until the end of the experiment.

Each of the models used, regardless of the trait analyzed, was characterized by high significance (Table 2). The fit of the data to the model (R^2) ranged from 0.1 for albumen weight in L class eggs to 0.71 for ApH of eggs of the same weight class. High R^2 values were also found for traits such as SG and ACD, regardless of the weight class of the eggs analyzed. The lowest values of the R^2 coefficient related to egg weight (0.17 and 0.2 for M and L grade eggs, respectively) and albumen weight (0.11 and 0.1 for M and L grade eggs, respectively). Student's *t*-tests comparing observed and predicted variables based on regression equations show no significant differences, confirming good model fitting.

3.2. Prediction of Egg Age based on the Dynamics of Changes in Egg Quality

The prediction of egg age based on the six trait values resulted in a model with a high fit index (R^2 = 0.72) (Table 3). Moreover, Mallows' statistic C(p) is below the degrees of freedom of the model. This also means a very good fitting of the model in terms of this criterion. Each of the characteristics used in the model showed highly significant influence on the model solution. The difference between the model predicted and observed egg storage days was not significant.

The residuals obtained from the regression model were assessed for lack of autocorrelation, their normal distribution and heteroscedasticity. The results obtained indicate that the assumptions of the regression analysis are met and the model fits the numerical data.

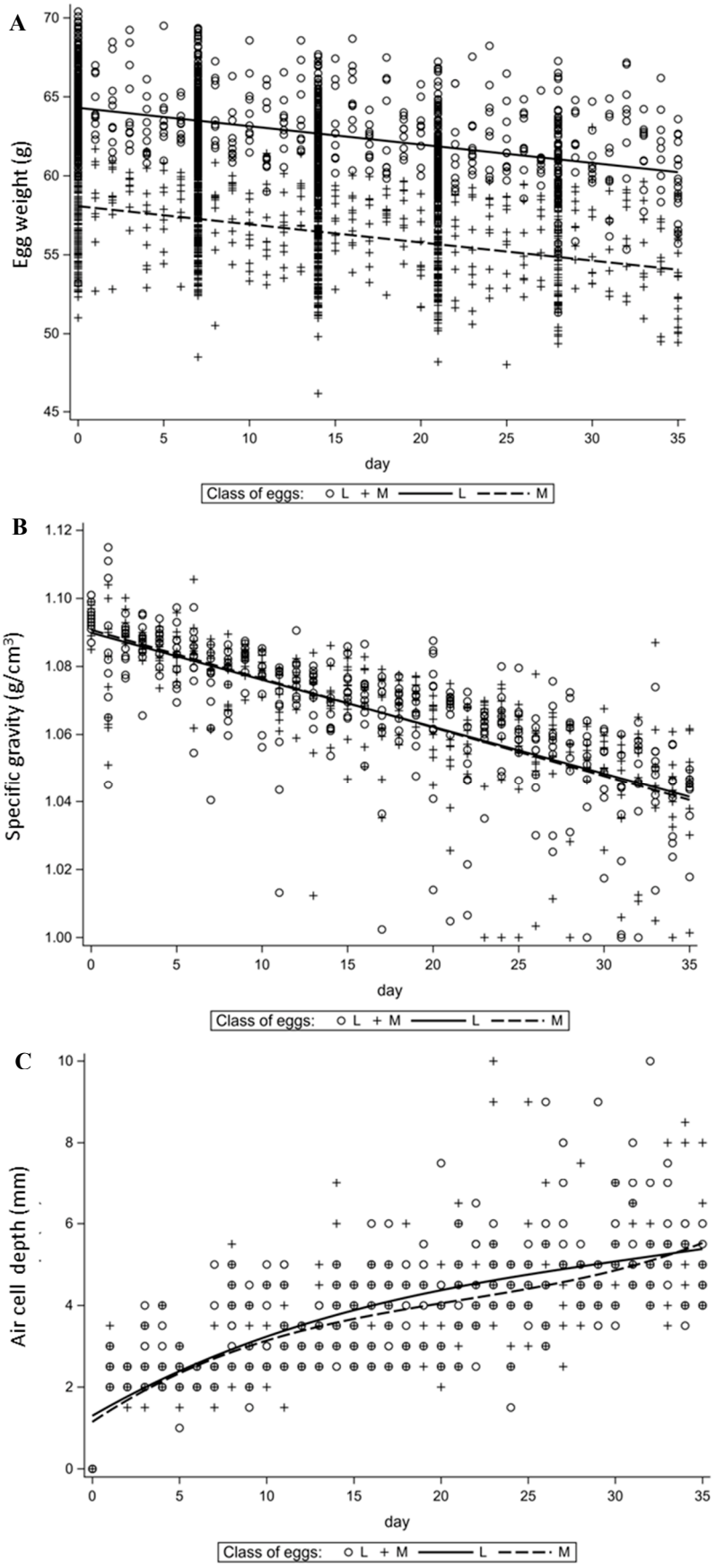

Figure 1. Changes in time of selected traits of whole egg. (**A**)—egg weight (EW), (**B**)—specific gravity (SG), (**C**)—air cell depth (ACD).

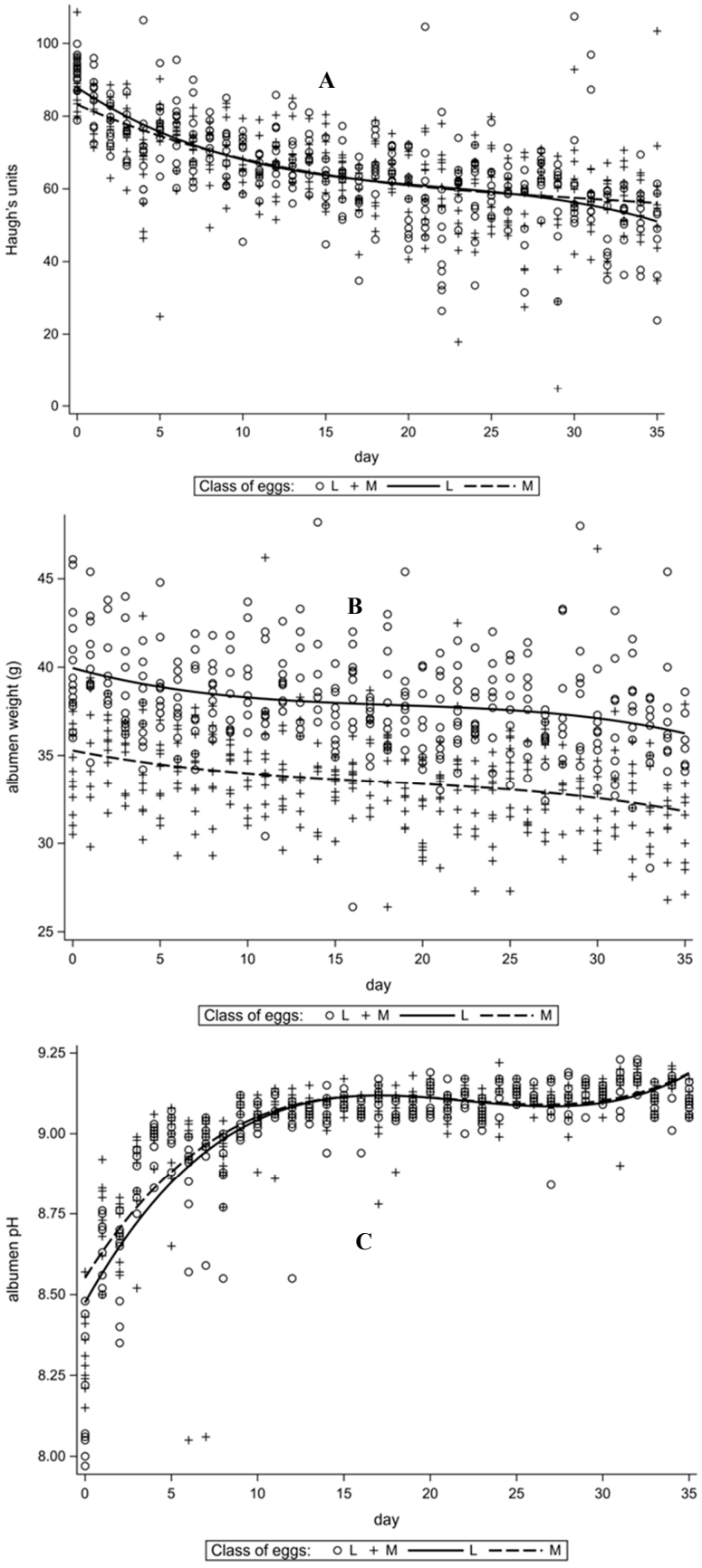

Figure 2. Changes in time of selected traits of egg albumen. (**A**)—Haugh units (HU), (**B**)—albumen weight (AW), (**C**)—albumen pH (ApH).

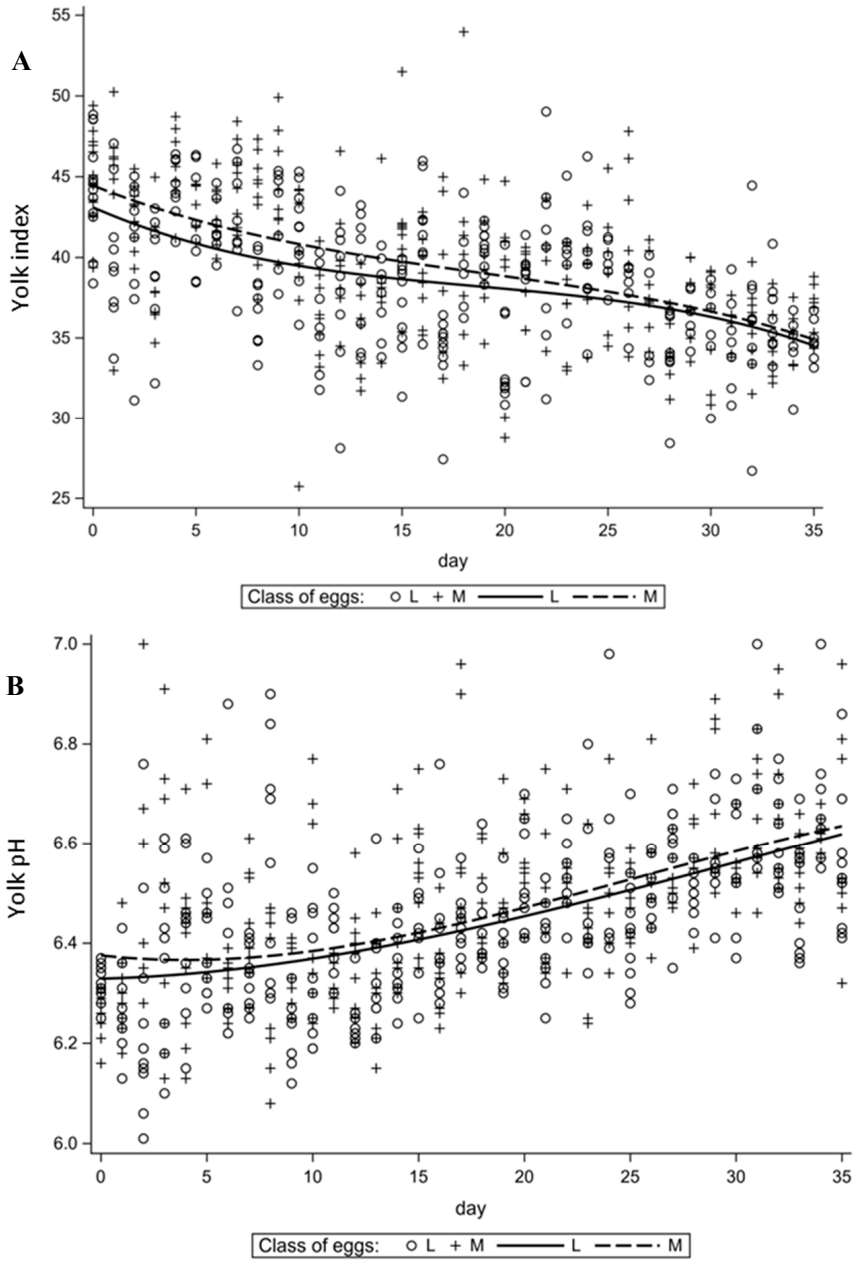

Figure 3. Changes in time of selected traits of egg yolk. (**A**)—yolk index (YI), (**B**)—yolk pH (YpH).

Table 2. Statistical models used for particular trait analysis.

