# ANALYSIS OF RAW AND COOKED BEEF LIPIDS BY CHEMICAL, INSTRUMENTAL AND SENSORY METHODS

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## SUMMARY

Lipids were extracted from raw, and cooked/stored ground beef patties with methylene chloride:methanol (2:1) and separated into neutral and polar fractions with a silicic acid column. These fractions were applied onto Chromarods S-III and analyzed by Iatroscan thin-layer chromatography equipped with a flame-ionization detector. Comparison of cooked beef stored for 0, 4 and 7 days at 4°C indicated that storage caused a decrease in total lipids, an increase in neutral lipids and a decrease in polar lipids. The mean percent fatty acid composition of neutral and polar lipids extracted from raw and freshly cooked beef patties showed that oleic acid was present at the highest concentration in the total lipid and the neutral lipid fractions, followed by palmitic and then stearic acids. In polar lipids, linoleic acid had the highest concentration followed by palmitic and oleic acids. Polar lipids also contain about 7 times the amount of arachidonic acid then either the total lipid or the neutral lipid fractions. Polyunsaturated fatty acids and specific phospholipids found in the polar fraction decreased during storage. These data suggest that polar lipids may be the major source of oxidative compounds that form during the meat flavor deterioration process.

## *Introduction*

Uncured cooked beef stored at 4°C for several days will develop off-flavors with a concurrent loss of desirable flavor. The results of this process is known as warmed-over flavor, or meat flavor deterioration, MFD (Spanier et al. 1989). During MFD, lipids oxidize and the intensities of many compounds increase. Specific compounds, such as hexanal and the 2,4-decadienals, have been used as marker compounds to follow the onset of MFD and to assess food quality (Dupuy et al. 1987; St. Angelo et al. 1987). Polar lipids that contain polyunsaturated fatty acids are more susceptible to oxidation than neutral lipids and thus, are proposed to be the major contributors to the compounds observed during the MFD process. The present investigation was begun to develop a method to identify and quantify lipid degradation in cooked beef during storage with Iatroscan thin layer chromatography interfaced with flame ionization detection. A second goal was to extract neutral and polar lipids from the raw and cooked/stored beef to determine their fatty acid contents.

## *Materials and Methods*

**Phospholipid standards**. Phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl glycerol (PG), lysophosphatidyl ethanolamine (LPE), lysophosphatidyl choline (LPC), phosphatidic acid (PA) and sphingomyelin (Sp), were purchased from Sigma Chemical Co., St. Louis, MO. Fatty acid methyl ester standards were purchased from Matreya, Inc., Pleasant Gap, PA. Silicic acid (200 mesh) was obtained from J. T. Baker, Inc., Phillipsburg, NJ. All other chemicals and organic solvents were of analytical grade.
**Sample preparation**. Top round steaks (*semimembranosus* muscle), choice cut, were purchased from a local supermarket the morning of sample preparation. After removal of visible fat, the lean meat was ground twice (1.0 cm plate followed by 0.8 cm plate) and separated into 85 g patties, which were cooked on a Farberware grill for 7 min/side. The fat content varied from 4 to 5 %. Several patties (standards) were cooked, placed in covered glass petri plates and stored frozen immediately. Other cooked patties were placed in covered petri plates and stored at 4°C up to 7 days to develop MFD. Experimental samples were raw, cooked, 0-day (assayed immediately after cooking) and those stored for 4 and 7 days. Samples were assayed by chemical, instrumental and sensory methods.

**Methodology**. Lipid samples were analyzed by the Iatroscan TH-10 TLC/FID (thin layer chromatography/flame ionization detector) Analyzer (ITFA), interfaced with an Hewlett-Packard 3390 Integrator (St. Angelo and James, 1993). Direct gas chromatography was performed with a Tracor 222 Gas Chromatograph, interfaced with an external closed inlet device (Scientific Instruments Service, River Ridge, LA) (Dupuy et al. 1987). The 2-thiobarbituric acid reactive substances (TBA) were measured at 532 nm with a Hewlett-Packard 8450-A diode array spectrophotometer (Tarladgis et al. 1960). Sensory profiles were by descriptive quantitative analysis (Meilgaard et al. 1987). Evaluations were by a trained panel of 12 using the spectrum intensity scale, 0-15, (Meilgaard et al. 1987).

