

Salicylaldehyde is a characteristic aroma component of buckwheat groats

Damjan Janež*, Samo Kreft

Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia

Received 13 September 2006; received in revised form 10 October 2007; accepted 17 December 2007

Abstract

Salicylaldehyde (2-hydroxybenzaldehyde) was identified as a characteristic component of buckwheat groats aroma by a sensory analysis guided fractionation of the extract. The extract with the strongest odour was prepared by petroleum ether extraction of water soaked groats. This extract was further extracted with sodium hydrogen carbonate solution and purified by a preparative layer chromatography and identified by NMR, MS and IR spectroscopy. A capillary electrophoresis method was developed and used to determine salicylaldehyde content in buckwheat groats and flour samples. Traditionally dehulled buckwheat grain, which had the strongest odour, contained the highest concentration (1.6 ppm) of salicylaldehyde with an odour activity value (OAV) of 216.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: *Fagopyrum esculentum*; Buckwheat groats; Aroma analysis; Odour

1. Introduction

Common buckwheat (*Fagopyrum esculentum* Moench) has been grown in Europe for centuries and is now an important alternative crop; its products have been associated with preventative nutrition (Bonafaccia, Marocchini, & Kreft, 2003; Skrabanja et al., 2004). In Central and Eastern Europe and in some parts of Japan, buckwheat grain is traditionally dehulled after hydrothermal pre-treatment (namely after cooking of grain in water) to obtain hard dehulled buckwheat grain (or kasha), which is afterwards cooked again and prepared for consumption (Skrabanja & Kreft, 1998; Skrabanja, Laerke, & Kreft, 1998). Groats can be further milled into various grades of flour. Hull (pericarp) can also be removed from buckwheat seeds by impact milling. The resulting groats are roller-milled and the product sifted to remove the fragmented hull (Steadman, Burgoon, Lewis, Edwardson, & Obendorf, 2001).

Storage and milling conditions greatly affect buckwheat aroma. Since the main characteristic aroma compounds of buckwheat were not known, some uncharacteristic volatiles like xylenes, ethylbenzene, acetaldehyde, methanol and ethanol (Ohinata, Karasawa, & Kurokouchi, 2001) and unidentified aldehydes and pentane (Przybylski, Woodward, Eskin, Malcolmson, & Mazza, 1995) were investigated to assess the aroma. There are no reports on the volatiles in buckwheat groats. Aroma of boiled buckwheat flour was investigated by Yajima et al., (1983) however, among 209 identified volatile compounds, none of them possessed the characteristic aroma of boiled buckwheat flour. In the report on headspace analysis of buckwheat flour, twenty-five compounds were listed as equally important for the aroma (Mazza et al., 1999). Volatile compounds of the distilled alcoholic beverage shochu were investigated and ethyl cinnamate was found as one of the most important aroma compounds (Sakaida et al., 2003).

The aim of this study was isolation and identification of compounds, responsible for the characteristic aroma of buckwheat groats, and development of a convenient analytical method for identification and quantification of those compounds.

* Corresponding author. Tel.: +386 1 47 69 583; fax: +386 1 42 58 031.
E-mail address: damjan.janez@ffa.uni-lj.si (D. Janež).

2. Experimental

2.1. Buckwheat

Samples of buckwheat groats and flour were purchased from a local store. Traditionally dehulled grains were from Ukraine and Slovenia, produced by Intes, Maribor and Bio-farm Rengeo, Šalovci, respectively. Impact milled grains were from China and from unknown country of origin, produced by Žito, Ljubljana and Mediacor, Šmarje pri Jelšah, respectively. Buckwheat flour was produced from Ukrainian buckwheat by Intes, Maribor, the flour from unknown country of origin was produced by Mlinotest, Ajdovščina.

2.2. Chemicals and chromatography materials

Solvents and reagents were purchased from the following producers: methanol, ethyl acetate (Fluka, Buchs, Switzerland), hexane, petroleum ether 40–60 °C, sodium hydrogen carbonate, hydrochloric acid 37% (Riedel de Haën, Seelze, Germany), deuterated chloroform with TMS (Euriso-Top, Saint-Aubin Cedex, France). Salicylaldehyde standard was purchased from Acros Organics, Geel, Belgium. TLC pre-coated glass plates Silica gel 60 F₂₅₄ and PLC pre-coated glass plates Silica gel 60 F₂₅₄, 2 mm were purchased from Merck KGaA, Darmstadt, Germany.

