Impacto de métodos alternativos de preservación de carne de novillos de diferentes sistemas de terminación sobre los atributos de calidad

Daniela Correa Nacimiento

Doctora en Ciencias Agrarias

Abril, 2025

Impacto de métodos alternativos de preservación de carne de novillos de diferentes sistemas de terminación sobre los atributos de calidad

Daniela Correa Nacimiento
Doctora en Ciencias Agrarias

Abril, 2025

Tesis aprobada por el tribunal integrado por Bach.Quím. (PhD.) Caterina Rufo, PhD. Nuria Panella-Riera e Ing. Agr. (PhD.) Fabio Montossi, el 16 de mayo de 2025. Autora: Ing. Agr. (MSc.) Daniela Correa. Director: Ing. Agr. (PhD.) Gustavo Brito. Codirectora: Ing. Agr. (PhD.) Marcia del Campo.

Agradecimientos

A INIA, por la financiación de este doctorado mediante una beca a la excelencia académica. A ANII por ser parte, junto a INIA, del financiamiento de mi pasantía en Teagasc (Irlanda). A Teagasc y todo el equipo que me hospedó por dos meses. A Fagro, por habilitar el título.

A mis tutores, Gustavo Brito y Marcia del Campo, mi agradecimiento especial por compartir amable y generosamente su calidez y enseñanza.

A Carlos Álvarez por formar parte del Comité de Seguimiento y ser de apoyo constante en todos los aspectos relacionado temas de la tesis, logísticos y académicos.

A María Font, presente siempre y dispuesta a ayudar aportando su rico conocimiento en los aspectos sensoriales de la carne y estadístico.

A Santiago Luzardo, que, además del apoyo en todas las mediciones realizadas en el laboratorio, ayudó en el diseño experimental, análisis estadístico y microbiológico.

A Carolina Realini, parte del Comité de Seguimiento de la tesis, que estuvo presente cada vez que fue convocada.

A todo el equipo de trabajo del Laboratorio de Tecnología de la Carne de INIA Tacuarembó: Guillermo de Souza, Sandra Alvarez, Adriana Soria, siempre con la mejor onda y buena actitud al trabajo para ayudarme en el experimento que insumió muchas horas de laboratorio.

Al personal de Laboratorio de Forestación de INIA Tacuarembó por el préstamo de las cámaras para la incubación de los microrganismos.

A Zully, a Fío, amigas, colegas en la maestría y al final como doctorandas por haber compartido todos estos años. A Zenia Barrios, por todo su apoyo desde siempre.

Finalmente, y más importantes: Delfi y Valen, mis hijos adorados, Álvaro, mi compañero de vida, por haber estado siempre apoyándome incondicionalmente.

Tabla de contenido

Resumen

Las prácticas de maduración y congelado (preservación) juegan un papel fundamental en la palatabilidad de la carne. Este estudio evaluó el efecto de los métodos de preservación (PM) sobre los atributos de calidad de la carne vacuna de novillos terminados (F) en pastura (P: n = 15) o grano (G: n = 15). Se utilizaron 60 bifes (*longissimus thoracis et lumborum*, *LTL*) de novillos de raza británica y menores de 2,5 años. Los PM fueron maduración seca en bolsa (DAb) y húmeda al vacío (WA) por 40 días + 180 días de congelado (Fr): (DAb + Fr) y (WA + Fr). Además de los métodos de maduración (AM), se consideró la combinación de ambos por un período total de 40 días, sin posterior congelado: DAb por 20 días + WA 20 días (DW) y WA 20 días + DAb 20 días (WD). La luminosidad (*L**) de la carne de los novillos terminados con G fue mayor que la de los terminados a P ($P < 0,01$). Considerando los PM, DAb + Fr mostró los valores más bajos ($P < 0,01$) de *L**, *a** y *b** comparándolo con DAb, WA y WA + Fr. Esta misma diferencia se observó en un mayor recuento de bacterias psicotróficas (PSY) y *Enterobacteriaceae* (ENT) que WA + Fr, DAb y WA ($P < 0,01$). Respecto a los AM, la WD presentó mayor recuento de bacterias PSY y bacterias totales (TBC) ($P < 0,05$) que los otros tres tratamientos (DAb, WA y DW). Una mayor concentración de ácidos grasos poliinsaturados (AGPI y AGPI n6) y ácido conjugado linoleico (ACL) ($P < 0,05$) fue observada en todos los AM de carne novillos terminados en P, mientras que los saturados y monoinsaturados (AGS y AGMI) fueron mayores en DW y WD de G ($P < 0,01$). Los análisis sensoriales reportaron calificaciones aceptables para todos los PM, presentando la DAb mayores concentraciones de aldehídos, lo que se asocia a sabores intensos de la carne, de preferencia en algunos mercados de excelencia. La WA podría satisfacer la demanda de los consumidores por terneza y jugosidad. Ambas ofrecen estrategias valiosas para producir carne de alta calidad para los mercados nacionales y de exportación. Diferentes métodos de maduración seguidos por un período de congelado podrían ser usados para exportar carne con atributos de calidad requeridos por el consumidor.

Palabras clave: vacunos, dieta de terminación, maduración seca en bolsa, congelación, atributos de calidad.

Summary

Aging and freezing practices play a fundamental role in meat palatability. This work studied the effects of preservation methods (PM) on meat quality attributes, from steers finished on pasture (P: n=15) or grain (G: n=15). 60 striploins (*longissimus thoracis et lumborum, LTL*) from British breed steers younger than 30 months were used. The PM assessed dry aging in a bag (DAb) and wet aging (WA) for 40 days, followed by frozen storage (Fr) for 180 days, in two conditions: (DAb+Fr) and (WA+Fr). Additionally, aging methods (AM) included combinations of both for a total period of 40 days without subsequent freezing: DAb for 20 days + WA for 20 days (DW) and WA for 20 days + DAb for 20 days (WD). Lightness (L^*) meat from G-finished steers was lighter than that on P-finished ($P<0.01$). Considering PM, DAb+Fr samples had the lowest L^*, a^*, and b^* values ($P<0.01$) compared to DAb, WA, and WA. This difference was also observed in higher counts of psychrotrophic bacteria (PSY) and *Enterobacteriaceae* (*ENT*) than WA+Fr, DAb, and WA ($P<0.01$). Regarding AM, WD presented higher counts of PSY and total bacteria (TBC) ($P<0.05$) than the other three treatments (DAb, WA, and DW). Greater concentrations of polyunsaturated fatty acid (PUFA, PUFA n6) and conjugated linoleic acid (CLA) ($P<0.01$) were observed in all AM from P-finished and in WD from G-finished steers. At the same time, saturated (SFA) and monounsaturated (MUFA) were greater in DW and WD from G-finished steers ($P<0.05$). Sensory analyses reported acceptable ratings for all PM, with DAb presenting higher aldehyde concentrations associated with intense meat flavors preferred in some premium markets. WA could satisfy consumer demand for tenderness and juiciness. Both offer valuable strategies for producing high-quality meat for domestic and export markets. Different aging methods, followed by a freezing period, can be used to export meat with the quality attributes that consumers require.

Keywords: beef, finishing diet, dry aging bag, freezing, quality attributes

1. Introducción

1.1. Contexto

El éxito de la cadena cárnica vacuna se fundamenta en proporcionar productos de alta calidad para satisfacer la demanda de los consumidores más exigentes. Desde un enfoque fucional y sensorial, la calidad de la carne hace referencia a los atributos que satisfacen las preferencias del consumidor, tales como la terneza, la jugosidad, el sabor, el color y la inocuidad (Huffman et al., 1996). La disposición a pagar más por un producto cárnico está impulsada por su palatabilidad, donde la terneza, la jugosidad y el sabor son los atributos más relevantes (Igo et al., 2013; Smith et al., 2008). Las prácticas de procesamiento de la carne, en particular la maduración y el congelado, cumplen un papel fundamental en su color y palatabilidad (Jeremiah et al., 1972).

1.1.1. Métodos de preservación de la carne

Diversas técnicas de preservación de la carne han sido desarrolladas para satisfacer la demanda del crecimiento de la población humana. Desde tiempos antiguos, los humanos han utilizado una variedad de técnicas para preservar el alimento y luego consumirlo (Pal y Devrani, 2018). La carne deteriorada pierde su textura, sabor y valor nutricional, y se produce un alimento no inocuo para consumo humano. En ausencia de técnicas de preservación adecuadas, el deterioro, la actividad microbiana, las reacciones enzimáticas y químicas y los cambios físicos son inevitables. Por lo tanto, la carne es preservada usando una variedad de métodos que incluyen enfriado o refrigeración, congelado, curado, ahumado, enlatado, procesos térmicos, deshidratación, irradiación, uso de químicos y aplicación de presión (Cassens, 1994; Cheftel, 1995; Norton et al., 2009; Zhou et al., 2010). Las estrategias de conservación mas utilizadas en carnes de exportación son el enfriado y el congelado.

1.1.1.1. Maduración de la carne y sus distintas formas

La maduración es el proceso inmediato al establecimiento del *rigor mortis* por el cual la carne mejora su terneza mediante la acción del sistema proteolítico propio del músculo. El proteoma constituye el componente principal del tejido muscular y

desempeña un papel fundamental en los cambios bioquímicos que ocurren durante la transformación del músculo en carne, durante el período post-mortem temprano y a lo largo del proceso de maduración de la carne. Este proceso influye sobre otros atributos como el color, la estabilidad oxidativa (Vitale et al., 2014), los compuestos volátiles precursores del sabor y la capacidad de retención del agua (CRA) (Huff-Lonergan y Lonergan, 2005). De esta forma, afecta la calidad y el valor comercial de la carne (Kim et al., 2018). Las condiciones óptimas para la maduración y el efecto que presentan algunos factores sobre las condiciones intrínsecas del producto final no han sido enteramente comprendidas. Es sabido que la duración, así como las condiciones de maduración de la carne, tienen un efecto diferencial sobre su calidad, dependiendo de factores tales como la especie animal, la raza, la edad, la dieta, el tipo de músculo y el grado de marmoreo o *marbling* (Kim et al., 2018). Por otro lado, los factores que más influyen en la vida útil de la carne refrigerada son la carga inicial de microrganismos, las condiciones de temperatura y humedad y el tipo de envasado (Nethra et al., 2023). Por lo tanto, para asegurar una calidad sensorial óptima, los procesos de maduración deben diseñarse a medida, considerando todos estos factores.

Tradicionalmente, la maduración se lograba dejando la canal, los cuartos de la canal o el corte de carne en una cámara en condiciones controladas de temperatura, humedad relativa y velocidad del aire. Al surgir la herramienta del envasado al vacío, los cortes seleccionados se pudieron madurar controlando la atmósfera dentro del envase y prevenir contaminación del ambiente. Tambien resultó en mejoras en el rendimiento, el procesamiento, el transporte y la vida útil de la carne. Actualmente, estos métodos de maduración son los que se continúan practicando en la industria frigorífica por los procesadores y vendedores minoristas. Ellos son los denominados *maduración seca* (*dry aging*: DA) y *maduración húmeda* (*wet aging*: WA). Ambos procesos mejoran la terneza de la carne, pero el sabor es el atributo dominante en el cual radica la diferencia entre ambos tipos de maduración (Iida et al., 2016; Kim et al., 2016).

Mientras que la WA consiste en el envasado al vacío y conservación de cortes de carne entre 0 °C y 3 °C de temperatura hasta congelarse o ser consumida, la DA tradicional o convencional consiste en el madurado de canales enteras o cortes, sin

envasado y conservados entre 0 °C y 3 °C con estricto control de temperatura, humedad y velocidad del aire. El período puede ir de 10 a 28 días e incluso más (Kim et al., 2018), con un menor rendimiento a medida que dicha duración es mayor. Esto es debido a la disminución de peso por pérdidas de humedad y recortes, pero que, por otro lado, produce una carne con sabor agregado único (Dashdorj et al., 2016). Se ha demostrado que una intensidad en la percepción del sabor de la carne vacuna (umami) es mayor con elevados niveles de *marbling* (equivalente a *abundant* en la escala USDA) y madurada en seco (Iida et al., 2016). Sin embargo, Berger et al. (2018) encontraron que la DA puede mejorar la calidad sensorial de la carne proveniente de animales alimentados con pastura y con bajo *marbling* (Slight, escala USDA), sin impactar negativamente sobre los aspectos microbiológicos.

Si bien el envasado al vacío se utiliza típicamente en WA, la industria cárnica ha introducido un sistema integrado de ambos procesos conocido como *maduración seca en bolsa* (*dry aging bag*: DAb). Este sistema utiliza bolsas altamente permeables al vapor de agua y produce un efecto como la DA tradicional. Sin embargo, mejora el rendimiento del corte (menores pérdidas de peso), limita la contaminación microbiana y proporciona eficiencia en la gestión del proceso, sin afectar la calidad del producto (Ahnström et al., 2006).

Los resultados obtenidos de los efectos de la maduración (DA y WA) sobre la calidad de carne han sido inconsistentes. Kim et al. (2017), combinando los dos procesos de maduración (las canales maduradas en seco y los bifes en húmedo), encontraron una mejora en el rendimiento de los cortes y una reducción de los costos operativos y de capacidad de frío sin comprometer el impacto positivo en la palatabilidad al consumo de la DA. Este sistema combinado se denomina *stepwise* (SW).

1.1.1.2. Congelado de la carne

El congelado es la técnica óptima para preservar la calidad original de la carne fresca. Previene el crecimiento microbiano e inhibe la actividad enzimática; además mantiene el mayor contenido de nutrientes durante el congelado (temperaturas -10 a -18 °C), con una pequeña pérdida durante el proceso de descongelado. Es uno de los

métodos más comunes y eficientes para preservar, almacenar y comercializar carne de alta calidad, evitar la estacionalidad de la oferta e incrementar la disponibilidad en el mercado de las carnes maduradas (Kim et al., 2017). Sin embargo, en la carne congelada se inician varios cambios físicos-químicos que podrían conducir al deterioro de la calidad. Por lo tanto, es reducida su percepción de aceptabilidad en general al ser comparada con la enfriada (Farouk et al., 2003). Estudios recientes han encontrado que los efectos adversos del congelado sobre la calidad de la carne pueden ser reducidos mediante una maduración adecuada previa al congelado, manteniendo los beneficios de esta última sobre la calidad (Farouk et al., 2012; Kim et al., 2011, Rehman et al., 2024).

1.1.2. Efecto de la dieta de terminación del animal

Múltiples factores influyen en la aceptabilidad global de la carne vacuna por parte del consumidor. Factores de producción animal como la edad a la faena, la nutrición, la genética, sexo/castración, así como los factores *post mortem*, influyen en la palatabilidad de la carne vacuna. De particular importancia es la dieta animal, la cual es conocida por el rol fundamental que juega e influye en las características de la canal y en los atributos de calidad de los productos cárnicos (French et al., 2001; Calkins y Hodgen, 2007). La dieta de terminación a base de granos mejora el sabor de la carne comparado con la carne de animales terminados en pasturas, principalmente debido a un aumento en la deposición de grasa intramuscular (GIM) o *marbling* (Schroeder et al., 1980; Hedrick et al., 1983). Los panelistas sensoriales a menudo utilizan términos como *sabor a pescado, a caza, a hierba* o *a leche* para describir los productos de carne vacuna provenientes de animales alimentados con pasto, en contraste con el término *grasa vacuna,* utilizado para describir la carne de animales alimentados con granos (Melton et al., 1990; Larick y Turner, 1990). Sin embargo, la investigación (Daley et al., 2010) sugiere que las dietas basadas en pasto pueden mejorar significativamente la composición de ácidos grasos (AG) y el contenido de antioxidantes de la carne bovina, aunque con impactos variables en la palatabilidad general. Las dietas a base de pasto mejoran los isómeros totales de ácido linoleico conjugado (CLA) (C18:2), el ácido transvaccénico (TVA) (C18:1 t11), un precursor

del CLA, y los AG omega-3 (n-3), en términos de g/g de grasa. Sin embargo, para nuestro trabajo en particular nos interesa estos últimos como precursores del acido graso linolénico (C18:3 n3) por ser un importante precursor del sabor y aroma de la carne. En otro aspecto, la proteómica ha sido reconocida por la mayoría de los investigadores, porque permite una mejor comprensión de los mecanismos de interés en la producción animal, incluidos los atributos de calidad de la carne. Estas moléculas (proteínas) impactan en los atributos de calidad, pero más importante aún, actúan como precursores del sabor (Kim et al., 2018).

1.1.3. Situación en Uruguay

El Uruguay exporta el 80 % de la carne bovina que produce, el 86 % de esta en forma de carne congelada y el 11 % enfriada. Esta última se exporta en su totalidad envasada al vacío (o madurado en húmedo), con un valor económico superior al de la carne congelada (INAC, 2020; INAC, 2019; MGAP, 2019). Aunque no existe una normativa específica que regule el proceso de la maduración en húmedo de manera detallada, se aplican las disposiciones generales del Reglamento Bromatológico Nacional y las normativas del Instituto Nacional de Carnes (INAC, 2023) relacionadas con la manipulación, almacenamiento y comercialización de carnes refrigeradas. El manejo de la carne congelada está regulado por las mismas normativas y en reglamento citado para la carne refrigerada, que establecen requisitos para su almacenamiento, transporte y comercialización, con el objetivo de garantizar la inocuidad y calidad del producto.

La investigación nacional se ha enfocado en evaluar el efecto de la maduración húmeda sobre la calidad de la carne (Brito et al., 2014; Franco et al., 2009) considerando además el sistema de terminación de los animales y las prácticas de manejo previo a la faena (Del Campo et al., 2010; Brito et al., 2009; Del Campo et al., 2008). También se ha determinado el valor nutricional (Cabrera et al., 2010; De la Fuente et al., 2009; Realini et al., 2004), la aceptabilidad del consumidor (Del Campo et al., 2018; Campo et al., 2006) y la calidad sensorial (Font-i-Furnols et al., 2006) de la carne, utilizando en todos los estudios el método de la maduración húmeda.

Sin embargo, en los últimos años ha habido un mayor interés en el proceso de maduración seca por parte de proveedores y minoristas en los países occidentales (Hanagasaki y Asato, 2018). Más recientemente, muchos de los países asiáticos (58 % del destino de exportación del Uruguay en 2020) han mostrado gran interés por este tipo de maduración (Dashdorj et al., 2016). Ademas este interés, aunque en volumen pequeño se ha desarrollado en el mercado interno. Las normativas de maduración en seco de carne en Utruguay, también se encuentran en el Reglamento Nacional de Carnicerías (Decreto N° 31/021, apartado 4.4.7 y 4.4.8). Las condiciones de maduración deben cumplir con los siguientes rangos de especificaciones: temperatura de -1°C a 4°C, humedad relativa menor o igual a 85% y velocidad de aire mayor o igual 0,5 m/s.

En este contexto, buscando alcanzar un producto prémium, la propuesta es evaluar los distintos sistemas de maduración considerando la alternativa de posterior congelado en carne de animales terminados a corral y en pasturas sobre aspectos fisicoquímicos, microbiológicos y sensoriales vinculados a la calidad de la carne. Los tipos de maduración a evaluar son húmedo (envasado al vacío), seco en bolsa y combinando (*stepwise*) en el mismo período de cuarenta días.

1.2. Hipótesis

Los métodos alternativos de preservación de carne (maduración seca en bolsa con o sin posterior congelado y el proceso *stepwise*) mejoran los atributos de palatabilidad como el sabor, en la carne de novillos provenientes de sistemas de engorde de pasto o de grano, al ser comparada con la maduración húmeda con y sin posterior congelado.

1.3 Objetivos

1.3.1. Objetivo general

Evaluar el efecto de diferentes procesos de maduración (seca en bolsa, húmedo y *stepwise*) con y sin posterior congelado sobre las características fisicoquímicas, microbiológicas y sensoriales de la carne de proveniente de novillos de dos sistemas de engorde: pasto vs. grano.

1.3.2. Objetivos específicos

1. Determinar el efecto de dos tipos de maduración, seca en bolsa vs. húmedo, durante un período de cuarenta días y posterior congelado durante 180 días, sobre las características de color, pérdidas por cocción, fuerza de corte, perfil y oxidación lipídica, microbiológicas y sensoriales (panel de consumidores) de la carne proveniente de novillos de dos sistemas de engorde (pasto vs. grano).

2. Determinar el efecto de dos secuencias de maduración (*stepwise*), veinte días seca en bolsa + veinte días húmeda vs. veinte días húmeda + veinte días seca en bolsa, comparado con los métodos en forma individual, sobre las características de color, pérdidas por cocción, fuerza de corte, perfil y oxidación lipídica, microbiológicas y sensoriales (panel de consumidores) de carne proveniente de novillos de dos sistemas de engorde (pasto vs. grano).

3. Determinar el efecto de dos tipos de maduración, seca en bolsa vs. húmedo, durante un período de cuarenta días, sobre los compuestos volátiles del sabor y su relación con los atributos sensoriales (panel de entrenados) de la carne proveniente de novillos de dos sistemas de engorde (pasto vs. grano).

4. Determinar el efecto de dos tipos de maduración, seca en bolsa vs. húmedo, durante un período de cuarenta días, sobre la proteómica del musculo *longissimus thoracis et lumborum* (*LTL*) proveniente de novillos de dos sistemas de engorde (pasto vs. grano).

1.4. Estructura general de la tesis

El capítulo 1 desarrolla la introducción al tema, la hipótesis, los objetivos y el esquema general de la tesis (Figura 1). La figura 1 esquematiza como se realizó (diseño) el trabajo para lograr los diferentes obejtivo planteados.

El Capítulo 2 ofrece un marco teórico robusto para entender por qué y cómo los métodos de maduración afectan la calidad de la carne. Los Capítulos 3 (OE-1) y 4 (OE-2) representan el eje central del experimento, donde se comparan los distintos métodos de preservación y maduración bajo dos dietas de terminación, evaluando variables fundamentales como color, terneza, ácidos grasos, oxidación lipídica, recuento microbiológico y como influyen en la aceptabilidad sensorial (sabor, terneza

y aceptabilidad global). En el Capítulo 5 (OE-3) se profundiza en la relación química-sensorial, explicando por qué ciertos métodos de maduración combinados con diferentes dietas de terminación resultan en sabores distintos a través del análisis de compuestos volátiles. Finalmente, el Capítulo 6 (OE-4) aborda el nivel más profundo haciendo una caracterización de los mecanismos moleculares subyacentes mediante proteómica, que podrían estar explicando los resultados encontrados, mediante las transformaciones musculares (músculo *Longissimus Thoracis et Lomborum*: LTL) post-mortem. En el Capítulo 7 unifica toda esta evidencia experimental para discutir cómo cada variable contribuye o no a validar la hipótesis central. Este diseño permite abordar la hipótesis de manera sólida y multidimensional, posicionando la tesis como un estudio completo que conecta tecnologías de maduración con impacto sensorial, microbiológico, químico y molecular de la carne.

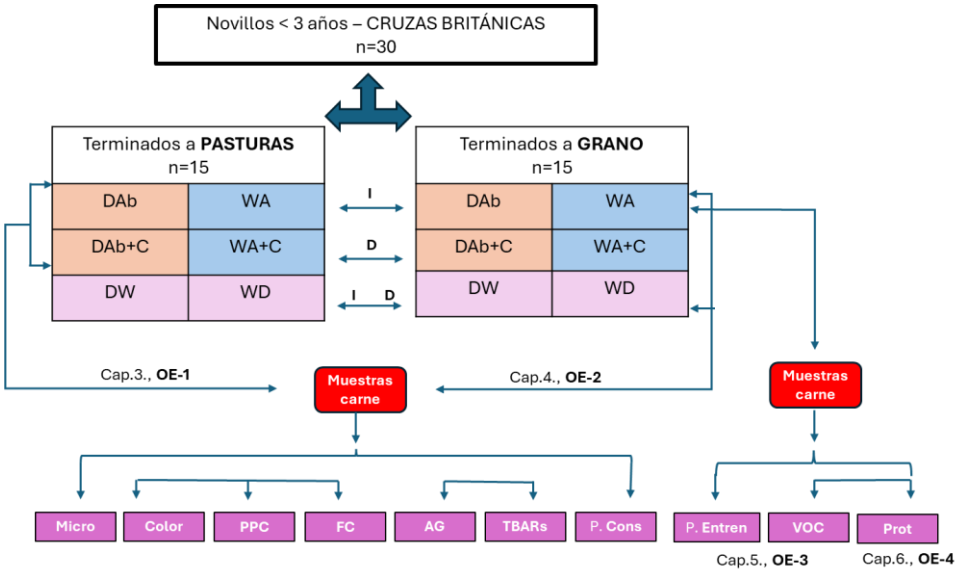


Figura 1. Esquema general de la estructura de la tesis de acuerdo con los factores estudiados: dieta de terminación (Pastura y Grano) y los métodos de preservación (**DAb**: maduración seca en bolsa- 40 días; **WA**: maduración húmeda- 40 días; **DAb+C**: DAb + congelado- 180 días; **WA+C**: WA + congelado- 180 días) y los métodos de maduración (DAb; WA; **DW**: DAb- 20 días + WA- 20 días; **WD**: WA- 20 días + DAb- 20 días). **I**: Bife izquierdo; **D**: Bife derecho. Las variables estudiadas fueron: **Color**, pérdidas por cocción (**PPC**), fuerza de corte (**FC**), perfil de ácidos grasos (**AG**), oxidación lipídica (**TBARs**), conteo microbiologico de superficie (**Micro**) y panel de consumidores (P. **Cons**.), compuestos volátiles (**VOC**), proteómica (**Prot**) y panel de entrenados (P. **Entren**)

En el capítulo 2 se presenta una revisión bibliográfica, a la cual se le otorgó mención por parte del Comité de Seguimiento en la defensa del proyecto de doctorado y fue sugerida para una publicación científica en la modalidad *review*. La misma será enviada a la revista arbitrada *Latin American Archives of Animal Production* (ALPA).

Los capítulos 3, 4, 5 y 6 desarrollan los objetivos especificos de la tesis 1, 2, 3 y 4, respectivamente.

El capítulo 3 contiene el artículo publicado en la revista arbitrada *Meat and Muscle Biology*. El objetivo fue aportar información sobre el efecto de dos tipos de maduración de la carne proveniente de animales terminados en dos tipos de dietas (pastura y grano), sobre las características fisicoquímicas, microbiológicas y sensoriales fueron registradas y analizadas en Uruguay (INIA Tacuarembó). https://doi.org/10.22175/mmb.17695

El capítulo 4 contiene el artículo publicado en la revista arbitrada *Meat and Muscle Biology*. El objetivo fue adicionar un novedoso método de maduración donde, además de estudiar los dos métodos anteriores, se evaluó una combinación de los dos (*stepwise*) en el mismo período de tiempo. https://doi.org/10.22175/mmb.18055

El capítulo 5 refiere a los compuestos volátiles del sabor y su relación con los atributos sensoriales de la carne. También será enviado a la revista arbitrada *Meat and Muscle Biology*.

En el capítulo 6 se presentan los resultados del análisis de proteómica efectuado en Teagasc (Irlanda) (al igual que el de volátiles). Se ha determinado enviar este artículo a la revista arbitrada *Food Research International*.

En el capítulo 7 se desarrolla la discusión general de todos los resultados abordado en el trabajo de tesis, para lograr una síntesis conjunta.

El capítulo 8 plantea las principales conclusiones de la tesis y, por último, en el capítulo 9 se presenta la bibliografía usada en los capítulos 1 y 7.

2. Aging methods on quality meat attributes: a review

Daniela Correa [1]*, Maria Font-i-Furnols[3], Marcia del Campo [1], Carlos Álvarez [2], and Gustavo Brito [1]

[1]INIA-Sistema Ganadero Extensivo, INIA Tacuarembó, Ruta 5, km 386, Tacuarembó, Uruguay

[2]Teagasc-Food Quality and Sensory Science Department, Teagasc Ashtown Food Research Centre, Ashtown, D15KN3K Dublin 15, Ireland

[3] IRTA- Food Technology, Granja Camps i Armet, 17121 Monells (Girona), Spain

*Corresponding author. Email: dcorrea@inia.org.uy

2.1. Introduction

Aging refers to the meat tenderization process that occurs through the action of endogenous muscle enzymes found in living animals, which assume a different role in post-mortem meat. Most of this factor tends to emerge in the biochemical changes in tissues during the post-mortem period, which begins immediately after the harvesting process starts and continues until the carcass is fabricated into wholesale or retail cuts (England et al., 2013). The two primary contributors to the muscle transformation of meat are fluctuations in tissue temperatures and pH, primarily due to the biochemical changes that take place post-mortem in the muscle (Graser, 2001).

Muscle pH values decline from a physiological pH of nearly 7.4 in living beef animals to around 5.4 or lower in the fresh meat cuts of most animals (Lomiwes et al., 2014). Temperature is also critical for preserving biological integrity. Body temperature and that of the corresponding carcass change from the homeostatic set point of approximately 38°C in living animals to very close to 0°C 24 hours after slaughter (Huff-Lonergan et al., 1996).

During the aging period, many changes occur in the properties of meat: the first occurs at the ultrastructural level in the muscle myofibril. The cathepsins were the first enzymatic system considered in studies focused on the mechanisms of meat

tenderization. Calpains received much more attention than cathepsins primarily due to their ability to alter the Z-line density, a modification often observed post-mortem, even though this change is not correlated with tenderness (Taylor et al., 1995). More recently, several lines of evidence have supported the potential role of the 20S proteasome in this process (Ouali et al., 2006; Taylor et al., 1995). Z disc degradation leads to weakening, fragmentation, and degradation associated with specific proteins, including desmin, nebulin, and titin, resulting in the formation of new polypeptides. However, the larger contractile proteins, myosin and actin, remain unaffected, even after 56 days of postmortem aging (Goll et al., 1991).

Immediately after slaughter, beef has been reported to elicit a sour taste and possess little beefy flavor even if cooked (Shahidi, 1994). Improvement of meat taste and flavor involves increased free amino acids and peptides in meats during post-mortem aging. Especially, the increase in free amino acids is thought to contribute to the enhancement of brothy taste, including umami flavor, while an increase in peptides is responsible for imparting mildness. The increase in peptides is caused by the action of cathepsins B and L, and calpains on muscle proteins, while the increase in free amino acids is caused by the action of aminopeptidases C, H, and P on the peptides during post-mortem aging (Nishimura, 1998).

Aging is influenced by the live animal's history before slaughter, including the effects of pre-slaughter stress; it can be affected even more significantly by the muscle's rigor temperature and the duration of aging. Additionally, the rate of aging varies both between and within muscles across different species, and even within the same animal (Devine and Dikeman, 2004). Post-mortem aging is a value-adding process that has been widely practiced by the global meat industry for years. Various aging methods are employed, ranging from traditional carcass hanging to packaging sub-primals or portion cuts in vacuum bags, followed by refrigerated storage for a specified period (Kim et al., 2018).

This review provides an overview of the techniques used to age meat and their effects on various meat attributes.

2.2. Packing options and preservation methods

There are numerous packaging options for raw, chilled, and processed meat that enhance the desired properties for storage and display. Most packaging options have been previously reviewed (McMillin, 2008), including air-permeable, vacuum, and modified atmosphere (MAP) systems with low or high oxygen levels. Air-permeable packaging uses films with holes, pores, or perforations that allow oxygen to diffuse from the atmosphere, leading to oxymyoglobin formation on the surface of the meat. Vacuum packaging employs negative pressure to remove ambient air by sealing formed packages while maintaining the vacuum state. Modified atmosphere packaging eliminates the gaseous environment and replaces it with a preferred gaseous atmosphere, typically involving blends rather than a single type of gas (McMillin, 2008).

While the food industry generally trends toward developing packaging materials with high barrier properties to extend shelf life, a novel and unconventional innovation has emerged: a plastic material designed with the opposite approach. Traditional high-quality foods—preserved through salting, drying, and smoking—often struggle to meet modern demands for rapid processing, enhanced food safety, and high product yields. A new type of food-processing bag has been introduced to address these challenges. This material features lower gas barrier properties, a high water vapor transmission rate, and a secure bacterial seal, making it particularly suitable for certain traditional products. Nonetheless, within the meat industry, the most commonly used aging methods—both by small-scale processors and large commercial packers—remain dry aging and wet aging (Ahnström et al., 2006).

2.2.1. Dry aging

Dry aging is a traditional method that involves storing carcasses, primals, or subprimals in cold rooms without protective packaging at temperatures of 0-3°C for several weeks (Savell, 2008). This method is commonly employed by small to medium-sized meat processors and purveyors serving upscale hotels, restaurants, and gourmet markets. Dry-aging is known to enhance the palatability attributes of meat, especially a unique dry-aged flavor producing descriptive determinants such as brown-

roasted, beefy/brothy, buttery, nutty, roasted nut and sweet (Campbell et al., 2001; Kim et al., 2016; O'Quinn et al., 2012; Warren and Kastner, 1992). Dry aging is considered an expensive process due to the higher product weight loss resulting from shrinkage and moisture loss, as well as labor costs associated with the aging process (Parrish et al., 1991; Savell, 2008).

2.2.2. Wet aging

In contrast to dry aging, wet aging is the dominant method for post-mortem aging. It is particularly effective when cuts are well vacuum-packaged and maintained at temperatures between -1 and 2 °C. Its benefits include a significant reduction in product weight loss, minimized trim loss, space efficiency, prolonged shelf-life while retaining palatability, decreased operational facility costs, and compatibility with automation and efficient product flow (Kim et al., 2018). Wet aging enhances tenderness but results in undesirable flavor characteristics such as bloody, serumy, metallic, and sour (Warren and Kastner, 1992).

2.2.3. Dry-aged bag

The dry-aging bag is an innovative packaging technology introduced in recent years that combines key features of both wet aging and traditional dry aging (Ahnström et al., 2006; DeGeer et al., 2009). This method allows for the development of dry-aged flavor characteristics while providing significantly higher saleable yields, primarily due to reduced weight loss during aging and decreased trim loss after aging (Dikeman et al., 2013; Eastwood et al., 2016). Dry-aged beef sub-primals in the specialized bag system showed similar sensory traits as traditionally dry-aged counterparts, while achieving substantially higher saleable yield through lower weight loss during aging, reduced trim loss after aging, and decreased microbial contamination (Ahnström et al., 2006; DeGeer et al., 2009; Dikeman et al., 2013; Stenströmet et al., 2014).

2.2.4. Stepwise aging

The stepwise aging system combines both methods mentioned, using the dry-aging method first on the carcass and then wet-aging in beef sub-primal cuts (Kim et al., 2017), or in cuts packaged in a water-permeable bag after wet-aging in a water-

impermeable barrier bag. Another stepwise option is to change the order of the methods by aging steaks, wet first and then dry (Ha et al., 2019; Campbell et al., 2001). Stepwise aging (dry-wet or wet-dry) could serve as an easily applicable processing strategy that might increase the saleable yield of dry-aged meat while reducing energy and operating costs compared to conventional carcass dry-aging, without compromising any of the positive impacts of dry-aging on eating quality attributes.

2.2.5. Freezing

Freezing is a preservation process where meat is cooled to temperatures below standard cooling (-1 °C), resulting in ice crystal formation. The freezing of large tissue pieces typically occurs under thermal gradients, with high freezing rates in the outer regions in contact with the refrigerant medium and decreasing toward the thermal center of the sample. Bevilacqua et al. (1979) and Bevilacqua and Zaritzky (1980) performed histological analyses of frozen meat tissues under conditions where heat flow was either parallel or perpendicular to the muscle fibers. Their findings revealed that intracellular ice formation took place only within a narrow zone adjacent to the area in direct contact with the cooling medium, where high freezing rates were applied. The authors defined the freezing rate (tc) as the time required to lower the temperature at a specific point in the tissue from −1 °C (the onset of freezing in beef) to −7 °C, at which approximately 80% of the water is converted to ice. As the freezing rate decreased, intracellular ice formation was no longer observed; instead, extracellular ice columns developed at the expense of intracellular water. This dehydration process led to distortion and irregularity in the shape of the muscle fibers.

Many studies have reported that freezing can increase meat tenderness before or after aging (Grayson et al., 2014; Lagerstedt et al., 2008; Shanks et al., 2002). However, freezing and thawing can increase weight loss by purging and reducing the sensory quality of meat due to lipid and protein oxidation caused by damaged muscle cells and tissues that release pro-oxidative enzymes (Kim et al., 2017). Other studies have examined the effect of freezing rate and the temperature at which meat is stored on meat quality (Boles and Swan, 1996; Petrovic et al., 1993). Although these results are not unanimous, they all agree that a higher freezing rate and a lower storage

temperature improve meat quality. However, Farouk et al. (2003) indicated that freezing at rates higher than those typically achieved through blast freezing (rapid air cooling) and storing at temperatures below -18 ° C may have little or no effect on the quality of frozen meat.

2.3. Effects of aging methods on Quality meat Attributes

Although inconsistent sensory results exist in the scientific literature, several groups have previously reported positive impacts of different aging methods on the eating quality attributes of beef loins, as summarized in Table 1.

Significant improvements in meat palatability attributes occur through cytoskeletal myofibrillar protein degradation by endogenous proteases during the aging process (Lepper-Blilie et al., 2016; Kemp et al., 2010; Huff-Lonergan and Lonergan, 2005; Kristensen and Purslow, 2001; Spanier et al., 1997).

Tenderness, juiciness, and flavor are currently the significant attributes of beef palatability, and tenderness is considered the most influential (Miller et al., 2001; Savell et al., 1987). However, to improve meat quality, significant changes occurred in other attributes, such as color, lipid and protein profiles, and microbial properties, during aging.

2.3.1. Tenderness

Both trained sensory panels and instrumental methods provide reliable assessments of relative differences in meat tenderness. The acceptability of a given level of tenderness can only be determined by the ultimate users: the consumers. More data is needed on the relationship between consumer impressions of meat tenderness and objective measures of tenderness (AMSA, 2016).

Most studies report no significant differences in shear force between the various aging methods, irrespective of cut type (short loins, strip loins, bone-in shell, boneless), marbling score (slight, small, moderate, modest), or gender (steers, heifers, cow) (Berger et al., 2018; Iida et al., 2016; Gudjónsdóttir et al., 2015; DeGeer et al., 2009; Smith et al., 2008; Dikeman et al., 2013). Furthermore, other studies have indicated that tenderness is likely influenced by the duration of the process, regardless

of the aging method employed. In this context, Lepper-Blilie et al. (2016) and Kahraman and Gurduz (2018) reported that tenderness improved linearly with the length of aging post-slaughter without statistical improvement (differences) attributable to the aging method during the period extending beyond 21 d and 28 d. Lepper-Blillie et al. (2016) compared short loin bone-in (BI) versus striploin boneless (BL), across two marbling scores (Sl50 and Sm50), maturity A50 to A100, in wet (W) and traditional dry (D), and reported that Warner-Bratzler shear force (WBSF) was not affected by aging method or loin type, although bone-in tended to have higher WBSF compared to boneless. Regardless of aging period and loin type, the loin was classified as very tender (<2.8 kgF) and it was noted that dry-aged beef boneless loins (longissimus dorsi) generally exhibited higher tenderness (sensory) than dry-aged bone-in, while wet-aging produced intermediate tenderness for both boneless and bone-in loins. Additionally, the authors pointed out that the slight further decrease in shear force after three weeks of dry aging cannot justify a prolonged aging period when considering the economic and flavor attribute data. Meanwhile, DeGeer et al. (2009) discovered that bone-in loins had higher moisture content than boneless ones, and they demonstrated a small, although not significant, tenderness (sensorial) advantage for dry aging bone-in steaks.

There is limited literature on the effects of stepwise aging (or combined aging) on meat quality attributes, particularly tenderness, and the available findings are somewhat contradictory. For instance, Zhang et al. (2019) reported no significant differences in instrumental tenderness (measured as shear force) between dry-aged beef aged continuously for 21 days and beef aged using a stepwise protocol. In contrast, Kim et al. (2017) found that stepwise aging significantly reduced shear force values (<2.66 kgF; $P < 0.05$) compared to traditional dry-aging methods (2.94 kgF), indicating enhanced tenderness. These divergent results may be attributed to differences in the type of beef used: lean bull beef with lower fat content (Zhang et al., 2019) and higher levels of intramuscular fat (USDA low-choice) (Kim et al., 2017). Such compositional differences could influence how muscle tissue responds to aging conditions and explain the inconsistency in tenderness outcomes.

2.3.2. Flavor

Meat flavor consists of taste and aroma—sensations caused by the chemoreception of compounds present in the meat. Considerable increases in beef flavor occur during aging due to the release of flavor-related compounds. Evaluating the precursors to these flavor compounds, including fatty acids (FA), reducing sugars, and free amino acids (FAA), offers insight into changes in eating quality resulting from different aging parameters (Foraker et al., 2020). However, the flavor of cooked meat originates from thermally induced reactions during heating, primarily the Maillard reaction and the degradation of lipids (Mottram and Elmore, 2010).

The primary precursor of meat flavor can be categorized into water-soluble components and lipid fragments. The primary water-soluble flavor precursors are believed to include free sugars, sugar phosphates, nucleotide-bound sugars, free amino acids, peptides, nucleotides, and other nitrogenous compounds, such as thiamine (Mottram, 1996). The degradation of lipids in cooked meat generates volatile compounds such as aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, and esters. Oxygenated heterocyclic compounds, including lactones and alkylfurans, arise from the oxidation of fatty acid components in lipid molecules (Martins et al., 2000; Yaylayan et al., 2000; Maga, 1994).

The increase in free amino acids in meats during post-mortem aging is mainly due to the actions of aminopeptidases C, H, and P on the peptides produced from meat proteins by means of cathepsins and calpains (Nishimura, 1998). Free amino acids and peptides play significant roles in eliciting the characteristic tastes of foods. Associated with a bitter taste are valine, tyrosine, isoleucine, phenylalanine, tryptophan, and leucine; with a sweet taste, alanine, methionine, glutamine, glycine, glucose, lactate, and succinate; with a sour taste, aspartic and glutamic acid, histidine, asparagine, succinate, and lactate; with a salty flavor, glutamic and aspartic acid; and with a savory/beefy taste (umami taste), glutamate (Glu), aspartate (Asp), carnosine, and inosine monophosphate (IMP) (Nishimura et al., 1988).

The umami/beefy taste and flavor characteristic of aged meat arises from a complex interaction among sulfur-containing amino acids, aspartic acid, glutamic acid, nucleotide compounds, and β-histidyl dipeptides (Dashdorj et al., 2015).

Monosodium glutamate (MSG), monosodium aspartate, and inosinic acid (IMP) are well known to contribution to umami in meat (Yamaguchi and Kirnizuka, 1979). Free amino acids, with the exception of Glu and Asp, do not evoke umami. However, they have been shown to synergistically enhance umami in the presence of IMP and MSG, even when their levels are below the threshold values (Yokotsuka et al., 1969).

Aging decreases the concentrations of glycogen and glucose 6-phosphate significantly from day 4 to day 15 (Meinert et al., 2009), while prolonged aging increases the ribose content of meat (Koutsidis et al., 2008b). As there is no increase in the concentration of total reducing sugars during the first 7 days of aging, but a significant increase afterward, another pool of metabolites could yield sugars upon enzymatic hydrolysis (Koutsidis et al., 2008b). It has been reported that a prolonged (over 28 days) wet aging regime has tremendously increased volatile compounds important to aroma development (Ba et al., 2014). Koutsidis et al. (2008a, 2008b) reported increased levels of 20 out of 22 measured amino acids during the wet aging period of 3-21 days (vacuum packed and stored at 4 °C). The amino acids that presented the most considerable fold change at 21 days compared to 3 days were serine, threonine, leucine, isoleucine, methionine, valine, and tryptophan. In contrast, alanine, b-alanine, glycine, and glutamine remained relatively stable.

According to Kim et al. (2016), the concentrations of eight of the thirty-two metabolites differed significantly between the dry-aged group and the wet-aged control group during 21 days of aging. Tryptophan, phenylalanine, valine, tyrosine, glutamate, isoleucine, and leucine were more abundant in the dry-aged beef samples than in the wet-aged samples, while IMP was more abundant in the wet-aged group. Kim et al. (2016) indicated that the primary contributor to higher levels of many metabolites in the dry-aged group is the concentration following the evaporation of water during the dry-aging treatment. The authors explained that these metabolites are linked to meat flavor as precursors in the Maillard reactions or through the Strecker degradation.

A sensory consumer panel (120 consumers) from New Zealand ranked higher flavor scores in the dry-aged beef compared to the wet-aged beef counterpart, despite the lower levels of IMP (Kim et al., 2016). This observation could suggest that

glutamate (and possibly other free amino acids observed), which was present in higher abundance in the dry-aged beef compared to the wet-aged counterpart, may play a more significant role in influencing consumer preference for beef flavor (Nishimura et al., 1988). The interaction between USDA quality grade (Choice vs. Select) and aging method (wet vs. dry) for the level of beef flavor was reported by Smith et al. (2008). In this study, consumers rated US Choice wet-aged steaks higher than US Select steaks from either the wet or dry treatments. However, dry-aged Select steaks did not differ in level of beef flavor ratings from the dry-aged Choice steaks.

While it is generally agreed that wet or dry aging improves meat flavor, not all studies report a noticeable positive impact of extended aging on meat flavor development (Lepper-Blilie et al., 2016; Brewer and Novakofski, 2008). Lepper-Blilie et al. (2016) found that the aging period affects the overall aged flavor of the loins across different aging methods (dry or wet) and loin types (short loins and strip loins). Panelist responses for loin steaks aged 42 and 49 days indicated higher (1 = extremely bland; 8 = extremely flavorful; AMSA, 2016) aged flavor compared to those aged for 14 and 21 days. Campbell et al. (2001) and Warren and Kastner (1992) discovered that dry aging of beef enhances beefy flavor compared to wet aging, in studies involving USDA Choice and Prime loins (IMF > 6%), revealing a stronger perception of beefy flavor. Spanier et al. (1997) reported that after 4 days (a short aging period) of wet aging at 4 °C, desirable flavor traits such as sweetness and beefy flavor improved, whereas longer aging periods resulted in an increase of undesirable traits like bitterness and sourness. Campo et al. (1999) also found that prolonged wet aging (up to 21 days) heightened the overall flavor intensity of livery odor development in loins from various breeds.

Several hundred volatile compounds derived from lipid degradation have been found in cooked meat, including aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, and esters. In general, these compounds result from the oxidation of the fatty acid components of lipids. Lipid oxidation in meat is a process whereby polyunsaturated fatty acid (PUFAs) reacts with reactive oxygen species (ROS) leading to a series of secondary reactions, which result in lipid degradation and the development of oxidative rancidity (Amaral et al., 2018). Such reactions may lead to

rancid off-flavors during long-term storage; however, in cooked meat, these reactions occur quickly and provide a different profile of volatiles that contribute to desirable flavors. Unsaturated fatty acids undergo autooxidation much more readily than those that are saturated. The autoxidation mechanism of free radicals in nature and the reaction have been extensively reviewed (Mottram and Elmore, 2010).

The extended lipid oxidation in post-mortem meat is highly dependent on the origin of the meat, type of muscle, species, and storage conditions (Estevez, 2015). A study on the muscles of pasture-fed cattle reported the presence of two to three times more PUFAs with three or more double bonds than those of grain-fed cattle. At the same time, there was lower lipid stability, except when there was α-tocopherol supplementation or a high level of antioxidants in the pasture-fed cattle. Although the high content of PUFA in meat is considered desirable from a nutritional point of view, it can affect oxidative meat stability. Dietary antioxidant supplementation is a common way to solve this problem (Li and Liu, 2012). On the other hand, Zhang et al. (2021) indicated that the stepwise (dry bag and wet) aging treatment did not significantly impact the oxidation of lipids and proteins. This may be due to the dry-aging bag barrier function reducing the potential oxidation (lower TBARS than dry-aging).

Generally, the odor threshold values for lipid-derived compounds are much higher than those for the sulfur- and nitrogen-containing heterocyclic compounds in meat volatiles derived from the water-soluble precursors. Therefore, the aroma significance of many of these lipid-derived volatiles is not as great as that for relatively low concentrations of the heterocyclic compounds (Mottram and Elmore, 2010).

Ryu et al. (2018) observed the microbiological composition of dry-aged meat. They noted that changes in the microflora occur during the aging process and that fungi could impact the quality of dry-aged meat, particularly its flavor. The influence of fungi and mold on flavor will be discussed in the section on Superficial Microbial Activity.

2.3.3. Color

Meat color is the most important quality trait that consumers use as an indicator of fresh and healthy food. The color of meat, as perceived by the consumer, is mainly

determined by factors such as the concentration and chemical form of myoglobin, the structure of the muscle tissue, and the muscle's ability to absorb or scatter incoming light (Cornforth, 1994). It is uncommon for all forms of myoglobin in meat to exist in the same state at the same time. Typically, two or more pigments will be present, but the dominant pigment will be the most prominent. For instance, fresh meat in a retail case usually displays the colors of oxymyoglobin and metmyoglobin; however, if oxymyoglobin is predominant, the meat will appear red. In contrast, if 60% of the pigments in a specific area become oxidized to metmyoglobin, that area will look brown (Bendall and Taylor, 1972).

The exact mechanisms by which aging influences the color and oxidative stability of muscles remain undetermined. Several intrinsic factors could be associated with prolonged aging, such as the accumulation of pro-oxidants (e.g., heme and non–heme iron) or the depletion of endogenous reducing compounds or antioxidants (Bekhit et al., 2013; Kim et al., 2011). Madhavi and Carpenter (1993) and Ma et al. (2017) reported decreased NAD+ concentration and metmyoglobin reductase activity in the beef psoas major and longissimus lumborum muscles over 21 days of aging. A decrease in reducing compounds such as α-tocopherol and β-carotene in Argentinian buffalo meat was noted during a 25-day aging period (Descalzo et al., 2008). During simulated retail display, Ma et al. (2017) suggested that instrumental color and oxidative stability are inversely associated with aging but are also muscle-specific.

Color coordinates (CIE L^* a^* b^* color system, 1976) are an objective form (spectro colorimeter) to measure the meat color. The L^*, a^*, and b^* values measure the lightness, redness, and yellowness, respectively. Studies have not found differences in the values of L^* (lightness) and those of a^* (redness) between the dry aging, dry aging bag and wet aging methods observed at 28 days but found an effect of aging time on lightness (Kahraman and Gurbuz, 2019; Li et al., 2013). During extended aging periods, the luminosity decreases in dry bag aging. However, other studies (Dikeman et al., 2013; Bertram et al., 2004) reported differences in luminosity between the three aging methods (wet, dry bag and dry) during 28 d of aging, where bag aging (wet and dry bag) recorded higher L^* values than dry by higher reflectance due to higher moisture content. Furthermore, wet and dry aging (35 days) did not affect

*L**, *a** or *b**, while *L** and *b** were lower and *a** was higher in dry aging and stepwise aging (wet then dry) than wet aging (56 days) (Ha et al., 2019).

2.3.4. Juiciness

Juiciness comes from two sources: moisture released by meat after the first bite and during chewing, and saliva moisture (Winger and Hagyard, 1994). These factors are influenced by the endpoint temperature at which meat is cooked and the intramuscular fat content (Gagaoua et al., 2019). Research has shown a positive impact of aging on the juiciness of beef steaks (Kim et al., 2018; Smith et al., 2008; Campbell et al., 2001; Campo et al., 1999); the improvement in tenderness with aging may have synergistic effects on perceived palatability, such as juiciness.

Berger et al. (2018) found that beef loin steaks from dry bag aging had greater juiciness compared to both dry and wet aging, as determined in a consumer sensory evaluation using meat from 100% grass-fed heifers. Vossen et al. (2022) reported improvements in tenderness and juiciness after three weeks of dry aging (75% RH, 2 and 6 °C). However, no clear trend for juiciness was observed, with the highest score coming at six weeks of dry aging (2 °C; 70 and 90% RH) and the lowest score at nine weeks. DeGeer et al. (2009) noted no differences in juiciness between traditional dry aging and dry bag aging, similar to other descriptive attribute sensory traits. The increase in juiciness with age may be attributed to a loss of water-holding capacity and the release of more juices as the meat is chewed, or it might result from the concentration of fat due to moisture loss during aging (Campbell et al., 2001).

2.3.5. Superficial microbial growth

Surface microbial contamination is the main cause of off-flavor development due to meat spoilage. Dainty et al. (1984) demonstrated that the initial signs of spoilage are characterized by the formation of fruity, sweet-smelling esters, which are subsequently followed by putrid sulfur compounds. Many unpleasant odors stem from the breakdown of proteins and amino acids by anaerobic bacteria. Volatile compounds produced include indole, methanethiol, dimethyl disulfide, and ammonia (Dainty, 1996). A negative correlation between moisture levels and the growth of

microorganisms (total aerobic count, yeasts, and molds) has also been reported (Gudjónsdóttir et al., 2015).

Analyses of the total aerobic bacteria indicated a significant increase in bacteria in traditional dry-aged beef during aging. Similar patterns were observed by Gudjónsdóttir et al. (2015) in yeasts and molds in traditional dry-aged beef. However, the microbial growth rate during dry aging is reduced or inhibited due to the low water activity of the dry-aged beef surface (Dashdorj et al., 2016; Smith et al., 2008). Some studies reported lower or similar total bacterial counts in dry-aged beef compared to wet-aged beef in the meat after trimming off the outer crust (Berger et al., 2018; Hulánková et al., 2018). Hulánková et al. (2018) found that the number of bacteria increased notably during the first two weeks of storage; after that, the count did not change significantly. The authors also explain that the decrease after approximately two weeks of storage could be due to surface drying during the aging period, as the water content decreased significantly. They note that this is produced by the growth inhibition caused by surface drying and storage temperatures low enough to retard growth (Campbell et al., 2001; Bernardo et al., 2020). However, other authors reported that dry-aged steaks present a higher aerobic plate count (APC) than vacuum-packaged ones for 14 days, and the dry aging period did not affect the aerobic count, remaining around 4 log CFU/g. Additional reports indicated APC ≥ 5 log CFU/g on the surface of traditional dry-aged samples aged at RH > 65% and 2 °C (Gudjónsdóttir et al., 2015; Li et al., 2013, 2014). After trimming, Bernardo et al. (2021) reported APC was <3 log CFU/g for 65 and 85% RH treatments.

For psychrotrophic (PSY) counts, the surface of samples aged in a dry-aging bag exhibited higher PSY counts than APC (Bernardo et al., 2021). Meanwhile, the samples from the traditional dry-aging process displayed a significant increase in the PSY count, reaching 6.22 log CFU/g. No difference was observed between the surface and internal dry-aged beef samples from special bags.

Campbell et al. (2001) reported that anaerobic lactic acid bacteria (LAB) increased during vacuum-packaged storage. Meanwhile, LAB counts remained below the detection limit (<1.0 log CFU/g) in dry and dry bag aging processes after 21 days at 65% RH according to Bernardo et al. (2021). Additionally, other studies have

observed that LAB counts decrease in samples dry-aged at relative humidity levels between 50% and 75% (Hulánková et al., 2018; Li et al., 2013; DeGeer et al., 2009; Campbell et al., 2001). However, Li et al. (2014) detected an increase in LAB counts reaching 4.4 and 3.2 log CFU/g after 19 days at 75% RH in samples of dry bags and traditional dry aging, respectively. Ryu et al. (2018) found LAB in both longissimus thoracis (LT) and biceps femoris (BF) muscles after 60 days of dry aging, with an increase in LAB count up to 6 log CFU/g with aging time.

A slight increase in the populations of yeast, psychotropic aerobic bacteria, and mold has been observed in dry-aged beef (Hulánková et al., 2018; Berger et al., 2018; Gudjónsdóttir et al., 2015; Li et al., 2013; Campbell et al., 2001), although these values remained within acceptable microbiological quality counts (less than 5 log CFU/g) (Feiner, 2006).

Samples of the meat surface from the dry aging bag showed higher mold and yeast counts compared to the meat surface samples from the traditional dry aging process. Molds were isolated in 33.3% of dry-aged samples and 66.6% of samples aged in special bags (Bernardo et al., 2021). However, other studies reported higher yeast counts in traditional dry aging than in dry aging using bags (Ahnstrom et al., 2006; DeGeer et al., 2009). Ryu et al. (2018) found that total bacteria and yeast/mold significantly increased in both longissimus thoracis (LT) and Biceps femoris (BF) samples for 50 days, while their growth declined after that period. Penicillium camemberti and Debaryomyces hansenii, which are used in cheese manufacturing, were observed to increase during the dry aging period (40-60 days). D. hansenii has been utilized to metabolize amino acids, produce volatile compounds, and enhance aroma in animal foods as a strategy (Flores et al., 2017). Molds (*Pilaira anomala*) and yeasts (Debaryomyces hansenii) are the primary microorganisms detected on the surface of dry-aged beef, according to Oh et al. (2019). Oh et al. (2019) isolated mold (P. anomala) and yeast (D. hansenii) from dry-aged beef and investigated their impact on flavor precursors, such as the levels of free fatty acids and free amino acids, through the lipolytic and proteolytic activities of the microorganisms. The results suggested that mold and yeast have significant but different roles regarding flavor precursors.

Therefore, the effect of the inoculation ratio of mold and yeast was analyzed to propose better applications of microorganisms for the dry aging process in the industry.

Zhang et al. (2020) hypothesized that the primary proteolytic and lipolytic processes during the early stage (1-2 weeks) of dry-aging are dominated by endogenous enzymes (mainly calpains and lipases). Subsequently, the growth of yeasts and molds introduces exogenous enzymes that trigger the secondary degradation of lipids and proteins over an extended aging period. These enzymatic reactions may result in distinct metabolite profiles (e.g., peptides and amino acids) as flavor precursors on the crust (Zhang et al., 2021; Kim et al., 2020) and in the inner section of the meat (Zhang et al., 2021; Lee et al., 2019), contributing to the development of a characteristic dry-aged flavor.

2.3.5. Proteomic overview

Proteomic changes occur continuously during beef storage or aging as the muscle transitions through and overcomes rigor mortis. Extracted muscle proteins are commonly categorized into two fractions: the sarcoplasmic fraction, which includes water-soluble proteins such as myoglobin, myokinase, and metabolic enzymes, and the myofibrillar fraction, which contains structural proteins like myosin, actin, troponin, and actinin, typically soluble in high-salt solutions or considered insoluble (Kim, Warner, and Rosenvold, 2014; Sierra et al., 2012). Sentandreu, Coulis, and Ouali (2002) noted that tenderness results from the degradation of myofibrillar structures by endogenous peptidases.

Proteomics has been widely utilized in meat science, especially in identifying biomarkers linked to beef tenderness (Gagaoua, Bonnet, & Picard, 2020b, 2021a; Picard & Gagaoua, 2020a; Zhu et al., 2021a). In addition to tenderness, proteomic techniques have also been employed to investigate other important meat quality attributes, such as color (Gagaoua et al., 2020a; Nair et al., 2018), water-holding capacity (Di Luca et al., 2016; Tao et al., 2021), and pH decline (Gagaoua et al., 2015b; Poleti et al., 2018).

Additionally, it has been used to assess how processing methods affect the proteomic profile of carcasses and meat cuts. However, only a few studies have compared aging methods between dry and wet aging.

Tabla 1. Summary of different aging methods and parameters used, and major findings reported in scientific studies. DA: Dry-aging; DAb: Dry-aging bags; Wet aging; WD: WA then DA. ST*: Semitendinosus*; LD: *Longissimus dorsi*; LTL: *Longissimus thoracis et lumborum*; GM: *gluteus medius*

Source	**Muscles**	**Methods**	**Aging Parameters**			
			Length (days)	**Temp (°C)**	**RH (%)**	**Air flow (m/s)**
Ahnström et al., 2006	LTL (strip loin)	DA-DAb	14, 21	2.5±0.3	87±2.6	-
Ba et al., 2014	LTL,LD-ST	WA	7, 28	4	-	-
Berger et al., 2018	LTL, loin	WA-DA-DAb	4 PM+28	2	78	<0.2
Bernardo et al., 2021	LTL	DA- DAb	21*vs* 42	2	65*vs*85	2.5
Brewer and Novakofski, 2008	LTL, loin	WA	0, 7, 14	4		
Campbell et al., 2001	LTL (strip/short loin)	WA-DA-WA	7-14; 7-14, 2, 9, 16	2	75	
Campo et al., 1990	LTL, different breeds	WA	3, 7, 10, 14, 21	4	-	-
DeGeer et al., 2009	LTL (strip shell strip loins)	DA vs DAb	21-28	2.2	50	UV
Descalzo et al., 2008	LTL, *buffalo*	WA	1, 15, 25	2±1	-	-
Dikeman et al., 2013	LTL, short loin	WA-DA-DAb	8+21	2.2	-	minimal
Foraker et al., 2020	LTL	WA-DA-WD	3, 14, 28, 35,49, 63; 21 DA;14+21 WD	3.1±1.2	70-90	-
Gudjónsdóttir et al., 2015	LTL, loin	DA, DAb	7, 14, 21	4	-	-
Ha et al., 2019	LTL	WA-DA-WD	7, 21, 35, 56; 35, 56 DA; 21+35 WD	2.1	89.4	0.75-1.2 /UV
Hulánková et al., 2018	LT	DA	12-36	1±1	85±2	0.5 ± 0.2
Iida et al., 2016	LTL, loin	DA	4, 11, 20, 30, 40, 50, 60	1-4	80-90	-
Kahraman and Gürbüz, 2019	LL	WA-DA-DAb	0, 7, 14, 21, 28	0.5 ± 0.5	80	-

Table 1 (*continued*)

Source	Muscles	Methods	Aging Parameters			
			Length (days)	Temp (°C)	RH (%)	Air flow (m/s)
Kim et al., 2011	LD, *ovine*	WA prior Fr		-1.5 ± 0.2		
Kim et al., 2016	LL	WA-DA	21	1 or 3		0.2 or 0.5
Kim, J.H. et al., 2017	SM (Top round, shank)	WA-DA	20, 40	1.0±0.5	80-85	0.5-1.5
Kim, J.H. et al., 2020	LTL	WA-DA	7, 30	1	85	0.5
Koutsidis et al., 2008b	LTL	WA	1, 3, 7, 14, 21	4	-	-
Lee et al., 2019	GM	DA	14 or 28	4	75	0, 2.5, 5
Lepper-Blilie et al., 2016	LTL (Strip/shortloin)	DA-WA	14, 21, 28, 35, 42,49	1	70	-
Li et al. 2013	GM, striploin	WA-DAb	21	2.8	91	-
Li et al., 2014	LTL, loin	WA-DA-DAb	10, 21	2.9	75	
Oh et al., 2019	LT	WA-DA		1-3	75	0, 2.5, 5
Ryu et al., 2018	LT	DA	40, 60		75-80	-
Smith et al., 2008	LT	WA-DA	14, 21, 28, 35	1.0±2	83±11	-
Spanier et al., 1997	SM	WA	-	4		-
Stenström et al., 2014	LTL	WA-DA-DAb	15	1.6	-	-
Vossen et al., 2022	LTL	DA	21, 42, 63	2-6; 2	75;70-90	-
Warren and Kastner, 1992	LTL, striploin	DA	3 PM+11	3.1 to 3.6	78±3	Air circulation every 30min
Zhang et al., 2019	LL	DAb+WA;DAb	7+14; 21	2±0.5	75±5	0.5, 1.5, 2.5; 0.5
Zhang et al., 2020	LL	DAb+WA;DAb	7+14; 21	2±0.5	75±5	0.5, 1.5, 2.5; 0.5

2.4. Conclusion

Meat aging is a crucial process that enhances tenderness, flavor, and overall palatability. It is primarily driven by biochemical and enzymatic activities that alter muscle structure and chemical composition after slaughter. Different aging methods influence this process. Dry aging enhances unique flavors (e.g., nutty, beefy) but is costly due to higher weight loss and labor requirements. It develops a distinct crust that may protect against microbial growth, but careful trimming is necessary to maintain quality. However, wet aging dominates the industry because of reduced weight loss, cost efficiency, and prolonged shelf life, although it may produce fewer desirable flavors (e.g., metallic, sour). Conversely, dry-aged bags combine the benefits of both methods, offering a higher yield and microbial safety while preserving sensory qualities similar to those of traditional dry aging. Alternating between dry and wet aging techniques can optimize yield and reduce energy costs without compromising quality by incorporating both methods in a stepwise aging process. The freezing process complements aging, influencing tenderness and sensory attributes. Freezing rates and storage conditions are pivotal in maintaining meat quality.

