Effect of Defatted Soybean Flour on the Flavour of Extruded Mixtures with Wheat Flour

JAN POKORNÝ, AMR FAROUK MANSOUR, FRANTIŠEK PUDIL and VÁCLAV JANDA

Department of Food Chemistry and Analysis – Institute of Chemical Technology, Prague, Czech Republic

Abstract

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Mixtures of wheat semolina, 5% glucose and 0–30% defatted soybean flour were used for the preparation of extruded samples using a pilot plant single screw extruder, maximum temperature being 140°C. The browning reactions were only moderate, the intensities of the trichromatic a* coordinate increased, and some changes were observed in chroma and the odour difference ΔE*. The sensory acceptability improved by increasing the content of soybean flour, the odour intensity increased, and some changes were observed in the sensory profile. Volatile substances were isolated using a SPME procedure, and the volatiles were separated using a Fisons GLC 8000 apparatus equipped with a mass spectrometer. Among the flavour active volatiles, pyrazines were the most prominent class of compounds, especially methyl and ethyl substituted derivatives. Pyrrole and furan substituted pyrazines were found only in small amounts because of the low extrusion temperature. Other furan and pyrrole derivatives had a lesser effect on flavour because of their relatively low amounts. Aldehydes, fatty acids, ketones, and other aliphatic derivatives contributed only a little to the resulting flavour. Compared to the mixtures of semolina and glucose only, the additions of defatted soybean flour moderately increased the number of substituted pyrazines detected in the extruded mixtures but increased substantially the pyrazine fraction in the total peak area. Among furan derivatives, 2-furancarboxaldehyde, 2-furanmethanol and 5-methyl-2-furyl alcohol belonged to the most prominent derivatives. Among other compounds, acetic acid, butyrolactone and maltol should be mentioned.

Keywords: extrusion; flavour; pyrazines; soybean flour; wheat flour

Baking is the most important technology for the processing of cereal grains, especially flours, but the requirements for energy and man power are rather high, so that the procedure is expensive and the products are not satisfactorily stable during storage. The extrusion cooking has become a favourite alternative for baking, particularly for the technological processing of cereal grains or grits. The extrusion time is very short and the extrusion temperature is substantially lower compared to the traditional baking, and thus the requirements for energy are considerably reduced. The resulting products have only a low water content due to which they can be stored for several weeks without being damaged by microorganisms.

A disadvantage of the extruded products is their weaker aroma and flavour intensity than is that of bread crust and of other traditional bakery products. It is due both to the lower temperature and the shorter extrusion time. The flavour intensity can be increased by the addition of suitable flavour precursors to the material before the extrusion. The flavour compounds result mostly from Maillard reactions of reducing sugars and free amino acids. The most important flavour products resulting from the browning reactions are different furan, pyran, pyrazine and pyrrole derivatives (ROTHE 1974). Pyrazines are the most important products in both bread crust and bread crumb (SIZER *et al*. 1975).

Previously, we reported about pyrazine formation in the course of extrusion cooking (FAROUK MANSOUR *et al*. 2000). We studied the effect of additions of glucose, fructose, and sucrose, combined with free amino acids, to wheat semolina. Heterocyclic oxygen and nitrogen containing derivatives belonged to the most important roasted-flavour compounds. The formation of furans, maltol,

pyrroles, and pyrazines was enhanced by the addition of reducing sugars and by the addition of amino acids. The results were previously published in greater detail (FAROUK MANSOUR *et al*. 2000, 2001).

Both reducing sugars and free amino acids are efficient precursors of Maillard reactions. Furan derivatives and maltol originate from sugar degradation products during extrusion cooking (RIHA & HO 1996) but pyrazines are the main sensory active products of Maillard reactions (YOO & HO 1997). Their content is higher in rye bread than in wheat bread (SCHIEBERLE & GROSCH 1987). Pyrazines, which possess very strong roasted bread crust and even burnt flavour notes, were isolated from bread (ROTHE 1974; RIHA & HO 1996; SCHIEBERLE & GROSCH 1984; OSNABRUGGE 1989). BALTES and BOCHMANN (1987) isolated many (more than 200) pyrazines from heated model mixtures of amino acids and sucrose as obtained at a temperature corresponding to that of coffee roasting.

