Volatile Flavor Constituents of Ovine Adipose Tissue

Fredric Caporaso,[1] John D. Sink,* Paul S. Dimick, Cynthia J. Mussinan, and Anne Sanderson

In an effort to enhance the utilization of sheep (*Ovis aries*) meat, this study was designed to isolate and identify compounds from ovine subcutaneous adipose tissue (*Panniculus adiposus*) which contribute to the characteristic flavor of the meat. Samples were taken from three Suffolk wether lambs and subsequently steam deodorized, extracted, fractionated, and analyzed. Olfactory and gas chromatographic (GC) analyses confirmed that the bulk of the flavor volatile compounds were in the neutral fraction, some in the acidic fraction, but little or none were detected in the basic fraction. Gas chromatographic–mass spectrometric (GC–MS) analysis of the neutral fraction resulted in the identification of 51 compounds: 26 aldehydes, 12 ketones, 6 lactones, 4 alcohols, and 3 cyclics. Thirty-nine of these compounds are being reported for the first time as constituents (some tentative) of ovine fat volatiles. Although no single neutral fraction compound identified could be shown to be primarily responsible for lamb/mutton flavor, 14 (10 aldehydes, 3 ketones, and 1 lactone) are suggested as important contributors to the overall flavor quality.

Generally, animal meat products are well-balanced nutritionally in energy, protein, minerals, and vitamins, and their palatability makes them a desirable food source for man. Thus, the decreasing trend in the utilization of sheep meat as a foodstuff deserves immediate attention in this age of critical world food supplies (USDA, 1976). From all reports, it appears that the distinctive flavor of sheep meat (Wasserman and Talley, 1968), especially that from older animals, is somewhat less acceptable and thus responsible for its low consumption rate by U.S. consumers (Ziegler and Daly, 1968).

Generally, the lipid-soluble flavor components are the most important (Sink, 1973) and many factors contribute to their expression (Sink and Caporaso, 1977). Although the lipid-soluble compounds responsible for the characteristic sheep meat (lamb or mutton) flavor are yet to be completely inventoried, current interest has focused attention on the acid fraction volatiles (Wong et al., 1975a,b). It was the purpose of this study to identify the volatile neutral fraction flavor components from ovine adipose tissue.

EXPERIMENTAL PROCEDURES

Sampling. Following standard exsanguination and chilling (3 °C) procedures, subcutaneous fat (*Panniculus adiposus*) samples (300 g) were taken from three castrate-male (wether) Suffolks (*Ovis aries*) raised under similar management conditions and nutritional regimes. Each sample was subsequently ground through a 3-mm plate, placed under nitrogen in an impermeable container, and frozen at −29 °C. The frozen samples were then gently melted (50 °C) and filtered through several layers of cheesecloth to separate the liquid fat from the tissue residue. Olfactory evaluation indicated the liquid fat did possess the distinctive sheep meat (lamb/mutton) character.

Steam Deodorization. One hundred grams of liquid fat was transferred into the 500-mL round-bottom flask (H) of the steam deodorization unit (Figure 1) and the unit assembled (adapted from that used by Dimick and Walker, 1967). The sample was heated to 163 °C to simulate oven temperature, and the absolute pressure on the system was reduced and maintained at 0.20 to 0.25 Torr. Heat was then applied to the steam generator (B) and steam was percolated through the sample for 3 h. Volatiles were collected in Dewar-type traps (I and J) which were maintained at −80 °C with dry ice–ethanol. After 3 h the heat was removed, the pressure was gradually increased back to atmospheric, the dry ice–ethanol traps allowed to thaw, and the apparatus disassembled. Olfactory evaluation indicated the residual fat had no lamb/mutton aroma.

Extraction. The collected volatiles in the traps were emptied into a 125-mL beaker. The 90°-angle two-way connecting tube and both traps were rinsed once with water and twice with distilled methylene chloride into the 125-mL beaker. The aqueous phase was saturated with sodium chloride and extracted in a 125-mL separatory funnel twice with distilled methylene chloride. The combined extract was carefully concentrated under a stream of nitrogen to approximately 100 μL. Olfactory evaluation indicated the concentrated extract possessed the lamb/mutton character.

