Comparison of the Aroma Characteristics of Acid-Hydrolyzed and Enzyme-Hydrolyzed Vegetable Proteins Produced from Soy

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The aroma volatiles of an acidic hydrolyzed vegetable protein (HVP) and an enzymatic hydrolyzed vegetable protein (EVP) were compared by gas chromatography−mass spectrometry (GC−MS) and gas chromatography−olfactometry (GCO). Major differences were found between the two hydrolysates. Furans and furanones, pyrroles, and sulfur-containing compounds were mainly present in HVP, whereas alcohols (including phenols) and pyrazines were mainly found in EVP. These differences reflected the two production methods. The high temperature and low pH during the acid hydrolysis of HVP favored the production of furans and the decomposition of sulfur amino acids, resulting in the production of sulfur-containing volatile compounds. A pH above 5 during the enzymatic hydrolysis facilitated the production of pyrazines. The alcohols in EVP may be formed by the added enzymes or by enzymes naturally present in the soy grits. Significant differences in the odors between the hydrolysates were detected by GCO.

Keywords: *Acid-hydrolyzed vegetable protein; enzyme-hydrolyzed vegetable protein; Maillard reaction; aroma; volatiles*

INTRODUCTION

Hydrolyzed vegetable protein (HVP) is a savory flavoring product produced by heating a protein source, such as soybean flour, wheat, or maize, with 4−6 M hydrochloric acid at 100−130 °C for 4−24 h (Manley et al., 1981; Dzanic et al., 1985; Weir, 1986). Enzyme-hydrolyzed vegetable protein (EVP), where the protein source is hydrolyzed by enzymes, is a newer alternative to HVP. Enzymatic hydrolysis is carried out at a pH between 5 and 7 at 50−55 °C for 10−24 h. Deactivation of the enzymes at 85 °C for 5 min is carried out after the hydrolysis (Pommer, 1995). The different production conditions result in two hydrolysates that are very different in color and flavor. The acidic hydrolysate is dark brown with a strong savory flavor, whereas the enzymatic hydrolysate is usually lighter in color and has a much less pronounced meaty or savory flavor (Weir, 1992).

Very little has been published comparing the volatile composition of acid and enzymatic hydrolysates. Misharina et al. (1987) reported on the volatile compounds in HVP and EVP heated with xylose and cysteine. The composition and sensory properties of HVP and EVP preparations similar to those used in the present work have been reported recently (Aaslyng et al., 1998). Some of the major volatile components were also identified, but a detailed comparison of the volatile compositions of the two hydrolysates was not undertaken. This paper compares the volatile compositions of EVP and HVP and identifies the compounds that make significant contributions to the aromas of these hydrolysates.

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MATERIALS AND METHODS

Chemicals. Untoasted defatted soy grits (Unisoy, 49.6% protein) were obtained from Loders Crocklaan (Holland); Flavourzyme (from *Aspergillus oryzae*, freeze-dried preparation, 3872 LAPU/g) and Alcalase 2.4L (from *Bacillus licheniformis*, 2.4 AU/g) were obtained from Novo Nordisk A/S (Bagsvaerd, Denmark); 1,2-dichlorobenzene (99%, spectrophotometric grade) was obtained from Aldrich Chemical Co. (Gillingham, U.K.); *n*-hexane was obtained from BDH Laboratory Supplies (Poole, U.K.).

Production of Hydrolysates. The acidic hydrolysate (HVP) was prepared by heating Unisoy 800 (100 g) with 240 mL of 4 M hydrochloric acid in a sealed glass bottle at 110 °C for 6 h (Aaslyng et al., 1998). After being cooled to room temperature, the mixture was neutralized to pH 6.5 with 4 M sodium hydroxide and centrifuged.

The enzymatic hydrolysate (EVP) was produced using the proteolytic enzymes Flavourzyme and Alcalase. Unisoy 800 (150 g) was mixed with 825 g of water and pasteurized at 85 °C for 5 min. After cooling to 50 °C, the pH was adjusted to pH 7.0 with 4 M sodium hydroxide. Flavourzyme (0.75 g) and Alcalase (0.75 g) were added to the mixture, which was allowed to stand at 50 °C for 5 h, after which time the pH was adjusted to 5 with 4 M hydrochloric acid. Sodium chloride (14.5 g) and Flavourzyme (0.38 g) were added, and the hydrolysis continued without pH adjustment for 24 h at 50 °C. Both hydrolysates were freeze-dried and stored at −18 °C until analysis.

