



## Identification of buckwheat (*Fagopyrum esculentum* Moench) aroma compounds with GC–MS

Damjan Janeš<sup>a,\*</sup>, Dragana Kantar<sup>b</sup>, Samo Kreft<sup>a</sup>, Helena Prosen<sup>b</sup>

<sup>a</sup> University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia

<sup>b</sup> University of Ljubljana, Faculty of Chemistry and Chemical Technology, Aškerčeva 5, 1000 Ljubljana, Slovenia

### ARTICLE INFO

#### Article history:

Received 13 February 2008

Received in revised form 7 April 2008

Accepted 15 May 2008

#### Keywords:

Buckwheat

*Fagopyrum esculentum* Moench

GC–MS

Aroma composition

### ABSTRACT

Buckwheat has a strong characteristic aroma, but its phytochemical background has not yet been fully elucidated. The aims of this study were identification and quantification of individual compounds responsible for the buckwheat aroma. Volatiles from a freshly ground buckwheat flour were extracted by different methods: direct extraction with petroleum ether, pentane or methanol, distillation with Clevenger apparatus and a headspace solid-phase microextraction method. The extracts were analysed by GC–MS with electron ionisation. Compounds were identified by MS and by comparison of retention times with reference compounds. Direct extraction with methanol and distillation proved to be very efficient. In these extracts twenty-five and thirty-five compounds were identified, respectively. The first extract contained more hydrophilic compounds and the latter more volatile compounds. Most of the compounds were quantified and their odour activity value (OAV) calculated. Only two compounds (salicylaldehyde and phenylacetaldehyde) were found in both extracts. The compounds with the highest contribution to the buckwheat aroma were: 2,5-dimethyl-4-hydroxy-3(2H)-furanone, (*E,E*)-2,4-decadienal, phenylacetaldehyde, 2-methoxy-4-vinylphenol, (*E*)-2-nonenal, decanal, hexanal and salicylaldehyde (2-hydroxybenzaldehyde).

© 2008 Elsevier Ltd. All rights reserved.

### 1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is a cool climate dicotyledonous plant of the family *Polygonaceae*, cultivated for food in most temperate countries (North America, Japan, Europe). Its grains are used as a cereal for the preparation of different dishes, e.g. noodles, bread, kasha (Mazza et al., 1999). Buckwheat products have been associated with preventative nutrition (Bonafaccia, Marocchini, & Kreft, 2003; Skrabanja et al., 2004). Besides high nutritional value, flavour is an important quality attribute of buckwheat grains. It is appetizing, cereal nutty-like in freshly harvested and freshly milled buckwheat or bland with a rancid tone in old buckwheat. Volatile constituents of the grain are believed to play a major role in its flavour, but there is little information about odour activity values of these components in buckwheat. Salicylaldehyde was shown to be a characteristic component of buckwheat aroma (Janeš & Kreft, 2008) and it was reported that nonanal, octanal and hexanal are important aroma compounds of buckwheat because of their low odour threshold values in water (Mazza et al., 1999). Yajima et al. (1983) identified 209 compounds in boiled buckwheat flour; however, none of them possessed the characteristic aroma of boiled buckwheat flour.

Storage and milling conditions greatly affect buckwheat aroma. Since the main characteristic aroma compounds of buckwheat were not known, some uncharacteristic volatiles, such as xylenes, ethylbenzene, acetaldehyde, methanol and ethanol (Ohinata, Karasawa, & Kurokouchi, 2001) and unidentified aldehydes and pentane (Przybylski, Woodward, Eskin, Malcolmson, & Mazza, 1995) were monitored to assess the aroma. In a recent study, volatile compounds of the distilled alcoholic beverage, shochu, were also investigated and ethyl cinnamate was found as one of the most important aroma compounds (Sakaida et al., 2003).