Similar processes occur during the extrusion cooking of cereal products (LUNDGREN *et al*. 1991/92). Under mild extrusion conditions used in our experiments, the production of volatile heterocyclic substances was much less intensive than in the course of model experiments or traditional baking. The number of oxygen and nitrogen containing heterocycles increased by the addition of reducing sugars and sucrose which is easily cleaved in to glucose and fructose during extrusion (FAROUK MANSOUR *et al*. 2001), and still more, by the simultaneous addition of sugars and amino acids (FAROUK MANSOUR *et al*. 2000).

Free pure amino acids and even protein hydrolysates are expensive as food additives, therefore, we looked for a suitable cheaper substitute. In this paper, we present our results on extruded mixtures in which free amino acids were replaced by defatted soy flour as an efficient protein supplement. Soy proteins also serve for the fortification of the product with essential amino acids.

MATERIALS AND METHODS

Materials. Wheat semolina, type T 600 (as specified by the respective Czech Standard) was produced as described in the above mentioned national standard. It contained 12.4% moisture, 0.4% ash, 8.6% protein and 0.3% fat, which are relatively. D-Glucose was purchased from Sigma-Aldrich (St. Louis, MN, USA). It was ground in a Moulinex mill (Moulinex, Paris, France). Full-fat soybean flour (oil content 20.5%) was obtained from the factory Soja, a. s., Kolín, and was ground in the factory. The moisture content was 7.6%. Defatted soybean flour was prepared from solvent extracted soybeans (the residual oil content 2.8%). It was ground in the above mentioned factory (moisture content 9.7%). Defatted soybean flour was stored before use in ground glass bottles in a refrigerator at 4–6°C.

Extrusion process. The material (5 kg of a mixture of semolina and soybean flour in defined weight ratios, with the addition of 0.25 kg glucose) was thoroughly mixed and then fed to the extruder. The extrusion conditions were identical in all samples (a single screw collet extruder VUMPP 83, manufactured in the Research Institute of Milling and Baking Technology in Prague, Czech Republic; screw type: 3-way; distance between flights: 36 mm; screw rotation: 5.85 Hz; dosing: 1.923; feed rate: only in the feed zone, defined by the dosing; residence time: 30 s; the maximum extrusion temperature: 140°C; dosing: 40 kg/h; shaping dies: diameter of 12 mm; distance dies: diameter of 88 mm; die temperature: 110°C). The extruded samples were immediately crushed and stored at room temperature in ground glass bottles filled up to the neck with the crushed material.

Extraction of volatile products. The Solid Phase MicroExtraction (SPME) procedure was used. A Carbowax™ divinylbenzene 65 µm fibre for Manual Holder (Red Label) was produced by Supelco (Bellefonte, CA, USA). The extraction time was 1 h at 85°C, and the desorption time was 2 min at 220°C. The fibre was then cleaned for 30 min at 220°C before extracting the next sample. The procedure is affected by the extraction conditions due to which, these were carefully controlled. The results apply, nevertheless, solely to the set of samples analysed, and to the solid phase used for extraction.

Gas-liquid chromatography. For gas chromatography (GLC), an apparatus GC 8000 (Fisons Instruments, Milan, Italy) was equipped with an autosampler HS 8000; injection temperature: 220°C; column: Supelco 60 m × 0.32 mm coated with Supelcowax 10; film thickness: 0.25 µm (Supelco, Bellefonte, CA, USA); column temperature programming: 50°C for 2 min, isothermal, then heating at 2 K/min to 220°C, and isothermally for 30 min at 220°C; carrier gas: helium; initial pressure: 100 kPa; the inject:split ratio: 1:25; FID detector; detector temperature: 220°C.

Mass spectrometric detection. For gas chromatography with the mass spectrometric detection (GC/MS), the Fisons MSD 8000 (Milan, Italy) mass spectrometer was used; the Manual SPME Injection Method was applied; the ionizing energy: 70 eV; identifications were based on the comparison with genuine standards, the MS Computer Library (NIST-Massalab-Software Package, Fisons, Milan, Italy), and the respective retention indices.

Colour evaluation. The samples of extrudates were finely ground for 30 s in a Moulinex processor (Moulinex, Paris, France). A CCD Fiber Optic Spectrophotometer S 200 (manufactured by Ocean Optic, Ltd., Dunedin, FL, USA) was used for the measuring of the reflected light. A standard light source D 65 was used; the reflexion assembly probe consisted of 7 fibres in a ferrule. The results were processed by the software Spectrawin Version 3.1. The following parameters were measured: L* – expressed the lightness (in %); the a* value – redness (positive) to greenness

(negative); b* value – the yellowness (positive) to blueness (negative); the hue angle h* is defined as $\tan^{-1} b^*/a^*$; the chroma $c^* = (a^{*2} + b^{*2})^{0.5}$; the colour difference: $\Delta E = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{0.5}$.