Isolation of Volatiles. Sodium chloride was added to EVP so that its concentration was the same as in HVP (39%). A solution (100 mL) of each hydrolysate (15%) was then made up in water and placed in a 250-mL conical flask.

Aroma isolates were collected on Tenax TA. The sample was held at 60 °C for 1 h while oxygen-free nitrogen, at 40 mL/min, swept the volatiles onto a glass-lined, stainless steel trap (105 mm × 3 mm i.d.) containing 85 mg of Tenax TA (Scientific Glass Engineering Ltd., Milton Keynes, U.K.). A standard (130 ng of 1,2-dichlorobenzene in 1 μL of *n*-hexane) was added to the trap at the end of the collection, and excess solvent and any water retained on the trap were removed by

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Table 1. Compounds Found in the Headspace Volatiles of Hydrolyzed Soy Protein Prepared Using Acid and Enzymatic Hydrolysis

compound[a]	LRI[b]	amount in headspace[c] HVP	amount in headspace[c] EVP	method of identification[d]	previously reported in soy and unfermented soy products[e]	previously reported in HVP or EVP
Aldehydes						
3-methylbutanal	670	1298 (47)	957 (209)	MS + LRI	X	X[f,g]
2-methylbutanal	678	635 (44)	587 (154)	MS + LRI	X	X[f]
pentanal	709	nd	55 (24)	MS + LRI	X	X[e]
(*E*)-2-methyl-2-butenal	755	24 (1)	18 (4)	MS + LRI	X	
3-methyl-2-butenal	800	24 (3)	33 (6)	MS + LRI		
hexanal	812	41 (1)	360 (84)	MS + LRI	X	X[f,h]
heptanal	914	33 (2)	32 (6)	MS + LRI	X	
(*E*)-2-heptenal	970	nd	21 (4)	MS + LRI	X	
benzaldehyde	988	105 (4)	281 (43)	MS + LRI	X	X[e,f,i]
octanal	1016	15 (0)	35 (5)	MS + LRI	X	X[e]
phenylacetaldehyde	1072	138 (23)	124 (35)	MS + LRI	X	X[e,i]
(*E*)-2-octenal	1076	nd	33 (10)	MS + LRI	X	
nonanal	1119	113 (5)	177 (36)	MS + LRI	X	
(*E*)-2-nonenal	1178	nd	52 (9)	MS + LRI	X	
decanal	1219	48 (1)	23 (5)	MS + LRI	X	
Ketones						
2,3-butanedione (diacetyl)	613	*j*	*j*	MS + LRI	X	X[f]
2-butanone	614	*j*	*j*	MS + LRI	X	X[e,f,i]
2-pentanone	698	25 (4)	9 (5)	MS + LRI	X	
3-pentanone	707	8 (0)	1 (1)	MS + LRI		
2,3-pentanedione	710	188 (21)	68 (33)	MS + LRI	X	
3-hydroxy-2-butanone (acetoin)	717	nd	54 (26)	MS + LRI		
3-methyl-2-pentanone	762	3 (0)	27 (8)	MS + LRI	X	