Fractionation. The concentrated extract from the total volatile fraction was separated into acidic, basic, and neutral fractions by counter-current extraction techniques (Alders, 1955), using 125-mL separatory funnels, as shown in Figure 2. Olfactory evaluation of the three fractions indicated the neutral fraction contained the most intense lamb/mutton character.

GC–MS Analyses. The neutral fraction was analyzed using a Hewlett-Packard Model 5750 gas chromatograph (GC), equipped with a flame-ionization detector, coupled with a Hitachi Model RMU-6E mass spectrometer (MS). The chromatographic column used was a 152.4 m × 0.76 mm stainless steel, open tubular, SF-96 coated capillary column. The oven temperature was programmed from 70 to 190 °C at 2 °C/min. The injector and detector temperatures were 220 °C and the helium flow rate was approximately 12 mL/min. The mass spectrometer was fitted with a Watson-Biemann helium separator (Watson and Biemann, 1965). The source temperature was 150 °C and the ionizing potential 70 eV. Identifications were based on the comparison of known and unknown mass spectra and confirmed wherever possible by determining

Department of Food Science, The Pennsylvania State University, University Park, Pennsylvania 16802 (F.C., J.D.S., P.S.D.) and Research and Development, International Flavors and Fragrances, Inc., Union Beach, New Jersey 07735 (C.J.M., A.S.).

[1]Present Address: Department of Food Science and Technology, University of Nebraska, Lincoln, Nebr. 68503.

the retention indices (I_E values) relative to a series of ethyl esters of normal alkanoic acids (Van den Dool and Kratz, 1963).

Olfactory Evaluation. Throughout this study, olfactory analyses were performed by six members of a trained sensory panel using profiling techniques (Amerine et al., 1965). At each step in the analyses, the panelists were asked to evaluate the sample(s) for the presence of the various lamb/mutton flavor notes. In the final analysis of possible molecular contributors, the panelists were asked to evaluate individual compounds after a preliminary GC exhaust port screening.

RESULTS AND DISCUSSION

The most frequent descriptors of the flavor notes associated with the total volatile fraction and the neutral fraction were lamby (sheeplike) and oxidized (fatlike). In addition, a heated adipose tissue sample also gave a characteristic aroma reminiscent of mushroom soup. The flavor intensity of the neutral fraction was observed to be strong. Although some slight aromas were evident in the acidic fraction, few to none were noted for the basic compounds.

The GC analyses of the neutral, acid, and basic fractions confirmed the olfactory evaluations in that the number of visible peaks observed were 71, 12, and none, respectively. Further GC–MS analyses of the neutral fraction resulted in the identification of 51 compounds (Table I): 26 aldehydes, 12 ketones, 6 lactones, 4 alcohols, and 3 cyclics. Although a number of these volatile constituents have been isolated and identified by others (Table II), 39 of the 51 are being reported for the first time and thus make a significant contribution to the known molecular inventory of lamb/mutton flavor.

Alkanals, alkenals, and alkadienals have been largely responsible for a wide range of oxidized flavors occurring in foods (Forss, 1972). Many terms are used to describe such flavors including green, reverted, rancid, metallic, oily, fishy, tallowy, and painty. As the term "oxidized" implies, most of these flavors are the result of oxidation, usually of C_{18} polyunsaturated fatty acids such as linoleic and linolenic, and the C_{20} unsaturated, arachidonic. However, Ziegler et al. (1967) have shown that these precursor fatty acids are usually present in small amounts. In many cases, single aliphatic aldehydes are responsible for characteristic flavor notes, e.g., *trans*-2,*trans*-6-nonadienal for a tallowy flavor in beef and mutton tallow (Hoffmann and Meijboom, 1968). The alkanals have low flavor thresholds and are more stable than many of the unsaturated aldehydes occurring in oxidized foods. The C_5–C_9 alkanals have green, oily, fatty, tallowy flavors, while C_{10}–C_{12} alkanals have citrus, orange peel flavors (Badings, 1970). Siek et al. (1971) reported that 1-alkanals above C_3 have flavor intensities which become stronger as the carbon chain length increases. They reported flavor thresholds for C_{5-7} 1-alkanals to be 4.2, 2.5, and 0.52 ppm, respectively, in water. The flavors of the *trans*-2,*cis*-4-hepta- and decadienals were described as being sour and sweet, respectively (Hoffmann, 1962). The trans-2,trans-4 isomers were described as rancid and fatty, respectively. The trans-2,cis-4 dienals (C_7–C_{10}) were approximately ten times as potent as their trans-2,trans-4 dienals. The C_{7-10} 2,4-alkadienals all have fatty, deep-fried odors.

