



TEXAS TECH UNIVERSITY

Libraries™

Influence of Aging Temperature and Duration on Flavor and Tenderness Development of Vacuum-Packaged Beef Longissimus

The Texas Tech community has made this publication openly available. [Please share](#) how this access benefits you. Your story matter to us.

Citation	Hernandez, M.S., Woerner, D.R., Brooks, J.C., Wheeler, T.L., & Legako, J.F.. 2023. Influence of Aging Temperature and Duration on Flavor and Tenderness Development of Vacuum-Packaged Beef Longissimus. <i>Meat and Muscle Biology</i> , 7(1). https://doi.org/10.22175/mmb.15710
Citable Link	https://hdl.handle.net/2346/96008
Terms of Use	© 2023 Sebastian Hernandez, et al. cc-by

Title page template design credit to [Harvard DASH](#).



Influence of Aging Temperature and Duration on Flavor and Tenderness Development of Vacuum-Packaged Beef *Longissimus*

M. Sebastian Hernandez¹, Dale R. Woerner¹, J. Chance Brooks¹, Tommy L. Wheeler², and Jerrad F. Legako¹

¹Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409, USA

²USDA-ARS, Roman L. Hruska Meat Animal Research Center, Clay Center, NE 68933, USA

*Corresponding author. Email: jerrad.legako@ttu.edu (Jerrad F. Legako)

Abstract: The objective of this study was to investigate the influence of beef wet-aging temperature and duration on beef palatability. Paired beef strip loins were obtained from USDA Choice carcasses ($n = 60$) at a commercial processing facility. Paired strip loins were assigned to a storage temperature (-2°C , 0°C , or 4°C). Strip loins were portioned into half loins and further assigned to an aging duration (14, 28, 42, or 56 d). Loins were aged in commercial upright refrigerators. After aging, loins were fabricated into 2.54-cm steaks and assigned to either volatile compound analysis, descriptive sensory analysis, or consumer sensory analysis. Data were analyzed as a split-plot in which carcass served as the whole plot and loin portion served as the subplot. An alpha of $P < 0.05$ was used. For descriptive sensory analysis, an interaction was observed for beef identity, bloody/serummy, fat-like, liver-like, bitter, sour, and musty/earthy ($P < 0.05$). Loins aged for 56 d at 4°C were the most intense for liver-like, sour, and musty/earthy notes compared with all other treatments ($P < 0.05$). An interaction was observed for consumer juiciness, tenderness, and overall liking ($P < 0.05$). Steaks from loins aged for 14 d at -2°C were rated the least for juiciness, tenderness, and overall liking ($P < 0.05$). Ethanol, acetic acid, 1-penten-3-ol, and 2-methylbutanal were each greatest in loins aged for 56 d ($P < 0.05$). Aging at 4°C yielded the greatest concentrations of ethanol and heptanoic acid ($P < 0.05$). Off-flavor development increased during extended aging but was dependent on storage temperature. Extended aging (>28 d) conducted at colder temperatures did not negatively influence palatability. Aging for 14 d at -2°C was detrimental to consumer liking. It may be concluded that both aging temperature and duration should be considered when seeking to optimize beef palatability.

Key words: wet aging, palatability, volatile compounds, taste, meat quality, storage

Meat and Muscle Biology 7(1): 15710, 1–14 (2023)

doi:10.22175/mmb.15710

Submitted 27 October 2022

Accepted 23 February 2023

Introduction

Wet aging is readily used by the beef industry to improve palatability. During the aging process, proteolytic activity degrades the protein structure, resulting in increased tenderness and the release of flavor-contributing metabolites such as free amino acids and sugars (Koutsidis et al., 2008; Huff-Lonergan et al., 2010; Foraker et al., 2020; Vierck et al., 2020).

Earlier works evaluating aging influences on palatability, namely tenderness, did not often evaluate

aging durations beyond 28 d (Jeremiah and Gibson, 2003; Bratcher et al., 2005; Wicklund et al., 2005; Gruber et al., 2006). This trend was in alignment with the 1998 National Beef Tenderness Survey, which reported post-fabrication times of 32 and 19 d for food-service and retail, respectively (Brooks et al., 2000). However, average post-fabrication aging times of subprimals in retail and foodservice have increased to 25.9 and 35.1 d, respectively (Martinez et al., 2017). Moreover, the maximum post-fabrication aging times have increased from 67 to 102 d (Brooks et al., 2000; Martinez et al., 2017). Extended aging is utilized to

ensure a tender product. However, extended aged product has been shown to produce off-flavors and aromas such as sour, oxidized, liver-like, metallic, and musty/earthy while decreasing beef flavor identity (Juárez et al., 2010; O'Quinn et al., 2016; Evers et al., 2020; Foraker et al., 2020). The development of these off-notes is frequently attributed to microbial growth and lipid oxidation (Watanabe et al., 2015; Foraker et al., 2020; Frank et al., 2020).

Storage temperature has been shown to influence proteolytic activity and resulting product tenderness (Whipple et al., 1990; King et al., 2003; King et al., 2009; Juárez et al., 2010; Hernandez et al., 2022). Reports of aging temperature influences on flavor development are very limited. Juárez et al. (2010) reported vacuum-packaged steaks aged at 5°C produced more intense off-flavors compared with steaks aged at 1°C. However, the off-flavors were not characterized. Additionally, Cassens et al. (2018) determined no differences in consumer flavor liking of steaks aged at either 0°C to 1.1°C or 3.3°C to 4.4°C. Hernandez et al. (2022) reported increased microbial growth and proteolytic activity in vacuum-packaged strip loins aged at 4°C. By-products of microbial growth and the release of beef flavor precursors may influence palatability (Hernandez et al., 2022).

Current literature has not fully captured the influence of storage temperature on beef flavor and tenderness development in wet-aged beef. Therefore, this study aims to evaluate the influence of wet-aging storage temperature during extended aging on beef flavor and tenderness development.

Materials and Methods

Product selection, subprimal aging, and fabrication

Product collection, aging, and fabrication are detailed in Hernandez et al. (2022). In brief, paired USDA Low Choice beef strip loins (Institutional Meat Purchasing Specifications #180) were collected from a commercial beef processing facility in 2 collection trips. Paired loins from an individual carcass were assigned to temperature (−2°C, 0°C, or 4°C). Aging duration was assigned to the portioned paired loins (14, 28, 42, or 56 d). Commercial upright refrigerators set to a respective temperature were used to aged vacuum-packaged loins (ESF1, Everest Refrigeration, Compton, CA). Refrigerator temperatures were monitored continuously using remote temperature recorders,

and mean temperatures and standard deviations are reported in Hernandez et al. (2022). At each aging duration interval, respective loins were fabricated into 2.54-cm steaks ($n = 5$), assigned to either cooked volatile analysis, descriptive sensory analysis, or consumer sensory analysis, vacuum packaged, and then frozen at −20°C until subsequent analysis.

Cooking procedure

Steaks were cooked as described in Hernandez et al. (2022). A combi-oven (Model SCC WE 61 E; Rational, Landsberg am Lech, Germany) was used to cook steaks to a medium degree of doneness (71°C). Temperature was monitored using a thermocouple (Rational) inserted into the geometric center of the steak. Raw and cooked weights and internal temperatures were recorded.

Consumer sensory analysis

Consumer sensory panels were conducted using modified methods from Legako et al. (2015) and Vierck et al. (2021). Untrained consumer panelists ($n = 200$) from Lubbock, Texas, evaluated 7 samples in groups of 20. A verbally anchored 100-point line scale was used to assess flavor, tenderness, juiciness, and overall liking through a tablet and digital ballot (Qualtrics, Provo, UT; iPad, Apple Inc., Cupertino, CA). Additionally, panelists were asked to specify acceptability and perceived quality. Each ballot consisted of a demographic sheet, a purchasing motivator sheet, and 7 sample ballots. Panelists were provided with distilled water, diluted apple juice, and unsalted crackers as palate cleansers. Prior to serving the first sample, panelists were served a “warm-up” sample to calibrate to the scale. Samples were randomized in an incomplete block design. Within each session, each of the 12 possible treatment combinations was evaluated twice. Each consumer evaluated 6 of the 12 possible treatment combinations with no duplicate treatment combination.