Trait	EWC	Model	Pr > F	R^2	MD (Observed – Expected)	SD	Pr > T	Model Type
EW	M	$EW = 58.06968 - 0.11497 * day$	<0.0001	0.17	−0.00001	2.496	0.9999	linear
	L	$EW = 64.30016 - 0.11649 * day$	<0.0001	0.20	−0.00002	2.285	0.9997	
SG	M	$SG = 1.09072 - 0.00144 * day$	<0.0001	0.53	0.000082	0.014	0.9124	linear
	L	$SG = 1.08987 - 0.00138 * day$	<0.0001	0.53	−0.00006	0.014	0.9353	
ACD	M	$ACD = 1.14811 + 0.28495 * day - 0.01024 * day^2 + 0.00016181 * day^3$	<0.0001	0.54	0.00179	1.072	0.9749	polynominal
	L	$ACD = 1.29760 + 0.24602 * day - 0.00586 * day^2 + 0.00006181 * day^3$	<0.0001	0.56	−0.00103	1.062	0.9854	
HU	M	$HU = 83.17175 - 1.99460 * day + 0.05648 * day^2 - 0.00061806 * day^3$	<0.0001	0.37	0.00126	10.460	0.9982	polynominal
	L	$HU = 87.84322 - 3.00134 * day + 0.12044 * day^2 - 0.00185 * day^3$	<0.0001	0.46	−0.00045	10.330	0.9993	
AW	M	$AW = 35.27816 - 0.19537 * day + 0.00798 * day^2 - 0.00014972 * day^3$	<0.0001	0.11	−0.00067	2.516	0.9961	polynominal
	L	$AW = 39.94734 - 0.27154 * day + 0.01282 * day^2 - 0.0002307 * day^3$	<0.0001	0.10	−0.00161	2.669	0.9911	
ApH	M	$ApH = 8.55088 + 0.08458 * day - 0.00407 * day^2 + 0.00006202 * day^3$	<0.0001	0.64	0.00112	0.115	0.8581	polynominal
	L	$ApH = 8.47459 + 0.09553 * day - 0.00457 * day^2 + 0.00006905 * day^3$	<0.0001	0.71	0.00156	0.110	0.7922	
YI	M	$YI = 44.44111 - 0.50472 * day + 0.01726 * day^2 - 0.00030335 * day^3$	<0.0001	0.32	−0.0007	3.768	0.9975	polynominal
	L	$YI = 43.08972 - 0.55522 * day + 0.02352 * day^2 - 0.00041845 * day^3$	<0.0001	0.25	0.000812	3.674	0.997	
YpH	M	$YpH = 6.37524 - 0.00472 * day + 0.00064422 * day^2 - 0.00000850 * day^3$	<0.0001	0.27	−0.00000718	0.145	0.9993	polynominal
	L	$YpH = 6.32879 + 0.00106 * day + 0.00032864 * day^2 - 0.00000350 * day^3$	<0.0001	0.31	−0.00003	0.135	0.9962	

EWC—egg weight class, EW—egg weight, SG—egg specific gravity, ACD—air cell depth, HU—Haugh units, AW—albumen weight, ApH—albumen pH, YI—yolk index, YpH—yolk pH, Pr > F—regression model significance, R^2—determination coefficient, MD—mean of differences (real value – expected value), SD—standard deviation of differences, Pr > T—significance of *t*-test for dependent samples.

Table 3. Statistical model used to predict the age of the eggs (in days).

Step	Variable Entered	Partial R^2	Model R^2	C(p)	Parameter Estimate	Pr > |t|
Inercept					17.90251	0.5232
1	SG	0.5176	0.5176	382.072	−0.12071	<0.0001
2	ApH	0.1096	0.6272	175.732	10.63105	<0.0001
3	YpH	0.0447	0.6719	92.7676	−0.33684	<0.0001
4	YI	0.0206	0.6926	55.5100	12.82286	<0.0001
5	HU	0.0182	0.7108	22.8532	1.11087	<0.0001
6	ACD	0.0094	0.7202	5.87	−152.42621	<0.0001

Paired *t*-test for day * estimated day: mean difference = −0.00031; SD = 5.49; Pr. > |t| = 0.999; SG—specific gravity, ApH—albumen pH, YpH—yolk pH, YI—yolk index, HU—Haugh units, ACD—air cell depth, mean different = day –estimated day.

4. Discussion

The prediction of egg age based on the analysis of its quality traits has been the subject of several works, varied by the measurement techniques used, as well as the traits that are involved in model construction. Thus far, analyses of this type have included S-ovoalbumine content [20], or albumen pH and Haugh units [21,22]. It seems that a more accurate prediction can be achieved by using not one, but at least several, features in the model, so perhaps the solution proposed in our study is characterized by a more comprehensive approach. Moreover, the authors of the mentioned works assumed a linear variation of traits during egg storage, while our study clearly shows a non-linear character of most of them.

Attempts have been made to use various methods to predict the magnitude of egg quality changes. Ragni et al. [23] used a technique for this purpose based on the dielectric properties of eggs and the variability of parameters during the storage period. The authors used the data obtained subsequently to determine a linear regression for changes in raw material quality, similar to that developed in their own study. A similar analytical method was also used by Soltani et al. [24], who additionally used neural networks to analyze the data obtained. Analyses performed using electronic nose and chemometric methods were also used to predict egg quality traits, including yolk index [25]. At the same time, the authors performed data analyses based on neural networks, which allowed determination of a model for changes in yolk index. An interesting method for analyzing eggs to predict their age was also proposed by Tan et al. [26], who analyzed changes in the center of gravity of eggs during storage using magnetic resonance. Based on the collected data, a model was developed to predict the age of eggs, which was characterized by a high fitting (R^2 = 0.961). The indicated solutions, although effective and reliable, required the use of specialized equipment. Therefore, despite considerable methodological advancement, it seems that practical use of the results obtained may be difficult. In our study, data were obtained using a minimum of available laboratory tools, giving a real chance for the models presented to be used in practice. From the model predicting the day of storage, measures related to the egg weight have been removed, making it more universal for different weight classes of eggs.

Changes in egg quality during their storage are influenced by factors affecting the laying hens. In order to be able to standardize the obtained results, it was necessary to unify the raw material itself. The study of Batkowska et al. [27] indicates that the intensity of quality changes in eggs with time after the oviposition may depend not only on the housing system of the birds, but also on the weight class of the eggs themselves. Therefore, two of the most popular egg weight classes on the EU market were chosen for the study. This is also related to consumer preferences, whose egg weight choices are driven by many factors, but in many countries, higher weight eggs are preferred [28]. At the same time, the final Model 3 proposed in our study omits egg weight, making it universal in this respect.

The egg weight loss and the related changes in specific gravity are among the most frequently reported observations in terms of storage studies of raw egg material regardless of its origin or storage conditions [7,29]. Weight loss occurs by evaporation of water through the shell pores, which are not uniform in number, size or distribution on the surface [30]. The shell pores play an important role in the incubation process, but in terms of table eggs, the porosity may affect the shelf life of the raw material. This is because excessive permeability and/or pore number increases egg weight loss through evaporation [31].

Many authors also identify the role of the most external layer of the egg, the cuticle, in the gas exchange process. The mucin layer protects eggs from microbial contamination and increases shell strength [32]. Although the effective protective function of the cuticle is only a few days [33], the need to keep it intact has restricted the washing of table eggs in EU countries [1]. In our study, weight loss was observed but there was no acceleration in its rate, which could only be indicated by the temporary protection of the eggs provided by the mucin layer. Furthermore, egg weight and specific weight were the only linear variables. On the other hand, Liu et al. [34] analyzed the effect of egg washing on their quality during storage, taking into consideration the cuticle covering of the shell. Their results indicate a faster deepening of the air cell in washed eggs, which may confirm the protective effect of the mucin layer.

Egg albumen also deteriorates during its storage. The most important factors include a drop in the Haugh units, a universal unit which takes into account the weight and height of the albumen. Our own research has shown a non-linear decrease in this value. The decrease in HU has also been noted by other authors [10,35]. Water loss, both through evaporation and due to its migration from the albumen to the yolk, is claimed to be the main factor influencing the change in dense albumen structure [36]. Additionally, other factors such as reduction of disulfide bonds or ovomucin depolymerization caused by alkaline hydrolysis are also indicated. The breakdown of bonds stabilizing the ovomucin–lysozyme complex is an important factor in this aspect [37]. A change in the structure of the mentioned complex contributes to the dilution of the albumen, and it has been found that the interaction between these two egg components is significantly weakened with increasing pH [38].

The change in albumen pH during storage is caused by the release of carbon dioxide through the shell pores, which contributes to the alkalization of the egg contents [39]. However, in our study it was found that the change in albumen pH is not linear. Underlying this relationship is the buffering capacity of the albumen itself. Studies of Heath [36] have shown that egg albumen has a buffering capacity based on a carbonate buffer. The highest buffering capacity was recorded for pH not exceeding 8. Increasing the pH decreased the buffering capacity of the albumen. These observations seem to be in line with those found in our study, especially since the pH recorded exceeded the values indicated in the cited work as the limit for the best buffering capacity of the albumen.

The changes in yolk quality parameters are mainly related to the movement of water between the particular morphological elements of the egg. It should be noted that this movement is mainly related to the penetration of water through the vitelline membrane from the albumen to the yolk [40]. One of the noticeable effects of water movement is the decrease in yolk shape index values observed by many authors, as well as in the strength of the vitelline membrane [10,17], which, in case of prolonged storage time, may lead to its rupture and the mixing of egg content elements. Our own research is consistent with the trend described, but it should be noted that these changes are not linear, as in the case of albumen traits, which suggests the presence of a relationship between the indicated egg quality traits.

5. Conclusions

The study showed that only two of the analyzed egg quality traits (egg weight and specific weight) showed linear variability during storage. The change in values of traits such as air cell depth, albumen height and Haugh units, albumen and yolk pH and yolk

index during the storage of eggs should be analyzed non-linearly, as indicated by high fit indices of regression equations.

The first part of the study allowed us to select easily measurable egg quality traits, whose values clearly change over time. The use of these traits, excluding those related to weight, resulted in a highly fitted multivariate predictive model for determining the age of eggs. The validity of all regression models used in this study was confirmed by estimating the expected values and comparing them to the real ones. The model is applicable when typical egg storage conditions are used, as in our work, but the influence of temperature on the dynamics of egg quality changes requires further research to determine temperature-dependent models.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ani11113192/s1, Table S1: The mean values of particular traits evaluated during the egg storage depending on the egg weight class and the time of storage.

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Dr inż. Tomasz Próchniak
Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13
20-950 Lublin

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Lublin, 15.11.2021

Dr inż. Tomasz Próchniak

Dr inż. Kornel Kasperek
Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13
20-950 Lublin

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Lublin, 15.11.2021

Dr inż. Kornel Kasperek

Dr hab. Justyna Batkowska prof. uczelni
Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13
20-950 Lublin

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The Impact of Package Type and Temperature on the Changes in Quality and Fatty Acids Profile of Table Eggs during Their Storage

Kamil Drabik, Tomasz Próchniak, Damian Spustek, Karolina Wengerska and Justyna Batkowska *

Institute of Biological Basis of Animal Production, University of Life Sciences in Lublin, 13 Akademicka St., 20-950 Lublin, Poland; kamil.drabik@up.lublin.pl (K.D.); tomasz.prochniak@up.lublin.pl (T.P.); damianspustek@gmail.com (D.S.); karolina.wengerska@gmail.com (K.W.)
* Correspondence: justyna.batkowska@up.lublin.pl

Abstract: The aim of the study was to evaluate the possibility of reducing changes in the quality of consumer hen eggs by storing them in various package type and under various temperature conditions (room and refrigeration). The material consisted of 960 chicken eggs packed in cardboard or plastic boxes, 10 pcs in each. Half of the packages were stored at room temperature (21 °C), the rest in the refrigerator (5 °C). The eggs were stored for 28 days qualitatively evaluated at 14-day intervals. The characteristics of whole egg (weight, specific weight, proportion of morphological elements, air cell depth) as well as of shell (weight, color, crushing strength, thickness, density, water conductivity), albumen (height, Haugh units, weight, pH) and yolk (weight, color, pH) were analyzed. The fatty acids profile of yolks was also evaluated as a freshness indicator. Packaging types available on the market, apart from its marketing and eggs protection function, can also influence the quality and stability of the product during storage. The use of plastic boxes can help to maintain higher eggs quality during the storage period, even after a significant extension of the storage time. Eggs stored in plastic boxes at room temperature had very similar results to those stored under refrigeration using conventional cardboard boxes. This effect is probably related to the lower permeability of plastic boxes in comparison to cardboard ones, but detailed research work in this direction is necessary to verify this relation.

