Volatile Lipid Oxidation Products of Wagyu and Domestic Breeds of Beef

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The influence of beef source, cooking method, and refrigerated storage time on the contents of volatile lipid oxidation products of Wagyu and domestic sources of beef was determined. Longissimus dorsi muscle from Japanese Wagyu, American Wagyu, Longhorn, Angus, and U.S. Choice carcasses was boiled or roasted and stored for 0 or 3 days at refrigerator temperatures. With storage time, the contents of lipid oxidation products increased significantly ($P < 0.05$). Interactions between beef source and storage time were significant ($P < 0.05$) for hexanal, 2-pentylfuran, octanal, nonanal, and other major lipid oxidation products. Beef source was shown to have a significant effect on the content of these compounds following 3 days of storage but not immediately following cooking (0 day). The contents of lipid oxidation products were higher in the Japanese and American Wagyu breeds than in other domestic beef sources. In general, cooking method had only minor influences on the content of lipid oxidation products.

Keywords: *Flavor; beef; breeds; lipid oxidation; Wagyu*

INTRODUCTION

Unique flavor and texture characteristics of Wagyu beef have contributed to interest in developing the Wagyu breed in the United States for domestic and export markets (Jussaume et al., 1990). Comparison of sensory characteristics of Japanese and American Wagyu beef to Angus, Longhorn, and U.S. Choice beef demonstrated the superior flavor and palatability characteristics of the Wagyu beef. The beef, prepared as steaks or shabu-shabu, the traditional Japanese style of boiled beef, was evaluated immediately following cooking (Busboom et al., 1993). The desirable juiciness and tenderness characteristics of Wagyu beef have been attributed to its high content of fat and intramuscular marbling (Jussaume et al., 1990). Cooked beef from the Wagyu breeds has significantly higher neutral lipid and unsaturated fatty acid contents in comparison to domestic beef sources (Boylston et al., 1995). However, because cooked Wagyu beef has a higher content of unsaturated fatty acids, it may have a greater tendency to develop undesirable flavors during storage through lipid oxidation reactions.

Aldehydes, ketones, and alcohols are among the volatile flavor compounds formed through lipid oxidation reactions. At high concentrations, these compounds contribute rancid, cardboardy, pungent, and other undesirable flavor characteristics to the beef rather than desirable beefy flavor notes (Chang and Peterson, 1977). Because of their low flavor threshold, carbonyl compounds generally have the greatest impact on the development of off-flavors in cooked meats, commonly referred to as "warmed-over flavor" (Forss, 1972; Dixon and Hammond, 1984). Oxidation of polyunsaturated fatty acids is the major mechanism for formation of these volatile compounds, with the polar lipid fraction identified as the primary source of these precursors (Love and Pearson, 1971). Higher contents of polyunsaturated fatty acids in the polar lipid fraction of different breeds of beef (Larick et al., 1989) and beef fed different diets (Larick and Turner, 1990) have been identified as sources of off-flavors and aftertastes in cooked beef.

The objective of this research was to determine the influence of beef source, cooking method, and storage time on the content and composition of volatile lipid oxidation products. Five beef sources, Japanese Wagyu, American Wagyu, Angus, Longhorn, and U.S. Choice; two cooking methods, boiling and roasting; and two storage times, 0 and 3 days, were compared.

MATERIALS AND METHODS

Beef. American Wagyu, Angus, and Longhorn cattle were raised at Washington State University, Department of Animal Sciences, as part of a feeding trial to evaluate the growth, feed efficiency, and final carcass characteristics of the three breeds. American Wagyu cattle were crossbreeds from purebred Wagyu and consisted of one 82.5% Wagyu and two 75% Wagyu cattle. Cattle were grouped by weight and fed a diet that consisted of ammoniated wheat straw, rolled barley, dehydrated alfalfa, and cracked corn for 524 days, typical of Japanese feeding practices (Busboom et al., 1993).

