Meat and Muscle Biology™

Flavor Development of Individually Vacuum-Packaged Beef Steaks During Extended Wet Aging†

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Abstract: The objective of the study was to determine the effect of extended aging on the flavor development of various muscles, individually stored in vacuum rollstock packaging. Strip loins, paired tenderloins, and top sirloin butts ($n = 48$) from USDA Low Choice carcasses (Small^{00} to Small^{100} marbling score, $n = 16$). Subprimals were wet-aged in the absence of light for 28 d postmortem before fabrication into 2.54 cm steaks representing the *longissimus lumborum* (LL), *psoas major* (PM), and *gluteus medius* (GM). Steaks were individually packaged in vacuum rollstock packaging and assigned to an additional aging time of 28, 35, 42, 49, or 56 d. Cut steaks ($n = 240$/test) were designated to trained descriptive panel analysis or volatile compound analysis. No interactions occurred for trained sensory analysis, but a main effect of days of age ($P \leq 0.033$) showed the greatest effect on negatively associated attributes, including liver-like, oxidized, fishy, bitter, and sour, after 42 d of aging. A main effect of muscle type also occurred ($P \leq 0.040$) for flavor attributes, in which GM and PM samples scored higher in off-flavor attributes compared with LL samples, including flavors such as liver-like, oxidized, and sour. An interaction between muscle type and days of age occurred for 2-pentyl-furan ($P = 0.021$). One compound—3 hydroxy-2 butanone—was affected by muscle type ($P = 0.009$). However, most compounds were affected by days of age ($P \leq 0.046$), in which compounds related to off-flavors increased in concentrations the most after 49 d. Additionally, discriminant function analyses were performed, suggesting the most effective aging time for individual steaks to be under 49 d when considering loadings for volatile compounds and flavor attributes corresponding with days of age. Overall, these data suggest individual packaging of GM, LL, and PM muscles is most optimal for up to 42 or 49 d of age without a large impact from the presence of off-flavors, thus providing food service establishments the opportunity to individually package beef steaks for an extended period while maintaining consumer satisfaction through optimal flavor.

Key words: aging, food service, trained descriptive panel, volatile compounds, vacuum packaged
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Introduction

Within a food service setting, consumers have very few interactions with raw products and are provided only with what they hope will be a positive eating experience. This allows the food service industry a unique opportunity to ease production and minimize waste by storing individually vacuum-packaged steaks. When the aging of whole subprimals may consume an excessive amount of refrigerated space, individually packaged steaks in a food service setting may ease storage methods and needs as well as prolong shelf life compared with traditionally wet-aged subprimals while still ensuring a positive eating experience.

Wet aging, characterized by the storing of raw products in vacuum packaging for an allotted amount of time, has traditionally been proven to enhance tenderness and flavor. However, the use of extended aging has the potential to produce undesirable flavors in whole subprimals, including sour or metallic.

Current studies have shown a decreased consumer liking to steaks wet-aged beyond 35 d (Lepper-Blilie et al., 2012; Pogorzelski et al., 2021). Consumers value a high-quality, unique eating experience, especially when provided by food service establishments (Polkinghorne and Thompson, 2010). Therefore, should the use of individually vacuum-packaged beef steak in food service be adopted, it is imperative to understand the optimal aging length in order to maintain desirable beef flavor and quality.

It is well established that muscle type may influence beef palatability following aging because of physiological differences including fatty acid composition, fiber type, and mitochondrial content (Hood, 1980; Hunt et al., 2016). Although other studies have evaluated the effect of packaging type and retail display on palatability of individual muscles (Ponce et al., 2019; Vierck et al., 2020; Barker et al., 2022), few, if any, have determined the effect of extended aging on individually vacuum-packaged muscles. Therefore, the objective of this study was to evaluate the quality of individual beef steaks packaged in vacuum rollstock packaging (VRP) for an extended period.

Materials and Methods

Product selection and subprimal fabrication

Product collection and fabrication were conducted following the methods of Barker et al. (2022). Modifications are as follows: strip loins (IMPS 180, NAMP 2014), top sirloin butts (IMPS 184, NAMP 2014), and paired tenderloins (IMPS 189A, NAMP 2014) were selected from USDA Low Choice (Small^{00} to Small^{100} marbling score) beef steer carcasses ($n = 16$) at a commercial processing facility. Carcass characteristics, including back fat thickness, ribeye area, marbling scores, lean maturity, and skeletal maturity, were collected by trained personnel for all carcasses and displayed in Table 1. Vacuum-packaged subprimals were wet-aged in darkness and under refrigeration (0°C to 4°C) for 28 d postmortem to mirror industry practice. Following 28 d of aging, all subprimals were fabricated to produce 2.54-cm-thick steaks ($n = 480$) representing the *longissimus lumborum* (LL), *psoas major* (PM), and *gluteus medius* (GM) per respective subprimal. From each subprimal, 10 steaks were produced. Fabricated steaks were individually allotted to VRP (Multivac Inc., Kansas City, MO) and randomly assigned to 1 of 5 aging treatments: 28, 35, 42, 49, and 56 d. Packaged steaks were stored under darkness and refrigerated conditions (0°C to 4°C; mean: 1.98°C ± 0.87°C) for their respective aging intervals and frozen at −20°C following aging.