Descriptive sensory analysis

Descriptive sensory analysis followed the American Meat Science Association Sensory Guidelines (AMSA, 2015). Panelists ($n = 9$) were trained to identify and quantify the intensity of 16 flavor and texture attributes from Adhikari et al. (2011) and AMSA (2015; Table 1). Attributes were rated on a 100-point scale where 0 = extremely dry/tough/not detectable and 100 = extremely juicy/tender/intense. Panelists were trained for 3 wk prior to testing. Steaks for descriptive sensory evaluation

Table 1. Definitions and standard references for descriptive beef flavor and texture attributes, where 0 = extremely dry/tough/not detectable and 100 = extremely juicy/tender/intense, from Adhikari et al. (2011) and AMSA (2015)

Attribute	Definition	Reference
Beef Identity	Amount of beef flavor in a sample	Swanson Beef Broth = 30 80% lean ground beef = 50 Beef brisket = 75
Bitter	The fundamental taste factor associated with a caffeine solution	0.01% caffeine solution = 15 0.02% caffeine solution = 25
Bloody/Serumy	The aromatics associated with blood on cooked meat products. Closely related to metallic	USDA Choice strip steak cooked to 60°C = 40
Brown/ Roasted	A round, full aromatic generally associated with beef suet that has been broiled	Beef suet = 50 80% lean ground beef = 60 Well-done strip steak = 65 90% lean ground beef = 30
Fat-Like	The aromatics associated with cooked animal fat	70% lean ground beef = 60 Hillshire Farm Lit'l Smokies = 44 Flat iron steak = 20
Liver-Like	The aromatics associated with cooked organ meat/liver	Calf liver = 90
Metallic	The impression of slightly oxidized metals such as iron, copper, and silver spoons	0.10% potassium chloride solution = 10 USDA Choice strip steak cooked to 60°C = 25 Dole canned pineapple juice = 38
Musty/ Earthy	Musty, sweet, decaying vegetation	Mushroom = 20
Oxidized	The aromatics associated with oxidized fats and oils. These aromatics may include cardboard, painty, varnish, and fishy.	Wesson vegetable oil microwaved for 3 min = 45 Wesson vegetable oil microwaved for 5 min = 60
Salty	The fundamental taste factor associated with a sodium chloride solution	0.15% sodium chloride solution = 10 0.25% sodium chloride solution = 45
Sour	The fundamental taste factor associated with citric acid	0.015% citric acid solution = 10 0.25% citric acid solution = 25
Sour Aromatics	The aromatics associated with sour substances	Buttermilk = 33

Table 1. (Continued)

Attribute	Definition	Reference
Umami	Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids, and other molecules called nucleotides	Unsalted beef broth = 30 0.035% Accent Flavor Enhancer solution = 50
Overall Tenderness	Amount of force required to masticate meat	Eye of round = 33 Strip steak = 55 Tenderloin = 90
Overall Juiciness	The amount of perceived moisture release	Well-done strip steak = 25 Medium strip steak = 50 Rare strip steak = 75

were cooked as previously described. Following cooking, steaks were wrapped in aluminum foil and held at 50°C to 55°C in a food service warmer (Cambro Manufacturing, Huntington Beach, CA). Steak exterior fat and heavy connective tissue was removed before cutting steaks into 1.27 × 1.27 × steak thickness cubes (1/2 Sensory Box, Tallgrass Solutions Inc., Manhattan, KS). Panelists evaluated a minimum of 2 steak cubes under red gel lights and recorded attribute ratings using a digital survey on a tablet (Qualtrics; iPad, Apple Inc.). Prior to the first sample and in between samples, panelists were instructed to cleanse their palate with apple juice, saltless crackers, and distilled water. Panelists were also provided an expectorant cup, napkin, and toothpick. Eight samples, in random order, were evaluated per session with a 10 min break between the 4th and 5th samples.

Cooked volatile compound analysis

Volatile compounds were measured similar to Gardner and Legako (2018) with modifications described in Hernandez et al. (2022). Five grams of cooked, homogenized, frozen sample were weighed into glass vials. Samples were spiked with an internal standard solution (1,2 dichlorobenzene, 2.5 µg/µL). Vials were sealed and loaded into a –20°C dry air-cooling block (MéCour Temperature Control, Groveland, MA). A Gerstel autosampler (Multipurpose Sampler; Gerstel, Inc., Linthicum, MD) removed samples from the cooling block and placed them in a 65°C agitator for extraction. An 85-µm film thickness carboxen polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA) was exposed in the headspace of the vial for solid phase microextraction (SPME) of volatile compounds. Separation and detection of volatile flavor compounds

was carried out using gas chromatography–mass spectrometry. After extraction, the SPME fiber was injected into the gas chromatograph (7890B series, Agilent, Santa Clara, CA) and desorbed onto a VF-5ms capillary column (30 m × 0.25 mm × 1 μm; Agilent J&W GC Columns). Separated compounds were introduced to the single quadrupole mass spectrometer (5977A, Agilent) through electron ionization at 70 eV. Mass range was determined at 45 to 500 m/z. Authentic standards (Sigma-Aldrich, St. Louis, MO) were used to confirm compound identities through retention time and fragmentation pattern of 1 target ion and 2 qualifying ions. A calibration curve and the internal standard were used for quantitation of volatile compounds (nanograms per gram of sample).

Statistical analysis

Univariate statistical analysis is detailed in Hernandez et al. (2022). Data were analyzed as a split-plot design in which carcass served as the whole plot and loin portion served as the subplot. Loin portion served as the experimental unit. For all analyses, carcass and collection trip were included in the model as random effects. For sensory data, panel session and feed order were included as random effects. Consumer acceptability and perceived quality data were analyzed with a binomial distribution. The PROC GLIMMIX procedure of SAS (v. 9.4; Cary, NC) was used to analyze data. An α of <0.05 was used for all analyses.

Interrelationships between sensory data and volatile data were explored using the partial least-squares regression function of XLSTAT (v. 2021.1; Addinsoft, Paris, France). Descriptive and consumer sensory attributes were the dependent variables, and volatile compounds were the independent variables. To ease interpretation of the biplot, only significant or trending ($P \leq 0.10$) volatile compounds from the prior analysis of variance were included in the model.

Results and Discussion

Consumer sensory evaluation

Consumer ($n = 200$) demographic and beef consumption habits are presented in Table 2. Most consumer panelists were female (56.78%), Caucasian (60.30%), married (52.26%), and 20 to 29 years old (27.14%) with some college or technical school education (33.16%). Consumers predominately came from households of 1 or 2 people (28.64%) with an annual

household income of \$50,000 to \$74,999 (18.09%). Most consumers ate beef 1 to 3 times a week (41.71%). Flavor was considered the most important palatability trait (46.23%) followed by tenderness (36.38%). Medium rare was the most preferred degree of doneness (42.21%) followed by medium (27.14%) and medium well (17.09%).

Consumer liking results are presented in Table 3. No significant main effects or two-way interactions were observed for flavor liking ($P > 0.05$). Two-way interactions were observed for juiciness, tenderness, and overall liking ($P < 0.05$). Steaks from loins aged for 14 d at -2°C were rated the lowest ($P < 0.05$) for juiciness liking compared with all treatments except for steaks aged for 28 d at -2°C ($P > 0.05$). Consumers rated steaks aged for 42 d at -2°C higher for juiciness compared with steaks aged for 42 d at 0°C ($P < 0.05$). Like juiciness scores, steaks aged for 14 d at -2°C were rated the lowest for tenderness compared with all other treatments ($P < 0.05$). Steaks aged at -2°C for 42 and 56 d were rated more tender than steaks aged for 28 d at -2°C and for 42 d at 0°C ($P < 0.05$). Steaks aged for 56 d at -2°C were rated higher for overall liking compared with steaks aged for 28 d at -2°C ($P < 0.05$). Consumers rated steaks aged for 14 d at -2°C the lowest for overall liking compared with all other treatments ($P < 0.05$).

A temperature × duration interaction was observed for consumer juiciness, tenderness, and overall acceptability ($P < 0.05$; Table 4). Steaks aged at -2°C for 14, 28, and 56 d; 0°C for 14, 42, and 56 d; and 4°C for 56 d had lower percentages of steaks deemed acceptable for juiciness ($P < 0.05$). Steaks aged for 42 d at -2°C had a higher percentage of steaks rated acceptable for juiciness than steaks aged for 14 d at 4°C ($P < 0.05$). Tenderness and overall acceptability were higher for steaks aged for 42 d at 4°C than steaks aged at 0°C regardless of duration ($P < 0.05$). Aging storage temperature influenced flavor acceptability ($P = 0.042$; Table 5). Steaks from loins aged at 4°C had a greater percentage of acceptable flavor ratings from consumers compared with steaks from loins aged at 0°C ($P < 0.05$). Flavor acceptability was also impacted by aging duration ($P = 0.021$; Table 5). Forty-two day-aged steaks had the greatest percentage of steaks rated as acceptable compared with all other aging durations ($P < 0.05$).

