Structures of Triacylglycerols in Bovine Bone Marrow

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Triacylglycerols of bone marrow from cervical and lumbar bones of grass-fed and feedlot steers and cows were isolated and subjected to structure analyses. Results obtained were similar with respect to bone, diet, and sex. Two classes of triacylglycerols containing either monounsaturation in the *sn*-2 position or diunsaturation comprised over 97% of the triacylglycerols studied. A major portion of marrow triacylglycerols consisted of 16:0–18:1–18:0, 16:0–18:1–18:1, 18:0–18:1–18:0, and 18:0–18:1–18:1.

Structures of triacylglycerols have been implicated in atherogenic and cholesterolemic effects of certain dietary lipids. The atherogenic potential of peanut oils is related to their triacylglycerol structures (Kritschevsky et al., 1971, 1973). Interesterified soybean oil–butterfat mixtures decreased serum cholesterol levels in humans (Mukherjee and Sengupta, 1981). Bovine bone marrow is a part of the American diet via soups, sauces, and mechanically separated meat, and there is a possibility that an increase in this food may occur, particularly with respect to the latter source. A Select Panel of the United States Department of Agriculture concerning mechanically separated meat noted a need for additional information concerning bovine marrow lipids (Kolbye and Nelson, 1977). The lipids in bovine bones (Mello et al., 1976) and marrows (Miller et al., 1982) have been characterized. This study reports the triacylglycerol structures in marrows of cervical and lumbar bones from grass and feedlot steers and cows. These two bones are typical bones used for production of mechanically separated beef.

EXPERIMENTAL PROCEDURES

Marrows from cervical and lumbar bones of five range and five feedlot steers and cows were separated and the lipid was extracted (Miller et al., 1982). Prior to this study the purified lipid solutions (hexane) had been stored for about 18 months at −25 °C, and it was necessary to determine whether hydrolysis had occurred during storage. The extent of hydrolysis was determined by analyzing free fatty acid levels in the lipid (Bergmann et al., 1980). Results from 40 marrow lipid samples were 3.2 ± 1.9 mmol of free fatty acids/100 g of lipid with octadecenoic acid as the standard. This value compares favorably with that obtained with lipid from freshly ground meat (2.3 ± 1.4 mmol/100 g, $n = 10$) and indicated little, if any, hydrolysis during storage. Prior to triacylglycerol analysis, aliquots of the marrow lipid samples were composited according to diet, sex, and bone by equal lipid weights.

The triacylglycerols in the composited samples were separated by thin-layer chromatography (TLC: silica gel G, 0.5-mm layer on glass, hexane–ether–glacial acetic acid at 80:20:1). Bands of triacylglycerols were located by spraying (0.01% methanolic 2′,7′-dichlorofluorescein) and ultraviolet visualization. The bands were scraped from the plate and eluted with 95:5 ethanol–ether, and the solvent was evaporated by rotary evaporation. Hexane was used to extract the triacylglycerols, and the hexane solution was passed through a 0.5 μm pore size solvent-inert membrane filter.

The isolated triacylglycerols were then subjected to argentation TLC. Glass plates with 0.5-mm layers of silica gel were prewashed (5 h) with 95% aqueous methanol, air- and oven-dried (110 °C), washed (5 h) with saturated methanolic silver chloride, air-dried, and stored in dark. Development was with chloroform–benzene–absolute ethanol (70:30:0.5). Six bands were consistently obtained with the samples by this procedure. Five triacylglycerol standards (Supelco, Inc., Bellefonte, PA): 1,2-di-16:0-3-18:0 (000); 1-16:0-2-18:1-3-18:0 (010); 1-16:0-2,3-di-18:1 (011); tri-18:1 (111), and tri-18:2 (222) were used for comparison. Bands were visualized, scraped, extracted, and purified as previously described. Prior to extraction, appropriate levels of tri-14:0 were added as internal standards for gas chromatographic analysis. Analysis of several total triacylglycerol samples indicated that little, if any, of this triacylglycerol was present.