2,3-hexanedione	796	25 (2)	nd	MS + LRI		
2-methylcyclopentanone	859	3 (0)	1 (0)	MS + LRI	X	
5-methyl-2-hexanone	862	5 (0)	nd	MS + LRI		
3-methylcyclopentanone	868	3 (0)	1 (0)	MS + LRI		
2-heptanone	900	31 (3)	39 (11)	MS + LRI	X	
2-methylcyclopent-2-en-1-one	922	23 (3)	nd	MS + LRI		
2,3-octanedione	995	15 (1)	31 (9)	ms + lri	X	
6-methyl-2,4-heptanedione	1036	nd	38 (5)	ms		
3-octen-2-one	1053	nd	30 (5)	ms + lri	X	
3,5,5-trimethyl-2-cyclohexen-1-one (isophorone)	1080	55 (4)	nd	MS + LRI		
acetophenone	1093	42 (3)	5 (1)	MS + LRI	X	X[e,i]
2-dodecanone	1410	86 (11)	nd	MS + LRI		
Alcohols and Phenols						
2-methyl-1-propanol	644	7 (0)	152 (42)	MS + LRI	X	X[f]
3-methyl-3-buten-1-ol	743	nd	25 (6)	ms + lri		
3-methyl-1-butanol	750	12 (0)	827 (222)	MS + LRI	X	X[e,f]
2-methyl-1-butanol	753	12 (1)	384 (97)	MS + LRI	X	X[f]
1-pentanol	780	22 (3)	115 (30)	MS + LRI	X	X[f]
1-hexanol	880	59 (6)	407 (114)	MS + LRI	X	X[e,f]
1-octen-3-ol	991	2 (0)	134 (32)	MS + LRI	X	
phenol	1002	4 (1)	73 (29)	MS + LRI	X	
2-ethyl-1-hexanol	1038	95 (10)	98 (36)	MS + LRI		
1-octanol	1083	nd	48 (17)	MS + LRI	X	X[e]
2-methoxyphenol (guaiacol)	1114	116 (9)	1968 (357)	MS + LRI	X	X[e,i]
4-vinylphenol	1247	nd	58 (21)	ms[k]	X	
1-decanol	1285	52 (2)	6 (2)	MS + LRI		
2-methoxy-4-vinylphenol	1344	1 (0)	218 (83)	ms[l] + lri	X	
1-undecanol	1387	95 (32)	nd	MS + LRI		
2-*tert*-butyl-4-methylphenol	1387	50 (5)	nd	ms		
1-dodecanol	1487	1395 (123)	10 (4)	MS + LRI		
Esters						
ethyl acetate	626	1 (0)	28 (9)	MS + LRI	X	X[e]
3-methylbutyl acetate	884	nd	81 (9)	MS + LRI	X	
methyl 4-oxopentanoate (methyl levulinate)	1002	71 (10)	nd	ms		
phenylethyl acetate	1279	tr	36 (9)	ms + lri		
4-vinylphenyl acetate 120, 91 (15), 91 (15), 43 (11), 121 (9), **162** (8), 65 (7)	1313	nd	31 (3)	se		
Furans and Furanoids						
tetrahydro-3-methylfuran	705	54 (5)	nd	ms		
2-ethylfuran	707	tr	22 (7)	MS + LRI	X	
dihydro-5-methyl-3[2*H*]furanone	822	29 (5)	nd	ms		
2-furfural	852	391 (40)	24 (4)	MS + LRI	X	X[e,f,i]
5-methyl-2[3*H*]-furanone	885	105 (20)	2 (0)	ms		X[e]
2-acetylfuran	929	1215 (137)	20 (3)	MS + LRI	X	X[e,f]
2-butyltetrahydrofuran	981	23 (1)	nd	ms		
5-methyl-2-furfural	982	129 (14)	2 (0)	MS + LRI	X	X[e,f,i]