Boneless loin sections were removed from carcasses at 3 days postmortem, vacuum packaged, and aged at 0 °C for 10 days (Busboom et al., 1993). For comparison, loin sections from Japanese Black Wagyu (purebred Wagyu, obtained from Japan) and U.S. Choice were obtained from commercial sources and vacuum packaged prior to storage. All loin sections were stored at −35 °C until analyzed. Steaks from four animals for Angus, Longhorn, and U.S. Choice and from three animals for Japanese Wagyu and American Wagyu were analyzed.

Cooking and Storage Treatments. Two cooking treatments, roasting and boiling, were compared. Loin sections were trimmed of excess subcutaneous fat prior to cooking. Steaks (2.5 cm thick) were roasted in a 175 °C oven to an internal temperature of 70 °C. The boiled beef was sliced into

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Table 1. Volatile Flavor Compounds[a] of Cooked Beef As Influenced by Beef Source and Storage Time

compound	day	Angus	American Wagyu	Longhorn	Japanese Wagyu	U.S. Choice
hexanal	0	1277.2^{bx}	718.6^{by}	427.3^{by}	1288.5^{by}	671.2^{bx}
	3	1249.8^{cx}	2895.2^{cx}	2132.1^{cx}	5103.7^{bx}	1385.1^{cx}
2-heptanone	0	21.4^{bx}	13.4^{by}	10.8^{bx}	21.9^{by}	10.7^{bx}
	3	27.1^{cx}	49.6^{bx}	21.3^{cx}	56.6^{bx}	18.7^{cx}
trans-2-heptenal	0	68.2^{bx}	44.3^{by}	27.6^{bx}	54.8^{by}	26.7^{bx}
	3	64.4^{cx}	131.7^{bx}	58.3^{cx}	133.2^{bx}	61.2^{cx}
2-pentylfuran	0	100.8^{bx}	33.8^{by}	28.0^{bx}	96.5^{by}	43.1^{bx}
	3	542.5^{bcx}	913.3^{bx}	374.6^{cx}	932.8^{bx}	240.3^{cx}
octanal	0	617.3^{bx}	464.6^{by}	297.2^{bx}	518.7^{bx}	218.8^{bx}
	3	543.4^{cdx}	1227.2^{bx}	531.8^{cdx}	1045.8^{bcx}	385.0^{dx}
2-octenal	0	112.3^{bx}	80.8^{by}	46.6^{bx}	86.6^{by}	41.8^{bx}
	3	115.7^{cx}	264.9^{bx}	103.8^{cx}	250.1^{bx}	93.6^{cx}
2-octen-1-ol	0	51.6^{bx}	26.0^{by}	20.3^{bx}	37.2^{by}	24.2^{bx}
	3	87.2^{bcx}	140.9^{bx}	65.8^{cx}	131.7^{bx}	62.5^{cx}
1-octanol	0	154.3^{bx}	91.2^{by}	82.5^{bx}	104.8^{bx}	65.7^{bx}
	3	155.8^{cdx}	305.0^{bx}	133.7^{dx}	222.5^{bcx}	122.9^{dx}
nonanal	0	741.0^{bx}	781.6^{by}	501.0^{bx}	657.7^{bx}	407.9^{bx}
	3	753.8^{cdx}	1766.8^{bx}	735.7^{cdx}	1387.1^{bcx}	463.5^{dx}
2-nonenal	0	155.5^{bx}	77.7^{by}	54.1^{bx}	78.7^{bx}	64.6^{bx}
	3	88.5^{cx}	250.1^{bx}	101.9^{bcx}	178.5^{bcx}	112.8^{bcx}
2,4-decadienal	0	31.7^{bx}	13.1^{by}	7.6^{bx}	18.0^{by}	19.7^{bx}
	3	31.3^{bcdx}	58.5^{bcx}	18.7^{dx}	63.0^{bx}	27.4^{cdx}

[a] Nanograms per gram of tissue, cooked weight. Interactions between beef source and storage time were significant ($P < 0.05$). Means for beef source effects with the same superscript (b−d) are not significantly different ($P > 0.05$). Means for storage time effects with the same superscript (x, y) are not significantly different ($P > 0.05$).