Only during the last 10 years has the importance of ketones in the flavor of nondairy foods been fully appreciated. Methyl ketones with an odd number of carbon atoms predominate in foods. Arctander (1969) reported flavor descriptions for several of the ketones: 2-heptanone (spicy), 2-nonanone (fatty), 2-decanone (citrus), 2-unde-

Table I. Volatile Compounds Identified in the Neutral Fraction of Cooked Ovine Fat

Compound	Presence reported[a]	Retention index,[b] I_E	Remarks[c]
Aldehydes			
Pentanal	–	2.79	R
Hexanal	–	4.00	R
Heptanal	–	5.11	R
Octanal	–	6.11	R
Nonanal	–	7.20	R
Decanal	–	8.12	R
Undecanal	x	9.16	T
Tridecanal	x	11.22	R
Tetradecanal	x	12.26	T
Pentadecanal	x		NRA
Heptadecanal	x		NRA
Octadienal	x		P
2-Hexenal	x	4.61	T
2-Heptenal	x	5.63	T
2-Octenal	x	6.65	R
2-Nonenal	–	7.66	R
2-Decenal	–	8.74	R
2-Undecenal	–	9.68	R
2-Tridecenal	x	11.78	R
2-Tetradecenal	x		NRA
2-Pentadecenal	x		NRA
2,4-Heptadienal	x	5.99	R
2,4-Heptadienal (isomer)	x		T
2,4-Decadienal	x	9.22	R
2,4-Decadienal (isomer)	x		T
2-Methyl-2-butenal	x	3.37	T
Ketones			
2-Heptanone	–		P
2-Nonanone	x	6.95	T
2-Decanone	x	8.00	T
2-Undecanone	x	9.00	R
2-Dodecanone	x	10.05	T
2-Tridecanone	x	11.06	R
2-Tetradecanone	x		NRA
2-Pentadecanone	x		NRA
2-Hexadecanone	x		NRA
2-Heptadecanone	x		NRA
3-Tetradecanone	x		P
4-Methyl-2-pentanone	x	3.22	R
Lactones			
γ-Heptalactone	x	7.46	T
γ-Octalactone	x	8.58	T
γ-Nonalactone	x	9.62	R
γ-Decalactone	–	10.68	R
γ-Tetradecalactone	–		P
γ-Butryrolactone (substituted)	x		P
Alcohols			
1-Pentanol	x	3.88	R
1-Hexanol	x	4.70	T
1-Heptanol	x	5.74	R
1-Octanol	x	6.78	R
Furans			
2-*n*-Pentylfuran	x	6.00	R
Hydrocarbons			
Toluene	x	3.72	R
Naphthalene	x	7.94	T

[a] Previous report indicated by a minus; first report (this study) indicated by an x. [b] Values are retention times or indices for pure compounds relative to a series of ethyl esters of normal alkanoic acids. [c] R = reference, unknown spectrum matches known reference spectrum; T = tentative, either mass spectrum or I_E value is slightly different than reference; NRA = no reference available, spectrum and I_E value are completely logical, but no reference spectrum was available for comparison; P = possible, interpretation difficult due to a weak or mixed spectrum.