Keywords: chicken eggs; cardboard egg box; plastic egg box; fridge temperature; room temperature; egg storage; fatty acid profile

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1. Introduction

Eggs, due to their balanced chemical composition and low price are one of the most important animal products in the human diet. Therefore, solutions are being sought to obtain eggs of the best quality or with improved nutritional value. The vast majority of works in this field focus on the modification of laying hen nutrition to increase the quality and improve the chemical composition of the obtained eggs [1,2].

At the same time, like all products available on the market, table eggs must meet the consumers' requirements. Analyzing data from available literature Rondoni et al. [3] pointed out that purchasing behavior is closely related to the place of research, but there are also common elements regarding egg appearance, freshness or packaging.

In addition to factors related to the bird housing system or egg appearance, one of the most important criteria for the selection of table eggs is their freshness [4,5]. Regardless of the origin of the eggs, with the time of storage, there is a deterioration of their quality and technological usefulness. In the EU, the marketing of table eggs is regulated by Commission and Council Regulation (EC) 589/2008 [6], which introduces a 28-day shelf life for Class A table eggs.

Moreover, this regulation limits the possibility of refrigerated storage of table eggs at the commercial stage, indicating such a possibility only for final consumers. Despite the positive effect of refrigeration methods on inhibiting negative changes in the quality of table eggs confirmed in studies [7,8], it is necessary to search for alternatives in this regard. So far, two main methods have been developed: coating egg shells with substances that limit water evaporation through shell pores [9–12] or using atmospheric modification [13]. These methods, although effective, are not currently of applicable importance.

Another element that may inhibit changes in the quality of consumer eggs is the type of packaging in which they were purchased. In the vast majority of cases consumer eggs are packed in small boxes (6 or 10 pieces each). Both cardboard and plastic packages are available on the market. It is, therefore, reasonable to find out whether the type of packaging (cardboard vs. plastic) is important in terms of egg storage.

The study aimed to evaluate the possibility of reducing changes in the quality of consumer hen eggs by storing them in various package types (cardboard or plastic egg box) and under various temperature conditions (room and refrigeration).

2. Materials and Methods

2.1. Material Preparation

The material for the study consisted of 960 L-class table eggs purchased from the laying hens stock kept in a cage system. Eggs were collected on the same day, numbered individually and then randomly divided into 4 treatments according to the scheme (Table 1). The quality of 60 eggs, treated as a control group was also evaluated on the same day (day 0), the rest of the eggs were stored in 10-piece egg boxes, made of plastic and/or cardboard, divided into two storage temperatures, room (21 °C) and refrigerated (5 °C).

Table 1. Schema of the experiment.

Package Type	Cardboard Box (CB)		Plastic Box (PB)	
Temperature	Room (R, 21 °C)	Fridge (F, 5 °C)	Room (R, 21 °C)	Fridge (F, 5 °C)
Time (Days)	RCB	FCB	RPB	FPB
0	60			
14	60	60	60	60
28	60	60	60	60
42	60	60	60	60
Total	240	240	240	240

RCB—cardboard box, room temperature (21 °C); FCB—cardboard box (5 °C), refrigeration temperature (5 °C), RPB—plastic box, room temperature (21 °C), FPB—plastic box, refrigeration temperature (5 °C).

2.2. Egg Quality Analyses

Egg quality was analyzed at 14-day intervals. An EQM (Egg Quality Measurement, TSS®, York, UK), Instron Mini 55 device (Instron®, Norwood, MA, USA) and pH-meter with combined glass electrode (Elmetron®, Zabrze, Poland) were used. The following experimental material characteristics were evaluated:

- whole egg—depth of the air cell (ACD), mass (EW), proportions of morphological elements (in relation to egg weight, EYP—yolk proportion in egg weight, EAP—albumen proportion in egg weight, ESP—shell proportion in egg weight).
- shell–colour (SC), weight (SW), thickness (ST), strength (SS), eggshell proportion egg weight.
- albumen–weight (AW), height (AH), pH (ApH).
- yolk–weight (YW), colour (YC, using 16-points DSM YolkFan™, DSM Nutritional Products, Basel, Switzerland), index (YI, as ratio of its height and diameter), pH (YpH).

Additional quality parameters, such as weight loss (WL) and specific mass of eggs (ESG, according to Archimedes principle), shell density (SD) [14] and its water vapor

conductance (ESC) (Ar et al., 1974) and Haugh's units (HU) [15] were calculated based on the obtained data.

2.3. Yolk Lipid Profile Analyses

On the day of the experiment, and after 28 days, 20 yolks/treatment were collected for further analyses. The samples were freeze-dried (Labconco Corporation, Kansas City, MO, USA), and then fatty acid profiles and cholesterol content were analyzed.

The fatty acid profile of egg yolks was analyzed using gas chromatography according to PN-EN ISO 5508: 1996 and PN-EN ISO 5509: 2001. The Varian 450-GC gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with the flame ionization detector (FID) equipped with capillary column Select™ Biodiesel for FAME (30 m 0.32 mm 0.25 µm) and autosampler CP-8400 were used for the analyses. Based on the proportions of particular fatty acids and their groups (Galaxie™ Chromatography Data System software), the following indexes were calculated: PI-peroxidizability index [16], AI –atherogenicity index and TI-thrombogenic index [17], DFA-desirable fatty acids [18], HFSA-hypercholesterolaemic saturated fatty acids [19] and h/H—hypocholesterolaemic/hypercholesterolaemic ratio [20].

2.4. Statistical Analyses

The data obtained were statistically analyzed using the SPSS 24.0 statistical package [21]. The Shapiro-Wilk test was used to assess the normal distribution of the traits. A two-factorial model of analysis of variance was then used, taking into account the type of packaging (P-cardboard or plastic), storage temperature (T-room or fridge) and the interaction between them. Group comparisons were made using Tukey's multiple comparisons test.

3. Results

3.1. Egg Quality

With the storage time of eggs, all their quality characteristics change. Table 2 shows the parameters of eggs external traits. Although all the eggs were classified as "L", they were slightly different in terms of weight, which explains the differences observed in the initial phase of the experiment. After 42 days of storage, it was found that eggs stored in plastic boxes at refrigeration temperature had significantly the highest weight. Observations on egg specific gravity (ESG) showed no significant differences after 28 days of storage regardless of the packaging used. The extended storage period showed that storage in plastic boxes allowed to inhibit changes in this range.

Similar observations also relate to egg weight loss during the storage. After 28 days, it was found that the lowest losses, regardless of storage temperature, were characteristic of eggs stored in plastic boxes. Prolongation of storage time indicated the continuation of this trend, with the highest weight loss registered in eggs stored at room temperature in cardboard boxes. As water loss is closely related to shell water conductivity, the data obtained for both 28 and 42 days of storage of table eggs indicate the same relationship as for WL.

Air cell depth (ACD) differed significantly between the experimental groups after both 28 and 42 days of storage. Significantly lower values in this regard were recorded for eggs stored in plastic boxes compared to those made of cardboard. At the same time, it should be noted that after 42 days of storage only in the case of eggs from the RCB group the air cell depth exceeded 6 mm, i.e., the limit value for consumer eggs of class A, was recorded.

Changes in egg quality also affected its content. It was found that the highest weight of albumen after 42 days of storage characterized eggs from the FPB group (Table 3). A similar trend was also observed for the albumen proportion in the whole egg weight.

Table 2. Changes in the characteristics of the whole egg depending on packaging and storage temperature.

Trait	Time (Days)	Treatment				Total	SEM	Factor (*p*-Value)		
		RCB	FCB	RPB	FPB			B	T	B × T
EW (g)	0	63.29 ^{a}	63.36 ^{a}	63.30 ^{a}	64.83 ^{b}	63.66	0.159			
	14	62.36 ^{a}	63.10 ^{ab}	63.11 ^{ab}	64.49 ^{b}	63.27	0.268	*0.042*	*0.041*	*0.187*
	28	61.56 ^{a}	61.85 ^{a}	62.34 ^{ab}	64.31 ^{b}	62.51	0.263	*0.020*	*0.001*	*0.080*
	42	59.95 ^{ab}	59.16 ^{a}	60.71 ^{b}	64.08 ^{c}	60.92	0.297	*0.004*	*0.000*	*0.000*
ESG (g/cm^3)	0	1.079					0.002			
	14	1.059 ^{a}	1.072 ^{b}	1.081 ^{c}	1.081 ^{c}	1.073	0.001	*0.007*	*0.000*	*0.000*
	28	1.078	1.054	1.072	1.076	1.070	0.011	*0.001*	*0.713*	*0.536*
	42	1.019 ^{a}	1.043 ^{b}	1.067 ^{c}	1.070 ^{c}	1.049	0.003	*0.001*	*0.000*	*0.020*
WL (%)	14	4.37 ^{c}	3.27 ^{b}	2.61 ^{a}	2.22 ^{a}	3.15	0.112	*0.000*	*0.000*	*0.006*
	28	6.56 ^{c}	4.33 ^{b}	3.00 ^{a}	2.87 ^{a}	4.15	0.215	*0.000*	*0.000*	*0.000*
	42	8.47 ^{c}	5.39 ^{b}	3.25 ^{a}	3.41 ^{a}	5.22	0.279	*0.000*	*0.000*	*0.000*
ESC (mg/day/torr)	14	2.28 ^{c}	1.73 ^{b}	1.37 ^{a}	1.17 ^{a}	1.64	0.057	*0.000*	*0.000*	*0.008*
	28	1.73 ^{c}	1.08 ^{b}	0.77 ^{a}	0.75 ^{a}	1.07	0.056	*0.000*	*0.000*	*0.000*
	42	1.48 ^{c}	0.91 ^{b}	0.54 ^{a}	0.60 ^{a}	0.90	0.049	*0.000*	*0.000*	*0.000*
ACD (mm)	14	3.83 ^{c}	2.80 ^{b}	2.10 ^{a}	2.23 ^{ab}	2.74	0.109	*0.005*	*0.000*	*0.000*
	28	5.20 ^{c}	3.98 ^{b}	2.80 ^{a}	2.55 ^{a}	3.63	0.159	*0.001*	*0.000*	*0.028*
	42	6.33 ^{c}	4.52 ^{b}	4.20 ^{a}	3.40 ^{a}	4.63	0.206	*0.001*	*0.000*	*0.172*

^{a,b,c}—means within row differ significantly at $p \leq 0.05$; SEM—standard error of mean; RCB—(21 °C); FCB—cardboard box (5 °C), refrigeration temperature (5 °C), RPB—plastic box, room temperature (21 °C), FPB—plastic box, refrigeration temperature (5 °C); B—egg box type, T—temperature; EW—egg weight, ESG—egg shell gravity, WL—weight loss, ESC—water vapor conductance, ACD—air cell depth.

Table 3. Changes in albumen characteristics concerning packaging and storage temperature.

Trait	Time (Days)	Treatment				Total	SEM	Factor (*p*-Value)		
		RCB	FCB	RPB	FPB			B	T	B × T
AW (g)	0	41.12					0.628			
	14	36.55	38.88	38.49	39.07	37.72	0.630	*0.048*	*0.994*	*0.886*
	28	36.43 ^{a}	38.28 ^{ab}	38.03 ^{ab}	39.14 ^{b}	37.97	0.352	*0.033*	*0.076*	*0.592*
	42	34.89 ^{a}	34.46 ^{a}	35.15 ^{a}	39.38 ^{b}	36.07	0.391	*0.002*	*0.000*	*0.000*
EAP (%)	0	63.77					0.548			
	14	58.61	60.63	60.06	60.56	59.10	0.929	*0.110*	*0.575*	*0.601*
	28	59.12	61.93	60.94	60.86	60.71	0.476	*0.152*	*0.693*	*0.129*
	42	58.27 ^{a}	56.95 ^{a}	57.93 ^{a}	61.46 ^{b}	58.75	0.326	*0.013*	*0.000*	*0.000*
AH (mm)	0	8.25					0.385			
	14	5.64 ^{a}	4.74 ^{a}	7.20 ^{b}	7.34 ^{b}	6.23	0.228	*0.335*	*0.000*	*0.188*
	28	3.08 ^{a}	3.68 ^{a}	6.74 ^{b}	6.60 ^{b}	5.02	0.213	*0.275*	*0.000*	*0.081*
	42	2.51 ^{a}	2.96 ^{a}	4.41 ^{b}	6.47 ^{c}	4.01	0.206	*0.000*	*0.000*	*0.000*
HU	0	88.67					2.124			
	14	68.17 ^{a}	63.32 ^{a}	83.12 ^{b}	83.46 ^{b}	74.51	1.665	*0.406*	*0.000*	*0.341*
	28	45.08 ^{a}	52.68 ^{b}	80.54 ^{c}	78.81 ^{c}	64.28	2.021	*0.152*	*0.000*	*0.024*
	42	35.08 ^{a}	42.34 ^{a}	61.06 ^{b}	78.32 ^{c}	53.13	2.417	*0.091*	*0.000*	*0.000*
ApH	0	8.51					0.075			
	14	9.19 ^{b}	9.13 ^{b}	8.95 ^{a}	8.88 ^{a}	9.04	0.023	*0.026*	*0.000*	*0.765*
	28	9.28 ^{b}	9.20 ^{b}	8.86 ^{a}	8.95 ^{a}	9.09	0.032	*0.899*	*0.000*	*0.054*
	42	9.12 ^{b}	9.05 ^{b}	8.82 ^{a}	8.74 ^{a}	8.95	0.030	*0.200*	*0.000*	*0.136*

$^{a, b, c}$—means within row differ significantly at $p \leq 0.05$; SEM—standard error of mean; RCB—cardboard box, room temperature (21 °C); FCB—cardboard box (5 °C), refrigeration temperature (5 °C), RPB- plastic box, room temperature (21 °C), FPB—plastic box, refrigeration temperature (5 °C); B—egg box type, T—temperature; AW—albumen weight, EAP—albumen proportion in egg weight, AH—albumen height, HU—Haugh's units; ApH—albumen pH.

