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Effect of Some Water-Soluble Components on Aroma of Heated Adipose Tissue

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The precursors of the characteristic aromas of heated pork, beef, and lamb adipose tissues were present in the lipids extracted with chloroform: methanol. Water-washing the extracts removed components involved in forming the characteristic odors. Amino acids and glucose were identified in the water-wash, which gave a nonspecific roast meat aroma on heating to dryness. Preliminary gas chromatographic studies of the extracted fats and the water-washes show primarily quantitative differences among the meat species.

In spite of a number of studies in recent years the flavor of meat and meat products is still a little understood subject. Not only are the specific flavor components essentially unknown but the site of developing flavor is still under discussion. Hornstein and Crowe (1964) stated that lean meat extracts of beef, pork, lamb, and even whale, heated at 100 °C, had the same basic meaty aroma, and postulated that species-specific aroma was developed in the fat. Wasserman and Gray (1965) confirmed that diffusates of dialyzed water extracts of lean beef, pork, and lamb developed a series of odors during boiling that culminated in a roast meat-like aroma, on dryness, with no species characteristics. Wasserman and Talley (1968) reported that chloroform:methanol extracts of beef and pork adipose tissue contained a water-soluble component(s) that influenced sensory identification of heated ground lean meat to which they were added.

Pippen *et al.* (1969) indicated that raw chicken fat, extracted from adipose tissue, did not develop characteristic odor on heating, but fat from cooked or roasted chicken did have typical chicken aroma. These authors suggest the components responsible for aroma originate in lean portion and are trapped or dissolved in the fat during cooking.

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Results of some preliminary studies on components of adipose tissue that may be involved in development of meat aroma are reported herein.

EXPERIMENTAL

Materials. Beef and pork subcutaneous adipose tissue and lamb kidney adipose tissue were obtained from local commercial sources.

Lipid Preparations. One-hundred grams of adipose tissue were homogenized for 4 min with 100 ml of cold chloroform:methanol (2:1) solution ($CHCl_3$:MeOH). Following vacuum filtration the residue was reextracted four times with 100 ml of solvent each time. The pooled extract was divided into two aliquots; one of these served as the unwashed lipid control. To the remaining aliquot an equal volume of water was added, and the mixture was shaken well and allowed to separate in the cold. The aqueous layer (subsequently referred to as "wash-water") was freeze-dried and the resultant white powder dissolved in 25 ml of water. The $CHCl_3$:MeOH solvent layer in all preparations was concentrated *in vacuo*. Final traces of solvent were removed by bubbling N_2 for approximately 1 hr through the lipid heated at 40 °C.

Analytical Procedures. Thin-layer chromatography was carried out on precoated Silica Gel-F-254 plates, 0.25-mm thick (EM Reagents Division, Brinkmann Instrument Co.). To remove the large concentrations of triglycerides present in the extracts, the plates were first developed in hexane:ether

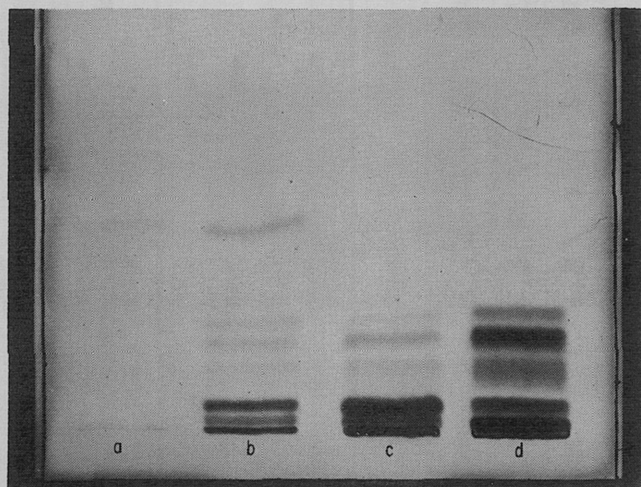

Figure 1. Aqueous separation of amino acids from adipose tissue lipids extracted with chloroform:methanol. a. washed lipid residue; b. initial unwashed lipid; c. aqueous layer; d. amino acid standards