Table 1. Least-squares means (± SEM[1]) of beef carcasses ($n = 16$) measurements

Carcass Characteristics	
Quality Attributes	
Lean maturity[2]	59 ± 4.2
Skeletal maturity[3]	58 ± 5.5
Marbling score[4]	463 ± 3.9
Yield Attributes	
Preliminary fat thickness, cm	2.9 ± 0.1
Adjusted fat thickness, cm	3 ± 0.1
Ribeye area, cm^2	92.7 ± 0.2

[1]Standard error of the mean.

[2]0–100 = A maturity, approximately 9–30 mo of age; 101–200 = B, approximately 30–42 mo of age; 201–300 = C, approximately 42–72 mo of age; 301–400 = D, approximately 72–96 mo of age; 401–500 = E, >96 mo of age.

[3]0–100 = A maturity, approximately 9–30 mo of age; 101–200 = B, approximately 30–42 mo of age; 201–300 = C, approximately 42–72 mo of age; 301–400 = D, approximately 72–96 mo of age; 401–500 = E, >96 mo of age.

[4]200 = Traces; 300 = Slight; 400 = Small; 500 = Modest; 600 = Moderate.

Cooking method

Steaks were tempered for 24 h at 2°C to 4°C prior to cooking. Following methods similar to Barker et al. (2022), steaks were cooked in electric rational ovens (Model SCC WE 62G, Rational USA, Rolling Meadows, IL). Raw and cooked weights and temperatures (Thermapen, Classic Super-Fast, ThermoWorks, American Fork, UT) were recorded for all samples.

Cooked homogenate

Samples assigned to volatile analysis were immediately processed following procedures described in Barker et al. (2022). Processed cubes were flash frozen by submersion in liquid nitrogen, pulverized to a free-flowing powder (Blixer 3 Food Processor, Robot Coupe, Ridgeland, MS), and stored at −80°C.

Descriptive trained sensory analysis

Descriptive trained sensory analyses were performed following the methods of Barker et al. (2022) and the American Meat Science Association sensory guidelines (American Meat Science Association, 2015). Panelists were trained for approximately 40 h to objectively identify descriptors described by Adhikari et al. (2011)

Table 2. Descriptive attributes and references

Flavor Attribute	Anchor	Location on Scale (0–100)
Beef flavor identity	Beef broth (heated to 74°C, served warm)	30
	80:20 ground chuck (71°C internally)	50
	Brisket (71°C internally)	75
Bloody/ serumy	USDA Choice strip steak (60°C internally)	40
Brown/ roasted	80:20 ground chuck (71°C internally)	40
	Well-done strip steak (77°C internally)	65
Fat-like	90:10 ground beef (71°C internally)	30
	70:30 ground beef (71°C internally)	60
Liver-like	Flat iron steak (71°C internally)	20
	Calf liver	90
Oxidized	Microwaved vegetable oil	30
	Cooked, stored (12 h at 4°C), and microwaved ground beef	60
Buttery	Unsalted butter, 0.1-cm-thick slice	65
Fishy	Cod liver oil	30
	Canned tuna	60
Umami	Beef broth (heated to 74°C, served warm)	30
Sour	0.015% citric acid solution	10
	0.050% citric acid solution	25
Salty	0.15% NaCl solution	10
	0.25% NaCl solution	45
Bitter	0.01% caffeine solution	15
	0.02% caffeine solution	25
Overall tenderness	Eye of round steak (77°C internally)	30
	Strip steak (71°C internally)	55
	Tenderloin steak (65°C internally)	90
Overall juiciness	Strip steak (85°C internally)	25
	Strip steak (71°C internally)	50
	Strip steak (60°C internally)	75

NaCl = sodium chloride.

(Table 2): beef flavor identity, brown/roasted, bloody/serumy, fat-like, liver-like oxidized, buttery, fishy, umami, earthy/musty, salty, bitter, and sour. Steaks from all carcasses, aging interval, and muscle types ($n = 240$) were randomly evaluated over 30 panel sessions. Panels were completed over a 15-d period.

Steaks were thawed and cooked following methods previously described and following postcooking procedures listed by Barker et al. (2022). Cooked samples were held (Cambro Ultra Heated Holding Pan Carrier, 214UPCH400, Cambro Manufacturing, Huntington Beach, CA) at 50°C to 55°C for no more than 5 min prior to serving. Prior to serving, cooked steaks were cube pieces measuring the steak thickness × 1.27 × 1.27 cm (1/2" sensory box, Tallgrass Solutions Inc., Manhattan, KS).

Volatile compound analysis

Volatile compound analyses ($n = 240$) were performed on cooked steaks following methods similar to Gardner and Legako (2018) and Barker et al. (2022). In brief, 5 g of homogenate was placed in 20-mL glass vials (Gerstel, Linthicum, MD), and 10 µL of an internal standard (1,2-dichlorobenzene, 25 ng/µL) was added before sealing. Vials were loaded onto a Gerstel autosampler for volatile extraction, separation, and measurement by head space solid phase microextraction with an 85-µm film thickness carboxen polydimethylsiloxane fiber (Supelco, Bellefonte, PA) and gas chromatography mass spectrometry (7890B series, Agilent, Santa Clara, CA; 5977A, Agilent). Volatile compound identities were confirmed by authentic standard (Sigma-Aldrich, St. Louis, MO), retention times, and comparing unique ion fragmentation patterns. Internal standard calibration was used to quantify compounds of interest, and data were expressed as quantity extracted in nanogram per sample weight in grams.

