Changes in the Content of Lipid Autoxidation and Sulfur-Containing Compounds in Cooked Beef during Storage

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Changes in the content of carbonyl- and sulfur-containing compounds were determined for ground beef patties stored at 4 °C for 0–4 days. Volatile compounds were isolated by using distillation–extraction techniques and analyzed by using gas chromatography and mass spectrometry. During storage, the content of the lipid autoxidation products showed a significant ($P < 0.05$) increase; the rate of formation of these compounds generally followed zero-order kinetics and was specific for each individual compound. The content of the heterocyclic sulfur compounds did not change significantly ($P > 0.05$) with storage. The increase in the content of the aliphatic and cyclic sulfur compounds with storage was significant ($P < 0.05$). Free-radical reactions, the proposed primary mechanism for lipid autoxidation, may also catalyze the degradation of sulfur-containing compounds and contribute to the increase in the content of the aliphatic and cyclic sulfur compounds.

The deterioration of meat flavor is a major problem related to the loss of flavor quality in refrigerated and frozen cooked meats and has been attributed primarily to lipid autoxidation (Love and Pearson, 1971). The rapid development of off-flavors in cooked meats was initially described as "warmed-over flavor" (WOF) by Tims and Watts (1958). However, the term "meat flavor deterioration" (MFD) has been suggested to better describe the complex series of chemical reactions that contribute to an overall increase in off-flavor notes and a loss in desirable meat flavor quality (Spanier et al., 1988, 1990). From a sensory point of view, these chemical reactions result in increases in the undesirable cardboard and painty notes and decreases in the desirable cooked beef brothy note (Johnsen and Civille, 1986; St. Angelo et al., 1987; Love, 1988). The intensity of the undesirable sensory notes has been positively correlated with the content of carbonyl compounds formed through lipid autoxidation reactions. The decrease in the intensity of desirable sensory notes may be attributed either to a decrease in the content of those compounds that contribute to desirable flavor or to a masking of the desirable flavor compounds by an increased content of undesirable flavor compounds (St. Angelo et al., 1987).

The primary mechanism for the degradation of desirable flavor in stored meats is lipid autoxidation mediated through free-radical reactions. Disruption of the muscle membrane system, through mechanical grinding, protein denaturation during cooking, and/or hydrolytic enzymatic activity, causes the release of free iron needed to catalyze lipid autoxidation (Sato and Hegarty, 1971; Igene and Pearson, 1979; Love, 1987). The lipid hydroperoxides, the initial products of lipid autoxidation reactions, are unstable and undergo further degradation through free-radical mechanisms to form aliphatic aldehydes, alcohols, ketones, and hydrocarbons. These secondary products of lipid autoxidation are the major contributors to off-flavor in meats (Lillard, 1987; Love, 1987). The lipid hydroperoxides can also interact with proteins or amino acids through reactions with the lipid free radicals, hydroperoxides, or nonradical secondary products. Cysteine and methionine side chains are especially susceptible to these oxidative free-radical reactions (Gardner, 1983; Lillard, 1987; Ladikos and Lougovois, 1990).

The total lipid content of ground beef ranges from 5.7 to 26.5 g/100 g of meat, with the phospholipids comprising 0.9–5.3 g/100 g total lipid. These ranges are dependent on the amount of adipose tissue present (Hood and Allen, 1971; Keller and Kinsella, 1973). Despite the low concentration of phospholipids in the tissue, the high degree of unsaturation of the phospholipid fatty acids and the close proximity of the phospholipids to proteins and other catalysts of lipid autoxidation make the phospholipids primary targets of lipid autoxidation reactions (Love and Pearson, 1971; Igene and Pearson, 1979; Willemot et al., 1985).

Over 600 compounds have been identified in meat (MacLeod and Seyyedian-Ardebili, 1981; Shahidi et al., 1986). Maillard reactions, fatty acid oxidation, and inter- and intramolecular cyclizations during cooking are involved in the synthesis of these flavor compounds (Keller and Kinsella, 1973; Wilson et al., 1973; Whitfield et al., 1988). Chang and Peterson (1977) have categorized the classes of compounds present in meat as contributors and noncontributors to desirable meat flavor. The contributors, which include acyclic sulfur compounds, aromatic and nonaromatic heterocyclic compounds, and lactones, are products of Maillard reactions (Chang and Peterson, 1977; Golovnja and Rothe, 1980; Shibamoto, 1980; Bailey, 1983; Shahidi et al., 1986). Cysteine and methionine are important precursors for the synthesis of thiophenes, thiazoles, and other sulfur-containing compounds (Baines and Mlotkiewicz, 1984). The noncontributors to meat flavor include hydrocarbons, saturated alcohols, carboxylic acids, esters, ethers, and carbonyl compounds (Chang and Peterson, 1977). The positive contributors to meat flavor are usually present at parts per billion levels, while the lipid autoxidation products and other noncontributors to meat flavor are present at parts per million levels. Many of these desirable flavor compounds have very low flavor thresholds. In gas chromatographic analysis, the undesirable compounds predominate over the desirable compounds and contribute to the difficulty associated with

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the identification of the desirable meat flavor compounds (St. Angelo et al., 1987).

