Chemical and Sensory Characterization of Hydrolyzed Vegetable Protein, a Savory Flavoring

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Three protein hydrolysates (HVPs) were produced from untoasted defatted soy by acidic hydrolysis (aHVP), enzymatic hydrolysis (eHVP), and enzymatic hydrolysis followed by a heat treatment with glucose (eHVPrea). The three HVPs were characterized by amino acid analysis, identification of volatile compounds, and sensory profiling. aHVP had a higher degree of hydrolysis compared with eHVP and eHVPrea which still contained peptides composed mainly of the smaller and the acidic amino acids. A total of 29 volatile compounds were identified by GC−MS. Furans and sulfides were primarily found in the acidic HVP, while alcohols and pyrazines primarily were found in eHVP/eHVPrea. Further Maillard reaction had occurred in eHVPrea compared to eHVP, but the sensory profile was not altered. The multivariate analysis of the sensory profile showed that the acidic HVP had increased intensity in the bouillon, soy, and lovage odor and taste characteristics compared with the two enzymatic HVPs.

Keywords: *HVP; amino acid composition; volatiles; flavor; sensory properties; soy; pyrazines; furans*

INTRODUCTION

Hydrolyzed vegetable protein (HVP) is a savory flavoring product used in a wide variety of food applications throughout the world. It is produced by hydrolysis of a protein source such as soybean flour, wheat, or maize (Weir, 1986). Traditionally this hydrolysis is carried out by acid, often using hydrochloric acid.

During the acidic hydrolysis some carcinogenic compounds such as mono- and dichloropropanols and monochloropropanediols can be produced (Nagodawithana, 1994), and furthermore the resulting salt content is very high (about 40%) (Manley et al., 1981). These disadvantages could be overcome by using enzymatic hydrolysis if a similar, or more desirable, flavor profile could be obtained.

Commercially, acidic hydrolysates are obtained by treating the protein source with 4−6 M HCl at 100−130 °C for 4−24 h followed by a neutralization with NaOH (Manley et al., 1981; Dzanic et al., 1985; Weir, 1986; Velisek et al., 1993). The enzymatic hydrolysis implies a much milder treatment, generally including a preliminary heat treatment to 85−90 °C for a few minutes, a pH adjustment to pH 5−7 depending on the optima of the enzymes, and a reaction time of 10−24 h at 50−55 °C. A pH adjustment during the hydrolysis to cover the whole pH spectrum of the enzymes may be included (Pommer, 1995). The acidic hydrolysate is usually dark-brown in color and has a strong savory flavor, whereas the enzymatic hydrolysate usually is lighter in color and has a much less-pronounced meaty or savory flavor (Weir, 1992).

The flavor of the HVPs is in general due to the content of free amino acids, smaller peptides, salt, and various volatile compounds. The free amino acids have some distinctive taste profiles, and especially glutamic acid is important because of its umami taste, also known as the fifth taste. This taste is very typical for traditionally produced HVP (Filer and Stegink, 1994; Maga, 1994). The taste of the smaller peptides is a function of their amino acid composition. A peptide containing hydrophobic amino acids has a bitter taste (Ney, 1979).

The free amino acids are also precursors in a variety of reactions which give rise to an extensive range of volatile flavors. The most studied of these reactions is the Maillard reaction (Weir, 1986, 1992; Nagodawithana, 1994). The chemical background of the Maillard reaction has been reviewed recently (Baltes, 1993; Mottram, 1994a).

Commercially the HVPs may be used directly or after further processing. For direct use it is desirable to be able to control the overall characteristics by directing both taste characteristic and chemical composition. When further processing leading to different specific meat flavors is included, the chemical composition is very important.

The aim of this work is to investigate and compare the chemical composition and sensory profile of three HVPs: a traditionally produced acidic hydrolysate (aHVP), an enzymatic hydrolysate (eHVP), and for the first time an enzymatic hydrolysate which has been reacted with glucose by heating (eHVPrea). The chemi-

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cal characterization focused on both amino acids and volatile compounds.

EXPERIMENTAL PROCEDURES

Chemicals. Untoasted, defatted soy grits (Unisoy 800, 52% protein determined by Kjeldahl) were obtained from Looders 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); Kjeldtabs CK were obtained from Thompson & Capper LTD (Cheshire, WA); *o*-phthaldialdehyde (OPA), acetonitrile, and triethylamine were obtained from Aldrich Chemie (Steinheim, Germany); phenyl isothiocyanate (PITC) was obtained from Pierce (Rockford, IL); L-2-aminobutyric acid and 4-methyl-2-pentanol were obtained from Fluka Chemie AG (Buchs, Switzerland); sample diluent for Pico Tag amino acid analysis was obtained from Waters Millipore Co. (Bedford, MA); trifluoroacetic acid (TFA) and monosodium glutaminate (MSG) were obtained from Sigma Chemical Co. (St. Louis, MO); Tenax AT 60−80 mesh was obtained from Buchem-BW (Apeldoorn, Holland). Water purified by MilliQ was used except where otherwise stated. Chemicals used for the production of hydrolysates were all analytical grade. All other chemicals were analytical or HPLC grade.

Production of Hydrolysates. Four separate batches were produced of each hydrolysate and mixed after drying. pH adjustments were controlled with a pH meter. Centrifugations were performed at room temperature, 3800 rpm for 15 min, in a Beckman centrifuge model J6-B (Palo Alto, CA).

Acidic Hydrolysate (aHVP): Unisoy 800 (100 g) was mixed with 240 mL of 4 M HCl and boiled in a closed glass bottle for 6 h at 110 °C. After cooling to room temperature the mixture was neutralized to pH 6.5 with 4 M NaOH. After centrifugation the precipitate was washed with 200 mL of tap water and centrifuged again. The combined hydrolysate and water were filtered and freeze-dried.