2.3. Extraction

Buckwheat groats were milled in a metal mill with a rotating knife to obtain particle size less than 0.2 mm.

In preliminary experiments four portions of milled groats (5 g) were extracted with 10 mL of petroleum ether, diethyl ether, acetone and methanol, respectively. Each extraction was performed with and without prior soaking of groats with 2 mL of water. For a selection of the optimal solvent for the extraction, sensory analysis was employed.

One kg of milled groats was thoroughly macerated with 400 mL of water and shaken with 2 L of petroleum ether at 200 rpm for 24 h. The mixture was filtered under reduced pressure and the residue on the filter was washed with 100 mL of petroleum ether. Petroleum ether extracts from four portions were combined and concentrated to approximately 100 mL using a Rotavapor (Büchi Labor Technik, Flawil, Switzerland) at 40 °C under reduced pressure. This solution was extracted three times with 30 mL of 10% sodium hydrogen carbonate solution. The preliminary experiments of sensory evaluation showed that acidic fraction of the crude extract possesses characteristic buckwheat aroma. Alkaline extracts were combined and extracted once with 30 mL of petroleum ether. Petroleum ether was discarded and the aqueous phase was acidified with 10% hydrochloric acid to pH 2 to convert the aroma compounds from their ionized to non-ionized form which were then extracted with the organic solvent in the next step.

Acidified solution was extracted three times with 30 mL of petroleum ether. The petroleum ether extracts were combined and concentrated to approximately 0.5 mL using the Rotavapor at 40 °C under reduced pressure.

2.4. Isolation and identification of odorants

Fractions of buckwheat extract were prepared by a preparative layer chromatography (PLC). Concentrated petroleum ether extract (0.5 mL) was sprayed with a sample applicator Linomat (Camag, Muttenz, Switzerland) on a preparative TLC plate in a form of an 18 cm long band. The plate was developed in a tank saturated with a mixture of hexane:ethyl acetate = 9:1 (v/v). Bands were marked by observing the dried plate under 254 and 366 nm UV light. Each band was scratched off the plate and suspended in 20 mL of methanol. The suspension was centrifuged at 5000 rpm and the clear methanol solution was decanted. The extraction procedure was repeated three times for each fraction of silica gel. Samples were concentrated to 10 mL with the Rotavapor at 40 °C under reduced pressure. One hundred microlitres of the concentrate was evaluated as described under sensory analysis. The fraction with a characteristic buckwheat groats odour was further carefully concentrated in a Speed Vac system (Savant Instruments, Inc., Farmingdale, NY, USA) at room temperature. ¹H NMR spectra were recorded with a Bruker Avance DPX 300 MHz spectrometer (Bruker Bio-Spin GmbH, Rheinstetten, Germany). The sample was dissolved in deuterated chloroform, containing TMS. Mass spectra were obtained with an Autospec Q (UG-Analytical) mass spectrometer (Micromass, Manchester, UK), using electron ionisation (EI) and fast atom bombardment (FAB) techniques. IR spectra were recorded with a Perkin Elmer 1600 Series FTIR spectrometer (Perkin Elmer, Wellesley, MA, USA) in a NaCl cell.

Solutions containing 1 g L⁻¹ of the isolated and the standard salicylaldehyde were prepared in methanol. Ten microlitres of each solution was applied to a TLC plate with the Linomat applicator in a form of 10 mm long band. The plate was developed with a mixture of hexane:ethyl acetate = 9:1 (v/v) in a saturated tank. After drying, the plate was examined under 254 and 366 nm UV light.

2.5. Sensory analysis

Five judges selected from the staff of the research centre, three males and two females, carried out the aroma evaluation. Each person sniffed and described the aroma sensation by writing a description on paper. A strip of filter paper (5 mm × 100 mm) was immersed in a solution of a sample. The paper was allowed to dry in the air for 5 min and then its aroma was evaluated. No formal checklist of terms was available, however, all judges went through a training session where a variety of buckwheat samples were sniffed and their aroma discussed. The panelists were not involved in any other aroma evaluation panel

for at least 30 preceding days. Panel sessions were held in a sensory panel room at 22 °C and relative humidity 60%.