This study was designed to determine the effects of refrigerated storage on the content and composition of the lipid autoxidation and sulfur-containing compounds in cooked beef patties. The effect of final internal temperature on the content and stability of these compounds was also examined. "Total volatile analysis" techniques, such as distillation–extraction, allow the qualitative and quantitative analysis of the composition of all volatile compounds in a sample (Weurman, 1969). This isolation technique provides the increased sensitivity needed to focus on the compounds that have the potential for making an impact on meat flavor. However, because heat is applied during the distillation, there is the potential for the formation of new compounds in the distillate in addition to those formed as a result of the initial cooking and storage treatments. Some of these new compounds would be formed from reactive components or precursors present in the meat after the initial cooking and storage. Therefore, changes in the reactive components and volatile compounds in the meat would be measured through the use of distillation–extraction techniques.

EXPERIMENTAL METHODS

Sample Preparation. Top round beef (*semimembranosus* muscle, USDA choice grade) was obtained from a local supermarket. Excess fat and the adductor muscle were removed and discarded. The lean muscle (4.25% lipid) was ground once each through plates with 1.0- and 0.8-cm holes by using a meat grinder (General). Patties (85 g) were cooked on a grill (Faberware, 180 °C) for either 7 or 8 min on each side. The mean final internal temperatures were 63.6 and 68.8 °C, respectively, and mean final cooked weights were 61.55 and 57.91 g, respectively, for the patties. The cooked patties were cooled and reground by passage through a plate with 0.8-cm holes to obtain more homogeneously distributed samples (Vercellotti et al., 1989). The cooked/reground beef patties (52 g) were tightly packed into glass Petri dishes, covered, and stored at 4 °C for 0, 1, 2, 3, and 4 days.

Volatile Analysis. The volatile compounds were isolated from the meat samples by using steam distillation–extraction techniques. A 300-g sample of beef and 900 g of deionized water were combined in a 2-L round-bottom flask. The volatiles were condensed in an ice–water-cooled condenser and trapped in an Erlenmeyer flask, surrounded with ice. The distillation was continued for 4 h after the sample had begun to reflux (45 min). The distillate (175–200 mL) was extracted with 6 × 25-mL aliquots of a methylene chloride/methanol (9:1 v/v, spectro-grade) solvent system (Vercellotti et al., 1989). Thiophenol (20 μg, internal standard) was added to the distillate prior to extraction. This compound was chosen as an internal standard because it is well separated from the other compounds of interest in the chromatogram and can be detected by the FID and FPD. The extract was dried over anhydrous sodium sulfate and concentrated to 500 μL. Identification of the volatile compounds was based on comparison of GC retention times and mass spectral confirmation of samples and pure commercial standards. Compounds for which authentic standards were not available were labeled as tentative.

The volatiles were separated on a cross-linked, 5% phenylmethyl silicone phase, fused silica capillary column (HG-5, 50 m, 0.32 mm o.d., 0.52-μm film thickness, Hewlett-Packard, Avondale, PA) installed in a gas chromatograph equipped with flame ionization (FID) and flame photometric (FPD) detectors (Model 5890A, Hewlett-Packard). The GC oven temperature was initially held at 35 °C for 15 min and then increased at a rate of 3 °C/min to a final temperature of 250 °C and held for 45 min. Injector and detector (FID and FPD) temperatures were set at 200, 250, and 220 °C, respectively. The extracts (2.0 μL) were injected by using a split injection, with a column flow rate of 1.1 mL/min, purge flow rate of 2.0 mL/min, split vent flow rate of 15.0 mL/min, and split ratio of 14:1.

Two standard curves for thiophenol using the FID and FPD were developed for the quantification of the volatile compounds in the samples in conjunction with the use of thiophenol as an internal standard. Area counts vs nanogram quantities (20–400 ng) was plotted for the quantification of the total volatile compounds by using the FID. Area counts vs the square root of nanogram quantities was plotted for the quantification of the sulfur-containing compounds by using the FPD. Peak areas of the volatile compounds were converted to nanogram amounts by using the standard curves developed for thiophenol for the respective detectors. The content of each of the volatile compounds in the meat sample was corrected by dividing the content of the volatile compound by the content of the internal standard for that specific analysis and multiplying by the ratio of the initial weight of the internal standard (20 μg) and meat sample (300 g). Volatile compounds quantified by using the FID and FPD are reported in parts per million and parts per billion, respectively.

A gas chromatograph–quadrupole mass spectrometer (Model 4500, Finnigan-MAT) interfaced with an Incos data system was used for confirmation of the identity of the volatile compounds in the extracts. GC conditions were as for the chromatographic analysis. The conditions for the mass spectrometer were set as follows: ionizing voltage, 70 eV; emission current, 0.3 mA; electron multiplier voltage, 1800 kV; ion source temperature, 150 °C; ionization chamber pressure, 6.0×10^{-6} atm; and scan range 33–250 m/z in 0.95 s with a 0.05-s hold.

