Possible Role of Adipose Tissue in Meat Flavor—The Nondialyzable Aqueous Extract

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Beef adipose tissue was either mixed with 3% NaCl or untreated, packaged in a nitrogen or air atmosphere, and stored at −23° C. Samples were extracted with borate buffer and dialyzed. The nondialyzable fraction was analyzed by chemical analysis and disc gel electrophoresis, followed by specific staining techniques. Nucleoproteins, glycoproteins, and lipoproteins were not detected, even though nucleic acids and other proteins were present. Neither nitrogen nor air appreciably influenced the protein or sulfhydryl groups. Salt treatments resulted in different protein distributional ratios and markedly increased sulfhydryl groups of samples stored under either air or nitrogen. Amino acid analysis showed that glutamic and aspartic acids, lysine, leucine, alanine, valine, threonine, serine, glycine, and proline each comprised over 5% of the total amino acid residues of the nondialyzable fraction. The possible role of components in the nondialyzable fraction to meat flavor is discussed.

Wasserman and Talley (1968) reported that roast beef containing 10% of beef adipose tissue was correctly identified 90.2% of the time as compared to only 42.5% for beef devoid of fat. This finding is in substantial agreement with earlier studies, indicating that meat flavor *per se* is in the water-soluble fraction of muscle (Zaika *et al.*, 1968; Wasserman and Gray, 1965; Macy *et al.*, 1964; Hornstein and Crowe, 1964; Kramlich and Pearson, 1958), whereas, the fatty tissues appear to be responsible for differences in the flavor of meat from various species (Wasserman and Talley, 1968; Hornstein and Crowe, 1960). The role of adipose tissue to meat flavor has received further support from Pepper and Pearson (1969), who have recently shown the water-soluble fraction of beef adipose tissue produced an appreciable quantity of hydrogen sulfide upon heating. Hydrogen sulfide had previously been identified in the volatiles from cooked beef (Hamm and Hoffmann, 1965; Kramlich and Pearson, 1958), pork (Hornstein and Crowe, 1960), lamb (Hornstein and Crowe, 1963; Jacobson and Koehler, 1963), and chicken (Klose *et al.*, 1966; Minor *et al.*, 1965).

The present study was undertaken to ascertain the nature of some of the constituents in the nondialyzable fraction from beef adipose tissue and their possible role in development of meat flavor. Since sausage products often contain high levels of fat and approximately 3% of added NaCl, ground beef adipose tissue with and without added salt was stored under air or nitrogen for 2 months at −23° C and then used to prepare the nondialyzable fraction. Thus, it was possible to determine the effects of storage under air or nitrogen with and without added NaCl.

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MATERIALS AND METHODS

Preparation of Samples. The trimmable subcutaneous and intermuscular fatty tissues obtained from three U.S. Choice grade steer carcasses were coarsely ground twice at 4° C with thorough mixing. The ground tissue was mixed for 1 min in an evacuated Hobart vertical cutter-mixer, and the mixer was brought to atmospheric pressure with nitrogen. The tissue was divided into four aliquots. Two aliquots were mixed with 3% solid, reagent grade sodium chloride. The four aliquots were placed in Cry-o-vac bags and one aliquot of both the salt-treated and untreated tissues was purged several times with nitrogen, evacuated, and sealed. The remaining salt-treated and untreated aliquots were similarly treated with air. All samples were stored at −23° C for 2 months.

All water used in this study was distilled, deionized, and saturated with toluene. All samples were inspected by oil immersion microscopy immediately before analyses to check for microbial contamination. All samples containing more than one or two organisms in a single microscopic field were discarded.

Fractionation and Concentration. Fractionation procedures were as outlined in Figure 1. Aliquots of adipose tissue (100 g) were successively homogenized with 125-, 75-, and 50-ml portions of borate buffer (pH 7.8; $\mu = 0.1$) containing 2 m*M* EDTA (ethylenediaminetetraacetic acid) for 1 min in an ice water bath with a Virtis 45 homogenizer. After each homogenization, the aqueous extract was collected. Upon the completion of homogenization, all solutions and the residual fatty tissues were combined and centrifuged at 27,000 × *g* for 20 min at 0° C. The aqueous fraction was collected and filtered through a milk filter and the filtrate was recentrifuged as previously described.