Enzymatic Hydrolysate (eHVP): Unisoy 800 (150 g) was mixed with 825 g of tap water and pasteurized at 85 °C for 5 min. After the mixture cooled to 50 °C, the pH was adjusted to pH 7.0 with 4 M NaOH. Flavourzyme (0.78 g) and Alcalase (0.78 g) were added, and the mixture was allowed to stand without pH adjustment at 50 °C. After 5 h the pH was adjusted to pH 5.0 with 4 M HCl, and 14.6 g of NaCl and 0.39 g of Flavourzyme were added. The hydrolysis continued without pH adjustment at 50 °C for a total of 24 h. The enzymes were deactivated at 85 °C for 5 min. After the mixture cooled to 50 °C, the pH was adjusted to pH 6.5 with 4 M NaOH. After centrifugation the precipitate was washed with 300 mL of tap water and centrifuged again. The combined hydrolysate and water were filtered and freeze-dried.

Enzymatic Hydrolysate Reacted with Glucose by Heating (eHVPrea): This hydrolysate was produced in the same way as the enzymatic hydrolysate until the drying step. Amino acid and peptide content were determined by Kjeldahl using factor 6.25 (Tecator Kjeltec system: digestion system 20 1015 and autosampler system 1035 analyzer, Tecator, Höganäs, Sweden). The hydrolysate (pH 6.5) was heated to 90 °C, and glucose equal to 0.5% of the protein content was added. After heating for 1 h at 95 °C, the reacted hydrolysate was cooled, filtered, and freeze-dried.

Chemical Characterization of Hydrolysates. *Content of Amino Acid and Peptide-Bound N*: The amino acid and peptide-bound N content was determined by Kjeldahl using factor 6.25.

Degree of Hydrolysis (DH): The DH was determined using *o*-phthaldialdehyde (OPA method) as described by Petersen et al. (1995).

Amino Acid Content: Amino acid analysis was performed on an HPLC-RP from Waters Chromatographics Division (Millipore Co., Bedford, MA). Technical details were as follows: a high-pressure pump (Waters M510), an injector (Waters M710B), a detector (Waters 486, 254 nm), and an amino acid analyzer column (Waters Pico Tag, 3.9 mm × 30 cm, C18 RP). Solutions used: Redry solution, 600 μL of methanol, 600 μL of 0.2 M sodium acetate, 300 μL of 99% triethylamine. Derivatization reagent: 1050 μL of methanol, 150 μL of water, 150 μL of 99% triethylamine, 150 μL of PITC. Mobile phases: (A) 19.0 g of sodium acetate·$3H_2O$, 1000 mL of water, 0.5 mL of triethylamine (99%), and 200 μL of EDTA, 1000 ppm, pH adjusted to pH 5.70 with acetic acid; (B) 600 mL of acetonitrile, 400 mL of water, and 200 μL of EDTA, 1000 ppm. Internal standard: L-2-aminobutyric acid (322.3 mg/100 mL), 10 mM HCl. Solutions were filtered through a 0.45-μm filter.

Total Amino Acids: Samples corresponding to 50 mg of protein were dissolved in 5 mL of 6 M HCl, and 1 mL of internal standard was added. After mixing the oxygen was removed by flushing with N_2 and the mixture was boiled for 16 h at 110 °C in sealed vials. The volume was adjusted to 25 mL with water.

Free Amino Acids: Samples corresponding to 0.8 g of protein and 1 mL of internal standard were dissolved in water to 25 mL total volume. All samples were filtered through a 0.45-μm filter, and 10 μL was dried under vacuum. The samples were redissolved in 30 μL of Redry solution and dried under vacuum again. Derivatization reagent (30 mL) was added, and after 20 min the samples were dried for 15 min under vacuum. Methanol (30 μL) was added, and the samples were dried under vacuum to full dryness. The samples were redissolved in 100 μL of sample diluent, and an 8-μL sample was injected by the autosampler. The flow was 1 mL/min for the first 30 min, increasing to 1.5 mL/min over 0.5 min and keeping this flow until 44 min. After that the flow decreased to 1 mL/min again over 1 min. The gradient was as follows: 89% A, 11% B; 80% A, 20% B during 1−5 min; 71% A, 29% B during 5−10 min; 65% A, 35% B during 10−13 min; 52% A, 48% B during 13−26 min; 100% B during 26−27 min keeping this until 38 min; 89% A, 11% B during 38−39 min keeping this until 45 min.

Molecular Weight Distribution: An HPLC-GPC system from Waters Chromatographics Division including a high-pressure pump (Waters M510), an injector (Waters WISP M710), a detector (Waters M440, 214 nm), and three GPC columns (TSK G 2000 SWXL, 7.8 mm × 300 mm) in series was used. Mobile phase: 0.05 M NaH_2PO_4·$2H_2O$, 0.5 M NH_4Cl, 0.1% TFA (v/v), and 25% (v/v) acetonitrile. The solution was filtered through a 0.45-μm filter. Samples corresponding to 4 mg of protein/mL were dissolved in the mobile phase and filtered through a 0.22-μm filter, and 20 μL was injected by the autosampler. The flow was 0.7 mL/min. Aspartame was used as standard.

Isolation of Volatiles by Dynamic Headspace Trapping: The procedure was modified after Poll and Hansen (1990). The hydrolysates were adjusted to the same NaCl content (39.2%), and a 15% solution in tap water was made. Two gas washing bottles were filled with 100 mL of the solution, and 0.5 mL of internal standard (4-methyl-2-pentanol, 50 ppm, in water) was added. Nitrogen was bubbled through the solutions at 200 mL/min at 60 °C for 1 h, and the volatiles were trapped on 300 mg of Tenax AT. Volatiles were collected from both traps in one vial by eluting with diethyl ether and at last concentrated to 40 mg by gently blowing N_2 over the surface.

