



Uniwersytet Przyrodniczy w Poznaniu
Wydział Nauk o Żywności i Żywieniu

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**Ocena wpływu warunków przechowywania na jakość
i stabilność oksydacyjną mięsa dzika i sarny**

Evaluation of the effect of storage conditions on the quality and oxidative
stability of wild boar and roe deer meat

Rozprawa doktorska w dziedzinie nauk rolniczych
w dyscyplinie technologia żywności i żywienia
Doctoral dissertation in the field of agricultural sciences
in the discipline of food science and nutrition

Promotor:

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Streszczenie

Ocena wpływu warunków przechowywania na jakość i stabilność oksydacyjną mięsa dzika i sarny

Mięso i przetwory mięsne stanowią ważną część diety ludzi na całym świecie. W obliczu rosnącej populacji światowej, kwestia zapewniania zdrowej i bezpiecznej żywności staje się jednym z najważniejszych wyzwań przemysłu mięsnego. Istotne jest więc monitorowanie jakości i bezpieczeństwa produktów przeznaczonych do spożycia. Zagrożenie stanowią m.in. procesy utleniania białek, które odgrywają kluczową rolę w kształtowaniu jakości mięsa i produktów mięsnych. Procesy oksydacyjne mogą prowadzić do zmian w smaku, zapachu, teksturze, barwie, wodochłonności oraz wartości odżywczej mięsa i produktów mięsnych. Spożywanie utlenionych białek może przyczyniać się do osłabienia układu odpornościowego, nerwowego i sercowo-naczyniowego, a także może być również związane z rozwojem chorób przewlekłych, takich jak nowotwory. W kontekście poszukiwania zrównoważonych źródeł żywności, dziczyzna zyskuje na znaczeniu, ze względu na swoje wysokie walory odżywcze oraz naturalne pochodzenie.

Celem badań było zbadanie wpływu metody i czasu przechowywania na właściwości fizykochemiczne i stabilność oksydacyjną mięsa dzika europejskiego (*Sus scrofa scrofa*) oraz sarny europejskiej (*Capreolus capreolus*). Badania obejmowały przechowywanie mięśni m. *longissimus thoracis et lumborum* (LTL), m. *biceps femoris* (BF) i m. *vastus lateralis* (VL) w warunkach próżniowych (VAC), w zmodyfikowanej atmosferze (MAP), oraz w komorze do sezonowania mięsa (DRY-AGED) przez 21 dni.

Wykazano, że mięso dzika i sarny charakteryzuje się wysoką zawartością białka i niską zawartością tłuszczu oraz korzystną kompozycją składników mineralnych. Znaczący wpływ na procesy oksydacyjne i agregację białek mają metody przechowywania, co z kolei negatywnie wpływa na jakość mięsa. Wysokotlenowe pakowanie MAP (80% O₂/20% CO₂) sprzyja intensywnemu utlenianiu białek i lipidów, co przekłada się na pogorszenie kruchości i barwy mięsa. Wykazano również, że wysoka zawartość tlenu istotnie wpływa na zdolność białek do agregacji poprzez tworzenie wewnątrzcząsteczkowych wiązań dwusiarczkowych. Najbardziej podatne na agregację były titina i miozyna, zidentyfikowano również białka odpowiadające na stres oksydacyjny.

Istnieje potrzeba badań nad mechanizmami utleniania białek w mięsie oraz nad wpływem tych procesów na stabilność oksydacyjną, funkcjonalność i wartość odżywczą produktów mięsnych. Rozprawa dostarcza istotnych informacji na temat wpływu metod przechowywania na jakość dziczyzny, co może przyczynić się do poprawy strategii przetwarzania i przechowywania tego surowca w przemyśle mięsnym.

Słowa kluczowe: dziczyzna, przechowywanie mięsa, oksydacja białek i lipidów, agregacja białek, proteomika.

Summary

Evaluation of the effect of storage conditions on the quality and oxidative stability of wild boar and roe deer meat

Meat and meat products are an important part of the diet of people all over the world. With a growing world population, the issue of ensuring healthy and safe food is becoming one of the meat industry's most important challenges. It is therefore important to monitor the quality and safety of products for consumption. Threats include protein oxidation processes, which play a key role in shaping the quality of meat and meat products. Oxidative processes can lead to changes in the taste, odour, texture, colour, water absorption and nutritional value of meat and meat products. Consumption of oxidised proteins can contribute to weakened immune, nervous and cardiovascular systems, and may also be associated with the development of chronic diseases such as cancer. In the context of the search for sustainable food sources, game meat is gaining importance due to its high nutritional value and natural origin.

The aim of this study was to investigate the effects of storage method and time on the physicochemical properties and oxidative stability of European wild boar (*Sus scrofa scrofa*) and European roe deer (*Capreolus capreolus*) meat. The study involved the storage of m. *longissimus thoracis et lumborum* (LTL), m. *biceps femoris* (BF) and m. *vastus lateralis* (VL) muscles under vacuum (VAC), modified atmosphere (MAP), and in a meat seasoning chamber (DRY-AGED) for 21 days.

Meat from wild boar and roe deer was shown to have a high protein and low-fat content and a favourable mineral composition. Oxidative processes and protein aggregation are significantly influenced by storage methods, negatively affecting meat quality. High-oxygen MAP packaging (80% O₂/20% CO₂) promotes intensive oxidation of proteins and lipids, which translates into a deterioration of meat tenderness and colour. The ability of proteins to aggregate and form intramolecular disulphide bonds under high oxygen content was also significantly affected. Titin and myosin were the most susceptible to aggregation, and proteins responding to oxidative stress were also identified.

There is a need for research into the mechanisms of protein oxidation in meat and the impact of these processes on the oxidative stability, functionality and nutritional value of meat products. The dissertation provides important information on the influence of storage methods on the quality of venison, which may contribute to improving the processing and storage strategies of this raw material in the meat industry.

Keywords: game meat, meat storage, protein and lipid oxidation, protein aggregation, proteomics.

Wykaz najczęściej używanych skrótów

ACT – aktyna

a_w – aktywność wody

BF – mięsień dwugłowy uda (m. *biceps femoris*)

CKM – kinaza kreatynowa

DNPH – 2,4-dinitrofenylohydrazyna

DRY-AGED / DA – przechowywanie w szafie do sezonowania mięsa

DTT – ditiotreitol

ICP-OES – spektrometria atomowa emisyjna z indukcyjnie sprzężoną plazmą

IDH – dehydrogenaza izocytrynianowa

LC-MS/MS – chromatografia cieczowa z tandemową spektrometrią mas

LTL – mięsień najdłuższy grzbietu (m. *longissimus thoracis et lumborum*)

MAP – pakowanie w atmosferze modyfikowanej

MAP(1) – atmosfera 80% O₂ /20% CO₂

MAP(2) – atmosfera 60% CO₂ /40% N₂

MHC – łańcuchy ciężkie miozyny

MYH1 – miozyna

OHZ – ośrodek hodowli zwierzyzny

PA – agregaty białkowe

RNS – reaktywne formy azotu

ROS – reaktywne formy tlenu

SERCA1 – ATPaza wapniowa typu 1 siateczki sarkoplazmatycznej/endoplazmatycznej

TCA – kwas trichlorooctowy

TBA – kwas 2-tiobarbiturowy

TBARS – substancje reaktywne kwasu tiobarbiturowego

TTN – titina

VAC – pakowanie próżniowe

VL – mięsień obszerny boczny (*m. vastus lateralis*)

WBSF – siła ścinająca Warnera-Bratzlera

SDS-PAGE – elektroforeza w żelu poliakrylamidowym w obecności dodecylsiarczanu sodu

SD – odchylenie standardowe

SEM – błąd standardowy średniej

LOD, DLs – granica wykrywalności

LOQ – granica oznaczalności

Wykaz artykułów naukowych wchodzących w skład zbioru

[P1] **Kasałka-Czarna, N.**, Bilska, A., Biegańska-Marecik, R., & Montowska, M. (2022). The effect of storage method on selected physicochemical and microbiological qualities of wild boar meat. *Journal of the Science of Food and Agriculture*, 102(12), 5250-5260. <https://doi.org/10.1002/jsfa.11878>.

IF₍₂₀₂₂₎ = 4.1; MNiSW = 100

[P2] **Kasałka-Czarna, N.**, Biegańska-Marecik, R., Proch, J., Orłowska, A., & Montowska, M. (2023). Effect of dry, vacuum, and modified atmosphere ageing on physicochemical properties of roe deer meat. *Polish Journal of Food and Nutrition Sciences*, 73(2), 175–186. <https://doi.org/10.31883/pjfn/163613>.

IF₍₂₀₂₃₎ = 2.3; MNiSW = 100

[P3] **Kasałka-Czarna, N.**, Stachniuk, A., Fornal, E., & Montowska, M. (2025). Proteomic analysis of wild boar meat: Effect of storage method and time on muscle protein stability. *Food Chemistry*, 464 (3), 141774. <https://doi.org/10.1016/j.foodchem.2024.141774>.

IF₍₂₀₂₅₎ = 8.5; MNiSW = 200

[P4] **Kasałka-Czarna, N.**, Stachniuk, A., Fornal, E., & Montowska, M. (2025). Protein aggregation during storage of roe deer meat: a proteomic study. *Journal of Agricultural and Food Chemistry*, 73, 8001–8014. <https://doi.org/10.1021/acs.jafc.4c12348>.

IF₍₂₀₂₅₎ = 5.7; MNiSW = 140

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1. Wstęp

Współczesne wyzwania związane z rosnącą populacją oraz zmianami demograficznymi mają istotny wpływ na przemysł spożywczy. Prognozy wskazują, że liczba ludności na świecie wzrośnie do 8,5 miliarda do 2030 roku oraz do 9,7 miliarda w 2050 roku (Wilmoth i in., 2022). Coraz większa liczba konsumentów podejmuje świadome decyzje żywieniowe, oczekując produktów zdrowych i funkcjonalnych, pozyskiwanych w sposób odpowiedzialny. W obliczu tych zmian przemysł mięsny staje przed wyzwaniem zapewnienia wysokiej jakości mięsa, jednocześnie minimalizując straty wartości odżywczych wynikające z procesów technologicznych. Jednym z głównych problemów wpływających na jakość mięsa są procesy oksydacyjne, które prowadzą do pogorszenia smaku, tekstury i wartości odżywczej, a także mogą wpływać na zdrowie konsumentów (Li i in., 2020). Utlenianie białek i lipidów zachodzi na różnych etapach przetwarzania i przechowywania mięsa, a jego intensywność zależy od takich czynników jak temperatura, czas przechowywania czy obecność tlenu (Nawaz i in., 2022). Procesy te, mimo ich negatywnych skutków, są nieodłącznym elementem dojrzewania mięsa i tworzenia aromatów (Domínguez i in., 2022).

Mięso dzikich zwierząt, takich jak dzik, sarna czy jelen, stanowi cenne źródło białka, nienasyconych kwasów tłuszczowych, witamin oraz minerałów. W Polsce, dzięki korzystnym warunkom środowiskowym, istnieje duży potencjał do zwiększenia udziału dziczyzny w rynku mięsnym, jednak obecnie spożycie dziczyzny odnotowuje się na marginalnym poziomie. Łowiectwo stanowi najbardziej pierwotny sposób pozyskiwania mięsa, współcześnie jednak, obejmuje cały proces ochrony i gospodarowania populacjami zwierząt dziko żyjących z uwzględnieniem warunków ich bytowania, ekologiczną gospodarką leśną oraz rolną. Biorąc pod uwagę, że populacje dzikich zwierząt są kontrolowane w ramach działań zarządzających ich liczebnością, wykorzystywanie ich mięsa do produkcji żywności stanowi podejście zgodne z zasadami zrównoważonego rozwoju. Wykorzystanie tych zasobów w sposób odpowiedzialny pozwala na pełniejsze spożytkowanie zwierząt, które są pozyskiwane w ramach regulacji populacji oraz przyczynia się do efektywnego wykorzystania zasobów naturalnych.

Wyraźne ograniczenie stanowi niska wiedza konsumentów na temat wartości odżywczej, ale także możliwości przechowywania i przetwarzania dziczyzny. Do

istotnych producentów dziczyzny w Europie zaliczyć można Niemcy, Francję, Szwecję oraz Polskę. Krajowa produkcja mięsa pochodzącego od dzikich gatunków zwierząt regularnie wzrasta, co uzależnione jest bezpośrednio od wielkości populacji takich gatunków jak: dzik, sarna oraz jelen. Mimo potencjału krajowego rynku mięsa zwierząt łownych zdecydowana większość eksportowana jest do Francji i Niemiec.

Mięso dzikich zwierząt jest szczególnie cenione ze względu na wartość odżywczą. Charakteryzuje się ono wysoką zawartością pełnowartościowego białka, które stanowi podstawowy składnik budulcowy tkanek organizmu człowieka. W porównaniu do mięsa zwierząt hodowlanych, dziczyzna zawiera znacznie mniej tłuszczu, co czyni ją atrakcyjnym produktem w diecie niskokalorycznej. Istotny jest również fakt, że ilość tłuszczu w mięsie zwierząt dziko żyjących ulega znacznym wahaniom ze względu na dostępność pokarmu w zależności od pory roku. Z punktu widzenia odżywczego, mięso dzikich zwierząt jest wartościowym elementem diety, ponieważ obfituje w witaminy z grupy B oraz minerały, takie jak żelazo, magnez, wapń czy cynk, które są kluczowe dla funkcjonowania organizmu człowieka. Zawartość łatwo przyswajalnego żelaza w mięsie dziczyzny ma szczególne znaczenie, zwłaszcza w kontekście zapobiegania anemii. Ponadto, mięso dziczyzny zawiera znaczne ilości nienasyconych kwasów tłuszczowych, w tym sprzężonych dienów kwasu linolowego (CLA), które wykazują właściwości przeciwzapalne, przeciwnowotworowe oraz wspomagające redukcję masy ciała. Dzikich zwierząt stanowi pokarm bezpieczny dla alergików oraz dzieci, ponieważ jest wolna od antybiotyków i związków wspomagających ich wzrost.

Procesy oksydacyjne są jednym z najistotniejszych zjawisk wpływających na jakość mięsa świeżego oraz jego przetworów. Utlenianie białek i lipidów prowadzi do zmian w smaku, teksturze, wartości odżywczej oraz bezpieczeństwie żywności (Li i in., 2020). Białka mogą łatwo reagować z związkami utleniającymi, jednak kinetyka ich reakcji w porównaniu z lipidami jest znacznie wolniejsza. W związku z powyższym jeszcze do niedawna procesom oksydacyjnym białek nie przypisywano dużego znaczenia (Lorenzo i in., 2018). Choć procesy te są mniej zbadane w odniesieniu do białek niż lipidów, ich znaczenie dla degradacji jakości mięsa jest równie istotne. Utlenianie lipidów może mieć jednak wpływ na kinetykę reakcji utleniania białek. Obecne w żywności białka mogą być utleniane przez bezpośrednie narażenie na reaktywne formy tlenu i azotu bądź pośrednio indukowane przez produkty uboczne utleniania cukrów i lipidów (Domínguez i in., 2022; Zhang i in., 2013). Za główne zmiany właściwości

biochemicznych białek powstałych wskutek utleniania uznaje się tworzenie związków karbonylowych, degradację oraz sieciowanie białek (Wang i in., 2020). Za główną przyczynę tych uważa się stres oksydacyjny (Davies, 2016). Karbonylacja białek jest uznawana za jedną z najbardziej znaczących, nieodwracalnych modyfikacji potranslacyjnych (Estévez i in., 2022).

Na poszczególnych etapach przetwarzania i przechowywania mięsa intensywność utleniania białek zależy m.in. od temperatury, czasu i obecności tlenu (Nawaz i in., 2022; Wang i in., 2020). Czas przechowywania mięsa ma istotny wpływ na jego jakość, wpływa m.in. na wartość pH, wodochłonność oraz ubytki termiczne, co podkreśla znaczenie tego czynnika w ocenie świeżości surowca (Zduńczyk i in., 2024). Zarówno metoda pakowania, jak i długość przechowywania decydują więc nie tylko o cechach fizykochemicznych mięsa, ale także o intensywności zachodzących w nim przemian oksydacyjnych. Oksydacja białek wpływa negatywnie na takie cechy mięsa jak kruchość, barwa czy zdolność wiązania wody, co z kolei obniża jego wartość ekonomiczną i odżywczą (Estévez, 2021; Li i in., 2020; Soladoye i in., 2015). Procesy te również zmniejszają strawność mięsa przez modyfikacje aminokwasów (Paredi i in., 2019; Xiong & Guo, 2021). Na zmiany oksydacyjne występujące w mięsie oprócz typu metabolicznego mięśni, ich składu chemicznego, decydujące jest również zastosowanie różnych technologii pakowania i przechowywania mięsa. Powszechnie praktykowane w przemyśle mięsnym pakowanie w atmosferze modyfikowanej (MAP) z wysokim stężeniem tlenu, choć skutecznie chroni przed rozwojem mikroorganizmów i utrzymuje atrakcyjną czerwoną barwę, przyspiesza utlenianie białek i lipidów. Od rodzaju metody pakowania i przechowywania w dużej mierze zależy trwałość mikrobiologiczna oraz barwa przechowywanego mięsa i produktów z niego otrzymanych (Górnicka i in., 2012). Biorąc pod uwagę wpływ pakowania na utlenianie białek mięsa, zdecydowanie zbyt mało uwagi poświęcono pakowaniu w wysokiej zawartości tlenu (Bao i in., 2016). Należy więc zastanowić się nad zasadnością stosowania atmosfery wysokotlenowej w pakowaniu mięsa różnych gatunków zwierząt.

Co istotne, zmiany oksydacyjne w obrębie białek stanowią również jedno z głównych źródeł substancji chemicznych w żywności o potencjale toksycznym (Barzegar i in., 2019; Nawaz i in., 2022; Papuc i in., 2017; Theron & Estévez, 2022). Badania na zwierzętach doświadczalnych jasno wskazują na rzeczywiste ryzyko związane z konsumpcją utlenionych białek. Długotrwałe narażenie na utlenione białka

oraz ich produkty uboczne może przyczyniać się do rozwoju chorób przewlekłych (Barzegar i in., 2019; Nawaz i in., 2022). Spożywanie utlenionych produktów mięsnych może prowadzić do zaburzeń w obrębie układu odpornościowego, nerwowego oraz sercowo-naczyniowego, zwiększając ryzyko chorób autoimmunologicznych, neurodegeneracyjnych i sercowo-naczyniowych (Wang i in., 2023). W spożywaniu utlenionych białek upatruje się także zwiększonego ryzyka rozwoju raka jelita grubego (Barzegar i in., 2019; Thøgersen i in., 2020). Estévez i Xiong (2019) podkreślają, że ignorowanie dowodów naukowych wskazujących na rolę utlenionych białek i aminokwasów w patogenezie wielu chorób jest nadal powszechne, co wymaga intensyfikacji badań w tym zakresie.

Mając na uwadze powyższe, wciąż brak jest wystarczających badań, które pozwalałyby na pełne zrozumienie wpływu procesów oksydacyjnych na jakość i bezpieczeństwo mięsa dzikich zwierząt. W szczególności, istnieje potrzeba badań dotyczących wpływu metod przechowywania, takich jak pakowanie w atmosferze modyfikowanej, na jakość tego typu mięsa. Czynniki fizykochemiczne biorące udział w inicjacji i propagacji utleniania białek w żywności wymagają dalszych, pogłębionych badań. Badania te mogą pozwolić na optymalizację procesów przechowywania, przetwarzania i dystrybucji dziczyzny, a tym samym na poprawę jej jakości i dostępności na rynku. Zrozumienie biochemicznych procesów zachodzących w mięsie dzikich zwierząt oraz ich wpływu na stabilność oksydacyjną, funkcjonalność oraz wartość odżywczą mięsa jest kluczowe dla przyszłości tego segmentu przemysłu mięsnego. W kontekście dalszych badań zauważa się rosnącą potrzebę zrozumienia mechanizmów molekularnych, które zachodzą pomiędzy składnikami diety podczas procesów trawienia, oraz rzeczywistego ryzyka związanego ze spożywaniem utlenionych białek. Ponadto, konieczne jest zaktualizowanie zaleceń żywieniowych, uwzględniając wpływ utlenionych białek na zdrowie konsumentów, zwłaszcza w kontekście ich roli w rozwoju różnych chorób przewlekłych.

2. Hipoteza badawcza i cel pracy

Przyjęto następującą hipotezę badawczą: „odpowiednie technologie pakowania i przechowywania mogą skutecznie ograniczać procesy oksydacji i agregacji białek w mięsie dzikich zwierząt, zastosowanie tych metod przyczyni się do zachowania stabilności oksydacyjnej mięsa oraz minimalizacji niekorzystnych zmian jakościowych, zapewniając wysoką jakość i bezpieczeństwo produktów mięsnych”.

W celu zweryfikowania powyższej hipotezy wyznaczono cel nadrzędny pracy oraz cele szczegółowe. Celem nadrzędnym niniejszej rozprawy doktorskiej była ocena jakości mięsa dzika i sarny oraz zmian w obrębie białek w wyniku przechowywania mięsa pakowanego próżniowo, w wysokotlenowej, modyfikowanej atmosferze gazów oraz w mięsie sezonowanym, ze szczególnym uwzględnieniem agregacji i oksydacji białek.

Ponadto, w pracy wyznaczono cele szczegółowe:

- 1) ocena jakości surowca mięsnego pochodzącego od dzika i sarny pozyskanego w jego naturalnym środowisku,
- 2) ocena bezpieczeństwa mikrobiologicznego mięsa dzika podczas przechowywania w próżni, w modyfikowanej atmosferze gazów oraz sezonowanego w komorze do sezonowania mięsa,
- 3) analiza jakości mięsa przechowywanego w próżni, w modyfikowanej atmosferze gazów oraz sezonowanego w komorze do sezonowania mięsa,
- 4) porównanie podatności oksydacyjnej analizowanych mięśni pod wpływem przechowywania,
- 5) badanie produktów utleniania białek mięśniowych, w tym tworzenia związków karbonylowych i sieciowania białek oraz produktów utleniania tłuszczu,
- 6) ocena i identyfikacja agregatów białkowych powstałych podczas przechowywania mięsa.

Przeprowadzenie tych badań pozwoliło na poszerzenie wiedzy na temat możliwych przemian biochemicznych zachodzących w mięsie dzika i sarny podczas przechowywania, co może przyczynić się do opracowania bardziej precyzyjnych strategii przetwarzania i przechowywania tego mięsa, co z kolei może zwiększyć konkurencyjność branży spożywczej i przyczynić się do rozwoju sektora mięsnego.

3. Materiał i metody

3.1 Materiał do badań

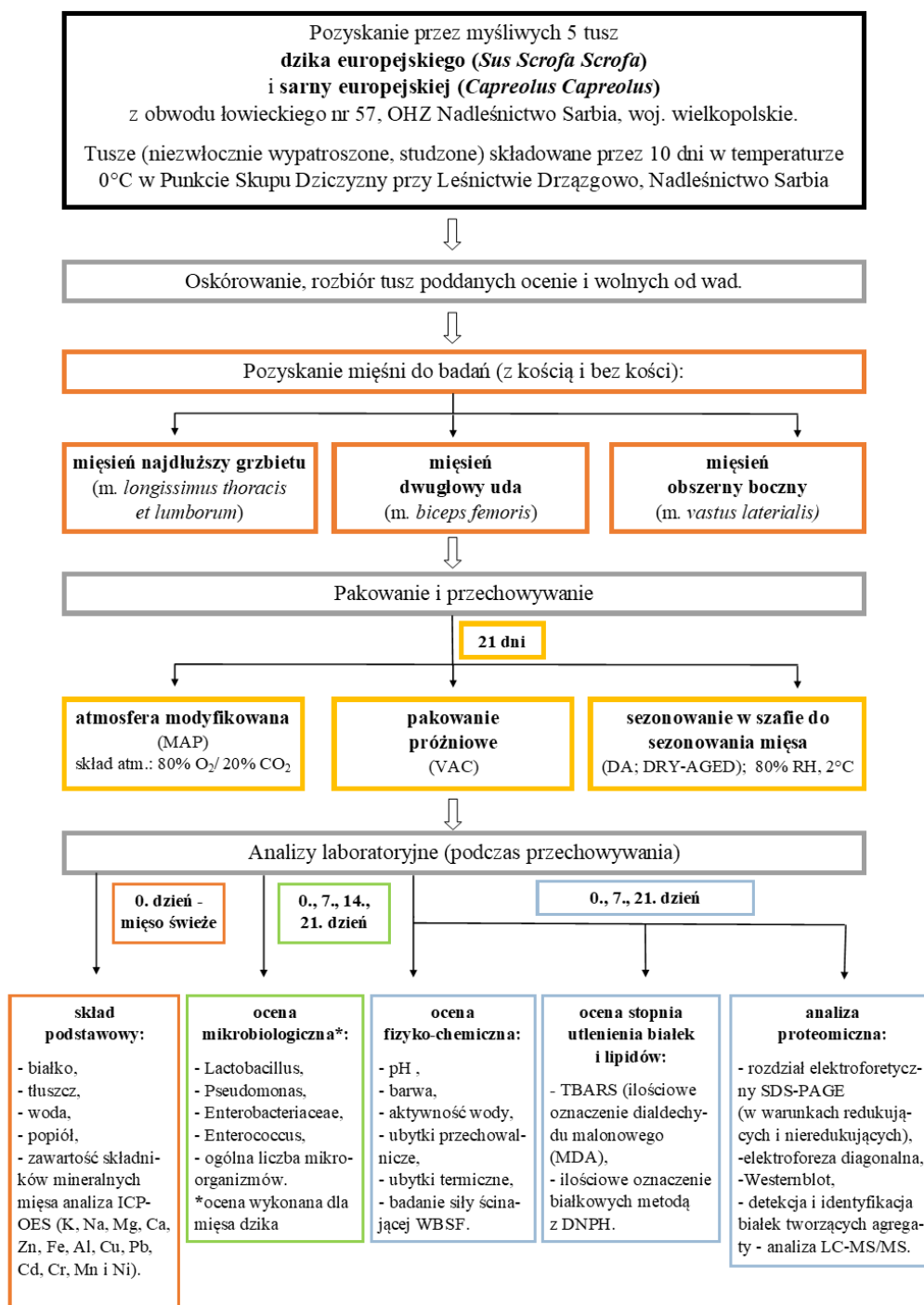
Materiał do badań stanowiły trzy mięśnie: m. *longissimus thoracis et lumborum*, m. *vastus lateralis* oraz m. *biceps femoris* pochodzące z 5 tusz dzika europejskiego (*Sus scrofa scrofa*) oraz sarny europejskiej (*Capreolus capreolus*) przechowywane za pomocą trzech metod. Tusze do badań pozyskane zostały przez myśliwych w lasach na terenie obwodu łowieckiego nr 57, OHZ Nadleśnictwo Sarbia, woj. wielkopolskie. Mięśnie przechowywano przez 21 dni pakowane próżniowo (VAC), w atmosferze modyfikowanej wysokotlenowej – 80% O₂/20% CO₂ (MAP/MAP(1)) oraz w komorze do sezonowania mięsa (DRY-AGED/ DA) [P1, P2, P3, P4]. Celem weryfikacji w P4 zastosowano dodatkowo atmosferę modyfikowaną o składzie 60% CO₂/40% N₂ (MAP(2)). Mięso pakowane próżniowo i w atmosferze modyfikowanej przechowywano w temperaturze $4 \pm 8^{\circ}\text{C}$, natomiast w komorze do sezonowania mięsa w temperaturze 2°C , o wilgotności 80%. Przedmiotem badań są próby pobrane w dniu pozyskania surowca oraz w 7. i 21. dniu przechowywania (przechowywane próżniowo, w atmosferze modyfikowanej oraz w szafie do sezonowania mięsa). W przypadku oceny mikrobiologicznej analizy przeprowadzono również w 14. dniu przechowywania [P1]. Próby natychmiast po otwarciu opakowań przeniesiono do szczelnie zamkniętych próbek i przechowywano w temp. -80°C .

3.2 Metody badawcze

Przygotowanie materiału do badań oraz metody analityczne zostały szczegółowo opisane w wskazanych publikacjach wchodzących w skład rozprawy doktorskiej [P1-P4]. W pracy zastosowano kompleksowy zestaw metod badawczych, obejmujących analizy fizykochemiczne, mikrobiologiczne oraz techniki elektroforetyczne i proteomiczne (LC-MS/MS), umożliwiające wieloaspektową ocenę zmian zachodzących w mięsie podczas przechowywania. Szczegółowy przebieg postępowania badawczego przedstawiono na rysunku 1.

3.2.1 Skład podstawowy

Ocena składu podstawowego: białko (metoda Kjeldahla; ISO 937:1978), zawartość wody (PN-ISO 1442:2000), tłuszcz (PN-ISO 1444:2000), popiół (PN-ISO 936:2000) [P1, P2].



Rys. 1. Schemat postępowania i przeprowadzonych analiz zrealizowanych w pracy doktorskiej.

3.2.2 Zawartość składników mineralnych mięsa

Zawartość 13. pierwiastków (K, Na, Mg, Ca, Zn, Fe, Al, Cu, Pb, Cd, Cr, Mn, Ni) oznaczono przy użyciu wysokorozdzielczej spektrometrii emisyjnej z plazmą sprzężoną indukcyjnie (ICP-HROES), stosując spektrometr PlasmaQuant PQ 9000 Elite (Analytik Jena, Niemcy) [P1, P2].

3.2.3 Ocena pH, barwy (CIELAB) i aktywności wody w mięsie

Pomiary pH wykonano za pomocą przenośnego miernika Handylab 2 (Schott AG, Mainz, Niemcy) wyposażonego w kombinowaną elektrodę pH Schott L68880 (PN-ISO 2917:2001). Barwę (CIE L^* , a^* , b^*) mierzono w trzech powtórzeniach na przekroju przy użyciu przenośnego kolorymetru Dr. Lange LMG161 (Dr. Bruno Lange GmbH & Co. KG, Berlin, Niemcy). Pomiar aktywności wody (a_w) mięsa wykonano przy użyciu aparatu LabMaster-aw neo (Novasina AG, Lachen, Szwajcaria) w temp. $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ [P1, P2].

3.2.4 Ubytki termiczne i przechowalnicze

Ubytki cieplne i przechowalnicze określono poprzez zważenie prób zarówno przed, jak i po obróbce termicznej lub po okresie przechowywania (1., 7., 21. dzień analizy). Wyniki przedstawiono jako procent początkowej masy próbki zmierzonej przed każdym etapem [P1, P2].

3.2.5 Siła cięcia Warnera-Bratzlera

Analizę przeprowadzono w temperaturze pokojowej za pomocą analizatora tekstury TA.XT.plus (Stable Micro Systems, Godalming, UK) wyposażonego w nóż Warnera-Bratzlera. Próby po ogrzaniu i schłodzeniu wycięto w kształcie prostopadłościanu ($1 \times 1 \times 3$ cm). Siłę cięcia, wyrażoną w niutonach (N), mierzono prostopadle do układu włókien mięśniowych z prędkością 100 mm/min. [P1, P2].

3.2.6 Ocena trwałości mikrobiologicznej

Oceny mikrobiologicznej dokonano dla mięsa dzika względem: ogólnej liczby mikroorganizmów (ISO 4833-1:2013), *Lactobacillus* (PN-ISO 15214:2002), *Pseudomonas* (ISO 13720:2010), *Enterobacteriaceae* (ISO 21528-1:2017) i *Enterococcus* (PN-A-82055-7:1997)) [P1].

3.2.7 Utlenianie lipidów

Utlenianie lipidów określono przez ilościowe oznaczenie dialdehydu malonowego (TBARS). Przygotowany roztwór z dodatkiem 5 ml 20 mM kwasu 2-tiobarbiturowego (TBA) ogrzewano w termobloku w 100°C przez 20 min., następnie schłodzono do temperatury pokojowej i zmierzono absorbancję przy 532 nm za pomocą spektrometru Ultrospec 2000 (Pharmacia Biotech, Uppsala, Szwecja). Wartości TBARS obliczono za pomocą krzywej standardowej i wyrażono jako mg równoważników dialdehydu malonowego ((mg MDA)/kg próby) [P1, P2].

3.2.8 Utlenianie białek (zawartość związków karbonylowych)

Ocenę oksydacyjnych modyfikacji białek mierzono na podstawie reaktywności grup karbonylowych białek powstałych podczas przechowywania mięsa przez derywatyzację z 2,4-dinitrofenylohydrazyną (DNPH). Mięso (1 g) poddano homogenizacji (Ultra Turrax) w buforze 20 mM fosforanu sodu i 6 M NaCl (pH 6,5). Homogenat przesączono, następnie białka z roztworu (2 x 0,4 ml) wytrącono za pomocą 10% TCA (1,5 ml) i inkubowano w 20°C przez 20 min, a następnie odwirowano przez 5 min w 4°C przy 10000 g. Usunięto supernatant, do jednego osadu w celu pomiaru stężenia białka dodano 1 ml 2 M HCl w celu, a do drugiego 1 ml 10 M DNPH w 2 M HCl celem pomiaru stężenia związków karbonylowych. Próby inkubowano przez 30 minut w temperaturze 37°C. Następnie, ponownie dodano 1 ml 10% TCA i odwirowano przez 5 minut w temperaturze 4°C i 10 000g. Aby usunąć nadmiar DNPH po odwirowaniu, osady przemyto 3x1 mL roztworem etanolu i octanu etylu (1:1, v/v). Osad wysuszono w temperaturze pokojowej przez 1 h, rozpuszczono w 1,5 ml 20 mM buforu fosforanu sodu zawierającym 6 M chlorowodorek guanidyny (pH 6,5) i inkubowano przez 30 minut w temperaturze 37°C. Absorbancję zmierzono (Ultrospec 2000), przy 280 nm (A280) i 370 nm (A370) [P1, P2].

3.2.9 Elektroforeza w żelu poliakrylamidowym (SDS-PAGE) w warunkach redukujących

Elektroforezę w żelu poliakrylamidowym z dodatkiem siarczanu dodecylu sodu (SDS-PAGE) przeprowadzono w celu porównania profili białkowych analizowanych prób w każdym wariancie przechowywania. Próby przygotowano w buforze zawierającym 8 M mocznik, 2 M tiomocznik, 0,05 mM Tris, 75 mM ditiotretitol (DTT), 3% SDS i 0,05% błękitu bromofenolowego przy pH 6,8. Białka o masie 15 µg naniesiono

na 15% żel poliakrylamidowy i rozdzielono przy natężeniu 20 mA na każdą płytkę (Hoefer SE250, GE Healthcare Bio-Sciences). Żele wybarwiono błękitem Coomassie brilliant blue R-250 oraz zeskanowano przy użyciu systemu Gel Doc XR+. Obrazy analizowano za pomocą programu Image Lab 6.0.1 (Bio-Rad Laboratories, Inc., CA, USA) [P3, P4].

3.2.10 Elektroforeza w żelu poliakrylamidowym (SDS-PAGE) w warunkach nieredukujących

Elektroforezę SDS-PAGE w warunkach nieredukujących przeprowadzono celem oceny polimeryzacji białek o wysokiej masie cząsteczkowej. Z każdej z prób odważono 0,04 g, a następnie poddano homogenizacji z 600 µl fosforanowego buforu ekstrakcyjnego (10 mM, 2% SDS, 25 mM NEM, pH 7,0). Wyekstrahowane białka rozpuszczono w buforze ładującym w stosunku 1:2. Rozdział prowadzono w 6% żelu poliakrylamidowym, w czasie 2 h 10 min, przy natężeniu 20 mA na każdą płytkę, dalsze procedury zostały przeprowadzone jak opisano powyżej [P3, P4].

3.2.11 Elektroforeza diagonalna-PAGE

Elektroforezę diagonalną-PAGE przeprowadzono w celu oceny indukowanej tlenem polimeryzacji białek o wysokiej masie cząsteczkowej zgodnie z procedurą opisaną przez Moczowską i in. (2017). Elektroforezę przeprowadzono w dwóch wymiarach, w pierwszym wymiarze białka rozdzielono w warunkach nieredukujących, w drugim etapie białka redukowano w buforze obciążającym zawierającym 20 mM DTT [P3, P4].

3.2.12 Western blotting

Celem wstępnej identyfikacji agregatów białkowych (PA) użyto analizy Western Blot zgodnie z procedurą opisaną przez Moczowska i in. (2017). Analizę Western Blotting prowadzono w obecności przeciwciała pierwszorzędowego miozyny (MF20, Developmental Studies HybridomaBank, University of Iowa). Detekcję prążków białkowych przeprowadzono w obecności wyznakowanego przeciwciała drugorzędowego (fosfatazy alkaicznej) oraz roztworu podstawowego NBT/BCIP, otrzymując barwny produkt. Membrany skanowano za pomocą zestawu z kamerą cyfrową Gel Doc XR+ System (Bio-Rad Laboratories, Inc., CA, USA) [P3, P4].

3.2.13 Trawienie trypsyną w żelu

Po zakończeniu elektroforezy SDS-PAGE w warunkach nieredukujących, wysokocząsteczkowe pasma białek i PA na żelu zostały zidentyfikowane i wycięte za pomocą sterylnego skalpela, pocięte na fragmenty (1–2 mm³) i poddane procedurze redukcji i alkilacji. Białka obecne w żelu strawiono dodając roztwór wodorowęglanu amonu zawierającym 50 ng/μl trypsyny (Promega GmbH, Mannheim, Niemcy) przez noc w 37°C. Uzyskany supernatant wysuszono próżniowo w koncentratorze miVacDuo (Genevac Ltd., Suffolk, Wielka Brytania). Przed wykonaniem analiz LC-MS/MS próby rozpuszczono w buforze A, zawierającym 5% acetonitrylu i 0,1% kwasu mrówkowego [P3, P4].

3.2.14 Analiza LC-MS/MS

Rozdział chromatograficzny przeprowadzono za pomocą ultrasprawnego chromatografu cieczowego serii 1290 Infinity, składającego się z pompy binarnej, termostatu i autosamplera, sprzężonego ze spektrometrem mas typu kwadrupol - analizator czasu przelotu jonów 6550 UHD iFunnel Q-TOF LC/MS (Agilent Technologies, Santa Clara, CA, USA). Związki zjonizowano techniką jonizacji przez elektrorozpylanie (ESI) przy użyciu źródła jonów Jet Stream. Rozdział chromatograficzny przeprowadzono przy użyciu kolumny analitycznej Agilent RRHD Eclipse PlusC18 (2,1 x 150 mm, wielkość cząstek 1,8 μm. Pozyskano jony dodatnie w zakresie m/z 100–1700 w trybie MS i m/z 40–1700 w trybie auto MS/MS (szybkość skanowania 5 skanów/s dla MS i 3 skany/s dla MS/MS). Wewnętrzna kalibracja masy odnosiła się do dwóch jonów referencyjnych przy m/z 121,0509 i 922,0098. Do przetwarzania danych użyto oprogramowania Agilent Mass Hunter (B.09.00) i oprogramowania jakościowego (B.10.00) [P3, P4].

3.2.15 Identyfikacja białek tworzących wysokocząsteczkowe agregaty

W celu identyfikacji białek uzyskane widma MS/MS zostały przeszukane przez bazę danych białek National Center for Biotechnology Information (NCBI, U.S. National Library of Medicine) przy użyciu oprogramowania Spectrum Mill MS Proteomics Workbench. Białka zidentyfikowano przy następujących parametrach wyszukiwania: specyficzność enzymatyczna dla trypsyny, maksymalnie 2 pominięte miejsca rozszczepienia, tolerancja masy produktu 50 ppm, ładunek peptydu max. 6+. Jako stałą modyfikację uwzględniono karbamidometylację, natomiast jako zmienną modyfikację

utlenianie metioniny. Zastosowano filtr jakości widma o czystości izolacji pików prekursora >70% oraz tolerancją masy prekursora 5 ppm. Wyniki wyszukiwania MS/MS zostały zweryfikowane przy użyciu trybu peptydowego oprogramowania Spectrum Mill uzyskując współczynnik wyniku fałszywego (FDR) max 1% dla progu identyczności i homologii [P3, P4].

3.2.16 Analiza statystyczna

Analizę statystyczną uzyskanych wyników przeprowadzono za pomocą programu Statistica 13.1 (StatSoft Inc., Tulsa, OK, USA). Zastosowano jednoczynnikową i dwuczynnikową analizę wariancji ANOVA i test porównań wielokrotnych Tukeya w celu wyznaczenia istotnych różnic między wartościami średnimi. Określenie siły związku pomiędzy badanymi cechami oszacowano na podstawie korelacji liniowej, testem Pearsona ($p < 0,05$).

Do tworzenia wykresów użyto oprogramowanie OriginPro 2023b (OriginLab Corporation, Northampton, MA, USA). Streszczenia graficzne w publikacjach utworzono przy użyciu BioRender.com.

4. Podsumowanie wyników analiz zawartych w publikacjach będących częścią rozprawy doktorskiej

4.1 Skład podstawowy i zawartość składników mineralnych

Na podstawie przeprowadzonych badań opisanych w publikacjach [P1 i P2] wykazano, że mięso pochodzące zarówno od dzika i sarny stanowi cenny surowiec pod względem wartości odżywczej. Wyniki analizy składu podstawowego przedstawiono w tabeli 1. Wykazano istotne statystycznie ($p < 0,05$) różnice w składzie chemicznym mięsa w zależności od analizowanego mięśnia. Mięso obu gatunków charakteryzowało się wysoką zawartością białka oraz niską zawartością tłuszczu, co jest istotnym atutem z perspektywy diety o obniżonej zawartości tłuszczu. Zawartość tłuszczu w mięsie sarny była istotnie niższa (1,71–2,58%) niż w mięsie dzika (3,68–5,27%). W mięsie dzika nie stwierdzono istotnych statystycznie różnic w zawartości białka pomiędzy mięśniami LTL, BF i VL, natomiast zawartość tłuszczu wykazała zróżnicowanie między analizowanymi próbami. W przypadku sarny najwyższą zawartość białka oraz najniższą zawartość tłuszczu odnotowano w mięśniu LTL.

Tabela 1. Skład podstawowy [%] mięsa dzika i sarny; średnia $\pm$ odchylenie standardowe.

Gatunek	Mięsień	Białko	Woda	Tłuszcz	Sucha masa	Popiół
Dzik (<i>Sus scrofa scrofa</i>)	LTL	21,91 $\pm$ 1,80	70,99 ^b $\pm$ 1,64	4,99 ^{ab} $\pm$ 1,14	24,02 ^a $\pm$ 0,58	1,14 $\pm$ 0,16
	BF	21,89 $\pm$ 0,78	71,86 ^b $\pm$ 2,06	5,27 ^a $\pm$ 2,01	22,87 ^{ab} $\pm$ 1,80	1,10 $\pm$ 0,20
	VL	21,68 $\pm$ 1,56	74,01 ^a $\pm$ 1,46	3,68 ^b $\pm$ 0,39	22,31 ^b $\pm$ 1,43	1,10 $\pm$ 0,17
Sarna (<i>Capreolus capreolus</i>)	LTL	22,86 ^a $\pm$ 0,41	73,89 $\pm$ 0,63	1,71 ^b $\pm$ 0,42	24,39 $\pm$ 0,46	1,41 $\pm$ 0,16
	BF	21,73 ^b $\pm$ 0,51	74,44 $\pm$ 0,81	2,26 ^a $\pm$ 0,40	23,29 $\pm$ 0,68	1,56 $\pm$ 0,17
	VL	21,65 ^b $\pm$ 0,67	74,26 $\pm$ 0,21	2,58 ^a $\pm$ 0,32	21,52 $\pm$ 0,25	1,50 $\pm$ 0,30

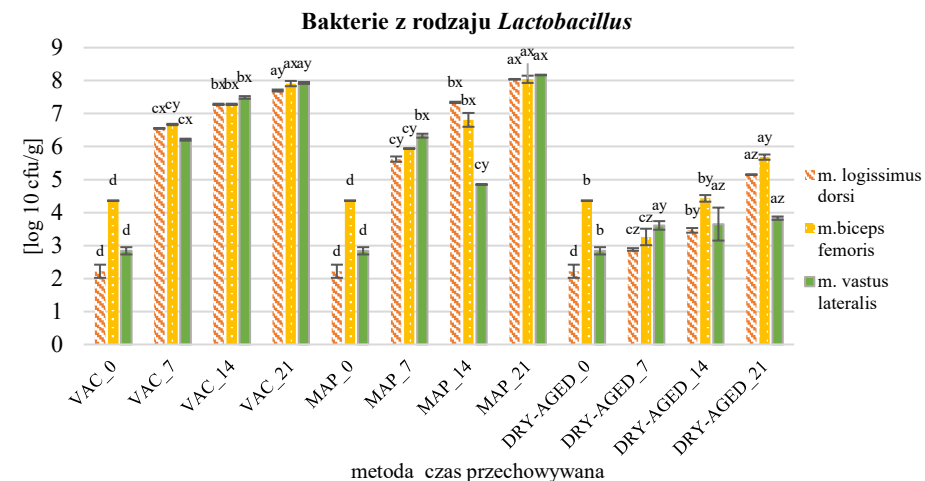
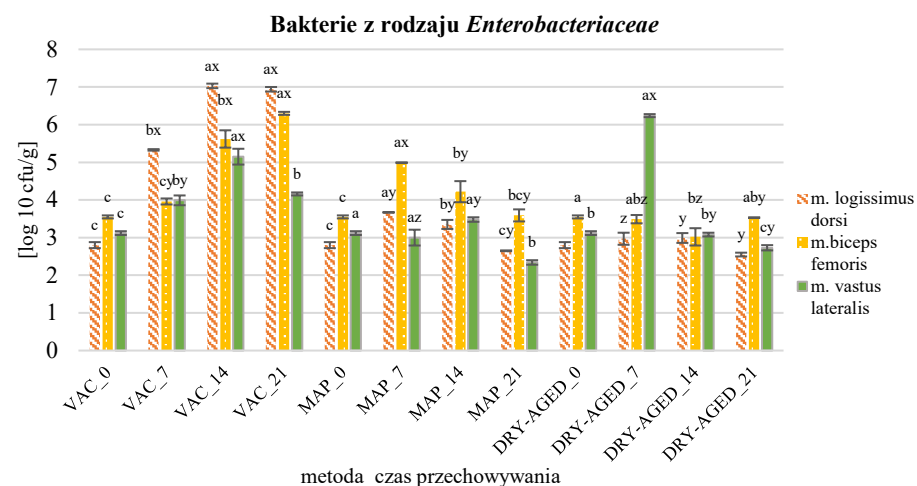
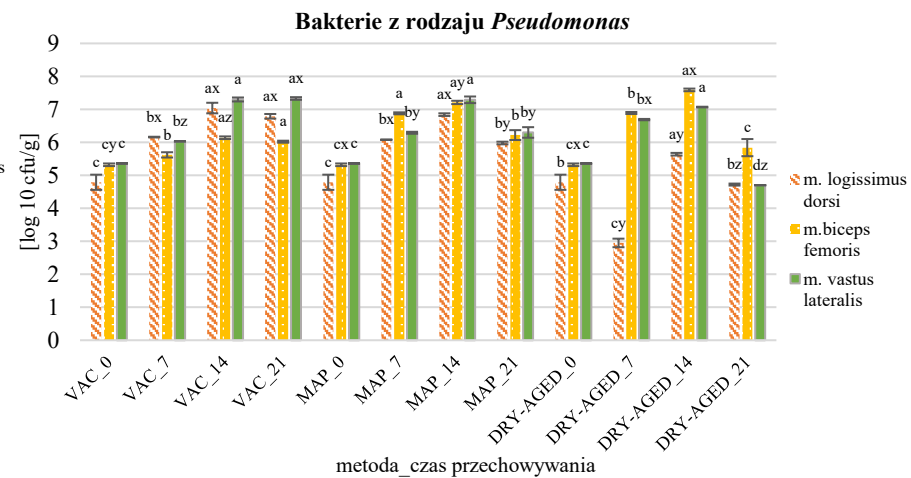
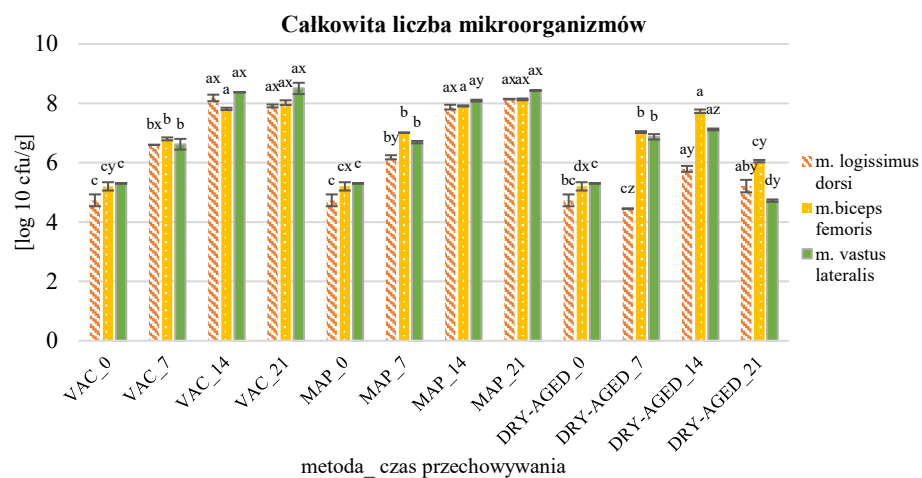
^{a,b,c} – średnie oznaczone różnymi literami w wierszu dla danego parametru wskazują na istotny wpływ rodzaju mięśnia w obrębie jednego gatunku ($p < 0,05$); LTL – m. *longissimus thoracis et lumborum* (mięsień najdłuższy grzbietu i lędźwi), BF – m. *biceps femoris* (dwugłowy uda), VL – m. *vastus lateralis* (obszerny boczny).

Zawartość składników mineralnych w mięsie dzika europejskiego została opisana w publikacji P1, natomiast w przypadku sarny europejskiej w publikacji P2. Wykazano istotne statystycznie różnice ($p < 0,05$) w zawartości poszczególnych pierwiastków między analizowanymi mięśniami (LTL, BF, VL) w obrębie obu gatunków. Mięso dzika i sarny stanowi cenne źródło makro- i mikroelementów, takich jak potas, sód, magnez, cynk czy żelazo (tab. 2). W mięsie dzika wykazano istotne statystycznie różnice ($p < 0,05$) między analizowanymi mięśniami w zakresie zawartości sodu, magnezu, cynku oraz miedzi. Z kolei w mięsie sarny istotne różnice stwierdzono dla sodu, wapnia, cynku, żelaza, glinu oraz miedzi. Mięso sarny było bogatsze w potas, magnez, wapń oraz żelazo. W żadnym z analizowanych mięśni w obrębie obu gatunków nie stwierdzono zawartości metali ciężkich takich jak kadm, chrom, mangan oraz nikiel. Skład mineralny różnił się w zależności od rodzaju mięśnia oraz gatunku zwierzęcia, co może mieć znaczenie z dietetycznego i technologicznego punktu widzenia.

Tabela 2. Zawartość składników mineralnych [mg/100g] w mięsie dzika i sarny; średnia $\pm$ odchylenie standardowe.