For the relevant assessment of buckwheat quality, main compounds responsible for the aroma must be known and an appropriate analytical method is needed. Analysis of food aroma should be approached by different state-of-the-art sampling or extraction techniques (solvent extraction, distillation, solid-phase microextraction (SPME), headspace techniques, adsorption traps) each of them having its inherent problems and possible bias (Belitz, Grosch, & Schieberle, 2004; Hofmann, Rothe, & Schieberle, 2005; Kaiser, 2006; Stephan, Bücking, & Steinhart, 2000). However, gas chromatography, with mass spectrometric detection (GC–MS) and comparison of spectra and retention indices of reference compounds, is the technique of choice for the analysis of volatile compounds (Molyneux & Schieberle, 2007; Zhou, Robards, Glennie-Holmes, & Helliwell, 1999). In this research three different methods of extraction were used together with GC–MS to find the most

\* Corresponding author. Tel.: +386 1 47 69 583; fax: +386 1 42 58 031.

E-mail address: [damjan.janes@ffa.uni-lj.si](mailto:damjan.janes@ffa.uni-lj.si) (D. Janeš).

suitable method for the identification and quantitative evaluation of odour-active compounds in buckwheat.

## 2. Materials and methods

### 2.1. Solvents, reference compounds and buckwheat sample

Solvents were purchased from the following producers: methanol (Fluka, Buchs, Switzerland), pentane, petroleum ether 40–60 °C, toluene (Riedel de Haën, Seelze, Germany). Reference compounds for confirmation of identity of volatile compounds were purchased from Sigma–Aldrich (Steinheim, Germany). A sample of dehulled buckwheat grains (groats) was produced in Slovenia by Bio-farm Rengeo, Šalovci. It was purchased from the local store.

### 2.2. Selection of solvent for extraction

Freshly ground buckwheat grains (5 g) were extracted with 10 ml of solvent (pentane, petroleum ether or methanol) for 5 min in an ultrasound bath and then 60 min on a shaker (300 rpm). In a parallel experiment, buckwheat was first soaked with 5 ml of water and then extracted with the respective solvent.

### 2.3. Extraction with methanol

Buckwheat grains were freshly ground and an aliquot of 250 g was extracted with 500 ml of methanol for 24 h on a shaker (250 rpm). The sample was filtered, rinsed with methanol and evaporated under reduced pressure to a volume of 30 ml.

### 2.4. Solid-phase microextraction (SPME)

DVB/CAR/PDMS (divinylbenzene, carboxen, polydimethylsiloxane, 50/30 µm) or PDMS (polydimethylsiloxane, 30 µm) fibres with manual holder from Supelco (Bellefonte, PA, USA) were used. Freshly ground buckwheat grains (4 g) and 1.5 ml of deionised water were measured into a 10 ml glass vial, crimped and kept at 50 °C for 20 min. The SPME fibre was inserted into the headspace and the compounds were sampled for 60 min. The fibre was subsequently inserted into the injector port of a gas chromatograph and desorbed for 10 min. Alternatively, the same quantity of ground buckwheat with 2 ml of deionised water was conditioned as described above. The SPME fibre was inserted in the liquid phase.

### 2.5. Distillation with a Clevenger apparatus

A modified procedure of the European Pharmacopoeia was used (Council of Europe, 2005). Briefly: a mixture of 250 g of buckwheat grains and 2 l deionised water was placed in a 4 l flask. Boiling time was 8 h. Toluene (1 ml) was used for the extraction of the compounds from the vapour.

### 2.6. GC–MS conditions

A gas chromatograph HP 5890 Series with a mass spectrometric detector (MSD) 5972 from Hewlett-Packard (Palo Alto, CA, USA) was used. Column: VOCOL, 60 m × 0.25 mm (i.d.), film thickness 1.5 µm (Supelco, Bellefonte, PA, USA). Temperature programme: starting temperature 50 °C (2 min), heating rate 5 °C min<sup>-1</sup>, final temperature 210 °C (40 min).  $T_{inj}$ : 250 °C;  $T_{det}$ : 280 °C; injection vol-

**Table 1**

Compounds found in the methanol extract with their concentrations in buckwheat, odour threshold value (OTV), odour activity value (OAV), recovery of standards by distillation and ACD/log $P$  (determined by Advanced Chemistry Development software)