Table 1. (Continued)

compound[a]	LRI[b]	amount in headspace[c] HVP	amount in headspace[c] EVP	method of identification[d]	previously reported in: soy and unfermented soy products[e]	previously reported in: HVP or EVP
Furans and Furanoids						
2-pentylfuran	999	nd	60 (26)	MS + LRI	X	
2-acetyl-5-methylfuran	1054	333 (36)	3 (0)	MS + LRI		X[e]
3-acetyl-2,5-dimethylfuran	1103	28 (2)	nd	MS + LRI		
an acetyldimethylfuran 123, **138** (38), 43 (15), 67 (8), 81 (8), 41 (7), 39 (6), 95 (6), 124 (6)	1144	25 (3)	nd	se		
5-methyl-2-propionylfuran	1151	219 (4)	2 (0)	MS + LRI		
an acetyldimethylfuran 123, **138** (45), 43 (14), 67 (11), 124 (7), 39 (5)	1177	46 (4)	nd	se		
an acetyltrimethylfuran 137, **152** (49), 43 (32), 67 (11), 109 (11), 95 (9), 138 (8)	1234	26 (3)	nd	se		
Pyrazines						
2,5(and/or 6)-dimethylpyrazine	930	6 (0)	230 (56)	MS + LRI	X	X[e–g,i]
ethylpyrazine	933	15 (2)	nd	MS + LRI	X	X[g,i]
trimethylpyrazine	1020	24 (5)	481 (177)	MS + LRI	X	X[f,i]
2-methyl-5(and/or 6)-vinylpyrazine	1038	nd	15 (5)	ms		X[i]
2,5-dimethyl-3-ethylpyrazine	1094	nd	51 (16)	MS + LRI	X	X[e,f,i]
a dimethylethylpyrazine	1102	34 (8)	nd	se	X	X[e]
tetramethylpyrazine	1105	nd	395 (199)	MS + LRI	X	X[e–g,i]
ethyltrimethylpyrazine	1173	nd	182 (79)	ms	X	X[e–g]
isopentyltrimethylpyrazine	1406	nd	91 (43)	se		
an MW 192 alkyldimethylpyrazine 122, 135 (19), 123 (12), 163 (5), 80 (5), **192** (3)	1492	nd	4 (1)	se		
Pyrroles						
2,5-dimethyl-1*H*-pyrrole	937	444 (48)	tr	ms		
an ethylmethylpyrrole 94, **109** (43), 93 (15), 108 (8), 95 (7), 106 (6)	1019	85 (8)	nd	se		
an ethyldimethylpyrrole 108, **123** (38), 93 (28), 106 (12), 107 (8), 94 (7), 109 (7), 122 (5)	1097	29 (3)	nd	se		
Sulfur Containing						
dimethyl disulfide	756	252 (14)	41 (16)	MS + LRI	X	X[e–g]
2-(methylthio)propanal 75, **104** (63), 47 (18), 49 (11)	825	tr	nd	se		
1-(methylthio)-2-propanone	863	10 (1)	tr	ms[m]		
tetrahydro-2-methylthiophene	866	15 (1)	nd	ms		X[e,g]
unknown, MW 113	923	2 (0)	nd	se		
3-(methylthio)propanal	928	35 (1)	25 (8)	MS + LRI		
dimethyl trisulfide	994	270 (9)	12 (5)	ms + lri		X[e–g]
2-acetylthiophene	1116	8 (1)	nd	MS + LRI		
benzyl methyl sulfide	1200	62 (2)	nd	ms		
dimethyl tetrasulfide	1260	28 (3)	nd	ms + lri		
benzothiazole	1271	32 (3)	3 (0)	MS + LRI	X	
Miscellaneous						
toluene	777	5 (1)	18 (7)	MS + LRI	X	
trimethyloxazole	857	2 (0)	79 (31)	MS + LRI		
1-dodecene	1195	52 (14)	nd	MS + LRI		
1-tetradecene	1398	64 (19)	nd	MS + LRI		
unknown, MW 206	1494	nd	2 (1)	se		

[a] Mass spectral data given for tentatively identified compounds, *m*/*z* (relative intensity). [b] Linear retention index on BPX-5 capillary column. [c] Amount in the headspace from 15 g of sample dissolved in 100 mL of water, reported as mean values of triplicate analyses with standard deviation in parentheses; nd, <0.1 ng/100 mL of solution; tr, <1 ng/100 mL of solution. [d] MS + LRI, mass spectrum and LRI agree with that of the authentic compound run under similar GC–MS conditions; ms + lri, mass spectrum and LRI agree with literature data; ms, mass spectrum agrees with literature spectrum; se, tentative identification based on interpretation of mass spectrum and comparison with similar compounds. Literature spectra from NIST/NIH/EPA Mass Spectral Database (1992) unless otherwise stated. Literature LRI from Kondjoyan and Berdagué (1996). [e] Nijssen et al. (1996). [f] Aaslyng et al. (1998). [g] Manley et al. (1981). [h] Roozen (1987). [i] Swaine (1993). [j] Quantification not possible due to coelution with solvent. [k] Walradt et al. (1971). [l] Stoll et al. (1967). [m] Nakamura et al. (1989).

purging the trap with nitrogen at 40 mL/min for 5 min. Three extractions were performed for each sample.

Gas Chromatography–Mass Spectrometry (GC–MS). Analyses were performed on a Hewlett-Packard 5972 mass spectrometer, fitted with a HP5890 series II gas chromatograph and a G1034C Chemstation. A CHIS injection port (Scientific Glass Engineering Ltd.) at 250 °C was used to thermally desorb the volatiles from the Tenax trap onto the front of a BPX5 fused silica capillary column (50 m × 0.32 mm i.d., 0.5 μm film thickness; Scientific Glass Engineering Ltd.). During the desorption period of 5 min, the oven was held at 0 °C. After desorption, the oven was heated at 40 °C/min to 40 °C and held for 2 min before heating at 4 °C/min to 280 °C. Helium at 8 psi was used as the carrier gas, resulting in a flow of 1.75 mL/min at 40 °C. A series of *n*-alkanes (C_6–C_{22}) was analyzed, under the same conditions, to obtain linear retention index (LRI) values for the hydrolysate aroma components.