Statistical Analysis. This experiment was designed as a 2 × 5 factorial, with two cooking treatments (7 and 8 min/side) and five storage times (0–4 days). The experiment was replicated four times. Interactions between storage time and cooking treatment were tested by using analysis of variance to ensure nonsignificance ($P > 0.05$) prior to further statistical analyses. Analysis of variance and least-squares means were used to determine the effect of storage time on the content of the volatile compounds. The rate of formation of the lipid degradation compounds was determined by using regression analysis. The effect of cooking treatment on the content of the lipid degradation and sulfur-containing compounds was determined by contrast of means (SAS, 1987).

RESULTS AND DISCUSSION

Effects of Storage Time. Lipid autoxidation in meats is initiated during the cooking process and continues throughout the storage period (Baines and Mlotkiewicz, 1984). The content of aldehydes, alcohols, ketones, and other lipid autoxidation products identified in the cooked beef patties (Table I) increased during refrigerated storage with significant ($P < 0.05$) variability as a function of storage time. Hexanal, a major lipid autoxidation product in the beef patties, is most frequently quantified as a measure of lipid autoxidation in foods (Melton, 1983). This compound is formed at rates significantly higher than the next eight major lipid autoxidation products (Figure 1, inset). Figure 1 focuses on the relationship between the content of the next eight most abundant lipid autoxidation products and storage time and expands the region of the curve between 0 and 200 ppm. The relationships between content and storage time were consistent for 28 selected compounds detected by the FID.

Linoleic, oleic, and arachidonic acids are the primary reactants for the formation of volatile compounds during lipid autoxidation in meats (Ladikos and Lougovois, 1990). The decomposition of the fatty acid hydroperoxides formed during lipid autoxidation involves a complex set of reactions resulting in the formation of numerous volatile compounds (Forss, 1972; Frankel, 1984). These factors, in addition to variability in the presence of catalysts, chelators, antioxidants, and other components, affect the rate of lipid autoxidation and complicate the determination of kinetic parameters (Labuza, 1971). Regression analysis, as a function of zero- and first-order kinetics, showed

Table I. Zero-Order Regression Analysis of the Effect of Refrigerated Storage Time on the Formation of Lipid Degradation Volatiles in Ground Beef Patties[a]

Kovats index	compd[b]	slope, ppm/day	intercept, ppm	r^c
705	pentanal	0.199	0.579	0.8017
809	hexanal	2.800	2.666	0.8964
902	heptanal	0.222	0.348	0.9054
961	2-heptenal	0.066	0.068	0.8769
1004	octanal	0.199	0.363	0.8999
1062	2-octenal	0.136	0.136	0.9044
1106	nonanal	0.257	0.503	0.8398
1164	2-nonenal	0.080	0.095	0.8691
1208	decanal	0.008	0.025	0.6859
1219	2,4-nonadienal	0.012	0.007	0.8401
1322	2,4-decadienal	0.030	0.026	0.8169
711	2-ethylfuran	0.062	0.045	0.7358
843	furfural	0.008	0.051	0.7656
993	2-pentylfuran	0.222	0.158	0.8538
781	pentanol	0.242	0.350	0.8256
862	3-hexen-1-ol	0.024	0.042	0.4382
877	hexanol	0.046	0.087	0.8402
973	heptanol	0.042	0.082	0.7857
983	1-octen-3-ol	0.196	0.246	0.8349
1071	2-octenol	0.079	0.061	0.7400
1073	1-octanol	0.085	0.170	0.8417
893	2-heptanone	0.100	0.086	0.9131
986	2,3-octanedione (t)	0.194	0.014	0.7938
1038	3-octen-2-one	0.020	0.052	0.8485
1094	2-nonanone	0.020	0.032	0.8352
1144	3-nonen-2-one	0.014	0.020	0.8506
966	benzaldehyde	0.013	0.099	0.5872
1051	phenylacetaldehyde	0.012	0.093	0.5198

[a] Data for four replications and two cooking times (7 and 8 min/side) have been pooled for the regression analysis. [b] Mass spectrum and Kovats retention index consistent with an authentic sample except where labeled tentative (t), where no authentic sample was available. [c] Correlation coefficient, trend of content vs time, is significant ($P < 0.0001$) for all compounds.

significant ($P < 0.0001$) trends for the formation of the lipid autoxidation compounds identified in this study (Table I). Data from the four replications were pooled because variability due to "replication" and "replication × storage time" effects were not significant ($P > 0.05$). In addition, because the "cooking time" and "cooking time × storage time" effects were not significantly different ($P > 0.05$) for the patties cooked to this narrow range of final internal temperatures (63.6–68.8 °C), these data sets were also combined.

The formation of the lipid autoxidation compounds favored zero-order kinetics for the 4-day storage period (Figure 1; Table I). The intercept and slope values are the initial content and rate of formation of the volatile compounds, respectively. For many of these compounds, the difference between the correlation coefficients for zero-order and first-order kinetics was less than 0.0500. Those compounds with higher correlation coefficients for first-order kinetics are summarized in Table II. The overriding dominance of lipid degradation compounds with tendencies to follow zero-order kinetics indicates that the content of these compounds was increasing at a linear rate throughout the storage period.

