

Volatile aroma components of soy protein isolate and acid-hydrolysed vegetable protein

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Abstract

The volatile aroma components of soy protein isolate (SPI) and acid-hydrolysed vegetable protein (aHVP) were compared by gas chromatography–mass spectrometry (GC–MS) and gas chromatography olfactometry (GCO). Major differences were found between the two soy-based products. Aliphatic aldehydes and ketones were mainly found in SPI, whereas pyrazines and sulphur-containing compounds were dominant in aHVP. Analyses of the non-volatile components showed that SPI was mainly protein (82.5%) with some lipid (3.5%), whereas aHVP contained no protein, only free amino acids (18.4%) and a trace quantity of lipid (0.4%). Polyunsaturates (47.8%), followed by saturates (24.9%) and monounsaturates (14.8%) dominated the fatty acid profile of the SPI lipid fraction. Both SPI and aHVP had a free fatty acid content <0.1%. Sensory analyses of aqueous suspensions of SPI and aHVP demonstrated significant differences in the odours of the two products. Compounds responsible for some of these differences were identified by GCO and GC–MS analyses of aqueous suspensions. The possible role of SPI and aHVP in the development of aroma in extrudates containing these soy products is discussed.

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1. Introduction

As part of our continuing study of the effect of different ingredients on the development of aroma in extruded products we chose to analyse the volatile components of two soybean-based ingredients, soy protein isolate (SPI) and acid-hydrolysed vegetable protein (aHVP). Both SPI and hydrolysed vegetable protein (HVP) have become important ingredients in extruded foods as a consequence of their availability at reasonable cost and demonstrated functional and nutritional values (Lusas & Riaz, 1995; Messina, 1995). SPI is used

for texturisation and to increase the protein content of foods (Lusas & Riaz, 1995) and HVP to promote cooked and roasted aromas through Maillard reactions (Aaslyng et al., 1998b). SPI is prepared from milled soybean white flakes or flours by solubilisation of the protein at pH 6.8–8 and 27–66 °C using aqueous sodium hydroxide or other alkaline agents approved for food use, followed by acidification to pH 4.5 with either hydrochloric or phosphoric acid (Lusas & Riaz, 1995). The resultant curd is concentrated by centrifugation after adjustment to pH 6.5–7.0 or spray-dried in its acidic form (Lusas & Riaz, 1995). HVP can be produced from soybean protein, either by acid or enzyme hydrolysis. Commercially, acidic hydrolysates are obtained by treating the protein with 4–6 M hydrochloric acid at 100–130 °C for 2–24 h followed by neutralisation with sodium hydroxide (Weir, 1986). Enzymatic hydrolysis

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involves a much milder treatment, including an initial heat treatment to 85–90 °C for several 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 (Pommer, 1995). The acid hydrolysate (aHVP) is usually dark-brown in colour and has a strong savoury flavour, whereas the enzymic hydrolysate (eHVP) is lighter in colour and has a much less pronounced flavour (Weir, 1992).

Currently, little is known of the effects that SPI and HVP, as ingredients, have on the aroma of extruded cereal-based foods. As manufactured, SPI has a slight green or bean-like aroma, whereas the aroma of HVP varies between bouillon/soy and malt/brown bread (Aaslyng et al., 1998b). Accordingly, the retention, by extruded foods, of compounds responsible for these ingredient aromas could influence the acceptability of the final product. In addition, the interaction of the cereal base, during extrusion with non-volatile components, such as amino acids and fatty acids present in these ingredients, could lead to the production of additional aroma compounds that would also influence the aroma of extruded foods.

Some work has been reported on the volatile components of acid- and enzyme-hydrolysed HVP; however, in that study the hydrolysates examined were produced under laboratory conditions (Aaslyng, Elmore, & Mottram, 1998a). By comparison, we have analysed the volatile components of commercially available samples of SPI and aHVP and have also determined the amino acid and fatty acid contents of these ingredients. This paper will consider the possible role that certain volatile components present in SPI and aHVP could have on the aroma of extruded products and will also outline the possible origin of such compounds in these ingredients.

2. Materials and methods

2.1. Raw materials, reagents and reference chemicals

The samples of SPI were obtained from ADM Protein Specialties P/L (Decatur, IL) and had a moisture content of 10.3% (w/w) and a pH of 6.6. The samples of aHVP were supplied by Halycon Proteins P/L (Dandenong, Australia) and were manufactured from soy protein. This material had a moisture content of 6.4% and a pH of 5.0. Distilled water was purified through a Milli-Q purification system (Millipore Corp., Bedford, MA). All inorganic chemicals were of analytical reagent grade and were purchased from Merck KGaA (Darmstadt, Germany). Authentic samples of reference compounds were either purchased from a range of laboratory chemical suppliers or obtained as gifts from flavour laboratories. Chlorododecane and chlorotetradecane were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI).

2.2. Lipid and free fatty acid analysis

Lipid and free fatty acid contents of samples of SPI and aHVP were determined by BRI Australia (North Ryde, Australia) using the following methods: bound and unbound lipids were determined, following acid hydrolysis of the samples and extraction with diethyl ether and petroleum ether (b.p.<60 °C) according to the Official Methods of AOAC (1995) 922.06. Unbound lipids were determined by extraction of the samples with diethyl ether, according to the Official Methods of AOAC (1995) 920.85. Free fatty acids were determined by titration as oleic acid, using the Official Methods of AOAC (1995) 920.85 and 940.28. All analyses were performed in triplicate and results are reported as mg/10 g sample.

2.3. Fatty acid composition of bound and unbound lipids

The fatty acid compositions of the lipid component of the SPI and aHVP samples were determined by Silliker Microtech Ltd (Regents Park, Australia) as their methyl esters using the Official Methods of AOAC (1995) 963.22. The bound and unbound lipids were isolated as previously described and were hydrolysed with methanolic sodium hydroxide. The salts of the fatty acids were converted to their methyl esters with boron trifluoride in methanol using the Official Methods of AOAC (1995) 991.39 and the esters quantified by gas chromatography (GC). The analyses were performed in triplicate and the results were reported as mg/10 g sample.

2.4. Total and water-soluble protein (amino acids)

Total and water-soluble protein (amino acids) in SPI and aHVP were determined by BRI Australia Ltd (North Ryde, Australia) using the Official Methods of AOAC (1995) 984.13 and 923.04, respectively. In both methods the nitrogen content was determined by titration, after digestion, of the samples in the presence of copper II sulphate and titanium dioxide and sulphuric acid. All analyses were performed in triplicate and % protein was obtained by multiplication of % nitrogen by 5.7.

2.5. Amino acid composition of SPI and aHVP

Amino acid analyses were performed by the Australian Proteome Analysis facility (North Ryde, Australia). Samples of SPI and aHVP were analysed for free amino acids without hydrolysis. However, to compare the amino acid composition of the proteins in SPI with the free amino acids in aHVP, a sample of SPI was also analysed after acid hydrolysis (110 °C/24 h). For the analyses of cysteine and methionine, the compounds were oxidised by performic acid to produce the cysteic and methionine

sulphone. α -Aminobutyric acid was used as the internal standard. Reaction of the amino acids with Waters AccQTag reagent (Waters Corp. Milford, MA) gave derivatives that were both detectable by UV light at 254 nm and fluorescence Ex 250, Em 395. The derivatised amino acids were separated using a Waters AccQ-Tag column. Each amino acid was quantified from calibration curves constructed from standards (Sigma Chemical Co. St Louis, MI) run at three different concentrations. All analyses were performed in duplicate and results are reported as mg/10 g sample.