When consumers were asked to designate samples as unsatisfactory, everyday quality, better than everyday quality, or premium quality, steaks aged at 4°C had the lowest percentage of unsatisfactory steaks compared with 0°C and -2°C ($P = 0.002$; Table 5). Steaks

Table 2. Consumer demographic information and beef consumption habits ($n = 200$)

Characteristic	Response	Percentage of Consumers	Characteristic	Response	Percentage of Consumers
Gender	Male	43.21	Annual Household Income	Under \$25,000	17.09
	Female	56.78		\$25,000–\$34,999	16.08
Household Size	1 person	14.57		\$35,000–\$49,999	11.06
	2 people	28.64		\$50,000–\$74,999	18.09
	3 people	15.08		\$75,000–\$99,999	14.57
	4 people	22.61		\$100,00–\$149,999	14.57
	5 people	13.07		\$150,000–\$199,999	5.03
	6 people	5.03		Over \$200,000	3.52
	>6 people	1.05	Education Level	Non-high school graduate	6.03
Marital Status	Single	47.74		High school graduate	13.57
	Married	52.26		Some college/technical school	33.16
Age	Under 20	4.52	College graduate	26.63	
	20–29	27.14	Postgraduate	20.60	
	30–39	21.61	Beef Consumption per Week	1–3 times	41.71
	40–49	21.61		4–6 times	36.18
50–59	10.55	7 or more		22.11	
Over 60	14.57	Most Important Palatability Trait	Flavor	46.23	
Ethnic Origin	African American		2.01	Tenderness	36.68
	Asian		0.50	Juiciness	17.09
	Caucasian/white	60.30	Preferred Degree of Doneness	Very rare	1.01
	Hispanic	32.66		Rare	6.03
	Mixed race	2.51		Medium rare	42.21
	Native American	0.50		Medium	27.14
	Other	1.51		Medium well	17.09
			Well done	5.53	
		Very well done	1.01		

Table 3. Interaction of consumer palatability attributes¹ of beef strip loins wet-aged in 3 temperature environments and 4 aging durations

Attribute	–2°C				0°C				4°C				SEM ²	P Value ³
	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d		
Flavor Liking	45.6	64.4	70.7	71.2	67.1	68.8	66.4	69.1	68.2	68.9	71.7	70.8	8.39	0.192
Juiciness Liking	36.4 ^d	52.4 ^{cd}	78.6 ^a	71.9 ^{ab}	68.1 ^{abc}	66.4 ^{abc}	61.1 ^{bc}	65.3 ^{abc}	65.0 ^{abc}	75.9 ^{ab}	71.3 ^{ab}	66.7 ^{abc}	8.97	<0.001
Tenderness Liking	40.8 ^c	60.2 ^b	76.4 ^a	77.4 ^a	63.9 ^{ab}	66.4 ^{ab}	59.8 ^b	72.7 ^{ab}	72.5 ^{ab}	75.2 ^{ab}	74.1 ^{ab}	73.8 ^{ab}	7.65	<0.001
Overall Liking	50.6 ^c	57.4 ^b	69.7 ^{ab}	76.1 ^a	68.2 ^{ab}	69.1 ^{ab}	62.1 ^{ab}	70.7 ^{ab}	69.7 ^{ab}	75.1 ^{ab}	67.4 ^{ab}	69.2 ^{ab}	3.20	0.004

¹0 = extremely bland/dry/tough/dislike, 100 = extremely flavorful/juicy/tender/like.

²Largest standard error of the least-squares means.

³Observed significance level.

^{a-d}Means in the same row without a common superscript differ ($P < 0.05$).

aged in –2°C and 0°C environments were similar ($P > 0.05$). Steaks aged for 42 d tended to have a lower percentage of unsatisfactory steaks ($P = 0.095$). A two-way interaction was observed for everyday quality, better than everyday quality, and premium quality ($P \leq 0.022$; Table 4). Steaks aged for 42 d at –2°C had a similar percentage of everyday quality ratings as steaks

aged for 28 d at 4°C ($P > 0.05$). When aged for 42 d, steaks aged in the 4°C environment had a greater percentage of everyday quality ratings compared with steaks aged at –2°C ($P < 0.05$). Consumers rated a greater percentage of steaks aged for 42 d at –2°C as better than everyday quality compared with steaks aged for any duration at 0°C ($P < 0.05$). Steaks aged for 42 d at

Table 4. Interaction of consumer acceptability and perceived quality levels¹ from beef strip loins wet-aged in 3 temperature environments² and 4 aging durations³

Attribute	−2°C				0°C				4°C				SEM ²	P Value ³
	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d		
Acceptability														
Juiciness Acceptability	65.7 ^e	66.1 ^e	91.6 ^a	72.8 ^{cde}	72.2 ^{de}	83.2 ^{abcd}	65.3 ^e	72.2 ^{cde}	79.1 ^{bcd}	84.2 ^{abc}	87.1 ^{ab}	72.5 ^{de}	0.38	<0.001
Tenderness Acceptability	72.1 ^e	80.8 ^{cde}	95.8 ^{ab}	83.2 ^{cde}	79.0 ^{de}	81.1 ^{cde}	77.7 ^{de}	89.4 ^{bc}	90.0 ^{abc}	87.0 ^{cd}	97.0 ^a	87.0 ^{cd}	0.59	0.002
Overall Acceptability	71.6 ^e	77.2 ^{cde}	91.6 ^{ab}	74.8 ^{de}	80.2 ^{cde}	81.1 ^{cde}	77.1 ^{cde}	81.9 ^{bcde}	84.1 ^{bcd}	86.2 ^{bc}	98.0 ^a	82.4 ^{bcde}	0.72	0.010
Perceived Quality														
Everyday Quality	49.0 ^{ab}	47.5 ^{ab}	25.5 ^c	44.4 ^{ab}	47.2 ^{ab}	46.5 ^{ab}	48.6 ^{ab}	43.5 ^{ab}	42.2 ^b	38.0 ^{bc}	56.8 ^a	46.8 ^{ab}	0.11	0.002
Better Than Everyday Quality	19.2 ^d	27.2 ^{bcd}	41.4 ^a	23.1 ^{cd}	21.9 ^{cd}	26.2 ^{bcd}	24.5 ^{cd}	18.2 ^d	37.9 ^{ab}	33.0 ^{abc}	25.2 ^{bcd}	29.0 ^{abcd}	0.26	0.022
Premium Quality	5.3 ^d	4.7 ^d	24.3 ^a	10.9 ^{bcd}	8.3 ^{cd}	7.6 ^{cd}	4.2 ^d	20.4 ^{ab}	9.9 ^{cd}	15.1 ^{abc}	11.3 ^{bcd}	8.5 ^{cd}	0.53	<0.001

¹Unsatisfactory, everyday quality, better than everyday quality, premium quality.

²Largest standard error of the least-squares means.

³Observed significance level.

^{a-c}Means in the same row without a common superscript differ ($P < 0.05$).

Table 5. Percentage of consumer acceptability ratings and perceived quality levels attribute from beef strip loins aged in 3 temperature environments and 4 aging durations

Attribute	Aging Duration				SEM ¹	P Value ²	Aging Temperature			SEM	P Value
	14 d	28 d	42 d	56 d			−2°C	0°C	4°C		
Acceptability											
Flavor Acceptability	81.7 ^b	84.3 ^b	90.1 ^a	81.4 ^b	0.26	0.021	83.5 ^{ab}	81.9 ^b	88.2 ^a	0.23	0.042
Perceived Quality											
Unsatisfactory	17.2	16.6	10.3	16.8	0.28	0.095	16.7 ^a	19.3 ^a	10.2 ^b	0.27	0.002

Note: Aging temperature × aging duration interaction not significant ($P > 0.05$).

¹Largest standard error of the least-squares means.

²Observed significance level.

^{a-c}Means in the same row without a common superscript differ ($P < 0.05$).

−2°C, 56 d at 0°C, and 28 d at 4°C produced similar percentages of steaks deemed premium quality ($P > 0.05$).

Consumer ratings showed minor differences across treatments with the exception of loins aged for 14 and 28 d at −2°C. Consumers clearly rated steaks aged at −2°C for either 14 or 28 d the lowest across all liking attributes. Tenderness may be the driver of these results based on slice shear force data presented in Hernandez et al. (2022), which showed that aging at −2°C or for 14 d produced minimal tenderness development. Hernandez et al. (2022) reported minimal proteolytic activity, i.e., desmin and troponin T degradation, in loins aged for 14 d at −2°C, suggesting insufficient tenderness development. However, Hernandez et al. (2022) also reported all steaks were considered “tender” or “very tender” according to slice shear force (SSF) values. Despite objective tenderness results, consumers were still able to discriminate against those steaks with greater SSF values. It should be noted that aging for 42 and 56 d at −2°C was able to develop tenderness comparable with aging

14 d at 0°C or 4°C. In regard to aging duration at 0°C or 4°C, minimal differences in all liking attributes were observed. Our results agree with Colle et al. (2015), who reported no differences in consumer acceptability, juiciness, and flavor liking in strip loins aged for 2 to 63 d. In the aforementioned study, subprimals were aged at 0°C, which would explain similarities with the present study. Regarding aging temperature, Cassens et al. (2018) reported no differences in consumer ratings between product aged at conventional or elevated storage temperatures. In the present study, the 0°C and 4°C treatments were closely aligned with those in Cassens et al. (2018).