Carbon number profiles (CNP) of the six triacylglycerol classes were obtained by gas chromatography. For this analysis, 0.5 m × 2 mm glass columns of 3% JXR on 100–200-mesh Gas-Chrom Q (Applied Sciences Laboratories, Inc., State College, PA) were programmed from 270 to 310 °C at 4 °C/min with injectors and detectors (flame ioniztion) at 310 °C. Nitrogen flow was about 45 mL/min. A standard mixture of triacylglycerols (Supelco, Inc., Bellefonte, PA) was prepared that contained CNP of 42 C, 44 C, 46 C, 48 C, 50 C, 52 C, and 54 C and was used to obtain detector response factors. After the quantity of each CNP had been calculated for each of the six classes within total triacylglycerols, the values were summed and the percentage distribution of each of the six classes was obtained.

Fatty acid methyl esters (FAME) were prepared by the procedure of MacGee and Allen (1977) using hexane to extract the FAME since concentration of many of the samples was necessary prior to gas chromatographic analysis. For this analysis, 1.8 m × 2 mm glass columns containing 10% SP2330 on 100–120-mesh Chromosorb (Supelco, Inc., Bellefonte, PA) were maintained at 175 °C with the injector at 200 °C and the detector at 245 °C. The nitrogen flow was about 20 mL/min. A standard FAME solution was prepared by using 16:0, 18:0, 18:1, 18:2, 18:3, and 20:1 (Supelco, Inc., Bellefonte, PA) and used to standardize calculations of fatty acid percentages.

RESULTS AND DISCUSSION

Five of the six bands obtained by argentation thin-layer chromatography corresponded to standards 000, 010, 011, 111, and 222. The sixth band occurring between 000 and 010 was later shown to contain most of the fatty acid 20:1 present in total triacylglycerols. Earlier results had shown that very little of this acid was present in the *sn*-1 position (Miller et al., 1982) and the unknown band was designated 001. The band corresponding to 222 was poorly resolved from the origin and was difficult to scrape from the plates.

Values obtained for the percentage distribution of the six classes of triacylglycerols from each cervical and lumbar

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Table I. Percentage Distribution of Six Classes of Triacylglycerols within Total Triacylglycerols of Cervical and Lumber Bone Marrow from Grass and Feedlot Steers and Cows[a]

triacylglycerol class	% distribution[b]
000	0.4 ± 0.3
001	0.3 ± 0.1
010	48.4 ± 7.2
011	48.8 ± 7.0
111	1.7 ± 0.7
222	0.4 ± 0.1

[a] Values represent averages and standard deviations obtained from four cervical and four lumbar bone marrow samples. [b] Triacylglycerol class refers to triacylglycerols separated by argentation thin-layer chromatography of total triacylglyerols. 000 = fully saturated, 001 = one double bond present, etc.

marrow sample were similar with respect to bone, diet, and sex. Therfore, the values were composited and appear in Table I. The two classes that contained either monounsaturation in the *sn*-2 position or diunsaturation comprised over 97% of the triacylglycerols studied. Almost 99% of the total Triacylglycerols could be accounted for by adding the class containing triunsaturation to these two classes.

The CNP obtained from the six classes were also similar with respect to bone, diet, and sex and were composited as before. These values appear in Table II. The smallest triacylglycerols occurred mainly in the 000 and 001 classes, but these two classes represented less than 1% of the total triacylglycerols. The two major classes, 010 and 011, contained mainly (95% or more) triacylglycerols of 50 C, 52 C, and 54 C.

Percentages of the major long chain fatty acids within each class are shown in Table III. As would be expected, saturated fatty acids predominated in the 000, 001, and 010 classes, whereas most of the unsaturated fatty acids were found in the other classes. This table allows an estimation of the efficiency of the methodology employed. The presence of 7.9% unsaturated fatty acids in the 000 class would suggest an accuracy of 92.1%. In the 010 class, monounsaturated fatty acids should comprise about 33% of the total instead of 38.1, and in the 011 class, both mono- and diunsaturated fatty acids should comprise about 67% instead of 67.8%. The most unsaturated class, 222, should contain little or no saturated fatty acids rather than the 27.5% observed. The band in the TLC plates corresponding to the 222 class represented only 0.4% of total triacylglycerols (Table I) and probably contained triacylglycerols with more than three double bonds.