From a certain perspective, the impact on meat attributes and tenderness significantly improves with aging, with duration being more influential than method. Extended aging beyond 21 to 28 days results in diminishing returns for tenderness improvements. Dry aging is more effective at enhancing umami and beefy flavor due to water loss and metabolite concentration, while wet aging can develop undesirable flavors over prolonged periods. Both dry and wet aging enhance juiciness, often through increased fat concentration and water release during consumption. Prolonged aging can lead to oxidative changes that affect visual appeal (color stability). Dry-aging methods exhibit differences in lightness (L*) due to moisture and surface drying effects.

Aging methods influence microbial growth. Dry aging fosters distinct microbial ecosystems that contribute to unique flavors, including molds and yeasts. Proper management of these microbes is essential for safety and quality.

The choice of aging method depends on the target market, economic constraints, and the desired quality attributes. Innovations such as dry-aging bags and stepwise aging provide flexible solutions for optimizing yield and sensory appeal. More studies are needed to clarify consumer preferences, long-term sensory changes, and microbial dynamics. Standardizing methodologies to evaluate tenderness, flavor, and safety across aging processes could help improve industry practices.

2.5. References

Ahnström, M.L., Seyfert, M., Hunt, M.C., and Johnson, D.E. 2006. Dry aging of beef in a bag highly permeable to water vapour. Meat Science, 73:674-679. https://doi.org/10.1016/j.meatsci.2006.03.006

Amaral, A. B., Silva, M. V. D., and Lannes, S. C. D. S. 2018. Lipid oxidation in meat: mechanisms and protective factors–a review. Food Science and Technology, 38:1-15. https://doi.org/10.1590/fst.32518

AMSA, 2016. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat, 2nd ed. American Meat Science Association, Champaign, pp. 106

Ba, H. V., Park, K., Dashmaa, D., and Hwang, I. 2014. Effect of muscle type and vacuum chiller ageing period on the chemical compositions, meat quality, sensory attributes and volatile compounds of Korean native cattle beef. Animal Science Journal, 85(2):164-173.https://doi.org/10.1111/asj.12100

Bendall, J. R., and Taylor, A. A. 1972. Consumption of oxygen by the muscles of beef animals and related species. II. Consumption of oxygen by post-rigor muscle. Journal of the Science of Food and Agriculture, 23(6):707-719.https://doi.org/10.1002/jsfa.2740230606

Bekhit, A.E.D.A, Hopkins, D.L., Fahri, F.T. and Ponnampalam, E.N. 2013. Oxidative Processes in Muscle Systems and Fresh Meat: Sources, Markers, and Remedies. Comprehensive Reviews in Food Science and Food Safety, 12(5):565-597. https://doi.org/10.1111/1541-4337.12027

Berger, J., Kim, Y. H. B., Legako, J. F., Martini, S., Lee, J., Ebner, P. E., and Zuelly, S. M. S. 2018. Dry aging improves meat quality attributes of pasture-fed beef

loins. Meat Science, 145:285-291. https://doi.org/10.1016/j.meatsci.2018.07.004

Bernardo, A. P. D. S., Da Silva, A. C. M., Ferreira, F. M. S., Do Nascimento, M. D. S., & Pflanzer, S. B. 2021. The effects of time and relative humidity on dry-aged beef: Traditional versus special bag. Food Science and Technology International, 27:626-634. DOI:10.1177/1082013220976487 journals.sagepub.com/home/fst

Bernardo, A.P., Da Silva, A.C., Francisco, V. C., Ribeiro, F. A., Nassu, R., Calkins, C. R., Do Nascimento, M. and Pflanzer, S. 2020. Effects of freezing and thawing on microbiological and physical-chemical properties of dry-aged beef. Meat Science, 161: 108003. https://doi.org/10.1016/j.meatsci.2019.108003

Bertram, H. C., Engelsen, S. B., Busk, H., Karlsson, A. H. and Andersen, H. J. 2004. Water properties during cooking of pork studied by low-field NMR relaxation: effects of curing and the RN-gene. Meat Science, 66:437-446. https://doi.org/10.1016/S0309-1740(03)00132-3

Bevilacqua, A., Zaritzky, N., and Calvelo, A. 1979. Histological measurements of ice in frozen beef. Journal of Food Technology, 14:237-251.https://doi.org/10.1111/j.1365-2621.1979.tb00868.x

Bevilacqua, A. and Zaritzky, N. 1980. Ice morphology in frozen beef. International Journal of Food Science and Technology, 15(6):589–597, https://doi.org/10.1111/j.1365-2621.1980.tb00978.x

Boles, J.A. and Swan, J.E. 1996. Effect of post-slaughter processing and freezing on the functionality of hot-boned meat from young bull. Meat Science, 44: 11-18. https://doi.org/10.1016/S0309-1740(96)00076-9

Brewer, S. and Novakofski, J. 2008. Consumer Sensory Evaluations of Aging Effects on Beef Quality. Journal of Food Science, 73:S78-S82. https://doi.org/10.1111/j.1750-3841.2007.00575.x

Campbell, R.E., Hunt, M.C., Levis, P. and Chambers, E. 2001. Dry-Aging Effects on Palatability of Beef Longissimus Muscle. Journal of Food Science, 66:196-199. https://doi.org/10.1111/j.1365-2621.2001.tb11315.x

Campo, M., Sañudo, C., Panea, B., Alberti, P., and Santolaria, P. 1999. Breed type and ageing time effects on sensory characteristics of beef strip loin steaks. Meat Science, 51:383-390.https://doi.org/10.1016/S0309-1740(98)00159-4

Cornforth, D. (1994). Color — its basis and importance. In: Pearson, A.M., Dutson, T.R. (eds) Quality Attributes and their Measurement in Meat, Poultry and Fish Products. Advances in Meat Research, vol 9. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-2167-9_2

Dainty, R. H. Edwards, R. A., Hibbard, C. M. 1984. Volatile compounds associated with the aerobic growth of some Pseudomonas species on beef. Journal of Applied Microbiologycal, 7:75-81. https://doi.org/10.1111/j.1365-2672.1984.tb02358.x

Dainty, R. H. 1996. Chemical/biochemical detection of spoilage. International Journal of Food Microbiology, 33:19-33. https://doi.org/10.1016/0168-1605(96)01137-3

Dashdorj, D., Amna, T., & Hwang, I. (2015). Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: An overview. European Food Research and Technology, 241:157-171. https://doi.org/10.1007/s00217-015-2449-3

Dashdorj, D. Tripathi, V. K., Cho, S., Kim, Y. and Hwang I. 2016. Dry aging of beef; Review. Journal of Animal Science and Technology, 58:20. doi 10.1186/s40781-016-0101-9

DeGeer, S. L., Hunt, M. C., Bratcher, C. L., Crozier-Dodson, B.A., Johnson, D. E., and Stika, J. F. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. Meat Science, 83:768-774. https://doi.org/10.1016/j.meatsci.2009.08.017

Descalzo, A. M., Rossetti, L., Sancho, A.M., Garcia, P.T. Biolatto, F. and Grigioni, G.M. 2008. Antioxidant consumption and development of oxidation during ageing of buffalo meat produced in Argentina. Meat Science, 79(3):582-588. https://doi.org/10.1016/j.meatsci.2007.10.020

Devine, C., and Dikeman, M. (Eds.). 2014. Encyclopedia of Meat Sciences. Elsevier.

Dikeman, M. E., Obuz, E., Gök, V., Akkaya, L., and Stroda, S. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef Longissimus lumborum steaks. Meat Science, 94: 228–233. https://doi.org/10.1016/j.meatsci.2013.02.002

Eastwood, L.C., Arnold, A.N., Miller, R.K., Gehring, K.B., Savell, J.W. 2016. Novel approach to aging beef: Vacuum-packaged foodservice steaks versus vacuum-packaged subprimals. Meat Science, 116:203-235. https://doi.org/10.1016/j.meatsci.2016.02.012

England, E. M., Scheffler, T. L., Kasten, S. C., Matarneh, S. K., and Gerrard, D. E. 2013. Exploring the unknowns involved in the transformation of muscle to meat. Meat Science, 95(4):837-843. https://doi.org/10.1016/j.meatsci.2013.04.031

Estévez, M. 2015. Oxidative damage to poultry: from farm to fork. Poultry Science, 94(6):1368-1378. https://doi.org/10.3382/ps/pev094

Farouk, M. M., Wielizcko, K.J., and Merts, I. 2003. Ultra-fast freezing and low temperatures are not necessary to maintain the functional properties of manufacturing beef. Meat Science, 66:171-179. https://doi.org/10.1016/S0309-1740(03)00081-0

Feiner, G. 2006. Meat products handbook: Practical science and technology. Elsevier, Amsterdam.

Flores, M., Moncunill, D., Montero, R., López-Díez, J. J., and Belloch, C. 2017. Screening of *Debaryomyces hansenii* strains for flavor production under a reduced concentration of nitrifying preservatives used in meat products. Journal of Agricultural and Food Chemistry, 65(19):3900-3909. https://doi.org/10.1021/acs.jafc.7b00971

Foraker, B. A., Gredell, D. A., Legako, J. F., Stevens, R. D., Tatum, J. D., Belk, K. E. and Woerner, D. R. 2020. Flavor, tenderness, and related chemical changes of aged beef strip loins. Meat and Muscle Biology, 4(28):1-18 doi:10.22175/mmb.11115

Gagaoua, M., Terlouw, C., and Picard, B. 2019. The associations between proteomic biomarkers and beef tenderness depend on the endpoint cooking temperature,

the country origin of the panelists and breed. Meat Science, 157:107871. https://doi.org/10.1016/j.meatsci.2019.06.007

Goll, D.E., Dayton, W.R., Singh, I., and Robson, R.M. 1991. Studies of the alpha-actinin/actin interaction in the Z-disk by using calpain. Journal of Biological Chemistry, 266:8501-8510.https://doi.org/10.1016/S0021-9258(18)93003-6

Grayson, A. L., King, D. A., Shackelford, S.D., Koohmaraie, M. and Wheeler, T. L. 2014. Freezing and thawing or freezing, thawing, and aging effects on beef tenderness. Journal of Animal Science, 92:2735-2740. doi:10.2527/jas2014-7613

Gudjónsdóttir, M., Gacutan, M. D.Jr., Mendes, A. C., Chronakis, I. S., Jespersen, L., and Karlsson, A. H. 2015. Effects of electrospun chitosan wrapping for dry-ageing of beef, as studied by microbiological, physicochemical and low-field nuclear magnetic resonance analysis. Food Chemistry, 184:167-175. https://doi.org/10.1016/j.foodchem.2015.03.088

Ha, M., McGilchrist, P., Polkinghorne, R., Huynh, L., Galletly, J., Kobayashi, K., Nishimura, T., Bonney, S., Kelman, K. R., and Warner, R. D. 2019. Effects of different ageing methods on colour, yield, oxidation and sensory qualities of Australian beef loins consumed in Australia and Japan. Food Research International, 125:108528. https://doi.org/10.1016/j.foodres.2019.108528

Huff-Lonergan, E. and Lonergan, S. M. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science, 71(1):194-204. https://doi.org/10.1016/j.meatsci.2005.04.022

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D.D., Parrish, F.C., Olson, Jr, D.G. and Robson, R.M. 1996. Proteolysis of Specific Muscle Structural Proteins by m-Calpain at Low pH and Temperature Is Similar to Degradation in Postmortem Bovine Muscle. Journal of Animal Science, 74:993-1008

Hulánková, R., Kameník, J., Saláková, A., Závodský, D., and Borilova, G. 2018. The effect of dry aging on instrumental, chemical and microbiological parameters of organic beef loin muscle. LWT - Food Science and Technology, 89:559-565. https://doi.org/10.1016/j.lwt.2017.11.014

Iida, F., Miyazaki, Y., Tsuyuki, R., Kato, K., Egusa, A., Ogoshi, H., and Nishimura, T. 2016. Changes in taste compounds, breaking properties, and sensory attributes during dry aging of beef from Japanese black cattle. Meat Science, 112:46-51. https://doi.org/10.1016/j.meatsci.2015.10.015

Kahraman, H. A. and Gürbüz, Ü. 2019. Effects of three aging methods on the Longissimus lumborum muscle from Holstein-Friesian steers. Medicine Veterinary, 75 (3):179-184. https://dx.doi.org/10.21521/mw.6182

Kemp, C.M., Sensky, P. L., Bardsley, R. G., Buttery, P. J. and Parr, T. 2010. Tenderness – An enzymatic view. Meat Science, 84:248-256.

Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., and Hunt, M.C. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies (Review). Meat Science, 144:74-90. https://doi.org/10.1016/j.meatsci.2018.04.031

Kim, Y. H. B., Frandsen, M., and Rosenvold, K. 2011. Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. Meat Science, 88:332-337. https://doi.org/10.1016/j.meatsci.2010.12.020

Kim, Y. H. B., Kemp, R., and Samuelsson, L. M. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. Meat Science, 111:168-176.

Kim, J. H., Kim, D. H., Ji, D., Lee, H. J., Yoon, D. K., and Lee, C. H. 2017. Effect of Aging Process and Time on Physicochemical and Sensory Evaluation of Raw Beef Top Round and Shank Muscles Using an Electronic Tongue. Korean Journal Food Science Animal Resource, 37(6):823-832 https://doi.org/10.5851/kosfa.2017.37.6.823

Kim, J. H., Kim, T. K., Shin, D. M., Kim, H. W., Kim, Y.B. and Choi, Y.S. 2020. Comparative effects of dry-aging and wet-aging on physicochemical properties and digestibility of Hanwoo beef. Asian-Australasian Journal of Animal Science. 33(3):501-505. https://doi.org/10.5713/ajas.19.0031

Koutsidis, G., Elmore, J.S., Oruna-Concha, M.J., Campo, M.M., Wood, J.D., and Mottram, D.S. 2008a. Water-soluble precursors of beef flavour: I. Effect of diet

and breed. Meat Science, 79:124-130. https://doi.org/10.1016/j.meatsci.2007.08.008

Koutsidis, G., Elmore, J.S., Oruna-Concha, M.J., Campo, M.M., Wood, J.D., Mottram, D.S. 2008b. Water-soluble precursors of beef flavour. Part II: Effect of *post-mortem* conditioning. Meat Science, 79:270-277. https://doi.org/10.1016/j.meatsci.2007.09.010

Kramlich, W. E., Pearson, A. M. and Tauber, F. W. 1975. Processed meats. The Avi Publishing Co. Inc., Westport, Conn

Kristensen, L., and Purslow, P. P. 2001. The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. Meat Science, 58(1):17-23. https://doi.org/10.1016/S0309-1740(00)00125-X

Lagerstedt, Å., Enfält, L., Johansson, L. and Lundström, K. 2008. Effect of freezing on sensory quality, shear force and water loss in beef *M. longissimus dorsi.* Meat Science, 80(2):457-461. https://doi.org/10.1016/j.meatsci.2008.01.009

Lee, H. J., Yoon, J. W., Kim, M., Oh, H., Yoon, Y., and Jo, C. 2019. Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. Meat Science, 153:152-158. https://doi.org/10.1016/j.meatsci.2019.03.019

Lepper-Blilie, A.N., Berg, E.P., Buchanan, D.S., and Berg, P.T. 2016. Effects of post-mortem aging time and type of aging on palatability of low marbled beef loins. Meat Science, 112:63-68. http://dx.doi.org/10.1016/j.meatsci.2015.10.017

Li, X., Babol, J., Wallby, A., and Lundström, K. 2013. Meat quality, microbiological status and consumer preference of beef gluteus medius aged in a dry ageing bag or vacuum. Meat Science, 95:229-234. https://doi.org/10.1016/j.meatsci.2013.05.009

Li, X., Babol, J., Bredie, W. L. P., Nielsen, B., Tománková, J. and Lundstrom. 2014. A comparative study of beef quality after ageing longissimus muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. Meat Science, 97:433-442. https://doi.org/10.1016/j.meatsci.2014.03.014

Li, Y., and S. Liu. 2012. Reducing lipid peroxidation for improving colour stability of beef and lamb: On-farm considerations. Journal of Science Food and Agriculture, 92:719 729. https://doi.org/10.1002/jsfa.4715

Lomiwes, D., Farouk, M.M., Wu, G., and Young, O.A. 2014. The development of meat tenderness is likely to be compartmentalised by ultimate pH. Meat Science, 96: 646-651. https://doi.org/10.1016/j.meatsci.2013.08.022

Ma, D., Y.H.B. Kim, Cooper, B.R., Oh, J.H., Chun, H., Choe, J., Schoonmaker, J. P., Ajuwon, K.M. and Min, B. 2017. Metabolomics Profiling to Determine the Effect of Postmortem Aging on Color and Lipid Oxidative Stabilities of Different Bovine Muscles. Journal of Agricultural and Food Chemistry, 65(31):6708–6716. https://doi.org/10.1021/acs.jafc.7b02175

Madhavi, D. L., Carpenter, C. E. 1993. Aging and Processing Affect Color, Metmyoglobin Reductase and Oxygen Consumption of Beef Muscles. Journal of Food Science, 58(5):939-942. https://doi.org/10.1111/j.1365-2621.1993.tb06083.x

Maga, J.A. 1994. Umami flavour of meat. In: Shahidi, F. (eds) Flavor of Meat and Meat Products. Springer, Boston, MA. pp. 98-115. https://doi.org/10.1007/978-1-4615-2177-8_6

Martins, S.I.F.S., Jongen, W.M.F., and van Boekel, M.A.J.S. 2000. A review of Maillard reaction in food and implications to kinetic modelling. Trends in Food Science & Technology, 11:364-373. https://doi.org/10.1016/S0924-2244(01)00022-X

McMillin, K.W. 2008. Where is MAP Going? A review and future potential of modified atmosphere packaging for meat. Meat Science, 80:43-65. https://doi.org/10.1016/j.meatsci.2008.05.028

Meinert, L., Schäfer, A., Bjergegaard, C., Aaslyng, M.D., and Bredie, W.L.P. 2009. Comparison of glucose, glucose 6-phosphate, ribose, and mannose as flavour precursors in pork; the effect of monosaccharide addition on flavour generation. Meat Science, 81:419-425.

Miller, M. F., Carr, M.A., Ramsey, C. B., Crockett, K. L., and Hoover, L. C. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79:3062-3068. https://doi.org/10.2527/2001.79123062x

Mottram, D.S. and Elmore, J.S. 2010. Control of the Maillard Reaction during the Cooking of Food. ACS Symposium Series. Vol. 1042. doi: 10.1021/bk-2010-1042.ch014

Mottram, D. S. 1996. The role of phospholipids in meat flavor: an overview. In Contribution of Low and Nonvolatile Materials to the Flavor of Foods; Pickenhagen, W., Ho, C.-T., Spanier, A. M., Eds.; Allured Publishing: Carol Stream, IL, pp 193-206.

Nishimura, T. 1998. Mechanism involved in the improvement of meat taste during post-mortem aging. Food Science and Technology International, Tokyo, 4:241-249. https://doi.org/10.3136/fsti9596t9798.4.241

Nishimura T, Rhue MR, Okitani A, and Kato H. 1988. Components contributing to the improvement of meat taste during storage. Agricultural and Biological Chemistry, 52:2323-2330

Oh, H., Lee, H.J., Lee, J., Jo, C. and Yoon, Y. 2019. Identification of Microorganisms Associated with the Quality Improvement of Dry-Aged Beef Through Microbiome Analysis and DNA Sequencing, and Evaluation of Their Effects on Beef Quality. Journal of Food Science, doi: 10.1111/1750-3841.14813

O'Quinn, T. G., Brooks, J. C., Polkinghorne, R. J., Garmyn, A.J., Johnson, B. J., Starkey, J. D., Rathmann, R.J. and Miller, M. F. 2012. Consumer assessment of beef strip loin steaks of varying fat levels. Journal of Animal Science, 90:626-634 doi:10.2527/jas.2011-4282

Ouali, A., Herrera-Mendez, C.H., Coulis, G., Becila, S., Boudjellal, A., Aubry, L., and Sentandreu, M. A. 2006. Revisiting the conversion of muscle into meat and the underlying mechanisms. Meat Science, 74:44-58. https://doi.org/10.1016/j.meatsci.2006.05.010

Parrish, F. C., Boles, J. A., Rust, R. E., and Olson, D. G. 1991. Dry and wet aging effects on palatability attributes of beef loin and rib steaks from three quality

grades. Journal of Food Science, 56:601-603. https://doi.org/10.1111/j.1365-2621.1991.tb05338.x

Petrovic, L., Grujic, R., and Petrovic, M. 1993. Definition of the optimal freezing rate-2. Investigation of the physico-chemical properties of beef M longissimus dorsi at different freezing rates. Meat Science, 33:319-331. https://doi.org/10.1016/0309-1740(93)90004-2

Ryu S., Park, M.R., Maburutse, B.E., Lee, W.J., Park, D.J., Cho, S., Hwang, I, Oh, S. and Kim, Y. 2018. Diversity and Characteristics of the Meat Microbiological Community on Dry Aged Beef. Journal of Microbiology and Biotechnology, 28(1):105-108, https://doi.org/10.4014/jmb.1708.08065

Savell, J.W., Branson, R.E., Cross, H.R., Stiffler, D.M., Wise, J.W., Griffin, D.B., and Smith, G.C. 1987. National Consumer Retail Beef Study: Palatability Evaluations of Beef Loin Steaks that Differed in Marbling. Journal of Food Science, 52 (3):517-519. https://doi.org/10.1111/j.1365-2621.1987.tb06664.x

Savell, J. 2008. Dry-aging of beef: Executive Summary. Beef Research. Available at https://www.beefresearch.org/Media/BeefResearch/Docs/dry_aging_of_beef_08-20-2020-28.pdf (Accessed 1 March 2024).

Shahidi, F. 1994. Flavor of meat and meat products-an overview. Book First edition 1994. Published by Blackie Academic and Professional, an imprint of Chapman & Hall, 2-6 Boundary Row, London SEl 8HN, UK.

Shanks, B. C., Wulf, D. M., and R. J. Maddock. 2002. Technical note: The effect of freezing on Warner-Bratzler shear force values of beef longissimus steaks across several postmortem aging periods. Journal of Animal Science, 80:2122-2125.

Smith, R., Nicholson, K., Nicholson, J., Harris, K., Miller, R., Griffin, D., and Savell, J. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. Meat Science, 79:631-639. https://doi.org/10.1016/j.meatsci.2007.10.028.

Spanier, A.M., Flores, M., McMillin, K.W., and Bidner, T.D. 1997. The effect of post-mortem aging on meat flavor quality in Brangus beef. Correlation of treatments, sensory, instrumental and chemical descriptors. Food Chemistry, 59:531-538. https://doi.org/10.1016/S0308-8146(97)00003-4

Stenström, H., Li, X., Hunt M.C, and Lundström, K. 2014. Consumer preference and effect of correct or misleading information after ageing beef longissimus muscle using vacuum, dry ageing, or a dry ageing bag. Meat Science, 96(2):661-666.

Taylor, R. G., G. H. Geesink, V. F. Thompson, M. Koohmaraie, and D. E. Goll. 1995. Is Z-disk degradation responsible for postmortem tenderization? Journal of Animal Science, 73:1351-1367. https://doi.org/10.2527/1995.7351351x

Vossen, E., Dewulf, L., Van Royen, G., Van Damme, I., De Zutter, L., Fraeye, I., and De Smet, S. 2022. Influence of aging time, temperature and relative humidity on the sensory quality of dry-aged Belgian Blue beef. Meat Science, 183:108659. https://doi.org/10.1016/j.meatsci.2021.108659

Warren, K.E., and Kastner, C.L. 1992. A comparison of dry-aged and vacuum-aged beef strip loins. Journal Muscle Foods, 3:151-157. https://doi.org/10.1111/j.1745-4573.1992.tb00471.x

Winger, R. J., and Hagyard, C. J. 1994. Juiciness–Its importance and some contributing factors. In 'Quality attributes and their measurement in meat, poultry and fish products'. (Eds AM Pearson, TR Dutson) pp. 94-124

Yamaguchi, S. and Kirnizuka, A. 1979. Psychometric studies on the taste of monosodium glutamate. In Glutamic Acid: Advances in Biochemistry and Physiology, ed. L.J. Filer, S. Garattini, M.R. Kare, W.A. Reynolds and R.J. Wurtman. Raven Press, New York, 35-54.

Yaylayan, V.A., Keyhani, A. and Wnorowski, A. 2000. Formation of Sugar-Specific Reactive Intermediates from 13C-Labeled l-Serines. Journal of Agricultural and Food Chemistry, 48(3):636-641. https://doi.org/10.1021/jf990687a

Yokotsuka, T., Saito, N., Okuhara, A., and Tanaka, T. 1969. Studies on the taste of alpha-amino acids. I. Ternary synergism of palatable taste of glycine. Agricultural Chemical Society Japan Journal.

Zhang, R., Yoo, M. J. Y., and Farouk, M. M. 2019. Quality and acceptability of fresh and long-term frozen in-bag dry-aged lean bull beef. J. Food Quality, 1975264: 1-15. https://doi.org/10.1155/2019/1975264

Zhang, R., Yoo, M. J. Y., and Farouk, M. M. 2020. Oxidative stability, proteolysis, and in vitro digestibility of fresh and long-term frozen stored in-bag dry-aged

lean beef. Food Chemistry, 344:128601. https://doi.org/10.1016/j.foodchem.2020.128601

Zhang, R., Ross, A.B., Yoo, M. J.Y., Farouk, M. M. 2021. Metabolic fingerprinting of in-bag dry- and wet-aged lamb with rapid evaporative ionisation mass spectroscopy. Food Chemistry, 347:128999. https://doi.org/10.1016/j.foodchem.2020.128999

3. Impact of Aging Methods and Frozen Storage on Beef Quality Attributes from Different Finishing Diets

Daniela Correa [1], Marcia del Campo [1], Santiago Luzardo [1], Guillermo de Souza [1], Carlos Álvarez [2], María Font-i-Furnols [3] and Gustavo Brito [1, *]

[1]INIA-Sistema Ganadero Extensivo, INIA Tacuarembó, Ruta 5, km 386, Tacuarembó, Uruguay

[2]Teagasc-Food Quality and Sensory Science Department, Teagasc Ashtown Food Research Centre, Ashtown, D15KN3K Dublin 15, Ireland

[3] IRTA- Food Technology, Granja Camps i Armet, 17121 Monells (Girona), Spain

*Corresponding author. Email: gbrito@inia.org.uy

3.1. Abstract

The effect of finishing diet (F: pasture or grain) and meat preservation methods (PM) was evaluated on beef's physicochemical, microbiological, and sensory attributes. The PM assessed dry aging in bag (DAb) and wet aging (WA) for 40 days, and then subjected them to frozen storage (Fr): (DAb+Fr) and (WA+Fr) for 180 days. Sixty striploins (Longissimus lumborum, LL) from British breed steers (n=15 from pasture and n=15 from grain-based diet) were used. Lightness (L*) was only affected by F, where meat from grain-fed steers was lighter than that fed on pasture ($P<0.01$). DAb meat had higher pH ($P<0.01$) and lower cooking losses ($P<0.01$) than WA. DAb+Fr had the highest Psychotrophic bacteria count (PSY) compared to WA+Fr, DAb, and WA ($P<0.01$). DAb and DAb+Fr increased Enterobacteriaceae (ENT) bacteria counts ($P<0.01$) compared to WA and WA+Fr. DAb+Fr samples had the lowest L*, a*, and b* values. No interaction between physicochemical characteristics (color coordinates, pH, cooking losses, and shear force) and surface microbiological load was observed ($P>0.05$). Greater polyunsaturated fatty acids (PUFA), PUFA n-3, conjugated linoleic acid (CLA; c9, t11-18:2) ($P <0.01$), PUFA/SFA ratio ($P <0.05$), and lower n-6: n-3 ratio ($P <0.01$) were observed in pasture-fed than grain-fed steers. The consumer sensory panel showed acceptable scores for all treatments, although

some differences between attributes were detected by cluster analysis. Different aging methods followed by a frozen storage period, could be used to produce and export meat with the required quality attributes to meet consumer expectations.

Keywords: beef; meat preservation; dry aging bag; finishing diet, quality attributes

3.2. Introduction

The beef industry's success depends on adding value to meat products, to satisfy consumer's demands. The willingness to pay for this product is inspired by several attributes, among which tenderness, juiciness, and flavor are the most relevant. Post-mortem aging improves tenderness (Campbell et al., 2001; Smith et al., 2008) through the action of the endogenous proteolytic system, influencing other attributes, such as flavor and overall palatability (Kim et al., 2020), affecting meat quality and its commercial value (Kim et al., 2018; Ha et al., 2019).

There are two fundamental ways to age beef: wet and dry. Wet aging involves vacuum packing meat into a highly moisture-impermeable bag and storing it under refrigerated conditions (-1 to 2 °C) for a specified time. Traditional dry aging exposes unpackaged meat directly to cooler conditions with strict temperature (0-4 °C), relative humidity (80-85%), and airflow control (0.5-2 m/s). This process implies higher costs associated with decreased yields and greater weight losses during aging and trimming (Parrish et al., 1991; Warren & Kastner, 1992) but it increases the intense beefy and roasted flavors (Iida et al., 2016; Ha et al., 2019; Li et al., 2021). In the last decades, dry aging in a highly moisture-permeable bag has been widely used. Such technology decreases trim loss and microbial contamination, thus maximizing yield (Ahnström et al., 2006). The dry aging bag acts as an oxygen barrier with the surrounding air, limiting oxidation and its associated consequences, which include oxidative deterioration and rancidity or off-flavor (Zhang et al., 2020; DeGeer et al., 2009).

Strategies to produce stable aged quality products (wet or dry) should be considered, especially if the products are meant to be traded globally where a long period of chilled/frozen storage is usually required. The advantages of frozen meat, rather than chilled, include increased storage time and greater flexibility in inventory

for retailers (Wheeler et al., 1990). It is thought that freezing reduces meat quality and although research findings are not conclusive (Luzardo et al., 2024; Bernardo et al., 2020; Coombs et al., 2017; Farouk et al., 2003), consumers tend to prefer meat that has not been frozen and thawed.

The beef cattle diet is known to play a pivotal role in influencing carcass composition and eating quality attributes of meat (Correa et al., 2020; Peripolli et al., 2018). Feeding beef cattle with grain before slaughter could improve beef flavor mainly due to an increase in the deposition of intramuscular fat compared with meat from pasture-fed animals (Brito et al., 2014). Previous research reported that the most significant difference in the flavors of meat from cattle fed on pasture or grain is due to fatty acid concentration (Realini et al., 2004) and composition as they are the primary source of aromatic compounds such as carbonyls (Melton, 1983), which plays an important role in the interaction and generation of volatile flavor compounds (Ba et al., 2016). Since consumer assessment is the golden standard for obtaining beef quality differentiation, it is important to understand the effect of aging and refrigeration on the sensory and acceptability of the product attributes by consumers.

Few studies connect the finishing diet to meat preservation methods, including aging types and freezing processes. Consequently, there remains a need to develop and assess effective storage strategies to mitigate the inconsistency of meat quality associated with preservation. We hypothesized that aging methods (dry bag vs. wet aging), with or without subsequent freezing, affect the meat quality of steers from pasture and grain finishing systems. The main goal of this study was to evaluate the impact of meat aging methods and subsequent frozen storage conditions on the physicochemical, microbiological, and sensory attributes of beef from steers finished on two different systems (pasture or grain).

3.3. Materials and methods

3.3.1. Raw materials and aging process

A total of 30 steers (under 30 months of age; British breed) finished (F) in the pasture (n=15) qualifying for UE Hilton quota (INAC, 2013) or grain (n=15) qualifying for UE 481 quota (MGAP, 2023) were slaughtered in a commercial meat

processing plant (hot carcass weight: 266.5 kg and 253.2 kg, respectively). Hilton quotas are selected cuts of beef from steers or heifers designed to produce superior quality beef raised exclusively on pasture, according to the Uruguayan grading system. Meanwhile, the "481" quota consists of beef cuts obtained from the carcasses of steers and heifers under 30 months of age, fed a diet containing no less than 62% concentrates for at least 100 days prior to slaughter. The steer herd originated from the same farm and was selected based on age, live weight, and fat cover to establish two similar groups. Sixty striploins (*Longissimus lumborum*, [LL]) were collected for analysis and assigned to an aging method (dry bag or wet) before being stored under frozen conditions. The striploin from the left side of each carcass was divided into three sections: the first section, measuring 7.5 cm in length, was used for fresh (initial or unaged) sample analysis; the second section, measuring 16 cm in length, was vacuum packaged in dry-aging bags (DAb; TUBLIN® 10 of 50 μm thick, polyamide mix with a water vapor transmission rate of 2.5 kg/50μ/m2/24 h at 38°C, 50% RH, TUB-EX ApS, Denmark); and the third section, measuring 14 cm in length, was vacuum packaged for wet-aging (WA; vacuum packaged in a barrier bag of 50 μm thickness; maximum oxygen transmission rate of 27 cm3/m2/24 h at 22-24°C and 0% RH, and moisture vapor transmission rate. of 5 g/m2/24 h at 38°C and 90% RH; Cryovac® Sealed Air Corp., BB 2620, Brazil.). The right striploin was processed in two sections following the same procedure for DAb (19 cm) and WA (17 cm) and after 40 days of aging were immediately frozen (Fr) at −20°C for 180 days to determine the effect of long-term frozen storage on the quality of dry bag and wet-aged beef (DAb+Fr and WA+Fr). The location of each meat portion from each striploin was alternated in cranial to caudal direction among carcasses. The striploin's portions were laid out on wire racks inside the chamber for 40 days.

During aging, the chamber was maintained at 2 ± 0.5°C and a relative humidity of 85 ± 5%, with air flow recorded at an average of 0.5 m/s. Temperature and relative humidity were recorded using three dataloggers (Electronic Temperature Instruments Ltd., UK), to obtain real-time information at different points in the chamber. The air velocity was recorded weekly in different chamber positions with a digital anemometer (HoldPeak 866A digi, China), averaging 0.5 m/s. The meat portions were relocated

into the chamber every 8 days to prevent any potential confounding effects of location within the chamber. After 40 days of aging, the left striploin portions (DAb and WA) were divided into steaks (2.5 cm) for different analyses. Meanwhile, the right striploin portions (DAb+Fr and WA+Fr) were split into steaks after the frozen periods (40 d aging + 180 d frozen) for different analyses.

3.3.2. Instrumental color

By determinate instrumental color (AMSA, 2012), one steak per loin section, from each treatment, was measured after blooming for 45 min under simulated retail display light at 4°C. Frozen samples were thawed at 4°C for 24 hours before determinations were carried out. The surface color was measured using a colorimeter (Minolta Chroma Meter CR-400; Konica Minolta Sensing Inc., Japan) fitted with an illuminant C, a standard observer of 2 grade and 8 mm of opening size and previously calibrated using a standard white tile. CIE L*a*b* (Commission Internationale de l'Eclairage, 1976) color space values: L* (lightness), a* (redness), and b* (yellowness) were taken per triplicate on the lean surface of each steak. Values were averaged to obtain a mean for each sample.

3.3.3. Cooking losses and Warner-Bratzler Shear Force

After color assessment, steaks were weighed before and after cooking to determine the cooking losses (CL) according to the American Meat Science Association (AMSA, 2016). The cooking loss percentage was calculated according to the following equation: (raw weight - cooked weight/raw weight) x 100. Steaks were cooked in a grill (GRP100 The Next Grilleration, Spectrum Brands, Inc., Miami, FL) until the internal core temperature of the steak reached 71 °C. The internal temperature was monitored using thermocouples Type T and a recording thermometer (Comark N9094, Comark Instruments Inc., UK).

Warner-Bratzler shear force (WBSF; KgF) was evaluated on six cores from each steak. Each cores was obtained parallel to the muscle fiber orientation using a 1.27 cm diameter hand-held coring device and sheared using a TA-XT Plus texture analyzer (Stable Micro System Ltd., UK) set with a "V" Warner Bratzler slot blade. Shear force values resulted from the average of the six cores per steak.

3.3.4. Fatty acid composition and thiobarbituric acid-reactive substance

Intramuscular fat (IMF) was determined using the chloroform-methanol lipid extraction procedure described by Bligh and Dyer (1959) and then the fatty acids profile was performed. The fatty acid profile was methylated with cold methanolic potash (IUPAC, 1987) and analyzed using a gas chromatograph (Shimadzu Nexis GC 2030 Tokyo, Japan). Fatty acids were identified using a 60 m SH-Rt-WAX capillary column (0.25 mm internal diameter and 0.25 μm thick film, Shimadzu, Columbia, Maryland, USA) where nitrogen was used as a carrier gas at 1 ml/min flow.

The injection volume was 1 μl, and a flame ionization detector (FID) was used. The detector was kept at 260 ºC, while the injector was at 230 ºC; the temperature ramp was 100 ºC for 0.5 min, increasing 10 ºC /min until it reached 120 ºC x 2 min, increasing 10 ºC / min until 220 ºC x 15 min, totalizing 29.5 min per sample. Fatty acids were identified by comparing retention times with those of a standard mixture of 37 FAME SupelcoTM 37 compounds (Sigma, St. Louis, USA), meanwhile conjugated linoleic acid (CLA; c9, t11-18:2) was identified using octadecadienoic acid, conjugated, methyl ester standard (No. O5632, Sigma, St. Louis, USA). Fatty acids were reported in mg/100 g of meat using methyl heneicosanoate (C21:0) as an internal standard. An internal standard, 1 ml of 1 mg methyl heneicosanoate (C21:0), was added before the addition of methylating reagents.

Lipid oxidation was determined by the thiobarbituric acid-reactive substance (TBARS) for the modified method of Ahn et al. (1998), at 0 d after the aging process (DAb and WA for 40 d), and after the frozen process (DAb+Fr and WA+Fr for 180 days). A 5 g meat sample was placed in a 50 ml test tube and homogenized with 15 ml of deionized distilled water (DDW) by using a tissue homogenizer (Wisd, HG-15A, Daihan Scientific) for 30 s. Meat homogenate (1 ml) was transferred to a disposable test tube (13x100 mm), and butyrated 16 hydroxyanisole (50 μl, 7.2%) and thiobarbituric acid/ trichloroacetic acid (TBA/TCA) solution (2 ml) were added. The mixture was vortexed and then incubated in a boiling water bath for 15 min for color development. After that, the samples were cooled in cold water for 10 min and then centrifuged for 15 min at 4000 rpm. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 ml DDW and 2 ml

TBA/TCA solution. Malonaldehyde (MDA) standard curves were prepared by using 1,1,3,3-tetra-ethoxypropane. The TBAR concentrations were calculated from the standard curve and expressed as milligrams of MDA per kg of meat.

3.3.5. Surface Microbial Counts

Microorganisms from the untrimmed surface of fresh beef samples were enumerated at 0 d, after the aging period (DAb and WA for 40 d), and after the frozen (DAb+Fr and WA+Fr for 180 days) storage period. Samples were analyzed for total bacterial count (TBC), Psychrotrophic microorganisms (PSY), and Enterobacteriaceae (ENT). At each sampling time, a 4 x 4 cm square was aseptically excised from the center of 10 steaks per treatment using disposable scalpels (Feather Sterile Scalpels 2975#21; Graham-Field Inc., Atlanta, GA) and placed into individual sterile Whirl-Pak bags (710 mL, 15x23 cm, 0.102 mm thick; Nasco Int., USA). The 4 x 4 cm squares for microbial analysis were homogenized with 90 mL of 0.1 % peptone water (Oxoid, UK), using a stomacher (BagMixer®400 P, Interscience, Saint Nom, France) for 2 min. Tenfold serial dilutions were prepared in test tubes with 9 ml of 0.1% buffered peptone water (BPW; Oxoid, UK). Appropriate dilutions were surface plated in duplicate onto two sets of Petrifilms (3M; USA); one set for the enumeration of mesophilic microbial populations and the second set for the enumeration of *psychrophilic* microorganisms. Appropriate dilutions were also duplicated onto a set of Petrifilm surfaces (3M; USA) for the ENT microbial population. Petrifilm was enumerated before incubation at 37°C for 48 h for TBC or 7°C for 10 d for PSY and 37°C 24 h for ENT.

3.3.6. Consumer Sensory Testing

Two analyses were carried out, one on fresh and the other on thawed samples. In each study, 10 sessions with 10 consumers (n=100, each one) were carried out, in September 2021 and March 2022, respectively, in the Meat and Technology Laboratory of INIA Tacuarembó. The consumer pool in both trials was homogeneous since they were recruited in the same region from a database of consumers (students, staff, and Campus interinstitutional members: INIA, University, MGAP, etc.) that represent the Uruguayan population demographics in terms of gender and age (Table

S1, Supplementary data). Moreover, consumers were selected since they eat meat as part of their diets. In the first sensory panel, consumers evaluated 4 samples, one of each combination of finishing system (grain and pasture) and aging type (DAb and WA). In the second panel, consumers assessed 4 samples stemming from the combination of finishing system (grain and pasture) and aging type, followed by a frozen period (DAb+Fr and WA+Fr).

Each consumer was asked to taste the samples following the order in each ballot, which was designed to avoid the first sample and carry-over effect (MacFie et al., 1989). Water and unsalted crackers were provided as palate cleansers. Before tasting, consumers were asked to answer a questionnaire with demographic (gender, age range, education level) and frequency in the consumption of different types of meat and to sign the consent form if they agreed to participate (Table S1, Supplementary data). After that, a ballot was provided to evaluate the steak samples' tenderness liking, flavor liking, and overall liking using a 9-point scale, where 1 represented “I like it extremely”, 2 “I like very much”, 3 “I quite like it”, 4 “I like it”, 5 “I neither like nor dislike”, 6 “I dislike it”, 7 “I quite dislike it”, 8 “I dislike very much” and 9 “I dislike it extremely”.

The day before the test, the steaks to be evaluated (4 per session, one for each treatment) were thawed at a 4°C chiller overnight. Before serving to consumers, samples were cooked in a grill (GRP100 The Next Grilleration, Spectrum Brands, Inc., Miami, FL) until the core (internal) temperature of the steak reached 71 °C (AMSA, 2016). Once cooked, steaks were trimmed of external fat and connective tissue and cut across the grain into a 1.3 ×1.3 × 2.0 cm piece, wrapped individually in coded aluminum foil, assigned to a cup, and kept warm in a heater/oven at 49°C for no more than 10 minutes until tasted. The procedures used for consumer sensory evaluation in this study were approved by the Institute of Agrifood Research and Technology (IRTA) Ethics Committee, with the internal code: CCSC 33/2023.

3.3.7. Statistical analysis

Data analysis was carried out using the SAS software v. 9.4 (SAS Institute Inc., Cary, NC, USA). All data were screened for normality using the UNIVARIATE

procedure and normalized as required using a log10 transformation, but estimates have been back-transformed to the response scale.

The experimental design was a split-plot, where each finishing diet served as a main plot (F: pasture or grain), and carcass sides (pair of loins) served as sub-plot for the preservation methods (PM: DAb, WA, DAb+Fr and WA+Fr). The statistical analysis was performed with a model including the fixed effects of F and PM, their interactions, and the random effect of the carcass using the MIXED procedure. The least-square mean (LSM) was calculated and means separation was performed ($P<0.05$) using the PDIFF option. Peak cooking temperature was used as a covariable for WBSF and cooking losses analysis.

Consumers' tenderness liking, flavor liking, and overall liking scores were evaluated using the MIXED procedure of SAS. The two trials were considered together because, although there could be confusion between consumer population and treatment (fresh vs frozen), the consumer population in both trials was homogeneous (Table S1, Supplementary data), consequently, it was assumed to be similar, allowing to take advantage of the full analysis. The model included the PM, and F as fixed effects and its interaction. Consumers were considered as random effects in the model. A tasting session was included as a blocking factor. A segmentation by CLUSTER was carried out to find groups of consumers with similar preferences, since when considered as a pool, differences are diluted, and they are difficult to determine. Segmentation was performed by using the CLUSTER procedure, applying Euclidian distance and the Ward method. The number of clusters to retain was based on the obtained dendrogram, considering the homogeneity within and among the segments and the principle of parsimony. An analysis of variance was carried out, considering PM, and F as fixed effects and their interaction, for the pooled sample and by cluster. A Tukey test was applied to find differences between least-squares means. The significance level was set at $P < 0.05$.

3.4. Results

3.4.1. Effects of preservation method and finishing system on instrumental color, pH, cooking losses, and shear force

The interaction for any of these parameters was not significant thus, results are presented independently by effects. Lightness (L*), redness (a*), and yellowness (b*) were affected by PM (Table 1). Lightness and a* were significantly greater in WA followed by DAb, DAb+Fr, and WA+Fr samples ($P < 0.01$). No difference in b* values was detected between DAb and WA samples ($P > 0.05$), lower values were observed in WA+Fr and DAb+Fr (the lowest values) ($P < 0.01$). Meat color from grain-fed steers resulted in greater L* values than pasture-fed animals ($P < 0.01$). The pH was affected by the aging method, DAb and DAb+Fr samples presented greater pH values than WA and WA+Fr samples ($P<0.01$). However, it is worth mentioning that the pH was below 5.8 in all treatments. The higher percentage of cooking losses (CL) was WA followed by WA+Fr, DAb, and DAb+Fr ($P<0.01$). Wet-aged and DAb samples presented lower WBSF values than WA+Fr and DAb+Fr samples ($P<0.021$). The finishing diet of the steers had no effect ($P> 0.05$) on pH, CL, or WBSF (Table 1).

Table 1. Effects (mean ± SEM) of preservation method (PM) and finishing diet (F) on meat quality parameters.

	PM					F		
Traits	**DAb**	**WA**	**DAb+Fr**	**WA+Fr**	**P-value**	**Pasture**	**Grain**	**P-value**
L*	40.5±0.4 b	41.8±0.4 a	38.2±0.4 d	39.7±0.4 c	<0.001	38.8±0.4	41.3±0.4	<0.001
a*	22.2±0.4 b	24.0±0.4 a	17.2±0.4 d	19.5±0.4 c	<0.001	20.7±0.3	20.8±0.3	0.783
b*	11.8±0.2 a	11.9±0.2 a	10.0±0.2 c	11.1±0.2 b	<0.001	11.1±0.1	11.3±0.1	0.424
pH	5.76±0.01 a	5.73±0.01 b	5.77±0.01 a	5.73±0.01 b	<0.001	5.73±0.02	5.76±0.02	0.294
CL (%)	17.7±0.4 c	23.0±0.4 a	15.2±0.4 d	21.4±0.4 b	<0.001	19.5±0.3	19.3±0.3	0.602
WBSF (kgF)	2.6±0.09 b	2.5±0.09 b	2.8±0.09 a	2.8±0.09 a	0.021	2.8±0.09	2.6±0.09	0.093

[1] DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DAb+Fr; Dry aging bag + 180 days frozen; WA+Fr; Wet aging + 180 days frozen. CL: cooking losses; WBSF: Warner Braztler Shear Force. Different letter in the same row denotes groups statistically different (*P<0.05*) among LSMeans. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s, and then frozen at -20 °C.

3.4.2. Effects of preservation method and finishing system on fatty acid profile and oxidation.

The initial (unaged) intramuscular fat (IMF) content of LL from pasture and grain finishing diets was 3.48% and 3.63% (no tabulated data), respectively, which were not significantly different ($P > 0.05$). However, after PM treatment, DAb+Fr showed a greater IMF than the other three treatments ($P<0.01$) (Table 2). The predominant fatty acid profile was analyzed and reported in Supplementary data (Table S2). Analyzing the fatty acid composition by effect (PM and F), PM did not affect any of the mentioned fatty acid groups and ratios ($P>0.05$) (Table 2). It was observed a greater concentration of polyunsaturated acid (PUFA), PUFA n-3, conjugated linoleic acid (CLA; c9, t11-18:2) ($P <0.01$), PUFA/SFA ratio ($P =0.028$) in pasture than grain-fed steers (Table 2). The n-6: n-3 fatty acids ratio was greater in grain-fed compared to pasture-fed steers ($P<0.01$). A significant interaction effect (PM*F) was detected on C20:0 and C22:0 fatty acids and in lipid oxidation evaluated through TBARS. Greater concentrations of TBARS were observed in DAb and WA from grain-fed and DAb from pasture-fed than other treatments. The lowest concentration was in WA+Fr from pasture-fed ($P<0.01$) (Table 3).

Table 2. Effects (mean ± SEM) of preservation method (PM) and finishing diet (F) on intramuscular fat and fatty acid composition.

	PM					F		
Traits	**DAb**	**WA**	**DAb+Fr**	**WA+Fr**	**P-value**	**Pasture**	**Grain**	**P-value**
IMF (%)	3.9±0.2 b	3.7±0.2 b	4.5±0.2 a	3.8±0.2 b	<.001	3.7±0.2	4.3±0.2	0.094
CLA (mg/100g meat)	21.2±1.9	23.0±1.8	19.7±1.9	20.6±1.8	0.494	26.9±1.9	15.3±1.9	<0.001
MUFA (mg/100g meat)	1826.5±111.5	1774.9±111.5	1633.5±118.0	1702.4±111.5	0.596	1632.1±95.0	1836.5±97.0	0.136
SFA (mg/100g meat)	1902.7±126.5	2003.0±122.2	1938±126.5	2024±120.4	0.835	1925.0±119.2	2009.4±118.8	0.617
PUFA (mg/100g meat)	253.8±15.3	239.2±14.4	216.2±13.8	246.3±14.9	0.304	268.9±11.5	211.5±9.3	<0.001
PUFA n-6 (mg/100g meat)	183.4±11.8	173.6±10.9	160.3±10.7	181.7±11.5	0.419	179.2±9.0	169.2±8.7	0.460
PUFA n-3 (mg/100g meat)	53.1±3.8	58.3±4.0	51.0±3.6	58.4±4.0	0.208	78.8±5.7	38.6±2.8	<0.001
n-6:n-3	3.1±0.17	2.9±0.16	3.1±0.17	3.1±0.17	0.049	2.1±0.15	4.5±0.34	<0.001
PUFA/SFA	0.12±0.007	0.12±0.007	0.12±0.007	0.12±0.007	0.570	0.14±0.01	0.11±0.008	0.028

DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DAb+Fr: Dry aging bag + 180 days frozen; WA+Fr: Wet aging + 180 days frozen. CLA: conjugated linoleic fatty acid (c9, t11-18:2); PUFA: sum of polyunsaturated fatty acid (PUFA n-6 + PUFA n-3); MUFA: monounsaturated fatty acid (C14:1 + C16:1 + C18:1n9); SFA: saturated fatty acid (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0); PUFA n-3 (C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3); PUFA n-6 (C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6); IMF: intramuscular fat. Different letter in the same row denotes groups statistically different ($P<0.05$) among LSMeans. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s, and then frozen at -20 °C.

Table 3. Effects (mean ± SEM) of the interaction between the preservation method (PM) and the finishing diet (F) on the Arachidic (C20:0), Behenic (C22:0), and Thiobarbituric acid-reactive substances (TBARS) concentrations

	Pasture				Grain				Significance		
Traits	**DAb**	**WA**	**DAb+Fr**	**WA+Fr**	**DAb**	**WA**	**DAb+Fr**	**WA+Fr**	**PM**	**F**	**PM*F**
C20:0 (mg/100g)	11.9±1.7a	13.4±1.9a	6.2±0.9 b	11.8±1.7 a	3.9±0.5 b	4.5±0.6 b	4.3±0.7 b	4.3±0.6 b	0.013	<.001	0.014
C22:0 (mg/100g)	4.4±0.5 a	5.1±0.5 a	2.6±0.3 b	2.9±0.3 b	2.7±2.3 b	3.0±0.3 b	2.9±0.3 b	3.0±0.3 b	0.012	0.024	<.001
TBARS (mg /kg)	0.391±0.04 a	0.260±0.02 b	0.293±0.03 b	0.133±0.01 c	0.379±0.03 a	0.479±0.04 a	0.254±0.02 b	0.307±0.03 b	<.001	<.001	0.001

DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DAb+Fr: Dry aging bag + 180 days frozen; WA+Fr: Wet aging + 180 days frozen. TBARS: Thiobarbituric acid-reactive substances (mg MDA/kg meat). Different letter in the same row denotes groups statistically different (P<0.05) among LSMeans. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s, and then frozen at -20 °C.

Table 4. Effects (mean ± SEM) of preservation method (PM) and finishing diet (F) on microbiological growth.

		PM					F		
Traits	**Initial**	**DAb**	**WA**	**DAb+Fr**	**WA+Fr**	**P-value**	**Pasture**	**Grain**	**P-value**
TBC (log10/cm2)	<1.0	4.35±0.2	3.86±0.2	4.19±0.2	4.41±0.2	0.137	4.22±0.2	4.19±0.2	0.890
PSY (log10/cm2)	<1.0	5.38±0.13 b	4.96±0.13 c	6.15±0.14 a	5.52±0.13 b	<.001	5.55±0.13	5.45±0.13	0.573
ENT (log10/cm2)	<1.0	3.33±0.2 a	2.72±0.2 ab	3.40±0.2 a	2.54±0.2 b	0.002	2.86±0.15	3.14±0.15	0.020

DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DAb+Fr; Dry aging bag + 180 days frozen; WA+Fr; Wet aging + 180 days frozen. TBC: total bacterial count; PSY: Psychotropic bacteria; ENT: Enterobacteriaceae bacteria. Different letter in the same row denotes groups statistically different (P<0.05) among LSMeans. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

3.4.3. Effects of preservation method and finishing system on surface microbial counts

Meat samples analyzed before aging presented surface microbial count below the detection limit (<2 log/cm^2). The preservation method did not affect the TBC number; the range was from 3.86 to 4.41 log CFU /cm^2 (P>0.05). However, PSY bacteria counts were more significant in aged frozen meat than in samples only aged (P<0.01). Lower ENT numbers were observed in WA and WA+Fr compared to DAb and DAb+Fr (P<0.01). In addition, meat from grain-fed steers presented a greater ENT count than that from pasture-fed steers (P<0.05) (Table 4).

3.4.4. Effects of preservation method and finishing system on consumer sensory panel

Meat from grain-fed steers was preferred in terms of overall liking, flavor liking, and tenderness liking than pasture-finished animals when all the consumers were considered (P<0.01) (Table 5). There was an interaction effect between the preservation method and finishing system on tenderness liking considering all the consumers (n=200), were more tender grain-fed than pasture-fed steers (P=0.031). All the consumers were segmented into three clusters depending on their acceptability scores. Cluster 1 (n=77) presented a PM*F interaction for both overall liking (P<0.01) and flavor liking (P<0.01). The less preferred samples were DAb and DAb+Fr from pasture-fed steers. Thus, Cluster 1 could be characterized by a higher preference for grain-fed steers independently of the preservation method, within pasture-fed beef, consumers preferred meat from WA (either fresh or frozen) in terms of overall flavor liking. They could be named "Grain-fed aged beef and pasture-fed WA beef likers". Cluster 2 consumers (n=43) presented a significant PM*F interaction for overall liking (P<0.01), tenderness liking (P=0.023), and flavor (P=0.033). Like Cluster 1, consumers' preference for the three attributes was greater in grain-fed samples, but regarding pasture-fed beef, their preference was mainly for DAb. However, consumers from Cluster 2 have scores closer to 'neither like nor dislike', thus, they were more hesitant to decide. They could be named "Grain-fed aged beef and pasture-fed DAb beef likers". On the other side, Cluster 3 (n=80) also presented PM*F for all the attributes evaluated (P<0.01). Consumers preferred (P < 0.05) WA and WA+Fr from grain-fed beef, while DAb+Fr from pasture-fed animals were also preferred for overall

liking. Regardless of these differences, consumers from Cluster 3 scored all the meat samples at good levels, between 2 to 3 on the scale, thus they can be named: “All types of beef likers”. Regarding socio-demographic characteristics no important differences have been found among clusters (Table S1, Supplementary data).

Table 5. Effect (mean ± SEM) of overall liking, tenderness, and flavor acceptability scores by consumers as a whole and segmented in clusters depending on the meat preservation method (PM) and the finishing diet (F) of steers.

	Pasture				Grain				Significance		
	DAb	WA	DAb+Fr	WA+Fr	DAb	WA	DAb+Fr	WA+Fr	PM	F	PM*F
Overall liking											
All consumers	3.7±0.14	3.7±0.14	3.6±0.14	3.7±0.14	3.5±0.14	3.1±0.14	3.3±0.14	3.3±0.14	0.444	<.001	0.196
Cluster 1	4.4±0.17a	3.6±0.17b	4.5±0.15a	3.5±0.15b	3.2±0.17c	3.3±0.17bc	3.2±0.15c	3.7±0.15b	0.048	<.001	<.001
Cluster 2	4.4±0.26c	5.5±0.26a	4.1±0.31c	5.4±0.31a	4.9±0.26b	4.1±0.26c	4.9±0.31b	4.7±0.31b	0.316	0.307	<.001
Cluster 3	2.6±0.17b	2.8±0.17b	2.4±0.18c	3.2±0.18a	2.9±0.17b	2.3±0.17c	2.8±0.18b	2.4±0.18c	0.487	0.255	<.001
Tenderness											
All consumers	3.3±0.14a	3.6±0.14a	3.2±0.14a	3.3±0.14a	2.7±0.14b	2.7±0.14b	2.9±0.14b	2.7±0.14b	0.740	<.001	0.031
Cluster 1	3.9±0.22	3.7±0.22	3.9±0.19	3.3±0.19	2.7±0.22	2.9±0.22	2.8±0.19	2.8±0.19	0.470	<.001	0.158
Cluster 2	3.8±0.31b	5.0±0.31a	3.7±0.36b	4.4±0.36a	3.3±0.31b	3.3±0.31b	3.7±0.36b	3.8±0.36b	0.062	0.001	0.023
Cluster 3	2.5±0.18a	2.7±0.18a	2.2±0.19b	2.8±0.19a	2.5±0.18a	2.0±0.18b	2.7±0.19a	2.1±0.19b	0.961	0.069	<.001
Flavor											
All consumers	3.7±0.14	3.7±0.14	3.7±0.14	3.7±0.14	3.5±0.14	3.3±0.14	3.4±0.14	3.4±0.14	0.735	0.001	0.923
Cluster 1	4.4±0.19a	3.6±0.19b	4.6±0.17a	3.4±0.17b	3.2±0.19b	3.4±0.19b	3.4±0.17b	3.7±0.17b	0.028	<.001	<.001
Cluster 2	4.3±0.30b	5.2±0.30a	4.0±0.35b	5.1±0.35a	4.8±0.30a	4.4±0.30b	4.5±0.35b	4.4±0.35b	0.334	0.600	0.033
Cluster 3	2.8±0.18ba	2.8±0.18ba	2.6±0.19b	3.4±0.19a	3.0±0.18a	2.5±0.18b	2.9±0.19ab	2.6±0.19b	0.184	0.365	0.006

DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DAb+Fr: Dry aging bag + 180 days frozen; WA+Fr: Wet aging + 180 days frozen. Scale 9 points: 1 “I like it extremely”, 2 “I like very much”, 3 “I quite like it”, 4 “I like it”, 5 “I neither like nor dislike”, 6 “I dislike it”, 7 “I quite dislike it”, 8 “I dislike very much” and 9 “I dislike it extremely”. Different letter in the same row denotes groups statistically different ($P < 0.05$) among LSMeans. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s, and then frozen at -20 °C.

3.5. Discussion

3.5.1. Effects of preservation method and finishing system on instrumental color, pH, cooking losses, and shear force

Consumers use the color of meat to determine its freshness and wholesomeness (Lee et al., 2013). It is the most significant characteristic influencing consumers' purchase decisions, even though preferences vary among consumers (Altmann et al., 2022; Realini et al., 2014). In line with Dikeman et al. (2013) and Gudjónsdóttir et al. (2015), WA meat was lighter (greater L* values) and less red (lower a* values) than DAb after 40 d aging and after aging and then frozen storage for 180 d. The lower lightness of DAb could be attributed to the moisture loss on the meat surface, resulting in more light absorption and a darker color (Kim et al., 2011). However, Li et al. (2014) did not find any effect of the aging method (dry, dry bag, and wet) on instrumental color values. Bernardo et al. (2020) did not find differences between traditional dry aging (chilled condition) and meat aging and then frozen conditions in the first 5 days of retail display in L* (32.7 vs. 30.8, respectively) values. However, a* (20.8 vs 16.6) and b* values (17.4 vs. 14.4) were lower in meat that was frozen compared to chilled striploin samples.

Meat from cattle raised on pasture is reported to be darker than meat from grain-finished animals measured by both objective and subjective methods (Vestergaard et al., 2000; Priolo et al., 2001; Gatellier et al., 2005). In our study, lower L* (lightness) values were observed in meat from pasture-fed steers. We hypothesize that a greater myoglobin concentration in pasture-fed animals would be responsible for less lightness of the meat since the final pH and IMF fat content did not differ between the two finishing systems. Muscles from grass-fed cattle have more myoglobin (more muscle activity), perhaps making them darker in appearance, and have greater mitochondrial-based oxidative enzyme content, less glycolytic enzyme content, and when subjected to an in vitro glycolysis system, produce less lactate (Apaoblaza et al., 2020).

The increase in pH after 40 days of aging compared to the initial value (data not shown) was more noticeable in DAb than WA, in both chilled-only and chilled and

then frozen samples. Still, in any case, pH was greater than 5.8, the threshold used in the beef market because it is associated with the inhibition of pathogenic and spoilage microorganisms. These results agreed with Zhang et al. (2019), who reported an increase in pH after 21 d of DAb beef. Other studies (Dikeman et al., 2013; Li et al., 2014; Obuz et al., 2014; Kim et al., 2017), indicated that pH increased in DAb and decreased in WA between 20 d to 40 d of aging. Later authors indicated that this increase in pH after DAb could be associated with the generation of nitrogenous compounds from proteolysis; meanwhile, the lower pH in WA would be caused by the greater accumulation of lactic acid.

Cooking losses decreased in DAb compared to WA, and this effect was in greater magnitude after frozen storage (DAb+Fr vs. WA+Fr), possibly due to the significant amount of moisture loss by evaporation during the dry aging process (Zhang et al., 2019; Juarez et al., 2011). In addition, freezing also reduces meat's water-holding capacity due to muscle fiber disruption by the ice crystal formation (Leygonie et al., 2012).