Comparison of the slope and intercept values for the lipid autoxidation compounds (Table I; Figure 1) shows a high degree of variability. Hexanal has a slope and intercept which are at least 5 times greater than that of the other volatile compounds. Comparison of the other major lipid autoxidation compounds reveals that the initial content (intercept) of a compound is not necessarily an indication of the rate of formation (slope) of the compound during storage (Table I; Figure 1) and the formation of lipid degradation compounds is not proportional. Ullrich

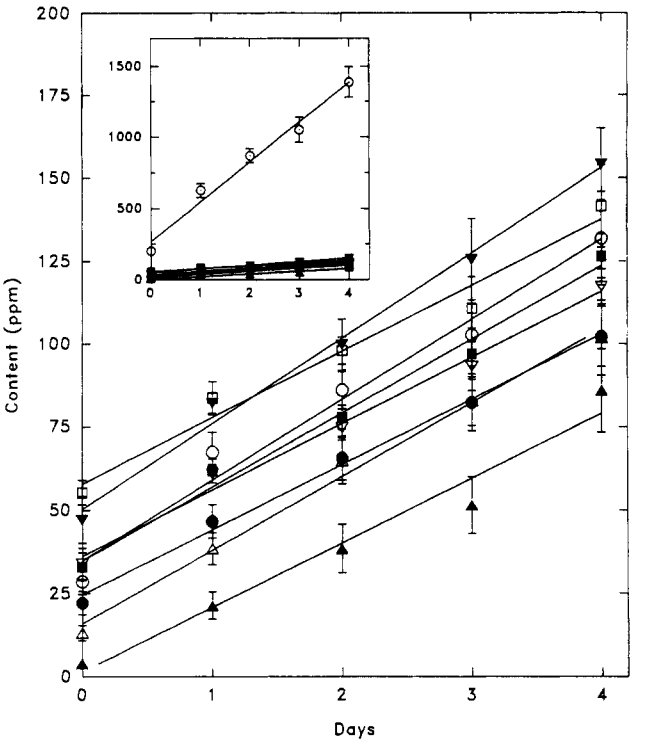

Figure 1. Relationship between the content of the nine most abundant lipid autoxidation products and storage time. Values are means ± standard error for four replications and two cooking times (7 and 8 min/side). (⊙, Hexanal; ○, pentanol; △, 2-pentylfuran; □, pentanal; ▽, octanal; ●, 1-octen-3-ol; ▲, 2,3-octanedione; ■, heptanal; ▼, nonanal.) (Inset) Plot of nine most abundant lipid autoxidation products. The main portion of the figure shows a plot of the inset with hexanal omitted.

Table II. First-Order Regression Analysis of the Effect of Storage Time on the Formation of Lipid Degradation Volatiles in Ground Beef Patties[a]

Kovats index	compd	slope, ln ppm/day	intercept ln ppm	intercept ppm	r^b
705	pentanal	0.212	−0.511	0.600	0.8205
1106	nonanal	0.286	−0.658	0.518	0.8486
1208	decanal	0.210	−3.684	0.025	0.7422
1219	2,4-nonadienal	0.498	−4.736	0.088	0.8805
1322	2,4-decadienal	0.403	−3.456	0.032	0.8757
711	2-ethylfuran	0.478	−3.023	0.048	0.8054
843	furfural	0.120	−2.972	0.051	0.7819
993	2-pentylfuran	0.497	−1.761	0.172	0.8690
862	3-hexen-1-ol	0.379	−3.438	0.032	0.6716
986	2,3-octanedione	0.708	−2.820	0.060	0.8718
1094	2-nonanone	0.314	−3.394	0.034	0.8404
1144	3-nonen-2-one	0.322	−3.831	0.022	0.8877
1051	phenylacetaldehyde	0.098	−2.381	0.092	0.5434

[a] Data for four replications and two cooking times (7 and 8 min/side) have been pooled for the regression analysis. [b] Correlation coefficient, trend of content vs time, is significant ($P < 0.0001$) for all compounds.

and Grosch (1987) made similar observations during the autoxidation of linoleic acid and concluded that the use of a single compound, such as hexanal, does not adequately assess the degree of lipid autoxidation and perceived flavor characteristics. The rate of formation of these compounds is independent of the initial concentration; thus, the total volatile content and distribution of volatiles change continuously during the 4-day storage time.

The compounds formed during lipid autoxidation do not contribute to desirable meaty flavor but, rather, impart green, rancid, fatty, pungent, and other off-flavor characteristics to the beef (Chang and Peterson, 1977). The

Table III. Flavor Thresholds of Classes of Lipid Autoxidation Compounds

class	threshold, ppm	ref[a]
hydrocarbons	90–2150	4
1-alkenes	0.02–9	4
substituted furans	2–27	4
2-phenylfuran	1–10	1
saturated alcohols	0.3–2.5	2
vinyl alcohols	0.05–3	2, 4
1-octen-3-ol	0.001	2
aldehydes	0.014–0.03	2
2-alkenals	0.04–2.5	3, 4
alkanals	0.04–1	3, 4
t,t-2,4-alkadienals	0.005–0.5	3, 4
t,c-2,4-alkadienals	0.002–0.6	4
methyl ketones	0.16–5.5	3
vinyl ketones	0.0002–0.007	2

[a] Compiled from the following: 1, Chang et al. (1966); 2, Forss (1972); 3, Dixon and Hammond (1984); 4, Frankel (1984).

actual flavor characteristics and threshold values of these lipid autoxidation compounds are unique (Forss, 1972; Meijboom and Jongenotter, 1981; Dixon and Hammond, 1984; Shahidi et al., 1986; Ullrich and Grosch, 1987; Gasser and Grosch, 1988). Table III provides a literature-derived overview of the relationship between classes of compounds and their flavor thresholds. Generally, the carbonyl compounds have the greatest impact on flavor due to their low flavor threshold in comparison to the hydrocarbons, substituted furans, and alcohols (Forss, 1972; Dixon and Hammond, 1984). For each of these classes of compounds, flavor threshold decreases with an increase in chain length (Forss, 1972; Meijboom and Jongenotter, 1981; Dixon and Hammond, 1984).