2.6. Quantification of salicylaldehyde

Buckwheat groats were roughly milled; 1 g was soaked with 1 mL of water and extracted five times with 1 mL of petroleum ether. Organic extracts were combined and extracted three times with 300 μ L of 0.01 M NaOH. Combined alkaline aqueous extracts were analysed by capillary electrophoresis (Hewlett Packard 3D system, electrophoresis buffer: 50 mM borate, pH 9.3; capillary: length 65 cm, inner diameter 50 μ m; injection: 40 mbar, 40 s; voltage: 30 kV; detection 235 nm, temperature 30 °C). Salicylaldehyde was used as an external standard.

To evaluate the efficiency of each of the two steps of the extraction procedure, the extracted groats were extracted once more by the same procedure as described in the previous paragraph. After the extraction with the alkaline water solution, the petroleum ether fraction was extracted again (three times with 1 mL of alkaline water solution). The overall efficiency was over 90%.

3. Results and discussion

Buckwheat grains contain up to 3.25% of lipids (Kim, Kim, Lee, & Park, 2001), which were extracted with petroleum ether, and they negatively affected the resolution of aroma compounds by column and thin layer chromatogra-

phy therefore isolation of the odorants by direct application of a chromatographic separation was virtually impossible. The problem was solved by extracting the mixture with an aqueous solution of sodium hydrogen carbonate, acidifying the alkaline solution and extracting again. The extract purified like this was then successfully separated by preparative TLC. Sensory evaluation guided fractionation led to the isolation of the characteristic buckwheat odour compound. The opinion of the panellists was in a mutual agreement at all levels. Sufficient amount of the compound was obtained for identification.

The isolated compound was a light yellow liquid with a strong phenolic and bitter almond-like odour, which became characteristic of buckwheat groats at a high dilution (2.5 ppm in water). Spectroscopic data of the isolated compound (see Figs. 1–4) were compared to the spectra of standard compound and to the literature data (NIST02; Sigma–Aldrich, 2007). All the information confirmed that the isolated compound was salicylaldehyde (2-hydroxybenzaldehyde).

The retardation factor (R_f) of the isolated compound and the standard salicylaldehyde was 0.35 on the plate, developed with a mixture of hexane:ethyl acetate = 9:1 (v/v) in the saturated tank. Both compounds absorbed light at 254 nm and had a bright blue fluorescence at 366 nm.

The experiments with extraction showed that petroleum ether is the optimal solvent for the extraction of the compound with the typical odour of buckwheat groats, but prior soaking of milled buckwheat groats with water is