Several studies have indicated no differences in WBSF due to the aging methods (WA vs DA and DAb) (Dikeman et al., 2013; Ahnstrom et al., 2006; Berger et al., 2018). All of them have an aging time ranging from 21 d to 28 d. However, Kahraman and Gurbuz (2019), reported greater WBSF values in DAb (3.77 kgF) than WA (3.27 kgF) in meat aged for 21 d and pointed out that striploin WBSF decreased as the aging time increased. Other studies observed a decrease in WBSF in dry aging (2.66 kgF) compared to stepwise aging (2.94 kgF) for 17 days (Kim et al., 2017). In our study, WA and DAb meat presented no difference in WBSF and the values were lower than frozen samples, however, all of them were below 3 kgF, a tenderness threshold for consumers beef acceptability (Miller et al., 2001).

3.5.2. Effects of preservation method and finishing system on fatty acid profile and oxidation

The effect of animal diet on fatty acid composition is demonstrated, e.g., animals fed on grass have higher omega-3 (n-3) fatty acids (Daley et al., 2010). The UK Department of Health (1994) recommended intakes of FAs with an n6: n3 ratio ≤4, which were reached in meat from pasture-fed steers and agreed with previous studies (Nuernberg et al., 2005; Brito et al., 2014). In the present study, the fatty acid profile (Supplementary Table S2) showed greater contents of myristic (C14:0), myristoleic (C14:1), palmitoleic (C16:1), oleic (C18:1), eicosadienoic (C20:2), and eicosatrienoic (C20:3) acids in the IMF of grain than in pasture-fed steers. Pasture-fed beef cattle had greater content of linolenic (C18:3), arachidic (C20:0), eicosapentaenoic (C20:5, EPA), and docosapentaenoic (C22:5, DPA) acids than grain-fed cattle. This information has been widely reported in previous studies (Realini et al., 2004; Ponnampalam et al., 2006; Jiang et al., 2010) where greater content of stearic (C18:0), linolenic (18:3) and arachidonic (C20:4) acids in pasture-fed animals was observed than in grain-fed animals.

Preservation methods increase IMF (%) in DAb+Fr, possibly due to water loss by evaporation during aging and frozen storage, which could also be associated with the lowest CL (%) (Zhang et al., 2019). However, the PM did not show an effect on most fatty acids identified, except for C20:0 and C22:0, which presented the highest values in samples from the pasture diet and during the aging process (DAb and WA). Berger (2017) reported no significant differences in fatty acid profile between aging methods (wet, dry, and dry bag aging) except for docosapentaenoic acid (DPA, 22:5n-3) which showed a greater concentration in DAb than the other two aging methods. The author did not have a clear explanation of how and why only affected the greater content of DPA of beef samples compared to other aging methods in the current study. Jiang et al. (2010), working with ground beef reported a greater concentration of C20:1n9 in un-aged samples than in dry-aged ones.

Lipid oxidation is a major cause of quality deterioration in meat and meat products. It leads to increase “rancidity” resulting in undesirable odors and flavors (Wang et al., 1997). In our study, a significant interaction between the preservation

method and the finishing system was detected. Greater oxidative stability of lipids (lower TBAR values) was observed in frozen stored beef regardless of the diet, except for WA from pasture-fed steers that had similar values to the other treatments. The lowest value was in WA+Fr from pasture-fed steers. Similar results were reported in other studies on beef comparing aged and frozen meat: 0.33 vs. 0.23 mg MDA/kg meat (Zhang et al., 2020; dry aging bag vs. stepwise (dry bag/wet) aging [21 days]: and then 12 months of frozen) and 0.24 vs. 0.25 mg MDA/kg meat (Bernardo et al., 2020; dry aged for 28 days vs. aged and then frozen for 1 month), respectively. During storage, MDA may further degrade into organic alcohols and acids or attach to free amino acids and proteins as MDA-amino-acids complex (Farouk et al., 2003), and these changes cannot be detected using the TBARS assay. Therefore, the variation during aging and frozen storage observed in the current studies could have resulted from different reaction rates between generation and degradation of MDA during storage. Differences observed in aging methods agree with previous findings that indicated less oxidation in dry aged loin steaks in bags compared with traditional dry aging (DeGeer et al., 2009), which would suggest a protective effect of dry aging in bags from the oxidative deterioration (Zhang et al., 2020). It has been shown that animals fed on pasture have greater concentrations of vitamin E in muscle than those grain-fed (Realini et al., 2004; Nuernberg et al., 2005; Descalzo et al., 2007; Daley et al., 2010; Bernardo et al., 2020), which delays the lipid oxidation and metmyoglobin formation (Schwarz et al., 1998; Zerby et al., 1999; Descalzo & Sancho, 2008). The range of TBARS (0.133–0.479 mg MDA/kg meat) found in this study, was lower than the threshold (2 mg MDA/kg meat) for the detection of rancid flavor by trained panelists (Campo et al., 2006).

3.5.3. Effects of preservation method and finishing system on superficial microbial counts.

Samples analyzed before aging (initial load) showed <1 log CFU/cm^2 for TBC and ENT. Dry aging bag and DAb+Fr increased ENT compared with WA after the frozen storage period. Li et al. (2013) did not find an impact of aging treatment on ENT counts when comparing DAb and WA for 14 days, the same as in our study when

the meat was aged for 40 days. Similar results were reported by Hulanková et al. (2018) and Ahnstrom et al. (2006) in dry aging meat for 14 days. Bacteria that develop in meat at chill temperatures and are kept in frozen conditions are regarded as psychrotrophic (Gonzalez-Gutierrez et al., 2020), including a huge list of bacteria. Despite the high number of microorganisms, only a few species dominate to cause spoilage because temperature, time of storage, and packaging atmosphere can affect both microbial growth and species selection during storage of fresh meat (Ercolini et al., 2006). In this sense, Borch *et al*. (1996) reported that the retail shelf-life of meat is estimated as the time required by the bacterial population to reach a level of 10^7 CFU/cm^2. Stanbridge and Davies (1998) also state that levels of PSY over 7 to 8 log/cm^2 trigger strange smells and surface sliminess in meat. In our study, the PSY numbers were less than 6.5 log CFU/$cm^{2,}$ not affecting meat quality attributes. However, an off flavor, which is a result of spoilage in meat, can be detected when the total bacteria count (TBC) is around 7 log CFU/cm^2 or g of meat product, although some negative changes can be observed much earlier with TBC numbers between 5 and 6 log CFU/cm^2 or g of meat product (Feiner, 2006). In this study, there was no effect of the aging method on TBC, suggesting that both methods are equally suitable for meat conditioning.

Some works have reported no significant differences in microbial counts between grain and pasture-fed beef (Duarte *et al*., 2022; Casas *et al*., 2021), just like in our study for TBC and PSY bacteria counts. However, greater ENT counts were observed in meat from grain-fed steers than in pasture-fed steers. It has been stated that high-grain diets can decrease ruminal *pH*, favoring the growth of acid-tolerant bacteria (Diez-Gonzalez *et al*., 1998), for example, *E. coli O157:H7*, a semi-acid-resistant pathogen that belongs to the ENT family. Nevertheless, Zhang *et al*. (2010) reported no differences in bacterial contamination between beef from grass and grain-fed cattle. These authors also stated that other aspects than diet may play an important role in microbial contamination of meat such as how beef is processed.

3.5.4. Effects of preservation method and finishing system on consumer sensory panel

Consumer preferences are very variable and dependent on an array of different factors (Font-i-Furnols & Guerrero, 2014). Thus, it is very common to find segments of consumers with different preferences, and it is important to identify them.

The sensory results showed that consumers from Cluster 1, i.e. "Grain-fed aged beef and pasture-fed WA beef likers" preferred aged meat from grain-fed animals, especially those from DAb aging, as well as WA meat from pasture-fed animals for overall liking and flavor liking. The last combination (WA from pasture-fed) is the most common meat consumed in Uruguay and is possibly recognized and more accepted by this consumer group. Wet-aged beef from fresh samples presented lower aging odor and flavor, higher herbs odor, lower hardness, and higher juiciness in mouth texture than DAb-aged beef (Panella-Riera *et al*., 2023), and this can be related to consumers' preferences for beef meat. On the other hand, Realini *et al.* (2009) found that, for a segment of consumers, meat from beef fed on a combination of concentrate and pasture was preferred to that from only pasture-fed animals. Similar results were found in lamb by Font i Furnols et al. (2006).

Consumers from Cluster 2 *i.e.* "Grain-fed aged beef and pasture fed DAb beef likers" are similar to those of Cluster 1 in the sense they liked pasture-fed beef, but are different from consumers of Cluster 1, in the sense that they preferred DAb (fresh or frozen) meat from pasture-fed steers instead of WA (fresh and frozen) samples from grain-fed steers, which had the highest scores (less like). Differences in preferences for the different sensory characteristics of meat (Panella-Riera *et al*., 2023) can explain these differences in acceptability.

In both cases, *i.e.* Cluster 1 and 2, results are surprising since Uruguayan consumers are used to eating beef from pasture-fed animals, and the habits greatly affect preferences (Font-i-Furnols and Guerrero, 2014; Font i Furnols *et al*., 2006). In fresh pasture-fed beef samples, both WA and DAb presented significantly higher abnormal and herb odors (Panella-Riera *et al*., 2023) that might have influenced consumer acceptance. Moreover, in non-aged beef samples, those from pasture had a higher beef odor and flavor intensity and higher mouth tenderness than those from concentrate plus hay-fed animals (Resconi *et al*., 2010), which also can influence

consumer's acceptability. A cluster with similar preferences as this one was found by Realini *et al.* (2009). Studies have reported that grain-fed cattle produce greater IMF in meat (Schroeder et al., 1980; Hedrick et al., 1983), and the dry aging process requires beef with a high content of IMF to help ensure products with consistent tenderness, flavor, and juiciness (Nishimura, 1998; Dashdorj *et al*., 2016). However, in this study, no differences in IMF were found for the F (grain *vs.* pasture) effect. Tenderness liking attribute tended to be best classified by this Cluster regarding the preservation methods in samples from grain-fed. This is in concordance with shear force values (< 2.95 kgF) found (Table 1).

Cluster 3 *i.e.* "all types of beef likers," is characterized by scoring all the samples close to "I like very much". They did not discriminate between the different treatments; they all liked it equally. Debate on the consumer preference and acceptability of dry, wet, and dry bag aging is ongoing. In concordance with our study, Berger *et al*. (2018) in samples from 100% grass-fed heifers (35 days aging, 2 °C, 78% RH and air flow of < 0.2 m/s), indicated that overall liking scores were not different across aging treatments (wet, dry, and dry bag). However, the same consumer panel identified beef aged in dry-aging bags as having higher tenderness and overall preference compared to aged beef in a typical vacuum bag, indicating that dry-aging in bags could be the preferred aging process for steaks from pasture-fed cattle like our Cluster 1, in overall and flavor liking. According to Zhang et al. (2022), tailored flavour profiles and consistent quality can be achieved by manipulating elements as animal sources, intramuscular fat content, use of novel dry-ageing techniques (e.g., in-bag dry-ageing and combined ageing regimes), and ageing parameters when designing dry-ageing strategies. Release of lipid-derived volatiles and flavour precursors further contributes to enhancing the flavour of cooked dry-aged meat.

The information obtained in this study indicates that the consumer sensory panel showed levels of overall liking for all treatments, among 2 ("I like very much") and 4 (I like it). Shear force values suggested high tenderness (< 3 kgF), lipid oxidation was below the rancid flavor threshold (2 mg MDA/kg meat), and PSY microbial growth was under 7 $\log_{10}/cm^2$. These findings suggest that any of the aging types and frozen conditions tested could produce meat quality within consumer satisfaction.

3.6. Conclusion

Even though differences were found in physicochemical characteristics among preservation methods, their magnitude would not have major implications for meat quality. The fatty acid profile of the intramuscular fat was more affected by the finishing diet than the preservation method. Dry aging (and also with subsequent frozen storage) showed an increase in the *Psychrotrophic* and *Enterobacteriaceae* counts compared to wet aging, despite the consumers scoring positively (at least "4 - I like it") the overall liking of all treatments. However, a better understanding of some preferences for treatment segmentation by clusters is necessary. Finally, frozen storage after aging beef would be a suitable strategy to supply high-quality meat for export markets; however, from a sensory perspective, it is suggested to conduct a consumer panel in countries with objective international markets. Therefore, further studies characterizing these positive benefits and the associated economic costs of the dry-aged bag at a higher resolution would be beneficial.

3.7. References

Ahn, D.U., Olson, D.G., Lee, J. I., Jo, C., Wu, C., and Chen, X. 1998. Packaging and irradiation effects on lipid oxidation and volatiles in pork patties. J. of Food Sci. 63:15-19. doi:10.1111/j.1365-2621.1998.tb15665.x

Ahnström, M.L., Seyfert, M., Hunt, M.C., and Johnson, D.E. 2006. Dry aging of beef in a bag highly permeable to water vapour. Meat Sci. 73: 674–679. https://doi.org/10.1016/j.meatsci.2006.03.006

Altmann, B. A., Anders, S., Risius, A., & Mörlein, D. 2022. Information effects on consumer preferences for alternative animal feedstuffs. Food Policy, 106, 102192. https://doi.org/10.1016/j.foodpol.2021.10219

American Meat Science Association (AMSA), 2016. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat, 2nd ed. Amer. Meat Sci. Assoc. Champaign, pp. 106

American Meat Science Association (AMSA), 2012. Research for Meat Color Measurement Guidelines. Revised December 2012. Amer Meat Sci. Assoc. 201 West Springfield Avenue, Suite 1202 Champaign, Illinois USA 61820

Apaoblaza, A., Gerrard, S.D., Matarneh, S.K., Wicks, J.C., Kirkpatrick, L., England, E.M., Scheffler, T.L., Duckett, S.K., Shi, H., Silva, S.L., Grant, A.L. & Gerrard, D.E. 2020. Muscle from grass- and grain-fed cattle differs energetically. Meat Sci. 161: 107996. https://doi.org/10.1016/j.meatsci.2019.107996

Ba, H. V., Oliveros, C. M., Park, K, Dashdorj, D. and Hwang, I. 2016. Effect of marbling and chilled ageing on meat-quality traits, volatile compounds and sensory characteristics of beef longissimus dorsi muscle. Anim. Prod. Sci. 57:(5) 981-992 https://doi.org/10.1071/AN15676

Berger, J. 2017. Effects of dry-aging on eating quality, physicochemical, and microbiological attributes of grass-fed beef loins. Master's thesis. Faculty of Purdue University. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Jordy+Berger%2C+2017&oq=j#:~:text=%2D%202017%20%2D-,docs.lib.purdue.edu,-Abstract%20The%20United

Berger, J., Kim, Y. H. B., Legako, J. F., Martini, S., Lee, J., Ebner, P. E., and Zuelly, S. M. S. 2018. Dry aging improves meat quality attributes of pasture-fed beef loins. Meat Sci. 145: 285-291. https://doi.org/10.1016/j.meatsci.2018.07.004

Bernardo, A.P., Da Silva, A.C., Francisco, V. C., Ribeiro, F. A., Nassu, R., Calkins, C. R., Do Nascimento M and Pflanzer S. 2020. Effects of freezing and thawing on microbiological and physical-chemical properties of dry-aged beef. Meat Sci. 161: 108003. https://doi.org/10.1016/j.meatsci.2019.108003

Bligh, E.G. & Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. Canadian J. Bioch. Physiol. 37:911-917. doi:10.1139/y59-099

Borch, E., Kant-Muermans, M.-L. and Blixt, Y. 1996. Bacterial spoilage of meat and cured meat products. Intern. J. of Food Microb. 33:103-120. doi:10.1016/0168-1605(96)01135-x

Brito, G., San Julián, R., La Manna, A., Del Campo, M., Montossi, F., Banchero, G., Chalkling, D. and Soares de Lima, J.M. 2014. Growth, carcass traits, and palatability: can the influence of the feeding regimes explain the variability found on those attributes in different Uruguayan genotypes? Meat Sci.98: 533-538. http://dx.doi.org/10.1016/j.meatsci.2014.07.003

Campbell, R.E., Hunt, M.C., Levis, P. & Chambers, E. 2001. Dry-Aging Effects on Palatability of Beef Longissimus Muscle. J. Food Sci., 66: 196–199. https://doi.org/10.1111/j.1365-2621.2001.tb11315.x

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D. and Richardson, R. I. 2006. Flavour perception of oxidation in beef. Meat Sci. 72: 303–311. https://doi.org/10.1016/j.meatsci.2005.07.015

Casas, D. E., Manishimwe, R., Forgey, S. J., Hanlon, K. E., Miller, M. F., Brashears, M. M. and Sanchez-Plata, M. X. 2021. Biomapping of Microbial Indicators on Beef Subprimals Subjected to Spray or Dry Chilling over Prolonged Refrigerated Storage. Foods. 10: 1403. https://doi.org/10.3390/foods10061403

Choe, J.-H., Stuart, A., and Kim, Y. H. B. 2016. Effect of different aging temperatures prior to freezing on meat quality attributes of frozen/thawed lamb loins. Meat Sci. 116, 158–164. doi:10.1016/j.meatsci.2016.02.014

Coombs CE, Holman BW, Friend MA, Hopkins DL. 2017. Long-term red meat preservation using chilled and frozen storage combinations: A review. Meat Sci. 125: 84-94. doi: 10.1016/j.meatsci.2016.11.025

Correa, D., Lema, M., Ravagnolo, O., Clariget, J., Luzardo, S and Brito, G. 2020. Effects of differences in level of post-weaning nutrition and in sire expected progeny differences for ribeye area on retail cuts yield in Hereford steers. Anim. Prod. Sci. https://doi.org/10.1071/AN19604

Daley, C. A., Abbot, A., Doyle, P. S., Nader, G. A. & Larson, S. 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition J. 9:10 http://www.nutritionj.com/content/9/1/10

Dashdorj, D. Tripathi, V. K., Cho, S., Kim, Y. & Hwang I. 2016. Dry aging of beef; Review. J. Anim. Sci. and Techn. 58: 20. doi 10.1186/s40781-016-0101-9

DeGeer, S. L., Hunt, M. C., Bratcher, C. L., Crozier-Dodson, B.A., Johnson, D. E., and Stika, J. F. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. Meat Sci. 83: 768–774. https://doi.org/10.1016/j.meatsci.2009.08.017

Descalzo, A. M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A.M., Carrete, J. and Pensel, N.A. 2007. Antioxidant status and odour profile in fresh beef from

pasture or grain-fed cattle. Meat Sci. 75(2): 299-307. doi: 10.1016/j.meatsci.2006.07.015

Descalzo, A. M., and Sancho, A. M. 2008. A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. Meat Sci. 79: 423-436. https://doi.org/10.1016/j.meatsci.2007.12.006

Diez-Gonzalez, F., Callaway, T. R., Kizoulis, M. G., & Russel, J. B. 1998. Grain feeding and the dissemination of acid-resistant Escherichia coli from cattle. Science. 281:1666–1668. DOI: 10.1126/science.281.5383.1666

Dikeman, M. E., Obuz, E., Gök, V., Akkaya, L., and Stroda, S. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef Longissimus lumborum steaks. Meat Sci. 94: 228–233. https://doi.org/10.1016/j.meatsci.2013.02.002

Duarte, T. L., Bolkenov, B., Klopatek, S. C., Oltjen, J. W., King, D. A., Shackelford, S. D., Wheeler, T. L., and Yang, X. 2022. Evaluating the Shelf Life and Sensory Properties of Beef Steaks from Cattle Raised on Different Grass Feeding Systems in the Western United States. Foods, 11, 2141. https://doi.org/10.3390/foods11142141

Ercolini, D., Russo, F., Torrieri, E., Masi, P., & Villani, F. 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Applied and environmental microbiology*, *72*(7), 4663-4671. https://doi.org/10.1128/AEM.00468-06

Farouk, M. M., Wielizcko, K.J., and Merts, I. 2003. Ultra-fast freezing and low temperatures are not necessary to maintain the functional properties of manufacturing beef. Meat Sci. 66: 171-179. https://doi.org/10.1016/S0309-1740(03)00081-0

Feiner, G. 2006. Meat products handbook: Practical science and technology. Elsevier, Amsterdam.

Font -i-Furnols, M., San Julián, R., Guerrero, L., Sañudo, C Campo. M.M., Olleta, J.L., Oliver, M.A., Cañeque, V., Álvarez, I., Díaz, M.T., Branscheid, W., Wicke, M., Nute, G.R. and Montossi, F.. 2006. Acceptability of lamb meat from different producing systems and ageing time to German, Spanish and British

consumers. Meat Sci. 72: 545-554. https://doi.org/10.1016/j.meatsci.2005.09.002

Font-i-Furnols, M., and Guerrero, L. 2014. Consumer preference, behavior and perception about meat and meat products: An overview. Meat Sci. 98: 361-371. https://doi.org/10.1016/j.meatsci.2014.06.025

Gatellier, P., Mercier, Y., Juin., H., & Renerre, M. 2005. Effect of finishing mode (pasture- or mixed-diet) on lipid composition, colour stability and lipid oxidation in meat from Charolais cattle. Meat Sci. 69: 175-186. doi:10.1016/j.meatsci.2004.06.022

Gill, C. O. 2014. Spoilage, factors affecting: Microbiological. Encyclopedia of Meat Sciences. 2nd ed. Elsevier, Amsterdam. pp. 388–393.

González-Gutiérrez, M., García-Fernández, C., Alonso-Calleja, C. and Capita, R. 2020. Microbial load and antibiotic resistance in raw beef preparations from northwest Spain. Food Sci. & Nutr. 8:777–785. DOI: 10.1002/fsn3.1319

Gudjónsdóttir, M., Gacutan, M. D.Jr., Mendes, A. C., Chronakis, I. S., Jespersen, L., and Karlsson, A. H. 2015. Effects of electrospun chitosan wrapping for dry-ageing of beef, as studied by microbiological, physicochemical and low-field nuclear magnetic resonance analysis. Food Chem 184: 167-, 175. https://doi.org/10.1016/j.foodchem.2015.03.088

Ha, M., McGilchrist, P., Polkinghorne, R., Huynh, L., Galletly, J., Kobayashi, K., Nishimura, T., Bonney, S., Kelman, K. R., Warner, R. D. 2019. Effects of different ageing methods on colour, yield, oxidation and sensory qualities of Australian beef loins consumed in Australia and Japan. Food Res. Int.125: 108528. https://doi.org/10.1016/j.foodres.2019.108528

Hedrick, H. B., Paterson, J. A., Matches, A. G., Thomas, J. D., Morrow, R. E., Stringer, W. G. & Lipsey, R. J. 1983. Carcass and Palatability Characteristics of Beef Produced on Pasture, Corn Silage and Corn Grain. J. Anim. Sci. 57: 91–801, https://doi.org/10.2527/jas1983.574791x

Hulánková, R., Kameník, J., Saláková, A., Závodský, D., and Borilova, G. 2018. The effect of dry aging on instrumental, chemical and microbiological parameters of

organic beef loin muscle. LWT - Food Sc. Techn. 89: 559–565. https://doi.org/10.1016/j.lwt.2017.11.014

Iida, F., Miyazaki, Y., Tsuyuki, R., Kato, K., Egusa, A., Ogoshi, H., and Nishimura, T. 2016. Changes in taste compounds, breaking properties, and sensory attributes during dry aging of beef from Japanese black cattle. Meat Sci. 112: 46–51. https://doi.org/10.1016/j.meatsci.2015.10.015

INAC (Instituto Nacional de Carne) 2013. Available at https://www.inac.uy/innovaportal/v/9944/14/innova.front/hilton [Accessed 29 March 2024]

IUPAC, 1987. Standard Methods for the Analysis of Oils, Fats and Derivatives. International Union of Pure and Applied Chemistry Division Commission on Oils, Fats and Derivatives, 7th ed Blackwell Jevent Publishers, Oxford

Juárez, M., Caine, W. R., Dugan, M. E. R., Hidiroglou, N., Larsen, I. L., Uttaro, B., and Aalhus, J. L. 2011. Effects of dry-ageing on pork quality characteristics in different genotypes. Meat Sci. 88: 117-121. https://doi.org/10.1016/j.meatsci.2010.12.011

Jiang, T., Bsuboom, J.R., Nelson, M.L., O´Fallon, J., Ringkob, T.P., Rogers-Klette, K.R., Joos, D. & Piper, K. 2010. The influence of forage diets and aging on beef palatability. Meat Sci. 86: 642-650. https://doi.org/10.1016/j.meatsci.2010.05.016

Kahraman, H. A. and Gürbüz, Ü. 2019. Effects of three aging methods on the Longissimus lumborum muscle from Holstein-Friesian steers. Med. Weter. 75 (3), 179-184. https://dx.doi.org/10.21521/mw.6182

Kahraman, H. A., and Gürbüz, Ü. 2018. Aging Applications on Beef Meat. Manas Journal of Engineering, 6(1), p7

Kim, Y. H. B., Frandsen, M., and Rosenvold, K. 2011. Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. Meat Sci. 88: 332–337. https://doi.org/10.1016/j.meatsci.2010.12.020

Kim, J. H., Kim, D. H., Ji, D., Lee, H. J., Yoon, D. K., and Lee, C. H. 2017. Effect of Aging Process and Time on Physicochemical and Sensory Evaluation of Raw Beef Top Round and Shank Muscles Using an Electronic Tongue. Korean J.

Food Science Animal Resource. 37(6): 823-832. https://doi.org/10.5851/kosfa.2017.37.6.823

Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., and Hunt, M.C. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies (Review). Meat Science. 144: 74-90. https://doi.org/10.1016/j.meatsci.2018.04.031

Kim, J. H., Kim, T. K., Shin, D. M., Kim, H. W., Kim, Y.B. and Choi, Y.S. 2020. Comparative effects of dry-aging and wet-aging on physicochemical properties and digestibility of Hanwoo beef. Asian-Australas J. Anim. Sci. 33: No. 3:501-505. https://doi.org/10.5713/ajas.19.0031

Lee, S. M., Lee, K. T., Lee, S. H., and Song, J. K. 2013. Origin of human colour preference for food. Journal of Food Engineering, 119(3) 508–515. https://doi.org/10.1016/j.jfoodeng.2013.06.021

Leygonie, C., Britz, T. J., and Hoffman, L. C. 2012. Impact of freezing and thawing on the quality of meat: Review. Meat Sci. 91: 93–98. https://doi.org/10.1016/j.meatsci.2012.01.013

Li, X., Babol, J., Wallby, A., and Lundström, K. 2013. Meat quality, microbiological status and consumer preference of beef gluteus medius aged in a dry ageing bag or vacuum. Meat Science. 95: 229–234. https://doi.org/10.1016/j.meatsci.2013.05.009

Li, X., Babol, J., Bredie, W. L. P., Nielsen, B., Tománková, J. and Lundstrom. 2014. A comparative study of beef quality after ageing longissimus muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. Meat Sci. 97: 433–442. https://doi.org/10.1016/j.meatsci.2014.03.014

Li, Z., Ha, M., Frank, D., McGilchrist, P., and Warner, R. D. 2021. Volatile profile of dry and wet aged beef loin and its relationship with consumer flavour liking. Foods, 10, 3113. https://doi.org/10.3390/foods10123113

Luzardo, S., Saadoun, A., Cabrera, M.C., Terevinto, A., Brugnini, G., Rodriguez, J., de Souza, G., Rovira, P., and Rufo, C. 2024. Effect of beef long-storage under different temperatures and vacuum-packaging conditions on meat quality,

oxidation processes and microbial growth. Journal of the Science of Food and Agriculture. 104(2): 1143-1153. doi: 10.1002/jsfa.12999

MacFie, H. J. and Bratchell, N.K. 1989. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. Journal of Sensory Studies. https://doi.org/10.1111/j.1745-459X.1989.tb00463.x

Melton S. L. 1983. Effect of forage feeding on beef flavor. Food Technology. 37: 239 248

MGAP (Ministerio de Ganaderia, Agricultura y Pesca) 2023. Available at https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/sites/ministerio-ganaderia-agricultura-pesca/files/2021-08/CarneAltaCalidad_8350_0621_UE_Ingles.pdf. [Accessed 1 March 2024]

Miller, M. F., Carr, M.A., Ramsey, C. B., Crockett, K. L., and Hoover, L. C. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79: 3062–3068. https://doi.org/10.2527/2001.79123062x

Nishimura, T. 1998. Mechanism involved in the improvement of meat taste during post-mortem aging. Food Science and echnology. 4: 241-249. https://doi.org/10.3136/fsti9596t9798.4.241

Nuernberg, K., Dannenberger, D., Nuernberg, G., Ender, K., Voigt, J. Scollan, N.D., Wood J.D., Nute, G.R., and Richardson, R.I. 2005. Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. Livest. Prod. Sci. 94: 137– 147. https://doi.org/10.1016/j.livprodsci.2004.11.036

Obuz, E., Akkaya, L., Gok, V., and Dikeman, N. E. 2014. Effects of blade tenderization, aging method and aging time on meat quality characteristics of Longissimus lumborum steaks from cull Holstein cows. Meat Sci. 96: 1227-1232. https://doi.org/10.1016/j.meatsci.2013.11.015

Panella-Riera, N., Correa, D., Brito, G., del Campo, M., Luzardo, S., and Font-i-Furnols, M. 2023. Sensory characteristics of wet and dry-bag aged beef from grain and pasture finished steers. Proceedings of the 69th International Congress of Meat Science and Technology (ICoMST), 21-25th August, Padova, Italy, p. 402-403.

Parrish, F. C., Boles, J. A., Rust, R. E., and Olson, D. G. 1991. Dry and wet aging effects on palatability attributes of beef loin and rib steaks from three quality grades. Journal of Food Science. 56: 601–603. https://doi.org/10.1111/j.1365-2621.1991.tb05338.x

Peripolli, E., Banchero, G., Pereira, A., Brito, G., La Manna, A., Fernandez, E., Montossi, F., and Baldi, F. 2018. Effect of growth path on the performance and carcass traits of Hereford steers finished on pasture or in feedlot. Animal Production Science. 58: 1341–1348. doi:10.1071/AN16061

Ponnampalam, E. N., Mann, N. J. and Sinclair, A. J. 2006. Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts: potential impact on human health. Asia Pacific Journal of Clinical Nutrition. 15 (1):21-29. apjcn.nhri.org.tw/server/APJCN/15/1/21.pdf

Priolo, A., Micol, D., and Agabriel, J. 2001. Effects of grass feeding systems on ruminant meat colour and flavour. A review. Animal Research. 50: 185–200 185 © INRA, EDP Sciences. DOI: 10.1051/animres:2001125

Realini, C. E., Kallas, Z., Pérez-Juan, M., Gómez, I., Olleta, J. L., Beriain, M. J., Albertí, P., and Sañudo, C. 2014. Relative importance of cues underlying Spanish consumers' beef choice and segmentation, and consumer liking of beef enriched with n-3 and CLA fatty acids. Food Quality and Preference, 33, 74-85. https://doi.org/https://doi.org/10.1016/j.foodqual.2013.11.007

Realini, C.E., Font i Furnols, M., Guerrero, L., Montossi, F., Campo, M.M., Sañudo, C., Nute, G.R., Alvarez, I., Cañeque, V., Brito, G., and Oliver, M.A. 2009. Effect of finishing diet on consumer acceptability of Uruguayan beef in the European market. Meat Science. 81: 499–506. https://doi.org/10.1016/j.meatsci.2008.10.005

Realini, C.E., Duckett, S.K., Brito, G.W., Dalla Rizza, M., and De Mattos, D. 2004. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. Meat Science. 66: 567–577. https://doi.org/10.1016/S0309-1740(03)00160-8

Resconi, V.C., Campo, M.M., Font i Furnols, M., Montossi, F., and Sañudo, C. 2010. Sensory quality of beef from different finishing diets. Meat Science. 86: 865-869. https://doi.org/10.1016/j.meatsci.2010.07.012

Schroeder, J. W., Cramer, D.A., Bowling R.A., and Cook, C. W. 1980. Palatability, shelf life and chemical differences between forage- and grain-finished Beef. Journal of Animal Science. 50: 852–859, https://doi.org/10.2527/jas1980.505852x

Smith, R., Nicholson, K., Nicholson, J., Harris, K., Miller, R., Griffin, D., and Savell, J. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. Meat Science. 79: 631–639. https://doi.org/10.1016/j.meatsci.2007.10.028

Schwarz, F. J., Augustini, C., Timm, M., Kirchgessner, M., and Steinhart, H. 1998. Effect of vitamin E on alpha-tocopherol concentration in different tissues and oxidative stability of bull beef. Livestock Production Science. 56: 165-171. https://doi.org/10.1016/S0301-6226(98)00189-4

Stanbridge, L.H., and Davies, A.R. 1998. The microbiology of chill-stored meat. In: A.R. Davies, R.J. Board and R.G. Board, editors, The microbiology of meat and poultry. Springer, New York. pp. 174-206

UK Department of Health. 1994. Report on health and social subjects. No. 46. Nutritional aspects of cardiovascular disease. London: HMSO.

Vestergaard, M., N. Oksbjerg, and P. Henckel. 2000. Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. Meat Science. 54:177–185. doi:10.1016/S0309-1740(99)00097-2

Wang, C., Zhu, L., and Brewer, M.S. 1997. Comparison of 2-Thiobarbituric acid reactive substances determination methods in various types of frozen, fresh meat. Journal of Food Lipids 4 87-96. https://doi.org/10.1111/j.1745-4522.1997.tb00083.x

Warren, K.E., and Kastner, C.L. 1992. A comparison of dry-aged and vacuum-aged beef strip loins. Journal of Muscle Foods. 3: 151–157. https://doi.org/10.1111/j.1745-4573.1992.tb00471.x

Wheeler, T. L., Savell, J. W., Cross, H. R., Lunt, D. K., and Smith, S. B. 1990. Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. Journal of Animal Science. 68: 4206–4220. https://doi.org/10.2527/1990.68124206x

Zerby, H. N., Belk, K. E. Sofos, J. N., McDowell, L. R., and Smith, G. C. 1999. Case life of seven retail products from beef cattle supplemented with alpha-tocopheryl acetate. Journal of Animal Science. 77:2458-2463. https://doi.org/10.2527/1999.7792458x

Zhang, R., Yoo, M. J.Y., and Farouk, M. 2021. Oxidative stability, proteolysis, and in vitro digestibility of fresh and long-term frozen stored in-bag dry-aged lean beef. Food Chemistry. https://doi.org/10.1016/j.foodchem.2020.128601

Zhang, R., Yoo, M. J. Y., and Farouk, M. M. 2019. Quality and acceptability of fresh and long-term frozen in-bag dry-aged lean bull beef. Journal of Food Quality, (1975264), 1–15. https://doi.org/10.1155/2019/1975264

Zhang, J., Wall, S. K., Xu, L., and Ebner, P. D. 2010. Contamination rates and antimicrobial resistance in bacteria isolated from “grass-fed” labeled beef products. Foodborne Pathogens Disease 7:1331-1336. https://doi.org/10.1089/fpd.2010.0562

3.8. Supplementary data

Table S1. Socio-demographic characteristics of the consumers (%).

Characteristics			All Consumers (n=100); aged	All Consumers (n=100); aged+frozen	All Consumers (n=200)	Clus.1 (n=77)	Clus.2 (n=43)	Clus.3 (n=80)
Sex		Male	45.0	56.0	55.5	53.0	53.5	58.8
		Female	55.0	44.0	44.5	47.0	46.5	41.2
Age		< 30 years	35.0	27.0	31.0	26.0	25.6	38.7
		30-50 years	53.0	60.0	56.5	63.6	62.8	46.3
		> 50 years	12.0	13.0	12.5	10.4	11.6	15.0
Educational level		Primary school	5.0	2.0	3.5	3.9	2.3	3.7
		Secondary school	25.0	27.0	26.0	28.6	25.6	23.8
		University	47.0	46.0	46.5	44.1	44.2	50.0
		Post-graduate	23.0	25.0	24.0	23.4	27.9	22.5
Frequency of fresh meat consumption	Pork	Never	24.3	19.0	21.5	16.9	30.2	21.2
		Once a month	39.4	52.0	45.5	44.1	48.8	45.0
		Every two weeks	22.2	13.0	17.5	19.5	9.4	20.0
		Every week	14.1	16.0	15.5	19.5	11.6	13.8
	Beef	Never	-	-	-	-	-	-
		Once a month	3.0	4.0	4.0	5.1	-	5.0
		Every two weeks	9.1	10.0	9.5	7.8	9.3	11.2
		Every week	87.9	86.0	86.5	87.1	90.7	83.8
	Chicken	Never	4.1	3.0	3.5	-	4.6	6.2
		Once a month	7.2	4.0	6.0	5.2	7.0	6.2
		Every two weeks	24.7	25.0	25.5	23.4	27.9	25.0
		Every week	63.9	68.0	65.5	71.4	60.5	62.6
	Lamb	Never	15.5	16.0	16.0	15.6	9.3	20.0
		Once a month	54.6	42.0	48.0	41.6	58.1	48.7
		Every two weeks	19.6	25.0	22.5	25.9	20.9	20.0
		Every week	10.3	17.0	13.5	16.9	11.7	11.3

Clus.1: Cluster 1; Clus.2: Cluster 2; Clus.3: Cluster 3.

Table S2. Effects (LSM and P-values) of preservation methods (PM) and finishing diet (F) PUFA (mg/100g meat) (Omega 6 and Omega 3), MUFA (mg/100g meat), and SFA (mg/100g meat).

	PM					**F**			**PM*F**
Traits	**DAb**	**WA**	**DAb+F**	**WA+F**	***P-value***	**Pasture**	**Grain**	***P-value***	***P-value***
PUFA	**253.8±15.3**	**239.2±14.4**	**216.2±13.8**	**246.3±14.9**	***0.304***	**268.9±11.5**	**211.5±9.3**	***<0.001***	***0.081***
PUFA n6	**183.4±11.8**	**173.6±10.9**	**160.3±10.7**	**181.7±11.5**	***0.419***	**179.2±9.0**	**169.2±8.7**	***0.460***	***0.138***
C18:2n6	123.0±7.9	117.3±7.4	111.1±7.5	124.5±7.9	*0.556*	122.9±6.5	114.9±6.2	*0.377*	*0.149*
C18:3n6	3.3±0.22	3.5±0.24	3.1±0.22	3.3±0.23	*0.501*	4.2±0.27	2.6±0.17	*<0.001*	*0.180*
C20:2n6	4.4±0.33	4.4±0.32	4.1±0.32	4.1±0.30	*0.770*	3.2±0.22	5.8±0.4	*<0.001*	*0.067*
C20:3n6	11.7±0.8	11.5±0.7	9.9±0.7	11.5±0.8	*0.241*	10.6±0.6	11.4±0.7	*0.390*	*0.091*
C20:4n6	39.1±2.9	36.1±2.6	30.7±2.4	36.6±2.7	*0.088*	36.9±2.4	34.1±2.3	*0.404*	*0.119*
PUFA n3	**53.1±3.8**	**58.3±4.0**	**51.0±3.6**	**58.4±4.0**	***0.208***	**78.8±5.7**	**38.6±2.8**	***<0.001***	***0.767***
C18:3n3	22.8±1.8	25.4±1.9	20.8±1.7	23.2±1.8	*0.286*	47.1±3.1	11.2±0.8	*<0.001*	*0.193*
C20:3n3	2.1±0.19	2.3±0.9	1.8±0.16	2.2±0.18	*0.172*	2.1±0.17	2.1±0.17	*0.800*	*0.089*
C20:5n3	10.0±0.9	10.0±0.9	8.0±0.8	9.4±0.9	*0.110*	11.8±1.2	7.3±0.8	*0.002*	*0.110*
C22:5n3	19.7±1.4	18.2±1.4	15.3±1.5	17.6±1.4	*0.164*	20.2±1.1	15.2±1.1	*0.002*	*0.095*
C22:6n3	3.4±0.3	3.2±0.3	3.6±0.3	4.0±0.3	*0.167*	3.3±0.2	3.7±0.2	*0.143*	*0.342*
n6:n3	**3.1±0.17**	**2.9±0.16**	**3.1±0.17**	**3.1±0.17**	***0.049***	**2.1±0.15**	**4.5±0.34**	***<0.001***	***0.767***

Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s, and then frozen at -20 °C.

Table S2 (Cont.). Effects (LSM and P-values) of preservation methods (PM) and finishing (F) PUFA (mg/100g meat) (Omega 6 and Omega 3), MUFA (mg/100g meat), and SFA (mg/100g meat).

CLA	**21.2±1.9**	**23.0±1.8**	**19.7±1.9**	**20.6±1.8**	***0.494***	**26.9±1.9**	**15.3±1.9**	***<0.001***	***0.218***
MUFA	**1826.5±111.5**	**1774.9±111.5**	**1633.5±118.0**	**1702.4±111.5**	**0.596**	**1632.1±95.0**	**1836.5±97.0**	**0.136**	**0.200**
C14:1	19.1±1.7	18.9±1.6	18.1±1.6	19.3±1.7	*0.858*	15.0±1.5	23.7±2.8	*0.002*	*0.283*
C16:1	30.2±2.1	31.1±2.1	29.5±2.1	30.9±2.1	*0.905*	26.5±1.8	34.9±2.4	*0.005*	*0.296*
C18:1n9	1685.0±100.7	1722.0±99.0	1583.0±104.7	1648.7±99.0	*0.756*	1543.0±86.0	1776.3±87.2	*0.060*	*0.291*
SFA	**1902.7±126.5**	**2003.0±122.2.0**	**1938±126.5**	**2024±120.4**	***0.835***	**1925.0±119.2**	**2009.4±118.8**	***0.617***	***0.447***
C10:0	1.8±0.13	1.9±0.13	1.9±0.13	2.1±0.13	*0.397*	2.0±0.13	1.9±0.12	*0.714*	*0.206*
C12:0	2.2±0.16	2.2±0.16	2.2±0.17	2.3±0.16	*0.870*	2.2±0.15	2.2±0.15	*0.927*	*0.149*
C14:0	95.2±7.1	98.8±7.3	94.3±7.3	100.3±7.4	*0.860*	89.9±7.0	105.0±8.2	*0.161*	*0.222*
C15:0	13.7±1.0	14.7±1.0	13.7±1.0	14.8±1.0	*0.684*	15.8±1.1	12.8±0.9	*0.040*	*0.169*
C16:0	1106.4±70.0	1150.1±68.9	1081.1±72.7	1136.1±68.9	*0.870*	1097.0±62.7	1139.8±63.5	*0.633*	*0.225*
C17:0	130.5±9.5	124.8±9.1	115.5±8.8	122.7±9.0	*0.527*	105.1±8.1	144.6±11.2	*0.005*	*0.215*
C18:0	594.8±41.6	636.8±43.8	584.2±42.4	609.0±41.9	*0.777*	643.9±41.2	570.1±36.9	*0.185*	*0.213*
C20:0	6.8±0.7	7.7±0.8	5.2±0.5	7.1±0.7	*0.017*	10.4±1.0	4.2±0.4	*<0.001*	***0.014***
C22:0	3.4±0.26	4.0±0.29	2.8±0.22	2.9±0.22	*0.001*	3.6±0.24	2.9±0.20	*0.024*	***<0.001***
C24:0	4.4±0.31	4.1±0.28	3.7±0.27	4.5±0.31	*0.168*	3.7±0.21	4.7±0.27	*0.008*	*0.226*
PUFA/SFA	**0.12±0.007**	**0.12±0.007**	**0.12±0.007**	**0.12±0.007**	***0.570***	**0.14±0.01**	**0.11±0.008**	***0.028***	***0.075***

DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DAb+Fr: Dry aging bag + 180 days frozen; WA+Fr: Wet aging + 180 days frozen. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s, and then frozen at -20 °C. CLA: conjugated linoleic fatty acid (*c*9, *t*11-18:2); PUFA: sum of polyunsaturated fatty acid (PUFA n-6 + PUFA n-3); MUFA: monounsaturated fatty acid (C14:1 + C16:1 + C18:1n9); SFA: saturated fatty acid (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0); PUFA n-3 (C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3); PUFA n-6 (C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6); PUFA/SFA: Polyunsaturated/Saturated fatty acids; IMF: intramuscular fat. Different letter in the same row denotes groups statistically different ($P < 0.05$) among LSMeans.

4. Effects of wet aging, dry bag aging, and stepwise aging methods on meat quality and sensory attributes of steaks from pasture and grain finished steers

Daniela Correa[a], Marcia del Campo[a], Santiago Luzardo[a], Guillermo de Souza[a], Carlos Álvarez[b], Maria Font-i-Furnols[c], Gustavo Brito[a*]

[a] INIA-*Sistema Ganadero Extensivo, INIA Tacuarembó, Ruta 5, km 386, Tacuarembó, Uruguay*

[b] *Teagasc-Food Quality and Sensory Science Department, Teagasc Ashtown Food Research Centre, Ashtown, D15KN3K Dublin 15, Ireland*

[c] *IRTA- Food Technology, Granja Camps i Armet, 17121 Monells (Girona), Spain*

* Corresponding author: e-mail address: gbrito@inia.org.uy

4.1. Abstract

The study aimed to evaluate the effect of two finishing diets (F: pasture or grain) and two method types: dry aging in bag (DAb) and wet aging (WA) for 40d; dry bag for 20 d + wet 20 d (DW) and wet 20 d + dry bag 20 d (WD), on physicochemical traits, microbiological loads, and sensory attributes of beef. Sixty striploins, consisting of the right and left Longissimus lumborum muscle (LL), from British crossbred steers, were employed, with 15 pairs of striploins obtained from pasture-finished and 15 pairs from grain-finished diet. Meat from grain-finished steers was lighter (greater L* values; $P<0.01$) than those finished on pasture. Meat aged using DAb presented lower cooking loss values ($P<0.01$) than WA. Stepwise WD aging increased Psychotrophic (PSY) and total bacterial count (TBC) ($P<0.05$) compared to the other three treatments. No AM*F interaction on the physicochemical characteristics (color, pH, cooking losses, and shear force) and the surface microbiological load was observed ($P>0.05$) except for a* and b* coordinates of lean color. There was a significant AM*F interaction effect on the fatty acid composition for conjugated linoleic acid (CLA; c9, t11-18:2), saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acid (PUFA), and PUFA n6. Greater concentrations of PUFA, PUFA n6, and CLA ($P <0.01$), were observed in all AM treatments from pasture-finished and in WD from grain-finished steers, meanwhile, SFA and MUFA were greater in DW and WD from grain-finished animals ($P <0.05$). A greater PUFA:SFA ratio ($P <0.05$) and lower

n6:n3 ratio ($P < 0.01$) were found in pasture-finished than grain-finished steers. Consumers preferred tenderness, flavor, and overall liking from DAb and WA samples ($P < 0.05$) over WD steaks. AM had the greatest influence on the physicochemical and microbial properties, while the finishing diet primarily affected the fatty acid composition and consumer preferences. All aging methods were acceptable to consumers but combining wet and dry aging in a bag did not enhance sensory appeal.

Keywords: Beef, Finishing diet, Stepwise aging, Dry aging bag, Meat quality attributes

4.2. Introduction

It is well-recognized that aging is a post-mortem practice for tenderization and flavor improvement of beef (Campbell et al., 2001; Smith et al., 2008; Kemp et al., 2010; Ba et al., 2016). In the meat industry, wet and dry aging are the most common processes to age beef. Wet aging involves placing the meat into a plastic bag, which acts as a barrier to moisture loss. Bags are vacuum-sealed and stored at refrigerated temperatures (-1 to 2 °C) for a specified length (Smith et al., 2008). Dry aging is a traditional aging method that exposes unpackaged meat directly to cooling conditions with strict temperature (0-4 °C), relative humidity (80-85%), and airflow control (0.5-2 m/s) (Savell, 2008). A third alternative is dry aging in a highly moisture-permeable bag, widely used in recent decades (Ahnström et al., 2006; Li et al.,2013; Zhang et al., 2019; Zhang et al., 2021). The adoption of water-permeable aging bags to produce dry-aged products is mainly to reduce microbial contamination, lipid oxidation, and trim loss when compared to the traditional out-of-bag dry-aging technique (DeGeer et al., 2009; Ahnström et al., 2006; Zhang et al., 2020). Kim et al. (2018) reported that savory/beef flavor increments increase during aging (particularly dry aging) because of flavor-related compound liberation. Another technique that combines the methods previously mentioned, dry aging in a bag with wet aging (stepwise process) is proposed since it produces microbiologically safe dry-aged beef compared to traditional dry-aged meat with maximized saleable meat yield (Zhang et al., 2019) maintaining traditional dry aging savory/beef flavor.

Carcass composition and eating quality attributes of beef meat are known to be influenced by the finishing diet (del Campo et al., 2008; Peripolli et al., 2018; Correa et al., 2020). Several studies reported that different nutritional-management approaches have shown that animals finished on high-concentrate diets displayed heavier carcasses and improved beef quality attributes such as tenderness, marbling, ribeye area, and backfat thickness than pasture-finished animals (Realini et al., 2004; Duckett et al., 2013; Ferrinho et al., 2020). Conversely, pasture-finished animals produced beef with lower concentrations of fat and cholesterol, and a higher percentage of n3 polyunsaturated fatty acids (PUFAs) and conjugated linoleic acid (CLA) than grain-finished animals (Aldai et al., 2011; Duckett et al., 2013; Brito et al., 2014; Ferrinho et al., 2020). Previous research (Melton, 1983; Nuernberg et al., 2005; Ha et al., 2019) reported that the greatest difference in the development of flavors of meat from cattle finished on pasture or grain is due to the concentration and composition of fatty acid as they are the primary source of aromatic compounds such as carbonyls.

In an international market where meat eaters are looking for new experiences and more intense flavors, it is important to investigate alternatives to fresh meat that are more attractive to them. It needs to be considered that eating quality differences between dry and wet aging have been consistently attributed to higher flavor and aroma intensities (Iida et al., 2016; Kim et al., 2016). Therefore, a marketing alternative to expanding the range of fresh meat products would be to implement dry-aging methods (dry aging bag) to capitalize on its benefits, to meet the expectations of more demanding consumers. We hypothesize that the dry aging bag and wet aging combination improve the physicochemical and organoleptic properties of the meat, adding the benefits of both aging methods compared to each method applied on its own.

Therefore, this study aimed to evaluate the effect of two different aging methods: dry bag aging (DAb) and wet aging (WA), and their combinations of DAb followed by WA (DW) and WA followed by DAb (WD) in meat from pasture-finished and grain-finished steers on physicochemical, microbiological, and consumer acceptance of sensory beef attributes.

4.3. Materials and methods

4.3.1. Raw materials and aging process

This study was carried out in complement to Correa et al. (2024), and the duplicities of the experimental design and sample set are acknowledged. Striploins (m. Longissimus lumborum, paired loins, n = 60) were obtained from thirty steers (under 30 months of age; British crossbred) finished (F) in a pasture (n=15) or grain (n=15). Animals raised on pasture are qualified for the Hilton quota and comprise select cuts from steers or heifers, ensuring the production of high-quality beef exclusively raised on pasture, following the Uruguayan grading system. Conversely, animals from grain feeding systems qualify for the UE 481 quota and consist of beef cuts from carcasses of steers and heifers under 30 months of age, which have been finished on a diet containing no less than 62% concentrates for a minimum of 100 days before slaughter. They were slaughtered in a commercial meat processing plant (hot carcass weight: 266.5 kg and 253.2 kg pasture and grain, respectively). Steers from the same commercial farm were selected considering age, live weight, and fat cover (INAC, 1997) to set up two similar groups. Thirty striploins (left and right Longissimus lumborum, LL) of each F system were obtained for analysis and assigned to an aging method. Both striploins of each carcass were divided into pieces, and the most cranial piece (10 cm) of each striploin was assigned to combined aging: 20 d dry aging bag followed by 20 d wet aging (DW) and 20 d wet aging followed by 20 d dry aging bag (WD). The remaining left striploin was divided into two pieces and assigned randomly to one of the following treatments: 40 d dry aging bag (DAb, 16 cm), and 40 d wet aging (WA, 14 cm), ensuring half of each treatment in each position of the striploin (caudal and cranial). The remaining right striploin was used for another study. The pH measurement was inserting the pH probe (HI 99163, Hanna Instruments Inc., Hoonsocket, USA) directly into the beef loin sections initially and at the end of the aging period. Dry aging was performed using a TUBLIN® bag (10 and 50 μm thick, polyamide mix with a water vapor transmission rate of 2.5 kg/50μ/m2/24 h at 38°C, 50% RH, TUB-EX ApS, Denmark) while a Cryovac® bag was employed for wet aging (50 μm thickness; maximum oxygen transmission rate of 27 cm3/m2/24 h at 22-24°C

and 0% RH and moisture vapor transmission rate of 5 g/m2/24 h at 38°C and 90% RH; Cryovac® Sealed Air Corp., BB 2620, Brazil).

Striploin pieces were placed on racks in a chilling chamber (at 2 ± 0.5°C and relative humidity of 85 ± 5%) during the aging period. Temperature and relative humidity were monitored using three dataloggers (Electronic Temperature Instruments Ltd., UK), in order to obtain real-time information at various locations inside the chamber. The air velocity was recorded weekly at different chamber positions with a digital anemometer (HoldPeak 866A digi, China) averaging 0.5 m/s. Meat pieces were relocated on the racks into the chamber every week to prevent any potential confounding effect of location within the cooler.

4.3.2. Instrumental color

One steak per loin section (DAb, WA, DW, and WD) was cut at the end of the aging period (40 days) and exposed to bloom for 45 minutes at 4°C (King et al., 2023). The surface color was measured using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing Inc., Japan), which was calibrated with a standard white tile. CIE L*a*b* (Commission Internationale de l'Eclairage, 1976) color space values—L* (lightness), a* (redness), and b* (yellowness)—were recorded (Illuminant C, 2-grade standard observer, 8 mm opening size) in triplicate on the lean surface of each steak. The values were averaged to obtain a mean for each sample. Total color change (Delta E) between treatments was calculated as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$ (King et al., 2023).

4.3.3. Cooking losses and Warner-Bratzler Shear Force

The same steaks (2.5 cm thick) used for color measurement were used for the cooking losses (CL) and the Warner-Bratzler shear force (WBSF). They were weighed before and after cooking to determine the cooking loss (CL) according to the American Meat Science Association protocol (AMSA, 2016). Steaks were cooked in a grill (GRP100 The Next Grilleration, Spectrum Brands, Inc., Miami, FL) until the internal core temperature reached 71 °C. The CL percentage was calculated with the following equation: ((raw weight - cooked weight)/raw weight) x 100.

After cooking, the steaks were cooled (5° C) for 12 h. Warner-Bratzler shear force (WBSF; kgF) was evaluated on six cores of 1.27 cm diameter from parallel to the longitudinal muscle fiber orientation using a hand-held coring device. The cylinders were sheared using a TA-XT Plus texture analyzer (Stable Micro System Ltd., UK) set with a "V" Warner Bratzler slot blade and 8 mm/sec speed. Shear force values resulted from the average of the six cores per steak.

4.3.4. Fatty acid composition and thiobarbituric acid-reactive substance

A second steak per loin section (DAb, WA, DW, and WD) was cut at the end of the aging period (40 days) for the intramuscular fat content (IMF), fatty acid composition, and lipid oxidation evaluation. The IMF was assessed using the lipid extraction method outlined by Bligh and Dyer (1959) involving chloroform-methanol, followed by the analysis of the fatty acid composition. Methylation of the fatty acids was carried out utilizing cold methanolic potash (IUPAC, 1987), and analysis was conducted via gas chromatography using a Shimadzu Nexis GC 2030 Tokyo, Japan instrument. Fatty acids were separated using a 60-meter SH-Rt-WAX capillary column (0.25 mm internal diameter, 0.25 μm film thickness, Shimadzu, Columbia, Maryland, USA), with nitrogen employed as the carrier gas at a flow rate of 1 mL/min.

A 1 μl injection volume was used with a flame ionization detector (FID). The detector temperature was maintained at 260 ºC, while the injector temperature was set to 230 ºC. The temperature ramp proceeded as follows: starting at 100 ºC for 0.5 min, it increased at a rate of 10 ºC/min until reaching 120 ºC for 2 min, then continued increasing at 10 ºC/min until reaching 220 ºC for 15 min, totaling 29.5 min per sample. Fatty acids were identified by comparing retention times with a standard mixture of 37 FAME SupelcoTM 37 compounds (Sigma, St. Louis, USA), while conjugated linoleic acid (CLA; c9, t11-18:2) was identified using an octadecadienoic acid, conjugated, methyl ester standard (No. O5632, Sigma, St. Louis, USA). Fatty acid content was reported in mg/100 g of meat using methyl heneicosanoate (C21:0) as an internal standard (1 ml of 1 mg), which was added before the addition of methylating reagents.

Lipid oxidation was assessed using the thiobarbituric acid-reactive substance (TBARS) method, as modified by Ahn et al. (1998). Briefly, minced samples of 5 g

were homogenized with 15 ml of deionized distilled water (DDW) using a tissue homogenizer (Wisd, HG-15A, Daihan Scientific) for 30 s. One milliliter of the meat homogenate was transferred to a disposable test tube (13 x 100 mm), and 50 µl of butyrate 16 hydroxyanisole (7.2%) and 2 ml of thiobarbituric acid/trichloroacetic acid (TBA/TCA) solutions were added. The mixture was vortexed and then incubated in a boiling water bath for 15 min for color development. The TBARS samples were cooled down in the ice bath for 10 min before centrifugation for 15 min at 4000 rpm 1789 xg. The supernatant solution was collected, and the absorbance was determined at 531 nm against a blank containing 1 ml DDW and 2 ml TBA/TCA solution. Standard curves of malonaldehyde (MDA) were prepared using 1,1,3,3-tetraethoxypropane. The amount of TBARS was expressed as milligrams of MDA/kg of meat.

4.3.5. Surface Microbial Counts

A third steak was cut at the end of the aging period (40 days) for the surface microbial count. The impact of aging treatments on microbial surface growth was assessed by measuring total bacterial count (TBC), Psychrotrophic microorganisms (PSY), and Enterobacteriaceae (ENT) concentrations. Microorganisms from the untrimmed surface of a 1.5 cm steak taken from the cranial end extreme of each piece were enumerated on day 0 and day 40 after the aging period. Individual beef samples (4 x 4 cm square) were aseptically excised from the center of 10 steaks per treatment using disposable scalpels (Feather Sterile Scalpels 2975#21; Graham-Field Inc., Atlanta, GA) and placed into individual sterile Whirl-Pak bags (710 mL, 15x23 cm, 0.102 mm thick; Nasco Int., USA). The 4 x 4 cm squares were placed into a stomacher bag (BagMixer® 400 P, Interscience, Saint Nom, France) and homogenized with 90 mL of 0.1% peptone water (Oxoid, UK) for 2 minutes for microbial analysis. The appropriate dilutions were surface-plated in duplicate onto two sets of Petrifilms (3M, Uruguay): one set was used for TBC enumerations, and the other for enumerating PSY. Dilutions were also duplicated onto Petrifilm surfaces (3M; Uruguay) to enumerate the ENT microbial population. For a clear identification, the Petrifilms were enumerated according to the treatments, before incubation at 37°C for 48 h for TBC or 7°C for 10 d for PSY and 37°C 24 h for ENT.

4.3.6. Consumer Sensory Testing

The fourth steak, cut after 40 days of aging, was designated for Consumer Sensory Testing. Meat samples were grilled on a grill (GRP100 The Next Grilleration, Spectrum Brands, Inc., Miami, FL) until the core temperature of the steak reached 71 °C (AMSA, 2016). After cooking, the steaks were trimmed of external fat and connective tissue, then cut across the grain into 1.3 × 1.3 × 2.0 cm pieces. They were wrapped individually in coded aluminum foil, assigned to a cup, and kept warm in a heater/oven at 49°C for no more than 10 minutes before tasting. Sensory evaluation was conducted by a Uruguayan consumer panel with 10 sessions of 10 consumers each (n=100). Each consumer evaluated 8 samples, served on two dishes, each containing 4 samples: 2 from each finishing system and 2 aging methods (WA/DAb or WD/DW). The order of the dishes was alternated among sessions. Furthermore, the sequence of sample presentation for each consumer and dish was arranged to avoid the first sample and carry-over effect (MacFie et al., 1989). Water and unsalted crackers were provided to consumers as palate cleansers. Before tasting, consumers were asked to complete a questionnaire regarding their demographics (gender, age range, education level) and frequency of consumption of different types of meat, and to sign the consent form if they agreed to participate (Table S1, Supplementary data). Each consumer was required to score each sample based on the acceptability of tenderness, flavor, and overall liking using a 9-point hedonic scale, as follows: 1-“I like it extremely”, 2-“I like very much”, 3-“I quite like it”, 4-“I like it”, 5-“I neither like nor dislike”, 6-“I dislike it”, 7-“I quite dislike it”, 8-“I dislike very much”, and 9-“I dislike it extremely.”

4.3.7. Data analysis

The experimental design was a split-plot, where each finishing diet served as a main plot (F: pasture or grain), carcasses (pair of loins) served as sub-plot for the aging methods (AM: DAb, WA, DW, and WD). The model included the fixed effects of F and AM, with their interactions, and the random effect of carcasses. These were analyzed using the MIXED procedure in SAS software (v. 9.4, SAS Institute Inc., Cary, NC, US). Data were checked for normality using the UNIVARIATE procedure and, when necessary, were normalized with a log10 transformation. The least-square

mean (LSM) was calculated and means separation was performed ($P<0.05$) using the PDIFF option. Peak cooking temperature was used as a covariable for WBSF and CL analysis.

For sensory evaluation, tenderness, flavor, and overall liking scores were analyzed using a model that included the fixed effects of AM, F, and their interaction and consumers were considered random effects. The tasting session was considered a blocking factor. It was carried out by CLUSTER to find groups of consumers in a segmentation with similar preferences since when considered as a pool, differences are diluted, and they are difficult to determine. The CLUSTER procedure was performed by segmentation applying Euclidian distance and the Ward method. The number of clusters to retain was based on the obtained dendrogram, considering the homogeneity within and among the segments and the principle of parsimony. An analysis of variance was carried out, considering fixed effects, AM and F, and their interaction for the pooled sample and by cluster. A Tukey test was applied to find differences between least-squared means. Significance was fixed at $P < 0.05$.

4.4. Results

4.4.1. Effects of aging method and finishing system on instrumental color, pH, cooking losses, and shear force

No interaction (AM*F; P >0.05) was observed for the final pH, CL, WBSF, and L* coordinate of meat color (Table 1). An interaction effect (P <0.05) for redness (a*) and yellowness (b*) (Figure S1, supplementary data), indicated the most significant values of a* in WA from grain-finished. In addition, for the b* coordinate, the most outstanding value was in DAb from pasture-finished. Lightness (L*) values were greater in WA than in DAb samples (P <0.05), and no differences were found between the two stepwise AM treatments (P > 0.05), presenting intermediate values not significantly different for WA or DAb (Table 1). Meat color from grain-finished steers resulted in lighter (greater L* values) than from pasture-finished animals (P <0.05). Delta E indicated values from 1.0 between DAb and WD to 2.7 between WA and DW; the difference value in the finishing diet was 2.2. The ultimate pH values were greater (P <0.05) in DAb than in WA and DW samples (Table 1). The CL was

lower in DAb samples than the other three AM, and no differences were observed between DW and WD aging methods (P>0.05), which was lower than WA. Warner Braztler shear force values did not differ among aging methods (P>0.05). The finishing diet of the steers did not affect ultimate pH, CL, and WBSF values (P> 0.05) (Table 1).