The aqueous fraction was vacuum filtered through What-

man No. 2 filter paper. Turbidity in the filtrate was removed by vacuum filtration through a 1.5-cm thick filter paper pad composed of a water slurry of pulverized Whatman No. 40 filter paper trimmings. The pad was sandwiched between two discs of Whatman No. 2 filter paper. The filtrate was collected in an ice bath and one drop of toluene was added to each 150-ml portion of filtrate to prevent microbial growth.

The filtrate was concentrated to 50–75 ml by pervaporation at 4° C and then dialyzed against 1-l. portions of water until 4 l. of diffusate had been collected. The dialysis tubing (1.53 and 2.10 cm diameter regenerated cellulose tubing) was purchased from E. H. Sargent and Co. and prior to using was boiled for 20 min in 2 m*M* EDTA and then thoroughly rinsed with water. The diffusate was collected and held for subsequent analyses.

The nondialyzable material was transferred to 1.53-cm diameter dialysis tubing and concentrated by dialysis for 24 hr at 4° C against 1 l. of 1 *M* decolorized sucrose which contained borate buffer and EDTA as described above. After concentration, the extract was centrifuged at 23,000 × *g* for 15 min at 4° C. The supernatant solution was placed in 6-oz Whirl-Pak bags, frozen, and stored at −23° C until utilized. The precipitate was discarded.

Chemical Analyses. Ether extract and moisture were determined according to Benne *et al.* (1956). Total nitrogen analyses were performed by a micro-Kjeldahl method (American Instrument Co., 1961). Sulfhydryl determinations were carried out as described by Pepper and Pearson (1969), except that 1 ml of 8.5 *M* urea was added to 2 ml of the protein–sucrose concentrate. Amino acid analyses were carried out on samples obtained by dialyzing 10-ml aliquots of the protein–sucrose concentrates in 2.1-cm diameter dialysis tubing (which was tied to allow a minimum of sample space) against water for 12 hr. The analyses were conducted according to Moore *et al.* (1958), except that the proteins were hydrolyzed in 6 *N* HCl at 110° C for 22 hr. Qualitative and quantitative amino acid analyses were accomplished using a Beckman (Model 120C) amino acid analyzer.

Disk Gel Electrophoresis. Separation of the nondialyzable components was carried out by disk gel electrophoresis. A modification of the method of Davis (1964) was used. A 5.0% spacer gel (0.75 cm) and a 7.5% running gel (5.0 cm) were made according to Jolley *et al.* (1967) using Cyanogum in place of acrylamide and bisacrylamide. A 0.025-ml aliquot of sucrose–protein concentrate was carefully layered on the surface of the space gel and a current of 2 mA per tube was applied to the system.

Specific Staining Techniques. The gels were stained for proteins, hemoproteins, sulfhydryl groups, glycoproteins, nucleoproteins, ribonucleic acids, lipoproteins, and esterase activity. Proteins were stained by immersing the gels in an Amido Black 10B solution for 20 min. The solution was composed of 250 ml of methanol, 50 ml of glacial acetic acid, and 2.0 g of Amido Black 10B. The gels were destained and stored in 7% acetic acid. Hemoproteins were detected according to the benzidine method of Smithies (1959). Sulfhydryl groups were determined by the method of Zwaan (1966), as modified by Pitt-Rivers and Schwartz (1967). However, Fast Violet B was found to be the most suitable coupling agent.

Glycoproteins were stained by Alcian blue (Gifford and Yuknis, 1965) and the acridine orange method of Richards *et al.* (1965) was used to detect nucleoproteins. Lipoproteins were determined by the Sudan Black B method of Gifford and Yuknis (1965) and the Oil Red O method of Beaton *et al.* (1961). Ribonucleic acid was detected according to Peacock and Dingman (1967). Esterase activity was ascertained according to Allen *et al.* (1965) using α-naphthyl butyrate.