Sensory analysis. The sensory analysis was carried out under conditions specified by the international standards (International Standardization Organization – ISO); general guidelines after ISO 6658 – 1985; in a test room provided with standard test booths (ISO 8589 – 1988); sensory assessors were selected, trained and monitored after ISO 8589 – 1988; unstructured graphical scales (ISO 4121.2 –1988) were presented as straight lines (70 mm long), provided with descriptors on either end to evaluate the brown colouration; 0 mm – very weak, 100 mm – very strong; odour acceptability: 0 mm – very little agreeable, 70 mm – very agreeable; odour intensity: 0 mm – very weak; 70 mm – very strong; texture (assessed by pressing the sample between fingers): 0 mm – pasty; 70 mm – very crispy. The sensory profile was based on free choice profiling, and the following descriptors were retained out of 32 collected descriptors: 1 = roasted, bread crust, roasted peanuts, ginger bread; 2 = burnt, caramel, bitter; 3 = woody, bark, resins; 4 = pasty, floury, bread crumb; 5 = spicy, sulphuric, onion, garlic; 6 = sharp, pungent, burning; 7 = fatty, oily, buttery; 8 = earthy, musty, moldy, sweat , wet dog; 9 = malty, cocoa, sweet; 10 = solvents, synthetic, chemicals; 11 = others (specify which). In the profile evaluation: 0 mm = absent; 70 mm = very strong. The colour intensity and the colour hue were assessed under the standard light source C (CIE, corresponding to the spectrum of the sun surface). Odour profiles were tested by sniffing from ground wide-neck 500 ml glass bottles.

RESULTS AND DISCUSSION

Effect of the amount of defatted soybean flour in mixtures with wheat flour on the browning intensity in the course of extrusion. Most sensory active compounds produced by extrusion are products of browning reactions, therefore, the degree of browning was studied both by sensory and instrumental methods, however, from different aspects. The sensory values were indicators of the overall browning reactions as they were expressed as absolute intensities of browning, the browning degree increased in mixtures containing higher amounts of soybean flour (Table 1). Sensory assessors were not asked to give their opinions on the colour hue. On the contrary, the instrumental data were expressed as trichromatic parameters related to the colour hue. The luminance values were adjusted to about 50% in all measurements.

The most interesting results are shown in Fig. 1. The data obtained by instrumental measurements concerned only the colour characteristics, not the colour intensity.

Small amounts (5–10%) of soybean flour increased the a* coordinate of the extruded sample (a shift from red to green) due to the addition of double bonds and auxochroms to the original system, but higher amounts had no further substantial effect. The b* coordinate did not significantly change with increasing amounts of soy flour. Changes of the hue angle were also insignificant because of a small impact of the coordinate b*. The chroma C* moderately increased, similarly as the coordinate a*. Changes of the colour difference ΔE were similar to those of the chroma. Their changes are caused mainly by the changes of the coordinate a*. Similar results were obtained in our previous experiments on the effect of sugars

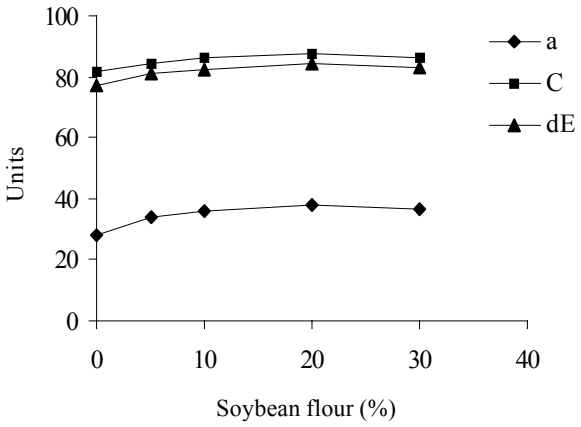

Fig. 1. Effect of the soybean flour content on the redness, chroma and colour difference of extruded products

Table 1. Some sensory characteristics of extruded mixtures containing soybean flour (%)

Sensory characteristic (mm of the scale)	0%	5%	10%	20%	30%
Brown colour intensity	40.5	32.5	37.7	47.0	59.1
Texture between fingers	42.6	49.5	50.1	50.9	50.7
Odour intensity	24.7	24.3	29.1	34.3	37.3
Roasted odour intensity	27.4	15.8	20.4	28.8	36.7
Caramel odour intensity	19.5	8.5	9.8	11.6	30.5
Crumb odour intensity	33.2	14.7	19.5	23.3	27.7

and amino acids (FAROUK MANSOUR *et al.* 2000, 2001). The intensity of browning reactions was only moderate because of the low extrusion temperature; the reaction time was also short.