Skład mineralny [mg/100g]	Dzik europejski (<i>Sus scrofa scrofa</i>)			Sarna europejska (<i>Capreolus capreolus</i>)		
	LTL	BF	VL	LTL	BF	VL
K	219,56 $\pm$ 17,55	234,80 $\pm$ 2,73	221,48 $\pm$ 2,89	258,69 $\pm$ 37,70	269,70 $\pm$ 24,30	253,90 $\pm$ 34,40
Na	46,67 ^b $\pm$ 1,51	42,83 ^b $\pm$ 4,54	60,25 ^a $\pm$ 0,84	49,26 ^a $\pm$ 1,35	42,04 ^b $\pm$ 0,23	42,51 ^b $\pm$ 0,55
Mg	14,71 ^b $\pm$ 0,03	16,22 ^a $\pm$ 0,21	13,92 ^c $\pm$ 0,06	21,25 $\pm$ 2,30	20,63 $\pm$ 0,26	20,59 $\pm$ 0,57
Ca	3,91 $\pm$ 1,35	4,11 $\pm$ 0,18	6,67 $\pm$ 0,12	6,65 ^b $\pm$ 1,10	7,26 ^a $\pm$ 0,71	5,64 ^c $\pm$ 0,39
Zn	3,42 ^a $\pm$ 0,23	1,46 ^c $\pm$ 0,12	2,51 ^b $\pm$ 0,02	3,00 ^a $\pm$ 0,02	2,71 ^b $\pm$ 0,07	2,65 ^b $\pm$ 0,15
Fe	1,64 $\pm$ 0,01	2,02 $\pm$ 0,21	1,91 $\pm$ 0,08	2,46 ^a $\pm$ 0,13	2,14 ^b $\pm$ 0,14	1,48 ^c $\pm$ 0,15
Al	0,51 $\pm$ 0,31	0,33 $\pm$ 0,46	0,35 $\pm$ 0,35	0,16 ^a $\pm$ 0,15	0,08 ^c $\pm$ 0,07	0,12 ^b $\pm$ 0,10
Cu	0,21 ^b $\pm$ 0,01	0,23 ^b $\pm$ 0,01	0,32 ^a $\pm$ 0,01	0,37 ^a $\pm$ 0,01	0,35 ^a $\pm$ 0,02	0,26 ^b $\pm$ 0,01
Pb	0,04 $\pm$ 0,01	0,04 $\pm$ 0,01	0,03 $\pm$ 0,01	0,03 $\pm$ 0,01	0,03 $\pm$ 0,01	0,03 $\pm$ 0,01
Cd	<0,003	<0,003	<0,003	<0,003	<0,003	<0,003
Cr	<0,003	<0,003	<0,003	<0,003	<0,003	<0,003
Mn	<0,003	<0,003	<0,003	<0,003	<0,003	<0,003
Ni	<0,003	<0,003	<0,003	<0,003	<0,003	<0,003

^{a,b,c} – średnie oznaczone różnymi literami w wierszu dla danego parametru wskazują na istotny wpływ rodzaju mięśnia w obrębie jednego gatunku ($p < 0,05$); LTL – m. *longissimus thoracis et lumborum* (najdłuższy mięsień grzbietu i lędźwi), BF – m. *biceps femoris* (dwugłowy uda), VL – m. *vastus lateralis* (obszerny boczny).



Rys. 2. Wpływ metody i czasu przechowywania na wzrost mikrobiologiczny w mięśniach; średnia $\pm$ SD. Różne litery w kolumnie (^{a, b, c}) dla danego parametru oznaczają istotny wpływ czasu w ramach metody ($p < 0,05$); różne litery w wierszu (^{x, y, z}) dla danego parametru wskazują na istotny wpływ metody w czasie przechowywania ($p < 0,05$).

4.2 Ocena bezpieczeństwa mikrobiologicznego mięsa dzika podczas przechowywania

Badania mikrobiologiczne przeprowadzone w ramach P1 miały na celu realizację celu szczegółowego 2, czyli ocenę bezpieczeństwa mikrobiologicznego mięsa dzika przechowywanego w próżni (VAC), atmosferze modyfikowanej (MAP) oraz sezonowanego na sucho (DRY-AGED) przez 21 dni, co ma kluczowe znaczenie dla trwałości i jakości mięsa (rys. 2). W żadnej z analizowanych prób nie stwierdzono wzrostu bakterii z rodzaju *Enterococcus*. Najniższą ogólną liczbę mikroorganizmów odnotowano w świeżym mięśniu LTL, nieznacznie wyższe wartości stwierdzono dla BF i VL. Spośród analizowanych metod DRY-AGED skutecznie ograniczał wzrost bakterii tlenowych, natomiast MAP i VAC nie wykazały istotnych różnic pod względem całkowitej liczby mikroorganizmów. W przypadku bakterii *Enterobacteriaceae* największy wzrost odnotowano w przypadku pakowania VAC. Przechowywanie MAP przyczyniło się do wzrostu liczebności bakterii *Lactobacillus*, które mogą mieć korzystny wpływ na jakość mięsa poprzez działanie antagonistyczne wobec bakterii patogennych, jednak ich nadmierny rozwój może prowadzić do pogorszenia jakości sensorycznej mięsa. Spośród analizowanych metod największą stabilność mikrobiologiczną zapewniało przechowywanie mięsa w szafie do sezonowania. Badania wykazały, że mięso dzika przechowywane we wszystkich analizowanych wariantach spełniało kryteria bezpieczeństwa mikrobiologicznego i zachowało przydatność do spożycia do 21 dnia przechowywania.

4.3 Zmiany we właściwościach fizykochemicznych i kruchości mięsa podczas przechowywania

W publikacjach P1 i P2 przedstawiono wyniki badań dotyczących właściwości fizykochemicznych oraz kruchości mięsa podczas przechowywania, realizując trzeci cel szczegółowy – ocenę jakości mięsa przechowywanego w różnych warunkach: próżniowo, w atmosferze modyfikowanej oraz sezonowanego w szafie do dojrzewania. Uzyskane wyniki umożliwiły porównanie różnic między mięśniami oraz wpływu czasu i metody przechowywania na badane parametry (tab. 3).

Wykazano istotny statystycznie wpływ ($p < 0,05$) zarówno metody, jak i czasu przechowywania na wartość pH mięsa. Początkowe wartości pH w mięsie obu gatunków wskazywały na dobrą jakość poubojową surowca, mieszcząc się w zakresie od 5,59 do

5,63 w zależności od analizowanego mięśnia. Największą stabilność wartości pH odnotowano w VAC, gdzie wahania w trakcie przechowywania były najmniejsze. Z kolei metoda DRY-AGED skutkowała wzrostem wartości pH zarówno w przypadku dzika, jak i sarny, co może wskazywać na zachodzące procesy enzymatyczne związane z dojrzewaniem mięsa. Najwyższe wartości pH odnotowano w BF po 21 dniach przechowywania, wynoszące odpowiednio 5,89 dla dzika i 5,78 dla sarny. Przechowywanie wysokotlenowe MAP prowadziło do istotnego obniżenia wartości pH. W przypadku mięsa dzika po 21 dniach wartości te wynosiły 5,31 dla LTL, 5,23 dla BF oraz 5,43 dla VL, natomiast w mięsie sarny były one najniższe dla mięśni LTL i BF, osiągając odpowiednio 5,51 i 5,44.

Istotne różnice wykazano dla parametrów barwy mięśni dzika i sarny pomiędzy badanymi wariantami w czasie przechowywania. Mięso dzika przechowywane w VAC wykazało najmniejsze zmiany parametrów barwy L^* (jasność), a^* (udział barwy czerwonej), b^* (udział barwy żółtej). Mięso przechowywane MAP charakteryzowało się istotnie wyższymi wartościami parametrów L^* i b^* oraz niższymi wartościami a^* , w przeciwieństwie do mięsa DRY-AGED, które wykazało odwrotne tendencje. W przypadku mięsa sarny również odnotowano znacznie niższe wartości a^* w próbach przechowywanych 21 dni w MAP w porównaniu do dwóch pozostałych metod. Zarówno w przypadku mięsa dzika oraz sarny, czas i metoda przechowywania miały istotny wpływ na aktywność wody. Najmniejsze zmiany tego parametru zaobserwowano w próbach przechowywanych próżniowo. Sezonowanie na sucho przez 21 dni przyczyniło się do największego spadku wartości a_w (0,9600-0,9690 w przypadku dzika oraz 0,9590-0,9656 dla sarny). Wysokotlenowa atmosfera gazów powodowała większe wahania a_w , szczególnie w mięsie sarny, gdzie po 21 dniach wartości a_w były najwyższe spośród analizowanych metod (0,9716-0,9737).

Analiza kruchości wykazała, że metoda przechowywania miała istotny wpływ na wartości siły cięcia (WBSF). DRY-AGED oraz VAC przyczyniły się do poprawy kruchości mięsa, co odzwierciedlało się w obniżeniu wartości WBSF po 21 dniach. Najniższe wartości siły cięcia odnotowano w DRY-AGED, gdzie wynosiły one 28,54–33,93 N dla dzika oraz 17,83–23,96 N dla sarny. Mięso przechowywane w VAC charakteryzowało się wartościami WBSF na poziomie 31,48–39,72 N dla dzika i 19,08–27,27 N dla sarny. Przechowywanie w atmosferze wysokotlenowej prowadziło do istotnego pogorszenia kruchości, zwiększając wartości WBSF do 50,70 N w przypadku

dzika i 27,13–29,55 N dla sarny, co wskazuje na wzrost twardości mięsa w wyniku utleniania białek.

Tabela 3. Wpływ czasu (0, 7, 21 dni) i metody przechowywania (VAC, MAP, DRY-AGED) oraz ich interakcji na wybrane właściwości mięśni *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) i *vastus lateralis* (VL) sarny europejskiej i dzika europejskiego.

Parametr	Gatunek	Mięsień	Czas przechowywania	Metoda przechowywania	Czas przechowywania x metoda przechowywania
pH	Dzik europejski, Sarna europejska	LTL	***	***	***
		BF	***	***	***
		VL	***	***	***
L^*	Dzik europejski	LTL	***	***	***
		BF	***	***	***
		VL	***	***	***
	Sarna europejska	LTL	NS	NS	NS
		BF	***	***	***
		VL	***	***	**
a^*	Dzik europejski	LTL	***	***	***
		BF	***	***	***
		VL	***	***	***
	Sarna europejska	LTL	***	NS	***
		BF	NS	***	***
		VL	NS	*	***
b^*	Dzik europejski	LTL	***	***	***
		BF	***	***	***
		VL	NS	***	*
	Sarna europejska	LTL	***	***	***
		BF	NS	***	***
		VL	***	***	**
Aktywność wody	Dzik europejski	LTL	***	***	***
		BF	***	***	***
		VL	***	***	***
	Sarna europejska	LTL	*	*	NS
		BF	***	***	***
		VL	***	***	***
Ubytki termiczne (%)	Dzik europejski	LTL	***	***	***
		BF	***	***	***
		VL	***	***	***
	Sarna europejska	LTL	**	***	***
		BF	***	***	***
		VL	***	***	***
Ubytki przechowalnicze (%)	Dzik europejski	LTL	***	***	***
		BF	***	***	***
		VL	***	***	***
	Sarna europejska	LTL	***	***	***
		BF	***	***	***
		VL	***	***	**
WBSF (N)	Dzik europejski, Sarna europejska	LTL	***	***	***
		BF	***	***	***
		VL	***	***	***
Utlenianie lipidów (TBARS)	Dzik europejski, Sarna europejska	LTL	***	***	***
		BF	***	***	***
		VL	***	***	***
Utlenianie białek (zawartość karbonyli białkowych)	Dzik europejski, Sarna europejska	LTL	***	***	***
		BF	***	***	***
		VL	***	***	***

* $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$; NS - nieistotne (dwuczynnikowa ANOVA).

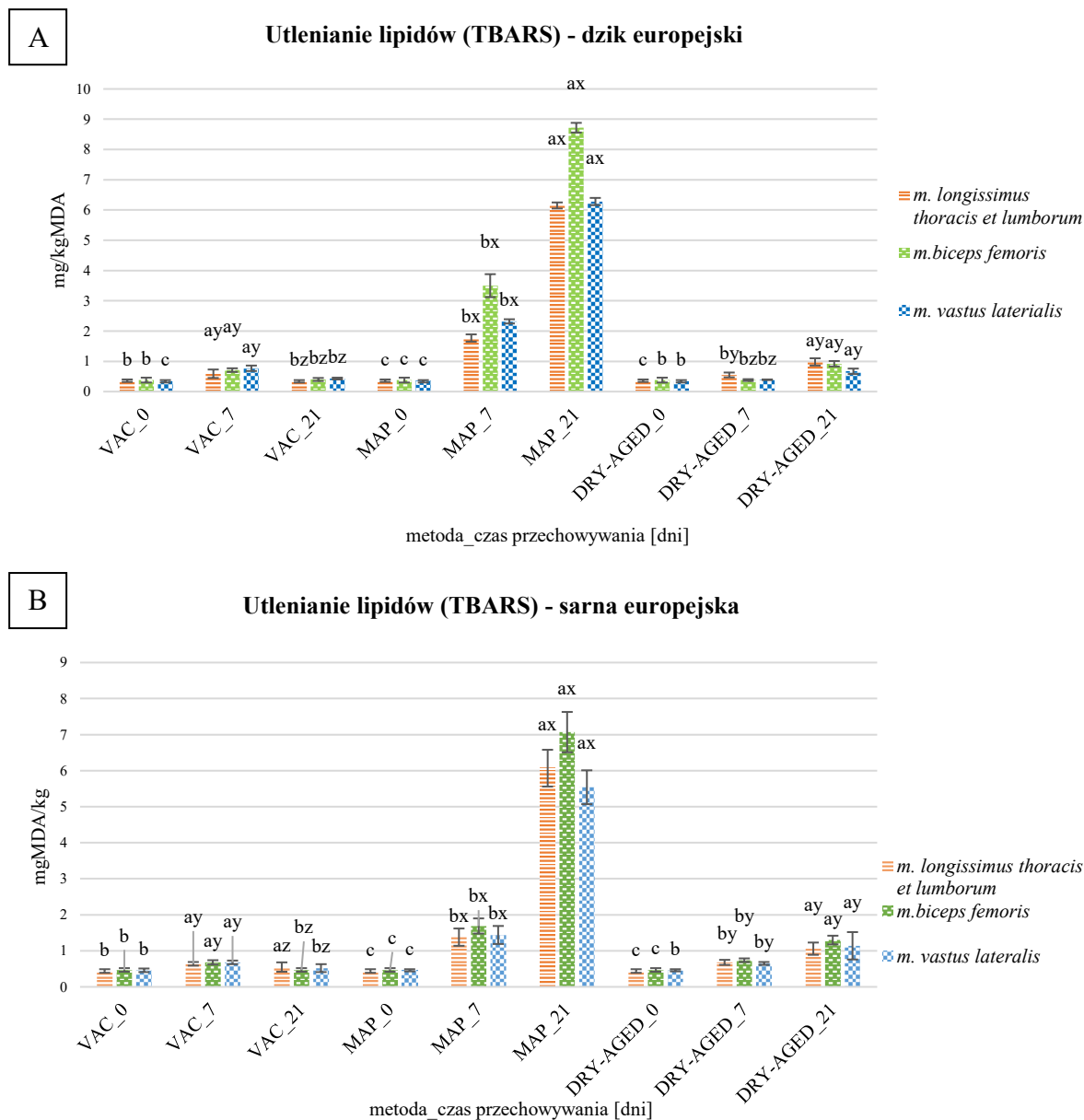
Wyniki badań przedstawione w publikacjach P1 i P2 wykazały istotny wpływ ($p < 0,05$) metody i czasu przechowywania na ubytki przechowalnicze, termiczne oraz kruchość mięsa dzika i sarny. Największe ubytki przechowalnicze odnotowano w próbach DRY-AGED, gdzie po 21 dniach wynosiły one od 25,63% do 32,89% dla dzika oraz od 23,59% do 42,04% dla sarny, w zależności od mięśnia. Mięso przechowywane w MAP wykazało mniejsze ubytki, osiągając po 21 dniach wartości od 6,49% do 8,78% w mięsie dzika i od 5,35% do 6,87% w mięsie sarny. Najmniejsze zmiany masy zaobserwowano w mięsie przechowywanym w VAC, gdzie ubytki nie przekraczały 6,84% dla dzika oraz 5,35% dla sarny.

Obróbka termiczna spowodowała największe ubytki masy w mięsie sezonowanym na sucho, osiągając wartości do 32,89% w przypadku dzika i 42,04% dla sarny. Mięso przechowywane w MAP traciło podczas obróbki mniej wody niż DRY-AGED, jednak więcej niż VAC, przy czym wartości ubytków termicznych po 21 dniach przechowywania wynosiły 7,49–8,78% dla dzika i 5,35–6,87% dla sarny. Najniższe ubytki termiczne odnotowano w próbkach VAC, gdzie wynosiły one od 4,60% do 6,84% dla dzika oraz od 3,96% do 5,23% dla sarny, co wskazuje na lepsze zatrzymanie wody w strukturze mięsa.

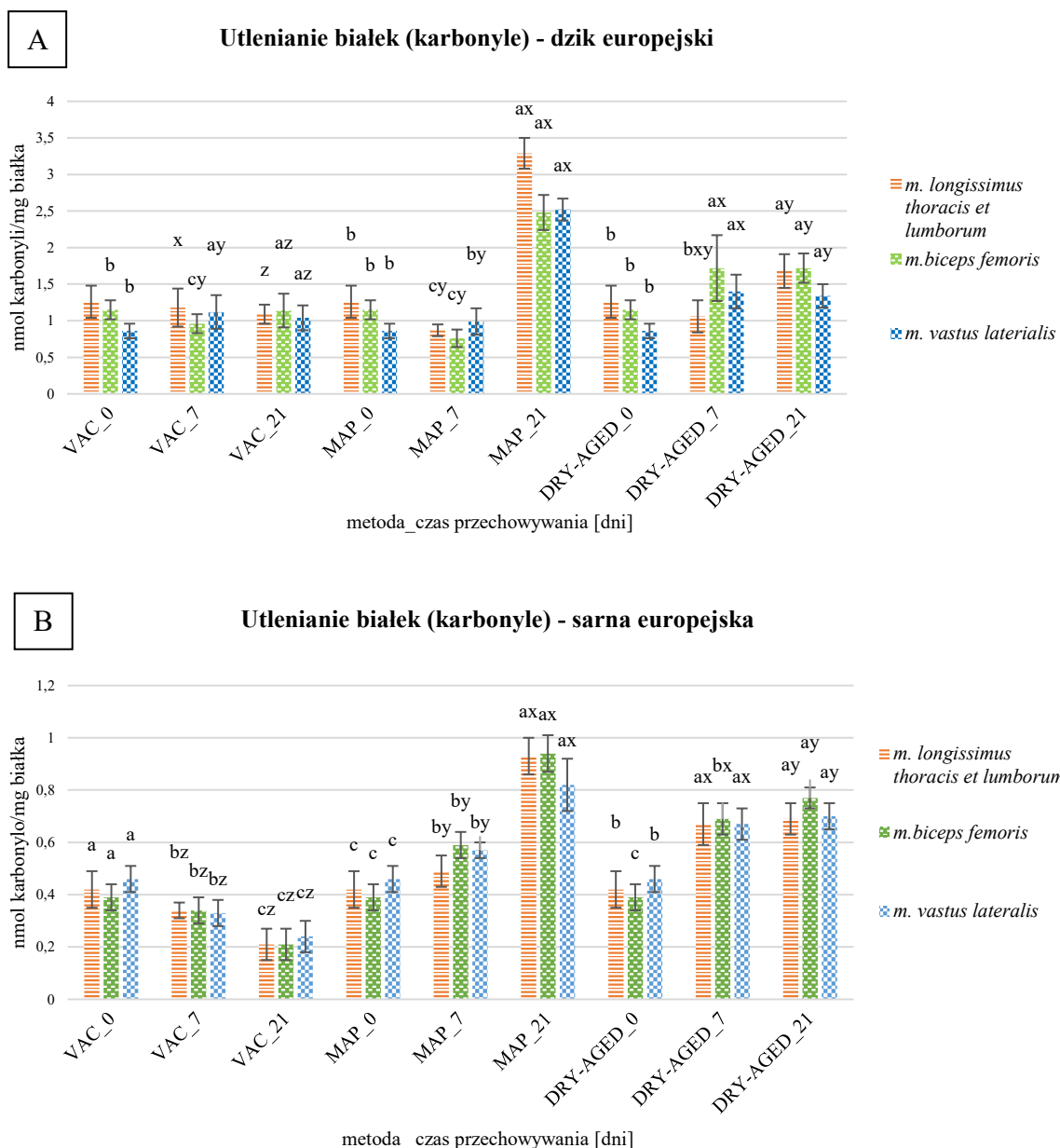
4.4 Wpływ metody przechowywania mięsa na stabilność oksydacyjną lipidów i białek

W publikacjach P1 i P2 przedstawiono wyniki badań dotyczących stabilności oksydacyjnej lipidów i białek podczas przechowywania, realizując czwarty cel szczegółowy – porównanie podatności oksydacyjnej analizowanych mięśni pod wpływem przechowywania. Wyniki wykazały istotny wpływ ($p < 0,05$) metody i czasu przechowywania na procesy utleniania lipidów i białek w mięsie dzika i sarny (rys. 3). Najwyższy poziom utleniania lipidów, mierzony jako zawartość substancji reaktywnych z kwasem tiobarbiturowym (TBARS), zaobserwowano w mięsie przechowywanym w MAP. Po 21 dniach przechowywania wartości TBARS wzrosły o 1512% dla dzika (rys. 3A) i 1263% dla sarny (rys. 3B), osiągając poziom 6,07–8,72 mg MDA/kg. Świadczy to o intensywnej peroksydacji lipidów i degradacji tłuszczów. Przechowywanie VAC oraz DRY-AGED skutecznie ograniczały oksydację lipidów. Wartości TBARS dla VAC były najniższe, nie przekraczając 0,56–0,77 mg MDA/kg, natomiast w przypadku DRY-AGED wynosiły 0,67–1,30 mg MDA/kg dla sarny oraz 0,67–0,98 mg MDA/kg dla

dzika, co wskazuje, że choć sezonowanie na sucho powodowało pewien wzrost utleniania lipidów, proces ten był znacznie mniej intensywny niż w warunkach wysokotlenowych MAP.



Rys. 3. Wpływ metody i czasu przechowywania na utlenianie lipidów w mięśniach dzika europejskiego (*Sus scrofa scrofa*) (A) i sarny europejskiej (*Capreolus capreolus*) (B); średnia $\pm$ SD. Różne litery w kolumnie (^{a, b, c}) dla danego parametru oznaczają istotny wpływ czasu w ramach metody ($p < 0,05$); różne litery w wierszu (^{x, y, z}) dla danego parametru wskazują na istotny wpływ metody w czasie przechowywania ($p < 0,05$).



Rys. 4. Wpływ metody i czasu przechowywania na utlenianie białek w mięśniach dzika europejskiego (*Sus scrofa scrofa*) (A) i sarny europejskiej (*Capreolus capreolus*) (B); średnia $\pm$ SD. Różne litery w kolumnie (a, b, c) dla danego parametru oznaczają istotny wpływ czasu w ramach metody ($p < 0,05$); różne litery w wierszu (x, y, z) dla danego parametru wskazują na istotny wpływ metody w czasie przechowywania ($p < 0,05$).

Podobne zależności zaobserwowano w przypadku utleniania białek, określonego na podstawie zawartości grup karbonylowych (nmol karbonyli na mg białka) (rys. 4). Po 21 dniach przechowywania w MAP wartości te wzrosły o 161% dla dzika i 155% dla sarny, osiągając poziom 2,48–3,29 nmol/mg białka, co wskazuje na intensywną degradację białek. W próbkach VAC utlenianie białek było najmniejsze, a wartości grup

karbonylowych nie przekraczały 0,26–1,09 nmol/mg białka. Sezonowanie na sucho prowadziło do umiarkowanego wzrostu utleniania białek – wartości karbonylowe wynosiły 1,34–1,72 nmol/mg białka dla sarny oraz 1,34–1,68 nmol/mg białka dla dzika, co było wyższe niż w VAC, ale niższe niż w MAP.

Uzyskane wyniki wskazują, że przechowywanie w wysokotlenowej atmosferze MAP znacząco przyspieszało procesy utleniania lipidów i białek, co mogło negatywnie wpływać na jakość i stabilność mięsa. Lipidy okazały się bardziej podatne na utlenianie niż białka, co potwierdzają wyższe wartości wskaźnika TBARS w porównaniu do zawartości grup karbonylowych. Najkorzystniejszym sposobem ograniczania niekorzystnych zmian oksydacyjnych było przechowywanie próżniowe, natomiast sezonowanie na sucho pozwalało na naturalny proces dojrzewania mięsa, jednocześnie ograniczając utlenianie białek i lipidów w porównaniu do wysokotlenowej atmosfery gazowej.

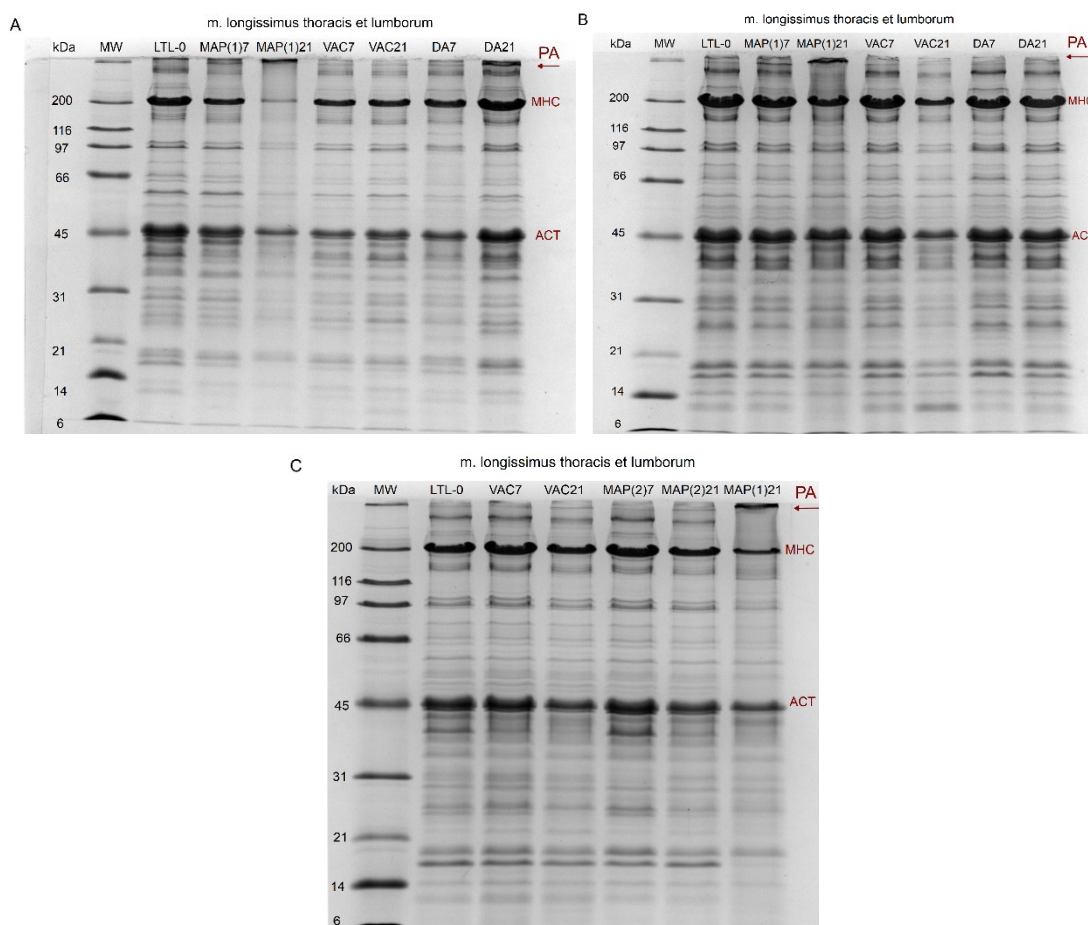
4.5 Ocena i identyfikacja agregatów białkowych powstałych podczas przechowywania mięsa

W celu pogłębionej analizy stabilności białek mięśniowych dzika i sarny zastosowano kompleksowe podejście proteomiczne. Uzyskane wyniki pozwoliły nie tylko na ocenę wpływu metody przechowywania na zmiany w strukturze białek, ale także na bezpośrednie porównanie wrażliwości proteomu mięsa obu gatunków na stres oksydacyjny. W publikacjach P3 i P4 przedstawiono wyniki badań dotyczących oceny i identyfikacji agregatów białkowych powstałych podczas przechowywania mięsa, realizując piąty i szósty cel szczegółowy. W przypadku sarny, mięśnie zapakowano w atmosferze modyfikowanej o dwóch składach gazów (MAP(1): 80% O₂/20% CO₂ oraz MAP(2): 40% CO₂/60% N₂), celem porównania.

4.5.1 Profile białkowe – ocena stopnia agregacji białek

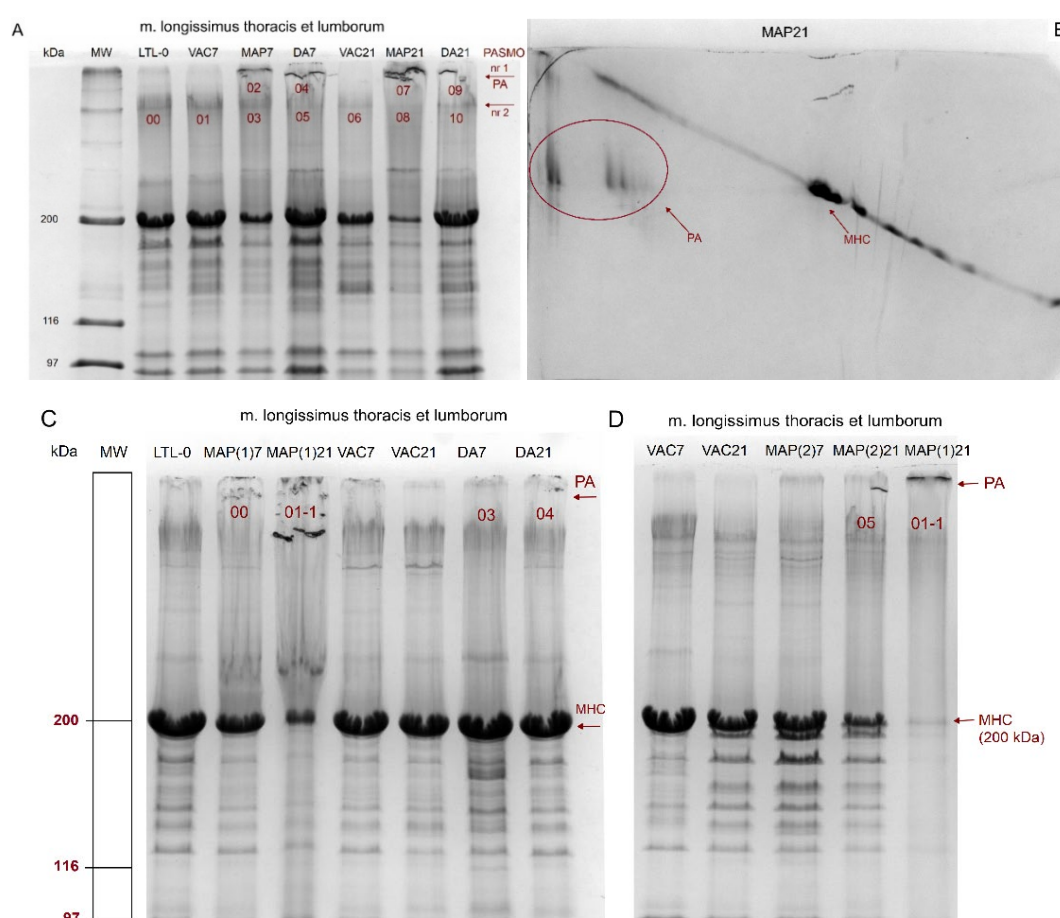
Profile wyekstrahowanych frakcji białkowych i rozkłady mas cząsteczkowych dla obu gatunków przedstawiono na rys. 5 (na przykładzie mięśnia LTL, 5A dla dzika oraz 5B dla sarny). Rys. 5C przedstawia porównanie profili białkowych LTL sarny, przechowywanego w MAP(2), MAP(1) oraz VAC. W przypadku obu gatunków, elektroforeza SDS-PAGE pozwoliła na wstępne potwierdzenie obecności PA o masie cząsteczkowej większej niż 200 kDa w MAP(1) oraz DA po 21 dniach przechowywania.

Ponadto, zarejestrowano znaczną degradację intensywności pasm reprezentujących ciężkie łańcuchy miozyny (MHC, 200 kDa) oraz aktynę (ACT, 45 kDa) w MAP(1). Próby przechowywane w VAC oraz w atmosferze MAP(2) zapewniły lepszą ochronę przed oksydacyjnym uszkodzeniem białek. Mięśnie pochodzące od dzika wykazały wyższy stopień agregacji białek w porównaniu z mięśniami sarny oraz charakteryzowały się większym stopniem degradacji miozyny i aktyny. Mimo tej różnicy, ogólna tendencja pozostała spójna: w obu badaniach, mięśniach przechowywanych przez 21 dni w MAP(1) występowały najbardziej zaawansowane zmiany oksydacyjne. Wyniki te są zgodne z badaniami nad oksydacją lipidów i białek, które wykazały zwiększoną podatność mięsa dzika na utlenianie w porównaniu do mięsa sarny [P1 i P2].



Rys. 5. Reprezentatywne rozdziały elektroforetyczne SDS-PAGE w warunkach redukujących dla wszystkich analizowanych metod przechowywania białek mięśni LTL; (A) dzika; (B,C) sarny. Agregaty białkowe (PA) znajdujące się na górze żelu SDS-PAGE i w dół od przekątnej są połączone wewnątrzcząsteczkowymi wiązaniami dwusiarczkowymi. MAP(1) – 80% O₂/20% CO₂; MAP(2) – 40% CO₂/60% N₂; VAC - próżnia; DA - szafa do sezonowania mięsa na sucho; 7, 21 - dni przechowywania; MHC - łańcuchy ciężkie miozyny; ACT - aktyna.

Analiza SDS-PAGE w warunkach nieredukujących (bez DTT) mięśni dzika (P3) i sarny (P4) wykazała, że warunki przechowywania istotnie wpływają na powstawanie PA w mięsie. Zidentyfikowano powstawanie wysokocząsteczkowych PA (>200 kDa), szczególnie intensywnych w MAP(1) (rys. 6). W przypadku mięśni dzika, agregaty były obecne już po 7 dniach, natomiast w mięsie sarny ich intensywność rosła z czasem i była najwyższa po 21 dniach. Co istotne, mięso dzika wykazało wyraźnie większą zdolność do tworzenia trwałych agregatów w przypadku sezonowania, niż mięso sarny, co może wynikać z różnic w strukturze białek. W przypadku obu gatunków pakowanie próżniowe zapobiegało tworzeniu agregatów białkowych.



Rys. 6. Reprezentatywne rozdziały elektroforetyczne w warunkach nieredukujących dla wszystkich analizowanych metod przechowywania białek mięśni LTL; (A) SDS-PAGE białek dzika; (B) elektroforeza diagonalna białek dzika przechowywanych w zmodyfikowanej atmosferze z 80% O₂ i 20% CO₂ przez 21 dni (MAP(1)21). Agregaty białkowe (PA) znajdujące się na górze żelu SDS-PAGE i w dół od przekątnej są połączone wewnątrzcząsteczkowymi wiązaniami dwusiarczkowymi. MAP(1) – 80% O₂/20% CO₂; MAP(2) – 40% CO₂/60% N₂; VAC - próżnia; DA - szafa do sezonowania mięsa na sucho; 7, 21 - dni przechowywania; MHC - łańcuchy ciężkie miozyny; ACT - aktyna.

W mięśniach przechowywanych w MAP(2) (w przypadku sarny) obecność agregatów była znikoma lub całkowicie nieobserwowalna, zarówno w 7. i 21. dniu przechowywania. W obu publikacjach (P3 i P4) zastosowano elektroforezę diagonalną (rys. 6B) w celu weryfikacji rodzaju wiązań stabilizujących agregaty białkowe powstające podczas przechowywania. Obserwacja pasm poniżej linii diagonalnej jednoznacznie potwierdziła, że agregaty te były stabilizowane głównie przez wiązania dwusiarczkowe, międzycząsteczkowe. Wyniki te wskazują, że atmosfera wysokotlenowa (MAP(1)) jest kluczowym czynnikiem inicjującym polimeryzację białek, prowadząc do trwałego sieciowania białek w warunkach stresu oksydacyjnego. Analiza Western Blot wykazała, że przeciwciało MF20 skutecznie rozpoznało miozynę w utworzonych PA, a także inne polimorficzne formy ciężkiego łańcucha miozyny i jego produkty degradacji [P3, P4].

4.5.2 Identyfikacja białek tworzących agregaty przy użyciu LC-MS/MS

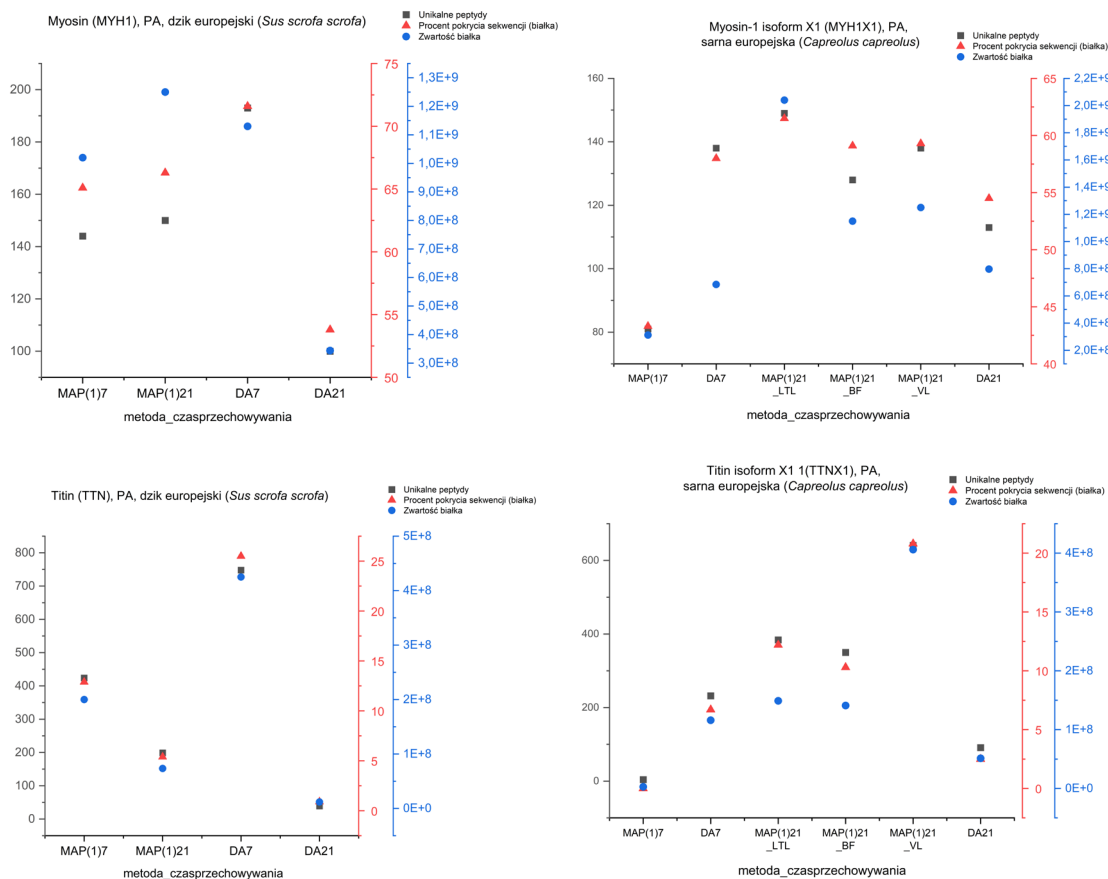
W celu realizacji szóstego celu szczegółowego, tj. identyfikacji agregatów białkowych powstałych podczas przechowywania mięsa dzika (P3) i sarny (P4), przeprowadzono analizę proteomiczną metodą LC-MS/MS. Do analizy wybrano pasma o wysokiej masie cząsteczkowej (>200 kDa) wyizolowane z żeli SDS-PAGE prowadzonej w warunkach nieredukujących (pasma 00-10; rys. 6). W przypadku mięśni dzika próby ujednolicono względem metod i czasu pakowania, oraz wycięto dwa pasma (PA – pasmo nr 1 oraz pasmo nr 2 (rys. 6A)). Dla mięśni pochodzących od sarny porównano również podatność oksydacyjną analizowanych mięśni pod wpływem przechowywania 21 dni MAP (rys. 6C, 01-1 dla LTL oraz adekwatnie dla BF i VL, tab. 4). W ramach przeprowadzonych analiz LC-MS/MS określono intensywność białek, liczbę unikalnych peptydów oraz procent pokrycia sekwencji białkowych. Uzyskane widma masowe zapewniły dobry procent pokrycia sekwencji analizowanych białek.

Warunki pakowania i przechowywania mięsa okazały się kluczowym czynnikiem determinującym intensywność utleniania i agregacji białek u obu gatunków. Ogólny profil białek tworzących agregaty był zbliżony u obu gatunków, jednak tempo i skala tych zmian różniły się – proces agregacji białek w mięsie dzika przebiegał szybciej i intensywniej niż w mięsie sarny (tab. 4, 5). W obu publikacjach [P3 i P4] potwierdzono, że mięso przechowywane w atmosferze wysokotlenowej (MAP(1)) wykazuje największą liczbę zidentyfikowanych białek w agregatach, co potwierdza silny wpływ tlenu jako czynnika indukującego utlenianie i sieciowanie białek. Dla prób pozyskanych od sarny

przechowywanych w atmosferze MAP(2) liczba białek wchodzących w skład agregatów była istotnie niższa. DA stanowiło przypadek pośredni – mięso przechowywane w kontrolowanej temperaturze, narażone na tlen z powietrza atmosferycznego (~20,9% O₂), wykazało umiarkowany poziom utleniania białek. Stwierdzono różnice między gatunkami: mięso dzika (tab. 4) było bardziej podatne na uszkodzenia oksydacyjne białek niż mięso sarny (tab. 5).

Tabela 4. Główne białka zidentyfikowane w agregatach białkowych (PA) powstałych w wyniku przechowywania mięsa dzika w zmodyfikowanej atmosferze z wysoką zawartością tlenu (80% O₂/20% CO₂; MAP(1)) oraz dojrzewania na sucho w komorze do sezonowania mięsa (DA) przez 7 i 21 dni.

Lp.	Białko	Skrót	Numer akcesyjny	MAP_7	MAP_21	DA_7	DA_21
1	Titin	TTN	XP_020931560.1	+	+	+	+
2	Myosin-1 isoform X1	MYH1	XP_020921683.1	+	+	+	+
3	Myosin-2 isoform X1	MYH2	XP_020921875.1	+	+	+	+
4	Myosin heavy chain slow isoform	MYH7	BAB20630.1	+	+	+	+
5	Nebulin	NEB	XP_020931125.1	+	+	+	+
6	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	+	+	+	-
7	Skeletal alpha actin	ACTA1	XP_020927589.1	-	+	-	-
8	Four and a half LIM domains protein 1 isoform X3	FHL1	XP_020935180.1	+	+	+	-
9	Keratin 1, partial	KRT1	BAF62303.1	+	+	+	+
10	Muscle creatine kinase	CKM	AAV28621.1	+	+	+	+
11	Obscurin isoform X1	OBSCN	XP_020939003.1	-	-	+	-
12	Glyceraldehyde-3-phosphate dehydrogenase isoform X1	GAPDH	prf 681085A	+	+	+	-
13	Actin alpha 2	ACTA2	ACT82246.1	+	-	-	+
14	Filamin-C isoform X1	FLNC	XP_020934608.1	+	+	+	-
15	Isocitrate dehydrogenase	IDH	NP_001157479.1	+	+	+	-
16	Muscle glycogen phosphorylase, partial	PYGM	XP_003122636.2	+	+	+	-
17	Myosin light chain 1/3, skeletal muscle isoform X1	MLC1F	ABK55642.1	+	+	+	-
18	Myosin-binding protein C, fast-type	MYBPC2	XP_003127419.3	+	+	+	-
19	Myomesin-2	MYOM2	XP_020931561.1	+	+	+	-
20	Myoglobin	MB	AAA31073.1	+	+	+	-
21	Beta-enolase 3	ENO3	ABC75829.1	-	+	-	-
22	Cardiac muscle ATP synthase H ⁺ transporting mitochondrial complex alpha subunit 1	ATP5A1	ACD02421.1	-	+	-	-
23	ADP/ATP translocase 4	SLC25A4	XP_003133393.1	-	+	+	-
Całkowita liczba białek, spośród wybranych tworzących PA				18	21	19	8



Rys. 7. Wyniki chromatografii cieczowej z tandemową spektrometrią mas dla dwóch najobficiej występujących białek strukturalnych w agregatach białkowych dzika europejskiego i sarny europejskiej – miozyny i titiny - uzyskane z wyciętych pasm białkowych oznaczonych na rysunku 6. Skróty: BF - *musculus biceps femoris*; DA - starzenie na sucho w szafie do sezonowania mięsa; LTL - *musculus longissimus thoracis et lumborum*; MAP(1) - atmosfera modyfikowana o składzie 80% O₂/20% CO₂; MAP(2) - atmosfera modyfikowana o składzie 40% CO₂/60% N₂; VL - *musculus vastus lateralis*. Dla każdego wariantu „7” i „21” oznaczają dni przechowywania.

Tendencja ta potwierdza większą podatność mięsa dzika na utlenianie białek, co może wynikać m.in. z wyższej zawartości tłuszczu śródmięśniowego u dzika sprzyjającej generowaniu reaktywnych form tlenu. Biorąc pod uwagę procent pokrycia i intensywności białek wykazano, że głównymi składnikami agregatów były białka strukturalne mięśni szkieletowych, w tym przede wszystkim miozyna-1 izoforma X1 oraz titina/titina izoforma X1 (rys. 7, tab. 4,5). Zawartość miozyny 1 w agregatach białkowych mięsa dzika mieściła się w zakresie od $3,44 \times 10^8$ (pokrycie sekwencji: 53,8%; DA_21) do $1,25 \times 10^9$ (66,3%; MAP(1)_21). Dla analizowanych mięśni sarny wartości te wynosiły odpowiednio: $2,45 \times 10^7$ i 13,4% (MAP(2)) oraz $2,04 \times 10^9$ i 61,5% (MAP(1)_21, LTL). Wyraźny spadek poziomu agregacji miozyny po 21 dniach mięsa dzika przechowywanego DA sugeruje, że proces ten jest dynamiczny i że miozyna ulega

istotnym zmianom strukturalnym w odpowiedzi na specyficzne warunki przechowywania. W przypadku titiny stwierdzono, że po 21 dniach przechowywania DA, jej udział w PA uległ wyraźnemu zmniejszeniu w mięsie dzika oraz nieznacznemu obniżeniu w mięsie sarny, niezależnie od warunków przechowywania, co najprawdopodobniej wynika z postępujących procesów proteolitycznych. Wymienione białka odgrywają kluczową rolę w utrzymaniu struktury i funkcji mięśni, dlatego uszkodzenia oksydacyjne wyrażone jako tworzenie agregatów i ich dalsza degradacja mogą prowadzić do zmian w teksturze mięsa, tj. utraty kruchości i innych właściwości mięsa np. barwy, co potwierdzają badania skupiające się na cechach fizykochemicznych.

Podczas przechowywania mięsa dzika (tab. 5) zaobserwowano istotne zmiany w trzech enzymach związanych z funkcją mięśni: kinazie kreatynowej (CKM), ATPazie wapniowej typu 1 siateczki sarkoplazmatycznej/endoplazmatycznej (SERCA1) i dehydrogenazie izocytrynianowej (IDH). Ich obecność w PA, szczególnie w warunkach wysokotlenowych, wskazuje na podatność tych enzymów na stres oksydacyjny. Strukturalne modyfikacje mogą prowadzić do utraty aktywności enzymatycznej, zaburzeń równowagi wapniowej oraz zakłóceń procesów energetycznych. IDH, kluczowy enzym szlaku dekarboksylacji oksydacyjnej, wykazał większą aktywność w MAP, co sugeruje jego udział w odpowiedzi na stres oksydacyjny. Wymienione białka były obecne również w PA w warunkach DA w 7. dniu przechowywania, natomiast po 21 dniach przechowywania odnotowano znaczną degradację utlenionych białek. W agregatach białek dzika identyfikowano również mitochondrialną syntazę ATP mięśnia sercowego (ATP5A1) oraz glikolityczną mięśniową enolazę 3 (ENO3), których modyfikacje oksydacyjne mogą mieć wpływ na jakość mięsa, w tym jego kruchość i trwałość. Zmiany te wskazują, że agregacja dotyczy nie tylko białek strukturalnych, ale także enzymów kluczowych dla homeostazy komórkowej [P3].

Tabela 5. Główne białka zidentyfikowane w agregatach białkowych (PA) powstałych w wyniku przechowywania mięsa sarny w zmodyfikowanej atmosferze z wysoką zawartością tlenu (80% O₂/20% CO₂; MAP(1)), w zmodyfikowanej atmosferze z dwutlenkiem węgla i azotem (40% CO₂/60% N₂; MAP(2)) oraz starzenia na sucho w komorze do sezonowania mięsa (DA) przez 7 i 21 dni. LTL – m. *longissimus thoracis et lumborum* (najdłuższy mięsień grzbietu), BF – m. *biceps femoris* (dwugłowy uda), VL – m. *vastus lateralis* (obszerny boczny).

Lp.	Białko	Skrót	Numer akcesyjny	MAP(1) 7	MAP(1) 21			DA 7	DA 21	MAP(2) 21
Mięsień					LTL	BF	VL			
1	Titin isoform X1	TTNX1	XP_017920706.1	+	+	+	+	+	+	+
2	Myosin-1 isoform X1	MYH1X1	XP_017920146.1	+	+	+	+	+	+	+
3	Myosin-2 isoform X1	MYH2X1	XP_020921875.1	-	-	-	-	+	-	-
4	Myosin heavy chain 2x	MYH2X	BAB40921.2	-	+	+	-	+	-	-
5	Nebulin isoform X31	NEB	XP_024836427.1	-	+	+	+	-	-	-
6	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_017895664.1	+	+	+	+	+	+	-
7	Actin, alpha skeletal muscle isoform X1	ACTA1	XP_020927589.1	+	-	-	-	+	-	-
8	Keratin 1, partial	KRT1	BAF62303.1	+	+	+	+	+	+	+
9	Collagen, type VI, alpha 1	COL6A1	DAA32939.1	-	-	-	-	+	+	-
10	Collagen alpha-3(VI) chain isoform X6	COL6A3	XP_017896230.1	-	-	-	-	+	+	-
11	Creatine kinase, muscle	CKM	AAI02908.1	-	+	+	+	-	-	-
12	Dehydrogenase, glyceraldehyde phosphate	GAPDH	AAI02590.1	-	+	+	+	-	-	-
13	Actinin, alpha 2	ACTN2	AAI02909.1	-	-	-	+	-	-	-
14	Filamin-C	FLNC	NP_001193919.1	-	+	-	+	-	-	-
15	Isocitrate dehydrogenase	IDH	CAA49207.1	-	+	+	+	-	-	-
16	Alpha-1,4-glucan orthophosphate glycosyl transferase	AGP	AAB46846.1	-	+	+	+	-	-	-
17	Myosin light chain, phosphorylatable, fast skeletal muscle	MLC2F	AAI20432.1	-	+	+	-	-	-	-
18	Myosin-binding protein C, fast-type	MYBOC2	XP_017918332.1	-	+	+	+	-	-	-
19	Myomesin-2	MYOM2	XP_020931561.1	-	+	+	+	-	-	-
20	Myoglobin	MB	AAI02987.1	-	+	+	-	-	-	-
21	Succinyl-CoA ligase	SUCLA	XP_017912243.1	-	+	+	-	-	-	-
22	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	ATP5A1	AAI16060.1	-	+	+	-	-	-	-
23	Adult beta-globin	HBA	ASH97668.1	-	+	+	-	-	-	-
Całkowita liczba białek, spośród wybranych tworzących PA				5	18	17	13	9	5	3

W przypadku mięsa sarny (tab. 5) zaobserwowano, że niektóre białka enzymatyczne ulegały agregacji wyłącznie w obecności tlenu, co czyni je potencjalnymi wskaźnikami stresu oksydacyjnego mięsa. Dotyczy to szczególnie CKM oraz IDH, których agregacja może świadczyć o przekroczeniu komórkowych mechanizmów obronnych wobec reaktywnych form tlenu. Obecność enzymów metabolicznych w agregatach wskazuje, że procesy oksydacyjne, również w przypadku sarny nie ograniczają się jedynie do białek strukturalnych, lecz obejmują też kluczowe komponenty odpowiedzialne za metabolizm komórkowy i homeostazę. Może to prowadzić do utraty aktywności enzymatycznej, zaburzeń funkcjonalnych mięśni oraz pogorszenia wartości odżywczej mięsa. Identyfikacja w agregatach także ATPazy siateczki sarkoplazmatycznej (SERCA1) oraz filaminy-C, potwierdza, że stres oksydacyjny obejmuje struktury odpowiedzialne za kurczliwość, integralność komórkową oraz transport jonów wapnia, co może istotnie wpływać na jakość technologiczną surowca.

Wśród analizowanych mięśni sarny, VL wykazał największą odporność na zmiany oksydacyjne oraz najmniejsze nasilenie niekorzystnych przemian podczas przechowywania. Analiza proteomiczna wykazała, że mięsień VL charakteryzował się najmniejszą liczbą białek zidentyfikowanych w agregatach białkowych w porównaniu do pozostałych mięśni (tab. 5). Potwierdzają to również najniższe wartości wskaźnika TBARS, jak i najniższe stężenia karbonylowych grup białkowych (rys. 3, rys. 4). Obserwacje te są zgodne z wynikami elektroforezy SDS-PAGE, która wykazała mniej intensywną agregację i degradację białek strukturalnych w VL, w porównaniu do mięśni BF i LTL. Mięsień VL charakteryzował się również ograniczoną obecnością enzymów takich jak kinaza kreatynowa (CKM) czy dehydrogenaza izocytrynianowa (IDH) w agregatach białkowych, co sugeruje mniejszy poziom stresu oksydacyjnego. Ponadto, wyniki proteomiczne nie wykazują obecności mioglobiny (MB) w agregatach białkowych VL w 21 dniu przechowywania, co może mieć istotne znaczenie z punktu widzenia barwy mięsa. Stabilność mioglobiny, jako głównej chromoproteiny mięsa odpowiedzialnej za intensywność barwy czerwonej (a^*), może wpływać na utrzymanie atrakcyjnego wyglądu produktu. Obniżona podatność VL na zmiany oksydacyjne może zatem przekładać się nie tylko na jego większą trwałość, ale także na korzystniejsze cechy sensoryczne i technologiczne [P4].

5. Podsumowanie i wnioski

W niniejszej rozprawie doktorskiej oceniono wpływ warunków przechowywania na jakość oraz stabilność oksydacyjną mięsa dzika (*Sus scrofa scrofa*) i sarny (*Capreolus capreolus*), z uwzględnieniem różnic pomiędzy trzema mięśniami: m. *longissimus thoracis et lumborum* (LTL), m. *biceps femoris* (BF) oraz m. *vastus lateralis* (VL). Przeprowadzone badania miały charakter interdyscyplinarny i obejmowały ocenę parametrów fizykochemicznych, mikrobiologicznych oraz proteomicznych, co pozwoliło na kompleksowe scharakteryzowanie zachodzących zmian.

Badania wykazały, że sposób przechowywania mięsa ma istotny wpływ na zachowanie jego jakości. Szczególnie niekorzystne zmiany oksydacyjne w obrębie białek i lipidów zaobserwowano w mięsie pakowanym w atmosferze modyfikowanej (MAP) o wysokiej zawartości tlenu (80% O₂/20% CO₂). Ich konsekwencją było pogorszenie kluczowych cech sensorycznych mięsa, w tym barwy, tekstury oraz wodochłonności. W warunkach pakowania próżniowego oraz sezonowania na sucho zaobserwowano korzystniejsze zachowanie jakości i cech technologicznych mięsa, mimo że w przypadku sezonowania obecny był tlen atmosferyczny. Stwierdzono również istotne różnice między analizowanymi mięśniami. Mięsień LTL wykazał największą podatność na zmiany oksydacyjne i agregację białek, podczas gdy mięsień VL charakteryzował się większą stabilnością, co może mieć praktyczne znaczenie przy projektowaniu warunków przechowywania i dalszego przetwarzania różnych elementów tuszy.

Uzyskane dane potwierdzają również, że procesy oksydacyjne mają złożony wpływ na jakość mięsa, obejmując nie tylko aspekty sensoryczne, ale także degradację struktury białek i ich potencjalne znaczenie zdrowotne. Analizy proteomiczne przeprowadzone z użyciem techniki LC-MS/MS pozwoliły na identyfikację białek podatnych na agregację oraz takich, które pełnią rolę w odpowiedzi komórek na stres oksydacyjny. Obecność utlenionych form titiny, miozyny oraz białek mitochondrialnych wskazuje na złożone mechanizmy degradacji i adaptacji, zachodzące w tkance mięśniowej podczas przechowywania.

Zrealizowane badania pozwoliły na udowodnienie tezy pracy oraz sformułowanie niżej przedstawionych wniosków odnoszących się do celów szczegółowych i wyników

prezentowanych w czterech publikacjach naukowych, stanowiących niniejszą rozprawę doktorską.

1. Mięso dzika i sarny, pozyskane ze środowiska naturalnego, charakteryzowało się wysoką zawartością białka, niskim udziałem tłuszczu oraz korzystnym profilem składników mineralnych, co czyni je wartościowym elementem diety.
2. Wszystkie zastosowane metody przechowywania zapewniły mikrobiologiczną stabilność mięsa dzika przez 21 dni.
3. Sposób przechowywania wpłynął istotnie na pH, barwę, termiczne i przechowalnicze ubytki masy oraz kruchość mięsa, przy czym najlepsze cechy technologiczne zachowało mięso dzika i sarny przechowywane w próżni oraz sezonowane.
4. Mięso dzika wykazało wyższą podatność na utlenianie białek i lipidów niż mięso sarny, szczególnie w warunkach wysokiego stężenia tlenu, gdzie odnotowano najwyższe wartości TBARS i związków karbonylowych.
5. Mięśnie *biceps femoris* (BF), *longissimus thoracis et lumborum* (LTL) oraz *vastus lateralis* (VL) wykazały zróżnicowaną podatność na zmiany oksydacyjne i technologiczne podczas przechowywania, a największą stabilność w zakresie parametrów jakościowych i mniejsze nasilenie utleniania stwierdzono w mięśniu VL.
6. Agregaty białkowe powstające w warunkach stresu oksydacyjnego zawierały głównie białka strukturalne (miozynę i titinę), enzymatyczne (kinazę kreatynową i dehydrogenazę izocytrynianową) oraz białka cytoszkieletowe (ATPazę wapniową typu 1 siateczki sarkoplazmatycznej/endoplazmatycznej, filaminę C).
7. Zmiany w składzie agregatów białkowych w zależności od czasu i metody przechowywania wskazują na dynamiczny charakter stresu oksydacyjnego i jego wpływ na właściwości funkcjonalne mięsa.
8. Postępująca agregacja białek strukturalnych podczas przechowywania miała negatywny wpływ na teksturę mięsa.

9. Minimalizacja dostępu tlenu podczas przechowywania może skutecznie ograniczać agregację oraz degradację białek i lipidów, poprawić stabilność mięsa dzika i sarny oraz zachować jego wysoką jakość technologiczną i odżywczą.

Podjęta problematyka jest istotna zarówno z punktu widzenia bezpieczeństwa żywności i jakości sensorycznej mięsa, jak i z perspektywy technologii przetwórstwa żywności. Dzikizna, jako surowiec o wysokiej wartości odżywczej i naturalnym pochodzeniu, może stanowić element zrównoważonej diety, zachowując odpowiednie warunki jej przechowywania i obróbki technologicznej. Uzyskane wyniki dostarczają nie tylko wiedzy poznawczej na temat zachodzących procesów biochemicznych w dziczyźnie, ale mogą także znaleźć zastosowanie w praktyce przemysłowej – szczególnie w zakresie oceny trwałości produktów mięsnych. Zidentyfikowane w pracy białka odpowiedzialne za odpowiedź na stres oksydacyjny mogą w przyszłości stanowić podstawę do opracowania markerów świeżości lub jakości mięsa.

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7. Oświadczenia o współautorstwie publikacji wchodzących w skład rozprawy doktorskiej

Publikacja 1: Kasalka-Czarna, N., Bilska, A., Biegańska-Marecik, R., & Montowska, M. (2022). The effect of storage method on selected physicochemical and microbiological qualities of wild boar meat. *Journal of the Science of Food and Agriculture*, 102(12), 5250-5260. <https://doi.org/10.1002/jsfa.11878>.

Publikacja 2: Kasalka-Czarna, N., Biegańska-Marecik, R., Proch, J., Orłowska, A., & Montowska, M. (2023). Effect of dry, vacuum, and modified atmosphere ageing on physicochemical properties of roe deer meat. *Polish Journal of Food and Nutrition Sciences*, 73(2), 175–186. <https://doi.org/10.31883/pjfn/163613>.

Publikacja 3: Kasalka-Czarna, N., Stachniuk, A., Fornal, E., & Montowska, M. (2025). Proteomic analysis of wild boar meat: Effect of storage method and time on muscle protein stability. *Food Chemistry*, 464 (3), 141774. <https://doi.org/10.1016/j.foodchem.2024.141774>.

Publikacja 4: Kasalka-Czarna, N., Stachniuk, A., Fornal, E., & Montowska, M. (2025). Protein aggregation during storage of roe deer meat: a proteomic study. *Journal of Agricultural and Food Chemistry*, 73, 8001–8014. <https://doi.org/10.1021/acs.jafc.4c12348>.



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Oświadczenie o współautorstwie

Niniejszym oświadczamy, że jesteśmy współautorami pracy:

Kasałka-Czarna, N., Bilaska, A., Biegańska-Marecik, R., Montowska, M. (2022). The effect of storage method on selected physicochemical and microbiological qualities of wild boar meat. Journal of the Science of Food and Agriculture, 102(12), 5250-5260. <https://doi.org/10.1002/jsfa.11878>

Jednocześnie wyrażamy zgodę na wykorzystanie przez mgr inż. Natalię Kasałka-Czarną publikacji w postępowaniu o nadanie stopnie doktora w dziedzinie nauk rolniczych w dyscyplinie technologia żywności i żywienia.

Indywidualny udział w powstawaniu pracy polegał na:

Autor	Udział	Udział procentowy	Podpis
mgr inż. Natalia Kasałka-Czarna	opracowanie metodyki; przeprowadzenie badań; analiza wyników; zarządzanie danymi; obliczenia statystyczne; interpretacja wyników i sformułowanie wniosków; wizualizacja; przygotowanie manuskryptu	65%	 PODPIS ZAUFANY NATALIA KASAŁKA-CZARNA 25.06.2025 10:24:14 (GMT+2) Dokument podpisany elektronicznie podpisem zaufanym
dr hab. inż. Agnieszka Bilaska	przeprowadzenie badań mikrobiologicznych; analiza wyników	10%	
dr hab. inż. Róża Biegańska-Marecik	przygotowanie prób – pakowanie w atmosferze	5%	 Signed by / Podpisano przez: Róża Maria Biegańska-Marecik Date / Data: 2025-06-25 09:33
prof. dr hab. Magdalena Montowska	autorstwo koncepcji; opracowanie metodyki; zarządzanie danymi; wizualizacja; recenzja i edycja manuskryptu; nadzór	20%	 Podpisano przez/ Signed by: MAGDALENA MONTOWSKA Data/ Date: 26.06.2025 11:17 mSzafir



Oświadczenie o współautorstwie

Niniejszym oświadczamy, że jesteśmy współautorami pracy:

Kasałka-Czarna, N., Biegańska-Marecik, R., Proch, J., Orłowska, A., & Montowska, M. (2023). Effect of dry, vacuum, and modified atmosphere ageing on physicochemical properties of roe deer meat. Polish Journal of Food and Nutrition Sciences, 73(2), 163-170. <https://doi.org/10.31883/pjfn/163613>

Jednocześnie wyrażamy zgodę na wykorzystanie przez mgr inż. Natalię Kasałka-Czarną publikacji w postępowaniu o nadanie stopnie doktora w dziedzinie nauk rolniczych w dyscyplinie technologia żywności i żywienia.

Indywidualny udział w powstawaniu pracy polegał na:

Autor	Udział	Udział procentowy	Podpis
mgr inż. Natalia Kasałka-Czarna	opracowanie metodyki; przeprowadzenie badań; analiza wyników; zarządzanie danymi; obliczenia statystyczne; interpretacja wyników i sformułowanie wniosków; wizualizacja; przygotowanie manuskryptu	60%	 PODPIS ZAUFANY NATALIA KASAŁKA-CZARNA 25.06.2025 10:22:30 [GMT+2] Dokument podpisany elektronicznie podpisem zaufanym
dr hab. inż. Róża Biegańska-Marecik	przygotowanie prób – pakowanie w atmosferze	5%	 Signed by / Podpisano przez: Róża Maria Biegańska-Marecik Date / Data: 2025-06-25 09:35 PODPIS ZAUFANY
dr Jędrzej Proch	badanie zawartości związków mineralnych; analiza wyników; zarządzanie danymi	10%	 JĘDRZEJ MATEUSZ PROCH 03.06.2025 18:16:45 [GMT+2] Dokument podpisany elektronicznie podpisem zaufanym
mgr Aleksandra Proch (de domo Orłowska)	badanie zawartości związków mineralnych; analiza wyników	5%	 PODPIS ZAUFANY ALEKSANDRA PROCH 03.06.2025 18:50:24 [GMT+2] Dokument podpisany elektronicznie podpisem zaufanym
prof. dr hab. Magdalena Montowska	autorstwo koncepcji; opracowanie metodyki; zarządzanie danymi; wizualizacja; recenzja i edycja manuskryptu; nadzór	20%	 Podpisano przez/ Signed by: MAGDALENA MONTOWSKA Data/ Date: 26.06.2025 11:13 mSzafir



Oświadczenie o współautorstwie

Niniejszym oświadczamy, że jesteśmy współautorami pracy:

Kasałka-Czarna, N., Stachniuk, A., Fornal, E., & Montowska, M. (2025). Proteomic analysis of wild boar meat: Effect of storage method and time on muscle protein stability. Food Chemistry, 464(3), 141774. <https://doi.org/10.1016/j.foodchem.2024.141774>

Jednocześnie wyrażamy zgodę na wykorzystanie przez mgr inż. Natalię Kasałka-Czarną publikacji w postępowaniu o nadanie stopnie doktora w dziedzinie nauk rolniczych w dyscyplinie technologia żywności i żywienia.

Indywidualny udział w powstawaniu pracy polegał na:

Autor	Udział	Udział procentowy	Podpis
mgr inż. Natalia Kasałka-Czarna	przeprowadzenie badań; analiza wyników; zarządzanie danymi; interpretacja wyników i sformułowanie wniosków; wizualizacja; przygotowanie manuskryptu	65%	 PODPIS ZAUFANY NATALIA KASAŁKA-CZARNA 25.06.2025 10:26:33 [GMT+2] Dokument podpisany elektronicznie podpisem zaufanym
dr hab. Anna Stachniuk	przeprowadzenie badań LC-MS; analiza wyników	10%	<i>Anna Stachniuk</i>
prof. dr hab. Emilia Fornal	analiza wyników LC-MS; zarządzanie danymi	5%	 Podpisano przez/ Signed by: MAGDALENA MONTOWSKA Data/ Date: 26.06.2025 11:15 mSzafir
prof. dr hab. Magdalena Montowska	autorstwo koncepcji; opracowanie metodyki; zarządzanie danymi; recenzja i edycja manuskryptu; nadzór	20%	


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Emilia Fornal
Data /Date: 2025.05.27 16:10
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Oświadczenie o współautorstwie

Niniejszym oświadczamy, że jesteśmy współautorami pracy:

Kasałka-Czarna, N., Stachniuk, A., Fornal, E., & Montowska, M. (2025). Protein aggregation during storage of roe deer meat: a proteomic study. Journal of Agricultural and Food Chemistry, 73, 8001–8014. <https://doi.org/10.1021/acs.jafc.4c12348>.

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The effect of storage method on selected physicochemical and microbiological qualities of wild boar meat

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Abstract

BACKGROUND: This study investigated the influence of the storage method on the physicochemical characteristics and microbial growth of *m. longissimus thoracis et lumborum* (LTL), *m. biceps femoris* (BF) and *m. vastus lateralis* (VL) of wild boar. Muscles were stored in a vacuum (VAC), in a modified high-oxygen atmosphere (MAP) or meat seasoning cabinet (DRY-AGED) for 21 days.

RESULTS: Wild boar meat was characterised by a high protein and low fat content and a good amount of potassium, sodium, calcium, magnesium, zinc and iron. Significantly higher ($P < 0.05$) pH values were noted for DRY-AGED muscles stored for 21 days (up to 5.89 for VL). On day 21, a significant decrease in pH was noted for all MAP muscles (down to 5.23 for BF). Storage losses due to desiccation and water loss were significantly higher for DRY-AGED samples and ranged from 25.63% to 32.89% on day 21. MAP affected protein and lipid oxidation, which was also reflected in Warner–Bratzler shear force VAC and DRY-AGED had positive results regarding tenderness, whereas on day 21 the MAP-stored meat had toughened significantly (from 35.3 N to 50.7 N in LTL). Lipids were oxidised much faster than proteins during prolonged storage in MAP. Compared to the other methods, DRY-AGED had the best effect on microbial growth.

CONCLUSION: These results indicate that the recommended methods for the storage of wild boar meat are either vacuum packing or dry ageing. The high oxygen content of MAP negatively affected the quality of wild boar meat and carried a risk of increased protein carbonylation.

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Supporting information may be found in the online version of this article.

Keywords: wild boar; protein and lipid oxidation; VAC; MAP; dry ageing; sustainable consumption

INTRODUCTION

World meat consumption is steadily increasing, yet growing awareness of health and environmental requirements are pushing consumers around the world to choose sustainable and unprocessed food. Due to the high content of saturated fatty acids in animal fats, which can contribute to diet-related diseases, consumers are looking for products with better quality and nutritional value. Also important is the ecological aspect, the origin of the product and natural production methods. Meat is considered to be a less sustainable food choice, as the production of meat imposes a heavy burden on the environment, compared to the cultivation of crops. Due to the above, many changes in dietary recommendations have been noted recently. However, a change in the proportion of plants and meat in the diet may be difficult to implement in societies with a strong tradition of meat consumption, especially in Central and Western Europe. Increased health awareness has therefore led to a demand for alternative, lower-fat and antibiotic-free types of meat.^{1,2} Game meat may be the solution. Due to greater awareness of the impact of food on human health, the interest of the modern consumer in game

meat is increasing.^{3,4} In Poland, one of the most common species is the wild boar. Wild boar meat is characterised by high quality, exceptional taste, and unique dietary and health-promoting properties. Importantly, wild boars are free from antibiotics and growth and development enhancers, and these animals are not exposed to the stress that is associated with industrial breeding.³ Compared to pork, wild boar meat is lower in fat, is richer in protein, polyunsaturated fatty acids, omega-3 and omega-6 fatty acids, and has a favourable ratio of n-6 to n-3 fatty acids. Another important aspect is the higher content of vitamins, folates and minerals in wild boar meat compared to pork.^{5,6} Despite its many

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advantages, the level of game meat consumption in Poland remains marginally low compared to the consumption of pork, poultry and beef. It is also much lower than in other European countries.⁷

Conditions in Poland allow for an increase in the share of wild game meat in the meat industry. However, most of the meat and processed meat is exported to Western European markets. Wild boar is one of the most numerous big game animal species in Poland. Research conducted in Poland and other European countries shows that the main consumers of game are hunters and their families. In Poland, in contrast to other European countries, according to the law the hunter cannot sell game, but such meat can be used for personal use or can be delivered to a collection point. Polish consumers can therefore purchase game meat at selected wholesalers or specialist retail stores, where prices are many times higher than the prices of other types of meat. Although the wholesale price in Poland is relatively low (0.9 EUR kg⁻¹ wild boar carcass), the prices of venison in retail stores are much higher; for example, the price for wild boar loin is approximately 18 EUR kg⁻¹ and for tenderloin approximately 23 EUR kg⁻¹.⁵ Polish consumers clearly indicate price and availability as the main factors, among others, influencing low consumption of venison.⁸ Hunting clubs and animal breeding centres have responded to this situation by conducting direct sales of game harvested in designated hunting districts. The game offered for direct sale is harvested in accordance with the provisions of hunting laws, and in the case of big game it is disembowelled under the applicable rules. In the case of wild boar carcasses, they must undergo appropriate veterinary examination, eliminating the threat of trichinosis. This solution is conducive to an increase in the consumption of wild game, about which there will be no uncertainty in terms of safety and origin.

Another factor indicated in studies on wild game consumption in many European countries is the lack of popularisation of wild game and poor knowledge of its storage and nutritional value.^{7,9} During storage, oxidative changes occur within both proteins and fats. Oxidative processes thus lead to the deterioration of meat functional properties as well as negatively affecting its nutritional value. By using appropriate packaging, storage or preservation technologies, oxidation processes can be effectively controlled.¹⁰⁻¹² Greater consumer awareness of the advantages of eating wild boar meat compared with farmed/domestic species, as well as obtaining new information on the quality of meat stored in various procedures, could contribute to an increase in the consumption of wild game meat in Poland and worldwide.

In this study, we aimed to evaluate methods of packaging/storage of wild boar meat to preserve its best characteristics and to avoid adverse health and nutritional effects caused by the oxidative processes of proteins and lipids. By using appropriate packaging, storage or preservation technologies, oxidation processes can be effectively controlled.¹⁰⁻¹² Recognition of differences in the biochemical processes and wild boar meat quality will allow for improvements in production and packaging techniques and, even further, the modification of nutrition strategies. These factors have been assessed for the main slaughter animal species, although studies for game species are still lacking. The article presents a qualitative evaluation of carcass elements of European wild boar (*Sus scrofa scrofa*), including monitoring physicochemical parameters, tenderness, oxidative changes and microbiological safety, during the storage of vacuum-packed meat, meat packed in a modified atmosphere and during the process of dry ageing meat.

MATERIALS AND METHODS

Obtaining and preparing muscle samples

The research materials included three muscles: m. *longissimus thoracis et lumborum* (LTL), m. *biceps femoris* (BF) and m. *vastus lateralis* (VL) from five carcasses of wild boar. Wild boars were hunt-harvested in Greater Poland, in one hunting district (Hunter District No. 57, OHZ, Sarbia Forest District). The study included muscles from the carcasses of animals aged 3–4 years. The age was determined on the basis of the development of the dentition. Based on the hunter's protocol, the timing of the shot was determined. After shooting, the carcass was eviscerated in the designated evisceration area (Drążgowo Forestry) to prevent internal infections. The carcass was then cooled and stored at the Game Buying Point (Drążgowo Forestry, Sarbia Forest District). All the hunted animals underwent veterinary inspection. The carcasses were stored at 0 °C for 5 days. This procedure is the standard approach in the ageing process, which is longer for venison than for slaughtered farm animals. A thorough evaluation and qualification were carried out after skinning. Carcasses with damage to elements as a result of the shooting, improperly cooled, or with an inappropriate pH value were eliminated in order to exclude meat defects. The carcasses were dissected to obtain the LTL, BF and VL muscles without bone and meat on the bone. The samples were vacuum packed and transported in a portable car refrigerator with a permanent power connection to the Department of Meat Technology, Poznan University of Life Sciences. Muscle from each boneless piece was divided into smaller samples (approximately 80 g); each sample in duplicate was vacuum-packed in barrier bags (VAC) using a MULTIVAC Sepp Haggemüller SE & Co. KG, Wolfertschwenden, Germany and in a modified atmosphere (80% O₂/20% CO₂) (MAP) using a Multivac T200 packaging machine and sealed with PET/PE film. Samples were stored at 4 ± 1 °C for 21 days. Carcass parts obtained on the bone were stored in a dry-ageing cabinet (DRY-AGED) at 2 °C and 80% humidity for 21 days. Samples were analysed before packing (0 days) and on days 7 and 21 of storage, for each variant. Microbiological safety was measured before packing (0 days) and on days 7, 14 and 21 of storage.

Proximate composition

The proximate composition was determined on fresh meat before packing, in triplicate. Samples for physicochemical analysis were homogenised using a Moulinex S 643.23 food processor. Protein (Kjeldahl method), moisture, fat (Soxhlet method) and ash were evaluated according to ISO standards PN-EN ISO 8968-1:2014-03, PN-ISO 1442:2000, PN-ISO 1444:2000 and PN-ISO 936:2000, respectively.

Mineral content

Accurately weighed, 0.500 ± 0.001 g dry samples were digested with 5.0 mL of 15.6 mol L⁻¹ HNO₃ (Merck, Darmstadt, Germany) in closed Teflon containers (55 mL) at 180 °C using the Mars 6 microwave digestion system (CEM, Matthews, NC, USA). After digestion, the samples were diluted with demineralised water to a final volume of 10 mL. The element concentration procedure was repeated as an outline by Zubaidi *et al.*¹³ The concentrations of minerals, i.e. K, Na, Mg, Ca, Zn, Fe, Al, Cu, Pb, Cd, Cr, Mn and Ni, were measured by inductively coupled plasma high-resolution optical emission spectrometry. A PlasmaQuant PQ 9000 Elite (Analytic Jena GmbH, Jena Germany) spectrometer was used.

pH, colour and water activity measurements

The pH, colour values and water activity were determined on fresh samples before packing (0 days of storage) and packed meat (VAC, MAP, DRY-AGED) stored for 7 and 21 days. The pH was measured using a portable HandyLab 2 m (Schott AG, Mainz, Germany) equipped with a Schott L68880 pH combination electrode (PN-ISO 2917:2001). Before measurements, the pH meter was calibrated at room temperature using pH buffers 4.01 and 7.00 (Testo Ltd, Alton, UK). Measurements were taken at room temperature (22–24 °C). Colour (CIE L^* , a^* , b^*) was measured in triplicate on the cross-section of freshly cut samples using a Dr Lange LMG161 portable colorimeter (Dr Bruno Lange GmbH & Co. KG, Berlin, Germany). The parameters were set to illuminant D65, observer angle of 10° and aperture size of 5.0 mm. Before measuring, the colorimeter was calibrated against a white standard (No. 3125). The water activity of the meat was measured using a LabMaster-aw neo instrument (Novasina AG, Lachen, Switzerland) at 25 ± 1 °C. Before measurements, the meter was calibrated with SAL-T 97% calibration standard with automatic recognition by an RFID tag.

Cooking and storage losses

Meat slices of 25 mm thickness were wrapped in aluminium foil and heated in a Rational Combi model SCC 61 convection oven (Landsberg am Lech, Germany). Heating was carried out at a temperature of 160 °C with air humidity in the oven chamber of 75% until reaching a core temperature of 72 °C. After cooling, the meat was packed. The cooking losses were determined after the samples had cooled down at room temperature. The cooking and storage losses were calculated by weighing the samples before and after thermal treatment or the storage period, respectively (measured before packing for fresh meat and after 7 and 21 days of storage for each packing method). The values are presented as the percentage of the mass noted before a given treatment. The storage losses were determined after 7 and 21 days of storage for each packing method.

Warner–Bratzler shear force

The Warner–Bratzler shear force (WBSF) test was carried out using a TA.XT.plus texture analyser (Stable Micro Systems, Godalming, UK) at room temperature. Meat tenderness (LTL, BF, VL) was assessed before packing and after 7 and 21 days of storage (VAC, MAP, DRY-AGED). Meat slices (2.5 cm thick) were cooked in a Rational Combi convection steam oven until the geometric centre of the sample was 72 °C. Cooled samples were cut into cuboids (1 × 1 × 3 cm). The shear force was measured perpendicularly to the arrangement of muscle fibres at a traverse speed of 100 mm min⁻¹. The force necessary to cut the meat sample was expressed as newtons per square centimetre.

Protein oxidation (carbonyl content)

The carbonyl content was measured before packing in fresh samples and in packed meat (VAC, MAP, DRY-AGED) stored for 7 and 21 days. Protein oxidation was measured by the total carbonyl content by derivatisation with 2,4-dinitrophenyl hydrazine (DNPH) according to Levine *et al.* (1994), with slight modifications. Meat (1 g) was homogenised 1:10 (w v⁻¹) in 20 mmol L⁻¹ sodium phosphate buffer containing 6 mol L⁻¹ NaCl (pH 6.5) using an Ultra Turrax homogeniser for 30 s. The homogenate was filtered through filter paper. Two aliquots of 0.4 mL were taken from the

homogenate and dispensed in 2 mL Eppendorf tubes. Proteins were precipitated by cold 10% trichloroacetic acid (TCA) (1.5 mL), incubated at 20 °C for 20 min, and subsequently centrifuged for 5 min at 4 °C at 10 000 × *g*. The supernatant was discarded and one pellet was treated with 1 mL of 2 mol L⁻¹ HCl for the protein concentration measurement and the other with 1 mL of 10 mol L⁻¹ DNPH in 2 mol L⁻¹ HCl for the carbonyl concentration measurement. Both samples were incubated for 30 min at 37 °C. Subsequently, samples were precipitated using 1 mL of 1 mol L⁻¹ TCA, centrifuged for 5 min at 4 °C at 10 000 × *g*, and washed three times with 1 mL ethanol–ethyl acetate (1:1, v/v) to remove excess DNPH. The pellets were then dried for 1 h at room temperature. Subsequently, the pellets were dissolved in 1.5 mL of 20 mmol L⁻¹ sodium phosphate buffer containing 6 mol L⁻¹ guanidine hydrochloride (pH 6.5), incubated for 30 min at 37 °C, and centrifuged for 5 min at 4 °C at 10 000 × *g* to remove insoluble fragments. The carbonyl concentration (nmol mg⁻¹ protein) was calculated from the absorbance of the samples at 280 and 370 nm using the following equation:¹⁴

$$\frac{\text{c hydrazone}}{\text{c protein}} = \frac{A_{370}}{22\,000 \times (A_{280} - A_{370} \times 0.43)} \times 10^6$$

Lipid oxidation

The thiobarbituric acid reactive substances (TBARS) value of wild boar was assessed according to the method described by Fiego *et al.*,¹⁵ with minor modifications. A milled sample of LTL, BF or VL (10 g) was homogenised at 10 000 × *g* for 60 s in 20 mL of 2 mol L⁻¹ TCA. After centrifuging at 3502 × *g* for 15 min at 4 °C, the mixture was filtered through qualitative filter paper by vacuum filtration. Then, 5 mL of the filtrate was mixed with the same volume of 20 mmol L⁻¹ 2-thiobarbituric acid. A solution was prepared by mixing 5 mL TCA and 5 mL 2-thiobarbituric acid solution as the blank. The sample and the blank were heated for 20 min in a boiling water bath and cooled to room temperature. Absorbance was measured at 532 nm. The TBARS value was measured before packing for fresh samples and in meat stored (VAC, MAP, DRY-AGED) for 7 and 21 days. Lipid oxidation was expressed as the level of malonic dialdehyde (MDA) [mg MDA kg⁻¹].

Microbiological growth

Microbiological safety was determined by the total count of microorganisms, as well as bacteria of the genera *Lactobacillus*, *Pseudomonas*, Enterobacteriaceae and *Enterococcus*. The count of microorganisms was evaluated according to ISO standards ISO 4833-1:2013, PN-ISO 15214:2002, ISO 13720:2010, ISO 21528-1:2017 and PN-A-82055-7:1997, respectively.

Statistical analysis

Data analysis was performed using Statistica 13.1 (StatSoft Inc., Tulsa, OK, USA) to evaluate the effect of storage method and storage period for meat (LTL, BF, VL) up to 21 days of storage. Analysis of variance and Tukey's test of multiple comparisons were used to determine significant differences between mean values. The results were considered to be statistically significant at $P < 0.05$. The results are expressed as mean ± standard deviation. The resulting values were imported into MS Excel 2019 (Microsoft, Redmond, WA, USA) for figure generation.

Table 1. Proximate composition of wild boar muscles; mean $\pm$ SD

Parameter (g kg ⁻¹)	Muscle		
	LTL	BF	VL
Protein	219.1 $\pm$ 18.0	218.9 $\pm$ 7.8	216.8 $\pm$ 15.6
Water	709.9b $\pm$ 16.4	718.6b $\pm$ 20.6	740.1a $\pm$ 14.6
Fat	49.9ab $\pm$ 11.4	52.7a $\pm$ 20.1	36.8b $\pm$ 3.9
Dry matter	240.2a $\pm$ 5.8	228.7ab $\pm$ 18.0	223.1b $\pm$ 14.3
Ash	11.4 $\pm$ 1.6	11.0 $\pm$ 2.0	11.0 $\pm$ 1.7

Different letters in a row (a, b, c) for a given parameter indicate a significant effect of the muscle ($P < 0.05$); LTL, longissimus thoracis et lumborum; BF, biceps femoris; VL, vastus lateralis.

RESULTS AND DISCUSSION

Changes in proximate composition, physicochemical properties and shear force

The proximate composition of the three examined muscles (LTL, BF, VL) is shown in Table 1. The wild boar meat was characterised by a high protein (216.8–219.1 g kg⁻¹) and low fat content (36.8–52.7 g kg⁻¹); as it is a low-fat product, it may be recommended in the modern diet. Protein is a less variable component than fat; thus no differences were noted between the protein content in the studied muscle. However, the water, fat and dry matter contents were affected by the type of muscle tested; that is, a significantly higher water content but lower fat and dry matter were observed in VL. Other authors determined similar water and even higher protein values for the LTL of wild boar; for instance, the protein ranged from 21.80% to 24.70%^{16,17} but according to Strazdina *et al.*⁶ LTL muscle contained 19.55–23.18% protein and 25.38% dry matter. The greatest differences were seen in the fat content in *m. longissimus* samples collected from Hungary in the late-winter period (3.5–5.3%) and from Latvia in the autumn–winter season (1.67–3.45%).^{18,19}

The content of selected minerals in LTL, BF and VL is presented in Supporting Information Table S1. The results show that wild boar meat is a very good source of potassium, sodium, calcium, magnesium, zinc and iron. The content of bioavailable iron ranged from 0.0164 to 0.0202 g kg⁻¹ of meat depending on the muscle analysed. The content of aluminium and copper as well as trace amounts of lead (0.0003–0.0004 g kg⁻¹) were also noted. No content of heavy metals such as cadmium, chromium, manganese and nickel was found (<0.00003 g kg⁻¹).

The physicochemical properties of the fresh muscles and muscles stored for 7 and 21 days under different conditions are presented in Table 2. A significant effect of storage time and method on pH values was demonstrated for all three of the muscles studied ($P < 0.05$). The pH value of DRY-AGED muscles increased during storage, while it decreased in the VAC and MAP samples relative to the fresh sample. Significantly higher pH values were noted for DRY-AGED muscles stored for both 7 and 21 days (pH ranged from 5.72 to 5.89 on day 21 for VL), while the pH of the analysed muscles stored in MAP on day 7 was lower and similar to that of the VAC samples. On day 21, a significant decrease in pH was noted for all MAP muscles; the lowest values were detected for BF and LTL MAP muscles (pH 5.23 and 5.31, respectively). VAC muscles had the least variation in the pH parameter. Of all the muscles, VL had the highest pH for each method during storage. According to Żmijewski and

Korzeniowski,¹⁷ the pH value measured 6 h post mortem in LTL muscle of wild boar was 5.57, while in ham muscles it was 5.59. After 24 h, the pH reached a minimum value (5.44 and 5.47) but after 6 days of maturation an increase in pH value to 5.60 was noted. This is consistent with the pH of our samples, which were obtained from carcasses aged for 7 days.

In terms of colour parameters, significant changes were found between the three storage methods after the storage period ($P < 0.05$). The meat lightness (L^*) increased relative to the fresh samples, except for LTL DRY-AGED, which was darker on day 21 of storage. For VL DRY-AGED no significant changes were observed. All DRY-AGED muscles on day 21 of storage were darker relative to day 7 of storage. For muscles stored in MAP and VAC, an increasing trend in the L^* parameter was noted on day 7 but only for MAP samples did the L^* increase after 21 days of storage. Each muscle, for each of the storage variants, was characterised by a lower proportion of red colour (a^*) compared to the fresh sample. An increase in the value of the a^* parameter from 7 to 21 days of storage was recorded for muscles stored in VAC and DRY-AGED, resulting in darker brownish colour due to changes in myoglobin form. The fresh wild boar meat had a characteristic red colour due to the high myoglobin content which, during storage in an atmosphere with a low oxygen concentration, exists mostly as purplish-red deoxymyoglobin (DeoxyMb), but further autoxidation of DeoxyMb to metmyoglobin (MetMb) leads to brown discoloration.²⁰ On day 21 of analysis, significantly lower a^* values were observed for samples stored in MAP compared to the other two methods, likely due to the formation of oxymyoglobin (OxyMb) under aerobic conditions in an atmosphere with a high concentration of oxygen. Samples stored in VAC and DRY-AGED were characterised by lower yellowness values (b^*), while meats stored in MAP were characterised by a significantly higher b^* component.

In general, MAP stored meat was characterised by significantly higher values of L^* and b^* and lower values of a^* parameters, contrary to DRY-AGED samples, which had higher values of a^* and lower values of L^* and b^* . The type of muscle had no significant effect on the above parameters. The results reported by Łopacka *et al.*²¹ for beef steaks confirm the effect of high-oxygen MAP on the increase of L^* parameter, which may be caused by conformational changes of proteins in the meat structure with prolonged storage time, resulting in greater light scattering. A similar trend was also observed for yellowing (b^*). MAP samples were characterised by a higher value of the b^* parameter. The colour of meat resulting from storage methods contributes significantly to consumers' perception of meat quality. Meat colour is influenced by many factors affecting the concentration and chemical form of myoglobin.^{11,22} Undesirable discoloration, caused by the conversion of OxyMb to DeoxyMb and OxyMb to MetMb, adversely affects meat colour. This conversion is caused by a reduction in haem redox stability. Unsaturated fatty acids in phospholipids and triacylglycerols are also oxidised, referred to as lipid oxidation. Biochemical reactions directly related to myoglobin and lipid oxidation generate products that accelerate mutual oxidation.^{11,23} Meat colour is also affected by factors such as pH, myoglobin concentration, oxygen penetration and the amount of free water on the meat surface.^{24,25} According to other reports, packaging in a high oxygen atmosphere (70–80%) is often used in the meat industry to preserve the bright red colour.^{11,26} However, the present analyses showed that storage of wild boar meat under high-oxygen conditions (80% O₂/20% CO₂), resulted in undesirable meat on day 21 of storage. MAP samples had the

Table 2. Physicochemical properties of muscles measured during storage at different conditions; mean $\pm$ SD

Parameter	Day	Muscle											
		LTL					BF					VL	
		VAC	MAP	DRY-AGED	VAC	MAP	DRY-AGED	VAC	MAP	DRY-AGED	VAC	MAP	
Storage method													
pH	0	5.59a ± 0.02	5.59a ± 0.02	5.59b ± 0.02	5.59a ± 0.05	5.59a ± 0.05	5.59c ± 0.05	5.59b ± 0.04	5.59a ± 0.04	5.59c ± 0.04	5.59c ± 0.04		
L*	7	5.56ay ± 0.05	5.55ay ± 0.07	5.71ax ± 0.06	5.59ay ± 0.08	5.58ay ± 0.09	5.72bx ± 0.04	5.65ay ± 0.05	5.64ay ± 0.11	5.82bx ± 0.06	5.82bx ± 0.06		
	21	5.46by ± 0.09	5.31bz ± 0.07	5.72ax ± 0.08	5.46by ± 0.06	5.23bz ± 0.03	5.89ax ± 0.03	5.57by ± 0.02	5.43bz ± 0.06	5.89ax ± 0.03	5.89ax ± 0.03		
	0	32.28b ± 3.19	32.28c ± 3.19	32.28b ± 3.19	29.70c ± 2.34	29.70b ± 2.34	29.70c ± 2.34	31.11b ± 2.17	31.11c ± 2.17	31.11 ± 2.17	31.11 ± 2.17		
a*	7	38.53a ± 4.81	38.98b ± 2.71	35.93a ± 2.89	38.53ax ± 4.81	35.61axy ± 3.42	33.28ay ± 1.31	34.96ax ± 1.94	33.39by ± 1.61	32.38y ± 0.86	32.38y ± 0.86		
	21	37.10ay ± 4.75	41.99ax ± 3.00	28.16cz ± 2.99	32.99by ± 2.54	36.28ax ± 1.43	31.56bz ± 1.36	35.80ax ± 3.18	36.87ax ± 1.84	30.05y ± 1.70	30.05y ± 1.70		
	0	9.73a ± 2.05	9.73a ± 2.05	9.73a ± 2.05	12.59a ± 2.30	12.59a ± 2.30	12.59a ± 2.30	13.12a ± 1.16	13.12a ± 1.16	13.12a ± 1.16	13.12a ± 1.16		
b*	7	5.61cy ± 0.94	6.30bx ± 1.30	7.56bx ± 2.27	9.14bx ± 1.25	7.36by ± 2.18	9.93cx ± 2.42	8.75by ± 1.34	8.76by ± 1.58	10.57bx ± 0.34	10.57bx ± 0.34		
	21	6.39by ± 1.23	3.74cz ± 0.97	8.33abx ± 1.28	10.06bx ± 1.20	5.78cy ± 1.00	10.55bx ± 1.91	10.05bx ± 1.59	6.39cy ± 1.23	10.61bx ± 1.63	10.61bx ± 1.63		
	0	12.19a ± 1.72	12.19 ± 1.72	12.19a ± 1.72	11.79a ± 1.30	11.79b ± 1.30	11.79a ± 1.30	11.23a ± 1.98	11.23c ± 1.98	11.23a ± 1.98	11.23a ± 1.98		
Water activity	7	9.41by ± 1.60	12.43x ± 1.62	9.36by ± 1.94	9.41by ± 1.60	13.04abx ± 1.90	10.52aby ± 0.74	10.64ay ± 1.54	13.42bx ± 2.25	9.05by ± 0.78	9.05by ± 0.78		
	21	9.16by ± 1.58	13.28x ± 4.80	7.83cy ± 1.18	11.53ay ± 1.47	15.42ax ± 5.06	10.29by ± 1.95	9.16by ± 1.58	16.38ax ± 0.36	9.76by ± 1.51	9.76by ± 1.51		
	0	0.975a ± 0.002	0.975a ± 0.002	0.975a ± 0.002	0.975a ± 0.001	0.975a ± 0.001	0.975a ± 0.001	0.974 ± 0.002	0.974a ± 0.002	0.974a ± 0.002	0.974a ± 0.002		
Cooking loss (%)	7	0.971bxy ± 0.002	0.971bx ± 0.003	0.969by ± 0.002	0.972bx ± 0.001	0.973bx ± 0.003	0.971by ± 0.002	0.973 ± 0.002	0.972b ± 0.002	0.971b ± 0.002	0.971b ± 0.002		
	21	0.972bx ± 0.001	0.971bx ± 0.002	0.960cy ± 0.002	0.972bx ± 0.002	0.970cy ± 0.002	0.969by ± 0.002	0.973x ± 0.003	0.971bxy ± 0.002	0.969by ± 0.004	0.969by ± 0.004		
	0	27.91c ± 2.76	27.91c ± 2.76	27.91b ± 2.76	32.07b ± 1.10	32.07c ± 1.10	32.07b ± 1.10	33.25b ± 1.55	33.25b ± 1.55	33.25 ± 1.55	33.25 ± 1.55		
Cooking loss (%)	7	35.33ax ± 2.00	35.31ax ± 1.47	30.24ay ± 1.51	39.14ax ± 2.72	34.24by ± 1.38	34.16 ay ± 1.96	40.80ax ± 2.99	34.73by ± 1.44	32.87y ± 2.89	32.87y ± 2.89		
	21	30.93bx ± 3.29	30.82bx ± 2.62	20.94cy ± 1.78	37.04ax ± 3.87	37.26ax ± 2.42	27.39cy ± 2.37	34.57by ± 3.05	39.64ax ± 3.19	30.94z ± 2.79	30.94z ± 2.79		

Different letters in a column (a, b, c) for a given parameter indicate a significant effect of time within the method ($P < 0.05$); different letters in a row (x, y, z) for a given parameter indicate a significant effect of the method with time ($P < 0.05$); LTL, *longissimus thoracis et lumborum*; BF, *biceps femoris*; VL, *vastus lateralis*.

highest b^* values and characteristic brown colour, which may be due to increased oxidation processes. The most attractive colour at 21 days of storage was retained in DRY-AGED meat, while VAC storage also showed better results compared to MAP.

Water activity (a_w) analysis showed a significant effect of storage time between fresh muscles and the muscles stored for 7 days ($P < 0.05$). The VAC samples expressed the least variation in the water activity parameter during storage, i.e. up to 0.973 for VL (highest a_w among the methods used), whereas seasoned muscles had the lowest a_w after time in storage. There was a significant decrease in a_w down to 0.960 on day 21 of analysis of the LTL DRY-AGED. This corresponds to the highest storage losses in seasoned muscles (Fig. 1). The results are consistent with other studies reporting that DRY-AGED meat has a lower moisture content than VAC and MAP meat.²⁷ Water activity affects biological processes in meat and affects microbial growth. Florek *et al.*²⁸ noted that VAC meat had higher water activity than DRY-AGED meat and that low a_w is a factor in the health and safety of DRY-AGED products.

Storage losses due to desiccation and water loss were significantly higher ($P < 0.05$) for DRY-AGED samples after 7 and 21 days of storage (Fig. 1, Supporting Information Table S2) and ranged from 15.92% to 19.42% on day 7 and from 25.63% to 32.89% on day 21 of storage. Storage losses on day 7 of the analysis for the other methods did not exceed 3.54% for VAC and 5.47% for MAP, while on day 21 they were 6.84% and 8.78%, respectively. Lund *et al.*²⁹ reported higher losses for high-oxygen MAP for pork compared to VAC. Storage losses can be directly related to economic parameters. DRY-AGED storage of wild boar meat reduces the overall process efficiency.

Cooking losses (Table 2) decreased during storage within a method. Significant differences were observed between samples stored in MAP and VAC *versus* DRY-AGED. The lowest percentage of thermal loss was noted for each of the DRY-AGED muscles. DRY-AGED meat loses most of its water by convection at an early stage, resulting in a characteristic crust that hardens over time.²⁷ Once the surface is dry, the crust hardens and the rate of water evaporation is driven mainly by diffusion. However, meat research shows that the ability to retain water increases with increasing pH. This is due to the structure of the protein, which is more open, allowing it to retain more water chemically bound by myofibrils. This was confirmed by our analysis, as despite the high water loss DRY-AGED meat

with the highest pH had high quality. It was noted that, within each method, VL had the highest thermal losses and LTL the lowest.

The results showed a significant effect of the packaging method and time ($P < 0.05$) on meat tenderness determined by WBSF values (Fig. 2 and Supporting Information Table S2). VAC and DRY-AGED had a positive effect on tenderness, while MAP had a negative effect during storage. The best tenderness after 21 days of storage was characteristic of seasoned muscles (DRY-AGED). The lowest variation in WBSF values was found for all vacuum-packed muscles. Favourable values below 40 N were already recorded on day 7 of analysis. MAP had a favourable effect on meat tenderness on day 7 of storage, but on day 21 the meat toughened significantly, e.g. from 35.3 N to 50.7 N in LTL muscle. A key factor for improving meat tenderness is ageing. Both VAC and DRY-AGED storage gave satisfactory results. However, it is noteworthy that BF and VL muscles showed satisfactory tenderness on day 7 for VAC, while DRY-AGED achieved the best results on day 21 of storage. Muscles with a predominance of white fibres are more susceptible to proteolysis during post mortem maturation and are more tender after heat treatment.^{30,31} According to Kirchofer *et al.*,³² LTL and BF belong to the white muscles, whereas VL belongs to the intermediate muscles. However, the results obtained indicate that the differences in tenderness were influenced by other factors – in this case, probably the rate of proteolysis and the oxygen concentration. The toughness of DRY-AGED meat improved significantly on day 21, obtaining products with the highest tenderness for all muscles analysed. Similar relationships were discussed in a review by Dashdorj *et al.*²⁷ However, meat stored under MAP on day 21 of analysis was less tender compared with 7 days of storage. Similar results were observed in an experiment conducted by Moczowska *et al.*,¹¹ where beef packed in a modified atmosphere showed lower tenderness after 14 days of storage; the lowest values were also obtained for BF muscle. Lagerstedt *et al.*³³ also reported increased WBSF in beef steaks packed in high-oxygen MAP. The poorer tenderness of stored meat in packaging with a high oxygen concentration may be due to an increase in the oxidation processes of fats and proteins, resulting in products with a lower myofibril fragmentation index and oxygen-induced protein aggregation.^{11,34} This relationship may also be influenced by factors related to muscle metabolism.

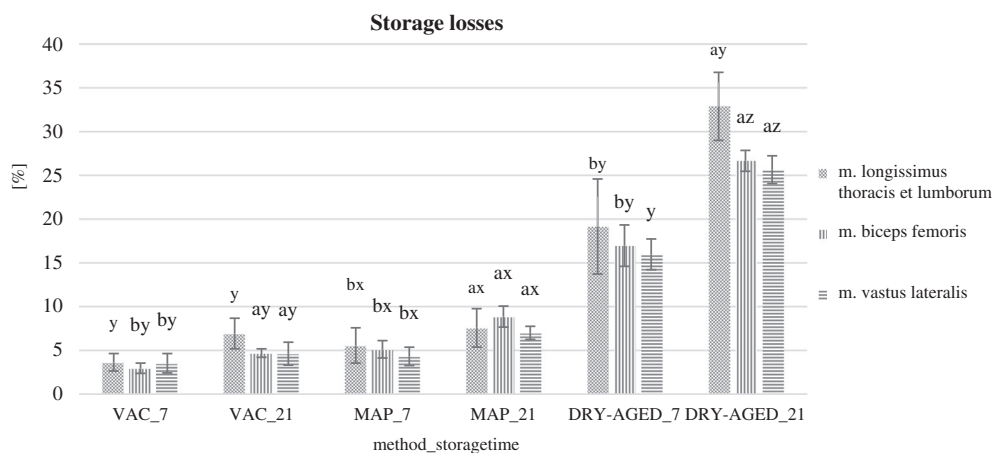


Figure 1. Effect of the method and storage time on storage losses in muscles; mean $\pm$ SD. Different letters in a column (a, b, c) for a given parameter indicate a significant effect of time within the method ($P < 0.05$); different letters in a row (x, y, z) for a given parameter indicate a significant effect of the method with time ($P < 0.05$).

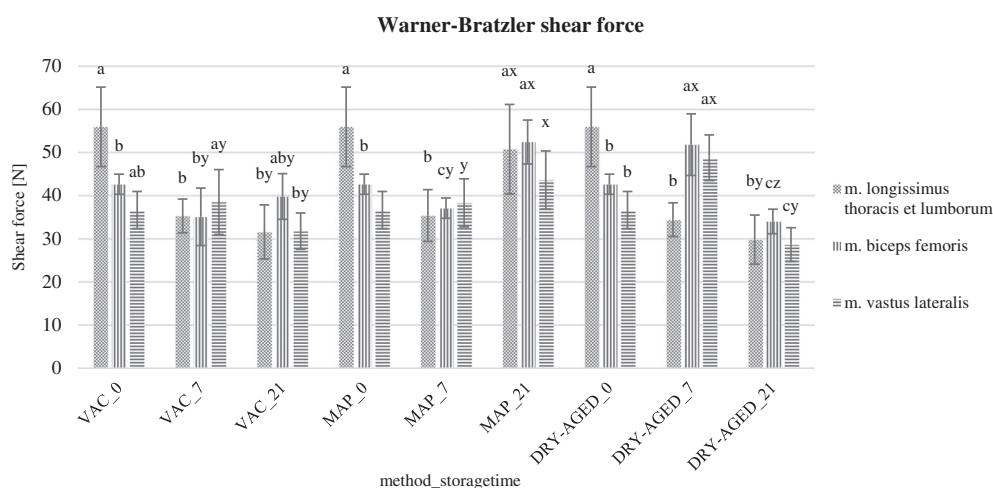


Figure 2. Effect of the method and storage time on Warner–Bratzler shear force in muscles; mean $\pm$ SD. Different letters in a column (a, b, c) for a given parameter indicate a significant effect of time within the method ($P < 0.05$); different letters in a row (x, y, z) for a given parameter indicate a significant effect of the method with time ($P < 0.05$).

Protein and lipid oxidation

Monitoring oxidative changes in proteins is an innovative topic among meat researchers. Carbonylation is considered one of the most interesting modifications in oxidised proteins.³⁵ Due to the scarcity of scientific research on the topic of oxidative processes within wild game meat, there is much room for future research. The effects of storage method and time on the oxidation levels of proteins and lipids in muscle (LTL, BF, VL) are shown in Fig. 3 and Supporting Information Table S3. There was a significant effect of time and storage method ($P < 0.05$) on the carbonyl content of muscles (expressed as nanomoles of carbonyl per milligram of protein). Oxidative changes progressed most strongly in MAP muscles. Vacuum packaging had no significant effect on the carbonyl content. With the DRY-AGED method, the greatest increase in protein oxidation was observed on day 7 of analysis. Also, in comparison to the other methods, on day 7 of analysis the level of oxidation was higher in DRY-AGED meat, while between the other two methods the difference was lower. On day 21 of analysis, the increase in protein oxidation that was observed in MAP samples ranged from 115% to 193% compared to fresh samples. For seasoned meat, the increase ranged from 33% to 63%. VAC caused a slight increase in carbonyl levels only for VL and BF (approximately 20%), while a 14% decrease was observed for LTL.

Specific protein carbonyls are active compounds that can be involved in many chemical reactions resulting in compromised meat quality. Altered protein conformation by carbonyls formed from specific amino acids leads to denaturation and the loss of protein functionality. A decrease in pH may also promote the formation of protein carbonyls. This relationship was noted in the present study on day 21 of analysis for MAP, where the significant increase in protein oxidation was in line with a decrease in pH. Moreover, the effect of packaging conditions on protein carbonylation is strongly species dependent.³⁵

Lindahl *et al.*³⁶ noted the effect of high-oxygen packaging on increased protein carbonylation for beef muscle, whereas the carbonyl level in high-oxygen MAP (70% O₂/30% CO₂) for LL pork meat stored for 7 days was not affected.²⁹ As reported in another study, 7 days storage of pork in MAP (80% O₂/20% CO₂) resulted

in greater, but still moderate (reaching approximately 2.40 nmol mg⁻¹ protein) formation of protein carbonyls.³⁷ In our study, the protein oxidation values for VAC samples were not much higher than those presented by the above authors for pork. Therefore, both lower oxygen, as well as iron content, might have influenced the lower progression of carbonyl formation in pork. The present study shows that wild boar meat stored in a high-oxygen atmosphere carries the risk of increased protein carbonylation.

An increase in lipid oxidation was detected in all samples after 7 days of storage. Significantly higher TBARS values (at 7 and 21 days of storage) were found in samples stored under high oxygen content (MAP) conditions. The smallest changes in lipids at 21 days of storage were observed for samples stored in VAC. Within the DRY-AGED muscles, changes occurred much more slowly compared to MAP. Lipids were oxidised much faster than proteins during prolonged storage in MAP. Haem proteins, especially myoglobin, promote increased lipid oxidation processes in meat. Higher concentrations of iron and myoglobin are associated with higher rates of lipid oxidation. In addition to the pro-oxidative metabolism of myoglobin, the dissociation of haem from myoglobin as well as iron from haem can also affect oxidative processes.²³ Importantly, decreasing the oxygen content in packaged pork resulted in the inhibition of both pork protein and lipid oxidation relative to 80% oxygen. It was also noted that values for carbonyls and TBARS were lower when pork was stored in air than in high-oxygen MAP.³⁷ Łopacka *et al.*²¹ observed that beef steaks packed in 80% O₂/20% CO₂ had significantly higher TBARS values compared to MAP packing. These observations show agreement with the present study, in that a high-oxygen atmosphere induced strong oxidation of wild boar proteins and lipids during storage. These changes in proteins and lipids during storage have a direct impact on the quality and tenderness of the raw material. The high oxygen concentration used in MAP packaging (80% O₂) had a significant effect on the course of oxidative transformations. The oxygen concentration present in the air, compared to MAP conditions, does not contribute to such advanced changes in the structure and quality of stored wild boar meat. Vacuum packaging reduced oxidative processes in wild boar proteins and lipids to the greatest extent.

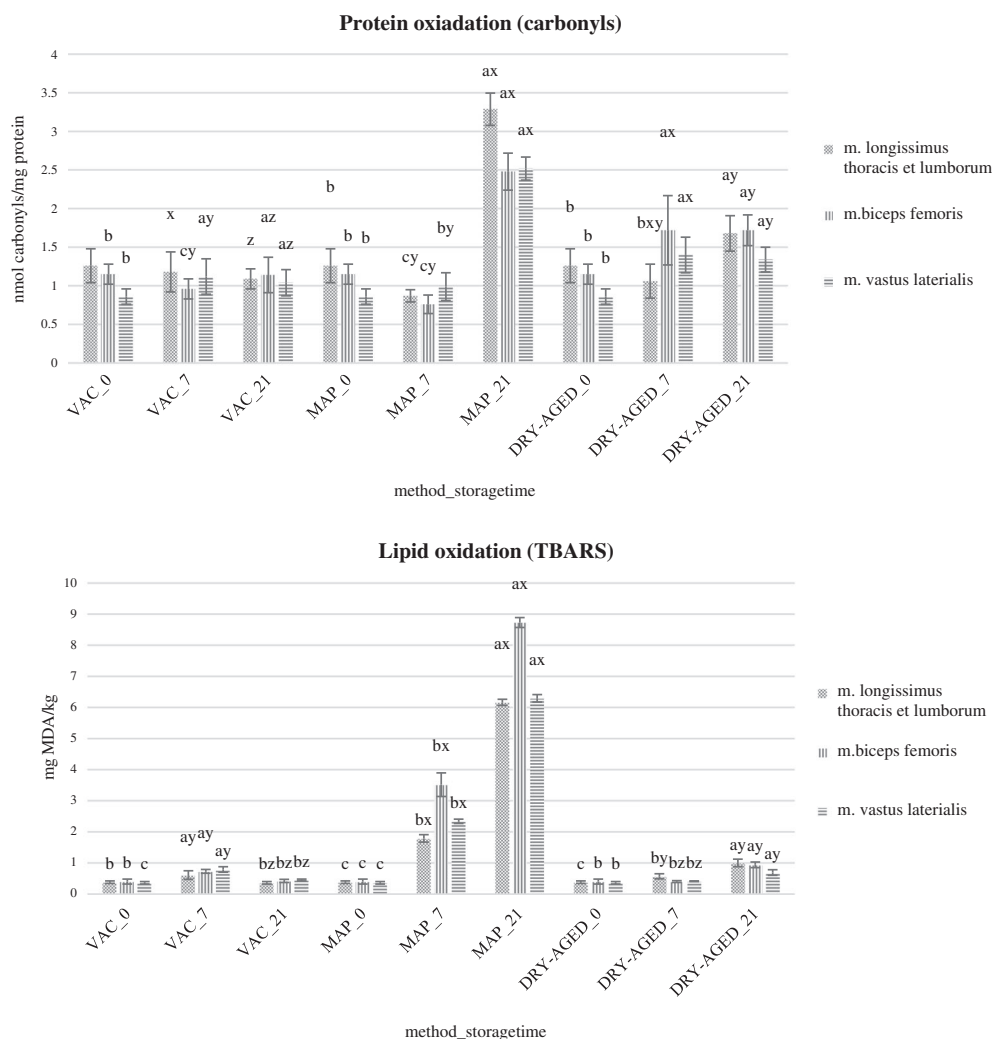


Figure 3. Effect of the method and storage time on protein and lipid oxidation in muscles; mean $\pm$ SD. Different letters in a column (a, b, c) for a given parameter indicate a significant effect of time within the method ($P < 0.05$); different letters in a row (x, y, z) for a given parameter indicate a significant effect of the method with time ($P < 0.05$).

Microbiological growth

Most of the game animals from which meat is obtained, after evisceration and the removal of contaminated tissues, are transported and stored in a cold store, where the required temperature and humidity conditions are maintained. Skinning and cutting may be delayed for several days and carried out at another location. These factors may have a direct impact on the microbiological aspects of wild boar meat. Nevertheless, wild boar meat, like livestock, is characterised by good microbiological quality.^{38,39} In our samples, the total microbial count for fresh meat was lowest for LTL, while it was slightly higher and comparable for BF and VL muscles. Figure 4 and Supporting Information Table S4 show the results of microbiological analyses for each muscle and storage period. Regarding the total count of microorganisms, the analyses show that seasoning (DRY-AGED method) significantly reduced the growth of aerobic microorganisms. This fact can be directly related to the highest storage losses as well as the lowest water activity values observed in DRY-AGED muscles. No significant differences were found between the other two methods (MAP and VAC). As reported by Ludwiczak *et al.*,³ the total

microbial count for wild boar *m. longissimus thoracis et lumborum* 48 h after slaughter was $4.52 \log_{10}$ CFU g^{-1} . For the muscle samples we studied, the values were slightly higher (4.73 – $5.30 \log_{10}$ CFU g^{-1}), which may be influenced by the type of muscle and time of storage after slaughter, as well as the conditions of the slaughter site.

Enterobacteriaceae counts for fresh meat ranged from $2.80 \log_{10}$ CFU g^{-1} for LTL to $3.55 \log_{10}$ CFU g^{-1} for BF. According to Russo *et al.*,³⁹ the average Enterobacteriaceae count for fresh LTL of wild boar muscle was $4.32 \log_{10}$ CFU g^{-1} , but these values can vary. Avagnina *et al.*⁴⁰ reported that the average value was $3.00 \log_{10}$ CFU g^{-1} . The differences may arise from uncontrolled conditions during carcass processing.⁴¹ DRY-AGED and MAP limited the growth of Enterobacteriaceae to the greatest extent, while the highest count of Enterobacteriaceae was determined for VAC. Differences were also noted between the analysed muscles.

In the retail environment, *Enterococcus* often contaminates meat.⁴² In the case of the wild boar muscles analysed, the results were negative for each of the samples (independent of the

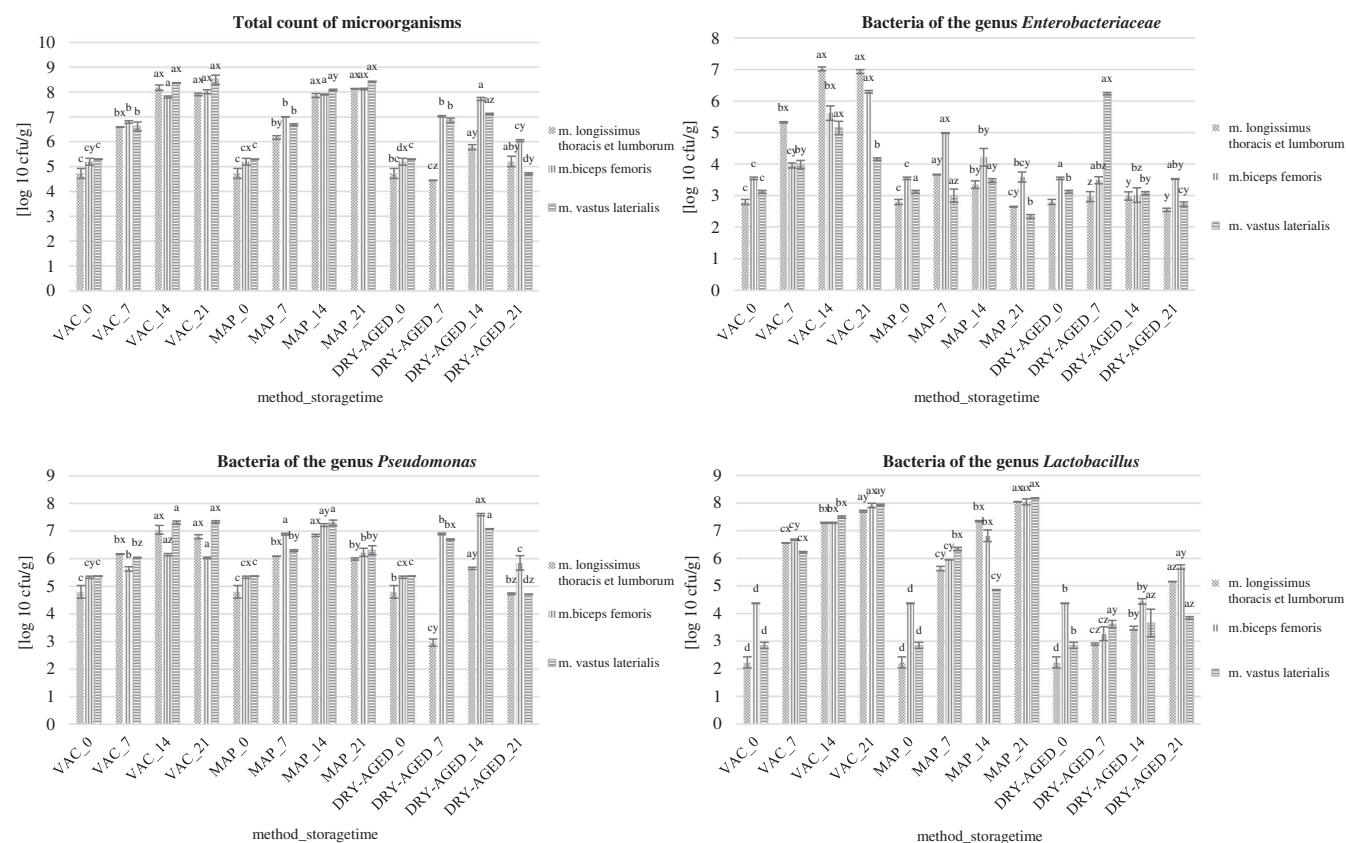


Figure 4. Effect of the method and storage time on microbiological growth in muscles; mean $\pm$ SD. Different letters in a column (a, b, c) for a given parameter indicate a significant effect of time within the method ($P < 0.05$); different letters in a row (x, y, z) for a given parameter indicate a significant effect of the method with time ($P < 0.05$).

method and storage time). This supports the high microbiological quality of the obtained muscles.

Bacteria belonging to the genus *Pseudomonas* may be the dominant microflora on the surface of raw meat that is stored under refrigerated conditions. These bacteria may account for 90% of the total count of microorganisms on the meat surface and cause adverse changes in the food, reducing the shelf life of the meat.⁴³ Of the three methods used, the lowest count of *Pseudomonas* bacteria was observed for DRY-AGED muscles after prolonged storage on day 21, in relation to day 14 of storage. No significant differences were observed in *Pseudomonas* counts for VAC and MAP. This may be due to the fact that *Pseudomonas* respire aerobically, using oxygen as the ultimate electron acceptor, but some also respire anaerobically by carrying out denitrification. Given this fact, seasoning meat potentially prolongs its shelf life and suitability for consumption.

DRY-AGED samples showed significantly lower growth of *Lactobacillus* than those stored in VAC and MAP. Meat stored in MAP was characterised by the highest number of *Lactobacillus* – slightly higher compared to VAC samples. Storage of wild boar meat under limited (VAC) or high oxygen (80%) MAP has been shown to adversely affect microbiological quality. DRY-AGED meat was therefore characterised by the highest microbiological stability. Bacteria of the genus *Lactobacillus* are lactic fermentation bacteria, naturally associated with long-term storage of fresh meat at low temperatures. Some of the bacteria associated with meat, particularly *Lactobacillus* strains, may have antagonistic effects against pathogenic and meat-perishing bacteria.³⁸

However, due to the increased growth of *Lactobacillus* bacteria, the quality and safety of meat may deteriorate more rapidly.

VAC protects meat from contact with oxygen from the air, which promotes the growth of some strains of *Pseudomonas* that can also breathe anaerobically by carrying out denitrification. This can directly affect the aroma, colour and presentation of meat products and, moreover, affect oxidative rancidity. However, psychrotrophic facultative anaerobic and aerobic bacteria can grow in vacuum-packed meat, which sometimes promotes the growth of Enterobacteriaceae. As reported by Hernández-Macedo *et al.*,⁴⁴ Enterobacteriaceae species that grow in vacuum-packed meat are able to grow at temperatures between 0 and 10 °C. In the present study, after the prolonged storage of VAC packed meat, the promotion of *Lactobacillus* and the potential risk associated with the growth of Enterobacteriaceae were observed. Compared to the other methods, the DRY-AGED method had the best effect on microbial growth.

CONCLUSIONS

Taking into account the health-promoting value of venison and its taste, it is worth making a change in diet in favour of increasing the consumption of wild boar meat. In terms of nutritional value, wild boar meat is characterised by a low fat content but a high content of protein. Wild boar meat is also rich in micro- and macro-elements. It has been shown that the method and time of storage have a direct effect on the quality of wild boar meat. The use of MAP caused an increase in protein carbonylation and

TBARS values, which may result in increased protein and lipid oxidation processes associated with negative changes in the technological and sensory properties of muscle during refrigerated storage. Overall, the physicochemical analysis and textural properties, and how they changed during storage, indicate that DRY-AGED and VAC stored muscles show no visible signs of deterioration and can be stored under refrigeration for up to 21 days. In the case of DRY-AGED samples, storage time had a positive effect on meat tenderness and colour. Meat seasoning also had a positive effect on microbial growth. The results indicate that the recommended storage methods for wild boar meat are either vacuum packing or dry-ageing in a meat seasoning cabinet. The high oxygen content of MAP negatively affected the quality of wild boar meat and carried a risk of increased protein carbonylation. Further studies should be conducted using other gas ratios for this species.

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CONFLICT OF INTEREST

The authors declare that they have no financial or commercial conflicts of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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Supplementary information

The effect of the storage method on the selected physicochemical and microbiological qualities of wild boar meat

Running title: The effect of the storage method on the quality of wild boar meat

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Table S1

The content of selected minerals in LTL, BF, and VL muscles; mean $\pm$ SD.

Content [g kg ⁻¹]	Muscle		
	LTL	BF	VL
K	2.1956 $\pm$ 0.1755	2.3480 $\pm$ 0.0273	2.2148 $\pm$ 0.0289
Na	0.4667 ^b $\pm$ 0.0151	0.4283 ^b $\pm$ 0.0454	0.6025 ^a $\pm$ 0.0084
Mg	0.1471 ^b $\pm$ 0.0003	0.1622 ^a $\pm$ 0.0021	0.1392 ^c $\pm$ 0.0006
Ca	0.0391 $\pm$ 0.0135	0.0411 $\pm$ 0.0018	0.0667 $\pm$ 0.0012
Zn	0.0342 ^a $\pm$ 0.0023	0.0146 ^c $\pm$ 0.0012	0.0251 ^b $\pm$ 0.0002
Fe	0.0164 $\pm$ 0.0001	0.0202 $\pm$ 0.0021	0.0191 $\pm$ 0.0008
Al	0.0051 $\pm$ 0.0031	0.0033 $\pm$ 0.0046	0.0035 $\pm$ 0.0035
Cu	0.0021 ^b $\pm$ 0.0001	0.0023 ^b $\pm$ 0.0001	0.0032 ^a $\pm$ 0.0001
Pb	0.0004 $\pm$ 0.0001	0.0004 $\pm$ 0.0001	0.0003 $\pm$ 0.0001
Cd	<0.00003	<0.00003	<0.00003
Cr	<0.00003	<0.00003	<0.00003
Mn	<0.00003	<0.00003	<0.00003
Ni	<0.00003	<0.00003	<0.00003

^{a,b,c} – Means with different letters in a row for a given parameter indicate a significant effect of the muscle (p<0.0005).

Table S2

Changes in storage loss and Warner-Bratzler shear force; mean $\pm$ SD.

Parameter	Day	Muscle								
		LTL			BF			VL		
		Packing method								
		MAP	DRY-AGED	VAC	MAP	DRY-AGED	VAC	MAP	DRY-AGED	VAC
Storage loss [%]	7	5.47 ^y ± 2.03	19.42 ^{bx} ± 5.45	3.54 ^{by} ± 1.01	5.03 ^{by} ± 1.00	16.92 ^{bx} ± 2.38	2.86 ^{by} ± 0.59	4.22 ^{by} ± 1.05	15.92 ^{bx} ± 1.76	3.43 ^y ± 1.11
	21	7.49 ^y ± 2.21	32.89 ^{ax} ± 3.91	6.84 ^{ay} ± 1.76	8.78 ^{ay} ± 1.21	26.65 ^{ax} ± 1.20	4.60 ^{az} ± 0.49	6.92 ^{ay} ± 0.75	25.63 ^{ax} ± 1.59	4.53 ^z ± 1.31
WBSF [N]	0	55.90 ^a ± 9.22	55.90 ^a ± 9.22	55.90 ^a ± 9.22	42.56 ^b ± 2.35	42.56 ^b ± 2.35	42.56 ^b ± 2.35	36.58 ± 4.31	36.58 ^b ± 4.31	36.58 ^{ab} ± 4.31
	7	35.30 ^b ± 6.01	34.32 ^b ± 3.92	35.21 ^b ± 3.92	37.01 ^{cy} ± 2.35	51.78 ^{ax} ± 7.16	35.01 ^{by} ± 6.67	38.24 ^y ± 5.59	48.74 ^{ax} ± 5.30	38.44 ^{ay} ± 7.53
	21	50.70 ^{ax} ± 10.40	29.71 ^{by} ± 5.69	31.48 ^{by} ± 6.28	52.37 ^{ax} ± 5.10	33.93 ^{cz} ± 2.84	39.72 ^{aby} ± 5.30	43.54 ^x ± 6.77	28.54 ^{cy} ± 3.92	31.68 ^{by} ± 4.51

^{a,b,c} – means with different letters in a column for a given parameter indicate a significant effect of time within the method ($p < 0.05$);

^{x,y,z} – means with different letters in a row for a given parameter indicate a significant effect of the method within time ($p < 0.05$).

Table S3

The effects of storage method and time on the oxidation levels of proteins and lipids in muscles; mean $\pm$ SD.

Parameter	Day	Muscle								
		LTL			BF			VL		
		Packing method								
		MAP	DRY-AGED	VAC	MAP	DRY-AGED	VAC	MAP	DRY-AGED	VAC
Lipid oxidation	0	0.36 ^c ± 0.04	0.36 ^c ± 0.04	0.36 ^b ± 0.04	0.38 ^c ± 0.08	0.38 ^b ± 0.08	0.38 ^b ± 0.08	0.34 ^c ± 0.04	0.34 ^b ± 0.04	0.34 ^c ± 0.04
	7	1.77 ^{bx} ± 0.12	0.55 ^{by} ± 0.08	0.59 ^{ay} ± 0.14	3.50 ^{bx} ± 0.07	0.38 ^{bz} ± 0.03	0.71 ^{ay} ± 0.06	2.32 ^{bx} ± 0.07	0.39 ^{bz} ± 0.01	0.77 ^{ay} ± 0.09
	21	6.15 ^{ax} ± 0.10	0.98 ^{ay} ± 0.12	0.34 ^{bz} ± 0.04	8.72 ^{ax} ± 0.16	0.92 ^{ay} ± 0.09	0.40 ^{bz} ± 0.05	6.28 ^{ax} ± 0.12	0.67 ^{ay} ± 0.09	0.43 ^{bz} ± 0.03
Protein oxidation	0	1.26 ^b ± 0.22	1.26 ^b ± 0.22	1.26 ± 0.22	1.15 ^b ± 0.13	1.15 ^b ± 0.13	1.15 ^b ± 0.13	0.86 ^b ± 0.10	0.86 ^b ± 0.10	0.86 ^b ± 0.10
	7	0.87 ^{cy} ± 0.08	1.06 ^{bxy} ± 0.22	1.17 ^x ± 0.26	0.76 ^{cy} ± 0.12	1.72 ^{ax} ± 0.45	0.96 ^{cy} ± 0.13	0.99 ^{by} ± 0.18	1.40 ^{ax} ± 0.23	1.12 ^{ay} ± 0.23
	21	3.29 ^{ax} ± 0.21	1.68 ^{ay} ± 0.23	1.09 ^z ± 0.13	2.48 ^{ax} ± 0.24	1.72 ^{ay} ± 0.20	1.41 ^{az} ± 0.23	2.52 ^{ax} ± 0.15	1.34 ^{ay} ± 0.16	1.04 ^{az} ± 0.17

^{a,b,c} – Means with different letters in a column for a given parameter indicate a significant effect of time within the method ($p < 0.05$);

^{x,y,z} – means with different letters in a row for a given parameter indicate a significant effect of the method within time ($p < 0.05$).

Table S4

The effect of the method and storage time on microbiological growth in muscles; mean $\pm$ SD.

Microbiological analysis [log10 cfu/g]	Day	Muscle								
		LTL			BF			VL		
		Packing method								
		VAC	MAP	DRY-AGED	VAC	MAP	DRY-AGED	VAC	MAP	DRY-AGED
Total count of microorganisms	0	4.73 ^c ± 0.20	4.73 ^c ± 0.20	4.73 ^{bc} ± 0.20	5.20 ^{cy} ± 0.14	5.20 ^{cx} ± 0.14	5.20 ^{dx} ± 0.14	5.30 ^c ± 0.01	5.30 ^c ± 0.01	5.30 ^c ± 0.01
	7	6.60 ^{bx} ± 0.00	6.18 ^{by} ± 0.07	4.45 ^{cz} ± 0.00	6.80 ^b ± 0.05	7.01 ^b ± 0.00	7.03 ^b ± 0.03	6.62 ^b ± 0.18	6.69 ^b ± 0.04	6.87 ^b ± 0.09
	14	8.18 ^{ax} ± 0.11	7.87 ^{ax} ± 0.08	5.79 ^{ay} ± 0.10	7.81 ^a ± 0.04	7.91 ^a ± 0.02	7.73 ^a ± 0.06	8.37 ^{ax} ± 0.01	8.09 ^{ay} ± 0.03	7.12 ^{az} ± 0.03
	21	7.94 ^{ax} ± 0.05	8.14 ^{ax} ± 0.01	5.21 ^{aby} ± 0.21	8.02 ^{ax} ± 0.08	8.13 ^{ax} ± 0.03	6.05 ^{cy} ± 0.04	8.50 ^{ax} ± 0.19	8.43 ^{ax} ± 0.01	4.72 ^{dy} ± 0.04
<i>Lactobacillus</i>	0	2.22 ^d ± 0.20	2.22 ^d ± 0.20	2.22 ^d ± 0.20	4.36 ^d ± 0.01	4.36 ^d ± 0.01	4.36 ^b ± 0.01	2.84 ^d ± 0.11	2.84 ^d ± 0.11	2.84 ^b ± 0.11
	7	6.55 ^{cx} ± 0.01	5.62 ^{cy} ± 0.08	2.88 ^{cz} ± 0.04	6.67 ^{cx} ± 0.02	5.94 ^{cy} ± 0.01	3.26 ^{cz} ± 0.25	6.21 ^{cx} ± 0.03	6.33 ^{bx} ± 0.06	3.61 ^{ay} ± 0.13
	14	7.28 ^{bx} ± 0.02	7.34 ^{bx} ± 0.02	3.46 ^{by} ± 0.07	7.28 ^{bx} ± 0.02	6.81 ^{bx} ± 0.21	4.43 ^{by} ± 0.10	7.49 ^{bx} ± 0.04	4.85 ^{cy} ± 0.01	3.65 ^{az} ± 0.15
	21	7.70 ^{ay} ± 0.03	8.04 ^{ax} ± 0.01	5.15 ^{az} ± 0.00	7.91 ^{ax} ± 0.08	8.04 ^{ax} ± 0.11	5.68 ^{ay} ± 0.08	7.93 ^{ay} ± 0.03	8.17 ^{ax} ± 0.01	3.83 ^{az} ± 0.05
<i>Enterococcus</i>	0	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-	-
	21	-	-	-	-	-	-	-	-	-
<i>Enterobacteriaceae</i>	0	2.80 ^c ± 0.08	2.80 ^c ± 0.08	2.80 ± 0.08	3.55 ^c ± 0.04	3.55 ^c ± 0.04	3.55 ^a ± 0.04	3.12 ^c ± 0.05	3.12 ^a ± 0.05	3.12 ^b ± 0.05
	7	5.33 ^{bx} ± 0.02	3.67 ^{ay} ± 0.01	2.97 ^z ± 0.16	3.96 ^{cy} ± 0.08	4.99 ^{ax} ± 0.01	3.49 ^{abz} ± 0.11	3.99 ^{by} ± 0.13	3.00 ^{az} ± 0.21	6.24 ^{ax} ± 0.04
	14	7.03 ^{ax} ± 0.06	3.35 ^{by} ± 0.12	2.99 ^y ± 0.13	5.62 ^{bx} ± 0.23	4.22 ^{by} ± 0.28	3.02 ^{bz} ± 0.23	5.15 ^{ax} ± 0.21	3.48 ^{ay} ± 0.06	3.08 ^{by} ± 0.05
	21	6.94 ^{ax} ± 0.06	2.65 ^{cy} ± 0.01	2.55 ^y ± 0.05	6.30 ^{ax} ± 0.04	3.59 ^{bcy} ± 0.16	3.53 ^{aby} ± 0.01	4.16 ^b ± 0.04	2.36 ^{bz} ± 0.06	2.73 ^{cy} ± 0.07
<i>Pseudomonas</i>	0	4.79 ^c ± 0.23	4.79 ^c ± 0.23	4.79 ^b ± 0.23	5.32 ^{cy} ± 0.04	5.32 ^{cx} ± 0.04	5.32 ^{cx} ± 0.04	5.36 ^c ± 0.00	5.36 ^c ± 0.00	5.36 ^c ± 0.00
	7	6.16 ^{bx} ± 0.01	6.08 ^{bx} ± 0.00	2.95 ^{cy} ± 0.13	5.62 ^b ± 0.08	6.88 ^a ± 0.03	6.89 ^b ± 0.03	6.03 ^{bz} ± 0.01	6.29 ^{by} ± 0.03	6.69 ^{bx} ± 0.02
	14	7.04 ^{ax} ± 0.16	6.84 ^{ax} ± 0.04	5.64 ^{ay} ± 0.04	6.14 ^{az} ± 0.04	7.21 ^{ay} ± 0.05	7.59 ^{ax} ± 0.04	7.30 ^a ± 0.06	7.29 ^a ± 0.10	7.07 ^a ± 0.01
	21	6.79 ^{ax} ± 0.07	5.98 ^{by} ± 0.04	4.72 ^{bz} ± 0.03	6.02 ^a ± 0.03	6.22 ^b ± 0.15	5.84 ^c ± 0.26	7.33 ^{ax} ± 0.04	6.30 ^{by} ± 0.16	4.70 ^{dz} ± 0.00

a,b,c – Means with different letters in a column for a given parameter indicate a significant effect of time within the method (p<0.05);

x,y,z – means with different letters in a row for a given parameter indicate a significant effect of the method within time (p<0.05).

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Effect of Dry, Vacuum, and Modified Atmosphere Ageing on Physicochemical Properties of Roe Deer Meat

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Consumers around the world are choosing sustainable and unprocessed foods. Roe deer meat, due to the natural origin, is a source of organic and healthy meat. The selection of suitable storage conditions and times plays an important role in a deterioration of the meat's functional and nutritional values. The knowledge about oxidation processes in roe deer meat stored and packed deploying different methods is limited. The main aim of this study was therefore to investigate the effect of storage method on the physicochemical properties of *musculus longissimus thoracis et lumborum* (LTL), *musculus biceps femoris* (BF), and *musculus vastus lateralis* (VL) of roe deer. The muscles were stored either dry (DRY-AGED), vacuum-packed (VAC), or packed under modified atmosphere (MAP) for 21 days. The quality of roe deer meat was assessed based on chemical composition, technological properties, pH values, water activity, colour, and oxidation processes of proteins and lipids. Roe deer meat had high protein (216.5–228.6 g/kg) and low fat content (17.1–25.8 g/kg). Both DRY-AGED and VAC contributed to improving meat tenderness during storage, while the Warner–Bratzler shear force of the MAP muscles increased. The high-oxygen conditions during MAP storage strongly induced the oxidation processes; an average increase of 1,263% for thiobarbituric acid reactive substance (TBARS) level and 155% for protein carbonyl content on day 21. Vacuum packaging and dry-ageing are recommended methods for storing roe deer meat. The high oxygen atmosphere negatively affected the quality of this game species. It carries the risk of increased oxidation of proteins and lipids which may promote the formation of potentially detrimental compounds.

Key words: roe deer, protein oxidation, lipid oxidation, vacuum, modified atmosphere, dry-ageing

INTRODUCTION

Today, despite its many advantages for human nutrition, meat is considered a less sustainable food choice due to growing concerns about health and the environment. Recently, many changes in dietary recommendations have been noticed, focusing much attention on the heavy environmental burden of meat production. In Central and Western Europe, there is a strong

tradition of meat consumption, but increased health awareness has led to a demand for alternative, low-fat, and antibiotic-free types of meat [Hoffman & Wiklund, 2006; Niewiadomska *et al.*, 2020]. In addition, consumers are looking for balanced and nutritious meat that can be included in a climate-friendly diet. When estimating the carbon footprint of farmed meat, many relative factors must be considered, ranging from feeding to energy,

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transportation, and slaughter. Therefore, the calculations for farmed meat are much higher than those for free-living game meat. For consumers who like meat, a pro-climate choice can be venison. In Poland, the most common species of deer is roe deer. Its meat is characterised by a unique taste and aroma but also by high quality and unique dietary and health-promoting properties. Roe deer meat is high in protein, unsaturated fatty acids, iron, and low in fat and saturated fatty acids [Cygan-Szczegielniak & Janicki, 2012a; Zomborszy *et al.*, 1996]. The high content of polyunsaturated fatty acids (PUFAs) is characteristic of the muscle tissue of game animals and much higher than that of farm animals [Klupsaite *et al.*, 2020; Razmaitė *et al.*, 2015]. Importantly, deer meat is free from antibiotics and growth and development enhancers, and animals are not exposed to the stress of commercial breeding [Cygan-Szczegielniak & Janicki, 2012b; Zomborszy *et al.*, 1996]. Another important aspect is the higher vitamin, folate, and mineral content of venison meat compared to many farmed species [Królikowski & Deptuła, 2020]. Despite its many advantages, the consumption of venison in Poland remains surprisingly low compared to pork, poultry, and beef. It is also significantly lower than in other European countries [Cygan-Szczegielniak & Janicki, 2012b; Królikowski & Deptuła, 2020; Kwiecińska *et al.*, 2018].

Venison meat and meat products obtained in Poland are largely exported to the markets of Western Europe; meanwhile, conditions in Poland could successfully contribute to an increase in the share of venison in the meat industry [Królikowski & Deptuła, 2020]. The high price of game meat and problems in maintaining quality during carcass processing and storage are the main reasons for low consumption [Kwiecińska *et al.*, 2018]. Most of the research carried out on the storage of meat has focused on the meat of farm animals [Daszkiewicz *et al.*, 2008; Vergara *et al.*, 2003]. The selection of suitable storage conditions and time plays an important role from the point of view of both consumer acceptance and the meat industry. However, incorrect storage can directly affect the deterioration of raw material quality [Kwiecińska *et al.*, 2018]. Meat is rich in many nutrients, but these compounds are prone to degradation processes, including oxidative ones especially in the case of proteins and lipids [Domínguez *et al.*, 2019].

Monitoring oxidative processes in food systems is important from the perspective of human nutrition and is therefore crucial for the meat industry [Lund *et al.*, 2011; Soladoye *et al.*, 2015]. Oxidative processes result in the deterioration of meat's functional properties and negatively affect its nutritional value [Estévez 2021; Kasalka-Czarna *et al.*, 2022; Li *et al.*, 2020; Soladoye *et al.*, 2015]. In addition to a reduction in the nutritional quality and bioavailability of oxidised proteins, the consumption of these modified proteins may be associated with an increased risk of certain disease states [Estévez & Xiong, 2019; Lund *et al.*, 2011; Soladoye *et al.*, 2015; Thøgersen *et al.*, 2020]. Soladoye *et al.* [2015] conducted a comprehensive review of the oxidative processes of proteins in processed meat, which highlighted the impact of the consumption of oxidised proteins in ageing and diseases associated with oxidative modifications of proteins and included

Alzheimer's disease, kidney failure, and diabetes, among others. Protein oxidation results in the formation of oxidised muscle protein products, but the characteristics of these products are highly dependent on the amino acids involved and mechanisms of oxidation process initiation. The oxidative changes of proteins include modifications of amino acid side chains, fragmentation, aggregation and polymerisation of proteins. Oxidative changes in proteins can be monitored by measuring the content of formed carbonyl groups [Estévez, 2011; Soladoye *et al.*, 2015]. Scientific studies have shown that processing and storage influence the development and intensity of oxidation processes in farmed species [Lund *et al.*, 2011]. In the case of game animals, which are characterised by a higher content of iron, myoglobin, and polyunsaturated fatty acids, the susceptibility to oxidation increases [Chaijan, 2008]. Despite this, there is still a scarcity of scientific research on oxidative processes within wild game meat. The main non-microbiological cause of meat deterioration is lipid oxidation, which is one of the main factors affecting meat quality, acceptability, and shelf life [Domínguez *et al.*, 2022]. Oxidative processes begin at slaughter and then intensify during processing, handling, and storage. The formation of lipid oxidation products results in discolouration, unpleasant odour and taste, and changes in texture [Chaijan, 2008]. In addition to reducing the nutritional value of meat, the aldehydes and oxysterols formed upon lipid oxidation exert pro-inflammatory, cytotoxic, and mutagenic effects [Domínguez *et al.*, 2022; Sottero *et al.*, 2019]. As Chaijan [2008] suggested, lipid oxidation leads to the formation of a wide range of aldehyde products that induce the oxidation of oxymyoglobin, resulting in an increase in the peroxidative activity of metmyoglobin. Oxidation processes can be effectively controlled by using appropriate packaging, storage or preservation technologies [Bao & Ertbjerg, 2019; Moczowska *et al.*, 2017; Wazir *et al.*, 2019].

The objective of this study was to use different storage methods and storage times for venison to preserve its quality (pH, colour, tenderness, water activity, water loss during cooking and storage) and to inhibit protein and lipid oxidation. Identifying differences in biochemical processes and quality of venison may allow improving production and packaging techniques and changing nutritional strategies for this type of meat. These factors have already been evaluated for common slaughter species, whereas studies on roe deer are still lacking.

MATERIALS AND METHODS

■ Sampling

Samples for the research were obtained from five carcasses of roe deer (*Capreolus capreolus*). Animals were hunted in Hunter District No. 57, OHZ, Sarbia Forest District (Poland). The selection was based on similarity of sex (male) and age (2–4 years), verifying the development of the dentition. Shooting and evisceration were performed under hunting protocol (internal carcass inspection report based on Regulation (EC) No 853/2004). The carcass was then cooled and stored at 0°C for 5 days at the Venison Buying Station (Drążgowo Forestry, Sarbia Forest District). This procedure is an ageing procedure, which is longer

for game meat than for slaughtered farm animals. Carcasses were assessed after skinning to exclude meat with defects (damaged elements by shooting, inappropriate pH value or improperly cooled meat). From each skinned roe deer carcass, three muscles were excised without bone and meat on the bone, namely *musculus longissimus thoracis et lumborum* (LTL), *musculus biceps femoris* (BF), and *musculus vastus lateralis* (VL). The cuts were vacuum-packed and transported to the Department of Meat Technology, Poznan University of Life Sciences, Poland in a portable car refrigerator with a permanent power supply (18445, Norauto, Warsaw, Poland). The muscles (without bone) were divided into equal, selected parts (approximately 80 g). Muscles (in triplicate) were vacuum-packed (VAC) in barrier bags (MULTIVAC Seep Haggemüller SE & Co. KG, Wolferschwenden, Germany) and in a modified atmosphere (MAP) (80% O₂, 20% CO₂) (Multivac T200 packaging machine) and sealed with polyethylene terephthalate/polyethylene (PET/PE) film. Samples were stored at 4±1°C for 21 days. Muscles on the bone were stored in a dry-ageing cabinet (DRY-AGED) at 2°C and 80% humidity for 21 days (Dry-Aged Cooler SW-28, Arnsberg, Germany). Samples were analysed immediately after preparation (0 days) and on the day 7 and 21 of storage, for each variant.

■ Proximate composition analysis

The proximate composition (protein, moisture, fat, and ash contents) was determined in triplicate for each muscle on fresh meat before packing. The composition was evaluated according to ISO standards. The protein content was calculated based on total nitrogen content and the 6.25 conversion factor for meat and dairy products following ISO 937:1978. In turn, respective Polish Standards: PN-ISO 1442:2000, PN-ISO 1444:2000, and PN-ISO 936:2000, were used for the other parameters tested. Samples for analysis were homogenised using a food processor (Moulinex S 643.23, Paris, France).

■ Analysis of the composition of minerals

Ash of raw meat samples (0.500±0.001 g) was digested with 5.0 mL of 65% HNO₃ (Merck, Darmstadt, Germany) in closed Teflon vessels (55 mL) at 180°C using the Mars 6 microwave digestion system (CEM, Matthews, NC, USA). Digested samples were diluted with high-pure deionized water (≥18 MΩ×cm resistivity) obtained from Milli-Q water purification system (Merck Millipore, Burlington, MA, USA) to a final volume of 10 mL. The procedure was reproduced after Zubaidi *et al.* [2021]. Contents of 13 minerals (K, Na, Mg, Ca, Zn, Fe, Al, Cu, Pb, Cd, Cr, Mn, and Ni) were determined in the samples with the inductively coupled plasma high-resolution optical emission spectrometry (ICP-HROES), using a PlasmaQuant PQ 9000 Elite spectrometer (Analytic Jena, Germany). The settings were as follows: radio frequency (RF) power 1.2 kW, auxiliary gas flow 0.5 L/min, nebulizer gas flow 0.5 L/min, plasma gas flow 12 L/min, and axial plasma view. High-resolution optics were echelle double monochromator and charge-coupled device (HR CCD), which was cooled to -10°C using the Peltier system. The following wavelengths of emission lines were applied: K 769.897 nm, Na 588.995 nm,

Mg 285.213 nm, Ca 315.887 nm, Zn 206.200 nm, Fe 238.204 nm, Al 396.152 nm, Cu 324.754 nm, Pb 220.353 nm, Cd 214.441 nm, Cr 267.716 nm, Mn 257.610 nm, and Ni 231.604 nm. Acquisition time was 1 s and signal was measured in 5 replicates. Argon was used as the internal standard (Ar 420.068 nm). Detection limits (DLs) were estimated at the level of 0.03 mg/kg dry weight (DW). ICP commercial analytical standards (Romil, Cambridge, UK) were used for calibration. Standard addition method was used in the quality control with acceptable recovery (80–120%). The uncertainty for the complete analytical process (including sample preparation) was at the level of 20%.

■ Water activity, pH and colour measurements

The water activity, pH and colour indices were measured on fresh meat before packing and on stored meat for 7 and 21 days under VAC, MAP, and DRY-AGED treatment. The water activity measurement was performed using a LabMaster-a_w neo instrument (Novasina AG, Lachen, Switzerland) at 25±1°C. The meter was calibrated with standard SAL-T 97% tagged with an automatic recognition system (RFID). The pH values were measured at room temperature (22–24°C) with a portable Handylab 2 pH meter equipped with an L68880 pH combination electrode (Schott AG, Mainz, Germany). The pH buffers 4.01 and 7.00 (Testo Ltd., Alton Hampshire, UK) were used for calibration. The colour on the cross-section of freshly cut samples was measured three times by the CIELab colour space using a Dr. Lange LMG161 portable colorimeter (Dr. Bruno Lange GmbH & Co. KG, Berlin, Germany). The values of lightness (L*), greenness/redness (a*), and blueness/yellowness (b*) were measured. The setting parameters were as follows: illuminant D65, aperture size of 5.0 mm, and observer angle of 10°. The instrument was standardised using a white standard (no. 3125).

■ Purge and cooking losses determination

The purge and cooking losses were expressed as a percentage, by weighing the samples before and after thermal treatment or the storage period. The purge losses were determined after 7 and 21 days of storage for each storage method while cooking losses were also determined for fresh meat. To determine cooking losses, 25 mm slices of meat wrapped in aluminium foil were heated to a core temperature of 72°C in a Rational combi model SCC 61 convection oven (Landsberg am Lech, Germany) at a temperature of 160°C and a 75% humidity in the cooking chamber. Cooking losses were determined on cooled samples at room temperature.

■ Warner-Bratzler shear force measurement

The samples cooked and cooled according to the procedure for cooking losses determination were cut into cuboids (1×1×3 cm). The Warner-Bratzler shear force (WBSF) for every muscle was tested before packing and after 7 and 21 days of storage (VAC, MAP, DRY-AGED). WBSF was measured by a TA.XT.plus texture analyser (Stable Micro Systems, Godalming, UK) using a Warner-Bratzler add-on device. The shear force expressed as N was measured perpendicularly to the arrangement of muscle fibres at a traverse speed of 100 mm/min.

■ Protein carbonyl content determination

Protein oxidation was determined by the total carbonyl content using 2,4-dinitrophenyl hydrazine (DNPH) derivatisation method according to Levine *et al.* [1994], with slight modifications. Fresh samples and packed meats (VAC, MAP, DRY-AGED) stored for 7 and 21 days were subjected to the analysis. One gram of meat was homogenised (Ultra Turrax) in 20 mM sodium phosphate buffer containing 6 M NaCl (pH 6.5) for 30 s, and immediately filtered through filter paper. Samples from homogenate (two aliquots of 0.4 mL) were precipitated by cold 10% trichloroacetic acid (TCA) (1.5 mL) and incubated at 20°C for 20 min. After incubation, the samples were centrifuged for 5 min at 4°C at 10,000×g. One of the pellets was treated with 1 mL of 2 M HCl (for the protein content measurement), the second with 1 mL of 10 M DNPH in 2 M HCl (for the carbonyl content measurement), and then were incubated for 30 min at 37°C. Afterward, the samples were treated with 1 mL of 10% TCA and centrifuged for 5 min at 4°C and 10,000×g. To remove excess DNPH after centrifugation, the precipitates were washed 3×1 mL with a solution of ethanol and ethyl acetate (1:1, v/v). The obtained pellets were then dried at room temperature for 1 h. The dried samples were dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine hydrochloride (pH 6.5) and incubated for 30 min at 37°C. To remove insoluble fragments, the samples were centrifuged for 5 min at 4°C at 10,000×g. The absorbance was measured (Ultrospec 2000, Pharmacia Biotech, Uppsala, Sweden) at 280 nm (A_{280}) and 370 nm (A_{370}) and carbonyl content (nmol mg protein) was calculated using Equation (1) [Levine *et al.*, 1994]:

$$\text{Carbonyl content} = \frac{A_{370}}{22,000 \times (A_{280} - A_{370} \times 0.43)} \times 10^6 \quad (1)$$

■ Determination of thiobarbituric acid reactive substance content

The thiobarbituric acid reactive substance (TBARS) content of LTL, BF, and VL was assessed according to the method described by Lo Fiego *et al.* [2004], with minor modifications. The analysis was performed for fresh samples and in meat stored (VAC, MAP, DRY-AGED) for 7 and 21 days. Raw meat samples from each muscle (10 g) were homogenised in 20 mL of 20% TCA at 10,000×g for 60 s and then centrifuged at 3,502×g for 15 min at 4°C. The supernatant was filtered through qualitative filter paper

by vacuum filtration. The volume of 5 mL of 20 mM 2-thiobarbituric acid was added to 5 mL of the filtrate. The blank was 5 mL of TCA and 5 mL of 2-thiobarbituric acid solution. Solutions were heated for 20 min in a boiling water bath and afterward cooled to 21°C. Absorbance was measured using an Ultrospec 2000 spectrophotometer (Pharmacia Biotech) at 532 nm. Results were expressed as mg of malonic dialdehyde (MDA) equivalents per kg of meat (mg MDA/kg).

■ Statistical analysis

Statistical analysis of the results was performed using Statistica 13.1 (StatSoft Inc., Tulsa, OK, USA). The statistics were consistent with the assumption of a normal distribution (verified by the Shapiro-Wilk test). One-way and two-way analysis of variance (ANOVA) was used. Tukey's Honestly Significant Difference (HSD) test was used to assess the significance of differences. The influence of the storage method of LTL, BF, and VL samples, as well as storage period up to 21 days on the quality characteristics, were assessed. The results were considered to be statistically significant at $p < 0.05$, and expressed as mean $\pm$ standard error of mean (SEM).

RESULTS AND DISCUSSION

■ The proximate composition, physicochemical properties, and shear force

The proximate composition of the three muscles tested (LTL, BF, VL) is shown in Table 1. Based on ANOVA, it was found that the type of muscle significantly ($p < 0.05$) affected the chemical composition (protein and fat content). The protein content of roe meat was 216.5–228.6 g/kg, while the fat content was 17.1–25.8 g/kg. Roe deer meat can therefore be categorised as low-fat meat. The muscle containing the most protein and the least fat was LTL. The protein content of VL and BF muscles was lower, while the fat content was higher than that of LTL. There were no statistically significant differences in moisture and ash contents between the muscles.

According to Ivanović *et al.*, [2020], the protein content of LTL from roe deer was 21.40%, while the fat content was 1.31%. Other authors [Daszkiewicz *et al.*, 2018] showed a similar protein content in this muscle, i.e. 229.76 g/kg, to that determined in the present study. As reported by Cygan-Szczegielniak & Janicki [2012a], the age of the animal had a direct effect on the proximate composition of the muscle derived from it. LTL muscle obtained from roe deer aged 2–3 had the highest protein content

Table 1. Proximate composition of roe deer muscles: *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF), and *vastus lateralis* (VL).

Component (g/kg)	LTL	BF	VL	<i>p</i>
Protein	228.6 $\pm$ 1.1 ^a	217.3 $\pm$ 1.3 ^b	216.5 $\pm$ 1.7 ^b	***
Moisture	738.9 $\pm$ 1.6	744.4 $\pm$ 2.1	742.6 $\pm$ 2.1	NS
Fat	17.1 $\pm$ 1.0 ^b	22.6 $\pm$ 1.0 ^a	25.8 $\pm$ 0.8 ^a	***
Ash	14.1 $\pm$ 1.6	15.6 $\pm$ 1.7	15.0 $\pm$ 2.0	NS

Results are shown as mean $\pm$ standard error of mean (SEM). Means with different letters (a,b) in a row indicate significant differences between the muscles ($p < 0.05$). * $p < 0.05$; ** $p < 0.01$; NS, non-significant (one-way ANOVA).

(24.02%) compared to the other age groups (4–5 and 6–7 year old), and a low fat content (0.43%). According to other studies, the fat content of roe deer in LTL ranged from 0.36 to 1.76% and depended on the season [Dominik *et al.*, 2012]. Compared to wild boar meat, which is also a representative of game, roe deer meat is richer in protein and has a lower fat content [Kasalka-Czarna *et al.*, 2022]. Nevertheless, the proximate composition of game meat is strongly influenced by diet, season, hunting, habitat, sex, and animal activity [Ivanović *et al.*, 2020].

Table S1 (in Supplementary Materials) shows the content of selected minerals in the LTL, BF, and VL. The highest contents were determined for potassium, sodium, magnesium, zinc and iron. The content of iron ranged from 14.8 to 24.6 mg/kg of meat. No heavy metals, such as cadmium, chromium, manganese, or nickel, were found. Roe deer meat was richer in potassium, magnesium, iron, and phosphorus than that of wild boar [Kasalka-Czarna *et al.*, 2022; Zomborszky *et al.*, 1996].

The physicochemical properties of muscles of roe deer (fresh and stored for 7 and 21 days in VAC, MAP and DRY-AGED variant) are presented in **Table 2**. Their values indicate a significant effect of storage time and method as well as their interaction on the pH of the tested muscles (**Table 3**). The pH values of fresh meat ranged from 5.53 to 5.63, depending on muscle type. Of all the roe deer muscles, VL had the highest pH for each storage method. Significantly higher pH values, compared to the other methods, were observed for DRY-AGED muscles on both day 7 and 21 of storage (pH ranged from 5.53 to 5.74 for LTL), while the pH of the muscles stored in MAP on day 7 was similar to the pH of the VAC samples, with exception of VL. On day 21, the highest decrease in pH was noted for BF in MAP (5.44). The VAC muscles featured the greatest fluctuations in pH values. Similar relationships between the effect of storage method and time were shown for the wild boar muscles in a similar experiment, but MAP storage resulted in a significant decrease in pH for each wild boar muscle (compared to 5.23 for BF) [Kasalka-Czarna *et al.*, 2022]. According to Daszkiewicz *et al.* [2018], the pH value measured 48–54 h *post-mortem* in LTL of roe deer was 5.52. However, for the LTL taken 24 h *post-mortem*, the pH was 5.72 [Ivanović *et al.*, 2020]. Other researchers have also shown that the pH of fresh meat from roe deer measured 120 h *post-mortem* ranged from 5.52 to 5.56, depending on the muscle metabolism [Dominik *et al.*, 2012]. A characteristic of game meat is the high content of lactic acid produced by glycolysis, which influences meat acidity. The pH values of venison meat measured up to 96 h *post-mortem* ranged from 5.4 to 5.6. These values also changed according to season, sex, and age [Dominik *et al.*, 2012]. Storage time resulted in differences in meat pH. Vergara *et al.* [2003] studied deer meat stored in a modified atmosphere in three gas mixtures (40% CO₂ + 60% N₂, 80% CO₂ + 20% O₂, and 80% CO₂ + 20% N₂). They noted that storage time affected the increase in pH for each method. In turn, Gill [1990], found that the pH of lamb meat decreased when it was packaged in a CO₂-rich atmosphere. Thus, depending on a given species, meat undergoes different changes with the same gas atmosphere in packaging.

The study demonstrated a significant effect of storage time and method as well as their interaction on the lightness values (L^*) of the BF and VL (**Table 3**). For VL, there was a weaker interaction between storage method and storage time than for BF. The value of L^* color parameter of meat on day 21 of MAP and VAC storage increased compared to the fresh samples (**Table 2**). For a^* and b^* values, an interaction was demonstrated between the storage method and time for each muscle (**Table 3**). Time did not significantly affect a^* values of BF and VL and b^* values of BF. The storage method also had no significant effect on a^* value of LTL. MAP had a negative effect on the a^* parameter (**Table 2**). Its value increased in DRY-AGED BF, whereas remained unchanged after 21-storage in VAC. On day 21 of the analysis, significantly lower a^* values were determined in the samples stored in MAP compared to the other two methods. This is most likely due to the formation of oxymyoglobin (OxyMb) under aerobic conditions in an atmosphere with a high oxygen concentration [Chaijan, 2008]. The samples stored in VAC and DRY-AGED had significantly lower values of yellowness (b^*) compared to the meat stored in MAP (**Table 2**). Vergara *et al.* [2003] found that the b^* value in deer meat increased when the O₂ content of the package was higher. The sharp changes in the b^* parameter values suggest that this gas was responsible for the browning of red deer meat. Oxygen is used to enhance fresh meat colour, as it maintains the characteristic red colour of oxymyoglobin, which is the oxidised form of myoglobin. However, at increased concentrations, oxygen can negatively affect meat colour [Świderski & Sadowska, 2011]. The increase in the b^* value observed during meat storage may be due to metmyoglobin formation [Daszkiewicz *et al.*, 2011]. Myoglobin is involved in the storage of oxygen in red muscles. The addition of oxygen can retard its formation, but when oxygen-free packaging is used, intense oxidative changes occur, which can contribute to the formation of its large amounts [Świderski & Sadowska, 2011]. A similar effect can be observed with vacuum packaging when oxygen is removed from the meat surface, which can also result in a change in meat colour towards browning. According to Daszkiewicz *et al.* [2011], no significant differences were found between the average values of the colour indices of roe deer meat after 7 days of storage in the case of using vacuum packaging and MAP (40% CO₂ + 60% N₂; 60% CO₂ + 40% N₂). The results also showed that, after 21 days of storage, the use of MAP with 60% N₂ had the smallest effect on colour changes. Other researchers have noted a similar relationship for deer meat [Vergara *et al.*, 2003]. The pH value also affects L^* values, and a lower pH increases protein denaturation, resulting in higher L^* values [Klupsaite *et al.*, 2020].

A significant effect of storage time and method on water activity (a_w) was shown for all muscles (**Table 3**). However, no significant interaction between method and storage time was observed for LTL. On day 7 of MAP storage, a_w values for all muscles were lower (0.9675 for LTL, 0.9690 for BF, and 0.9686 for VL) compared to fresh meat and meat stored for 21 days (**Table 2**). No similar relationship was observed for VAC and DRY-AGED. After 21 days, a significant decrease in a_w was determined in DRY-AGED

Table 2. Physicochemical properties of roe deer muscles: *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF), and *vastus lateralis* (VL) during storage under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing.

Parameter	Storage time (day)	LTL			BF			VL		
		MAP	DRY-AGED	VAC	p-Method	MAP	DRY-AGED	VAC	p-Method	p-Method
pH	0	5.53±0.03 ^b	5.53±0.03 ^b	5.53±0.03	NS	5.54±0.01 ^a	5.54±0.01 ^c	5.54±0.01 ^b	NS	5.63±0.01 ^c
	7	5.59±0.00 ^y	5.70±0.02 ^{ax}	5.58±0.02 ^y	***	5.57±0.01 ^{ay}	5.68±0.01 ^{bx}	5.60±0.01 ^{ay}	***	5.69±0.01 ^{bx}
	21	5.51±0.01 ^{by}	5.74±0.02 ^{ax}	5.54±0.02 ^y	***	5.44±0.03 ^{bx}	5.78±0.02 ^{ax}	5.54±0.01 ^{by}	***	5.80±0.02 ^{ax}
p-Time		**	***	NS	***	***	***	***	***	*
L*	0	24.41±0.66 ^b	24.41±0.66 ^b	24.41±0.66 ^b	NS	28.33±0.60 ^b	28.33±0.60 ^a	28.33±0.60 ^b	NS	26.13±1.28 ^b
	7	31.01±0.15 ^{ax}	26.87±0.35 ^y	29.79±0.48 ^{ax}	***	31.45±0.48 ^{ax}	28.89±0.51 ^{ay}	30.20±0.49 ^{axy}	**	28.72±0.54 ^{ay}
	21	31.26±0.47 ^{ax}	24.83±0.56 ^y	28.17±0.70 ^{axy}	***	30.40±0.38 ^{ax}	24.55±0.44 ^{by}	30.54±0.35 ^{ax}	***	27.97±0.30 ^y
p-Time		***	NS	***	***	***	***	**	***	NS
a*	0	14.23±0.59 ^a	14.23±0.59 ^a	14.23±0.59 ^a	NS	11.25±0.29 ^a	11.25±0.29 ^b	11.25±0.29 ^a	NS	11.67±1.09 ^a
	7	12.21±0.13 ^{bx}	12.25±0.34 ^{bx}	10.21±0.49 ^{by}	***	11.52±0.14 ^{ax}	11.53±0.31 ^{bx}	9.95±0.42 ^{by}	***	11.53±0.31 ^{ax}
	21	10.28±0.60 ^{cy}	11.71±0.28 ^{bxy}	12.66±0.34 ^{ax}	***	8.00±0.32 ^{bx}	13.94±0.55 ^{ax}	11.05±0.16 ^{ay}	***	13.96±0.55 ^{ax}
p-Time		***	***	***	***	***	***	**	***	*
b*	0	13.59±0.43 ^b	13.59±0.43 ^a	13.59±0.43 ^a	NS	12.87±0.16 ^c	12.87±0.16	12.87±0.16 ^a	NS	11.89±0.42
	7	15.12±0.11 ^{ax}	12.1±0.23 ^{by}	11.41±0.40 ^{by}	***	14.53±0.14 ^{ax}	12.57±0.30 ^y	11.97±0.25 ^{by}	***	14.67±0.18 ^{ax}
	21	14.23±0.55 ^{bx}	10.29±0.23 ^{cz}	11.77±0.29 ^{by}	***	14.93±0.40 ^{ax}	13.05±0.46 ^y	11.71±0.19 ^{bx}	***	13.28±0.48
p-Time		*	***	***	***	***	NS	***	***	NS
Water activity (-)	0	0.9754±0.0001 ^a	0.9754±0.0001 ^a	0.9754±0.0001 ^a	NS	0.9727±0.0006 ^a	0.9727±0.0006 ^a	0.9727±0.0006 ^a	NS	0.9733±0.0009 ^a
	7	0.9675±0.0003 ^b	0.9620±0.0025 ^b	0.9712±0.0005 ^b	NS	0.9690±0.0005 ^{by}	0.9653±0.0007 ^{bx}	0.9711±0.0004 ^{ax}	***	0.9686±0.0009 ^{by}
	21	0.9737±0.0005 ^{ax}	0.9590±0.0036 ^{cy}	0.9700±0.0007 ^{bx}	***	0.9716±0.0003 ^{ax}	0.9644±0.0009 ^{bx}	0.9686±0.0008 ^{by}	***	0.9718±0.0004 ^{ax}
p-Time		***	***	***	***	***	***	***	***	***
Cooking loss (%)	0	23.03±0.51 ^c	23.03±0.51 ^a	23.03±0.51	NS	28.52±0.34 ^b	28.52±0.34 ^b	28.52±0.34 ^b	NS	22.45±0.35 ^b
	7	32.15±0.52 ^{ax}	21.10±0.26 ^{bx}	22.59±0.35 ^y	***	34.55±0.34 ^{ax}	30.00±0.24 ^{ay}	29.31±0.38 ^{by}	***	34.76±0.37 ^{ax}
	21	29.42±0.45 ^{bx}	23.12±0.29 ^y	22.21±0.55 ^y	***	33.80±0.49 ^{ax}	27.17±0.42 ^{cy}	32.14±0.46 ^{ax}	***	31.85±0.39 ^{bx}
p-Time		***	***	NS	***	***	***	***	***	***

Results are shown as mean ± standard error of mean (SEM). Means with different superscripts a–c in a column for a given parameter differ significantly within storage time ($p < 0.05$). Means with different superscripts x–z in a row for a given muscle differ significantly within storage methods ($p < 0.05$). p-Time, effect of time within method; p-Method, effect of method within time; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, non-significant (one-way ANOVA). L*, lightness; a*, greenness/redness; b*, blueness/yellowness.

Table 3. Effect of time (0, 7, 21 days) and method of storage (dry, vacuum, and modified atmosphere ageing) and their interaction on selected properties of roe deer muscles: *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF), and *vastus lateralis* (VL).

Parameter	Muscle	Storage time	Storage method	Storage time × storage method
pH	LTL	***	***	***
	BF	***	***	***
	VL	***	***	***
L^*	LTL	NS	NS	NS
	BF	***	***	***
	VL	***	***	**
a^*	LTL	***	NS	***
	BF	NS	***	***
	VL	NS	*	***
b^*	LTL	***	***	***
	BF	NS	***	***
	VL	***	***	**
Water activity	LTL	*	*	NS
	BF	***	***	***
	VL	***	***	***
Cooking loss	LTL	**	***	***
	BF	***	***	***
	VL	***	***	***
Purge losses	LTL	***	***	***
	BF	***	***	***
	VL	***	***	**
WBSF	LTL	***	***	***
	BF	***	***	***
	VL	***	***	***
TBARS content	LTL	***	***	***
	BF	***	***	***
	VL	***	***	***
Protein carbonyl content	LTL	***	***	***
	BF	***	***	***
	VL	***	***	***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, non-significant (two-way ANOVA). L^* , lightness; a^* , greenness/redness; b^* , blueness/yellowness; WBSF, Warner–Bratzler shear force; TBARS, thiobarbituric acid reactive substances.

meats, e.g., from 0.9754 to 0.9590 for LTL. This decline corresponds to the highest purge losses (Figure 1). The highest a_w was determined for the samples stored for 21 days in MAP, especially for LTL (0.9737). For vacuum packaging, the decrease in water activity was not significant after longer storage. Meat seasoning had a positive effect on a_w , which was also observed for wild boar

meat [Kasalka-Czarna et al., 2022]. The growth of microorganisms and the rate of biochemical processes are strongly dependent on water activity. Additionally, according to Florek et al. [2019], DRY-AGED meat has a lower water content than those stored in VAC, which affects, among others, its microbiological safety during storage. According to other reports, for the same storage conditions (MAP, VAC, DRY-AGED), at 7, 14 and 21 days of storage, wild boar meat was microbiologically safe [Kasalka-Czarna et al., 2022].

In terms of cooking losses, the interaction effect of storage time × storage method (Table 3) on LTL, BF and VL was significant ($p < 0.001$). Significant effect of time and storage method was found for BF and VL ($p < 0.001$; $p < 0.001$) and LTL ($p < 0.01$; $p < 0.001$), respectively. Cooking losses in fresh meat were 23.03% in LTL, 28.52% in BF, and 22.45% in VL (Table 2). The lowest percentage of thermal loss after 7 days of storage was noted in the DRY-AGED (21.10% for LTL, 30.00% for BF, and 23.28% for VL) and VAC (22.59% for LTL, 29.31% for BF and 23.03% for VL) muscles. For VAC, cooking losses significantly increased on day 21 of storage relative to the fresh sample for VL and BF. Significant differences were noted at day 7 of MAP storage for LTL and VL compared to both the fresh sample and the sample stored for 21 days. The increase of the thermal losses for MAP stored samples indicates a decrease in water retention in the muscle, which may have a direct effect on its texture. This was not observed for VAC in muscles from wild boar, in which cooking losses decreased during storage [Kasalka-Czarna et al., 2022]. According to Daszkiewicz et al. [2011], roe deer meat stored (m. *longissimus dorsi*) under vacuum was characterised by increased cooking losses compared to the modified atmosphere with oxygen exclusion. Despite this, no significant differences were observed in cooking losses between MAP and VAC. Therefore, the composition of the modified atmosphere can have an impact on cooking losses. In the early phase of storage, a characteristic crust develops on the surface of DRY-AGED meat due to water loss [Dashdorj et al., 2016]. The increase in water retention capacity is strongly correlated with an increase in meat pH. As a result of the increase in pH, the protein structure became more open and myofibrillar proteins could bind more water. Despite high storage losses and high water loss, DRY-AGED meat with the highest pH is of high quality [Dashdorj et al., 2016]. The same result was obtained from wild boar meat analyses (on day 21 of storage of LTL, pH values were 5.31 (MAP), 5.46 (VAC), 5.72 (DRY-AGED), and cooking losses were 30.82%, 30.92%, 20.94%, respectively) [Kasalka-Czarna et al., 2022].

Significant ($p < 0.001$) effect of storage time and storage method on purge losses was found in the study (Table 3). The interaction effect of storage time × storage method (Table 3) on LTL, BF and VL was significant ($p < 0.001$ for LTL and BF; $p < 0.01$ for VL). Purge losses were highest on day 21 of storage for each muscle and storage method (Figure 1, Table S2). The lowest purge losses were determined for MAP and VAC. For LTL on day 21 of storage, there was an increase of 2.3% for MAP and 1.27% for VAC relative to day 7 of storage. For LTL DRY-AGED, the difference was 15.92% (26.12% on day 7 and 42.04% on day 21).

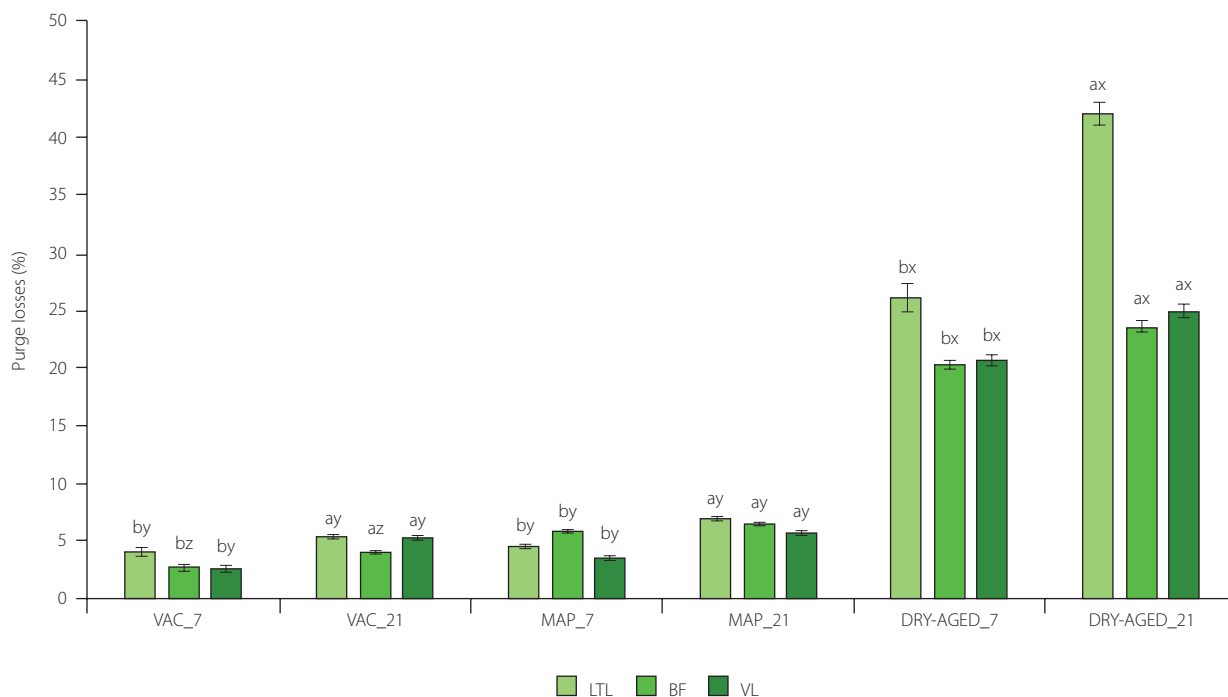


Figure 1. Purge losses in muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of roe deer during storage (at day 7 and 21) under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing. Results are shown as mean and standard error of mean (SEM). Different a and b letters above bars indicate a significant effect of time within the method ($p < 0.05$). Different x–z letters above bars indicate a significant effect of the method within time ($p < 0.05$).

After 7 and 21 days of storage, the purge losses of DRY-AGED meat due to drying and water loss were significantly four to even seven times higher. Daszkiewicz *et al.* [2008] reported lower losses for roe deer LTL in VAC (1.50% after 7 and 2.17 after 21 days of storage) and observed that the use of modified atmosphere packaging with 60% CO₂ + 40% N₂ resulted in reduced purging. The extent of purge losses can have some economic consequences, as, e.g., DRY-AGED storage of roe deer meat dramatically reduces the overall process efficiency. A higher storage loss was recorded within each method for roe deer compared to wild boar meat [Kasalka-Czarna *et al.*, 2022].

The study demonstrated a significant ($p < 0.001$) effect of storage time and method as well as their interaction on the WBSF of the LTL, BF and VL (Table 3). VAC and DRY-AGED had a positive effect on tenderness at day 21 of storage (except for LTL, DRY-AGED and VL), whereas MAP adversely affected this quality attribute during meat storage (Figure 2, Table S2). For LTL muscle stored as DRY-AGED, the lowest values were reached on day 7 of storage (16.91 N). For each of the MAP muscles, WBSF values increased on day 21 relative to the fresh sample. The highest tenderness of BF and VL muscles was reached after 21 days of storage for DRY-AGED (17.83 N and 19.08 N, respectively). For LTL, the lowest values were observed on day 7 of storage for both DRY-AGED and VAC (16.91 N and 16.53 N, respectively). Klupsaitė *et al.* [2020] showed that roe deer meat analysed 24 h *post-mortem* had the highest tenderness compared to other game species (wild boar, deer, and beaver), and its tenderness was satisfactory (1.17 kg/cm² for *m. glutenus medius*). In another study, cutting force was determined for LTL from roe deer 24 h *post-mortem* at

47.21 N [Dominik *et al.*, 2012]. Therefore, the carcass skin maturation treatment applied in this study for 5 days had a major effect on the tenderness of roe deer meat. The muscles showed satisfactory tenderness on the day the samples were obtained. According to Daszkiewicz *et al.* [2008], in LTL from roe deer, VAC storage for 21 days had a positive effect on tenderness (16.24 N). The researchers used modified atmosphere packaging, but with the elimination of oxygen (40% CO₂ + 60% N₂ and 60% CO₂ + 40% N₂); the analyses showed a positive effect on tenderness development. Other researchers have also reported that high oxygen concentrations may contribute to increased WBSF. This may be due to increased oxidation of proteins and fats, resulting, for example, in the formation of oxygen-induced protein aggregates [Clausen *et al.*, 2009; Moczowska *et al.*, 2017]. In pork and beef stored in a high-oxygen atmosphere, myosin heavy chains form intermolecular bonds that are not observed without the presence of oxygen. This may contribute to the deterioration of tenderness [Lund *et al.*, 2007]. In addition to the method and time of storage, these differences can also be influenced by different muscle metabolisms. In another study on beef stored in a high-oxygen atmosphere, a reduction in tenderness was confirmed due to cross-linking of myosin through disulphide bond formation [Kim *et al.*, 2010].

■ Protein and lipid oxidation

Two-way ANOVA showed the significant effect of storage time and method and the effect of storage time × storage method (Table 2) on protein and lipid oxidation in LTL, BF and VL ($p < 0.001$). During VAC storage, the content of protein carbonyl

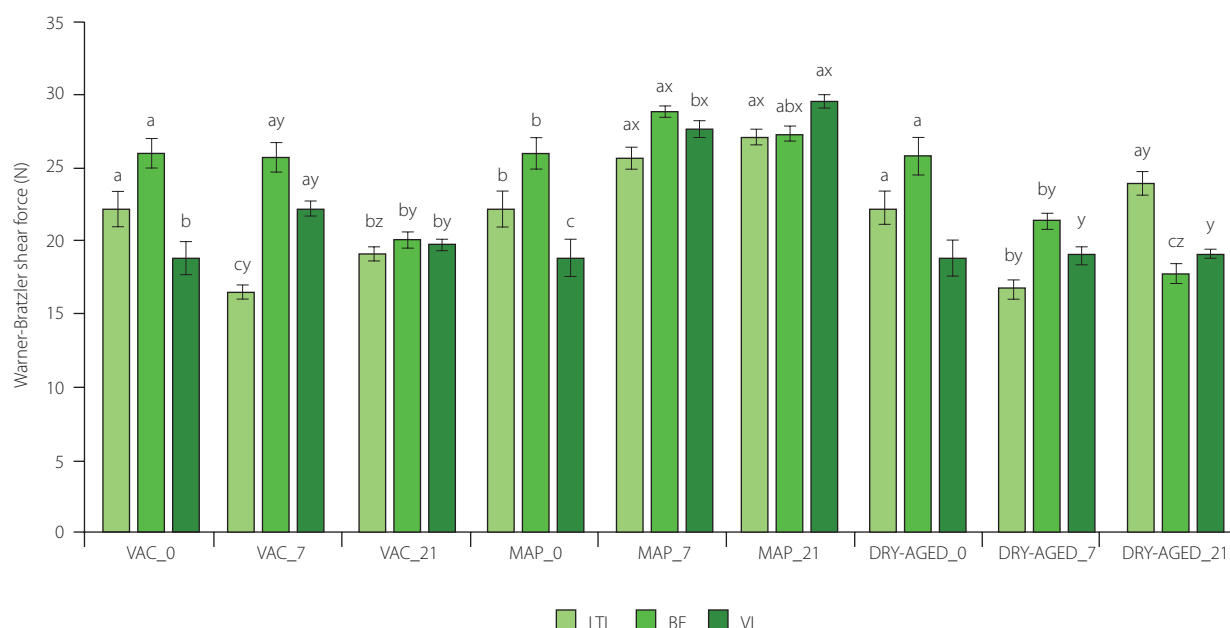


Figure 2. Warner–Bratzler shear force determined for muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of roe deer during storage (at day 0 (fresh), 7 and 21) under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing. Results are shown as mean and standard error of mean (SEM). Different a–c letters above bars indicate a significant effect of time within the method ($p < 0.05$); Different x–z letters above bars indicate a significant effect of the method within time ($p < 0.05$).

groups decreased in all muscles at both 7 and 21 days of storage compared to the fresh sample (Figure 3, Table S3). On day 21 of VAC storage, the protein carbonyl content ranged from 0.21 to 0.26 nmol/mg protein. On day 7 of storage, the highest levels of carbonyls were determined for all DRY-AGED muscles. They remained stable, and no considerable increase was observed on day 21, except for BF. Oxidative changes in MAP-stored muscles up to day 7 were less intense than in the DRY-AGED muscles. On day 21 of analysis, however, the increase in protein oxidation observed in MAP samples was the highest, i.e., 121.43% for LTL, 141.03% for BF, and 78.26% for VL, compared to the fresh samples.

As a result of protein oxidation, carbonyl group content for all roe deer muscles within each method did not exceed 0.94 nmol/mg protein (21 days MAP, BF). In a similar study on wild boar meat, the carbonyl contents for fresh LTL, BF, and VL muscles were significantly higher (1.26, 1.15, and 0.86 nmol/mg protein, respectively), while MAP storage contributed to protein oxidation products of 2.48–3.29 nmol/mg [Kasalka-Czarna et al., 2022]. As reported by Lindahl et al. [2010], greater protein carbonylation in beef muscle was observed with high-oxygen packaging. In other studies, carbonyl levels in pork LTL stored for 7 days in MAP with a high oxygen content (70% O₂ + 30% CO₂) did not change significantly and were slightly higher than for VAC samples. After 14 days, a decrease in carbonyl levels was noted for VAC and a slight increase for MAP samples [Lund et al., 2007]. As reported by Wang et al. [2019], 7 days of pork storage in MAP (80% O₂ + 20% CO₂) resulted in enhanced (reaching approximately 2.40 nmol/mg protein) formation of protein carbonyls.

The formation of protein carbonyls may also be promoted by a decrease in pH [Estévez, 2011]. In the current study, it was noted

that on day 21 of MAP storage, a significant increase in protein oxidation was consistent with a decrease in pH. A deterioration in tenderness, colour, and odour of MAP-stored roe meat was observed in the present study. There are many hypotheses on the consequences of protein oxidation on meat flavour due to the formation of carbonyl groups [Lund et al., 2011]. According to Ventanas et al. [2007] and Fuentes et al. [2010], the formation of protein carbonyls can affect the taste and aroma of meat. In contrast, other authors demonstrated the effect of protein oxidation on changes in meat colour [Domínguez et al., 2019; Estévez & Cava, 2004]. This may explain the changes observed in our study for the *b** parameter values of meat during storage in MAP.

The levels of TBARS in fresh roe meat were 0.44, 0.47, and 0.46 mg MDA/kg for LTL, BF, and VL, respectively (Figure 3, Table S3). Daszkiewicz et al. [2018] reported 0.92 mg MDA/kg for LTL roe deer, but Klupsaitė et al. [2020] detected 0.30 mg MDA/kg for *m. gluteus medius*. These values are strongly dependent on muscle metabolism and proximate composition. After 7 days of storage, an increase in lipid oxidation was detected in all samples. Significantly higher TBARS values were noted (after 7 and 21 days of storage) in the samples stored under high oxygen conditions (MAP). The least changes in lipids after 21 days of storage were observed in the samples stored in VAC. In the DRY-AGED muscles, the changes, although significant, occurred more slowly compared with MAP. TBARS values in roe deer meat increased faster than the protein carbonyl content during prolonged storage in MAP (an average increase of 1263% for TBARS and 155% for carbonyls on day 21). In our previous study, we observed that changes within TBARS occurred equally rapidly upon the storage of wild boar meat in a high-oxygen atmosphere [Kasalka-Czarna et al., 2022].

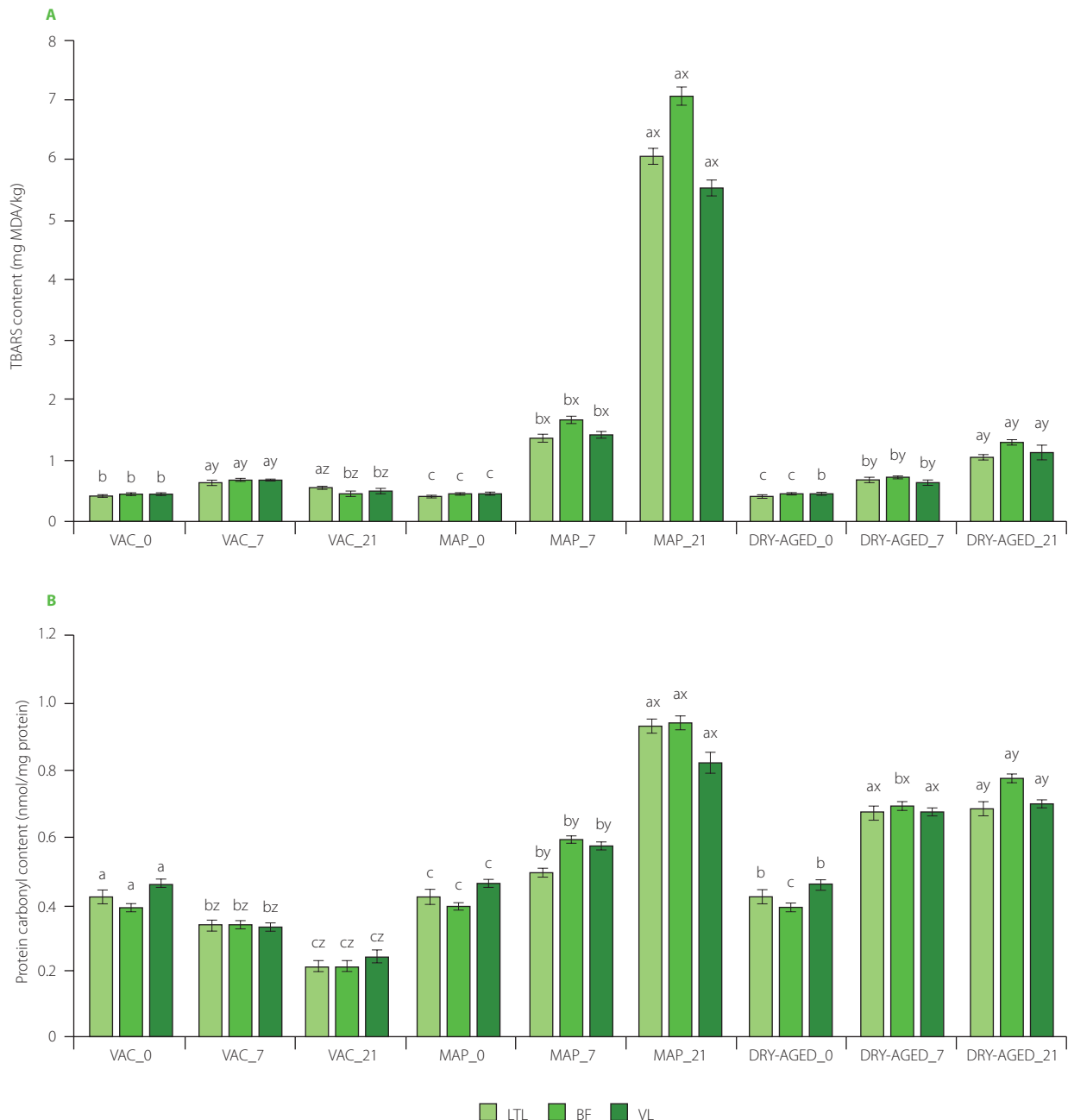


Figure 3. Contents of thiobarbituric acid reactive substances, TBARS (**A**) and protein carbonyls (**B**) in muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of roe deer during storage (at day 0 (fresh), 7 and 21) under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing. Results are shown as mean and standard error of mean (SEM). Different a–c letters above bars for a given parameter indicate a significant effect of time within the method ($p < 0.05$). Different x–z letters above bars for a given parameter indicate a significant effect of the method within time ($p < 0.05$). MDA, malonic dialdehyde.

Interestingly, TBARS values were not considerably different for roe deer meat compared to wild boar meat. The LTL from wild boar on day 21 of MAP storage contained 6.15 mg MDA/kg, while the LTL from roe deer had 6.07 mg MDA/kg. High TBARS values result in a rancid aroma or noticeable colour change [Domínguez *et al.*, 2019]. The acceptable MDA content for meat products, where no rancid aroma is perceptible, is 2.0–2.5 mg MDA/kg meat [Campo *et al.*, 2006]. Importantly, reducing the oxygen in packaged pork results in the inhibition of both pork protein and lipid oxidation compared with 80% oxygen atmosphere. When the pork was stored in the air, the carbonyl and TBARS contents were lower

than in high oxygen MAP [Wang *et al.*, 2019]. Similarly, beef steaks stored in a high oxygen atmosphere (80% O₂ + 20% CO₂) had higher TBARS values compared to the VAC-packed ones [Łopacka *et al.*, 2016].

CONCLUSIONS

In conclusion, a significant effect of storage time and method (dry, vacuum, and modified atmosphere ageing) of muscles of roe deer on pH, water activity, thermal loss, storage loss, WBSF and lipid and protein oxidation was found in the present study. A significant interaction effect of storage time × storage method was also

observed for all traits examined in BF and VL. For LTL, no significant interaction effect was found only for L^* values and water activity. The high-oxygen atmosphere induces the formation of protein carbonyls and lipid oxidation products. The use of MAP also caused negative changes in the technological properties and colour parameters of the muscles during storage. From the point of view of physicochemical analysis and texture properties, muscles stored in the DRY-AGED and VAC system showed no visible signs of deterioration and could be stored in the refrigerator for up to 21 days. The storage time had a positive effect on the tenderness of the meat and the colour of the DRY-AGED samples. The results indicate that the recommended methods for storing roe meat for 21 days are VAC and DRY-AGED. Further research should be carried out using other gas indicators for MAP packaging for this species. Due to its very low fat content and high protein content, roe meat is a valuable component of a balanced diet. Compared to other game species, venison meat is also rich in micro- and macronutrients.

SUPPLEMENTARY MATERIALS

The following are available online at <http://journal.pan.olsztyn.pl/Effect-of-Dry-Vacuum-and-Modified-Atmosphere-Ageing-on-Physicochemical-Properties,163613,0,2.html>; Table S1. The content (mg/kg) of selected minerals in muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of the roe deer. Table S2. Storage loss and Warner-Bratzler shear force (WBSF) for muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of roe deer during storage under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing. Table S3. Contents of thiobarbituric acid reactive substances (TBARS) and protein carbonyls in muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of roe deer during storage under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing.

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CONFLICT OF INTERESTS

The authors declare that they have no financial or commercial conflicts of interest.

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Effect of Dry, Vacuum, and Modified Atmosphere Ageing on Physicochemical Properties of
Roe Deer Meat by *Natalia Kasalka-Czarna, Róża Biegańska-Marecik, Jędrzej Proch,*
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Supplementary Material

Table S1. The content (mg/kg) of selected minerals in muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of the roe deer. Table S2. Storage loss and Warner-Bratzler shear force (WBSF) for muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of roe deer during storage under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing. Table S3. Contents of thiobarbituric acid reactive substances (TBARS) and protein carbonyls in muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of roe deer during storage under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing.

Table S1. The content (mg/kg) of selected minerals in roe deer muscles: *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF), and *vastus lateralis* (VL).

Mineral	LTL	BF	VL	
K	2,587±97	2,697±63	2,539±89	NS
Na	492.6±3.5 ^a	420.4±0.6 ^b	425.1±1.4 ^b	***
Mg	212.5±6.0	206.3±0.7	205.9±1.5	NS
Ca	66.5±3.0 ^b	72.6±1.8 ^a	56.4±1.0 ^c	***
Zn	30.0± 0.0 ^a	27.1±0.2 ^b	26.5±0.4 ^b	***
Fe	24.6±0.3 ^a	21.4±0.3 ^b	14.8± 0.4 ^c	***
Cu	3.7±0.0 ^a	3.5±0.0 ^a	2.6±0.0 ^b	***
Al	1.6±0.4 ^a	0.8±0.0 ^c	1.2±0.2 ^b	***
Pb	0.3±0.0	0.3±0.0	0.3±0.0	NS
Cd	<0.03	<0.03	<0.03	NS
Cr	<0.03	<0.03	<0.03	NS
Mn	<0.03	<0.03	<0.03	NS
Ni	<0.03	<0.03	<0.03	NS

Results are shown as mean ± standard error of mean (SEM). Means with different letters (a–c) in a row indicate significant differences between the muscles ($p<0.05$). * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS, non-significant (one-way ANOVA).

Table S2. Storage loss and Warner-Bratzler shear force (WBSF) determined for roe deer muscles: *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF), and *vastus lateralis* (VL) during storage under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing.

Parameter	Storage time (day)	LTL				BF				VL			
		MAP	DRY-AGED	VAC	<i>p</i> -Method	MAP	DRY-AGED	VAC	<i>p</i> -Method	MAP	DRY-AGED	VAC	<i>p</i> -Method
Purge losses (%)	7	4.57±0.14 ^{by}	26.12±1.19 ^{bx}	4.08±0.30 ^{by}	***	5.78±0.14 ^{by}	20.28±0.35 ^{bx}	2.75±0.19 ^{bz}	***	3.61±0.09 ^{by}	20.64±0.52 ^{bx}	2.69±0.13 ^{by}	***
	21	6.87±0.09 ^{ay}	42.04±0.9 ^{ax}	5.35±0.07 ^{ay}	***	6.49±0.12 ^{ay}	23.59±0.45 ^{ax}	3.96±0.10 ^{az}	***	5.71±0.11 ^{ay}	24.98±0.68 ^{ax}	5.23±0.15 ^{ay}	***
<i>p</i> -Time		***	***	***		***	***	***		***	***	***	
WBSF (N)	0	22.13±1.18 ^b	22.13±1.18 ^a	22.13±1.18 ^a	NS	26.00±1.07 ^b	26.00±1.07 ^a	26.00±1.07 ^a	NS	18.86±1.18 ^c	18.86±1.18	18.86±1.18 ^b	NS
	7	25.67±0.70 ^{ax}	16.91±0.54 ^{by}	16.53±0.43 ^{cy}	***	28.84±0.36 ^{ax}	21.43±0.51 ^{by}	25.83±0.84 ^{ay}	***	27.68±0.51 ^{bx}	19.10±0.29 ^y	22.26±0.48 ^{ay}	***
	21	27.13±0.57 ^{ax}	23.96±0.76 ^{ay}	19.21±0.43 ^{bz}	***	27.27±0.62 ^{abx}	17.83±0.62 ^{cz}	20.19±0.36 ^{by}	***	29.55±0.45 ^{ax}	19.08±0.29 ^y	19.86±0.32 ^{by}	***
<i>p</i> -Time		***	***	***		*	***	***		***	NS	***	

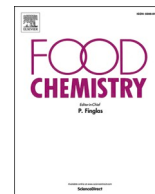
Results are shown as mean ± standard error of mean (SEM). Means with different superscripts a–c in a column for a given parameter differ significantly within storage time ($p<0.05$). Means with different superscripts x–z in a row for a given muscle differ significantly within storage methods ($p<0.05$). *p*-Time, effect of time within method; *p*-Method, effect of method within time; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS, non-significant (one-way ANOVA).

Table S3. Contents of thiobarbituric acid reactive substances (TBARS) and protein carbonyls in muscles *longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF) and *vastus lateralis* (VL) of roe deer during storage under modified atmosphere (MAP), dry (DRY-AGED) and vacuum (VAC) ageing.

Parameter	Storage time (day)	LTL				BF				VL			
		MAP	DRY-AGED	VAC	<i>p</i> -Method	MAP	DRY-AGED	VAC	<i>p</i> -Method	MAP	DRY-AGED	VAC	<i>p</i> -Method
TBARS content (mg MDA/kg)	0	0.44±0.01 ^c	0.44±0.01 ^c	0.44±0.01 ^b	NS	0.47±0.01 ^c	0.47±0.01 ^c	0.47±0.01 ^b	NS	0.46±0.01 ^c	0.46±0.01 ^b	0.46±0.01 ^b	NS
	7	1.38±0.06 ^{bx}	0.68±0.02 ^{by}	0.64±0.03 ^{ay}	***	1.69±0.05 ^{bx}	0.74±0.01 ^{by}	0.68±0.02 ^{ay}	***	1.44±0.06 ^{bx}	0.65±0.01 ^{by}	0.68±0.01 ^{ay}	***
	21	6.07±0.13 ^{ax}	1.06±0.04 ^{ay}	0.56±0.03 ^{az}	***	7.07±0.15 ^{ax}	1.30±0.03 ^{ay}	0.52±0.03 ^{bz}	***	5.54±0.12 ^{ax}	1.14±0.09 ^{ay}	0.52±0.03 ^{bz}	***
<i>p</i> -Time		***	***	***		***	***	***		***	***	***	
Protein carbonyl content (nmol/mg protein)	0	0.42±0.02 ^c	0.42±0.02 ^b	0.42±0.02 ^a	NS	0.39±0.01 ^c	0.39 ^c ± 0.01	0.39±0.01 ^a	NS	0.46±0.01 ^c	0.46±0.01 ^b	0.46±0.01 ^a	NS
	7	0.49±0.01 ^{by}	0.67±0.02 ^{ax}	0.34±0.01 ^{bz}	***	0.59±0.01 ^{by}	0.69 ^{bx} ± 0.01	0.34±0.01 ^{bz}	***	0.57±0.01 ^{by}	0.67±0.01 ^{ax}	0.33±0.01 ^{bz}	***
	21	0.93±0.02 ^{ax}	0.69±0.01 ^{ay}	0.26±0.02 ^{cz}	***	0.94±0.02 ^{ax}	0.77 ^{ay} ± 0.01	0.21±0.02 ^{cz}	***	0.82±0.03 ^{ax}	0.70±0.01 ^{ay}	0.24±0.02 ^{cz}	***
<i>p</i> -Time		***	***	***		***	***	***		***	***	***	

Results are shown as mean ± standard error of mean (SEM). Means with different superscripts a–c in a column for a given parameter differ significantly within storage time ($p < 0.05$). Means with different superscripts x–z in a row for a given muscle differ significantly within storage methods ($p < 0.05$). *p*-Time, effect of time within method; *p*-Method, effect of method within time; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, non-significant (one-way ANOVA). MDA, malonic dialdehyde.

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Proteomic analysis of wild boar meat: Effect of storage method and time on muscle protein stability

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ABSTRACT

Oxidation processes affect proteins from various molecular pathways and are crucial for wild boar meat quality, shelf life and human health. This study investigated the effects of different storage methods on the formation and composition of oxygen-induced protein aggregates in the muscles of European wild boar (*Sus scrofa scrofa*). Vacuum packaging (VAC), modified atmosphere packaging (MAP) and dry-ageing (DA) were compared over a 21-day storage period. The results showed significant differences in protein aggregation depending on the method and storage time. The most intense protein aggregation occurred in the MAP (80 % O₂), while air DA (20.9 % O₂) resulted in intermediate levels of protein aggregation. Crucial myofibrillar proteins involved in aggregate formation were titin, myosin isoforms (MYH1, MYH2 and MYH7) and nebulin, which were cross-linked with small sarcoplasmic enzymes, such as muscle creatine kinase, isocitrate dehydrogenase and ATPase 1. High-oxygen storage conditions also promoted the oxidation of ATP synthase, beta-enolase 3, ADP/ATP translocase and myoglobin.

1. Introduction

Raw meat, due to its rich composition, high water content and favourable pH, is among the most susceptible foods to spoilage. In addition to microbial growth, mechanisms related to lipid and protein oxidation and enzymatic autolysis are responsible for physicochemical changes in meat that often reach levels unacceptable to consumers (Soladoye, Aalhus, & Dugan, 2024). The susceptibility of meat to oxidative changes is strongly influenced by the species, origin and age of the animal, type of muscle and product composition. Protein oxidation also occurs to a great extent during meat storage and processing (Goethals et al., 2020; Kasałka-Czarna, Bilska, Biegańska-Marecik, & Montowska, 2022; Moczowska, Póttorak, Montowska, Pospiech, & Wierzbińska, 2017; Soladoye et al., 2024; Wazir et al., 2019). The selection of appropriate storage conditions and time is important from the point of view of meat safety, quality and consumer acceptance.

Pork, poultry and, to a lesser degree, beef are the primary types of meat consumed in Poland. Even though wild game meat is characterised by a high protein content, a low fat content, the presence of unsaturated fatty acids, vitamins and valuable minerals and meets the characteristics of sustainable food, its consumption in Poland is at a marginal level.

However, national meat production from wild animal species is regularly increasing and is directly related to the size of the population of such species as wild boar, roe deer and red deer. According to estimates of Statistics Poland (Forestry Statistical Yearbook, 2023), most roe deer (202.0 thousand) and wild boar (160.5 thousand) were shot in the 2022/2023 season. Poland, along with Germany, France and Sweden, is among the most important producers of wild game in Europe. Conditions in Poland are conducive to increasing the share of wild game meat in the meat industry, but the high price of meat and problems with maintaining its high quality during carcass processing are the main reasons for low domestic consumption. Lack of knowledge regarding the nutritional value of this meat and its storage and processing options also remains a significant constraint. During the storage and distribution of game meat, a series of significant biochemical changes occur, which can positively or negatively affect the quality of the raw material.

Currently, the most common storage methods in the meat industry, with the distinction of the game market, are vacuum packaging (wet-ageing), MAP and meat seasoning (dry-ageing) (Kasałka-Czarna et al., 2022; Kasałka-Czarna, Biegańska-Marecik, Proch, Orłowska and Montowska, 2023). High-oxygen-modified atmosphere packaging (HiOx-MAP) is a common method for extending the minimum shelf life of fresh

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red meat (Kolbeck, Reetz, Hilgarth, & Vogel, 2019). Despite common practices, high oxygen concentrations reduce meat quality due to the oxidation of proteins and lipids, resulting in reduced nutritional value and water-holding capacity, deterioration of meat tenderness and the formation of undesirable odour and flavour compounds (Kasalka-Czarna et al., 2022; Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007; Moczowska et al., 2017). Previous studies conducted on beef and pork have proven that packaging systems with a high oxygen content can promote increased degradation, protein oxidation and aggregation processes (Lagerstedt, Lundström, & Lindahl, 2011; Li, Guo, Shen, Pan, & Dong, 2022; Lund et al., 2007; Moczowska et al., 2017; Shen et al., 2022). Oxidation also induces the formation of cross-links, fragmentation, and changes in the secondary and tertiary conformations of the protein structure (Davies, 2016; Jongberg, Lund, Waterhouse, & Skibsted, 2011; Nawaz et al., 2022; Wang et al., 2020; Zhang, Xiao, & Ahn, 2013). The formed cross-links contribute to protein aggregation, reducing their solubility (Nawaz et al., 2022).

For a protein to perform its proper functions in the human body, it should be maintained in as unmodified a chemical and structural form as possible (Nawaz et al., 2022). The oxidation of intracellular proteins and the formation of protein aggregates promote the loss of cellular function and reduce the ability to respond to physiological stress (Squier, 2001). Nevertheless, it is important to point out that, in addition to reducing the nutritional quality and bioavailability of oxidised proteins, the consumption of these modified proteins may be associated with an increased risk of some disease states in the human body, such as cancer, and may also contribute to weakness in the immunological, nervous and cardiovascular systems (Barzegar, Kamankesh, & Mohammadi, 2019; Nawaz et al., 2022; Wang, Yang, Xu, Wang, & Wang, 2023). In the context of these challenges, proteomic research can play a key role in better understanding the proteins involved in oxidative processes in game meat and help develop effective and proper storage methods.

Studies on the storage of wild game meat taking into account oxidative changes and protein modifications are limited and focus primarily on the effect of oxidative stress on lipid stability as a factor affecting meat quality. Researchers compared the oxidative stability of lipids during meat storage, showing that long-term vacuum storage at -18°C reduced lipid oxidation in fallow deer and wild boar meat (Reitznerová et al., 2023). Ludwiczak, Bykowska-Maciejewska, Składanowska-Baryza, and Stanisław (2019) presented the effect of vacuum packing of wild fallow deer meat at $+2^{\circ}\text{C}$ (14 days) and -20°C (60 days). The analysis of basic composition, pH, colour and tenderness proved that there is a negative effect on meat quality during frozen storage compared to refrigerated storage. As with wild fallow deer, VAC refrigerated storage (14 days at $+2^{\circ}\text{C}$) had no impact on the chemical composition of the wild boar meat, and there was no indication of any negative effect of this storage method on meat quality (Ludwiczak et al., 2019). Similarly, Florek, Skalecki, Domaradzki, Wolan, and Ryszkowska-Siwko (2017) conducted an evaluation of the nutritional value and physico-chemical properties of frozen wild boar and red deer shoulder muscles, indicating the potential for vacuum freezing for 60 days. Despite the widespread use of MAP in the meat industry, there is a gap in the research on game meat.

We conducted the first large-scale proteomic analysis focusing on the formation of protein aggregates induced by the oxidation of wild boar meat proteins. Protein oxidation is a significant challenge for the meat industry and is a research topic for scientists working not only in human nutrition but also in the field of medical sciences. There is a need for more research into the mechanisms of protein oxidation in wild boar meat and the impact of these processes on the oxidative stability, functionality and nutritional value of wild boar meat products.

The study aimed to evaluate the protein aggregation ability of proteins of European wild boar (*Sus scrofa scrofa*) meat as a result of storage of meat in a modified gas atmosphere and meat seasoned in a meat seasoning cabinet considering oxidative processes. The comparative method was vacuum packaging. A comprehensive analysis using liquid

chromatography coupled with tandem mass spectrometry (LC-MS/MS) was applied to identify all proteins forming high-molecular-weight protein aggregates and to determine protein changes in the muscle during storage. The focus was on selecting the best method of storing game meat to avoid adverse health and nutritional effects caused by protein degradation and aggregation processes. Understanding the biochemical processes in wild game meat will make it possible to improve production techniques and modify nutritional strategies. These factors are verified for the main slaughter animal species, while studies on game species are lacking.

2. Materials and methods

2.1. Sample preparation

Meat samples of European wild boar (*Sus scrofa scrofa*) were obtained from hunting circuit No. 57, Sarbia Forest District, Poland. The wild boars were hunted by an experienced marksmen in accordance with the internal carcass inspection report under Regulation (EC) No. 853/2004, and the meat was subjected to a veterinary inspection. After shooting, the carcasses were eviscerated in the designated evisceration area (Drżążkowo Forestry) to prevent internal infections. The carcass was then cooled and stored at 0°C for 5 days at the Game Buying Point (Drżążkowo Forestry, Sarbia Forest District). The muscles for the study were received from 5 carcasses of animals aged 3–4 years. Three muscles *m. longissimus thoracis et lumborum* (LTL), *m. vastus lateralis* (VL), and *m. biceps femoris* (BF) were excised from each skinned wild boar (WB) carcass, both boneless and as relevant cuts/muscles on the bone. The muscles were stored for 21 days using three methods: vacuum packaging (VAC), in a modified atmosphere (MAP) and in a meat seasoning cabinet (dry-ageing, DA). Each muscle (without bone) was subdivided into equal, designated portions of approximately 80 g, packed in VAC and in MAP and stored at a temperature ranging from 4 to 8°C . The muscles were vacuum sealed in triplicate using barrier bags (MULTIVAC Seep Haggemüller SE & Co. KG, Wolferschwenden, Germany), in MAP consisting of 80 % O_2 and 20 % CO_2 (Multivac T200 packaging machine) and sealed with polyethylene terephthalate/polyethylene (PET/PE) film with an oxygen transmission rate of $80\text{ cm}^3/\text{m}^2\cdot\text{d}\cdot\text{bar}$ and a water vapour transmission rate of $<3.0\text{ g}/\text{m}^2\cdot\text{d}$. The meat on the bone subjected to seasoning was stored at 2°C with 80 % humidity (DA) for the same 21-day period in the seasoning cabinet (Dry-Aged Cooler SW-28, Arnsberg, Germany). The subjects of the study were samples taken on the day of carcass cutting (FRESH) and on the 7th and 21st days of storage (stored under vacuum, in MAP and in a meat seasoning cabinet). Immediately after the packages were opened, the samples were transferred to sealed tubes and stored at -80°C .

2.2. Sodium dodecyl Sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions

Myofibrils isolated from meat stored under different conditions and for different times were prepared according to the methodology described by Montowska, Fornal, Piątek, and Krzywdzińska-Bartkowiak (2019). The sample (2 mg) was incubated at 98°C for 3 min in lysis buffer (containing 8 M urea, 2 M thiourea, 0.05 mM Tris, 75 mM dithiothreitol (DTT), 3 % SDS and 0.05 % bromophenol blue at pH 6.8). Proteins of 15 μg were applied to a 15 % polyacrylamide gel and separated (Hoefer SE250, GE Healthcare Bio-Sciences) at a constant current of 20 mA per gel. After separation, the gels were stained with Coomassie brilliant blue dye, and images were recorded using a Gel Doc XR+ system (Bio-Rad Laboratories, Inc., CA, USA). Image analysis was performed using Image Lab 6.0.1 software (Bio-Rad Laboratories), and the molecular weights of the protein bands were determined using a reference standard with a wide range from 200 to 6.5 kDa (Bio-Rad Laboratories).

2.3. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under non reducing conditions

SDS-PAGE was performed under non-reducing conditions to evaluate the polymerisation of high-molecular-weight proteins. A meat sample of 40 mg was homogenised in 600 µl of phosphate extraction buffer (10 mM, 2 % SDS, 25 mM NEM, pH 7.0) with Ultra-Turrax T25 (IKA Labortechnik, Staufen, Germany) for 2 × 20 s at 9500 rpm and for 15 s at 13,500 rpm. The sample was centrifuged at 14,000 rpm for 10 min at 10 °C. The supernatant was stored at −80 °C for further analysis. The samples (15 µg of protein) were diluted 1:2 in 2× SDS-PAGE loading buffer (125 mM Tris-HCl, 4 % w/v SDS, 20 % v/v glycerol and 0.004 % w/v bromophenol blue, at pH = 6.8), heated at 95 °C for 5 min, loaded onto 6 % polyacrylamide gel and separated for 2 h and 10 min in a Hoefer SE250 apparatus (GE Healthcare Bio-Sciences) at a constant current of 20 mA per gel. Further procedures were conducted as described above. In addition, diagonal-PAGE electrophoresis was performed to assess oxygen-induced polymerisation of high-molecular-weight proteins according to a procedure described by [Moczowska et al. \(2017\)](#). In this type of electrophoresis, in the first dimension, proteins are separated under non-reducing conditions, and before the second dimension, they are reduced in a loading buffer containing 20 mM DTT.

2.4. Western blotting analysis

The detection and identification of aggregate-forming proteins was performed by western blotting analysis according to the procedure applied by [Moczowska et al. \(2017\)](#). Changes in myosin degradation and aggregation were monitored using the appropriate myosin primary antibody (MF20, Developmental Studies HybridomaBank, University of Iowa). The procedure consists of two major parts. The first step was electrophoretic SDS-PAGE separation of high-molecular-weight meat proteins under non-reducing conditions with masses in the range of >97 kDa extracted from samples for each method and storage time. The separated proteins were semi-dry transferred to the polyvinylidene diamine (PVDF) membrane. Detection of protein striations was performed using a labelled secondary antibody tracer, alkaline phosphatase (S3841, Promega GmbH), which resulted in a coloured product at the antibody binding site to the protein. The gels and membranes were photographed using the Gel Doc XR+ System (Bio-Rad Laboratories, Inc., CA, USA) and analysed with Image Lab 6.0.1 software (Bio-Rad Laboratories).

2.5. In-gel protein digestion

Protein bands containing high-molecular-weight proteins and protein aggregates (PA) obtained from SDS-PAGE gels under non-reducing conditions were cut into small pieces (1–2 mm³) with a sterile scalpel and placed in 1.5-ml tubes, and a wash solution (50 % methanol, 5 % acetic acid in Milli-Q water) was added. The samples were shaken overnight at room temperature (RT) using a Vortex-Genie 2 mixer (Scientific Industries, Inc., USA). On day two, the washing solution was removed, and a fresh aliquot was added for 2–3 h at RT. For dehydration, acetonitrile (ACN) and 10 mM DTT were added to reduce the proteins, and the samples were incubated for 30 min at RT. Proteins were alkalinised with 50 mM iodoacetamide in the dark for 30 min at RT. Then, the samples were dehydrated with ACN, and 100 mM ammonium bicarbonate was added for rehydration and incubated for 10 min at RT, followed by another dehydration step with ACN for 5 min. Trypsin reagent was prepared using 50 mM ammonium bicarbonate containing trypsin at a final concentration of 50 ng/µl, and samples were digested at 37 °C overnight. After digestion, 50 mM ammonium bicarbonate was added and incubated for 10 min, and the supernatant was collected. Extraction buffer (50 % acetonitrile and 5 % formic acid in Milli-Q water) was added to the remaining gel pieces, and after 10 min of

incubation, the supernatant was collected. This step was repeated with extraction buffer II (85 % acetonitrile and 5 % formic acid in Milli-Q water). All the resulting supernatants were combined in a single Eppendorf tube. The samples were then dried under vacuum in a miVac Duo Concentrator (Genevac Ltd., UK) and resuspended in buffer A (98 % Milli-Q water, 5 % acetonitrile and 0.1 % formic acid) before UHPLC-Q-TOF-MS/MS analysis.

2.6. LC-MS/MS analysis

Chromatographic separation was performed using an Agilent 1290 Infinity LC column equipped with an Agilent RRHD Eclipse Plus analytical column (2.1 × 150 mm, 1.8 µm). Mobile phases A (0.1 % formic acid in water) and B (0.1 % formic acid in acetonitrile) were used, with a column flow rate of 0.3 ml/min. The gradient program included the following steps: 0–2 min, 3 % B; 2–40 min, increasing to 35 % B; 40–45 min, reaching 40 % B; 45–50 min, rising to 90 % B; and 50–55 min, maintaining 90 % B, followed by a 5-min post-run at 3 % B. An injection volume of 10 µl was used, and the column temperature was maintained at 40 °C.

MS analysis was performed on an Agilent Technology 6550 iFunnel Q-TOF mass spectrometer equipped with a Jet Stream ion source operating in positive electrospray ionisation (ESI+) mode. The operating parameters were set as follows: ion source gas temperature (N₂) at 250 °C at a flow rate of 14 l/min; nebuliser pressure at 35 psi; sheath gas temperature at 250 °C; sheath gas flow rate at 11 l/min; capillary voltage maintained at 3500 V. The nozzle voltage and fragmentator voltage were set at 1000 V and 400 V, respectively. Positive ions were acquired in the range of 100–1700 *m/z* in MS scan mode and MS/MS auto mode, with an MS scan rate of 1 scan/s and MS/MS auto scan rate of 9 scans/s for MS and 7 scans/s for MS/MS. Internal mass calibration used reference ions at *m/z* 121.0509 and *m/z* 922.0098. Agilent Mass Hunter Data Acquisition software (B.09.00) and Qualitative software (B.10.00) were used to process the data.

2.7. Protein identification

The MS/MS spectra obtained were searched in the NCBI protein database for protein identification using the Spectrum Mill MS Proteomics Workbench. Proteins were identified with the following search parameters: enzyme specificity for trypsin of two missed cleavages, product mass tolerance of 50 ppm, and maximum precursor charge of 6+. Among the fixed post-translational modifications of proteins, carbamidomethylation was selected, while methionine oxidation was chosen as a variable modification. A spectral quality filter was used with a precursor isolation purity of >70 % and a precursor mass tolerance of 5 ppm. MS/MS search results were validated using Spectrum Mill's automatic threshold strategy and peptide mode, aiming to achieve an FDR of 1 %.

2.8. Data analysis

OriginPro 2023b (OriginLab Corporation, Northampton, MA, USA) was used to create the graphs. The graphical abstracts were created using [BioRender.com](#).

3. Results and discussion

3.1. Formation of high-molecular-weight protein structures

To analyse protein changes in samples stored by different methods and at different storage times, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out under both reducing and non-reducing conditions. To verify disulfide bond formation, diagonal electrophoresis was used. A comparison of the protein profiles for each method and storage time is shown in [Fig. 1A](#) for the representative LTL

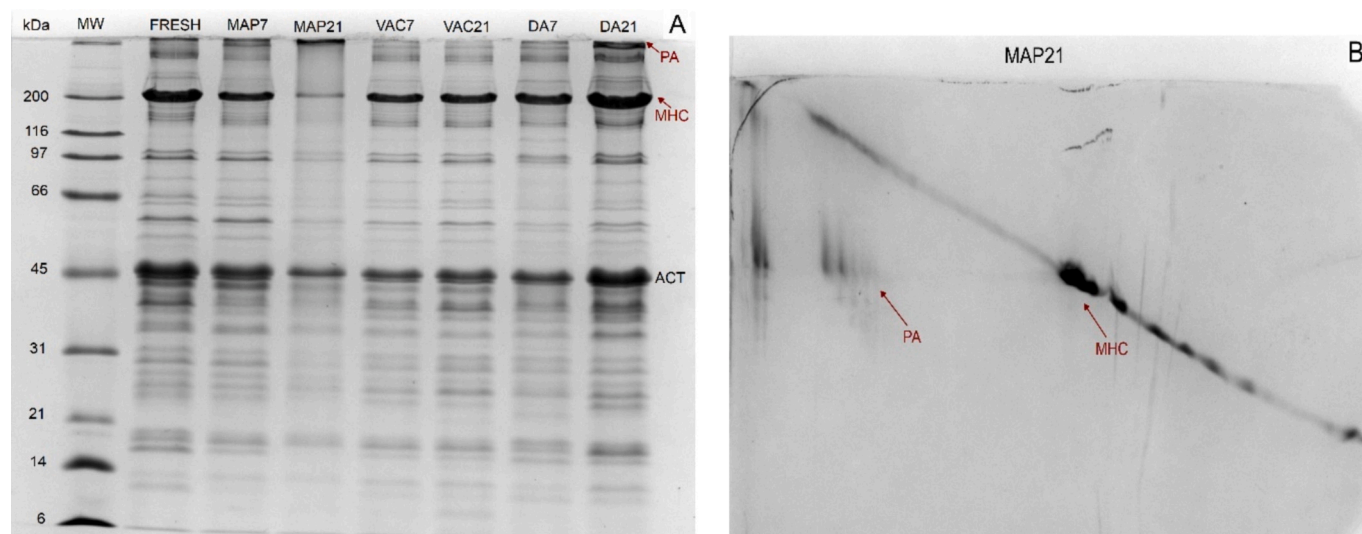


Fig. 1. Representative electrophoretic separations of LTL muscle proteins; (A) SDS-PAGE gel under reducing conditions for all analysed storage methods; (B) Diagonal-PAGE gel on the 21st day of storage MAP. Protein aggregates (PA) located on top of the SDS-PAGE gel and downwards from the diagonal are linked via intramolecular disulfide bonds. MAP – modified atmosphere; VAC – vacuum; DA – dry-ageing meat seasoning cabinet; 7, 21 – days of storage; MHC – myosin heavy chain; ACT – actin.

muscle. Protein aggregates (PA) above 200 kDa in both MAP and DA samples stored for 21 days were initially detected using SDS-PAGE separation. It was also observed that the intensity of bands representing myosin heavy chains (MHC, 200 kDa) and actin (ACT, 45 kDa) decreased considerably with storage time under high-oxygen MAP conditions; for instance, MHC intensity decreased by an average of 49.50 % in MAP21 compared to FRESH.

Electrophoretic separation showed a comparable ability to aggregate proteins for LTL muscle compared to VL and BF, but in the previous study, a slightly greater impact of protein carbonyl formation during storage of LTL wild boar meat was reported (Kasalka-Czarna et al., 2022). Previously, Zakrys-Waliwander, O'Sullivan, O'Neill, and Kerry (2012) reported the presence of high-molecular-weight myosin aggregates in beef steaks packed in a high- O_2 atmosphere, which were absent in vacuum-packed steaks. For rabbit meat stored for 14 days in the refrigerator, electrophoretic studies showed that MHC and troponin T are sensitive to oxidation, while the actin protein is less susceptible to oxidative processes (Nakyinsige et al., 2015). In another study, researchers examined the effects of different packaging methods on beef steaks and found that those stored under high oxygen MAP (78.8 % O_2 /8.8 % CO_2 /2.4 % N_2) conditions showed higher levels of protein oxidation, while those stored under high carbon dioxide MAP (60 % CO_2 /40 % N_2) conditions had the lowest levels of oxidation. These findings correlate with our observations, especially under high oxygen conditions, highlighting the impact of packaging methods on protein stability during storage (Lai, Wang, Zhang, Zhou, & Zhu, 2023).

Diagonal electrophoretic separation enables the determination of a protein's ability to build disulfide cross-links under reducing conditions. Disulfide bonds can be formed between cysteine residues and thiol groups in the same protein (intramolecular) or between proteins (intermolecular). The proteins located on the diagonal line are non-oxidised and thus lack disulfide bonds. Proteins whose separation occurs downward from the diagonal contain intramolecular disulfide bonds. By contrast, proteins that migrate upward from the diagonal form intermolecular disulfide bonds (Moczkowska et al., 2017). Diagonal-PAGE analysis (Fig. 1B) showed intermolecular cross-linking of MHC during storage of WB meat in the oxygen atmosphere (MAP). Modification of thiols by oxygen-reactive forms within cysteine may contribute to neurodegenerative disorders (Ahmad et al., 2017).

SDS-PAGE analysis under non-reducing conditions allowed the separation of high-molecular-weight proteins and the visualization of bands

>200 kDa. Protein aggregates (Fig. 2) were formed in three examined muscles, i.e. LTL (Fig. 2A), BF (Fig. 2C), and VL (Fig. 2D) stored in MAP and DA on both the 7th and the 21st day of storage. The most abundant PAs were noted on day 21 of MAP storage. In this study, we showed that packaging wild boar meat in MAP with 80 % oxygen resulted in the development of high-molecular-weight protein polymers. The results obtained herein are consistent with previous studies conducted on pork packed in a high-oxygen atmosphere, in which increased oxidation and polymerisation of myosin were observed (Kim, Huff-Loneragan, Sebra- nek, & Lonergan, 2010; Lund et al., 2007).

The MF20 antibody was used in western blot analysis to detect myosin in PA. Western blot analysis showed that the antibody successfully recognised myosin in the formed aggregates, as well as other polymorphic forms of myosin heavy chain and its degradation products. Particular attention was paid to protein band No. 1 (Ba1), which was formed by polymerisation (Fig. 2B). The presence of myosin-containing aggregates in meat stored in both MAP and DA after 7 and 21 days was confirmed.

In a study conducted on beef (Moczkowska et al., 2017), a high oxygen concentration affected protein oxidation, as reflected in myosin oxidative crosslinking. SDS-PAGE, diagonal electrophoresis and immunoblotting showed the formation of protein aggregates, which can adversely affect meat tenderness and depend on the packaging method and type of muscle. In another study of beef steaks stored in a high-oxygen atmosphere (80 % O_2 /20 % CO_2) and under vacuum, it was presented that a high oxygen content affects changes in lipid oxidation, causes a decrease in colour stability, tenderness and juiciness and results in an unpleasant taste. SDS-PAGE, immunoblotting and diagonal electrophoresis showed oxidative cross-linking of MHC in MAP-stored meat (Kim et al., 2010). Electrophoretic studies of chemically oxidised myofibrils taken from pig *m. longissimus dorsi* showed that myosin is the protein most susceptible to oxidation, and polymerisation and aggregation can make myofibrillar proteins less susceptible to proteolysis (Lund et al., 2007; Morzel, Gatellier, Sayd, Renerre, & Laville, 2006). In previous studies on wild boar protein carbonylation, it was found that VAC and DA positively influenced tenderness, whereas MAP had a negative impact during storage. The best tenderness after 21 days of storage was observed in DA, which may indicate the positive effect of mild protein oxidation (Kasalka-Czarna et al., 2022). Similarly, a high-oxygen atmosphere reduced the tenderness and juiciness of pig *m. longissimus dorsi* (Lund et al., 2007). SDS-PAGE analysis of myofibrillar

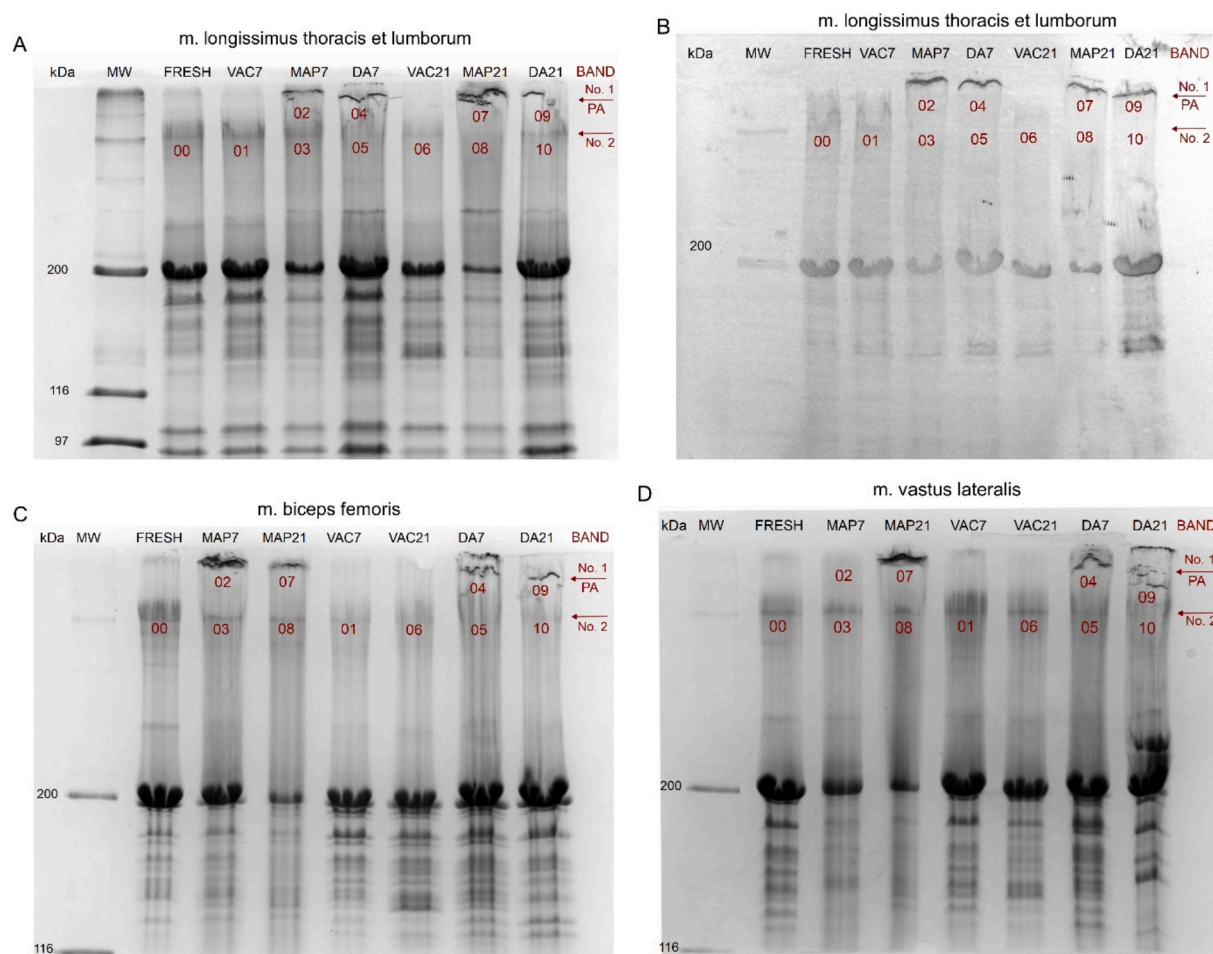


Fig. 2. Representative SDS-PAGE gel muscle proteins under non-reducing conditions for all analysed storage methods; (A, B) LTL muscle and corresponding western blotting using monoclonal anti-myosin sarcomere MHC antibody; (C) BF muscle; (D) VL muscle. Protein bands No. 1 and No. 2 excised from particular lanes for LC-MS/MS analysis are labelled with numbers 00 to 10. MAP – modified atmosphere; VAC – vacuum; DA – dry-ageing; 7, 21 – days of storage; MHC – myosin heavy chain.

Table 1

Main proteins identified in protein aggregates (band no. 1) resulting from storing meat in modified atmosphere packaging (MAP) and meat seasoning cabinet (DA) for 7 and 21 days.

No.	Protein	Abbrev.	Accession No.	MAP_7	MAP_21	DA_7	DA_21
1	Titin	TTN	XP_020931560.1	+	+	+	+
2	Myosin-1 isoform X1	MYH1	XP_020921683.1	+	+	+	+
3	Myosin-2 isoform X1	MYH2	XP_020921875.1	+	+	+	+
4	Myosin heavy chain slow isoform	MYH7	BAB20630.1	+	+	+	+
5	Nebulin	NEB	XP_020931125.1	+	+	+	+
6	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	+	+	+	–
7	Skeletal alpha actin	ACTA1	XP_020927589.1	–	+	–	–
8	Four and a half LIM domains protein 1 isoform X3	FHL1	XP_020935180.1	+	+	+	–
9	Keratin 1, partial	KRT1	BAF62303.1	+	+	+	+
10	Muscle creatine kinase	CKM	AAV28621.1	+	+	+	+
11	Obscurin isoform X1	OBSCN	XP_020939003.1	–	–	+	–
12	Glyceraldehyde-3-phosphate dehydrogenase isoform X1	GAPDH	prf 681085 A	+	+	+	–
13	Actin alpha 2	ACTA2	ACT82246.1	+	–	–	+
14	Filamin-C isoform X1	FLNC	XP_020934608.1	+	+	+	–
15	Isocitrate dehydrogenase	IDH	NP_001157479.1	+	+	+	–
16	Muscle glycogen phosphorylase, partial	PYGM	XP_003122636.2	+	+	+	–
17	Myosin light chain 1/3, skeletal muscle isoform isoform X1	MLC1F	ABK55642.1	+	+	+	–
18	Myosin-binding protein C, fast-type	MYBPC2	XP_003127419.3	+	+	+	–
19	Myomesin-2	MYOM2	XP_020931561.1	+	+	+	–
20	Myoglobin	MB	AAA31073.1	+	+	+	–
21	Beta-enolase 3	ENO3	ABC75829.1	–	+	–	–
22	Cardiac muscle ATP synthase H ⁺ transporting mitochondrial F1 complex alpha subunit 1	ATP5A1	ACD02421.1	–	+	–	–
23	ADP/ATP translocase 4	SLC25A4	XP_003133393.1	–	+	+	–
Total number of proteins				18	21	19	8

proteins isolated from chicken breasts and thighs showed increased levels of MHC cross-linking in samples packaged in HiOxy MAP compared to an anaerobic atmosphere, which, in the case of breasts, was reflected in sensory quality (Jongberg, Lund, & Skibsted, 2017).

3.2. Identification of proteins that form aggregates using LC-MS/MS

Selected proteins from SDS-PAGE gels under non-reducing conditions (Fig. 2) were identified using LC-MS/MS. Proteins were cut from bands No. 1 and No. 2 (Ba1 and Ba2). The proteins present in Ba1 are PA (formed at days 7 and 21 for MAP and DA). No differences in intensity were observed between the proteins of the lower fraction (Ba2) which therefore was cut for each storage variant. Table 1 shows the presence or absence of up to 23 of the main proteins identified in protein aggregates. Detailed data obtained by LC-MS/MS, including the protein intensity, number of unique peptides and per cent coverage, can be found in Table S1 in the Supplementary material. The mass spectra obtained provided good per cent coverage of the protein sequences and many unique peptides. Four main proteins were identified in all types of aggregates: titin, myosin isoforms (MYH1, MYH2, MYH7), nebulin and muscle creatine kinase, with a molecular weight range from 3847.4 kDa down to 43.3 kDa. The main proteins identified in excised Ba2 obtained for all analysed storage methods for fresh meat and stored for 7 and 21 days are shown in Table 2 and Table S2.

For the three selected most abundant myofibrillar proteins, i.e. titin, myosin-1 isoform X1 and nebulin, a comparison of the number of unique peptides, percentage of sequence coverage and protein abundance detected during storage and depending on the method are shown in Fig. 3. Considering the per cent coverage and protein abundance (Table S1, Fig. 3), the main aggregate-forming protein in wild boar meat is myosin. In DA7, the myosin-1 isoform X1 (MYH1) represents the most significant part (protein abundance of 1.13E+09 and coverage of 71.6

%), while in the MAP7, MYH1 has an abundance of 1.02E+09 and 65.1 % coverage. On day 21, myosin remains the dominant protein, particularly in MAP21, where its abundance increases to 1.25E+09, and coverage reaches 66.3 %. In addition, the high content of myosin-1 proteins was also noted in Ba2, particularly in the context of MAP (Table S2). A previous study showed that myosin crosslinking is caused by cysteine and methionine oxidation as induction of an oxidising environment (Lu, Luo, & Lametsch, 2018). Myosin remained the main aggregate-forming protein throughout the storage of WB meat. MAP and, to a lesser extent, DA affect the aggregation of this protein. The clear decrease in the level of aggregation after 21 days of DA suggests that this process is dynamic and that myosin undergoes significant structural changes in response to specific storage conditions.

The current study showed that myosin forms aggregates with titin and nebulin (Ba1), especially during the first week of DA and MAP; however, after 21 days, the presence of titin decreased significantly under both storage conditions likely due to proteolytic processes occurring during storage. Consequently, cumulation of titin into smaller polypeptide fragments (Ba2) is observed under VAC conditions and on day 7 of DA. VAC promoted an increase in the number of unique peptides relative to FRESH (403) at both day 7 (971) and day 21 (986) of storage as well as a high level of percentage coverage relative to the other variants.

In the case of nebulin, the noted changes under different storage conditions indicate its dynamic nature. The level of aggregation is varied at different seasoning dates (DA7 and DA21) and in a high-oxygen atmosphere (MAP7 and MAP21), suggesting an influence of not only the method but also the storage time (Table S1, Fig. 3). Meat seasoning may cause a significant decrease in the level of nebulin aggregation at later stages (DA21). The observation of less aggregation in MAP and a higher amount of its non-aggregated form in Ba2 may indicate a more favourable environment for maintaining the structural integrity of

Table 2

Main proteins identified in excised band No. 2 obtained for all analysed storage methods for fresh meat and stored for 7 and 21 days.

No.	Protein	Abbrev.	Accession No.	FRESH	VAC_7	DA_7	MAP_7	VAC_21	DA_21	MAP_21
1	Titin	TTN	XP_020931560.1	+	+	+	+	+	+	+
2	Myosin-2 isoform X1	MYH2	XP_020921875.1	+	+	+	+	+	+	+
3	Myosin-1 isoform X1	MYH1	XP_020921683.1	+	+	+	+	+	+	+
4	Myosin heavy chain slow isoform	MYH7	BAB20630.1	+	+	+	+	+	+	+
5	Nebulin	NEB	XP_020931125.1	+	+	+	+	+	+	+
6	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	SERCA1	XP_020941340.1	+	+	+	+	+	+	+
7	Actin, alpha skeletal muscle isoform X1	ACTA1	XP_020927589.1	+	+	+	+	+	+	+
8	Four and a half LIM domains protein 1 isoform X3	FHL1	XP_020935180.1	+	+	+	+	+	+	+
9	Keratin 1, partial	KRT1	BAF62303.1	+	+	+	+	+	+	+
10	Muscle creatine kinase	CKM	AAV28621.1	+	+	+	+	+	+	+
11	Obscurin isoform X1	OBSCN	XP_020939003.1	+	+	+	+	+	+	+
12	Myosin heavy chain 2b	MYH2B	BAA82145.1	+	+	+	+	+	+	+
13	Keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	+	+	+	+	+	+	+
14	Unnamed protein product	Unnamed	CAA33170.1	+	+	+	+	+	+	+
15	Dehydrogenase, glyceraldehyde phosphate	GAPDH	prf 681085 A	+	+	+	+	+	+	+
16	Actin alpha 2	ACTA2	ACT82246.1	+	+	+	+	+	+	+
17	Filamin-C isoform X1	FLNC	XP_020934608.1	+	+	+	+	+	+	+
18	Isocitrate dehydrogenase	IDH	NP_001157479.1	+	+	+	+	+	+	+
19	Glycogen phosphorylase, muscle form	PYGM	XP_003122636.2	+	+	+	+	+	+	+
20	MLC1f	MLC1f	ABK55642.1	+	+	+	+	+	+	+
21	Myosin-binding protein C, fast-type	MYBPC2	XP_003127419.3	+	+	+	+	+	+	+
22	Myomesin-2	MYOM2	XP_020931561.1	+	+	+	+	+	+	+
23	Myoglobin	MB	AAA31073.1	+	+	+	+	+	+	+
24	Myosin-6 isoform X1	MYH6	XP_020954892.1	+	+	+	+	+	+	+
25	Myosin-8 isoform X1	MYH8	XP_020921928.1	+	+	+	+	+	+	+
26	Fructose-bisphosphate aldolase A isoform X1	ALDOA	XP_020943654.1	+	+	+	+	+	+	+
27	Myosin regulatory light chain 2	MYL2	AAV28622.1	+	+	+	+	+	+	+
28	ADP/ATP translocase 4	SLC25A4	XP_003133393.1	+	+	+	+	+	+	+
29	glyceraldehyde-3-phosphate dehydrogenase isoform X1	GAPDHX1	XP_020946773.1	+	+	+	+	+	+	+
30	Poly	Poly	XP_020954765.1	+	+	+	+	+	+	+
31	Cardiac muscle ATP synthase H+ transporting mitochondrial F1 complex alpha subunit 1	ATP5A1	ACD02421.1	+	+	+	+	+	+	+
32	Beta-enolase 3	ENO3	ABC75829.1	+	+	+	+	+	+	+
Total proteins				11	15	12	25	9	8	22

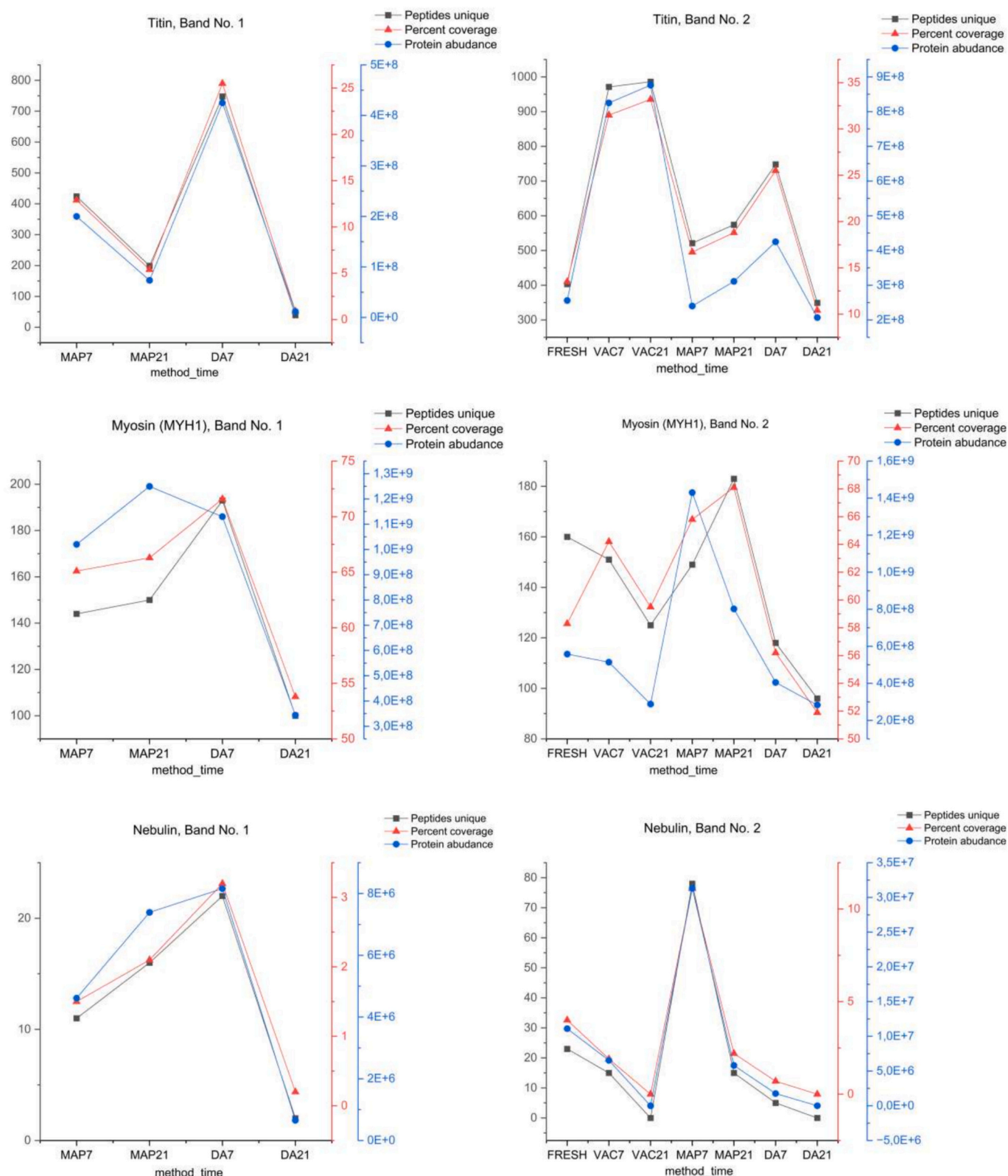


Fig. 3. LC-MS/MS output results for the top three myofibrillar proteins, i.e. titin (TTN), myosin-1 isoform X1 (MYH1) and nebulin (NEB) obtained from the excised protein bands labelled in Fig. 2A. The graph shows the number of matched unique peptides, per cent coverage of the entire amino acid sequence and protein abundance (intensity). MAP - modified atmosphere; VAC - vacuum; DA - dry-ageing in meat seasoning chamber; 7, 21 - storage days.

nebulin. Nebulin appears to be a less susceptible protein to oxidation than myosin, although its proportion in aggregates increased slightly after 21 days; as with myosin, non-aggregated nebulin was progressively degraded during storage regardless of the method.

The three aforementioned proteins play a crucial role in maintaining muscle structure and function; therefore, oxidative damage expressed as the aggregate formation and their further degradation can lead to changes in meat texture, i.e. loss of firmness, tenderness and other sensory properties of meat, which negatively affects meat quality, as confirmed by previous studies focusing on the physical and chemical characteristics of WB meat stored under the same conditions (Kasalka-Czarna et al., 2022). The toughness increased significantly in MAP21 (50.7 N) compared to MAP7 (35.3 N) in LTL muscle as a result of the increase in carbonyl content by 193 % compared to fresh WB samples. The toughness of BF meat stored with DA was highest (51.7 N) on day 7 but significantly improved (33.9 N) on day 21 of storage. Degradation products of large structural proteins (>100 kDa) during post-mortem ageing of Angus bovine muscle (*m. longissimus dorsi*) have been identified previously using mass spectrometry (Wu, Clerens, & Farouk, 2014); however, the current study indicates the precise composition of oxygen-induced protein aggregates during storage, which other researchers have not yet reported.

During WB meat storage, significant changes have been noted in three key enzymes associated with muscle function: creatine kinase muscle (CKM), sarcoplasmic/endoplasmic reticulum ATPase 1 isoform X1 (SERCA1) and isocitrate dehydrogenase (IDH) (Fig. 4). Structural changes in enzyme proteins can lead to loss of their biological function, which can affect metabolic processes in muscle, including changes related to meat ageing and catalysis of post mortem chemical reactions. Storing meat under high-oxygen conditions negatively affected CKM and IDH. CKM is one of the smallest proteins (43.29 kDa) identified in PA in MAP and DA, which suggests its ability to form large protein aggregates. CKM plays a crucial role in energy processes, which may be an adaptive behaviour in response to different storage conditions. This symptom may also be important in the context of meat quality and potential structural changes during storage, serving as an indicator of physical stress or muscle damage (Daroit & Brandelli, 2008). High-oxygen conditions induced the entanglement of sarcoplasmic proteins within PA in higher Ba1 and, to a much lesser extent, in Ba2. Within Ba2, a few peptides unique to VAC7 were also noted, and importantly for DA21 and VAC21, CKM was not identified in the lower protein Ba2 (Table 2).

During storage for 7 days, IDH was identified for both MAP and DA in PA, while further storage in DA further inactivated this protein. Given that a high oxygen content promoted increased IDH activity at each of the storage times, special attention should be focused on the role of this protein in protection against oxidative stress. IDH is an NADP⁺-dependent enzyme responsible for catalysing the oxidative decarboxylation of isocitrate to α -ketoglutarate in the mitochondrial matrix and is crucial for the production of NADPH to limit the accumulation of mitochondrial reactive oxygen species (ROS) (Al-Khallaf, 2017; Lee et al., 2020).

SERCA1 is an enzyme involved in mechanisms that pump Ca²⁺ from the cytoplasm into the lumen of the sarcoplasmic reticulum leading to muscle relaxation after contraction. Importantly, SERCA1 is essential for normal development, while changes in SERCA1 activity have been observed in animal models of ageing and ageing human myocardium (Desmond, Muriel, Markwardt, Rizzo, & Bloch, 2015; Periasamy & Kalyanasundaram, 2007). This protein was detected in PA for MAP7, MAP21 and DA7 samples, while it was not present in aggregates in DA21 (Fig. 4, Table S1). Within the proteins obtained from Ba2, SERCA1 was present for all analysed variants, but its abundance was reduced by half in MAP21 compared to Ba1, likely due to prolonged aggregation of the protein and lower susceptibility of highly oxidised PA to proteolytic degradation. In the case of DA21 in Ba2, the presence of SERCA1 also reached a marginal level. In response to oxidative stress, the functions of SERCA1, which is susceptible to oxidative modifications, may be

impaired, which may result in elevated cytosolic Ca²⁺ concentrations (Xu & Van Remmen, 2021). A study of the effect of peroxynitrite on SERCA1 activity in correlation with enzymatic carbonylation showed a decrease in its activity and was associated with an increase in protein carbonyls (Žižková et al., 2014). Wang et al. (2019) detected lower SERCA1 activity in PSE pork (pale, tender and exudative) compared to RFN meat (red, firm and non-exudative), proving that high levels of S-nitrosylation can cause a calcium imbalance in the cytoplasm, leading to accelerated pH decline and PSE meat development. A previous study showed the effect of the storage method and time on the quality of wild boar meat, which included, among others, a significant drop in pH at day 21 of MAP storage, i.e. from 5.59 down to 5.23 for BF and 5.31 for LTL (Kasalka-Czarna et al., 2022).

The three main MAP-specific proteins whose levels changed during WB meat storage were cardiac muscle ATP synthase H⁺ transporting mitochondrial F1 complex alpha subunit 1 (ATP5A1), beta-enolase 3 (ENO3) and ADP/ATP translocase (SLC25A4) (Tables 1, S1 and Tables 2, S2). ATP synthase is a mitochondrial complex necessary to produce ATP and sustain mitochondrial function, and its dysfunction has been linked to various pathological conditions (Althaher & Alwahsh, 2023; Ebanks & Chakrabarti, 2022). The ATP synthase enzyme, due to its structure, provides multiple targets for oxidative modification (Ebanks & Chakrabarti, 2022). ATP5A1 is the source of power for most of the cell's energy needs through oxidative phosphorylation. Medical attention to this enzyme is particularly important, as modifications of this enzyme can lead to cardiovascular or neurodegenerative diseases, obesity or type II diabetes (Althaher & Alwahsh, 2023; Dautant et al., 2018; Ebanks & Chakrabarti, 2022; Tran, Langlais, Hoffman, Roust, & Katsanos, 2019). In the case of rat studies, inhibition of ATP5A1 activity has been shown to lead to increased oxidative stress (Doenst, Nguyen, & Abel, 2013). The approximately 30-kDa mitochondrial ADP/ATP transporter performs one of the most important transport activities in eukaryotic cells by importing ADP into the mitochondrial matrix, where it can be converted to ATP and export ATP to carry out energy-requiring metabolic processes in the cell (Ruprecht & Kunji, 2019). As mentioned, another glycolytic metabolic enzyme that showed variable oxidative damage during storage under high oxygen conditions was ENO3. According to proteomic studies correlated with beef tenderness, it was observed that decreased oxidative damage of ENO3 promoted beef tenderness (Malheiros et al., 2019).

Proteins identified solely for MAP 7 and 21 in Ba2 were filamin-C isoform X1, isocitrate dehydrogenase, glycogen phosphorylase and MLC1f (Table S2). The increased oxidative activity of these proteins may indicate their involvement in the stress response or muscle damage. High-oxygen storage conditions also promoted the oxidation of myoglobin (MB), which was identified in PA on days 7 and 21 of MAP storage and in Ba2 on day 21. For the DA method, myoglobin formed aggregates on day 7 of storage (Tables 1, S1 and Tables 2, S2). MB is an oxygen-binding haeme protein present in the heart and skeletal muscle, susceptible to oxidative modifications at both the inter- and intramolecular levels (Mannino et al., 2020). Oxidation of myoglobin and lipids in meat contributes to changes in meat colour and odour. The central iron atom in the haeme group is responsible for the change from red OXY-MB to brown METH-MB due to oxidation (Faustman, Sun, Mancini, & Suman, 2010). An increase in non-haeme iron indicates haeme degradation, which is closely correlated with an increase in protein and lipid oxidation products (Liu, Liu, Zheng, & Ma, 2022). According to Wang et al. (2020), the iron-catalysed oxidation system and the metmyoglobin oxidation system also induced S—S crosslinking of MHC and MLC. The present findings are consistent with previously reported significantly higher values of *L*^{*} and *b*^{*} and lower values of *a*^{*} parameters in MAP-stored WB meat opposite to DA samples (Kasalka-Czarna et al., 2022).

Collagen alpha-1(VI), alpha-2(VI) and alpha-3(VI) chains (COL6A1, COL6A2, COL6A3) were detected solely in PA at day 7 of DA storage (Table S1), but their presence could occur as a result of proteolytic

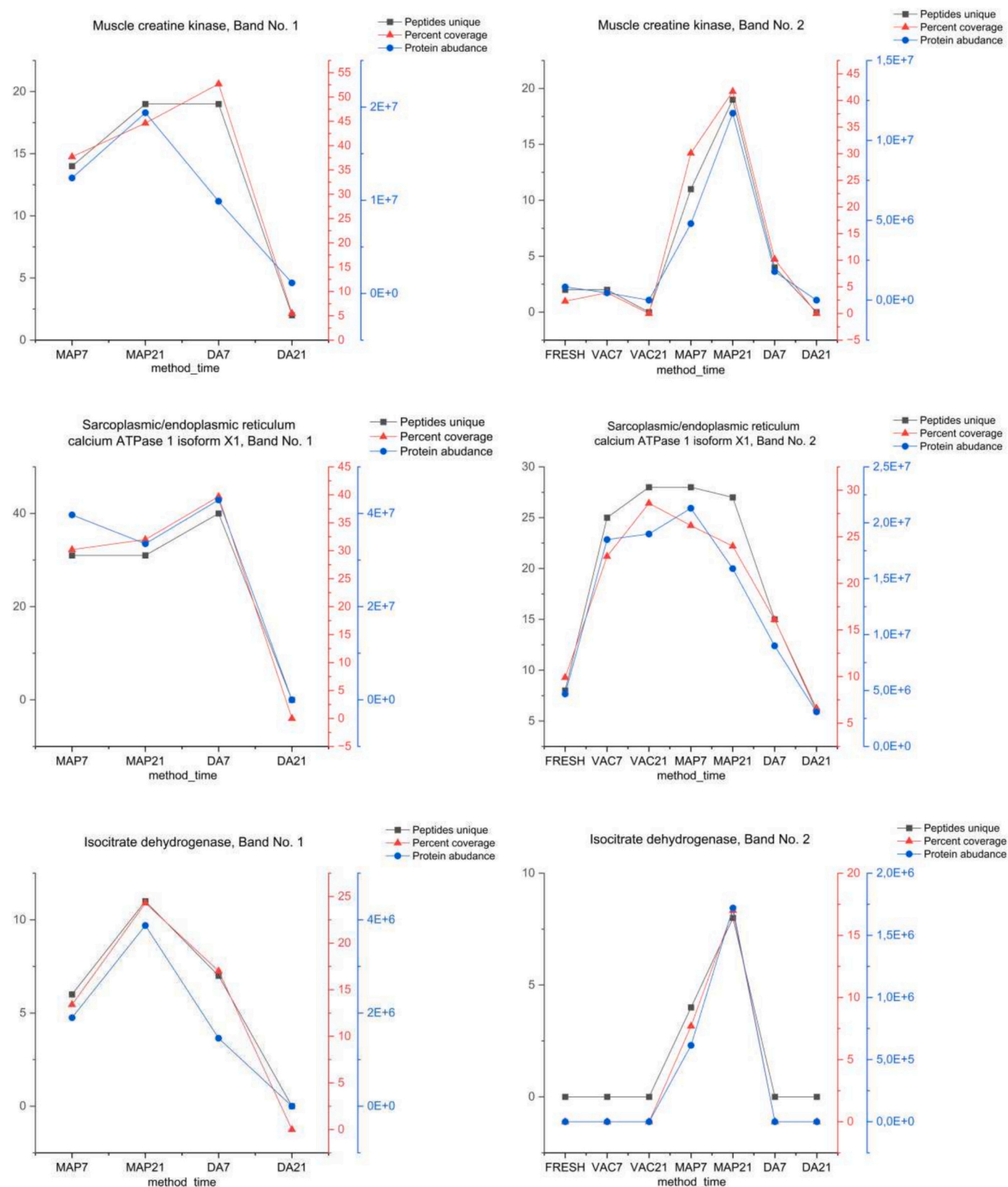


Fig. 4. LC-MS/MS output results for the selected three enzymes associated with muscle function, i.e. muscle creatine kinase (CKM), sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1 (SERCA1), and isocitrate dehydrogenase (IDH)) obtained from excised protein bands labelled in Fig. 2A. The graph shows the number of matched unique peptides, per cent coverage of the entire amino acid sequence and protein abundance (intensity). MAP – modified atmosphere; VAC – vacuum; DA – dry-ageing in meat seasoning cabinet; 7, 21 – days of storage.

processes since smaller fragments may be more capable of aggregation. Modifications within collagen can lead to changes in meat quality, including toughness. The solubility of collagen molecules depends on cross-links between collagen molecules in connective tissue. These changes in collagen solubility during heat treatment have a remarkable effect on meat texture (Khalid et al., 2023). Oxidative damage within COL6A1, COL6A2 and COL6A3 may also explain the result of the Warner-Bratzler shear force test, which showed that meat in DA was less tender on day 7 than on day 21 of storage (Kasalka-Czarna et al., 2022).

Researchers also highlight the need to verify the molecular mechanisms acting between dietary components during digestion and to confirm the actual risks associated with the consumption of oxidised proteins. Attention is also drawn to the need to clarify toxic doses and update dietary recommendations related to consuming oxidation-prone and highly processed foods (Estévez & Xiong, 2019). Importantly, Kanner et al. (2017) highlight the concept of 'postprandial oxidative stress' (POS) as a heightened susceptibility to oxidative damage after eating a lipid-rich meal, attributing it primarily to various meat products; among others, the high concentration of myoglobin in red meat has also been pointed out as a contributor to lipid peroxidation, and thus it is worth noting the contribution of proteins that also cause POS (Jakobsen et al., 2017). Frame et al. (2020) studied the effect of oxidised protein in livestock diets on oxidative status and performance noting differences in yield, gut morphology and digestibility, likely caused by reduced protein availability. As highlighted by Estévez and Xiong (2019), ignoring scientific evidence that proves the role of oxidised proteins and amino acids in the pathogenesis of many diseases is common. Importantly, food components are further oxidised after food ingestion during digestion, absorbed in the intestine and destructed in the bloodstream (de La Pomélie, Santé-Lhoutellier, Sayd, & Gatellier, 2018; Kanner et al., 2017). Therefore, the present study underlines the increased formation of protein aggregates of different protein functional groups primarily due to oxidation in high-oxygen gas atmospheres, as well as the scarcity of research in oxidised proteins of animal origin during storage.

4. Conclusions

Wild boar meat oxygen-induced protein aggregation has undergone significant changes depending on the storage conditions and duration and significantly impacts meat quality and storage stability. The greatest diversity of proteins was observed in the aggregates formed under MAP conditions, and their number increased over time, reaching higher levels than under air DA storage. Myosin showed the greatest ability to aggregate and remain in this form, especially under MAP conditions after 21 days of storage. The proportion of titin in protein aggregates decreased after 21 days under both MAP and DA conditions. Nebulin showed dynamic changes under different storage conditions, and its level of aggregation decreased in the later stages of storage under DA conditions. High-oxygen packaging also resulted in the aggregation of sarcoplasmic enzymes such as muscle creatine kinase (CKM) and isocitrate dehydrogenase (IDH). No significant correlation was found between VAC and protein aggregation, indicating its effectiveness in preserving meat quality during longer storage times. The formation of oxygen-induced protein aggregates affects structural and functional changes in muscle proteins, as well as meat quality parameters, including texture. These results suggest that further research is necessary to understand the mechanisms involved in meat protein aggregation and to optimise storage conditions. Research should continue into storage atmospheres and seasoning methods and determine optimal storage times, focusing on health aspects to assess the potential health effects of consuming accumulated protein aggregates. Further attention needs to be focused on the resistance of protein aggregates to thermal processing (cooking, roasting and frying) and their digestibility concerning human health issues.

CRedit authorship contribution statement

Natalia Kasalka-Czarna: Writing – original draft, Visualization, Investigation, Formal analysis. **Anna Stachniuk:** Investigation, Formal analysis. **Emilia Fornal:** Formal analysis. **Magdalena Montowska:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

The following are available online: Table S1. Proteins identified in protein aggregates (band No. 1) resulting from storing meat in modified atmosphere packaging (MAP) and meat seasoning cabinet (DA) for 7 and 21 days; Table S2. Proteins identified in excised band No. 2 obtained for all analysed storage methods (vacuum packaging (VAC), modified atmosphere (MAP) and meat seasoning cabinet (DA)) for fresh meat and stored for 7 and 21 days.

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Supplementary information

Proteomic analysis of wild boar meat: Effect of storage method and time on muscle protein stability

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Table S1. Proteins identified in protein aggregates (band No. 1) resulting from storing meat in modified atmosphere packaging (MAP) and meat seasoning cabinet (DA) for 7 and 21 days.

No.	Identified protein	Protein name abbreviation	Accession No.	Protein abundance	Percent coverage	Peptides unique	Unique score	MW
MAP_7								
1	titin	TTN	XP_020931560.1	2.00E+08	12.9	424	6179.07	3847396
2	myosin-1 isoform X1	MYH1	XP_020921683.1	1.02E+09	65.1	144	2473.01	224112
3	myosin heavy chain 2b	MYH2B	BAA82145.1	9.12E+08	62.8	134	2265.48	224145
4	myosin-2 isoform X1	MYH2	XP_020921875.1	9.27E+08	59.6	133	2272.27	224073
5	myosin heavy chain slow isoform	MYH7	BAB20630.1	4.00E+08	53.0	104	1766.89	224093
6	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	3.97E+07	30.2	31	516.81	111415
7	unnamed protein product	Unnamed	CAA33170.1	1.37E+07	12.8	13	199.63	116445
8	muscle creatine kinase	CKM	AAV28621.1	1.24E+07	37.7	14	213.68	43286.5
9	dehydrogenase, glyceraldehyde phosphate	GAPDH	prf 681085A	9.30E+06	34.6	11	155.07	35937.4
10	actin alpha 2	ACTA2	ACT82246.1	7.28E+06	24.1	8	124.75	42407.6
11	nebulin	NEB	XP_020931125.1	4.61E+06	1.5	11	124.00	860740
12	filamin-C isoform X1	FLNC	XP_020934608.1	2.35E+06	2.5	8	99.20	295331
13	isocitrate dehydrogenase	IDH	NP_001157479.1	1.90E+06	13.4	6	83.90	51309.6
14	keratin 1, partial	KRT1	BAF62303.1	1.64E+06	7.9	5	80.41	65371.1
15	glycogen phosphorylase, muscle form four and a half LIM domains protein 1 isoform X3	PYGM	XP_003122636.2	1.42E+06	7.2	6	76.45	97725
16	MLC1f	MLC1F	ABK55642.1	1.21E+06	14.0	3	49.57	21031.6
17	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	1.75E+06	3.7	3	49.12	62871.9
18	myosin-binding protein C, fast-type	MYBPC2	XP_003127419.3	6.95E+05	2.4	3	42.97	128559
19	myomesin-2	MYOM2	XP_020931561.1	5.33E+05	1.5	3	36.04	167906
20	myoglobin	MB	AAA31073.1	8.59E+05	13.6	2	35.75	17084.3
DA_7								
1	titin	TTN	XP_020931560.1	4.25E+08	25.5	748	11575.9	3847396
2	myosin-1 isoform X1	MYH1	XP_020921683.1	1.13E+09	71.6	193	3379.89	224112
3	myosin-2 isoform X1	MYH2	XP_020921875.1	9.78E+08	71.0	183	3143.41	224073
4	myosin heavy chain 2b	MYH2B	BAA82145.1	9.80E+08	69.5	179	3083.39	224145
5	myosin heavy chain slow isoform	MYH7	BAB20630.1	6.31E+08	67.2	161	2745.75	224093
6	myosin-6 isoform X1	MYH6	XP_020954892.1	5.28E+08	51.1	128	2205.6	227006
7	myosin-8 isoform X1	MYH8	XP_020921928.1	7.44E+08	47.0	121	2050.93	223966
8	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	4.29E+07	39.7	40	647.48	111415
9	unnamed protein product	Unnamed	CAA33170.1	1.83E+07	20.7	21	323.7	116445
10	skeletal alpha actin	ACTA1	AAC48692.1	7.91E+07	52.5	18	315.94	42392.6
11	muscle creatine kinase	CKM	AAV28621.1	9.90E+06	52.7	19	314.94	43286.5
12	nebulin	NEB	XP_020931125.1	8.16E+06	3.2	22	284.22	860740
13	glycogen phosphorylase, muscle form dehydrogenase, glyceraldehyde phosphate	GAPDH	prf 681085A	1.35E+07	43.3	14	200.04	35937.4
14	myomesin-2	MYOM2	XP_020931561.1	2.23E+06	6.7	9	141.87	167906
15	keratin 1, partial	KRT1	BAF62303.1	3.68E+06	9.6	7	125.67	65371.1
16	MLC1f	MLC1F	ABK55642.1	3.23E+06	51.0	7	119.64	21031.6
17	filamin-C isoform X1	FLNC	XP_020934608.1	2.30E+06	3.7	10	116.63	295331
18	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	3.64E+06	9.2	7	115.79	62871.9
19	isocitrate dehydrogenase	IDH	NP_001157479.1	1.46E+06	17	7	97.09	51309.6
20	alpha-actinin-3 isoform X1	ACTN3	XP_005660702.1	1.36E+06	6.2	6	92.96	106846
21	alpha-actinin-2	ACTN2	NP_001230595.1	5.84E+05	2.6	2	26.87	104300
22	four and a half LIM domains protein 1 isoform X3	FHL1	XP_020935180.1	4.84E+06	19.3	5	70.66	36107.6
23	myosin-binding protein C, fast-type	MYBPC2	XP_003127419.3	7.22E+05	2.8	3	59.60	128559
24	myosin-binding protein C, slow-type isoform X1	MYBPC1	XP_005664259.1	5.17E+05	2.6	3	39.82	138134
25	myoglobin	MB	AAA31073.1	1.75E+06	27.9	4	50.25	17084.3
26	fructose-bisphosphate aldolase A isoform X1	ALDOA	XP_020943654.1	6.12E+05	9.3	4	48.37	47597.2
27	collagen alpha-1(VI) chain	COL6A1	XP_020926753.1	3.37E+05	2.2	2	37.95	109758
28	collagen alpha-2(VI) chain	COL6A2	XP_020938158.1	3.39E+05	2.0	2	35.16	110511
29	obscurin isoform X1	OBSCN	XP_020939003.1	7.95E+05	0.2	3	33.72	926507
30	myosin regulatory light chain 2	MYL2	AAV28622.1	3.29E+05	11.8	2	30.47	19092.3
31	L-lactate dehydrogenase A chain isoform X1	LDHA	XP_013849955.2	2.47E+06	5.5	3	30.28	46551.2
32	troponin I	TNNI	AAAY96419.1	9.55E+05	11.5	2	27.59	21504.4
33	collagen alpha-3(VI) chain	COL6A3	XP_020931400.1	2.84E+05	0.8	2	24.31	337207

35	ADP/ATP translocase 1	SLC25A4	XP_003133393.1	2.84E+05	6.0	2	22.47	33254.1
MAP_21								
1	titin	TTN	XP_020931560.1	7.34E+07	5.4	199	2725.26	3847396
2	myosin-1 isoform X1	MYH1	XP_020921683.1	1.25E+09	66.3	150	2623.35	224112
3	myosin-2 isoform X1	MYH2	XP_020921875.1	1.12E+09	64.0	148	2550.87	224073
4	myosin heavy chain slow isoform	MYH7	BAB20630.1	9.31E+08	65.9	146	2524.69	224093
5	myosin heavy chain 2b	MYH2B	BAA82145.1	1.01E+09	62.0	133	2313.73	224145
6	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	3.35E+07	32.0	31	496.62	111415
7	glycogen phosphorylase, muscle form	PYGM	XP_003122636.2	1.18E+07	27.6	21	331.92	97725
8	muscle creatine kinase	CKM	AAV28621.1	1.94E+07	44.6	19	300.66	43286.5
9	nebulin	NEB	XP_020931125.1	7.39E+06	2.1	16	214.95	860740
10	dehydrogenase, glyceraldehyde phosphate	GAPDH	prf 681085A	2.17E+07	43.3	13	204.72	35937.4
11	actin, alpha skeletal muscle isoform X1	ACTA1	XP_020927589.1	1.70E+07	32.5	10	158.95	45704.5
12	isocitrate dehydrogenase	IDH	NP_001157479.1	3.88E+06	24.3	11	152.86	51309.6
13	four and a half LIM domains protein 1 isoform X3	FHL1	XP_020935180.1	9.87E+06	34.3	10	144.66	36107.6
14	alpha-actinin-2	ACTN2	NP_001230595.1	3.87E+06	9.0	8	133.23	104300
15	cardiac muscle ATP synthase H+ transporting mitochondrial F1 complex alpha subunit 1	ATP5A1	ACD02421.1	2.64E+06	10.8	7	87.12	59800.7
16	myosin regulatory light chain 2	MYL2	AAV28622.1	1.55E+06	27.8	5	79.90	19092.3
17	MLC1f	MLC1F	ABK55642.1	3.99E+06	24.4	5	70.56	21031.6
18	ADP/ATP translocase 1	SLC25A4	XP_003133393.1	1.48E+06	11.7	4	58.32	33254.1
19	filamin-C isoform X1	FLNC	XP_020934608.1	8.46E+05	1.3	4	51.73	295331
20	Chain B. Porcine Hemoglobin (beta Subunit)	HBB	pdb 1QPW B	1.07E+06	25.3	3	49.38	16092.1
21	beta-enolase 3	ENO3	ABC75829.1	1.76E+06	7.8	4	44.21	47471.5
22	keratin 1, partial	KRT1	BAF62303.1	8.90E+05	4.1	3	43.50	65371.1
23	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	1.76E+06	3.7	3	38.34	62871.9
24	myoglobin	MB	AAA31073.1	2.04E+06	13.6	2	34.20	17084.3
25	myosin-binding protein C, fast-type	MYBPC2	XP_003127419.3	6.12E+05	1.7	2	31.99	128559
26	myomesin-2	MYOM2	XP_020931561.1	6.05E+05	1.1	2	29.59	167906
DA_21								
1	myosin heavy chain slow isoform	MYH7	BAB20630.1	8.58E+08	61.3	124	2186.92	224093
2	myosin-1 isoform X1	MYH1	XP_020921683.1	3.44E+08	53.8	100	1709.17	224112
3	myosin-6 isoform X1	MYH6	XP_020954892.1	6.29E+08	46.6	98	1705.28	227006
4	myosin heavy chain 2b	MYH2B	BAA82145.1	3.28E+08	52.5	95	1616.59	224145
5	myosin-2 isoform X1	MYH2	XP_020921875.1	2.92E+08	46.5	92	1521.73	224073
6	titin	TTN	XP_020931560.1	1.19E+07	0.9	39	507.80	3847396
7	actin alpha 2	ACTA2	ACT82246.1	7.32E+06	24.1	8	127.69	42407.6
8	keratin 1, partial	KRT1	BAF62303.1	5.56E+06	7.9	5	91.08	65371.1
9	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	4.09E+06	5.2	4	62.62	62871.9
10	muscle creatine kinase	CKM	AAV28621.1	1.14E+06	5.5	2	33.35	43286.5
11	nebulin	NEB	XP_020931125.1	6.54E+05	0.2	2	22.74	860740

Table S2. Proteins identified in excised band No. 2 obtained for all analysed storage methods (vacuum packaging (VAC), modified atmosphere (MAP) and meat seasoning cabinet (DA)) for fresh meat and stored for 7 and 21 days.

No.	Identified protein	Protein name abbreviation	Accession No.	Protein abundance	Percent coverage	Peptides unique	Unique score	MW
FRESH								
1	titin	TTN	XP_020931560.1	2.56E+08	13.5	403	5859.46	3847396
2	myosin-2 isoform X1	MYH2	XP_020921875.1	6.21E+08	65.3	176	2749.50	224073
3	myosin-1 isoform X1	MYH1	XP_020921683.1	5.58E+08	58.3	160	2494.99	224112
4	myosin heavy chain slow isoform	MYH7	BAB20630.1	4.82E+08	58.5	148	2366.10	224093
5	embryonic skeletal myosin heavy chain 3, partial	MYH3	ART85712.1	1.87E+08	20.5	61	875.20	223954
6	nebulin	NEB	XP_020931125.1	1.11E+07	4.0	23	314.07	860740
7	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	4.70E+06	9.9	8	120.62	111415
8	actin, alpha skeletal muscle isoform X1	ACTA1	XP_020927589.1	2.77E+06	11.2	4	59.32	45704.5
9	four and a half LIM domains protein 1 isoform X3	FHL1	XP_020935180.1	1.85E+06	11.3	3	41.51	36107.6
10	keratin 1, partial	KRT1	BAF62303.1	1.16E+06	4.7	3	39.30	65371.1
11	muscle creatine kinase	CKM	AAV28621.1	8.29E+05	2.3	2	30.60	43286.5
12	obscurin isoform X1	OBSCN	XP_020939003.1	1.57E+06	0.2	2	27.22	926507
VAC_7								
1	titin	TTN	XP_020931560.1	8.25E+08	31.5	971	15266.1	3847396
2	myosin-1 isoform X1	MYH1	XP_020921683.1	5.14E+08	64.2	151	2508.54	224112
3	myosin-2 isoform X1	MYH2	XP_020921875.1	4.21E+08	59.9	137	2240.78	224073
4	myosin heavy chain 2b	MYH2B	BAA82145.1	4.27E+08	56.7	127	2090.54	224145
5	myosin-8 isoform X1	MYH8	XP_020921928.1	3.10E+08	40.8	92	1492.47	223966
6	myosin heavy chain slow isoform	MYH7	BAB20630.1	1.34E+08	33.6	67	1052.10	224093
7	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	1.85E+07	22.9	25	402.47	111415
8	nebulin	NEB	XP_020931125.1	6.54E+06	1.9	15	205.65	860740
9	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	4.46E+06	9.2	7	105.83	62871.9
10	keratin 1, partial	KRT1	BAF62303.1	7.93E+06	8.2	6	104.63	65371.1
11	actin alpha 2	ACTA2	ACT82246.1	1.41E+06	14.8	5	67.65	42407.6
12	dehydrogenase, glyceraldehyde phosphate	GAPDH	prf 681085A	7.14E+05	9.3	3	41.76	35937.4
13	obscurin isoform X1	OBSCN	XP_020939003.1	4.30E+05	0.1	2	22.20	926507
14	muscle creatine kinase	CKM	AAV28621.1	4.62E+05	3.9	2	17.75	43286.5
MAP_7								
1	titin	TTN	XP_020931560.1	2.40E+08	16.7	521	7759.34	3847396
2	myosin-1 isoform X1	MYH1	XP_020921683.1	1.43E+09	65.8	149	2704.29	224112
3	myosin-2 isoform X1	MYH2	XP_020921875.1	1.29E+09	65.3	148	2609.98	224073
4	myosin heavy chain 2b	MYH2B	BAA82145.1	1.30E+09	64.7	142	2562.73	224145
5	myosin heavy chain slow isoform	MYH8	BAB20630.1	8.06E+08	61.2	127	2229.04	224093
6	myosin-8 isoform X1	MYH7	XP_020921928.1	1.01E+09	43.3	98	1729.4	223966
7	nebulin	NEB	XP_020931125.1	3.13E+07	11.1	78	1112.47	860740
8	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	2.13E+07	26.2	28	413.51	111415
9	unnamed protein product	Unnamed	CAA33170.1	6.45E+06	9.2	11	159.59	116445
10	filamin-C isoform X1	FLNC	XP_020934608.1	6.79E+06	10.2	25	366.28	295331
11	glycogen phosphorylase, muscle form	PYGM	XP_003122636.2	5.23E+06	22.9	18	264.54	97725
12	actin alpha 2	ACTA2	ACT82246.1	8.82E+06	38.4	11	180.09	42407.6
13	dehydrogenase, glyceraldehyde phosphate	GAPDH	prf 681085A	1.07E+07	38.8	11	174.75	35937.4
14	muscle creatine kinase	CKM	AAV28621.1	4.79E+06	30.1	11	169.84	43286.5
15	ADP/ATP translocase 1	SLC25A4	XP_003133393.1	3.47E+06	25.8	8	122.29	33254.1
16	keratin 1, partial	KRT1	BAF62303.1	3.45E+06	8.2	6	109.82	65371.1
17	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	3.89E+06	8.4	7	104.20	62871.9
18	myosin-binding protein C, slow-type isoform X1	MYBPC1	XP_005664259.1	1.71E+06	4.8	6	89.11	138134
19	myosin-binding protein C, fast-type	MYBPC2	XP_003127419.3	7.73E+05	2.4	3	47.99	128559
20	four and a half LIM domains protein 1 isoform X3	FHL1	XP_020935180.1	3.16E+06	21.6	6	80.47	36107.6
21	myomesin-2	MYOM2	XP_020931561.1	4.99E+05	2.4	4	48.93	167906
22	MLC1f	MLC1F	ABK55642.1	7.44E+05	14.0	3	44.82	21031.6
23	isocitrate dehydrogenase	IDH	NP_001157479.1	6.15E+05	7.7	4	42.14	51309.6
24	fructose-bisphosphate aldolase A isoform X1	ALDOA	XP_020943654.1	6.71E+05	5.9	2	27.10	47597.2
25	poly	Poly	XP_020954765.1	5.14E+05	1.6	2	23.20	127667

26	cardiac muscle ATP synthase H+ transporting mitochondrial F1 complex alpha subunit 1	ATP5A1	ACD02421.1	3.91E+05	3.6	2	17.66	59800.7
DA_7								
1	titin	TTN	XP_020931560.1	5.65E+08	24.7	751	11621.5	3847396
2	myosin-1 isoform X1	MYH1	XP_020921683.1	4.05E+08	56.2	118	1979.61	224112
3	myosin-2 isoform X1	MYH2	XP_020921875.1	3.57E+08	51.5	111	1842.84	224073
4	myosin heavy chain 2b	MYH2B	BAA82145.1	3.81E+08	52.1	103	1740.91	224145
5	myosin heavy chain slow isoform	MYH7	BAB20630.1	2.28E+08	42.7	85	1402.14	224093
6	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	9.00E+06	16.1	15	248.11	111415
7	keratin 1, partial	KRT1	BAF62303.1	4.73E+06	8.2	6	103.58	65371.1
8	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	3.54E+06	7.0	6	78.52	62871.9
9	actin alpha 2	ACTA2	ACT82246.1	2.37E+06	14.8	5	71.98	42407.6
10	nebulin	NEB	XP_020931125.1	1.76E+06	0.7	5	66.59	860740
11	muscle creatine kinase	CKM	AAV28621.1	1.79E+06	10.2	4	61.34	43286.5
12	keratin, type I cytoskeletal 18	KRT18	XP_005652636.3	1.21E+06	3.7	2	25.08	47417.2
13	glyceraldehyde-3-phosphate dehydrogenase isoform X1	GAPDH	XP_020946773.1	3.39E+05	6.7	2	24.88	37475.1
VAC_21								
1	titin	TN	XP_020931560.1	8.76E+08	33.2	986	15783.20	3847396
2	myosin-1 isoform X1	MYH1	XP_020921683.1	2.88E+08	59.5	125	2159.01	224112
3	myosin-2 isoform X1	MYH2	XP_020921875.1	2.45E+08	52.5	113	1939.50	224073
4	myosin heavy chain 2b	MYH2B	BAA82145.1	2.47E+08	54.4	108	1855.87	224145
5	myosin heavy chain slow isoform	MYH7	BAB20630.1	9.43E+07	34.9	65	1051.87	224093
6	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	1.90E+07	28.6	28	435.96	111415
7	keratin 1, partial	KRT1	BAF62303.1	5.09E+06	11.4	8	147.82	65371.1
8	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	3.27E+06	8.4	7	102.26	62871.9
9	keratin, type I cytoskeletal 14	KRT14	XP_020922659.1	2.04E+06	7.1	5	75.16	51934.8
10	actin alpha 2	ACTA2	ACT82246.1	1.83E+06	12.2	4	53.68	42407.6
MAP_21								
1	titin	TTN	XP_020931560.1	3.11E+08	18.8	574	8661.26	3847396
2	myosin-1 isoform X1	MYH1	XP_020921683.1	8.02E+08	68.1	183	3184.28	224112
3	myosin heavy chain 2b	MYH2B	BAA82145.1	7.28E+08	64.7	170	2907.40	224145
4	myosin-2 isoform X1	MYH2	XP_020921875.1	7.34E+08	65.2	170	2903.17	224073
5	myosin heavy chain slow isoform	MYH7	BAB20630.1	5.45E+08	60.7	134	2344.80	224093
6	myosin-6 isoform X1	MYH6	XP_020954892.1	4.71E+08	46.7	106	1857.79	227006
7	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	1.59E+07	24.0	27	425.15	111415
8	muscle creatine kinase	CKM	AAV28621.1	1.17E+07	41.7	19	297.18	43286.5
9	glycogen phosphorylase, muscle form	PYGM	XP_003122636.2	6.58E+06	24.3	20	290.58	97725
10	muscle glycogen phosphorylase, partial	PYGB	ABF81978.1	6.62E+06	28.1	20	284.02	84434
11	nebulin	NEB	XP_020931125.1	5.80E+06	2.2	15	190.90	860740
12	actin alpha 2	ACTA2	ACT82246.1	5.80E+06	27.0	9	143.54	42407.6
13	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	5.79E+06	11.9	10	138.91	62871.9
14	keratin 1, partial	KRT1	BAF62303.1	7.57E+06	10.1	7	123.60	65371.1
15	dehydrogenase, glyceraldehyde phosphate	GAPDH	prf 681085A	4.43E+06	26.5	9	114.26	35937.4
16	isocitrate dehydrogenase	IDH	NP_001157479.1	1.72E+06	17.0	8	99.98	51309.6
17	MLC1f	MLC1F	ABK55642.1	1.86E+06	24.4	5	71.72	21031.6
18	beta-enolase 3	ENO3	ABC75829.1	1.84E+06	15.2	6	66.38	47471.5
19	myoglobin	MB	AAA31073.1	5.46E+05	13.6	2	33.11	17084.3
20	cardiac muscle ATP synthase H+ transporting mitochondrial F1 complex alpha subunit 1	ATP5A1	ACD02421.1	7.74E+05	3.6	2	29.76	59800.7
21	myosin regulatory light chain 2	MYL2	AAV28622.1	1.80E+05	11.8	2	29.01	19092.3
22	keratin, type I cytoskeletal 18	KRT18	XP_005652636.3	1.39E+06	3.7	2	25.40	47417.2
23	ADP/ATP translocase 4	SLC25A4	XP_003129255.3	4.93E+05	4.9	2	23.35	35999
24	filamin-C isoform X1	FLNC	XP_020934608.1	3.92E+05	0.6	2	22.41	295331
DA_21								
1	titin	TTN	XP_020931560.1	2.07E+08	10.4	349	5149.08	3847396
2	myosin-1 isoform X1	MYH1	XP_020921683.1	2.83E+08	51.9	96	1653.87	224112
3	myosin heavy chain 2b	MYH2B	BAA82145.1	2.64E+08	49.4	89	1543.14	224145
4	myosin-2 isoform X1	MYH2	XP_020921875.1	2.36E+08	45.6	86	1453.69	224073
5	myosin heavy chain slow isoform	MYH7	BAB20630.1	5.93E+07	13.1	26	432.23	224093
6	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_020941340.1	3.10E+06	6.6	6	89.55	111415
7	keratin 1, partial	KRT1	BAF62303.1	4.38E+06	7.9	5	76.31	65371.1
8	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	1.96E+06	3.7	3	42.43	62871.9

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Protein Aggregation during Storage of Roe Deer Meat: a Proteomic Study

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ABSTRACT: Understanding the biochemical changes associated with protein aggregation in meat during storage is critical to improving food safety, quality assurance, and consumer satisfaction in the global meat market. The study evaluated oxygen-induced protein aggregation in the meat of European roe deer (*Capreolus capreolus*) stored for up to 21 days under refrigeration. Three packaging methods were compared: modified atmosphere packaging with two different gas compositions (MAP(1): 80% O₂/20% CO₂ and MAP(2): 40% CO₂/60% N₂) and vacuum packaging (VAC). Additionally, meat dry aging (DA) was included in the study for comparison. Structural proteins most susceptible to aggregation included titin isoform X1 and myosin 1 isoform X1, and among sarcoplasmic proteins, sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1. In addition, specific proteins involved in aggregate formation under high oxygen conditions were identified, mainly nebulin, isocitrate dehydrogenase, glyceraldehyde phosphate dehydrogenase, and creatine kinase. The extent of their aggregation varied with the type of muscle analyzed, with the most pronounced changes observed in MAP(1) after 21 days. Oxidation plays a crucial role in determining meat quality and shelf life; based on our findings, VAC is recommended for roe deer meat storage.

KEYWORDS: roe deer meat, oxidative stability, vacuum packaging, modified atmosphere packaging, dry-aging, proteomics, protein aggregation, LC-MS/MS

1. INTRODUCTION

In the European Union, the changes in eating habits and dietary strategies are an important topic. At present, animal products are the main source of protein, but meat consumption is expected to fall by 1.6 kg per person by 2035.¹ The predicted decline in beef and pork consumption is led by growing environmental awareness and concern for animal welfare, while poultry consumption is forecast to be higher.¹ According to other estimates, global meat consumption is expected to increase by around 0.5 kg per person in 2033.² Per capita consumption in Europe between 2021 and 2023 averaged 51.4 kg, while in North America and Oceania it was 78.5 and 55.6 kg, respectively.² Given these market dynamics, game could potentially grow in popularity as an alternative source of meat that combines environmental benefits with health and taste. Despite its many advantages, game meat is not highly consumed globally and in European countries (Statista Search Department. 2024). Among the Scandinavian countries, in 2020, Norwegians consumed approximately 5.2 tons of game meat. In 2021, the per capita consumption of game meat was 1.9 kg in Sweden and only 0.1 kg in Finland.^{3–5} According to 2020 data, the majority of Japanese consumers had never eaten venison, while the respondents who had eaten venison consumed it once every few years. To promote venison as a source of meat, Japan's Ministry of Agriculture, Forestry and Fisheries introduced a certification system for venison processors in 2018 to complement the certification of local systems.⁶ In Poland, game meat consumption is marginal at 0.08 kg/person.⁷

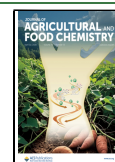
Game meat production mostly occurs through hunting, which has a lower environmental impact than farmed meat.⁸ Although game consumption is low, consumers are showing increasing interest in game species, pointing to them as an alternative source of protein due to their health, ethical, and environmental benefits.^{9–11} Considering the environmental, economic, and social aspects, game meat is likely to play an important role to meet the goals set out in the United Nations Sustainable Development Goals and the European Green Deal.¹² However, food safety, including the supply chain, storage, and processing, is still one of the main reasons why wild game consumption is low.¹² For example, among South African consumers, there was a dearth of knowledge regarding game meat or the key characteristics that determine the choice of game meat.⁹ Importantly, in Poland, purchasing chilled game meat cuts is extremely difficult, as they are not available in retail chains. Most often, only large frozen cuts are offered, which complicates the purchasing process. Therefore, it is necessary to carry out game meat research, including the determination of the appropriate storage strategies that ensure the sustainability, safety, and high quality of the raw material.¹³ The choice of the storage method depends on a number of

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factors, including the type of meat, the production conditions and technology, the shelf life, the type and time of distribution, and the impact of packaging on oxidative stability. At present, there are numerous packaging systems, although VAC and MAP are mainly used in the meat industry. In the case of gas-atmosphere packaging, the composition of the gases (oxygen, carbon dioxide, and nitrogen) is a decisive factor, significantly impacting the shelf life of the products. Packaging with an oxygen-modified atmosphere is very common for fresh red meat packaging.¹⁴

A significant challenge in the meat industry are oxidative changes in proteins.^{15–18} Protein and lipid oxidation contribute to the deterioration of meat quality by reducing its nutritional value and water-holding capacity, impairing tenderness, and leading to the formation of undesirable flavor and aroma compounds.^{13,19–22} According to Estévez and Xiong,²³ oxidized proteins are potential promoters of oxidative stress, and their consumption may have detrimental health effects and may be involved in the pathogenesis of many diseases. Proteins are key biomolecules that must adopt a stable structure to perform their biological functions.²⁴ Therapeutic interventions targeting these proteins show promise, but further research is required to elucidate the complex nature of these conditions.^{24–26}

Most of the available studies have focused on lipid oxidation during storage of meat and meat products. Packaging systems affect modifications within proteins, and studies conducted on beef and pork have shown that packaging systems with a high oxygen content can promote increased degradation, oxidation, and aggregation of proteins.^{20,27} However, most research on protein oxidation has focused on the impact of meat processing rather than the conditions of refrigerated storage.²⁸ Additionally, findings from studies on farmed animals cannot be directly extrapolated to game species, as wild animals live in significantly different environmental conditions, including diet, physical activity, and exposure to stress. The aspects of protein oxidation and aggregation have not yet been sufficiently clarified, although the application of methods based on mass spectrometry technology carries enormous research potential.²³ Physicochemical changes during storage of venison have been only partially characterized.^{13,19,29–33} In our previous study, we characterized the physicochemical changes in roe deer meat during storage, including pH, color (CIELab parameters: *L*, *a*, *b**), protein oxidation (carbonyl content), lipid oxidation (TBARS content), water activity (all), shear force (WBSF), purge and cooking losses, as well as proximate and mineral composition.¹³ The only proteomic studies on protein aggregation in wild game meat are from our previous work on wild boar meat.³⁵ We observed that MAP with high oxygen content negatively affects PA formation. However, due to differences in muscle composition, oxidative stability and metabolic traits between wild boar and roe deer meat, differences in the extent and mechanisms of protein oxidation can be expected, which may affect overall meat quality and storage stability.³⁴ Additionally, our recently published comparative proteomic analysis of the *m. longissimus thoracis et lumborum* (LTL) of domestic pig and wild boar showed that, despite their close affinity, there was a set of differentially abundant proteins, involved in muscle contraction and various enzymatic activities, between domesticated and wild animals.³⁵ These differences may influence the protein aggregation phenomenon. The continuation of research on the storage methods of European roe deer meat is essential for

deepening our understanding of the mechanisms that determine protein quality and stability in wild ruminant meat.

The present study aimed to evaluate the effects of different storage methods on protein aggregation in European roe meat (*Capreolus capreolus*). The storage methods analyzed included vacuum packaging (VAC), modified atmosphere packaging (MAP) with two gas compositions (MAP(1): 80% O₂/20% CO₂ and MAP(2): 40% CO₂/60% N₂) and meat dry aging (DA). We identified aggregate-forming proteins and determined changes in muscle proteins during storage using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis for fresh samples and stored for 7 and 21 days. We also selected potential proteins susceptible to oxidation, which consequently affects meat quality during storage. Understanding the biochemical changes associated with protein aggregation in meat during storage is critical to improve food safety and consumer satisfaction and for quality assurance in the global meat market. Our study adds to the limited research on game meat storage and contributes valuable knowledge to support sustainable practices and innovation in the meat processing industry.

2. MATERIALS AND METHODS

2.1. Sample Preparation. Samples were obtained from five European roe deer (*C. capreolus*) aged 2–4 years, which were hunted in hunting circuit no. 57, Sarbia Forest District, Poland. They were hunted by an experienced marksman in accordance with the internal carcass inspection report under Regulation (EC) No. 853/2004. The carcasses were eviscerated after shooting at the designated evisceration site (Drzazgowo Forestry) to prevent meat spoilage and stored at 0 °C for 5 days. Three muscles were examined in this study: *musculus longissimus thoracis et lumborum* (LTL), *musculus vastus lateralis* (VL), and *musculus biceps femoris* (BF). Each muscle (without bones) was divided into portions weighing approximately 80 g and stored for 21 days using three types of storage, namely, VAC, MAP, and DA. The muscle samples were consistently collected from the same anatomical location to ensure uniformity and minimize variability. All samples were stored in triplicate, vacuum-stored using barrier bags (MULTIVAC Seep Haggenmüller SE & Co. KG, Wolferschwenden, Germany). MAP samples were stored in two gas mixtures: in MAP(1) consisting of 80% O₂ and 20% CO₂, and MAP(2) consisting of 40% CO₂ and 60% N₂ (Multivac T200), and sealed with polyethylene terephthalate/polyethylene (PET/PE) film with an oxygen transmission rate of 80 cm³/m²·d·bar and a water vapor transmission rate of <3.0 g/m²·d. Samples were stored at 4 ± 1 °C for 21 days without light. Meat on the bone was subjected to the DA process at 2 °C and 80% humidity under natural daylight in a meat aging chamber (Dry-Aged Cooler SW-28, Dry Ager, Arnsberg, Germany). The samples (from the outer part of the muscle) for analysis were taken from fresh meat and on days 7 and 21 of storage. Immediately after opening the packages, the samples were transferred to sealed tubes and stored at –80 °C until LC-MS/MS analyses.

2.2. Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE) under Reducing Conditions. Electrophoretic separations were performed according to the methodology described by Montowska et al.³⁶ Two milligrams of each meat sample were incubated at 98 °C for 3 min in lysis buffer (containing 8 M urea, 2 M thiourea, 0.05 mM Tris, 75 mM dithiothreitol [DTT], 3% SDS, and 0.05% bromophenol blue, at pH 6.8). Fifteen micrograms of protein from each sample were onto a 15% polyacrylamide gel and separated (Hoefer SE250, GE Healthcare Bio-Sciences, Bridgewater, MA, USA) at a constant current of 20 mA per gel. After separation, the gels were stained with Coomassie brilliant blue dye, and images were recorded using a Gel Doc XR+ system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Image analysis was performed using Image Lab 6.0.1 software (Bio-Rad Laboratories), with a reference standard

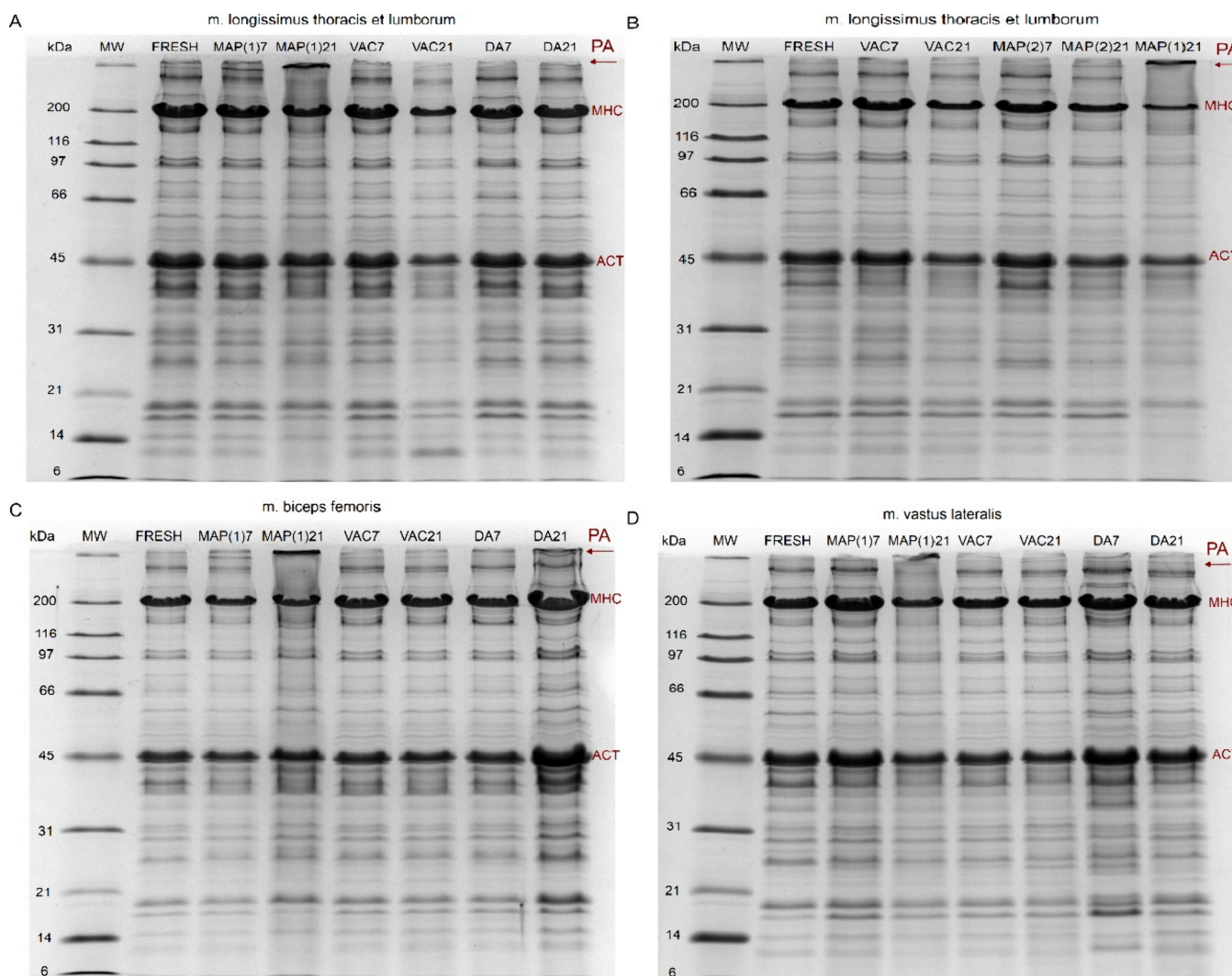


Figure 1. Representative sodium dodecyl sulfate–polyacrylamide gel electrophoresis protein profiles obtained under reducing conditions for the analyzed storage methods for the (A, B) *musculus longissimus thoracis et lumborum*, (C) *musculus biceps femoris*, and (D) *musculus vastus lateralis*. Abbreviations: ACT – actin; DA – dry-aging in a meat seasoning cabinet; MAP(1) – modified atmosphere 1 (80% O₂/20% CO₂); MAP(2) – modified atmosphere 2 (40% CO₂/60% N₂); MHC – myosin heavy chain; PA – protein aggregation; VAC – vacuum. For each sample, ‘7’ and ‘21’ indicate the days of storage.

ranging from 200 to 6.5 kDa (Bio-Rad Laboratories) to determine the molecular mass of each band.

2.3. SDS-PAGE under Nonreducing Conditions. The polymerization of high-molecular-weight proteins was evaluated with SDS-PAGE performed under nonreducing conditions. Initially, 40 mg of the meat sample was homogenized in 600 μ L of phosphate extraction buffer pH 7.0 (10 mM, 2% SDS, and 25 mM NEM) using an Ultra-Turrax T25 (IKA Labortechnik, Staufen, Germany) for two 20 s cycles at 9,500 rpm, followed by centrifugation for 15 s at 13,500 rpm. The homogenate was centrifuged at 14,000 rpm for 10 min at 10 $^{\circ}$ C, and the resulting supernatant was stored at -80 $^{\circ}$ C for later analysis. The samples (15 μ g of protein) were diluted 1:2 in 2 $\times$ SDS-PAGE loading buffer (125 mM Tris–HCl, 4% [w/v] SDS, 20% [v/v] glycerol, and 0.004% [w/v] bromophenol blue, at pH 6.8) and heated at 95 $^{\circ}$ C for 5 min. Fifteen micrograms of protein were loaded into a 6% polyacrylamide gel and separated for 130 min in a Hoefer SE250 apparatus (GE Healthcare Bio-Sciences) at a constant current of 20 mA per gel. Subsequently, the gels were stained with Coomassie brilliant blue and scanned using a Gel Doc XR+ system (Bio-Rad Laboratories).

Diagonal PAGE was performed to assess oxygen-induced polymerization of high-molecular-weight proteins, following the methodology described by Moczkowska et al.²⁰ Electrophoresis was performed in

two steps: proteins were separated under nonreducing conditions for the first dimension, and then they were reduced in a loading buffer containing 20 mM DTT for the second dimension.

2.4. Western Blotting. Aggregate-forming proteins were detected and identified according to Moczkowska et al.²⁰ High-molecular-weight meat proteins (>97 kDa) were separated with SDS-PAGE under nonreducing conditions. The separated proteins were transferred by semidry transfer to a polyvinylidene diamine (PVDF) membrane. Changes in myosin degradation and aggregation were evaluated using an antimyosin heavy chain (MHC) monoclonal antibody (MF 20) of confirmed species reactivity to mammals (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA). Alkaline phosphatase (S3841, Promega GmbH, Fitchburg, WI, USA) was used as a secondary antibody marker. The Gel Doc XR+ system and the Image Lab 6.0.1 software (Bio-Rad Laboratories) were used for membrane evaluation.

2.5. In-Gel Protein Digestion. Protein aggregates (PAs) obtained from SDS-PAGE gels under nonreducing conditions were cut into small pieces (1–2 mm³), which were placed in 1.5 mL tubes and washed with a solution consisting of 50% methanol and 5% acetic acid in Milli-Q water. Using a Vortex-Genie 2 mixer (Scientific Industries, Inc., Bohemia, NY, USA), the samples were shaken

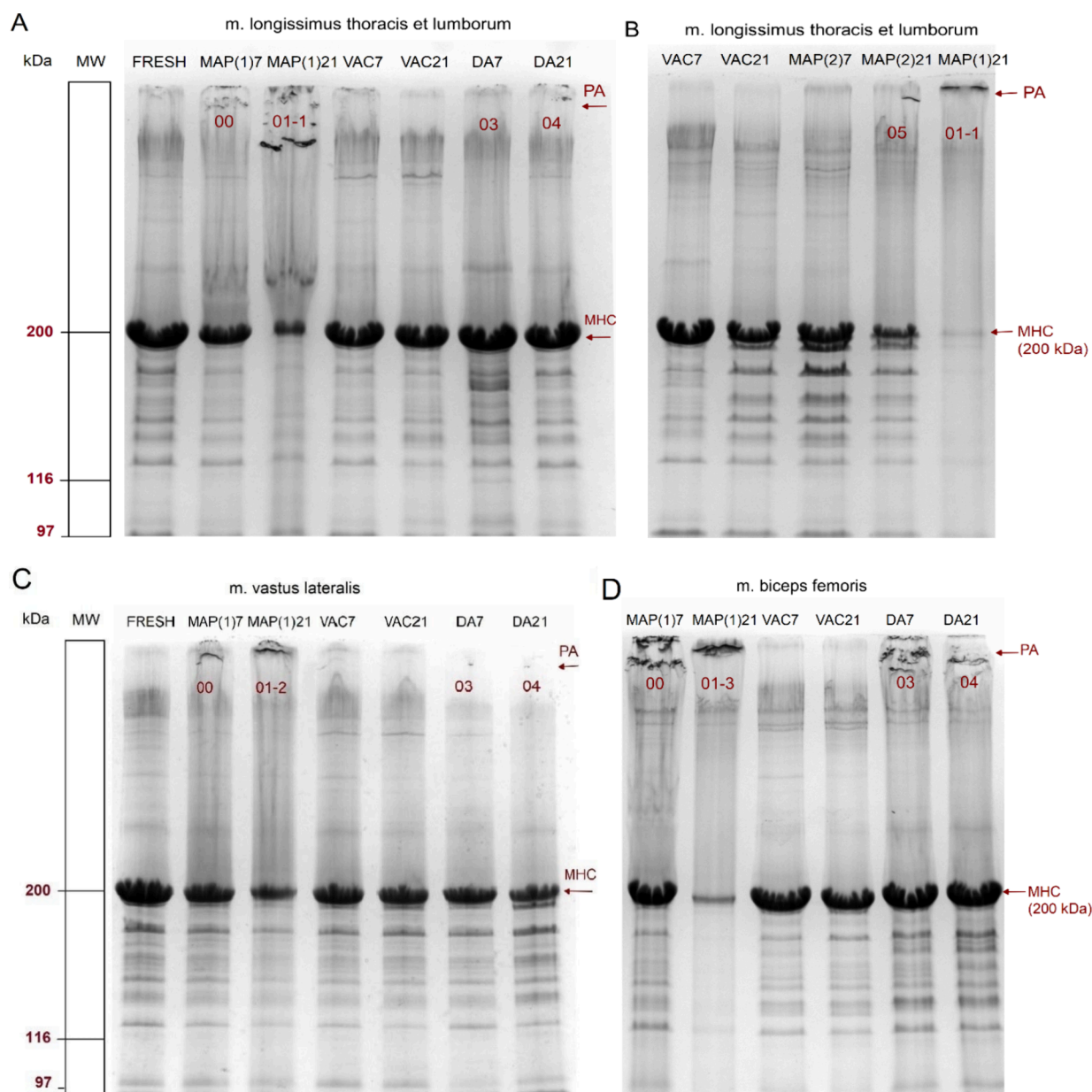


Figure 2. Representative sodium dodecyl sulfate–polyacrylamide gel electrophoresis protein profiles obtained under nonreducing conditions for the (A, B) *musculus longissimus thoracis et lumborum*, (C) *musculus biceps femoris*, and (D) *musculus vastus lateralis*. Protein bands excised from particular lanes for subsequent analysis are labeled with numbers from 00 to 05. Abbreviations: DA – dry-aging in a meat seasoning cabinet; MAP(1) – modified atmosphere 1 (80% O₂/20% CO₂); MAP(2) – modified atmosphere 2 (40% CO₂/60% N₂); MHC – myosin heavy chain; PA – protein aggregation; VAC – vacuum. For each sample, '7' and '21' indicate the days of storage.

overnight at room temperature. The next day, the rinse solution was removed and the sample was washed again (2–3 h) with a fresh rinse solution at room temperature. Acetonitrile (ACN) and 10 mM DTT were added, and the mixture was incubated for 30 min at room temperature. Proteins were alkylized with 50 mM iodoacetamide 30 min at room temperature in the dark. The samples were dehydrated with ACN; then, the samples were incubated with 100 mM ammonium bicarbonate for 10 min at room temperature (to rehydrate), and then again dehydrated with ACN (5 min). Trypsin with a final concentration of 50 ng/μL was prepared with 50 mM ammonium bicarbonate, and the samples were digested at 37 °C overnight. The next day, 50 mM ammonium bicarbonate was added and incubated (10 min), and the supernatant was collected. Extraction buffer (50% ACN and 5% formic acid in Milli-Q water) was added to the remaining gel pieces, and the supernatant was collected after incubation for 10 min. This step was repeated with

extraction buffer II with a higher ACN content (85%). All of the supernatants were pooled into one Eppendorf tube. Then, the samples were vacuum-dried in a miVac Duo Concentrator (Genevac Ltd., Suffolk, UK) and resuspended in buffer A (98% Milli-Q water, 5% ACN, and 0.1% formic acid) before being subjected to liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

2.6. LC-MS/MS Analysis. The chromatographic separation was performed on a 1290 Infinity LC system using an RRHD Eclipse Plus analytical column (2.1 × 150 mm, 1.8 μm; Agilent Technology, Santa Clara, CA, United States). A column flow rate of 0.3 mL/min was set with mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in ACN). The gradient program comprised the following steps: 0–2 min, 3% B; 2–40 min, increasing to 35% B; 40–45 min, reaching 40% B; 45–50 min, increasing to 90% B; and 50–55 min, maintaining 90% B, followed by a 5 min post-run at 3% B. An injection volume of

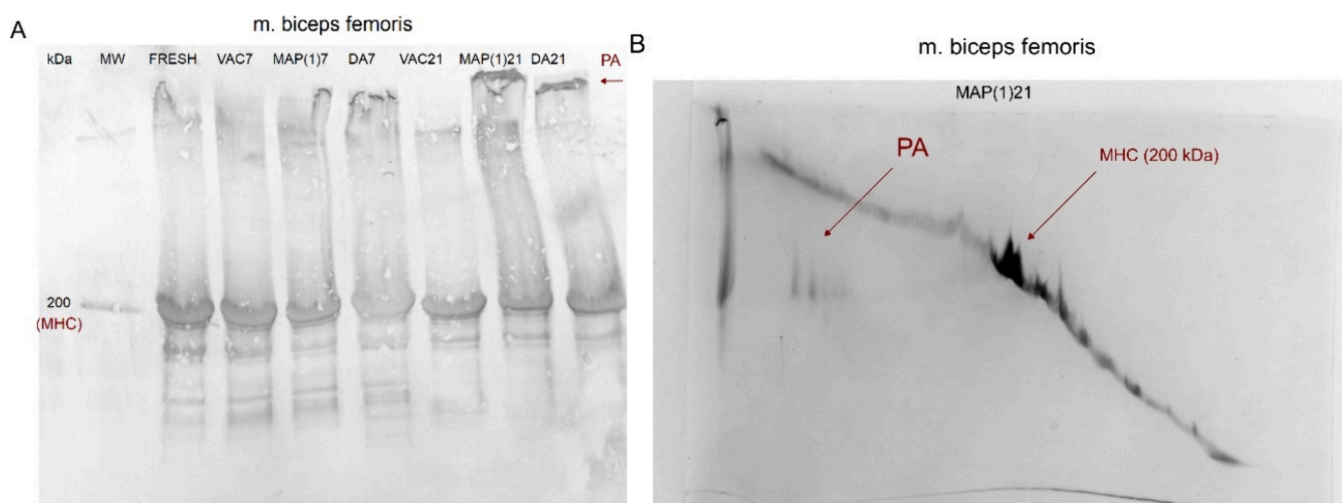


Figure 3. Representative separation of *musculus biceps femoris* (BF) proteins. (A) Western blotting using a monoclonal antimyosin heavy chain (MHC) antibody. (B) Diagonal polyacrylamide gel electrophoresis of BF proteins stored under a modified atmosphere with 80% O₂ and 20 CO₂ for 21 days (MAP(1)21). Protein aggregates (PA) linked via intramolecular disulfide bonds are labeled in panels A and B. Abbreviations: DA – dry-aging in a meat seasoning cabinet; VAC – vacuum. For each sample, ‘7’ and ‘21’ indicate the days of storage.

10 μ L was used, and the column temperature was maintained at 40 $^{\circ}$ C.

MS/MS analysis was carried out on a 6550 iFunnel Q-TOF mass spectrometer (Agilent Technology) equipped with a Jet Stream ion source operating in the positive electrospray ionization mode (ESI+). The ion source gas temperature (N₂) was 250 $^{\circ}$ C at a flow rate of 14 L/min, the nebulizer pressure was 35 psi, the mantle gas temperature was 250 $^{\circ}$ C, the mantle gas flow rate was 11 L/min, and the capillary voltage was maintained at 3500 V. The jet voltage and the fragmentation voltage were set at 1000 and 400 V, respectively. Positive ions were acquired in the 100–1700 m/z range in the MS scan mode (at 9 scans/second) and the MS/MS auto mode (at 7 scans/s). Reference ions at m/z 121.0509 and 922.0098 were used for internal mass calibration. The Mass Hunter data acquisition software (B.09.00) and Qualitative software (B.10.00) (Agilent Technology) were used for data processing.

2.7. Protein Identification and Data Analysis. The Spectrum Mill MS Proteomics Workbench (Agilent Technology, Santa Clara, CA, United States) was employed for protein identification based on the MS/MS spectra, using the National Center for Biotechnology Information (NCBI) protein database. Proteins were detected using the following search criteria: trypsin enzyme specificity allowing for a maximum of two missed cleavages, product mass tolerance of 50 ppm, and a maximum precursor charge of 6+. Carbamidomethylation was selected as a fixed post-translational modification of the protein, while methionine oxidation was selected as a variable modification. A spectral quality filter was used with a precursor isolation purity of >70% and a precursor mass tolerance of 5 ppm. The MS/MS search results were validated by using Spectrum Mill’s automatic threshold strategy and peptide mode to obtain a 1% false-discovery rate (FDR).

The assessment of the composition of protein aggregates was based on the total protein spectral intensity (protein abundance), the sequence coverage of validated proteins, and the number of unique peptides identified from the spectra associated with the protein calculated with the Spectrum Mill software, where total protein spectral intensity is total intensity of all peptides assigned to a given protein, percent coverage is percent of the protein sequence covered by the identified peptides. Peptide intensities are calculated from extracted ion chromatograms from the precursor ions. OriginPro 2023b (OriginLab Corporation, Northampton, MA, USA) was used to create the graphs. BioRender.com was used to generate the graphical abstract.

3. RESULTS AND DISCUSSION

3.1. High-Molecular-Weight PAs. Figure 1A,C, and D show a comparison of the LTL, BF, and VL protein profiles, respectively, under reducing conditions. Additionally, Figure 1B compares the separation of MAP(2) LTL proteins with MAP(1), VAC, and fresh LTL proteins. The initial screening showed that high-molecular-weight proteins such as MHC were most susceptible to oxidative changes, especially for the MAP(1) after 21 days of storage: there was a considerable decrease in MHC intensity relative to the fresh sample, and PAs were apparent at the top of the lane. Actin was less susceptible to oxidative changes. There was more pronounced protein aggregation of wild boar meat in our previous study compared with the present results, with a greater degree of myosin and actin degradation.³⁴ Despite this difference, the overall relationship remained consistent: in both studies, the MAP(1) muscle showed the most oxidative changes after 21 days of storage.

Oxidation of myosin and actin causes myofibrillar weakness, affects myosin–actin interactions, and reduces the force generated by these proteins.³⁷ Among the myofibrillar proteins of porcine *musculus longissimus dorsi* (LD), myosin has been shown to be the most susceptible to oxidation.³⁸ According to Bao et al.,³⁹ actin, MHC, MLC (myosin light chain), and tropomyosin were among the oxidation-affected muscle proteins. Myosin is highly susceptible to various oxidative modifications (oxidation of cysteine, methionine, lysine, and asparagine residues) during thermal processing and induction of an oxidative environment.^{40,41}

With SDS-PAGE under nonreducing conditions, we detected PAs > 200 kDa (Figure 2). There were PAs in the MAP(1) and DA LTL (Figure 2A), BF (Figure 2C), and VL (Figure 2D) after 7 and 21 days of storage. Of note, the MAP(2) LTL showed PAs only after 21 days of storage. In general, for each analyzed muscle, MAP(1) after 21 days of storage had the most PAs. The fresh and VAC samples did not show even traces of aggregate formation. Packing roe meat in a high-oxygen atmosphere induced the development of high-molecular-weight PAs, while an atmosphere containing nitrogen and carbon dioxide significantly reduced their formation.

Table 1. Proteins Identified in Protein Aggregates (PA) Resulting from Storing Meat in a Modified Atmosphere with High Oxygen (80% O₂/20% CO₂; MAP(1)), in a Modified Atmosphere with Carbon Dioxide and Nitrogen (40% CO₂/60% N₂; MAP(2)), and Dry Ageing in a Meat Seasoning Cabinet (DA) for 7 and 21 Days

no.	identified protein	protein abbreviation	accession no.	species	protein abundance	percent coverage	unique peptides	unique score	molecular weight (Da)
MAP(1), 7 days of storage									
1	myosin-1 isoform X1	MYH1X1	XP_017920146.1	<i>Capra hircus</i>	3.12×10^{08}	43.3	81	223992.90	223992.9
2	myosin-2	MYH2	XP_017920148.1	<i>Capra hircus</i>	2.58×10^{08}	34.4	65	1115.36	223843.2
3	myosin-4	MYH4	XP_017920145.1	<i>Capra hircus</i>	2.34×10^{08}	33.6	60	1051.42	224017.1
4	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_017895664.1	<i>Capra hircus</i>	4.60×10^{06}	7.0	6	112834.20	116445.0
5	keratin 1, partial	KRT1	BAF62303.1	<i>Sus scrofa</i>	4.74×10^{06}	7.9	5	65371.10	43286.5
6	titin isoform X1	TTNX1	XP_017920706.1	<i>Capra hircus</i>	2.73×10^{06}	0.0	4	3933082.70	42407.6
7	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	<i>Sus scrofa</i>	1.34×10^{06}	3.7	3	62871.90	860740.0
DA, 7 days of storage									
1	titin isoform X1	TTNX1	XP_017920706.1	<i>Capra hircus</i>	1.16×10^{08}	6.7	232	3439.24	3933082.7
2	myosin-1 isoform X1	MYH1X1	XP_017920146.1	<i>Capra hircus</i>	6.84×10^{08}	58	138	2447.62	223992.9
3	myosin-2 isoform X1	MYH2X1	XP_020921875.1	<i>Sus scrofa</i>	5.62×10^{08}	48.8	114	2011.45	224073.2
4	myosin-4	MYH4	XP_017920145.1	<i>Capra hircus</i>	4.99×10^{08}	44.6	95	1692.11	224017.1
5	myosin-8	MYH8	XP_013827646.2	<i>Capra hircus</i>	4.30×10^{08}	37.0	84	1469.91	223719.6
6	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_024840649.1	<i>Bos taurus</i>	2.15×10^{07}	26.3	27	439.61	112671.1
7	keratin 1, partial	KRT1	BAF62303.1	<i>Sus scrofa</i>	7.76×10^{06}	10.1	8	149.04	65371.1
8	keratin, type II cytoskeletal 1b	KRT1B	XP_017903203.1	<i>Capra hircus</i>	1.59×10^{06}	3.4	3	56.25	64038.5
9	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	<i>Sus scrofa</i>	6.19×10^{06}	10.7	8	118.48	62871.9
10	actin, alpha skeletal muscle isoform X1	ACTA1	XP_020927589.1	<i>Sus scrofa</i>	1.72×10^{06}	11.2	4	60.74	45704.5
11	collagen, type VI, alpha 1	COL6A1	DAA32939.1	<i>Bos taurus</i>	1.12×10^{06}	3.1	3	52.39	109809.8
12	collagen alpha-3(VI) chain isoform X6	COL6A3X6	XP_017896230.1	<i>Capra hircus</i>	5.40×10^{05}	0.6	3	39.69	354480.2
13	collagen alpha-2(VI) chain isoform X1	COL6A2X1	XP_017907820.1	<i>Capra hircus</i>	6.17×10^{05}	2.0	2	38.95	110588.7
MAP(1), 21 days of storage, <i>musculus longissimus thoracis et lumborum</i>									
1	titin isoform X1	TTNX1	XP_024835650.1	<i>Bos taurus</i>	1.49×10^{08}	12.2	384	5857.47	3919245.8
2	myosin-1 isoform X1	MYH1X1	XP_017920146.1	<i>Capra hircus</i>	2.04×10^{09}	61.5	149	2679.67	223992.9
3	myosin heavy chain 2x	MYH2X	BAB40921.2	<i>Bos taurus</i>	1.97×10^{09}	61.3	145	2607.39	223899.0
4	myosin-2	MYH2	XP_017920148.1	<i>Capra hircus</i>	1.81×10^{09}	61.0	138	2494.22	223843.2
5	myosin heavy chain 2a	MYH2A	BAB40920.1	<i>Bos taurus</i>	1.74×10^{09}	58.4	138	2491.57	224227.6
6	myosin-4	MYH4	XP_017920145.1	<i>Capra hircus</i>	1.54×10^{09}	48.6	112	2028.29	224017.1
7	myosin-7	MYH7	XP_017910093.1	<i>Capra hircus</i>	5.97×10^{08}	45.4	87	1464.53	223973.0
8	MYH3 protein	MYH3	AAI49228.1	<i>Bos taurus</i>	7.18×10^{08}	24.4	59	995.16	224710.4
9	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_017895664.1	<i>Capra hircus</i>	3.18×10^{07}	33.4	33	518.39	112834.2
10	creatine kinase, muscle	CKM	AAI02908.1	<i>Bos taurus</i>	2.15×10^{07}	46.1	15	263.70	43216.4
11	myomesin-2	MYOM2	NP_001033229.2	<i>Bos taurus</i>	3.04×10^{06}	9.4	13	212.75	169545.2
12	isocitrate dehydrogenase (NADP+)	IDH	CAA49207.1	<i>Bos taurus</i>	4.80×10^{06}	26.7	12	196.84	51279.6
13	alpha skeletal actin precursor	ACTA1	AAA82873.1	<i>Bos taurus</i>	7.03×10^{06}	38.4	11	185.21	42477.7
14	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	ATP5A1	AAI16060.1	<i>Bos taurus</i>	3.66×10^{06}	21.8	11	177.14	59834.7
15	alpha-1,4-glucan orthophosphate glycosyl transferase	AGP	AAB46846.1	<i>Bos taurus</i>	2.93×10^{06}	11.8	8	120.41	97748.1
16	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	<i>Sus scrofa</i>	3.11×10^{06}	9.6	8	119.10	62871.9
17	adult beta-globin	HBA	ASH97668.1	<i>Capreolus capreolus</i>	2.68×10^{06}	49.6	7	110.61	16132.2
18	keratin 1, partial	KRT1	BAF62303.1	<i>Sus scrofa</i>	5.09×10^{06}	7.9	5	86.88	65371.1
19	dehydrogenase, glyceraldehyde phosphate	GAPDH	AAI02590.1	<i>Bos taurus</i>	1.61×10^{06}	21.9	7	85.53	35937.4
20	myosin light chain, phosphorylatable, fast skeletal muscle	MLC2F	AAI20432.1	<i>Bos taurus</i>	9.73×10^{05}	28.2	5	79.25	19126.3
21	myosin-binding protein C, fast-type	MYBPC2	XP_003127419.3	<i>Sus scrofa</i>	1.42×10^{06}	3.5	4	72.52	128559.1
22	nebulin isoform X31	NEB	XP_024836427.1	<i>Bos taurus</i>	1.07×10^{06}	0.5	5	70.70	932075.6
23	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	OGDH	AAI18107.1	<i>Bos taurus</i>	9.51×10^{05}	4.0	4	62.46	116947.0
24	myosin light chain 1, alkali	MYL1	AAI26717.1	<i>Bos taurus</i>	2.61×10^{06}	20.3	4	56.00	21045.6
25	myoglobin	MB	AAI02987.1	<i>Bos taurus</i>	1.55×10^{06}	29.8	4	55.66	17206.4
26	succinyl-CoA ligase	SUCLA	XP_017912243.1	<i>Capra hircus</i>	6.62×10^{05}	5.3	3	48.52	50532.6
27	filamin-C	FLNC	NP_001193919.1	<i>Bos taurus</i>	5.95×10^{05}	0.9	3	37.56	289900.1
28	translocase	SLC25A4	AAA30768.1	<i>Bos taurus</i>	2.89×10^{05}	7.0	2	30.41	33195.1
29	heart aconitase precursor	HAP	AAA30987.1	<i>Sus scrofa</i>	3.65×10^{05}	1.9	2	26.35	86501.0
30	nesprin-1 isoform X1	SYNE1	XP_024852834.1	<i>Bos taurus</i>	5.70×10^{05}	0.2	2	17.10	1019729.8

Table 1. continued

no.	identified protein	protein abbreviation	accession no.	species	protein abundance	percent coverage	unique peptides	unique score	molecular weight (Da)
MAP(1), 21 days of storage, <i>musculus biceps femoris</i>									
1	titin isoform X1	TTNX1	XP_024835650.1	<i>Bos taurus</i>	1.41×10^{08}	10.3	350	5228.91	3919245.8
2	myosin-1 isoform X1	MYH1X1	XP_017920146.1	<i>Capra hircus</i>	1.15×10^{09}	59.1	128	2291.86	223992.9
3	myosin heavy chain 2x	MYH2X	BAB40921.2	<i>Bos taurus</i>	1.12×10^{09}	58.9	125	2236.23	223899.0
4	myosin-2	MYH2	XP_017920148.1	<i>Capra hircus</i>	9.75×10^{08}	56.6	117	2067.74	223843.2
5	myosin heavy chain 2a	MYH2A	BAB40920.1	<i>Bos taurus</i>	9.00×10^{08}	55	116	2055.99	224227.6
6	myosin-4	MYH4	XP_017920145.1	<i>Capra hircus</i>	8.56×10^{08}	46.3	98	1770.42	224017.1
7	myosin-7 isoform X1	MYH7	XP_024853058.1		3.12×10^{08}	40.8	76	1277.19	224033.2
8	MYH3 protein	MYH3	AAI49228.1	<i>Bos taurus</i>	4.29×10^{08}	23.3	52	874.08	224710.4
9	myosin-7B	MYH7B	XP_010809975.2	<i>Bos taurus</i>	7.28×10^{07}	4	11	167.23	222565.9
10	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_017895664.1	<i>Capra hircus</i>	4.00×10^{07}	33.6	33	553.14	112834.2
11	creatine kinase, muscle	CKM	AAI02908.1	<i>Bos taurus</i>	3.23×10^{07}	48	17	300.92	43216.4
12	isocitrate dehydrogenase (NADP+)	IDH	CAA49207.1	<i>Bos taurus</i>	4.44×10^{06}	27.8	12	196.23	51279.6
13	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	ATPSA1	AAI16060.1	<i>Bos taurus</i>	2.97×10^{06}	21.6	11	170.83	59834.7
14	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	<i>Sus scrofa</i>	5.76×10^{06}	13.2	9	147.34	62871.9
15	keratin 1, partial	KRT1	BAF62303.1	<i>Sus scrofa</i>	1.22×10^{07}	10.6	7	121.35	65371.1
16	alpha skeletal actin precursor	ACTA1	AAA82873.1	<i>Bos taurus</i>	4.97×10^{06}	21.2	7	114.57	42477.7
17	myomesin-2	MYOM2	NP_001033229.2	<i>Bos taurus</i>	1.88×10^{06}	5	8	102.48	169545.2
18	dehydrogenase, glyceraldehyde phosphate	GAPDH	AAI02590.1	<i>Bos taurus</i>	2.76×10^{06}	18	5	66.18	35937.4
18	myosin, light chain 1, alkali	MYL1	AAI26717.1	<i>Bos taurus</i>	2.41×10^{06}	20.3	4	65.89	21045.6
19	myosin light chain, phosphorylatable, fast skeletal muscle	MLC2F	AAI20432.1	<i>Bos taurus</i>	7.20×10^{05}	23.5	4	64.14	19126.3
20	succinyl-CoA ligase	SUCLA	XP_017912243.1	<i>Capra hircus</i>	1.39×10^{06}	7.5	4	56.33	50532.6
21	adult beta-globin	HBA	ASH97668.1	<i>Capreolus capreolus</i>	1.03×10^{06}	22.7	3	53.71	16132.2
22	myoglobin	MB	AAI02987.1	<i>Bos taurus</i>	9.92×10^{05}	20.7	3	47.74	17206.4
23	alpha-1,4-glucan orthophosphate glycosyl transferase	AGP	AAB46846.1	<i>Bos taurus</i>	7.49×10^{05}	4	3	46.50	97748.1
24	myosin-binding protein C, fast-type	MYBOC2	XP_017918332.1	<i>Capra hircus</i>	9.22×10^{05}	2.1	3	44.43	128491.4
25	nebulin isoform X31	NEB	XP_024836427.1	<i>Bos taurus</i>	5.66×10^{05}	0.3	3	38.81	932075.6
26	translocase	SLC25A4	AAA30768.1	<i>Bos taurus</i>	2.87×10^{05}	5.3	2	24.15	33195.1
MAP(1), 21 days of storage, <i>musculus vastus lateralis</i>									
1	titin isoform X1	TTNX1	XP_024835650.1	<i>Bos taurus</i>	4.06×10^{08}	20.8	641	10078.23	3919245.8
2t	itin	TTN	XP_020931560.1	<i>Sus scrofa</i>	3.08×10^{08}	16	494	7656.20	3847396.4
3	myosin-1 isoform X1	MYH1X1	XP_017920146.1	<i>Capra hircus</i>	1.25×10^{09}	59.3	138	2474.92	223992.9
4	myosin-1	MYH1	spIQ9BE40.2	<i>Bos taurus</i>	1.32×10^{09}	58.4	134	2417.61	223899.0
5	myosin heavy chain 2a	MYH2	BAB40920.1	<i>Bos taurus</i>	1.08×10^{09}	57.1	131	2304.43	224227.6
6	myosin-4	MYH4	XP_017920145.1	<i>Capra hircus</i>	9.79×10^{08}	47.8	107	1927.44	224017.1
7	myosin-7	MYH7	XP_017910093.1	<i>Capra hircus</i>	4.44×10^{08}	54.1	103	1779.82	223973.0
8	MYH3 protein	MYH3	AAI49228.1	<i>Bos taurus</i>	4.32×10^{08}	24.7	57	953.33	224710.4
9	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_024840649.1	<i>Bos taurus</i>	5.66×10^{07}	34.6	37	602.49	112671.1
10	creatine kinase, muscle	CKM	AAI02908.1	<i>Bos taurus</i>	1.35×10^{07}	45.6	15	244.90	43216.4
11	alpha skeletal actin precursor	ACTA1	AAA82873.1	<i>Bos taurus</i>	3.45×10^{06}	21.2	7	104.99	42477.7
12	four and a half LIM domains protein 1 isoform X3	FHL1	XP_020935180.1	<i>Sus scrofa</i>	3.50×10^{06}	21.6	6	100.77	36107.6
13	myomesin-2	MYOM2	NP_001033229.2	<i>Bos taurus</i>	1.58×10^{06}	4.3	6	90.82	169545.2
14	filamin-C	FLNC	NP_001193919.1	<i>Bos taurus</i>	1.42×10^{06}	2.3	7	87.08	289900.1
15	nebulin isoform X31	NEB	XP_024836427.1	<i>Bos taurus</i>	1.37×10^{06}	0.6	6	83.65	932075.6
16	actinin, alpha 2	ACTN2	AAI02909.1	<i>Bos taurus</i>	1.30×10^{06}	6.2	5	81.23	104347.3
17	keratin 1, partial	KRT1	BAF62303.1	<i>Sus scrofa</i>	1.87×10^{06}	6.6	4	65.63	65371.1
18	isocitrate dehydrogenase (NADP+)	IDH	CAA49207.1	<i>Bos taurus</i>	8.44×10^{05}	7.9	4	57.85	51279.6
19	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	<i>Sus scrofa</i>	1,640,000	6.3	5	57.31	62871.9
20	dehydrogenase, glyceraldehyde phosphate	GAPDH	AAI02590.1	<i>Bos taurus</i>	946,000	11.4	4	51.78	35937.4
21	alpha-1,4-glucan orthophosphate glycosyl transferase	AGP	AAB46846.1	<i>Bos taurus</i>	487,000	3.9	3	39.74	97748.1
22	obscurin isoform X1	OBSN	XP_017907006.1	<i>Capra hircus</i>	670,000	0.3	3	35.92	1024788.4
23	myosin, light chain 1, alkali	MYL1	AAI26717.1	<i>Bos taurus</i>	435,000	10.4	2	29.19	21045.6
DA, 21 days of storage									
1	myosin-1 isoform X1	MYH1X1	XP_017920146.1	<i>Capra hircus</i>	7.97×10^{08}	54.5	113	2033.22	223992.9
2	myosin-1	MYH1	spIQ9BE40.2	<i>Bos taurus</i>	8.24×10^{08}	53	108	1943.12	223899.0

Table 1. continued

no.	identified protein	protein abbreviation	accession no.	species	protein abundance	percent coverage	unique peptides	unique score	molecular weight (Da)
DA, 21 days of storage									
3	myosin heavy chain 2a	MYH2A	BAB40920.1	<i>Bos taurus</i>	6.77×10^{08}	50.1	100	1783.54	224227.6
4	myosin heavy chain 2b	MYH4	BAA82145.1	<i>Sus scrofa</i>	6.53×10^{08}	43.9	86	1567.47	224145.2
5	myosin-8	MYH8	XP_013827646.2	<i>Capra hircus</i>	5.04×10^{08}	35.5	71	1282.44	223719.6
6	MYH3 protein	MYH3	AAI49228.1	<i>Bos taurus</i>	2.84×10^{08}	19.4	43	722.08	224710.4
7	myosin-6	MYH6	XP_024853710.1	<i>Bos taurus</i>	2.18×10^{08}	15.4	33	560.12	224644.6
8	titin isoform X1	TTNX1	XP_017920706.1	<i>Capra hircus</i>	5.13×10^{07}	2.5	91	1308.74	3933082.7
9	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1	SERCA1	XP_024840649.1	<i>Bos taurus</i>	1.20×10^{07}	18.4	17	265.70	112671.1
10	collagen alpha-3(VI) chain isoform X6	COL6A3X6	XP_017896230.1	<i>Capra hircus</i>	6.96×10^{06}	4.6	15	215.51	354480.2
11	collagen, type VI, alpha 1	COL6A1	DAA32939.1	<i>Bos taurus</i>	4.81×10^{06}	9.3	8	142.53	109809.8
12	collagen alpha-2(VI) chain isoform X1	COL6A2 × 1	XP_005202110.1	<i>Bos taurus</i>	2.72×10^{06}	7.3	8	117.55	110517.7
13	keratin, type I cytoskeletal 10 isoform X1	KRT10	XP_003131508.1	<i>Sus scrofa</i>	4.23×10^{06}	8.1	6	91.02	62871.9
14	keratin 1, partial	KRT1	BAF62303.1	<i>Sus scrofa</i>	6.38×10^{06}	7.9	5	84.82	65371.1
15	collagen alpha-1(I) chain isoform X1	COL1A1	XP_024835395.1	<i>Bos taurus</i>	9.34×10^{05}	1.8	2	33.94	143293.8
16	collagen alpha-1(XII) chain isoform X1	COL12A1	XP_005210721.2	<i>Bos taurus</i>	5.16×10^{05}	0.6	2	33.66	340114.8
17	alpha skeletal actin precursor	ACTA1	AAA82873.1	<i>Bos taurus</i>	5.98×10^{05}	6.6	2	23.65	42477.7
MAP(2), 21 days of storage									
1	myosin-1 isoform X1	MYH1X1	XP_017920146.1	<i>Capra hircus</i>	2.45×10^{07}	13.4	25	397.65	223992.9
2	myosin-2	MYH22	DAA18727.1	<i>Bos taurus</i>	2.38×10^{07}	11.7	20	326.20	224241.6
3	titin isoform X1	TTNX1	XP_017920706.1	<i>Capra hircus</i>	1.47×10^{06}	0	3	40.53	3933082.7
4	keratin 1, partial	KRT1	BAF62303.1	<i>Sus scrofa</i>	2.08×10^{06}	4.1	3	39.68	65371.1

Daszkiewicz et al.³² reported the effect of a modified atmosphere with 40% CO₂ and 60% N₂ on quality characteristics, including weight loss during storage, chemical composition, and shear force. Importantly, our findings extend the knowledge on this topic and provide additional protein analysis.

Based on electrophoretic separation under both reducing and nonreducing conditions (Figures 1 and 2), the VL appears to be the least susceptible to protein changes, including aggregation. The reason for the differences in response to different storage conditions may be due to muscle metabolism, considering the type of fibers and the number of mitochondria, which are the main site of endogenous reactive oxygen species (ROS) production.⁴² Meat quality is strongly correlated with the muscle fiber type, although the mechanisms remain unclear.⁴³ Muscle fibers are classified based on MHC isoforms (types I, IIA, IIB, and IIX) and are divided into oxidative (slow contractile – type I) and glycolytic (fast contractile – type IIA and IIB) fibers depending on their contractile and metabolic properties. The BF is classified as a fast/glycolytic muscle.⁴³ According to Ruusunen and Puolanne,⁴⁴ the LD in wild pigs has a more balanced proportion of type IIA and IIB fibers and a higher oxidative capacity, while the LD in domestic pigs is dominated by a higher number and larger cross-section of type IIB fibers, which is associated with a faster growth rate and a more glycolytic nature of the muscle. Curry et al.⁴⁵ reported that in the fallow deer (*Dama dama*), the VL showed greater resistance to oxidative processes than the LD, due to differences in muscle fiber composition. The VL contains more type I oxidative fibers, which are more resistant to oxidative stress, while the LD has a predominance of type II glycolytic fibers, which are more susceptible to oxidation. Overall, the VL has better oxidative potential and metabolic stability compared with the LD.⁴⁵ Hence, the VL seems to be the least susceptible to oxidation due to the predominance of oxidative fibers, while the BF and LD, with a predominance of glycolytic fibers, are more vulnerable to oxidative processes,

consistent with the electrophoretic protein profiles presented in Figure 2.

Western blotting with the MF20 antibody confirmed the presence of myosin in PAs in the MAP(1) and DA samples and revealed other polymorphic forms of MHC and its degradation products (Figure 3A). The ability of myosin to form disulfide bonds was apparent based on diagonal electrophoresis for the MAP(1) samples after 21 days of storage (Figure 3B). In that panel, PAs containing intramolecular disulfide bonds between cysteine and thiol groups are marked. As reported by Lu et al.,⁴⁶ cysteine present in the head of myosin forms sulphinic and sulfonic acids under oxidative conditions, while cysteine in the tail of myosin participates in the formation of disulfides. Methionine present in the myosin tail is also susceptible to oxidative processes. Myosin cross-linking is favored by changes in thiol groups.⁴⁷

3.2. Identification of Aggregate-Forming Proteins Using LC-MS/MS. We excised selected bands from the nonreducing gels (Figure 2) and subjected them to protein identification using LC-MS/MS. Table 1 shows all proteins identified in PAs for each method, excluding VAC and MAP(2) after 7 days of storage, because we did not identify PAs in those samples. Factors such as a low oxygen content and naturally occurring autolysis slow down the formation of PAs as a result of the removal of structurally similar molecules assembled from submolecular structures.⁴⁸ The MAP(1) LTL, VL, and BF samples showed differences after 21 days of storage, consistent with the differences we observed in the nonreducing gels (Figure 2, bands 01–1, 01–2, and 01–3, respectively). After 21 days of storage, PAs increased under high-oxygen conditions; however, the three studied muscles showed differences in the number of PAs formed, with 30 proteins for the LTL, 26 proteins for the BF, and 23 proteins for the VL (Table 1). MS confirmed that the VL was the least susceptible to the formation of PAs, perhaps because less intense oxidative and biochemical processes occur in this muscle. Importantly, these results are consistent with a

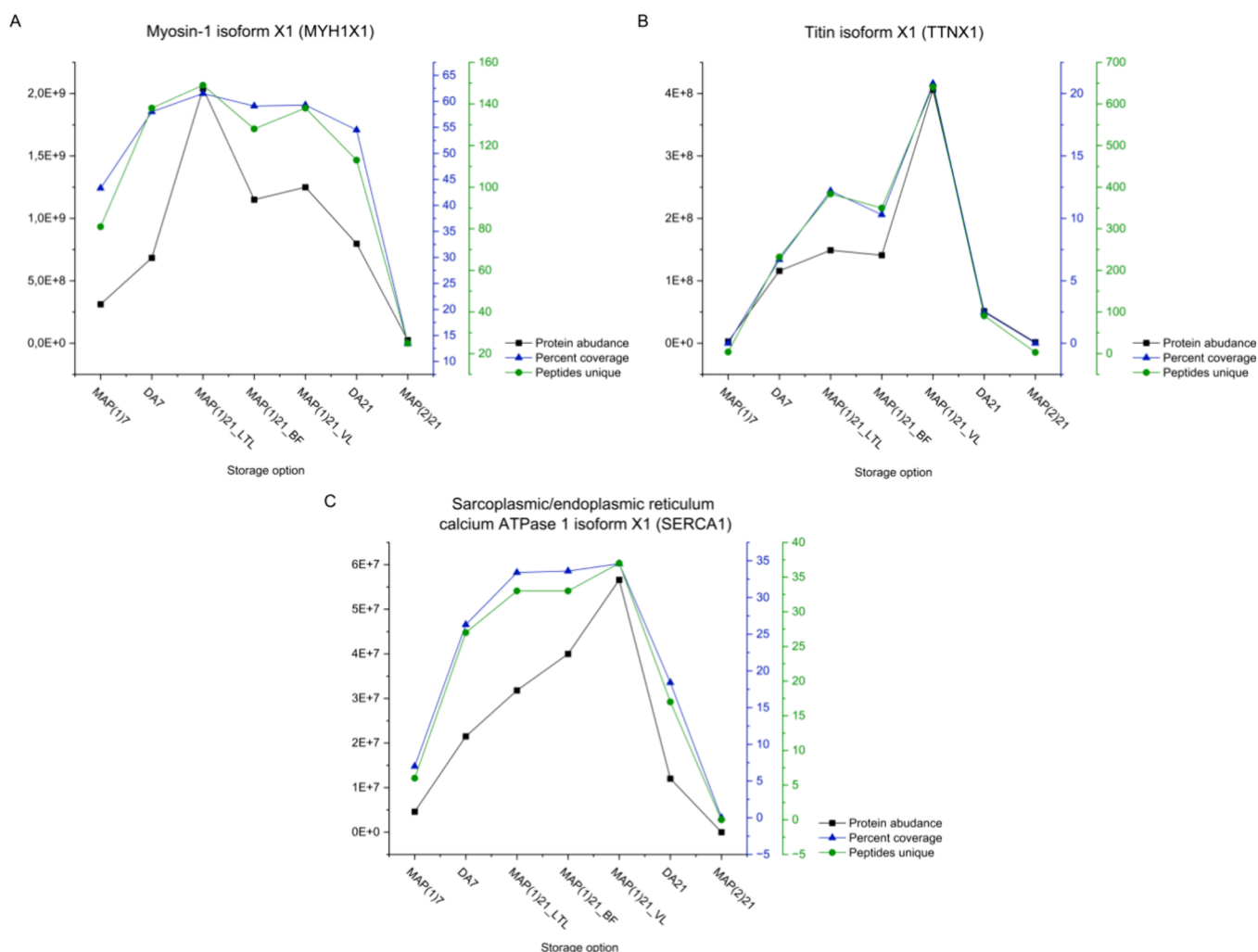


Figure 4. Liquid chromatography–tandem mass spectrometry results for the three most abundant structural proteins in protein aggregates – (A) myosin-1 isoform X1 (MYH1X1), (B) titin X1 (TTNX1), and (C) sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1 (SERCA1) – obtained from the excised protein bands labeled in Figure 2A. Abbreviations: BF – *musculus biceps femoris*; DA – dry-aging in a meat seasoning cabinet; LTL – *musculus longissimus thoracis et lumborum*; MAP(1) – modified atmosphere 1 (80% O₂/20% CO₂); MAP(2) – modified atmosphere 2 (40% CO₂/60% N₂); VL – *musculus vastus lateralis*. For each sample, ‘7’ and ‘21’ indicate the days of storage.

previous study that analyzed the formation of protein carbonyls, for which the same relationship was noted for all three muscles subjected to MAP(1) for 21 days.¹³

Despite the differences between the muscles during storage, the storage method had a significant effect on PA formation. There were significantly more proteins in PAs for the MAP(1) samples after 21 days of storage compared with 7 days of storage, suggesting an increase in oxidation with storage time. MAP(2) did not have an effect on protein changes: only four proteins formed PAs after 21 days of storage (Table 1), suggesting a protective effect of nitrogen that does not promote ROS formation, while little oxidative damage could be induced by reactive nitrogen species (RNS).^{49–51} VAC followed by MAP(2) reduced oxidative processes and PA formation to the greatest extent compared with MAP(1) and DA. The composition of PAs for the DA samples differed from the other packing methods based on the presence of collagens. Among other identified proteins, we noted structural cytoskeletal proteins, metabolic enzymes, and other functional (transport and regulatory) proteins (Table 1). Overall, the PAs mainly included structural and metabolic proteins. Among the

structural proteins, myosin-1 isoform X1 (MYH1X1) and titin isoform X1 (TTNX1) were the most common.

There was a dynamic decrease in titin aggregation during DA storage based on total protein intensity and the number of unique peptides we detected (Table 1 and Figure 4B), likely caused by changes related to proteolysis and degradation due to the activity of muscle enzymes. Similarly, in wild boar meat, titin was quantitatively the second most abundant protein in PA in DA samples after 21 days of storage, which also indicates intense proteolysis during storage.³⁴ Storage in MAP(1) for 7 days did not significantly affect titin aggregation, but storage in MAP(1) for 21 days, especially in the VL (Figure 4B). In the PAs resulting from the samples stored in MAP(2) for 21 days, we identified TTNX1, but its abundance was relatively low and there were only three unique peptides, indicating the least protein structure interference. Titin tends to form different types of intermolecular interactions, leading to the formation of oligomers and aggregates.⁵²

Among the various myosin isoforms, MYH1X1 (223.99 kDa) had the highest peptide number and percentage coverage in each storage variant (Table 1). These data suggest that myosin plays a significant role in the aggregation process, but

we noted that the pattern of aggregation varied significantly depending on the storage conditions, as indicated by the abundance of MYH1X1 and the presence of other proteins. For the MAP(1) and DA samples, there was high MYH1X1 abundance after 7 and 21 days of storage. Considering the total intensity and the number of unique peptides (Table 1 and Figure 4A), DA showed a considerable reduction in aggregation on day 21, while MAP(1) promoted oxidative damage to MYH1X1 at a similar level for all three analyzed muscles. MAP(2) reduced protein aggregation, resulting in a lower abundance of MYH1X1 after 21 days of storage.

During storage of wild boar meat, MYH1X1 was also susceptible to aggregation. However, protein aggregation in wild boar meat was faster and more intense than in roe deer meat. Previous research has shown that the fat content of roe deer LTL is 17.1 g/kg, significantly lower than that of wild boar (49.9 g/kg), which may contribute to differences in protein carbonylation.^{13,19,34} Furthermore, it was reported that for roe deer LTL meat stored in a high-oxygen MAP for 21 days, protein carbonylation was 0.82 nmol/mg protein and TBARS (the thiobarbituric acid reactive substances) was 5.54 mg MDA/kg (the level of malonic dialdehyde), lower than the values of 3.29 nmol/mg and 6.15 mg MDA/kg, respectively, for wild boar LTL meat.^{13,19} Lipid oxidation can affect the kinetics of protein oxidation, and proteins present in food can be oxidized by direct exposure to ROS and RNS or indirectly induced via byproducts of sugar and lipid oxidation.^{53,54} Moczowska et al.²⁰ also reported PAs in a high-oxygen atmosphere; they consisted of MHC and had an adverse effect on beef tenderness. According to Chernukha et al.,⁴⁸ cross-linkage and aggregation of muscle proteins, especially myosin, leads to a deterioration of meat quality, including a reduced water-binding capacity of the meat, tenderness, and juiciness, and adversely affects the taste of the meat.

We identified the sarcoplasmic/endoplasmic reticulum calcium ATPase 1 isoform X1 (SERCA1) protein, which plays a key role in regulating muscle calcium levels, in PAs for all storage variants except MAP(2) (Table 1 and Figure 4C). There was an increase in SERCA1 aggregation in each analyzed muscle for MAP(1) after 21 days of storage relative to 7 days of storage (6 unique peptides), while DA showed an inverse relationship (27 unique peptides after 7 days of storage, and 17 unique peptides after 21 days of storage). In our previous study, we detected SERCA1 in PAs from wild boar meat stored under high-oxygen conditions for 7 days (31 unique peptides) and 21 days (31 unique peptides), and in meat subjected to DA for 7 days (40 unique peptides), whereas it was not present in PAs from meat subjected to DA for 21 days.³⁴ Some ROS activities can activate signaling pathways that affect the expression, phosphorylation, or function of calcium-regulating proteins.⁵⁵ As evidenced by Johnson et al.,⁵⁶ specific proteins in the sarcoplasmic proteome, including SERCA1, can serve as markers to predict pork quality, particularly in terms of the water holding capacity.

Aggregation of structural and contractile proteins, such as myosin and titin, can alter the textural properties of meat, affecting tenderness and quality. Understanding the changes associated with protein aggregation can be used to develop improved storage and processing techniques to maintain meat quality. Previous physicochemical studies of roe meat have demonstrated that the storage method and time affect meat tenderness. Specifically, MAP(1) had a negative effect on this quality factor, while meat seasoning improved tenderness for

the BF and VL after 21 days of storage (17.83 and 19.08 N, respectively) compared with 7 days of storage (21.43 and 19.10 N, respectively). Due to the high water loss of the LTL after 21 days of storage, the desired tenderness was achieved after 7 days of storage (16.91 N).¹³ Changes in collagen alpha-1(VI), alpha-2(VI) isoform X1, and alpha-3(VI) isoform X6 (COL6A1, COL6A2X1, and COL6A3X6, respectively), which were detected only after 7 and 21 days of DA storage, and collagen alpha-1(XII) isoform X1 and alpha-1(I) isoform X1 (COL12A1 and COL1A1, respectively), detected after 21 days of DA storage, may also promote tenderness (Table 1).

Figure 5 shows the 10 proteins that formed aggregates mainly for the MAP(1) samples after 21 days of storage, which

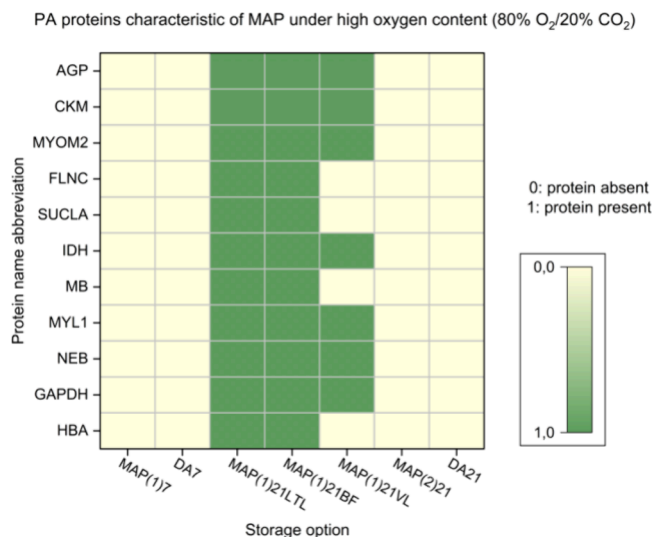


Figure 5. Selected proteins identified in protein aggregates formed in the *musculus longissimus thoracis et lumborum* (LTL), the *musculus biceps femoris* (BF), and the *musculus vastus lateralis* (VL) stored in a high-oxygen atmosphere. Abbreviations: AGP – alpha-1,4-glucan orthophosphate glycosyl transferase; CKM – creatine kinase, muscle; DA – dry-aging in a meat seasoning cabinet; FLNC – filamin-c; GAPDH – glyceraldehyde phosphate dehydrogenase; HBA – adult beta-globin; IDH – isocitrate dehydrogenase; MAP(1) – modified atmosphere 1 (80% O₂/20% CO₂); MAP(2) – modified atmosphere 2 (40% CO₂/60% N₂); MB – myoglobin; MYL1 – myosin light chain 1 alkali; MYOM2 – myomesin-2; NEB – nebulin; SUCLA – succinyl-CoA ligase; PAs – protein aggregates. For each sample, ‘7’ and ‘21’ indicate the days of storage.

were not present for the other storage variants. They included the metabolic proteins alpha-1,4-glucan orthophosphate glycosyltransferase (AGP) and succinyl-CoA ligase (SUCLA); proteins responsible for oxidative stress and energy metabolism, namely creatine kinase (CKM) and isocitrate dehydrogenase (IDH); proteins responsible for muscle structure and function, specifically myomesin-2 (MYOM2), filamin-C (FLNC), myosin light chain 1 alkali (MYL1), nebulin isoform X31 (NEB); and proteins involved in oxygen transport, including myoglobin (MB) and adult beta-globin (HBA). These 10 proteins that are present in PAs prove that the metabolic and structural processes of cells can be significantly altered in response to specific oxidative conditions. The MAP(1) conditions favored aggregation of enzymes associated with muscle function in each analyzed muscle.

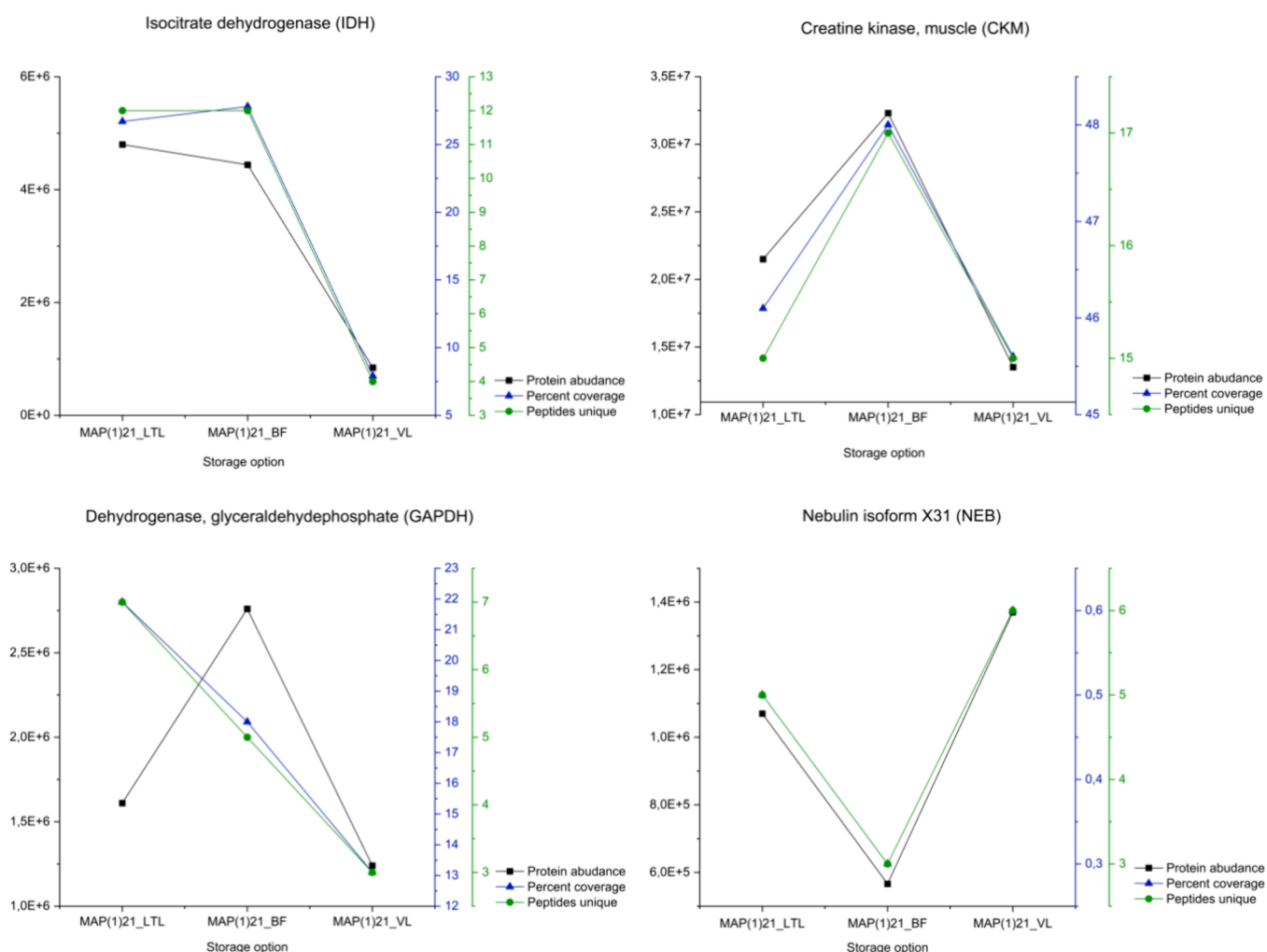


Figure 6. Liquid chromatography–tandem mass spectrometry results for selected proteins characteristic of storage in a modified high-oxygen atmosphere. The graph shows the number of matched unique peptides, the percent coverage, and protein abundance. Abbreviations: BF – *musculus biceps femoris*; DA – dry-aging in a meat seasoning cabinet; LTL – *musculus longissimus thoracis et lumborum*; MAP(1) – modified atmosphere 1 (80% O₂/20% CO₂); MAP(2) – modified atmosphere 2 (40% CO₂/60% N₂); VL – *musculus vastus lateralis*. For each sample, ‘7’ and ‘21’ indicate the days of storage.

Figure 6 shows the main meat proteins that formed aggregates only for the MAP(1) samples after 21 days of storage and the difference in LC-MS/MS outputs between the studied muscles. Among these proteins, CKM displayed the highest intensity, which was also related to the percentage of sequence coverage. According to our earlier study,³⁴ wild boar meat was more susceptible to oxidation of CKM (the MAP and DA storage conditions and times for that study were the same as the present study). MAP with a high-oxygen content promoted protein aggregation at a similar level to roe deer meat, with a similar high protein concentration, indicating a direct effect of the storage method. This enzyme is used as a common indicator of physical stress or muscle damage.⁵⁷ A study of sheep meat showed no correlation between creatine kinase activity and meat quality, but did show the influence of preslaughter stress, breed, and season.⁵⁸ Creatine kinase was also associated with changes in the quality characteristics of chicken meat that was vacuum sealed and stored under refrigeration for 7 days.⁵⁹

The highest aggregation of NEB was for the MAP(1) VL stored for 21 days, with six identified peptides (Figure 6 and Table 1). In the case of wild boar muscles, there were more

intense aggregation changes in both high-oxygen MAP and DA, indicating its dynamic nature, as dry aging contributed to a significant decrease in the level of NEB aggregation at day 21 of storage.³⁴ The inverse relationship between CKM and NEB levels (Figure 6 and Table 1) may suggest that the two proteins compete for similar structural or environmental conditions during aggregation or it may be the result of differences in muscle fiber composition. Aggregation of structural proteins such as titin and NEB may lead to less involvement of enzymes such as CKM in muscle metabolism. When structural components aggregate in response to oxidative stress, they can use up cellular resources, thereby reducing their activity. NEB is an actin-binding protein found in various cytoskeletal structures and tissues, with a role responsible for stabilizing the length of actin filaments.⁶⁰ The high aggregation of titin and NEB in response to oxidative stress in the VL can weaken the muscle structure, consistent with a previous study showing that after MAP(1) storage for 21 days, VL tenderness deteriorated and was lower compared with the LTL and the BF.¹³

IDH increased at all storage times in response to a high-oxygen atmosphere; however, the oxidative stress response was

lowest for the VL compared with the LTL and BF (Figure 6 and Table 1). During the storage of wild boar meat for 7 days, there were IDH aggregates for the MAP(1) and DA samples, while after 21 days, IDH only appeared in the MAP(1) samples.³⁴ IDH may be a specific indicator of the oxidative stress response. IDH is responsible for the two-step oxidative catalysis of the decarboxylation of isocitrate to 2-oxoglutarate (α -ketoglutarate), while also producing NADPH from NADP⁺ and has been identified as a potent oncometabolite in cancer cells.⁶¹ Therefore, further research is needed on the effect of its consumption with food versus accumulation in the human body. As reported by Tan et al.,⁴³ the quality of meat is strongly dependent on the type of muscle fibers, but knowledge of the proteins that regulate muscle fiber types in pigs and other common slaughter and game species is incomplete. The aggregation of structural proteins and enzymes, such as CKM and IDH, differed in the BF, LTL and VL, reflecting their different metabolic properties. In the VL, which showed the highest aggregation of NEB and titin, structural proteins are particularly involved, which favors metabolic stability. In contrast, in the BF and LTL, greater aggregation of energetic proteins such as CKM or IDH, may limit their metabolic function, leading to lower muscle capacity. These differences in protein aggregation are likely the result of different muscle fiber structures, which affects meat quality and its resistance to oxidative stress.

An increased oxygen content also promoted aggregation of GAPDH, a glycolytic enzyme present in the cytoplasm (Figure 6). In response to ROS, GAPDH may undergo structural modifications leading to its aggregation, thus disrupting cellular homeostasis.⁶² The aggregation of proteins including GAPDH is observed in the development of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Specifically, the involvement of GAPDH is extremely important in the pathogenesis of these diseases, and GAPDH deposits are observed in post-mortem samples of diagnosed patients.⁶³ This raises another question about the accumulation of ingested protein aggregates and their impact on downstream metabolic pathways.

MYOM2 also showed increased abundance under the influence of oxidative stress. Of the three analyzed muscles, the LTL had a slightly higher unique peptide count (13) and protein abundance of 3.04×10^{06} (Figure 4 and Table 1). MYOM2 was also identified in beef exudate taken at 23 days postslaughter and stored under vacuum.⁶⁴ MB oxidation was also promoted by high oxygen storage conditions, which we identified in PAs of LTL and BF after 21 days of storage (Table 1). Our results are in line with previous studies on meat color in roe deer meat stored in MAP(1), which showed a negative effect of time and method of storage on L^* , a^* , and b^* parameters.¹³ For wild boar meat, we identified MB in PAs after 7 and 21 days of MAP storage and after 7 days of DA storage.³⁴

Storage in a modified atmosphere with a high oxygen content (80% O₂ and 20% CO₂) resulted in the formation of high-molecular-weight PAs. MHC showed the greatest ability to form PAs after 21 days of storage. The proportion of titin in PAs increased after 21 days of storage in MAP(1), especially in the VL, while in DA the proportion decreased after 21 days of storage compared with 7 days of storage. In MAP(2), there was negligible myosin and marginal titin in PAs. SERCA1 showed dynamic changes in DA – its level of aggregation decreased as the storage time increased – while in MAP(1)

aggregation intensified over time in each analyzed muscle. MAP(1) also caused aggregation of NEB and sarcoplasmic enzymes such as CKM and IDH. There were no PAs in the VAC samples, showing the effectiveness of this method to preserve the oxidative stability of meat. Structural and functional changes in muscle proteins resulting from oxidant-induced protein aggregation promote adverse changes in meat quality, including texture and color. Considering the effect of packaging on the oxidation of meat proteins, far too little attention has been paid to packaging in a high-oxygen atmosphere. Therefore, further research should be conducted on the effect of PA consumption on health and functioning in the human body. In this regard, it is necessary for medical and food science researchers to work together to gain a comprehensive understanding of this issue.

■ ASSOCIATED CONTENT

Data Availability Statement

Data will be made available on request.

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Notes

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