Compound	Concentration in buckwheat [ppb]	OTV (Ref.) [ppb in water]	OAV	Recovery of refer. comp. by distillation (%)	ACD/log $P^d$
2,5-Dimethyl-4-hydroxy-3(2H)-furanone <sup>A</sup>	6238	0.03–60 <sup>a,b</sup>	2.08 × 10 <sup>5</sup> to 104	0	0.34
Phenylacetaldehyde	1959	4 <sup>b</sup>	490	–	1.78
2-Methoxy-4-vinylphenol	345.7	0.75 <sup>b</sup>	461	0	1.93
Salicylaldehyde	1216	30 <sup>b</sup>	40.5	–	1.61
Furfural <sup>A</sup>	1116	280–8000 <sup>b</sup>	3.98–0.139	0	0.73
2,5-Dimethylpyrazine <sup>A</sup>	206.9	80–1800 <sup>b</sup>	2.59–0.115	2.4	0.64
Furfuryl alcohol <sup>A</sup>	793.4	1000–2000 <sup>b</sup>	0.793–0.397	0	0.20
5-Methylfurfural <sup>A</sup>	204.9	500 <sup>c</sup>	0.410	4.2	1.19
2,6-Dimethoxyphenol <sup>A</sup>	162.0	400 <sup>b</sup>	0.405	0	0.77
2-Methylpyrazine	708.2	60,000 <sup>c</sup>	0.118–7.08 × 10 <sup>-3</sup>	0.98	0.18
Phenol	552.9	5500 <sup>b</sup>	0.101	0.26	1.48
Benzoic acid	785.7	85,000 <sup>b</sup>	9.24 × 10 <sup>-3</sup>	0	1.90
2-Cyclopentene-1,4-dione <sup>A</sup>	304.1	Not available	–	0	–1.22
Geranic acid <sup>A</sup>	858.4	Not available	–	0	3.46
5-(Hydroxymethyl)furfural <sup>A</sup>	5008	Not available	–	0	–0.49
Isobutyric acid <sup>A</sup>	390.1	Not available	–	0	0.59
3-Methoxycatechol <sup>A</sup>	4010	not available	–	0	0.70
2-Methylbutanal <sup>A</sup>	2184	Not available	–	0	1.25
3-Methylbutanal	3515	Not available	–	0	1.25
4-Cyclopentene-1,3-dione <sup>A</sup>	Not quantified	–	–	–	–1.22
Guaiacol	Not quantified	–	–	–	1.19
4-Hydroxybenzaldehyde <sup>A</sup>	Not quantified	–	–	–	1.39
4-(2-Hydroxyethyl)-5-methylthiazole <sup>A</sup>	Not quantified	–	–	–	0.04
Isovaleric acid	Not quantified	–	–	–	1.13
Vanillin <sup>A</sup>	Not quantified	–	–	–	1.19

<sup>A</sup> Newly identified.

<sup>a</sup> Belitz et al., 2004.

<sup>b</sup> Burdock, 2005.

<sup>c</sup> Ho, Wan Aida, Maskat, & Osman, 2007.

<sup>d</sup> ChemZoo Corporation, 2007.

**Table 2**  
Compounds found in distilled fraction with their concentrations in buckwheat, odour threshold value (OTV), odour activity value (OAV), and ACD/log*P* (determined by Advanced Chemistry Development software)