In addition, a sample of soy flour was analysed, after hydrolysis and derivatisation, by gas chromatography–mass spectrometry (GC–MS) (School of Food Biosciences, University of Reading) to identify any non-essential amino acids present in soybean products.

2.6. Determination of reducing sugars in SPI and aHVP

Reducing sugars were determined colorimetrically after reaction with 3,5-dinitrosalicylic acid (Aldrich Chemical Co. Inc., Milwaukee, WI), according to the method of Englyst and Hudson (1987). The absorbances of the solutions were measured at 540 nm using a Pharmacia LKB Biochrom 4060 UV–visible spectrometer (Pharmacia Biotech, Uppsala, Sweden). A calibration curve was plotted for standard solutions of glucose and the reducing sugar contents of SPI and aHVP were expressed as mg glucose/10 g sample. All analyses were performed in triplicate.

2.7. Assessment of sample odours

Each sample (10 g) of SPI and aHVP was placed in a screw-capped glass jar and wetted with water (30 ml) just before sensory assessment. The samples were assessed at room temperature by a small trained panel using a range of pre-selected descriptive terms.

2.8. Collection of volatile components

A sample (10 g) of SPI or aHVP was transferred to a 250 ml conical flask fitted with a 30 mm screw joint, a Teflon seal, a Dreschel head and a magnetic stirrer bar. The sample was mixed with water (80 ml) and chlorododecane (100 ng in 100 μ l ethanol) was added as an internal standard to estimate the degree of recovery of the volatile compounds. A pre-conditioned, glass-lined stainless-steel tube (115 mm long \times 0.75 mm i.d.) packed with 10 mg Tenax TA (Scientific Glass Engineering Pty Ltd, Melbourne, Australia) was attached by a stainless-steel reducing union fitted to the Dreschel head outlet. During collection of the volatile components, the aqueous mixture was stirred slowly and the volatiles were swept from the flask onto the absorbent in the trap using a flow of oxygen-free nitrogen (40 ml/min). The collec-

tion was continued for 1 h, during which time the flask and sample were maintained at 37 °C in a water bath. The trap was maintained at room temperature. At the end of the collection the trap was removed and connected directly to the nitrogen supply (40 ml/min) for 5 min to remove residual moisture. An internal standard, chlorotetradecane (100 ng in 1 μ l pentane) was added for quantification purposes to the front of the trap just before analysis by GC–MS.

For the odour assessment of compounds by gas chromatography olfactometry (GCO), volatiles from three different quantities of SPI and aHVP (10, 1 and 0.1 g) were collected. The headspace technique was the same as that used for the GC–MS analyses, except that chlorotetradecane was not added to the trap before olfactory analysis.

2.9. Analysis of volatiles by gas chromatography–mass spectrometry

Analyses were performed on a Hewlett–Packard HP5890 series II gas chromatograph (Hewlett–Packard, Palo Alto, CA) fitted with a CHIS injection port (Scientific Glass Engineering, Melbourne, Australia) and coupled to a Hewlett–Packard HP5972 mass spectrometer controlled by a G 1701 BA ChemStation. The GC was fitted with a Hewlett–Packard HP5-Trace Analysis GC column (25 m \times 0.2 mm i.d. 1 μ m film thickness) and a pre-column retention gap (30 cm \times 0.32 mm i.d.), uncoated but deactivated (Fisons Instruments, Mainz, Germany). The absorbed volatiles were desorbed onto the front of the pre-column by heating the Tenax trap for 10 min at 280 °C and cooling the pre-column to –78 °C with solid carbon dioxide. During the desorption, the GC was held at 40 °C, then heated at 5 °C/min to 280 °C and held at this temperature for 5 min. Helium (0.6 ml/min) was the carrier gas. A series of *n*-alkanes (C₅–C₂₄) was analysed, under the same conditions each day, to obtain the linear retention index (LRI) values for the volatile components.

The MS was operated in the electron impact mode with an electron energy of 70 eV and an emission current of 50 μ A. The ion source temperature was 250 °C. A continuous scan mode was employed over a mass range of 35–400 amu, at a rate of 1 s/decade. 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 LRI values with either those of authentic standards or published values. The relative concentrations of individual compounds were determined by comparing the peak area of the compound in each chromatogram with that of the chlorotetradecane internal standard (100 ng) and assuming all response factors were 1. The relative concentrations of these compounds are reported as ng/10 g samples.

Compounds described as “trace” were present at concentrations of <0.5 ng/10 g samples. The reported concentrations are the average of three separate volatile isolations taken from each sample.

2.10. Gas chromatography olfactometry

A Hewlett–Packard HP 5890 series II Plus gas chromatograph equipped with a CHIS injection port and a humidified odour port (Scientific Glass Engineering, Melbourne, Australia) was used for the odour evaluations. The pre-column, column and GC oven conditions were the same as those used for the GC–MS analyses. The column effluent (0.6 ml/min) was split at a ratio of 1:8 (v/v) between the flame ionisation detector and the odour port. Both the detector and the connecting line to the port were held at 250 °C and the make-up gas for the detector and port was nitrogen (30 ml/min). Humidified air (40 ml/min) was added to the GC effluent at the odour port.

Five trained assessors separately evaluated the aromas of the eluted components from each of the three extracts (10, 1 and 0.1 g samples). The assessors described in their own words the odours perceived, and these descriptors were recorded, alongside the retention time of the odour. All odours reported here were described by at least three assessors. The assessors also rated the intensity of each odour according to a scale, including, low, moderate, strong and very strong. Retention data of the eluted compounds were obtained as LRI values by the analysis of a solution of *n*-alkanes (C₅–C₂₄) at the beginning and end of the days of olfactory analyses.

3. Results and discussion

3.1. Non-volatile components of SPI and aHVP

Data from the analyses of the non-volatile components of SPI and aHVP are recorded in Tables 1–3. Results in Table 1 shows that the free fatty acid and unbound lipid content of both soy products were very similar. However, after acid hydrolysis, the SPI was found to contain bound lipids (300 mg/10 g), whereas these additional compounds were not detected in the aHVP (Table 1). Analysis of the fatty acid composition

Table 1
Lipid content of SPI and aHVP

| Lipid | SPI (mg/10 g) | aHVP (mg/10 g) |
|------------------|---------------|-----------------|
| Free fatty acids | <10 | <10 |
| Unbound lipids | 50 | 40 |
| Bound lipids | 300 | nd ^a |

^a nd, not detected at a detection limit of 1 mg/10 g.