3 mm thick slices and cooked in boiling water for 60 s. Following cooking, the beef samples were analyzed immediately (0 day) or wrapped in aluminum foil and stored for 3 days at 3 °C.

Analysis of Volatile Flavor Compounds. Volatile flavor compounds were isolated using purge-and-trap headspace analysis techniques. HPLC grade solvents and prepurified nitrogen were used for the volatile flavor analysis. Traps consisted of Tenax GC (300 mg, 2,6-diphenyl-*p*-phenylene oxide, Alltech Associates Inc., Deerfield, IL) packed into silanized glass tubing (9 mm o.d.) with silanized glass wool. Traps were rinsed with 15 mL of methanol and purged with prepurified nitrogen (25 mL/min) at 280 °C for 2 h to activate prior to use.

Cooked beef was ground once through a meat grinder with a 1 cm plate. A 100 g sample of the ground beef was placed in a 500 mL, double-neck, round-bottom flask with 100 mL of deionized water. Tetradecane (12.5 μg, internal standard) was added to each sample prior to purging. Samples were purged with nitrogen at a flow rate of 100 mL/min. A vacuum was applied to the system through a Tenax trap placed at the center neck. Flasks were placed in a water bath (70 °C) and purged for 4 h. Volatile flavor compounds were eluted from the traps with 15 mL of hexane and concentrated to 200 μL in a stream of nitrogen.

Volatile flavor compounds were separated on a 5% phenyl, 95% methyl polysiloxane capillary column (SE-54, 30 m, 0.32 mm i.d., 0.25 mm film thickness, Alltech Associates, Inc.) installed in a gas chromatograph (Model 3400, Varian Associates, Inc., Walnut Creek, CA) equipped with a flame ionization detector and an on-column injection port. The temperature program was 35 °C for 15 min, increased to 160 °C at 1.5 °C/min, and held for 30 min. The detector temperature was set at 250 °C. The injector program was 50 °C for 3 min, then increased to 250 °C at 150 °C/min, and held for 30 min. Flow rates were as follows: carrier He, 2.0 mL/min; He make-up gas, 20 mL/min; H_2, 30 mL/min; and air, 300 mL/min. Head pressure was set at 10 psi.

Area counts vs nanogram quantities (6.25−200 ng) were plotted for the internal standard, tetradecane. Peak areas for all compounds were converted to nanogram quantities based on the standard curve for tetradecane. Contents of the individual volatile compounds were calculated on the basis of recovery of the internal standard and quantities of beef tissue and internal standard used in the isolation. Identification of volatile compounds was based on comparison of retention times of samples and commercial standards (Alltech Associates, Inc.; Aldrich Chemical Co., Milwaukee, WI) and mass spectrometry.

A gas chromatograph−quadrupole mass spectrometer (Model HP-5988A, Hewlett-Packard, Inc., Palo Alto, CA) was used to confirm the identity of volatile compounds. Column and oven conditions were as for the gas chromatographic analysis. Conditions for the mass spectrometer were as follows: electron ionization voltage, 70 eV; ionizing current, 0.3 mA; electron multiplier voltage, 1800 kV; ion source temperature, 200 °C; ionization chamber pressure, 1×10^{-6} Torr; and scan range, m/z 33−250.

Statistical Analysis. The experiment was designed as a three-way factorial, with beef source, cooking method, and storage time as the main factors. Analysis of variance and least-squares means were used to determine the influence of these factors on the content of volatile flavor compounds. Animals within each breed were treated as a nested variable. The significance of interactions between these main factors was also determined, and data were pooled for volatile flavor compounds in which interactions were not significant ($P > 0.05$) (SAS, 1987).

RESULTS AND DISCUSSION

The compounds identified and quantitated in this study were primarily aldehydes, ketones, alcohols, and other products of lipid oxidation reactions. These compounds are decomposition products of fatty acid hydroperoxides formed during the oxidation of linoleic, oleic, and arachidonic acids, the predominant unsaturated fatty acids in meats (Forss, 1972; Frankel, 1984; Ladikos and Lougovois, 1990). Interactions between storage time and beef source were significant for 11 lipid oxidation products (Table 1). For the remaining volatile lipid oxidation products, the interactions between storage time and beef source were not significant and the data were pooled to focus on the specific treatment effects (Tables 2 and 3). The interactions between cooking method and either storage time or beef source were not significant, and the data for all lipid oxidation products were pooled (Table 4).