Gas Chromatography−Mass Spectrometry: To identify the volatiles analytical separation was performed on a Hewlett-Packard G1800A GCD system gas chromatograph (Delaware). Technical details were as follows: column, DB-Wax, 30 m, i.d. = 0.250 mm; film thickness, 0.25 μm; injector temperature, 250 °C. The mass spectra were obtained on an electron ionization detector. The carrier gas was He at approximately 1 mL/min. Temperature program: 40 °C for 10 min, 40−240 °C at 3 °C/min, 240 °C in 30 min. Three samples of each hydrolysate were analyzed.

Gas Chromatography−FID: To quantify the volatiles analytical separation was performed on a Hewlett-Packard series II plus 5890 gas chromatograph (Delaware) with a FID

detector, 250 °C. Column, injection, carrier gas, and temperature program were the same as above. Two samples of eHVP and eHVPrea and three samples of aHVP were analyzed.

Sensory Profiling. *Panel*: The sensory profiling was carried out at the Sensory Laboratory at The Royal Veterinary and Agricultural University, Copenhagen, Denmark (RVAU). The panel was composed of seven trained assessors from the RVAU and one semitrained assesor with product knowledge from Novo Nordisk A/S. The panel from the RVAU had all followed a basic training after the ISO standard 8586-1:1993 *Sensory Analysis − General Guidance for Selection, Training and Monitoring of Assessors* and ASTM STP 758 *Guidelines for the Selection and Training of Sensory Panel Members*. They were students and inhabitants from Frederiksberg, Copenhagen, between 20 and 60 years old.

Training: The descriptors for the sensory profile analysis were initially discussed byproduct experts from Novo Nordisk A/S and the panel leaders. From the initial set of descriptors a set of reference solutions was made.

The profiling panel was introduced to the three samples. eHVP and eHVPrea being very similar were tested in a triangle test, and a significant difference ($p < 0.05$) was found. The panel proposed some descriptors. Their descriptors were generally very close to the product experts descriptors. The first two of four training sessions included discussion with respect to the reference solutions. The final profiling descriptor set was (1) odor—bouillon, soy sauce, smoky, malt/brown bread, lovage/vegetable; (2) taste—sweet, lovage/vegetable, salt, bouillon, MSG, smoky, soy sauce, bitter.

Reference Solutions: The reference solutions were made mainly for the odor descriptors except the MSG which was solely a taste descriptor. The odor references were made empirically in order to obtain the most characteristic flavor. The following references was prepared. Bouillon: one bouillon cube (10 g, beef flavor, from Knorr, CPC-foods A/S (Skovlunde, Denmark) consisting of a.o. MSG, yeast extract, and beef extract) dissolved in water was filtrated and diluted, and some of the dissolved references (malt, smoky, and soy) were added. Soy sauce: soy sauce (Kikkoman PTE CTD, Singapore) was very diluted with water. Smoky: prepared from water used for boiling smoked bacon. Malt: prepared from broken barley sugar in water. Bread: boiling water was poured on light black bread. Lovage: prepared from lovage leaves. MSG: 2.5 g/L of tap water.

Samples: The hydrolysates were adjusted to the same NaCl content, and a 1% dilution was made; 30 mL was served at 22 °C.

Profiling: In the final profiling a reference sample of the acidic hydrolysate was served first. The HVPs were served for each assessor twice a day in 3 days. The samples were coded with 3-digit numbers and served in a randomized order.

Data Analysis. The data were analyzed using Excell (Microsoft) for the general data handling, SAS statistical system (SAS Institute Inc.) for the univariate analysis of the sensory data, and Unscrambler (Camo, Norway) for the multivariate data analysis of the GC data and the sensory data. The GC data were analyzed by principal component analysis (PCA). Each measurement was treated as an independent object giving seven objects (three aHVP, two eHVP, two eHVPrea). The sensory data were analyzed by PCA and partial least-squares regression (PLS). The average of the assessors for each day and replicate were used giving 18 objects (3 hydrolysates, 3 days, 2 replicates). The data analysis was performed with standardized variables and full cross-validation (Martens and Martens, 1986).

RESULTS

The general chemical composition of the HVPs in this study are listed in Table 1. Two major differences seemed to be present between aHVP and eHVP/eHVPrea. One was the NaCl content which was much larger in aHVP. This resulted in a larger total N as percent of dry matter and therefore a larger protein content in the two enzymatic hydrolysates. The other was degree of hydrolysis (DH) which was higher in aHVP resulting in fewer peptides. The amount of free amino acids as percent of dry matter (DM) was still almost equal for the three hydrolysates. The major difference between aHVP and the data reported by Manley et al. (1981), who do not mention the protein source, seemed to be the content of Glu which was less in aHVP. This could be due to a different protein source as soy is very low in Glu content compared to other protein sources, such as wheat gluten, or extra Glu added in the hydrolysate of Manley et al. (1981).