Table 2
Fatty acid content of SPI and aHVP lipids

| Fatty acid | SPI (mg/10 g) | aHVP (mg/10 g) |
|-------------------------|---------------|-----------------|
| Myristic acid (14:0) | 0.35 | nd ^a |
| Palmitic acid (16:0) | 66.9 | ND ^b |
| Margaric acid (17:0) | 0.7 | nd |
| Stearic acid (18:0) | 18.6 | ND |
| Arachidic acid (20:0) | 0.7 | nd |
| Palmitoleic acid (16:1) | 0.35 | nd |
| Oleic acid (18:1) | 44.1 | ND |
| Eloiodic acid (18:1) | 6.65 | ND |
| Eicosenoic acid (20:1) | 0.7 | nd |
| Linoleic acid (18:2) | 151.2 | ND |
| Linolenic acid (18:3) | 15.8 | ND |

^a nd, not detected at a detection limit of 50 µg/10 g.

^b ND, not determined.

Table 3
Amino acid composition of SPI and aHVP

| Amino acid | SPI ^{a,b} (mg/10 g) | aHVP ^{c,d} (mg/10 g) |
|---------------|------------------------------|-------------------------------|
| Aspartic acid | 770 | 49.0 |
| Glutamic acid | 1368 | 327 |
| Asparagine | nd ^e | 2.4 |
| Serine | 368 | 125 |
| Glycine | 287 | 72.8 |
| Threonine | 297 | 82.2 |
| Tyrosine | 300 | 39.0 |
| Cysteine | 95 | 16.8 |
| Alanine | 280 | 240 |
| Proline | 376 | 218 |
| Valine | 360 | 90.1 |
| Methionine | 102 | 33.0 |
| Isoleucine | 373 | 48.2 |
| Leucine | 587 | 183 |
| Phenylalanine | 411 | 117 |
| Tryptophan | nd | 20.0 |
| Histidine | 207 | 43.6 |
| Arginine | 626 | 80.6 |
| Lysine | 445 | 47.2 |

^a After acid hydrolysis of protein.

^b Protein content 82.5%, free amino acids not detected.

^c Free amino acid content.

^d Free amino acids content 18.4%, protein not detected.

^e nd, not detected at a detection limit of 50 µg/10 g.

of the total lipids of the soy products showed that the SPI lipids contained 11 fatty acids, of which 24.9% were saturated, 14.8% monounsaturated and 47.8% polyunsaturated acids (Table 2). By comparison, no quantitative data was obtained from the analysis of the aHVP lipids, although six fatty acids were detected (Table 2). Consequently, it has been assumed that the lipid isolated from the aHVP was principally non-saponifiable material. The high proportion of polyunsaturated fatty acids (PUFA) in the SPI isolate would suggest that most of the bound lipids in this material were phospholipids (Mottram, 1998). Although it was not possible to quantify the individual fatty acids present in the aHVP iso-

late, the detection of six acids common to those of SPI would suggest that these two materials have similar free fatty acid compositions.

Results in Table 3 show that SPI contained 82.5% protein but no free amino acids, whereas aHVP contained 18.4% free amino acids but no protein. Analysis of the amino acids present in SPI protein, after acid hydrolysis, showed that this protein was principally made up of 17 amino acids. Those amino acids present in greatest concentrations were glutamic acid, aspartic acid, arginine, leucine, lysine and phenylalanine. By comparison, analysis of the free amino acids in aHVP showed that this material contained 19 amino acids. Those acids not present in the SPI protein were asparagine and tryptophan (Table 3). The major amino acids present in the aHVP were glutamic acid, alanine, proline, leucine, serine and phenylalanine. Accordingly, the amino acid compositions of the SPI protein and aHVP free acids were very similar.

Reducing sugars were not detected in either soy product.

3.2. Volatile components of SPI and aHVP

Data from the analysis of the volatile components of SPI and aHVP are reported in Table 4. A total of 95 compounds, of which 30 were tentatively identified, were found in the two soy products. Thirty three were found in the SPI and 78 in the aHVP. However, quantitatively, the SPI (6607 ng/10 g) had a higher concentration of compounds than the aHVP (1776 ng/10 g). The major difference between the contents of the SPI and aHVP was in the level of lipid-derived compounds. Such compounds accounted for 5716 ng/10 g (86.5% w/w of total volatiles) in the SPI but only 126 ng/10 g (7.1% w/w of the total volatiles) in the aHVP. These quantities of lipid-derived compounds are consistent with the levels of total fatty acids found in the two soy products (Table 2) and indicate that SPI, at some earlier stage of production, had a much higher proportion of free fatty acids than found in the final product (Table 1). By comparison, the major compounds in the aHVP were derived from amino acids. Such compounds accounted for 1156 ng/10 g (65% w/w of total volatiles) in the aHVP but only 240 ng/10 g (3.6% w/w of total volatiles) in the SPI. These quantities of amino acid-derived compounds are also consistent with the levels of free amino acids found in the two soy products (Table 3).

3.3. Lipid derived compounds

Of the 33 compounds found in SPI, 19 were derived from lipid degradation, including aliphatic aldehydes (11), ketones (4) and alcohols (2). The additional compounds were benzaldehyde and 2-pentylfuran. By comparison, of the 78 compounds found in the aHVP,

only 12 were derived from lipid degradation, including aliphatic aldehydes (6) ketones (4), alcohols (1) and benzaldehyde (Table 4). The major lipid-derived compounds in SPI were hexanal (2800 ng/10 g), 2-pentylfuran (1200 ng/10 g), pentanal (700 ng/10 g) and 2-heptanone (670 ng/10 g) whereas, in aHVP, the corresponding major compounds were benzaldehyde (200 ng/10 g), 2-butanone (48 ng/10 g), nonanal (26 ng/10 g) and octanal (11 ng/10 g). Furthermore, in the aHVP, hexanal and 2-heptanone were only minor components (8 and 2 ng/10 g) and pentanal and 2-pentylfuran were absent. Another major difference between the lipid-derived volatiles in these products was the presence of 2-alkenals (2) and 2,4-alkadienals (3) in the SPI, compared with their absence in the aHVP. All of the aliphatic aldehydes (alkanals, 2-alkenals and 2,4-alkadienals) are derived from the oxidation of oleic, linoleic and linolenic acids (Badings, 1970). In particular, hexanal would be derived from the oxidation of linoleic and linolenic acids, two of the major fatty acids found in SPI (Table 2). The absence of quantifiable levels of these fatty acids in the aHVP accounts for the low levels of aldehydes found in this material (Table 4).

The aromatic aldehyde, benzaldehyde, was present in both SPI and aHVP in comparable concentrations (Table 4). Two pathways have been proposed for the formation of this compound in food; one involves lipid oxidation (Bruechert et al., 1988) and the other Strecker degradation of phenylglycine (Vernin & Párkányi, 1982). Accordingly, it would appear that, in SPI, benzaldehyde is derived from lipid oxidation of linoleic acid (Bruechert et al., 1988) whereas, in aHVP, the likely source could be an amino acid such as phenylglycine (Vernin & Párkányi, 1982).