Descriptive sensory attributes

Aging temperature × aging duration interactions were observed for beef flavor identity, bloody/serumy, fat-like, liver-like, bitter, sour, and musty/earthy ($P < 0.05$; Table 6). Steaks aged for 42 d at −2°C, 14 or 56 d at 0°C, and 28 d at 4°C ($P > 0.05$) were more intense for

Table 6. Interaction of descriptive beef flavor attributes¹ from beef strip loins wet-aged in 3 temperature environments and 4 aging durations

Attribute	−2°C				0°C				4°C				SEM ²	P Value ³
	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d	14 d	28 d	42 d	56 d		
Beef ID	48.8 ^{bcd}	49.3 ^{abcd}	50.4 ^a	49.7 ^{abc}	49.7 ^{abc}	48.5 ^{cd}	49.1 ^{abcd}	50.2 ^{ab}	48.5 ^{cd}	50.4 ^a	48.1 ^d	48.1 ^d	0.60	0.003
Bloody/Serum	3.6 ^{cd}	4.1 ^{bcd}	5.1 ^{abc}	4.6 ^{abcd}	5.9 ^a	3.9 ^{cd}	4.7 ^{abcd}	4.8 ^{abcd}	4.6 ^{abcd}	5.7 ^{ab}	3.2 ^d	4.9 ^{abcd}	1.39	0.026
Fat-Like	11.7 ^{cde}	12.0 ^{bcd}	12.8 ^{ab}	12.0 ^{bcd}	12.2 ^{abcd}	12.6 ^{abc}	11.8 ^{bcd}	11.2 ^e	13.2 ^a	11.7 ^{cde}	11.7 ^{cde}	11.4 ^{de}	0.45	0.004
Liver-Like	1.9 ^d	2.3 ^{bcd}	1.6 ^d	2.0 ^d	2.3 ^{cd}	2.0 ^d	2.1 ^{cd}	3.4 ^b	1.9 ^d	3.3 ^{bc}	3.3 ^{bc}	5.8 ^a	0.42	<0.001
Bitter	2.3 ^{cde}	1.9 ^{cde}	1.8 ^{de}	3.0 ^{bc}	1.8 ^{de}	2.6 ^{bc}	2.4 ^{cde}	4.0 ^{ab}	1.4 ^e	2.48 ^{cd}	4.3 ^a	5.1 ^a	0.45	0.002
Sour	2.2 ^{bc}	1.9 ^{bc}	1.5 ^c	1.7 ^c	1.6 ^c	2.0 ^{bc}	1.5 ^c	1.6 ^c	2.2 ^{bc}	1.9 ^{cb}	2.7 ^b	3.9 ^a	0.42	0.044
Musty/Earthy	1.5 ^d	1.3 ^d	1.5 ^d	1.8 ^{cd}	1.3 ^d	1.4 ^d	1.2 ^d	2.5 ^{bc}	1.0 ^d	2.0 ^{bcd}	2.8 ^b	4.2 ^a	0.39	0.008

¹From Adhikari et al. (2011).²Largest standard error of the least-squares means.³Observed significance level.^{a-d}Means in the same row without a common superscript differ ($P < 0.05$).

beef identity compared with steaks aged for 42 or 56 d at 4°C ($P < 0.05$). When aging for 14 d, steaks aged at 0°C were more intense for bloody/serumy notes compared with −2°C ($P < 0.05$). Fat-like scores were similar for steaks aged for 14 d at 4°C, 14 and 28 d at 0°C, and 42 d at −2°C ($P > 0.05$). Steaks aged for 56 d at 4°C were the most intense for liver-like compared with all other treatments ($P < 0.05$). Moreover, steaks aged for 28 and 42 d at 4°C were similar to each other ($P > 0.05$) and were more intense for liver-like than steaks aged for 14 d at 4°C ($P < 0.05$). Aging steaks for 56 d at 0°C and 28 d at −2°C produced similar liver-like intensities as aging for 28 or 42 d at 4°C ($P > 0.05$). Bitter intensity was similar in steaks aged for 42 and 56 d at 4°C and steaks aged for 56 d at 0°C ($P > 0.05$). Steaks aged for 28 d at 4°C had increased bitter intensity compared with aging for 14 d at 4°C ($P > 0.05$) but were less intense than steaks aged for 42 and 56 d at 4°C ($P < 0.05$). Steaks aged for 56 d at 4°C were the most sour compared with all other treatments ($P < 0.05$). Steaks aged for 42 and 56 d at −2°C and for 14, 42, and 56 d at 0°C were similar in sour intensity ($P > 0.05$) but were lower in intensity compared with steaks aged for 42 d at 4°C ($P < 0.05$). Musty/earthy was most intense in steaks aged for 56 d at 4°C compared with all other treatments ($P < 0.05$). Within the 0°C treatment, steaks aged for 56 d had the most intense musty/earthy notes compared with other durations ($P < 0.05$). No differences in musty/earthy intensity were observed across duration within the −2°C treatment ($P > 0.05$). Steaks aged for 42 d at 4°C produced more intense musty/earthy notes than steaks aged for 56 d at −2°C ($P < 0.05$).

Neither aging temperature nor duration impacted overall juiciness or brown/roasted ($P > 0.05$; Table 7). Aging temperature influenced umami, sour aromatic,

and salty attributes ($P < 0.05$). Umami was more intense in steaks aged in −2°C compared with those aged in 4°C ($P < 0.05$). The 4°C aged steaks were the most intense for sour aromatic ($P < 0.05$) compared with −2°C and 0°C aged steaks, which were similar ($P > 0.05$). Steaks aged in the −2°C environment were saltier than 4°C steaks ($P < 0.05$). The 4°C steaks were more tender than −2°C and 0°C ($P = 0.017$). Aging duration influenced umami, metallic, salty, oxidized, and overall tenderness ($P < 0.05$). Steaks aged for 56 d possessed the lowest umami intensity ($P < 0.05$). Aging for 14, 28, and 42 d showed no differences in umami scores ($P > 0.05$). Steaks aged for 14, 28, and 56 d were similar in metallic intensity ($P > 0.05$). Aging for 42 d produced lower metallic intensity compared with aging for 14 or 56 d ($P < 0.05$). Fifty-six day-aged steaks produced the lowest saltiness ($P < 0.05$) compared with all other treatments, which were similar ($P > 0.05$). Oxidized was more intense in 56 d-aged steaks compared with 14 and 28 d ($P < 0.05$). Aging for 42 and 56 d produced steaks of similar tenderness ($P > 0.05$). Contrastingly, aging for 56 d produced more tender steaks compared with those aged for 14 and 28 d ($P < 0.05$).

In the present study, both storage temperature and aging duration influenced flavor and tenderness development. Off-flavor development during extended aging was dependent on storage temperature in many cases. Colder temperatures inhibited off-flavor development even during extended aging, whereas aging at 4°C promoted off-flavor development. These results follow trends similar to those in Juárez et al. (2010). The prior study reported increased off-flavor intensity in steaks aged at 5°C and for extended periods of time. However, no interaction was observed, and specific

Table 7. Least-squares means of descriptive beef flavor and texture attribute¹ scores from beef strip loins aged in 3 temperature environments and 4 aging durations

Attribute	Aging Duration				SEM ²	P Value ³	Aging Temperature				SEM ²	P Value
	14 d	28 d	42 d	56 d			-2°C	0°C	4°C			
Brown/Roasted	48.2	48.2	49.0	48.5	0.38	0.384	48.5	48.6	48.3	0.34	0.756	
Umami	11.0 ^a	11.1 ^a	10.7 ^a	9.7 ^b	0.31	<0.001	11.1 ^a	10.7 ^{ab}	10.1 ^b	0.32	0.021	
Metallic	4.0 ^a	3.7 ^{ab}	3.3 ^b	4.3 ^a	0.36	0.036	3.5	3.8	4.2	0.36	0.164	
Sour Aromatic	2.2	1.9	1.9	2.4	0.21	0.095	1.9 ^b	2.0 ^b	2.5 ^a	0.19	0.009	
Salty	1.4 ^a	1.5 ^a	1.5 ^a	1.0 ^b	0.15	0.006	1.6 ^a	1.3 ^{ab}	1.2 ^b	0.14	0.029	
Oxidized	1.2 ^b	1.1 ^b	1.4 ^{ab}	1.8 ^a	0.21	0.005	1.2	1.4	1.6	0.20	0.138	
Overall Tenderness	55.7 ^c	56.6 ^{bc}	58.2 ^{ab}	58.3 ^a	0.79	0.003	56.6 ^b	56.0 ^b	59.0 ^a	0.87	0.017	
Overall Juiciness	52.4	51.5	50.9	50.8	0.87	0.190	51.5	51.4	51.3	0.83	0.959	

Note: Aging temperature × aging duration interaction not significant ($P > 0.05$).

¹From Adhikari et al. (2011).

²Largest standard error of the least-squares means.

³Observed significance level.