Table I. Amino Acid Composition of the Water-Soluble Fraction from Chloroform:Methanol Extracts of Adipose Tissue

	Amino acid components of adipose tissue		
	Pork	Beef	Lamb
	μM/100 g of tissue		
Cysteic acid	2.35	1.95	1.5
Tau	19.40	9.20	34.70
Urea	44.60	39.15	...
Asp	0.85	3.60	3.25
Asp–NH_2 + Thr	10.00	3.85	8.85
Ser	7.00	3.90	8.40
Glu	4.50	4.90	8.25
Pro	7.05	4.45	10.35
Gly	16.85	13.20	35.25
Ala	39.55	25.75	45.40
Val	5.80	3.90	8.65
Met	1.65	0.90	2.3
Ile	5.45	6.55	11.4
Leu	8.10	4.35	11.3
Tyr	4.20	1.65	4.45
Phe	2.95	2.50	5.80
NH_3	3.65	2.90	1.15
Lys	0.75	0.95	1.90
Anserine	0.80	0.50	1.55
Carnosine	5.90	0.85	0.75
Arg	1.70	0.80	0.11

(95:5). After drying, the plates were developed with chloroform:methanol:water (65:25:4). Amino acids were detected with ninhydrin (0.25% in 95% ethanol) and anisidine–phthalic acid reagent (equal volumes of 0.2 *M* solutions in 95% ethanol) was used for the sugars.

Quantitative amino acid analyses were carried out with the Piez and Morris (1960) one-column system using a Phoenix Analyzer.

Pyrolysis and gas chromatography of the volatiles was performed with a Loenco pyrolyzer fitted to an F&M 810 gas chromatograph, using the flame ionization detector. Quantities of the lipid or wash-water preparation were freeze-dried in glass U-tubes and flash-heated at 225°C for 3 min in the pyrolyzer. The volatiles were collected in the pyrolyzer trap during this time and then injected as a plug on the column. A 6-ft × $^1/_8$-in. i.d. stainless steel column packed with 15% Carbowax 20M-TPA on 60–80 mesh Gas Chrom P was used.

Table II. Sensory Evaluation of Heated Chloroform:Methanol Extracts of Adipose Tissue

	% Correct identification[a]	
Tissue	Unwashed	Washed[b]
Beef	28	47
Pork	50	33
Lamb	62	36

[a] Panel consisted of 24 members. [b] Extracted lipids were washed with water.

Chromatograph operating conditions were: helium carrier gas flow rate, 25 ml/min; injection port temperature, 210°C; detector temperature, 220°C; and oven temperature isothermal at 68°C for 5 min and then programmed at 6°C/min to 163°C and held.

Sensory evaluation was conducted by heating small quantities of materials in 10-ml beakers on a hot plate calibrated at 225°C. The panelists smelled the aromas as they developed. An untrained panel of approximately 25 people from the laboratory was asked to identify the aromas as those of beef, pork, and lamb, using their memory of these odors as standards. In addition, an expert panel was formed whose six members developed a vocabulary to describe the odors developed on heating the samples. Sensory evaluation was difficult because the character of aroma changed with time, reflecting occurrence of reactions and volatilization of components with different vapor pressures; panelists' replies, therefore, depended on the time at which they sniffed the vapors. Following each judging session a discussion period was held to reach a consensus as to the odors smelled. The tests were not designed for statistical evaluation.

RESULTS

Approximately 96% of beef, pork, or lamb adipose, consisting of both nonpolar lipids and some classes of polar compounds, is soluble in $CHCl_3$:MeOH. Water-soluble components were removed from the $CHCl_3$:MeOH solution on washing. The major component of this fraction is a group of amino acids, as shown in Figure 1 for the pork preparation. Similar results were obtained with beef and lamb lipid washes.

Quantitative analyses of amino acids in water-soluble fraction from beef, pork, and lamb lipids are shown in Table I. The concentrations of most of the amino acids in the beef preparation were lower than those of either pork or lamb, and the highest concentrations were present in the lamb water-wash. Considerably more taurine and glycine were present in the lamb than in beef or pork preparation. Pork wash-water contained almost as much tyrosine and asparagine–threonine, and approximately fifteen times as much arginine, as lamb. Pork also contained considerably more of carnosine than either the beef or lamb preparations.

Glucose was the only carbohydrate identified in the water-soluble material from the three lipid preparations, although fructose, ribose, and the sugar phosphates are also known to be normal cell constituents. The concentration of these compounds may be below the level of detectability of the tlc method.

Heated adipose tissue produced aroma components that were characteristic of the type of meat and were identifiable by an untrained panel. Pork and lamb lipids, extracted from tissue with $CHCl_3$:MeOH, could be identified by more than half the panel, but beef lipid was recognized by a smaller number of judges (Table II) primarily because of its bland aroma

and lack of characteristic notes. On continued heating beef lipid aroma resembled hot fat, whereas the aromas of pork and lamb were recognizable for the entire heating period.

Following water-wash to remove polar components, fewer panelists correctly identified heated pork and lamb lipids but, paradoxically, washed beef lipid was correctly identified more frequently than the unwashed preparation.

Descriptive analyses of the aromas of the lipid preparations by the trained panelists were difficult to analyze. The characteristics by which pork lipid was recognized were not the aromas of pork chops or any pork-type meat, but notes that were called "piggy," "sour," or "goaty." These notes were observed in both the unwashed and washed lipids. Beef lipid odors were the most difficult to characterize. The unwashed lipid had a slightly roast meat-like aroma on heating, but the washed lipid smelled waxy. The beef odors were the least intense of the three types of lipid studied.

The judges on the trained panel agreed that the identification of the washed lipids was based principally on aromas produced during the early stages of heating. When the lipids became very hot, to the point of formation of brown color in unwashed lipid samples, definite aroma differences between washed and unwashed lipids were observed. The unwashed preparation had meaty aromas, while the washed lipids were more like hot fat or fat in which vegetables had been fried.

On heating the water-washes, containing the water-soluble components of the three types of adipose tissues, a basic roast meat aroma was obtained. This was similar to the aroma obtained from diffusates of water extracts of lean meat. Only the water-wash of the lamb lipid preparation was identifiable by the aroma.

Preliminary gas chromatographic studies of the volatiles produced by heating unwashed and washed pork lipids suggest differences are generally quantitative (Figure 2). However, since the chromatographic peaks may contain more than one component, reduction in size of a peak could be due to qualitative changes in compounds formed on heating. The large increase in the peak at 2–3 min in the washed material cannot be explained at this time.

Chromatograms of the volatiles produced on heating wash-waters of beef, pork, and lamb are compared in Figure 3. The profiles appear to be qualitatively similar, although those of pork and lamb are more obviously alike. This may be a matter of concentration, as beef components appear to be present in lower concentrations. The pyrolyzed residue of lamb wash-water contains several components eluting at 33–35 min that are either absent in the other lipid preparations or formed in lower concentrations.

Identification of components in the heated lipids or wash-water preparations has not been undertaken at this time. The concentration of detectable material released is very small and the large quantities of water formed during heating interfered with trapping and isolation procedures. Many odors were noted as material eluted from the chromatograph, but none were characteristic of the specific meat, or even of meat itself.

SUMMARY AND DISCUSSION

Adipose tissues from beef, pork, and lamb, and the $CHCl_3$: MeOH extracts of these tissues produce aromas when heated that are identifiable with the species of the animal. Washing the extracts with water, however, renders identification considerably more difficult, while the aroma of the water extracts is similar to that of the diffusates of water extracts of the lean meats themselves. Amino acids and glucose were identified in the water-soluble fraction.