Compound	Concentration in buckwheat [ppb]	OTV (Ref.) [ppb in water]	OAV	ACD/log <i>P</i> <sup>c</sup>
( <i>E,E</i> )-2,4-Decadienal	317	0.07–10 <sup>a,b</sup>	4523–31.7	3.18
( <i>E</i> )-2-Nonenal	24.2	0.1 <sup>a,b</sup>	242	3.17
Decanal	11.7	0.1–5 <sup>a,b</sup>	117–2.34	4.09
Hexanal	376	4.1–22.8 <sup>a,b</sup>	91.7–16.5	1.97
Salicylaldehyde	2246	30 <sup>b</sup>	74.9	1.61
Nonanal	58.3	1–8 <sup>a,b</sup>	58.3–7.29	3.56
Octanal	22.1	0.7–6.4 <sup>b</sup>	31.6–3.46	3.03
2-Pentylfuran	150	6 <sup>b</sup>	25.0	3.97
Phenylacetaldehyde	54.5	4 <sup>b</sup>	13.6	1.78
2-Heptanone	10.4	1–1330 <sup>b</sup>	10.4–7.80 × 10 <sup>-3</sup>	1.97
Heptanal	30.5	3–60 <sup>b</sup>	10.2–0.508	2.50
( <i>E</i> )-2-Octenal	28.8	3–4 <sup>a,b</sup>	9.58–7.19	2.64
Limonene	16.2	4–229 <sup>b</sup>	4.05–0.0707	4.45
1-Heptanol <sup>A</sup>	9.63	3 <sup>b</sup>	3.21	2.47
( <i>E</i> )-2-Octenol <sup>A</sup>	11.6	4–14 <sup>a,b</sup>	2.89–0.826	2.64
Benzaldehyde	169	100–4600 <sup>b</sup>	1.69–0.0367	1.64
Alpha-pinene	3.51	2.5–62 <sup>b</sup>	1.41–0.0566	4.37
( <i>E</i> )-2-Heptenal	13.2	13–51 <sup>a,b</sup>	1.01–0.259	2.11
1-Octanol	16.3	42–48 <sup>b</sup>	0.388–0.340	3.00
Geranylacetone <sup>A</sup>	17.0	60 <sup>b</sup>	0.283	4.13
2-Octanone	5.40	41–62 <sup>b</sup>	0.132–0.0871	2.50
1-Nonanol	6.01	50–90 <sup>b</sup>	0.120–0.0667	3.53
6-Methyl-5-heptene-2-one	0.561	50 <sup>b</sup>	0.0112	2.09
2-Decanone	7.76	Not available	–	3.56
Dibutyl maleate <sup>A</sup>	28.4	Not available	–	3.81
2-Methoxybenzaldehyde <sup>A</sup>	17.3	Not available	–	1.72
( <i>E</i> )-2-Undecenal <sup>A</sup>	6.83	Not available	–	4.23
Acetophenone <sup>A</sup>	Not quantified	–	–	1.67
Borneol <sup>A</sup>	Not quantified	–	–	2.71
Cymene	Not quantified	–	–	4.02
1,3-Dimethoxybenzene <sup>A</sup>	Not quantified	–	–	1.93
( <i>E,E</i> )-2,4-Heptadienal	Not quantified	–	–	1.59
Lauric acid <sup>A</sup>	Not quantified	–	–	5.03
Myristic acid	Not quantified	–	–	6.09
2-Nonanone	Not quantified	–	–	3.03

<sup>A</sup> Newly identified.

<sup>a</sup> Belitz, Grosch, & Schieberle (2004).

<sup>b</sup> Burdock (2005).

<sup>c</sup> ChemZoo Corporation (2007).

ume: 1 µl. Carrier gas: He, flow 1 ml min<sup>-1</sup>. MS conditions: electron impact mode, total ion current (TIC) recorded. Mass spectra of the compounds were compared to the spectra from the NIST02 spectral library and with mass spectra of reference compounds. Identities of all compounds presented in Tables 1 and 2 were confirmed by comparison of their retention times with the retention times of reference compounds.

For quantification, calibration curves were made and linearity was calculated (for thirty-six compounds  $R > 0.99$ , for nine compounds  $R > 0.98$  and for one compound  $R > 0.96$ ). Only the peaks above the quantification limit and within the linear range were quantified. The odour activity values (OAV) were calculated by dividing the measured concentrations by the published odour threshold values (OTV).

### 3. Results and discussion

Comparison of the chemical composition of extracts of ground buckwheat grains in pentane, petroleum ether and methanol revealed the greatest number of the compounds to be in the methanol extract (Fig. 1). With SPME, petroleum ether and pentane extraction, only a few of these compounds were observed (e.g. salicylaldehyde, benzaldehyde, acetophenone, hexanal) and the intensity of the chromatographic peaks was much lower compared to the methanol extract.