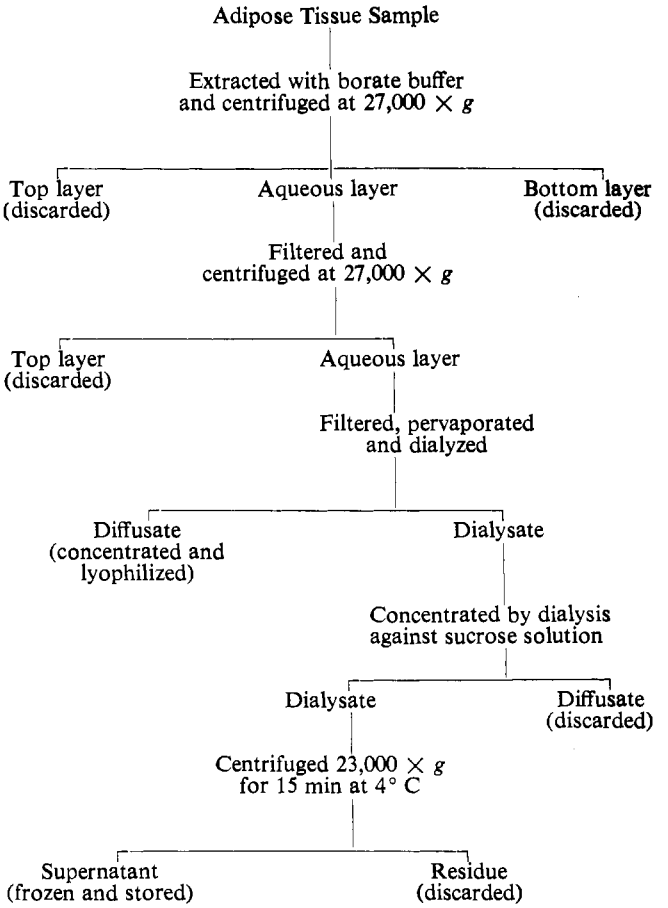

Figure 1. Fractionation procedure utilized for flavor precursor analysis of adipose tissue

Preparation of Beef Muscle Control. A sample of beef muscle was prepared in order to compare the water-soluble extracts from adipose tissue with those from muscle. The procedures utilized for the beef muscle control sample were as nearly like that of adipose tissue as possible, but were altered slightly to solve problems encountered during separation and disk gel electrophoresis. Beef muscle (10.0 g) was trimmed of all fat and combined with 0.3 *M* sucrose solution of borate buffer (pH 7.8) (20 ml) as previously described. The mixture was blended at 4° C and used within 2 days to develop disk gel electrophoretic patterns.

RESULTS AND DISCUSSION

Chemical Analysis. The moisture, fat, and protein content of the ground raw beef adipose tissue averaged 10.35, 86.07, and 3.27%, respectively. The same starting material was utilized for preparing all nondialyzable fractions in this study. Duplicate samples from the original adipose tissue sample always appeared to behave the same. Other samples would vary depending upon the source of raw material, but it is believed the results obtained herein are representative of the general classification of adipose tissue, and that variation in original composition would largely result in quantitative rather than qualitative differences.