Effect of the amount of defatted soybean flour in mixture with wheat flour on sensory odour characteristics of extruded samples. The odour intensity increased with the increasing percentage of soybean flour in the extruded mixture (Table 1). The odour acceptability moderately improved by the addition of soybean flour as the amount of browning precursors increased (Fig. 2). In the sensory profile, intensities of spicy, oily and sharp odour notes also increased (Fig. 2). In the case of roasted, caramel, and bread crumb odour notes, the sample containing 5% soybean flour was the least intensive, less than the extruded wheat flour, but with the further increasing of the percentage of soybean flour in the mixture, the intensity increased again (Table 1). Two opposing processes obviously proceed during the extrusion, i.e. the binding of volatile products on proteins contained in soybean flour, and the formation of flavour compounds from the flavour precursors present in the original soybean flour. The effect of large amounts of soybean flour may be partially due to the presence of α-galactosides (KADLEC 2000), about 8%, which are decomposed into simple sugars under the extrusion conditions and could thus participate in browning reactions.

Effect of the amount of soybean flour (%) on the number of sensory active substances and on their amounts. The results are given in Table 1. The number of substances detected by the analytical method described above obviously depends on the sensitivity of the determination. The absence of a substance in our list does not mean that the substance was not really present but that its content was too low to be detected by our experimental method. The number of nitrogen heterocycles slightly increased

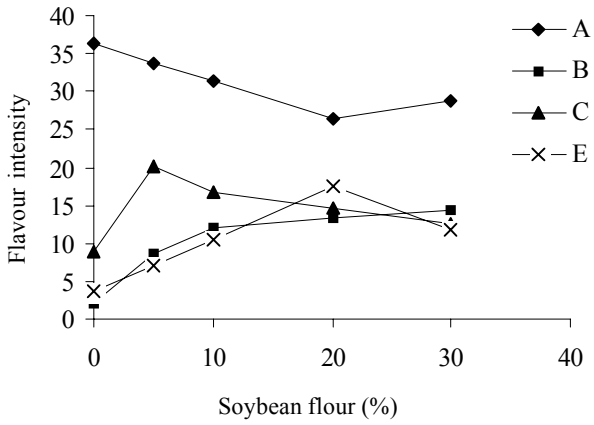

Fig. 2. Effect of soybean flour on some odour characteristics of extruded products (A = roasted, B = caramel, C = woody, E = spicy)

with the increasing content of soybean flour (20 in the mixture containing only wheat and glucose, 28 in the mixture with 5% soybean flour, 30 in all mixtures containing higher additions of soybean flour). No such effect was observed in the case of oxygen heterocycles, as expected, because they originated from sugars, the contents of which were nearly the same (5%) in all mixtures. The additions of soybean flour suppressed the number of aliphatic aldehydes and ketones as these carbonylic substances were probably bound during the extrusion to the lysine residues in protein (containing about 3.1% lysine in defatted soybean flour) in the form of imines. Some flavour compounds may also be combined with starch giving rise to nonvolatile adducts during the extrusion (MAIER *et al.* 1987).

Additions of 5–30% soybean flour to the extruded mixture increased the content of pyrazines, pyridines and

Table 2. Effect of the addition of defatted soybean flour (in %) on the number of volatile compounds isolated and identified in the extruded mixtures

Class of compounds	0%	5%	10%	20%	30%
Pyrazines	16	21	22	22	22
Pyridines	0	2	2	1	1
Pyrroles	4	5	6	7	7
Furans	12	11	12	14	12
Pyrones	2	2	2	2	2
Carbocyclics	4	2	2	2	3
Sulphur compounds	0	0	0	0	0
Aliphatic aldehydes	7	4	6	4	4
Aliphatic ketones	6	3	3	2	3
Total identified	72	76	84	80	75

Table 3. Effect of an addition of soybean flour (%) on the amount of sensory active substances expressed as peak areas

