Journal of Cereal Science 69 (2016) 25–31

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

The effect of processing on the phytosterol content in buckwheat groats and by-products

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ARTICLE INFO

Article history:
Received 27 May 2015
Received in revised form
1 February 2016
Accepted 7 February 2016
Available online 16 February 2016

Keywords:
Processing
Buckwheat groats
Buckwheat by-products
Phytosterols

ABSTRACT

The research focused on the influence of the process of roasting on the content of fat, protein, ash and phytosterols in buckwheat grains, as well as its industrial by-products (broken roasted buckwheat groats, roasted buckwheat hulls, buckwheat bran) and the end product (whole roasted buckwheat groats). The highest content of phytosterols was observed for buckwheat bran (0.90 mg/g of product), while the lowest – for roasted hull (0.36 mg/g of product). Varying phytosterol profiles were determined for the tested fractions and the fat obtained from these fractions. Of all phytosterols researched in buckwheat products, sitosterol was the prevalent type (from 15.61 to 29.56 mg/g of fat), which constituted over 57% of all phytosterols found in fat extracted from roasted buckwheat hull. A unique type of phytosterol, not found in any other cereals – cycloartenol – was identified in all tested samples. Its highest concentrations were found in roasted buckwheat hulls (5.35 mg/g of fat) and roasted buckwheat grains (2.34 mg/g of fat). These differences among phytosterol profiles in fractions obtained and fats extracted during this research can be used to guide the process of designing dietary supplements or food products aimed for people suffering from specific disorders.

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1. Introduction

Phytosterols are compounds found in plants; they resemble cholesterol, while their chemical composition is close to human steroids, but they cannot transform into active steroids. They constitute, among other compounds, cell membranes in plant cells, and play a vital role in nutrition (Marangoni and Poli, 2010). Due to their ability to attach to intestinal epithelium receptors, they limit the absorption of cholesterol in the human body. Phytosterols bind bile acids and reduce the risk of high blood levels of total cholesterol without affecting the levels of HDL cholesterol (Harrabi et al., 2008; Hovenkamp et al., 2008; Marangoni and Poli, 2010; Wong, 2014). Approximately 40 different compounds are classified as phytosterols; however, the most commonly found ones include: sitosterol, campesterol, β-sitosterol and stigmasterol. Manufacturers of food more and more often fortify their products with plant sterols. Among the main sources of these compounds are oils, leguminous plants, sesame, sunflower and corn (Harrabi et al., 2008), and – of all cereals – wheat (Chen et al., 2013; Liu, 2007; Nurmi et al., 2012). The recommended daily intake of plant sterols is 800 mg. In highly developed countries, the actual consumption is at about 50% of the recommended level, hence the efforts to supplement it.

Buckwheat products are a source of biologically active nutrients, such as antioxidants, vitamins, amino acids, dietary fibre and phytosterols (Cercaci et al., 2007; Dziedzic et al., 2012; Qin et al., 2013). Consumption of these products reduces the absorption of cholesterol in intestines (Bajguz and Tretyn, 2003; Ferretti et al., 2010; Kaloustian et al., 2008; Marangoni and Poli, 2010). Phytosterol content in buckwheat varies, and depends on the stage of plant development and its morphological part. The most common sterol isolated from buckwheat grains is β-sitosterol, constituting as

Abbreviations: BB, buckwheat bran; BGR, raw buckwheat grains; d, distance; l, linkage; max, maximum linkage Euclidean distance; PC, principal component; PCA, principal component analysis; RBBGT, roasted broken buckwheat groats; RBGR, roasted buckwheat grains; RBGT, roasted buckwheat groats; RBH, roasted buckwheat hull.

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http://dx.doi.org/10.1016/j.jcs.2016.02.003
0733-5210/Published by Elsevier Ltd.

much as 70% of all phytosterols found in buckwheat. Hulled buckwheat grains were found to contain β-sitosterol, campesterol and traces of stigmasterol (Krkošková and Mrázová, 2005).

Biologically active compounds found in buckwheat are currently the subject of many research studies. Their beneficial influence in the prevention of diseases of civilization has been well documented. Production of buckwheat groats includes processes such as roasting, hulling, conditioning and sorting. It is vital, therefore, to arrange these processes in an optimal sequence, which will lead to the highest possible content of biologically active nutrients in the final product. Different types of technological treatment applied in the food industry can lead to diminished levels of phytosterols in the end product (Kmiecik et al., 2011), so the evaluation of these types of treatment from the point of view of health enhancing properties of obtained buckwheat groats is essential.

2. Materials and method

2.1. Reagents and standard

Methanol, chloroform, petroleum ether and n-hexane were purchased from PoCh S.A. (Gliwice, Poland). Sylon BTZ (BSA + TMCS + TMSI, 3:2:3, v/v/v), campesterol, stigmasterol, sitosterol, sitostanol and cycloartenol were acquired from Sigma Aldrich (St. Louis, USA), avenasterol and D-7-stigmasterol from Red Analytical (Cambridgeshire, UK), and MTBE (Methyl-tert-Butyl Ether) from J. T. Baker (Center Valley, USA).