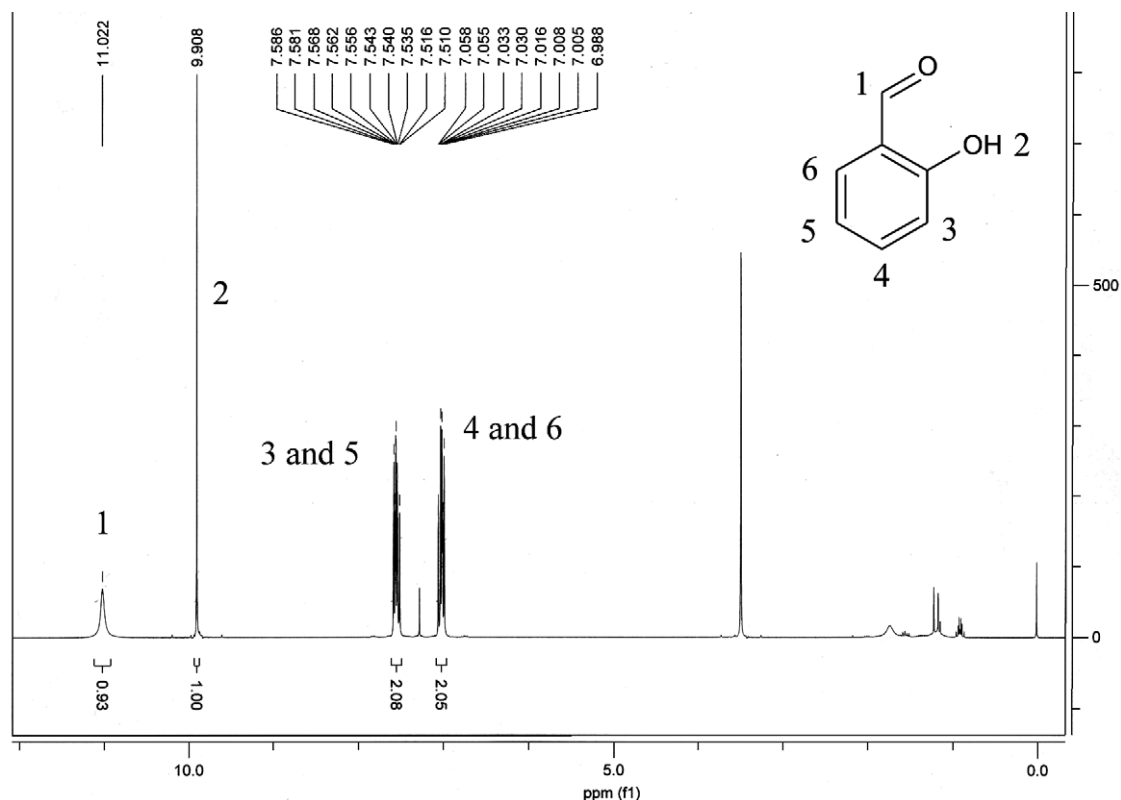


Fig. 1. ¹H NMR spectrum (300 MHz, CDCl₃) of the isolated compound (salicylaldehyde) with assigned peaks of protons.

File:KBR-10 Ident:4 Acq: 9-FEB-2006 10:08:29 +0:22 Cal:GLY
AutoSpecE CI+ Magnet Bpl:867248 TIC:2914480 Flags:HALL
File Text:EI