Statistical Analysis

Data from descriptive trained sensory panels and volatile flavor compound analyses were analyzed as a split plot design, with muscle serving as the whole plot and days of age representing the subplot. Individual package served as the experimental unit for the subplot. Product collection replication, trained panel session, and trained panel date were all incorporated into the model as random effects. Peak temperature was also included as a covariate. Probability values (P values) less than or equal to $\alpha = 0.05$ were considered statistically significant. Denominator degrees of freedom were estimated using the Kenward-Roger adjustment. All data were evaluated using the PROC GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Cary, NC).

Additionally, discriminant function analyses (DFA) were performed to generate visual plots of variables of interest (days of age and muscle type) on the sensory attributes and volatile compounds evaluated in a multivariate space. Statistical analyses were performed in R statistical software (v. 4.0.2, R Core Team, 2020) following the procedures described by Barker et al. (2022). Highly correlated values were removed at $r > 0.50$ (R statistical software, v. 4.0.2, R Core Team, 2020). Significant effects were determined at $P < 0.05$.

Results and Discussion

Descriptive trained sensory analysis

There were no interactions between aging time and muscle type for any flavor attributes detected by trained panelists ($P \geq 0.062$). However, there was a main effect for days of age on flavor development for 11 out of 16 attributes evaluated ($P \leq 0.033$) (Table 3). As days of age increased, positively associated attributes, including beef flavor, buttery, and brown/roasted, decreased ($P < 0.05$) in score at day 49. Meanwhile, these attributes did not differ ($P > 0.05$) among samples between 28 and 42 d. Similar studies have found a decrease in signature beef flavors following extended aging beyond 35 d (Lepper-Blilie et al., 2016; Foraker et al., 2020). When considering extended aging of beef steaks, vacuum packaging may be detrimental to consumer perception as signature beef flavors decline with age (Garmyn et al., 2020). Contrastingly, those flavors that are often considered negative (i.e., liver-like, oxidized, fishy, sour, etc.) were more prevalent to panelists by day 42 ($P < 0.05$). Overall juiciness and tenderness did not differ over the aging period ($P > 0.05$). Furthermore, umami flavors did not change during aging durations, disagreeing with others suggesting umami notes tend to increase during refrigerated aging (Dashdorj et al., 2015). The increased presence of off-flavors, specifically sour notes, may be due to the accumulation of lactic acid bacteria, commonly developed in extended vacuum-packaged aging (Pierson et al., 1970; Egan, 1983). Additionally, the increased presence of off-flavors may lead to a masking effect, recognized in prior studies in which positive notes, such as umami, are often hidden by more offensive notes (Stutz et al., 1991; Jackson et al., 1992). Although this study did not evaluate the development of flavors prior to 28 d, a companion paper evaluated similar attributes following 14 d of subprimal aging, in addition of up to 10 d of retail display under vacuum as individual steaks (Barker et al., 2022). The results in said study also recognized a decrease in umami notes at 6 d in retail display (20 d postmortem and under vacuum), and an increase in off-flavors – liver-like and sour – as soon as 2 d in retail display (16 d postmortem and under vacuum).

Muscle type influenced 11 out of 16 attributes evaluated by trained panelists ($P \leq 0.040$) (Table 4). Two attributes most often associated with positive eating experiences in beef – beef flavor ID and brown/roasted – were highest in LL steaks ($P < 0.05$), whereas GM and PM steaks did not differ for beef flavor ID ($P > 0.05$) and were lower than LL steaks ($P < 0.05$). For other positive attributes, including umami, salty, and overall juiciness, values across muscles did not differ ($P > 0.05$). Undesirable flavors like liver-like, oxidized, earthy/musty, and sour were highest in PM steaks ($P < 0.05$), intermediate in GM steaks, and lowest in the LL ($P < 0.05$). The PM is often considered a bland muscle, lacking strong flavors under normal conditions. However, as a muscle made up of predominantly red muscle fibers, the PM is more receptive to oxidation, proving that extended aging may accelerate the development of off-flavors as oxidation of those fibers occurs (O'Keeffe and Hood, 1982). Furthermore, the lack of stability in the PM is often noticed during retail display, when the PM continues to prove itself as a muscle