Table 1. Chemical Characteristics of HVP[a]

	aHVP	eHVP	eHVPrea	HVP (Manley et al., 1981)
total N, % of DM[b]	5.9	8.8	8.6	5−7
NaCl, % of DM	41.3	13.3	14.5	35−45
free amino acids, % of DM	30.1	32.9	29.8	23
free amino acids, % of total amino acid	81.6	59.8	55.1	
Glu, % of DM	6.6	3.0	3.2	12
peptides, % of DM	5.9	23.7	24.6	7
salt-free non-amino acid matters,[c] % of DM	21.8	31.7	31.8	11−34
DH,[d] %	78.2	65.6	63.8	
M_n[e]	258	202	202	
M_w[f]	257	410	423	

[a] The hydrolysates are produced form untoasted defatted soy grits. aHVP, acidic hydrolysate; eHVP, enzymatic hydrolysate using two proteolytic enzyme mixtures (Flavorzyme and Alcalase, both from Novo Nordisk A/S, Denmark); eHVPrea, enzymatic hydrolysate using the same procedure as eHVP, but added glucose and heated for 1 h. [b] DM, dry matter. [c] Salt-free non-amino acid matters are calculated as 100 − (total N × 6.25 + NaCl). [d] DH, degree of hydrolysis. [e] M_n, median molecular weight. [f] M_w, average molecular weight.

The content of salt-free non-amino acid dry matter was larger for the enzymatic hydrolysates than for the acidic. They are, however, in the range of the data reported by Manley et al. (1981). It is not known exactly what the other compounds are, but different kinds of carbohydrates are possible.

The yield of the hydrolysis was 98% in aHVP and 58% in eHVP when looking at dry matter content of hydrolysate:Unisoy, but due to a very high NaCl content in aHVP (Table 1), the yield was more equal when looking at the total N content of hydrolysate:Unisoy being 70% in aHVP compared to 62% in eHVP (results not shown).

The distribution of free amino acids can influence the flavor of HVPs. The distribution of free amino acid as percent of total amino acid content is given in Figure 1. Glu and Asp were present as free amino acids to a much larger extent in aHVP compared to eHVP and eHVPrea. One reason is that Gln and Asn have been deaminated to Glu and Asp during the acidic hydrolysis. The contents of free Pro and Gly and to a lesser extent Ala and Ser were higher in aHVP compared to eHVP and eHVPrea. Trp and Cys were destroyed during the acidic hydrolysis. Some of the Trp seemed also to have been destroyed during the heat treatment of eHVPrea.

The proportion of free amino acid to total amino acid (faa:taa), showing how much of each amino acid is still present as peptides, is given in Figure 2. In general, the peptide bonds including the small (Pro, Gly, Ala, Ser) and the acidic (Glx, Asx) amino acids were difficult for the enzymes to hydrolyze as the faa:taa was only between 0.2 and 0.6. The acidic hydrolysis yielded a

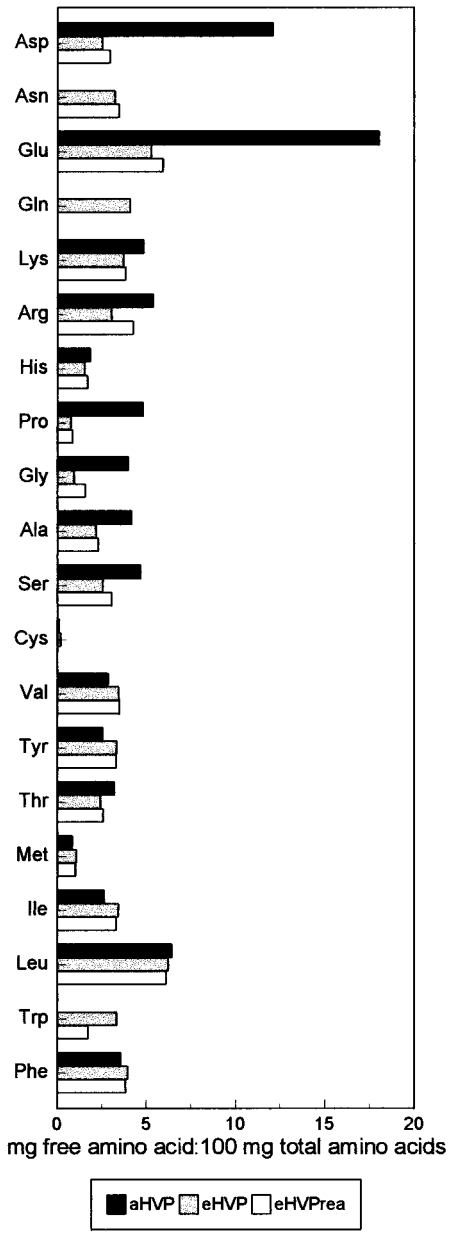

Figure 1. Free amino acids in HVP as mg/100 mg of protein. aHVP, acidic hydrolysate; eHVP, enzymatic hydrolysate using two proteolytic enzyme mixtures (Flavourzyme and Alcalase, both from Novo Nordisk A/S, Denmark); eHVPrea, enzymatic hydrolysate using the same procedure as eHVP, but added glucose and heated for 1 h.

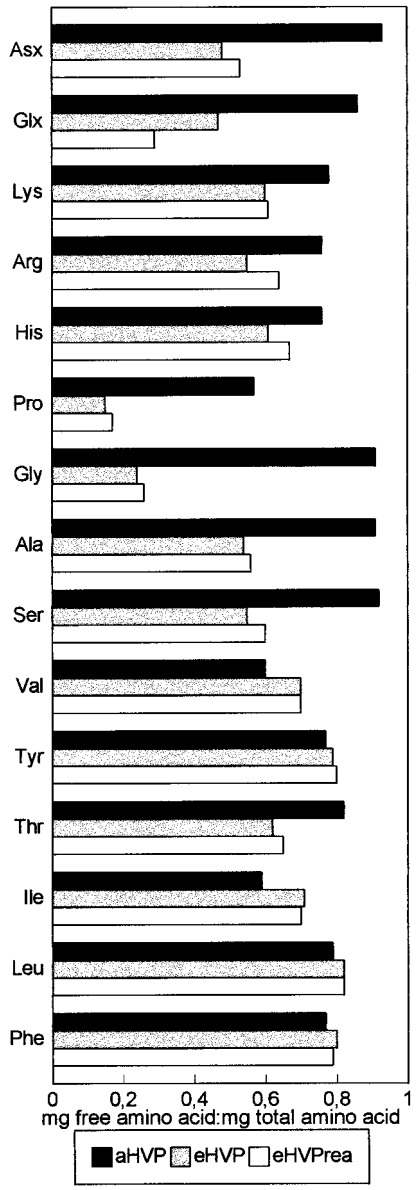

Figure 2. Ratio of free amino acid to total amino acid in HVP. The proportion of an amino acid that is present as free amino acid describes the effectiveness of the actual hydrolysis against peptide bonds including the amino acid in question.