Since adipose tissue is composed of metabolically active cells, it is not surprising we identified a number of amino acids and at least one sugar component in extracts of tissue. Additional carbohydrates, proteins, and other normal cell con-

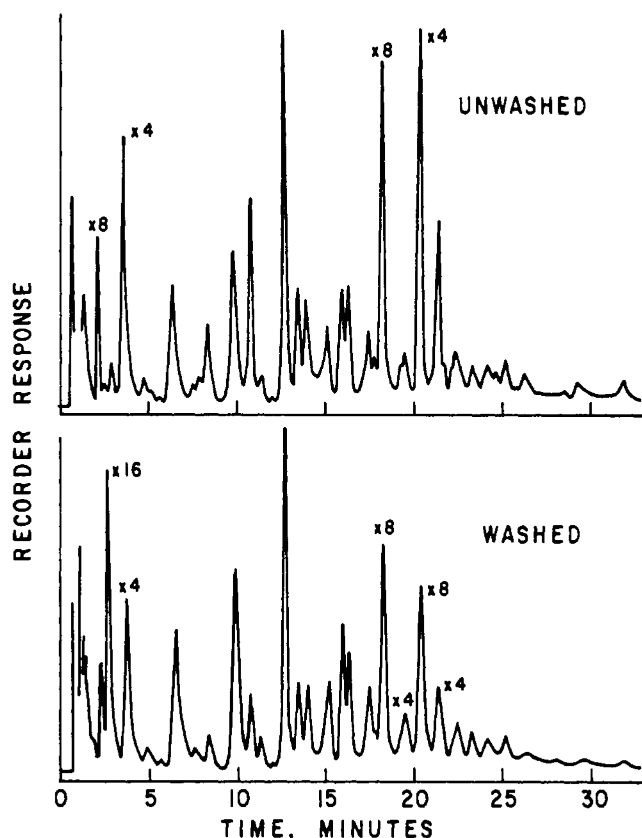

Figure 2. Gas chromatographic profiles of heated washed and unwashed pork lipids

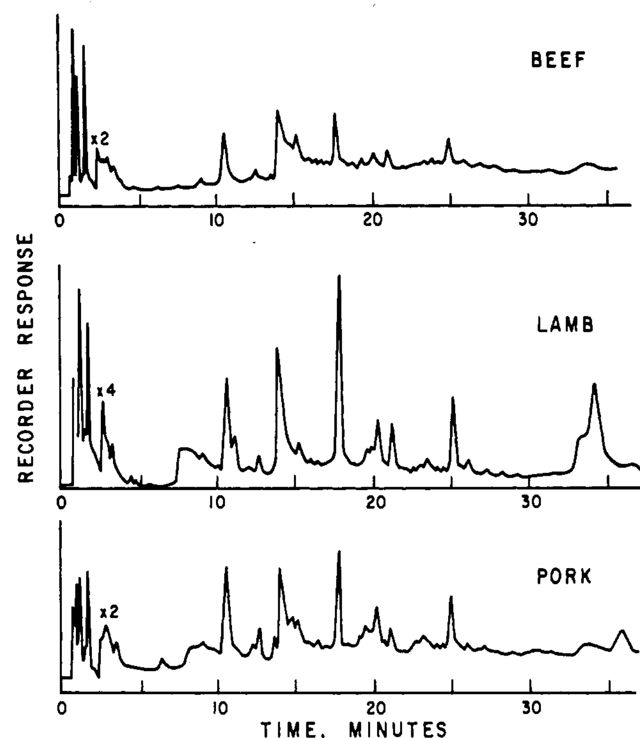

Figure 3. Gas chromatographic profiles of water-soluble material from adipose tissue lipids extracted with chloroform: methanol

stituents could be expected as well. However, there are literature reports on the role of "fats," or lipids, in flavor of meat in which involvement of these extraneous factors was not considered. Yamato *et al.* (1970), for instance, state that "although raw beef fat does not possess a meaty aroma, it is produced on heating." These authors heated bovine leaf fat for 5 hr at 150°C. Since "bovine leaf fat" is adipose tissue, the origin of some of the compounds identified as coming from fat may be open to question.