Four aliphatic ketones were found in each soy product but only 2-heptanone was common to both materials (Table 4). As with the aliphatic aldehydes, far greater quantities of ketones were found in the SPI (749 ng/10 g) than in aHVP (59 ng/10 g). The major ketone formed in SPI was 2-heptanone. During lipid oxidation, 2-alkanones can be formed from alkanals by their reaction with methyl free radicals (Mookherjee, Deck, & Chang, 1965). The high concentrations of hexanal and 2-heptanone found in SPI support this reaction pathway, as does the co-occurrence of significant levels of octanal and 2-nonanone, and nonanal and 2-decanone in this material. The other major ketone found in SPI, (*E,E*)-3,5-octadien-2-one, had been shown to be an autoxidation product of linolenic acid but as a result of further isomerization or by other reactions (Badings, 1970). The major ketone found in aHVP was 2-butanone. This compound may be formed by lipid oxidation (Selke, Rohwedder, & Dutton, 1975) or by thermal decomposition of glucose (Umano, Hagi, Nakahara, Shyoji, & Shibamoto, 1995). As 2-butanone was not found in SPI, a material with a relatively high lipid content, it would

Table 4
Relative concentrations of headspace volatiles of SPI and aHVP

| Identity | LRI ^a | SPI ng/10 g sample ^b (SD) | aHVP | Method of identification ^c |
|---|------------------|---|----------|---------------------------------------|
| <i>Lipid-derived</i> | | | | |
| <i>Aldehydes</i> | | | | |
| Pentanal | 722 | 700 (170) | – | MS+LRI |
| Hexanal | 817 | 2800 (360) | 8 (5.0) | MS+LRI |
| Heptanal | 907 | 110 (13) | 4 (2.8) | MS+LRI |
| Octanal | 1008 | 34 (4.4) | 11(4.2) | MS+LRI |
| Nonanal | 1105 | 23 (3.1) | 26 (3.6) | MS+LRI |
| Decanal | 1204 | 4 (0.73) | 9 (3.0) | MS+LRI |
| Undecanal | 1304 | – | 1 (0.8) | MS+LRI |
| (<i>E</i>)-2-Octenal | 1062 | 23 (2.6) | – | MS+LRI |
| (<i>E</i>)-2-Nonenal | 1162 | 5 (0.98) | – | MS+LRI |
| (<i>E,E</i>)-2,4-Heptadienal | 1018 | 3 (0.21) | – | MS+LRI |
| (<i>E,E</i>)-2,4-Nonadienal | 1217 | 3 (0.83) | – | MS+LRI |
| (<i>E,E</i>)-2,4-Decadienal | 1317 | 6 (0.91) | – | MS+LRI |
| <i>Aromatic aldehydes</i> | | | | |
| Benzaldehyde ^d | 979 | 360 (59) | 200 (26) | MS+LRI |
| <i>Ketones</i> | | | | |
| 2-Butanone ^e | <650 | – | 48 (33) | MS+LRI |
| 2-Pentanone | 699 | – | 7 (2.1) | MS+LRI |
| 2-Hexanone | 802 | – | 2 (0.2) | MS+LRI |
| 2-Heptanone | 898 | 670 (120) | 2 (0.3) | MS+LRI |
| 2-Nonanone | 1093 | 26 (4.0) | – | MS+LRI |
| (<i>E,E</i>)-3,5-Octadien-2-one | 1100 | 38 (6.0) | – | MS+LRI |
| 2-Decanone | 1192 | 15 (2.3) | – | MS+LRI |
| <i>Alcohols</i> | | | | |
| 1-Hexanol | 880 | 32 (5.0) | – | MS+LRI |
| 1-Octen-3-ol | 984 | 24 (3.8) | 8 (2.0) | MS+LRI |
| <i>Furans</i> | | | | |
| 2-Pentylfuran | 995 | 1200 (240) | – | MS+LRI |
| <i>Sugar-derived</i> | | | | |
| <i>Furans</i> | | | | |
| 2-Furfural | 841 | – | 1 (0.08) | MS+LRI |
| 1-(2-Furanyl)-ethanone | 916 | – | 16 (3.4) | MS |
| Dihydro-5-methyl-2(3 <i>H</i>)-furanone | 965 | – | 8 (4.4) | MS |
| 2-Acetyl-5-methylfuran | 1042 | – | 7 (1.0) | MS+LRI |
| <i>Amino acid-derived</i> | | | | |
| <i>Aldehydes</i> | | | | |
| 2-Methylpropanal | <650 | – | 31 (3.9) | MS+LRI |
| 3-Methylbutanal | 669 | 97 (29) | 460 (16) | MS+LRI |
| 2-Methylbutanal | 677 | – | 110 (13) | MS+LRI |
| Phenylacetaldehyde | 1063 | – | 57 (15) | MS+LRI |
| <i>Maillard reaction-derived heterocyclic, aliphatic and aromatic compounds</i> | | | | |
| <i>Oxazoles</i> | | | | |
| 4,5-Dimethyloxazole | 796 | – | 1 (0.21) | MS+LRI |
| 2,4,5-Trimethyloxazole | 848 | – | 1 (0.5) | MS+LRI |
| <i>Pyrroles</i> | | | | |
| 1 <i>H</i> -pyrrole | 771 | – | 6 (5.1) | MS+LRI |
| 1-Ethyl-1 <i>H</i> -pyrrole | 829 | – | tr | MS+LRI |
| 2-Methyl-1 <i>H</i> -pyrrole | 853 | – | 1 (0.48) | MS+LRI |
| <i>Pyrazines</i> | | | | |
| Methylpyrazine | 839 | – | 3 (0.42) | MS+LRI |

Table 4 (continued)