**Lipid extraction and separation**. A modified Folch procedure was used for extraction of total lipids as described by St. Angelo and James (1993). The modified extraction solvent was methylene chloride/methanol (2:1, v/v). The sample to solvent ratio was 1:10. Total lipids were fractionated by chromatography on a mini-column of silicic acid. Neutral and polar fractions obtained were chromatographically pure as judged by ITFA.

**Chromarod calibration**. Two sets of 10 Chromarods each were spotted with 3.0 ug of PC and calibrated as described previously (St. Angelo and James, 1993). This method involved a two-step solvent system comprised of benzene/chloroform/formic acid (50:20:1.5, v/v/v) in the first phase and chloroform/methanol/ 29.3% ammonium hydroxide (50:50:5) in the second phase. From the 20 rods tested, a matched set of 10 were selected statistically ($P>0.05$). This interrod precision is important to obtain accurate results without an internal standard (Parrish and Ackman, 1983, 1985), and to eliminate the need for response factor calculations for each rod.

Standard curves were run under the two-step system described above. Commercial standards, PG, PE, PC, LPE, LPC and Sp, in amounts from 1-5 µg of each phospholipid were used. Regression analyses were run for the linear function: $y = ax + b$, where y is area count and x is concentration in mg. From this equation, correlation coefficients ($r^2$ values) were calculated and reported as previously described (St. Angelo and James, 1993). The matched set of 10 Chromarods were used to calculate the linearity and precision of the FID response at micro-quantities of phospholipids.

**Analysis of beef lipids**. A two-step solvent system was used to separate lipids extracted from raw and cooked ground beef patties stored for 0, 4 and 7 days at 4°C. Experimental samples were applied at the point of origin on the rods. The rods were air dried and then developed in the first solvent system for 30 min, then air dried for 5 min. The neutral lipids migrated during this development phase; polar lipids remained at the point of origin. The rods were not scanned at this point. After drying, the rods were developed in the second solvent system for 15 min. The polar lipids were separated during this phase of the development. Upon completion of the two-stage development process, and the drying of the rods, the rods were scanned using ITFA. This procedure was repeated thrice/sample.

To quantitate individual phospholipids in the extracts of ground beef patties, standard solutions of phospholipids (PE, PC, PG, LPE, LPC, Sp), ranging from 1 to 5 µg, were analyzed by Iatroscan TLC/FID. The developing method was the same as that used in calibrating the rods. Calibration curves for the standards were linear throughout the concentration applied, although the y axis intercept was slightly below "zero".

*Results and Discussion*

MFD is a highly complex process that involves a loss of desirable flavor notes, such as beefy/meaty, brothy, browned/caramel, and sweet accompanied by an increase in off-flavor notes, such as, painty, cardboardy, sour and bitter. Other markers of MFD are an increase in TBA numbers and an increase in the intensities of hexanal and total volatiles (St. Angelo, 1992). The intensities of these markers for the cooked beef patties are shown in Table 1. The 0-day controls had high intensities for the desirable sensory markers, BM, BRO and BRC, low intensities for the off-flavor markers, PTY, CBD, SUR and BTR, and low intensities for the lipid oxidation chemical markers, TBA, hexanal and total volatiles. These results are characteristic of those found in meat that has not undergone the MFD process and has retained the desirable beefy taste as judged by a trained taste panel using descriptive analysis (St. Angelo, 1992). On the other hand, the opposite was observed in the MFD sample that was stored for 4 days. The desirable sensory notes had lower intensities than those in the 0-day controls, whereas the intensities of the off-flavor notes and the chemical markers were higher than those of the control samples. The 7-day samples were not tasted by the sensory panel, but the chemical indicators were determined. Results indicated that in the 7-day samples, lipid oxidation, and thus MFD, was greater than in the samples stored for 4 days.