Table 1. Effects (mean ± SEM) of aging method (AM) and finishing diet (F) and their interaction (AM*F) on meat quality parameters.

	Aging (AM)						**Finishing (F)**				**AM*F**
Traits	DAb	WA	DW	WD	SEM	*P-value*	Pasture	Grain	SEM	*P-value*	*P-value*
*L**	40.5 b	41.8 a	40.9 ab	41.3 ab	0.4	*0.033*	40.0	42.2	0.4	*<0.001*	*0.350*
*a**	22.2 b	24.0 a	21.7 b	22.3 b	0.4	*<0.001*	22.4	22.7	0.4	*0.551*	*0.011*
*b**	11.8 a	11.9 a	10.7 b	11.1 b	0.2	*<0.001*	11.3	11.4	0.3	*0.444*	*0.029*
*Delta E**	2.22	0	2.74	1.94	-	-	-	2.22	-	-	-
pH	5.7 a	5.73 b	5.71 b	5.74 ab	0.01	*0.002*	5.72	5.75	0.02	*0.246*	*0.869*
CL (%)	17.9 c	23.4 a	20.2 b	20.2 b	0.5	*<0.001*	20.6	20.2	0.5	*0.593*	*0.246*
WBSF (kgF)	2.6	2.5	2.6	2.5	1.0	*0.318*	2.7	2.5	1.2	*0.457*	*0.198*

DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DW: Dry aging bag 20d + WA 20d; WD: Wet aging 20d + Dry aging bag 20d. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. CL: cooking losses; WBSF: Warner Braztler Shear Force. Different letters in the same row denotes groups' statistically differences (P<0.05) among LSMeans. *For Delta E calculations WA was used as a reference. L* indicates lightness and a* and b* are chromaticity coordinates. a* and b* are color directions: +a* is the red axis, -a* is the green axis, +b* is the yellow axis and -b* is the blue axis

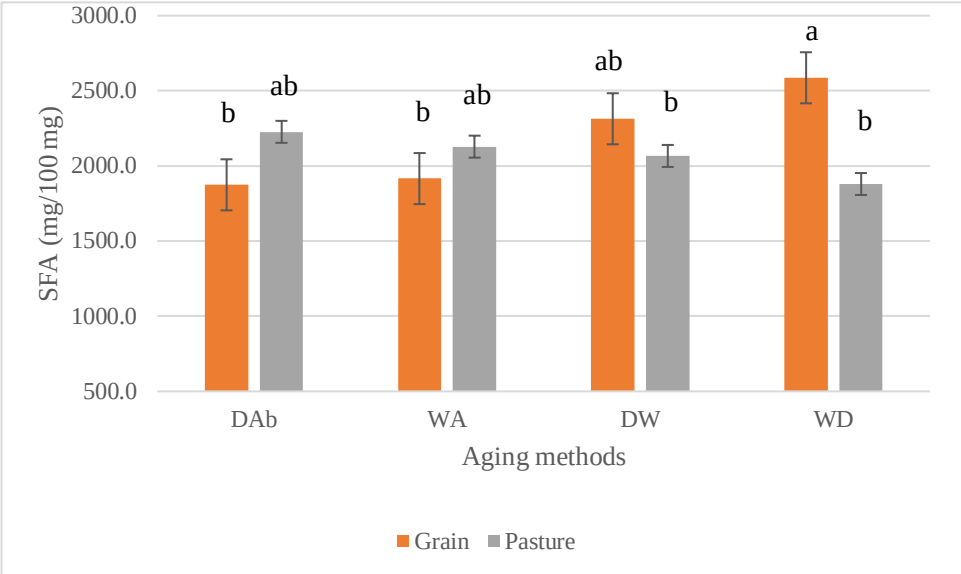
4.4.2. Effects of aging method and finishing system on fatty acid profile and oxidation

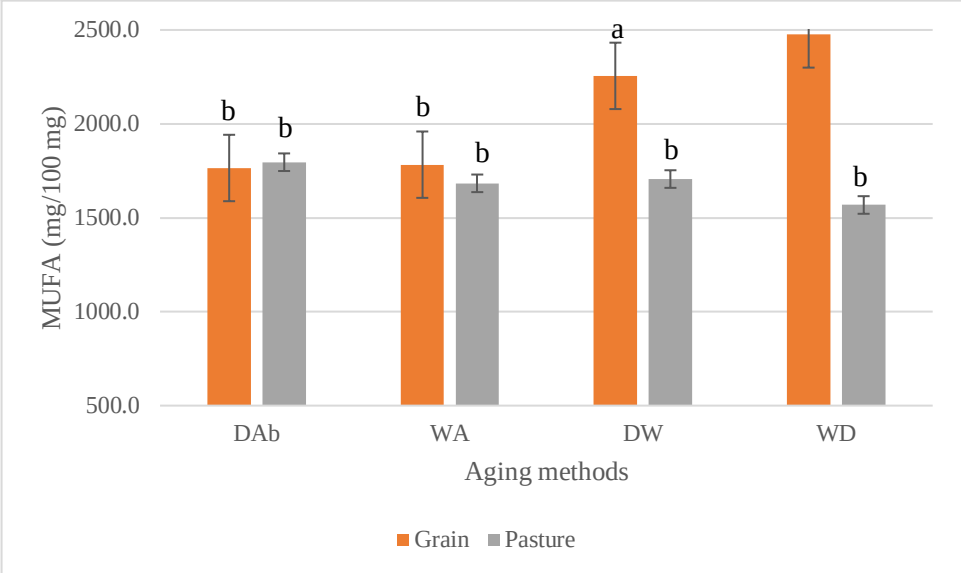
No interaction (AM*F; P>0.05) was observed in IMF (%), PUFA n3, n6:n3, and PUFA: SFA (Table 2); the other fatty acids are presented in supplementary data (Table S2). After the aging period, DW presented higher IMF (%) values than WA, with intermediate values in DAb and WD (P<0.01). Polyunsaturated fatty acid n3 and PUFA: SFA ratio increased, and n6:n3 ratio decreased in pasture-finished steers (P>0.05). The combination of aging methods and finishing diet impacted (AM*F; P<0.05) most of the fatty acids (Figures 1 and 2, and in supplementary data Table S3). Saturated FA and MUFA presented the highest values in DW and WD from grain-finished steers (P<0.05; Figure 1). In addition, PUFA (Figure 1) and CLA (Figure 2) presented the greatest content in pasture-finished animals regardless of treatments, and PUFA n6 in DAb from pasture (Figure 2). Although no interaction between aging methods and finished diet was observed for the sum of PUFA n3, there was interaction (P<0.05) for C18:3n3 and C20:3n3. The linolenic acid (C18:3n3) presented the highest values in aging treatments from pasture-finished and the eicosatrenoic acid (C20:3n3) did in DAb from pasture-finished and WD from grain-finished steers (Table S3). Regarding lipid oxidation, AM*F interaction (Figure 3; P < 0.05) was observed. Both stepwise aging (WD and DW), and WA from grain-finished steer meat presented greater TBARS values than the other treatments.

Table 2. Effects (mean ± SEM) of the aging method (AM) and the finishing diet (F) and their interaction (AM*F) on intramuscular fat and the fatty acid profile

	Aging						**Finishing**				**AM*F**
Trait	DAb	WA	DW	WD	*SEM*	*P-value*	Pasture	Grain	*SEM*	*P-value*	*P-value*
IMF (%)	3.9 ab	3.7 b	4.2 a	4.0 ab	0.2	*0.030*	3.7	4.2	*0.2*	*0.082*	0.623
PUFA n3 (mg/100g meat)	61.2	61.5	58.1	66.5	4.3	*0.750*	89.6	41.2	*4.0*	*<0.001*	0.053
n6:n3	3.1	2.9	3.1	3.0	0.17	*0.477*	2.1	4.5	*0.23*	*<0.001*	0.336
PUFA:SFA	0.12	0.12	0.11	0.12	0.007	*0.377*	0.14	0.10	*0.01*	*0.006*	0.083

DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DW: Dry aging bag 20d + wet aging 20d; WD: Wet aging + dry aging bag 20d. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. IMF (%): Intramuscular Fat; PUFA: sum of polyunsaturated fatty acid (PUFA n6 + PUFA n3); SFA: saturated fatty acid (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0); PUFA n3 (C18:3n3 + C20:3n3 + C20:5n3 + C22:5n3 + C22:6n3); PUFA n6 (C18:2n6 + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6). A letter in the row denotes groups' statistical differences (P<0.05) among LSMeans.


3000.0
2500.0
2000.0
1500.0
1000.0
500.0
SFA (mg/100 mg)
b
ab
b
ab
ab
b
a
b
DAb
WA
DW
WD
Aging methods
Grain
Pasture


2500.0
2000.0
1500.0
1000.0
500.0
MUFA (mg/100 mg)
b
b
b
b
a
b
b
DAb
WA
DW
WD
Aging methods
Grain
Pasture

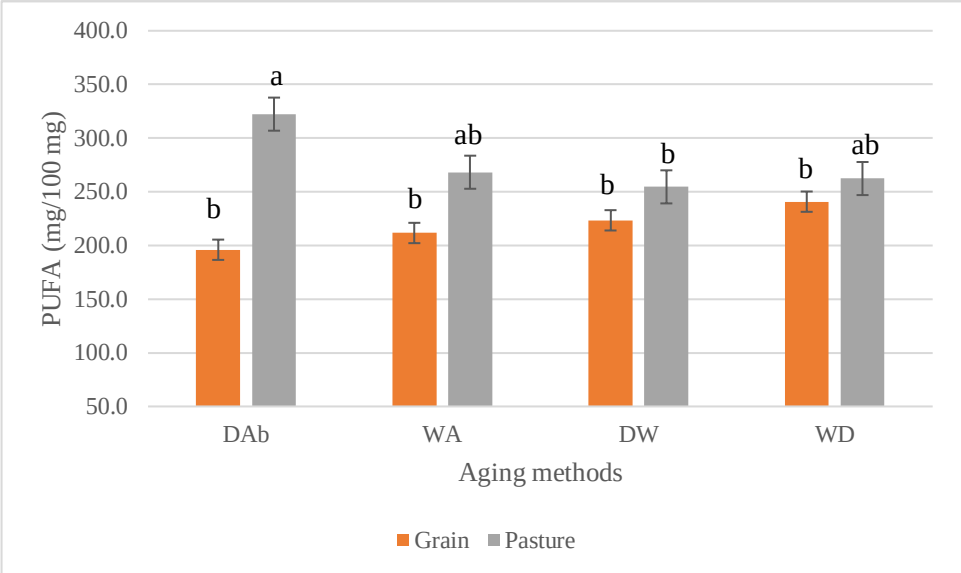

400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
PUFA (mg/100 mg)
b
a
b
ab
b
b
b
ab
DAb
WA
DW
WD
Aging methods
Grain
Pasture

Figure 1. Interaction between aging methods and finishing diet on SFA, MUFA, and PUFA (mg/100 g meat). DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DW: DAb 20d + WA 20d; WD: WA 20d + DAb 20d. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. SFA: saturated fatty acid (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0); MUFA: monounsaturated fatty acid (C14:1 + C16:1 + C18:1n9); PUFA: sum of polyunsaturated fatty acid (PUFA n6 + PUFA n3); PUFA n3 (C18:3n3 + C20:3n3 + C20:5n3 + C22:5n3 + C22:6n3); PUFA n6 (C18:2n6 + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6). Different letters denote groups' statistical differences (*P<0.05*) among LSMeans.

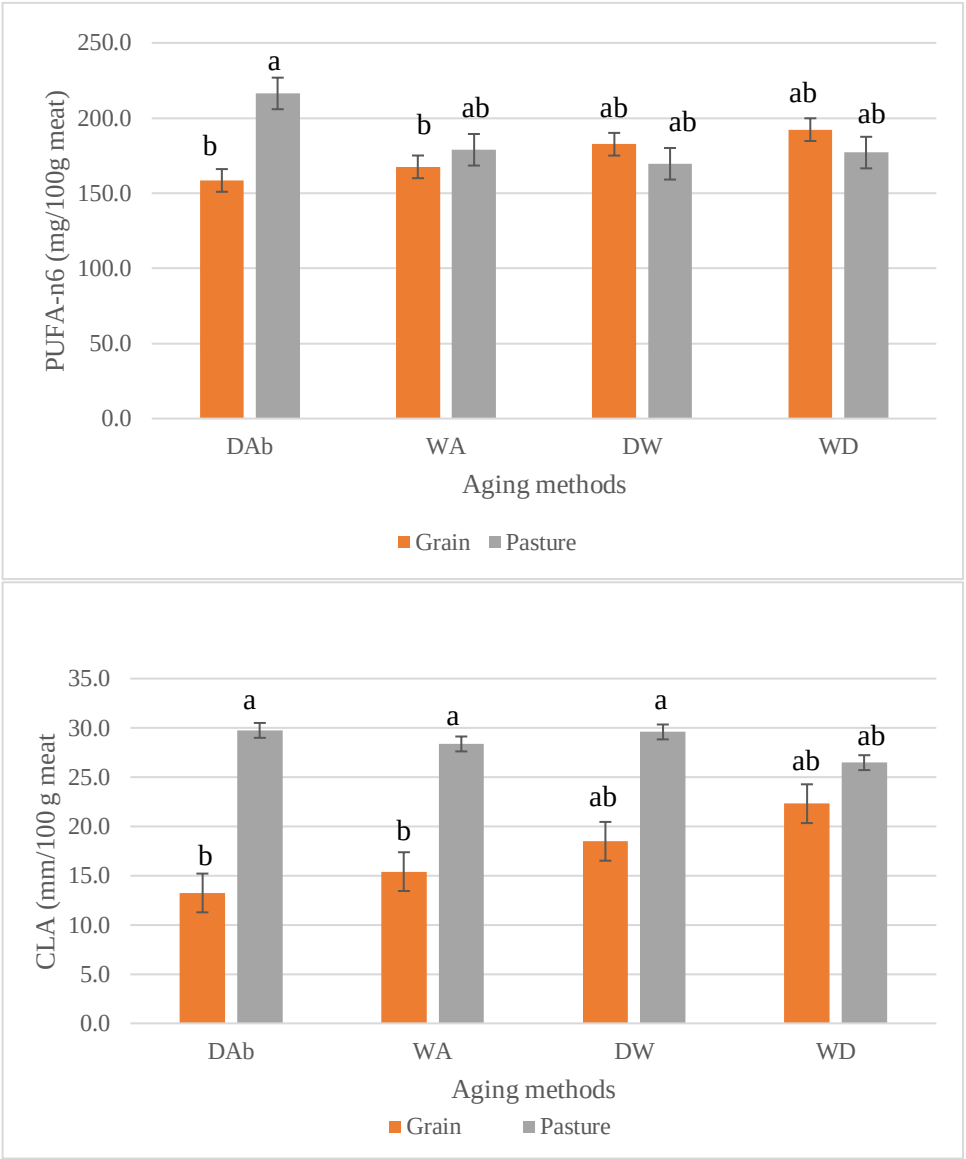


Figure 2. Interaction between aging methods and finishing diet on PUFA n6 and CLA (mg/100 g meat). DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DW: Dry aging bag 20d + wet aging 20d; WD: Wet aging + dry aging bag 20d. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. CLA: conjugated linoleic fatty acid (*c*9, *t*11-18:2); PUFA n6 (C18:2n6 + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6). Different letter denotes groups' statistical differences (*P<0.05*) among LSMeans.

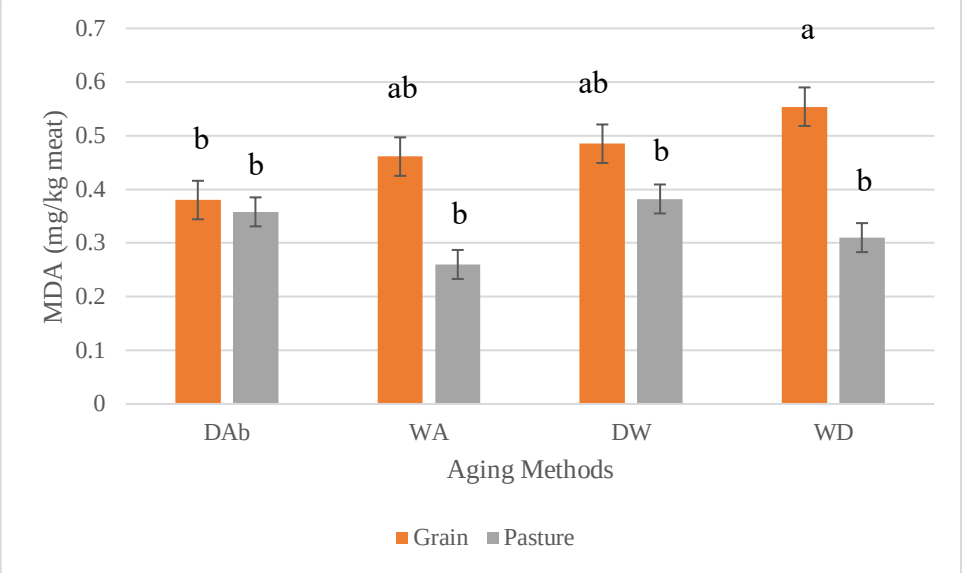


Figure 3. Interaction between aging methods and finishing diet on thiobarbituric acid-reactive substances (TBARS) concentrations (mg MDA /kg meat). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DW: Dry aging bag 20d + wet aging 20d; WD: Wet aging + dry aging bag 20d. Different letter denotes groups' statistical differences (*P<0.05*) among LSMeans.

4.4.3. Effects of aging method and finishing system on superficial microbial counts

Before aging, the microbial counts were below the detection limit (<1 log/cm2). There was no interaction between AM * F (P>0.05). The aging method affected the TBC (P = 0.023) and PSY (P < 0.01). WD had the highest values, followed by DAb, and then WA and DW. No differences were observed for ENT load (P=0.105). The finished diet had no significant effect (P > 0.05) on TBC, PSY, and ENT counts in meat aged by the different methods (Table 3).

Table 3. Effects (mean ± SEM) of aging method (AM) and finishing diet (F) and their interaction (AM*F) on microbiological growth.

	Aging						Finishing				AM*F
Trait	DAb	WA	DW	WD	*SEM*	*P-value*	Pasture	Grain	*SEM*	*P-value*	*P-value*
TBC (log_{10}/cm^2)	4.3 ab	3.9 b	4.1 b	4.6 a	*0.19*	*0.023*	4.3	4.2	*0.17*	*0.837*	*0.361*
PSY (log_{10}/cm^2)	5.4 b	5.0 c	5.0 c	6.5 a	*0.11*	*<0.001*	5.5	5.4	*0.10*	*0.903*	*0.890*
ENT (log_{10}/cm^2)	3.3	2.7	3.4	2.8	*0.23*	*0.105*	3.0	3.1	*0.17*	*0.471*	*0.324*

DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DW; Dry aging bag 20d+ Wet aging 20d; WD; Wet aging 20d + Dry aging bag 20d. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. TBC: total bacterial count; PSY: Psychotropic bacteria; ENT: Enterobacter bacteria. Different letter in the same row denotes groups statistically different (P<0.05) among LSMeans

Table 4. Effects (mean ± SEM) of aging method (AM) and finishing diet (F) and their interaction (AM*F) on sensory attributes (All consumers)

	Aging						Finishing				AM*F
Trait	DAb	WA	DW	WD	*SEM*	*P-value*	Pasture	Grain	*SEM*	*P-value*	*P-value*
Overall liking	3.6 b	3.4 b	3.6 ba	4.0 a	*0.11*	*<0.001*	3.8	3.5	*0.1*	*<0.001*	*0.209*
Tenderness	3.0 b	3.1 b	3.3 ba	3.6 a	*0.12*	*<0.001*	3.6	2.9	*0.1*	*<0.001*	*0.196*
Flavor	3.6 b	3.5 b	3.7 b	4.0 a	*0.12*	*<0.001*	3.9	3.6	*0.1*	*0.015*	*0.780*

DAb: Dry aging bag (40 days); WA: Wet aging (40 days); DW: dry aging bag 20d + wet aging 20d; WD: wet aging 20d + dry aging bag 20d. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. Scale 9 points: 1 “I like it extremely”, 2 “I like very much”, 3 “I quite like it”, 4 “I like it”, 5 “I neither like nor dislike”, 6 “I dislike it”, 7 “I quite dislike it”, 8 “I dislike very much” and 9 “I dislike it extremely”. Different letter in the same row denotes groups statistically different (P <0.05) among LSMeans

4.4.4. Effects of aging method and finishing system on meat acceptability

The results of the consumer study are presented in Tables 4 and 5, considering all consumers as a single pool or segmented into clusters, respectively. Analyzing all consumers (n=100) no interaction between AM and F was observed in any of the attributes evaluated ($P>0.05$; Table 4). Regarding AM, the least preferred meat was WD for overall preference, tenderness, and flavor liking, while the most preferred were DAb and WA. On the other hand, meat from grain-finished steers was preferred compared to grass-finished animals. When all consumers are considered as a pool, the differences are diluted and difficult to determine. Therefore, the consumers were segmented into three clusters based on their acceptability scores (Table 5). For cluster 1 (n=31), the lowest acceptability ($P < 0.05$) of meat was from DW and pasture diet for the three attributes. Cluster 1 could be characterized by a higher preference for grain-finished steers and wet aging methods (WA, DW, and WD), thus, they could be named "Grain-finished wet aging beef likers". For Cluster 2, the least preferred option (n=27) was WD from the grain diet steers, based on overall liking and flavor. Also, Cluster 2 could be characterized by a higher preference for grain-finished steers, especially those with DAb. They could be named "Grain-finished dry-aged beef likers". In cluster 3 (n=42), the biggest group, the least preferred samples for overall and flavor liking came from WD and pasture. In contrast, the most preferred options for overall acceptability were DW from pasture and WA from grain, as well as DW from pasture-fed steers for flavor liking. However, no significant differences in scores have been found between samples, and most of them are within the "like it" score, making it difficult to classify them. Thus, they can be named "Undefined beef likers".

Table 5. Effects (mean ± SEM) of the interaction between the aging method (AM) and finishing diet (F) on sensory attributesaccording to the consumer's clusters

Trait	Pasture				Grain				SEM	Significance		
	DAb	WA	DW	WD	DAb	WA	DW	WD		**AM**	**F**	**AM*F**
						Overall liking						
Cluster 1	3.4 b	3.0 bc	4.5 a	3.5 b	3.7 b	2.5 c	2.6 c	3.2 b	0.2	<.001	<.001	<.001
Cluster 2	3.0 b	3.1 b	2.6 bc	3.3 ab	2.0 c	2.4 bc	2.8 b	3.6 a	0.2	<.001	0.039	0.002
Cluster 3	4.3 b	4.7 b	4.0 b	5.0 a	4.3 b	4.0 b	4.5 ab	4.5 ab	0.2	0.031	0.235	0.009
						Tenderness						
Cluster 1	2.9 bc	3.2 b	4.2 a	3.4 ab	2.7 bc	2.2 c	2.5 bc	2.8 bc	0.2	0.025	<.001	0.007
Cluster 2	2.4	2.9	2.9	3.1	1.8	1.9	2.4	2.8	0.2	0.002	<.001	0.476
Cluster 3	4.1	4.3	3.8	4.1	3.3	3.4	3.6	4.5	0.2	0.026	<.001	0.354
						Flavor						
Cluster 1	3.4 ab	2.9 b	4.4 a	3.6 ab	3.6 ab	2.8 b	2.9 b	3.3 b	0.3	0.028	<.001	<.001
Cluster 2	3.2 ab	2.9 bc	2.6 bc	3.2 ab	2.2 c	2.5 bc	3.0 b	3.8 a	0.2	0.334	0.600	0.033
Cluster 3	4.2 b	4.6 ab	4.0 b	4.9 a	4.3 ab	4.1 ab	4.5 ab	4.5 ab	0.2	0.184	0.365	0.006

DAb: Dry aging bag; WA: Wet aging; DW: dry aging bag 20d + wet aging 20d; WD: wet aging 20d + dry aging bag 20d. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. Scale 9 points: 1 “I like it extremely”, 2 “I like very much”, 3 “I quite like it”, 4 “I like it”, 5 “I neither like nor dislike”, 6 “I dislike it”, 7 “I quite dislike it”, 8 “I dislike very much” and 9 “I dislike it extremely”. Different letter in the same row denotes groups statistically different (P <0.05) among LSMeans.

4.5. Discussion

4.5.1. Effects of aging method and finishing system on instrumental color, pH, cooking losses, and shear force

The physicochemical traits were influenced by the aging method, except for WBSF. Regarding the finishing diet, the higher L* values observed in WA may be explained by the greater reflectance associated with higher moisture levels (Bertram et al., 2004). The combination of AM*F affected the a* parameter, with the lowest redness values found in DAb from grain-fed, followed by DW from pasture and grain-fed. This may be attributed to the lower water content resulting from the dry bag aging process and decreased absorption on the meat's surface, which appears dark red (Kim, 2011). Concerning the b* coordinate, the values corresponded with a*, indicating that the lowest values were in DW and WD, regardless of the diet. Previous studies evaluated different aging methods, reporting that the highest b* value was in the dry + wet combination (De Faria Vilella et al., 2019), whereas other studies found no differences in b* between treatments (Zhang et al., 2020; Kim et al., 2017).

Regarding diet, studies have indicated that beef sourced from pasture-raised cattle appears darker compared to meat from animals finished on concentrated diets, as assessed through both objective (lightness) and subjective (brightness) criteria (Priolo et al., 2001; Gatellier et al., 2005). Muscle from grass-finished cattle possesses more myoglobin, which may cause it to appear darker due to having greater mitochondrial-based oxidative enzyme content, fewer glycolytic enzymes, and producing less lactate when subjected to an in vitro glycolysis system (Apaoblaza et al., 2020). In addition, Apaoblaza et al. (2020) reported greater values for meat a* and L* coordinates from grain-finished compared to their forage-finished counterparts. Several factors contribute to this difference, such as variations in ultimate pH, myoglobin, and intramuscular fat content, which seem to play a major role (McKeith et al., 2016). However, in our study, neither the final pH nor the intramuscular fat content differed between the finishing systems. Perhaps other characteristics could have affected the meat color, for example, the myoglobin concentration that was not evaluated in this study (Apaoblaza et al., 2020). The present study indicates that the

aging combination methods decreased the color parameters (a* and b*). Although statistically correct, from a technical point of view, there was not a significant difference in the coordinates of color. Theoretically, ΔEs of less than 1.0 are not detectable unless the samples are side by side. This parameter was calculated by comparing aged treatments, which are useful for establishing tolerances for variation in color between samples (King et al., 2023). No value was higher than 2.7; it's an instrumental difference, but not evident to consumers or the naked eye, indicating that only experienced observers could notice a color difference (Mokrzycki and Tatol, 2011).

After 40 days of aging, the pH was higher in DAb than in WA. Several studies reported an increase in pH after 21 days of dry bag aging beef and a decrease in WA between 20 days and 40 days of aging (Dikeman et al., 2013; Li et al., 2014; Obuz et al., 1014; Kim et al., 2017; Zhang et al., 2019). These authors indicated that this increase in pH following dry bag aging could be associated with the generation of nitrogenous compounds resulting from proteolysis, while the lower pH in wet aging would be attributed to a higher accumulation of lactic acid. Additionally, Triki et al. (2018) reported that pH increases during chilled storage of meat were associated with the production of nitrogenous basic compounds due to microbial spoilage, and that this was influenced by the type of packaging.

In agreement with our findings, Laster et al. (2008) found greater cooking yields in traditional dry-aged (without packaging) striploin steaks than in wet-aged. On the other hand, de Faria Vilella et al. (2019) reported no significant differences in cooking loss between the unaged and aged samples (wet, dry, and combined) during 28 days. The difference from the previous studies is the dry aging method: dry bag versus traditional dry aging, implying less loss and greater cooking yields in dry bag aging. The reduced cooking loss (CL) in DAb compared to WA is attributed to differences in moisture loss through evaporation during the dry bag aging process, as previously explained (Zhang et al., 2019; Juarez et al., 2011). Aligned with the previous studies, the combined aging process (DW and WD) presented intermediate values between DAb and WA, with no differences between them.

Previous studies had indicated that beef loins assigned to stepwise dry/wet-aging had lower WBSF values (2.66 kgF) compared to the loins assigned to conventional dry-aging (2.94 kgF) (Kim *et al.*, 2017). However, de Faria Vilella *et al*. (2020) reported no differences in WBSF due to the aging methods (wet, dry, and their combination). The discrepancy between studies could be due to sample management because in the former the dry aging process (traditional) was in the carcass and for wet aging the loin was removed from the carcass and vacuum-packaged and frozen by 30 days; in the latter study, the aging process was steak in-bone for dry and wet. Consistent with most experiments (Dikeman *et al*., 2013; Ahnstrom *et al*., 2006; Berger *et al*., 2018), our study showed no differences in WBSF between aging methods (DAb, WA, DW, and WD), and the values were below three kgF, indicating that the products could be considered as moderately tender (Smith *et al.*, 2008).

4.5.2. Effects of aging method and finishing system on fatty acid profile and oxidation

Previous studies reported no interaction between diet and aging treatments on the fatty acid composition of beef (Jiang et al., 2010). However, our work showed an interaction effect on SFA, MUFA, and PUFA. Regarding the finishing diet, no differences were found in SFA and MUFA concentrations. The higher levels of IMF in DW compared to WA could be attributed to the loss of water during the dry aging period. However, this alone does not sufficiently explain this change, as the IMF content of DAb steaks was not significantly different from that of WD steaks. In line with this, Wood et al. (2008) reported that the amount of intramuscular fat influences the fatty acid composition of beef due to increased SFA deposition as total fat rises. Polyunsaturated fatty acid content was higher in DAb, WA, and WD from pasture-finished animals and corresponded with PUFA n6 and C18:3n3 concentrations. However, the total PUFA n3, the n6:n3 ratio, and PUFA:SFA did not show an interaction between AM and F, instead indicating an increase in the PUFA n3 and n6:n3 ratio observed in pasture-finished compared to grain-finished steers. The nutrient composition of animal diets alters the fatty acid profile of meat, making it more appealing for consumers considering health concerns and meat flavor, particularly with grass-finished animals (Nuernberg et al., 2005; Melton et al., 1983).

In terms of human health, a significant aspect is the concentrations of omega 6 (n6) and omega 3 (n3) fatty acid families (Daley et al., 2010). The Department of Health (1994) of the United Kingdom has published recommended intakes of fatty acids with an n6:n3 ratio ≤4, which was achieved in our study of meat from pasture-finished steers, consistent with earlier studies (Nuernberg et al., 2005; Brito et al., 2014). The higher content of CLA, PUFA, and PUFA n3 aligns with the findings of Realini et al. (2004), Ponnampalam et al. (2006), and Jiang et al. (2010). Regarding meat flavor, linolenic fatty acid (C18:3n3) is an important precursor (Ba et al., 2012), and its higher concentration in beef from pasture-finished animals negatively impacted desirable beef flavor (Melton et al., 1982). However, the odor detection threshold values for lipid-derived compounds are significantly higher than those for sulfur and nitrogen-containing heterocyclic compounds formed from water-soluble precursors via the Maillard reaction (Ba et al., 2012). Therefore, the aromatic significance of many lipid-derived compounds is not as substantial as that of relatively low concentrations of the heterocyclic compounds.

In agreement with Berger et al. (2018) and Zhang et al. (2020), our study found that the composition of fatty acid groups in intramuscular fat—SFA, MUFA, and PUFA—did not differ among AM. Conversely, Kim et al. (2017) reported that SFA, MUFA, and PUFA content in wet-aged beef was greater than in dry-aged beef after 40 days of aging in top round and shank (muscle from the leg). Kim et al. (2017) also documented lower C18:3n3 content in dry aging compared to wet aging, indicating its negative effect on flavor when reacting with volatile compounds from the cooking process. The difference in our findings may be attributed to the fact that dry aging in the bag allows for less exposure to oxygen compared to traditional dry aging, suggesting that the oxidative stability of FAs would be less affected by AM.

In summary, the fatty acid profile was primarily affected by the finishing diet, with grass-finished meat steers exhibiting the highest concentrations of PUFA, particularly n-3 PUFA, indicating their importance for human health and meat flavor.

Lipid oxidation is a process in which polyunsaturated fatty acids react with reactive oxygen species, leading to a series of secondary reactions. These reactions result in lipid degradation and the development of oxidative rancidity (Park et al.,

2006). This process significantly contributes to the gradual reduction of the sensory and nutritional quality of meat. In our study, the AM*F interaction impacted the oxidative stability of lipids, with the highest TBARS value found in WD from grain-finished steers. No differences in TBARS values were observed among AM from pasture-finished steers; however, there was a trend toward increasing TBARS contents in samples from pasture-finished and dry-bag aging. Zhang et al. (2020) reported no differences in TBARS between dry aging bags versus stepwise [21 days] for samples from pasture-finished cattle. Similarly, Ha et al. (2019) reported no difference in TBARS between dry and wet aging after 35 days of storage. Our findings may be attributed to a higher IMF concentration in meat from a grain-finished steer aged with dry bags and the AM combination (DW and WD). Furthermore, the IMF contents found in DAb from pasture-finished steers relate to the fatty acid profile, as these contain higher PUFA n-3 concentrations, which indicate greater oxidation instability. As is well known, lipid oxidation requires oxygen to produce oxidized products such as aldehydes (Nam and Ahn, 2003). Nam et al. (2001) noted increasing TBARS values and off-flavors in aerobically packaged meats. DeGeer et al. (2009) indicated that steaks from loin dry-aged in a bag exhibited less oxidation compared to those in traditional dry aging. Zhang et al. (2020) suggest that using aging bags prevents oxidative deterioration. This study did not examine traditional dry aging; however, the use of dry-aging bags can help explain the observed lower oxidation.

On the other hand, it has been reported that animals finished on pasture have greater concentrations of vitamin E in muscle than those grain-finished (Realini et al., 2004; Nuernberg et al., 2005; Daley et al., 2010), which delays lipid oxidation and metmyoglobin formation (Schwarz et al., 1998; Zerby et al., 1999; Descalzo & Sancho, 2008). Although vitamin E content was not measured in this study, its likely greater concentration in the muscle of grass-finished animals would explain, at least in part, the lower TBARS values compared to grain-finished cattle.

4.5.3. Effects of aging method and finishing system on superficial microbial counts

In the manner of Li et al. (2013) and Ahnstrom et al. (2006), in our study, AM did not impact the ENT counts. Enterobacteriaceae is a specific family of bacteria that

includes several pathogens and serves as a fecal contamination indicator, with a threshold of 4 to 5 log CFU/cm² according to EU microbiological regulatory criteria (Rinn et al., 2024). TBC and PSY counts had the same behavior and their highest count was observed in WD, and the lowest load values were in WA and DW. Campbell et al. (2001) reported no trend in microflora when beef was stored under vacuum after dry aging. Therefore, vacuum packaging (the first step in WD) may create a microclimate with high humidity, which is ideal for the growth of psychrophilic bacteria (Gardner, 1981). Psychrophilic bacteria are particularly relevant for products stored under chilling conditions, as these microorganisms can still multiply (Gonzalez-Gutierrez et al., 2019). Thus, meat juices are a suitable breeding substrate for microorganisms. In WD, bacteria from wet aging are not eliminated during dry aging, instead, some may survive and multiply. As a result, a higher microbial load in WD is observed compared to wet or dry bag aging alone. Previous studies indicated that levels of 6 to 8 log CFU/g of microorganisms (PSY) are sufficient to produce off-odors and appearance defects in meat, and that these values trigger strange smells and sliminess in meat (Ercolini et al., 2006; Stanbridge and Davies, 1998). Moreover, off-flavor, a spoilage result in meat, can be detected when the total bacteria count (TBC) is around 7 log CFU/cm2 or g. However, some negative changes can be observed much earlier with TBC numbers between 5 and 6 log CFU/cm2 or g of meat product (Feiner, 2006). In our study, the highest TBC and PSY counts in WD aging reached almost the loads to produce off-flavor (4.6 and 6.5 log CFU/cm2 TBC and PSY, respectively). However, it seems that consumers did not detect off-flavors, as they scored the meat samples at least as “I like it” for flavor.

Regarding finishing diets, other studies have reported insufficient differences in microbial counts between grain and pasture-finished beef in concordance with our results (Duarte et al., 2022; Casas et al., 2021; Zhang et al., 2010). These authors stated that other aspects, such as how beef is processed, may play a more important role in microbial contamination of meat than diet. The microbial count for the three families studied was just below the thresholds allowed from both safety and off-flavor perspectives.

4.5.4. Effects of aging method and finishing system on consumer sensory panel

Consumers (n=100) preferred meat from DAb or WA, while the less acceptable beef was from WD. Berger et al. (2018) reported no difference in overall liking scores for the meat samples from grass-finished heifers across aging treatments (wet, dry, and dry bag). In addition, Ha et al. (2019) reported higher acceptability for eating attributes (tenderness, juiciness, flavor, and overall liking) in wet-then-dry-aged beef in Japanese consumers. In terms of finishing diet, consumers preferred meat from grain-finished steers for all three evaluated attributes. This difference might not be due to the IMF content because no differences were found in this variable associated with the animal diet. Panelists have shown that fat flavor intensity was higher in beef from steers finished on concentrates than grass-finished steers, even though fat contents were similar (Melton et al., 1982). These authors suggested that differences were because of diet on the fatty acid composition of beef, especially polyunsaturated fatty acid content (PUFA) that elicit undesirable aroma flavors due to their PUFA-derived products lower or inhibit the formation of some heterocyclic Maillard products (Ames et al., 2001). In our study, flavor differed between pasture-finished beef and grain-finished beef and C18:3n3 was higher in beef from forage treatments compared to the grain diet treatments but the effect was limited since all aging treatments received high acceptability (≤4 "I like it") for tenderness, flavor, and overall liking.

When the consumer data was analyzed in Clusters (Font-i-Furnols et al., 2009), the interaction AM*F was observed. In Cluster 1, i.e. "Grain-finished wet aged beef likers" the least preferred sample was from DW and pasture-finished animals, for overall, tenderness and flavor liking. Meanwhile, in Cluster 2, i.e. "Grain-finished dry aged beef likers" the least preferred beef was from WD aging and grain-finished animals. Thus, it can be hypothesized that the stepwise aging procedure (DW or WD) could produce changes in the tenderness and flavor of the beef that influence clusters 1 and 2 consumer acceptability (decreasing it). One possible reason for this could be lipid oxidation (Figure 3), because the consumers might have detected off-flavor in stepwise AM, particularly in the WD. In addition, WD presented microbial loads close to the threshold for off-flavor development. However, further insight would be needed, to fully understand the possible reason for this lower acceptability in the sensory

characteristics of WD-aged beef. On the other hand, Ha et al. (2019) working with Australian consumers, reported opposite results since wet-then-dry aging (21 d wet + 35 d dry) had better acceptability scores for flavor and overall liking compared to wet-aged beef. Differences between studies may be due to consumer preferences by country (Font i Furnols et al., 2006), type of beef (Campo et al., 1999), production system (Priolo et al., 2002), and aging process (Brewer & Novakofski, 2008; Lepper-Blilie et al., 2016). Consumers from clusters 1 and 2 preferred beef from grain-finished over pasture-finished steers. These results are surprising since Uruguayan consumers are used to eating beef from pasture-finished animals, and the habits greatly affect preferences (Font-i-Furnols and Guerrero, 2014; Font i-Furnols et al., 2006). Meanwhile, in Cluster 3, regardless of some differences, consumers scored all meat samples between 4 to 5 for overall and flavor liking, closer to 'neither like nor dislike', thus, they hesitated more when making their decisions.

Despite slight differences between treatments and consumer groups, aged beef was well accepted under instrumental meat quality assessment and microbiological data.

4.6. Conclusion

Aging methods significantly influenced the physicochemical and microbial characteristics, while the finishing diet affected the fatty acid composition and consumer panel results. Meat quality characteristics, such as color (except L* values), pH, CL, and WBSF, did not show differences due to the finishing diet or its interaction with the aging method. Under the conditions of this study, all aging methods were acceptable from the consumer's perspective. However, combining both aging techniques (wet and dry in a bag) would not be a suitable alternative for enhancing consumers' meat sensory acceptability. Either dry bag or wet-aging from grain-finished steers appears valuable for the Uruguayan consumer panel.

Further research is necessary to identify and develop an in-depth understanding of the safety and quality of extended aging methods in fresh beef.

4.7. References

Ahn, D.U., Olson, D.G., Lee, J. I., Jo, C., Wu, C., and Chen, X. 1998. Packaging and irradiation effects on lipid oxidation and volatiles in pork patties. J. of Food Sci. 63:15-19.

Ahnström, M.L., Seyfert, M, Hunt, M.C., and Johnson, D.E. 2006. Dry aging of beef in a bag highly permeable to water vapour. Meat Sci., 73, 674–679.

Ames, J. M., Guy, R. C. E., and Kipping, G. J. (2001). Effect of pH and temperature on the formation of volatile compounds in cysteine/reducing sugar/starch mixtures during extrusion cooking. J. of Agric. and Food Chem. 49, 1885-1894.

AMSA, 2012. Meat Color Measurement Guidelines. American Meat Science Association 201 West Springfield Avenue, Suite 1202 Champaign, Illinois USA 61820 800-517-2672 http://www.meatscience.org

AMSA, 2016. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat, 2nd ed. Amer. Meat Sci. Assoc., Champaign, pp. 106

Aldai, N., Dugan, M. E. R., Kramer J. K. G., Martinez A., Lopez-Campos, O., Mantecon, A., R. and Osoro, K. 2011. Length of concentrate finishing affects the fatty acid composition of grass-finished and genetically lean beef: an emphasis on trans-18:1 and conjugated linoleic acid profiles. Animal 5, 1643–1652. doi:10.1017/S1751731111000607

Apaoblaza, A., Gerrard, S.D., Matarneh, S.K., Wicks, J.C., Kirkpatrick, L., England, E.M., Scheffler, T.L., Duckett, S.K., Shi, H., Silva, S.L., Grant, A.L. and Gerrard, D.E. 2020. Muscle from grass- and grain-finished cattle differs energetically. Meat Sci. 161, 107996.

Ba, H. V., Oliveros, C. M., Park, K, Dashdorj, D. and Hwang, I. 2016. Effect of marbling and chilled ageing on meat-quality traits, volatile compounds and sensory characteristics of beef *longissimus dorsi* muscle. *Anim. Prod. Sci.* 57(5) 981-992 https://doi.org/10.1071/AN15676

Ba, H.V., Hwang, I., Jeong, D. and Touseef, A. 2012. Principle of Meat Aroma Flavors and Future Prospect. *In*: *IntechOpen* Chapter 7. http://dx.doi.org/10.5772/51110

Berger, J. 2017. Effects of dry-aging on eating quality, physicochemical, and microbiological attributes of grass-finished beef loins. Master's thesis. Faculty of Purdue University. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Jordy+Berger%2C+2017&oq=j#:~:text=%2D%202017%20%2D-,docs.lib.purdue.edu,-Abstract%20The%20United

Berger, J., Kim, Y. H. B., Legako, J. F., Martini, S., Lee, J., Ebner, P. E., and Zuelly, S. M. S. 2018. Dry aging improves meat quality attributes of pasture-finished beef loins. Meat Sci, 145 (2018) 285-291.

Bertram, H. C., Engelsen, S. B., Busk, H., Karlsson, A. H. and Andersen, H. J. 2004. Water properties during cooking of pork studied by low-field NMR relaxation: effects of curing and the RN-gene. Meat Scie. 66 437-446.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. *Can. J. Bioch. Physiol.*, 37:911-917

Brito, G., San Julián, R., La Manna, A., Del Campo, M., Montossi, F., Banchero, G., Chalkling, D. and Soares de Lima, J.M. 2014. Growth, carcass traits, and palatability: can the influence of the feeding regimes explain the variability found on those attributes in different Uruguayan genotypes? Meat Scie. 98, 533-538.

Bruns, K.W., Pritchard, R.H, Boggs, D.L. 2004.The relationships among body weight, body composition, and intramuscular fat content in steers. J Anim Sci. 82:1315-22.

Campbell, R.E., Hunt, M.C., Levis, P. and Chambers, E. 2001. Dry-Aging Effects on Palatability of Beef Longissimus Muscle. J. of Food Scie. 66, 196–199.

Campo, M., Sañudo, C., Panea, B., Alberti, P., and Santolaria, P. (1999). Breed type and ageing time effects on sensory characteristics of beef strip loin steaks. Meat Scie. 383–390.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D. and Richardson, R. I. 2006. Flavour perception of oxidation in beef. Meat Scie. 72, 303–311.

Casas, D. E., Manishimwe, R., Forgey, S. J., Hanlon, K. E., Miller, M. F., Brashears, M. M. and Sanchez-Plata, M. X. 2021. Biomapping of Microbial Indicators on

Beef Subprimals Subjected to Spray or Dry Chilling over Prolonged Refrigerated Storage. *Foods* 2021, 10, 1403

Correa, D., Lema, M., Ravagnolo, O., Clariget, J., Luzardo, S and Brito, G. 2020. Effects of differences in level of post-weaning nutrition and in sire expected progeny differences for ribeye area on retail cuts yield in Hereford steers. Anim. Prod. Scie. https://doi.org/10.1071/AN19604

Correa, D., del Campo, M., Luzardo, S., De Souza, G., Álvarez, C., Font-i-Furnols, M. and Brito, G. 2024. Impact of Aging Methods and Frozen Storage on Meat Quality Attributes of Beef from Different Finishing Diets. Meat and Muscle Biol. https://doi.org/10.22175/mmb.17695

Daley, C. A., Abbot, A., Doyle, P. S., Nader, G. A. and Larson, S. 2010. A review of fatty acid profiles and antioxidant content in grass-finished and grain-finished beef. Nutrition Journal 2010, 9:10 http://www.nutritionj.com/content/9/1/10

de Faria Vilella, G., Gomes, C.L. Battaglia, C.T. Pacheco, M.T.B., da Silva, V.S.N., Rodas-González, A. and S.B. Pflanzer. 2019. Effects of combined wet- and dry-aging techniques on the physicochemical and sensory attributes of beef ribeye steaks from grain-finished crossbred Zebu steers. Can. J. Anim. Sci. 99: 497–504 dx.doi.org/10.1139/cjas-2018-0127

DeGeer, S. L., Hunt, M. C., Bratcher, C. L., Crozier-Dodson, B.A., Johnson, D. E. and Stika, J. F. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. Meat Scie., 83, 768–774.

Department of Health. 1994. Report on health and social subjects. No. 46. Nutritional aspects of cardiovascular disease. London: HMSO.

Descalzo, A. M., and Sancho, A. M. 2008. A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. Meat Scie. 79: 423-436.

Dikeman, M. E., Obuz, E., Gök, V., Akkaya, L., and Stroda, S. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef Longissimus lumborum steaks. Meat Scie. 94, 228–233.

Duarte, T. L., Bolkenov, B., Klopatek, S. C., Oltjen, J. W., King, D. A., Shackelford, S. D., Wheeler, T. L., and Yang, X. 2022. Evaluating the Shelf Life and Sensory Properties of Beef Steaks from Cattle Raised on Different Grass Feeding Systems in the Western United States. *Foods* 2022, 11, 2141. https://doi.org/10.3390/foods11142141

Duckett, S. K., Neel, J. P. S., Lewis, R. M, Fontenot, J. P. and Clapham W. N. 2013. Effects of forage species or concentrate finishing on animal performance, carcass, and meat quality. Jour. of An. Scie. 91, 1454–1467. doi:10.2527/jas.2012-5914

Faustman, C., and Cassens. R. G. 1990. The biochemical basis for discoloration in fresh meat: a review. *Jour. of Muscle Foods*. 1: 217-243.

Ferrinho, A. M, Peripolli, E., Banchero, G., Cravo Pereira, A. S., Brito, G., La Manna, A., Fernandez, E., Montossi, F., Kluska, S., Freitas Mueller, L., Berchielli, T.T. and Baldi, F. 2020. Effect of growth path on carcass and meat-quality traits of Hereford steers finished on pasture or in feedlot. Anim. Prod. Scie. 60, 323–332. doi:10.1071/AN18075

Font-i-Furnols, M., San Julián, R., Guerrero, L., and Sañudo C. 2006. Aceptabilidad de consumidores españoles, alemanes e ingleses frente a la carne de ovino de diferentes sistemas productivos y tiempos de maduración. In: Jornada sobre presentación de resultados del proyecto, Madrid (Ed.). Sañudo C, Montossi F. Madrid: Inia (España). p. 53-55

Font-i-Furnols, M., and Guerrero, L. 2014. Consumer preference, behavior and perception about meat and meat products: An overview. Meat Sci. 98:361–371. https://doi.org/10.1016/j.meatsci.2014.06.025

Foraker, B. A., Gredell, D. A., Legako, J. F., Stevens, R. D., Tatum, J. D., Belk, K. E. and Woerner, D. R. 2020. Flavor, tenderness, and related chemical changes of aged beef strip loins. Meat Muscle Biol. 4(1): 28, 1–18 doi:10.22175/mmb.11115

Feiner, G. 2006. Meat products handbook: *Practical science and technology*. Elsevier.

Gardner, G. A. 1981. Brochothrix thermosphacta (Microbacterium thermosphactum) in the spoilage of meats: a review, p. 139-173. In T. A. Roberts, G. A. Hobbs, J.

H. B. Christian, and N. Skovgaard (eds..), Psychrotrophic microorganisms in spoilage and pathogenicity. Academic Press, London, England.

Gatellier, P., Mercier, Y., Juin., H., and Renerre, M. 2005. Effect of finishing mode (pasture- or mixed-diet) on lipid composition, colour stability and lipid oxidation in meat from Charolais cattle. Meat Scie. 69: 175-186. doi:10.1016/j.meatsci.2004.06.022

Gill, C. O. 2014. Spoilage, factors affecting: Microbiological. Encyclopedia of Meat Sciences (2nd ed.). 3. Encyclopedia of Meat Scie. (pp. 388–393). Elsevier.

González-Gutiérrez, M., García-Fernández, C., Alonso-Calleja, C. and Capita, R. 2020. Microbial load and antibiotic resistance in raw beef preparations from northwest Spain. Food Scie Nutr. 8:777–785. https://doi.org/10.1002/fsn3.1319

Ha, M., McGilchrist, P., Polkinghorne, R., Huynh, L., Galletly, J., Kobayashi, K., Nishimura, T., Bonney, S., Kelman, K. R., and Warner, R. D. 2019. Effects of different ageing methods on colour, yield, oxidation and sensory qualities of Australian beef loins consumed in Australia and Japan. Food Res. Inter.125 (2019) 108528. https://doi.org/10.1016/j.foodres.2019.108528

Iida, F., Miyazaki, Y., Tsuyuki, R., Kato, K., Egusa, A., Ogoshi, H., and Nishimura, T. 2016. Changes in taste compounds, breaking properties, and sensory attributes during dry aging of beef from Japanese black cattle. Meat Sci. 112:46–51. https://doi.org/10.1016/j.meatsci.2015.10.015

INAC, 2024. Instituto Nacional de Carne. Exportación https://www.inac.uy/innovaportal/v/23295/10/innova.front/exportacion

IUPAC, 1987. Standard Methods for the Analysis of Oils, Fats and Derivatives. International Union of Pure and Applied Chemistry Division Commission on Oils, Fats and Derivatives, 7th ed Blackwell Jevent Publishers, Oxford

INAC, 1997. Resolución 65/997 Del Instituto Nacional de Carnes Del 14 de Abril de 1997. 1997. Available online: https://www.Inac. Uy/Innovaportal/File/2024/1/-Res-65-997.Pdf (accessed on 01 October 2024).

Jiang, T., Bsuboom, J.R., Nelson, M.L., O´Fallon, J., Ringkob, T.P., Rogers-Klette, K.R., Joos, D. and Piper, K. 2010. The influence of forage diets and aging on beef palatability. Meat Sci 86, 642-650

Juárez, M., Caine, W. R., Dugan,M. E. R., Hidiroglou, N., Larsen, I. L., Uttaro, B., and Aalhus, J. L. 2011. Effects of dry-ageing on pork quality characteristics in different genotypes. Meat Sci. 88:117–121. https://doi.org/10.1016/j.meatsci.2010.12.011

Kemp, C.M., Sensky, P. L., Bardsley, R. G., Buttery, P. J. and Parr, T. 2010. Tenderness – An enzymatic view. Meat Sci. 84, 248-256.

Kern, S.A., Pritchard, R.H., Blair, A.D., Scramlin, S.M., and Underwood, K.R.2014. The influence of growth stage on carcass composition and factors associated with marbling development in beef cattle. J Anim Sci. 92:5275-84.

Kim, Y. H. B. 2011. "Advances technology to improve meat color" In Control of Meat Quality. S. T. Joo, Ed., pp.31-60. Research Signpost, Trivanfrum, India.

Kim, Y. H. B., Kemp, R., and Samuelsson, L. M. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. Meat Scie. 111, 168–176.

Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., and Hunt, M.C. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies (Review). Meat Scie. 144 74-90.

Kim, J. H., Kim, D. H., Ji, D., Lee, H. J., Yoon, D. K., and Lee, C. H. 2017. Effect of Aging Process and Time on Physicochemical and Sensory Evaluation of Raw Beef Top Round and Shank Muscles Using an Electronic Tongue. Korean Jour. Food Scie. An. 37(6): 823~832 (2017) https://doi.org/10.5851/kosfa.2017.37.6.823

Kim, J. H., Kim, T. K., Shin, D. M., Kim, H. W., Kim, Y.B. and Choi, Y.S. 2020. Comparative effects of dry-aging and wet-aging on physicochemical properties and digestibility of Hanwoo beef. Asian-Aus. Jour. Anim. Scie. Vol. 33, No. 3:501-505. https://doi.org/10.5713/ajas.19.0031

King, D. A., Hunt, M. C., Barbut, S., Claus, J. R., Cornforth, D. P., Joseph, P., Kim, Y. H. B., Lindahl, G., Mancini, R. A., Nair, M. N., Merok, K. J., Milkowski, A., Mohan, A., Pohlman, F., Ramanathan, R., Raines, C.R., Seyfert, M., Sørheim, O., Suman, S. P. and Weber, M. 2023. American Meat Science Association

Guidelines for Meat Color Measurement. Meat Muscle Biol. 6(4): 12473, 1–81. doi:10.22175/mmb.12473

Laster, M.A., Smith, R.D., Nicholson, K.L., Nicholson, J.D., Miller, R.K., Griffin, D.B., Harris, K.B., and Savell, J.W. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer sensory attribute evaluations of steaks from ribeyes, strip loins, and top sirloins from two quality grade groups. Meat Scie. 80(3): 795-804. doi: 10.1016/j.meatsci.2008.03.024.

Li, X., Babol, J., Wallby, A., and Lundström, K. 2013. Meat quality, microbiological status and consumer preference of beef gluteus medius aged in a dry ageing bag or vacuum. Meat Scie. 95, 229–234.

Li, X., Babol, J., Bredie, W. L. P., Nielsen, B., Tománková, J. and Lundstrom. 2014. A comparative study of beef quality after ageing longissimus muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. Meat Scie. 97 (2014) 433–442

MacFie, H. J., and Bratchell, N. K. 1989. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. Jour. of Sensory Stud. https://doi.org/10.1111/j.1745-459X.1989.tb00463.x

McKeith, R. O., King, D. A., Grayson, A. L. Shackelford, S. D., Gehring, K. B., Savell, J.W. and Wheeler, T. L. 2016. Mitochondrial abundance and efficiency contribute to lean color of dark cutting beef. Meat Scie. 116, 165-173.982.

Melton, S. L., Black, J. M., Davis, G. W., and Backus, W. R. 1982. Flavor and Selected Chemical Components of Ground Beef from Steers Backgrounded on Pasture and Finished Corn up to 140 Days. J. of Food Scie. Volume 47 -699

Melton S. L. 1983. Effect of forage feeding on beef flavor. Food Tech. 37 239 248

Mokrzycki, W. S., and Tatol, M. 2011. Color differenceΔ EA survey. *Mach. Graph. Vis*, *20*(4), 383-411.

MGAP (Ministerio de Ganadería, Agricultura y Pesca). 2023. Available at https://www.gub.uy/ministerio-ganaderia-agriculturapesca/sites/ministerio-ganaderia-agricultura-pesca/files/202108/CarneAltaCalidad_8350_0621_UE_Ingles.pdf. (Accessed 1 March 2024).

Nam, K. C., and Ahn, D. U. 2003. Double-packaging is effective in reducing lipid oxidation and off-odor volatiles of irradiated raw turkey meat. Poultry Scie. 82(9),
1468-1474.

Nam, K. C., Ahn, D. U., Du, M., and Jo, C. 2001. Lipid oxidation, color, volatiles, and sensory characteristics of aerobically packaged and irradiated pork with different ultimate pH. J. of Food Scie. 66, 1220-1225.

Nuernberg, K., Dannenberger, D., Nuernberg, G., Ender, K., Voigt, J. Scollan, N.D., Wood J.D., Nute, G.R., and Richardson, R.I. 2005. Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. Livest. Prod. Scie. 94, 137– 147

Obuz, E., Akkaya, L., Gok, V., and Dikeman, N. E. 2014. Effects of blade tenderization, aging method and aging time on meat quality characteristics of Longissimus lumborum steaks from cull Holstein cows. Meat Scie. 96, 1227-1232.

Park, D., Xiong, Y. L., Alderton, A. L., and Ooizumi, T. 2006. Biochemical changes in myofibrillar protein isolates exposed to three oxidizing systems. J. Agric. Food Chem. 54(12), 4445–4451.

Peripolli, E., Banchero, G., Pereira, A., Brito, G., La Manna, A., Fernandez, E., Montossi, F., and Baldi, F. 2018. Effect of growth path on the performance and carcass traits of Hereford steers finished on pasture or in feedlot. *Anim. Prod. Scie*. 58, 1341–1348. doi:10.1071/AN16061

Ponnampalam, E. N., Mann, N. J. and Sinclair, A. J. 2006. Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts: potential impact on human health. Asia Pacific J. of Clinical Nutr. 2006;15 (1):21-29

Priolo, A., Micol, D., and Agabriel, J. 2001. Effects of grass feeding systems on ruminant meat colour and flavour. A review. Anim. Res. 50 185–200 185 © INRA, EDP *Sciences*

Realini, C.E., Duckett, S.K., Brito, G.W., Dalla Rizza, M., De Mattos, D. 2004. Effect of pasture *vs.* concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. Meat Scie. 66: 567–577

SAS Institute Inc (2012) 'SAS/STAT 9.2 User's Guide.' (SAS Institute Inc.: Cary, NC, USA)

Rinn, N., Braun, A.S., Müller, A., Wadepohl, K., Gerulat, B., Kumm, F., Yue, M. and Kehrenberg, C. 2024. Microbiological quality of raw beef imported into the European Union from third countries. Food Control 160: 110358

Savell, J. 2008. Dry-aging of beef: Executive Summary. Texas, Estados Unidos: Center for Research and Knowledge Management. National Cattlemen's Beef Association. http://www.beefresearch.org/cmdocs/beefresearch/dry%20Aging%20of%20beef.pdf

Smith, R., Nicholson, K., Nicholson, J., Harris, K., Miller, R., Griffin, D., and Savell, J. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. Meat Scie. 79, 631–639

Schwarz, F. J., Augustini, C., Timm, M., Kirchgessner, M., and Steinhart, H. 1998. Effect of vitamin E on alpha-tocopherol concentration in different tissues and oxidative stability of bull beef. *Livest. Prod. Scie.* 56, 165-171.

Stanbridge, L.H., and Davies, A.R. 1998. The microbiology of chill-stored meat. In A.R. Davies, R.J. Board and R.G. Board (Eds.), Microb. of Meat and Poultry (pp. 174-)

Triki, M., Herrero, A.M., Jiménez-Colmenero, F. and Ruiz-Capillas, C. 2018. Quality Assessment of Fresh Meat from Several Species Based on Free Amino Acid and Biogenic

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I., and Whittington, F.M. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Scie. 78: 343–358

Zerby, H. N., Belk, K. E. Sofos, J. N. McDowell L. R., and Smith, G. C. 1999. Case life of seven retail products from beef cattle supplemented with alpha-tocopheryl acetate. J. Anim. Scie. 77:2458-2463.

Zhang, R., Yoo, M. J.Y., and Farouk, M. 2020. Oxidative stability, proteolysis, and in vitro digestibility of fresh and long-term frozen stored in-bag dry-aged lean beef. Food Chem. https://doi.org/10.1016/j.foodchem.2020.128601

Zhang, R., Yoo, M. J. Y., and Farouk, M. M. 2019. Quality and acceptability of fresh and long-term frozen in-bag dry-aged lean bull beef. J. of Food Quality, 2019 (1975264), 1–15.

Zhang, J., Wall, S. K., Xu, L., and Ebner, P. D. 2010. Contamination rates and antimicrobial resistance in bacteria isolated from "grass-finished" labeled beef products. Foodborne Pathogens Disease 7:1331-1336.

Statement of authorship

We hereby declare that we are the sole authors of this original article and have not used any sources other than those identified as references. We declare that we have not submitted this original article to any other journals and that it is not under consideration for publication elsewhere.

CRediT author statement

Daniela Correa: Methodology, Investigation, Forma analysis, Data curation, Writing – original draft, Visualization**. Marcia del Campo**: Conceptualization, Funding acquisition, Review & editing. **Santiago Luzardo**: Conceptualization, Methodology, Visualization, Writing – review & editing. **Guillermo de Souza**: Data curation. **Carlos Álvarez:** Conceptualization**,** Visualization, Writing – review & editing. **Maria Font i-Furnols**: Formal analysis, Writing – review & editing. **Gustavo Brito**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition

Declaration of Competing Interest

We confirm that there are no known conflicts of interest associated with this publication and there have been no significant financial support for this work that could have influenced its outcome.

Data availability

Data will be made available on request.

Acknowledgments

The authors would like to thank INIA Uruguay for funding Project N-24992 “New aging alternatives and preservation methods of beef from different finishing systems,” which made this study possible. D. C. acknowledges her INIA Academic Excellence Award assistantship. Thanks to the staff of the Meat Technology Laboratory at INIA Tacuarembó for their dedication and effort in all the analyses and services performed. Also, the authors would like to acknowledge Tacuarembó MARFRIG’s staff.