faa:taa around 0.9 for these amino acids showing almost complete hydrolysis. In eHVP and eHVPrea the faa:taa was around 0.7 for peptides composed of the larger hydrophobic amino acids. For peptides composed of the basic amino acids, the faa:taa was only around 0.6. In aHVP faa:taa was about 0.8 for both large hydrophobic amino acids and basic amino acids, which was lower compared to the acidic and small amino acids in aHVP. For the large hydrophobic amino acids, the proportion of free to total amino acids was in the same area for all three HVPs.

Table 2 shows the results of the GC analysis for volatile compounds. All compounds with a retention time less than 3.0 min on the GC−MS were removed as they were confounded with the solvent. A total of 105 compounds were detected on the GC−FID. On the GC−MS, 29 compounds were tentatively identified. The presence of nine compounds was confirmed by the retention time of reference samples.

The GC data have been analyzed by principal component analysis (PCA) (data not shown). Of the total variance 76% could be explained by two principal components (PC). PC1 explaining 46% of the total variance discriminated between aHVP and eHVP/eHVPrea, whereas PC2 explaining 30% of the total variance discriminated between eHVP and eHVPrea but did not explain very much of the variance of aHVP. The analysis showed that some volatiles seemed to be characteristic for the aHVP whereas others seem to be characteristic for the eHVP and eHVPrea. This is summarized in Table 3 for the identified peaks.

Results from the sensory profiling showed that besides characteristics such as bouillon and soy taste and odor the assessors also described characteristics such as lovage/vegetable odor and taste and malt/bread odor. The average score can be seen in the profiling wheel in Figure 3. The wheel shows a very distinctive difference between aHVP and eHVP/eHVPrea for bouillon and soy

Table 2. Volatile Compounds[a] Found in HVP

chemical group	chemical compound	t_R (min)	area relative to IS			identified by	
			aHVP	eHVP	eHVPrea	MS[b]	t_R
aldehydes	2-methylbutanal	3.18	64.8	43.5	82.5	+++	
	3-methylbutanal	3.24	124.6	77.2	156.3	+++	
	hexanal	8.19	1.6	26.2	24.4	+++	+
	3-methylthiopropanal	28.50	1.9	0.5	0.5	++	+
	benzaldehyde	31.24	26.9	68.6	92.5	+++	+
alcohols	ethanol	3.63	7.1	9.9	18.4	+++	
	2-methyl-1-propanol	9.24	0	22.7	14.4	+++	
	2-methyl-1-butanol[c]	16.70		+	+	+	
	3-methyl-1-butanol	16.77	0	101.1	51.7	+++	+
	1-pentanol	19.24	2.7	49.9	17.3	+++	+
	1-hexanol	24.56	6.7	181.7	54.9	++	+
ketones	2-butanone	3.00	36.7	11.4	20.8	+++	+
	2,3-butanedione	4.42	56.5	85.3	112.4	++	
pyrazines	2,5(or 2,6)-dimethylpyrazine	22.29	0	13.3	48.7	+++	
	2,6(or 2,5)-dimethylpyrazine	22.64	0	17.8	36.3	+++	
	2,3-dimethylpyrazine	23.45	0	5.5	12.2	++	
	trimethylpyrazine	26.38	1.5	59.0	160.1	+++	
	3-ethyl-2,5-dimethylpyrazine	28.31	10.6	26.2	69.9	+++	
	a pyrazine MW 136[d]	29.03	0	8.7	3.4	+	
	tetramethylpyrazine	29.64	1.1	52.3	137.2	+++	
	6-ethyl-2,3,5-trimethylpyrazine[e]	31.38		+	+	+++	
	a pyrazine MW 178 or 164[f]	37.20	0	10.2	16.1	++	
	a pyrazine MW 192 or 178[g]	39.72	0	11.2	25.3	++	
furans	furfural (2-furancarboxaldehyde)	29.0	213.8	0	2.1	+++	+
	1-(2-furanyl)ethanone	30.73	22.5	0	0	+++	
	5-methyl-2-furancarboxaldehyde	33.57	47.2	0	0	+++	
sulfides	dimethyl disulfide	7.6	4.4	0	0	+++	+
	dimethyl trisulfide	24.67	3.1	0	0	+++	
	dimethyl sulfoxide[d]	33.67	+			+++	

[a] The compounds have been identified using GC−MS and by retention time (t_R) of commercial reference compounds. Internal standard (IS): 4-methyl-2-pentanol, 50 ppm, in water. [b] The quality of the matching of the MS with the MS of the database: +++, $Q \geq 90$; ++, $80 \leq Q < 90$; +, $Q < 80$. [c] On the GC for quantification 2-methyl-1-butanol and 3-methyl-1-butanol were not separated. The area for 3-methyl-1-butanol includes both. [d] *m/z* 43(21), 54(26), 42(41), 136(57), 57(64), 135(100). [e] Not possible to make an exact quantification of the compound. [f] *m/z* 123(9), 121(10), 39(11), 135(11), 42(12), 122(100). [g] *m/z* 41(8), 42(9), 149(10), 137(12), 53(18), 136(100).