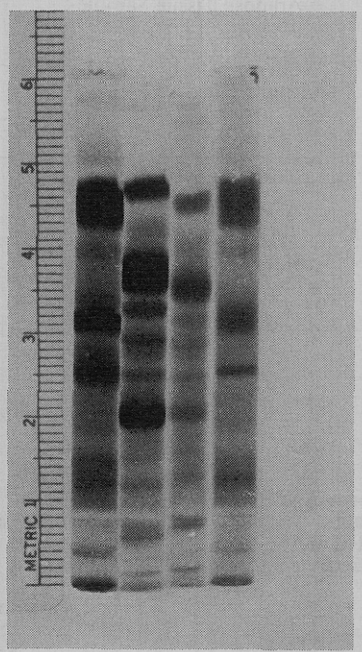

Figure 2. Comparison between protein and sulfhydryl distribution in muscle and adipose tissue extracts. Left to right: (1) adipose tissue protein; (2) muscle protein; (3) muscle SH-groups; and (4) adipose tissue SH-groups

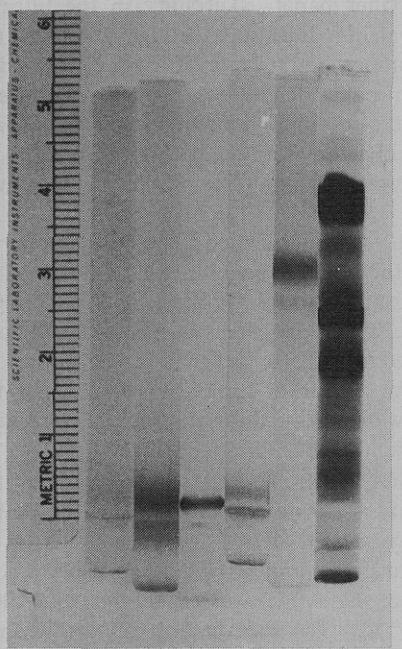

Figure 3. Staining for specific groups on disc gel from nondialyzable fraction of adipose tissue (7.5% running gels). The adipose tissue had previously been stored in air without added salt. Left to right: (1) ribonucleic acid stain; (2) nucleoprotein stain; (3) esterase stain; (4) glycoprotein stain; (5) heme proteins in muscle extract; and (6) protein stain for adipose tissue

Table I. The Amino Acid Composition of Nondialyzable Aqueous Fractions of the Various Treatments

	Treatments, % amino acids			
	N_2[a]	Air[b]	Air–S[c]	N_2–S[d]
Lysine	9.10	9.00	9.24	9.52
Histidine	2.80	2.67	2.84	3.41
Arginine	3.85	3.77	4.06	4.10
Aspartic acid	10.43	10.48	10.56	10.96
Threonine	5.99	5.92	6.11	1.80
Serine	6.24	5.75	5.86	0.43
Glutamic acid	12.54	12.79	12.72	16.55
Proline	5.12	5.07	5.11	6.27
Glycine	5.64	5.43	5.80	6.32
Alanine	8.44	8.06	8.04	8.80
Half-cystine[e]	2.90	3.23	2.80	2.28
Valine	6.83	6.93	6.88	7.83
Methionine[e]	0.25	1.07	0.66	0.90
Isoleucine	3.27	3.29	3.34	3.36
Leucine	9.82	9.87	9.58	10.70
Tyrosine	2.55	2.53	2.42	2.21
Phenylalanine	4.24	4.12	3.99	4.54

[a] Adipose tissue stored under nitrogen. [b] Adipose tissue stored under air. [c] Adipose tissue with 3% NaCl stored under air. [d] Adipose tissue with 3% NaCl stored under nitrogen. [e] The values for half cystine and methionine are probably low since acid hydrolysis causes some destruction of S-containing amino acids.

Table II. Chemical Analyses of Water-Soluble Nondialyzable Adipose Tissue Components

Treatments[a]	Protein, mg/ml	Sulfhydryl mμmol/g protein
N_2	9.81	9.05
Air	9.30	8.17
N_2S	12.16	16.00
Air–S	11.61	15.79

[a] Treatment codes are as follows: N_2 = Sample stored under nitrogen without added NaCl; Air = Sample stored under air without added NaCl; N_2S = Sample stored under nitrogen with 3% added NaCl; and Air–S = Sample stored under air with 3% added NaCl.