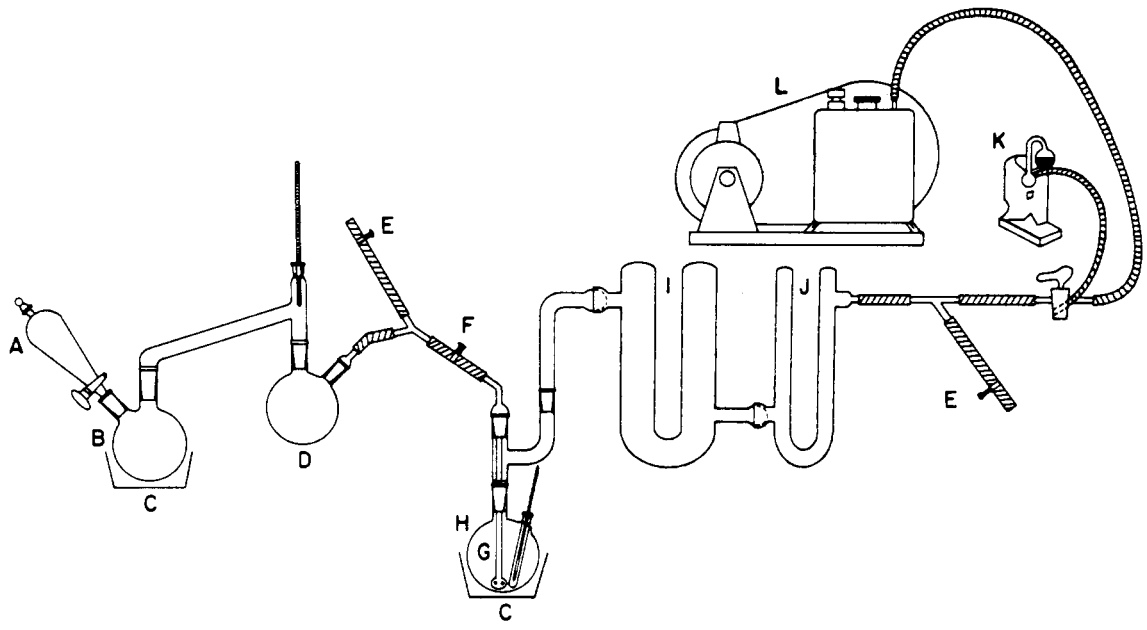

Figure 1. Apparatus for the isolation of total volatiles from liquid ovine fat: (A) water reservoir flask, (B) steam generator, (C) heating mantle, (D) steam condensation flask, (E) vacuum controlling pinch clamp, (F) steam controlling pinch clamp, (G) steam aspirator, (H) sample flask, (I) 3-L cold trap, (J) 1-L cold trap, (K) vacuum gauge, (L) vacuum pump.

Table II. Volatile Compounds Identified in Lamb and/or Mutton by Other Researchers

Compound	Reference
Aldehydes	
Acetaldehyde	Hornstein and Crowe (1963)
Propionaldehyde	Jacobson and Koehler (1963)
Butanal	Jacobson and Koehler (1963)
Pentanal	Jacobson and Koehler (1963)
Hexanal	Hornstein and Crowe (1963)
Heptanal	Jacobson and Koehler (1963)
4-*trans*-Heptenal	Hoffmann and Meijboom (1968)
4-*cis*-Heptenal	Hoffmann and Meijboom (1968)
Octanal	Jacobson and Koehler (1963)
Nonanal	Hornstein and Crowe (1963)
2-Nonenal	Hornstein and Crowe (1963)
2-*trans*,6-*trans*-Nonadienal	Hoffmann and Meijboom (1968)
2-*trans*,6-*cis*-Nonadienal	Hoffmann and Meijboom (1968)
Decanal	Jacobson and Koehler (1963)
2-Decenal	Hornstein and Crowe (1963)
2-*trans*,4-*trans*-Decadienal	Park et al. (1974)
2-Undecenal	Hornstein and Crowe (1963)
Ketones	
Acetone	Cramer (1974)
2-Butanone	Cramer (1974)
2-Pentanone	Jacobson and Koehler (1963)
3-Pentanone	Cramer (1974)
2-Hexanone	Jacobson and Koehler (1963)
2-Heptanone	Jacobson and Koehler (1963)
2-Octanone	Jacobson and Koehler (1963)
2-Nonanone	Jacobson and Koehler (1963)
2-Decanone	Jacobson and Koehler (1963)
2-Methylcyclopentanone	Jacobson and Koehler (1963)
Lactones	
γ-Decalactone	Watanabe and Sato (1968)
γ-Dodecalactone	Watanabe and Sato (1968)
γ-Dodec-*cis*,6-enolactone	Park et al. (1974)
γ-Tetradecalactone	Watanabe and Sato (1968)
δ-Decalactone	Watanabe and Sato (1968) Dimick et al. (1966)
δ-Dodecalactone	Watanabe and Sato (1968) Dimick et al. (1966)
δ-Tetradecalactone	Watanabe and Sato (1968) Dimick et al. (1966)
δ-Hexadecalactone	Watanabe and Sato (1968) Dimick et al. (1966)
Alcohols	
Butanol	Cramer (1974)
Ethanol	Cramer (1974)