The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current

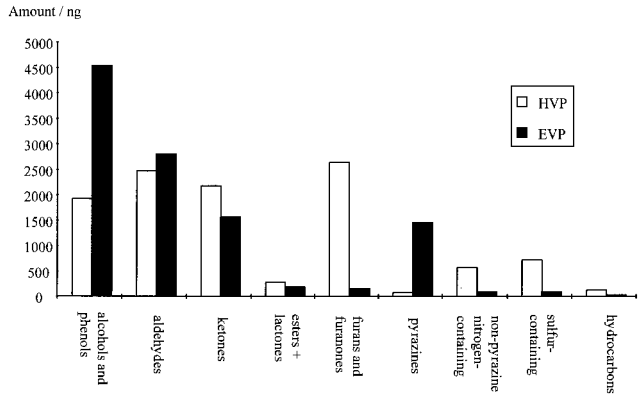

Figure 1. Quantities of volatile compounds by chemical class found in the headspace volatiles of hydrolyzed soy protein, prepared using acid and enzymatic hydrolysis.

of 50 μA. The mass spectrometer scanned from *m*/*z* 29 to *m*/*z* 400 at 1.9 scans/s. Compounds were identified by first comparing their mass spectra with those contained in the NIST/EPA/NIH Mass Spectral Database or in previously published literature, followed by comparison of linear retention index (LRI) values with either those of authentic standards or published values.

Gas Chromatography−Olfactometry (GCO). The samples were analyzed on a Hewlett-Packard 5890 gas chromatograph, using the same BPX5 fused silica capillary column as for the GC−MS analyses. The column effluent was split equally between a flame ionization detector and a humidified odor port. Gas chromatographic conditions were the same as for the GC−MS experiment.

Three assessors evaluated the aromas of the components of each hydrolysate once. The assessors described in their own words the odors perceived, and these descriptions were recorded, alongside the retention time of the odor. All odors reported here were detected by at least two assessors.

RESULTS AND DISCUSSION

The two hydrolysates were very different in appearance. HVP was dark brown with a very strong savory aroma, whereas EVP was yellow with more vegetable-like aroma notes. A total of over 80 volatiles were identified in the headspace volatiles of the HVP and EVP. Of these, 57 volatiles were present in the HVP headspace and 53 volatiles were present in the EVP headspace at or above a concentration of 20 ng/100 mL in the hydrolysate solution. These compounds are listed in Table 1 together with selected compounds present at lower concentrations that were of sensory significance. The total quantities of the compounds, arranged according to chemical classes, are shown in Figure 1.

Furfurals, furanones, other furans, pyrroles, and sulfur-containing compounds were present primarily in HVP, whereas alcohols (including phenols) and pyrazines were more dominant in EVP. Aldehydes, ketones, and esters were generally present in similar quantities in the two hydrolysates. These differences reflected the different production conditions.

The principal flavor precursors in hydrolyzed vegetable protein are amino acids and traces of reducing sugars from the hydrolysis of carbohydrates present in the soy raw material. An important flavor forming reaction is therefore the Maillard reaction. This is promoted by higher temperatures but can be inhibited at low pH, where the amino group is protonated. More Maillard reaction products, such as furfurals, furans, and sulfur-containing volatiles, were found in HVP than in EVP, reflecting the higher processing temperature. Previous studies showed that EVP contained some residual cysteine, whereas HVP contained none (Aaslyng et al., 1998). This confirmed that cysteine was less reactive at the lower temperature and higher pH conditions found in EVP, resulting in lower levels of sulfur volatiles.