5. Volatile compounds and sensory characterization of beef from different finishing diets aged in dry-bag and wet during 40 d

Daniela Correa[1], Gustavo Brito[1], Marcia del Campo[1], Santiago Luzardo[1], Guillermo de Souza[1], Carlos Álvarez[2], Iwona Skibinska[2,] and María Font-i-Furnols[3*]

[1]INIA-Sistema Ganadero Extensivo, INIA Tacuarembó, ruta 5, km 386, Tacuarembó, Uruguay

[2]Teagasc-Food Quality and Sensory Science Department, Teagasc Ashtown Food Research Centre, Ashtown, D15KN3K Dublin 15, Ireland

[3] IRTA-Food Technology, Granja Camps i Armet, 17121 Monells (Girona), Spain

*Corresponding author. Email: maria.font@irta.cat

5.1. Abstract

This study evaluated the effects of two finishing diets (F: pasture or grain) and two meat aging methods (AM): wet aging (WA) for 40 days and dry aging in the bag (DAb) for 40 days, on the volatile compounds and sensory attributes of beef. Sixty striploins, consisting of the right and left longissimus lumborum muscle (LL) from British crossbred steers, were used, with 15 pairs of striploins obtained from pasture-finished and 15 pairs from grain-finished diets. Aldehydes, carboxylic acids, and ketone groups increased ($P<0.05$) in beef aged in dry bags compared to the wet-aged meat. The aldehyde group (2-propenal, 2-decanal, decanal, dodecanal, heptanal, pentanal, tri-decanal, and undecanal) and carboxylic acids (decanoic acid, dodecanoic acid, hexanoic acid, octanoic acid, and pentanoic acid) were higher in pasture-fed compared to grain-fed beef ($P<0.05$). No differences in ketones were observed between pasture-fed and grain-fed aged meat. The pasture diet increased the herbal odor ($P=0.015$), abnormal odor ($P=0.021$), and milky flavor ($P=0.013$), while it decreased the metallic flavor ($P=0.002$). The DAb method increased the beef and age-intensity odor while decreasing the liver odor. Hardness, fibrousity, masticability, and residues increased in DAb ($P<0.05$), whereas initial juiciness, crumbliness, and final juiciness increased in WA ($P<0.05$). The intensity of the age and bitterness flavors

increased, while those of metallic, liver, and astringent flavors decreased in DAb ($P<0.05$). For markets valuing intense, aged beef flavors, dry aging with DAb is preferable. However, for consumers prioritizing tenderness and juiciness, wet aging may be more suitable.

Keywords: Beef, Finishing diet, Dry aging bag, Volatiles compound, Meat flavor, Trained panel

5.2. Introduction

Meat is a complex, heterogeneous mixture of volatile compounds that contribute to sensory perception overall (Merkle et al., 2015). Flavor, which includes both taste and aroma, is a primary factor influencing consumers' acceptance of foods (Maughan et al., 2012). While the foundation of meat flavor, such as in beef, is established during the primary production phase through the selection of breed and feed, it is also influenced by the slaughtering process and the aging and cooking processes of the meat (Aaslying et al., 2017).

Among the various factors influencing meat flavor, the animal's diet is the most important, particularly its lipid content, as it serves as the primary source of aromatic volatile compounds (Karabagias, 2018). Raw meat constitutes a rich matrix of non-volatile precursors (amino acids, peptides, reducing sugars, vitamins, nucleotides, and fatty acids) and a diverse array of volatile organic compounds (VOCs), some of which are responsible for its flavor (Aaslying et al., 2017). This may lead to flavor changes in the meat after cooking as well as the formation of free radicals in lipids. Including forage in the diet of beef cattle should enhance n-3 fatty acid concentrations since forages are a good source of 18:3n-3 (Scollan et al., 2001). Elevated levels of n-3 PUFA promote a more intense meat flavor, and the thermally induced oxidation of these less stable PUFA during cooking may contribute to this effect (Sañudo et al., 2000). Steaks from grass-fed beef have been described as having stronger barny, bitter, gamey, and grassy flavor notes —all of which are classified as negative attributes— compared to grain-fed beef, while also being less juicy and receiving lower umami recognition from panelists (Maughan et al., 2012).

Aging is the process of storing meat in a controlled environment for a certain period to increase the palatability of meat (Jin and Yim, 2020). Aging can be especially beneficial for beef with low consumer preference, providing an additional value. There are two forms of aging: wet and dry (Kim et al., 2017). In wet aging, meat is vacuum-packaged and stored in a refrigerating condition (Jin and Yim, 2020). On the other hand, dry aging involves holding the meat unpacked in the open air (Lee et al., 2019). A new packaging bag technology, designated for dry aging (UMAi), has been introduced. These oxygen-permeable bags have a very high one-way water vapor transmission rate to the exterior, simulating traditional dry aging (Berger et al., 2018; Kim et al., 2018; Setyabrata et al., 2021). In-the-bag dry-aging of *longissimus lumborum* (LL) has been found to lower livery flavor and amount of perceived connective tissue and increase juiciness, brown-roasted aroma (Barragán-Hernández et al., 2022), tenderness, umami taste, and buttery/fatty flavors (Li et al., 2014) compared to wet-aging when evaluated by trained panelists. Therefore, using these dry-ageing bags could allow processors to dry-age meat in coolers at conventional temperatures and humidity, without adapting to cooler conditions (Dashdorj et al., 2016), and facilitate transoceanic shipment of chilled beef.

There is a need to characterize the flavor of Uruguayan beef in steers from pasture or grain finishing diet and vacuum-packaged beef exposed to extended periods of post-mortem aging, especially in the export market where the product is destined to be consumed. The main goal of this study was to evaluate the effect of extended meat aging methods, dry bag and wet on the volatile compounds, and their relationship with the sensory attributes of *longissimus lumborum* from steers finished on two different final diets (pasture or grain).

5.3. Materials and methods

5.3.1. Raw materials and aging process

This study was carried out in complement to Correa et al. (2024), and the duplicities of the experimental design and sample set are acknowledged. Thirty steers (under 30 months of age; British breed) finished (F) in the pasture (n=15) or grain (n=15) were slaughtered in a commercial meat processing plant (hot carcass weight:

266.5 kg and 253.2 kg, respectively). Animals finished in pasture belong to a production system where steers are selected to produce superior quality beef cuts that will have been raised exclusively on pasture, following the Uruguayan grading system (Hilton quota; INAC, 2013). On the other hand, grain-finished animals refer to beef cuts obtained from carcasses of steers under 30 months old, that have been fed on a diet containing not less than 62% of concentrates, at least for 100 days prior to slaughter ("481" quota; MGAP, 2023). Sixty striploins (*longissimus lumborum*, LL) were obtained for analysis and assigned to an aging method: dry bag or wet. The striploin from the left side of each carcass was divided into two sections or pieces, a section of 16 cm of length was vacuum packaged in dry-aging bags (DAb; TUBLIN® 10 of 50 μm thick, polyamide mix with a water vapor transmission rate of 2.5 kg/50μ/m2/24 h at 38°C, 50% RH, TUB-EX ApS, Denmark) and a second section of 14 cm of length was vacuum packaged for wet-aged (WA; vacuum packaged was a barrier bag of 50 μm thickness; maximum oxygen transmission rate of 27 cm3/m2/24 h at 22-24°C and 0% RH and moisture vapor transmission rate of 5 g/m2/24 h at 38°C and 90% RH; Cryovac® Sealed Air Corp., BB 2620, Brazil.). The location of each meat portion from each striploin was alternated in cranial to caudal direction among carcasses. The striploin's portions were laid out on wire racks inside the chamber for 40 days. During aging, the chamber was set up at 2 ± 0.5°C and a relative humidity of 85 ± 5%. Temperature and relative humidity were recorded using three dataloggers (Electronic Temperature Instruments Ltd., UK), to obtain real-time information at different points in the chamber. The air velocity was recorded weekly in different chamber positions with a digital anemometer (HoldPeak 866A digi, China), averaging 0.5 m/s. The meat portions were relocated into the chamber every 8 days to prevent any potential confounding effects of location within the chamber. After 40 days of aging, the left striploin portions (DAb and WA) were divided into steaks (2.5 cm) for different analyses. Steaks for trained sensory panel analyses and volatile compounds were vacuum packaged in conventional bags and frozen at -20 °C for shipping to IRTA (Spain) and Teagasc Moorepark (Ireland), respectively, until analysis.

5.3.2. Volatile compound analyses

Ten grams of finely chopped beef and 100ul of internal standard (4-methyl-2-pentanol at 50ppm) were placed in a 20 ml clear crimp vial and capped for sample preparation and extraction. Vials were placed in Markes International HiSorb Agitator, with a temperature of 72°C. The meat was cooked for 60 minutes at 72°C. Markes HiSorb probe (polydimethylsiloxane absorptive phase HS-P1, conditioned before and after use) was inserted through the septum of the cap and exposed to the meat headspace. The extraction was performed at 40°C for 60 minutes at 250 rpm. After that, the extraction probes were gently washed with distilled water, dried, and placed in empty stainless steel thermal desorption tubes, which were then capped. Thermal desorption tubes with the probes were placed on the Markes International TD Autosampler tray and introduced to the Thermal Desorption Unit. Samples were analyzed in triplicate.

5.3.3. Gas Chromatography-Mass Spectrometry Method

A Unity 2 Thermal Desorption Unit (Markes International Ltd) was used to concentrate the volatiles and remove any excess moisture before direct transfer to a Gas Chromatograph-Mass Spectrometer (Agilent 7890A GC and Agilent 5977B MSD). Unity 2 was operated with a purge gas of nitrogen at 50 psi. Tubes were dry-purged for 2 min using a 1:20 split. A two-stage desorption was employed; the first stage desorption was performed at 150 °C, and the second stage desorption was performed at 280 °C and held for 5 min. A 1/10 split was used for the final tube desorption. The trap used was a materials emissions trap held at 30 °C during tube desorption with a gas flow of 50 mL/min. Before trap desorption, a 2-minute pre-trap fire purge was performed with a 1/50 split. Trap desorption was performed at 30º-280ºC at a rate of 24 °C/min and held for 5 min with a 1/10 split.

The column used was a capillary DB-624 UI (60m x 0.3mm x 1.8µm) (Agilent Technologies Ltd.) with helium as the carrier gas. The column temperature started at 40ºC, held for 5 min, increased to 230ºC at 5ºC/min and held at 230ºC for 35 min (total run time of 78 min). The injector temperature was set at 250ºC. Desorbed volatile compounds were injected in splitless mode with a consistent pressure of 23 psi. An

MS quadrupole detector generated the volatile compound's mass spectra with an ionization voltage of 70 eV, 3.32 scans/s, and a scanning mass range of 35-350 amu. The ion source temperature was 230ºC and the interface temperature was set at 280°C. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library and an in-house library with target and qualifier ions using MassHunter Qualitative Analysis software. Spectral deconvolution was also performed to confirm the identification of compounds using AMDIS. Linear retention indices were determined using the method of Van Den Dool & Kratz (1963).

An auto-tune of the GCMS was carried out before the analysis to ensure optimal GCMS performance. A set of external standards was run at the start and end of the sample set and abundances were compared to known amounts to ensure both separation and MS detection were performing within specification.

5.3.4. Trained sensory panel

A panel of 9 trained panelists carried out the sensory characterization of the samples. Samples were evaluated in 15 sessions, with 4 samples each, one of each combination of finishing diet and aging type. Meat samples, previously thawed during 24 h at 4ºC, were tempered for 2 h and then cooked in a sandwich grill pre-heated at 200ºC until reaching 63ºC of core temperature, which was measured with a TP type K probe (Instrumentos Testo SA, Cabrils, ES). The cooked loins were cut into 10 portions, wrapped in aluminum foil, and coded with a 3-digit random number. The position of each portion was recorded to ensure that, in each session, each panelist received portions from the same loin position, to avoid positional effects. The position assigned to each random panelist was changed in each session. The wrapped portions were placed in a heater to keep them warm until served to each panelist, within 15 minutes after cooking. The order of evaluation of each sample was designed to avoid the first sample and carry-over effect (MacFie et al., 1989). The final attributes were decided after discussion in four training sessions. Finally, the intensity of 6 odors (beef, aging, dairy, liver, herbs and abnormal), 6 textures (hardness, initial and final juiciness, fibrosity, crumbliness and masticability) and 6 flavors (beef, aging, dairy, metallic,

liver and acid) attributes were evaluated in a 10-point scale from 0 (no perception) to 10 (extremely intense perceptions).

5.3.5. Statistical analysis

Results were expressed as abundance values, equivalent to peak areas. For volatile compounds, the carcass was included as a random effect. Least-squares means were generated for all analyses utilizing generalized linear mixed models (PROC GLIMMIX) of SAS software (SAS version 9.4, SAS Institute Inc., Cary, NC, US) and separated with the PDIFF function, with significance defined as $\alpha = 0.05$.

The GLM procedures of SAS were applied to the previously standardized and averaged score of each sensory attribute. The model included the finishing diet and the aging type as fixed effects and the session as a blocking variable. The interaction was not included because it is not significant. The Tukey test was applied to find significant differences between treatments at a level of 0.05.

Principal component analysis (PCA) was conducted on the correlation matrix using the SAS FACTOR procedure, including the VOC and sensory traits.

5.4. Results

5.4.1. Volatile compounds

Forty-six volatile organic compounds (VOC) in dry-bag and wet-aged beef from grass and grain-fed steers were identified after 40 days of aging (Table 1). They were assigned to the following chemical groups: aldehydes (n=15), alcohols (n = 10), carboxylic acid (n=8), ketones (n = 5), furans, esters, and benzene (n = 6), and terpene and S-containing compounds (n = 2). Each volatile compound resulted from either the Maillard reaction or lipid degradation.

Table 1. Identified volatile compounds in a dry bag or wet-aged loins during 40 days of aging from steers finishing in pasture or grain.

Class	Name	CAS	LRI	Ref RI	rt	Reference odor descriptor
Alcohol	Ethanol	64-17-5	506	506	7,893	sweet, alcoholic, medicinal
Alcohol	Isopropyl Alcohol	67-63-0	543	539	9,263	alcoholic, musty, woody
Alcohol	2-Butanol	78-92-2	647	648	13,631	fruity
Alcohol	1-Butanol, 3-methyl-	123-51-3	785	784	19,869	whiskey, fusel, alcoholic, fruity, banana
Alcohol	1-Pentanol	71-41-0	815	815	21,186	fermented, yeasty, balsamic, fusel, winey (fruity, alcoholic, green, woody)
Alcohol	1-Hexanol	111-27-3	915	916	25,302	green, floral
Alcohol	1-Hexanol, 2-ethyl-	104-76-7	1077	1077	31,203	citrus, floral, green, fresh
Alcohol	Phenol	108-95-2	1095	1112	31,854	sweet, tarry, chemical, phenolic
Alcohol	1-Octanol	111-87-5	1118	1118	32,588	waxy, green, citrus, floral, sweet, fatty, coconut
Alcohol	Tetradecanol*	112-72-1	1726		51,359	fruity, waxy, orris, coconut
Aldehyde	2-Propenal*	107-02-8	524		8,567	almond, cherry
Aldehyde	Propanal, 2-methyl-	78-84-2	595	592	11,243	banana, malty, chocolate-like, cocoa

Aldehyde	Butanal	123-72-8	630	622	12,807	pungent, cocoa, musty, green, malty, bready
Aldehyde	Butanal, 3-methyl-	590-86-3	692	692	15,718	malty, cheese, green, dark chocolate, cocoa
Aldehyde	Pentanal	110-62-3	735	733	17,647	pungent, almond, malty
Aldehyde	Hexanal	66-25-1	839	839	22,199	green, grassy, herbal, lemon, tallow
Aldehyde	Heptanal	111-71-7	943	943	26,362	fatty/oily, green, citrus, rancid
Aldehyde	Benzaldehyde	100-52-7	1032	1032	29,626	bitter almond, sweet cherry
Aldehyde	Octanal	124-13-0	1047	1047	30,166	waxy, citrus, orange peel, soapy, green, herbal, fresh, fatty
Aldehyde	Nonanal	124-19-6	1151	1150	33,662	green, citrus, fatty, floral
Aldehyde	Decanal	112-31-2	1254	1255	36,894	soapy, floral, waxy, citrus
Aldehyde	Undecanal	112-44-7	1358	1354	39,906	waxy, soap, floral, aldehydic, citrus, green, fatty
Aldehyde	Dodecanal*	112-54-9	1461		42,716	soapy, waxy, aldehydic, citrus, green, floral
Aldehyde	Tridecanal*	10486-19-8	1562		45,575	fresh, clean, aldehydic, soapy, citrus, petal, waxy, grapefruit peel
Aldehyde	2-Decenal, E-*	3913-81-3	1330		39,13	waxy, fatty, earthy, green
Benzene	Toluene	108-88-3	794	794	20,306	nutty, bitter, almond, plastic
Benzene	1,3-Di-tert-butylbenzene*	1014-60-4	1288		37,913	
Carboxylic acid	Acetic acid	64-19-7	687	690	15,474	pungent, vinegar, sour
Carboxylic acid	Butanoic acid	107-92-6	861	864	23,111	rancid, cheesy, strong, sweaty
Carboxylic acid	Pentanoic acid*	109-52-4	955		26,814	putrid, acidic, sweaty, rancid, sharp, cheese-like, sour milky, tobacco, fruity

Carboxylic acid	Hexanoic acid	142-62-1	1050	1052	30,255	acidic, sweaty, cheesy, sharp, goaty
Carboxylic acid	Octanoic acid	124-07-2	1243	1244	36,54	goaty, waxy, soapy, cheesy, rancid, pungent, sweat
Carboxylic acid	Nonanoic acid	112-05-0	1341	1347	39,416	waxy, dirty, cheese, cultured, dairy
Carboxylic acid	Decanoic acid	334-48-5	1439	1440	42,142	rancid, sour, fatty, citrus
Carboxylic acid	Dodecanoic acid*	143-07-7	1636		47,943	mild, fatty, coconut, bay oil
Ester	Methyl butanoate	623-42-7	749	754	18,276	fruity
Esters	Methyl hexanoate	106-70-7	951	949	26,65	ethereal, fruity, pineapple, apricot, strawberry, tropical, fruit, banana, bacon
Furan	Furan*	110-00-9	519		8,37	ethereal
Furan	Furan, 2-pentyl-	3777-69-3	1013	1012	28,961	green bean, vegetable, earthy, metallic
Ketone	Acetone	67-64-1	534	533	8,938	solvent, ethereal, sour milk, apple
Ketone	2,3-Butanedione	431-03-8	632	631	12,9	buttery, strong
Ketone	2-Butanone	78-93-3	639	639	13,233	buttery, sour milk, etheric
Ketone	Acetoin	513-86-0	777	784	19,544	buttery, creamy, dairy, milky, fatty
Ketone	2-Heptanone	110-43-0	935	936	26,039	fruity, spicy, sweet, herbal, woody (cheesy, blue cheese, roquefort)
Sulfur	Carbon disulfide	75-15-0	549	546	9,499	cabbage, sulphur, fruity, burnt
Terpene	a-Pinene	80-56-8	956	950	26,847	pine, camphoreous, earthy, woody

CAS: Chemical CAS (Chemical Abstract Service) (Blanks relate to isomers where we could not be 100% sure of identification and therefore could not provide full identification. **LRI**: Linear retention indices as determined by using the method of Van Den Dool & Kratz (1963). **Ref LRI**: These values were obtained from published papers or NIST 2014; NA: No published reference available to date (not many published as yet on a DB624 column); *tentative identification, might be an isomer of this chemical compound.

5.4.1.1. Alcohols

Alcohol contents did not present significant interaction between AM * F (P>0.05), except for 1-hexanol, 1-hexanol 2-ethyl and 1-pentanal (Table 2). The highest 1-hexanol and 1-hexanol 2-ethyl concentrations were in DAb samples from pasture and grain-fed steers, while, for 1-pentanol, the highest concentrations were in DAb from pasture-fed beef. 1-Octanol, phenol, and tetradecanol contents increased in DAb beef, and isopropyl alcohol increased in wet-aged beef (P<0.05). Ethanol and 2-butanol concentrations increased, and phenol decreased in beef finished on a grain diet compared to pasture-fed.

5.4.1.2. Aldehydes, Carboxylic Acids, and Ketones

Aldehydes, carboxylic acids, and ketone groups did not present any significant interaction AM * F (P>0.05) (Table 3). All volatile compound concentrations from these groups increased in beef aged in dry bags compared to the wet-aged meat. Some volatile compounds from the aldehydes group (2-propenal, 2-decanal E, decanal, dodecanal, heptanal, pentanal, tri-decanal, and undecanal) and carboxylic acids (decanoic acid, dodecanoic acid, hexanoic acid, octanoic acid, and pentanoic acid) increased in pasture-fed compared to grain-fed beef (P<0.05). No differences in ketones were observed between pasture-fed and grain-fed aged meat.

5.4.1.3. Benzenes, Esters, Furans and Others

Toluene, furan, and carbon disulfide presented significant interaction AM * F (P<0.05) (Table 4). Furan (P=0.016) and carbon disulfide (P=0.005) presented the highest and the lowest concentrations in DAb from pasture-fed steer meat, respectively. However, toluene presented the lowest concentration in wet aging from grain-fed steer beef (P=0.023). 1,3, Di,tert, butylbenzene, furan 2- pentyl and a-pinene concentrations increased in dry bag aging (P<0.05), and methyl hexanoate increased in WA from pasture-fed steers (P<0.05). Most dry bag and wet aging samples were discriminated against when a PCA was performed based only on the volatile compounds, and a second biplot-PCA discriminated the volatile compounds that significantly contributed to this difference (Figures S1 and S2, supplementary data).

Table 2. Effects (mean ± SEM) of aging method (AM) and finishing diet (F) on alcohol compounds (UA 10^{-5}/g of meat wet basis).

Compound	Aging		Finishing		Significance		
	DAb	WA	Pasture	Grain	AM	F	AM* F
			Alcohols				
1-Butanol 3 methyl	1.46±0.27	No-est	No-est	0.41±0.11	<.001	0.585	No-est
1-Hexanol	0.75±0.71	0.25±0.02	0.43±0.04	0.44±0.04	<.001	0.863	0.040
1-Hexanol 2 ethyl	1.71±0.13	1.22±0.13	1.53±0.13	1.4±0.13	0.010	0.453	<.001
1-Octanol	1.90±0.11	0.34±0.03	0.69±0.06	0.58±0.05	<.001	0.196	0.608
1-Pentanol	0.91±0.11	0.35±0.04	0.72±0.08	0.43±0.05	<.001	0.004	0.040
2_Butanol	2.38±0.41	-	1.57±0.37	3.60±0.91	-	0.035	-
Ethanol	5.17±1.01	3.60±0.71	2.83±0.56	6.55±1.28	0.203	0.004	0.593
Isopropyl Alcohol	1.36±0.22	2.54±0.42	1.81±0.31	1.92±0.31	0.011	0.803	0.310
Phenol	0.95±0.07	0.55±0.04	0.83±0.06	0.63±0.05	<.001	0.019	0.421
Tetradecanol	1.62±0.20	0.42±0.05	0.80±0.05	0.86±0.10	<.001	0.670	0.783

DAb: Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. Different letters in the same row denote statistically different groups (*P<0.05*) among LSMeans.

Table 3. Effects (mean ± SEM) of aging method (AM) and finishing diet (F) on aldehydes, carboxylic acids, and ketones compounds (UA 10^{-5}/g of meat wet basis).

	Aging		Finishing		Significance		
Compound	**DAb**	**WA**	**Pasture**	**Grain**	**AM**	**F**	**AM*F**
			Aldehydes				
2_Propenal	1.92±0.21	1.19±0.13	1.91±0.21	1.20±0.13	0.004	0.005	0.499
2 Decenal E	0.79±0.09	0.32±0.04	0.61±0.07	0.42±0.05	<.001	0.020	0.954
Benzaldehyde	3.94±0.32	2.02±0.16	2.95±0.24	2.70±0.22	<.001	0.441	0.784
Butanal	1.28±0.09	0.87±0.09	1.16±0.09	0.99±0.09	0.002	0.178	0.903
Butanal 3 methyl	0.85±0.09	0.33±0.03	0.54±0.06	0.52±0.05	<.001	0.804	0.505
Decanal	2.99±0.29	0.95±0.09	2.01±0.19	1.42±0.14	<.001	0.013	0.911
Dodecanal	0.77±0.06	0.40±0.03	0.64±0.05	0.49±0.04	<.001	0.037	0.758
Heptanal	4.78±0.42	1.88±0.17	3.44±0.31	2.62±0.23	<.001	0.034	0.877
Hexanal	4.18±0.34	2.12±0.17	3.13±0.25	2.84±0.23	<.001	0.396	0.289
Nonanal	22.70±1.71	7.06±0.53	13.80±1.04	11.61±0.87	<.001	0.114	0.513

Octanal	4.54±0.36	1.58±0.12	3.00±0.24	2.39±0.19	<.001	0.051	0.691
Pentanal	2.94±0.26	1.27±0.11	2.36±0.21	1.59±0.14	<.001	0.003	0.875
Propanal 2 methyl	0.36±0.04	0.19±0.02	0.26±0.03	0.27±0.03	<.001	0.720	0.374
Tridecanal	0.61±0.06	0.38±0.04	0.56±0.05	0.42±0.04	<.001	0.039	0.287
Undecanal	0.84±0.07	0.40±0.04	0.68±0.06	0.49±0.04	<.001	0.015	0.831
Carboxylic Acids							
Acetic acid	5.40±0.05	3.03±0.27	4.37±0.38	3.74±0.33	<.001	0.218	0.820
Butanoic acid	6.97±1.11	0.31±0.50	5.88±0.94	3.74±0.59	0.001	0.051	0.393
Decanoic acid	3.73±0.69	0.87±0.16	2.52±0.46	1.29±0.24	<.001	0.013	0.507
Dodecanoic acid	2.20±0.31	0.59±0.08	1.40±0.19	0.93±0.13	<.001	0.042	0.874
Hexanoic acid	6.10±0.93	2.28±0.36	5.14±0.80	2.71±0.42	<.001	0.006	0.766
Nonanoic acid	5.74±0.60	1.39±0.16	3.21±0.36	2.48±0.29	<.001	0.120	0.859
Octanoic acid	4.50±0.85	1.10±0.21	3.29±0.63	1.51±0.28	<.001	0.005	0.329
Pentanoic acid	1.11±0.16	0.42±0.09	0.92±0.15	0.51±0.10	<.001	0.029	0.578

			Ketones				
2, 3, Butanedione	7.00±1.40	1.12±0.37	2.89±0.72	2.72±0.80	<.001	0.876	0.970
2, Butanone	69.29±5.10	1.57±012	10.54±0.78	10.33±0.76	<.001	0.844	0.053
2, Heptanone	0.67±0.08	0.28±0.04	0.47±0.06	0.40±0.05	<.001	0.380	0.714
Acetoin	11.37±1.96	3.20±0.58	6.27±1.11	5.81±1.03	<.001	0.765	0.550
Acetone	74.10±0.44	5.28±0.31	6.62±0.39	59.11±0.35	<.001	0.180	0.060

DAb: Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. Different letters in the same row denote statistically different groups ($P<0.05$) among LSMeans.

Table 4. Effects (mean ± SEM) of aging method (AM) and finishing diet (F) on benzenes, esters, furans and other compounds (UA 10^{-5}/g of meat wet basis).

	Aging		Finishing		Significance		
Compound	**DAb**	**WA**	**Pasture**	**Grain**	**AM**	**F**	**AM*F**
			Benzenes				
1,3, Di,tert,butylbenzene	1.60±0.21	0.92±0.13	1.18±0.16	1.24±0.17	0.006	0.794	0.308
Toluene	0.71±0.08	0.32±0.04	0.78±0.08	0.29±0.04	<.001	<.001	0.023
			Esters				
Methyl_butanoate	0.61±0.07	0.64±0.06	0.56±0.06	0.70±0.06	0.770	0.143	0.642
Methyl hexanoate	0.83±0.08	0.94±0.08	0.60±0.08	1.17±0.08	0.393	<.001	0.691
			Furans				
Furan	0.13±0.02	0.10±0.01	0.14±0.02	0.09±0.01	0.104	0.050	0.016
Furan 2, pentyl	1.64±0.22	0.62±0.08	1.12±0.15	0.90±0.12	<.001	0.250	0.855
			Others				
Carbon disulfide	3.75±0.67	4.58±1.03	4.99±1.11	3.51±0.63	0.496	0.257	0.005
-Pinene	0.20±0.02	0.15±0.01	0.18±0.02	0.17±0.02	0.014	0.660	0.602

DAb: Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s. Different letters in the same row denote groups statistically different (*P<0.05*) among LSMeans.

5.4.2. Trained sensory panel

Sensory-trained attributes are presented in Table 5. Regarding the finishing diet, the pasture increased the herbal odor ($P = 0.015$), abnormal odor ($P = 0.021$), and milky flavor ($P = 0.013$), whereas it decreased the metallic flavor ($P = 0.002$). Concerning the aging methods, DAb increased the beef and age-intensity odor and decreased liver odor. The AM affected all the texture attributes, hardness, fibrousity, masticability, and residues increased in DAb ($P<0.05$), whereas initial juiciness, crumbliness, and final juiciness increased in WA ($P<0.05$). The intensity of the age and bitterness flavors increased, and those of metallic, liver, and astringent flavors decreased in DAb ($P<0.05$).

5.4.3. Trained sensory panel and volatile compound relationships

The PCA analysis illustrates the relationship between the VOC and the sensory panel attributes (Figure 1). The first factor (F1) explained 35.55% of the accounted variance, enabling the segmentation of the samples based on their AM, with DAb samples positioned on the right side and WA samples on the left side. A greater contribution of VOC is seen in the positive section of axis 1 (F1), indicating that the highest concentration of VOC is linked to the DAb treatments, which aligns with the results presented in Table 2. Only methyl-hexanoate, methyl-butanoate, and isopropyl alcohol were found to be lower in DAb samples. Furthermore, the second axis (F2, 11.17% of the accounted variance) does not provide a clear separation of the VOCs based on the animals' finishing diet, meaning that VOCs cannot be distinguished between those fed on pasture versus grain.

Regarding the sensory variables, F1 mainly separates aged odor and flavor (right part of the axis), liver odor and metallic flavor (left part). However, the second axis (F2) was related in the positive part with the texture attributes hardness, masticability, fibrosity, and residue, and bitter flavor, and in the negative part, with the texture attributes initial and final juiciness and crumbliness, and with astringent and intensity beef flavor and herbal odor attributes.

Table 5. Effects (mean ± SEM) of aging method (AM) and finishing diet (F) on odor, flavor and texture profile.

Descriptors	Aging		Finishing		Significance		
	DAb	WA	Pasture	Grain	AM	F	PM*F
			Odor				
Beef intensity	5.9±0.1	5.6±0.1	5.7±0.1	5.8±0.1	0.022	0.850	0.858
Aged intensity	1.5±0.1	0.7±0.1	1.3±0.1	1.0±0.1	<.001	0.081	0.484
Milky	1.4±0.1	1.5±0.1	1.4±0.1	1.5±0.1	0.318	0.459	0.782
Liver	0.6±0.07	0.9±0.07	0.7±0.07	0.8±0.07	0.001	0.162	0.840
Herbal	0.9±0.1	1.1±0.1	1.2±0.1	0.8±0.1	0.210	0.015	0.231
Brou	0.5±0.07	0.7±0.07	0.6±0.07	0.6±0.07	0.136	0.711	0.394
Abnormal	0.9±0.1	0.9±0.1	1.2±0.1	0.6±0.1	0.858	0.021	0.785
			Texture				
Hardness	4.6±0.1	4.0±0.1	4.5±0.2	4.1±0.2	0.003	0.242	0.326
Juiciness initial	3.4±0.1	3.9±0.1	3.7±0.1	3.6±0.1	<.001	0.578	0.519
Fibrous	3.8±0.1	3.4±0.1	3.7±0.2	3.6±0.2	0.014	0.587	0.639

Crumbling	3.0±0.1	3.3±0.1	3.1±0.1	3.2±0.1	0.023	0.771	0.897
Juiciness final	3.7±0.09	4.1±0.09	4.0±0.1	3.9±0.1	<.001	0.668	0.716
Masticability	4.6±0.1	4.2±0.1	4.5±0.2	4.3±0.2	<.001	0.468	0.266
Residue	2.8±0.1	2.4±0.1	2.7±0.1	2.6±0.1	0.004	0.614	0.149
		Flavor					
Beef intensity	5.2±0.07	5.3±0.07	5.2±0.08	5.2±0.08	0.130	0.807	0.858
Aged intensity	1.3±0.1	0.8±0.1	1.2±0.1	0.9±0.1	0.013	0.112	0.574
Milky	1.1±0.07	1.3±0.07	1.3±0.07	1.0±0.07	0.149	0.013	0.974
Metallic	1.4±0.07	1.8±0.01	1.4±0.08	1.8±0.08	0.002	0.005	0.392
Liver	1.4±0.1	1.7±0.1	1.5±0.2	1.5±0.2	0.020	0.968	0.952
Acid	1.6±0.08	1.5±0.08	1.6±0.09	1.6±0.09	0.614	0.811	0.739
Astringent	1.5±0.07	1.7±0.07	1.6±0.09	1.5±0.09	0.012	0.268	0.892
Bitter	1.3±0.08	1.0±0.08	1.01±0.1	1.2±0.1	0.007	0.647	0.949

DAb: Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

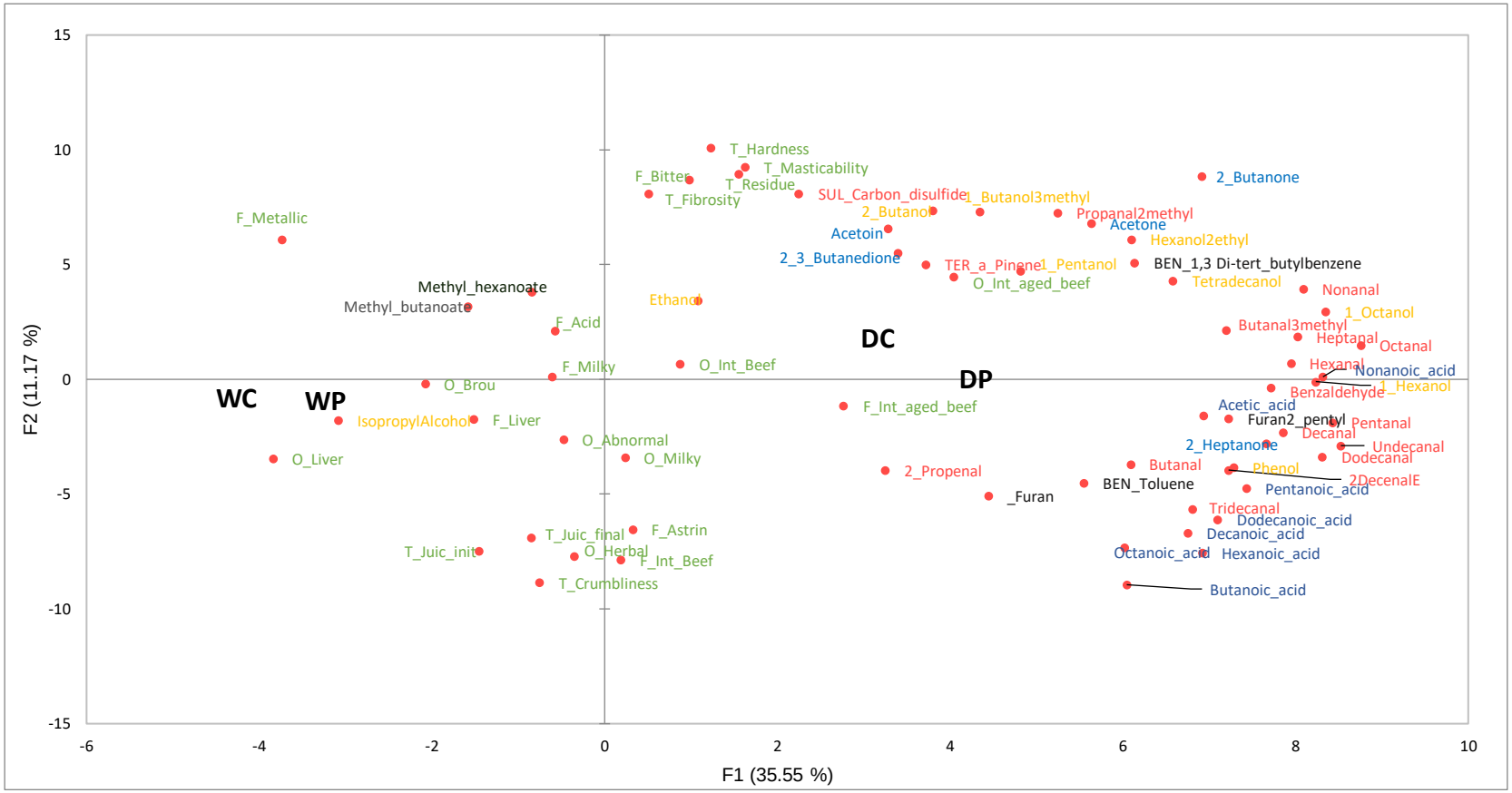


Figure 1. Principal component analysis (PCA) of DAb-grain (DC), DAb-pasture (DP), WA-grain (WC), and WA-pasture (WP) meat samples based on volatile compounds (different colors) and sensory attributes (green color). DAb: Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

5.5. Discussion

Many studies have examined the effect of diets on volatile flavor compounds in cooked meat (Evers et al., 2020; Elmore et al., 1999; Larick et al., 1987). The most significant difference in meat flavors from cattle fed grass versus grain- based diets is attributed to the concentration and type of fatty acids, which serve as the primary carbonyl source (Melton, 1983). The fatty acid profile in this study showed differences (data not shown) based on the finishing diet. However, no significant differences were noted in intramuscular fat (IMF) percentages between pasture- and grain- fed steers, which were 3. 3.7% and 4. 4.3%, respectively (Correa et al., 2024). The key difference between the diets was the level of PUFA n- 3, with linolenic acid (C 18: 3 n- 3) exhibiting four times more muscle content in pasture- fed than in grain- fed steers (Correa et al., 2024). Of the 46 volatile compounds identified, 16 were influenced by the diet. We attribute the low number of volatile compounds responding to the finishing diet to the lack of difference in intramuscular fat content between pasture-fed and grain- fed steer samples. However, among the 16 VOCs affected by the animal diet, 15 increased in pasture- fed steers, while only ethanol decreased in pasture- fed compared to grain- fed steers. Regarding ethanol, Evers et al. (2020) reported no differences in ethanol content between grass- and grain- fed beef. Most of the remaining 15 VOCs impacted by the diet were aldehydes (9). Under the similar cooking conditions utilized in this study, Elmore et al. (1999) demonstrated that fatty acids and aldehydes were released from the phospholipids of various types of meat, comprising the most significant class of volatiles in cooked beef. The same authors indicated that the concentrations of saturated and monounsaturated straight- chain aliphatic aldehydes, derived from lipids, greatly increased in beef with higher PUFA content. We attribute the higher concentration of aldehydes to the increased muscle content of linolenic acid (C 18: 3 n- 3) in pasture- fed steers. Aldehydes are likely the most intriguing lipid- derived volatiles, as they possess low odor threshold values and may enhance the flavor of the cooked beef samples. Consistent with our findings, Suzuki and Bailey (1985) noted that higher concentrations of pentanoic, nonanoic, decanoic, and dodecanoic acids were produced in the meat fat from grass- fed animals.

The aging conditions (e.g., oxygen availability, temperature, humidity, and aging time) under which beef is aged influence the meat's ultimate flavors (Watanabe et al., 2015). Particularly, aging in an oxygen environment causes a burnt, toasted off-odor. Additionally, dry aging enhances beef flavor attributes more than aging in a vacuum or carbon dioxide (Campbell et al., 2001; Jeremiah & Gibson, 2003).

In our study, the most abundant alcohol compound was ethanol; although no significant differences were found between DAb and WA, the highest value was recorded in DAb. Li et al. (2021) reported significantly higher concentrations of alcohols, particularly ethanol, in wet-aged beef compared to traditional dry-aged counterparts at 35 and 56 days of age. Conversely, Barragán-Hernández et al. (2022) noted that using dry-aging bags for 28 days affected only a few individual volatiles. at the same time, alcohols and ketones were more concentrated in dry-aging bags than in wet-aged meat. Furthermore, Barragán-Hernández et al. (2022) explained that alcohols and ketones are produced from the oxidation of fatty acid lipids. Among alcohols, 1-octanol, 1-pentanol, and 1-octen-3-ol are known to be formed from C18:1n-9 and C18:2n-6 fatty acids, respectively (Van Ba et al., 2013). In this study, the C18:1n-9 and C18:2n-6 content samples did not show significant differences between DAb and WA (Correa et al., 2024). Mikami et al. (2021) indicated that linoleic acid (C18:2n-6) serves as a precursor of volatiles produced by fungi during the dry-aging process.

Aldehydes (heptanal, hexanal, nonanal, octanal), carboxylic acids (acetic acid, butanoic acid, decanoic acid, hexanoic acid, nonanoic acid, octanoic acid), and ketones (2, 2,3- butanedione, 2- butanone, acetoin, and acetone) exhibited the highest abundances and increased in DAb compared to WA. Aldehydes are the primary secondary lipid oxidation products and contributors to dry bag aging (Ruiz, Muriel, & Ventanas, 2002). In meat, aldehydes are mainly formed during lipid oxidation and the Strecker degradation of amino acids, including butanal, 3- methyl, and benzaldehyde (Legako et al., 2016). According to the present research, Barragán- Hernández et al. (2022) reported that aldehydes had higher concentrations in dry bag aging compared to wet aging. Hexanal is known to form from the oxidation/degradation of C 18: 2 n-6, while heptanal, octanal, and nonanal are derived from C 18: 1 n- 9 (Elmore et al., 2002; Van Ba et al., 2013). Previous studies found greater concentrations of aldehydes

in traditionally dry- aged beef than in wet- aged beef (Ha et al., 2019; Lee et al., 2021), while Setyabrata et al. (2021) did not report any differences in aldehyde concentrations between traditional and in- the- bag dry- aged and wet- aged beef. The differences in results across studies may be attributed to the aging duration: according to Ha et al. (2019), most volatile compounds increased in concentration with longer aging time and different aging methods. Most of the studies lasted 28 days, whereas our studies and Ha et al. (2019) utilized an aging time of 40 days. Li et al. (2021) reported that the concentrations of heptanal, octanal, and nonanal increased with aging time during dry aging (35 vs 56 days). Ma et al. (2012) noted that an increase in low molecular weight aldehydes did not occur until three weeks of storage, and lipid oxidation would be relatively suppressed for up to three weeks under vacuum conditions. In this study, compared to wet aging, the increase in these volatile compounds in dry bags corresponds with the lipid oxidation results (TBARS) observed in the same samples, as noted by Correa et al. (2024). Hexanal, heptanal, octanal, and nonanal are commonly reported lipid- derived volatiles in cooked beef, and their odors are described as “green, " “fatty,” and “sweet,” serving as indicators of lipid oxidation in meat (Frank et al., 2016).

Concerning sensory profile, DAb increased beef and aged identity odor, and aged identity flavor, whereas it decreased the intensity of liver odor and metallic flavor. Also, Li et al. (2014) and Foraker et al. (2020) found that in-the-bag or traditionally dry-aged LTL was less liver-like and metallic than wet-aged samples after different aging periods (8-63 d).

5.6. Conclusions

The dry-aging bag method yielded higher concentrations of volatile organic compounds, including aldehydes, carboxylic acids, and ketones, compared to wet aging. These compounds are associated with desirable flavor attributes such as aged identity and beef intensity.

Pasture-fed beef exhibited higher concentrations of lipid-derived volatile organic compounds, particularly aldehydes, than grain-fed beef, which showed lower

concentrations but slightly higher intramuscular fat content, which might balance sensory attributes.

The aging methods displayed significant differences in texture: dry bag-aged beef exhibited greater hardness and fibrousness, while WA beef maintained higher juiciness. Dry aging bags enhanced the intensity of beef and aged odors and flavors while diminishing liver and metallic tastes. These characteristics align with consumer preferences for the flavors of aged beef.

Principal component analysis distinguished dry bag and wet-aged samples-based on their volatile profiles and sensory attributes. Volatile compounds, especially aldehydes and-alcohols, were closely linked to the sensory perception of aged beef.

The study supports using dry-aging bags as a practical alternative, enhancing the flavor and sensory qualities of beef while providing flexibility in export markets. For markets that value intense, aged beef flavors, dry aging with DAb is preferable. However, for consumers who prioritize tenderness and juiciness, wet aging may be more suitable.

5.7. References

Barragán-Hernández, W., Leighton, P.L.A., López-Campos, O., Segura, J., Aalhus, J.L. and Prieto, N. 2022. Effect of in-the-bag dry-ageing on meat palatability and volatile compounds of cull cows and youthful steers. Meat Scie. 188:108800. https://doi.org/10.1016/j.meatsci.2022.108800

Berger, J., Kim, Y. H. B., Legako, J. F., Martini, S., Lee, J., Ebner, P. E., and Zuelly, S. M. S. 2018. Dry aging improves meat quality attributes of pasture-fed beef loins. Meat Sci. 145: 285-291. https://doi.org/10.1016/j.meatsci.2018.07.004

Campbell, R. E., Hunt, M. C., Levis, P., and Chambers, E. I. V. 2001. Dry-aging effects on palatability of beef longissimus muscle. J. of Food Scie. 66:196-199. https://doi.org/10.1111/j.1365-2621.2001.tb11315.x

Correa, D., del Campo, M., Luzardo, S., De Souza, G., Álvarez, C., Font-i-Furnols, M., and Brito, G. 2024. Impact of aging methods and frozen storage on meat quality attributes of beef from different finishing diets. Meat Muscle Biol. 8:17695, 1–16. https://doi.org/10.22175/mmb.17695

Dashdorj, D. Tripathi, V. K., Cho, S., Kim, Y. and Hwang I. 2016. Dry aging of beef; Review. J. Anim. Sci. and Techn. 58: 20. doi 10.1186/s40781-016-0101-9

Elmore, J. S., Campo, M. M., Enser, M., and Mottram, D. S. 2002. Effect of lipid composition on meat-like model systems containing cysteine, ribose, and polyunsaturated fatty acids. J. Agric. Food Chem., 50(5):1126-1132. https://doi.org/10.1021/jf0108718

Elmore, J. S., Mottram, D. S., Enser, M. and Wood, J. D. 1999. Effect of the Polyunsaturated Fatty Acid Composition of Beef Muscle on the Profile of Aroma Volatiles. J. Agric. Food Chem. 47: 1619-1625

Evers, D. L., Garmyn, A. J., Legako, J. F., Woerner, D. R. and Miller, M. F. 2020. Flavor Characterization of Grass- and Grain-Fed Australian Beef Longissimus Lumborum Wet-Aged 45 to 135 Days. Meat Muscle Biol. 4(1): 22, 1-14. doi:10.22175/mmb.11043

Frank, D., Joo, S. T., and Warner, R. 2016. Consumer acceptability of intramuscular fat. Korean J. Food Scie. of An. Resour. 36(6):699-708. https://doi.org/10.5851/kosfa.2016.36.6.699

Ha, Y. Hwang, I., Ba, H. V., Ryu, S., Kim, Y., Kang, S. M., Kim, J., Kim,Y., and Cho, S. 2019. Effects of Dry- and Wet-ageing on Flavor Compounds and Eating Quality of Low Fat Hanwoo Beef Muscles. Food Sci. Anim. Resour. 39(4):655-667. DOI https://doi.org/10.5851/kosfa.2019.e58

INAC. Resolución 65/997 Del Instituto Nacional de Carnes Del 14 de Abril de 1997. 1997. Available online: https://www.Inac. Uy/Innovaportal/File/2024/1/-Res-65-997.Pdf (accessed on 01 October 2024)

Jeremiah, L. E., and Gibson, L. L. 2003. The effects of postmortem product handling and aging time on beef palatability. Food Research Inter. 36:929-941.

Jin, S.K. and Yim, D.G. 2020. Comparison of effects of two aging methods on the physicochemical traits of pork loin. Food Sci. Anim. Resour. 40(5):844-851.doi: 10.5851/kosfa.2020.e22

Karabagias IK. 2018. Volatile profile of raw lamb meat stored at 4 ±1 ◦C: The potential of specific aldehyde ratios as indicators of lamb meat quality. Foods. 7(3):40.

Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., and Hunt, M.C. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies (Review). Meat Sci. 144:74-90. https://doi.org/10.1016/j.meatsci.2018.04.031

Kim, J.H.; Kim, D.H.; Ji, D.S.; Lee, H.J.; Yoon, D.K., and Lee, C.H. 2017. Effect of aging process and time on physicochemical and sensory evaluation of raw beef top round and shank muscles using an electronic tongue. Korean J. Food Sci. Anim. Resour. 37:823-832.

Lee, H.J.; Yoon, J.W.; Kim, M.; Oh, H.; Yoon, Y., and Jo, C. 2019. Changes in microbial composition on the crust by different airflow velocities and their effect on sensory properties of dry-aged beef. Meat Sci. 153:152-158. https://doi.org/10.1016/j.meatsci.2019.03.019

Lee, D.; Lee, H.J.; Yoon, J.W.; Kim, M., and Jo, C. 2021. Effect of Different Aging Methods on the Formation of Aroma Volatiles in Beef Strip Loins. Foods, 10: 146. https://doi.org/10.3390/foods10010146

Li, X., Babol, J., Bredie, W. L. P., Nielsen, B., Tománková, J. and Lundstrom. 2014. A comparative study of beef quality after ageing *longissimus* muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. Meat Sci. 97: 433-442. https://doi.org/10.1016/j.meatsci.2014.03.014

Li, Z. Ha, M., Frank, D., MacGilchrist, P. And Warner, R.D. 2021. Volatile Profile of Dry and Wet Aged Beef Loin and Its Relationship with Consumer Flavour Liking. Foods. 10:3113. https://doi.org/10.3390/foods10123113

Ma, Q. L., Hamid, N., Bekhit, A. E. D., Robertson, J., and Law, T. F. 2012. Evaluation of pre-rigor injection of beef with proteases on cooked meat volatile profile after 1 day and 21 days post-mortem storage. Meat Sci. 92(4): 430-439.https://doi.org/10.1016/j.meatsci.2012.05.006

MacFie, H. J., Bratchell, N., Greenhoff, K., and Vallis, L. V. 1989. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. J. Sens. Stud. 4:129-148. https://doi.org/10.1111/j.1745-459X.1989.tb00463.x

Maughan C, Tansawat R, Cornforth D, Ward R, and Martini S. 2012. Development of a beef flavor lexicon and its application to compare the flavor profile and consumer acceptance of rib steaks from grass- or grain-fed cattle. Meat Sci. 90(1):116-21. https://doi.org/10.1016/j.meatsci.2011.06.006

Merkle S, Kleeberg K, and Fritsche J. 2015. Recent developments and applications of solid phase microextraction (SPME) in food and environmental analysis—a review. Chromatography. 2(3):293-381. https://doi.org/10.3390/chromatography2030293

Melton, S. L. 1983. Effect of forage feeding on beef flavor. Food Techn. 37(5): 239-248.

Larick, D. K., Hedrick, H. B., Bailey, M. E., Williams, J. E., Hancock, D. L., and Garner, G. B. 1987. Flavor constituents of beef as influenced by forage- and grain-feeding. J. of Food Scie. 52(2):245-251. https://doi.org/10.1111/j.1365-2621.1987.tb06585.x

Legako, J.F.; Dinh, T.T.N.; Miller, M.F.; Adhikari, K., and Brooks, J.C. 2016. Consumer palatability scores, sensory descriptive attributes, and volatile compounds of grilled beef steaks from three USDA Quality Grades. Meat Sci. 112:77-85. https://doi.org/10.1016/j.meatsci.2015.10.018

Scollan, N.D., Choi, N.J., Kurt, E., Fisher, A.V., Enser, M., and Wood, J.D., 2001. Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. Br. J. Nutr. 85:115-124.

Setyabrata, D., Xue, S., Vierck, K., Legako, J., Ebner, P., Zuelly, S. and Kim, Y. H. B. 2021. Impact of Various Dry-Aging Methods on Meat Quality and Palatability Attributes of Beef Loins (M. longissimus lumborum) from Cull Cow. Meat Muscle Biol. 6(1):13025, 1-15. doi:10.22175/mmb.13025

Suzuki, J., and Bailey, M. E. 1985. Direct sampling capillary GLC analysis of flavor volatiles from ovine fat. J. Agri. Food Chem. 33(3):343-347. https://doi.org/10.1021/jf00063a006

Wehrens, R., Weingart, G., and Mattivi, F. 2014. metaMS: An open-source pipeline for GC-MS-based untargeted metabolomics. J. Chromatogr. B Analyst. Technol, Biomed, Life Sci. 966:109-116. https://doi.org/10.1016/j.jchromb.2014.02.051

5.8. Supplementary data

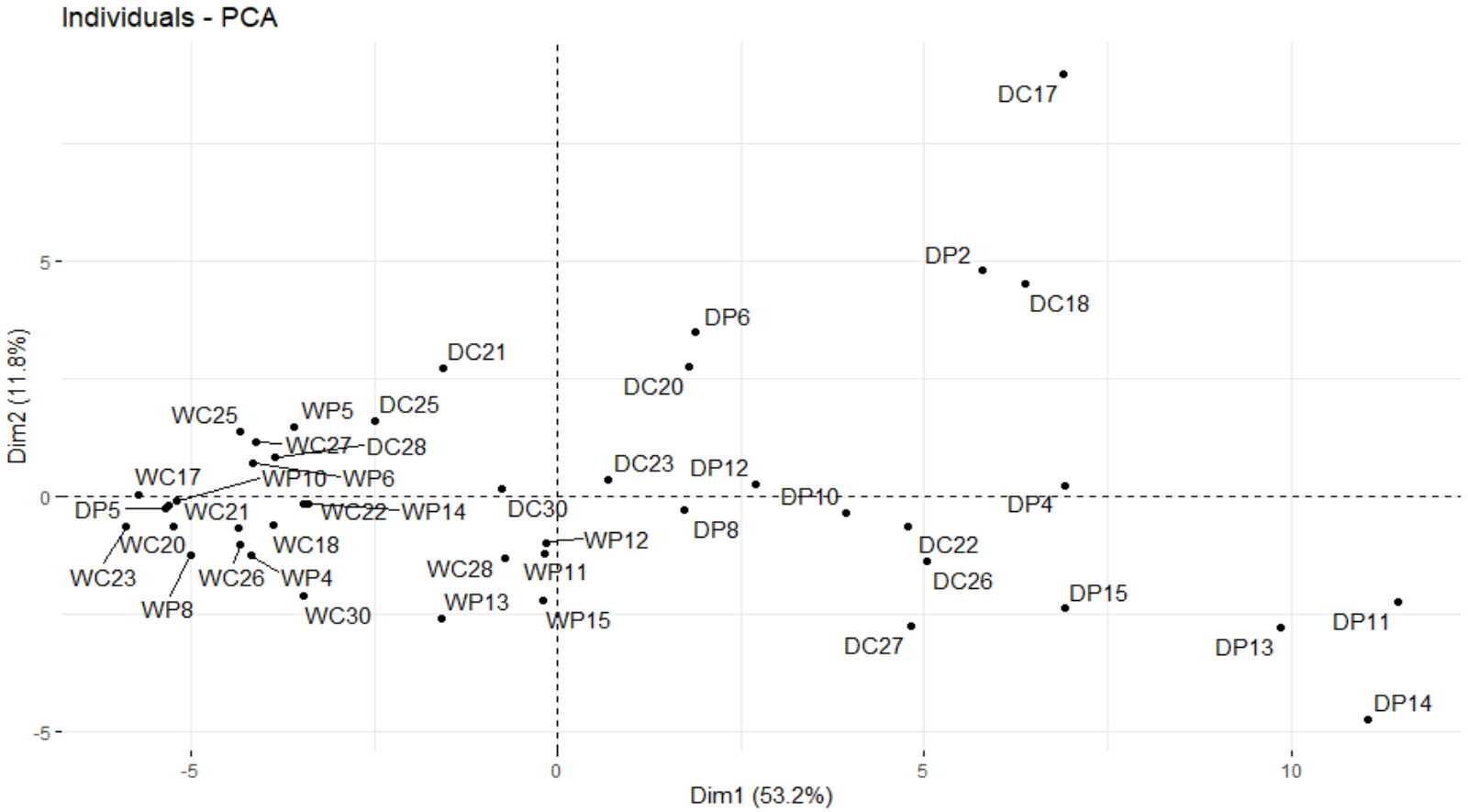


Figure S1. Principal component analysis (PCA) of DAb-grain (DC), DAb-pasture (DP), WA-grain (WC), and WA-pasture (WP) meat samples based on volatile compounds. DAb: Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

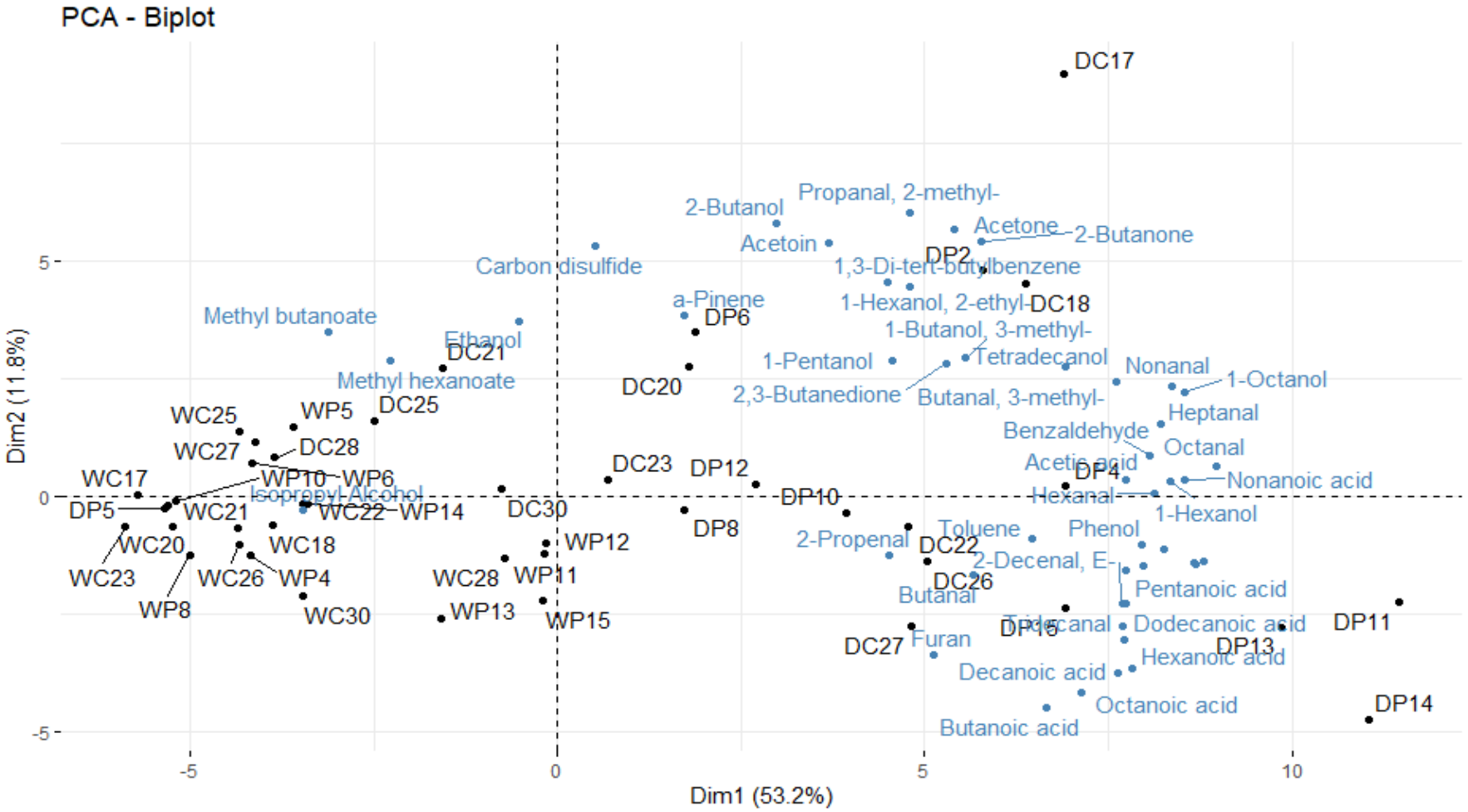


Figure S2. Principal component analysis (PCA) of DAb-grain (DC), DAb-pasture (DP), WA-grain (WC) and WA-pasture (WP) meat samples based on volatile compounds

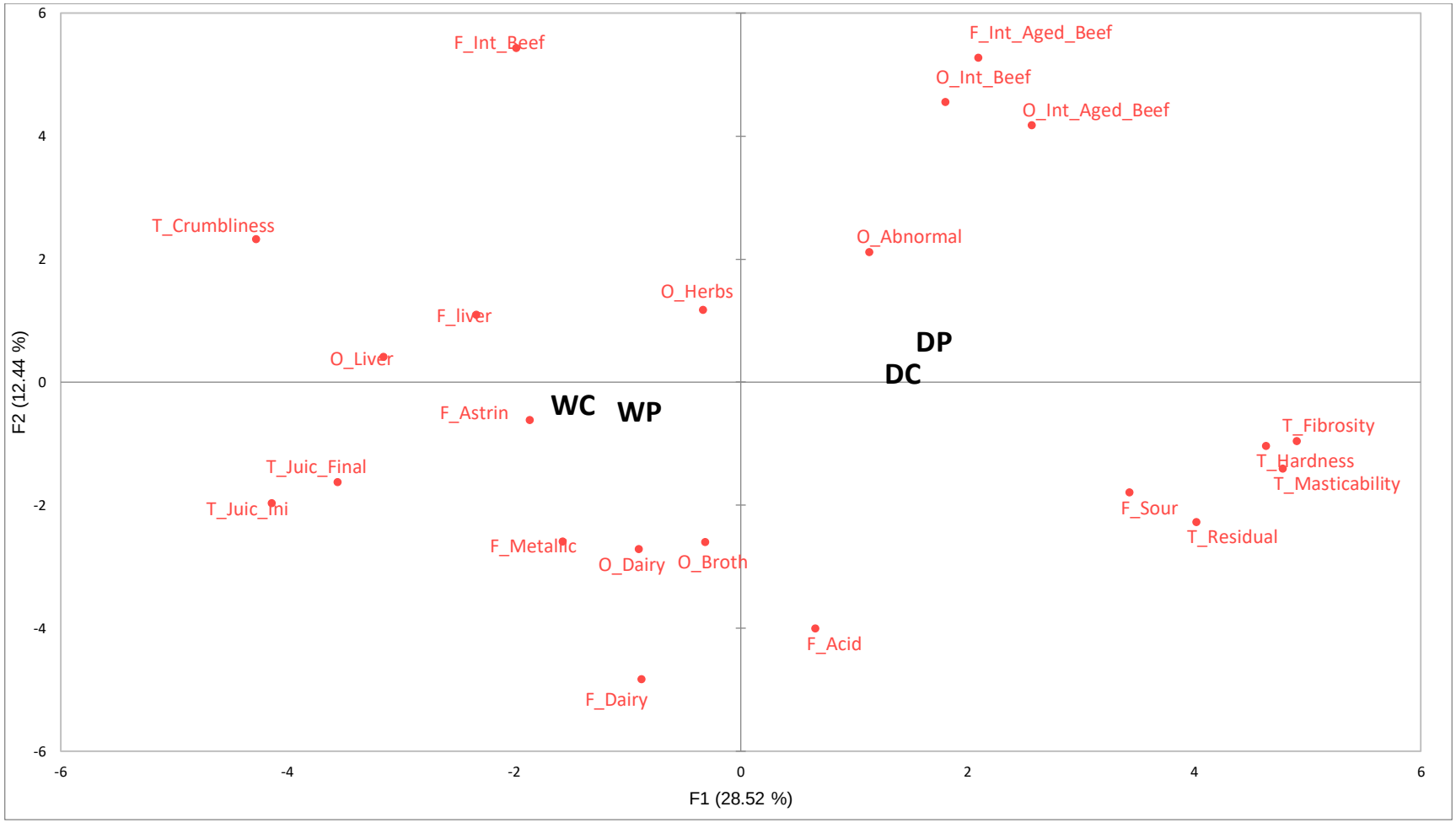


Figure S3. Principal component analysis (PCA) of DAb-grain (DC), DAb-pasture (DP), WA-grain (WC) and WA-pasture (WP) meat samples based on sensory panel descriptors. DAb: Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