The aldehydes are major contributors to the loss of desirable flavor in meats because of their high rate of formation during lipid autoxidation and low flavor threshold (Ullrich and Grosch, 1987). Hexanal is the major autoxidation product of linoleic acid (Badings, 1970; Ullrich and Grosch, 1987). Several of the aldehydes quantified (Table I and II) already exceeded their flavor threshold at day 0 and, thus, made an immediate negative impact on the flavor quality of the meat. The rate of formation (slope) of the saturated aldehydes was greater than that of the unsaturated aldehydes during refrigerated storage of meat and lipid autoxidation. The unsaturated aldehydes undergo further oxidation to shorter chain aldehydes, while the saturated aldehydes are more stable and accumulate (Grosch, 1982; Frankel, 1984). In model systems, the 2,4-dienals and 2-enals oxidize faster than linoleate or linolenate (Grosch, 1982). Hexanal and 2-octenal are among the products formed during the autoxidation of 2,4-decadienal (Josephson and Lindsay, 1987).

The alcohols contribute measurably less to the undesirable flavor quality of meat than the aldehydes due to the relatively higher flavor thresholds of the alcohols (Table III). Pentanol and 1-octen-3-ol, the major alcohols formed during the storage of the beef patties, are products of linoleate autoxidation (Forss, 1972; Ullrich and Grosch, 1987). These compounds do not exceed their threshold until about day 3 of the storage period. Hexanol, heptanol, and octanol are products of oleate autoxidation (Forss, 1972) and are present in lower concentrations in the beef patties that have been stored for 0–4 days.

2-Heptanone and 2,3-octanedione were among the ketones produced during the refrigerated storage of the cooked beef. 2,3-Octanedione is unique in that the content of this compound is negligible at day 0 but is produced rapidly with storage. Ketones are products of lipid oxidation and have previously been identified in the off-flavors of animal and vegetable fats. However, the mechanism for the formation of these compounds is less clear (Forss, 1972; Shahidi et al., 1986).

The hydrocarbons have high flavor thresholds and make minimal contributions to desirable or undesirable flavors (Min et al., 1979; Frankel, 1985). For this reason, the hydrocarbon compounds that were identified in this study were not quantified.

2-Pentylfuran has been suggested to be an autoxidation product of linoleic acid. Its distinctive beany and grassy flavor characteristics have been associated with flavor reversion in soybean oil (Chang et al., 1966; Forss, 1972; Baines and Mlotkiewicz, 1984; Whitfield et al., 1988).

Benzaldehyde, phenylacetaldehyde, furfural, and 2-ethylfuran are mainly products of Maillard reactions. Furfural is formed through a series of reactions formed from the Amadori product of ribose (Whitfield et al., 1988) and has a pungent, but sweet, caramel-like aroma (Fors, 1983). Benzaldehyde and phenylacetaldehyde are Strecker degradation products of phenylglycine and phenylalanine, respectively (Mottram and Edwards, 1983). Thermal oxidation of linoleic acid is a suggested mechanism for the formation of benzaldehyde (Kawada et al., 1967); however, temperatures in this study were not high enough for this reaction to occur. The thermal degradation of carbohydrates and Maillard reactions is the predominant pathway for the formation of 2-ethylfuran, which is characterized by its burnt, sweet, caramel-like aroma (Shibamoto, 1980; Vernin and Vernin, 1982). Although these compounds are not recognized as products of lipid autoxidation, by use of distillation techniques, significant ($P < 0.05$) increases in the content of these compounds, with storage time, were noted. Free-radical-induced scission and other hydrolytic reactions can occur in food systems (Gardner, 1983; Ladikos and Lougovois, 1990; Spanier et al., 1990) and may contribute to the increase in the content of free amino acids during storage and the subsequent increase in the content of the Maillard reaction products during distillation.

The sulfur-containing compounds are among the important contributors to desirable meat flavor (Chang and Peterson, 1977). Although these compounds are generally present in the low parts per million to the parts per billion range, there is the potential for these compounds to have a high impact on flavor because of their low flavor threshold. The use of the FPD in conjunction with the FID allows sulfur-containing compounds to be quantified without interference from the more abundant carbonyl compounds (Reineccius and Anandaraman, 1984). However, the instability of the sulfur-containing compounds (Golovnja and Rothe, 1980) and the nonlinear response of the FPD (Withycombe et al., 1976) contribute to difficulties associated with the analysis of sulfur-containing compounds and account for some of the variability in the analyses. About 25–30 compounds were detected in the FPD profile. Of these, the identity of 10 compounds, which have been identified previously in meats, was confirmed through mass spectrometry and/or the comparison of retention times of pure standards. The effects of storage time on the content of these compounds will be discussed (Table IV). Interactions between "storage time" and "cooking time" were not significantly different ($P > 0.05$) for the sulfur-containing compounds; thus, the data for the two cooking times were combined to determine the effect of storage time on these compounds.