Below pH 5, the formation of pyrazines in the Maillard reaction is not favored. Therefore, they were not found to any extent in HVP, whereas the higher pH of the enzymatic hydrolysis favored the production of pyrazines. A number of aldehydes are produced during the Maillard reaction from the Strecker degradation of amino acids. These include 2- and 3-methylbutanal and phenylacetaldehyde. More such compounds might be expected in the HVP because of the higher processing temperature, but similar quantities were found in HVP and EVP. Two possible explanations can be offered. First, the lower pH of the HVP would make the amino acid less reactive, and second, the aldehydes formed could undergo further reactions to give nonvolatile products, including colored melanoidins, that would occur more readily at higher temperatures. Other aliphatic aldehydes with straight chains were formed from lipid oxidation. These tended to be found at higher levels in the EVP. This could be due to enzyme-induced lipid oxidation or due to loss from the HVP by further reactions.

Aliphatic straight-chain alcohols also derive from lipids, and they followed the same trends as the aliphatic aldehydes. However, branched aliphatic alcohols, such as 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol, were major volatile components of EVP but were present at much lower levels in HVP. Similarly, significant quantities of phenols and methoxyphenols were isolated from the EVP. The most likely explanation for these compounds is that they are products of enzymatic reactions involving the added enzymes or enzymes naturally present in the soy grits.

Gas Chromatography−Olfactometry. Table 2 shows the odor active regions of the chromatogram. Twenty regions were described in HVP and 18 were described in EVP. Eleven of these regions were present at similar LRI values in both hydrolysates. In many cases they possessed similar aromas in both hydrolysates due to the presence of the same compounds. However, some of these regions gave different odor descriptions, usually because the relative concentrations of the compounds differed. Other regions were specific to one or other hydrolysate, and in many cases, these regions related to compounds that were specific to either HVP or EVP.

The odors of the early regions were similar in both hydrolysates. The odor of the first region was very strong in both but eluted very early in the chromatogram, and compounds could not be detected by GC−MS. The odor of the second region was also very strong, but the two compounds identified in this region coeluted with the solvent (from the added internal standard) and could not be quantified. Since 2,3-butanedione is described as having a buttery odor (Arctander, 1969; Furia and Bellanca, 1971; Bauer et al., 1990) and 2-butanone has been described as a key volatile in HVP (Swaine, 1993), it is likely that one or both were responsible for the odor in this region. Both have been found in heated soybean extract (Coleman et al., 1996) and may originate from the soy grits and not from reaction during

Table 2. Odors Found during GCO Analysis of HVP and EVP

	description of odor found		
LRI region	HVP	EVP	possible compound(s)
597−612	cake, malty, toffee	malty, buttery, toffee	unknown
613−614	toffee, caramel, buttery	butter, toffee	2,3-butanedione
			2-butanone
663−670	cookie, cake crust, green, fermented fruit	malty, dried food, green, chewing gum	3-methylbutanal
675−678	malty		2-methylbutanal
702−710	butter, toffee, fatty	toffee, sweet, buttery	3-pentanone
			2,3-pentanedione
723−737		brandy, alcoholic, sweet	3-hydroxy-2-butanone
775−777		hot plastic, paint	toluene
			1-pentanol
809−810		apples, green, grass	hexanal
820−822	sulfur, burnt rubber		2-(methylthio)propanal
855−860	pungent, raw onion		unknown
848−866	raw, musty, sweet	sweet, floral, fruit/wine gum	trimethyloxazole
			2-methylcyclopentanone
			5-methyl-2-hexanone
			1-(methylthio)-2-propanone
			tetrahydro-2-methylthiophene
			3-methylcyclopentanone
874−882	raw, stale, hot plastic, fatty	floral, toffee, fruit	unknown
905−907	boiled vegetable, stale		2-heptanone
916−923	cheesy, biscuit		2-methyl-2-cyclopenten-1-one
			unknown, MW 113
924−935	fatty, dumplings, stale, oily, boiled food	fatty, earthy, oily	2-acetylfuran
			3-(methylthio)propanal
			2,5(and/or 6)-dimethylpyrazine
934−940		stale, biscuit	unknown
930−950	chicken meat, fat, sage, mouse		2,5-dimethyl-1*H*-pyrrole
983−1013	stagnant water, acidic, stale	earthy, mushroom, stale, cabbage-like, fatty, rotten	1-octen-3-ol
			dimethyl trisulfide
1037		phenolic, earthy, rotten	6-methyl-2,4-heptanedione
			2-ethyl-1-hexanol
			2-methyl-5(and/or 6)-vinylpyrazine
1054−1055	fatty, mushroom		2-acetyl-5-methylfuran
1068−1082	rose	rose	phenylacetaldehyde
1096−1102	hazelnuts, floral	vegetable, earthy	acetophenone
			3-ethyl-2,5-dimethylpyrazine
1112−1118	phenolic, plastic, slight pastry	medical, phenolic, chocolate	2-methoxyphenol
			2-acetylthiophene
			nonanal
1178−1180		cucumber, vegetable	(*E*)-2-nonenal
1197−1198	vegetable, cucumber, meatlike		benzyl methyl sulfide
1435−1440	malty, HVP-like		unknown
1495		hot donuts, earthy, HVP-like	pyrazine, MW 192
			unknown, MW 206