Table 3. Main effect of days of age[1] on significant[2] flavor attributes

	Days of Age						
Attribute	28	35	42	49	56	SEM[3]	*P* Value
Beef flavor ID	49.6^{a}	49.6^{a}	49.8^{a}	48.6^{b}	46.0^{c}	0.64	<0.001
Brown/roasted	45.7^{a}	46.3^{a}	46.1^{a}	45.1^{a}	42.6^{b}	1.14	0.020
Liver-like	8.9^{b}	8.6^{c}	8.9^{b}	10.6^{b}	12.2^{a}	1.14	0.007
Oxidized	7.5^{c}	7.9^{bc}	8.0^{bc}	8.9^{b}	11.4^{a}	0.58	<0.001
Metallic	12.3^{c}	12.3^{c}	13.0^{c}	15.5^{b}	18.5^{a}	0.58	<0.001
Fishy	2.7^{b}	2.1^{b}	3.0^{b}	4.5^{a}	5.4^{a}	0.64	0.004
Buttery	5.1^{a}	4.8^{a}	4.2^{a}	2.9^{b}	2.9^{b}	0.55	<0.001
Earthy/musty	4.9^{c}	5.8^{bc}	6.8^{b}	6.4^{bc}	8.8^{a}	0.76	<0.001
Salty	3.1^{b}	3.3^{b}	3.2^{b}	3.6^{b}	5.0^{a}	0.63	0.012
Bitter	6.2^{b}	6.2^{b}	7.2^{ab}	6.9^{ab}	7.7^{a}	0.92	0.033
Sour	8.3^{d}	9.3^{d}	11.5^{c}	16.4^{b}	24.1^{a}	0.92	<0.001

[1]28, 35, 42, 49, and 65 d.

[2]Significant attributes determined at $P < 0.05$.

[3]Standard error of the least-squares mean (largest).

[a–d]Means within rows lacking a common superscript differ ($P < 0.05$).

Table 4. Main effect of muscle type[1] on significant[2] flavor attributes

	Muscle Type				
Attribute	GM	LL	PM	SEM[3]	*P* Value
Beef flavor ID	48.6[b]	50.0[a]	47.5[b]	0.55	<0.001
Brown/roasted	45.9[a]	46.8[a]	43.7[b]	1.02	0.001
Fat-like	13.6[b]	14.3[a]	15.2[a]	0.51	0.001
Liver-like	10.9[a]	6.8[b]	11.7[a]	0.57	<0.001
Oxidized	9.1[a]	7.4[b]	9.4[a]	1.04	0.001
Metallic	15.4[a]	13.9[b]	13.8[b]	0.47	0.007
Fishy	4.2[a]	2.9[b]	3.6[ab]	0.5	0.040
Buttery	3.3[b]	4.4[a]	4.3[a]	0.43	0.040
Earthy/musty	6.3[b]	5.7[ab]	7.5[a]	0.96	0.004
Sour	15.5[a]	11.7[b]	14.0[a]	0.71	<0.001
Overall tenderness	52.7[b]	53.8[b]	69.9[a]	0.83	<0.001

[1]*Gluteus medius* (GM), *longissimus lumborum* (LL), *psoas major* (PM).

[2]Significant attributes determined at $P < 0.05$.

[3]Standard error of the least-squares mean (largest).

[a,b]Means within rows lacking a common superscript differ ($P < 0.05$).

lacking color stability (O'Keefe and Hood, 1982; Lanari and Cassens, 1991). Although this study did not evaluate color of the muscle described, it may be worth noting that discoloration or color stability may be related to flavor development because discoloration and lipid oxidation may be products of myoglobin oxidation (Mancini and Hunt, 2005; Suman and Joseph, 2013). It is still unclear if lipid or myoglobin oxidation occurs first, but the two often go together, and where there is oxidation, there are typically off-flavors. Furthermore, studies have noted an increase in undesirable liver-like flavors in cuts like the PM and GM as iron content and myoglobin concentrations increase (Yancey et al., 2006). Interestingly, there were no interactive effects between muscle type and aging. Thus, this implies that each factor strongly influences flavor but that the impacts of aging were similar in nature for each muscle.

Volatile compound analysis

An interaction occurred between days of age and muscle type ($P = 0.021$, standard error of the mean = 0.34) (Figure 1) for one compound, 2-pentyl furan. The compound 2-pentyl-furan has been described as an oxidation product of linoleic acid (Ho and Chen, 1994; Mallick et al., 2021). At 28 d of aging, 2-pentyl furan was at its highest concentration ($P < 0.05$) in all 3 muscles but decreased at 35 d, with similar concentrations ($P > 0.05$) for the remainder of the aging duration. Mallick et al. (2021) further suggests that the oxidation of lipids prior to cooking may dictate 2-pentyl-furan products. It may be possible that degradation of lipids has occurred to a point that the production of this compound is no longer possible to the same extent with prolonged aging methods.

Of the 56 compounds evaluated in samples, a main effect of days of aging ($P \leq 0.046$) occurred for 22 compounds (Table 5). Of the 22 compounds affected, 16 were lipid-derived compounds, whereas the remaining 6 were Maillard reaction derived. Compounds including ethanol, nonanoic acid, and decane showed increased ($P < 0.05$) concentrations as aging duration increased. Ethanol is a common product of vacuum packaging because of the fermentation of lactic acid bacteria (Argyri et al., 2015). In a similar study, Barker et al. (2022) also recognized substantial increases in ethanol concentrations with vacuum-packaged display up to 10 d. Furthermore, Shahidi and Pegg (1994) further suggested that aldehydes, including decane, and carboxylic acids like nonanoic acid are sensitive to oxidation and are excellent indicators of flavor