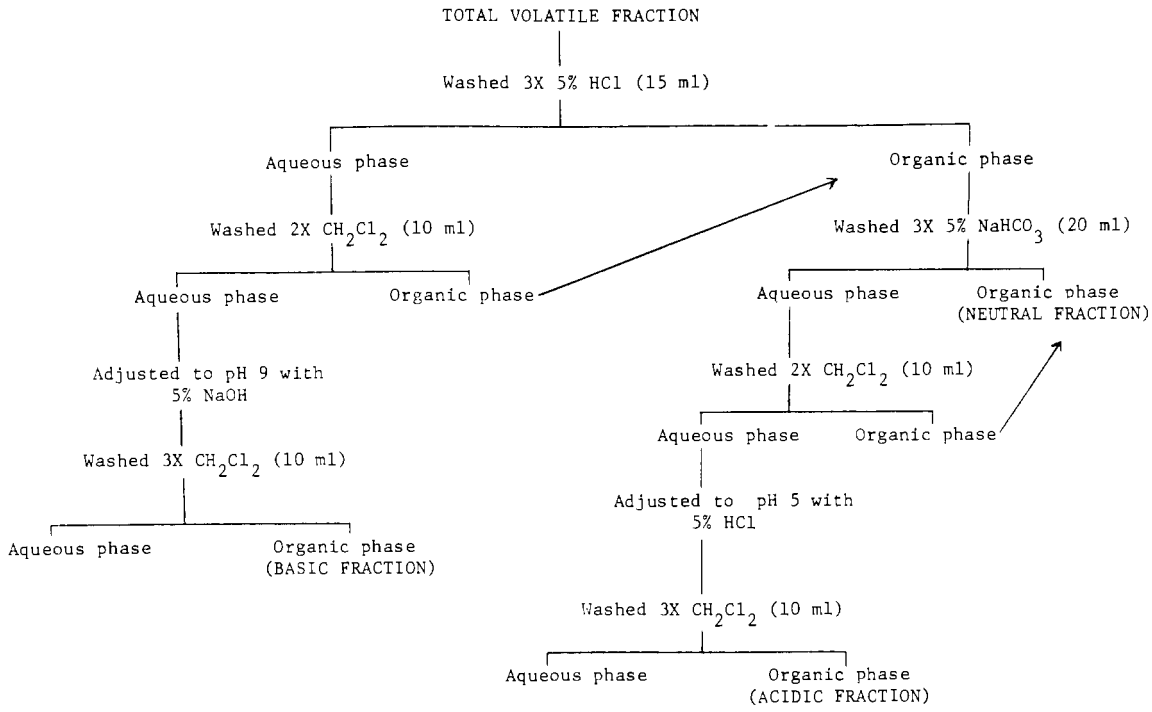

Figure 2. Scheme of separation of total volatile fraction into acidic, basic, and neutral fractions.

Table III. Possible Contributors to Lamb/Mutton Flavor

Aldehydes	Ketones	Lactones
Pentanal	2-Nonanone	γ-Octalactone
Hexanal	2-Dodecanone	
Heptanal	2-Tridecanone	
Octanal		
Nonanal		
2-Octenal		
2,4-Heptadienal		
2,4-Heptadienal (isomer)		
2,4-Decadienal		
2,4-Decadienal (isomer)		

canone (fruity), 2-dodecanone (waxy), and 2-tridecanone (oily). Siek et al. (1971) noted that C_7–C_{13} 2-alkanones had flavor thresholds ranging from 0.15 to 0.65 ppm in water.

γ- and δ-lactones have notable flavor significance in food products and have been used as flavoring components for over 20 years. Flavor descriptions of several γ-lactones isolated in this study were previously evaluated by Arctander (1969). They are: γ-heptalactone (sweet), γ-octalactone (oily), γ-nonalactone (musk), and γ-decalactone (fruity). Reported flavor thresholds (ppm) in water vary widely among compounds and investigators such as: γ-heptalactone, 0.52 (Siek et al., 1971); γ-octalactone, 0.04 (Keith and Powers, 1968), 0.095 (Siek et al., 1971), 0.007 (Buttery et al., 1971); γ-nonalactone, 0.065 (Siek et al., 1971); γ-decalactone, 0.09 (Keith and Powers, 1968), 0.088 (Siek et al., 1971).

The flavor significance of the other compounds identified in this study has yet to be determined.