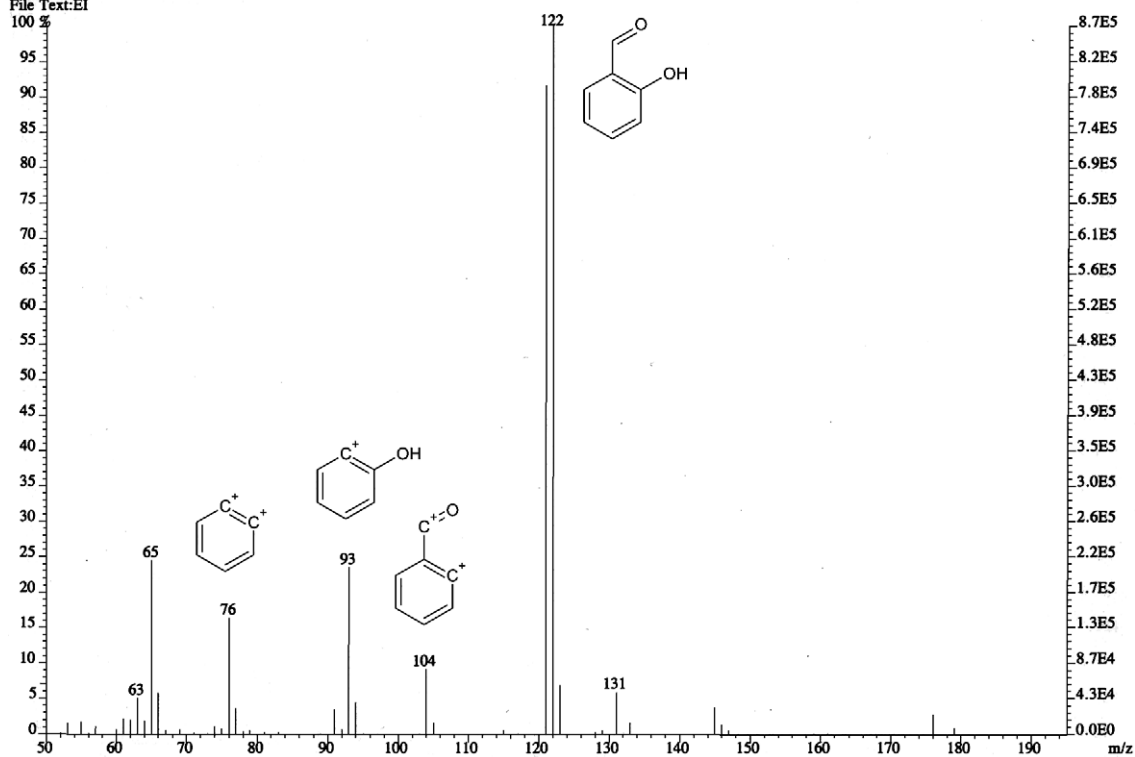


Fig. 2. Mass spectrum (CI⁺) of the isolated compound (salicylaldehyde) with assigned peaks of possible fragments.

File:KBR-10-FAB Ident:3 Acq: 6-FEB-2006 15:31:50 +0:18 Cal:GLY
AutoSpecE FAB+ Magnet Bpl:719640 TIC:4134814 Flags:HALL
File Text:FAB

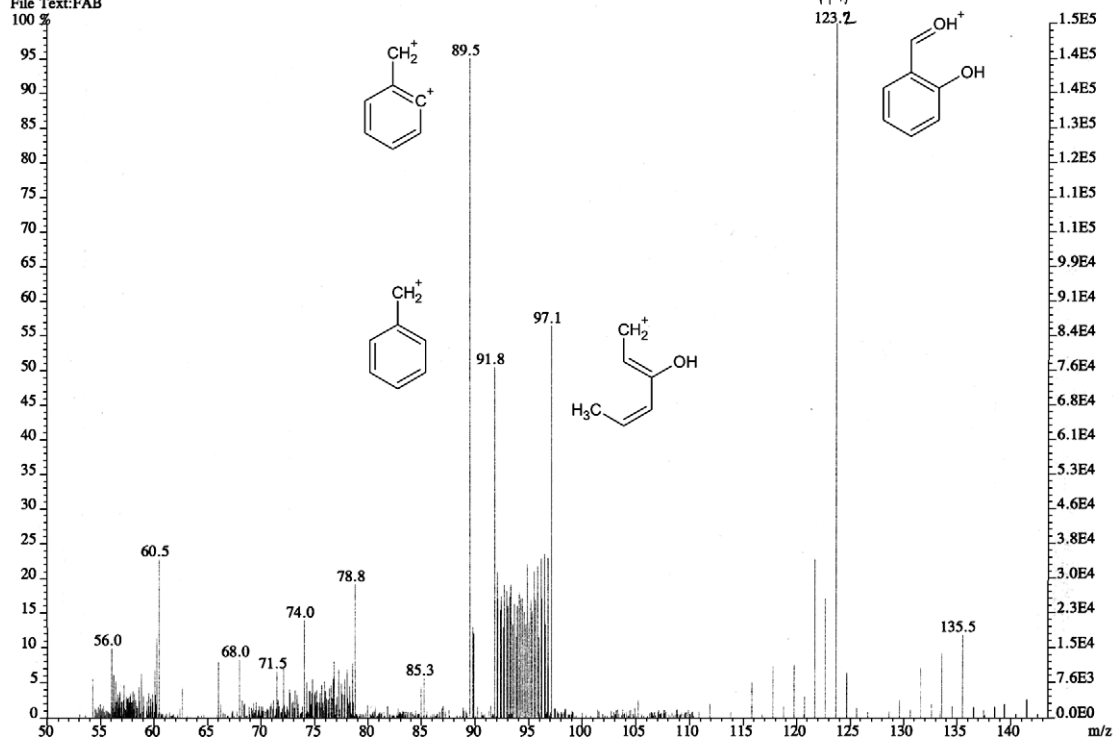


Fig. 3. Mass spectrum (FAB⁺) of the isolated compound (salicylaldehyde) with assigned peaks of possible fragments.