Table S1. Correlation coefficients of volatile compounds (alcohols) with descriptive attributes of *longissimus lumborum* beef steaks from pasture and grain-fed steers aged in DAb and WA. DAb: Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

	Odor						Texture					Flavor					
Variables	Beef	Aging	Liver	Herbs	Brou	Abnormal	Hardness	Juiciness-in	Juiciness-fin	Masticability	Residue	Beef	Aging	Metallic	Liver	Crumbliness	Bitter
1-Butanol3methyl	0,112	**0,459**	-0,262	-0,301	-0,219	-0,074	0,126	-0,177	-0,200	0,072	0,058	-0,008	0,148	0,037	-0,098	-0,231	0,061
1-Pentanol	-0,194	0,267	-0,288	-0,018	-0,084	0,050	0,482	-0,191	-0,129	0,399	0,297	-0,160	0,206	-0,189	-0,009	0,096	0,262
1-Hexanol	0,079	**0,356**	-0,332	-0,047	-0,211	-0,045	0,128	-0,172	-0,082	0,196	0,194	-0,041	0,251	-0,294	-0,087	0,058	0,143
1-Hexanol2ethyl	-0,088	**0,345**	-0,218	-0,283	-0,123	-0,148	0,132	-0,045	-0,012	0,190	0,146	-0,267	0,063	0,039	-0,055	-0,147	0,223
Phenol	-0,006	**0,355**	-0,332	-0,093	-0,047	-0,147	-0,142	0,097	0,041	-0,123	-0,137	0,072	0,198	-0,426	-0,112	0,210	-0,060
1-Octanol	0,062	**0,454**	-0,294	-0,148	-0,248	-0,072	0,170	-0,204	-0,130	0,155	0,127	-0,041	0,192	-0,283	-0,032	-0,075	0,147
Tetradecanol	-0,010	**0,400**	-0,286	**-0,369**	-0,252	-0,208	0,164	-0,181	-0,152	0,090	0,080	-0,077	0,258	-0,220	-0,165	-0,223	0,105

Table S2. Correlation coefficients of volatile compounds (aldehydes) with descriptive attributes of *longissimus lumborum* beef steaks from pasture and grain-fed steers aged in DAb and WA. DAb: Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

	Odor						Texture					Flavor					
Variables	Beef	Aging	Liver	Herbs	Brou	Abnormal	Hardness	Juiciness-in	Juiciness-fin	Masticability	Residue	Beef	Aging	Metallic	Liver	Crumbliness	Bitter
2-Propenal	-0,120	0,200	-0,200	0,049	-0,230	0,173	-0,127	-0,028	0,005	-0,166	-0,234	0,307	**0,522**	-0,329	-0,118	0,266	-0,163
Butanal	0,070	0,237	**-0,433**	0,163	-0,042	-0,148	0,107	0,032	0,123	0,178	0,103	0,030	**0,335**	**-0,625**	**-0,488**	0,010	0,009
Pentanal	0,141	**0,448**	**-0,445**	0,085	-0,122	-0,061	0,161	-0,157	-0,093	0,218	0,191	0,023	0,299	**-0,529**	-0,220	0,112	0,131
Hexanal	0,056	**0,335**	**-0,383**	-0,018	-0,056	-0,128	0,256	-0,149	-0,082	**0,349**	0,308	-0,121	0,228	-0,328	-0,216	0,076	0,176
Heptanal	0,069	**0,415**	**-0,470**	-0,028	-0,128	-0,064	0,166	-0,155	-0,121	0,140	0,156	-0,029	0,262	**-0,367**	-0,141	0,044	0,128
Octanal	0,129	**0,458**	**-0,355**	-0,094	-0,213	-0,071	0,170	-0,218	-0,141	0,199	0,173	-0,028	0,247	-0,322	-0,116	-0,038	0,122
Nonanal	0,106	**0,489**	**-0,341**	-0,162	-0,231	-0,061	0,210	-0,225	-0,162	0,191	0,147	-0,061	0,224	-0,203	-0,016	-0,124	0,144
Decanal	0,261	**0,416**	-0,315	0,084	-0,183	0,069	0,077	-0,175	-0,089	0,169	0,138	0,079	0,268	**-0,349**	-0,045	0,002	0,029
Undecanal	0,147	**0,434**	**-0,394**	0,036	-0,158	-0,036	0,043	-0,064	-0,007	0,089	0,060	0,178	**0,389**	**-0,474**	-0,187	0,085	-0,003
Dodecanal	0,132	**0,391**	**-0,344**	0,023	-0,176	-0,071	0,038	-0,053	0,013	0,081	0,045	0,187	**0,387**	**-0,451**	-0,149	0,059	-0,040
Tridecanal	0,070	0,299	-0,296	0,059	-0,131	0,014	-0,062	0,038	0,069	-0,025	-0,085	**0,337**	**0,471**	**-0,436**	-0,133	0,168	-0,168
2DecenalE	0,261	0,318	-0,305	0,103	-0,136	-0,085	-0,058	-0,128	-0,100	0,071	0,106	0,119	0,294	**-0,377**	-0,070	0,171	0,041

Table S3. Correlation coefficients of volatile compounds (carboxylic acids) with descriptive attributes of *longissimus lumborum* beef steaks from pasture and grain-fed steers aged in DAb and WA. DAb: Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

Variables	Odor						Texture					Flavor					
	Beef	Aging	Liver	Herbs	Brou	Abnormal	Hardness	Juiciness-in	Juiciness-fin	Masticability	Residue	Beef	Aging	Metallic	Liver	Crumbliness	Bitter
Acetic acid	-0,115	0,262	-0,201	-0,085	**-0,379**	-0,073	-0,210	0,024	-0,005	-0,137	-0,176	-0,013	0,094	-0,302	-0,034	0,096	-0,102
Butanoic acid	0,069	0,138	-0,185	0,225	-0,197	0,082	-0,152	0,015	0,094	-0,020	-0,032	0,214	0,150	**-0,428**	-0,086	0,275	-0,246
Pentanoic acid	-0,034	0,127	**-0,362**	0,139	-0,156	-0,016	-0,027	0,007	0,062	0,034	0,124	-0,043	0,088	**-0,528**	-0,191	0,166	0,051
Hexanoic acid	0,009	0,156	-0,301	0,264	-0,266	0,088	-0,023	-0,003	0,104	0,029	0,050	0,190	0,230	**-0,527**	-0,136	0,186	-0,128
Octanoic acid	0,002	0,024	-0,225	0,267	-0,239	0,129	-0,062	-0,045	0,076	0,036	0,113	0,077	0,080	**-0,509**	-0,165	0,162	-0,046
Nonanoic acid	0,082	**0,338**	-0,326	-0,103	-0,235	-0,043	0,113	-0,156	-0,090	0,131	0,166	-0,050	0,193	**-0,377**	-0,108	-0,077	0,146
Decanoic acid	0,081	0,105	-0,254	0,236	-0,244	0,132	-0,020	-0,088	0,028	0,086	0,142	0,098	0,142	**-0,508**	-0,168	0,108	-0,030
Dodecanoic acid	0,063	0,113	-0,218	0,194	-0,233	0,092	-0,027	-0,073	0,005	0,056	0,129	0,071	0,126	**-0,480**	-0,153	0,088	-0,035

Table S4. Correlation coefficients of volatile compounds (benzene, ketones, furans, sulfur, and terpene) with descriptive attributes of *Longissimus lumborum* beef steaks from pasture and grain-fed steers aged in DAb and WA. Dry aging bag (40 days); WA: Wet aging (40 days). Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

Variables	Odor						Texture					Flavor					
	Beef	Aging	Liver	Herbs	Brou	Abnormal	Hardness	Juiciness-in	Juiciness-fin	Masticability	Residue	Beef	Aging	Metallic	Liver	Crumbliness	Bitter
Toluene	-0,074	0,212	-0,234	0,205	-0,030	0,045	-0,073	0,252	0,168	-0,063	-0,019	0,162	0,136	**-0,420**	0,126	**0,363**	-0,022
1-3-Di-tert-butylbenzene	-0,085	**0,358**	-0,240	-0,209	-0,158	-0,103	0,120	-0,053	-0,055	0,071	0,049	-0,101	-0,031	-0,096	-0,063	0,018	0,189
Methyl butanoate	**-0,353**	-0,328	0,097	-0,278	0,216	**-0,367**	0,000	**0,350**	**0,364**	-0,101	-0,011	-0,127	-0,200	0,239	-0,112	-0,005	0,072
Methyl hexanoate	-0,034	-0,231	0,205	**-0,342**	-0,033	**-0,411**	-0,037	0,029	0,149	0,052	0,057	-0,289	-0,209	0,198	-0,057	-0,033	0,195
Furan	0,187	**0,395**	-0,225	0,083	0,029	-0,218	-0,099	0,174	0,065	0,003	-0,081	0,192	0,262	**-0,348**	**-0,341**	0,101	-0,128
2-Butanone	0,100	**0,501**	**-0,401**	-0,262	-0,254	-0,087	**0,408**	**-0,396**	-0,313	**0,363**	**0,380**	-0,242	0,206	-0,149	-0,189	-0,231	**0,365**
Acetoin	0,091	**0,332**	-0,126	-0,199	-0,175	-0,085	-0,008	-0,043	-0,047	-0,049	-0,062	0,015	-0,051	0,230	0,147	-0,118	-0,059
2-Heptanone	0,136	0,296	-0,311	-0,036	-0,090	-0,054	0,030	-0,107	-0,005	0,116	0,118	0,124	**0,355**	**-0,370**	-0,311	0,124	0,051
Carbon disulfide	-0,073	0,027	-0,026	-0,250	-0,054	-0,129	0,022	-0,058	0,011	0,070	0,148	-0,218	-0,287	0,244	0,142	-0,027	0,269
a-Pinene	-0,242	0,224	-0,245	-0,253	-0,071	**-0,398**	**0,329**	0,107	0,003	0,191	0,247	-0,053	0,116	-0,187	-0,099	-0,010	0,139

6. Effect of the aging methods and finishing diet in the *longissimus thoracis et lomborum* muscle of steers- a proteomics approach

Daniela Correa[1], Gustavo Brito[1], Marcia del Campo[1], Santiago Luzardo[1], Guillermo de Souza[1], Carlos Álvarez[2], María Font-i-Furnols[3], Laura Alessandroni[4], Mohammed Gagaoua[5]*.

[1]INIA-Sistema Ganadero Extensivo, INIA Tacuarembó, Ruta 5, km 386, Tacuarembó, Uruguay

[2]Teagasc-Food Quality and Sensory Science Department, Teagasc Ashtown Food Research Centre, Ashtown, D15KN3K Dublin 15, Ireland

[3] IRTA-Food Technology, Granja Camps i Armet, 17121 Monells (Girona), Spain

[4] INICAM-School of Pharmacy, University of Camerino, Via Sant'Agostino 1, Camerino 62032, Italy

[5] INRAE-Equipe Alimentation, Environnement, Qualité des Produits pour les Systèmes d'Elevage de Monogastriques. G16, Le Clos, 35590 Saint-Gilles, France

*Corresponding author. Email: mohammed.gagaoua@inrae.fr

6.1. Introduction

The beef industry continually faces the challenge of producing high-value products with consistent quality that meet consumers' expectations (McCarthy et al., 2017; Gagaoua et al., 2019). There is a need to develop efficient and non-invasive tools to identify animals or carcasses with desired quality attributes before or after slaughter (Ouali et al., 2013). With this goal in mind, over the past two decades, high-throughput molecular techniques, collectively known as proteomic approaches, have been applied in meat science (Di Luca et al., 2016; Picard and Gagaoua, 2020; Munekata et al., 2021). Proteomics studies on beef tenderization, color, and marbling (Gagaoua, Hughes et al., 2020; Gagaoua, Bonnet et al., 2020b) enable the simultaneous examination of hundreds or thousands of muscle proteins to characterize the changes occurring during the post-mortem period in meat of varying quality, leading to the

identification of potential protein markers of eating quality (Gagaoua, Terlouw et al., 2021).

The quality characteristics of beef depend on intrinsic factors such as muscle type, sex, and breed, as well as extrinsic factors like pasture- or grain-based fattening (Henchion et al., 2017; Verbeke et al., 2010). Since grain-fed cattle differ substantially from pasture or forage-based systems in average daily gain, we may observe changes in muscle metabolism that contribute to the development of final eating quality traits, such as color or tenderness. Beef from cattle finishing in feedlot systems (grain-fed) is brighter red and more tender than beef from cattle finishing in extensive systems (pasture-based). In contrast, pasture- or forage-finishing cattle produce fresh meat that is often darker in appearance and less tender, which may be attributed to a pronounced oxidative metabolism in the muscles of grass-fed animals (Apaoblaza et al., 2020). However, the finishing regime type is often confounded with weight gain because cattle on intensive feeding programs tend to grow at faster rates. Regardless, understanding the changes in muscle that occur with the feeding paradigm may provide insight into the mechanisms underlying beef quality development.

The duration of aging after rigor mortis, which is part of the production process, is an additional key factor influencing eating quality, which occurs during the processing stage at slaughterhouses or cutting plants (Bischof et al., 2021; Kim et al., 2017). The aging process can be classified into two main categories: wet- and dry aging (Terjung et al., 2021; de Faria Vilella et al., 2019). In dry aging, meat is unpacked and exposed to environmental conditions, allowing moisture to evaporate. Dry-aged beef is characterized by its unique flavor and product quality (DeGeer et al., 2009). In contrast, wet aging, also called vacuum aging, is widely used in the meat industry due to its low loss in production yield and convenience in storage and transport (Warren and Kastner, 1992). However, the dry aging process, which utilizes a high-permeable water vapor bag (dry aging bag), was introduced to the market to enhance the yield of the traditional unpackaged dry aging process. Meat aged in a dry aging bag is expected to have the same sensory quality as traditional, unpackaged dry-aged meat, but with reduced aging and trim losses, a lower risk of contamination, and

fewer requirements for environmental control (Ahnström et al., 2006; DeGeer et al., 2009).

Meat quality results from many biochemical and physical mechanisms in muscles starting right after animal bleeding and continuing during aging periods (Gagaoua, Schilling et al., 2022). Recently, the application of proteomics in meat research has yielded valuable insights into conventional methods also called the hypothetico-deductive (H-D) (Purslow et al., 2021), hence contributing significantly to a better understanding of the complexity of the muscle proteome and its importance in determining meat quality (Gagaoua, Duffy et al., 2022).

Research has been done to characterize the proteome through different aging methods (Kim et al., 2020; Álvarez et al., 2023) at various post-mortem times and to characterize different animal growth rates and finishing regimen types (Antonelo et al., 2022; Gagaoua et al., 2017), but the literature lacks studies about the impact of the extended aging process combined with different finishing diets on muscle proteomic profiles.

This study aims to identify differentially abundant proteins (DAPs) in the *longissimus thoracis et lumborum* (LTL) muscle of steers from concentrate- or pasture-finished animals using two aging methods: dry bag and wet, over a 40-day period.

6.2. Materials and methods

6.2.1. Raw materials and aging process

This study was carried out in complement to Correa et al. (2024) as a part of a large experiment, and the duplicities of the experimental design and sample set are acknowledged. A total of 30 steers (under 30 months of age; British breed) finished (F) in the pasture (P; n = 15) qualifying for UE Hilton quota (INAC, 2013) or concentrate (C; n = 15) qualifying for UE 481 quota (MGAP, 2023) were slaughtered in a commercial meat packing plant (hot carcass weight: 266.5 kg: P and 253.2 kg: C). The steer herd originated from the same farm and was selected based on age, live weight, and fat cover to establish two similar groups. Sixty striploins (*longissimus thoracis et lumborum*, LTL) were obtained for analysis and assigned to an aging

method: dry bag (DAb) or wet (WA). The striploin from the left side of each carcass was divided into two sections or pieces; a section 16 cm in length was vacuum packaged in dry-aging bags (D; TUBLIN® 10 of 50 μm thick, polyamide mix with a water vapor transmission rate of 2.5 kg/50 μ/m2/24 h at 38°C, 50% RH, TUB-EX ApS, Denmark) and a second section of 14 cm of length was vacuum packaged for wet-aged (W; vacuum packaged was a barrier bag of 50 μm thickness; maximum oxygen transmission rate of 27 cm3/m2/24 h at 22-24°C and 0% RH and moisture vapor transmission rate of 5 g/m2/24 h at 38°C and 90% RH; Cryovac® Sealed Air Corp., BB 2620, Brazil). The location of each meat portion from each striploin was alternated in cranial to caudal direction among carcasses. The striploin's portions were laid out on wire racks inside the chamber during an aging period of 40 d. At the end of the aging period, a steak from each piece was retired, frozen (-20 °C) and shipped to Teagasc National Food Research Center in Ashtown, Dublin (Ireland). Once the samples arrived, they were stored at -80 °C for further analysis.

6.2.2. Protein extraction and quantification

Frozen muscle tissue samples (150-200 mg) were homogenized in 3 mL of fresh extraction buffer consisting of 8.3 M urea, 2 M thiourea, 1% DL-Dithiothreitol (DTT), 2% CHAPS and Pharmalyte® 3-10 (Bouley et al., 2004) using a T18 digital ULTRA-TURRAX® (IKA®, Staufen, Germany) which was run at 20,000 rpm for 60 s (2 × 30 s). The homogenates were incubated on ice for 30 minutes and centrifuged for 30 minutes at 10000 xg at 4 °C (Lamri, Della Malva, Djenane, López-Pedrouso et al., 2023). The supernatant was transferred into Eppendorf tubes and stored at -80 °C until protein quantification. Protein concentrations were determined using the Bradford method (Bradford, 1976), and bovine serum albumin (Thermo Scientific, Rockford, IL, USA) was employed as a standard. Absorbance was measured at 595 nm using a spectrophotometer (UV-1700, Pharmaspec, SHIMADZU) to determine protein concentrations in the protein extracts.

6.2.3. One-dimensional (1D) sodium dodecyl Sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for shotgun proteomics

The protein concentrations of the muscle extracts were adjusted with MilliQ water, and an equal volume of Laemmli buffer was added (Sigma-Aldrich, St. Louis, MO, USA) to ensure a final protein mass of 40 µg in each well. The samples were heated in a standard block heater (SBH130D/3, Bibby Scientific Limited, Staffordshire, UK) at 80 °C for 15 min. A volume of 15 µL of each sample was loaded in freshly prepared 12% resolving and 4% stacking gels for 1D SDS-PAGE using a Mini-PROTEAN® Tetra Cell System (Bio-Rad, Hercules, CA, USA) with a run time of 15 min at 4 W to concentrate the proteins. Protein bands for shotgun proteomics were prepared as described by Lamri et al. (2023). Subsequent digestion of the protein bands and mass spectrometry analysis were performed following the protocol described by Chantada-Vázquez et al. (2021). Modified porcine trypsin (Promega, Madison, WI, USA) was used to digest the dried gel bands at a concentration of 20 ng/µL in 20 mM ammonium bicarbonate at 37 °C for 16 h. The resulting peptides were extracted with 60% acetonitrile in 0.5% formic acid, vacuum-concentrated in a SpeedVac and stored at 20 ° for further LC-MS/MS analysis.

6.2.4. Generation of the reference spectral library and protein quantification by SWATH-MS

To generate the MS/MS spectral libraries and obtain a good representation of the peptides and proteins in all samples, vials from each group (i.e., dry aged condition) were prepared and analyzed by a shotgun data-dependent acquisition (DDA) approach using a micro-LC-MS/MS. Briefly, a peptides mixture of 4 µL per condition was separated on a micro-LC system Ekspert nLC425 (Eksigen, Dublin, CA, USA) using an Eksigent C18 column (150 × 0.30 mm, 3 mm particle size and 120 Å pore size) (Eksigent, Sciex). The eluting peptides were directly injected into a hybrid quadrupole-TOF mass spectrometer coupled with a Triple TOF 6600 (Sciex, Redwood City, CA, USA), which operated with a data-dependent acquisition system in positive ion mode. The MS raw files were used for peptides and protein identification employing the ProteinPilot software (version 5.0.1, Sciex) and the bovine-specific Uniprot database

(https://www.uniprot.org/). The false discovery rate (FDR) was set to 1% for both peptides and proteins. For the relative quantification of peptides and proteins, 4 μL of peptides from each sample were analyzed using a data-independent acquisition (IDA) method, known as SWATH (sequential window acquisition of all theoretical mass spectra)-MS method. Samples were analyzed using the LC-MS equipment and LC gradient. Sixty samples from 4 treatments (2 finishing diets x 2 aging types, 15 animals) were analyzed. The data extraction from the SWATH runs was performed by PeakView v.2.2 (Sciex, Framingham, MA, USA) employing the SWATH Acquisition MicroApp v.2.0. This application processed the data based on the spectral library created and used the following parameters: 10 peptides /protein and 7 fragments/peptide and FDR below 1%. Protein quantification was calculated by summing the peak areas of the corresponding peptides.

6.2.5. Statistical analysis

SWATH-MS analysis allowed the identification and quantification of 653 proteins and 1684 peptides at an FDR of 1%. Data processing and statistical analysis were performed using the MetaboAnalyst platform (https://www.metaboanalyst.ca/). Proteins with >50% missing values were removed. The remaining missing proteins were estimated using the k-nearest neighbor (KNN) algorithm. Data was normalized using a log10 transformation and Pareto scaling approach. Pairwise (two groups) and multiple (four groups) comparisons were performed to analyze the proteomic data. In the case of pairwise comparisons, a volcano plot with a 1.5-fold change (FC) and a P-value of 0.01 was used. For multiple comparisons, a partial least squares discriminant analysis (PLS-DA) with a fixed VIP (variable importance in projection) threshold ≥2 was employed.

6.2.6. Bioinformatics analysis

To benefit from the most complete annotation available, the bovine Uniprot IDs of the 653 proteins were converted into the orthologous human EntrezGeneID (Gagaoua, Terlouw et al., 2021), using the UniprotKB database (https://www.uniprot.org/). Both bovine and human Uniprot IDs were indexed in the databases and used for bioinformatics analysis. The Metascape tool

(https://metascape.org/) was used to perform Gene Ontology (GO) enrichment analysis and identify the most significant and enriched GO terms, allowing for the exploration of the potential functions and biological processes of the differentially abundant proteins (DAPs) identified in the proteome of dry-aged beef. Bioinformatics analysis was performed as described by Gagaoua, Terlouw et al. (2021) with some brief modifications. The current study considered the GO terms with a *P*-value <0.01, a minimum overlap of 2, and an enrichment factor > 1.5 (ratio between the observed counts and the counts expected by chance). Representative terms with a similarity score > 0.3 were clustered based on their membership similarities and visualized in network layouts. Additionally, Cytoscape v.3.9.1 was utilized to enhance the visualization and exploration of the enriched networks.

6.3. Results

A total of 653 proteins were identified and quantified by SWATH-MS analysis. The following sections will present the answer to the following points: 1) the impact of finishing diet on the proteomic profile of dry-aged in a bag meat finishing (PDAb vs CDAb), 2) the impact of each finishing diet on wet-aged meat (PWA vs CWA), 3) the overall impact of aging type on finishing meat from pasture finishing animals (PWA vs PDAb) and 4) the overall impact of aging type on meat coming from concentrate finishing animals (CWA vs CDAb).

6.3.1. Differentially abundant proteins (DAPs) comparing the dry aging meat within each pasture and concentrate finished diet

A volcano plot (Fig. 1- A and Table 1) assessed differential protein expression, displaying the changes due to the finished diet in dry bag-aged samples. On the left side, the blue points represent the significantly overabundant proteins in pasture-dry bag aging (38 proteins); on the right, the red points correspond to the proteins overabundant in concentrate-dry bag aging (68 proteins). The biological pathway and cluster enrichment analysis performed on the 106 DAPs (Fig. 1- B) revealed 20 significant Gene Ontology (GO) terms identified in LTL muscle tissue of steers, of which four belong to the pasture finished diet aged in dry bag treatment (PDAb). The

three most abundant GOs: GO: 0055001 “muscle cell development,” GO: 0006091 “generation of metabolites and energy,” and GO: 0006163 “purine nucleotide metabolic process” were associated with concentrate finished diet-dry bag aging treatment (CD). The network layout (Fig. 1-C) between the representative enriched GO terms illustrates the extent of the enrichment or the clusters contributing to each term. Partial least squares-discriminant analysis (PLS-DA) allowed us to discriminate between the two finished diets in dry-bag aging samples: concentrate-dry bag aging (CD) and pasture-dry bag aging (PDAb) (Fig. 2-A). The top 20 most important proteins (Fig. 2-B), according to the VIP (variable importance in projection), revealed two proteins (MYH7B and IPO5) to be more abundant in samples from pasture and 18 proteins (PGK1.1, TNNT3, TPM1, ATP2A3, PKM2, GAPDH, CASQ1, TUBA4A, TPM1, PGK1, ATP5F1A, TUBB2A, MYBPC1, PKM, PYGM, LGALS-1.1, SYPL2, AMPD1) more abundant in samples from concentrate. Among them, PGK1.1 (VIP > 3.5) was the significant protein in abundance (putative biomarker) with a higher VIP score, being overabundant in the CD samples.

Table 1. Significant and differentially abundant proteins between pasture dry-aged (PDAb) and concentrate dry-aged beef (CDAb) samples from the shotgun SWATH-MS proteomics analysis. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

Uniprot ID	**Full protein names**	**Gene names**	**Log2 (FC)**	**-Log10 (p-value)**
Q3T0P6	Phosphoglycerate kinase 1	*PGK1*	3.82	6.14
Q5KR49	Tropomyosin alpha-1 chain	*TPM1*	2.46	6.47
P81948	Tubulin alpha-4A chain	*TUBA4A*	2.11	4.01
Q8MKI3	Troponin T, fast skeletal muscle	*Tnnt3*	1.84	4.37
A0A3Q1MTP9	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	*ATP2A3*	1.81	4.74
P14618	Pyruvate kinase PKM	*PKM2*	1.80	4.97
P31415	Calsequestrin-1	*CASQ1*	1.66	7.32
E1BJB1	Tubulin beta-2A chain	*TUBB2A*	1.52	5.00
Q5KR49	Tropomyosin alpha-1 chain	*TPM1*	1.52	8.04

P23109	AMP deaminase 1	*AMPD1*	1.44	3.75
P19483	ATP synthase subunit alpha, mitochondrial	*ATP5F1A*	1.42	4.57
P10096	Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	1.41	3.39
Q00872	Myosin-binding protein C, slow-type	*MYBPC1*	1.33	8.04
A0AAA9TL30	Synaptophysin-like protein 2	*SYPL2*	1.32	5.10
Q3T149	Heat shock protein beta-1	*HSPB1*	1.29	4.87
F1MNF8	Tubulin alpha chain	*TUBA1A*	1.28	2.56
A0A077S2W5	lysozyme	*LYZF5*	1.27	2.00
Q9XSG3	Isocitrate dehydrogenase [NADP] cytoplasmic	*IDH1*	1.19	2.50
E1BAJ4	Starch binding domain 1	*STBD1*	1.18	2.60
Q04760	Lactoylglutathione lyase	*GLO1*	1.12	3.13
Q3ZC55	Alpha-actinin-2	*ACTN2*	1.12	6.33
Q5KR47	Tropomyosin alpha-3 chain	*TPM3*	1.11	5.83
P13639	Elongation factor 2	*EEF2*	1.11	3.16
E1BNV1	Myosin binding protein C2	*MYBPC2*	1.10	4.50
E3Q1Q2	Galectin	*LGALS-11*	1.09	2.88
A5D984	Pyruvate kinase	*PKM*	1.06	3.38
P11966	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	*PDHB*	1.05	2.61
P12344	Aspartate aminotransferase, mitochondrial	*GOT2*	1.04	2.77
P79334	Glycogen phosphorylase, muscle form	*PYGM*	1.04	2.61
Q8SQ24	Myozenin-1	*MYOZ1*	1.03	4.04
P10790	Fatty acid-binding protein, heart	*H-FABP*	1.01	2.60
P02510	Alpha-crystallin B chain	*CRYAB*	1.00	4.24

A0AAA9S5Q9	Four and a half LIM domains protein 1	*FHL1*	0.97	3.57
Q08DP0	Phosphoglucomutase-1	*PGM1*	0.96	5.45
P15246	Protein-L-isoaspartate(D-aspartate) O-methyltransferase	*PCMT1*	0.96	3.32
P00829	ATP synthase subunit beta, mitochondrial	*ATP5F1B*	0.95	3.66
O60662	Kelch-like protein 41	*KLHL41*	0.92	3.15
P00442	Superoxide dismutase [Cu-Zn]	*SOD1*	0.92	2.73
F1MPR3	Calcium-transporting ATPase	*ATP2A2*	0.91	7.90
E1BNE7	Caveolae associated protein 1	*CAVIN1*	0.90	4.36
Q2TBU0	Haptoglobin	*HP*	0.90	2.19
Q0P571	Myosin regulatory light chain 2, skeletal muscle isoform	*MYLPF*	0.89	5.35
P19120	Heat shock cognate 71 kDa protein	*HSPA8*	0.88	6.47
P35573	Glycogen debranching enzyme	*AGL*	0.88	4.70
A7YY67	10-formyltetrahydrofolate dehydrogenase	*ALDH1L1*	0.85	2.64
Q148D5	Succinate--CoA ligase [ADP-forming] subunit beta, mitochondrial	*SUCLA2*	0.84	3.92
Q5E947	Peroxiredoxin-1	*PRDX1*	0.84	3.42
F1MRQ7	SH3 domain binding glutamate rich	*SH3BGR*	0.82	2.71
Q0III9	Alpha-actinin-3	*ACTN3*	0.78	3.59

Q3SZE5	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform	*MYL2*	0.78	3.39
Q29RI2	phosphorylase kinase	*PHKG1*	0.77	2.81
A6QLL8	Fructose-bisphosphate aldolase	*ALDOA*	0.77	4.40
Q17QE2	LIM and cysteine-rich domains protein 1	*LMCD1*	0.75	2.98
Q3Y5Z3	Adiponectin	*ADIPOQ*	0.73	3.31
O46629	Trifunctional enzyme subunit beta, mitochondrial	*HADHB*	0.73	3.23
P68530	Cytochrome c oxidase subunit 2	*COX2*	0.73	2.55
Q27975	Heat shock 70 kDa protein 1A	*hsp70*	0.72	2.73
A0A1K0FUD3	Globin C1	*GLNC1*	0.71	5.17
Q0VD16	Dual specificity mitogen-activated protein kinase kinase 1	*MAP2K1*	0.68	2.60
Q9TT36	Thyroxine-binding globulin	*SERPINA7*	0.67	2.30
P02070	Hemoglobin subunit beta	*HBB*	0.67	3.43
Q3ZBT1	Transitional endoplasmic reticulum ATPase	*VCP*	0.65	2.59
Q3SZB4	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	*ACADM*	0.64	4.24
Q5E9F7	Cofilin-1	*CFL1*	0.64	2.15
A3KN12	Adenylosuccinate lyase	*ADSL*	0.62	2.56
E1BF23	Myomesin 2	*MYOM2*	0.61	5.44
P80473	Myomesin-1	*MYOM1*	0.61	2.40
P02763	Alpha-1-acid glycoprotein 1	*Agp*	-0.62	2.15
E1BH94	Peptidoglycan recognition protein 2	*PGLYRP2*	-0.63	2.39

Q2KJB3	Angiopoietin-like 3	*ANGPTL3*	-0.65	2.02
A6QQ49	Pepsin A	*PGA5*	-0.66	2.26
F1MC13	Laminin subunit alpha 5	*LAMA5*	-0.70	2.71
Q2KJF1	Alpha-1B-glycoprotein	*A1BG*	-0.72	2.43
Q148M6	Matrix remodeling-associated protein 8	*MXRA8*	-0.74	2.86
Q9XTA2	Prolyl endopeptidase	*PREP*	-0.75	2.48
A2VE41	EGF containing fibulin extracellular matrix protein 1	*EFEMP1*	-0.80	2.50
P12763	Alpha-2-HS-glycoprotein	*AHSG*	-0.81	2.53
Q2HJ60	Heterogeneous nuclear ribonucleoproteins A2/B1	*HNRNPA2B1*	-0.82	2.00
P78417	Glutathione S-transferase omega-1	*GSTO1*	-0.88	2.10
Q32LG3	Malate dehydrogenase, mitochondrial	*MDH2*	-0.91	2.44
Q4PJW3	Lanosterol 14-alpha demethylase	*CYP51A1*	-0.93	3.59
E1BLA8	Golgi membrane protein 1	*GOLM1*	-0.94	2.30
E1B9K4	Hemicentin 2	*HMCN2*	-0.95	2.52
Q6IM73	Reticulon	*RTN2*	-0.95	2.98
P41361	Antithrombin-III	*SERPINC1*	-0.96	6.02
F1MQ74	Sushi domain containing 1	*SUSD1*	-0.98	4.17
Q29437	Primary amine oxidase, liver isozyme	*SAO*	-0.99	4.46
E1BCW0	HGF activator	*HGFAC*	-1.01	3.98
Q6H320	Glandular kallikrein	*KLK1*	-1.02	2.81
A7YW45	Protein arginine N-methyltransferase 5	*PRMT5*	-1.03	2.57
O18738	Dystroglycan 1	*DAG1*	-1.10	2.68
A0A3Q1LMV9	Epiplakin 1	*EPPK1*	-1.12	2.76

A7E337	phosphoinositide 5-phosphatase	*OCRL*	-1.14	2.64
A4IFA5	VASN protein	*VASN*	-1.15	2.92
Q5E9Z9	Nectin-4	*NECTIN4*	-1.35	3.87
Q28035	Glutathione S-transferase A1	*GSTA1*	-1.36	3.74
Q9BE40	Myosin-1	*MYH1*	-1.38	2.46
Q3SZN8	Ribonuclease inhibitor	*RNH1*	-1.48	2.58
Q3SZJ7	Lysosomal-associated membrane protein 2	*LAMP2*	-1.50	2.85
Q8WML4	Mucin-1	*MUC1*	-1.73	2.58
E3Q1Q2	Galectin	*LGALS-11*	-1.75	3.12
Q3ZBS7	Vitronectin	*VTN*	-1.76	2.29
Q2KHW2	CD83 molecule	*CD83*	-2.12	2.54
F1MPE5	Importin 5	*IPO5*	-2.20	3.12
E1BPX8	Myosin heavy chain 7B	*MYH7B*	-2.40	3.46

Proteins with positive log2(FC) values are more abundant in CD samples

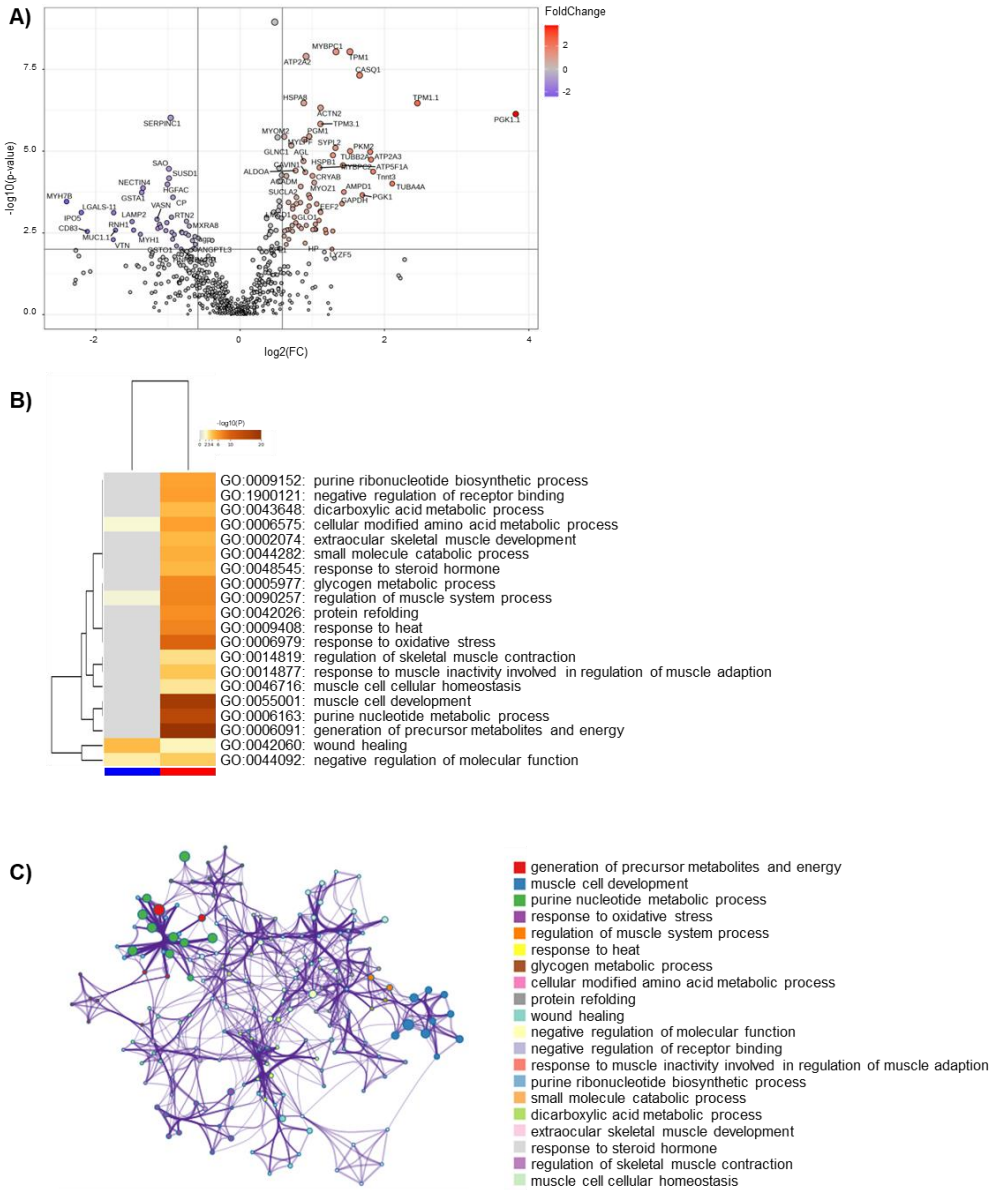


Figure 1. Statistical and bioinformatics analyses of the differentially abundant proteins (DAPs) in dry-aged beef between the two production systems. A) Volcano plot of the DAPs highlighting the 106 proteins that were significantly different between the pasture dry-aged (PD = PDAb) compared to the concentrated dry-aged beef production system (CD = CDAb). The down-abundant (n = 38) and the up-abundant (n = 68) proteins in CD (CDAb) samples are shown in blue and red colors, respectively. B) Bioinformatics enrichment analyses based on Gene Ontology (GO) terms, using the 106 DAPs including the redundant proteoforms. C) Network layout based on the enriched terms of the 106 proteins. Each term is represented by a circle

node, with its size proportional to the number of input genes that fall under that term, and its color represents the cluster identity. Terms with a similarity score > 0.3 are linked by an edge. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

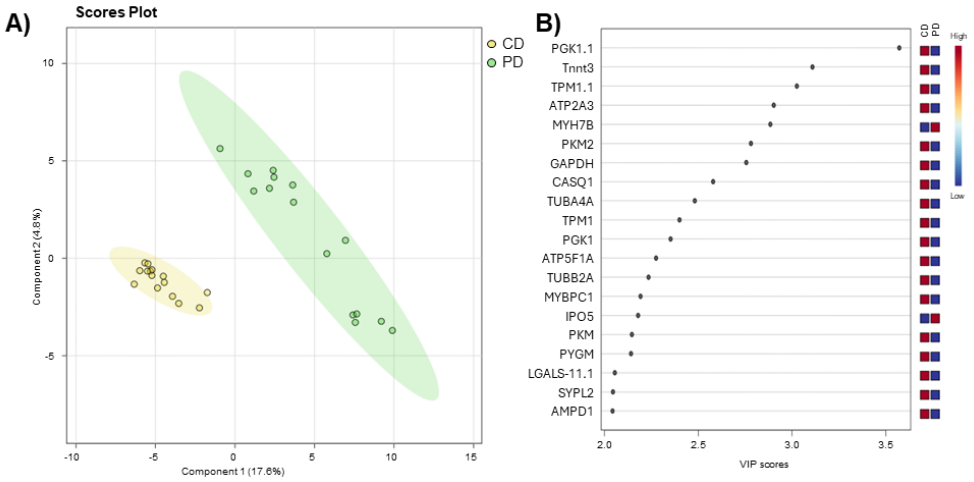


Figure 2. Discriminant analysis within the dry-aged meat proteomes. A) Partial least squares-discriminant analysis (PLS-DA) score plot of dry-aged meat proteome distribution according to production system, CD (CDAb), and PD (PDAb). B) Variable importance in projection (VIP) plot, top 20 values. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

6.3.2. Differentially abundant proteins (DAPs) comparing the wet aging meat within each pasture and concentrate finished diet

Eighteen significantly different proteins were identified through volcano plot analysis between pasture and concentrate-finishing diets after wet-aging (Fig. 3-A and Table 2). Three proteins were more abundant (down-regulated) in the pasture and 15 proteins were more abundant (up-regulated) in concentrate. The biological pathways and cluster enrichment analysis performed on the 18 DAPs (Fig. 3-B, C), revealed 7 significant enriched terms, all in concentrate-wet aging. The most abundant were GO: 0030049 “muscle filament sliding”, GO: 0042026 “protein refolding” and GO:0120163 “negative regulation of cold-induced thermogenesis”. No common terms were found between the pasture and concentrate-finishing diet in wet aging treatments. Partial least squares-discriminant analysis (PLS-DA) allowed to discriminate the two finishing diets in wet aging samples: concentrate-wet (CW) and pasture-wet (PW)

(Fig. 4-A). The top 20 most (Fig. 4-B) important proteins according to the VIP revealed three proteins (RPL23AY, ROR2 and GOT2) to be more abundant from pasture origin meats and 17 proteins (TPM1.1, ID.1, CRYAB, ATP5F1B, HSPB6, bmmp-9, TPM1, MASP2, HINT1, MAPK14, MYH2, DEFB1, ACTN3, PTMA, PITPNA, PCMT1, TPM3.1) from concentrate, where TPM1.1 (VIP > 4.0) was protein finishing most relevant protein to discriminate between feeding regimes after wet aging.

Table 2. Significant and differentially abundant proteins between pasture wet-aged (PW) and concentrate wet-aged beef (CW) samples from the shotgun SWATH-MS proteomics analysis. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

Uniprot ID	**Full protein names**	**Gene names**	**Log2 (FC)**	**-Log10 (p-value)**
Q5KR49	Tropomyosin alpha-1 chain	*TPM1*	2.41	5.86
P02510	Alpha-crystallin B chain	*CRYAB*	2.24	4.37
A4IF68	DNA-binding protein inhibitor ID-1	*ID*	1.52	5.78
P00829	ATP synthase subunit beta, mitochondrial	*ATP5F1B*	1.15	4.39
Q148F8	Heat shock protein beta-6	*HSPB6*	1.08	6.47
P46159	Beta-defensin 1	*DEFB1*	1.00	2.31
Q5KR49	Tropomyosin alpha-1 chain	*TPM1*	0.94	5.18
P62958	Adenosine 5'-monophosphoramidase HINT1	*HINT1*	0.94	3.14
E1BJ49	MBL-associated serine protease 2	*MASP2*	0.92	2.50
Q9N282	MMP-9	*bmmp-9*	0.83	2.85

A0AAA9TIQ1	Protease-associated domain containing 1	*PRADC1*	0.81	2.03
Q0III9	Alpha-actinin-3	*ACTN3*	0.74	3.35
P00514	cAMP-dependent protein kinase type I-alpha regulatory subunit	*PRKAR1A*	0.64	2.25
A0JNJ5	Myosin light chain 1/3, skeletal muscle isoform	*MYL1*	0.63	3.07
Q32LG3	Malate dehydrogenase, mitochondrial	*MDH2*	0.60	3.05
F1MMT2	Laminin subunit alpha 2	*LAMA2*	-0.59	3.26
P41563	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	*IDH3A*	-0.69	2.90
Q9BE39	Myosin-7	*MYH7*	-0.75	2.37

Proteins with positive log2(FC) values are more abundant in CW samples

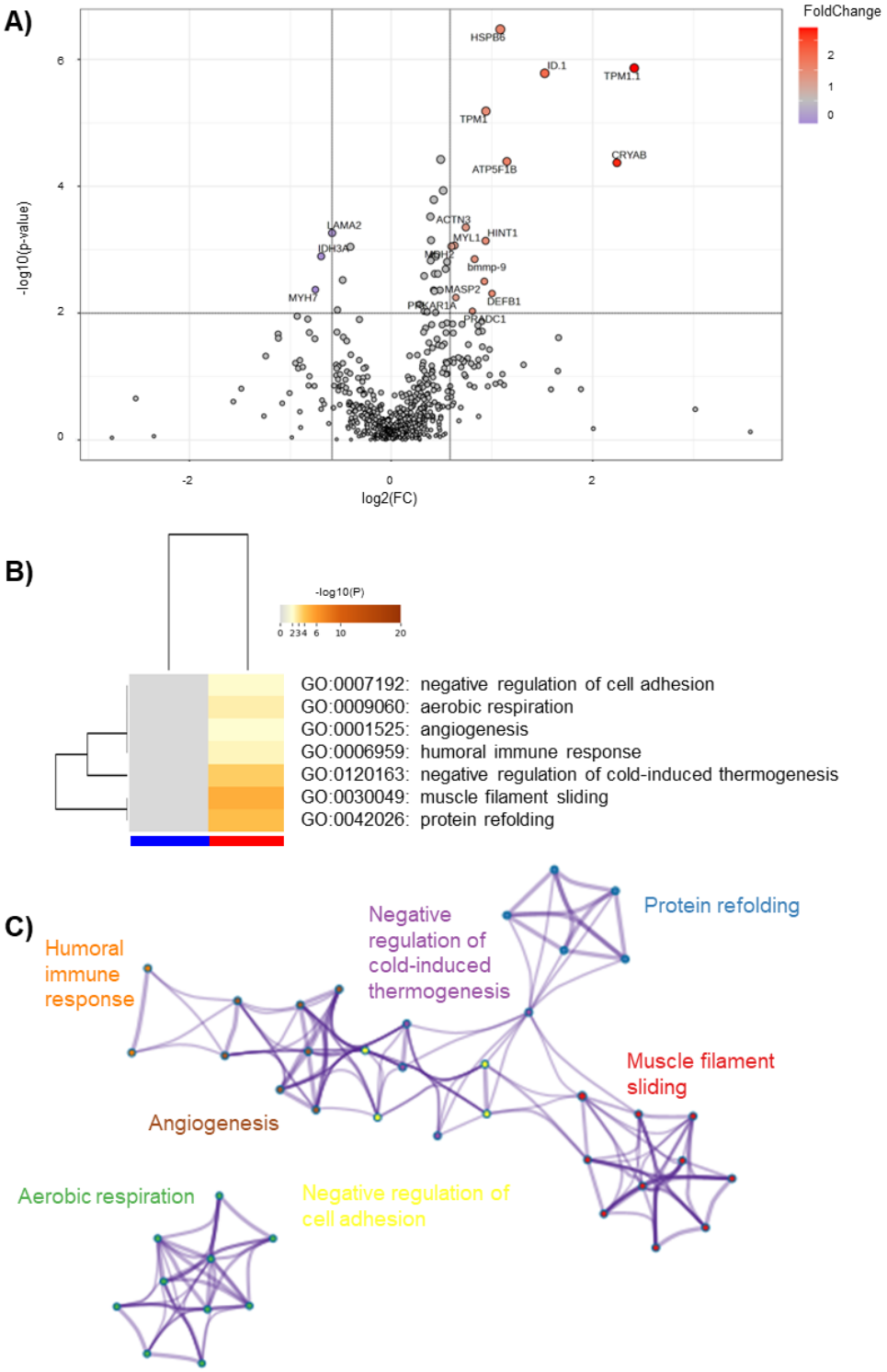


Figure 3. Statistical and bioinformatics analyses of the differentially abundant proteins (DAPs) in wet-aged beef between the two production systems. A) Volcano plot of the DAPs highlighting the 18 proteins that were significantly different between

the pasture wet aged (PW) compared to concentrate wet aged (CW). The down-abundant (n = 3) in PW and the up-abundant (n = 15) in CW samples are shown in blue and red colors, respectively. B) Bioinformatics enrichment analyses based on Gene Ontology (GO) terms, using the 18 DAPs, including the redundant proteoforms. C) Network layout based on the enriched terms of the 18 proteins. Each term is represented by a circle node, where its size is proportional to the number of input genes fall under that term, and its color represents the cluster identity. Terms with a similarity score > 0.3 are linked by an edge. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

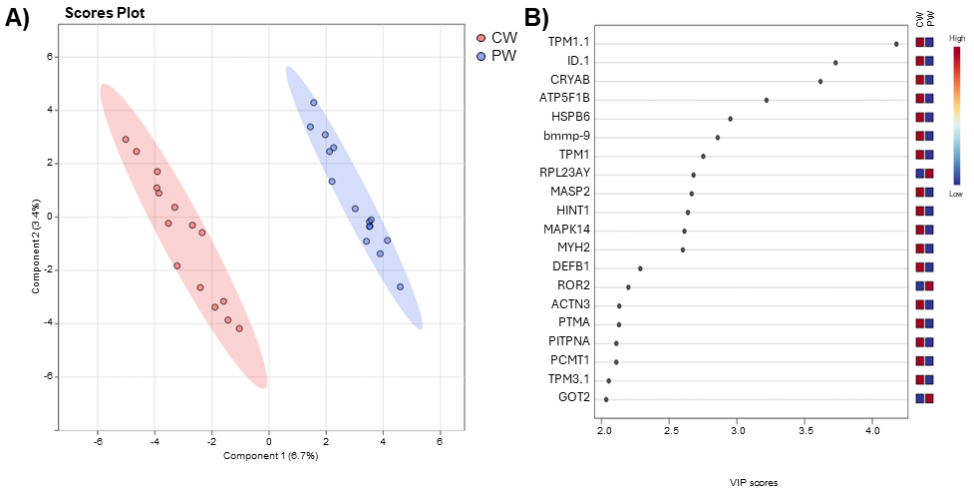


Figure 4. Discriminant analysis within the wet aging meat proteomes. A) Partial least squares-discriminant analysis (PLS-DA) score plot of wet-aged meat proteome distribution according to the production system, CW, and PW. B) Variable importance in projection (VIP) plot top 20 values. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

6.3.3. Differentially abundant proteins (DAPs) comparing the pasture finished diet within different aging types

A total of 68 proteins were significantly different, identified through volcano plot analysis between dry bag (PD = PDAb) and wet aging (PW = PWA) from the pasture-finished diet (Fig. 5-A and Table 3). Eleven proteins were more abundant (down) in PWA and 57 (up) in PDAb. The bioinformatics enrichment analyses (Gene Ontology, KEGG, Reactome) with 68 DAPs (Fig. 5-B), revealed 20 significantly

enriched GO terms, where only one term was specific for PW (GO: 1904851 “positive regulation of establishment of protein localization to telomere”), another one was sharing with PC cluster (GO:0007507 “heart development”) and the rest of the GO terms belonging to PC, where the most abundant were GO: 0055001 “muscle cell development” and GO: 0006091 “generation of precursor metabolite of energy”. The networking (Fig. 5-C) layout based on enriched terms of the 68 proteins also indicated “muscle cell development” and “generation of precursor metabolite of energy” as the biggest term and cluster identity. The PLS-discriminant analysis (Fig. 6-A) allowed the discrimination of the two combinations of treatment: dry bag and wet aging from pasture-fed steer samples. The plot shows a clear separation of samples into pasture between dry bag and wet aging. Analyzing the 20 top proteins (VIP scores, Fig. 6-B) from the combination revealed two more abundant proteins in PW (RPL23AY and FHL1) and 18 in PD (MYH2, ID, EEF2, SCGB1D, ATP2A3, ATP5F1A, TNNT3, PGK1, LGALS-11, GSTP1, KLHL41, PYGM, GAPDH, ACTIN3, TPM1, COL6A3, EEF1G and CRYAB).

Table 3. Significant and differentially abundant proteins between pasture dry-aged (PDAb) and pasture wet-aged beef (PWA) samples from the shotgun SWATH-MS proteomics analysis. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

Uniprot ID	**Full protein names**	**Gene names**	**Log2 (FC)**	**-Log10 (p-value)**
Q8MKI3	Troponin T, fast skeletal muscle	*TNNT3*	3.92	2.78
P10096	Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	3.88	2.39
A0JNP2	Secretoglobin family 1D member	*SCGB1D*	3.68	3.97
P79334	Glycogen phosphorylase, muscle form	*PYGM*	3.23	2.90
E1BMQ6	Calcium-transporting ATPase	*ATP2A3*	3.18	3.45
Q3SYU2	Elongation factor 2	*EEF2*	2.72	4.79

E3Q1Q2	Galectin	*LGALS-11*	2.71	3.47
Q3T0P6	Phosphoglycerate kinase 1	*PGK1*	2.52	3.69
P19483	ATP synthase subunit alpha, mitochondrial	*ATP5F1A*	2.46	5.67
A0AAA9S4Q2	Bardet-Biedl syndrome 5 protein homolog	KBTBD10	2.31	4.59
P41134	DNA-binding protein inhibitor ID-1	*ID*	2.25	8.70
P28801	Glutathione S-transferase P	*GSTP1*	2.22	4.34
Q5KR49	Tropomyosin alpha-1 chain	*TPM1*	2.13	3.96
Q3ZBS7	Vitronectin	*VTN*	1.86	2.59
Q9ULJ8	Neurabin-1	*NEB.1*	1.67	2.58
Q3SZV3	Elongation factor 1-gamma	*EEF1G*	1.62	2.97
Q0III9	Alpha-actinin-3	*ACTN3*	1.61	5.68
Q9BE41	Myosin-2	*MYH2*	1.57	3.22
P80473	Myomesin-1	*MYOM1*	1.57	3.87
E1BA44	Latent transforming growth factor beta binding protein 4	*LTBP4*	1.56	2.75
Q5E9F7	Cofilin-1	*CFL1*	1.54	2.36
Q2KIV8	Glutathione S-transferase	*GSTM3*	1.47	2.39
F1MLX6	AMP deaminase	*AMPD1*	1.40	2.98
Q3ZBH0	T-complex protein 1 subunit beta	*CCT2*	1.39	2.31
P02510	Alpha-crystallin B chain	*CRYAB*	1.37	4.41
P16116	Aldo-keto reductase family 1 member B1	*AKR1B1*	1.36	2.20
Q05JF3	Calsequestrin	*CASQ1*	1.33	3.19
Q04760	Lactoylglutathione lyase	*GLO1*	1.31	4.05
E1BI98	Collagen type VI alpha 1 chain	*COL6A1*	1.29	2.45
Q5E947	Peroxiredoxin-1	*PRDX1*	1.28	3.43
P00829	ATP synthase subunit beta, mitochondrial	*ATP5F1B*	1.28	2.95

E1BB91	Collagen type VI alpha 3 chain	*COL6A3*	1.24	5.53
P22226	Cathelicidin-1	*CATHL1*	1.20	3.73
P19803	Rho GDP-dissociation inhibitor 1	*ARHGDIA*	1.15	3.77
Q8SPX7	Lambda-crystallin homolog	*CRYL1*	1.14	3.69
Q2KJJ9	Fructose-1,6-bisphosphatase isozyme 2	*FBP2*	1.14	3.35
E1BNV1	Myosin binding protein C2	*MYBPC2*	1.14	3.34
O77834	Peroxiredoxin-6	*PRDX6*	1.13	2.17
O46629	Trifunctional enzyme subunit beta, mitochondrial	*HADHB*	1.08	3.42
Q2HJH1	Aspartyl aminopeptidase	*DNPEP*	1.07	3.06
A7E2Z4	Cadherin related family member 5	*CDHR5*	1.02	2.47
Q9XSJ4	Alpha-enolase	*ENO1*	0.99	2.30
Q32KY0	Apolipoprotein D	*APOD*	0.99	2.21
O18963	Cytochrome P450 2E1	*CP*	0.98	3.00
Q3SWX4	Glioblastoma amplified sequence	*NIPSNAP2*	0.97	3.63
A6QNM9	SLC25A12 protein	*SLC25A12*	0.94	2.09
E1BPV6	Smoothelin like 1	*SMTNL1*	0.94	2.76
A7E3S8	Heat shock 70kD protein binding protein	*ST13*	0.93	3.55
Q3ZBH2	NAD(P)H dehydrogenase [quinone] 1	*NQO1*	0.91	2.19
A4IFC3	Polyadenylate-binding protein	*PABPC4*	0.90	2.41
Q0VBZ3	Synaptophysin-like 2	*SYPL2*	0.82	2.14
Q3T0R7	3-ketoacyl-CoA thiolase, mitochondrial	*ACAA2*	0.81	2.48
Q148M6	Matrix remodeling-associated protein 8	*MXRA8*	0.77	2.69

P15497	Apolipoprotein A-I	*APOA1*	0.73	2.13
P48818	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	*ACADVL*	0.66	2.42
G3X778	Phosphorylase b kinase regulatory subunit	*PHKA1*	0.63	2.06
A3KN12	Adenylosuccinate lyase	*ADSL*	0.59	2.35
F1MPE5	Importin 5	*IPO5*	-0.60	2.12
Q3T0K2	T-complex protein 1 subunit gamma	*CCT3*	-0.63	2.39
Q32KV0	Phosphoglycerate mutase 2	*PGAM2*	-0.77	2.76
A5D7F4	PCDHGC3 protein	*PCDHGC3*	-0.79	2.17
G3N3C9	LIM domain binding 3	*LDB3*	-0.83	3.74
F1MWD3	T-complex protein 1 subunit epsilon	*CCT5*	-0.85	2.96
A0A1K0FUF3	Myoglobin	*GLNG*	-0.97	3.97
P62803	Histone H4	*H4*	-1.09	5.46
E1BM57	NHL repeat containing 3	*NHLRC3*	-1.14	2.79
Q13643	Four and a half LIM domains protein 3	*FHL1*	-1.32	7.29
S5G966	Large ribosomal subunit protein uL23	*RPL23AY*	-1.58	3.52

Proteins with positive log2(FC) values are more abundant in PDAb samples

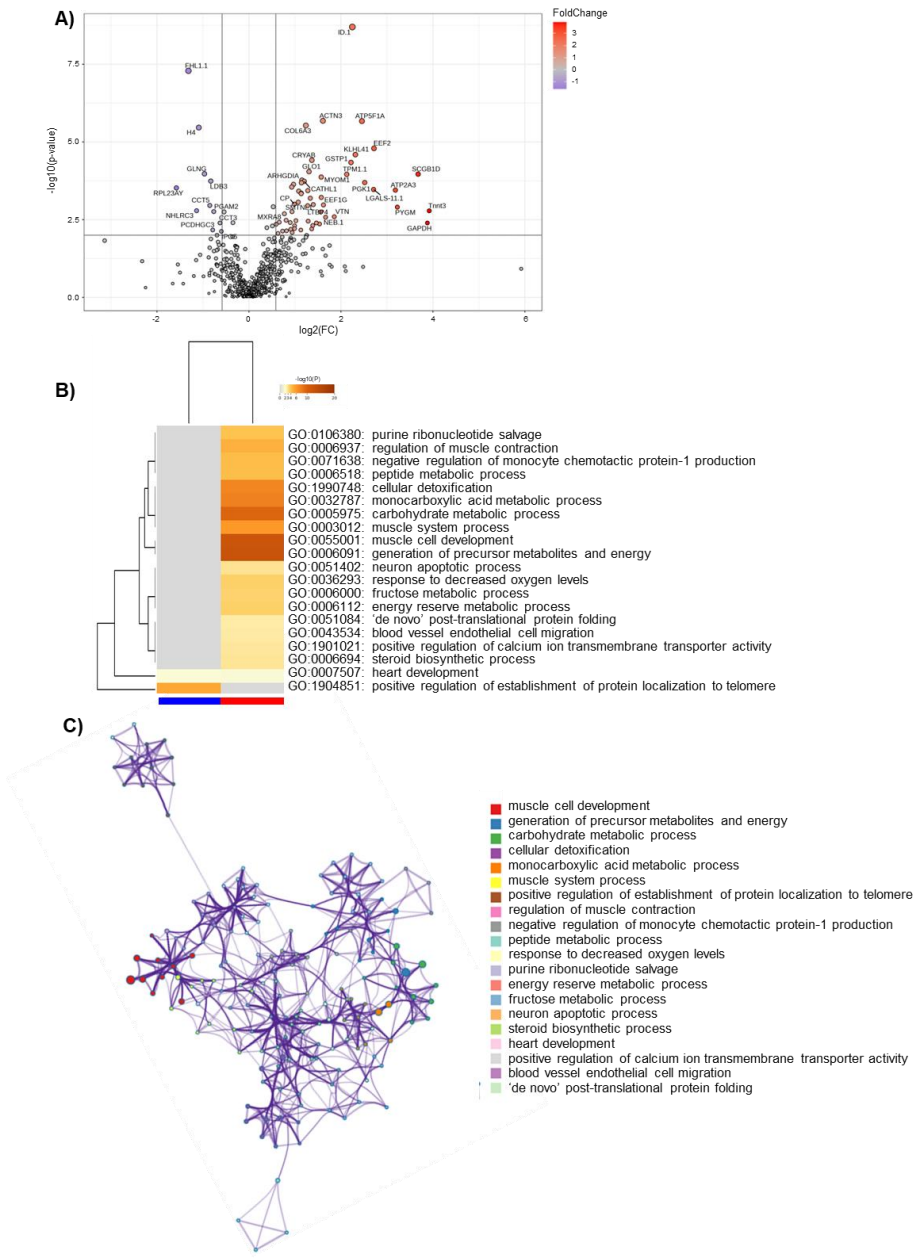


Figure 5. Statistical and bioinformatics analyses of the differentially abundant proteins (DAPs) in the pasture feed system between different aging types. A) Volcano plot of the DAPs highlighting the 68 proteins that were significantly different between pasture dry-aged (PD = PDAb) compared to pasture wet-aged (PW = PWA). The down-abundant (n = 11) in PWA and the up-abundant (57) in PDAb samples are shown in blue and red colors, respectively. B) Bioinformatics enrichment analyses based on Gene Ontology (GO) terms, using the 68 DAPs including the redundant proteoforms. C) Network layout based on the enriched terms of the 68 proteins. Each term is

represented by a circle node, with its size proportional to the number of input genes fall under that term, and its color represents the cluster identity. An edge links Terms with a similarity score > 0.3. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

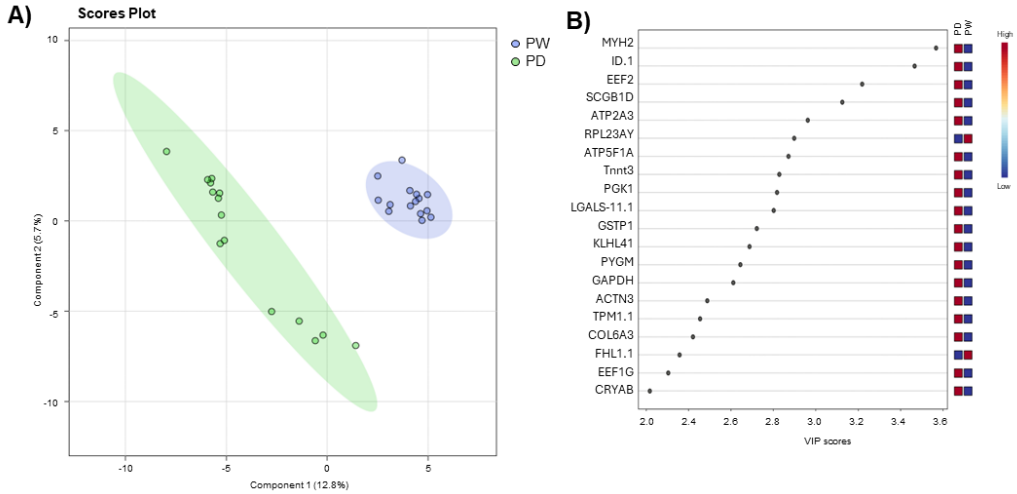


Figure 6. Discriminant analysis within the pasture-fed meat proteomes. A) Partial least squares-discriminant analysis (PLS-DA) score plot of pasture-fed meat proteome distribution according to aging type, PD (PDAb), and PW (PWA). B) Variable importance in projection (VIP) plot, top 20 values. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

6.3.4. Differentially abundant proteins (DAPs) comparing the concentrate-finished diet with different aging type

A total of 155 significantly different proteins were identified through volcano plot analysis between dry bag (CDAb) and wet aging (CWA) from the concentrate-finished diet (Fig. 7-A and Table 4). Fifty-eight proteins were more abundant (down) in wet aging from the concentrate-finished diet, and 97 proteins were more abundant (up) in dry bag aging from concentrate samples. The bioinformatics enrichment analyses (Gene Ontology) with 155 DAPs (Fig. 7-B and C) revealed 20 significantly enriched GO terms, where 9 terms were from CWA, from which two terms were specific for CWA (GO: 0007162 “negative regulation of cell adhesion” and GO: 0010756 “positive regulation of plasminogen activation”. Among the 18 GO terms from CDAb, the most important in abundance were: GO: 0003012 “muscle system

process", GO: 0006091 "generation of precursor metabolites and energy", GO: 0055001 "muscle cell development", and GO: 0019752 "carboxylic acid metabolic process". The PLS-discriminant analysis allowed the discrimination of the two combinations of treatment: CDAb and CWA samples. The plot (Fig. 8-A) shows a clear separation of samples into concentrate between dry bag and wet aging. Analyzing the 20 top proteins (VIP scores, Fig. 8-B) from the combination revealed three more abundant proteins in CWA (MYH7B, MASP2 and IPO5) and 17 in CDAb (TNNT3, ATP2A3, GADPH, PGK1, LGALS-11, PYGM, SCGB1D, EEF2, PGK1, KLHL41, ATP5F1A, CASQ1, CFL1, PKM2, TUBB2A, AMPD1 and MYOM1).