Researchers appreciate the complex nature of lamb/mutton flavor. Recently, a group of branched and unsaturated, medium chain length (eight–ten carbons) fatty acids from the acidic fraction of cooked mutton have been implicated in the undesirable sheep-meat flavor (Wong et al., 1975a), especially 4-methyloctanoic acid (Wong et al., 1975b). However, these researchers also suggest other factors in the nonacid fraction of cooked fat volatiles contribute to mutton flavor. We suggest the neutral fraction and its constituents are significant contributors to lamb/mutton flavor and list (Table III) the 14 "key" compounds based on our GC–MS and olfactory analyses: 10 aldehydes, 3 ketones, and 1 lactone.

LITERATURE CITED

Alders, L., "Liquid-Liquid Extraction", Elsevier, New York, N.Y., 1955.

Amerine, M. A., Pangborn, R. M., Roessler, E. B., "Principles of Sensory Evaluation of Food", Academic Press, New York, N.Y., 1965.

Arctander, S., "Perfume and Flavor Chemicals", published by author, Montclair, N.J., 1969.

Badings, H. T., *Ned. Melk- Zuiveltijdschr.* **24**, 147 (1970).

Buttery, R. G., Seifert, R. M., Guadagni, D. G., Ling, L. C., *J. Agric. Food Chem.* **19**, 524 (1971).

Cramer, D. A., *N.M., State Univ., Exp. Stn., Bull.*, 616 (1974).

Dimick, P. S., Patton, S., Kinsella, J. E., Walker, N. J., *Lipids* 1, 387 (1966).

Dimick, P. S., Walker, N. J., *J. Dairy Sci.* **50**, 97 (1967).

Forss, D. A., in "Progress in the Chemistry of Fats and Other Lipids", XIII, Part 4, Pergamon Press, New York, N.Y., 1972, p 177.

Hoffmann, G., *J. Am. Oil Chem. Soc.* **39**, 439 (1962).

Hoffmann, G., Meijboom, P. W., *J. Am. Oil Chem. Soc.* **45**, 468 (1968).

Hornstein, I., Crowe, P. F., *J. Agric. Food. Chem.* 11, 147 (1963).

Jacobson, M., Koehler, H. H., *J. Agric. Food Chem.* 11, 336 (1963).

Keith, E. S., Powers, J. J., *J. Food Sci.* **33**, 213 (1968).

Park, R. J., Murray, K. E., Stanley, G., *Chem. Industry* 4 May, 380 (1974).

Siek, T. J., Albin, I. A., Sather, L. A., Lindsey, R. C., *J. Dairy Sci.* **54**, 1 (1971).

Sink, J. D., *J. Am. Oil Chem. Soc.* **50**, 470 (1973).

Sink, J. D., Caporaso, F., *Meat Sci.* 1, 119 (1977).

U.S. Department of Agriculture, "Foreign Agricultural Circular Livestock and Meat", FAS, FLM 2-76, 1976.

Van den Dool, H., Kratz, P. D., *J. Chromatogr.* 11, 463 (1963).

Wasserman, A. E., Talley, F., *J. Food Sci.* **33**, 219 (1968).

Watanabe, K., Sato, Y., *Agric. Biol. Chem.* **32**, 1318 (1968).

Watson, J. T., Biemann, K., *Anal. Chem.* **37**, 844 (1965).

Wong, E., Nixon, L. N., Johnson, C. B., *J. Agric. Food Chem.* **23**, 495 (1975a).
Wong, E., Johnson, C. B., Nixon, L. N., *N.Z. J. Agric. Res.* **18**, 261 (1975b).
Ziegler, J. H., Daly, M. J., *Proc. Recip. Meat Conf.* **21**, 168 (1968).
Ziegler, J. H., Miller, R. C., Stanislaw, C. M., Sink, J. D., *J. Anim. Sci.* **26**, 58 (1967).

Received for review February 9, 1977. Accepted July 18, 1977. Paper No. 5218 in the journal series of the Pennsylvania Agricultural Experiment Station.