hydrolysis. Both compounds were found with moderate odor dilution values in heated yeast extract (Münch et al., 1997).

3-Methylbutanal was identified at a high concentration in the third region, and as it was present in concentrations above its threshold (Fors, 1983), it is likely to be responsible for the odor. 3-Methylbutanal is moderately important in yeast extract aroma (Münch et al., 1997) and a character impact component of malted barley (Beal and Mottram, 1994). "Malt" was an important descriptor of these hydrolysates (Aaslyng et al., 1998), and it is likely to be a key component in the flavor of both HVP and EVP. 2-Methylbutanal also has a malt-like aroma and was present at concentrations well above its threshold value in both HVP and EVP. It was clearly detected in HVP by GCO, but it is difficult to explain why this odor was not detected in EVP.

2,3-Pentanedione was the compound most likely to be responsible for the odor between LRI 702 and LRI 710. It has been reported as possessing a musk-like aroma (Furia and Bellanca, 1971), although Arctander (1969) states that the aroma is oily−buttery.

Several odorous compounds were present in both EVP and HVP between LRI 924 and LRI 935. Methional (3-methylthiopropanal) was at similar levels in both hydrolysates. It is typically described as potato-like (Guadagni et al., 1972), meat-like (Furia and Bellanca, 1971), or bouillon/onion-like (Arctander, 1969). This is different from the odor described by GCO in this region, although "boiled food" may correspond with methional. Methional is the Strecker aldehyde of methionine. It is important in the aromas of heated beef and chicken and in yeast extract (Gasser and Grosch, 1988, 1990; Münch et al., 1997). It has also been found in HVP and EVP heated with a mixture of glucose and xylose (Umano et al., 1995). 2-Acetylfuran was present at much higher concentrations in HVP than in EVP. It has been described as balsamic-sweet (Arctander, 1969; Bauer et al., 1990). 2,5-Dimethylpyrazine and/or 2,6-dimethylpyrazine were detected in EVP at 230 ng and HVP at 6 ng in the headspace. Both of these dimethylpyrazines have a high threshold (van Gemert and Nettenbreijer, 1977; Fors, 1983) and are unlikely to make a significant contribution to the odors of the hydrolysates.

In the odor active region between LRI 983 and LRI 1013, a relatively high concentration of 1-octen-3-ol was found in EVP, with traces of dimethyl trisulfide, whereas

the reverse was found in HVP. 1-Octen-3-ol is described as mushroom-like (Bauer et al., 1990), herbaceous, earthy (Furia and Bellanca, 1971), sweet-earthy, herbaceous, fungus- or fermentation-like, whereas dimethyl trisulfide is described as onion-like (Arctander, 1969). Both of these compounds appeared to contribute to the odor in this region.

Phenylacetaldehyde, a Strecker aldehyde of phenylalanine, was the only compound detected between LRI 1068 and LRI 1082 where the odor in both HVP and EVP was described as "roses". Its aroma is floral, hyacinth-like (Arctander, 1969; Furia, 1971; Bauer, 1990). It has a relatively high odor dilution value in heated beef (Gasser and Grosch, 1988) but a low value in heated chicken and in yeast extract (Gasser and Grosch, 1990; Münch et al., 1997). Its isomer, acetophenone, was found in HVP at levels nearly 10 times greater than in EVP, and it may have contributed to the floral note of HVP in the odor active region immediately following phenylacetaldehyde. It is described as a key odorant in HVP (Swaine, 1993). The odor of EVP differed in character, probably due to 3-ethyl-2,5-dimethylpyrazine, which also eluted in this region but was present at much lower levels in HVP.