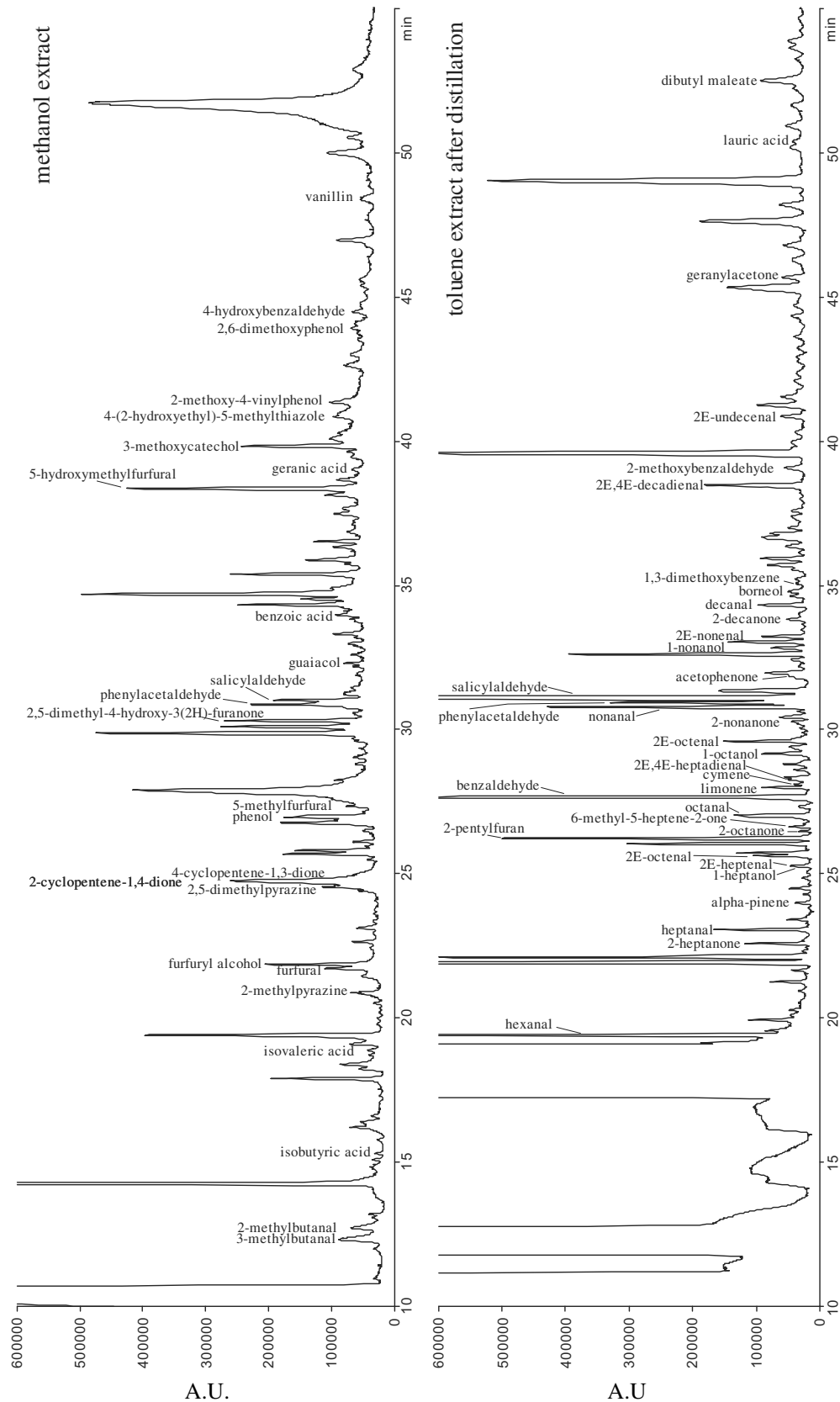
The compounds in the concentrated methanol extract were identified by comparison of their mass spectra with the spectra

from the library. The identities of twenty-five of them were later confirmed with reference compounds and nineteen of them were also quantified. Table 1 presents the compounds identified in methanol extract and their odour activity values (OAV) calculated from their concentrations and odour threshold values (OTV) obtained from the literature. Compounds with OAV higher than 1 contribute to the odour of the sample. During the evaporation of methanol from the extract, a separation of lipids occurred. Lipids were removed by filtering the refrigerated sample. Possibly, some nonpolar aroma components were lost in this way.

In the extract obtained by distillation of boiled buckwheat with the Clevenger apparatus, thirty-five different compounds were identified and twenty-seven of them quantified (Fig. 1, Table 2). Only two of these compounds were also identified in the methanol extract: salicylaldehyde (2-hydroxybenzaldehyde) and phenylacetaldehyde.

The efficiency of the extraction was verified with a repeated extraction of buckwheat after the first extraction. Over 94% of compounds were extracted in the first extraction with both methods (distillation, methanol extraction).