| Identity | LRI ^a | SPI ng/10 g sample ^b | aHVP (SD) | Method of identification ^c |
|---|------------------|------------------------------------|--------------|---------------------------------------|
| 2,5- or 2,6-Dimethylpyrazine | 916 | – | 11 (1.2) | MS + LRI |
| 2-Ethyl-5-methylpyrazine | 1000 | – | 13 (1.9) | MS + LRI |
| 2-Methyl-5-(1-methylethyl)-pyrazine | 1059 | – | 3 (0.57) | MS |
| 2,5-Dimethyl-3-ethylpyrazine | 1081 | – | 26 (4.1) | MS + LRI |
| 2-Methyl-6-propylpyrazine | 1090 | – | 10 (1.8) | MS |
| 2,3-Diethyl-6-methylpyrazine | 1122 | – | 2 (0.56) | MS + LRI |
| 3,5-Diethyl-2-methylpyrazine | 1126 | – | 5 (1.1) | MS + LRI |
| 2,5 or 2,6-Dimethyl-3-propylpyrazine | 1131 | – | 11 (1.3) | MS |
| 3,5-Dimethyl-2-(2-methylpropyl)- pyrazine | 1141 | – | 1 (0.4) | MS |
| 2,3,5-Trimethyl-6-propylpyrazine | 1171 | – | 2 (0.07) | MS |
| 6-Methyl-2-(3-methylbutyl)-pyrazine | 1248 | – | 5 (0.97) | MS |
| 2,5-Dimethyl-3-(3-methylbutyl)-pyrazine | 1308 | – | 9 (1.7) | MS |
| 2,3,5-Trimethyl-6-(3-methylbutyl)-pyrazine | 1381 | – | 2 (0.36) | MS |
| <i>Thiazoles</i> | | | | |
| 2,4,5-Trimethylthiazole | 990 | – | 2 (0.42) | MS + LRI |
| <i>Thiophenes</i> | | | | |
| 3-Methylthiophene | 805 | – | 2 (1.6) | MS |
| 2-Pentylthiophene | 1164 | 3 (0.62) | 1 (0.03) | MS + LRI |
| 3-Phenylthiophene | 1446 | – | 3 (0.15) | MS |
| <i>Aliphatic sulphur-containing compounds</i> | | | | |
| Dimethyl sulphide | <650 | – | 3 (2.8) | MS + LRI |
| (Methylthio)-ethane | <650 | – | 12 (8.0) | MS + LRI |
| Dimethyl disulphide | 756 | 140 (42) | 130 (38) | MS + LRI |
| 1-(Methylthio)-butane | 847 | – | 1 (0.55) | MS |
| Methyl ethyl disulphide | 861 | – | 1 (0.56) | MS + LRI |
| 3-Methyl-1-(methylthio)-butane | 869 | – | 2 (0.34) | MS |
| 1-(Methylthio)-pentane | 902 | – | 7 (0.45) | MS |
| Dimethyl trisulphide | 975 | – | 110 (2.1) | MS + LRI |
| 1-(Methylthio)-hexane | 1004 | – | 1 (0.11) | MS |
| Dimethyl tetrasulphide | 1240 | – | 10 (1.1) | MS + LRI |
| Ethanethioic acid, S-methyl ester | 766 | – | 42 (0.58) | MS |
| Thiocyanic acid, methyl ester | 723 | – | 18 (1.2) | MS |
| <i>Aromatic sulphur-containing compounds</i> | | | | |
| Benzyl methyl sulphide | 1167 | – | 2 (0.18) | MS + LRI |
| <i>Other sources</i> | | | | |
| <i>Aldehydes</i> | | | | |
| 2-Butyl-2-octenal | 1367 | 23 (7.4) | – | MS + LRI |
| 5-Methyl-2-phenyl-2-hexenal | 1486 | – | 16 (2.6) | MS + LRI |
| <i>Ketones</i> | | | | |
| 2,4-Dimethyl-3-pentanone | 806 | – | 3 (0.89) | MS |
| 5-Methyl-2-hexanone | 857 | – | 32 (1.6) | MS + LRI |
| Acetophenone | 1063 | – | 21 (2.2) | MS + LRI |
| 1-Phenyl-1-propanone | 1072 | – | 2 (0.94) | MS |
| 1-Phenyl-1-butanone | 1285 | – | 1 (0.47) | MS |
| (E,E)-6,10-dimethyl-5,9-undecadien-2-one | 1443 | – | tr | MS |
| <i>Phenols</i> | | | | |
| 3-Methylphenol | 846 | – | 1 (0.1) | MS + LRI |
| <i>Furans</i> | | | | |
| 2-Furancarboxitrile | 822 | – | 4 (0.42) | MS |
| 3-Phenylfuran | 1228 | – | 1 (0.05) | MS |
| <i>Hydrocarbons</i> | | | | |
| Toluene | 784 | 93 (8.7) | 170 (18) | MS + LRI |

(continued on next page)

Table 4 (continued)

| Identity | LRI ^a | SPI ng/10 g sample ^b (SD) | aHVP | Method of identification ^c |
|-------------------------------|------------------|---|-----------|---------------------------------------|
| Ethylbenzene | 869 | – | 8 (1.2) | MS+LRI |
| 1,4-Dimethylbenzene | 878 | 6 (2.2) | 4 (0.510) | MS+LRI |
| Styrene | 900 | – | 17 (2.5) | MS+LRI |
| Propylbenzene | 958 | – | 7 (1.5) | MS+LRI |
| 1,2,3-Trimethylbenzene | 999 | – | 1 (0.8) | MS+LRI |
| Limonene | 1034 | 4 (0.26) | 2 (0.66) | MS+LRI |
| Undecane | 1100 | – | tr | MS+LRI |
| Dodecane | 1200 | 3 (0.29) | 2 (0.33) | MS+LRI |
| Tridecane | 1300 | 1 (0.85) | – | MS+LRI |
| <i>Miscellaneous</i> | | | | |
| 2-Methylpropanenitrile | <650 | – | 2 (0.38) | MS |
| 1-Tert-butoxy-2-methoxyethane | 844 | 4 (2.3) | – | MS |
| 1-Nitropentane | 947 | 53 (22) | – | MS |
| Benzonitrile | 994 | – | 4 (0.49) | MS |
| 1-Nitrohexane | 1050 | 100 (15) | – | MS |
| 1-Nitroheptane | 1150 | 2 (0.35) | – | MS |
| Butyl butanoate | 1370 | 2 (0.9) | 3 (1.8) | MS+LRI |

(The GC correction factors for each component are considered to be 1:1. Consequently, the listed quantities are considered as semi-quantitative.)

^a Linear Retention Index.

^b Concentrations (ng/10 g) obtained by comparing GC–MS peak area with that from 100 ng chlorotetradecane internal standard added to the Tenax trap after volatile collection; the average of triplicate analyses are shown; (–) not detected (limit of detection approximately 0.1 ng/10 g sample); (tr) volatiles in concentrations of <0.5 ng/10 g.

^c MS+LRI, identified by comparison of mass spectrum and LRI with those of an authentic compound or previously published data; MS, mass spectrum agrees with the reference spectrum from the NIST/EPA/NIH Mass Spectral Database.

^d Benzaldehyde may be lipid- or amino acid-derived.

^e 2-Butanone may be lipid- or sugar-derived.

appear that the most likely source of this compound in aHVP was carbohydrate.

Two aliphatic alcohols, 1-hexanol and 1-octen-3-ol were found in SPI but only 1-octen-3-ol was found in aHVP (Table 4). Both alcohols are derived from lipid oxidation, 1-hexanol from tristearin (Selke et al., 1975) and 1-octen-3-ol from linoleic acid (Badings, 1970). The higher levels of alcohols found in SPI (56 ng/10 g) compared with that of aHVP (8 ng/10 g) are in keeping with the relative lipid contents of these soy products.

2-Pentylfuran was found in SPI in major concentrations (1200 ng/10 g) but was not detected in aHVP (Table 4). This compound is an identified oxidation product of methyl linoleate (Frankel, Neff, & Selke, 1981; Neff, Frankel, Selke, & Weisleder, 1983). Accordingly, its absence from aHVP, when other oxidation products of linoleic acid are present, suggests that the precursor of 2-pentylfuran forms alternative products in the presence of free amino acids (Table 3).

3.4. Sugar-derived compounds

Four furan derivatives derived from sugars were found in minor concentrations in aHVP but were not detected in SPI (Table 4). All of these compounds are thermal decomposition products of glucose (Umano

et al., 1995) and their presence indicates that aHVP initially contained some reducing sugars. 2-Butanone, although reported as a lipid-derived component, is also an identified thermal decomposition product of glucose (Umano et al., 1995). This compound was also only found in aHVP (Table 4).