Class of compounds	0%	5%	10%	20%	30%
Pyrazines	2.5	3.3	3.1	4.9	6.4
Pyridines	0	0.01	0.20	0.12	0.11
Pyrroles	0.40	0.83	1.30	2.19	2.28
Furans	12.80	20.55	15.50	20.11	19.02
Pyrones	6.80	26.66	25.50	25.65	21.61
Carbocyclics	0.30	0.49	0.30	0.57	0.78
Aliphatic aldehydes	2.30	1.38	1.60	1.30	1.26
Aliphatic ketones	3.30	0.52	0.30	0.38	0.48

pyrroles (from 2.9% in the original sample and 4.1% in the sample containing 5% soybean flour to 8.8% in the sample containing 30% soybean flour). No systematic change was observed in the case of oxygen containing heterocycles except that any addition of soybean protein increased the contents of the oxygen heterocycles. The change might be due to α-galactosides present in soybeans as mentioned above. The contents of aliphatic aldehydes and ketones decreased with increasing content of soybean flour in the extrudate. Obviously, the carbonyl groups reacted with amine groups of the lysine residues (more than 3% of total amino acids combined in protein) forming non-volatile products.

Effect of addition of defatted soybean flour on the content of oxygen containing heterocyclic compounds in extruded products. As shown above, the content of defatted soybean flour had no pronounced effect on the total content of different oxygen containing heterocyclic classes. It does not mean, however, that there was no effect on the proportions of the individual oxygen heterocyclic species. In Table 4, the influence of soybean flour on the contents of volatile furans and furanones is shown. Furans and a furanone were also detected in model mixtures of D-glucose and DL-alanine heated to 100–140°C (KIM *et al.* 1988). The most important compound of this class is 2-tetrahydrofuranone but its relative content decreased substantially by the addition of soybean flour, probably due to the reaction with free amine groups of combined lysine. On the contrary, the contents of 2,5-dimethyl-4-hydroxy-3(2H)-furanone and of 2-furanmethanol, and to a lesser extent, that of 2-methyl-2-furfuryl alcohol increased. In agreement with

Table 4. Effect of defatted soybean flour (%) on the contents of individual species of different furan and furanone compounds in extruded products

Identified furans and furanones	0%	5%	10%	20%	30%
2-Pentylfuran	0.28	0.02	0.15	0.09	0.14
2-Methyl-3(2H)-furanone	0	0	0	0.25	0.02
2-Furancarbocaldehyde	4.69	9.39	5.17	6.83	9.88
1-(2-Furanyl)-ethanone	0.51	0.60	0.56	0.81	0.74
2-Methyl-2-furfural	0.52	1.27	0.92	2.85	2.97
2-Tetrahydrofuranone	70.58	52.61	31.64	29.28	27.55
2-Furanmethanol	4.48	6.30	8.13	17.87	20.48
2-Methyl-2-furanyl alcohol	4.60	9.12	6.31	8.39	7.68
2-Heptylfuran	0.59	traces	0.59	0.06	traces
1-(2-Furanyl)-1,2-propandione	0.74	0.64	3.00	0.83	0.81
1-(5-Methyl-2-furanyl)-1,2-propandione	0.09	0.14	0.22	0.36	0.20
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	7.71	13.47	15.83	25.39	25.91
Dihydro-5-propyl-2(3H)-furanone	0	0	0	2.10	0
s-(+)-3-Hydroxytetrahydrofuran	5.21	6.45	27.80	4.89	3.60

Table 5. Effect of additions of soybean flour (%) on the contents of individual pyrazine species in resulting extruded products

Pyrazine species	0%	5%	10%	20%	30%
Pyrazine	12.14	16.70	13.36	8.65	7.28
Methylpyrazine	32.94	51.61	49.19	40.91	36.77
2,5-Dimethylpyrazine	2.53	3.28	3.03	3.84	5.26
2,6-Dimethylpyrazine	6.26	7.65	7.37	9.31	10.10
Ethylpyrazine	7.22	1.75	4.78	3.31	3.13
2,3-Dimethylpyrazine	2.18	1.65	2.65	1.56	1.78
2-Ethyl-6-methylpyrazine	1.16	3.66	1.08	1.81	1.70
2-Ethyl-5-methylpyrazine	1.13	1.75	0.60	0.49	0.75
Trimethylpyrazine	22.80	0.28	3.78	0.26	0.27
2-Ethyl-3-methylpyrazine	0	0	1.44	0.11	0.17
Vinylpyrazine	1.69	1.23	1.86	1.45	0.88
3-Ethyl-2,5-dimethylpyrazine	0.45	0.50	0.81	0.81	0.36
2-Vinyl-6-methylpyrazine	0.10	0.33	1.01	0.52	0.20
2-Vinyl-5-methylpyrazine	0.07	0.14	0.52	0.25	0.10
2-(1-Propenyl)pyrazine	0	0.10	0.81	0.44	0.11
Acetylpyrazine	16.80	7.14	2.71	5.77	20.29
5-Methyl-2-acetylpyrazine	23.57	0.24	0.22	5.86	0.35
6-Methyl-2-acetylpyrazine	6.73	0.32	0.52	7.18	0.61
Quinoxaline	0	0.45	1.97	0.34	0.85
2-Furanylpyrazine	0	0.59	1.14	6.00	8.05
5-Methyl-2-furanylpyrazine	0	0.33	0.53	0.55	0.53
6-Methyl-2-furanylpyrazine	0	0.26	0.49	0.52	0.45