Table 3. Characteristic Volatile Compounds in HVP[a]

aHVP	eHVP/eHVPrea
2-butanone	ethanol
dimethyl disulfide	2,3-butanedione
dimethyl trisulfide	hexanal
3-methylthiopropanal	2-methyl-1-propanol
furfural	3-methyl-1-butanol
1-(2-furanyl)ethanone	1-pentanol
5-methyl-2-furancarboxaldehyde	2,5-dimethylpyrazine
	2,6-dimethylpyrazine
	2,3-dimethylpyrazine
	1-hexanol
	trimethylpyrazine
	3-ethyl-2,5-dimethylpyrazine
	some pyrazine
	tetramethylpyrazine
	benzaldehyde
	2-butyl-3,5-dimethylpyrazine
	6-butyl-2,3,5-trimethylpyrazine

[a] The compounds identified on GC−MS were statistically analyzed by a PCA (plots not shown). The analysis showed that the aroma compounds discriminated most between aHVP and eHVP/eHVPrea.

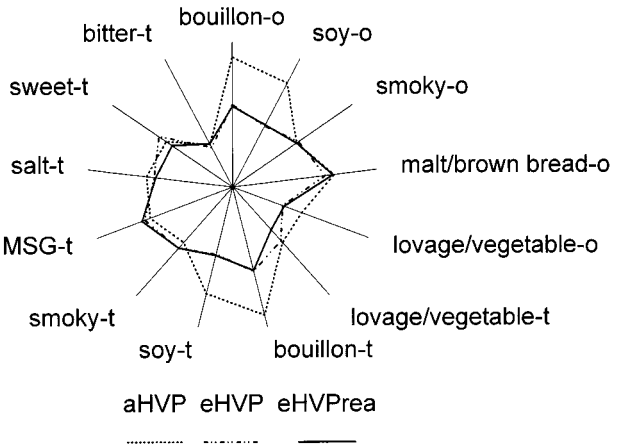

Figure 3. Profiling wheel of HVP: o, odor characteristic; t, taste characteristic. aHVP, acidic hydrolysate; eHVP, enzymatic hydrolysate using two proteolytic enzyme mixtures (Flavourzyme and Alcalase, both from Novo Nordisk A/S, Denmark); eHVPrea, enzymatic hydrolysate using the same procedure as eHVP, but added glucose and heated for 1 h.

taste and odor, whereas the difference for the other characteristics was less clear. This was confirmed in an analysis of variance where aHVP was found significantly different from eHVP and eHVPrea for these four attributes ($p < 0.001$ for soy taste, soy odor, and bouillon odor; $p < 0.01$ for bouillon taste).

The sensory profiling data have also been analyzed by PCA and PLS. Figure 4 shows the loading plots of the PLS analysis. It was seen from the loading plot (Figure 4) that factor 1 explaining 54% of the variation roughly depended on the flavor characteristics whereas factor 2 explaining 22% of the variation depended more on the basic tastes especially sweet and bitter. It was also seen on the loading plot that factor 1 depended on the product variation separating aHVP from eHVP/eHVPrea whereas factor 2 seemed more to be a day and replicate variation. In general the differences between eHVP and eHVPrea are hidden in the day/replicate variation, and the sensory characteristics important in discriminating between these two products can therefore not be found.

Combining the attributes and products (Figure 4) showed that the flavor characteristics soy and bouillon

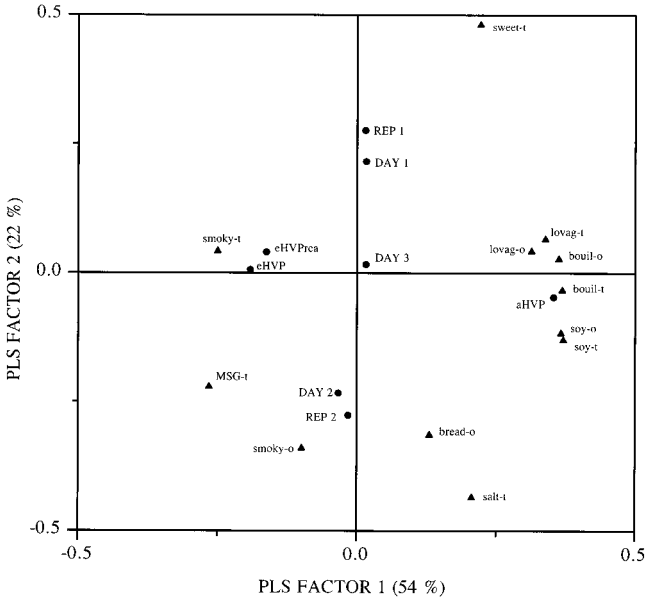

Figure 4. Loading plot of PLS analysis of sensory profiling data of HVP. The data were analyzed using the average of the assessors for each day and replicate. The horizontal axis is factor 1; the vertical axis is factor 2. If a variable has a high loading with respect to one of the factors—being situated far from 0—this variable explains much of the variation in the material. Factor 1 explains 54% of the total variation; factor 2 explains 22% of the total variation.

odor and taste, lovage/vegetable taste, and to a lesser extent lovage odor were important for aHVP whereas smoky taste and MSG taste were important for eHVP/eHVPrea in the discrimination between products.

DISCUSSION

The general chemical composition of the three HVPs (Table 1) was very close to the content reported in the literature for HVP (Manley et al., 1981; Swaine, 1993). Also the amino acid distribution of the HVPs (Figure 1) seemed very similar to earlier reports on commercial acidic HVP (Dzanic et al., 1985). The HVPs prepared in this study can therefore be expected to be comparable to commercial products.