The compounds that were only found in the methanol extract, but not in the distilled fraction were hydrophilic (ACD/log*P* < 2, determined by Advanced Chemistry Development software) and were probably consequently flushed out of the toluene layer by the water circulating in the steam distillation apparatus. Only lipophilic compounds (ACD/log*P* > 2) were efficiently extracted after distillation. To verify this hypothesis, an additional experiment



**Fig. 1.** Chromatograms of buckwheat methanol extract and toluene extract after distillation with assigned peaks. Unassigned high peaks are siloxane artefacts from the column.

was conducted, where a mixture of standard substances that were found in the methanol extract was dissolved in water and distilled

in the Clevenger apparatus in the same way as for the buckwheat sample. Only small fractions of the compounds were detected in

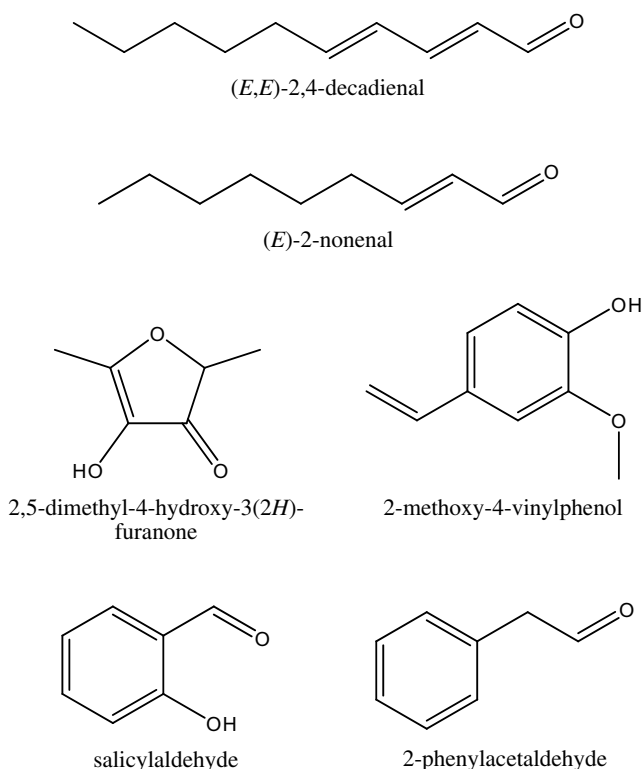


Fig. 2. Compounds with the highest odour activity value (OAV) in buckwheat.

the toluene layer (Table 1). Highly reactive compounds, e.g. 2-methoxy-4-vinylphenol, can polymerize at elevated temperature and this may be the reason why it was not found in the toluene extract after distillation.

There are two possible reasons, why the substances that were observed in the distilled fraction were not observed in the methanol extract: (1) some of the compounds may be formed during the distillation process or (2) the concentration of compounds in the methanol extract concentrated to 30 ml may be below the detection limit. During distillation, the compounds from 250 g of buckwheat were concentrated in 1 ml of toluene. In the methanol extract, the compounds from the same amount of buckwheat were concentrated in 30 ml of solvent. Possible losses of the compounds, identified in the distilled fraction during the concentration of methanol extract, were checked by dissolving these compounds in 500 ml of methanol and concentrating the solution under reduced pressure to 40 ml. The recoveries were in the range of 23–66%. Different analytical results obtained for different extracts can be explained by degradation or/and polymerisation of phenylacetaldehyde during distillation and by loss of salicylaldehyde during evaporation of methanol. Since degradation, polymerisation, and evaporation may all contribute to underestimation of content, it may be considered, that the higher analytical result is closer to the true content of salicylaldehyde and phenylacetaldehyde in buckwheat, and even this might be underestimated.

Fourteen compounds were found having OAV higher than 10. As can be seen from Tables 1 and 2, two compounds with the highest OAV are 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone and (*E,E*)-2,4-decadienal (Fig. 2). The first has a sweet, fruity, strawberry, hot sugar, caramel or burnt pineapple aroma (Burdock, 2005) and the latter has a deep frying odour (Belitz et al., 2004) or a powerful green odour at low concentration and a sweet, orange-like odour at high concentration (Burdock, 2005).