2.2. Plant material

The material used for the research consisted of buckwheat grains (*Fagopyrum esculentum* of Kora variety) and products resulting from its processing at "Podlaskie Zakłady Zbożowe" – a milling company in Białystok, Poland. Raw buckwheat grains (**BGR**) were roasted (130 °C, p = 5 bar, t = 60′), and then hulled. Using these treatments, the following products were obtained: roasted buckwheat grains (**RBGR**), roasted broken buckwheat groats (**RBBGT**), roasted buckwheat groats (**RBGT**), roasted buckwheat hulls (**RBH**), and buckwheat bran (**BB**) – Fig. 1. The samples for the research were taken in three independent trials, over the three days of the duration of the technological process, and then averaged.

2.3. Chemical composition

Dry matter content was determined according to AOAC 2001.12, 2001. Ash content was determined by comparing test sample weight prior to and after incineration (ICC 104/1:1990, 1990). Protein content was determined by classic Kjeldahl method. The conversion rate of 6.25 was used to convert nitrogen content to protein (AOAC 992.23, 1992). Measurements were conducted using Kjeltec equipment from Foss Tecator (Sweden). The analysis was carried out in triplicates.

2.4. Total content of lipid

The total content of lipids was determined by using petroleum ether, and the solvent was evaporated using the gravimetric method (AOAC 996.01, 2000). Soxtec HT6 equipment from Foss Tecator (Sweden) was used for the extraction.

2.5. Lipid extraction

Total lipids were extracted using chloroform/methanol mixture (Folch et al., 1957). Each sample (2.5 g) was crushed and then incubated with a mixture of $CHCl_3$–MeOH (2:1, v/v) for 1 h. Water (20%) was added and the system was thoroughly mixed to remove water-soluble substances. The chloroform fraction was collected and the solvent was evaporated to obtain lipid fraction.

2.6. Sterol content

Lipid extracts (0.05 g) with 2 mL of 1 M methanolic KOH were incubated in room temperature for 18 h. The unsaponifiable fraction was extracted with a mixture of hexane: MTBE (1:1 v/v), then the solvent was evaporated using nitrogen and silylated using Sylon BTZ (AOCS, 6-91, 1997). The analysis was conducted in triplicates.

2.7. GC-analysis

Chromatographic analysis was conducted using Hewlett–Packard 6890 chromatograph equipped with flame ionization detector and DB-35MS capillary column (25 m × 0.2 mm × 0.33 m; J&W Scientific, Folsom, CA). Sterols were separated using programmed oven temperatures: the temperature program was initially set to 100 °C for 5 min, then raised at a rate of 25 °C/min to 250 °C and held for 1 min; in the next phase the temperature was raised by 3 °C/min up to 290 °C and held for 20 min. Injector and detector temperature was 300 °C. Splitless injection was used. Hydrogen was used as the carrier gas, at a flow rate of 1.5 ml/min 5α-cholestan was used for the internal standard, and sterols were identified based on retention time of standards and literature data (AOCS 6-91, 1997).

2.8. Statistical analysis

Measurements of the total content of protein, lipids, ash and phytosterols were carried out in triplicates. Hierarchical cluster analysis was performed using Ward amalgamation rule with the Euclidean distance (d) measure. The tree plots were scaled to a standardized scale (dlink/dmax*100). Non-hierarchical cluster analysis (k-means clustering) was performed to form a grouping of buckwheat grain, groats, hull and bran samples according to the parameters of material. V-fold cross-validation algorithm was used to determine the best number of clusters. Principal component analysis (PCA) technique was used to reduce dimensionality of data and to present the samples in a new coordinate system. Statistical analysis was performed using Statistica software, Version 10, StatSoft Inc., OK-US.

3. Results and discussion

Total content of protein, lipids, ash and phytosterols was shown in Table 1. Roasting (130 °C, p = 3–5.5 bar, t = 60′) led to an increase in the total protein content of buckwheat grains by 12.1%, which can be directly attributed to the loss of water. Among all tested samples, the highest protein content was found in buckwheat bran (BB) – 13.8%, while the lowest in roasted buckwheat hull (RBH) – 5.4%. The highest content of lipids was observed in BB (3.5%), while the lowest in RBH (0.7%). The investigated industrial processing – roasting of buckwheat grains – had no significant impact on the levels of lipid content, but affected the content of phytosterols in individual samples. The highest total phytosterol content was found for lipids extracted from RBH (51.7 mg/g of lipid), and the lowest – in those extracted from BB and whole roasted buckwheat groats (RBGT), 26.1 and 26.8 mg/g of lipid, respectively. RBH was found to contain the lowest levels of lipids. An increase in the total content of researched compounds was observed after roasting (RBGR) – from 29.81 to 36.27 mg/g of lipid. The highest content of phytosterols was found for BB (0.90 mg/g of product), while the lowest for RBH (0.36 mg/g of product), which can be directly