About 97% of total marrow triacylglycerols consisted of 010 and 011 classes (Table I), and about 70–90% of these two classes contained triacylglycerols of 52 and 54 CNP (Table II). Previous data (Miller et al., 1982) showed that the fatty acids 16:0, 18:0, and 18:1 were present at about 25, 21, and 41% of the total fatty acids, respectively, in these marrow triacylglycerols. Furthermore, 16:0 predominated in the *sn*-1 position, 18:0 in the *sn*-1 and -3 positions, and 18:1 in the *sn*-2 and -3 positions. Therefore, a major portion of these bovine bone marrow triacylglycerols consisted of 16:0–18:1–18:0, 16:0–18:1–18:1, 18:0–18:1–18:0, and 18:0–18:1–18:1. In an extensive study of peanut oil triacylglycerol structures, Manganaro et al. (1981) found appreciable levels of 16:0–18:1–18:1 and 18:0–18:1–18:1 in the oils studied. However, the peanut oils with the highest levels of these two triacylglycerols exhibited the lowest atherogenic potential. It has been suggested that 14:0, one of the predominant saturated fatty acids in butterfat, is a factor in the cholesterolemic acition of buterfat, particularly if 14:0 is present in the *sn*-2 position of trisaturated triacylglycerols (Mukherjee and Sengupta, 1981). Trisaturated triacyglycerols comprise less than 1% of marrow triacylglycerols (Table I), and the amount of triacylglycerols with CNP of 44–48 C present in the trisaturated class is low (Table II). Consequently, the major classes of triacylglycerols present in bovine bone marrow are classes that have not been related to atherogenicity when present in peanut oil, and little, if any, of the classes in butterfat suggested as being cholesterolemic are present in bovine bone marrow.

Table II. Percentage Distribution of Carbon Number Profiles in Six Triacylglycerol Classes of Total Triacylglycerols of Bone Marrow from Grass and Feedlot Steers and Cows[a]

carbon no. profile	triacylglycerol class[b]					
	000	001	010	011	111	222
44 C	0.5 ± 0.2	0.7 ± 0.3				
46 C	3.6 ± 1.2	2.4 ± 0.9	0.6 ± 0.1			
48 C	16.9 ± 2.6	8.3 ± 2.2	4.4 ± 1.1	1.5 ± 0.4	1.7 ± 0.5	2.3 ± 0.9
50 C	38.7 ± 3.1	30.7 ± 1.6	26.0 ± 6.5	8.8 ± 1.6	7.5 ± 1.8	11.1 ± 2.9
52 C	32.2 ± 2.1	42.7 ± 5.7	48.3 ± 4.6	62.4 ± 9.2	51.9 ± 5.6	47.8 ± 3.8
54 C	8.1 ± 3.1	15.2 ± 4.6	20.7 ± 7.2	27.3 ± 3.3	38.9 ± 8.6	38.8 ± 4.0

[a] Values represent averages and standard deviation of four cervical and four lumbar bone marrow samples. [b] Triacylglycerol class refers to triacylglycerols separated by argentation thin-layer chromatography of total triacylglycerols. 000 = fully saturated, 001 = one double bond present, etc.

Table III. Fatty Acid Percentages in Six Triacylglycerol Classes of Total Triacylglycerols in Bone Marrow of Range and Feedlot Steers and Cows[a]

fatty acid	triacylglycerol class[b]					
	000	001	010	011	111	222
16:0	43.0 ± 1.7	35.5 ± 1.5	30.4 ± 2.8	19.7 ± 1.2	12.4 ± 1.0	17.3 ± 2.4
18:0	49.1 ± 6.1	38.9 ± 8.1	31.5 ± 1.9	12.1 ± 2.3	7.7 ± 2.4	10.2 ± 1.7
16:1	0.7 ± 0.6	0.6 ± 0.8	0.9 ± 0.2	1.6 ± 0.4	2.5 ± 0.4	1.9 ± 0.4
18:1	6.4 ± 3.9	21.9 ± 6.1	36.6 ± 1.0	62.4 ± 3.1	58.7 ± 8.4	44.9 ± 5.1
20:1		1.8 ± 0.6	0.6 ± 0.2	0.2 ± 0.2	0.5 ± 0.9	
18:2	0.8 ± 0.2	1.3 ± 0.2	0.2 ± 0.2	3.4 ± 1.2	17.4 ± 5.6	14.9 ± 3.2
18:3				0.6 ± 0.5	0.8 ± 0.1	10.8 ± 2.2

[a] Values represent averages and standard deviations of four cervical and four lumbar bone marrow samples. [b] Triacylglycerol class refers to triacylglycerols separated by argentation thin-layer chromatography of total triacylglycerols: 000 = fully saturated, 001 = one double bond, etc.