Table 4. Significant and differentially abundant proteins between concentrate dry-aged (CDAb) and concentrate wet-aged beef (CWA) samples from the shotgun SWATH-MS proteomics analysis. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

Uniprot ID	**Full protein names**	**Gene names**	**Log2 (FC)**	**-Log10 (p-value)**
Q8MKI3	Troponin T, fast skeletal muscle	*TNNT3*	5.40	15.16
P10096	Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	5.32	21.89
Q93084	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	*ATP2A3*	5.07	14.73
Q3T0P6	Phosphoglycerate kinase 1	*PGK1*	4.69	7.45
Q3T0P6	Phosphoglycerate kinase 1	*PGK1*	4.38	12.13
O75390	Citrate synthase, mitochondrial	*CS*	3.95	2.60
E3Q1Q2	Galectin	*LGALS-11*	3.84	15.11
P79334	Glycogen phosphorylase, muscle form	*PYGM*	3.71	22.52
P13639	Elongation factor 2	*EEF2*	3.54	10.99
P19483	ATP synthase subunit alpha, mitochondrial	*ATP5F1A*	3.45	16.17

F2Z4J1	Histone H2A	*H2AC6*	3.41	2.04
A0JNP2	Secretoglobin family 1D member	*SCGB1D*	3.35	11.46
O60662	Kelch-like protein 41	*KLHL41*	3.34	20.07
P31415	Calsequestrin-1	*CASQ1*	3.13	18.93
P14618	Pyruvate kinase PKM	*PKM2*	2.83	10.24
P23109	AMP deaminase 1	*AMPD1*	2.76	10.40
P81948	Tubulin alpha-4A chain	*TUBA4A*	2.71	5.23
F1MNF8	Tubulin alpha chain	*TUBA1A*	2.63	6.37
E1BJB1	Tubulin beta-2A chain	*TUBB2A*	2.59	9.33
Q5E9F7	Cofilin-1	*CFL1*	2.53	11.12
P28801	Glutathione S-transferase P	*GSTP1*	2.47	6.93
Q9GKN8	Prolargin	*PRELP*	2.26	3.65
Q04760	Lactoylglutathione lyase	*GLO1*	2.23	7.93
P20929	Nebulin	*NEB*	2.19	5.05
Q5KR49	Tropomyosin alpha-1 chain	*TPM1*	2.17	4.94
E1BNV1	Myosin-binding protein C2	*MYBPC2*	2.16	12.10
P80473	Myomesin-1	*MYOM1*	2.12	18.36
Q0VBZ3	Synaptophysin-like 2	*SYPL2*	2.10	8.36
E1BNE7	Caveolae-associated protein 1	*CAVIN1*	2.09	12.90
Q29RI2	phosphorylase kinase	*PHKG1*	1.81	11.26
P41563	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	*IDH3A*	1.81	11.84
Q3ZBA8	Protein NDRG2	*NDRG2*	1.78	5.97
P06394	Keratin, type I cytoskeletal 10	*KRT10*	1.75	2.44
Q9BE41	Myosin-2	*MYH2*	1.75	3.61
P00442	Superoxide dismutase [Cu-Zn]	*SOD1*	1.69	5.74
P12344	Aspartate aminotransferase, mitochondrial	*GOT2*	1.67	4.64

Q0III9	Alpha-actinin-3	*ACTN3*	1.65	11.44
P16116	Aldo-keto reductase family 1 member B1	*AKR1B1*	1.60	8.00
P10790	Fatty acid-binding protein, heart	*FABP3*	1.51	5.04
Q9BE39	Myosin-7	*MYH7*	1.50	8.13
Q5E947	Peroxiredoxin-1	*PRDX1*	1.50	7.88
P11966	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	*PDHB*	1.49	4.19
Q3SZB4	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	*ACADM*	1.48	10.53
Q00872	Myosin-binding protein C, slow-type	*MYBPC1*	1.48	11.01
Q0VC12	Protein YIPF3	*YIPF3*	1.42	2.22
Q3T149	Heat shock protein beta-1	*HSPB1*	1.39	6.40
E1BAJ4	Starch binding domain 1	*STBD1*	1.38	3.16
Q148D5	Succinate--CoA ligase [ADP-forming] subunit beta, mitochondrial	*SUCLA2*	1.37	8.88
P25708	NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial	*NDUFV1*	1.34	3.24
P19803	Rho GDP-dissociation inhibitor 1	*ARHGDIA*	1.33	4.56
Q3T0R7	3-ketoacyl-CoA thiolase, mitochondrial	*ACAA2*	1.32	5.38
A6QNM9	SLC25A12 protein	*SLC25A12*	1.30	4.12
A4IFC3	Polyadenylate-binding protein	*PABPC4*	1.28	5.33
Q99JY0	Trifunctional enzyme subunit beta, mitochondrial	*HADHB*	1.27	6.95

E1BI98	Collagen type VI alpha 1 chain	*COL6A1*	1.26	2.43
E1BB91	Collagen type VI alpha 3 chain	*COL6A3*	1.23	6.50
Q3Y5Z3	Adiponectin	*ADIPOQ*	1.22	6.53
P41134	DNA-binding protein inhibitor ID-1	*ID.1*	1.20	14.33
P45879	Non-selective voltage-gated ion channel VDAC1	*VDAC1*	1.20	7.38
Q2KHU5	ADP-ribose glycohydrolase MACROD1	*MACROD1*	1.19	2.26
P45879	Voltage-dependent anion-selective channel protein 1	*VDAC1*	1.17	6.13
Q3ZC55	Alpha-actinin-2	*ACTN2*	1.17	8.54
A7E3S8	Heat shock 70kD	*ST13*	1.15	4.86
Q27965	Heat shock 70 kDa protein 1B	*HSPA1B*	1.12	7.80
P27214	Annexin A11	*ANXA11*	1.11	6.97
Q08DP0	Phosphoglucomutase-1	*PGM1*	1.11	12.83
Q13642	Four and a half LIM domains protein 1	*FHL1*	1.10	8.64
P00829	ATP synthase subunit beta, mitochondrial	*ATP5F1B*	1.08	9.20
Q17QE2	LIM and cysteine-rich domains protein 1	*LMCD1*	1.06	5.84
Q07536	Methylmalonate-semialdehyde/malonate-semialdehyde dehydrogenase [acylating], mitochondrial	*ALDH6A1*	1.05	2.31
AGL	Glycogen debranching enzyme	*AGL*	1.02	7.16

F1MPR3	Calcium-transporting ATPase	*ATP2A2*	0.99	9.12
Q2TBU0	Haptoglobin	*HP*	0.98	2.56
Q3ZBT1	Transitional endoplasmic reticulum ATPase	*VCP*	0.97	3.78
P48818	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	*ACADVL*	0.95	5.51
F1MRQ7	SH3 domain binding glutamate-rich protein	*SH3BGR*	0.95	3.46
Q3SZV3	Elongation factor 1-gamma	*EEF1G*	0.93	3.04
Q3SYZ8	PDZ and LIM domain	*PDLIM3*	0.91	4.90
FBP2	Fructose-1,6-bisphosphatase isozyme 2	*FBP2*	0.91	6.16
Q9XSJ4	Alpha-enolase	*ENO1*	0.88	4.12
P19120	Heat shock cognate 71 kDa protein	*HSPA8*	0.85	9.49
P15497	Apolipoprotein A-I	*APOA1*	0.85	4.96
G3X778	Phosphorylase b kinase regulatory subunit	*PHKA1*	0.85	6.01
Q2HJD1	VIP36-like protein	*LMAN2*	0.85	2.16
P31081	60 kDa heat shock protein, mitochondrial	*HSPD1*	0.84	4.03
P80209	Cathepsin D	*CTSD*	0.83	2.43
A6QPQ2	Serpin A3-8	*SERPINA3-8*	0.81	3.30
A6QLL8	Fructose-bisphosphate aldolase	*ALDOA*	0.80	2.52
Q5KR47	Tropomyosin alpha-3 chain	*TPM3*	0.79	6.99
Q3ZCI9	T-complex protein 1 subunit theta	*CCT8*	0.78	3.58
Q8SQ24	Myozenin-1	*MYOZ1*	0.75	2.79
Q76LV2	Heat shock protein HSP 90-alpha	*HSP90AA1*	0.73	2.51

G3X6N3	Serotransferrin	*TF*	0.69	2.34
P07107	Acyl-CoA-binding protein	*DBI*	0.68	2.03
E1BF23	Myomesin 2	*MYOM2*	0.65	7.05
Q58CP0	Isocitrate dehydrogenase [NAD] subunit gamma, mitochondrial	*IDH3G*	0.63	2.78
Q17R06	Ras-related protein Rab-21	*RAB21*	0.60	2.01
A5PKM0	Glutathione S-transferase	*GSTM2*	-0.60	3.91
Q562R1	Beta-actin-like protein 2	*ACTBL2*	-0.64	2.30
Q2KJF1	Alpha-1B-glycoprotein	*A1BG*	-0.65	2.86
Q2KJ23	Bridging integrator 1	*BIN1*	-0.68	4.71
Q29437	Primary amine oxidase, liver isozyme	*SAO*	-0.68	4.13
P62803	Histone H4	*H4*	-0.68	4.05
A0A3Q1M390	Immunoglobulin-like and fibronectin type III domain containing 1	*IGFN1*	-0.71	2.28
Q5E9Z9	Nectin-4	*NECTIN4*	-0.74	5.39
Q9N282	MMP-9	*bmmp-9*	-0.74	2.05
Q5EA88	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	*GPD1*	-0.75	6.58
Q687I9	Purine nucleoside phosphorylase	*EDIP*	-0.79	4.10
A8YXZ2	C8G protein	*C8G*	-0.81	2.16
F1MZX6	Myosin heavy chain 13	*MYH13*	-0.81	2.49
Q92859	Neogenin	*NEO1*	-0.85	2.40
Q9XSC6	Creatine kinase M-type	*CKM*	-0.87	4.45
P19534	Cadherin-2	*CDH2*	-0.87	4.21
Q9MZ13	Voltage-dependent anion-selective channel protein 3	*VDAC3*	-0.89	3.11
A6QNS6	NID1 protein	*NID1*	-0.93	3.48

Q0P5D6	Retinoic acid receptor responder (Tazarotene induced) 1	*RARRES1*	-0.95	3.00
F1MYN5	Fibulin-1	*FBLN1*	-0.97	3.26
A0A3Q1LMV9	Epiplakin 1	*EPPK1*	-0.98	2.88
G3MZK7	Troponin C, skeletal muscle	*TNNC2*	-1.02	2.90
Q2KJ16	Phosphorylase b kinase gamma catalytic chain, liver/testis isoform	*PHKB*	-1.04	2.39
P04467	Calbindin	*CALB1*	-1.08	2.30
Q9XTA3	Myocilin	*MYOC*	-1.08	2.52
B8Y898	Malic enzyme	*ME1*	-1.11	2.01
F1MR86	Four and a half LIM domains 1	*FHL1*	-1.11	9.05
A1L5C6	HPN protein	*HPN*	-1.12	2.03
Q0VC16	Transport and Golgi organization protein 1 homolog	*MIA3*	-1.16	2.86
Q9GMB8	Serine--tRNA ligase, cytoplasmic	*SARS*	-1.19	3.77
E1BM57	NHL repeat containing 3	*NHLRC3*	-1.23	2.53
E1BCW0	HGF activator	*HGFAC*	-1.26	8.26
P79107	Low-affinity immunoglobulin gamma Fc region receptor III-A	*FCGR3A*	-1.26	2.97
P41361	Antithrombin-III	*SERPINC1*	-1.27	10.83
Q05204	Lysosome-associated membrane glycoprotein 1	*LAMP2*	-1.32	2.39
Q29630	CD74 molecule	*CD74*	-1.32	2.31
E1BG25	Melanotransferrin	*MELTF*	-1.33	2.49
E1BGJ5	CD93 molecule	*CD93*	-1.35	2.74
Q13951	Core-binding factor subunit beta	*CBFB*	-1.36	2.86

E3Q1Q2	Galectin	*LGALS-11*	-1.41	3.89
P02465	Collagen alpha-2(I) chain	*COL1A2*	-1.41	3.84
F1MQ74	Sushi domain containing 1	*SUSD1*	-1.43	2.20
Q6IM73	Reticulon	*RTN2*	-1.53	3.77
Q8SPF8	BPI fold-containing family B member 1	*BPIFB1*	-1.56	2.41
Q32PI5	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	*PPP2R2A*	-1.59	2.18
Q8WML4	Mucin-1	*MUC1*	-1.63	2.83
A6QLR9	Mitogen-activated protein kinase 14	*MAPK14*	-1.83	2.62
Q9BE40	Myosin-1	*MYH1*	-1.83	11.23
Q32LG3	Malate dehydrogenase, mitochondrial	*MDH2*	-1.87	5.58
F1MM32	Sulfhydryl oxidase	*QSOX1*	-2.17	5.29
P80929	Angiogenin-2	*ANG2*	-2.20	4.33
A5PJM4	Importin 5	*IPO5*	-2.36	7.45
E1BJ49	MBL associated serine protease 2	*MASP2*	-2.46	8.29
Q2KHW2	CD83 molecule	*CD83*	-2.70	6.45
Q3ZCJ2	Aldo-keto reductase family 1 member A1	*AK1*	-2.70	4.41
S5G966	Large ribosomal subunit protein uL23	*RPL23AY*	-2.72	3.28
E1BPX8	Myosin heavy chain 7B	*MYH7B*	-2.83	8.06
A7E2Y1	Myosin-7B	*TMEM151A*	-2.91	3.10

Proteins with positive log2(FC) values are more abundant in CDAb samples.

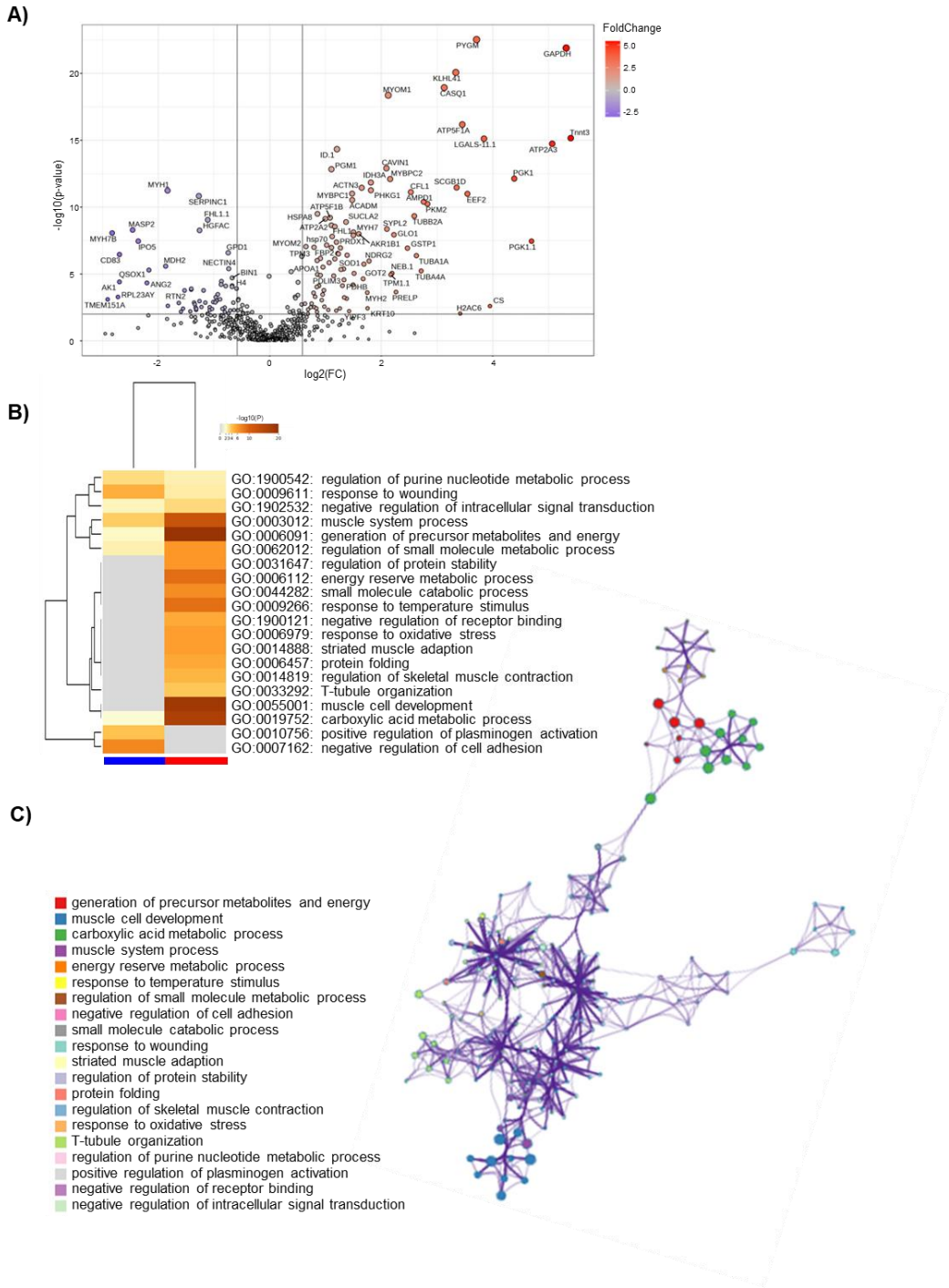


Figure 7. Statistical and bioinformatics analyses of the differentially abundant proteins (DAPs) in concentrate feed systems between different aging types. A) Volcano plot of the DAPs highlighting the 155 proteins that were significantly different between the concentrate dry-aged (CDAb) compared to concentrate wet-aged (CWA). The down-abundant (n = 58) in CWA and the up-abundant (n = 97) in CDAb

samples are shown in blue and red colors, respectively. B) Bioinformatics enrichment analyses based on Gene Ontology (GO) terms, using the 155 DAPs including the redundant proteoforms. C) Network layout based on the enriched terms of the 155 proteins. Each term is represented by a circle node, with its size proportional to the number of input genes fall under that term, and its color represents the cluster identity. Terms with a similarity score > 0.3 are linked by an edge. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

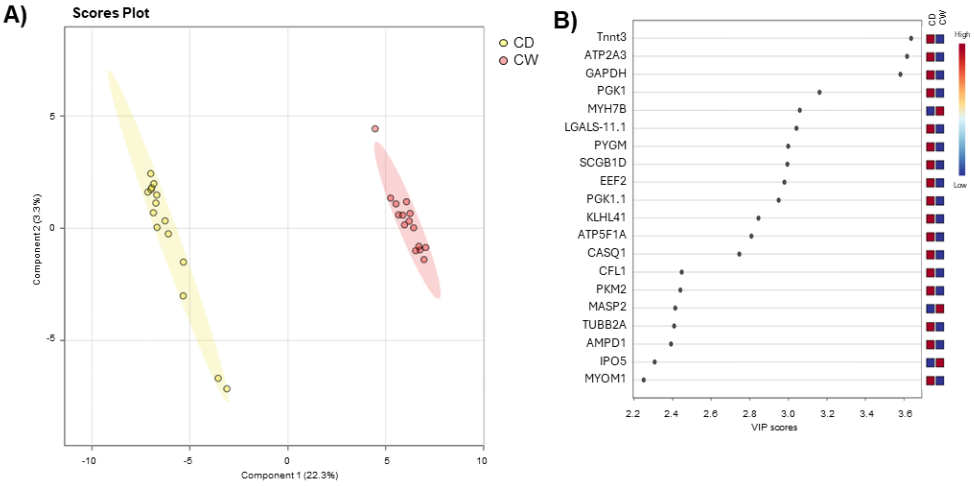


Figure 8. Discriminant analysis within the concentrated fed meat proteomes. A) Partial least squares-discriminant analysis (PLS-DA) score plot of concentrated fed meat proteome distribution according to aging type: CD (CDAb) and CW (CWA). B) Variable importance in projection (VIP) plot top 20 values. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

6.3.5. Putative biomarkers identification between the four groups analyzed: pasture (dry bag and wet) and concentrate (dry bag and wet)

The PLS-discriminant analysis allowed the discrimination of the four combinations of treatment: PDAb, PWA, CDAb, and CWA samples (Fig. 9-A). Analyzing the 20 top proteins (VIP scores, Fig. 9-B) from the combination revealed 17 more abundant proteins in CDAb, two in PWA (RPL23AY and IPO5), and one in CWA (MYH7B). The PLS-discriminant analysis also allowed the discrimination of the aging types: dry bag and wet aging samples (Fig. 1S-A), and the finished diet: pasture and concentrate (Fig. 2S-A). The top 20 proteins (VIP scores, Fig. 1S-B) in

aging types revealed two abundant proteins in wet (MYH7B and RPL23AY), whereas 18 abundant proteins in dry bag aging (ATP2A3, TNNT3, GAPDH, EEF2, SCGB1D, PGK1, LGALS-11, KLHL41, PYGM, ATP5F1A, MYH2, GSTP1, CASQ1, PGK1, CFL1, ID, MYOM1 and AMPD1). The top 20 proteins (VIP scores, Fig. 2S-B) in the finished diet revealed three abundant proteins in the pasture (MYH7B, RPL23AY and IPO5) and 17 abundant proteins in concentrate (TPM1.1, PGK1, TPM1, CRYAB, ATP5F1B, TNNT3, TPM3, PKM2, ID, TUBA4A, HSPB6, PCMT1, GAPDH, FHL1, PYGM, PGM1 and ATP5F1A).

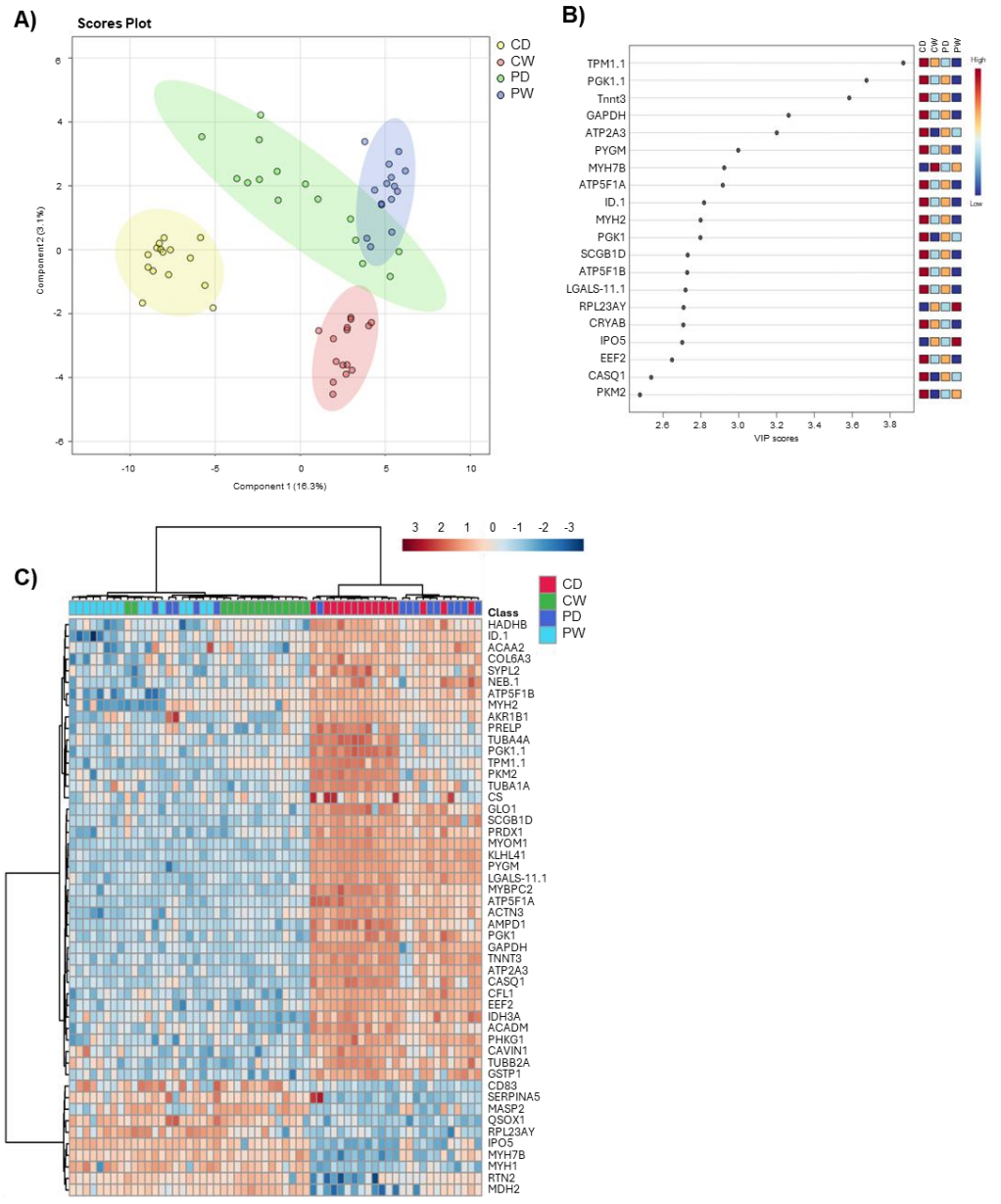


Figure 9. Discriminant analysis within the four analyzed groups concentrated fed meat proteomes: CD (CDAb), CW (CWA), PD (PDAb), and PW (PWA). A) Partial least squares-discriminant analysis (PLS-DA) score plot of meat proteome distribution according to aging type and production system. B) Variable importance in projection (VIP) plot top 20 values. C) Heatmap of top 50 PLS-DA VIP analyzed by hierarchical clustering. Each row represents a single protein. Each column represents an individual sample. Protein expression values were log2-normalized, and cluster analysis was performed using Z-score. Red indicates a low expression level; green indicates a high expression level. Meat was aged at 2 ± 0.5°C, 85 ± 5% RH, and air flow of 0.5 m/s.

6.4. Discussion

The present study evaluated proteomic changes in response to two different finishing diets (concentrate vs. pasture) and two aging methods (dry bag vs. wet) over a 40-day period. The results highlight the impact of post-mortem processing on proteomic changes and their potential influence on eating quality (Bischof et al., 2022). The analysis identified key differentially abundant proteins (DAPs) and focused on the 20 proteins with the highest variable importance in projection (VIP).

6.4.1. Effect of Aging Methods on the Proteomic Muscle *LTL*

The change in the proteome is a continuous process resulting from proteolytic activity that occurs during tenderization to counteract rigor mortis (Bischof et al., 2022). The study revealed notable differences between dry bag and wet aging, with most VIP proteins being more abundant in dry-aged samples. Structural muscle proteins (7), energy metabolism proteins (7), and other pathways (6) were identified. In wet-aged, key proteins, including MYH7B (muscle structure-related) and RPL23AY (oxidative stress-related). The most relevant six proteins related to dry bag aging are involved in energy metabolism (ATP2A3, GAPDH, and PGK1), structural muscle cells (TNNT3), and other pathways (EEF2 and SCGB1D). TNNT3 has been extensively used to monitor the extent of proteolysis during aging, as it is a marker of proteolysis and beef tenderness (Gagaoua, Troy et al., 2021). It was one of the most significant proteins in dry-aged samples. Energy metabolism proteins (GAPDH,

PGK1) indicated metabolic shifts due to oxygen deprivation in the direction of a glycolytic pathway and increased aerobic and oxidative pathways (Gagaoua, Terlouw et al., 2021).

6.4.1.1. Proteome muscle difference between pasture and concentrate finished diet aged in a dry bag

Muscle Structure-Related Proteins. Nine of the 20 VIP proteins were associated with muscle structure, with eight more abundant in concentrate-fed samples. troponin T (TNNT3) and tropomyosin (TPM1) are part of a complex, being crucial for muscle contraction, and responding to Ca2+ ion concentration (Cooper & Hausman, 2000). The breakdown of key myofibrillar and cytoskeletal proteins due to endogenous proteolytic systems' action is central to meat tenderization (Ertbjerg, 2022). MYH7B, a slow-twitch myosin belonging to the thick filament of the sarcomere, was more abundant in pasture-fed cattle, indicating a preference for oxidative metabolism (Gerrard & Grant, 2006). These findings align with prior research indicating increased oxidative metabolism enzymes in pasture compared to concentrate-fed cattle (Shibata et al., 2009).

Energy Metabolism-Related Proteins. The post-mortem anoxic condition shifts energy metabolism toward glycolytic and oxidative pathways (Gagaoua, Terlouw et al., 2021). The most relevant proteins (PYGM, GAPDH, PGK1, PKM and PKM2) belonging to glycolytic metabolism (Gagaoua, Terlouw et al., 2021) and ATP metabolism (ATP2A3, ATP5F1A and AMPD1) were observed in higher abundance in concentrate compared to pasture-finished diet in dry bag aging. PYGM is a key enzyme that regulates glycogen breakdown in the muscle and has been reported in several dark-cutting proteomics studies with about 50% reduction in expression in dark-cutting muscles in comparison to normal-pH beef (Kiyimba et al., 2023; Fuente-García et al., 2021; Gagaoua, Warner et al., 2021; Wu et al., 2020). GAPDH, PGK1, and PKM exhibited significantly higher expression in fast-twitch fibers characterized by high glycolytic activity (Okomura et al., 2005). They were identified in dry-aged concentrate-fed samples as a potential biomarker highlighting their crucial role in beef tenderness (Gagaoua, Terlouw et al., 2021). Prior studies indicated that PGK1

correlates negatively with shear force, supporting its role in tenderness development (Silva et al., 2019). PKM was mainly positively associated with tenderness for bulls and steers compared to heifers (Gagaoua, Terlouw et al., 2021). PKM catalyzes the dephosphorylation of phosphoenolpyruvate to pyruvate, and a higher PKM abundance is likely to reflect a potentially higher production of pyruvate. Furthermore, it was shortlisted as a beef color biomarker (Gagaoua, Hughes, et al., 2020). Proteins related to the ATP metabolism system, such as ATP2A3 play a role in calcium ion regulation within muscle cells, essential for muscle contraction and relaxation; this could be significant to beef quality because it helps to maintain calcium homeostasis, which affects protease activity in the aging process and could also impact muscle tenderness post-mortem. AMPD1 plays a crucial role in the energy metabolism of skeletal muscle cells in cattle and other species, as a potential activator for rate-limiting enzymes in the glycolytic pathway (Baker et al., 2010). This process is relevant during periods of high energy demand, such as intense exercise, because it promotes the regeneration of ATP from ADP via the myokinase reaction (Ronca and Raggi, 2018). Antonelo et al. (2022) identified more abundant AMPD1 and PYGM in feedlot high-growth animals than in feedlot low-growth animals, for non-aged meat. The overabundance of the glycolytic pathway and ATP metabolism-related proteins in concentrate compared to pasture finishing diet could be due to increased energy demands on muscle cells (England et al., 2016).

Protein biosynthesis, apoptosis, and transport proteins. The current study shows an abundance of IPO5, a nuclear transport protein, in pasture compared to a concentrate-finished diet aged in a dry bag. Li et al. (2021) described a large dataset of multi-tissue cattle gene expressions and reported that IPO5 was one of the top five molecular markers for predicting meat quality at a given time. In contrast, LGALS-1, which is more abundant in CDAb than in PDAb, reported interactions between LGALS-11 and SOD1 (oxidative stress protein) in an integrative "omics" ('integromics') study by Gagaoua, Terlouw et al. (2021), using a meta-analysis approach with data collected from the longissimus muscle.

6.4.1.2. Proteome muscle difference between pasture and concentrate finished diet in wet aging meat

Muscle Structure-Related Proteins. Eighteen DAPs were identified, with significant GO terms including “muscle filament sliding” and “protein refolding.” TPM1 remains abundant in the dry aging combination, while TPM3, MHY2, and ACTN3 emerge as newly identified structural-related proteins. In previous findings, TPM1 and TPM3 have been identified as critical for sarcomere contraction and tenderness biomarkers (Gagaoua et al., 2021).

Energy Metabolism-Related Proteins. Two energy metabolism proteins, ATP5F1B (oxidative phosphorylation) and GOT2 (TCA cycle), were more abundant in concentrate-fed samples. GOT2 has been linked to oxaloacetate production and beef color stability (Ramanathan et al., 2021), and it is a potential biomarker of the dark-cutting beef defect (Gagaoua, Terlouw et al., 2021); however, contrasting results have been reported (Sentandreu et al., 2021). ATP5F1B is again more abundant in concentrate with wet aging than CDAb. The association of ATP5F1B with mitochondrial function aligns with its proposed role in dark-cutting beef, a condition resulting from an elevated ultimate pH (Bismarck et al., 2023). However, mitochondrial activity persists when pH remains high, leading to increased oxygen consumption and reduced partial pressure (Gagaoua, Warner et al., 2021).

Heat Shock Proteins (HSPs) and Other Pathways. The wet aging process induced, probably, oxidative stress responses, with small HSPs (CRYAB, HSPB6; overabundance in CW) playing a protective role (Picard and Gagaoua, 2017). HSPs are mostly chaperones, essential for normal cell function, and enable cells to resist stress (Fink, 1999). Their relationships with tenderness were positive and negative, possibly due to other factors such as differences in pre-slaughter stress levels, breed, gender, and/or the type of isoform involved (Mato et al., 2019; Picard & Gagaoua, 2020). Small HSPs can bind to myofibrils (Lomiwes et al., 2014; Ma & Kim, 2020), thereby protecting skeletal muscle through structural protein complexes. These proteins have also been identified to be significant for beef color development and stability (Gagaoua, Hughes et al., 2020). Other proteins observed, such as ID and DEFB1, possibly belong to the term “negative regulation of cold-induced

thermogenesis". Negative regulation of this process involves any factor that reduces or inhibits this heat production, including changes in the activity of genes, proteins, or specific metabolic pathways (Ji et al., 2022).

6.4.2. Effect of Finishing Diet on the Proteomic Muscle *LTL*

Diet significantly impacts skeletal muscle biochemistry and beef quality (Apaoblaza et al., 2020). A proteomic analysis of concentrate- and pasture-finished diets revealed differences in muscle structure proteins (8), energy metabolism proteins (7), and other pathways (5). The most abundant proteins were from the concentrate finishing diet, where TPM1 and TNNT3 (muscle structure), PGK1 and ATP5F1A (energy metabolism), and CRYAB (HSPs) emerged as the key top five proteins, while MYH7B, IPO5, and RPL23AY were abundant in the pasture-finishing diet.

6.4.2.1. Proteome muscle differences between dry and wet aging in pasture-finished diet

Muscle Structure-Related Proteins. MYH2, TNNT3, ACTN3, TPM1, and FHL1 were more abundant in dry-aged pasture-fed beef. The first four proteins were identified as abundant in dry aging or concentrate in their various combinations and are noted for their new abundance, while the FHL1 protein is present in pasture-wet aging (PW). Many studies (Beldarrain et al., 2018; Boudon et al., 2020; D'Alessandro, Marrocco et al., 2012; D'Alessandro, Rinalducci et al., 2012; Gagaoua, Bonnet, et al., 2020; Laville et al., 2009; Malheiros et al., 2019) reported that all three subunits of the troponin complex are more abundant in tender than in tough beef. This suggests a more active proteolytic process in dry aging compared to wet aging, contributing to tenderness. The most relevant protein was MYH2, which was observed in higher abundance in the dry bag than in wet aging in the pasture. When compared to different diets under the same aging type (WA), the overabundance was found in concentrate-finished diet (CWA) samples, highlighting the consistency of low abundance (or down-regulation) in pasture and wet (PWA). MHY2 was identified as a candidate biomarker of meat tenderness (Della Malva et al., 2022; Gagaoua, Terlouw et al., 2021). FHL1 facilitates a complex association of signaling proteins with the actin cytoskeleton, and the proteolysis of FHL1 is related to the release of α-actinin

(ACTN3: identified as a negative biomarker in Gagaoua et al., 2018 and Boudon et al., 2020) from myofibrils and contributes to the weakening of the Z-disc during meat tenderization (Morzel et al., 2004).

Energy Metabolism-Related Proteins. In dry-aged samples, ATP metabolism proteins (ATP2A3, ATP5F1A) and glycolytic enzymes (PGK1, GAPDH, PYGM) were abundant. In contrast, the last glycolytic proteins, which were overabundant in PD, were also reported as abundant in CDAb compared to pasture-dry aging (PDAb). This highlights the importance of dry aging, suggesting a glycolytic shift that potentially contributes to differences in pH and tenderness (Dhar-Chowdhury et al., 2005), as previously discussed. The low abundance of PGYM and other glycogenolytic enzymes suggests a limited capacity for the muscles to mobilize and utilize glycogen, resulting in greater pH levels post-mortem. PGK1 and GADPH exhibited much higher expression in fast-twitch fibers, which are characterized by high glycolytic activity (Okumura et al., 2005).

Heat Shock Proteins and Oxidative Stress Proteins. CRYAB was overabundant in the dry-aged bag compared to the wet in the pasture, and the previous combination treatment was overabundant in concentrate compared to the pasture-finished diet aged in wet. The combination PWA was low in abundance in both situations. It is a small HSP with a role in muscle-to-meat conversion and meat tenderness or other quality traits that have been extensively studied (Gagaoua, Hughes et al**.,** 2020; Lomiwes et al., 2014; Ma & Kim, 2020; Picard & Gagaoua, 2017). RPL23AY, in contrast to CRYAB, was identified as overabundant in pasture and wet aging compared to CWA and PDAb. RPL23AY is known to respond to oxidative stress, which increases post-mortem due to oxygen depletion (Ubaida-Mohien et al., 2019). This may suggest that several proteins experience changes during post-mortem metabolism, as a resilient response of muscle cells to energy deprivation (Ouali et al., 2013). Extensive research in meat science has focused on identifying various proteins involved in structural integrity, metabolic pathways, and oxidative stress as potential indicators of beef quality attributes, such as tenderness, marbling, and flavor. However, RPL23AY protein has not been directly established as a biomarker for beef quality in some proteomics studies (Gagaoua, Bonnet et al., 2020; Bonnet et al., 2020).

6.4.2.2. Proteome muscle differences between dry and wet aging in concentrate-finished diet

Muscle Structure-Related Proteins. TNNT3 and KLHL41 were more abundant in dry-aged than wet from concentrate-fed beef, while MYH7B was more abundant in wet than dry bag aging in this diet. Similar to the complex of tropomyosin and troponin, KLHL41 is an integral protein of the thin filament (Gagaoua, Terlouw et al., 2021) that interacts with proteins like nebulin to ensure proper alignment and stabilization of muscle fibers. This could have implications for meat tenderness, a key attribute of meat quality.

Energy Metabolism-Related Proteins. GAPDH, PGK1, and PKM2 were the most abundant glycolytic proteins in dry-aged concentrate-fed samples. PKM2, involved in pyruvate production, has been suggested as a biomarker for meat color stability (Ramanathan et al., 2019). ATP2A3 transports calcium ions from the cytosol into the sarcoplasmic/endoplasmic reticulum lumen, contributing to calcium sequestration in muscular excitation/contraction. Among the energy metabolism proteins identified as candidate biomarkers of energy metabolism-related in goats (Lamri, Della Malva, Djenane, Albenzio et al., 2023), ATP5F1A was also identified with water holding capacity (WHC) and drip loss (negative correlation). It has been intensively documented and is thought to be primarily related to the impact on the denaturation of proteins due to the acidic conditions in the post-mortem muscle (Huff-Lonergan and Lonergan, 2007).

Other pathways: immunology, binding, apoptosis, and transport proteins. MASP2 and IPO5 were two of three proteins (20-VIP) identified as abundant in wet compared to dry bag aging in the concentrate-finished diet. Previously, when comparing only the finishing diet, IPO5 was overabundant in pasture compared to concentrate, and this result was repeated when aged in the dry bag. This denotes the low abundance in concentrate and dry bag aged combination. SCGB1D, EEF2, and LGALS-11.1 were abundant in a dry bag compared to wet aging in a concentrate-finished diet. However, they were also identified as abundant in dry bags compared to wet aging in the pasture-finished diet.

6.5. Conclusion

The study identified proteomic shifts associated with aging methods and finishing diets. Dry bag aging resulted in a higher abundance of proteins related to muscle structure, energy metabolism, and proteolysis, likely enhancing tenderness. Wet aging led to the expression of incremental heat shock proteins (HSPs) and oxidative stress-related proteins, suggesting a response to environmental stressors during the aging process.

Concentrate-finished beef exhibited greater glycolytic enzymes, which may contribute to improved tenderness and color stability, denoting a shift toward anaerobic metabolism. Pasture-finished beef increased oxidative metabolism proteins, aligning with the greater physical activity of grass-fed cattle, which may result in darker meat and slightly lower tenderness.

TPM1, PGK1, TNNT3, GAPDH, ATP2A3, and PYGM in concentrate and dry aging were the top six proteins, followed by MYH7B overabundance in concentrate and wet aging. Finally, RPL23AY and IPO5 were the most important in pasture and wet aging. These proteins emerged as key biomarkers of diet and aging effects, warranting further investigation into their role in beef quality.

This research provides a comprehensive understanding of how aging and diet influence beef quality at the proteomic level. However, future studies should further explore the identified biomarkers and validate their role in predicting beef quality across different breeds and production systems.

6.6. References

Ahnström, M. L., Seyfert, M., Hunt, M. C., & Johnson, D. E. (2006). Dry aging of beef in a bag highly permeable to water vapour. *Meat Science*, 73(4), 674-679.https://doi.org/10.1016/j.meatsci.2006.03.006

Álvarez, S., Mullen, A. M., Álvarez, C., Hamill, R. M., O'Neill, E., & Gagaoua, M. (2023). Impact of sampling location and aging on the Longissimus thoracis et lumborum muscle proteome of dry-aged beef. *Meat Science*, 205, 109315. https://doi.org/10.1016/j.meatsci.2023.109315

Antonelo, D. S., Gómez, J. F., Silva, S. L., Beline, M., Zhang, X., Wang, Y., ... & Balieiro, J. C. (2022). Proteome basis for the biological variations in color and tenderness of *longissimus thoracis* muscle from beef cattle differing in growth rate and feeding regime. *Food Research International*, 153, 110947. https://doi.org/10.1016/j.foodres.2022.110947

Apaoblaza, A., Gerrard, S. D., Matarneh, S. K., Wicks, J. C., Kirkpatrick, L., England, E. M., ... & Gerrard, D. E. (2020). Muscle from grass-and grain-fed cattle differs energetically. *Meat Science*, 161, 107996. https://doi.org/10.1016/j.meatsci.2019.107996

Baker, J. S., McCormick, M. C., & Robergs, R. A. (2010). Interaction among skeletal muscle metabolic energy systems during intense exercise. *Journal of Nutrition and Metabolism* (1) 905612.https://doi.org/10.1155/2010/905612

Beldarrain, L. R., Aldai, N., Picard, B., Sentandreu, E., Navarro, J. L., & Sentandreu, M. A. (2018). Use of liquid isoelectric focusing (OFFGEL) on the discovery of meat tenderness biomarkers. *Journal of Proteomics*, 183, 25-33. https://doi.org/10.1016/j.jprot.2018.05.005

Bischof, G., Witte, F., Terjung, N., Heinz, V., Juadjur, A., & Gibis, M. (2022). Metabolic, proteomic and microbial changes postmortem and during beef aging. Critical Reviews in *Food Science and Nutrition*, 64(4), 1076-1109. https://doi.org/10.1080/10408398.2022.2113362

Bischof, G., Witte, F., Terjung, N., Januschewski, E., Heinz, V., Juadjur, A., & Gibis, M. (2021). Analysis of aging type-and aging time-related changes in the polar fraction of metabolome of beef by 1H NMR spectroscopy. *Food Chemistry*, 342, 128353. https://doi.org/10.1016/j.foodchem.2020.128353

Bismarck, S., Baldassini, W., Neto, O. M., Chardulo, L. A., Torres, R., Pereira, G., ... & Gagaoua, M. (2023). Post-mortem muscle proteome of crossbred bulls and steers: Relationships with carcass and meat quality. *Journal of Proteomics*, 278, 104871. https://doi.org/10.1016/j.jprot.2023.104871

Bonnet, M., Soulat, J., Bons, J., Léger, S., Dekoning, L., Carapito, C. & Picard, B. (2020). Quantification of biomarkers for beef meat qualities using a combination

of parallel 2 reaction monitoring- and antibody-based proteomics. *Food Chemistry*, 317, 126376. 10.1016/j.foodchem.2020.126376.

Boudon, S., Ounaissi, D., Viala, D., Monteils, V., Picard, B., & Cassar-Malek, I. (2020). Label free shotgun proteomics for the identification of protein biomarkers for beef tenderness in muscle and plasma of heifers. *Journal of Proteomics*, 217, 103685. https://doi.org/10.1016/j.jprot.2020.103685

Bouley, J., Chambon, C., & Picard, B. (2004). Mapping of bovine skeletal muscle proteins using two-dimensional gel electrophoresis and mass spectrometry. *Proteomics*, *4*(6), 1811-1824. https://doi.org/10.1002/pmic.200300688

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Chantada-Vázquez, M.d.P., García Vence, M., Serna, A., Núñez, C., Bravo, S.B. (2021). SWATH-MS Protocols in Human Diseases. In: Carrera, M., Mateos, J. (eds) Shotgun Proteomics. Methods in Molecular Biology, vol 2259. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-1178-4_7

Cooper, G. M., & Hausman, R. (2000). A molecular approach. The Cell. 2nd ed. Sunderland, MA: Sinauer Associates, 1394-403.

D'Alessandro, A., Rinalducci, S., Marrocco, C., Zolla, V., Napolitano, F., & Zolla, L. (2012). Love me tender: An Omics window on the bovine meat tenderness network. *Journal of proteomics*, 75(14), 4360-4380. https://doi.org/10.1016/j.jprot.2012.02.013

D'Alessandro, A., Marrocco, C., Rinalducci, S., Mirasole, C., Failla, S., & Zolla, L. (2012). Chianina beef tenderness investigated through integrated Omics. *Journal of Proteomics*, 75(14), 4381-4398. https://doi.org/10.1016/j.jprot.2012.03.052

De Faria Vilella, G., Gomes, C.L. Battaglia, C.T. Pacheco, M.T.B., da Silva, V.S.N., Rodas-González, A. & S.B. Pflanzer. (2019). Effects of combined wet- and dry-aging techniques on the physicochemical and sensory attributes of beef ribeye

steaks from grain-finished crossbred Zebu steers. *Canadian Journal of Animal Science* 99: 497-504. dx.doi.org/10.1139/cjas-2018-0127

DeGeer, S. L., Hunt, M. C., Bratcher, C. L., Crozier-Dodson, B. A., Johnson, D. E., & Stika, J. F. (2009). Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. *Meat Science*, 83(4), 768-774. https://doi.org/10.1016/j.meatsci.2009.08.017

Dhar-Chowdhury, P., Harrell, M. D., Han, S. Y., Jankowska, D., Parachuru, L., Morrissey, A., ... & Coetzee, W. A. (2005). The glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate isomerase, and pyruvate kinase are components of the KATP channel macromolecular complex and regulate its function. *Journal of Biological Chemistry*, 280(46), 38464-38470. https://doi.org/10.1074/jbc.M508744200

Di Luca, A., Hamill, R. M., Mullen, A. M., Slavov, N., & Elia, G. (2016). Comparative proteomic profiling of divergent phenotypes for water holding capacity across the post mortem ageing period in porcine muscle exudate. *PloS One*, 11(3), e0150605. https://doi.org/10.1371/journal.pone.0150605

England, E. M., Matarneh, S. K., Oliver, E. M., Apaoblaza, A., Scheffler, T. L., Shi, H., & Gerrard, D. E. (2016). Excess glycogen does not resolve high ultimate pH of oxidative muscle. *Meat Science*, 114, 95-102. https://doi.org/10.1016/j.meatsci.2015.10.010

Ertbjerg, P. (2022). Current understanding on the role of proteolysis on meat quality. In P. Purslow (Ed.), New Aspects of Meat Quality (pp. 95-114). Woodhead Publishing. https://doi.org/10.1016/B978-0-323-85879-3.00022-2

Fuente-García, C., Sentandreu, M. A., Aldai, N., Oliván, M., & Sentandreu, E. (2021). Proteomic pipeline for biomarker hunting of defective bovine meat assisted by liquid chromatography-mass spectrometry analysis and chemometrics. *Journal of Proteomics*, 238, 104153. https://doi.org/10.1016/j.jprot.2021.104153

Gagaoua, M., Monteils, V., Couvreur, S., & Picard, B. (2017). Identification of biomarkers associated with rearing practices, carcass characteristics, and beef quality: An integrative approach. *Journal of Agricultural and Food Chemistry*, 65(37), 8264-8278. https://doi.org/10.1021/acs.jafc.7b03239

Gagaoua, M., Bonnet, M., De Koning, L., & Picard, B. (2018). Reverse phase protein array for the quantification and validation of protein biomarkers of beef qualities: The case of meat color from Charolais breed. *Meat Science*, *145*, 308–319. https://doi.org/10.1016/j.meatsci.2018.06.039

Gagaoua, M., Picard, B., & Monteils, V. (2019). Assessment of cattle inter-individual cluster variability: the potential of continuum data from the farm-to-fork for ultimate beef tenderness management. *Journal of the Science of Food and Agriculture*, 99(8), 4129-4141.https://doi.org/10.1002/jsfa.9643

Gagaoua, M. Bonnet, M. & Picard, B. (2020). Protein Array-Based Approach to Evaluate Biomarkers of Beef Tenderness and Marbling in Cows: Understanding of the Underlying Mechanisms and Prediction. *Foods* 2020, 9(9), 1180. https://doi.org/10.3390/foods9091180

Gagaoua, M., Hughes, J., Terlouw, E.M. C., Warner, R., D., Purslow P., Lorenzo, J. M., & Picard, B. (2020). Proteomic biomarkers of beef colour. *Trends in Food Science & Technology*. 101 234-252.https://doi.org/10.1016/j.tifs.2020.05.005

Gagaoua, M., Terlouw, E. C., Mullen, A. M., Franco, D., Warner, R. D., Lorenzo, J. M., ... & Picard, B. (2021). Molecular signatures of beef tenderness: Underlying mechanisms based on integromics of protein biomarkers from multi-platform proteomics studies. *Meat Science*, 172, 108311. https://doi.org/10.1016/j.meatsci.2020.108311

Gagaoua, M., Troy, D., & Mullen, A. M. (2021). The extent and rate of the appearance of the major 110 and 30 kDa proteolytic fragments during post-mortem aging of beef depend on the glycolysing rate of the muscle and aging time: An LC–MS/MS approach to decipher their proteome and associated pathways. *Journal of Agricultural and Food Chemistry*, 69(1), 602-614. https://doi.org/10.1021/acs.jafc.0c06485

Gagaoua, M., Warner, R. D., Purslow, P., Ramanathan, R., Mullen, A. M., López-Pedrouso, M., … Terlouw, E. M. C. (2021). Dark-cutting beef: A brief review and an integromics meta-analysis at the proteome level to decipher the

underlying pathways. *Meat Science*, 181, 108611. https://doi.org/10.1016/j.meatsci.2021.108611

Gagaoua, M., Duffy, G., Alvarez, C., Burgess, C. M., Hamill, R., Crofton, E., Botinestean, C., Ferragina, A., Cafferky, J., Mullen, A. M., & Troy, D. (2022). Current research and emerging tools to improve fresh red meat quality. *Irish Journal of Agricultural and Food Research*, *61*(1), 145–167. https://www.jstor.org/stable/27225939

Gagaoua, M., Schilling, W.M., Zhang, X., & Suman, S.P. (2022). Applications of proteomics in meat research. Elsevier Ltd. https://doi.org/10.1016/B978-0-323-85125-1.00123-XHenchion, M. M., McCarthy, M., & Resconi, V. C. (2017). Beef quality attributes: A systematic review of consumer perspectives. *Meat Science*, 128, 1-7. https://doi.org/10.1016/j.meatsci.2017.01.006

Huff-Lonergan, & E. Lonergan, S.M. (2007) New frontiers in understanding drip loss in pork: recent insights on the role of postmortem muscle biochemistry, J. Anim. Breed. Genet. 124 (s1) 19-26.https://doi.org/10.1111/j.1439-0388.2007.00683.x

INAC (Instituto Nacional de Carne) 2013. Hilton. Reglamento (EU) 593/2013. Available at https://www.inac.uy/innovaportal/v/9944/14/innova.front/hilton [Accessed 29 March 2024]

Ji, K.; Jiao, D.; Yang, G.; Degen, A.A.; Zhou, J.; Liu, H.; Wang, W.& Cong, H. (2022). Transcriptome analysis revealed potential genes involved in thermogenesis in muscle tissue in cold-exposed lambs. *Front. Genet*, 13, 1017458. https://doi.org/10.3389/fgene.2022.1017458

Kim, J. H., Kim, T. K., Shin, D. M., Kim, H. W., Kim, Y. B., & Choi, Y. S. (2020). Comparative effects of dry-aging and wet-aging on physicochemical properties and digestibility of Hanwoo beef. *Asian-Australasian Journal of Animal Sciences*, 33(3), 501. doi: 10.5713/ajas.19.0031

Kim, Y. H. B., Meyers, B., Kim, H. W., Liceaga, A. M., & Lemenager, R. P. (2017). Effects of stepwise dry/wet-aging and freezing on meat quality of beef loins. *Meat Science*, 123, 57-63.https://doi.org/10.1016/j.meatsci.2016.09.002

Kiyimba, F., Cassens, D., Hartson, S. D., Rogers, J., Habiger, J., Mafi, G. G., & Ramanathan, R. (2023). Integrative proteomics and metabolomics profiling to

understand the biochemical basis of beef muscle darkening at a slightly elevated pH. *Journal of Animal Science*, 101, skac376. https://doi.org/10.1093/jas/skac376

Lamri, M., Della Malva, A., Djenane, D., Albenzio, M., & Gagaoua, M. (2023). First insights into the dynamic protein changes in goat semitendinosus muscle during the post-mortem period using high-throughput proteomics. *Meat Science, 202*, Article 109207. https://doi.org/10.1016/j.meatsci.2023.109207

Lamri, M., Della Malva, A., Djenane, D., López-Pedrouso, M., Franco, D., Albenzio, M., ... & Gagaoua, M. (2023). Towards the discovery of goat meat quality biomarkers using label-free proteomics. *Journal of Proteomics*, 278, 104868. https://doi.org/10.1016/j.jprot.2023.104868

Laville, E., Sayd, T., Morzel, M., Blinet, S., Chambon, C., Lepetit, J., ... & Hocquette, J. F. (2009). Proteome changes during meat aging in tough and tender beef suggest the importance of apoptosis and protein solubility for beef aging and tenderization. *Journal of Agricultural and Food Chemistry*, 57(22), 10755-10764. https://doi.org/10.1021/jf901949r

Li, P., Zhu, Y., Kang, X., Dan, X., Ma, Y., & Shi, Y. (2021). An integrated approach in gene-expression landscape profiling to identify housekeeping and tissue-specific genes in cattle. *Animal Production Science*, 61(16), 1643-1651. https://doi.org/10.1071/AN20689

Lomiwes, D., Farouk, M. M., Wiklund, E., & Young, O. A. (2014). Small heat shock proteins and their role in meat tenderness: A review. *Meat Science*, 96(1), 26-40. https://doi.org/10.1016/j.meatsci.2013.06.008

Ma, D., & Kim, Y. H. B. (2020). Proteolytic changes of myofibrillar and small heat shock proteins in different bovine muscles during aging: Their relevance to tenderness and water-holding capacity. *Meat Science*, 163, 108090. https://doi.org/10.1016/j.meatsci.2020.108090

Malheiros, J. M., Braga, C. P., Grove, R. A., Ribeiro, F. A., Calkins, C. R., Adamec, J., & Chardulo, L. A. L. (2019). Influence of oxidative damage to proteins on meat tenderness using a proteomics approach. *Meat Science*, 148, 64-71. https://doi.org/10.1016/j.meatsci.2018.08.016

Mato, A., Rodriguez-Vazquez, R., Lopez-Pedrouso, M., Bravo, S., Franco, D., & Zapata, C. (2019). The first evidence of global meat phosphoproteome changes in response to pre-slaughter stress. *BMC Genomics*, *20*, 590. https://doi.org/10.1186/s12864-019-5943-3

McCarthy, S. N., Henchion, M., White, A., Brandon, K., & Allen, P. (2017). Evaluation of beef eating quality by Irish consumers. *Meat Science*, 132, 118-124. https://doi.org/10.1016/j.meatsci.2017.05.005

MGAP (Ministerio de Ganadería, Agricultura y Pesca) 2023. Carne de alta Calidad UE. Available at https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/sites/ministerio-ganaderia-agricultura-pesca/files/2021-08/CarneAltaCalidad_8350_0621_UE_Ingles.pdf. [Accessed 1 March 2024]

Morzel, M., Chambon, C., Hamelin, M., Sante-Lhoutellier, V., Sayd, T., & Monin, G. (2004). Proteome changes during pork meat ageing following use of two different pre-slaughter handling procedures. *Meat Science*, *67*, 689–696. https://doi.org/10.1016/j.meatsci.2004.01.008

Munekata, P. E., Pateiro, M., López-Pedrouso, M., Gagaoua, M., & Lorenzo, J. M. (2021). Foodomics in meat quality. Current Opinion in Food Science, 38, 79-85. https://doi.org/10.1016/j.cofs.2020.10.003

Okumura, N., Hashida-Okumura, A., Kita, K., Matsubae, M., Matsubara, T., Takao, T., & Nagai, K. (2005). Proteomic analysis of slow-and fast-twitch skeletal muscles. *Proteomics*, 5(11), 2896-2906. https://doi.org/10.1002/pmic.200401181

Picard, B., & Gagaoua, M. (2017). Proteomic investigations of beef tenderness. *Proteomics in Food Science*, 177-197. https://doi.org/10.1016/B978-0-12-804007-2.00011-4

Picard, B., & Gagaoua, M. (2020). Meta-proteomics for the discovery of protein biomarkers of beef tenderness: An overview of integrated studies. *Food Research International*, 127, 108739. https://doi.org/10.1016/j.foodres.2019.108739

Ouali, A., Gagaoua, M., Boudida, Y., Becila, S., Boudjellal, A., Herrera-Mendez, C. H., & Sentandreu, M. A. (2013). Biomarkers of meat tenderness: present knowledge and perspectives in regard to our current understanding of the mechanisms involved. *Meat Science*, 95(4), 854-870. https://doi.org/10.1016/j.meatsci.2013.05.010

Purslow, P. P., Gagaoua, M., & Warner, R. D. (2021). Insights on meat quality from combining traditional studies and proteomics. *Meat Science*, 174, 108423. https://doi.org/10.1016/j.meatsci.2020.108423

Ramanathan, R., Nair, M. N., Wang, Y., Li, S., Beach, C. M., Mancini, R. A., Belskie, K. & Suman, S. P. (2021) Differential Abundance of Mitochondrial Proteome Influences the Color Stability of Beef Longissimus Lumborum and Psoas Major Muscles. *Meat and Muscle Biology* 5(1), 16, 1-16. doi: https://doi.org/10.22175/mmb.11705

Ramanathan, M.N. Nair, M.C. Hunt, Suman, S.P. (2019). Mitochondrial functionality and beef color: Apeptidase dehydrogenase review of recent research, S. African *Journal of Animal Science*. 49 9. https://doi.org/10.4314/sajas.v49i1.2

Ronca, F., & Raggi, A. (2018). Role of the HPRG component of striated muscle AMP deaminase in the stability and cellular behaviour of the enzyme. *Biomolecules*, 8(3), 79. https://doi.org/10.3390/biom8030079

Sentandreu, E., Fuente-García, C., Pardo, O., Oliván, M., León, N., Aldai, N., … Sentandreu, M. A. (2021). Protein biomarkers of bovine defective meats at a glance: Gel-free hybrid Quadrupole-Orbitrap analysis for rapid screening. Journal of Agricultural and Food Chemistry, 69(26), 7478-7487. https://doi.org/10.1021/acs.jafc.1c02016

Shibata, M., Matsumoto, K., Oe, M., Ohnishi-Kameyama, M., Ojima, K., Nakajima, I., ... & Chikuni, K. (2009). Differential expression of the skeletal muscle proteome in grazed cattle. Journal of Animal Science, 87(8), 2700-2708. doi:10.2527/jas.2008-1486

Silva, L. H., Rodrigues, R. T., Assis, D. E., Benedeti, P. D., Duarte, M. S., & Chizzotti, M. L. (2019). Explaining meat quality of bulls and steers by differential

proteome and phosphoproteome analysis of skeletal muscle. *Journal of Proteomics*, 199, 51-66. https://doi.org/10.1016/j.jprot.2019.03.004

Terjung, N., Witte, F., & Heinz, V. (2021). The dry aged beef paradox: Why dry aging is sometimes not better than wet aging. *Meat Science*, 172, 108355. https://doi.org/10.1016/j.meatsci.2020.108355

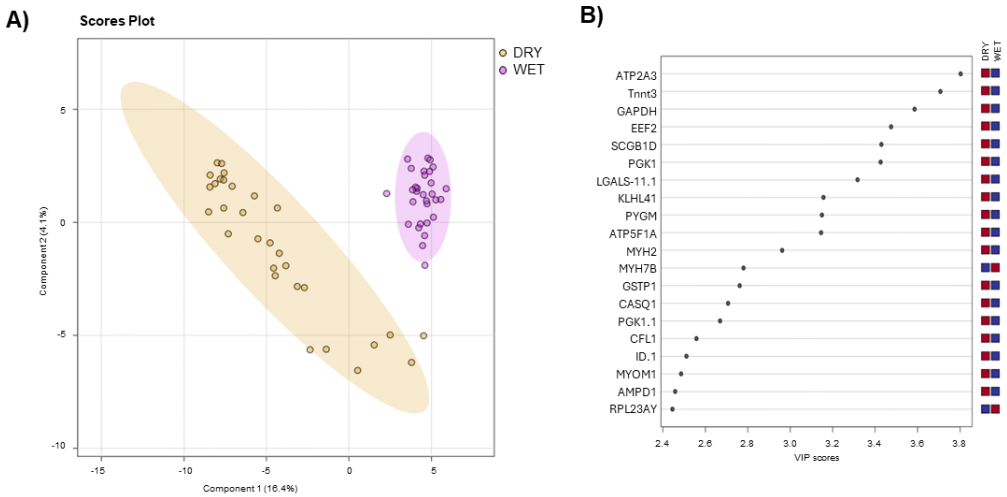
Ubaida-Mohien, C., Gonzalez-Freire, M., Lyashkov, A., Moaddel, R., Chia, C. W., Simonsick, E. M., ... & Ferrucci, L. (2019). Physical activity associated proteomics of skeletal muscle: being physically active in daily life may protect skeletal muscle from aging. *Frontiers in Physiology*, 10, 312. https://doi.org/10.3389/fphys.2019.00312

Verbeke, W., Pérez-Cueto, F. J., de Barcellos, M. D., Krystallis, A., & Grunert, K. G. (2010). European citizen and consumer attitudes and preferences regarding beef and pork. *Meat Science*, 84(2), 284-292. https://doi.org/10.1016/j.meatsci.2009.05.001

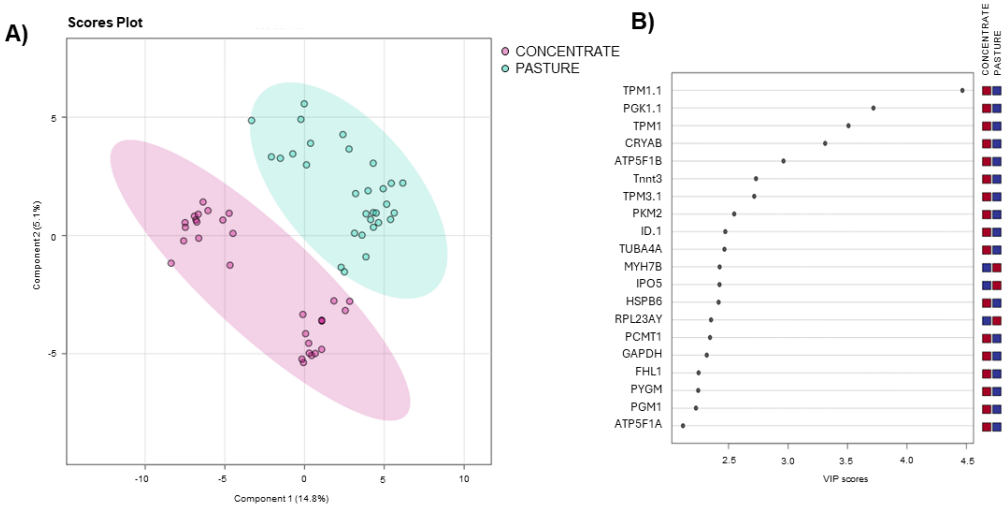
Warren, K. E., & Kastner, C. L. (1992). A comparison of dry-aged and vacuum-aged beef strip loins 1. *Journal of Muscle Foods*, 3(2), 151-157. https://doi.org/10.1111/j.1745-4573.1992.tb00471.x

Wu, S., Han, J., Liang, R., Dong, P., Zhu, L., Hopkins, D. L., ... & Luo, X. (2020). Investigation of muscle-specific beef color stability at different ultimate pHs. *Asian-Australasian Journal of Animal Sciences*, 33(12), 1999. doi: 10.5713/ajas.19.0943

6.7. Supplementary data



Supplementary figures – 1S - Discriminant analysis within the 30 dry-aged and the 30 wet-aged samples proteomes. A) Partial least squares-discriminant analysis (PLS-DA) score plot of meat proteome distribution according to aging type. B) Variable Importance in Projection (VIP) plot Top 20 values.