Table I presents the amino acid composition of the nondialyzable material from aqueous extraction of beef adipose tissue. Glutamic acid accounted for over 12.5% of the total amino acid residues in all samples, but was approximately 16.5% in the sample with added NaCl followed by storage under nitrogen. Aspartic acid composed approximately 10.5% of the amino acid residues in the nondialyzable fraction and was followed by leucine with over 9.5%, alanine with over 8.0%, threonine with approximately 6.0% (except for the sample with added NaCl and stored under nitrogen which had 1.8%), serine with over 5.75% (except for the sample with 3% added NaCl and stored under nitrogen which comprised only 0.43% of the total), glycine with about 5.5%, and proline with over 5.0% of the total. The major changes in the proportion of individual amino acids occurred for the salt-treated samples during storage under nitrogen, but there were both increases and declines, depending upon the particular amino acid. Since analyses were not made on fresh unstored adipose tissue, it is difficult to explain the reasons for the changes in concentration. However, it is possible that the addition of NaCl to adipose tissue followed by storage under nitrogen could protect certain proteins from oxidation, thereby changing the soluble protein profile and the amino acid residue distribution.

Table II shows the protein and sulfhydryl values for the different treatments. Results suggest that there may be a slight atmospheric effect on sulfhydryl groups for both the salt-treated and salt-free samples, since the level of sulfhydryls was lower when the samples were stored under air than under nitrogen. However, the most striking finding was the almost twofold greater sulfhydryl content of the samples containing salt as compared to the salt-free samples. These results are in agreement with the findings of Mabrouk and Dugan (1960), who reported that the adddition of NaCl to a linoleic acid and methyl linoleate buffered emulsion exerted an inhibitory effect on oxidation of the system by decreasing

oxygen solubility in the emulsion. However, other studies (Ellis *et al.*, 1968; Gaddis, 1952; Chang and Watts, 1950) have shown that addition of NaCl accelerates autoxidation of adipose tissue, which continues during freezer storage (Ellis *et al.*, 1968; Gaddis, 1952). Although the effects of NaCl on autoxidation of emulsion systems containing unsaturated fatty acid derivatives is not clear, NaCl appears to stabilize protein sulfhydryl groups in protein–fat–water emulsion systems.

The reason for the higher sulfhydryl content of the salt-treated samples is not clear, but may be due to saturation of the water content with NaCl and subsequent displacement of dissolved gases including oxygen, thus retarding oxidation. Another possible explanation may be emulsion breakdown preventing any prooxidant effect from the interaction of products of oxidation with the other nondialyzable components.

Specific Staining. The results of staining for sulfhydryls and proteins on the disk gels of the variously tested nondialyzable fractions from adipose tissue are shown in Figure 2. A control sample prepared from beef muscle is also included in Figure 2 so that comparisons can be made. Examination of the patterns shows that there are some bands common to both adipose tissue and muscle for sulfhydryls and proteins, yet they are more dissimilar than similar. Attention is specifically called to the broad band at approximately 3.6 cm in the muscle samples, which was absent in adipose tissue. The dissimilarities between disc gel patterns of adipose tissue and muscle indicate that some of the proteins are different and are characteristic of either muscle or adipose tissue, but are not common to both. This would suggest that both adipose tissue and muscle could make characteristic contributions to the development of meat flavor aside from those derived from lipid material.

Figure 3 indicates that the disc gel pattern from adipose tissue gave positive tests for ribonucleic acids, nucleoproteins, and glycoproteins. In addition, esterase activity was also identified on the same general area of the gel. To ascertain whether or not glycoproteins and nucleoproteins were independently present or only associated with the esterase, the proteins were chromatographed with a 5% running gel to improve the separation. Although all proteins migrated further on the 5% than in the 7.5% running gel, the material staining positively for glycoproteins and ribonucleic acids did not move any further in the 5% gel. They stained extremely light with Amido Black, which is a strong protein dye. However, they gave a very strong reaction with methylene blue, which is specific for ribonucleic acids. Thus, it was concluded that glycoproteins and nucleoproteins were not present and that the glycoprotein and nucleoprotein stains were positive due to the carbohydrates and nucleic acid residues associated with ribonucleic acids.

