JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of the Aroma of a Meatlike Process Flavoring from Soybean-Based Enzyme-Hydrolyzed Vegetable Protein

YI-FANG G. WU† AND KEITH R. CADWALLADER*,‡

School of Human Ecology, Louisiana Tech University, Ruston, Louisiana 71272, and Department of Food Science and Human Nutrition, University of Illinois, 1302 West Pennsylvania Avenue, Urbana, Illinois 61801

Defatted soybean meal was converted into enzyme-hydrolyzed vegetable protein (E-HVP) using the proteolytic enzyme Flavorzyme. Total free amino acids increased by 40-fold after enzyme hydrolysis, with leucine being the most abundant, followed by phenylalanine, lysine, glutamine/glutamic acid, and alanine. Volatile components from a meatlike process flavoring made from E-HVP were isolated by direct solvent extraction (DSE)-high vacuum transfer (HVT), dynamic headspace sampling and static headspace sampling and analyzed by gas chromatography (GC)-mass spectrometry and GC-olfactometry. Aroma extract dilution analysis was used to establish a flavor dilution chromatogram of the DSE-HVT extract. Results of these complementary techniques indicated the importance of odorants of high (hydrogen sulfide and methanethiol), intermediate (2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-furanmethanethiol, and 3-(methylthiol)propanal) and low volatility (maltol and Furaneol) in the overall aroma of the meatlike process flavoring.

KEYWORDS: Soybean; enzyme-hydrolyzed vegetable protein; volatile; flavoring; aroma extract dilution analysis

INTRODUCTION

Thermally generated imitation meat flavors are often described in the patent literature as "process flavorings". Knowledge of the identities of the volatile aroma components of model systems that simulate the cooking of meat is important for developing process flavors with authentic meatlike qualities, especially for species specific flavors such as beef, chicken, and pork. The volatile components of cooked meat model systems have been studied by a number of researchers. Mussinan and Katz (*1*) identified a total of 24 and 15 sulfur-containing compounds in a hydrolyzed vegetable protein (HVP)/L-cysteine/D-xylose system and an L-cysteine/D-xylose system, respectively. Farmer et al. (*2*) found that the volatile compounds of a cysteine and ribose reaction mixture were dominated by sulfur-containing heterocyclic compounds such as 2-methyl-3-furanthiol, 2-furanmethanethiol, 2-thiophenethiol, and 3-mercapto-2-pentanone. Hofmann and Schieberle (*3*) studied the aroma components of a thermally treated aqueous solution of cysteine/ribose and found that 2-furanmethanethiol, 2-methyl-3-furanthiol, 2-thenyl mercaptan, and ethyl mercaptan were the main contributors to the overall roasty, meatlike aroma. However, the predominant aroma components of a thermally treated enzyme-HVP (E-HVP)/L-cysteine/D-ribose system have not been determined.

Wu et al. (*4*) correlated response surface methodology to sensory data to find the optimum reaction conditions for production of a meatlike process flavoring (chicken or beeflike flavor). Optimum conditions were pH 6, at 99 °C, for 1.5 h, using 5×10^{-4} mol of ribose and 5×10^{-4} mol of cysteine. In the present study, volatile compounds from a meatlike flavoring made from E-HVP were isolated by direct solvent extraction-high vacuum transfer (DSE-HVT), dynamic headspace sampling (DHS), and static headspace sampling (H) and analyzed by gas chromatography-mass spectrometry (GS-MS) and GC-olfactometry (GCO). Aroma extract dilution analysis (AEDA) was used to establish a flavor dilution (FD) chromatogram of DSE-HVT extract.

AEDA has been widely used to identify key odorants in foods (*5*−*7*). In AEDA, serial dilutions of a flavor extract are evaluated by GCO. The highest dilution at which an aroma active compound is detected is a measure of its potency and is referred to as the FD factor for that compound (*8*). However, this dilution technique is limited to the analysis of only intermediate and high-boiling components. To overcome this limitation, results of AEDA have been complemented by results of GCO of decreasing DHS and H volumes (*9*, *10*). The aims of the following study were (i) to apply AEDA, GCO-DHS, and GCO-H for the identification of predominant odorants and (ii) to relate amino acid composition and sensory aroma profiles to predominant odorants in a meatlike process flavoring made by thermal reaction of a mixture of E-HVP, L-cysteine, and D-ribose.

MATERIALS AND METHODS

Chemicals. Flavorzyme was purchased from Novo Nordisk Bioindustrials, Inc. (Danbury, CT). L-Cysteine and D-(−)-ribose were from

* To whom correspondence should be addressed. Tel.: 217-333-5803. Fax: 217-333-1875. E-mail: cadwlldr@uiuc.edu.
† Louisiana Tech University.
‡ University of Illinois.

10.1021/jf0114076 CCC: $22.00
Published on Web 04/04/2002

Sigma Co. (St. Louis, MO). All other chemical reagents were obtained from Sigma or Fisher Scientific (Pittsburgh, PA). The authentic aroma compounds (listed in **Tables 2** and **3**) were purchased from commercial sources: **1**−**4**, **6**−**9**, **11**, **12**, **18**, **20**, **23**, **27**, **28**, **31**−**33**, **36**, **39**, **40**, **42**, **44**, and **47** were from Aldrich Chemical Co. (St. Louis, MO); **19** and **46** were from Lancaster Synthesis, Inc. (Windham, NH). 2-Acetyl-1-pyrroline (**21**) was obtained from Dr. R. Buttery (USDA, ARS, WRRC, Albany, CA). *β*-Damascenone (**37**) was a gift from Firmenich Inc. (Princeton, NJ). 3-Mercapto-2-butanone (**17**) and 2-thenyl mercaptan (**34**) were synthesized as described by Hofmann and Schieberle (*3*); 3-mercapto-2-pentanone (**22**) and (*Z*)-1,5-octadien-3-one (**24**) were synthesized as previously described (*11*, *12*), respectively.

Samples. Defatted soybean meal (DSM, protein content 48%) was obtained from Archer Daniels Midland (Clarksdale, MS). Preparation of E-HVP was conducted as previously described (*4*). The product made under optimum reaction conditions was designated process flavoring R4. An unheated R4 (UR4) also was prepared. The general reaction procedure for preparation of the process flavoring was described in a previous paper (*4*).

Content of Free Amino Acids. *Sample Preparation.* A 3 g sample (in duplicate) of either E-HVP or autoclaved DSM (ADSM) plus 4.2 mg of norvaline (internal standard) was homogenized for 2 min in 25 mL of 6% (w/v) aqueous perchloric acid. The sample was centrifuged at 3000*g* for 30 min and then vacuum-filtered (Whatman 40 filter paper, Fisher). The precipitate was resuspended and homogenized for 2 min in 15 mL of 6% (w/v) aqueous perchloric acid, centrifuged at 3000*g* for 15 min, and then vacuum-filtered as above. The combined filtrates were neutralized with aqueous 1 M KOH, and the solution was vacuum-filtered (Whatman 40) and freeze-dried. The freeze-dried sample (200 mg) was dissolved in 2 mL of dry methanol, and then, 600 μL of acetyl chloride was slowly added. The solvent was evaporated under a stream of nitrogen. The solid residue was mixed with 1 mL of trifluoroacetic anhydride and 1 mL of dichloromethane (CH_2Cl_2), and the mixture reacted for 1 h at 75 °C. The solvent was evaporated under a gentle stream of nitrogen. The residue was dissolved in 200 μL of CH_2Cl_2 and stored at −20 °C until analyzed by GC-MS (*13*, *14*).

Quantification of Free Amino Acids. Amino acids were identified based on comparison of their retention times (RT), the relative intensities of their characteristic *m*/*z*, and mass spectra with those of authentic reference compounds analyzed under identical experimental conditions. Quantification of the individual amino acids was done using calibration curves of amount ratios (amino acid/norvaline) vs peak area ratios (amino acid/norvaline) generated under identical experiment conditions. The concentration of an amino acid in the sample was calculated as follows:

$$\text{concentration (mg/g)} = \frac{X \times 4.2 \text{ mg of norvaline}}{3\text{g}}$$

where X is the amount ratio calculated using the appropriate calibration equation for each amino acid.

DSE-HVT. The process flavoring sample (100 mL) plus 20 mL of redistilled CH_2Cl_2 and 9.82 μg of internal standard (100 μL of a 0.0982 mg/mL solution of 3-heptanol in CH_2Cl_2) were added to a glass 250 mL centrifuge bottle equipped with a PTFE-lined cap (Fisher). The bottle (positioned horizontally) was shaken at 23 °C at medium speed on an orbital shaker (Bellco Biotechnology Co., Vineland, NJ) for 30 min and then centrifuged at 3500*g* for 30 min in a Sorval RC-5B centrifuge (Dupont, Wilmington, DE). After centrifugation, the solvent (CH_2Cl_2) layer (bottom) was collected and the extraction procedure was repeated twice as above, except that the volume of solvent was 10 mL. The CH_2Cl_2 layers were pooled and stored in a −20 °C freezer overnight to facilitate removal of excess water as ice crystals. The extract was dried over 10 g of anhydrous sodium sulfate. Before analysis, it was necessary to separate volatile material from nonvolatile residue by HVT as follows: the sample (about 40 mL) was placed in a 250 mL round-bottom flask and immersed in a Dewar containing liquid nitrogen until the sample was frozen. The flask was connected to a rough pump/diffusion pump vacuum system fitted with a receiving tube and waste tube. The unit was similar to the apparatus described by Sen et al. (*15*). The high vacuum connector consisted of a HI-VAC valve (Kontes, Vineland, NJ). The receiving and waste tubes were immersed in Dewars containing liquid nitrogen throughout the distillation period. The sample was maintained at room temperature (~23 °C) and high vacuum (5×10^{-5} Torr) until most of the solvent was evaporated (2 h) and then heated to 60 °C and the distillation continued for an additional 2 h. After completion of the distillation, the HI-VAC valve was closed. The system was removed from the vacuum source and vented via the HI-VAC valve. Volatile extract was recovered from the receiving tube and then dried over anhydrous sodium sulfate (10 g).

DSE-HVT extract was concentrated to l mL under a gentle stream of nitrogen. A 100 μL aliquot of the aroma extract was serially diluted (1:3 ratio; i.e., one part extract + two parts of redistilled CH_2Cl_2) for AEDA. The remaining 900 μL of the extract was further concentrated to 25 μL under a gentle stream of nitrogen for analysis by GC-MS. DSE-HVT extracts were prepared in duplicate and stored in amber 2 mL glass vials equipped with PTFE-lined screw caps at −20 °C until analyzed.

GC-MS. GC-MS was performed with a Hewlett-Packard (HP) 5890 series II GC/HP 5972 mass selective detector system (Hewlett-Packard, Palo Alto, CA). Two microliters of each extract was injected (splitless mode; 200 °C injector temperature; 30 s valve delay) into a 60 m × 0.25 mm i.d. × 0.25 μm film thickness (d_f) DB-5MS capillary column (J & W Scientific, Folson, CA) for analysis of the amino acid extracts. One microliter of each DSE-HVT extract was injected into a 60 m × 0.25 mm i.d. × 0.25 μm d_f DB-WAX or DB-5MS capillary column (J & W Scientific) in the splitless injection mode (200 °C injector temperature). The oven temperature was programmed from 40 to 200 °C at a rate of 3 °C/min with initial and final hold times of 5 and 60 min, respectively. Helium was the carrier gas at a constant flow rate of 1.0 mL/min (linear velocity = 25.0 cm/s). The MSD interface temperature was 280 °C, ionization voltage was set at 70 eV, mass range was 33−350 amu, and electron multiplier (EM) voltage was 200 V above the Autotune value. Injections were performed in duplicate.

AEDA. The FD factor for a compound was defined as the ratio of its concentration in the initial extract to its concentration in the most dilute extract in which odor was detected by the GCO (*16*). The data were summarized by generation of an FD chromatogram, which is a plot of FD factors for the odor active substances against their retention indices (RI). The FD chromatogram presented herein was that of one expert panelist.

Serial dilutions (1:3) of DSE-HVT extracts were analyzed on a GCO system consisting of a Varian 3300 (or 3400) GC (Varian Instrument Group, Walnut Creek, CA) equipped with a flame ionization detector (FID) and a sniffing port. One microliter of each sample was injected (splitless mode, 200 °C injector temperature) into a 30 m × 0.32 mm i.d. × 0.25 μm d_f DB-WAX or DB-5MS capillary column (J & W Scientific). The oven temperature was programmed from 40 to 200 °C at a rate of 10 °C/min with initial and final hold times of 5 and 30 min, respectively. FID and sniffing port transfer line temperatures were 250 °C.

DHS GC-MS. Analysis of the dynamic headspace samples was performed with an HP 5890 series II GC connected to a Tekmar 3000 Purge and Trap (P&T) concentrator/cryofocusing module (Tekmar Co., Cincinnati, OH). Process flavoring sample R4 (1 mL) or UR4 (1 mL) was placed in a 25 mL headspace sampling tube (15.2 cm × 1.6 cm i.d.). The sample was preheated at 60 °C for 5 min. The volatiles were purged at 60 °C with helium (40 mL/min) for 0.25, 1.25, or 6.25 min, which corresponded to purge volumes of 10, 50, and 250 mL, respectively. Volatiles were trapped onto a Tenax-TA trap (Tekmar Co.) maintained at 0 °C. After sample purging was completed, the trap was dry purged for 5 min to remove moisture. Trapped volatiles were then thermally desorbed (180 °C for 1 min) and subsequently cryofocused at −120 °C onto a 15 cm section of 0.53 mm i.d. deactivated fused silica capillary column. Transfer lines and valves were maintained at 175 °C. Trap pressure control was set at 4 psi during purging. Helium flow, passing through a Tenax trap at 20 mL/min and a cryofocusing trap at 1.4 mL/min during thermal desorption, was controlled by the split/splitless electronic pressure control pneumatics of the GC. Cryofocused volatiles were desorbed (180 °C for 1 min) directly into the analytical GC column. All other GC and GCO

conditions were as described above. A fresh sample was used for each analysis. Between each run, the system was cleaned by performing a blank run in which clean glassware was purged and the Tenax TA trap subsequently baked at 225 °C for 10 min. The DHS GC-MS system was the same as the GC-MS system previously described except that injection was performed by DHS system. Prepurge time and purge time were 2 and 20 min, respectively, for DHS GC-MS.

GCO of Static Headspace Samples (GCO-H). Process flavoring sample R4 (1 mL) or UR4 (1 mL) was placed into a 250 mL round-bottom flask, sealed with a septum, and incubated for 20 min in a 60 °C water bath. Headspace volumes (0.2, 1, or 5 mL) were withdrawn from the flask using a preheated (80 °C) gastight syringe and then immediately injected at a velocity of 5 mL/min into an HP 5890A GC equipped with a packed column inlet modified for capillary GC injection (Uniliner Sleeve Adaptor, Restek Corp., Bellefonte, PA). During injection, a 15 cm section of precolumn (0.53 mm i.d. deactivated fused silica capillary) was cooled in liquid nitrogen in order to cryofocus the volatiles at the head of the column. After injection was complete, the column section was removed from the liquid nitrogen and the GC was heated rapidly. The run was started when oven temperature reached 40 °C. The capillary columns used for GCO-H were 30 m × 0.53 mm i.d. × 1 μm d_f DB-WAX (J & W Scientific) and 30 m × 0.53 mm i.d. × 1.5 μm d_f DB-5MS (J & W Scientific). A fresh sample was used for each analysis. Other GC conditions were the same as previously described above for GCO-DHS.

Identification of Aroma Compounds. Positive identifications of volatile components were made by matching RI (*17*), mass spectra, and aroma properties of unknowns with those of authentic standard compounds analyzed under identical experimental conditions. Tentative identifications were based either on standard MS library information (Wiley 138K Mass Spectral Database, Wiley and Sons, 1990) or by comparison of RI values and odor properties of unknowns with those of authentic standards.

Sensory Evaluation and Statistical Analysis. Panelists (8 men and 2 women) were selected based on interest and time availability and were university employees and students ranging in age from 22 to 65 years. Sensory evaluation was done according to the procedure of Wu et al. (*4*). Each sample (R4 and UR4) was prepared in duplicate. Panelists were instructed to evaluate the two sets of samples with a 3 min break between each set. Differences between samples for each sensory attribute were evaluated by paired samples *t*-test procedure. All analyses were performed using SAS, Version 7 (*18*).

RESULTS AND DISCUSSION

Free Amino Acid Composition. The free amino acid composition of ADSM before and after (E-HVP) enzyme hydrolysis is given in **Table 1**. The content of total free amino acids in ADSM was low (2.94 mg/g) and only asparagine/aspartic acid and glutamine/glutamic acid were detected. After enzyme hydrolysis, the total free amino acids increased in concentration to 129.6 mg/g, indicating that a high degree of DSM proteolysis is brought about by the use of Flavorzyme.

Leucine (26.63 mg/g) was the most abundant free amino acid found in E-HVP, comprising over 20% of total free amino acids. Other grains such as maize, sorghum, and millet also contain high levels of leucine (*19*). Other abundant (>10% of the total) amino acids found in E-HVP included phenylalanine and lysine, which collectively accounted for 24% of the total free amino acids. These amino acids may play important roles in process flavorings, since potent odorants 3-methylbutanal and phenylacetaldehyde can arise from Strecker degradation of leucine and phenylalanine, respectively (*20*, *21*). Lysine can undergo thermal reactions leading to formation of alkylpyrazines and 2-acetyl-1-pyrroline (*22*). The remaining amino acids were at lower concentrations. Methionine was the only sulfur-containing amino acid detected. Despite its low level, methionine may have an important influence on Maillard type flavors since its breakdown via Strecker degradation yields numerous sulfur-containing compounds, such as dimethyl disulfide, dimethyltrisulfide, 3-(methylthio)propanal, and methanethiol (*22*).

Table 1. Composition of Free Amino Acids in ADSM and E-HVP

		concn (mg/g)[a]	
no.	amino acid	ADSM[b]	E-HVP[c]
1	alanine	ND[d]	11.86
2	glycine	ND	3.43
3	threonine	ND	5.11
4	valine	ND	6.43
5	serine	ND	7.97
6	leucine	ND	26.63
7	isoleucine	ND	1.18
8	cysteine	ND	ND
9	aspartic acid/asparagine	2.10	4.45
10	proline	ND	5.03
11	hydroxy proline	ND	ND
12	glutamic acid/glutamine	0.83	12.30
13	methionine	ND	5.32
14	phenylalanine	ND	17.79
15	tyrosine	ND	8.76
16	lysine	ND	13.34
17	arginine	ND	ND
18	cystine	ND	ND
	total	2.94	129.60

[a] Concentration on wet basis. [b] ADSM, autoclaved DSM. [c] E-HVP. [d] ND, amino acid was not detected.

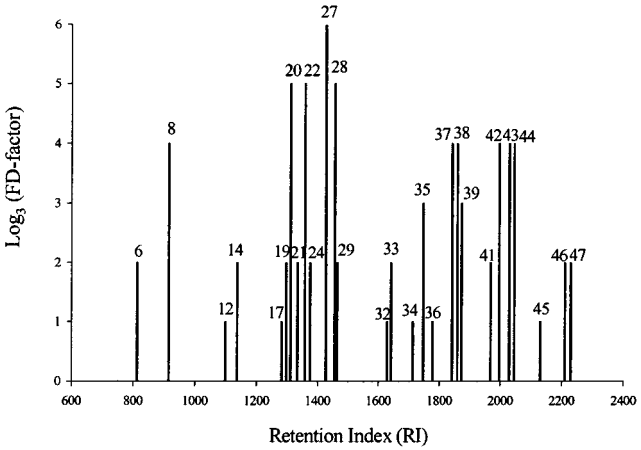

Figure 1. FD chromatogram of volatile flavor compounds in DSE-HVT extract (analysis was performed on DB-5ms column; peak numbers correspond to those in **Table 2**).

Aroma Compounds Isolated by DSE-HVT. A combined total of 47 odorants were detected by all techniques in the process flavoring sample (R4) and are shown in **Tables 2** and **3**. Of these, 33, 22, and 21 odorants were found by GCO of AEDA, GCO-DHS, and GCO-H, respectively. Additional experiments involving GCO-DHS and GCO-H were performed in order to directly compare the aroma impact components of the heated process flavoring R4 and the unheated UR4.

Results of AEDA are shown in **Figure 1**. The FD chromatogram (**Figure 1**) provides a general idea of the odor intensity of each aroma compound in R4. 2-Furanmethanethiol (2-furfurylthiol, **27**) was the most intense aroma active compound in R4 ($\log_3$FD factor = 6), followed by 2-methyl-3-furanthiol (**20**), 3-mercapto-2-pentanone (**22**), and 3-(methylthiol)propanal (methional, **28**) ($\log_3$FD factor = 5). 2-Furfurylthiol was previously described as sulfurous/coffeelike and was dominant in boiled yeast extract (*23*). Moreover, Hoffmann and Schieberle (*24*) demonstrated that under dry heating, cysteine in the presence of glucose resulted in a decreased generation of

2-furfurylthiol but an increased generation with ribose present, indicating that different mechanisms were involved in the formation of odorants with these two carbohydrates. Previously, 2-furfurylthiol and 2-methyl-3-furanthiol were shown to be important odorants in commercial meat flavoring, as well as cooked beef, chicken broth, and roasted coffee (*7*, *25*−*27*). 2-Methyl-3-furanthiol has an extremely low odor threshold of 0.005−0.01 ppb (*6*), which is probably why it was detected by GCO despite its low abundance. 3-Mercapto-2-pentanone exhibited a catty/urine odor in a thermally treated mixture of cysteine and carbohydrates (*24*). As compared to the ribose/cysteine mixture, the glucose/cysteine and rhamnose/cysteine mixtures led to a decrease in the flavor contribution of 3-mercapto-2-pentanone in aqueous solution and under dry-heating conditions (*24*). The last compound in this category, methional, had a low odor detection threshold of 0.2 ppb (*28*). Methional was described as having a pleasant cooked potato-like aroma. This compound can be formed via the Maillard reaction (specifically Strecker degradation) from methionine (*29*) and is also formed in milk when exposed to light (*30*).

In addition to the above, other potent odorants with high $\log_3$FD factors (= 4) included 3-methylbutanal (**8**), β-damascenone (**37**), 3-hydroxy-2-methyl-4-pyrone (maltol, **42**), 4-hydroxy-2,5-dimethy-3(2*H*)-furanone (Furaneol, **44**), and two unknowns (**38** and **43**). 3-Methylbutanal, with a dark chocolate aroma, had been previously identified in Arabica coffee (*7*) and in cod and trout (*9*). β-Damascenone, with a characteristic tea/applesauce aroma, has a low odor threshold in water (2pg/g) (*31*). This compound has been found to predominate in diverse foods such as fresh raspberries (*32*), tomato paste (*31*), grapes (*33*), apples (*34*), and drinks such as coffee, black tea, and beer (*15*, *35*). In natural products such as grapes, β-damascenone is thought to be formed from the hydrolysis of glycosides. The same phenomenon is suspected in heated tomato products and has been reported in heat-treated black tea (Darjeeling) infusions (*31*, *36*). Furaneol had a strawberry-like note at low concentrations and exhibited a burnt sugarlike note at higher concentrations. It was reported to be a character impact aroma of pineapple (*37*), strawberry (*38*), and muscadine grape juice (*39*). Furaneol has been found in heated foods such as beef broth and stewed beef (*40*, *41*).

Compounds **35** and **39** were detected at a $\log_3$FD factor of 3. Guaiacol (**39**), characterized by a smoky aroma, has a very low threshold value, 0.02 ppt in water, (*42*) and contributes to the odor character of tequila and lychee (*43*, *44*).

Ten odorants had $\log_3$FD factors of 2. 2-Methylpropanal (**6**) eluted very early during GCO and imparted a dark chocolate odor. The lipid-derived odorant, 1-octen-3-one (**19**), has been reported to be a predominant odor active compound in alligator tail meat aroma (*45*). 2-Acetyl-1-pyrroline (**21**) contributed a popcornlike note. It was demonstrated that 2-acetyl-1-pyrroline is a thermally generated compound from the reaction of certain carbonyls with proline and lysine (*22*) or from reaction of 2-oxopropanal with either proline or ornithine (*46*). This compound has been identified as a character impact odorant in numerous cooked foods such as cooked rice (*47*), bread (*5*), cooked blue crab (*48*), and cooked tail meat of spiny lobster (*49*). Phenylacetaldehyde (**33**) was associated with a floral, honeysuckle aroma character and is known to be a Strecker degradation product of the amino acid phenylalanine (*21*, *23*). Odorant **46** was identified as *p*-vinyl guaiacol and had a cloves/curry aroma note. This compound was considered to be an off-flavor in orange juice (*50*). *o*-Aminoacetophenone (**47**) had a nonequivocal MS signal and was tentatively identified by matching its RI and odor quality with those of the standard compound and had a grape, sweet, and foxy aroma note. This compound was hypothesized to be important in the aroma of muscadine grape juice (*39*) and has been identified as causing a undesirable flavor in fermented tuna sauce (*51*) and milk products (*52*, *53*).

Compounds **12**, **17**, **32**, **34**, **36**, and **45** were detected at a $\log_3$FD factor of 1. The compounds 3-mercapto-2-butanone (**17**) and 2-thenyl mercaptan (**34**) were characterized by rubber/sour/meaty and stewed meat/vitamin aroma notes, respectively. Both compounds were found in thermally treated ribose/cysteine mixtures (*24*). 2-Acetyl-2-thiazoline (**36**) was first identified in roast beef and overcooked beef (*54*). This compound, with its roasty, popcornlike odor, has recently been indicated as a potent odorant in several processed meat products including chicken broth (*27*), roast beef (*55*), stewed beef (*41*), and boiled trout (*56*). It was reported that 2-acetyl-2-thiazoline is formed from the reaction of 1-mercapto-2-aminoethane (cysteamine) and 2-oxopropanal (methylglyoxal) via 2-(1-hydroxyethyl)-4,5-dihydrothiazole as the key intermediate (*57*).

The last group of aroma compounds is listed in **Table 2**. All had $\log_3$FD factors less than 1 and were only detected in the concentrated sample extract. 2,3-Butanedione (**9**) and (*E*,*Z*)-2,6-nonadienal (**31**) had buttery and stale/hay/cucumber aroma properties, respectively. 2,3-Butanedione, with a threshold of 2.6 ppb in water (*58*), is a characteristic component of cooked foods and is thermally generated through the Maillard reaction (*59*). Lipid-derived compound (*E*,*Z*)-2,6-nonadienal is often found in seafood and can be derived from Ω-3 fatty acids (*60*). The odor description of hexanal (**11**) was green/grassy. Cadwallader et al. (*61*) reported that this compound contributed to off-odor of alligator meat. 2-Phenylethanol (**40**) was described as floral/rosey. This compound contributes to muscadine juice aroma (*39*) and was important in apricot (*62*).

Aroma Compounds in the Headspace of Process Flavoring R4 and Unheated R4 (UR4). In general, DSE-HVT is a good method to isolate aroma compounds of intermediate and low volatility. However, because this technique requires solvent extraction, distillation, and evaporation steps, volatile components, as well as their relative abundance, could be changed, and some highly volatile components might be lost altogether. Application of GCO on decreasing H and DHS of the sample can help overcome these problems.

A total of 22 and 16 odorants were detected by GCO-DHS in R4 and UR4 (**Table 3**). In GCO-DHS (or GCO-H), the odorants detected in the lowest headspace volume should have a greater odor impact than those found only in the higher headspace volumes. A total of 11 predominant odorants (**2**−**5**, **8**, **9**, **19**, **23**, **27**, **28**, and **34**) were detected in R4, while only 4 (**2**, **5**, **23**, and **27**) were detected in UR4. These results indicated that some odorants are already present in UR4 and that the total numbers and intensities of odorants are increased during heating.

With the exception of **6**, **8**, **9**, **19**, **21**, **22**, **27**, **28**, and **34**, the other compounds were not detected by AEDA of DSE-HVT extracts. Methanethiol (**2**) and acetaldehyde (**3**) were described as having rotten/sulfurous and solvent/sweet aroma notes, respectively. Methanethiol and acetaldehyde were also reported in cod (*9*) and stewed beef juice, with odor detection threshold values of 0.2 and 25 ppb in water, respectively (*41*). Dimethyl sulfide (**4**) had a cornlike note and was identified in cod along with methanethiol and acetaldehyde (*9*). 2-Methylbutanal (**7**) had a similar dark chocolate-like odor note as 2-methylpropanal (**6**) and 3-methylbutanal (**8**). Dimethyltrisulfide (**23**), one of the sulfur-containing compounds identified in this study, imparted

Table 2. Aroma Active Compounds Detected during Aroma Extraction Dilution Analysis (AEDA) of Process Flavoring Sample R4

no.[a]	compd name	RI[b] DB-WAX	RI[b] DB-5MS	odor description
6	2-methylpropanal[d]	813	<600	dark chocolate
8	3-methylbutanal[c]	915	652	dark chocolate
9	2,3-butanedione[d]	980	608	buttery
11	hexanal[c]	1078	803	green, grass
12	3-methyl-thiophene[c]	1099	770	sour, plastic water bottle
14	unknown	1137	ND[e]	sour, rubber, stale
17	3-mercapto-2-butanone[d]	1282	817	rubber, sour, meaty
19	1-octen-3-one[d]	1297	981	mushroom
20	2-methyl-3-furanthiol[d]	1311	868	vitamin, meaty
21	2-acetyl-1-pyrroline[d]	1334	925	popcorn
22	3-mercapto-2-pentanone[d]	1358	903	catty, urine
24	(*Z*)-1,5-octadien-3-one[d]	1375	984	metallic
27	2-furanmethanethiol[c]	1427	912	coffee, sulfurous
28	3-(methylthiol)propanal[c]	1456	910	potato
29	unknown	1464	1110	roasted potato
30	unknown	1555	1055	sulfurous, stewed meat
31	(*E*,*Z*)-2,6-nonadienal[d]	1579	1150	stale, hay, cucumber
32	2-acetyl-3-methylpyrazine[d]	1628	1080	burnt
33	phenyl acetaldehyde[c]	1641	1050	floral, honeysuckle
34	2-thenyl mercaptan[d]	1713	1107	stewed meat, vitamin
35	unknown	1747	1175	saffron rice, chicken broth
36	2-acetyl-2-thiazoline[d]	1778	1118	popcorn
37	β-damascenone[d]	1842	1389	ice tea, apple sauce
38	unknown	1859	n.d.	stewed chicken
39	guaiacol[c]	1873	1102	smoky
40	2-phenylethanol[c]	1919	1116	floral, rosey
41	unknown	1968	n.d.	stewed meat
42	maltol[cf]	1996	1172	burnt sugar, sweet hay
43	unknown	2029	1415	unripe apple, green, sour
44	Furaneol[cg]	2045	1055	burnt sugar, strawberry
45	unknown	2130	n.d.	burnt sugar
46	*p*-vinyl guaiacol[c]	2210	1350	cloves, curry
47	*o*-aminoacetophenone[d]	2229	1308	grape, sweet, foxy

[a] Numbers correspond to those in **Table 3** as well as **Figure 1**. [b] RIs calculated from GCO results. [c] Compound positively identified. [d] Compound tentatively identified by matching its RIs and odor quality with those of standard compound. [e] Not detected by GCO. [f] Maltol, 3-hydroxy-2-methyl-4-pyrone. [g] Furaneol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone.

a cabbagelike aroma. This compound was also found in boiled crayfish tail meat and other crustaceans and was considered to have a negative impact on seafood aroma (*63*). Because of possible losses from irreversible adsorption or degradation in the purge-and-trap system, the GCO-DHS technique was unable to detect 2-methyl-3-furanthiol (**20**) and β-damascenone (**37**), which were detected as potent odorants by both AEDA and GCO-H.

With the exception of compounds **20** and **37**, results of GCO-H were similar to those of GCO-DHS. A total of 21 and 11 odorants were detected by GCO-H in R4 and UR4, respectively (**Table 3**). The most intense odorants detected in R4 were compounds **1**−**3**, **8**, **19**, **20**, **22**, **27**, **28**, and **37**. Compounds **1**, **2**, **23**, and **27** were the only predominant odorants in UR4. These results confirmed the findings of GCO-DHS that heating increased the total number and intensities of aroma compounds in the process flavoring.

Relationship and Changes in Sensory Profiles and Aroma Active Compounds by Heat Processing. Heating of process flavoring sample R4 increased the aroma intensity of six of the 10 attributes (including overall aroma intensity), with statistically significant differences notable in four (**Figure 2**). However, the aroma intensities of four of the 10 attributes decreased after heat treatment, with statistically significant differences noted in two of these, although each of these attributes was inappropriate for desired headspace of a meatlike process flavoring (**Figure 2**). Therefore, heat is a necessary factor for generation of the highest overall aroma acceptability for process flavoring R4 and elicits more intense odor compounds in the process flavoring confirmed by DHS and H data, resulting in a more complex flavor profile (Maillard type flavors) as compared to UR4. A comparison of sensory profile and headspace aroma analysis (DHS, H) before and after heating suggests that for the attributes that decreased intensity after heating, the increase in the overall intensity and headspace volatile complexity tended to mask the attributes, such as applesauce-like (β-damascenone), which may not have actually decreased following heating but perhaps was simply dominated by other aroma notes.

Use of the three aroma isolation techniques (DSE-HVT, DHS, H) coupled with the results of GCO demonstrated that compounds of low, intermediate, and high volatility contributed to the overall aroma of the meatlike process flavoring. The descriptive aroma profiles of R4 and UR4 for each attribute are shown in **Figure 2**. Specific aroma compounds can be related to the sensory descriptive terms generated in our previous report (*4*). For example, H_2S and 3-(methylthiol)propanal might be responsible for the egglike and potato-like aroma terms, respectively. Furthermore, the burnt sugar notes of maltol and Furaneol might contribute to the terms beany and molasses-like. Presence of 2-methyl-3-furanthiol and 2-furanmethanethiol probably contributed to the terms chickeny, beefy, and roasted. Similarly, the Brussels sprout term might have been due to the presence of dimethyltrisulfide, while the term applesauce-like could have been due to β-damascenone and an unknown compound **43**, which were described as tea/applesauce and unripe apple/green/sour, respectively. A sensory evaluation of process flavoring R4 revealed that chicken and beef odor notes contributed to the overall aroma quality (**Figure 2**). These data

Table 3. Aroma Active Compounds Detected by GC-Olfactometry of Decreasing Dynamic (Purge Volume) and Static (Headspace Volume) Headspace Samples of Process Flavoring Sample R4 and Unheated Process Flavoring Sample R4 (UR4)

		RI[b]			purge volume (mL)[c]		headspace volume (mL)[d]	
no.[a]	compd name	DB-WAX	DB-5MS	odor description	R4	UR4	R4	UR4
1	hydrogen sulfide[f]	<600	<600	cooked egg	50	50	0.2	0.2
2	mMethanethiol[e]	643	<600	rotten, garbage	10	10	0.2	0.2
3	acetaldehyde[e]	677	<600	solvent, sweet	10	250	0.2	ND[h]
4	dimethyl sulfide[e]	720	<600	corn	10	50	1	ND
5	unknown	757	<600	gasoline, pungent, sulfurous	10	10	ND	ND
6	2-methylpropanal[g]	812	<600	dark chocolate	50	250	1	1
7	2-methylbutanal[e]	908	661	dark chocolate	50	ND	1	5
8	3-methylbutanal[e]	912	651	dark chocolate	10	250	0.2	ND
9	2,3-butanedione[g]	981	608	buttery	10	ND	1	ND
10	unknown	1044	ND	sour, rubbery	250	ND	5	ND
13	unknown	1114	ND	sour, rubbery, skunky	50	50	5	1
15	unknown	1145	ND	sour, rubbery, gasoline	50	50	1	1
16	unknown	1171	ND	rancid, stale, crabby, rotten	50	50	ND	ND
18	octanal[c]	1289	1007	sweet, winelike	50	ND	ND	ND
19	1-octen-3-one[g]	1302	979	mushroom	10	50	0.2	1
20	2-methyl-3-furanthiol[g]	1310	869	vitamin, meaty	ND	ND	0.2	5
21	2-acetyl-1-pyrroline[g]	1336	923	popcorn	50	250	1	ND
22	3-mercapto-2-pentanone[g]	1360	ND	catty, urine	50	ND	0.2	ND
23	dimethyltrisulfide[g]	1380	964	cabbage	10	10	1	0.2
25	unknown	1418	ND	burnt agar	50	ND	ND	ND
26	unknown	1411	1083	burnt, burnt agar	ND	ND	5	ND
27	2-furanmethanethiol[g]	1430	915	coffee, sulfurous	10	10	0.2	0.2
28	3-(methylthiol)propanal[g]	1455	908	potato	10	50	0.2	1
31	(*E*,*Z*)-2,6-nonadienal[g]	1579	1150	stale, hay, cucumber	n.d.	n.d.	5	ND
34	2-thenyl mercaptan[g]	1710	1105	stewed meat, vitamin	10	50	ND	ND
37	β-damascenone[g]	1840	1388	ice tea, applesauce	ND	ND	0.2	ND

[a] Numbers correspond to those in **Table 2** as well as **Figure 1**. [b] RIs calculated from GCO-DHS or GCO-H results. [c] Lowest purge gas volume required for compound detection by GCO. [d] Lowest static headspace volume required for compound detection by GCO. [e] Compound positively identified. [f] Compound tentatively identified by matching its retention time and odor quality with those of standard compound. [g] Compound tentatively identified by matching its RI and odor quality with those of standard compound. [h] Not detected.

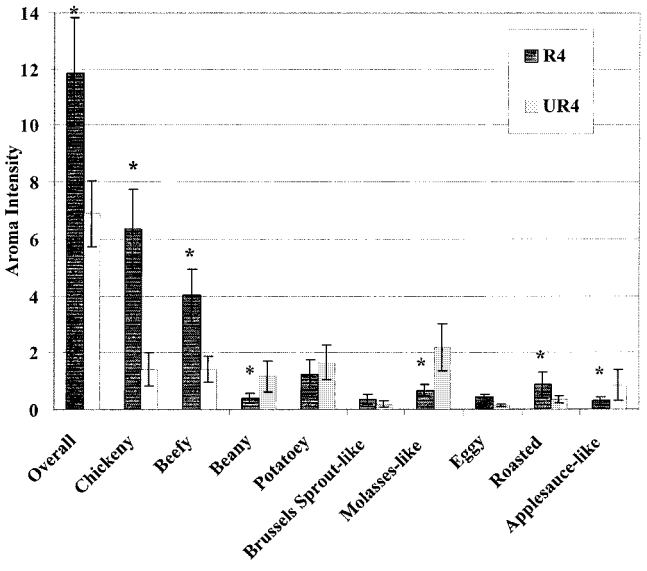

Figure 2. Descriptive aroma profiles of R4 (10% E-HVP plus 5×10^{-4} mol of ribose and 5×10^{-4} mol of cysteine, pH 6, heated at 99 °C for 1.5 h) and unheated R4 (UR4). The asterisk indicates the significant difference ($p < 0.05$) between R4 and UR4. Vertical bars represent standard errors.

were consistent with that obtained by AEDA, DHS, and H for R4 process flavoring. Results are based on the consistently high FD factors of 2-methyl-3-furanthiol (**20**), 2-furanmethanethiol (**27**), and an unknown odorant **38**, which elicited vitamin/meaty, coffee/sulfurous, and stewed chicken attributes, respectively.

ABBREVIATIONS USED

E-HVP, enzyme-hydrolyzed vegetable protein; DSE-HVT, direct solvent extraction-high vacuum transfer; DHS, dynamic headspace sampling; H, static headspace sampling; GC-MS, gas chromatography-mass spectrometry; GCO, gas chromatography-olfactometry; AEDA, aroma extract dilution analysis; FD, flavor dilution; DSM, defatted soybean meal; UR4, unheated process flavoring R4; ADSM, autoclaved DSM; RT, retention time; RI, retention index; FID, flame ionization detector.

LITERATURE CITED

(1) Mussinan, C. J.; Katz, I. Isolation and identification of some sulfur chemicals present in two model systems approximating cooked meat. *J. Agric. Food Chem.* **1973**, *21*, 43−45.

(2) Farmer, L. J.; Mottram, D. S.; Whitfield, F. B. Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid. *J. Sci. Food Agric*. **1989**, *49*, 347−368.

(3) Hofmann, T.; Schieberle, P. Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution techniques. *J. Agric. Food Chem.* **1995**, *43*, 2187−2194.

(4) Wu, Y.-F.; Baek, H. H.; Cadwallader, K. R. Development of a meatlike process flavoring from soybean-based enzyme-hydrolyzed vegetable protein (E-HVP). *J. Food Sci*. **2000**, *65*, 1220−1227.

(5) Schieberle, P.; Grosch, W. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm.-Unters. Forsch.* **1987**, *185*, 111−113.

(6) Gasser, U.; Grosch, W. Identification of volatile flavour compounds with high aroma values from cooked beef. *Z. Lebensm.-Unters. Forsch.* **1988**, *186*, 111−113.

(7) Blank, I.; Sen, A.; Grosch, W. Potent odorants of the roasted powder and brew of Arabica coffee. *Z. Lebensm.-Unters. Forsch.* **1992**, *195*, 239−245.

(8) Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68−73.

(9) Milo, C.; Grosch, W. Detection of odor defects in boiled cod and trout by gas chromatography-olfactometry of headspace samples. *J. Agric. Food Chem.* **1995**, *43*, 459−462.

(10) Cadwallader, K. R.; Baek, H. H. Aroma-impact components in cooked tailmeat of freshwater crayfish (*Procambarus clarkii*). In *Food Flavors: Formation, Analysis and Packaging Influences*; Contis, E. T., Ho, C.-T., Mussinan, C. J., Parliment, T. H., Shahidi, F., Spanier, A. M., Eds.; Elsevier: Amsterdam, 1998; pp 271−278.

(11) Asinger, F.; Schäfer, W.; Herkelmann, G.; Römgens, M.; Reintges, B. D.; Scharein, G.; Wegerhoff, A. Effect of sulfur and ammonia on tridecanone-(7)- and pentanone-(2). *Justus Liebigs Ann. Chem*. **1964**, *672*, 156−178.

(12) Ullrich, F.; Grosch, W. Identification of the most intense volatile flavor compounds formed during autoxidation of linoleic acid. *Z. Lebensm.-Unters. Forsch.* **1987**, *184*, 277−282.

(13) Kawashima, K.; Yamanaka, H. Free amino acids responsible for the browning of cooked scallop adductor muscle. *Jpn. Fish. Sci.* **1996**, *62*, 293−296.

(14) Lizarraga-Guerra, R.; Lopez, M. G. Content of free amino acids in Huitlacoche (*Ustilago maydis*). *J. Agric. Food Chem.* **1996**, *44*, 2556−2559.

(15) Sen, A.; Schieberle, P.; Grosch, W. Quantitative determination of (*E*)-*β*-damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757−759.

(16) Blank, I.; Grosch, W. Evaluation of potent odorants in dill seed and dill herb (*Anethum graveolens* L.) by aroma extract dilution analysis. *J. Food Sci.* **1991**, *56*, 63−67.

(17) Van den Dool, H.; Kratz, P. D. A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463−471.

(18) *SAS User's Guide: Statistics*, Version 7; SAS Institute, Inc.: Cary, NC, 1998.

(19) Kent, N. L. Chemical composition of cereals. In *Technology of Cereals*; Kent, N. L., Ed.; Pergamon Press: Oxford, U.K., 1984; pp 34−35.

(20) Coleman, W. M.; White, J. L., Jr.; Perfetti, T. A. Characteristics of heat-treated aqueous extracts of peanuts and cashews. *J. Agric. Food Chem.* **1994**, *42*, 190−194.

(21) Hofmann, T.; Münch, P.; Schieberle, P. Quantitative model studies on the formation of aroma-active aldehydes and acids by Strecker-type reactions. *J. Agric. Food Chem*. **2000**, *48*, 434−440.

(22) Griffith, R.; Hammond, E. G. Generation of Swiss cheese flavor components by the reaction of amino acids with carbonyl compounds. *J. Dairy Sci.* **1989**, *72*, 604−613.

(23) Münch, P.; Hofmann, T.; Schieberle, P. Comparison of key odorants generated by thermal treatment of commercial and self-prepared yeast extracts: Influence of the amino acid composition on odorant formation. *J. Agric. Food Chem.* **1997**, *45*, 1338−1344.

(24) Hofmann, T.; Schieberle, P. Identification of key aroma compounds generated from cysteine and carbohydrates under roasting conditions. *Z. Lebensm.-Unters. Forsch.* **1998**, *207*, 229−236.

(25) Gasser, U. Flavor volatiles of beef and chicken broths. Ph.D. Dissertation, Technical University of Munich, 1990.

(26) Gasser, U.; Grosch, W. Aroma extract dilution analysis of commercial meat flavorings. *Z. Lebensm.-Unters. Forsch.* **1990**, *190*, 511−515.

(27) Gasser, U.; Grosch, W. Primary odorants of chicken broth. *Z. Lebensm.-Unters. Forsch.* **1990**, *190*, 3−8.

(28) Guadagni, D.; Buttery, R.; Turnbaugh, J. Odor thresholds and similarity ratings of some potato chip components. *J. Sci. Food Agric.* **1972**, *23*, 1435−1444.

(29) Tressl, R.; Helak, B.; Martin, N.; Kersten, E. Formation of amino acid specific Maillard products and their contribution to thermally generated aromas. In *Thermal Generation of Aroma*; Parliament, T. H., McGorrin, R. J., Ho, C., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 156−171.

(30) Badings, H. T. Milk. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991.

(31) Buttery, R. G.; Teranishi, R.; Ling, L. C.; Turnbaugh, J. G. Quantitative and sensory studies on tomato paste volatiles. *J. Agric. Food Chem.* **1990**, *38*, 336−340.

(32) Roberts, D. D.; Acree, T. E. Effects of heating and cream addition on fresh raspberry aroma using a retronasal aroma simulator and gas chromatography. *J. Agric. Food Chem.* **1996**, *44*, 3919−3925.

(33) Shure, K. B.; Acree, T. E. Changes in the odor-active compounds in *Vitis labruscana* Cv. Concord during growth and development. *J. Agric. Food Chem.* **1994**, *42*, 350−353.

(34) Roberts, D. D.; Mordehai, A. P.; Acree, T. E. Detection and partial characterization of eight *β*-damascenone precursors in apples (*Malus domestica* Borkh. Cv. Empire). *J. Agric. Food Chem.* **1994**, *42*, 345−349.

(35) Deibler, K. D.; Acree, T. E.; Lavin, E. H. Aroma analysis of coffee brew by gas chromatography-olfactometry. In *Food Flavors: Formation, Analysis and Packaging Influences*; Contis, E. T., Ho, C.-T., Mussinan, C. J., Parliment, T. H., Shahidi, F., Spanier, A. M., Eds.; Elsevier: Amsterdam, 1998; pp 69−78.

(36) Kumazawa, K.; Masuda, H. Change in the flavor of black tea drink during heat processing. *J. Agric. Food Chem.* **2001**, *49*, 3304−3309.

(37) Rodin, J. O.; Himel, C. M.; Silverstein, R. M.; Leeper, R. W.; Grortner, W. A. Volatile flavor and aroma components of pineapple. I. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3(2H)-furanone. *J. Food Sci.* **1965**, *30*, 280−285.

(38) Pickenhagen, W.; Velluz, A.; Passerat, J.-P.; Ohloff, G. Estimation of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol) in cultivated and wild strawberries, pineapples and mangoes. *J. Sci. Food Agric.* **1981**, *32*, 1132−1134.

(39) Baek, H. H.; Cadwallader, K. R.; Marroquin, E.; Silva, J. L. Identification of predominant aroma compounds in muscadine grape juice. *J. Food Sci.* **1997**, *62*, 249−252.

(40) Tonsbeek, C. H. T.; Plancken, A. J.; van de Weerdhof, T. Components contributing to beef flavor. Isolation of 4-hydroxy-5-methyl-3(2*H*)-furanone and its 2,5-dimethyl homologue from beef broth. *J. Agric. Food Chem.* **1968**, *16*, 1016−1021.

(41) Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analysis and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862−2866.

(42) Shimoda, M.; Nakada, Y.; Nakashima, M.; Osajima, Y. Quantitative comparison of volatile flavor compounds in deep-roasted and light-roasted sesame seed oil. *J. Agric. Food Chem.* **1997**, *45*, 3193−3196.

(43) Benn, S. M.; Peppard, T. L. Characterization of tequila flavor by instrumental and sensory analysis. *J. Agric. Food Chem.* **1996**, *44*, 557−566.

(44) Ong, P. K. C.; Acree, T. E. Gas chromatography/olfactory analysis of lychee (*Litchi chinesis* Sonn.). *J. Agric. Food Chem.* **1998**, *46*, 2282−2286.

(45) Baek, H. H.; Cadwallader, K. R. Aroma volatiles in cooked alligator meat. *J. Food Sci.* **1997**, *62*, 321−325.

(46) Schieberle, P. The role of free amino acids present in yeast as precursors of the odorants 2-acetyl-1-pyrroline and 2-acetyl-tetrahydropyridine in wheat bread crust. *Z. Lebensm.-Unters. Forsch.* **1990**, *191*, 206−209.

(47) Buttery, R. G.; Ling, L. C.; Juliano, B. O.; Turnbaugh, J. G. Cooked rice aroma and 2-acetyl-1-pyrroline. *J. Agric. Food Chem.* **1983**, *31*, 823−826.

(48) Chung, H. Y.; Cadwallader, K. R. Aroma extract dilution analysis of blue crab claw meat volatiles. *J. Agric. Food Chem.* **1994**, *42*, 2867−2870.

(49) Cadwallader, K. R.; Tan, Q.; Chen, F.; Meyers, S. P. Evaluation of the aroma of cooked spiny lobster tail meat by aroma extract dilution analysis. *J. Agric. Food Chem.* **1995**, *43*, 2432−2437.
(50) Tatum, J. H.; Nagy, S.; Berry, R. E. Degradation products formed in canned single-strength orange juice during storage. *J. Food Sci.* **1975**, *40*, 707−709.
(51) Cha, Y. J.; Cadwallader, K. R. Aroma-active compounds in skipjack tuna sauce. *J. Agric. Food Chem.* **1998**, *46*, 1123−1128.
(52) Arnold, R. G.; Libbey, L. M.; Day, E. A. Identification of components in the stale flavor fraction of sterilized concentrated milk. *J. Food Sci.* **1966**, *31*, 566−573.
(53) Karagül-Yüceer, Y.; Drake, M. A.; Cadwallader, K. R. Aroma-active components of nonfat dry milk. *J. Agric. Food Chem.* **2001**, *49*, 2948−2953.
(54) Hartmann, G. J.; Jin, Q. Z.; Collins, G. J.; Lee, K. N.; Ho, C.-T.; Chang, S. S. Nitrogen-containing heterocyclic compounds identified as volatile flavor constituents of roasted beef. *J. Agric. Food Chem.* **1983**, *31*, 1030−1033.
(55) Cerny, C.; Grosch, W. Evaluation of potent odorants in roasted beef by aroma extract dilution analysis. *Z. Lebensm.-Unters. Forsch.* **1992**, *194*, 322−325.
(56) Milo, C.; Grosch, W. Changes in the odorants of boiled trout (*Salmo fario)* as affected by the storage of the raw material. *J. Agric. Food Chem.* **1993**, *41*, 2076−2081.
(57) Hofmann, T.; Schieberle, P. Studies on the formation and stability of the roast-flavor compound 2-acetyl-2-thiazoline. *J. Agric. Food Chem*. **1995**, *43*, 2946−2950.
(58) Fors, S. Sensory properties of volatile Maillard reaction products and related compounds. In *Maillard Reaction in Food and Nutriiition*; Waller, G. R., Feather, M. S., Eds.; American Chemical Society: Washington, DC, 1983; pp 185−286.
(59) Hodge, J. E. Origin of flavour in foods. Nonenzymic browning reactions. In *Chemistry and Physiology of Flavours*; Schultz, H. W., Day, E. A., Libbey, L. M., Eds.; AVI Publishing: Westport, CT, 1967; pp 465−491.
(60) Josephson, D. B.; Lindsay, R. C. Enzymic generation of volatile aroma compounds from fresh fish. In *Biogeneration of Aroma*; Parliament, T. H., McGorrin, R. J., Ho, C.-T., Eds.; American Chemical Society: Washington, DC, 1986; pp 386-395.
(61) Cadwallader, K. R.; Baek, H. H.; Chung, H. Y.; Moody, M. W. Contribution of lipid-derived components to the flavor of alligator meat. In *Lipids in Food Flavors*; Ho, C.-T., Hartman, T. G., Eds.; American Chemical Society: Washington, DC, 1994; pp 186−195.
(62) Chairote, G.; Rodriguez, F.; Crouzet, J. Characterization of additional volatile flavor components of apricot. *J. Food Sci.* **1981**, *46*, 1898−1901, 1906.
(63) Vejaphan, W.; Hsieh, T. C.-Y.; Williams, S. S. Volatile flavor components from boiled crayfish (*Procambarus clarkii*) tail meat. *J. Food Sci.* **1988**, *53*, 1666−1670.

Received for review October 25, 2001. Revised manuscript received February 28, 2002. Accepted March 1, 2002. Funding provided by the Mississippi Soybean Promotion Board. This is manuscript no. J9595 of the Mississippi Agricultural and Forestry Experiment Station (MAFES).

JF0114076