^{a-c}Means in the same row within the same main effect without a common superscript differ ($P < 0.05$).

flavor notes were not explored, unlike the present study. Evers et al. (2020) reported increases in various off-flavors such as liver-like, metallic, bitter, rancid, and sour as days of age increased. Foraker et al. (2020) reported sour and musty/earthy attributes were most intense in beef strip loins aged for 49 and 63 d. These results are congruent with the present study wherein sour and musty/earthy were the most intense in subprimals aged for 42 and 56 d. However, these results were dependent on storage temperature. In vacuum-packaged beef, increases in off-flavors have been attributed to microbial growth and metabolism (Foraker et al., 2020; Frank et al., 2020). Hernandez et al. (2022) reported increased spoilage organism growth in extended aged strip loins stored at 4°C. Increased microbial growth would explain the increase in off-flavor development. Although lipid oxidation has been suggested to contribute to off-flavor development, the present study suggests microbial growth is the primary contributor of off-flavors in vacuum-packaged beef subprimals. Frank et al. (2020) reported similar concentrations of aldehydes at various aging points (84 to 140 d), suggesting vacuum packaging protected subprimals from further oxidation. Sour and bitter basic tastes in aged beef are often associated with metabolic by-products of spoilage organisms. However, the accumulation of taste-active compounds, i.e., free amino acids, could also be contributing to the increased sour and bitter intensities. Descriptive tenderness results mirror SSF values reported in Hernandez et al. (2022). Previous works reported ultimate sensory tenderness at 28 d of age (Juárez et al., 2010; Lepper-Blilie et al., 2016; Foraker et al., 2020). However, in the present study, ultimate descriptive sensory tenderness

was realized at 42 d. The degradation of desmin and troponin T peaked at 42 and 56 d, respectively, and resulted in SSF values peaking at 42 d (Hernandez et al., 2022).

Volatile flavor compounds

Aging temperature × aging duration interactions were determined for 2 volatile flavor compounds: butanoic acid, methyl ester and dimethyl-disulfide ($P < 0.05$; Figures 1 and 2). Aging loins for 42 d at 4°C produced the greatest concentration of butanoic acid, methyl ester compared with all other treatments ($P < 0.05$), which were all similar ($P > 0.05$). Loins aged for 14 d at -2°C; 14, 42, and 56 d at 0°C; and 14 and 28 d at 4°C produced similar concentrations

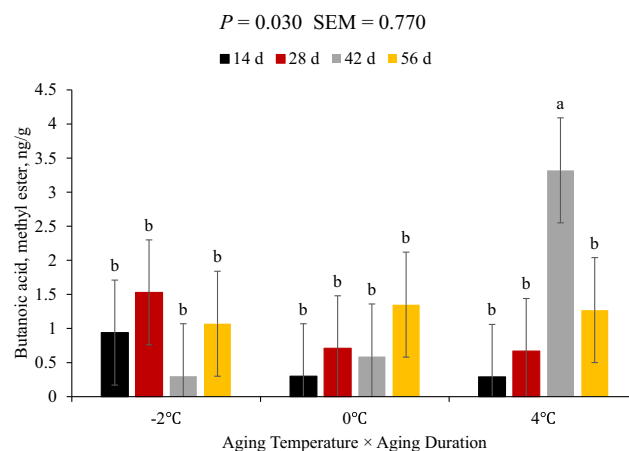


Figure 1. Interaction of butanoic acid, methyl ester concentration (nanograms/grams) from beef strip loins wet-aged in 3 temperature environments and 4 aging durations. ^{a,b}Means without a common superscript differ ($P < 0.05$).

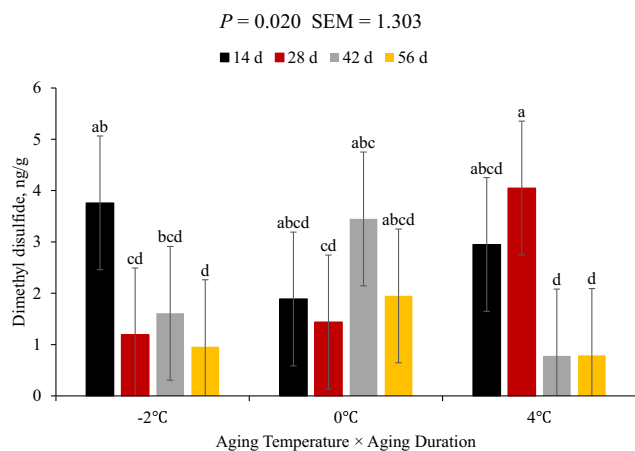


Figure 2. Interaction of butanoic acid, methyl ester concentration (nanograms/grams) from beef strip loins wet-aged in 3 temperature environments and 4 aging durations. ^{a–d}Means without a common superscript differ ($P < 0.05$).

of dimethyl-disulfide ($P > 0.05$). Moreover, loins aged for 14 d at 2°C and 28 d at 4°C possessed greater concentrations of dimethyl-disulfide compared with loins aged for 28 and 56 d at –2°C, 28 d at 0°C, and 42 and 56 d at 4°C ($P < 0.05$).

Aging duration influenced content of ethanol, 1-penten-3-ol, toluene, acetic acid, and heptanoic acid ($P < 0.05$; Table 8). With the exception of toluene, each of these compounds gradually increased as aging duration increased. Aging for 14, 28, and 42 d produced similar concentrations of ethanol ($P > 0.05$). A dramatic increase in ethanol was observed at 56 d ($P < 0.05$). Aging for 56 d produced a greater concentration of 1-penten-3-ol compared with aging for 14 d ($P < 0.05$). 1-Penten-3-ol concentrations at 28 and 42 d of age were similar to both 14 and 56 d aged loins ($P > 0.05$). Both 14 and 56 d aged loins produced the greater concentrations of toluene compared with loins aged for 28 d ($P < 0.05$). Moreover, aging for 42 and 56 d produced similar concentrations of toluene ($P > 0.05$). Similar to ethanol, aging for 56 d produced the greatest concentration of acetic acid ($P < 0.05$) compared with all other treatments, which were similar ($P > 0.05$). Aging for 14 d produced the lowest concentration of heptanoic acid ($P < 0.05$) compared with the remaining treatments, which were similar ($P > 0.05$). Of the Maillard reaction-derived volatile compounds, Strecker aldehydes, methional and 2-methylbutanal, were influenced by aging duration ($P < 0.05$; Table 10). Methional concentrations were the greatest in loins aged for 28, 42, and 56 d compared with loins aged for 14 d ($P < 0.05$). Loins aged for 56 d produced greater concentrations of 2-methylbutanal compared with loins aged for 14 d ($P < 0.05$). Loins aged for 28 and 42 d

were intermediate and similar to both loins aged for 14 and 56 d ($P > 0.05$).

Aging temperature influenced ethanol and heptanoic acid ($P < 0.05$; Table 8). Aging at 4°C resulted in a substantial increase of ethanol production compared with –2°C and 0°C ($P < 0.05$). Likewise, heptanoic acid concentration was the greatest in loins aged at 4°C ($P < 0.05$) compared with loins aged at –2°C and 0°C, which were similar ($P > 0.05$). Aging temperature did not influence Maillard reaction-derived volatiles ($P > 0.05$; Table 9).

Few compounds were influenced by aging duration, aging temperature, or their interaction. However, compounds that were found to be significant were drivers of off-flavor development. These compounds are known products of microbial metabolism (Casaburi et al., 2015). Multiple studies have observed an increase in ethanol concentration beginning at 49 d of age and up to 140 d (Evers et al., 2020; Foraker et al., 2020; Frank et al., 2020; Li et al., 2021). Ethanol has been suggested to be a by-product of microbial metabolism, specifically lactic acid producing bacteria (Hernández-Macedo et al., 2012; Casaburi et al., 2015). Moreover, lactic acid bacteria have been associated with acetic acid production (Hernández-Macedo et al., 2012; Casaburi et al., 2015). Li et al (2021) reported an increase in acetic acid during wet aging. Ethanol and acetic acid are associated with sharp, medicinal, sour, vinegar-like aromas (Casaburi et al., 2015; Kerth and Miller, 2015). This increase in ethanol and acetic acid is congruent with the spoilage organism growth data reported in Hernandez et al. (2022). As previously discussed, off-flavor development in vacuum-packaged beef subprimals is primarily driven by microbial growth rather than lipid oxidation. This was evident by the lack of differences in aldehyde concentration, namely hexanal. Aldehydes are very reactive and could be utilized by microorganisms in the production of organic acids (Resconi et al., 2018; Li et al., 2021). Nonetheless, this agrees with Foraker et al. (2020), who reported limited lipid oxidation through minimal differences in fatty acids and oxidation derived volatiles. However, toluene, a hydrocarbon suggested to be a lipid oxidation product, increased from day 28 to 56. Watanabe et al. (2015) reported increased toluene concentration as aging duration increased and cited lipid oxidation as the mechanism.

It was hypothesized that there would be an increase of volatile production because of an increase in flavor precursors, namely free amino acids and reducing sugars. However, there were few significant differences

Table 8. Least-squares means of lipid-derived volatile flavor compound concentration from beef strip loins aged in 3 temperature environments and 4 aging durations

Volatile Compound (ng/g)	Aging Duration						Aging Temperature				
	14 d	28 d	42 d	56 d	SEM ³	<i>P</i> Value ⁴	–2°C	0°C	4°C	SEM	<i>P</i> Value
<i>Alcohols</i>											
Ethanol	16.54 ^b	24.39 ^b	31.09 ^b	77.98 ^a	12.480	<0.001	26.53 ^b	28.10 ^b	57.87 ^a	11.040	0.049
1-Hexanol	1.29	1.56	1.63	1.90	0.236	0.302	1.68	1.46	1.65	0.210	0.739
1-Octanol	7.70	7.72	7.70	7.54	0.096	0.319	7.56	7.73	7.70	0.088	0.185
1-Octen-3-ol	2.21	2.44	2.12	2.23	0.887	0.717	2.22	2.26	2.27	0.885	0.983
1-Pentanol	1.67	2.07	1.84	1.99	0.385	0.656	1.94	1.90	1.83	0.367	0.940
1-Penten-3-ol	0.31 ^b	0.54 ^{ab}	0.62 ^{ab}	0.83 ^a	0.122	0.019	0.62	0.43	0.67	0.107	0.268
2,3-Butanediol	15.53	7.37	9.21	25.68	5.277	0.050	12.99	9.81	18.29	4.640	0.426
<i>Aldehydes</i>											
Decanal	0.16	0.17	0.19	0.16	0.084	0.969	0.19	0.15	0.17	0.081	0.839
Dodecanal	0.90	0.93	0.91	0.82	0.229	0.504	0.94	0.89	0.85	0.228	0.556
Heptanal	14.22	15.50	14.27	15.95	2.110	0.911	16.07	13.83	15.05	1.801	0.674
Hexanal	42.38	48.20	38.82	45.82	11.915	0.652	45.17	43.32	42.93	11.561	0.940
Nonanal	0.32	0.43	0.44	0.44	0.074	0.453	0.46	0.35	0.41	0.067	0.282
Octanal	7.76	8.26	7.63	8.16	2.481	0.983	8.96	6.61	8.29	0.302	0.439
Pentanal	7.53	8.71	6.83	7.76	1.677	0.574	8.05	7.56	7.51	1.608	0.883
<i>Carboxylic Acids</i>											
Acetic Acid	89.87 ^b	106.15 ^b	120.17 ^b	299.05 ^a	53.130	0.014	128.04	112.34	221.05	46.286	0.207
Butanoic Acid	5.09	5.47	5.89	7.46	0.990	0.305	5.76	5.03	7.14	0.917	0.242
Heptanoic Acid	0.49 ^b	0.87 ^a	0.88 ^a	1.10 ^a	0.109	0.001	0.76 ^b	0.70 ^b	1.04 ^a	0.093	0.022
Hexanoic Acid	0.13	0.14	0.15	0.20	0.031	0.372	0.17	0.11	0.17	0.029	0.208
Nonanoic Acid	1.99	1.89	1.86	2.48	0.371	0.339	2.15	2.04	1.98	0.341	0.874
Octanoic Acid	1.82	1.75	1.64	1.76	0.372	0.916	1.93	1.76	1.54	0.359	0.211
<i>Esters</i>											
Hexanoic Acid, Methyl Ester	0.55	0.83	1.06	1.03	0.436	0.526	0.86	0.79	0.95	0.418	0.904
Octanoic Acid, Methyl Ester	21.05	21.11	20.12	20.55	3.100	0.986	22.31	19.66	20.16	2.900	0.544
<i>Furans</i>											
2-Pentyl Furan	0.99	0.97	0.83	0.90	0.147	0.621	0.96	0.95	0.86	0.128	0.648
<i>Hydrocarbons</i>											
Benzene	0.18	0.20	0.18	0.28	0.135	0.400	0.26	0.19	0.17	0.134	0.354
Ethylbenzene	0.09	0.09	0.13	0.13	0.031	0.468	0.14	0.09	0.10	0.028	0.199
Nonane	2.14	2.38	2.09	2.31	0.312	0.896	2.36	2.08	2.25	0.267	0.750
Octane	2.88	3.03	3.20	4.29	1.127	0.271	4.06	2.87	3.12	1.098	0.252
Tetradecane	1.51	1.32	1.32	1.47	0.291	0.665	1.56	1.39	1.26	0.281	0.230
Toluene	43.44 ^a	2.48 ^c	15.80 ^{bc}	30.28 ^{ab}	22.356	<0.001	21.29	25.38	22.34	22.190	0.850
d-Limonene	0.30	0.40	0.39	0.41	0.067	0.493	0.43	0.32	0.37	0.060	0.277
p-Xylene	0.04	0.04	0.04	0.05	0.011	0.413	0.05	0.04	0.04	0.011	0.091
<i>Ketones</i>											
Butyrolactone	3.03	2.84	2.94	3.55	0.581	0.724	3.64	2.81	2.82	0.528	0.300
2-Butanone	13.49	14.60	14.61	20.02	3.662	0.299	17.23	13.48	16.33	3.438	0.533
2-Heptanone	0.62	0.72	0.70	0.76	0.140	0.717	0.75	0.65	0.69	0.132	0.651
2-Pentanone	0.33	0.37	0.42	0.47	0.069	0.305	0.46	0.35	0.39	0.070	0.447
2-Propanone	29.42	31.33	30.52	45.82	6.190	0.132	35.58	27.86	39.38	5.605	0.296

Note: Aging temperature × aging duration interaction not significant ($P > 0.05$).

¹–2°C, 0°C, 4°C

²14, 28, 42, 56 d.

³Largest standard error of the least-squares means.

⁴Observed significance level.

^{a-c}Means in the same row without a common superscript differ ($P < 0.05$).

Table 9. Least-squares means of Maillard reaction-derived volatile flavor compound concentration of beef strip loins aged in 3 temperature environments and 4 aging durations

Volatile Compound (ng/g)	Aging Duration						Aging Temperature				
	14 d	28 d	42 d	56 d	SEM ¹	P Value ²	–2°C	0°C	4°C	SEM	P Value
<i>Ketones</i>											
2,3-Butanedione	14.36	13.95	15.09	13.79	3.686	0.986	17.61	12.46	12.82	3.476	0.253
3-Hydroxy-2-Butanone	25.89	21.72	19.26	22.00	5.081	0.696	30.16	17.94	18.55	5.146	0.088
<i>Pyrazines</i>											
Methylpyrazine	0.24	0.34	0.33	0.41	0.074	0.296	0.36	0.26	0.37	0.066	0.360
Trimethylpyrazine	2.51	2.83	2.63	2.83	0.392	0.919	2.98	2.47	2.65	0.335	0.537
2,5-Dimethylpyrazine	0.59	0.81	0.79	0.86	0.147	0.394	0.85	0.65	0.79	0.133	0.350
2-Ethyl-3,5-Dimethylpyrazine	3.61	3.57	3.57	3.94	0.650	0.942	3.97	3.62	3.41	0.592	0.663
<i>Strecker Aldehydes</i>											
Acetaldehyde	138.47	105.78	103.82	125.60	21.090	0.583	129.28	107.82	118.15	18.620	0.714
Benzaldehyde	17.70	17.00	14.84	16.57	3.470	0.542	16.90	14.71	15.72	3.390	0.434
Butyraldehyde	13.21	14.33	14.33	19.66	3.578	0.299	16.90	13.24	16.01	3.350	0.539
Methional	0.64 ^b	1.23 ^a	1.28 ^a	1.70 ^a	0.208	0.004	1.13	1.00	1.51	0.177	0.109
Phenylacetaldehyde	0.35	0.19	0.35	0.37	0.106	0.531	0.21	0.34	0.40	0.093	0.271
3-Methylbutanal	1.51	2.25	2.38	3.09	0.618	0.171	2.68	1.60	2.63	0.593	0.228
2-Methylbutanal	0.76 ^b	1.54 ^{ab}	1.84 ^{ab}	2.57 ^a	0.418	0.018	1.83	1.19	2.01	0.369	0.259
<i>Sulfur Containing</i>											
Carbon Disulfide	35.04	26.27	30.20	44.39	19.768	0.330	38.60	31.61	31.72	19.376	0.672
Dimethyl Sulfide	4.85	5.80	4.91	6.00	0.841	0.532	5.81	4.75	5.61	0.783	0.481
Methanethiol	3.88	3.74	3.57	4.70	0.490	0.339	4.47	3.78	3.73	0.440	0.475
<i>Thiophenes</i>											
2-Methyl Thiophene	0.006	0.005	0.004	0.006	0.002	0.518	0.006	0.005	0.006	0.002	0.858

Note: Aging temperature × aging duration interaction not significant ($P > 0.05$).

¹Largest standard error of the least-squares means.