3.5. Amino acid-derived compounds

Only three of the 33 compounds identified in SPI were derived from either the Strecker degradation of amino acids or Maillard reaction, whereas some 39 of the 78 compounds found in aHVP were derived from these sources (Table 4). In SPI, the two compounds were 3-methylbutanal from the Strecker degradation of leucine and dimethyl disulphide from the Strecker or thermal degradation of cysteine or methionine (Vernin & Párkányi, 1982). These three amino acids were not found free in SPI but were all present in the protein fraction of this material (Table 3). By comparison, the aHVP contained four Strecker aldehydes as well as 11 methylsulphide derivatives and two thioacids (Table 4). The additional Strecker aldehydes, 2-methylpropanal, 2-methylbutanal and phenylacetaldehyde were derived from valine, isoleucine and phenylalanine (Vernin & Párkányi, 1982). All six amino acids involved in the

formation of these aldehydes and of the methylsulphide derivatives were present in the free state in aHVP (Table 3). As previously discussed, benzaldehyde can be formed by the Strecker degradation of phenylglycine (Adamic, Rössner, Velišek, Cejpek, & Šavel, 2001); however, analysis of soy flour for non-essential amino acids showed that phenylglycine was not present in this soybean-derived material. Accordingly, the origin of benzaldehyde in aHVP remains uncertain.

Excluding benzaldehyde, the other Strecker aldehydes, together with methylsulphide derivatives and thioacids, account for 89% of the amino acid-derived compounds in aHVP. The remaining 11% are heterocyclic compounds, made up of oxazoles (2), pyrroles (3), pyrazines (14), thiazoles (1) and thiophenes (Table 4). All of these compounds are formed directly from amino acid degradation products (Vernin & Párkányi, 1982), by the interaction of these degradation products with sugars (Vernin & Párkányi, 1982) or lipid-derived compounds (Whitfield, 1992). Accordingly, the failure to detect nitrogen-containing heterocyclic compounds in SPI (Table 4) can be attributed to the absence of free amino acids in this material.

Most heterocyclic compounds found in aHVP have been identified as components of cooked and heat-processed foods (MacLeod & Seyyedain-Ardebili, 1981) and as reaction products of amino acid/sugar model systems (Whitfield, Mottram, Brock, Puckey, & Salter, 1988). This particularly applies to the five major pyrazines, 2,5 (or 2,6)-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 2-methyl-6-propylpyrazine and 2,5 (or 2,6)-dimethyl-3-propylpyrazine found in aHVP (Table 4). These compounds account for 59% of the heterocyclic compounds derived from the Maillard reaction found in aHVP.

3.6. Compounds of unidentified origins

In SPI, compounds of unidentified origin account for 4.4% of the total volatile content whereas, in aHVP, they account for 17%. Major contributors to this class in SPI are 1-nitrohexane, toluene and 1-nitropentane and in aHVP they are toluene, 5-methyl-2-hexanone, acetophenone and styrene (Table 4). Toluene has been identified as a thermal degradation product of lipids (Min, Ina, Peterson, & Chang, 1977) and carotenoids (Bruechert et al., 1988); however, the relatively high concentrations of this compound found in SPI and aHVP suggest that it, together with the other aromatic hydrocarbons found in these materials (Table 4), are derived from external sources. SPI contains three nitroalkanes with 5, 6 and 7 carbon atoms (Table 4). Since the three major aliphatic aldehydes present in SPI are pentanal, hexanal and heptanal, it is possible that these compounds are the precursors of the nitroalkanes in this material. Of the remaining compounds, 2-butyl-2-

octenal in SPI and 5-methyl-2-phenyl-2-hexenal in aHVP appear to be Aldol condensation products of aldehydes, derived from lipid and amino acid degradations. However, it is difficult to explain the origins of 5-methyl-2-hexanone and acetophenone in aHVP.

3.7. Sensory analyses of volatile components of SPI and aHVP

Preliminary sensory assessment of the samples of SPI showed they had a weak green aroma with an underlying bitter fatty note. By comparison, the samples of aHVP had an intense green, garlic and onion-like aroma, somewhat similar to that of chicken broth. In an attempt to identify those compounds contributing to these aromas, samples of SPI and aHVP were analysed by GCO at three dilutions. Those compounds detected at the greatest dilution could be expected to most influence the aroma of these soy products. The results of these analyses are discussed below.

3.8. Aroma profile of SPI

The GCO analyses of the volatile components of SPI led to the detection of 37 odour points of which 17 had LRI values that corresponded to identified compounds (Table 5). Accordingly, it would appear that many of the odorous compounds were present at concentrations below their GC–MS detection limits. None of the aromas were described as ‘very strong’ but 14 were described as ‘strong’. These odours corresponded to the LRI values of six identified compounds, dimethyl disulphide, hexanal, 1-hexanol, 2-heptanone, 1-octen-3-ol, 2-pentylfuran, and eight unidentified compounds. Descriptors used by the panel for most of these compounds were in agreement with those reported in the literature (Aldrich, 1998; Badings, 1970; Fors, 1983). However, it is accepted that, for some odours, the compounds identified might not be responsible for the perceived odour. At one tenth dilution, 25 odours were detected, of which seven had intensities described as strong (Table 5). Two of the odours with strong intensities had LRI values that corresponded to hexanal and 2-pentylfuran. The other five odours could not be associated with any identified compound. At this dilution, another six odours had intensities described as medium. Compounds associated with three of these odours were 3-methylbutanal, dimethyl disulphide and 1-octen-3-ol. At 100th dilution, the number of odours detected had declined to 14 of which two were described as strong and five described as of medium intensity. One of the strong odours had the same LRI as hexanal, and three of the odours with medium intensities corresponded to dimethyl disulphide, 1-octen-3-ol and 2-pentylfuran. The compounds responsible for the remaining two odours of medium intensity could not be identified.