Although the specific esterase was not identified, its presence indicated that the extraction and concentration procedures used for adipose tissue were relatively mild. This would suggest the water-soluble proteins found in the adipose tissue extracts were indigenous and not breakdown products due to the method of extraction and concentration.

Tests for lipoproteins by staining all gels with both Sudan Black B and Oil Red O gave negative results. Thus, it was concluded that lipoproteins were not present in the nondialyzable fraction from the aqueous extract of beef adipose tissue.

Figure 4 presents the protein and sulfhydryl disc gel pattern for the nondialyzable extract from aqueous extraction of beef adipose tissue. Neither air nor nitrogen storage appeared to alter the protein or sulfhydryl patterns. However, patterns from samples stored with salt were markedly different from those stored without salt. Both the protein and sulfhydryl bands at 2.1 cm (Figure 4) were more intensely stained in the samples stored with salt than in those of the salt-free treatments. Conversely, the protein and sulfhydryl bands at 2.6 cm were more distinct in the salt-free treatments. In addition, the bands at 1.2 cm in gels of the salt-free treatment are more distinct than similar bands for the salt treatment. Protein staining showed the bands at 5.8 cm to be more intensely stained for the salt treatment, but sulfhydryl groups were not present in any of the samples at this point.

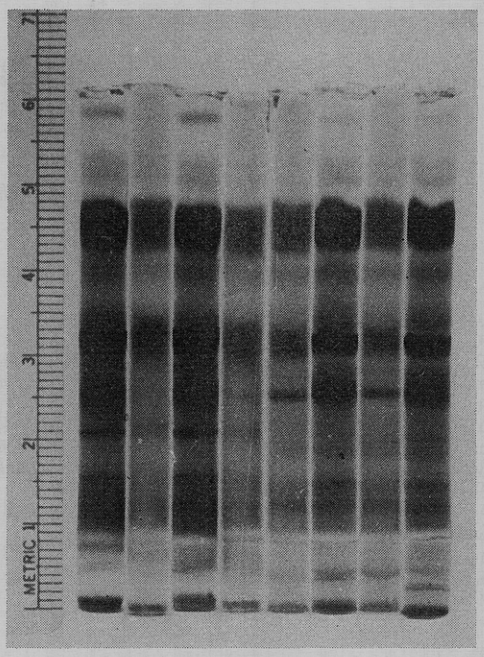

Figure 4. Gel patterns for water-soluble fractions of adipose tissue subjected to storage under nitrogen (N_2) or air (air) without salt and under nitrogen (N_2S) or air (air–S) with salt. Left to right, adipose tissue extract patterns: (1) N_2S–protein stain; (2) N_2S–SH stain; (3) Air–S–protein stain; (4) Air–S–SH stain; (5) Air–SH stain; (6) Air–protein stain; (7) N_2–SH stain; and (8) N_2–protein stain

Although earlier studies have indicated that both lean tissue (Hornstein and Crowe, 1964; Kramlich and Pearson, 1958) and adipose tissues (Pepper and Pearson, 1969; Wasserman and Talley, 1968; Hornstein and Crowe, 1960) may contribute to meat flavor, the emphasis has been upon the contributions of lipids *per se* from adipose tissue and no attention has been given to the water-soluble material in this fraction. Results of this study indicate that there are some differences in proteins present in lean and adipose tissues. This suggests that the water-soluble fraction from adipose tissue may make distinct and characteristic contributions to meat flavor above and beyond those related to the lipid fraction.

Another interesting possibility for an additional role of adipose tissue to meat flavor could be through the greater number of SH-groups as a result of added salt. Since salt is commonly added during cooking, it is possible that salt may retard the oxidation of sulfhydryl groups sufficiently to allow the parent components to be evolved as volatile sulfur-containing compounds, which have been shown to play a major role in the development of cooked meat flavor (Minor *et al.*, 1965).

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