²Observed significance level.

^{ab}Means in the same row within the same main effect without a common superscript differ ($P < 0.05$).

among Maillard reaction-derived volatiles. Free amino acids and sugars are substrates for the Maillard reaction and have been known to increase during postmortem aging (Koutsidis et al., 2008; Foraker et al., 2020; Hernandez et al., 2022). The increase of methional concentration after 14 d can be explained by the increased concentration of free methionine. During Strecker degradation, free methionine degraded into methional (Mottram, 1998). Moreover, the increase in 2-methylbutanal during extended aging can be attributed to increased free isoleucine, which is then degraded during Strecker degradation (Mottram, 1998; Frank et al., 2020). However, 2-methylbutanal has also been reported to be produced by microbial metabolism of *Enterococcus* spp. (Casaburi et al., 2015). The increase of these specific flavor precursors is reported in Hernandez et al. (2022). The lack of differences in other Maillard-derived volatiles is consistent with the minor differences observed in the descriptive flavor attributes associated with the Maillard reaction, i.e., brown/roasted.

Interrelationships between volatile flavor compounds and sensory outcomes

To visualize interrelationships between volatile flavor compounds and descriptive and consumer sensory results, partial least-squares regression was conducted. Data are presented in a biplot (Figure 3). Factors 1 and 2 accounted for 37.1% and 48.4% of the variation of the dependent variables (descriptive and consumer sensory attributes), respectively. Axis t1 showed a clear separation between positive and negative flavor attributes. Ethanol (medicinal) and acetic acid (sour, vinegar-like) were closely associated with off-flavors, musty/earthy, sour, sour aromatic, musty/earthy, bitter, liver-like, and oxidized. Moreover, these compounds and attributes were clustered with loins aged for 56 d at 4°C. These relationships are similar to those found in the univariate data. Overall liking and flavor liking were associated with butanoic acid, methyl ester (sweet, fruity), 1-penten-3-ol (green), 2-methylbutanal (malty, musty, brothy), and methional

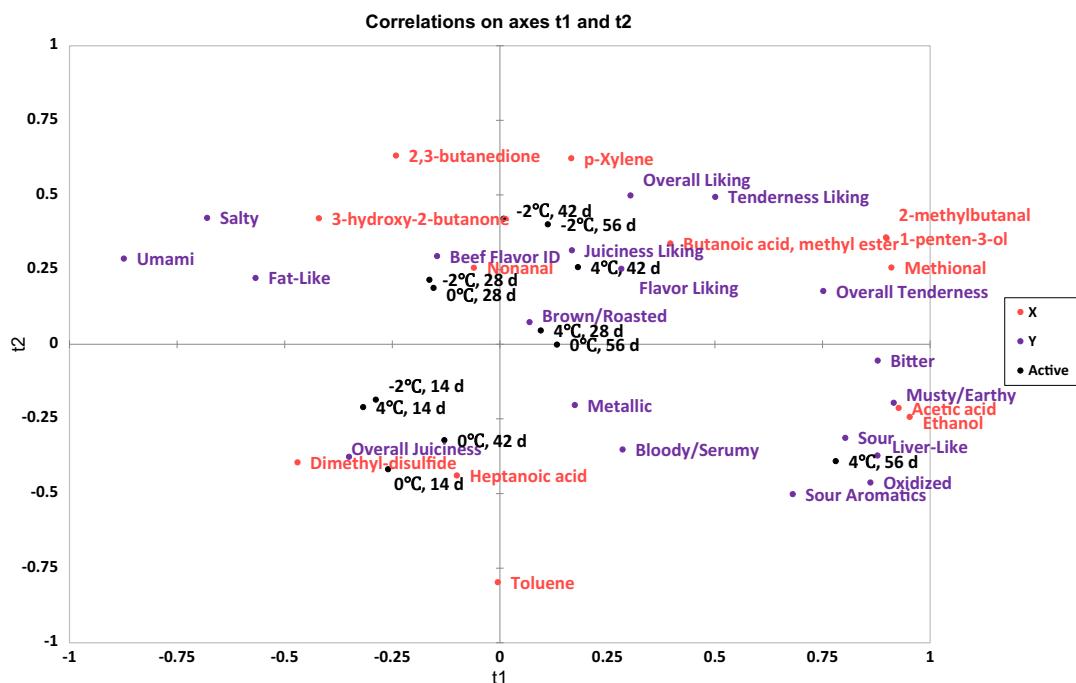


Figure 3. Partial least-squares regression biplot of volatile flavor compounds (red), descriptive and consumer sensory attributes (purple), and treatments (black).

(meaty, savory). 3-Hydroxy-2-butanone (buttery) was associated with fat-like, salty, and umami attributes. Consumer liking attributes were associated with loins aged for either 42 or 56 d at -2°C , loins aged for 56 d at 0°C , and loins aged for 28 or 42 d at 4°C . Moreover, brown/roasted and descriptive tenderness were associated with consumer liking attributes.

The partial least-squares regression model illustrated various relationships between volatile flavor profile, descriptive sensory attributes, and consumer liking attributes. Acetic acid and ethanol were drivers of off-flavor development, which reinforces the development of off-flavors in vacuum-packaged subprimals and is primarily caused by spoilage organism growth. Methional has been cited as an important contributor of beef flavor (Kerscher and Grosch, 2000). Moreover, Li et al. (2021) reported a positive correlation between 2-methylbutanal and flavor liking. It was interesting to observe the association of flavor and overall liking with volatile compounds derived from microbial growth and lipid degradation. As previously discussed, lipid oxidation was not a driver of off-flavor development in the present study. Therefore, it is probable that the formation of 1-penten-3-ol was through lipid thermal degradation, which has been suggested to produce volatile compounds with desirable aromas (Mottram, 1998; Kerth and Miller, 2015). The association of 3-hydroxy-2-butanone and 2,3-butanedione with fat-like

flavor is consistent with previous studies, in which these compounds were correlated with buttery/beef fat flavors (O'Quinn et al., 2016). When considering the univariate consumer sensory results, off-flavors detected by the descriptive panel did not influence consumer liking. Furthermore, both the univariate and multivariate data show multiple treatment combinations performed similarly to one another.

Conclusions

Overall, aging temperature and duration have a clear influence on beef tenderness and flavor development. Extended aging and elevated temperatures promoted tenderness development. Aging temperature and duration influenced flavor development to an extent. Off-flavor development increased during extended aging but was dependent on storage temperature. The likely mechanism of off-flavor development was through microbial growth and metabolism. Alcohols and carboxylic acids were drivers of off-flavor development. Aging beef subprimals at -2°C for 14 d was detrimental to consumer liking. These results imply that processors and retailers should consider minimum aging durations within colder storage regimens to ensure palatability. Conversely, when relatively warmer storage temperatures are utilized,

processors and retailers should consider maximum aging durations in order to minimize development of detrimental off-flavors during wet aging.

Acknowledgements

This project was funded by the Beef Checkoff. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture (USDA). The USDA is an equal opportunity provider and employer.