Table 5
GCO analysis of volatile components at different concentrations of SPI

| LRI ^a | Aroma description | Aroma intensity ^b | | | Major compound in region of aroma ^c |
|------------------|--|------------------------------|-------|---------|--|
| | | 10 (g) | 1 (g) | 0.1 (g) | |
| 612 | Fresh green peas | S | – | – | Unknown |
| 633 | Sharp and bitey, soda water-like | M | M | M | Unknown |
| 638 | Sulphury, frankfurt-like on dilution | S | W | – | Unknown |
| 669 | Smooth, malty | M | M | – | 3-Methylbutanal |
| 722 | Nutty, sweet and green on dilution | W | W | W | Pentanal |
| 742 | Painty, crushed ants on dilution | M | S | W | Unknown |
| 756 | Unpleasant, stink bug, sulphury, crushed ant on dilution | S | M | M | Dimethyl disulphide |
| 792 | Herbaceous, grass-like, sweet on dilution | S | W | – | Unknown |
| 817 | Green, bitey, very sweet, dripping-like on dilution | S | S | S | Hexanal |
| 822 | Biscuit-like, vanilla, apple-like on dilution | S | S | – | Unknown |
| 839 | Crushed ant, eugenol-like, green on dilution | S | M | W | Unknown |
| 861 | Rock melon, tropical, metallic on dilution | W | W | W | Unknown |
| 866 | Garlic-like | W | – | – | Unknown (methyl ethyl disulphide) |
| 874 | Metallic, sweet and herbaceous | W | – | – | Unknown |
| 880 | Old, musty, grass-like, unpleasant | S | – | – | 1-Hexanol |
| 898 | Sweet, banana-like, musty on dilution | S | W | – | 2-Heptanone |
| 907 | Must, sweet, fruity | W | W | – | Heptanal |
| 970 | Garlic-like | M | – | – | Unknown (dimethyl trisulphide) |
| 979 | Metallic and herbaceous | W | – | – | Benzaldehyde |
| 984 | Medicinal, mushroom-like on dilution | S | M | M | 1-Octen-3-ol |
| 995 | Green | S | S | M | 2-Pentylfuran |
| 1008 | Stink bug | M | – | – | Octanal |
| 1025 | Eucalyptus oil | S | S | W | Unknown |
| 1062 | Metallic, vegetable-like on dilution | M | M | – | Unknown |
| 1065 | Frankfurt-like | W | W | – | Unknown |
| 1103 | Sulphury, sweet meat, cheese-cracker-like on dilution | M | W | – | Unknown |
| 1128 | Celery, cauliflower | W | W | W | Unknown |
| 1159 | Biscuit-like, vanilla-like on dilution | M | W | W | Unknown |
| 1162 | Grass | W | W | – | (E)-2-Nonenal |
| 1164 | Bitter green | W | – | – | 2-Pentylthiophene |
| 1217 | Cauliflower, wet carpet, mouldy, green on dilution | M | W | – | (E,E)-2,4-Decadienal |
| 1247 | Biscuit-like, biscuit- and cardboard-like on dilution | S | S | M | Unknown |
| 1283 | Hot oven, tomato-like on dilution | M | W | – | Unknown |
| 1304 | Biscuit | S | S | S | Unknown |
| 1317 | Bread, cheese-like on dilution | W | W | – | (E,E)-2,4-Decadienal |
| 1342 | Biscuit | W | – | – | Unknown |
| 1566 | Musty biscuit | W | W | – | Unknown |

^a LRI, Linear Retention Index.

^b VS, very strong; S, strong; M, moderate; W, weak; –, not present.

^c Aroma description need not necessarily relate to the compound(s) identified in this region of the chromatogram.

All 14 odorous compounds detected at the one hundredth dilution could be expected to influence the aroma of SPI. In particular, hexanal and 2-pentylfuran would be associated with the green aroma of this material. However, it is unclear which compounds contribute to the perceived bitter fatty notes of SPI. Further studies are required to identify the other 10 compounds detected by odour at this dilution.

3.9. Aroma profile of aHVP

Some 55 odour points were detected in the GCO analyses of the volatile compounds of aHVP, of which 37 had LRI values that corresponded to identified compounds (Table 6). Consequently, as with SPI, it would appear that many of the odorous compounds in aHVP

were also present at concentrations below their GC–MS detection limits. Eight of the odours were described as ‘very strong’ and 16 as ‘strong’. Of the odours with ‘very strong’ intensities, five had LRI values that corresponded to identified compounds, including 3-methylbutanal, 1-(methylthio)-butane, methyl ethyl disulphide, phenylacetaldehyde, 2,5 or (2,6)-dimethylpyrazine, and 2,3,5-trimethyl-6-(3-methylbutyl)pyrazine. By comparison, 11 of the 16 odour points with ‘strong’ intensities had the same LRI values as identified compounds (Table 6). At one tenth dilution, 39 odours were detected, of which 15 had intensities described as ‘strong’. Most of these odours had been previously described as ‘very strong’ or strong in the undiluted sample (Table 6). Ten of these odours had LRI values that corresponded to identified compounds. Prominent among

Table 6
GCO analysis of volatile components at different concentrations of aHVP

| LRI ^a | Aroma description | Aroma intensity ^b | | | Major compound in region of aroma ^c |
|------------------|---|------------------------------|-------|---------|--|
| | | 10 (g) | 1 (g) | 0.1 (g) | |
| 605 | Sweet, fruity | M | – | – | 2-Butanone |
| 669 | Pungent, sweet biscuit on dilution | VS | M | M | 3-Methylbutanal |
| 677 | Toast | M | W | – | 2-Methylbutanal |
| 752 | Anty | VS | S | M | Unknown |
| 756 | Garlic | S | S | M | Dimethyl disulphide |
| 762 | Boiled meat, car oil | S | – | – | Unknown |
| 771 | Sweet, nutty, sharp | S | S | – | Pyrrrole |
| 780 | Floral | W | – | – | Unknown |
| 783 | Beef stock, garlic-like | VS | S | W | Unknown |
| 792 | Acrylic, sharp | M | S | S | Unknown |
| 799 | Cooked fish | W | M | – | Unknown |
| 805 | Green, painty | S | S | W | 3-Methylthiophene |
| 806 | Caramel, ketone | S | S | W | 2,4-Dimethyl-3-pentanone |
| 817 | Green, aldehyde | M | – | – | Hexanan |
| 822 | Boiled meat, nutty on dilution | M | M | – | 2-Furancarboxitrile |
| 833 | Geranium, green | M | – | – | Unknown |
| 839 | Roasted peanuts, boiled potato on dilution | S | S | M | Methylpyrazine |
| 847 | Garlic, stench | VS | M | M | 1-(Methylthio)-Butane |
| 861 | Sulphur, stench, meaty on dilution | VS | W | – | Methyl ethyl disulphide |
| 900 | Musty, mouldy, damp, cardboard | S | – | – | Unknown |
| 902 | Potato skin, green, mouldy bread | M | M | – | 1-(Methylthio)-pentane |
| 907 | Grassy, fatty aldehyde | S | W | – | Heptanal |
| 916 | Peeled potato | M | – | – | 2,5- or 2,6-Dimethylpyrazine |
| 930 | Butter, fatty aldehyde | M | – | – | Unknown |
| 940 | Fatty, green, raw potato on dilution | M | M | – | Unknown |
| 942 | Raw potato, butter | M | W | – | Unknown |
| 975 | Bitter vegetable, unpleasant fatty, garlic-like on dilution | M | M | M | Dimethyl trisulphide |
| 979 | Peanuts, green, bitter | S | S | – | Benzaldehyde |
| 984 | Mushroom | M | M | W | 1-Octen-3-ol |
| 990 | Sulphury, meaty, oniony, garlic-like on dilution | S | S | M | 2,4,5-Trimethylthiazole |
| 1000 | Rubber, glue, cement, drain odour | M | S | W | 2-ethyl-5-Methylpyrazine |
| 1004 | Green, meaty, fatty | S | – | – | 1-(Methylthio)-Hexane |
| 1008 | Citrus, sweet | S | – | – | Octanal |
| 1034 | Leaf, acrylic | W | – | – | Limonene |
| 1053 | Peas, green, floral, rose-like on dilution | M | M | – | Acetophenone |
| 1063 | Floral heavy | VS | M | – | Phenylacetaldehyde |
| 1105 | Vegetable, fatty acrylic, wet dog | M | W | – | Nonanal |
| 1122 | Roasted peanuts, oil | M | – | – | 2,3-Diethyl-6-Methylpyrazine |
| 1126 | Chicken soup, lard, fish, savoury cheese, green on dilution | M | W | W | 3,5-Diethyl-2-methylpyrazine |
| 1131 | Nutty, battered fish | VS | M | – | 2,5 or 2,6-Dimethyl-3-pyropylpyrazine |
| 1072 | Pleasant green, fatty, wet dog-like on dilution | S | S | S | 1-Phenyl-1-propanone |
| 1141 | Vegemite | M | W | – | 3,5-Dimethyl-2-(2-methylpropyl)-pyrazine |
| 1240 | Onion, green beans, fishy | W | W | – | Dimethyl tetrasulphide |
| 1304 | Rubbery, furniture polish | M | – | – | Undecanal |
| 1308 | Buttered vegetables, boiled meat | W | – | – | 2,5-Dimethyl-3-(3-methylbutyl)-pyrazine |
| 1381 | Green, butter, garlic-like on dilution | VS | S | W | 2,3,5-Trimethyl-6-(3-methylbutyl)-pyrazine |
| 1426 | Burnt rubber, biscuit-like on dilution | M | M | W | Unknown |
| 1446 | Meaty, fried onion and sulphury on dilution | S | M | M | 3-Phenylthiophene |
| 1460 | Cooked onion, sulphur, rubber | S | S | – | Unknown |
| 1486 | Hot chocolate drink | W | W | W | 5-Methyl-2-phenyl-2-hexenal |
| 1523 | Heated lubricating oil, acrid, sharp | M | – | – | Unknown |
| 1553 | Onion, hot oil, fish-like, herbaceous, sulphury | M | W | – | Unknown |
| 1626 | Meaty, fatty, wet socks | M | – | – | Unknown |
| 1639 | Unpleasant, fatty, green | S | M | – | Unknown |
| 1857 | Biscuit, sharp musty flour, musty and metallic on dilution | M | S | W | Unknown |