Supplementary figures – 2S - Discriminant analysis within the 30 concentrate-fed and the 30 pasture-fed samples proteomes. A) Partial least squares-discriminant analysis (PLS-DA) score plot of meat proteome distribution according to production systems. B) Variable Importance in Projection (VIP) plot: Top 20 values.

7. Discusión general

7.1. Introducción

La comprensión de los procesos alternativos de conservación/preservación de carne, así como el uso de la maduración (maduración seca en bolsa con o sin posterior congelado y el proceso *stepwise*), es de suma relevancia dado que, en su ausencia, el deterioro, la actividad microbiana, las reacciones enzimáticas y químicas y los cambios físicos son inevitables. Por lo tanto, la carne es preservada usando una variedad de métodos que incluyen enfriado o maduración, congelado, procesos térmicos, deshidratación, irradiación, entre otros (Rahman et al., 2023; Zhou et al., 2010; Cheftel et al., 2000; Cassens, 1994).

La maduración de la carne consiste en un proceso fundamental que mejora la terneza, sabor y palatabilidad de la carne (Miller et al., 2001; Maga, 1994; Savell et al., 1987) a través de la degradación de proteínas miofibrilares del citoesqueleto mediante proteasas endógenas del músculo (Lepper-Blilie et al., 2016; Kemp et al., 2010; Huff-Lonergan y Lonergan, 2005). Además, el hecho de que la carne provenga de animales de diferentes orígenes, esto es, terminados a pasto o grano, determina diferentes características en ella (Calkins y Hodgen, 2007; Melton et al., 1983).

Estos factores fueron evaluados en un experimento utilizando carne de animales de diferentes dietas de terminación (grano y pastura) a la que se le aplicó distintos métodos de preservación, maduración en seco en bolsa, maduración húmeda y la combinación (*stepwise*) de ambos métodos, por un período de cuarenta días y posterior congelado en los dos primeros. El objetivo fue evaluar el efecto de los factores mencionados sobre las características fisicoquímicas, microbiológicas y sensoriales de la carne (músculo *longissimus lumborum y thoracis* [*LTL*]).

7.2. Evaluación conjunta

La evaluación conjunta estará presentada a través de 1) análisis del efecto de los métodos de maduración, seco en bolsa (DAb), húmeda (WA) y combinada (*stepwise*: DW y WD), y la dieta de terminación (pastura vs. grano) y 2) análisis del efecto de los métodos de maduración, seca en bolsa (DAb) y húmeda (WA) vs. maduración seca en

bolsa + congelado (DAb + Fr) y húmeda + congelado (WA + Fr), y la dieta de terminación (pastura vs. grano).

7.2.1. Métodos de maduración y la dieta de terminación sobre las características fisicoquímicas, microbiológicas y atributos sensoriales (panel entrenado y consumidores)

Los métodos de maduración (DAb, WA, DW y WD) afectaron significativamente a las características fisicoquímicas, con excepción de la fuerza de corte (WBSF). Estos datos sugieren que, en iguales condiciones, en maduraciones largas (40 días) es indiferente usar seco en bolsa, húmeda o combinado, dado que la mayor disminución en fuerza de corte (mayor degradación de la fibra muscular) se produce en los primeros veinte días de maduración (Utama et al., 2020; Lepper Blilie, 2016). Respecto al color, hubo una mayor luminosidad (L^*) en las muestras maduradas en húmedo (WA) respecto a los demás métodos de maduración; esto puede haberse dado por el aumento en la reflectancia debido a la mayor retención de humedad (Bertram et al., 2004). Sin embargo, los parámetros a^* y b^* presentaron diferente respuesta de acuerdo con la procedencia de la carne. En el caso del parámetro a^*, los valores fueron menores cuando la carne provenía de animales terminados en pastura, tanto madurados en DAb como en WD. Esto puede ser atribuido al menor contenido de agua de las muestras maduradas mediante el proceso seco en bolsa, por una menor absorción de la luz por la superficie de la carne, lo que le da una apariencia más oscura (Kim et al., 2011). El parámetro b^* mostró valores más bajos en los tratamientos *stepwise*, DW y WD, con los dos tipos de dieta. Resultados similares fueron reportados por De Faria Vilella et al. (2019), pero contrastaron con otros estudios (Zhang et al., 2020; Kim et al., 2017) que no encontraron diferencias entre los métodos de maduración. Los mayores valores de L^* encontrados en la carne de animales terminados en grano se relacionan con que estas tienden a ser más claras, probablemente por un mayor contenido de grasa intramuscular y reducidos niveles de mioglobina comparado a la de animales terminados en pastura (Priolo et al., 2001; Apaoblaza et al., 2020).

Las diferencias en pH fueron levemente superiores en los tratamientos madurados en seco en bolsa (y con su combinación) comparados con WA. Varios estudios (Zhang et al., 2019; Kim et al., 2017; Li et al., 2014; Dikeman et al., 2013) reportaron incrementos en pH después de los veintiún días de maduración seca en bolsa y una disminución de este en WA entre los veinte y cuarenta días de madurado. En la maduración seca, estos resultados están asociados a la generación de compuestos nitrogenados producto de la proteólisis, también puede reducir la estabilidad oxidativa, facilitando la formación de compuestos como aldehídos y cetonas, lo que puede acortar la vida útil si no se controlan adecuadamente las condiciones de almacenamiento. Mientras que la disminución en WA podría ser causa de una mayor acumulación de ácido láctico (Zhang et al., 2019; Li et al., 2014), favorece la estabilidad microbiológica, ya que inhibe el crecimiento de microorganismos patógenos y deteriorantes. Esto se traduce en una vida útil más prolongada, aunque puede haber una menor intensidad en el desarrollo de ciertos compuestos de sabor.

De acuerdo con varios experimentos (Berger et al., 2018; Dikeman et al., 2013; Ahnstrom et al., 2006), este estudio no presentó diferencias en fuerza de corte entre los diferentes métodos de maduración (DAb, WA, DW y WD). Además, todos los valores estuvieron por debajo de 3 kgF, lo que indica que la carne puede ser considerada tierna (Miller et al., 2001).

A diferencia de Jiang et al. (2010), en el presente se observó interacción entre los métodos de maduración y la dieta de terminación para los grupos de ácidos grasos SFA, MUFA y PUFA, así como para PUFA-n6 y el CLA. Los mayores valores de SFA y MUFA se presentaron en los tratamientos combinados (DW y WD) de la carne proveniente de animales terminados en grano. En estos tratamientos también se observaron los mayores contenidos de grasa intramuscular (IMF). Wood et al. (2008) reportaron que la cantidad de IMF influye en la composición de ácidos grasos de la carne vacuna, dado que, al aumentar la grasa total, incrementa la cantidad de SFA en la misma medida. Sin embargo, en PUFA y PUFA n6 los mayores valores se presentaron en los tratamientos DAb y WA provenientes de carne de novillos terminados en pastura. Por otro lado, la suma de PUFA n3, n6:n3 y PUFA:SFA no fueron afectados por la interacción, observándose un incremento en PUFA n3 y

PUFA:SFA y disminución del ratio n6:n3 en las muestras provenientes de pasturas. El ácido linolénico (C18:3n3) es un importante precursor del sabor (Ba et al., 2012) y su mayor concentración en carne procedente de animales alimentados en pastura ha presentado un impacto negativo sobre el sabor deseado de la carne (Melton et al., 1983). La diferencia más importante entre dietas estuvo dada por PUFA n3, donde el ácido linolénico presentó cuatro veces más contenido en músculo de los animales terminados en pasturas en relación con aquellos terminados en grano. Al relacionar esta información con los resultados de los VOC precursores del sabor, observamos que, de los 46 compuestos volátiles identificados, dieciséis de ellos fueron afectados por la dieta del animal. Este bajo número de VOC afectados por la dieta fue atribuido a la poca diferencia de IMF entre las dietas de terminación: pastura (3,7 %) vs. grano (4,3 %). Sin embargo, de los dieciséis VOC afectados, quince de ellos aumentaron y solo el etanol disminuyó en músculo de animales terminados en pastura. La mayoría (nueve) de los quince VOC pertenecieron a la clase aldehídos.

En el músculo vacuno, C18:2 n6 y C18:3 n3 están presentes en los triacilglicéridos, así como en los lípidos polares (Marmer et al., 1984). Los fosfolípidos (lípidos polares) son considerados el primer objetivo de las reacciones de la oxidación lipídica porque, además de su alto grado de insaturación, estos son expuestos a proteínas y otras catálisis de oxidación de lípidos como componentes de membrana en contacto con el citosol (Boylston et al., 1996). Bajo condiciones de cocción similares al presente trabajo, Fogerty et al. (1990) reportó que los ácidos grasos y los aldehídos fueron liberados desde los fosfolípidos de varios tipos de carnes. Debido a que los ácidos grasos libres se oxidan mucho más fácilmente que las fracciones de ácidos grasos de los lípidos saturados, la rápida hidrólisis de los fosfolípidos puede explicar en parte por qué desempeñan un papel mucho más importante en la formación del sabor de la carne que los triacilglicéridos (Mottram y Edwards, 1983). Los volátiles derivados de lípidos comprenden aldehídos, alcoholes, hidrocarburos y ketonas, todos con cadena alquílicas lineales que contienen cinco o más átomos de carbono. Todos estos volátiles son formados por oxidación térmica de cadena de ácidos grasos de triacilglicéridos y fosfolípidos (Mottram, 1996). Esto sugiere que los PUFA inducen un incremento en la degradación termal del ácido linolénico por el alto nivel de

aldehídos en la carne proveniente de animales terminados en pastura (Elmore et al., 1999). Los aldehídos son los volátiles derivados de lípidos más interesantes, ya que tienen valores de umbral de olor bajos y pueden contribuir al sabor de las muestras de carne cocida. En nuestro estudio, todos los aldehídos identificados fueron más abundantes en las muestras DAb que en las de WA y solo el 2-propenal, 2-decenal E, decanal y dodecanal fueron más abundantes en pastura en relación con la dieta de terminación a grano. Sin embargo, la oxidación lipídica presentó interacción entre el método de maduración y el tipo de dieta animal, donde, independientemente del método de maduración, los valores fueron más bajos en pastura que en la dieta a grano. De acuerdo con lo discutido en los párrafos anteriores, esperaríamos que estos valores fueran más altos debido al mayor contenido de PUFA. Sin embargo, estudios anteriores (Daley et al., 2010; Nuernberg et al., 2005; Realini et al., 2004) han reportado altas concentraciones de vitamina E en muestras procedentes de pasturas. Aunque esta variable no fue estudiada en este trabajo, asumimos que su efecto podría haber enlentecido el proceso de oxidación de los lípidos y la formación de metmioglobina (Descalzo y Sancho, 2008), y les otorga una mayor estabilidad oxidativa a las muestras procedentes de animales terminados en pastura.

Desde el punto de vista microbiológico de la superficie de la carne, solo los métodos de maduración afectaron a las TBC y a las PSY. Las mayores cargas se encontraron (al igual que la oxidación lipídica) en el tratamiento WD. Sin embargo, los valores alcanzados no superaron los umbrales de detección de malos olores (*off-flavors*) por los consumidores, de acuerdo con lo reportado por la bibliografía para TBC (7 log CFU/cm^2; Feiner, 2006) y para PSY (6-8 log CFU/g; Stanbridge y Davies, 1998). Además, esto lo podemos corroborar con los resultados del panel de consumidores, donde todas muestras fueron juzgadas como «me gusta» a «me gusta mucho».

De todas maneras, cuando se analizaron en forma conjunta los resultados de todos los consumidores, las muestras de carne mejor valoradas fueron aquellas maduradas en DAb o WA, y las menos aceptadas, las DW y WD, para los tres atributos de calidad sensorial estudiados: aceptabilidad global, terneza y sabor. Sin embargo, Berger et al. (2018) no reportaron diferencias en aceptabilidad global entre los métodos

de maduración (*wet*, *dry* y *dry bag*), en muestras de carne de vaquillonas alimentadas en pastura. De acuerdo con la dieta de terminación de los animales, las muestras más preferidas fueron aquellas provenientes de terminación en grano en comparación con las de pasturas. Estas diferencias no podrían ser explicadas por el contenido de IMF, dado que no hubo diferencias significativas entre las distintas dietas. Es reportado que una mayor IMF determina mayor jugosidad y está positivamente correlacionado con la palatabilidad (Kim et al., 2018; Smith et al., 2008). Sin embargo, sí existieron diferencias en cuanto al perfil de ácidos grasos, según la dieta de terminación, de acuerdo con lo mencionando anteriormente. La dieta proveniente de pastura con mayor contenido de PUFA n3, especialmente C18:3 n3, podría estar provocando a la carne, sabores y aromás no tan deseados como la carne de animales con dieta de granos, debido a que los productos derivados de los PUFA reducen o inhiben la formación de productos heterocíclicos de las reacciones de Maillard (Ames et al., 2001). Estos compuestos volátiles del sabor, compuestos heterocíclicos oxigenados, tales como lactonas y alquilfuranos, que han sido encontrados en la carne cocida, provienen de la oxidación de los ácidos grasos (Yaylayan et al., 2000; Maga, 1994). De acuerdo con los resultados del panel entrenado, las muestras de carne procedentes de animales terminados en pastura presentaron un incremento en olor a hierba y anormal, en sabor a leche y disminuyeron el sabor a metálico en comparación con las de grano.

La maduración seca en bolsa aumentó la intensidad del olor a madurado y a carne, mientras que disminuyó la intensidad de olor a hígado y sabor metálico. Leighton et al. (2023), en concordancia con nuestros resultados, reportó que la maduración seca en bolsa aumentó el sabor intenso a carne y sabor salado (*salty taste*), mientras que disminuyó la intensidad del sabor amargo y agrio-lácteo y rancio/cartón en churrasco del corte paleta (*clod heart*). Además, Li et al. (2014) y Foraker et al. (2020) encontraron que en DAb o seco tradicional el músculo LTL fue menos amargo (*sour*) que en las muestras WA después de diferentes períodos de maduración (8-63 días), probablemente debido al crecimiento limitado de bacterias ácido-lácticas. Dado que los compuestos que producen el sabor agrio/ácido/amargo son solubles en agua (Dashdorj et al., 2015), posiblemente se pierden durante el proceso de purga del madurado en seco (Castejón et al., 2015). Respecto a la textura, que es el atributo que

se relaciona con la terneza de la carne, los resultados del panel de entrenados indicaron un incremento en la dureza, fibrosidad, masticabilidad y residuos, así como una disminución en la jugosidad inicial y final y desmenuzamiento, en las muestras DAb con relación a las WA. Si relacionamos los atributos de textura con los resultados de fuerza de corte, que es una forma de estimar la terneza de la carne, registrados en este trabajo, podemos decir que, aunque en forma objetiva no se detectaron diferencias entre los métodos de maduración, el panel de entrenado tuvo una mayor sensibilidad en detectar, en todos los atributos referidos a la textura, diferencias entre la DAb y la WA. Estas discrepancias entre el análisis sensorial y las mediciones de fuerza de corte podrían deberse a los complejos movimientos de masticación involucrados en el análisis sensorial, la presencia de saliva (Szczesniak, 1987) y el efecto de lubricación de la grasa intramuscular durante el procesamiento oral (Frank et al., 2016). De hecho, Shackelford et al. (1995) informaron que la fuerza de corte explicaba solo una pequeña proporción (R^2 = 0,11) de la varianza en las puntuaciones de terneza de los panelistas entrenados en churrasco de corte de la paleta madurados en húmedo. A su vez, el panel de consumidores (no entrenados) no detectó diferencias en terneza entre DAb y WA, pero sí le atribuyeron un puntaje menor (menos tierno) a los tratamientos combinados (DW y WD), aunque todos estuvieron dentro de los umbrales de aceptación. Los consumidores no entrenados tienden a ser menos sensibles a las diferencias de palatabilidad que los panelistas entrenados (Rodas-González et al., 2009).

7.2.2. Métodos de maduración más congelado y la dieta de terminación sobre las características fisicoquímicas y microbiológicas, perfil y oxidación lipídica y panel de consumidores

Pocos estudios han relacionado el tipo de dieta de terminación de los animales con los métodos de preservación de la carne, tales como maduración y congelado. En la hipótesis de trabajo se planteó que los métodos de maduración (DAb y WA) con o sin subsecuente congelado afectan la calidad de carne de novillos procedente de diferentes dietas de terminación en pastura o en grano.

El color es la característica más importante que influye en las decisiones de compra de los consumidores, aunque las preferencias varían entre ellos (Altmann et

al., 2022; Realini et al., 2014). Todos los parámetros del color (*a**, *b** y *L**) fueron afectados por el método de preservación; además, la luminosidad (*L**) fue afectada por la dieta animal. La carne madurada al vacío (WA) fue la más brillosa (mayor *L**) y menos roja (mayor *a**) que la DAb luego de los cuarenta días de maduración y también después de los 180 días de congelado, atribuido posiblemente a menor perdida de humedad en la superficie de la carne, lo que resultó en una mayor absorción de la luz y un color más claro (Kim et al., 2011). Bernardo et al. (2020) no encontraron diferencias entre la maduración en seco tradicional vs. el mismo madurado más el congelado de treinta días, con cinco días de exhibición en góndola de comercios minoristas en los valores de *L**. Sin embargo, los valores de *a** y *b** fueron más bajos en la carne congelada que la madurada en muestras de bife angosto (*striploin*) en el mismo trabajo. Esto puede deberse a la existencia de un sistema de enzimas capaces de reducir la metmioglobina a mioglobina (*metmyoglobin reducing activity*-MRA) propuesto por Livingston y Brown (1981). Cuando la carne es madurada o congelada, la actividad de MRA disminuye y ocurre una acumulación acelerada de metmioglobina en la superficie de la carne. La carne de los animales procedente de dieta en pastura presentó menores valores de *L** posiblemente debido a la mayor concentración de mioglobina por una mayor actividad muscular, lo que hizo que el corte de carne presente una apariencia más oscura y menos brillosa (Apaoblaza et al., 2020). Otros factores que influyen en el color de la carne son el pH y la IMF, que en nuestro experimento no fueron diferentes entre dietas.

El pH presentó la misma tendencia discutida en el punto 6.2.1., con valores levemente mayores, aunque significativos, en DAb que en WA y se mantuvieron luego del proceso de congelado. En todos los casos, los valores de *pH* estuvieron por debajo de 5,8. Las pérdidas por cocción disminuyeron en DAb frente al WA y esta diferencia se acentuó en las muestras congeladas. Esto puede deberse a las pérdidas por purga durante el proceso de la maduración en seco en bolsa (Zhang et al., 2019). Además, el congelado reduce la capacidad de retención de agua en el músculo debido a la ruptura de las fibras musculares por la formación de los cristales de hielo (Leygonie et al., 2012).

La fuerza de corte de la carne WA y DAb (WBSF) no presentó diferencias entre ellas, como fue mencionado en el análisis de los diferentes métodos de maduración, y los valores fueron más bajos que en las muestras congeladas. Existe un consenso general en la literatura con respecto a que la terneza de la carne aumenta con el congelado y descongelado (Lagerstedt et al., 2008; Farouk et al., 2003; Wheeler et al., 1990). Sin embargo, se ha reportado que el aumento de la terneza está correlacionado con la duración del congelado y el grado de maduración previo al congelado. El efecto de ablandar la carne con el congelado se anularía cuando la carne se ha madurado lo suficiente antes de la congelación (Vieira et al., 2009). Esta es la situación de nuestros resultados, dado que todas las muestras estuvieron por debajo de 3 kgF, umbral de terneza para la aceptabilidad de la carne por parte del consumidor (Miller et al., 2001).

A diferencia de lo que ocurrió entre los métodos de maduración, el método de preservación afectó el porcentaje de IMF, lo que indico mayores valores en las muestras maduradas en seco en bolsa con posterior congelado. Esto se puede haber debido a las pérdidas por evaporación producidas durante el proceso de maduración y congelado, lo cual está asociado con los valores más bajos de pérdidas por cocción (% CL). Sin embargo, el perfil de ácidos grasos para los grupos SFA, MUFA y PUFA (n6 y n3), así como CLA y las relaciones n6:n3 y PUFA:SFA, no fueron afectados por el método de preservación. Por el contrario, Zhang et al. (2021) reportaron una disminución de SFA y un incremento en los PUFA en muestras maduradas en seco en bolsa (veintiún días) y congeladas durante doce meses con su contraparte no congelada. Las diferentes dietas de terminación no afectaron el porcentaje de IMF, pero sí incrementaron los valores de CLA PUFA, PUFA n3 y la relación PUFA:SFA y disminuyeron la n6:n3 en las muestras de dietas terminadas en pastura.

Los métodos de preservación y la dieta animal presentaron interacción en oxidación lipídica, al igual que entre los métodos de maduración. Los valores más bajos de oxidación lipídica fueron observados en las muestras congeladas independientemente de la dieta de terminación. Estos coinciden con resultados reportados en otros estudios que comparan carne madurada con congelada (Zhang et al., 2021; Bernardo et al., 2020). Durante el almacenamiento (madurado y congelado), el MDA puede degradarse aún más en alcoholes y ácidos orgánicos o unirse a

aminoácidos y proteínas libres como un complejo MDA-aminoácidos (Farouk et al., 2003) y, con estos cambios, el MDA no puede ser detectado por el TBARS. Por lo tanto, la variación observada en el presente trabajo durante la maduración y el congelado podría haber sido el resultado de diferentes reacciones de generación y degradación de MDA durante el proceso de preservación. Se ha reportado que la temperatura óptima para el almacenamiento de la carne congelada es de -40 °C, ya que solo un porcentaje muy pequeño de agua queda descongelada en este punto (Estévez, 2011). La fracción de agua no congelada también es importante en términos de oxidación, ya que pueden ocurrir reacciones químicas durante el período de congelado que inician la oxidación lipídica primaria (peroxidación) en la carne. Esto puede provocar una oxidación lipídica secundaria radical durante el descongelado (Owen y Lawrie, 1975), lo que produce cambios adversos en el color, el olor, el sabor y la inocuidad de la carne. Como fue discutido, se ha demostrado que los animales alimentados con pastura tienen mayores concentraciones de vitamina E en músculo que los alimentados con granos (Bernardo et al., 2020; Daley et al., 2010; Descalzo et al., 2007; Nuernberg et al., 2005; Realini et al., 2004), lo que retrasa la oxidación lipídica y la formación de metmioglobina (Descalzo y Sancho, 2008; Zerby et al., 1999; Schwarz et al., 1998). El rango de TBARS (0,133-0,479 mg MDA/kg de carne) encontrado en este estudio fue menor que el umbral (2 mg MDA/kg de carne) para la detección de sabor rancio por panelistas entrenados (Campo et al., 2006).

Según la literatura, ni el congelado ni el descongelado parecen reducir la cantidad de microorganismos viables presentes en la carne. Sin embargo, durante la congelación, el deterioro microbiano se enlentece significativamente, ya que los microorganismos entran en estado latente. Sin embargo, recuperan su actividad durante el descongelado (Löndahl y Nilaaon, 1993). Esto es especialmente preocupante cuando se emplea la descongelación al aire. Por esta razón, las buenas prácticas de higiene y manipulación son aún más importantes para la carne que se congela y descongela en comparación con la que se vende fresca (Pham, 2004). En el presente trabajo, el método de preservación afectó a las bacterias psicotróficas (PSY) y a las enterobacterias (ENT). El tipo de maduración seco en bolsa presentó las cargas más altas de estos microorganismos (PSY y ENT) y luego este evento se acentuó

durante el congelado en el caso de PSY. Las cargas de PSY son particularmente relevantes para productos que son mantenidos en condiciones de enfriado, dado que esos microorganismos aún se pueden reproducir en esas condiciones (González-Gutiérrez et al., 2020). A esto se suman las condiciones para el crecimiento microbiano que pueden ocurrir durante la descongelación de la carne, debido a la disrupción celular y la destrucción de las fibras musculares causadas por la congelación (Choe et al., 2016), y, con el aumento de la temperatura, el exudado se libera y crea un entorno ideal para el crecimiento microbiano (Gill, 2014; Löndahl y Nilaaon, 1993). De todas formas, las cargas microbianas no superan los umbrales establecidos para producir malos olores y defectos de apariencia en la carne. Estos valores umbrales fueron de 6 a 8 log CFU/g para PSY (Ercolini et al., 2016), 5 a 6 log CFU/cm^2 o gramos de carne para TBC (Feiner, 2006) y 4 a 5 log UFC/cm^2 para ENT de acuerdo con los criterios regulación microbiológicos de la UE (Rinn et al., 2024). Mayores valores de ENT fueron observados en las muestras de carne procedentes de dietas de terminación en grano. En este estudio no hubo efecto matadero ya que los animales se faenaron en el mismo matadero y en el mismo momento, y la mayor cantidad de ENT podría esxplicarse por las dietas, las altas en grano pueden disminuir el pH ruminal, lo que favorece el crecimiento de las enterobacterias tolerantes a los pH mas bajos (Diez-González et al., 1998); por ejemplo, *E. coli O157:H7*, un patógeno semirresistente a los ácidos que pertenece a la familia ENT.

Las preferencias de los consumidores son muy variables y dependen de diversos factores (Font-i-Furnols y Guerrero, 2014). Por ello, es muy común encontrar y es importante identificar segmentos de consumidores con diferentes preferencias. En este trabajo se identificaron tres segmentos o *clusters* de consumidores: 1- los que preferían la carne madurada proveniente de la dieta de grano o de pastura, pero madurada WA, sin distinción entre con o sin congelado. La última combinación (WA de animales alimentados con pasto) es la carne más consumida en Uruguay y posiblemente sea la más reconocida y aceptada por este grupo de consumidores. De acuerdo con el panel de entrenados, la carne madurada WA presentó menor olor y sabor a maduración, mayor olor a hierbas, menor dureza y mayor jugosidad en la textura en boca que la carne de res madurada con DAb; esto puede estar relacionado con las preferencias de

los consumidores por la carne vacuna. 2- Los que preferían la carne madurada proveniente de la dieta de grano o de pastura y madurada en DAb, sin distinción entre con o sin congelado. Las diferencias en las preferencias por las distintas características sensoriales de la carne observadas en el panel de entrenado pueden explicar estas diferencias en la aceptabilidad. 3- Los que no tenían ninguna preferencia por los distintos tipos de carnes. Estos no discriminaban entre los diferentes tratamientos y calificaban como buenos a todos los tipos de carne. Esta información indica que el panel de consumidores mostró niveles de agrado general para todos los tratamientos, entre 2 («me gusta mucho») y 4 («me gusta»). Estos concuerdan con los valores obtenidos en fuerza de corte que sugirieron cortes tiernos (<3 kgF), con la oxidación de lípidos, que estuvo por debajo del umbral de sabor rancio (2 mg MDA/kg de carne), y con el crecimiento microbiano, que no superaron los umbrales establecidos para producir malos olores y superficies de la carne viscosa. Estos hallazgos sugieren que cualquiera de los tipos de maduración y condiciones de congelación probados podría producir una calidad de carne dentro de la satisfacción del consumidor.

8. Conclusion

1. Los métodos de maduración tuvieron un efecto más pronunciado en las características fisicoquímicas y microbiológicas de la carne, mientras que la dieta de terminación influyó principalmente en la composición de ácidos grasos y atributos sensoriales. A pesar de algunas diferencias en el color de la carne, pH, pérdida por cocción y fuerza de corte (WBSF), estas variaciones no afectaron significativamente la percepción del consumidor. Es importante destacar que todos los métodos de maduración fueron considerados aceptables por parte de los consumidores, donde la maduración seca en bolsa realzó los sabores a carne madurada, mientras que la maduración húmeda preservó mejor la jugosidad y terneza. No obstante, en el panel de consumidores por grupo (*clusters*) se detectaron preferencias por algunos de los métodos y dietas evaluados. Mediante la evaluación sensorial con panel entrenado, se detectaron diferencias en la textura, en la que la maduración seca en bolsa mostró una mayor dureza y fibrosidad, mientras que la húmeda mantuvo una mayor jugosidad.

2. Desde una perspectiva microbiológica, la maduración en seco (y su combinación con congelado) resultó en mayores conteos de bacterias psicotrofas y enterobacterias en comparación con la maduración húmeda. Sin embargo, estos niveles se mantuvieron dentro de los umbrales aceptables y no afectaron negativamente las preferencias de los consumidores.

3. Los compuestos orgánicos volátiles fueron significativamente influenciados por el proceso de maduración y se observó que la maduración seca en bolsa generó mayores concentraciones de aldehídos, ácidos carboxílicos y cetonas, asociados con sabores a carne madurada deseables. La carne de animales terminados en pastura presentó mayor concentración de compuestos volátiles derivados de lípidos, mientras que la de animales terminados a grano mostró menores niveles de dichos compuestos, pero un contenido levemente mayor de grasa intramuscular, pudiendo equilibrar los atributos sensoriales.

4. Los resultados sugieren que tanto la maduración húmeda como la maduración seca en bolsa son opciones viables para los consumidores uruguayos, aunque la combinación de ambas (seca en bolsa más húmeda y viceversa) no proporciona beneficios sensoriales adicionales. La maduración seca en bolsa es particularmente

adecuada para mercados que prefieren sabores intensos a carne madurada, mientras que la maduración húmeda puede ser más atractiva para consumidores que priorizan la terneza y jugosidad. Asimismo, el congelado después de la maduración surge como una estrategia viable para mantener la calidad de la carne en los mercados de exportación, lo que proporciona flexibilidad en el almacenamiento y transporte, sin variar sus atributos de calidad.

5. En general, estos resultados respaldan la selección estratégica de los métodos de maduración y preservación en función de las preferencias de los consumidores del mercado objetivo. Se requiere más investigación para profundizar en el impacto de los métodos de maduración y conservación prolongada en la seguridad, calidad y segmentación del consumidor y garantizar características óptimas de la carne para los mercados nacionales e internacionales.

9. Implicancias

Desde el punto de vista de la producción animal, la terminación de los novillos con 100 dias alimentados a granos no implicó diferencias en términos del contenido de grasa intramuscular, con relación a la terminación a pasto. Expresar diferencias en el contenido de grasa intramuscular y que las mismas provengan de sistemas de terminación a grano podrían exaltar el efecto en el sabor de la carne cuando se emplean metodos de maduración en seco.

En cuanto a la producción de carne, este experimento no evaluó puntualmente el rendimiento carnicero (pérdidas de pesos de los cortes) determinado por los métodos de preservación (maduración y congelado). Sí fueron medidas las pérdidas por cocción, observando menores pérdidas en la maduración en seco mas congelado. Sin embargo, la literatura reporta menor rendimiento para las carnes maduradas en seco ya que tienen mayor perdida de agua. Habría que evaluar de acuerdo con el mercado objetivo (interno o externo), si los aspectos positivos de la carne madurada en seco (con o sin congelado) son tan valorados por el consumidor de forma que pague esas diferencias por pérdidas en rendimiento, en términos económicos. Se deberían hacer estudios de paneles de consumidores y evaluar su disposición a pagar mas, por este tipo de producto. En el panel de consumidores uruguayos, se identificó una segregación de consumidores con diferentes preferencias por las carnes de las distintas combinaciones de productos (dieta x maduración), aunque todas fueron valoradas de forma positiva.

Otro aspecto considerado en el estudio fue el tiempo de maduración, los 40 días fueron pensados tratando de optimizar dos aspectos, la duración promedio del transporte de los productos cárnicos uruguayos a sus principales mercados (Europa y Asia) y la expresión del sabor por incorporar tecnologías de preservación de la carne.

Del presente trabajo se destaca la confirmación de resultados del punto de vista de calidad de carne por introducir la maduración en seco en bolsa en carne proveniente de animales terminados con grano al comparar con la implementación tradicional de maduración humeda y dietas pastoriles. Además, la congelación de la carne por 180 días mantuvo los atributos de calidad de la carne fresca.

10. Bibliografía general

Ahnström, M. L., Seyfert, M., Hunt, M. C. y Johnson, D. E. (2006). Dry aging of beef in a bag highly permeable to water vapour. *Meat Science*, *73*(4), 674-679. https://doi.org/10.1016/j.meatsci.2006.03.006

Altmann, B. A., Anders, S., Risius, A. y Mörlein, D. (2022). Information effects on consumer preferences for alternative animal feedstuffs. *Food Policy*, *106*, 102192. https://doi.org/10.1016/j.foodpol.2021.102192

Ames, J. M., Guy, R. C. E. y Kipping, G. J. (2001). Effect of pH and temperature on the formation of volatile compounds in cysteine/reducing sugar/starch mixtures during extrusion cooking. *Journal of Agriculture and Food Chemistry*, *49*(4), 1885-1894. https://doi.org/10.1021/jf0012547

Apaoblaza, A., Gerrard, S. D., Matarneh, S. K., Wicks, J. C., Kirkpatrick, L., England, E. M., Scheffler, T. L., Duckett, S. K., Shi, H., Silva, S. L., Grant, A. L. y Gerrard, D. E. (2020). Muscle from grass- and grain-fed cattle differs energetically. *Meat Science*, *161*, 107996. https://doi.org/10.1016/j.meatsci.2019.107996

Ba, H. V., Ryu, K. S. y Hwang, I. H. (2012). Flavor characteristics of hanwoo beef in comparison with other Korean foods. *Asian-Australian Journal of Animal Science*, *25*(3), 435-446. 10.5713/ajas.2011.11286

Berger, J., Kim, Y. H. B., Legako, J. F., Martini, S., Lee, J., Ebner, P. E. y Zuelly, S. M. S. (2018). Dry aging improves meat quality attributes of pasture-fed beef loins. *Meat Science*, *145*, 285-291. https://doi.org/10.1016/j.meatsci.2018.07.004

Bernardo, A. P., Da Silva, A. C., Francisco, V. C., Ribeiro, F. A., Nassu, R., Calkins, C. R., Do Nascimento, M. y Pflanzer, S. (2020). Effects of freezing and thawing on microbiological and physical-chemical properties of dry-aged beef. *Meat Science*, *161*, 108003. https://doi.org/10.1016/j.meatsci.2019.108003

Bertram, H. C., Engelsen, S. B., Busk, H., Karlsson, A. H. y Andersen, H. J. (2004). Water properties during cooking of pork studied by low-field NMR relaxation: effects of curing and the RN-gene. *Meat Science*, *66*(2), 437-446. https://doi.org/10.1016/S0309-1740(03)00132-3

Boylston, T. D., Morgan, S. A., Johnson, K. A., Wright, R. W., Busboom, J. R. y Reeves, J. J. (1996). Volatile lipid oxidation products of Wagyu and domestic breeds of beef. *Journal of Agricultural and Food Chemistry*, *44*(4), 1091-1095.

Brito, G., San Julián, R., La Manna, A., Del Campo, M., Montossi, F., Banchero, G., Chalkling, D. y Soares de Lima, J. M. (2014). Growth, carcass traits, and palatability: can the influence of the feeding regimes explain the variability found on those attributes in different Uruguayan genotypes? *Meat Science*, *98*(3), 533-538. https://doi.org/10.1016/j.meatsci.2014.07.003

Brito, G., Chalkling, D., Simeone, A., Franco, J., Beretta, V., Beriau, E., Iriarte, J. M., Tucci, D. y Montossi, F. (2009). Effect of finishing systems on meat quality and fatty acid composition of Uruguayan steers. 55th ICoMST, Denmark (pp. 45-47) (Meat and Nutrition session).

Cabrera, M. C., Ramos, A. L. L. D. P., Saadoun, A. y Brito, G. (2010). Selenium, copper, zinc, iron and manganese content of seven meat cuts from Hereford and Braford steers fed pasture in Uruguay. *Meat Science*, *84*(3), 518-528.

Calkins, C. R. y Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, *77*(1), 63-80. https://doi.org/10.1016/j.meatsci.2007.04.016

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D. y Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science*, *72*(2), 303-311. https://doi.org/10.1016/j.meatsci.2005.07.015

Castejón, D., García-Segura, J. M., Escudero, R., Herrera, A. y Cambero, M. I. (2015). Metabolomics of meat exudate: Its potential to evaluate beef meat conservation and aging. *Analytica Chimica Acta*, *901*, 1-11. https://doi.org/10.1016/j.aca.2015.08.032

Cassens, R. G. (1994). *Meat Preservation, Preventing Losses and Assuring Safety*. Food and Nutrition Press; Inc. Trumbull.

Cheftel, J. C. (1995). Review: High-pressure, microbial inactivation and food preservation. *Food Science Technology International*, *1*, 75-90.

Cheftel, J. C., Lévy, J. y Dumay, E. (2000). Pressure-Assisted Freezing and Thawing: Principles and Potential Applications. *Food Reviews International*, *16*(4), 453-483. http://dx.doi.org/10.1081/FRI-100102319

Choe, J. H., Stuart, A. y Kim, Y. H. B. (2016). Effect of different aging temperatures prior to freezing on meat quality attributes of frozen/thawed lamb loins. *Meat Science*, *116*, 158-164. 10.1016/j.meatsci.2016.02.014

Daley, C. A., Abbot, A., Doyle, P. S., Nader, G. A. y Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal*, *9*(10). http://www.nutritionj.com/content/9/1/10

Dashdorj, D., Amna, T. y Hwang, I. (2015). Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: An overview. European *Food Research and Technology*, *241*, 157-171. https://doi.org/10.1007/s00217-015-2449-3

Dashdorj, D., Tripathi, V. K., Cho, S., Kim, Y. y Hwang, I. (2016). Dry aging of beef; Review. *Journal of Animal Science and Technology*, *58*(20), 1-11. 10.1186/s40781-016-0101-9

De Faria Vilella, G., Gomes, C. L., Battaglia, C. T., Pacheco, M. T. B., Da Silva, V. S. N., Rodas-González, A. y Pflanzer, S. B. (2019). Effects of combined wet- and dry-aging techniques on the physicochemical and sensory attributes of beef ribeye steaks from grain-finished crossbred Zebu steers. *Canadian Journal Animal Science*, *99*(3), 497-504. https://doi.org/10.1139/cjas-2018-0127

Del Campo, M., San Julián, R., Brito, G. y De Souza, G. (2018, agosto). *Castrated vs not Castrated Beef Cattle: Instrumental Quality and Meat Consumers Perception*. International Congress of Meat Science and Technology. Melbourne, Australia.

Del Campo, M., Brito, G., Soares de Lima, J. M., Hernández, P. y Montossi, F. (2010). Finishing diet, lairage time and temperament effects in carcass and meat quality traits in steers. *Meat Science*, *86*(4), 908-14. https://doi.org/10.1016/j.meatsci.2010.07.014

Del Campo, M., Brito, G., De Lima, J. S., Martins, D. V., Sañudo, C., San Julián, R., Hernández, P. y Montossi, F. (2008). Effects of feeding strategies including different proportion of pasture and concentrate, on carcass and meat quality traits in Uruguayan steers. *Meat Science*, *80*(3), 753-760. https://doi.org/10.1016/j.meatsci.2008.03.026

De la Fuente, J., Díaz, M. T., Alvarez, I., Oliver, M.A., Font-i-Furnols, M., Sañudo, C., Campo, M. M., Montossi, F., Nute, G. R. y Cañeque, V. (2009). Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. *Meat Science*, *82*(3), 331-337. https://doi.org/10.1016/j.meatsci.2009.02.002

Descalzo, A. M. y Sancho, A. M. (2008). A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. *Meat Science*, *79*(3), 423-436. https://doi.org/10.1016/j.meatsci.2007.12.006

Descalzo, A. M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A. M., Carrete, J. y Pensel, N. A. (2007). Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. *Meat Science*, *75*(2), 299-307. https://doi.org/10.1016/j.meatsci.2006.07.015

Diez-González, F., Callaway, T. R., Kizoulis, M. G. y Russel, J. B. (1998). Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. *Science*, *281*(5383), 1666-1668. 10.1126/science.281.5383.1666

Dikeman, M. E., Obuz, E., Gök, V., Akkaya, L. y Stroda, S. (2013). Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef *Longissimus lumborum* steaks. *Meat Science*, *94*(2), 228-233. https://doi.org/10.1016/j.meatsci.2013.02.002

Elmore, J. S., Mottram, D. S., Enser, M. y Wood, J. D. (1999). Effect of the Polyunsaturated Fatty Acid Composition of Beef Muscle on the Profile of Aroma Volatiles. *Journal Agriculture Food Chemistry*, *47*(4), 1619-1625. https://doi.org/10.1021/jf980718m

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science*, *89*, 259-279.

Farouk, M. M., Mustafa, N. M., Wu, G. y Krsinic, G. (2012). The "sponge effect" hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with ageing. *Meat Science*, *90*(3), 670-677. https://doi.org/10.1016/j.meatsci.2011.10.012

Farouk, M. M., Wielizcko, K. J. y Merts, I. (2003). Ultra-fast freezing and low temperatures are not necessary to maintain the functional properties of

manufacturing beef. *Meat Science*, *66*, 171-179. https://doi.org/10.1016/S0309-1740(03)00081-0

Feiner, G. (2006). *Meat products handbook: Practical science and technology*. Elsevier.

Font-i-Furnols, M. y Guerrero, L. (2014). Consumer preference, behavior and perception about meat and meat products: An overview. *Meat Science*, *98*(3), 361-371. https://doi.org/10.1016/j.meatsci.2014.06.025

Fogerty, A. C., Whitfield, F. B., Svoronos, D. y Ford, G. L. (1990). Changes in the composition of the fatty acids and aldehydes of meat lipids after heating. *International Journal of Food Science & Technology*, *25*(3), 304-312.

Foraker, B. A., Gredell, D. A., Legako, J. F., Stevens, R. D., Tatum, J. D., Belk, K. E. y Woerner, D. R. (2020). Flavor, tenderness, and related chemical changes of aged beef strip loins. *Meat and Muscle Biology*, *4*(1), 28, 1-18. https://doi.org/10.22175/mmb.11115

Franco, J., Bianchi, G., Feed, O., Garibotto, G., Ballesteros, F., Bentancur, O., Carrere, M. y Chiruchi, J. (2009). Efecto de la estimulación eléctrica de la canal sobre la calidad de la carne de vacunos de pastoreo. *ITEA*, *105*(4), 313-323.

Frank, D., Joo, S. T. and Warner, R. (2016). Consumer acceptability of intramuscular fat. Korean J. Food Sci. Anim. Resour., 36(6), 699-708. https://doi.org/10.5851/kosfa.2016.36.6.699

French, P., O'riordan, E. G., Monahan, F. J., Caffrey, P. J., Mooney, M. T., Troy, D. J. y Moloney, A. P. (2001). The eating quality of meat of steers fed grass and/or concentrates. *Meat Science*, *57*(4), 379-386. https://doi.org/10.1016/S0309-1740(00)00115-7

Gill, C. O. (2014). Spoilage, factors affecting: Microbiological. In *Encyclopedia of Meat Sciences* (2a ed.), Academic Press: Cambridge, MA, USA; (pp. 388-393).

González-Gutiérrez, M., García-Fernández, C., Alonso-Calleja, C. y Capita, R. (2020). Microbial load and antibiotic resistance in raw beef preparations from northwest Spain. *Food Science and Nutrition*, *8*(2), 777-785. https://doi.org/10.1002/fsn3.1319

Hanagasaki, T. y Asato, N. (2018). Changes in free amino acids and hardness in round of Okinawan delivered cow beef during dry-and wet-aging processes. *Journal of Animal Science and Technology*, *60*, 1-9.

Hedrick, H. B., Paterson, J. A., Matches, A. G., Thomas, J. D., Morrow, R. E., Stringer, W. G. y Lipsey, R. J. (1983). Carcass and Palatability Characteristics of Beef Produced on Pasture, Corn Silage and Corn Grain. *Journal of Animal Science*, *57*(4), 91-801. https://doi.org/10.2527/jas1983.574791x

Huffman, K. L., Miller, M. F., Hoover, L. C., Wu, C. K., Brittin, H. C., y Ramsey, C. B. (1996). Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. *Journal of animal science*, *74*(1), 91-97. DOI: 10.2527/1996.74191x · Source: PubMed

Huff-Lonergan, E. y Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, *71*(1) 194-204. https://doi.org/10.1016/j.meatsci.2005.04.022

Iida, F., Miyazaki, Y., Tsuyuki, R., Kato, K., Egusa, A., Ogoshi, H. y Nishimura, T. (2016). Changes in taste compounds, breaking properties, and sensory attributes during dry aging of beef from Japanese black cattle. *Meat Science*, *112*, 46-51. https://doi.org/10.1016/j.meatsci.2015.10.015

Instituto Nacional de Carnes (INAC). (2023). Reglamento Nacional de Carnicerias. https://www.inac.uy/innovaportal/file/18947/1/reglamento-nacional-de-carnicerias-16.4.24.pdf

Instituto Nacional de Carnes (INAC). (2020). Exportaciones. https://www.inac.uy/innovaportal/v/23295/10/innova.front/exportacion#:~:text=En%20cuanto%20a%20los%20destinos,(56%20millones%20de%20d%C3%B3lares).

Instituto Nacional de Carnes (INAC). (2019). Comunicaciones. https://www.inac.uy/innovaportal/v/19609/37/innova.bs/japon:-valioso-mercado-restringido-por-condiciones-arancelarias

Jeremiah, L. E., Carpenter, Z. L. y Smith, G. C. (1972). Beef color as related to consumer acceptance and palatability. *Journal of Food Science*, *37*(3), 476-479.

Jiang, T., Bsuboom, J. R., Nelson, M. L., O'Fallon, J., Ringkob, T. P., Rogers-Klette, K. R., Joos, D. y Piper, K. (2010). The influence of forage diets and aging on beef palatability. *Meat Science*, *86*(3), 642-650. https://doi.org/10.1016/j.meatsci.2010.05.016

Kemp, C. M., Sensky, P. L., Bardsley, R. G., Buttery, P. J. y Parr, T. (2010). Tenderness – An enzymatic view. *Meat Science*, *84*(2), 248-256. https://doi.org/10.1016/j.meatsci.2009.06.008

Kim, J. H., Kim, D. H., Ji, D., Lee, H. J., Yoon, D. K. y Lee, C. H. (2017). Effect of aging process and time on physicochemical and sensory evaluation of raw beef top round and shank muscles using an electronic tongue. *Korean Journal Food Science Animal Resource*, *37*(6), 823-832. https://doi.org/10.5851/kosfa.2017.37.6.823

Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E. y Hunt, M. C. (2018). Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies (Review). *Meat Science*, *144*, 74-90. https://doi.org/10.1016/j.meatsci.2018.04.031

Kim, Y. H. B. (2011). Advances technology to improve meat color. En S. T. Joo, (ed.), *Control of Meat Quality* (pp. 31-60). Research Signpost.

Kim, Y. H. B., Frandsen, M. y Rosenvold, K. (2011). Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. *Meat Science*, *88*(3), 332-337. https://doi.org/10.1016/j.meatsci.2010.12.020

Kim, Y. H. B., Kemp, R. y Samuelsson, L. M. (2016). Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. *Meat Science*, *111*, 168-176.

Lagerstedt, Å., Enfält, L., Johansson, L. y Lundström, K. (2008). Effect of freezing on sensory quality, shear force and water loss in beef *M. longissimus dorsi*. *Meat Science*, *80*(2), 457-461. https://doi.org/10.1016/j.meatsci.2008.01.009

Larick, D. K. y Turner, B. E. (1990). Flavor characteristics of forage-and grain-fed beef as influenced by phospholipid and fatty acid compositional differences. *Journal of Food Science*, *55*(2), 312-317.

Leygonie, C., Britz, T. J. y Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science*, *91*(2), 93-98. https://doi.org/10.1016/j.meatsci.2012.01.013

Lepper-Blilie, A. N., Berg, E. P., Buchanan, D. S. y Berg, P. T. (2016). Effects of post-mortem aging time and type of aging on palatability of low marbled beef loins. *Meat Science*, *112*, 63-68. http://dx.doi.org/10.1016/j.meatsci.2015.10.017

Li, X., Babol, J., Bredie, W. L. P., Nielsen, B., Tománková, J. y Lundstrom. (2014). A comparative study of beef quality after ageing *longissimus* muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. *Meat Science*, *97*(4), 433-442. https://doi.org/10.1016/j.meatsci.2014.03.014

Livingston, D. J. y Brown, W. D. (1981). The chemistry of myoglobin and its reactions. *Food Technology*, *35*, 238-252.

Löndahl, G. y Nilaaon, T. (1993). Storage of Frozen Foods. En *Encyclopaedia of Food Science and Nutrition* (2.ª ed., pp. 2732-2735). Academic Press.

Maga, J. A. (1994). Umami flavour of meat. En *Flavor of meat and meat products*. (pp. 98-115). Springer.

Marmer, W. N., Maxwell, R. J. y Williams, J. E. (1984). Effects of dietary regimen and tissue site on bovine fatty acid profiles. *Journal of Animal Science*, *59*(1), 109-121.

Melton, S. L. (1983). Effect of forage feeding on beef flavor. *FoodTechnology*, *37*, 239-248.

Melton, S. L. (1990). Effects of feeds on flavor of red meat: A review. *Journal of Animal Science*, *68*(4421).

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L. y Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science*, *79*(12), 3062-3068. https://doi.org/10.2527/2001.79123062x

Mottram, D. S. (1996). The role of phospholipids in meat flavor: an overview. En W. Pickenhagen, C.-T., Ho y A. M. Spanier (eds.), *Contribution of Low and Nonvolatile Materials to the Flavor of Foods* (pp. 193-206). Allured Publishing.

Mottram, D. S. y Edwards, R. A. (1983). The role of triglycerides and phospholipids in the aroma of cooked beef. *Journal of the Science of Food and Agriculture*, *34*(5), 517-522. https://doi.org/10.1002/jsfa.2740340513

Nethra, P. V., Sunooj, K. V., Aaliya, B., Navaf, M., Akhila, P. P., Sudheesh, C., ... & George, J. (2023). *Critical factors affecting the shelf life of packaged fresh red meat—a review. Meas Food 10: 100086*. https://doi.org/10.1016/j.meafoo.2023.100086

Norton, T., Delgado, A., Hogan, E., Grace, P. y Sun, D. W. (2009). Simulation of high-pressure freezing processes by enthalpy method. *Journal of Food Engineering*, *91*(2), 260-268.

Nuernberg, K., Dannenberger, D., Nuernberg, G., Ender, K., Voigt, J., Scollan, N. D., Wood J. D., Nute, G. R. y Richardson, R. I. (2005). Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. *Livestock Production Science*, *94*(1-2), 137-147. https://doi.org/10.1016/j.livprodsci.2004.11.036

Owen, J. E. y Lawrie, R. A. (1975). The effect of an artificially induced high pH (hydrogen-ion concentration) on the susceptibility of minced porcine muscle to undergo oxidative rancidity under frozen storage. *Journal of Food Technology*, *10*(2), 169-180.

Pal, M. y Devrani, M. (2018). Application of Various Techniques for Meat Preservation. *Journal of Experimental Food Chemistry*, *4*(1), 134. 10.4172/2472-0542.1000134

Pham, Q. T. (2004). Thawing. En W. K. Jensen (ed.), *Encyclopedia of meat sciences* (vol. 3, pp. 1150-1156). Elsevier Academic Press.

Priolo, A., Micol, D. y Agabriel, J. (2001). Effects of grass feeding systems on ruminant meat colour and flavour. A review. *Animal Research*, *50*(3), 185-200.

Rahman, M. M., Hashem, M. A., Azad, M. A. K., Choudhury, M. S. H. y Bhuiyan, M. K. J. (2023). Techniques of meat preservation- A review. *Meat Research*, *3*(3), 55. https://doi.org/10.55002/mr.3.6.73

Realini, C. E., Kallas, Z., Pérez-Juan, M., Gómez, I., Olleta, J. L., Beriain, M. J., Albertí, P. y Sañudo, C. (2014). Relative importance of cues underlying Spanish consumers' beef choice and segmentation, and consumer liking of beef enriched with n-3 and CLA fatty acids. *Food Quality and Preference*, *33*, 74-85. https://doi.org/10.1016/j.foodqual.2013.11.007

Realini, C. E., Duckett, S. K., Brito, G. W., Dalla Rizza, M. y De Mattos, D. (2004). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science*, *66*(3), 567-577. https://doi.org/10.1016/S0309-1740(03)00160-8

Reglamento Brimatológico Nacional. 2019. https://www.impo.com.uy/bases/decretos-originales/31-2021/1

Rehman, S. U., Seo, J. K., Romanyk, M., Shin, D. J. y Kim, Y. H. B. (2024). Impacts of Stepwise Aging/Freezing Process and Repeated Freezing on Meat Quality, Physicochemical and Biochemical Properties, and Sensory Attributes of Beef Loins. *Meat and Muscles Biology*, *8*(1), 18294, 1-18. doi:10.22175/mmb.18294

Rinn, N., Braun, A. S., Müller, A., Wadepohl, K., Gerulat, B., Kumm, F., Yue, M. y Kehrenberg, C. (2024). Microbiological quality of raw beef imported into the European Union from third countries. *Food Control*, *160*, 110358. https://doi.org/10.1016/j.foodcont.2024.110358

Rodas-González, A., Huerta-Leidenz, N., Jerez-Timaure, N. y Miller, M. F. (2009). Establishing tenderness thresholds of Venezuelan beef steaks using consumer and trained sensory panels. *Meat Science*, *83*(2), 218-223. https://doi.org/10.1016/j.meatsci.2009.04.021

Savell, J. W., Branson, R. E., Cross, H. R., Stiffler, D. M., Wise, J. W., Griffin, D. B. y Smith, G. C. (1987). National Consumer Retail Beef Study: Palatability Evaluations of Beef Loin Steaks that Differed in Marbling. *Journal of Food Science*, *52*(3), 517-519. https://doi.org/10.1111/j.1365-2621.1987.tb06664.x

Shackelford, S. D., Wheeler, T. L. y Koohmaraie, M. (1995). Relationship between shear force and trained sensory panel tenderness ratings of 10 major muscles from *Bos indicus* and *Bos taurus* cattle. *Journal of Animal Science*, *73*(11), 3333-3340. https://doi.org/10.2527/1995.73113333x

Schroeder, J. W., Cramer, D. A., Bowling R. A. y Cook, C. W. (1980). Palatability, shelf life and chemical differences between forage- and grain-finished Beef. *Journal of Animal Science*, *50*(5), 852-859. https://doi.org/10.2527/jas1980.505852x

Schwarz, F. J., Augustini, C., Timm, M., Kirchgessner, M. y Steinhart, H. (1998). Effect of vitamin E on alpha-tocopherol concentration in different tissues and oxidative stability of bull beef. *Livestock Production Science*, *56*(2), 165-171. https://doi.org/10.1016/S0301-6226(98)00189-4

Smith, R., Nicholson, K., Nicholson, J., Harris, K., Miller, R., Griffin, D. y Savell, J. (2008). Dry versus wet aging of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. *Meat Science*, *79*(4), 631-639. https://doi.org/10.1016/j.meatsci.2007.10.028

Szczesniak, A. S. (1987). Correlating sensory with instrumental measurements-an overview of recent developments. *Journal of Texture Studies*, *18*(1), 1-15. https://doi.org/10.1111/j.1745-4603.1987.tb00566.x

Vieira, C., Diaz, M. T., Martínez, B. y García-Cachán, M. D. (2009). Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. *Meat Science*, *83*(3), 398-404. https://doi.org/10.1016/j.meatsci.2009.06.013

Wheeler, T. L., Savell, J. W., Cross, H. R., Lunt, D. K. y Smith, S. B. (1990). Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. *Journal of Animal Science*, *68*(12), 4206-4220. https://doi.org/10.2527/1990.68124206x

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I. y Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, *78*(4), 343-358.

Yaylayan, V. A., Keyhani, A. y Wnorowski, A. (2000). Formation of Sugar-Specific Reactive Intermediates from 13C-Labeled l-Serines. *Journal of Agricultural and Food Chemistry*, *48*(3), 636-641. https://doi.org/10.1021/jf990687a

Zerby, H. N., Belk, K. E. Sofos, J. N. McDowell L. R. y Smith, G. C. (1999). Case life of seven retail products from beef cattle supplemented with alpha-tocopheryl

acetate. *Journal of Animal Science*, *77*(9), 2458-2463. https://doi.org/10.2527/1999.7792458x

Zhou, G. H., Xu, X. L. y Liu, Y. (2010). Preservation technologies for fresh meat – A review. *Meat Science*, *86*(1), 119-128.

Zhang, R., Yoo, M. J. Y. y Farouk, M. M. (2019). Quality and acceptability of fresh and long-term frozen in-bag dry-aged lean bull beef. *Journal of Food Quality*, (1975264), 1-15. https://doi.org/10.1155/2019/1975264

Zhang, R., Yoo, M. J. Y. y Farouk, M. M. (2020). Oxidative stability, proteolysis, and in vitro digestibility of fresh and long-term frozen stored in-bag dry-aged lean beef. *Food Chemistry*, *344*, 128601. https://doi.org/10.1016/j.foodchem.2020.128601

Zhang, R., Ross, A. B., Yoo, M. J. Y. y Farouk, M. M. (2021). Metabolic fingerprinting of in-bag dry- and wet-aged lamb with rapid evaporative ionisation mass spectroscopy. *Food Chemistry*, *347*, 128999. https://doi.org/10.1016/j.foodchem.2020.128999