The odor of the region between LRI 1112 and LRI 1118 was very intense in both hydrolysates. 2-Methoxyphenol and nonanal were present in both. Nonanal was found at similar levels in both hydrolysates, whereas 2-methoxyphenol was present in EVP at much higher levels than in HVP. 2-Methoxyphenol is described as slightly phenolic (Furia and Bellanca, 1971), smoke-like, and medicinal (Arctander, 1969) and probably made a significant contribution to the odors of both HVP and EVP. Such odors may be considered as unacceptable in hydrolysates. Nonanal is described as fatty/floral (Arctander, 1969), fatty/rose-like (Bauer et al., 1990), and fatty with floral, citrus notes (Furia and Bellanca, 1971). It may also contribute to the odor of this region. 2-Acetylthiophene was also detected in HVP at 8 ng/100 mL of hydrolysate solution.

Some of the odor regions that were specific to HVP, appeared to contain sulfur compounds. In the narrow region LRI 820−822, the sulfur, burnt rubber odor was possibly due to a compound whose mass spectrum indicated the presence of sulfur and a molecular weight of 104, which was present at less than 1 ng in the headspace. Two sulfur compounds, 1-(methylthio)-2-propanone and tetrahydro-2-methylthiophene, were present at levels of 10 and 15 ng in the headspace of HVP, respectively, in the region LRI 848−866 and may have contributed the musty character noted in the GCO of HVP. Benzyl methyl sulfide was another sulfur compound only found in HVP. It eluted in the odor region LRI 1197−1200.

2-Acetyl-5-methylfuran was detected in the headspace of HVP at a level of 333 ng, but only trace amounts were found in EVP. It was probably responsible for the odor detected at LRI 1054 in the HVP. It has previously been identified in HVP (Weir, 1986). Although heterocyclic nitrogen compounds were mostly found only in EVP, the exceptions were alkylpyrroles, which were only isolated from HVP. They included a compound tentatively identified as 2,5-dimethyl-1*H*-pyrrole, which appeared to contribute to the odor detected in the region LRI 930−950.

The compounds that appeared to be responsible for the odor active regions specific to EVP were principally aldehydes and pyrazines. Hexanal eluted in the odor active region at an LRI of about 810. It was present at a high concentration in EVP, compared to HVP, and the odor description was in good agreement with the literature (Arctander, 1969; Furia and Bellanca, 1971; Bauer et al., 1990). Hexanal probably causes undesirable odors in EVP. It is derived from lipid oxidation and has been correlated with beany taste in enzymatic protein hydrolysates (Roozen, 1989). Another lipid oxidation product, (*E*)-2-nonenal, was probably responsible for the odor in the region LRI 1178−1180 in EVP. It may also contribute to undesirable odors in EVP.

Pyrazines appeared to be responsible for the earthy aromas of the three odor active regions at LRI 1037, LRI 1096−1102, and LRI 1495 that were only detected in EVP. 2-Methyl-5-vinylpyrazine and/or 2-methyl-6-vinylpyrazine were identified in the first region at a level of 15 ng in the EVP headspace but were absent in HVP. 3-Ethyl-2,5-dimethylpyrazine was identified in the second region at 51 ng, compared to 5 ng in the headspace of HVP. It has been identified in soy sauce but not in HVP (Manley et al., 1981). Two compounds, one of molecular weight 192 and the other of 206, were detected in the third region. The first is probably an alkyldimethylpyrazine; the identity of the second is uncertain. An HVP note was detected by the assessors in this region.

Conclusion. Significant differences were found between the aroma compositions of soy protein hydrolysates prepared by enzymatic and acid hydrolysis. Manipulation of the hydrolysis conditions for EVP will be necessary in order to increase the levels of desirable aroma components, such as sulfur compounds and Strecker aldehydes, and to reduce possible off-odors, such as hexanal and 2-methoxyphenol. Although some pyrazines were produced more readily in EVP, these introduced earthy notes rather than desirable roasted, savory characteristics.

The desirable compounds in HVP derive from amino acids, either via Strecker degradation or decomposition of sulfur-containing amino acids. Sulfur-containing aroma compounds are almost absent in EVP, although it may be possible to adjust processing conditions to produce such compounds from the key amino acid cysteine, which is present in EVP. Strecker aldehyde concentrations in EVP could be increased by altering pH and temperature.

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