Literature Cited

- Adhikari, K., E. Chambers, IV, R. Miller, L. Vázquez-Araújo, N. Bhumiratana, and C. Philip. 2011. Development of a lexicon for beef flavor in intact muscle. *J. Sens. Stud.* 26:413–420. <https://doi.org/10.1111/j.1745-459x.2011.00356.x>
- AMSA. 2015. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. 2nd ed. American Meat Science Association, Champaign, IL.
- Bratcher, C. L., D. D. Johnson, R. C. Littell, and B. L. Gwartney. 2005. The effects of quality grade, aging, and location within muscle on Warner–Bratzler shear force in beef muscles of locomotion. *Meat Sci.* 70:279–284. <https://doi.org/10.1016/j.meatsci.2005.01.013>
- Brooks, J. C., J. B. Belew, D. B. Griffin, B. L. Gwartney, D. S. Hale, W. R. Henning, D. D. Johnson, J. B. Morgan, F. C. Parrish, Jr., J. O. Reagan, and J. W. Savell. 2000. National Beef Tenderness Survey–1998. *J. Anim. Sci.* 78:1852–1860. <https://doi.org/10.2527/2000.7871852x>
- Casaburi, A., P. Piombino, G.-J. Nychas, F. Villani, and D. Ercolini. 2015. Bacterial populations and the volatiles associated to meat spoilage. *Food Microbiol.* 45:83–102. <https://doi.org/10.1016/j.fm.2014.02.002>
- Cassens, A. M., A. N. Arnold, R. K. Miller, K. B. Gehring, and J. W. Savell. 2018. Impact of elevated aging temperatures on retail display, tenderness, and consumer acceptability of beef. *Meat Sci.* 146:1–8. <https://doi.org/10.1016/j.meatsci.2018.07.024>
- Colle, M. J., R. P. Richard, K. M. Killinger, J. C. Bohlscheid, A. R. Gray, W. I. Loucks, R. N. Day, A. S. Cochran, J. A. Nasados, and M. E. Doumit. 2015. Influence of extended aging on beef quality characteristics and sensory perception of steaks from the gluteus medius and *longissimus lumborum*. *Meat Sci.* 110:32–39. <https://doi.org/10.1016/j.meatsci.2015.06.013>
- Evers, D. L., A. J. Garmyn, J. F. Legako, D. R. Woerner, and M. F. Miller. 2020. Flavor characterization of grass- and grain-fed Australian beef *longissimus lumborum* wet-aged 45 to 135 days. *Meat Muscle Biol.* 4:22. <https://doi.org/10.22175/mmb.11043>
- Foraker, B. A., D. A. Gredell, J. F. Legako, R. D. Stevens, J. D. Tatum, K. E. Belk, and D. R. Woerner. 2020. Flavor, tenderness, and related chemical changes of aged beef strip loins. *Meat Muscle Biol.* 4:28. <https://doi.org/10.22175/mmb.11115>
- Frank, D., J. Hughes, U. Piyasiri, Y. Zhang, M. Kaur, Y. Li, G. Mellor, and J. Stark. 2020. Volatile and non-volatile metabolite changes in 140-day stored vacuum packaged chilled beef and potential shelf life markers. *Meat Sci.* 161:108016. <https://doi.org/10.1016/j.meatsci.2019.108016>
- Gardner, K., and J. F. Legako. 2018. Volatile flavor compounds vary by beef product type and degree of doneness. *J. Anim. Sci.* 96:4238–4250. <https://doi.org/10.1093/jas/sky287>
- Gruber, S. L., J. D. Tatum, J. A. Scanga, P. L. Chapman, G. C. Smith, and K. E. Belk. 2006. Effects of postmortem aging and USDA quality grade on Warner–Bratzler shear force values of seventeen individual beef muscles. *J. Anim. Sci.* 84:3387–3396. <https://doi.org/10.2527/jas.2006-194>
- Hernandez, M. S., D. R. Woerner, J. C. Brooks, T. L. Wheeler, and J. F. Legako. 2022. Influence of aging temperature and duration on spoilage organism growth, proteolytic activity, and related chemical changes in vacuum-packaged beef *longissimus*. *Meat Muscle Biol.* 6:13724. <https://doi.org/10.22175/mmb.13724>
- Hernández-Macedo, M., C. J. Contreras-Castillo, S. M. Tsai, S. H. Da Cruz, C. I. G. L. Sarantopoulos, M. Padula, and C. T. S. Dias. 2012. Gases and volatile compounds associated with micro-organisms in blown pack spoilage of Brazilian vacuum-packed beef. *Lett. Appl. Microbiol.* 55:467–475. <https://doi.org/10.1111/lam.12004>
- Huff-Lonergan, E., W. Zhang, and S. M. Lonergan. 2010. Biochemistry of postmortem muscle – Lessons on mechanisms of meat tenderization. *Meat Sci.* 86:184–195. <https://doi.org/10.1016/j.meatsci.2010.05.004>
- Jeremiah, L. E., and L. L. Gibson. 2003. The effects of postmortem product handling and aging time on beef palatability. *Food Res. Int.* 36:929–941. [https://doi.org/10.1016/S0963-9969\(03\)00102-9](https://doi.org/10.1016/S0963-9969(03)00102-9)
- Juárez, M., I. L. Larsen, L. L. Gibson, W. M. Robertson, M. E. R. Dugan, N. Aldai, and J. L. Aalhus. 2010. Extended ageing time and temperature effects on quality of sub-primal cuts of boxed beef. *Can. J. Anim. Sci.* 90:361–370. <https://doi.org/10.4141/CJAS09079>
- Kerschler, R., and W. Grosch. 2000. Comparison of the aromas of cooked beef, pork and chicken. In: *Weurman flavour research symposium; frontiers of flavor science*. Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany. p. 17–20.
- Kerth, C. R., and R. K. Miller. 2015. Beef flavor: a review from chemistry to consumer. *J. Sci. Food Agr.* 95:2783–2798. <https://doi.org/10.1002/jsfa.7204>
- King, D. A., M. E. Dikeman, T. L. Wheeler, C. L. Kastner, and M. Koohmaraie. 2003. Chilling and cooking rate effects on some myofibrillar determinants of tenderness of beef. *J. Anim. Sci.* 81:1473–1481. <https://doi.org/10.2527/2003.8161473x>
- King, D. A., T. L. Wheeler, S. D. Shackelford, K. D. Pfeiffer, R. Nickelson, and M. Koohmaraie. 2009. Effect of blade tenderization, aging time, and aging temperature on tenderness of beef *longissimus lumborum* and *gluteus medius*. *J. Anim. Sci.* 87:2952–2960. <https://doi.org/10.2527/jas.2009-1803>
- Koutsidis, G., J. S. Elmore, M. J. Oruna-Concha, M. M. Campo, J. D. Wood, and D. S. Mottram. 2008. Water-soluble precursors of beef flavour. Part II: Effect of post-mortem conditioning.

- Meat Sci. 79:270–277. <https://doi.org/10.1016/j.meatsci.2007.09.010>
- Legako, J. F., J. C. Brooks, T. G. O'Quinn, T. D. J. Hagan, R. Polkinghorne, L. J. Farmer, and M. F. Miller. 2015. Consumer palatability scores and volatile beef flavor compounds of five USDA quality grades and four muscles. *Meat Sci.* 100:291–300. <https://doi.org/10.1016/j.meatsci.2014.10.026>
- Lepper-Bllilie, A. N., E. P. Berg, D. S. Buchanan, and P. T. Berg. 2016. Effects of post-mortem aging time and type of aging on palatability of low marbled beef loins. *Meat Sci.* 112:63–68. <https://doi.org/10.1016/j.meatsci.2015.10.017>
- Li, Z., M. Ha, D. Frank, P. McGilchrist, and R. D. Warner. 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>
- Martinez, H. A., A. N. Arnold, J. C. Brooks, C. C. Carr, K. B. Gehring, D. B. Griffin, D. S. Hale, G. G. Mafi, D. D. Johnson, C. L. Lorenzen, R. J. Maddock, R. K. Miller, D. L. VanOverbeke, B. E. Wasser, and J. W. Savell. 2017. National Beef Tenderness Survey—2015: Palatability and shear force assessments of retail and foodservice beef. *Meat Muscle Biol.* 1:138–148. <https://doi.org/10.22175/mmb2017.05.0028>
- Mottram, D. S. 1998. Flavour formation in meat and meat products: a review. *Food Chem.* 62:415–424. [https://doi.org/10.1016/S0308-8146\(98\)00076-4](https://doi.org/10.1016/S0308-8146(98)00076-4)
- O'Quinn, T. G., D. R. Woerner, T. E. Engle, P. L. Chapman, J. F. Legako, J. C. Brooks, K. E. Belk, and J. D. Tatum. 2016. Identifying consumer preferences for specific beef flavor characteristics in relation to cattle production and postmortem processing parameters. *Meat Sci.* 112:90–102. <https://doi.org/10.1016/j.meatsci.2015.11.001>
- Resconi, V. C., M. Bueno, A. Escudero, D. Megalhaes, V. Ferreira, and M. M. Campo. 2018. Ageing and retail display time in raw beef odour according to the degree of lipid oxidation. *Food Chem.* 242:288–300. <https://doi.org/10.1016/j.foodchem.2017.09.036>
- Vierck, K. R., J. F. Legako, J. K. Kim, B. J. Johnson, and J. C. Brooks. 2020. Determination of package and muscle-type influence on proteolysis, beef-flavor-contributing free amino acids, final beef flavor, and tenderness. *Meat Muscle Biol.* 4:26. <https://doi.org/10.22175/mmb.10933>
- Vierck, K. R., J. F. Legako, and J. C. Brooks. 2021. Evaluation of dry-heat cookery method on volatile flavor compound development and consumer evaluation of six beef muscles. *Meat Muscle Biol.* 5:20. <https://doi.org/10.22175/mmb.11710>
- Watanabe, A., G. Kamada, M. Imanari, N. Shiba, M. Yonai, and T. Muramoto. 2015. Effect of aging on volatile compounds in cooked beef. *Meat Sci.* 107:12–19. <https://doi.org/10.1016/j.meatsci.2015.04.004>
- Whipple, G., M. Koohmaraie, M. E. Dikeman, and J. D. Crouse. 1990. Effects of high-temperature conditioning on enzymatic activity and tenderness of *Bos indicus longissimus* muscle. *J. Anim. Sci.* 68:3654–3662. <https://doi.org/10.2527/1990.68113654x>
- Wicklund, S. E., C. Homco-Ryan, K. J. Ryan, F. K. Mckeith, B. J. Mcfarlane, and M. S. Brewer. 2005. Aging and enhancement effects on quality characteristics of beef strip steaks. *J. Food Sci.* 70:S242–S248. <https://doi.org/10.1111/j.1365-2621.2005.tb07164.x>