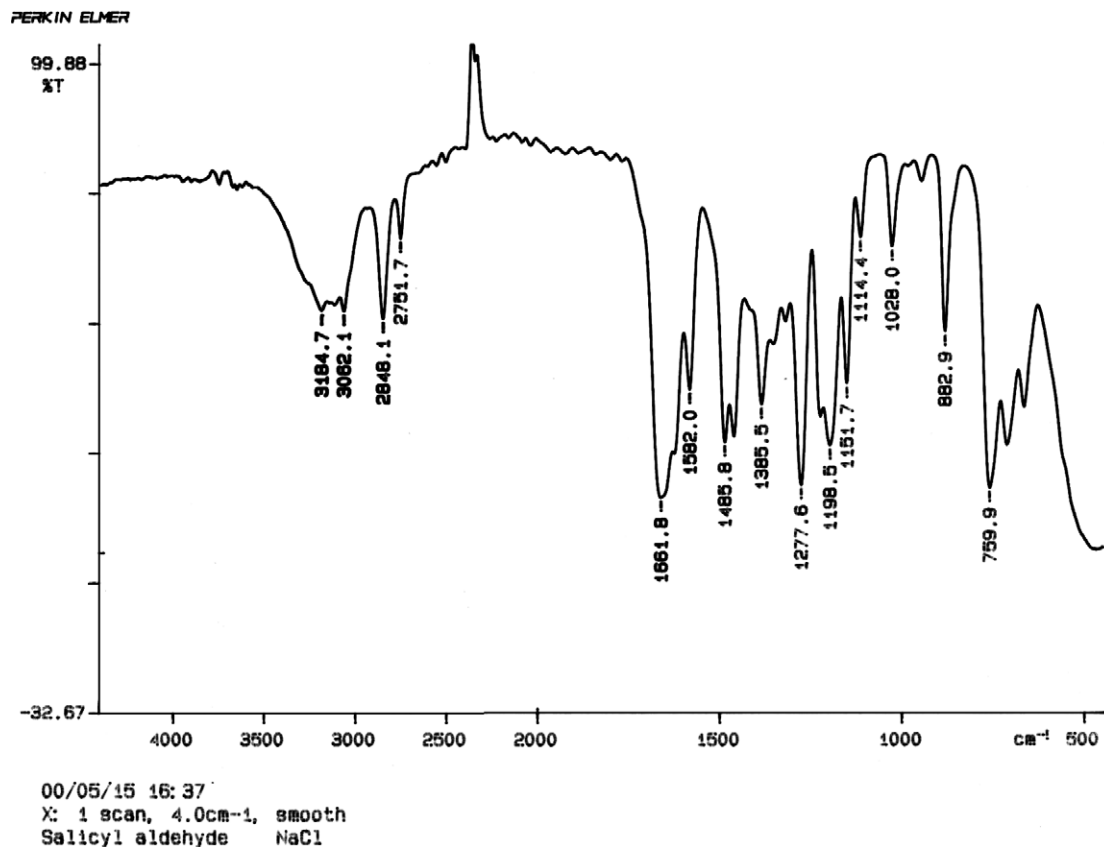


Fig. 4. IR spectrum of the isolated compound (salicylaldehyde).

essential for the extraction of salicylaldehyde. Possible explanation can be that water disturbs the interactions between salicylaldehyde and macromolecules in buckwheat and releases the salicylaldehyde. Belitz, Grosch, and Schieberle (2004) and Reineccius (2006) report on several interactions of aroma compounds with lipids, carbohydrates, and proteins in foods.

Concentrations of salicylaldehyde in different samples of buckwheat groats and flour, determined by capillary electrophoresis of the extracts, are presented in Table 1. Capillary electrophorograms are given in Fig. 5.

GC–MS was previously used for detection but not for quantification of salicylaldehyde in buckwheat (Yajima et al., 1983). Salicylaldehyde was not detected in the buckwheat distilled alcoholic beverage shochu, where ethyl cinnamate was found as one of the most important aroma compounds (Sakaida et al., 2003) and it is not mentioned

in the report on headspace analysis of buckwheat flour among twenty-five compounds listed as equally important for the aroma (Mazza et al., 1999). The exact quantitative analysis of volatiles is relatively difficult if not using the isotope dilution analysis (IDA), especially for the compounds which occur only in traces in food (Werkhoff, Brennecke, Bretschneider, & Bertram, 2002). Capillary electrophoresis is not as sensitive an analytical method as GC–MS, but in this research it proved to be suitable for the analysis of salicylaldehyde.