Storage Time. Refrigerated storage of cooked beef resulted in an increase in the content of lipid oxidation products (Tables 1 and 2). The increase in the content of undesirable volatile flavor compounds during refrigerated storage has been demonstrated through sensory evaluation, determination of TBA-reactive substances,

Table 2. Volatile Flavor Compounds[a] of Cooked Beef As Influenced by Storage Time

	storage time	
compound	0 days	3 days
3-hexanone	255.5[b]	186.7[b]
2-hexanone	46.6[b]	34.6[b]
octane	47.0[b]	32.1[c]
3-hexanol	14.5[b]	4.6[c]
furfural	4.7[c]	12.4[b]
trans-2-hexenol	15.5[b]	17.2[b]
1-hexanol	35.2[c]	75.7[b]
heptanal	454.1[c]	738.8[b]
benzaldehyde	309.2[b]	200.1[c]
1-heptanol	368.9[b]	238.0[c]
1-octen-3-ol	210.5[c]	273.1[b]
2,3-octanedione	249.3[c]	540.7[b]
2-octanone	4.3[b]	13.4[b]
decane	6.2[c]	16.5[b]
decanal	21.5[b]	26.2[b]
2-decenal	78.8[c]	139.5[b]
2-undecenal	45.8[c]	83.9[b]

[a] Nanograms per gram of tissue, cooked weight. Interactions between beef source and storage time were not significant ($P > 0.05$). Data were pooled to determine the effects of storage time on content of volatile flavor compounds. Means with the same superscript (b, c) are not significantly different ($P > 0.05$).

Table 3. Volatile Flavor Compounds[a] of Cooked Beef As Influenced by Beef Source

	beef source				
compound	Angus	American Wagyu	Longhorn	Japanese Wagyu	U.S. Choice
3-hexanone	168.1[b]	341.3[b]	155.7[b]	292.2[b]	148.1[b]
2-hexanone	27.7[c]	81.8[b]	23.6[c]	44.9[bc]	24.9[c]
octane	26.7[cd]	69.6[b]	22.3[d]	56.2[bc]	23.0[d]
3-hexanol	4.0[d]	14.9[bc]	5.5[cd]	15.6[b]	7.8[bcd]
furfural	8.4[b]	9.2[b]	5.5[b]	15.0[b]	4.7[b]
trans-2-hexenol	9.0[c]	20.2[c]	7.1[c]	37.9[b]	7.5[c]
1-hexanol	60.3[b]	66.8[b]	43.1[b]	71.4[b]	35.8[b]
heptanal	673.3[bc]	782.9[b]	429.9[bc]	779.9[b]	316.3[c]
benzaldehyde	174.3[c]	360.4[b]	179.2[c]	412.7[b]	146.6[c]
1-heptanol	223.4[c]	419.6[b]	213.8[c]	488.4[b]	172.0[c]
1-octen-3-ol	224.1[cd]	352.2[b]	156.2[d]	336.9[bc]	139.7[d]
2,3-octanedione	418.1[bc]	521.5[b]	217.2[c]	515.5[b]	302.6[bc]
2-octanone	4.7[c]	20.0[b]	4.4[c]	10.2[bc]	5.1[c]
decane	8.8[b]	11.3[b]	5.5[b]	24.5[b]	6.9[b]
decanal	21.2[c]	37.0[b]	15.2[c]	27.5[bc]	18.3[c]
2-decenal	126.3[b]	136.5[b]	69.9[b]	117.3[b]	95.8[b]
2-undecenal	77.9[b]	75.7[b]	42.8[b]	58.6[b]	69.1[b]

[a] Nanograms per gram of tissue, cooked weight. Interactions between beef source and storage time were not significant ($P > 0.05$). Data were pooled to determine the effects of storage time on content of volatile flavor compounds. Means with the same superscript (b–d) are not significantly different ($P > 0.05$).

and quantitation of volatile flavor compounds (Huang and Greene, 1978; Drumm and Spanier, 1991; Stapelfeldt et al., 1993). Therefore, the primary emphasis of the discussion of results of this research will focus on the effects of beef source and cooking method and the interaction of these effects with storage time.