Four heterocyclic compounds, 4-methylthiazole, 2-acetylthiazole, benzothiazole, and 2-furylmethanethiol, were identified among the sulfur-containing compounds.

Table IV. Effect of Refrigerated Storage Time on the Content of Selected Sulfur-Containing Compounds

Kovats index	compd[a]	content,[b] ppb				
		day 0	day 1	day 2	day 3	day 4
756	1,1-ethanedithiol (t)	67.294[a]	87.033[ab]	78.974[ab]	100.235[b]	133.571[c]
823	4-methylthiazole	8.370[a]	7.638[ab]	5.738[b]	6.614[ab]	6.667[ab]
854	1-(methylthio)ethanethiol (t)	16.546[c]	26.297[c]	27.551[be]	43.971[b]	61.580[a]
911	methional	13.329[ab]	16.123[a]	10.268[b]	13.461[ab]	16.292[ab]
916	2-furylmethanethiol	7.753[bc]	9.128[ab]	6.565[c]	8.215[abc]	10.283[a]
979	dimethyl trisulfide	12.714[a]	5.871[a]	6.442[a]	5.490[a]	5.445[a]
1024	2-acetylthiazole	7.261[ab]	9.490[a]	6.920[b]	7.843[ab]	8.139[ab]
1156	dimethyltrithiolane (t)	93.090[b]	107.585[b]	81.178[b]	168.008[ab]	267.639[a]
1243	benzothiazole	5.443[ab]	5.632[ab]	4.729[b]	6.290[a]	6.502[a]

[a] Mass spectrum and Kovats retention index consistent with an authentic sample except where labeled tentative (t), where no authentic sample was available. [b] Means within rows with the same superscript are not significantly different ($P > 0.05$). Values are means of four replications and two cooking times (7 and 8 min/side).

4-Methylthiazole and 2-acetylthiazole have been described as having nutty, cereal aromas, benzothiazole as having heated rubber aromas, and 2-furylmethanethiol as having pungent, garlic aromas (Vernin and Vernin, 1982; Farmer et al., 1989). These compounds are formed during cooking and are products of Maillard or thiamin degradation reactions. The sulfur amino acids are important amine sources, and the reducing sugars and lipid autoxidation products are important carbonyl sources for the Maillard reaction (Schutte, 1974; Baines and Mlotkiewicz, 1984; Farmer et al., 1989). As analyzed by use of distillation techniques, these heterocyclic compounds appear to be fairly stable to the effects of storage and free-radical degradation reactions. The content of these compounds changed only slightly during the 4-day storage period (Table IV).

Several aliphatic and cyclic sulfur compounds are formed by the polymerization of small sulfur-containing compounds produced from the degradation of sulfur amino acids (Baines and Mlotkiewicz, 1984). The flavor characteristics of 3,5-dimethyl-1,2,4-trithiolane and 1-(methylthio)ethanethiol are unique in that they are concentration dependent. Both compounds have a meaty aroma at dilute concentrations, but when present at higher concentrations, dimethyltrithiolane has a sulfide aroma (Herz and Chang, 1970) and (methylthio)ethanethiol has an oniony aroma (Brinkman et al., 1970). 3,5-Dimethyl-1,2,4-trithiolane, 1-(methylthio)ethanethiol, and 1,1-ethanedithiol are formed from methanethiol, acetaldehyde, and hydrogen sulfide, Strecker degradation products of cysteine, methionine, and alanine (Chang et al., 1968; Brinkman et al., 1972; Schutte and Koenders, 1972; Boelens et al., 1974; Nixon et al., 1979; Bailey, 1983; Baines and Mlotkiewicz, 1984). Hydrogen sulfide is also produced by free-radical reactions (Schutte, 1974). The abundance of free radicals during storage and the susceptibility of the sulfur amino acids to radical damage (Gardner, 1983) would contribute to an increase in hydrogen sulfide content with storage. The significant ($P < 0.05$) increases in the contents of ethanedithiol, (methylthio)ethanethiol, and dimethyltrithiolane during storage may be partially attributed to the elevated hydrogen sulfide content. During the distillation, hydrogen sulfide may react with other constituents of the meat to form these compounds.

Methional is the Strecker degradation product of methionine and has a warm meat- or souplike aroma (Gasser and Grosch, 1988). During the 4-day storage period, the content of this compound did not change significantly. Because methional is not the final degradation product, it is possible that during storage the increase in methional content due to an enhanced availability of methionine or its subsequent oxidation to methional is balanced by degradation to the low-boiling compounds, methanethiol, dimethyl disulfide, dimethyl sulfide, and acrolein (Schutte, 1974; Baines and Mlotkiewicz, 1984).