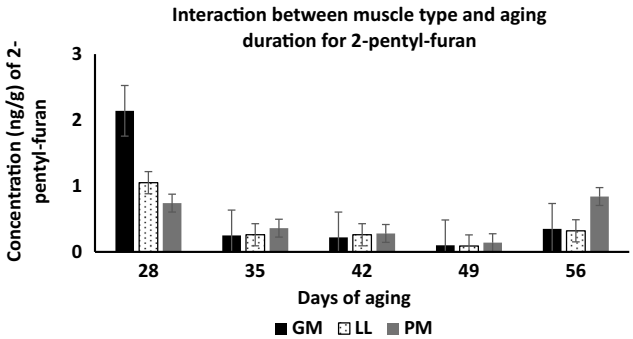

Figure 1. Two-way interaction ($P = 0.021$) between muscle type (*gluteus medius* (GM); *longissimus dorsi* (LL); and *psoas major* (PM)) and aging duration (28, 35, 42, 49, and 56 d) for the compound 2-pentyl-furan.

Table 5. Main effect of days of age on significant[1] volatile flavor compounds

Compounds		Days of Age					SEM[2]	*P* Value
		28	35	42	49	56		
Lipid and/or Microbial-Derived Compounds								
Alcohols	Ethanol	10.38^{b}	12.64^{b}	15.62^{b}	30.49^{b}	90.42^{a}	14.81	<0.001
	1-Pentanol	4.87^{a}	3.90^{ab}	2.49^{bc}	2.93^{bc}	1.78^{c}	0.68	0.004
	1-Octanol	1.92^{ab}	0.91^{b}	1.98^{ab}	0.43^{b}	3.67^{a}	0.73	0.016
n-Aldehydes	Pentanal	28.65^{a}	19.51^{b}	11.11^{b}	13.18^{b}	13.44^{b}	3.23	<0.001
	Hexanal	80.87^{a}	54.94^{ab}	40.09^{b}	37.68^{b}	54.59^{ab}	10.04	0.021
	Octanal	13.67^{ab}	9.58^{b}	12.71^{ab}	6.94^{b}	19.71^{a}	3.07	0.034
	Nonanal	20.98^{abc}	13.67^{bc}	26.67^{ab}	9.22^{c}	37.66^{a}	6.69	0.011
	Decanal	1.76^{a}	0.42^{b}	1.13^{ab}	0.32^{b}	1.66^{a}	0.32	0.001
	Dodecanal	0.49^{ab}	0.36^{b}	0.81^{a}	0.22^{b}	0.82^{a}	0.12	0.001
Alkenes	Toluene	0.95^{bc}	0.99^{bc}	0.78^{c}	2.66^{a}	1.63^{b}	0.29	<0.001
	p-Xylene	0.14^{b}	0.22^{b}	0.22^{b}	0.24^{b}	0.45^{a}	0.05	0.001
	D-limonene	0.05^{b}	0.09^{b}	0.56^{a}	0.07^{b}	0.50^{a}	0.11	<0.001
Carboxylic Acids	Acetic acid	108.44^{b}	140.75^{b}	212.34^{b}	266.19^{b}	956.49^{a}	212.4	0.028
	Nonanoic acid	0.57^{b}	0.29^{b}	0.61^{b}	0.24^{b}	1.19^{a}	0.18	<0.001
Hydrocarbons	Decane	0.26^{b}	0.24^{b}	0.40^{b}	0.24^{b}	0.74^{a}	0.09	<0.001
	Tetradecane	0.71^{ab}	0.28^{c}	0.47^{bc}	0.23^{c}	0.94^{a}	0.12	<0.001
Maillard Reaction Compounds								
Ketones	3 hydroxy-2 butanone	64.09^{a}	70.55^{a}	43.39^{b}	18.26^{c}	3.88^{c}	6.79	<0.001
Pyrazines	2-ethyl-3,5/6-dimethylpyrazine	0.20^{a}	0.16^{ab}	0.15^{ab}	0.10^{bc}	0.09^{c}	0.02	0.001
Strecker Aldehydes	3-methylbutanal	4.42^{bc}	7.22^{ab}	7.47^{ab}	11.03^{a}	3.16^{c}	1.44	0.002
	2-methylbutanal	2.24^{b}	4.39^{ab}	4.67^{ab}	6.63^{a}	3.38^{b}	0.92	0.009
	Phenylacetaldehyde	0.34^{b}	0.23^{b}	0.58^{ab}	0.24^{b}	1.25^{a}	0.26	0.028
Sulfur-Containing	Dimethyl-disulfide	0.04^{b}	0.12^{b}	0.14^{ab}	0.30^{a}	0.19^{ab}	0.06	0.046

[1]Significant compounds determined at $P < 0.05$.

[2]Standard error of the least-squares mean (largest).