Beef Source. The significance of interactions between beef source and storage time was dependent upon the specific volatile flavor compounds (Tables 1 and 3). Of particular interest are those volatile flavor compounds in which the interactions between beef source and storage time were significant (Table 1). These compounds, which include hexanal, 2-pentylfuran, octanal, and nonanal, are among the major volatile flavor compounds formed through lipid oxidation reactions. Drumm and Spanier (1991) demonstrated that the rate of formation of lipid oxidation products was dependent on the specific compound. Comparison of the rates of

Table 4. Volatile Flavor Compounds[a] of Cooked Beef As Influenced by Cooking Method

	cooking method	
compound	boiled	roasted
3-hexanone	237.5[b]	204.7[b]
2-hexanone	42.9[b]	38.2[b]
octane	37.2[b]	42.0[b]
hexanal	1673.6[b]	1896.1[b]
3-hexanol	9.0[b]	10.2[b]
furfural	8.2[b]	8.9[b]
trans-2-hexenol	15.0[b]	17.7[b]
1-hexanol	49.0[b]	62.0[b]
2-heptanone	22.0[b]	28.6[b]
heptanal	505.2[c]	687.7[b]
trans-2-heptenal	58.5[b]	75.6[b]
benzaldehyde	232.2[b]	277.0[b]
1-heptanol	271.0[b]	335.9[b]
1-octen-3-ol	211.9[c]	271.7[b]
2,3-octanedione	306.0[c]	484.0[b]
2-pentylfuran	370.2[b]	290.9[b]
2-octanone	9.8[b]	8.0[b]
decane	11.2[b]	11.6[b]
octanal	512.1[b]	657.2[b]
2-octenal	101.3[c]	137.9[b]
2-octen-1-ol	54.2[c]	75.2[b]
1-octanol	126.5[c]	161.1[b]
nonanal	754.1[c]	885.1[b]
2-nonenal	98.6[b]	133.9[b]
decanal	21.6[b]	26.1[b]
2-decenal	87.0[c]	131.3[b]
2,4-decadienal	22.9[c]	34.9[b]
2-undecenal	54.0[b]	75.7[b]

[a] Nanograms per gram of tissue, cooked weight. Interactions between beef source and cooking method were not significant ($P > 0.05$). Data were pooled to determine the effects cooking method on content of volatile flavor compounds. Means with the same superscript (b, c) are not significantly different ($P > 0.05$).

formation of lipid oxidation products (Drumm and Spanier, 1991) to the results from this study showed that the interactions between beef source and storage time were significant ($P < 0.05$) for those lipid oxidation products that are formed at a faster rate than the other lipid oxidation products (Drumm and Spanier, 1991).

The lipid oxidation process is initiated during cooking with the release of iron from the myoglobin molecule during heating (Sato and Hegarty, 1971; Igene and Pearson, 1979; Baines and Mlotkiewicz, 1984; Love, 1987). Although the lipid and fatty acid contents of the five different beef sources differed (Boylston et al., 1995), the contents of lipid oxidation products of the five beef sources did not differ ($P > 0.05$) at day 0. Immediately following cooking, the lipid oxidation reactions had not proceeded to an extent that differences in the contents of the lipid oxidation products, for which the interaction between beef source and storage time was significant, were detectable.