The DHs of eHVP and eHVPrea (Table 1) were higher than normally found in enzymatic hydrolysates (Munk Nielsen, 1997). This is due to a new available enzyme mixture (Flavourzyme) containing both endo- and exopeptidase activity instead of only exopeptidase activity. It seems that the enzyme mixture is lacking some activity to hydrolyze peptides composed of smaller amino acids including Gly, Pro, Glu/Gln, and Asp/Asn (Figure 2), and the DHs were therefore still lower than that of aHVP (Table 1). The amount of free amino acids as percent of protein was smaller in eHVP and eHVPrea compared to aHVP, and therefore the amount of peptides was larger (Table 1). The peptides must have been quite small as the DH was higher than the amount of free amino acids as percent of total amino acid content. This was confirmed by the molecular weigth distribution where the median molecular weight in eHVP and eHVPrea was about 200 Da, equal to dipeptides or free amino acids, whereas the average molecular weight was about 410 Da, equal to tetra-, tri-, or dipeptides. In aHVP both the median and average MWs were about 257 Da, equal to dipeptides. The average and median molecular weights in eHVP and eHVPrea were different showing that still some larger peptides were present in the eHVP and eHVPrea compared to the aHVP.

Another difference between aHVP and eHVP/eHVPrea was the content of Glu (Figure 1). Glu is very important in generating the desired taste umami, whereas Gln does not have this taste. In aHVP all Gln's were deaminated to Glu's because of the acidic conditions during the hydrolysis. This deamination had not taken place in eHVP/eHVPrea. It is possible to deaminate Gln to Glu enzymatically by using a glutaminase, but apparently neither Flavourzyme nor Alcalase has this activity. It is not known why Gln was missing in eHVPrea. In the sensory profiling (Figure 3) the panel gave eHVP and eHVPrea almost the same score for MSG taste (umami) as aHVP despite the lower Glu content. The explanation could be that MSG taste was difficult to recognize and that the panel instead associated it with the smoky taste as these two tastes were situated together on the loading plot (Figure 4).

Earlier enzymatic HVPs were often described as being bitter compared to the more aromatic acidic hydrolysates (Lalasidis et al., 1978; Konrad and Lieske, 1979). The bitterness was due to smaller peptides with hydrophobic amino acids, especially Leu in the C-terminal (Ishibashi et al., 1987; Nishimura and Kato, 1988). Hydrophobic amino acids were more bitter when present in a dipeptide compared to the free amino acids (Matoba and Hata, 1972). In this investigation no bitterness was found in either of the hydrolysates. This was in agreement with the results from the amino acid analysis where the amount of peptide-bound Leu was even smaller for eHVP/eHVPrea than for aHVP (Figure 2). With enzyme mixtures such as Flavourzyme, bitterness of enzymatic HVPs is apparently no longer a problem.

The complex differences between the flavor of the three HVPs were probably more due to the volatiles than to the amino acids. Still the amino acid composition can have an influence since different amino acids can give rise to different volatiles. For example Cys, Met, and Pro have been reported to be important precursors for meat flavor (Gasser and Grosch, 1988), and it is known that Gly can increase the formation of a compound like furfural (Whitfield et al., 1988), whereas both Gly and Lys can increase the pyrazine formation (Meynier and Mottram, 1995). Thr has been described as a precursor for 3-hydroxy-4-methyl-5-ethyl-2(5*H*)-furanone which on its own or after decomposition seems to have a typical HVP flavor (Sulser et al., 1967) and which has been found in acidic HVP (Manley et al., 1980).

The composition and the amount of volatiles differed between aHVP and eHVP/eHVPrea, whereas the difference between eHVP and eHVPrea was related to the amount of the various volatiles (Table 2). In general the heating with glucose had increased the amount of volatiles. As can be seen from Table 2 aldehydes and ketones were found in all three hydrolysates, whereas alcohols and pyrazines primarily were detected in the two enzymatic hydrolysates, and furans and sulfides were primarily in the acidic hydrolysate. This is in agreement with the current concepts of Maillard reactions. Furans are formed at low pH (pH less than 5), and the pH occurring during the acidic hydrolysis was very low. The pyrazines are created at a more neutral pH (higher than pH 5), and this was the condition during the heating of the enzymatic hydrolysates where

the pH was 6.5. The alcohols could be enzymatically produced from the aldehydes by alcohol dehydrogenase. The furans and thiols were found to be quantitatively important for describing the volatile composition of aHVP, whereas the alcohols and pyrazines were found to be quantitatively important for describing the volatile composition of eHVP/eHVPrea (Table 3).

The aldehydes are typical Maillard reaction products from the Strecker degradation (Mottram, 1994b). They are generally described as green, fatty, and fruity. 3-Methylthiopropanal coming from Met is special in having a potato flavor (Nursten, 1980; Danehy, 1986; Mottram, 1992). Despite the potato flavor 3-methylthiopropanal has been found as an important component of beef flavor (Gasser and Grosch, 1988). It has also been reported in heat-treated HVP (Misharina et al., 1987). Hexanal was also described in the beef flavor but with a low importance for the total flavor (Gasser and Grosch, 1988). Benzaldehyde has been reported both in heated aqueous soy extract (Coleman et al., 1996) and in heated HVP (Misharina et al., 1987). The compounds 2- and 3-methylbutanal have been found in heated aqueous soy extract (Coleman et al., 1996). They are reaction products of the amino acids Ile and Leu. They were more abundant in eHVPrea and aHVP compared to eHVP, and this could indicate that further Maillard reactions have taken place in these two hydrolysates.