^a LRI, Linear Retention Index.

^b VS, very strong; S, strong; M, moderate; W, weak; –, not present.

^c Odour description need not necessarily relate to the compound(s) identified in this region of the chromatogram.

these compounds were dimethyl disulphide, pyrrole, 3-methylthiophene, 2,4-dimethyl-3-pentanone, methylpyrazine, benzaldehyde, 2,4,5-trimethylthiazole, 2-ethyl-5-methylpyrazine, 1-phenyl-1-propanone and 2,3,5-trimethyl-6-(3-methylbutyl)-pyrazine. At one hundredth dilution, 20 odours were detected of which 15 had LRI values that corresponded to identified compounds (Table 6). Only two of these odours were described as 'strong', whereas another eight were of medium intensity. One of the strong odours had the same LRI value as 1-phenyl-1-propanone. Of those odours with medium intensity, seven had the same LRI values as 3-methylbutanal, dimethyl disulphide, methylpyrazine, 1-(methylthio)-butane, dimethyltrisulphide, 2,4,5-trimethyl-thiazole and 3-phenylthiophene.

All 20 odorous compounds detected at one hundredth dilution could be expected to influence the aroma of aHVP. The green note associated with aHVP would appear to be principally due to the presence of 1-phenyl-1-propanone. As for the onion-garlic note, at least four of the identified compounds, including dimethyl disulphide, 1-(methylthio)-butane, dimethyl trisulphide and 2,4,5-trimethylthiazole, would be major contributors. Furthermore, the chicken broth note associated with this material could be influenced by the presence of 3,5-diethyl-2-methylpyrazine (Table 6). These six compounds would appear to be the major contributors to the aroma of aHVP. Further studies are required to identify the other five compounds detected at this dilution.

3.10. Possible role of SPI and aHVP in extruded products

During the commercial production of some extruded products, SPI may be added at levels between 1% and 5% and aHVP at levels of 1% or less. Accordingly, the addition of these ingredients could affect the flavour of the extruded product, either as a result of the presence of odorous compounds in the SPI or aHVP, or from the generation of such compounds during extrusion from non-volatile precursors present in these ingredients. Consequently, all 14 compounds detected by aroma at one hundredth dilution of SPI and all 20 compounds similarly detected at one hundredth dilution of aHVP could influence the aroma of extruded products if these ingredients were added at 1% levels. Of the aroma compounds identified in SPI, two of these, hexanal and 2-pentylfuran, detected as strong and medium intensity odours at this dilution (Table 5), could be expected to play dominant roles in any green odour perceived in an extruded product. Similarly, those compounds identified in aHVP and detected as strong or medium intensity odours at one hundredth dilution, such as 1-phenyl-1-propanone, 3-methylbutanal, dimethyl disulphide and 3,4,5-trimethylthiazole (Table 6) could play equally important roles in any green, pungent or garlic-like odours present in the final product. Admit-

tedly, the concentrations of some of these compounds could be reduced during the extrusion process but they must still be considered as possible contributing aromas to any cereal extrudate containing either SPI or aHVP.

Non-volatile components present in SPI and aHVP could also contribute to the aroma of a product, either by decomposition or reaction with other ingredients during extrusion. Accordingly, the addition of 1% SPI to a cereal for extrusion would increase the free and bound lipid contents of the mixture by 3.5 mg/10 g, of which 47.8% of the fatty acids present would be polyunsaturated (Tables 1 and 2). Free fatty acids liberated by hydrolysis of the lipids during extrusion would, on oxidation, result in increased levels of carbonyl compounds, such as hexanal (Badings, 1970), and alkylfurans, particularly 2-pentylfuran (Frankel et al., 1981; Neff et al., 1983). These compounds could be expected to further increase the green aroma of the extruded product. Similarly, the addition of 1% aHVP would increase the free amino acid content of the mixture by 18.3 mg/10 g (Table 3). Such amino acids in the presence of reducing sugars from the cereal component of the mixture would undergo Strecker degradations (Vernin & Párkányi, 1982) and Maillard reactions (Vernin & Párkányi, 1982) to produce a wide range of odorous compounds. In particular, these would be the Strecker aldehydes, such as 3-methylbutanal, and the Maillard reaction products, such as dimethyl disulphide and 3,4,5-trimethylthiazole. Such compounds would further increase the pungent or garlic-like aroma of the extruded product. Whether the presence of such compounds, either present or subsequently derived from the added SPI or aHVP, would have a favourable effect or not on the aroma of the extrudate, would depend very much on the desired flavour of the final product.

4. Conclusion

Significant differences were found between the volatile and non-volatile compositions of SPI and aHVP. These differences could influence the aroma of an extrudate containing as little as 1% of either of these ingredients. The presence of SPI in such products could be expected to increase the lipid-derived volatile content of the extrudate, whereas aHVP would increase the amino acid-derived volatiles, such as Strecker aldehydes and sulphur compounds. The roles of such aroma compounds in an extrudate are still to be evaluated. Accordingly, in future studies we intended to investigate the effect that each of these ingredients has on aroma development in extruded cereal products.

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