The content of dimethyl trisulfide, which has a cabbagelike, sulfurous aroma (Gasser and Grosch, 1988), did not change significantly ($P > 0.05$) with storage, although the trend indicates a decrease in content from day 0. Dimethyl disulfide, an oxidation product of methanethiol, can react to form dimethyl trisulfide and dimethyl sulfide. Subsequently, dimethyl trisulfide may be degraded to hydrogen sulfide, carbonyl sulfide, and methanethiol (Schutte, 1974; Baines and Mlotkiewicz, 1984).

Further research is needed to determine the effect of storage time and meat flavor deterioration on the content and composition of the volatile compounds that contribute to desirable and undesirable meat flavors. Generally, the heterocyclic compounds have been identified as contributors to desirable meat flavor (Golovnja and Rothe, 1980; Bailey, 1983; Shahidi et al., 1986). The content of the heterocyclic compounds quantified in this study did not change significantly during the 4-day storage period. On the other hand, the content of the lipid autoxidation products and aliphatic and cyclic sulfur compounds did increase during the storage period. The increase in the content of these two groups of compounds may be attributed to similar mechanisms (i.e., free-radical degradation reactions) and account for the formation of undesirable flavors in meats during storage. Thus, the decrease in desirable flavor notes observed during the refrigerated storage of cooked meat (Johnsen and Civille, 1986; St. Angelo et al., 1987; Love, 1988) may be attributed to the masking of desirable flavor notes by the increased content of undesirable flavor compounds rather than a degradation of desirable flavor compounds.

Effect of Final Internal Temperature. Cooking method and final internal temperature have an important effect on the formation and stability of the volatile compounds in meats (MacLeod and Coppock, 1977; MacLeod and Ames, 1986; Spanier et al., 1988, 1990). The formation of the Maillard reaction products (MRP) is enhanced at the higher cooking temperatures. Several of these MRP, such as reductones, have antioxidative characteristics (Nursten, 1981). These compounds, whether formed during cooking (Huang and Greene, 1978; Spanier et al., 1988) or added back to the meat products (Bailey, 1988, and references cited within), inhibit the rate of lipid autoxidation and deterioration of meat flavor. The rate of lipid autoxidation is also enhanced in meats cooked to a higher internal temperature. The enhanced myoglobin degradation (Spanier et al., 1990) and subsequent release of free iron (Mottram, 1985; Tanchotikul et al., 1989) and the disruption of the muscle membrane to expose the lipids to oxygen and catalysts (Lillard, 1987) augment the rate of lipid autoxidation and deterioration of meat flavor.

Table V. Effect of Cooking Time on the Content of Lipid Degradation Compounds in Cooked Beef Patties[a]

Kovats index	compd	content, ppm, at cooking time, min/side, of 7[b]	8[c]	$P_r > F$[d]
705	pentanal	0.955	1.001	0.6890
809	hexanal	8.259	8.272	0.9930
902	heptanal	0.780	0.805	0.8222
961	2-heptenal	0.201	0.199	0.9542
1004	octanal	0.735	0.786	0.6151
1062	2-octenal	0.400	0.414	0.8456
1106	nonanal	0.975	1.060	0.5527
1164	2-nonenal	0.247	0.262	0.7242
1208	decanal	0.039	0.044	0.3363
1219	2,4-nonadienal	0.031	0.031	0.9935
1322	2,4-decadienal	0.083	0.089	0.7747
711	2-ethylfuran	0.174	0.166	0.8340
843	furfural	0.066	0.067	0.9077
993	2-pentylfuran	0.597	0.605	0.9487
781	pentanol	0.840	0.826	0.9186
862	3-hexen-1-ol	0.093	0.086	0.6397
877	hexanol	0.178	0.179	0.9473
973	heptanol	0.162	0.171	0.7153
983	1-octen-3-ol	0.644	0.632	0.9083
1071	2-octenol	0.215	0.221	0.8834
1073	1-octanol	0.330	0.350	0.6628
893	2-heptanone	0.285	0.287	0.9732
986	2,3-octanedione	0.422	0.383	0.7353
1038	3-octen-2-one	0.090	0.095	0.6613
1094	2-nonanone	0.069	0.073	0.6751
1144	3-nonen-2-one	0.045	0.048	0.7013
966	benzaldehyde	0.117	0.132	0.1149
1051	phenylacetaldehyde	0.106	0.127	0.0115

[a] Values are means of four replications and five storage times (0–4 days). [b] Mean final internal temperature, 64 °C. [c] Mean final internal temperature, 69 °C. [d] Significance probability that contents for the two cooking times are equal.

Table VI. Effect of Cooking Time on the Content of Sulfur-Containing Compounds in Cooked Beef Patties[a]

Kovats index	compd	content, ppb, at cooking time, min/side, of 7[b]	8[c]	$P_r \geq F$[d]
756	1,1-ethanedithiol	84.09	102.75	0.1071
823	4-methylthiazole	6.26	7.75	0.0326
854	1-(methylthio)ethanethiol	29.34	41.03	0.1029
911	methional	10.92	16.87	0.0034
916	2-furylmethanethiol	7.21	9.57	0.0063
979	dimethyl trisulfide	7.81	6.41	0.6288
1024	2-acetylthiazole	7.32	8.54	0.0878
1156	dimethyltrithiolane	111.61	175.39	0.0944
1243	benzothiazole	5.31	6.13	0.3671

[a] Values are means of four replications and five storage times (0–4 days). [b] Mean final internal temperature, 64 °C. [c] Mean final internal temperature, 69 °C. [d] Significance probability that contents for the two cooking times are equal.