The two observed ketones (Table 2) have been described in heated aqueous soy extract (Coleman et al., 1996), and 2,3-butanedione has also been found in heated HVP (Misharina et al., 1987). The odor threshold value is in the parts per million level (Fors, 1983), and they could therefore be important for the total flavor of the HVPs.

Many different pyrazines are detected from eHVP and eHVPrea (Table 2). The general odor of pyrazines is nutty, roasted, earthy, and popcorn-like (Mauron, 1981; Fors, 1983). They seem to be key components in the discrimination between aHVP and eHVP/eHVPrea (Table 3). They also seem to be more abundant in eHVPrea compared to eHVP (Table 2) as another indication that further Maillard reactions have taken place during the glucose/heating treatment. Due to a high threshold value for the methylpyrazines (Fors, 1983), they are not expected to be very important for the general flavor of the HVPs. The ethyl-substituted pyrazines have a much lower threshold (Fors, 1983) and could therefore be of significance. The compounds 2,3-, 2,5-, and 2,6-dimethylpyrazine and tetramethylpyrazine have been reported in acidic HVPs (Manley et al., 1981) and in model systems containing different amino acids heated with different carbohydrates or other reactants (Danehy, 1986; Wong and Bernhard, 1988; Shu and Ho, 1989; Meynier and Mottram, 1995). Trimethylpyrazine has not been reported before in HVP but has been found in soy sauce (Manley et al., 1981). 3-Ethyl-2,5-dimethylpyrazine has been found in soy sauce (Manley et al., 1981), whereas 6-ethyl-2,3,5-trimethylpyrazine has hitherto not been described in HVP or similar products.

Furfural and 5-methyl-2-furfural are described as having a caramel, sweet, and fruity odor (Mottram, 1994b), and they have been found in both heated HVP and heated aqueous soy extract (Weir, 1986; Misharina et al., 1987; Coleman et al., 1996). On their own they might not have any great influence on the overall flavor of aHVP, but they are important precursors for thiophenes which—if they have a thiol group in position 3—are known to be very important for meat flavor due to an extremely low odor threshold value in the parts per billion level (Fors, 1983; Mottram, 1992; Mottram, 1994b). Even though thiophenes have not been identified in this study, they could easily have been present in quantities large enough to have a major impact on the flavor because of the low odor threshold.

The aliphatic sulfides, having either a meaty odor or an onion/cabbage odor with low odor threshold values (Gasser and Grosch, 1988; Mottram, 1992; Manley and Ahmedi, 1995), have been reported in meat (Gasser and Grosch, 1988), heated aqueous soy extract (Coleman et al., 1996), soy sauce (Manley et al., 1981), heated HVP (Misharina et al., 1987), and HVP (Manley et al., 1981). Due to the low odor threshold value, they could be expected to have a major influence on the flavor of the aHVP.

From the analysis of volatiles it could be seen that the 1-h heating of eHVP in the presence of a low amount of glucose had resulted in an increased amount of Maillard reaction product. Even though eHVP and eHVPrea were significantly different in a triangel test ($p < 0.05$), the heating had not altered the sensory profile to any extent (Figure 3). This could be due to the conditions during the reaction, which were performed in a commercial realistic way. If a pentose instead of a hexose had been chosen, both the qualitative and quantitative composition of the volatiles could have been altered (May, 1991). Higher temperature (Shu and Ho, 1989) and lower pH (Shu et al., 1985; Shu and Ho, 1989; Mottram, 1994a; Meynier and Mottram, 1995) could likewise have influenced the volatile production in order to prepare an HVP more like the aHVP.

Even though aHVP had the most intense flavor especially concerning the soy sauce and bouillon characteristics (Figure 3), it was not possible to detect and identify many volatile compounds from it (Table 2). This could be due to a low threshold of the important aroma components combined with the liquid desorption technique used.

The furfurals and aliphatic sulfides detected in aHVP have been reported respectively in heated aqueous soy extract (Weir, 1986; Misharina et al., 1987; Coleman et al., 1996) and soy sauce (Manley et al., 1981) and could therefore be key components in the difference in soy flavor between aHVP and eHVP/eHVPrea. The aliphatic sulfides detected in aHVP have also been reported in meat (Gasser and Grosch, 1988). In combination with the higher amount of Glu in aHVP compared to eHVP/eHVPrea, the aliphatic sulfides could provide an explanation of the variation in bouillon flavor.

Factor 2 from the sensory PLS analysis was mainly due to replicate variation (Figure 4). The basic tastes such as bitter and sweet had a high loading at factor 2 (Figure 4). This combination shows that the sensory panel seemed to have difficulties in assessing these tastes, possibly because the products were neither very bitter nor very sweet or because of the complexity of the hydrolysates. The flavor characteristics except smoky odor and bread odor had a low loading at factor 2 showing that they seemed much easier to replicate.

A sensory profile of HVPs has not been described hitherto (Figure 3). HVPs are traditionally divided into pale and dark HVPs with pork, beef, or chicken characteristics where the specific meat characters are

obtained by selection of raw materials and production conditions both during and after the hydrolysis step (Munk Nielsen, 1997). This study shows that characteristics such as lovage, malt/bread, and smoky can also be descriptors of HVPs. The differences between the tested products were however due to the descriptors bouillon, soy, and lovage/vegetable odor (Figure 4).

This experiment shows that it is possible to alter the chemical and flavor characteristics of HVPs by using different kinds of hydrolyses and production conditions after the hydrolysis step. Further studies of how to obtain a flavor profile of a reacted enzymatic hydrolysate more similar to the acidic hydrolysate and to meat flavor are needed as well as studies of the relations between the chemical and sensory characteristics of HVPs.

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