It was also observed that albumen height and Haugh's unit number decreased during eggs' storage, regardless of the packaging type of temperature applied. At the same time, it should be noted that for both AH and HU the highest quality was found of eggs stored at refrigeration temperature in plastic packaging s, while the lowest values for both traits

were recorded for the RCB group. Importantly, eggs stored at room temperature in plastic packs had better quality compared to those stored under refrigeration in cardboard packs.

In terms of changes in albumen pH, it was found that eggs from the RPB and FPB groups had a lower pH only after 14 days of the experiment compared to those stored in cardboard packaging, and this trend was maintained until the end of the storage time. Although there were no significant differences in yolk weight during egg storage (Table 4), the proportion of this egg morphological element, after 42 days of the experiment was considerably the lowest in the FPB group, while the other groups did not differ statistically.

Table 4. Changes in the characteristics of yolk depending on packaging and storage temperature.

Trait	Time (Days)	Treatment				Total	SEM	Factor (p-Value)		
		RCB	FCB	RPB	FPB			B	T	B × T
YW (g)	0	15.50					0.236			
	14	17.87	17.02	16.96	17.18	17.80	0.551	*0.206*	*0.516*	*0.609*
	28	16.89	15.62	16.14	16.90	16.39	0.278	*0.646*	*0.634*	*0.072*
	42	17.17	17.52	17.42	16.58	17.15	0.160	*0.173*	*0.615*	*0.059*
EYP (%)	0	24.10					0.437			
	14	28.64	26.57	26.52	26.65	27.97	0.242	*0.134*	*0.686*	*0.719*
	28	27.48	25.22	25.95	26.28	26.23	0.457	*0.295*	*0.800*	*0.161*
	42	28.67 ^{b}	29.70 ^{b}	28.69 ^{b}	25.86 ^{a}	28.16	0.295	*0.019*	*0.001*	*0.000*
YC (pkt.)	0	12.10					0.376			
	14	11.55 ^{ab}	10.75 ^{a}	11.50 ^{ab}	11.70 ^{b}	11.38	0.130	*0.236*	*0.077*	*0.050*
	28	11.45 ^{ab}	11.15 ^{a}	11.95 ^{ab}	12.00 ^{b}	11.64	0.121	*0.593*	*0.005*	*0.455*
	42	10.05 ^{ab}	9.83 ^{a}	11.31 ^{b}	11.15 ^{ab}	10.53	0.197	*0.283*	*0.000*	*0.600*
YI	0	0.414					0.009			
	14	0.362 ^{a}	0.365 ^{a}	0.402 ^{b}	0.400 ^{b}	0.383	0.006	*0.949*	*0.002*	*0.814*
	28	0.275 ^{a}	0.279 ^{a}	0.390 ^{b}	0.385 ^{b}	0.331	0.009	*0.931*	*0.000*	*0.667*
	42	0.258 ^{a}	0.267 ^{a}	0.359 ^{b}	0.405 ^{c}	0.325	0.010	*0.340*	*0.000*	*0.000*
YpH	0	6.12					0.012			
	14	6.21	6.25	6.17	6.18	6.20	0.020	*0.585*	*0.221*	*0.673*
	28	6.46 ^{b}	6.32 ^{b}	6.35 ^{b}	6.06 ^{a}	6.30	0.034	*0.001*	*0.003*	*0.179*
	42	6.50 ^{b}	6.54 ^{b}	6.35 ^{ab}	6.25 ^{a}	6.42	0.030	*0.451*	*0.000*	*0.064*

^{a,b,c}–means within row differ significantly at $p \leq 0.05$; SEM—standard error of mean; RCB—cardboard box, room temperature (21 °C); FCB—cardboard box (5 °C), refrigeration temperature (5 °C), RPB—plastic box, room temperature (21 °C), FPB—plastic box, refrigeration temperature (5 °C); B—egg box type, T—temperature; YW—yolk weight, EYP—yolk proportion in egg weight, YC—colour, YI—yolk index, YpH—yolk pH.

The yolk shape index decreased during storage regardless of temperature or type of packaging. After 28 days of storage, it was found that eggs from groups stored in plastic boxes were characterized by significantly higher shape index values compared to those stored in cardboard ones.

The yolk pH was also significantly affected by time. After 28 days of the experiment, eggs from the FPB group had the lowest values, while the other study groups did not differ significantly.

In terms of egg quality, it was found that almost all whole egg traits (Table 2) were significantly influenced by temperature, type of packing as well as the interaction of both factors. Slightly different observations were made for egg content quality traits (Tables 3 and 4). Most of them remained significantly influenced by the storage temperature. Definitely fewer traits showed a dependence on the type of packaging and the interaction of both experimental factors.

3.2. Fatty Acids Profile

From the yolk samples taken at the time of analysis, the fatty acid composition was determined (Table 5) and their indices were calculated (Table 6). None of the saturated fatty acids (SFA) showed variation with temperature or type of packaging. Similar observations were also made for monounsaturated fatty acids.

Table 5. Fatty acid profile of egg yolk in relation to packaging and storage temperature.

Time (Days)	0	28				Total	SEM	Factor (*p*-Value)		
Treatment		RCB	FCB	RPB	FPB			B	T	B × T
SFA										
C14:0	0.320	0.308	0.348	0.322	0.308	0.321	0.018	*0.337*	*0.064*	*0.661*
C15:0	0.072	0.074	0.070	0.066	0.066	0.069	0.003	*0.806*	*0.185*	*0.425*
C16:0	23.370	19.334	23.512	22.320	22.384	22.184	0.954	*0.582*	*0.013*	*0.504*
C17:0	0.180	0.634	0.162	0.168	0.186	0.266	0.087	*0.439*	*0.716*	*0.057*
C18:0	6.724	5.408	6.524	6.700	6.806	6.432	0.296	*0.858*	*0.760*	*0.639*
C20:0	0.018	0.016	0.014	0.016	0.012	0.015	0.002	*0.630*	*0.923*	*0.772*
MUFA										
C14:1n5	0.068	0.132	0.092	0.074	0.064	0.086	0.014	*0.347*	*0.295*	*0.052*
C16:1n7	2.848	6.846	3.840	3.276	2.650	3.892	0.769	*0.747*	*0.092*	*0.017*
C18:1 n9 c and C18:1 n9 t	38.932	33.114	40.416	42.372	40.190	39.005	1.396	*0.625*	*0.300*	*0.228*
C20:1n9	0.246	0.178	0.224	0.216	0.214	0.216	0.010	*0.289*	*0.450*	*0.257*
C22:1n9	0.004	0.004	0.012	0.004	0.000	0.005	0.002	*0.709*	*0.120*	*0.184*
PUFA										
C18:2 n6 c and C18:2 n6 t	20.608	24.066	17.280	17.166	20.016	19.827	1.005	*0.987*	*0.920*	*0.048*
C18:3 n6 γ	0.078	4.250	0.092	0.078	0.090	0.918	0.832	*0.409*	*0.242*	*0.371*
C18:3 n3 α	0.610 ^{ab}	0.360 ^{a}	0.804 ^{b}	0.728 ^{ab}	0.596 ^{ab}	0.620	0.049	*0.259*	*0.674*	*0.026*
C20:2 n6	0.232	0.150	0.146	0.144	0.194	0.173	0.012	*0.239*	*0.268*	*0.537*
C20:3 n6	0.102	0.064	0.106	0.094	0.112	0.096	0.008	*0.030*	*0.139*	*0.270*
C20:4 n6	1.412 ^{ab}	0.938 ^{a}	1.258 ^{ab}	1.292 ^{ab}	1.494 ^{b}	1.279	0.064	*0.040*	*0.025*	*0.457*
C20:3 n3	0.008	0.004	0.012	0.008	0.004	0.007	0.002	*0.756*	*0.605*	*0.263*
C22:2 n6	0.040	0.046	0.054	0.060	0.058	0.052	0.004	*0.414*	*0.114*	*0.280*
C22:6 n3	0.000 ^{a}	0.024 ^{ab}	0.164 ^{ab}	0.182 ^{ab}	0.350 ^{b}	0.144	0.041	*0.128*	*0.092*	*0.859*

^{a,b}–means within row differ significantly at $p \leq 0.05$; RCB—cardboard box, room temperature (21 °C); FCB—cardboard box (5 °C), refrigeration temperature (5 °C), RPB—plastic box, room temperature (21 °C), FPB—plastic box, refrigeration temperature (5 °C); B—egg box type, T—temperature; C14:0—myristic acid, C15:0— pentadecanoic acid, C16:0—palmitic acid, C17:0—margaric acid, C18:0—stearic acid, C20:0—arachidic acid, C14:1 n5—tetradecenoic acid, C16:1 n7—palmitoleic acid, C18:1 n9 c and C18:1 n9 t— oleic acid, C20:1 n9—eicosenoic acid, C22:1 n9—erucic acid, C18:2 n6 c and C18:2 n6 t—linoleic acids (LA) cis and trans, respectively, C18:3 n6-γ—linolenic acid (GLA), C18:3 n3-α—linolenic acid (ALA), C20:2 n6—eicosadienoic acid, C20:3 n6—dihomo-γ-linolenic acid, C20:4 n6- arachidonic acid (AA), C20:3 n3–eicosatrienoic acid, C22:2 n6—docosadienoic acid, C22:6 n3—docosahexaenoic acid (DHA). SFA—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids.

Table 6. Fatty acid indexes of egg yolk depending on packaging and storage temperature.

Time (Days)	0	28				Total	SEM	Factor (*p*-Value)		
Treatment		RCB	FCB	RPB	FPB			B	T	B × T
SFA	30.680	31.386	30.630	29.588	29.762	30.409	0.287	*0.567*	*0.055*	*0.417*
MUFA	42.154	42.662	44.584	45.942	43.118	43.692	0.530	*0.699*	*0.507*	*0.086*
PUFA	23.090	22.114	19.916	19.752	22.914	21.557	0.594	*0.658*	*0.742*	*0.096*
n3	0.618 ^{ab}	0.514 ^{a}	0.980 ^{b}	0.918 ^{ab}	0.950 ^{b}	0.796	0.056	*0.031*	*0.086*	*0.053*
n6	22.472	21.600	18.936	18.834	21.964	20.761	0.590	*0.792*	*0.849*	*0.060*
n9	39.182	39.690	40.652	42.592	40.404	40.504	0.497	*0.643*	*0.298*	*0.221*
PI	29.178	38.570	26.967	26.959	31.726	30.680	2.496	*0.118*	*0.119*	*0.248*
AI	0.374	0.319	0.387	0.360	0.358	0.359	0.016	*0.595*	*0.016*	*0.694*
TI	0.880	0.747	0.864	0.824	0.823	0.827	0.037	*0.311*	*0.040*	*0.321*
DFA	71.968	70.184	71.024	72.394	72.838	71.682	0.344	*0.890*	*0.014*	*0.483*
HSFA	23.626	19.646	23.860	22.642	22.692	22.493	0.968	*0.558*	*0.012*	*0.499*
h/H	2.623	2.495	2.533	2.732	2.761	2.634	0.040	*0.714*	*0.019*	*0.958*

^{a,b}—means within row differ significantly at $p \leq 0.05$; RCB—cardboard box, room temperature (21 °C); FCB—cardboard box (5 °C), refrigeration temperature (5 °C), RPB—plastic box, room temperature (21 °C), FPB—plastic box, refrigeration temperature (5 °C); B—egg box type, T—temperature; SFA—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids, PI—peroxidizability index, AI—atherogenicity index, TI—thrombogenic index, DFA—desirable fatty acids, HFSA—hypercholesterolaemic saturated fatty acids, h/H—hypocholesterolaemic/hypercholesterolaemic ratio.