the earlier data (YAYLAYAN *et al.* 1992), hydroxymethylfuraldehyde and maltol were important sugar degradation products in extruded mixtures. No systematic changes were observed in most other compounds investigated.

Effect of addition of defatted soybean flour on the content of individual pyrazines in extruded products. The content of free amino acids in the extruded systems was quite small but some amino acids bound in protein are also reactive, particularly asparagin and glutamin. The ε-amine group of lysine is less reactive than the α-amine group (HWANG *et al.* 1994) but it can still act as a precursor of pyrazines.

Pyrazines are the most important sensory active heterocycles in extruded cereal products as their perception thresholds are very low, from 1 mg/kg in the case of alkylpyrazines to 0.01 μg/kg for alkoxypyrazines (FORS & OLOFSSON 1985; SHIBAMOTO 1986). Alkylpyrazines possess typical roasted aroma notes (KOEHLER *et al.* 1971). They were present in all samples of our experimental series in relatively large amounts, especially methyl and trimethylpyrazine (Table 5). Several vinyl and allyl derivatives and acetyl derivatives were also present. The acetyl derivatives are typical representatives of bread crust aroma (FADEL & HEGAZY 1993). The contents of individual pyrazine species (Table 4) show no systematic relationship with the content of soybean flour, except in the case of furanylpyrazines. They were not detected in a mixture of semolina with glucose but did increase with the increasing addition of soybean flour up to 9.03%.

Conclusion

The addition of defatted soybean flour to wheat flour (semolina) extrudates improves sensory characteristics by increasing the amount of sensory active volatiles resulting from browning reactions.

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Souhrn

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Směsi pšeničné mouky s 5 % glukosy a 0–30 % odtučněné sójové mouky byly zpracovány extruzí na poloprovozním jednošnekovém zařízení při maximální teplotě 140 °C. Vliv sójové mouky se projevil jen málo na intenzivnějším hnědnutí a na zvýšení hodnoty souřadnice a* trichromatického hodnocení. Senzorická analýza vykázala určité mírné zvýšení příjemnosti vůně, zesílení intenzity vůně a některé změny senzorického profilu. Těkavé látky byly izolovány metodou SPME (mikroextrakce na tuhé fázi) a rozděleny na plynovém chromatografu Fisons GLC 8000 s hmotnostním detektorem. Ze skupin těkavých látek byly nejvýznamnější pyraziny, zvláště methylované a ethylované deriváty. Jejich podíl na celkových těkavých látkách rostl s rostoucím podílem sójové mouky v extrudátu. Pyrrolové a furanové deriváty pyrazinů byly vzhledem k nízké teplotě zjištěny jen v malém množství, ostatní furanové a pyrrolové deriváty mají jen nepatrný vliv na celkové aroma. Také aldehydy, mastné kyseliny, ketony a další alifatické sloučeniny se na celkovém aromatu podílely jen málo. Ve srovnání s extrudovanými směsmi pšeničné mouky a glukosy

bylo v extrudovaných směsích se sójovou moukou přítomno jen málo dalších sloučenin, ale značně narostl podíl frakce pyrazinů. Z furanových derivátů se jako nejvýznamnější ukázaly 2-furankarboxaldehyd, 2-furanmethanol a z pyronových derivátů maltol. Kromě těchto sloučenin stojí za zmínku kyselina octová a butyrolakton.

Klíčová slova: extruze; aroma; pyraziny; sójová mouka; pšeničná mouka

Corresponding author:

Prof. Ing. JAN POKORNÝ, DrSc., Ústav chemie a analýzy potravin, Vysoká škola chemicko-technologická, Technická 5, 166 28 Praha 6, Česká republika

tel.: + 420 224 353 264, fax: + 420 233 339 990; e-mail: jan.pokorny@vscht.cz