While it is difficult to conclude from the data reported in this paper how the lipids and water-soluble components of adipose tissue interact to produce the characteristic meaty aroma, several mechanisms are suggested, as follows. *Species-specific compounds are formed on heating lipids.* The studies described here and the work of Pippen *et al.* (1969) with raw chicken lipids, however, suggest this does not occur. Pork was identified from its heated lipids, but the aroma produced was not characteristic of pork food products; it was a "piggy" odor, possibly from thermal degradation of unsaturated fatty acids in the lipid. *Interaction between compounds produced on heating both lipid and lean meat.* Pippen and Mecchi (1969) suggested H_2S produced from amino acids or proteins reacted with carbonyls from thermal degradation of fatty acids to form odors that contribute to chicken aroma. *Interaction directly between lean and lipids.* Browning reaction may occur directly between amino groups of the lean meat and carbonyl compounds in the lipids to produce odorous compounds. *Fats serve as solvent.* Aroma components, developed by thermal degradation of or reactions occurring in lean meat, may be dissolved in the lipid fraction. Pippen *et al.* (1969) claim this is the role of fat in chicken aroma. Other preformed fat-soluble components may be liberated as heat increases their vapor pressure. The "boar" aroma, described by Patterson (1968) as odor of 5α-androst-16-ene-3-one, becomes evident when some pork fats are heated.

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Shallow-Fried Beef: Additional Flavor Components

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A flavor concentrate of shallow-fried beef was isolated and fractionated into 17 parts by silicic acid column chromatography. Two fractions possessed characteristic flavors, one reminiscent of heated beef odor and the other of sour cream-like odor after the roasted flavor, based on the basic compounds, was removed. The flavor components in these fractions were isolated in pure state by gas chromatography. Identification was based on gas chromatographic retention times and mass spectra. Several alcohols, methional, 2-acetylfuran, 2-furfuryl methyl ketone, 1-methyl-2-acetylpyrrole, benzothiazole, acetol acetate, and *O*-hydroxyacetophenone were identified.

This series of investigations is ultimately aimed at identifying the volatile components responsible for beef flavor. The first two papers (Watanabe and Sato, 1968a,b) described γ- and δ-lactones developed from beef fat. And then the various flavor compounds from heated beef fat were identified and their possible contribution to the heated beef fat flavor was discussed (Watanabe and Sato, 1971a). In a recent report, the heated flavor from the mixture of beef lean meat and fat was investigated, and some alkyl-substituted pyrazines and pyridines which might contribute to the roasted flavors were investigated (Watanabe and Sato, 1971b). This paper deals with the identification of potentially significant flavor compounds developed from the shallow-fried beef. Identification was based on gas chromatographic retention times and mass spectra.

Many literature references about work done elsewhere on beef flavor directly related to the present study have been cited previously (Watanabe and Sato, 1971b).

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EXPERIMENTAL

Materials and Preparation of Flavor Components. The lean meat and fat used in this investigation were veal shoulder (20.0 kg) and kidney fat (2.0 kg). These samples were cut into small pieces and heated under the conditions of shallow frying. The procedures for the sample preparation and heating were essentially the same as those reported previously (Watanabe and Sato, 1971b). The flavor concentrate obtained was fractionated by silicic acid column chromatography. This procedure was used in the separation of flavor components obtained from green tea, and their better separations were described (Yamanishi *et al.*, 1970). The 23 × 3 cm column was packed with 90 g of silicic acid (Mallinckrodt, 100 mesh). The column was successively eluted with hexane (250 ml, Fr. 1), hexane (250 ml, Fr. 2), various hexane solutions containing ethyl ether in the concentration described below, 1% (200 ml, Fr. 3), 2% (200 ml, Fr. 4), 3% (200 ml, Fr. 5), 4% (200 ml, Fr. 6), 5% (200 ml, Fr. 7), 6% (200 ml, Fr. 8), 8% (200 ml, Fr. 9), 10% (200 ml, Fr. 10), 20% (200 ml, Fr. 11), 20% (200 ml, Fr. 12), 20% (200 ml, Fr. 13), 30% (200 ml, Fr. 14), and 50% (200 ml, Fr. 15), ethyl ether (200 ml, Fr. 16), and, lastly, methanol (200 ml, Fr. 17). All of the solvents used were