Among PUFAs, 3 polyunsaturated fatty acids were found to change with storage time. Among them, arachidonic acid (C20:4 n6) and docosahexaenoic acid (C22:6 n3) remained at significantly highest levels in egg yolks stored under refrigeration in plastic boxes compared to the other experimental groups.

Concerning fatty acid indices, significant differences were observed only in the case of the n3 group (Table 6). The highest content of n3 fatty acids was found in yolks of eggs stored in refrigerator (FCB and FPB). The values obtained for these groups did not differ significantly from those obtained for fresh eggs (0 days).

4. Discussion

Natural changes in the raw material of eggs are the result of biophysical and chemical changes taking place in the egg content from the moment when the eggs are laid. However, although time is one of the basic elements influencing changes in the quality of table eggs, factors related to the egg itself or environmental conditions are also important. Egg weight loss during storage occurs regardless of the environmental conditions or protective treatments applied [9,22–24]. At the same time, it should be noted that the intensity of these changes can be reduced by decreasing the intensity of gas exchange between the external environment and the egg contents. The most common practice is to moderate the storage temperature, which reduces the loss of egg mass. Several studies indicate the effectiveness of this method [8,25], which is also confirmed in our research. Interestingly, the type of packaging also significantly contributed to the inhibition of egg weight loss or air chamber deepening, allowing similar results for cardboard boxes for refrigeration temperature and plastic ones at room temperature. This was probably due to the difference in the structure of the material, thus greater access of air to eggs stored in cardboard boxes. These observations seem all the more relevant given that the legislation adopted in the EU reserves the refrigerated storage of eggs to the final consumer only. The use of suitable packaging, which is already available on the market, could therefore be an alternative to reduce the storage temperature of the egg raw material.

The reduction of the weight loss of eggs during storage also contributed to the inhibition of other changes in their quality. One of the basic characteristics analyzed in this respect is the air cell depth. The changes in this parameter observed in our study are similar to those noticed in previous studies [8]. At the same time, it should be emphasized that the limit value of 6 mm [6] was exceeded only in the case of eggs stored for 42 days at room temperature in cardboard boxes, while in the remaining cases, even the extended period of storage did not influence its deepening to a higher degree than assumed in the legislation.

Quality changes also affect particular elements of the egg content. With time, there is a loss of albumen mass both through evaporation and the diffusion that occurs from the albumen to the yolk [26]. These changes entail further changes, such as decreases in albumen height or related to Haugh units. Early studies [27] indicated that it is the loss of albumen mass that is one of the most important factors in changing its structure. At present, an equally important role is attributed to albumen alkalisation through the release of carbon dioxide as a result of carbonic acid dissociation [28], which in turn leads to the weakening of bonds within the ovomuccin-lysosome complex, which is one of the factors responsible for maintaining the correct structure of dense albumen. Our studies, and those presented by other authors, agree on the role of time in the occurrence of these changes, as well as proving that lowering the storage temperature can effectively reduce their intensity [7,29].

Quality changes during egg storage also affect the yolk. Both our own studies and reports by other authors indicate an increase in yolk mass by diffusion of water from the albumen [26], but also an increase in the proportion of egg mass or a decrease in the shape index value [30]. Since these changes adversely affect the quality of the raw materials obtained, it is necessary to inhibit these processes. One of the most popular methods in this respect is lowering the storage temperature, which at the same time is one of the most effective, which is confirmed by the study of Keener et al. [25], as well as the results

obtained by us. It should also be noted that the use of plastic boxes proved to be even more effective than lowering the temperature for eggs stored in classical cardboard boxes.

Apart from changes in quality characteristics and those affecting the technological value of eggs, their chemical composition may also change during storage. Since egg yolk is a valuable source of polyunsaturated fatty acids, lipid peroxidation during storage is a particularly important issue in this respect. The high UFA content in yolk makes it all the more vulnerable to oxidation [31]. With this in mind, it is necessary to ensure that the oxidative stability of the harvested eggs is as high as possible. Many works focus in this respect on the use of feed additives of antioxidant nature such as cinnamaldehyde [32] or plant extracts of thyme or oregano [33]. In our study, no similar additives were used and the study material was unified, so the variability observed was solely due to the storage methods used.

The available literature does not provide information on the effect of storage on changes in the fatty acid profile of egg yolks. Admittedly, some works indicate that dimaldehyde content rises with storage time and temperature [33], while according to other authors similar differences are almost imperceptible [34]. Although in our study the level of MDA was not analyzed, the observed differences in the concentration levels of arachidonic acid and ALA may indicate the occurrence of lipid oxidation [33]. Regardless of the background of this variation, it was found that apart from lowering the temperature, also the use of plastic boxes reduces the intensity of these changes.

The fatty acid profile provides valuable nutritional and health-promoting information, however, in terms of consumer safety of the eggs, fatty acid indices are considerably better. The most commonly assessed are the SFA/PUFA ratio and the much more accurate AI and TI, which determine indices of atherogenicity and thrombogenicity respectively [17].However, these indices are mostly mentioned in the context of changes occurring in eggs due to supplementation of birds with various lipid-like substances, e.g., natural vegetable oils [35,36].The number of studies analysing such changes over time is very limited. The results obtained in our study for fatty acid indices for term 0 are partly in line with those described by other authors, such as Attia et al. [37], who show that eggs originating from different purchase locations, were characterized by significant variability for this trait. These observations may explain the differences between the results of our study and those presented for control groups (i.e., birds fed a standard feed mixture) by other authors. For example, Omari et al. [38] found similar relationships between particular indices, but the results presented by them differ significantly from those obtained in our study. As the subject of the study was the type of packaging and storage temperature of the raw material, it should be noted that the only differences were observed in the case of PUFAs and especially the group of n3 acids, i.e., those susceptible to oxidation [39]. In the case of other indices, no significant differences were found, which may suggest that in the case of variation in the profile of fatty acids and their indices, storage is a factor of relatively minor importance in the case of shelled eggs.

5. Conclusions

Packaging types available on the market, apart from its marketing and eggs protection function, can also influence the quality and stability of the product during storage. Research has shown that the use of plastic boxes can help to maintain higher eggs quality during the storage period, even after a significant extension of the storage time.

The study showed that whole egg characteristics (weight, specific gravity, air cell depth) changed significantly less in groups stored at refrigeration temperature, but it was also found that a similar effect could be obtained at room temperature using plastic egg boxes. Despite the prolonged storage time and varied storage conditions, it was found that storage had little effect on the fatty acids profile and their indices, and the only change over time was observed for PUFAs (mainly n3), which content changed in the least in the group of eggs stored in plastic boxes and at reduced temperature.

Importantly, eggs stored in plastic boxes at room temperature had very similar results to those stored under refrigeration using conventional cardboard boxes. This gives real hope for their use on a wider scale as an alternative to refrigerated storage, which in the EU is reserved exclusively for the final consumer. This effect is probably related to the lower permeability of plastic boxes in comparison to cardboard ones, but detailed research work in this direction is necessary to verify this relationship.

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Dr inż. Tomasz Próchniak
Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13
20-950 Lublin

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Lublin, 15.11.2021

Dr inż. Tomasz Próchniak

Inż. Damian Spustek
Kierunek: Agroleśnictwo
Studia stac., II°
Wydział Agrobioinżynierii
Uniwersytet Przyrodniczy w Lublinie

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Lublin, 15.11.2021

Damian Spustek

Inż. Damian Spustek

Mgr inż. Karolina Wengerska
Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13
20-950 Lublin

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Lublin, 15.11.2021

Karolina Wengerska

Mgr inż. Karolina Wengerska

Dr hab. Justyna Batkowska prof. uczelni
Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13
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Dr hab. Justyna Batkowska prof. uczelni

Citric acid as a factor limiting changes in the quality of table eggs during their storage

Kamil Drabik, Justyna Batkowska,[1] Tomasz Próchniak, and Beata Horecka

Institute of Biological Basis of Animal Production, University of Life Sciences in Lublin, 20-950 Lublin, Poland

ABSTRACT The aim of the experiment was to evaluate the potential use of citric acid as a modifier of quality changes in table eggs during their storage. About 780 table hen eggs were collected on the same day. They were numbered individually and placed on trays 30 pcs on each. Control group (CA0) consisted of eggs unmodified with any additional substances. In experimental groups CA10 and CA15, eggshells were sprayed with the aqueous solution of citric acid (10 and 15% concentration, respectively). At the start of the experiment, only quality traits of eggs from the control group were analyzed. The remaining eggs were stored at 14°C and 70% RH (typical storage conditions). Their quality was evaluated after 7, 14, 21, and 28 d. The depth of the air cell, egg weight and specific gravity, traits of shell (permeability, strength, weight, thickness, density), and egg content (pH of yolk and albumen, Haugh units, yolk weight and color) were evaluated each time. The use of citric acid decreased the severity of qualitative changes. Citric acid–treated eggs demonstrated smaller weight loss, shallower air cell, higher structural albumen, less-intensive water diffusion from albumen to yolk indicating the improved resistance of the vitelline membrane. Owing to the fact that citric acid is accepted and recognized as a safe food preservative is a relatively cheap and available substance, it seems that it can be used to inhibit quality changes in table eggs during their storage.

Key words: eggs storage, pores sealing, eggshell structure

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INTRODUCTION

The basic quality of table eggs depends on a variety of factors (genotype, age, rearing system, nutrition, etc.). Regardless of the initial values of the eggs' quality characteristics, negative changes emerge over time. One of the fundamental transformations is the natural weight loss of eggs due to the gaseous exchange (water evaporation) between the egg content and the external environment. This process also affects other egg quality traits, including the air cell depth. These changes are all the more important as they form the basis of the current EU legislation. Commission Regulation (EC) No. 589/2008 (Commission Regulation (EC), 2008) specifies a minimum shelf-life for table eggs at 28 d and a maximum air cell depth of 6 mm. More importantly, the regulation stipulates that the refrigerated storage of eggs is reserved exclusively for final consumers.

Despite the confirmed positive results of cold storage in inhibiting the egg quality deterioration (Jin et al., 2010; Brodacki et al., 2019), this restriction makes it necessary to seek alternative solutions to this problem. Methods inhibiting the quality changes of table eggs may be divided into 2 main groups: the modification of the atmosphere in the storage container and the use of eggshell coating substances.

In case of MAP (modified atmosphere packaging), standard gas mixtures (Rocculi et al., 2009) as well as pure gases in high concentrations (Pasquali et al., 2012; Jin et al., 2019) have already been used for atmosphere modification. Studies carried out by Aygun and Sert (2013) have also shown the viability of extending storage time by means of vacuum packaging.

However, the use of atmospheric modifications requires specialized technological solutions. Therefore, substances coating the eggshell are used much more frequently. So far, the use encompassed both vegetable and mineral oils (Jirangrat et al., 2010; Ryu et al., 2011; Eke et al., 2013; Nongtaodum et al., 2013), chitosan and its emulsions with oils (Torrico et al., 2010, 2014; Jo et al., 2011), propolis (Copur et al., 2008), and many others. In general, eggshell coating results in the reduction of gaseous exchange (water evaporation) from the

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[1]Corresponding author: justyna.batkowska@up.lublin.pl

egg. According to Campo et al. (2000), the gaseous exchange between the egg content and external environment is almost 2 times greater than in reverse direction. Therefore, limiting the shell pores' permeability has the effect of inhibiting the fundamental changes in the eggs' quality during their storage, as confirmed by the aforementioned articles.

Apart from the eggshell becoming sealed by coating, we also considered changing the structure of the shell itself, which should reduce the gas exchange as well. Owing to the high calcium content in the eggshell, the use of acids to seal the shell pores seems plausible. As a result of the reaction between calcium and acid, the surface thickness of the shell will probably decrease. However, the pores should be filled with the products of this reaction. One such acid may be citric acid (*Acidum citricum*) which is a natural component of any living organism, where it plays an important role in the carbohydrates metabolism. In addition, in the ionic form, that is, citrate, it is an important indirect product in the Krebs cycle. As one of the fruit acids, citric acid is mainly found in citrus fruits such as lemons and oranges. However, the chief means of obtaining this preservative is by cultivating *Aspergillus niger* (Dhillon et al., 2013).

Importantly, hitherto studies have shown the effectiveness of citric acid in the disinfection of Japanese quail hatching eggs (He et al., 2020). At the same time, the authors observed a decrease in the thickness of eggshells indicating the occurrence of the reaction described above.

The aim of the experiment was to evaluate the potential use of citric acid as a substance inhibiting negative changes in the quality of table eggs during storage.