Since the extracts obtained by methanol extraction and by distillation and extraction in toluene produced different aroma profiles, it can not be excluded that some other compounds, important for buckwheat aroma could be identified in the future by using alternative extraction approaches. The aroma profile of buckwheat proved to be very complex. By using different extraction techniques, many compounds, not previously found in buckwheat, were identified and several of them have high odour activity values.

## References

- Belitz, H.-D., Grosch, W., & Schieberle, P. (2004). Aroma compounds. In *Food chemistry* (3rd ed., pp. 342–408). Berlin: Springer.
- Bonafaccia, G., Marocchini, M., & Krefl, I. (2003). Composition and technological properties of the flour and bran from common and tartary buckwheat. *Food Chemistry*, 80(1), 9–15.
- Burdock, G. A. (2005). *Fenaroli's handbook of flavor ingredients* (5th ed.). Boca Raton: CRC Press.
- ChemZoo Corporation, ChemSpider Beta. (2007). <<http://www.chemspider.com>> Accessed 28.11.07.
- Council of Europe (2005). Determination of essential oils in vegetable drugs. *European pharmacopoeia 5.0.* (Vol. 1, pp. 217–218). Strasbourg: European Directorate for the Quality of Medicines.
- Ho, C. W., Wan Aida, W. M., Maskat, M. Y., & Osman, H. (2007). Changes in volatile compounds of palm sap (*Arenga pinnata*) during the heating process for production of palm sugar. *Food Chemistry*, 102(4), 1156–1162.
- Hofmann, T., Rothe, M., & Schieberle, P. (2005). State-of-the-art in flavour chemistry and biology. In *Proceedings of the 7th wartburg symposium*. Eisenach: Deutsche Forschungsanstalt für Lebensmittelchemie.
- Janeš, D., & Krefl, S. (2008). Salicylaldehyde is a characteristic aroma component of buckwheat groats. *Food Chemistry*, 10.1016/j.foodchem.2007.12.032.
- Kaiser, R. (2006). Introduction to the world of natural scents. In *Meaningful scents around the world* (pp. 1–14). Zürich: Verlag Helvetica Chimica Acta.
- Mazza, G., Cottrell, T., Malcolmson, L., Girard, B., Oomah, B. D., & Eskins, M. A. M. (1999). Headspace gas chromatography and sensory analyses of buckwheat stored under controlled atmosphere. *Journal of Food Quality*, 22, 341–352.
- Molyneux, R. J., & Schieberle, P. (2007). Compound identification: A journal of agricultural and food chemistry perspective. *Journal of Agricultural and Food Chemistry*, 55, 4625–4629.
- Ohinata, H., Karasawa, H., & Kurokouchi, H. K. (2001). Influence of milling methods on buckwheat aroma. In *The proceeding of the 8th ISB* (pp. 694–697).
- Przybylski, R., Woodward, L., Eskin, N. A. M., Malcolmson, L. J., & Mazza, G. (1995). Effect of buckwheat storage and milling on aroma compounds. *Current Advances in Buckwheat Research*, 783–787.
- Sakaida, H., Nakahara, N., Watashi, N., Kai, T., Nakashima, Y., Sakakibara, Y., et al. (2003). Characteristic flavor of buckwheat *Shochu* and comparison of volatile compounds from variety cereal *Shochu*. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 50(12), 555–562.
- Skrabanja, V., Krefl, I., Golob, T., Modic, M., Ikeda, S., Ikeda, K., et al. (2004). Nutrient content in buckwheat milling fractions. *Cereal Chemistry*, 81(2), 172–176.
- Stephan, A., Bücking, M., & Steinhart, H. (2000). Novel analytical tools for food flavours – review. *Food Research International*, 33, 199–209.
- Yajima, I., Yanai, T., Nakamura, M., Sakakibara, H., Uchida, H., & Hayashi, K. (1983). Volatile flavor compounds of boiled buckwheat flour. *Agricultural and Biological Chemistry*, 47, 729–738.
- Zhou, M., Robards, K., Glennie-Holmes, M., & Helliwell, S. (1999). Analysis of volatile compounds and their contribution to flavor in cereals. *Journal of Agricultural and Food Chemistry*, 47, 3941–3953.