The highest concentration of salicylaldehyde (1.6 ppm) was determined in traditionally dehulled buckwheat from the Ukraine. The odour activity value (OAV) of salicylaldehyde in this sample was 216. It was calculated by dividing the measured concentration by the odour threshold value (OTV = 7.4 ppm) published by Alevantis (2003). Groats from Slovenia, dehulled in the same manner contained 0.55 ppm of salicylaldehyde. Samples of groats prepared with impact milling had much lower concentrations of salicylaldehyde (0.14 and <0.1 ppm). The traditional dehulling process, which includes boiling the buckwheat grains with water, might be the reason for the increased concentration of salicylaldehyde. This can be explained by hydrolysis of salicylaldehyde glycosides and/or migration of salicylaldehyde from husks to the endosperm. Salicylaldehyde was found in high concentration in buckwheat husks (Yajima et al., 1983). Glycosides of salicylaldehyde were found in plants, for example in *Filipendula ulmaria* L. (Wichtl,

Table 1
Concentration of salicylaldehyde in different samples of buckwheat groats and flour, determined by a capillary electrophoresis of extracts

Sample	Method of preparation	Origin	Concentration of salicylaldehyde (ppm)
Groats	Traditional dehulling	Ukraine	1.6
		Slovenia	0.55
	Impact milling	China	<0.10
Flour	Milling, after impact milling	Unknown	0.14
		Ukraine	0.18
		Unknown	<0.10
		Unknown	<0.10

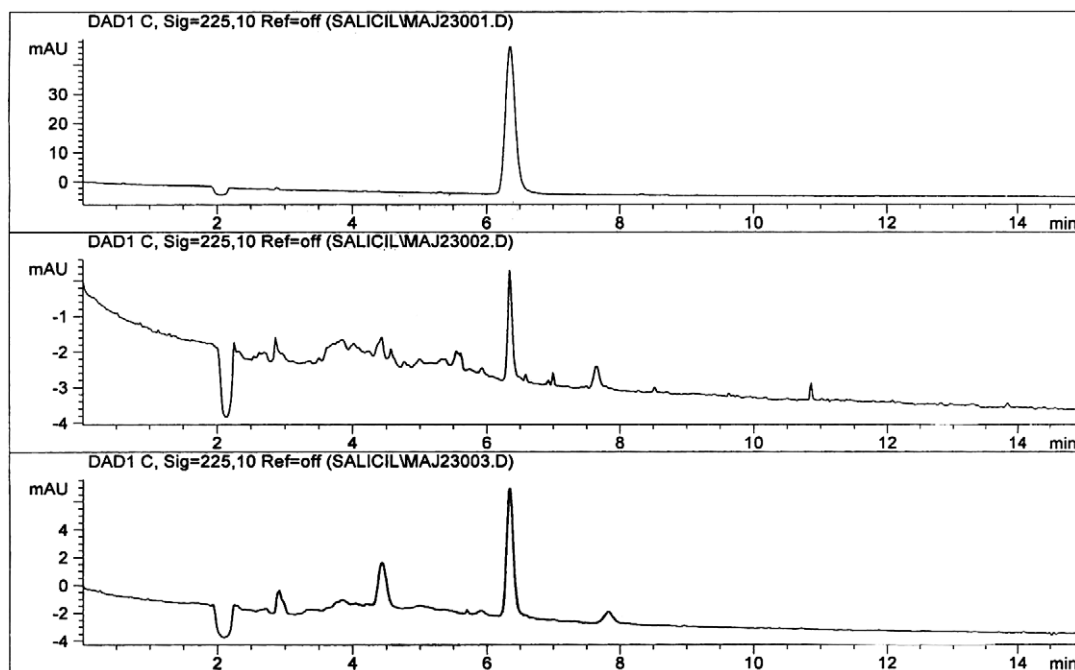


Fig. 5. Electrophorograms of salicylaldehyde standard (upper), extracts from Slovenian (middle) and Ukrainian (bottom) buckwheat groats. The identity of peaks was confirmed also by comparing the UV spectra.