Typical ITFA chromatograms of total lipid extracted from cooked ground beef stored at 4°C for 0, 4 and 7 days are shown in Figure 1. Changing profiles in the chromatograms demonstrate the complexity of the lipids and the changes they undergo under refrigerated storage conditions. All but two of the major peaks were

identified. The first was near the origin (the area where the sample was applied to the rod) at a scanning retention time of 0.46 min, and the second appeared at a retention time of 21 min. ITFA chromatograms (not included) of neutral and polar lipids extracted from cooked ground beef stored at 4°C for 0, 4 and 7 days and separated on silicic acid columns were highly comparable to those obtained from the chromatogram of the total lipid profile, shown in Figure 1.

As observed in Figure 1, the changes that occurred during storage appeared ever so slight. However, by compilation of the differences in the peak areas from replicates, the slight changes were more noticeable. For example, total lipid extracted from the fresh cooked ground beef pattie was 4.28%; the total neutral lipid content was 3.19% whereas the polar lipid was 0.99%. As MFD increased with storage of 4 and 7 days, total lipids decreased, 4.18% and 4.08%, respectively. The neutral lipids increased to 3.23% to 3.36% respectively, but the polar lipids decreased to 0.79% and 0.73%, respectively. These values are in agreement with those previously reported (Hornstein et al. 1961; Dugan, 1987). Additionally, the increase in neutral lipids has been previously reported by Willemot et al. (1985) on refrigerated storage of pork. Likewise, the decrease in phospholipids during refrigerated storage of cooked pork was reported by Willemot et al. (1985), and of cooked beef by Igene and Pearson (1979). Both groups reported that the phospholipids were the main source of lipid oxidation during the MFD process. Furthermore, as lipid oxidation increased, Keller and Kinsella (1973) reported that phospholipid content decreased.

The concentration of individual phospholipids extracted from the polar fraction of raw and cooked/stored patties is shown in Table 2. In raw beef patties, PC was the phospholipid with the highest concentration (36.7%) followed by LPE (18.8%) and PE (16.6%). Upon cooking and storage, 0 and 4 days, the mean percent of all phospholipids decreased except PG.

Since lipid changes occur during storage, fatty acid profiles were examined to determine which fatty acids were affected. Neutral fatty acid methyl esters of raw ground beef patties were found to contain approximately 38% oleic acid and 27.5% palmitic acid, the fatty acids present in the highest concentrations. With cooking and storage for 4-days, these values fell to 31% for oleic acid and to 24.5% for palmitic acid. Other changes that were observed during storage were stearic acid from 11.4% (raw) to 10.5% (cooked/stored 4 days), palmitoleic acid from 4.5% to 3.9%, myristic acid from 3.4% to 2.3%, and heptadecanoic acid from 1.5% to 1.1%. In the polar fatty acid methyl ester fractions, linoleic acid was present at a concentration of 22.4% followed by palmitic (19.8%), oleic (16.1%), stearic (10.8%) and arachidonic (7.2%). The cooked/stored values for these fatty acids were linoleic (21.8%), palmitic (21.7%), oleic (15.4%), stearic (10.7%) and arachidonic (5.0%). The fatty acid methyl ester data were further analyzed by measuring their degree of saturation. As expected, the fresh cooked neutral lipid fraction contained 44% saturated fatty acids (SAT), 42% monounsaturated (MUFA) but only 4% polyunsaturated (PUFA). The fresh cooked polar fraction contained 46% SAT, 18% MUFA and 34% PUFA. With cooking and storage, the neutral fraction dropped to 41% MUFA; SAT and PUFA fractions showed little change. However, in the polar fraction, PUFA content decreased to 32%.

*Conclusion*

These results showed that upon cooking and storage at 4°C, MFD developed, desirable flavor notes decreased, off-flavor notes increased and lipid oxidation increased. The neutral lipid fraction contained very little PUFA (4.6% at 0-day and 3.9% at 4-day storage) when compared to the polar lipid fractions (34% and 32%, respectively). With storage, the polar PUFA decreased by 2%. Specific phospholipids also decreased with storage. These differences may account for the origin of the secondary reaction products observed during the MFD process.

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