Registry No. 16:0-18:1-18:0, 84412-85-1; 16:0–18:1–18:1, 14960-35-1; 18:0–18:1–18:0, 2846-04-0; 18:0–18:1–18:1, 79517-07-0.

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Determination of Sodium Chloride in Meat by Near-Infrared Diffuse Reflectance Spectroscopy

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Near-infrared (NIR) spectroscopy was used to measure the amount of salt (NaCl) in canned cured hams. Calibration with 20 samples produced a high correlation ($r = 0.96$) between salt contents determined by chemical analysis and by NIR using the second derivative of log (1/reflectance) values at 1806 nm. Salt contents of nineteen unknown samples were predicted with a standard error of prediction of 0.17% NaCl. Salted beef, salted fresh ham (uncured), and salted water model systems demonstrated that the ability to measure salt by NIR is due to the shift in the water spectrum caused by salt-induced changes in the amount of hydrogen bonding.

Near-infrared (NIR) reflectance spectroscopy is a growing technique for the rapid compositional analysis of foods. The method was developed to replace slow, laborious, conventional methods such as the Kjeldahl method for protein analysis. Ben-Gera and Norris (1968) used transmission spectroscopy in the NIR range to determine the fat and moisture contents of meat products. More recently, Kruggel et al. (1981), Martens et al. (1981), and Lanza (1983) used NIR reflectance spectroscopy to determine the moisture, fat, and protein contents of fresh meats.

We now describe the use of NIR reflectance to measure the amount of salt (NaCl) added to meat products. We used canned cured ham to develop a calibration of NIR data to chemically determine salt values, and we prepared salted beef, salted fresh ham, and salted water samples to study the spectrochemical basis of the method.

The NIR reflectance analysis of salt in meat is based on the change in the water component of the meat spectrum. Bernal and Fowler (1933) showed that the addition of electrolytes change the spectrum of water in the infrared overtone region. Luck (1974) showed that temperature variations caused similar spectral changes and linked these changes to variations in the amount of hydrogen bonding. We show here that the best calibration of NIR data to salt content occurs at a point in the meat spectrum where the salt-induced changes in the water spectrum can be mathematically isolated from other spectral variations.

Because the NIR method can measure several constituents quickly, cheaply, accurately, and simulanteously, it can become a valuable tool for the quality control of processed meats.

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Table I. Salt and Fat Contents of Sample Sets

	no. of samples	% NaCl (w/w)	% fat (w/w)
fresh ham	20	0–4.8	7.4, 20.9, 24.9
beef	26	0–10.7	16.9, 17.4, 28.8
cured ham	20	2.3–3.5	1.4–6.5
cured ham	19	2.3–3.5	1.4–6.5
water	13	0–5	

EXPERIMENTAL SECTION

Samples. Duplicate finely ground canned cured ham samples were supplied and analyzed by the USDA Food Safety and Inspection Service (FSIS) using the AOAC procedure for salt determination in meats (AOAC, 1980, 24.011). We prepared model salted meat samples of fresh ham (uncured) and ground beef by first grinding samples to a paste in a Robot-Coupe food processor and then adding known percentages (w/w) of dry NaCl gravimetrically. The amount of fat in the beef samples was varied by choosing different types of ground beef. The amount of fat in the fresh ham samples was varied by carving portions of a fresh ham to include more or less fat prior to grinding. The fat levels were varied to ensure that the addition of salt could be measured independently of varying levels of protein, water, and fat. Aqueous salt solutions were prepared (w/w) with certified ACS grade NaCl and distilled deionized water. The salt and fat contents of the samples are summarized in Table I.

Instrumentation. Diffuse reflectance (R) was measured on all meat samples with a Neotec Model 6350 research composition analyzer. This instrument is a computer-based system with a single-beam scanning monochromator and a lead sulfide detector. The monochro-

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