2002). The concentration of salicylaldehyde in buckwheat groats depends on the origin and the method of preparation. Low concentrations of salicylaldehyde in samples of buckwheat flour (0.18 and <0.1 ppm) were expected. Salicylaldehyde, being a volatile compound, can be lost through evaporation during storage of the flour. Also, it can oxidise to odourless salicylic acid.

Acknowledgements

The skilful laboratory assistance of Bojana Roškar and Metka Šmid is greatly appreciated.

References

- Alevantis (2003). *Building material emissions study* (p. 84). Sacramento California: Integrated Waste Management Board.
- Belitz, H.-D., Grosch, W., & Schieberle, P. (2004). Aroma compounds: Interactions with other food constituents. In *Food Chemistry* (3rd ed., pp. 394–398). Berlin: Springer.
- Bonafaccia, G., Marocchini, M., & Kreft, I. (2003). Composition and technological properties of the flour and bran from common and tartary buckwheat. *Food Chemistry*, 80(1), 9–15.
- Kim, S. L., Kim, S. K., Lee, Y. H., & Park, C. H. (2001). Varietal difference of fatty acid composition and vitamin E content in buckwheat grains. In *The proceeding of the 8th ISB* (pp. 523–531).
- Mazza, G., Cottrell, T., Malcolmson, L., Girard, B., Oomah, B. D., & Eskins, M. A. M. (1999). Headspace gas chromatography sensory analysis of buckwheat stored under controlled atmosphere. *Journal of Food Quality*, 22, 341–352.
- NIST02 Mass Spectral Library (2002). Fiveash Data Management, Inc.
- Ohinata, H., Karasawa, H., & Kurokouchi, H.K. (2001). Influence of milling methods on buckwheat flavor. 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 flavor compounds. *Current Advances in Buckwheat Research*, 6, 783–787.
- Reineccius, G. (2006). Flavor release from foods. In *Flavor Chemistry and Technology* (2nd ed., pp. 139–159). Boca Raton: CRC Press.
- Sakaïda, H., Nakahara, N., Watashi, N., Kai, T., Nakashima, Y., Sakakibara, Y., Nishiyama, K., Fukuda, N., & Suiko, N. (2003). Characteristic flavor of buckwheat Shochu and comparison of volatile compounds from variety cereal Shochu. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 50(12), 555–562.
- Sigma–Aldrich (2007). Product database: <<http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/03273>> (accessed: 03.10.2007).
- Skrabanja, V., & Kreft, I. (1998). Resistant starch formation following autoclaving of buckwheat (*Fagopyrum esculentum Moench*) groats. An in vitro study. *Journal of Agricultural and Food Chemistry*, 46, 2020–2023.
- Skrabanja, V., Golob, T., Modic, M., Ikeda, S., Ikeda, K., et al. (2004). Nutrient content in buckwheat milling fractions. *Journal of Cereal Chemistry*, 81(2), 172–176.
- Skrabanja, V., Laerke, H. N., & Kreft, I. (1998). Effects of hydrothermal processing of buckwheat (*Fagopyrum esculentum Moench*) groats on starch enzymic availability in vitro and in vivo. *Journal of Cereal Science*, 28(2), 209–214.
- Steadman, K. J., Burgoon, M. S., Lewis, B. A., Edwardson, S. E., & Obendorf, R. L. (2001). Buckwheat seed milling fractions: Description, macronutrient composition and dietary fibre. *Journal of Cereal Science*, 33(3), 271–278.
- 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.
- Werkhoff, P., Brennecke, S., Bretschneider, W., & Bertram, H.-J. (2002). Modern methods for isolating and quantifying volatile flavor and fragrance compounds. In R. Marsili (Ed.), *Flavor Fragrance and Odor Analysis* (pp. 139–204). New York: Marcel Dekker.
- Wichtl, M. (2002). Spiraeae flos. In M. Wichtl, (Ed.), *Teedrogen und Phytopharmaka*, 4. Aufl. (4th ed.) (pp. 587–589). Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH.