Volatile Compounds in Flavor Concentrates Produced from Crayfish-Processing Byproducts with and without Protease Treatment

H. H. Baek and K. R. Cadwallader*

Department of Food Science, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803-4200

Crayfish-processing byproducts (CPBs) were hydrolyzed using alkaline protease Optimase APL-440 under optimum conditions. Volatile components of flavor concentrates prepared by atmospheric evaporation (100 °C) and vacuum evaporation (60 °C) were analyzed and compared by gas chromatography/mass spectrometry and gas chromatography/olfactometry. Concentrations of 12 pyrazines in flavor concentrates increased significantly ($p < 0.05$) after enzymatic hydrolysis. Concentrations of dimethyl disulfide, dimethyl trisulfide, and benzaldehyde also increased after enzymatic hydrolysis, whereas lipid decomposition products decreased significantly. Levels of thermally generated volatiles were much higher after atmospheric evaporation. A greater number of aroma-active compounds were detected in CPB hydrolysates than in unhydrolyzed CPBs.

Keywords: *Crayfish byproducts; hydrolysis; protease; volatile; pyrazines; olfactometry*

INTRODUCTION

Annually, over 38 600 tons of crayfish-processing byproducts (CPBs) are produced from Louisiana crayfish-processing plants (Meyers et al., 1990). In recent years, significant strides have been taken for better utilization of CPBs, such as for the recovery of astaxanthin pigment (Meyers, 1987) and chitin (No and Meyers, 1989a,b). However, not all components of CPBs are being utilized to their full potential since important and potentially recoverable flavors and precursors remain (No and Meyers, 1989b; Tanchotikul and Hsieh, 1989; Cha et al., 1992). It is possible to achieve more complete utilization of CPBs if these flavors and precursors are recovered prior to pigment and chitin extraction.

Proteolytic enzymes have been used extensively in the seafood industry as processing aids and for modification of marine raw materials (Gildberg, 1993). Use of proteases in production of flavor extracts is an emerging trend (In, 1990; Pan, 1990). Recently, the enzymatic hydrolysis of CPBs has been evaluated, and conditions have been optimized (Baek and Cadwallader, 1995). Use of protease treatment plays an important role in the recovery of water soluble compounds as well as in aroma formation. Amino acids and peptides released by protease action may react during thermal processing to form cooked meat aroma (Rizzi, 1989). The production of certain meat and savory flavors employs this technology (Dziezak, 1986). Volatiles derived from amino acids make substantial contributions to many seafood aromas, such as lobster (Cadwallader et al., 1995) and crab (Chung and Cadwallader, 1994).

General schemes for the production of seafood flavor extracts have been described by Ochi (1980). Commercial seafood flavorings are produced in the form of either concentrated liquids, pastes, or spray-dried powders. These flavorings have existing and expanding international markets such as in the surimi industry. The method and conditions of concentration/dehydration are important factors affecting the volatile composition of the flavor extract (Cha et al., 1992).

The objective of the present study was to employ gas chromatography/mass spectrometry (GC/MS) and gas chromatography/olfactometry (GC/O) to evaluate the effects of protease treatment and concentration method on the volatile composition of flavor concentrates made from CPBs.

EXPERIMENTAL PROCEDURES

Materials. Live crayfish (*Procambarus clarkii*) were purchased from a seafood processor in Baton Rouge, LA, and temporarily stored in a 4 °C walk-in cooler. After being washed in tap water, live crayfish were boiled for 7 min at 100 °C (Marshall et al., 1987). After cooling, tail meat was removed from the boiled crayfish by hand to collect CPBs. CPBs were composed of claw, viscera, and shell. CPBs were ground using a Waring Blendor with the addition of distilled water to make a final concentration of 75% (w/v). Ground CPBs were vacuum-packaged in poly(ethylene) bags (≈1 kg/bag; Koch Supplies, Inc., Kansas City, MO) and then stored at −20 °C.

Authentic flavor compounds were purchased from commercial sources or were generous gifts from Aldrich Chemical Co. (Milwaukee, WI). Optimase APL-440 was obtained from Solvay Enzymes, Inc. (Elkhart, IN).

Enzymatic Hydrolysis of CPBs. After thawing at 4 °C overnight, 1 kg of CPBs was placed into a 1-L jacketed reaction vessel (Cat. No. 991780, Wheaton, Millville, NJ). A stirrer was attached to thoroughly mix CPBs during reaction under optimal reaction conditions (pH 8−9, 65 °C, and 2.5 h reaction time) with 0.3% of Optimase APL-440 (Baek and Cadwallader, 1995). CPB pH was intrinsically optimal (≈9), and pH was not adjusted. Control consisted of CPBs subjected to the same conditions as above except for the absence of Optimase APL-440. Each hydrolysis was performed in duplicate.

Preparation of Flavor Concentrates. CPB hydrolysate was placed into a 5-L round bottom flask containing 1 L of boiling water to inactivate protease, and an additional 1 L of distilled water was added to the flask. Aqueous extraction of flavor compounds was carried out for 2 h after returning to a boil. A heating mantle was used to heat the flask. This method is common for obtaining seafood extracts (Ochi, 1980).

* Corresponding author: Department of Food Science and Technology, Mississippi State University, Box 9805, Mississippi State, MS 39762 [phone, (601) 325-3200; fax, (601) 325-8728; e-mail, krcmail@ra.msstate.edu].

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For control, Optimase APL-440 was added to boiling water 20 min before unhydrolyzed CPBs were placed into the flask and then extracted as described above. After cooling to 4 °C, extracts were filtered through cheese cloth (2 layers) and then No. 41 filter paper (Whatman Ltd., Maidstone, England).

It was our intention to study the effects of protease treatment and evaporation method on the volatile composition of flavor extracts, and no attempt was made to determine the effect of pH on volatile formation and composition. Filtrates were concentrated using two evaporation methods: atmospheric evaporation and vacuum evaporation. For atmospheric evaporation, an externally heated stainless steel container was used to concentrate the filtrate. Vacuum evaporation was performed at 60 °C using a Rotavapor (Büchi, Switzerland). It took approximately 1 h for atmospheric evaporation and 6 h for vacuum evaporation to concentrate to 25−30 °Brix, which was measured using a hand refractometer (Cambridge Instruments Inc., Buffalo, NY).

Vacuum Simultaneous Steam Distillation/Solvent Extraction (V-SDE). A standard SDE apparatus (Cat. No. K-5230101-0000, Kontes, Vineland, NJ) was modified as described by Cadwallader et al. (1994) to perform under vacuum (≈30 in.Hg, bp 60−65 °C) in order to minimize artifact formation during extraction. Each concentrate was placed into a 5-L round bottom flask and brought to 2 L with distilled water. Glass beads and 45.4 μg of 2,4,6-trimethylpyridine (TMP) as the internal standard were added to the flask. Air was evacuated for 30 min prior to heating the sample flask. Extraction was carried out for 4 h using 100 mL of redistilled dichloromethane as solvent. V-SDE extracts were kept at −20 °C overnight to facilitate water removal. Volume of each V-SDE extract was reduced to 10 mL under a gentle stream of nitrogen, dried over 3 g of anhydrous sodium sulfate, and then further reduced to 100 μL prior to analysis.

Gas Chromatography/Mass Spectrometry (GC/MS). A Hewlett-Packard (Palo Alto, CA) GC/mass selective detector (HP5790 GC/5970B MSD) was used to analyze V-SDE extracts. A 5-μL aliquot of each V-SDE extract was injected in the splitless mode. Volatile components were separated using a fused silica gel capillary column (Supelcowax 10, 60 m × 0.25 mm i.d. × 0.25-μm film thickness; Supelco, Inc., Bellefonte, PA). Other conditions were the same as described by Cadwallader et al. (1994).

Compound Identification. Compound identifications were based on comparison of retention indices (RI) (van den Dool and Kratz, 1963) and mass spectra of unknowns with those of authentic standard compounds. Tentative identifications were based on matching mass spectra of unknowns with those in the Wiley/NBS mass spectral database (Hewlett-Packard Co., 1988).

Quantification of Compounds. Positively identified compounds were quantified using calibration curves of amount ratios (compound/internal standard) vs peak area ratios (compound/internal standard) under identical experimental conditions. Peak areas of coeluting compounds and compounds in low abundance were calculated by mass chromatography (Hites and Biemann, 1970). Concentration of a compound in the sample was calculated as follows:

$$\text{concentration (ppb)} = \frac{\text{amount ratio} \times 45\,400 \text{ ng of TMP}}{750 \text{ g}}$$

Gas Chromatography/Olfactometry (GC/O). Sensory properties of individual volatile compounds in V-SDE extracts of flavor concentrates were evaluated by sniffing the GC effluent. Prior to sniffing, each extract was diluted to possess the same intensity of the internal standard peak. Each sample was evaluated by an expert panelist who was asked to record the description and intensity (strong, medium, and weak) of each odorant detected. The GC/O system was the same as described by Cadwallader et al. (1994).

Statistical Analysis. Randomized block design (RBD) with 2 × 3 factorial arrangement was used, with each replication as a block. Statistical analysis of data was conducted using the general linear model (GLM) procedure (SAS Institute, Inc., 1985). Least-squares means for volatile compounds were calculated and tested by least significant difference method.

RESULTS AND DISCUSSION

Flavor Concentrates from CPB Hydrolysate and CPB Control. A 3-fold higher volume of flavor concentrate (based on soluble solids content) was obtained by enzymatic hydrolysis of CPBs compared with control. Hydrolysate pH (9.13) was slightly lower than that of the control (9.33) due to protein hydrolysis. At pH values above 7.5−7.8, hydrolysis of protein is accompanied by a decrease in pH due to the release of H^+ from carboxyl groups (Adler-Nissen, 1986).

Effect of Protease Treatment on Volatile Compounds in CPBs. Twenty-seven out of 54 compounds detected in CPBs were significantly affected by enzymatic hydrolysis ($p < 0.05$), most of which were thermally generated or lipid-derived (Table 1).

Twelve pyrazines were positively identified in CPBs, and their concentrations increased significantly ($p < 0.05$) after enzymatic hydrolysis, especially when followed by atmospheric evaporation. 2,5-Dimethylpyrazine was the most abundant among the pyrazines detected in both hydrolysate and the control. Pyrazines were previously reported to play an important role in the flavor of crayfish hepatopancreatic tissue, tail meat, and processing byproducts (Kinlin et al., 1974; Vejaphan et al., 1988; Tanchotikul and Hsieh, 1989; Cha et al., 1992).

Other significant increases ($p < 0.05$) after enzymatic hydrolysis were found for dimethyl disulfide and dimethyl trisulfide. These sulfur-containing compounds may contribute to the overall aroma quality of flavor extracts because of their low threshold values of 12 and 10 ppb, respectively (Buttery et al., 1976). Dimethyl disulfide may have been thermally generated from 3-(methylthio)propanal (methional), a Strecker degradation product of methionine (Ballance, 1961). Mussinan and Katz (1973) demonstrated the thermal generation of dimethyl trisulfide from cysteine. Therefore, higher levels of these two compounds in the enzyme hydrolysate may be attributed to increases in free methionine and cysteine.

Benzaldehyde, which has a nutty almond/fruity aroma, was the most abundant compound in the hydrolysate and increased significantly ($p < 0.05$) after enzymatic hydrolysis. Hayashi et al. (1990) reported that the level of benzaldehyde increased in heated crab leg meat enriched with any of four amino acids (taurine, proline, alanine, or phenylalanine); however, no mechanism was proposed. Even though the concentration of benzaldehyde in hydrolysate of CPBs was much higher than in the control, its contribution to the overall aroma is questionable because of its relatively high threshold value of 350 ppb (Buttery et al., 1988).

These results suggest that an increase in precursors (amino acids and peptides) by enzymatic hydrolysis of CPBs led to the increase in thermally generated volatiles. Both amino acids and peptides are believed to generate Maillard reaction type volatiles (Rizzi, 1989; Ho et al., 1992). In addition to increased precursors, ammonia liberation via deamidation of asparagine and glutamine in proteins or hydrolysates might contribute to the formation of pyrazines in hydrolysates (Izzo and Ho, 1992, 1993; Hwang et al., 1993). Deamidation of proteins could have occurred by protease action (Kato et al., 1987; Shih, 1990).

Levels of (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-2,6-nonadienal, 1-octen-3-ol, 2,3-pentanedione, and

Table 1. Comparison of Volatile Compounds in Flavor Concentrates Prepared from CPBs with and without Protease Treatment with Respect to Concentration Method

			concentration (ppb)								
			N[a]			A[b]			V[c]		
no.	compd name	RI[d]	H[e]	C[f]	H/C[g]	H	C	H/C	H	C	H/C
					Aldehydes I						
3	(*E*)-2-butenal[h]	1036	20	21	1.0	8.0	0.8	10.0[i]	3.5	2.5	1.4
7	hexanal	1079	12	6.9	1.7	nd[j]	nd		32	33	1.0
8	(*E*)-2-methyl-2-butenal	1091	5.6	15	0.4	tr[k]	tr		tr	tr	
13	heptanal	1182	nd	nd		0.9	0.1	9.0	3.5	1.9	1.8
15	(*Z*)-4-heptenal[h]	1239	nd	nd		tr	nd		7.7	2.4	3.2[i]
34	(*E*)-2-octenal[l]	1421	tr	nd		nd	nd		nd	nd	
39	2-furancarboxaldehyde	1468	tr	tr		tr	tr		tr	tr	
46	benzaldehyde[h]	1520	280	49	5.7[i]	980	80	12.3[i]	230	63	3.7[i]
					Aldehyde II—Dienals						
32	(*E*,*E*)-2,4-hexadienal[m]	1406	nd	0.9	*i*	nd	nd		nd	tr	
42	(*E*,*E*)-2,4-heptadienal[m]	1494	0.2	6.6	0.03[i]	nd	1.2	*i*	0.04	2.3	0.02[j]
48	(*E*,*Z*)-2,6-nonadienal[m]	1585	nd	0.8		nd	0.2		0.1	2.7	0.04[j]
49	(*E*,*E*)-2,4-octadienal	1588	nd	nd		nd	nd		tr	tr	
53	(*E*,*E*)-2,4-decadienal	1809	nd	nd		nd	tr		nd	2.3	
					Alcohols						
10	1-Penten-3-ol	1154	20	35	0.6	0.6	0.1	6.0	0.2	0.6	0.3
19	(*Z*)-2-penten-1-ol	1309	14	33	0.4	nd	nd		nd	nd	
33	(*E*)-2-hexen-1-ol	1406	2.3	1.8	1.3	nd	nd		tr	tr	
37	1-octen-3-ol[m]	1450	3.9	5.7	0.7	nd	nd		tr	0.2	
41	2-ethyl-1-hexanol	1489	3.1	4.2	0.7	8.2	1.5	5.5	5.2	7.5	0.7
					Ketones						
1	2-pentanone	976	(0.06)[n]	(0.08)	0.8	(0.05)	(0.02)	2.5	(0.03)	(0.02)	1.3
2	2,3-butanedione	985	1.9	2.2	0.9	35	11	3.2	9.3	8.7	1.1
4	2,3-pentanedione[m]	1055	0.3	3.9	0.1[i]	1.8	0.7	2.6	0.8	1.5	0.5
6	2-hexanone	1078	(0.01)	(0.03)	0.3	(0.01)	(0.003)	3.3	(0.002)	(0.01)	0.2
9	(*E*)-3-penten-2-one[h]	1123	4.4	3.0	1.5	2.8	tr		0.8	0.1	8.0
12	2-heptanone	1179	6.1	36	0.2	3.4	0.4	8.5[i]	0.9	1.4	0.6
18	2-octanone	1284	1.6	2.7	0.6	0.2	0.03	6.7	0.2	0.2	1.0
24	6-methyl-5-hepten-2-one	1338	nd	(0.02)		(0.002)	nd		tr	nd	
30	2-nonanone	1391	1.5	2.8	0.5	0.4	tr		0.04	0.2	0.2
35	2-cyclohexen-1-one	1433	0.8	2.5	0.3	12	0.3	40.0	0.5	0.8	0.6
43	2-decanone[m]	1495	(0.03)	(0.05)	0.6	tr	(0.002)		(0.001)	(0.01)	0.1
45	(*E*,*Z*)-3,5-Octadien-2-one[l]	1517	(0.02)	(0.05)	0.4	nd	nd		nd	nd	
47	(*E*,*E*)-3,5-Octadien-2-one[l]	1575	(0.16)	(0.22)	0.7	nd	nd		nd	tr	
					Sulfur-Containing Compounds						
5	dimethyl disulfide[h]	1070	13	3.1	4.2[i]	220	11	20.0[i]	60	11	5.5[i]
27	dimethyl trisulfide[h]	1380	4.8	0.8	6.0	77	8.7	8.9[i]	27	8.7	3.1
50	2-acetylthiazole[m]	1646	14	19	0.7[i]	tr	nd		tr	nd	
52	2-thiophenecarboxaldehyde	1699	(0.05)	(0.05)	1.0	nd	nd		tr	tr	
					Pyridines						
11	pyridine	1173	9.8	11	0.9	0.7	nd		0.1	0.1	1.0
17	2-ethylpyridine[h]	1277	(0.03)	(0.01)	3.0	tr	tr		nd	nd	
26	2,4,6-trimethylpyridine (IS)	1362	61	61	1.0	61	61	1.0	61	61	1.0
					Pyrazines						
14	pyrazine[h]	1204	5.5	7.1	0.8	3.7	nd	*i*	0.2	tr	
16	methylpyrazine[h]	1259	16	11	1.5	58	1.0	58.0[i]	2.7	1.0	2.7[i]
20	2,5-dimethylpyrazine[h]	1316	230	89	2.6	860	57	15.1[i]	57	58	1.0
21	2,6-dimethylpyrazine[h]	1322	2.0	1.0	2.0	2.5	0.1	25.0[i]	0.2	0.2	1.0
22	ethylpyrazine[h]	1328	2.0	1.8	1.1	2.8	0.2	14.0[i]	0.2	0.1	2.0
25	2,3-dimethylpyrazine[h]	1341	0.4	0.3	1.3	1.5	nd	*i*	tr	nd	
28	2-ethyl-6-methylpyrazine[h]	1380	1.3	1.2	1.1	0.8	nd	*i*	0.1	nd	
29	2-ethyl-5-methylpyrazine[h]	1385	18	2.9	6.2	44	8.6	5.1	8.2	8.7	0.9
31	trimethylpyrazine[h]	1399	16	5.5	2.9	44	1.0	44.0[i]	3.2	2.1	1.5
36	2-ethyl-3,6-dimethylpyrazine[h]	1439	4.0	1.1	3.6[i]	12	0.7	17.1[i]	3.6	1.9	1.9
38	2-ethyl-3,5-dimethylpyrazine[h]	1455	1.2	0.1	12.0[i]	2.2	nd	*i*	0.6	0.4	1.5
40	tetramethylpyrazine[h]	1469	tr	nd		nd	nd		nd	nd	
					Miscellaneous						
23	2-acetyl-1-pyrroline	1337	tr	tr		tr	nd		nd	nd	
44	1*H*-pyrrole	1514	58	83	0.7	22	6.2	3.5[i]	9.6	14	0.7[i]
51	α-terpineol	1694	1.0	1.5	0.7	nd	nd		nd	nd	
54	*β*-ionone[m]	1938	2.2	4.9	0.4	nd	tr		nd	0.3	
55	phenol	2003	3.9	2.2	1.8	tr	0.2		tr	tr	

[a] Not concentrated. [b] Concentrated by atmospheric evaporation. [c] Concentrated by vacuum evaporation. [d] Retention index. [e] Hydrolysate. [f] Control. [g] Concentration ratio. [h] Effect of Optimase APL-440 on the increase of concentration was significant ($p < 0.05$). [i] Significant difference between hydrolysate and control ($p < 0.05$). [j] Not detected. [k] Trace. [l] Tentatively identified. [m] Effect of Optimase APL-440 on the decrease of concentration was significant ($p < 0.05$). [n] Numbers in parentheses represent peak area ratios (peak area of compound/peak area of TMP).

2-decanone, which may have been derived via lipid oxidation, decreased significantly ($p < 0.05$) after enzymatic hydrolysis, whereas concentrations of other lipid-derived volatiles remained unchanged in the hydrolysate. Similar results have been reported (Cha et al., 1992; Kim et al., 1994). This may be explained by the fact that lipid decomposition products can react with Maillard reaction intermediates to form heterocyclic compounds (Ho et al., 1989; Shibamoto and Yeo, 1992). Another possible explanation for a decrease in dienals might be the antioxidative effect of Maillard reaction products (Waller et al., 1983; Lingnert and Eriksson, 1983). Usually, lipid oxidation products are considered off-flavors, but these are characteristic volatile compounds of various seafoods (Josephson, 1991) and play an important role in the flavor of crayfish hepatopancreatic tissue (Kinlin et al., 1974). It is not clear how the decrease of lipid oxidation products influenced the overall aroma quality of flavor concentrates.

Concentrations of some thermally generated volatiles decreased or did not change after enzyme hydrolysis. 2-Acetylthiazole, which has a cracker- or popcorn-like aroma (Teranishi and Buttery, 1985), can be generated by thermal reaction (Schutte, 1974). Its concentration decreased significantly after enzymatic hydrolysis ($p < 0.05$). The reason for its decrease is not clear. The amount of 1*H*-pyrrole, a thermally generated volatile (Maga, 1981), did not change significantly ($p < 0.05$) after enzymatic hydrolysis. This compound can be formed by heating hydroxyproline and glucose (Kobayashi and Fujimaki, 1965). Thus, hydroxyproline may not have been liberated by enzymatic hydrolysis of CPBs since this amino acid is a component of collagen, which is resistant to protease attack (Stryer, 1988).

Effect of Concentration Methods on Volatile Compounds in CPBs. CPB hydrolysates were concentrated to produce flavor concentrates by evaporation. A number of volatile flavor compounds disappeared after atmospheric evaporation or vacuum evaporation when compared with volatiles in CPB hydrolysates before concentration (Table 1). Pyrazines were the major volatile compounds in concentrates of CPB hydrolysate. Concentrations of most pyrazines decreased after vacuum evaporation, whereas their levels increased after atmospheric evaporation. However, when these concentrates are considered as infeeds for spray drying, the concentration method might not be critical for formation of pyrazines because of subsequent heat exposure during spray drying. Additionally, encapsulation during spray drying may preserve these important aromas (Reineccius, 1988).

GC/O of Flavor Concentrates of CPBs. Sometimes instrumental data do not necessarily correspond to sensory data, that is, a compound present at a high concentration will not necessarily provide an intense aroma. Furthermore, the sensitive human olfactory system makes it possible to detect aroma-active compounds which cannot be easily detected by instrumental means. Therefore, GC/O was conducted in order to verify instrumental data and to detect additional aroma-active compounds present at levels below intrumental detection limits (Table 2).

A considerable number of sulfury, nutty and baked potato, crabby and grainy, and raw marine-like notes were detected by GC/O. More aroma-active compounds were present in CPB hydrolysate than in control (Figure 1), which may be due to improved flavor extractability or an increase in aroma precursors by enzymatic hydrolysis. Predominant aroma-active compounds in hydrolysate were dimethyl trisulfide (**27**, cooked cabbage) and several unknowns having sweet/grainy (p), sulfury (z and ac), mushroom (ab and an), and raw marine-like (ao) odors, while three compounds (**27**, p, and ae) were predominant in control. 2-Acetyl-1-pyrroline (2-AP, **23**, popcorn) and 2-ethyl-3,5-dimethylpyrazine (**38**, nutty and baked potato) probably contributed to the overall aroma quality because of their desirable aroma properties. These two compounds were detected at the same intensities in both hydrolysate and control.

Several aroma-active compounds disappeared after vacuum evaporation of CPB hydrolysate (Figure 2), while flavor concentrates prepared by atmospheric evaporation exhibited a higher aroma intensity. The most intense compounds after atmospheric evaporation were 2-AP (**23**), dimethyl trisulfide (**27**), and an unknown (ao). 2,3-Butanedione (**2**), dimethyl disulfide (**5**), and 2-AP were not detected after vacuum evaporation.

Table 2. Aroma-Active Compounds in Flavor Concentrates of CPBs

peak[a]	compd name	RI[b]	odor description
2	2,3-butanedione	985	buttery
a	unknown	1015	sour, onion
5	dimethyl disulfide	1070	sour, sulfury
b	unknown	1092	sour, sulfury
c	unknown	1148	chocolate
d	unknown	1156	chocolate
e	unknown	1167	rancid, pungent
15	(*Z*)-4-heptenal	1239	baked potato, rancid
f	unknown	1293	mushroom
g	unknown	1302	nutty, peanut
23	2-acetyl-1-pyrroline	1337	popcorn
27	dimethyl trisulfide	1380	cooked cabbage, sour
34	(*E*)-2-octenal[c]	1421	raw peanut skin
h	unknown	1429	mushroom
i	unknown	1445	nutty, peanut skin
38	2-ethyl-3,5-dimethylpyrazine	1455	nutty, baked potato
j	unknown	1469	nutty, stale
k	unknown	1483	nutty, baked potato
l	unknown	1520	sulfury, sour
m	unknown	1570	sweet, grainy
n	unknown	1605	sour
o	unknown	1639	sulfury, sour
50	2-acetylthiazole	1646	popcorn, chocolate
p	unknown	1662	sweet, grainy
q	unknown	1668	grainy, nutty, crabby
r	unknown	1673	nutty, meaty
s	unknown	1691	nutty, crabby
t	unknown	1717	fishy, fresh fish
u	unknown	1731	crabby, grainy
v	unknown	1747	cucumber
w	unknown	1751	fishy, fresh fish
x	unknown	1768	sweet, grainy
y	unknown	1800	plastic, sweet
z	unknown	1814	burnt, sulfury
aa	unknown	1857	catty
ab	unknown	1881	mushroom
ac	unknown	1897	sulfury, vegetable-like
ad	unknown	1935	sulfury, sour
ae	unknown	1935	sweet, crabby, grainy
af	unknown	2004	sulfury, sour
ag	unknown	2014	skunky
ah	unknown	2025	sweet
ai	unknown	2057	sweet, grainy
aj	unknown	2079	sewage
ak	unknown	2135	mothball
al	unknown	2170	sweet, melon
am	unknown	2201	sweet, floral
an	unknown	2214	cooked mushroom
ao	unknown	2220	naphthalene, raw marine

[a] Numeric numbers correspond to those in Table 1. [b] Retention index. [c] Tentatively identified.

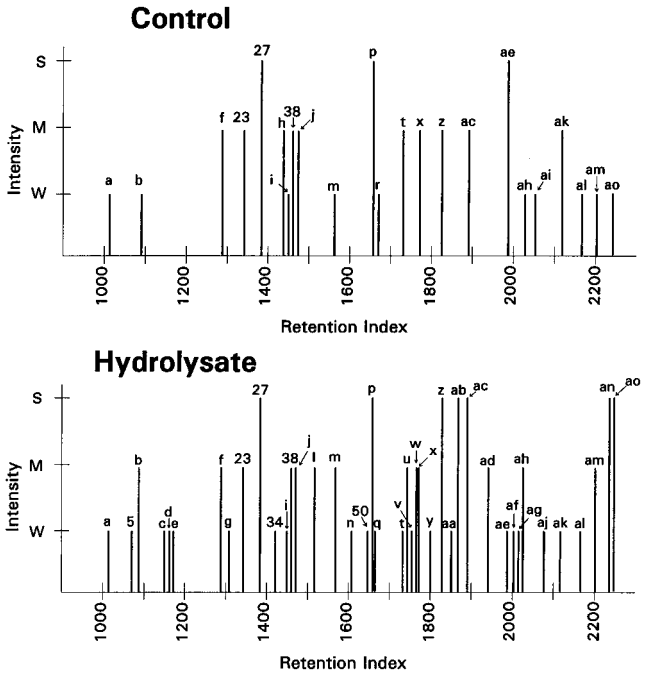

Figure 1. Aromagrams of volatile flavor compounds in CPB control and CPB hydrolysate (peak numbers and marks correspond to those in Table 2; S, strong; M, medium; W, weak).

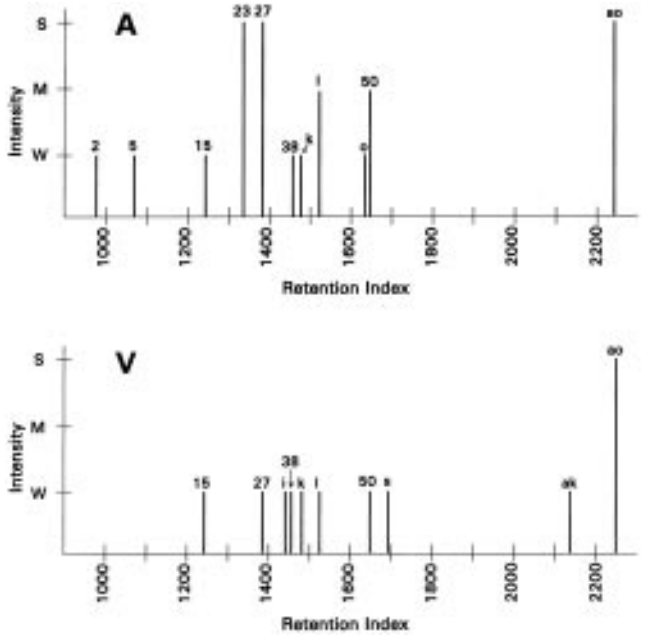

Figure 2. Aromagrams of volatile flavor compounds in flavor concentrates of CPB hydrolysate: (A) prepared by atmospheric evaporation and (V) prepared by vacuum evaporation (peak numbers and marks correspond to those in Table 2; S, strong; M, medium; W, weak).

The loss of 2-AP was thought to be undesirable to the overall aroma quality of the vacuum-evaporated flavor concentrate.

CONCLUSIONS

Enzymatic hydrolysis significantly affected thermally generated aromas and lipid decomposition products. Concentrations of 12 pyrazines in CPBs increased significantly ($p < 0.05$) after enzymatic hydrolysis. Concentrations of dimethyl disulfide, dimethyl trisulfide, and benzaldehyde, which are thermally generated, also increased after enzymatic hydrolysis, whereas lipid degradation products decreased significantly.

A number of volatile compounds disappeared after either atmospheric evaporation or vacuum evaporation. Pyrazines constituted the major class of volatile compounds in flavor concentrates prepared by either atmospheric evaporation or vacuum evaporation. The concentration of pyrazines increased after atmospheric evaporation of CPB hydrolysate. 2-Ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine may play important roles in flavor concentrates because of their low threshold values.

Results of GC/O compared favorably with the GC/MS data in that a larger number of aroma-active compounds were detected in CPB hydrolysate than in control. GC/O further confirmed that the overall aroma of the flavor concentrate prepared by atmospheric evaporation was more intense than that of the concentrate prepared by vacuum evaporation.

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