Major chemical changes, which include changes in protein and amino acid contents and enzyme activity, begin to occur between 60 and 77 °C (Spanier et al., 1990). As temperature increases up to this range, lipid autoxidation also increases; beyond this range, lipid autoxidation decreases. The decrease in the rate of lipid autoxidation is attributed to the formation of MRP with antioxidative characteristics at the elevated temperatures (Huang and Greene, 1978; Bailey et al., 1987; Lillard, 1987).

The formation of the sulfur-containing compounds, benzaldehyde, and phenylacetaldehyde appears to be more directly influenced by temperature than are the lipid degradation compounds (Tables V and VI) by using this isolation method. Because no statistically significant ($P > 0.05$) interactions between cooking time and storage time were noted, the values presented in these tables are means over the 4-day storage period. With the exception of dimethyl trisulfide, the patties cooked to the higher temperature (69 °C) had a higher content of the sulfur-containing compounds than the patties cooked to the lower temperature (64 °C). The differences in content of sulfur-containing compounds as a function of final internal temperature were significant ($P < 0.05$) for 4-methylthiazole, methional, and 2-furylmethanethiol. Therefore, it is assumed that the content of the reductones and other MRP with antioxidative properties would also be elevated, although these compounds were not quantified. The content of lipid degradation compounds of patties cooked to different temperatures was not significantly ($P > 0.05$) different. The additional MRP formed at higher temperature may not have been sufficient to slow the rate of lipid autoxidation, thereby resulting in no effect on the content of the lipid degradation compounds. On the other hand, the free iron content may have been elevated in the patties cooked to the higher temperature, and, therefore, these two opposing effects nullified each other. The opposing effects of the role of temperature on the stability of reactive components and volatile compounds in cooked beef patties may be better understood by cooking the beef patties to temperatures that encompassed a wider range than included in this study.

MRP and other volatile compounds that make a positive impact on meat flavor are present in extremely low quantities (Chang and Peterson, 1977; St. Angelo et al., 1987). Total volatile analysis techniques, including distillation–extraction techniques, give the increased sensitivity, in comparison to that of "headspace analysis techniques", needed for the isolation of these volatile compounds (Weurman, 1969). However, the distillation method also contributes to the potential formation of new compounds during the isolation, thereby possibly masking the effects of final internal temperature on the formation and stability of volatile compounds. The trends indicated that a majority of the sulfur-containing compounds were present in higher quantities in the patties cooked to the higher final internal temperature. Further research in this area and the use of headspace concentration, supercritical fluid extraction, or other isolation and concentration techniques will better define the effect of final internal temperature on the formation and stability of these compounds.

CONCLUSIONS

Meat flavor deterioration is a dynamic process in which free-radical reactions are involved in the deterioration of meat flavor observed during storage. These reactions affect the stability of the lipids and sulfur-containing compounds and lead to the formation of undesirable flavors. The compounds that are formed as a result of these degradation reactions have unique flavor characteristics and rates of formation and contribute to the overall loss in desirable meaty flavor. The heterocyclic compounds, which contribute to desirable meaty flavor, appear to be less susceptible to free-radical degradation reactions and the effects of meat flavor deterioration.

ACKNOWLEDGMENT

We thank Dr. Bryan Vinyard for his assistance with the statistical analyses and Ms. Myrna Franklin, Cheryl Richard, and Donna Sullen for their help in sample preparation.

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Received for review March 29, 1990. Revised manuscript received July 30, 1990. Accepted August 9, 1990.

Registry No. Pentanal, 110-62-3; hexanal, 66-25-1; heptanal, 111-71-7; 2-heptenal, 2463-63-0; octanal, 124-13-0; 2-octenal, 2363-89-5; nonanal, 124-19-6; 2-nonenal, 2463-53-8; decanal, 112-31-2; 2,4-nonadienal, 6750-03-4; 2,4-decadienal, 2363-88-4; 2-ethylfuran, 3208-16-0; furfural, 98-01-1; 2-pentylfuran, 3777-69-3; pentanol, 71-41-0; 3-hexen-1-ol, 544-12-7; hexanol, 111-27-3; heptanol, 111-70-6; 1-octen-3-ol, 3391-86-4; 2-octenol, 22104-78-5; 1-octanol, 111-87-5; 2-heptanone, 110-43-0; 2,3-octanedione, 585-25-1; 3-octen-2-one, 1669-44-9; 2-nonanone, 821-55-6; 3-nonen-2-one, 925-78-0; benzaldehyde, 100-52-7; phenylacetaldehyde, 122-78-1; 1,1-ethanedithiol, 69382-62-3; 4-methylthiazole, 693-95-8; 1-(methylthio)ethanethiol, 31331-53-0; methional, 3268-49-3; 2-furylmethanethiol, 98-02-2; dimethyl trisulfide, 3658-80-8; 2-acetylthiazole, 24295-03-2; dimethyltrithiolane, 23654-92-4; benzothiazole, 95-16-9.