[a–c]Means within rows lacking a common superscript differ ($P < 0.05$).

deterioration as they may contribute to rancid or sour notes (Burdock, 2005). Interestingly, in the current study, concentrations of both decane and nonanoic acid remained steady from day 28 to 49 ($P > 0.05$) but increased almost 3 times the original concentrations by day 56 ($P < 0.05$). Acetic acid also showed substantial increases in concentration over the aging period ($P < 0.05$) while also being the most prevalent compound when comparing the main effect. Like ethanol, acetic acid is often associated with vacuum-packaged products and the production of sour notes (O'Quinn et al., 2012). However, whereas sour flavors only appeared to increase by day 56 for trained panelists, acetic acid showed a steady numerical increase, although statistical significance was not shown until day 56 ($P < 0.05$). Contrastingly, compounds that are most often associated with positive eating experiences and beef flavor, like 3-methylbutanal and 3-hydroxy-2-butanone, decreased as aging increased ($P < 0.05$). Although the masking effect is theorized extensively in terms of distinguishable flavor, this provides evidence of a clear decrease in these Maillard reaction–derived compounds, aiding in positive attributes. However, many studies have also suggested that 3-hydroxy-2-butanone is also correlated with the oxidation unsaturated fatty acids (El-Magoli et al., 1996; Stetzer et al., 2008). Because the final stage of lipid oxidation is termination, it may be possible that the production of 3-hydroxy-2-butanone had increased to its full potential and could no longer increase in concentration by 56 d.

Only one compound—3-hydroxy-2-butanone—was affected by muscle type ($P = 0.009$) (Table 6). The LL showed the highest concentration of the compound ($P < 0.05$), whereas the GM and PM were similar ($P > 0.05$). Previous studies have recognized 3-hydroxy-2-butanone's contribution to buttery flavors in samples with higher fat content (El-Magoli et al., 1996). Although all cuts analyzed in the current study came from the same carcasses and were all low Choice quality, there may be slight fat differences among muscles contributing to differences in concentrations. Nonetheless, intramuscular fat content in prior studies has shown no differences in GM and LL steaks (Hunt

Table 6. Main effect of muscle type on significant[1] volatile flavor compounds

	Muscle Type				
Compound	GM	LL	PM	SEM[2]	*P* Value
3 hydroxy-2 butanone	36.26[b]	53.01[a]	30.83[b]	5.25	0.009

[1]Significant compounds determined at $P < 0.05$.

[2]Standard error of the least-squares mean (largest).

[a,b]Means within rows lacking a common superscript differ ($P < 0.05$).

GM = *gluteus medius*, LL = *longissimus lumborum*, PM = *psoas major*.

et al., 2016) or LL steaks versus other round and chuck cuts (Nyquist et al., 2018) of the same quality grade.

Discriminant function analysis

A DFA was utilized to determine maximum variation between groups while minimizing variation within groups. Variables that were highly correlated were removed from the functions, and those attributes that were less correlated remained. Loadings and standardized coefficients were determined for each treatment DFA, and models were developed according to each fixed effect (muscle type and aging duration) to further interpret relationships among variables. Both fixed effects, muscle type and aging duration, varied among treatments ($P < 0.001$) within their respective model.

Muscle type

The DFA corresponding to muscle type showed the canonical correlations on DF1 explained 75.7% of variation, whereas DF2 explained 24.3% of the variation ($P < 0.001$) (Figure 2A and 2B). The DFA explained 100% of the variation between treatments overall. The DF1 was most effective at explaining variation and showed maximal separation between the LL and GM from the PM. On the DF2 function, the LL and GM were maximally separated from each other, with the PM lying between the 2 factors.

The DF1 function explained much of the variation between the 2 functions. However, all the variables in the output loaded heavily on the function around the LL and GM, apart from overall tenderness, which loaded heavily on the function corresponding with the PM. Of the flavor attributes selected by the model, many could be considered negative and include sour, liver-like, oxidized, and bitter. Acetic acid was a notable volatile compound present on the DF1 function. Acetic acid is a contributor to sour flavors in beef, especially when vacuum packaged because of lactic acid bacteria (Seideman et al., 1976; O'Quinn et al., 2012). In a similar study, results from Barker et al. (2022) were similar for muscle loadings, in which acetic acid also corresponded to the GM and LL but not the PM.

Although the DF1 function explained more variation, differences between loadings on the DF2 function were clearer. The GM and LL on the function were maximally separated, with the PM between the two. Negatively associated attributes, such as sour, oxidized, liver, and fishy, weighed heaviest on the side of the function corresponding with the GM. On the opposite side of the function, positive attributes like beef flavor ID, umami, buttery, and fat-like corresponded with the canonical correlations, LL and PM. These flavors may be associated with the volatile compounds that also loaded heavily with the standardized coefficients. Ethyl benzene is a lipid oxidation product responsible for roast beef flavors (MacLeod and Ames, 1986) and may be contributing the umami and beef flavors in the PM and LL samples. Other compounds loading on the function most heavily with the PM, including 2,3-butandiol and acetic acid, suggest anaerobic fermentation occurring in those samples. As aging under vacuum increases, spoilage organisms tend to increase, eventually contributing to a loss of product quality and consumer acceptance. A study examining the shelf life of tenderloins under vacuum using total viable microorganism counts, pH, and mass spectrometry recognized a decrease in pH for tenderloins under vacuum from 0 to 21 d of aging (Mansur et al., 2019). Although the study did not hold steaks under vacuum as long as the current study, it can be speculated that lactic acid bacteria, common in vacuum-packaged beef, are responsible for the production of fermentation products and organic acids (Jalilsood et al., 2015). Hernandez et al. (2022) further confirms this during long aging methods, in which lactic acid bacteria increased over a 56-d aging period under vacuum. Furthermore, Hernandez et al. (2022) showed a substantial increase in acetic acid concentrations with extended wet aging of subprimals beyond 14 d. These data suggest extended aging in GM steaks produces fewer desirable flavors and associated compounds compared with LL and PM samples.

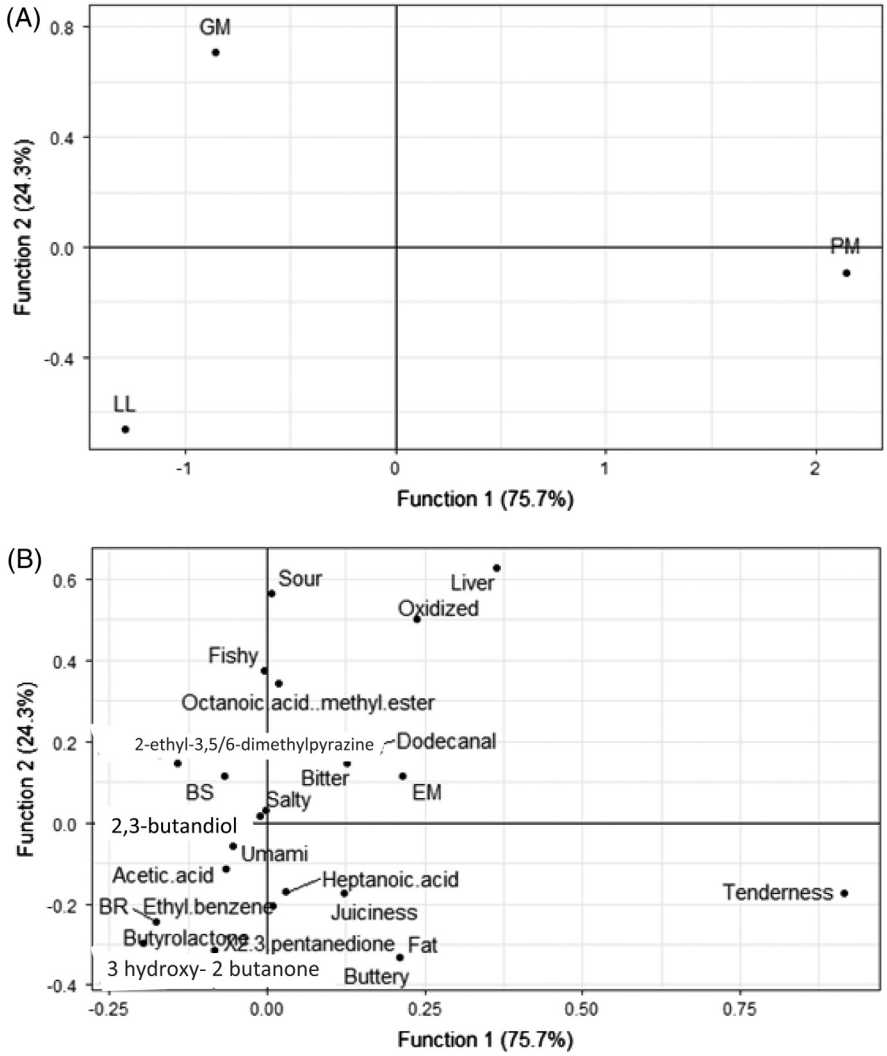

Figure 2. (A) Plot of muscle types (*gluteus medius* (GM), *longissimus lumborum* (LL), and *psoas major* (PM)) on the discriminant functions derived from the combination of trained sensory panels and volatile flavor compounds ($P < 0.001$). (B) Loadings of sensory attributes and volatile flavor compounds corresponding to the plot of muscle type.

Aging duration

The DFA corresponding to aging duration showed the canonical correlations on DF1 explained 75.7% of variation, whereas DF2 explained 24.3% of the variation ($P < 0.001$) (Figure 3A and 3B). The DFA explained 100% of the variation between treatments overall. The DF1 was most effective at explaining variation and showed maximal separation between days 56 and 28, with days of age following sequential order across the function. On the DF2 function, days 56 and 49 were maximally separated from each other, with days 42, 35, and 28 falling between the 2 factors.

The DF1 function explained most of the variation for days of aging. Although days 56 and 28 were maximally separated across the function, the loadings corresponding to these 2 d also drastically differed from each other. The loadings corresponding with the canonical correlations, 28 and 35 d, are primarily composed of positively associated attributes and volatile compounds. Positive palatability traits such as buttery, beef flavor ID, fat-like, and overall juiciness were most associated with the shortest aging treatments, suggesting a desirable eating experience is still maintainable after 28 and 35 d of vacuum packaging. Furthermore, numerous Maillard reaction–derived volatile compounds, such as 2-ethyl-3,5/6-dimethylpyrazine and 3-hyrdroxy-2-butanone, loaded heavily corresponding with days 28 and 35 on the DF1 function. In general, pyrazines like 2-ethyl-3,5/6-dimethylpyrazine have been extensively associated with roasted foods resulting from the condensation of sugars and amino acids (Hodge, 1953; Mussinan et al., 1973). With increased aging, free amino acid availability also increases (Koutsidis et al., 2008; Foraker at al., 2020;