Following 3 days of storage, however, significant differences in the content of lipid oxidation products as influenced by beef source were noted (Table 1). In general, the contents of these compounds were significantly higher ($P < 0.05$) for American and Japanese Wagyu beef samples than for Angus, Longhorn, or U.S. Choice beef samples. The one exception was for hexanal, a major product of lipid oxidation reactions, which was present in significantly higher contents in Japanese Wagyu samples than in American Wagyu, Angus, Longhorn, and U.S. Choice samples. Contents of volatile lipid oxidation products were also higher in the American and Japanese Wagyu than in the other beef sources for those compounds in which the interactions between storage time and beef source were not signifi-

cant (Table 3). The contents of the lipid oxidation compounds in the beef samples following 3 days of refrigerated storage reflected differences in the content of unsaturated fatty acids of the neutral lipid fraction of those samples. The susceptibility of beef to lipid oxidation is a function of the content of unsaturated fatty acids present. The neutral lipid contents of American and Japanese Wagyu beef (23.5−23.9 g of lipid/100 g of cooked weight) were previously found to be significantly higher than those of the other three beef sources (9.5−15.4 g of lipid/100 g of cooked weight; Boylston et al., 1995).

Polar lipids have been identified as the primary targets of lipid oxidation reactions because of their high degree of unsaturation and close proximity to proteins and other catalysts of lipid oxidation (Love and Pearson, 1971; Igene and Pearson, 1979; Willemot et al., 1985). The polar lipid content of the five different beef sources included in this study ranged from 1.01 to 1.27 g of lipid/100 g of cooked weight, with no definitive relationship between beef source and fatty acid content (Boylston et al., 1995). On the other hand, the Wagyu breeds, which had the highest content of unsaturated fatty acids in the neutral lipid fraction, in comparison to the other beef sources, in general, had higher contents of lipid oxidation products. The dramatic differences in the contents of unsaturated fatty acids in the neutral lipid fraction of these five beef sources appear to be a major factor contributing to greater formation of volatile lipid oxidation products in the Wagyu beef samples following refrigerated storage.

Cooking Method. The contents of a majority of the volatile lipid oxidation products were not significantly different for roasted and boiled beef (Table 4), but roasted beef had significantly higher ($P < 0.05$) contents of heptanal, 2,3-octanedione, 2-octenal, 2-octen-1-ol, and 2-decenal than boiled beef. The higher content of lipid oxidation products in the roasted beef can be attributed, in part, to the higher content of neutral lipid unsaturated fatty acids in the roasted beef, as compared to the boiled beef (Boylston et al., 1995). Research by MacLeod and Coppock (1977) showed that sensory panelists could detect differences in the flavor characteristics of boiled and roasted beef. Other researchers have also demonstrated that cooking method has a significant influence on warmed-over flavor development. Meat cooked to high internal temperatures for extended cooking times develops less warmed-over flavor than meat cooked for shorter cooking times and lower internal temperatures (Huang and Greene, 1978; Satyanarayan and Honikel, 1992). Maillard reaction products, which form to a greater extent in meats cooked to higher internal temperatures and for longer times, have antioxidative properties to slow the rate of lipid oxidation in meats (Sato and Hegarty, 1971; Huang and Greene, 1978; Bailey et al., 1987). Due to the influence of time and temperature on the formation of Maillard reaction products, these compounds are present in higher concentrations in roasted beef than in boiled beef (Bailey, 1994). The opposing effects of a higher lipid content and higher content of Maillard reactions products in the roasted beef, as compared to the boiled beef, could contribute to the observed similarity in the profile of lipid oxidation products for roasted and boiled beef samples.

Lipid oxidation products, which at high concentrations can contribute to undesirable flavor characteristics in cooked meats, were present in significantly higher concentrations in roasted and boiled beef from Japanese and American Wagyu than from Longhorn, Angus, or U.S. Choice following refrigerator storage. Differences in the contents of these volatile compounds were attributed to higher neutral lipid and unsaturated fatty acid contents of the Wagyu breeds. Sensory evaluation panels have indicated a preference for Wagyu beef in comparison to domestic beef sources when evaluated immediately following cooking (Busboom et al., 1993). In this study, contents of lipid oxidation products in the five beef sources were not significantly different immediately following cooking. Differences in the content of heterocyclic compounds, which contribute to desirable roasted beefy flavor notes, may contribute to the higher acceptability of the Wagyu beef. Further research is necessary to determine the relationship between beef source and content of volatile flavor compounds that contribute to desirable beef flavor characteristics.

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