Allyl Isothiocyanate and Allyl Cyanide Production in Cell-Free Cabbage Leaf Extracts, Shredded Cabbage, and Cole Slaw

Leslie G. West, Arthur F. Badenhop, and Jerry L. McLaughlin*

Using cell-free extracts, it was demonstrated that the myrosinase in cabbage leaves (*Brassica oleracea* L. var. *capitata L.*), responsible for hydrolyzing sinigrin to allyl isothiocyanate and allyl cyanide, had a pH optimum near neutrality; was activated by *l*-ascorbic acid by factors of 13.6 at pH 7.0 and 10.3 at pH 4.45 (the pH of cole slaw); and was activated by *d*-ascorbic acid and ascorbigen by factors of 6.0 and 2.9 at pH 4.45, respectively. Activation by *l*-ascorbic acid was terminated by oxidation with ferric ion. Cole slaw was studied for 22 days and allyl isothiocyanate concentration was found to be almost six times greater in the food product than in the shredded cabbage control. Allyl cyanide concentration in cole slaw was approximately one-tenth of the concentration of allyl isothiocyanate. Vitamin C levels in cole slaw were sufficiently high initially to activate the myrosinase, but they declined rapidly. The concentration of allyl isothiocyanate and allyl cyanide was found to be equal in shredded cabbage.

The effects of food processing on the enzymatic hydrolysis of glucosinolates present in cabbage to yield different types and abnormal amounts of natural flavorants and toxicants has received renewed interest (Daxenbichler et al., 1977). Such studies are of importance since the usual environment for the enzymatic formation of these secondary metabolites is considerably altered by the addition of the necessary ingredients to make food products.

As reported in this paper, procedures were developed to study cell-free cabbage leaf extracts to determine the properties of the cabbage myrosinase which hydrolyzes the natural glucosinolate, sinigrin; the concentrations of allyl isothiocyanate and allyl cyanide were quantitated in shredded cabbage and cole slaw. This work was initially prompted by customer complaints about variation in the flavor of commercially prepared cole slaw, and the well-known flavoring properties of these volatile components suggested closer examination of their formation.

EXPERIMENTAL SECTION

Preparation of Cell-Free Extracts. Fifty grams of dried and pulverized cabbage leaves (white cabbage purchased from local supermarkets and freeze-dried at 0 °C) were extracted with ice-cold acetone until no more color was removed. The defatted plant material was homogenized with 600 mL of pH 6.5 citrate phosphate buffer for 40 s in a Waring blender. The extract was expressed through tissue paper and centrifuged at 23 500*g* for 30 min. Control samples consisting of only the supernatant cell-free preparation or sinigrin in buffer revealed no hydrolysis products upon GLC analysis.

Department of Foods and Nutrition (L.G.W.), Department of Horticulture (A.F.B.), and Department of Medicinal Chemistry and Pharmacognosy (J.L.M.), School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907.

Procedure for Conducting Cell-Free Extract Experiments. One milligram-sinigrin samples were weighed using a Cahn Microbalance. Samples were transferred quantitatively to a microvial; 250 μL of cell-free extract was added. The vial was shaken gently to dissolve the sinigrin, and incubation was begun. When room temperature incubation for the desired time was complete, a minute quantity of the liquid was quickly removed with a small capillary, and the pH was checked using narrow range pH paper. Any sample in which the pH had changed during incubation was rejected.

Methylene chloride (250 μL) was added to the vial followed by either 50 μL or 100 μL (depending upon the particular experiment) of a known quantity of freshly distilled ethyl isothiocyanate (Aldrich Chemical Co.) in methylene chloride as the internal standard. The vials were all shaken at constant agitation for 3 min. After centrifugation for 5 min, the methylene chloride layer was removed with a glass syringe and placed in a microvial at 0 °C until GLC analysis.

For those experiments involving enzyme activators, aqueous solutions of the suspected activator were prepared in concentrations such that 50 μL added to the 250 μL of cell-free solution resulted in the desired concentration of activator without exceeding the buffering capacity of the extract.

GLC Analysis for Allyl Isothiocyanate and Allyl Cyanide. Two microliter portions of the methylene chloride extracts were subjected to GLC with a 6 ft × 0.25 in. × 2 mm glass column packed with 20% FFAP on Chromosorb W, H/P, 100/120 mesh size (Varian Instruments). A Varian Model 1400 gas chromatograph equipped with a flame ionization detector was used for the chromatography. The detector and injector temperatures were at 160 °C, and the column temperature was programmed starting at 60 °C and rising to 110 °C at a rate of 2 °C/min. The column was allowed to cool after