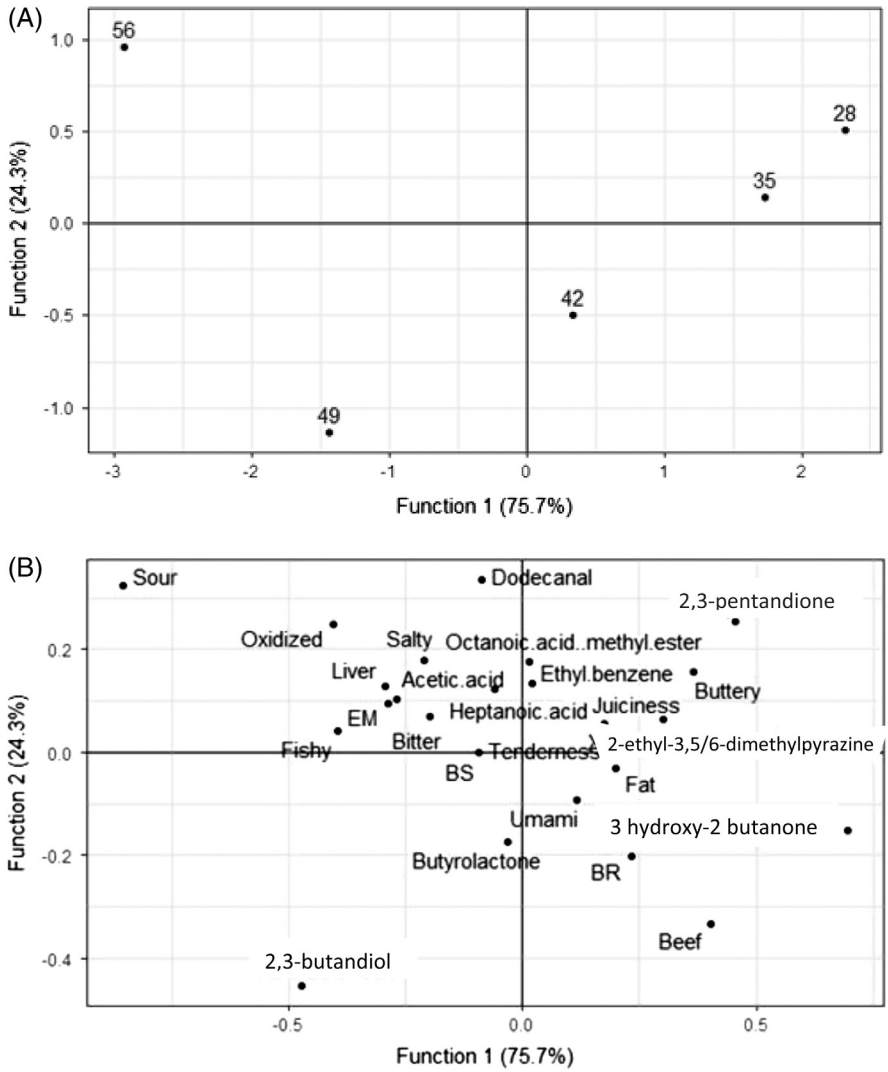

Figure 3. (A) Plot of aging duration (28, 35, 42, 49, and 56 d) on the discriminant functions derived from the combination of trained sensory panels and volatile flavor compounds ($P < 0.001$). (B) Loadings of sensory attributes and volatile flavor compounds corresponding to the plot of display duration.

Hernandez et al., 2022), therefore suggesting increased Maillard reaction products and thus a beefier, roasted flavor with increased aging. However, loadings on the function suggest that as aging increases beyond 42 d, off-flavors such as liver-like, earthy/musty, bitter, fishy, and oxidized become more present. This could be due to a masking effect theory, suggesting that positive notes are hidden as negative ones become more prevalent (Stutz et al., 1991; Jackson et al., 1992).

The DF2 function explains less variation in the function. However, it may provide a better timeline for individual vacuum-packaged aging by showing maximal separation between days 56 and 49. Many positive flavor attributes and volatile compounds are centered at the intersection of the 2 functions, loading heavily in correspondence with days 42, 35, and 28. On the bottom of the function, fewer flavor attributes correspond with day 49. However, those that do—beef flavor ID and brown/roasted—are considerably more positive than those loading heavily on the function with 56 d. As aging increases beyond 49 d and up to 56 d, the function shows more attributes associated with negative eating experiences—sour, oxidized, and liver-like—suggesting that individual vacuum-packaged steaks begin to noticeably decline in flavor following 49 d of age.

Conclusions

These data show that although individual aging of vacuum-packaged steaks does not pose any detrimental effects to eating quality and muscle integrity, extended aging does. Although differences in evaluated flavor attributes and volatile compounds were minor, the

results advocate against long-term aging to a certain point because they follow similar trends exhibited in prior research mentioned throughout. Additionally, the use of DFA provided a visual explanation for the data presented, again suggesting the most optimal aging reaches up to approximately 42 d, with potential to extend up to 49 d. Individual steaks aged individually beyond 56 d showed the greatest decline in positive flavor attributes and desirable volatile flavor compounds, concluding that aging to this degree would be detrimental to the product and consumer satisfaction.

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