MATERIALS AND METHODS

Preliminary Experiment

The aim of the preliminary experiment was to establish the effective concentration of the experimental factor (citric acid, CA). The material consisted of hen eggs from Polbar breed, belonging to Polish genetic resources added to the World Watch List for Domestic Animal Diversity by the Food and Agricultural Organization and maintained at the Laura Kaufman Didactic and Research Station of Small Animals, belonging to the University of Life Sciences in Lublin (Poland) (Gryzińska et al., 2015). Fifty eggs were collected on the same day and divided into equal groups. Zero factor was used in the control group (CA0). In other groups (CA5, CA10, CA15, CA20), eggs were sprayed with the citric acid solution at a concentration of 5, 10, 15, and 20%, respectively. All aqueous solutions were prepared using percentage by weight (*w/v*) formula. After preparation, the pH of obtained solutions were measured (average of 3 samples). In particular groups, it amounted to 1.95, 1.80, 1.72, and 1.51, respectively. After natural drying, eggs were placed on trays (10 pcs each) in the blunt-end-up position and stored at room conditions (21°C; 45% of relative humidity) for 28 d.

Weekly changes of the egg weight and air cell depth were recorded. The analyses covered selected quality parameters such as eggshell strength (Instron 55 Mini apparatus), shell thickness (by micrometer, part of EQM—Egg Quality Measurement electronic set, TSS), albumen and yolk acidity (by pH meter with a combined glass electrode). The collected eggshells were subjected to a microscopic analysis using a stereoscopic microscope (Olympus SZX16, magnification 8 ×).

Main Experiment

The material for the main experiment consisted of 780 brown-shelled table hen eggs (Tetra SL, commercial stock, cage system), individually numbered and placed on trays 30 pcs on each. All eggs were collected and subjected to experimental procedure on the same day. The control group (CA0) consisted of eggs not coated with any additional substances. As experimental factor 2 the most effective concentrations of citric acid solution were chosen based on the results of preliminary study. In groups CA10 and CA15, eggshells were sprayed with the aqueous solution of citric acid at 10 and 15% concentration, respectively. The schema of the experiment is presented in Table 1. At the start of the experiment, quality traits of eggs from the control group were analyzed exclusively. The remaining eggs were stored at 14°C and 70% RH (typical storage conditions) and their quality was evaluated after 7, 14, 21, and 28 d.

The following experimental material characteristics were evaluated:

- whole egg—depth of the air cell (**ACD**, visually using the template), mass (EW, using an electronic scale with an accuracy of 0.01 g), proportions of morphological elements (in relation to egg weight: **YP**, yolk proportion in egg weight; **AP**, albumen proportion in egg weight; SP, shell proportion in egg weight),
- shell—weight (**SW**, using an electronic scale with an accuracy of 0.01 g), thickness (**ST**, by EQM micrometer screw, on the "equator"), strength (**SS**, Instron 55 Mini apparatus, along the long axis, blunt end up),
- albumen—weight (**AW**, using an electronic scale with an accuracy of 0.01 g), height (**AH**, EQM detector), pH (**ApH**, pH meter with a combined glass electrode),
- yolk—weight (**YW**, using an electronic scale with an accuracy of 0.01 g), color (**YC**, using 16-points Roche, DSM), index (**YI**, as ratio of its height and diameter), pH (**YpH**, pH meter with a combined glass electrode).

Table 1. Schema of the main experiment (number of eggs).

	Treatments		
		Sprayed with citric acid	
	Control	10%	15%
Time (days)	CA0	CA10	CA15
0	60		
7	60	60	60
14	60	60	60
21	60	60	60
28	60	60	60
Total	360	240	240

Based on the obtained data, additional quality parameters were calculated, such as

- weight loss (**WL**), using an electronic scale with an accuracy of 0.01 g,
- specific mass of eggs (**ESG**), as per Archimedes' principle based on egg weight measured in the air ("dry egg weight") and in the water ("wet egg weight"),
- shell density (**SD**) calculated in accordance with the formula proposed by Shafey (2002),
- water vapor permeability (ESC, egg shell conductance) calculated in accordance with the method proposed by Ar et al. (1974) with adjustment to eggs storage conditions (temperature, humidity), expressed in SI units,
- Haugh units (**HU**, according to Williams, 1992).

The samples collected from equator parts of shells (surface and cross-section) were subjected to a microscopic analysis. Micrographs were taken using a scanning electron microscope FEI QUANTA 200 SEM (Hillsboro, OR) operated at 25 kV.

The obtained data were statistically analyzed using the SPSS 24.0 statistical package (IBM Corp., 2016; IBM Corporation, 2016). The normality of data distribution was tested using Shapiro-Wilk test. The groups in preliminary experiment were compared using the one-way analysis of variance with Tukey's post hoc test at the significance level $P \leq 0.05$. In the main research, the two-factorial analysis of the model including the influence of time (**T**) and citric acid coverage (**CA**) as well as the interaction between both factors was conducted.

RESULTS

Preliminary Experiment

On the basis of the preliminary study (Table 2), no significant differences in egg weight were found regardless of the experimental group. Significant differences were found in the range of egg weight loss in the preliminary study, with the highest value being observed for eggs from the control group and the lowest recorded for 10 and 15% citric acid concentrations. Importantly, eggs coated with 5% citric acid did not differ significantly from those in the control group. A similar trend was observed in the analysis of the change in air cell depth. After 28 d of storage, the highest values, exceeding the limits set by the Commission Regulation (EC) No. 589/2008, were found in the CA0 group. It should also be noted that eggs from groups CA5 and CA20 did not differ significantly from the control group. On the other hand, for concentrations of 10 and 15%, these values were significantly lower.

No significant differences in eggshell quality parameters (strength and thickness) were noted. This observation is important because it indicates that there is no negative impact of the applied experimental factor. In addition, changes in the pH of morphological elements indicate only a surface reaction of citric acid, regardless of its concentration.

An additional element of the work involved the photography of shells' surface treated with various concentrations of citric acid with the use of stereoscopic microscope (Figure 1). It was observed that eggs treated with the acid (especially 10 and 15%) had a higher sheen and their surface looked smoother. These observations confirm the occurrence of reactions between shell components (mainly calcium) and the experimental factor. It was also found that the eggshells of CA20 group had marks of minor damage, which may suggest an excessively high concentration of the acid.

The results obtained in the preliminary experiment showed that the most effective concentration of citric acid seems to be 10 or 15%. It does not deteriorate the eggshell quality as well as contributes to egg content preservation.

Main Experiment

The results concerning the quality characteristics of the whole egg are presented in Table 3. A significant influence of time was found in all groups covered by the

Table 2. The results of the preliminary experiment, characteristics of whole egg depending on the experimental group.

Characteristic	T (days)	CA0	CA5	CA10	CA15	CA20	SEM
EW (g)	0	36.96	36.69	36.85	35.84	37.73	0.295
	7	36.06	36.24	36.22	35.46	37.21	0.283
	14	35.40	35.93	35.73	35.21	36.84	0.277
	21	34.54	35.51	35.12	34.89	36.38	0.275
	28	33.94	35.21	34.70	34.67	36.06	0.278
WL (%)	0-28	8.168^{y}	$5.013^{x,y}$	3.559^{x}	4.556^{x}	4.297^{x}	0.397
ACD (mm)	7	3.30^{y}	$2.56^{x,y}$	2.13^{x}	$2.44^{x,y}$	$2.50^{x,y}$	0.122
	14	4.60	3.75	3.38	3.81	4.11	0.145
	21	6.10^{y}	$4.94^{x,y}$	3.81^{x}	4.31^{x}	4.44^{x}	0.183
	28	7.00^{y}	$5.75^{x,y}$	4.81^{x}	5.19^{x}	$5.39^{x,y}$	0.210
SS (N)	28	37.90	41.54	43.19	46.59	41.24	1.817
ST (mm)		0.301	0.291	0.294	0.302	0.287	0.003
ApH		9.35	9.32	9.23	9.33	9.30	0.018
YpH		7.11	7.17	6.95	7.17	6.21	0.214

^{x,y}means within the row differ significantly at $P \leq 0.05$.

Abbreviations: ACD, air cell depth; ApH, albumen pH; CA, citric acid; EW, egg weight; SS, shell strength; ST, shell thickness; WL, weight loss; YpH, yolk pH.

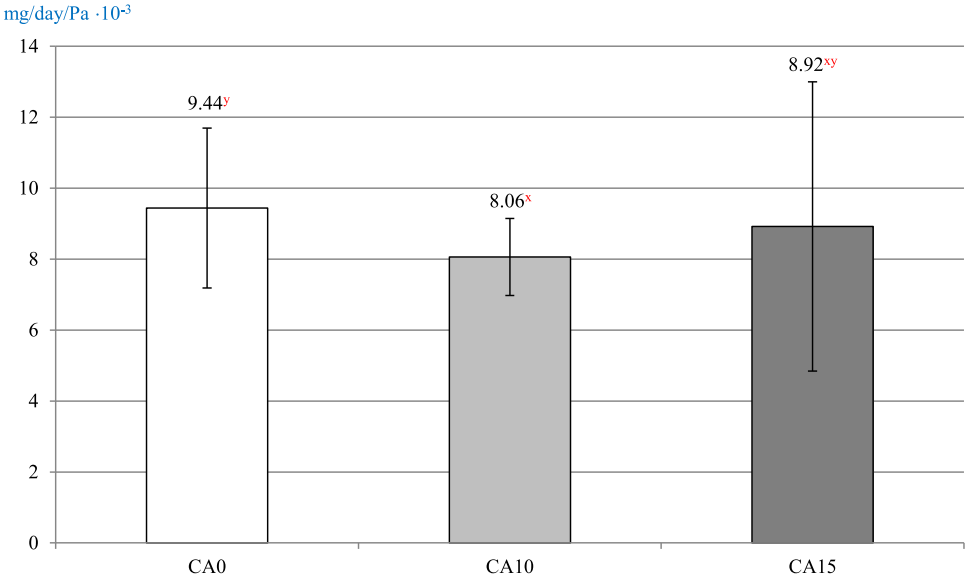

Figure 1. The eggshell permeability after 28 d of storage depending on the specific concentration of citric acid applied on the shell, ^{x,y} means within the row differ significantly at $P \leq 0.05$.

experiment. One of the fundamental changes in the quality of eggs during the storage is their weight loss. The first significant differences were observed as early as after 14 d of the experiment, with the highest weight loss being observed in eggs from the control group, and the lowest in those sprayed with 10% citric acid solution. CA15 group did not differ significantly from the other groups included in the experiment. After 28 d, it was found that eggs from the control group were characterized by the highest weight loss. The experimental groups showed significantly lower values of this parameter and did not differ from each other. It should also be noted that WL was influenced by both factors (time and citric acid) as well as their interactions. The ESG was significantly influenced by time and the experimental factor, with no interaction between them; however, no differences were observed between groups at the end of the study.

The air cell depth changed during egg storage. The first time-specific differences between the groups were observed after merely 7 d of the trial. On both the 14th and 21st day of storage, the eggs from the CA0 group were characterized by significantly deeper air cell compared with both experimental groups. Interestingly, no significant differences between the groups were found after 28 d of storage. As in the case of WL,

Table 3. Characteristics of the whole egg depending on the specific concentration of citric acid applied on the shell.

		Group					Factors		
Characteristic	Time (days)	CA0	CA10	CA15	Total	SEM	CA	T	CA × T
EW (g)	0	60.76	61.53	60.82	61.04^{c}	0.402	[1]	[1]	-
	7	59.29	60.88	60.22	$60.04^{b,c}$	0.399			
	14	57.65	59.51	58.73	$58.67^{a,b}$	0.391			
	21	57.07	59.21	58.41	58.56^{a}	0.398			
	28	56.53^{x}	58.88^{y}	$58.05^{x,y}$	58.18^{a}	0.397			
ESG (g/cm^3)	0	1.088			1.088^{e}	0.004	[1]	[1]	-
	7	1.071^{x}	1.079^{y}	1.079^{y}	1.076^{d}	0.001			
	14	1.069	1.073	1.074	1.072^{c}	0.001			
	21	1.058^{x}	1.066^{y}	1.070^{y}	1.064^{b}	0.001			
	28	1.054	1.055	1.052	1.054^{a}	0.001			
WL (%)	0–7	1.085	1.019	0.991	1.032^{b}	0.026	[1]	[1]	[1]
	7–14	2.774^{y}	2.247^{x}	$2.559^{x,y}$	2.527^{c}	0.075			
	14–21	1.012^{y}	0.512^{x}	0.477^{x}	0.657^{a}	0.069			
	21–28	0.952^{y}	0.558^{x}	0.625^{x}	0.712^{a}	0.033			
	0–28	5.703^{y}	4.278^{x}	4.551^{x}	4.844	0.126			
ACD (mm)	7	2.683^{z}	1.450^{x}	1.733^{y}	1.955^{a}	0.086	[1]	[1]	[1]
	14	4.093^{y}	2.383^{x}	2.517^{x}	2.998^{b}	0.115			
	21	4.567^{y}	3.650^{x}	3.383^{x}	3.867^{c}	0.102			
	28	4.700	4.400	4.550	4.517^{d}	0.082			

^{x,y}means within the row differ significantly at $P \leq 0.05$; ^{a,b} means within the column differ significantly at $P \leq 0.05$.

Abbreviations: ACD, air cell depth; CA, citric acid; ESG, egg specific gravity; EW, egg weight; T, time; WL, weight loss.

[1]The influence of factor significant at $P \leq 0.05$.

a significant influence of both experimental factors and their interactions was found.

Eggshell permeability was also analyzed (Figure 1). It was found that the highest value of this parameter characterized eggs from the control group (P = 0.032), with significantly lower values for CA10 and CA15 groups.

The results of the other eggshell quality analysis are presented in Table 4. The experiment revealed that the application of citric acid did not deteriorate the shell's strength. Moreover, after 7 d of storage, the strength of eggshells from groups CA10 and CA15 was significantly improved in comparison with those from group CA0. However, these observations were not confirmed at the end of the trial (after 28 d).

No significant differences were observed in the weight of the eggshell, as well as its proportion in the total egg weight, regardless whether citric acid was used or concerning its specific concentration.

The thickness and density of the shell was observed to be significantly influenced by experimental factors and their interactions (P = 0.005). It was found that citric acid at a concentration of 15% reduced the thickness of the shell while increasing its density comparing to both other groups.

Storage time significantly affected all the characteristics of albumen quality (Table 5). The albumen height differed significantly between treatments after 21 d of egg storage. The highest albumen was noted in eggs from CA10 group, but it did not differ from the control one. Lower values were recorded for the CA15 group. This relation was not confirmed after 28 d of egg storage. Taking into account the relation between albumen height and Haugh units, the same effect as observed for Haugh's unit.

A significant effect of storage time was also proved by the range of albumen weight and its percentage proportion in the egg weight. However, no variability between experimental groups was found within the individual time points.

Groups did not differ significantly with respect to changes in the albumen pH during storage. However, a significant influence of time and interactions of experimental factors on changes in the albumen pH was observed.

Similarly as in the case of egg albumen, the quality characteristics of yolk (Table 6) were also determined by storage time. However, no significant differences were noted in its weight or percentage share in the egg weight regardless of the experimental group.

Significant differences in the yolk shape index were demonstrated as early as after 14 d of the experiment. Higher values of this parameter characterized eggs treated with citric acid. On the 21st day of the experiment, no differences between CA0 and CA10 groups were registered. However, after 28 d of storage, differences between the control and experimental groups were statistically significant with higher YI values for the latter ones.

Table 4. Characteristics of the eggshells depending on the specific concentration of citric acid applied on the shell.

Characteristic	Time (days)	Group				SEM	Factors		
		CA0	CA10	CA15	Total		CA	T	CA × T
SS (N)	0	58.54			58.54^{a}	2.516	-	[1]	[1]
	7	53.65^{x}	61.82^{y}	61.10^{y}	$58.86^{a,b}$	1.116			
	14	60.35	61.88	62.03	61.42^{b}	0.469			
	21	61.18	62.37	61.27	61.61^{b}	0.438			
	28	59.55	56.63	58.33	58.17^{a}	1.520			
SW (g)	0	8.193			8.193^{b}	0.152	-	-	-
	7	7.750	7.987	7.913	$7.883^{a,b}$	0.078			
	14	7.611	7.817	7.793	7.740^{a}	0.113			
	21	7.898	7.938	7.947	$7.928^{a,b}$	0.083			
	28	7.805	7.830	7.693	$7.776^{a,b}$	0.070			
SP (%)	0	13.48			13.48^{b}	0.186	-	[1]	-
	7	12.83	13.04	13.11	$12.99^{a,b}$	0.117			
	14	12.69	13.03	12.90	12.87^{a}	0.169			
	21	13.29	13.17	13.40	$13.29^{a,b}$	0.093			
	28	13.30	13.20	13.20	$13.23^{a,b}$	0.086			
ST (mm)	0	0.332			$0.332^{b,c}$	0.008	[1]	[1]	[1]
	7	0.329^{x}	$0.336^{x,y}$	0.343^{y}	$0.336^{b,c}$	0.005			
	14	0.324^{x}	0.361^{y}	0.357^{y}	0.347^{c}	0.004			
	21	0.282^{x}	$0.303^{x,y}$	0.322^{y}	0.302^{a}	0.005			
	28	0.323^{y}	0.326^{y}	0.299^{x}	$0.316^{a,b}$	0.003			
SD (g/cm^3)	0	3.41			3.41^{b}	0.097	[1]	[1]	[1]
	7	3.46^{y}	3.24^{x}	3.19^{x}	$3.30^{a,b}$	0.031			
	14	3.20	2.99	3.01	3.07^{a}	0.048			
	21	4.14^{y}	$3.69^{x,y}$	3.47^{x}	3.78^{c}	0.086			
	28	3.40^{x}	3.35^{x}	3.63^{y}	$3.46^{b,c}$	0.035			

^{x,y}means within the row differ significantly at $P \leq 0.05$; ^{a,b} means within the column differ significantly at $P \leq 0.05$.

Abbreviations: CA, citric acid; SD, shell density; SP, shell proportion in egg weight; SS, shell strength; ST, shell thickness; SW, shell weight; T, time.

[1]The influence of factor significant at $P \leq 0.05$.

Table 5. Characteristics of the egg albumen depending on the specific concentration of citric acid applied on the shell.

		Group					Factors		
Characteristic	Time (days)	CA0	CA10	CA15	Total	SEM	CA	T	CA × T
AH (mm)	0	8.22			8.22^{c}	0.198	-	[1]	-
	7	5.69	5.44	5.74	5.62^{b}	1.433			
	14	4.94	4.90	5.48	5.11^{b}	0.110			
	21	4.52^{y}	4.63^{y}	4.37^{x}	4.51^{a}	0.094			
	28	4.11	4.22	4.11	4.15^{a}	0.095			
HU	0	90.9			90.9^{c}	1.174	-	[1]	-
	7	73.4	70.8	74.2	72.8^{b}	0.827			
	14	67.5	66.7	71.3	68.5^{b}	0.948			
	21	64.2^{y}	65.7^{y}	62.1^{x}	64.0^{a}	0.973			
	28	60.3	64.2	59.2	61.2^{a}	1.593			
AW (g)	0	37.27			37.27^{b}	0.734	-	[1]	-
	7	35.98	36.47	35.88	$36.11^{a,b}$	0.373			
	14	36.45	36.40	36.06	$36.30^{a,b}$	0.405			
	21	35.26	35.22	34.78	35.09^{a}	0.336			
	28	34.89	34.86	34.87	34.87^{a}	0.378			
AP (%)	0	61.15			61.15^{b}	0.531	-	[1]	-
	7	59.47	59.34	59.19	59.33^{a}	0.285			
	14	59.9	60.53	59.59	$60.01^{a,b}$	0.367			
	21	59.81	58.87	59.61	59.43^{a}	0.392			
	28	58.79	58.32	58.62	58.58^{a}	0.265			
ApH	0	8.25			8.25^{a}	0.039	-	[1]	[1]
	7	8.92	9.02	8.97	8.97^{b}	0.020			
	14	9.09	9.03	9.02	9.05^{b}	0.014			
	21	9.09	9.09	9.09	9.09^{b}	0.006			
	28	9.11	9.09	9.11	9.10^{b}	0.006			

[x, y]means within the row differ significantly at $P \leq 0.05$; [a, b] means within the column differ significantly at $P \leq 0.05$.

Abbreviations: CA, citric acid; AH, albumen height; AP, albumen proportion in egg weight; ApH, pH of albumen; AW, albumen weight; T, time.

[1]The influence of factor significant at $P \leq 0.05$.

Table 6. Characteristics of the egg yolk depending on the specific concentration of citric acid applied on the shell.

		Group					Factors		
Characteristic	Time (days)	CA0	CA10	CA15	Total	SEM	CA	T	CA × T
YW (g)	0	15.37			15.37^{a}	0.267	-	[1]	-
	7	15.88	15.74	15.70	15.77^{b}	0.141			
	14	16.65	16.42	16.55	$16.54^{a,b}$	0.160			
	21	16.87	16.50	16.59	16.65^{b}	0.153			
	28	17.13	16.72	16.72	$16.86^{a,b}$	0.272			
YP (%)	0	25.37			25.37^{a}	0.525	-	[1]	-
	7	27.70	27.62	27.70	27.67^{b}	0.246			
	14	27.41	26.44	27.51	27.12^{b}	0.291			
	21	26.90	27.96	26.99	27.28^{b}	0.383			
	28	27.91	28.48	28.20	28.20^{b}	0.263			
YI	0	44.47			44.47^{c}	0.579	-	[1]	-
	7	41.91	40.33	42.99	41.74^{b}	0.725			
	14	38.11^{x}	39.00^{y}	40.18^{y}	39.10^{b}	0.373			
	21	37.53^{x}	37.52^{x}	38.37^{y}	37.81^{b}	0.376			
	28	33.99^{x}	36.53^{y}	36.02^{y}	35.51^{a}	0.334			
YC (pts)	0	13.48			13.84^{b}	0.162	-	[1]	-
	7	12.80	12.20	12.63	12.54^{a}	0.122			
	14	12.46	12.14	12.30	12.30^{a}	0.088			
	21	12.53	12.55	12.23	12.44^{a}	0.087			
	28	12.50^{y}	$12.13^{x,y}$	11.83^{x}	12.15^{a}	0.107			
YpH	0	6.29			6.29^{a}	0.013	-	[1]	-
	7	6.37^{y}	6.35^{y}	6.26^{x}	$6.33^{a,b}$	0.013			
	14	6.38	6.36	6.39	6.38^{b}	0.013			
	21	6.42^{x}	6.58^{y}	6.46^{x}	6.49^{c}	0.017			
	28	6.54	6.48	6.51	6.51^{c}	0.021			

[x,y]means within the row differ significantly at $P \leq 0.05$; [a,b] means within the column differ significantly at $P \leq 0.05$.

Abbreviations: CA, citric acid; T, time; YC, yolk color; YI, yolk index; YP, yolk proportion in egg; YpH, pH of yolk; YW, yolk weight.

[1]The influence of factor significant at $P \leq 0.05$.

As far as the yolk color is concerned, significant differences between the groups were noted. The significantly darkest yolk color after 28 d of egg storage characterized eggs from group CA0, whereas the brightest one was recorded for eggs from CA15 group.

The yolk pH was determined by the time of egg storage. However, significant differences between the experimental groups were recorded exclusively after 7 and 21 d of storage. Eggs from CA15 group were characterized by lower pH, with higher values recorded for CA10 group.

The scanning electron microscopy technique (Figure 2) revealed that pores were closed in eggshells treated with citric acid. In addition, in the case of eggshells from CA15 group, a higher integrity of the surface layer was found. Figure 3 shows the effect of various concentrations of citric acid on the eggshell surface and its cross-section. On the CA0 micrograph, the permeable eggshell pore is visible in the cross-section. The CA10 demonstrates the eggshell pore sealed at the external surface due to the effect of citric acid, whereas CA15, the changes in eggshell integrity.

DISCUSSION

Storage time is one of the fundamental factors affecting the eggs' quality deterioration (Samli et al., 2005; Brodacki et al., 2019). One of the main changes is the loss of egg weight due to water evaporation. Therefore, the reduction of gas exchange (water evaporation) allows the process of the eggs' "aging" to be inhibited.

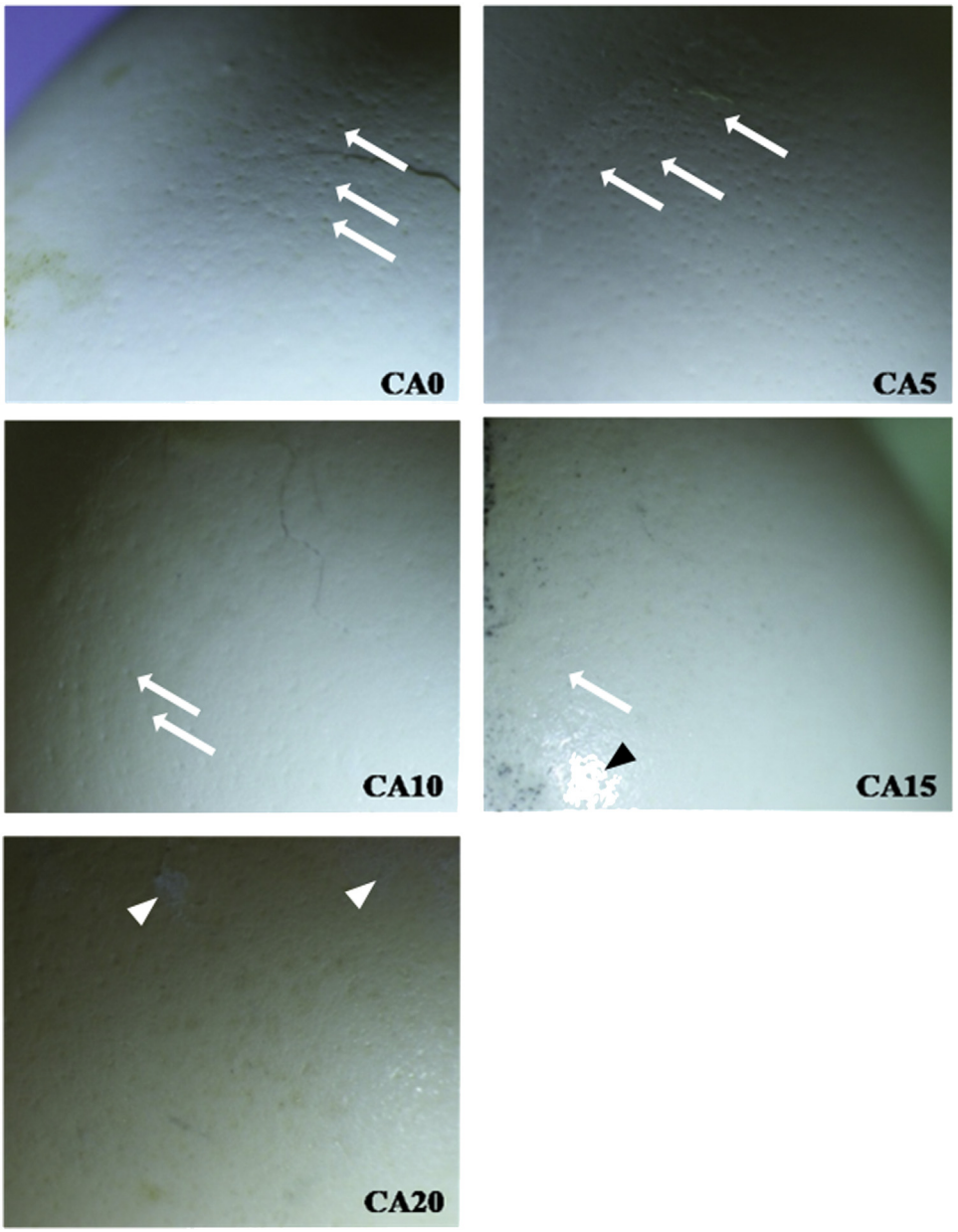

Figure 2. The effect of various concentrations of citric acid on the eggshell surface (magnification 8 ×). CA0, control group; CA5, CA10, CA15, CA20, the concentration of citric acid at 5, 10, 15, and 20%, respectively; CA0 and CA5, well visible shell pores without visible changes (white arrows); CA10, pores are shallower (white arrows); CA15, almost no visible pores (white arrow) and visible smoothing of the shell surface (black arrow head); CA20, the citric acid salts visible as the form of white powder (white arrow heads).

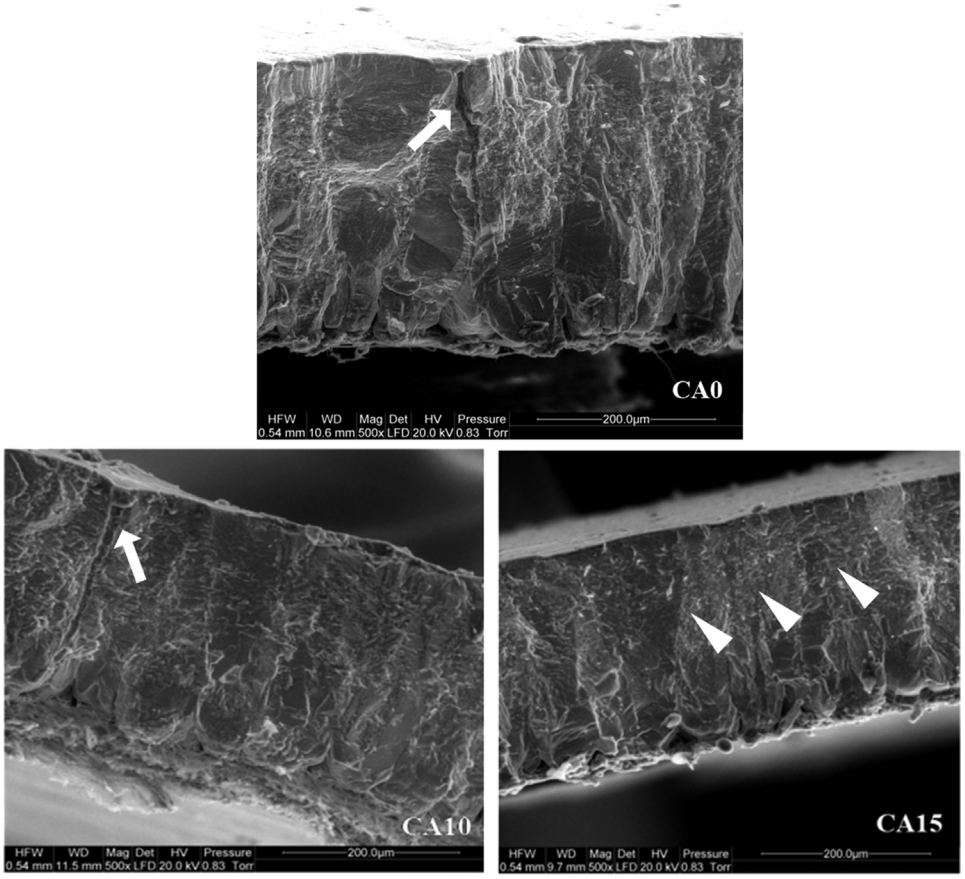

Figure 3. The effect of various concentrations of citric acid on the eggshell surface (scanning microscope). CA0, control group; CA10, CA15, the concentration of citric acid at 10 and 15%, respectively; CA0, the permeable eggshell pore visible in cross-section (white arrow); CA10, the eggshell pore sealed at the external surface due to the effect of citric acid (white arrow); CA15, visible changes in the integrity of the eggshell (white arrow heads).

This is confirmed by the results of own research and data presented by other authors (Caner, 2005; Jo et al., 2011). Pires et al. (2019) demonstrated that eggshells coating with substances of various origin (mineral oil or rice protein) reduces the weight loss. In addition, authors showed that the effectiveness of rice protein depended on its dose. The type of the substance used was an important inhibitor in this respect. The data presented by Caner and Yüceer (2015) showed significant differences in egg weight loss during 28 d of storage depending on the substance such as whey protein isolate, whey protein concentrate, zein and shellac. The best results were obtained after the use of shellac. However, it should be noted that these substances covered only the surface of the eggshell, while in own research the experimental agent reacted with it. Owing to the high calcium content in the eggshell, citric acid reacted with it giving citrates. This led to a change in the outer structure of the eggshell and to seal off the shell pores with citric acid salts.

The egg weight loss during storage also affects the depth of the air cell. In previous studies (Drabik et al., 2018), as well as in this research, a positive effect of coating eggshells with preserving agents was found to limit the air cell deepening during eggs' storage. This dependence is directly related to the water permeability of the shell, which both in our own research as well as in the aforementioned study was reduced by shells being coated with substances that seal the pores.The eggshell has a natural mucine layer to protect the eggs from microbial penetration (De Reu et al., 2006), but as the time passes from egg laying, the eggshell pores become unsealed (Rodrigez - Navarro et al., 2013). At the same time, studies show that the egg washing procedure limited the thickness of the mucin layer, which, however, did not significantly affect the depth of the air chamber during egg storage (Liu et al., 2016). The Rodrigez - Navarro et al. (2013) study showed that eggshell cuticle is composed of 2 basic layers: the outer layer, rich in proteins, and the inner one, made of sulfated polysaccharides and phosphates. Citric acid, as a weak organic acid, should not react with any of the cuticle components, even in relatively high concentrations like those used in our study. It can therefore be assumed that the action of the acid focused on the inorganic eggshell layer, whereas the mucine layer was only affected to the extent resulting from the test substance application procedure (spraying).

Although the substance used reacts with shell-building elements, no significant quality deterioration was found. In terms of the shell quality, one the most important features is its strength. Jones and Musgrove (2005) demonstrated that storage time does not significantly affect the shell strength, which was also confirmed by own research. However, it should be noted that

certain methods of limiting changes in the eggs' quality during the storage may have a negative impact on this parameter. For example, it has been shown that the use of vacuum packing (Aygun and Sert, 2013) can cause shell cracking, while some of the substances increased shells' strength (Caner and Canzis, 2008; Biladeau and Keener, 2009). In the case of own research, the absence of significant differences between the groups is a positive effect as it confirms the surface action of citric acid without damaging the shell structure.

Storage time of table eggs strongly affects the albumen quality, which is confirmed by studies by Samli et al. (2005), Brodacki et al. (2019), as well as by own research. One of the fundamental qualitative changes during the storage of table eggs is a decrease in the height of dense albumen and the associated number of Haugh units. Studies have shown a relationship between ovomucin and albumen height, which changes with storage time. In accordance with the data presented by Wang et al. (2019), there is a negative correlation between the content of ovomucin and pH. Considering the above, it may be concluded that changes in the albumen alkalinity will significantly reduce the value of other egg quality assessment parameters. Alkalization of albumen is a natural phenomenon associated with the release of carbon dioxide from egg content (Monira et al., 2003). Therefore, the use of sealing compounds allows limiting the loss of CO_2 from the egg content, which contributes to maintaining a higher albumen quality. This relationship is confirmed by numerous works (Biladeau and Keener, 2009; Pires et al., 2020), as well as by own research, where significant differences in albumen height and Haugh units after 21 days of storage were noted. At the same time, it should be noted that the use of citric acid had no direct effect on the albumen pH at the beginning of the experiment, which only confirms its surface effect. The subsequent variability is the effect of limiting the possibility of releasing carbon dioxide from the egg content by limiting the permeability of the shell pores.

During egg storage, the weight of yolk increases because of the diffusion of water from albumen to yolk (Menezes et al., 2012). This process leads additionally to the change of yolk color and shape index. In our own research, as well as in studies presented by other authors using chitosan, whey protein concentrate, soybean oil (Wardy et al., 2010), and propolis (Copur et al., 2008), an increase in yolk mass during egg storage was found. It is noteworthy that the use of a protective factor inhibited the increase of this characteristic. The yolk shape index was also reduced. The use of citric acid allowed inhibiting this process, which is indicated by significantly higher values after 28 d of storage in the CA10 and CA15 groups. A similar effect can be obtained using other shell coating substances such as chitosan in conjunction with organic acids (Caner and Cansiz, 2007) or soybean protein isolate in conjunction with montmorillonite (Xu et al., 2017). Similarly to changes in albumen quality, the traits of yolk changed not directly as a result of citric acid, but through its effect on the permeability of the eggshell pores. Limiting weight loss, release of carbon dioxide and water vapor from the egg content also contributed to limiting changes of yolk traits, so citric acid inhibited changes in the quality of this egg element only indirectly through direct action on the eggshells.

CONCLUSION

The use of citric acid led to a reduction of qualitative changes in eggs demonstrated by reducing the weight loss, shallower air cell, higher structural albumen, less-intensive water diffusion from albumen to yolk indicating the improved resistance of vitelline membrane.

Owing to the fact that citric acid is accepted and recognized as a safe preservative and is a relatively cheap and available substance, it seems that it can be used to limit the quality changes in table eggs during their storage.

Because of the protective effect and the lack of damage signs of the eggshell surface, it seems that the recommended concentration of citric acid used as a coating factor during eggs storage may be 10%.

DISCLOSURES

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 article.

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Dr inż. Tomasz Próchniak
Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13
20-950 Lublin

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Lublin, 15.11.2021

Dr inż. Tomasz Próchniak

Dr hab. Justyna Batkowska prof. uczelni
Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13
20-950 Lublin

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Lublin, 15.11.2021

Dr hab. Justyna Batkowska prof. uczelni

Dr inż. Beata Horecka
Instytut Biologicznych Podstaw Produkcji Zwierzęcej
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13
20-950 Lublin

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Lublin, 15.11.2021

Beata Horecka

Dr inż. Beata Horecka