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Defatted strawberry seeds as a source of phenolics, dietary fiber and minerals

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ABSTRACT

Strawberry seeds obtained from industrial press cake from three harvest seasons and defatted by supercritical CO_2 were analysed for dry substance, protein, fat, ash, minerals, saccharides, total dietary fiber and polyphenols. The defatted seeds were composed mostly of dietary fibers (on average 728 g/kg dry matter) and proteins (167 g/kg dry matter). The sum of polyphenols determined by HPLC varied from 15.6 to 17.5 g/kg dry matter and did not vary significantly with production year. There are two main groups of polyphenols among determined compounds: ellagitannins and flavanols, which mean content are 8.50 and 5.82 g/kg dry matter, respectively. Total contents of ellagitannins in seeds varied significantly with production year, but not flavanols. High contents of dietary fiber, makes it reasonable to consider the use of degreased seeds in food production, as source of dietary fiber or polyphenol preparations.

1. Introduction

Strawberry attracts much attention from consumers due to its high sensory values, rich composition in bioactive substances and proven health-beneficial properties. Both fresh and processed strawberries are rich sources of polyphenols – 95–788 mg/100 g, and the highest polyphenol contents were recorded in strawberries harvested in Poland (Aaby, Skrede, & Wrolstad, 2005; Cheel, Theoduloz, Rodríguez, Caligari, & Schmeda-Hirschmann, 2007; Klopotek, Otto, & Böhm, 2005; Oszmiański, Wojdyło, & Matuszewski, 2007; Roussos, Denaxa, & Damvakaris, 2009; Da Silva Pinto, Lajolo, & Genovese, 2008; Skupień & Wójcik-Stopczyńska, 2006). The highest concentrations of polyphenols c.a. 1429 mg/100 g are found in strawberry seeds, while strawberry flesh contains only 375–998 mg/kg of polyphenols (Cheel et al., 2007). Proanthocyanidins represent the highest share in strawberry flavonoids, and the predominant phenolic acid is ellagic acid, especially linked at form of ellagitannins, which make up 50% of polyphenols (Häkkinen et al., 1999; Oszmiański et al., 2007). The content of ellagic acid is ten times higher in seeds, comparing to pure flesh (Da Silva Pinto et al., 2008). Ellagitannins are esters of hexahydroxylodiphenyl acid (HHDPA) and a monosaccharide, usually D-glucose. Health-beneficial properties of ellagitannins are believed to be linked to the capacity of these molecules to deliberate free ellagic acid, and further metabolism of the acid in human and animal organisms. Hydrolysis of ellagitannins in a digestive tract results in prolonged liberation of ellagic acid to blood (Heber, 2008). The acid can be thus regarded as the chemical marker of hydrolysable tannins presence in fruit material, and also a biomarker in research on bioavailability of diet ellagitannins (Seeram, Lee, & Heber, 2004).

Average yearly processing of strawberries into mash and concentrated juices in Poland is c.a. 30.000 tons. During processing, residual press cake represents 10–15% of initial fruit weight (Klimczak, Król, & Rozpara, 2011). Press cake resulting from juice production and waste from mash preparation are characterized by relatively high share of fruit in total weight. Seeds (nuts) only represent 1% of fruit weight, but they contain up to 11% of total phenolics present in strawberry fruits (Klimczak & Król, 2010).

In accordance with environmental regulations all waste should be utilized or directed to recycling (Nawirska, 2007; Nawirska, Sokół-Łętowska, & Kucharska, 2007). The waste from strawberry

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pressing and mash production is not effectively used to date. Some is used in production of infusions (Nawirska, 2007). Another option is incorporation in gluten-free bread with increased amount of dietary fiber and polyphenols (Korus et al., 2012). Strawberry seeds as a by-product of industrial juice production contain circa 20% of oil rich in polyunsaturated fatty acids and antioxidants like tocopherols (Johansson, Laakso, & Kallio, 1997; Pieszka, Tombarkiewicz, Roman, Migdał, & Niedziółka, 2013). Increasing interest in vegetable oils, mainly in the pharmaceutical and cosmetic industries, uses such by-product as raw material. At the same time large quantities of next by-product after oil extraction remain, the composition, properties and possible ways of applications of which are not well researched.

To our knowledge, there is no publication on composition of defatted strawberry seeds and their use in food. This paper aims to assess this co-product as a source of nutritionally important components, like phenolics, dietary fibre and minerals.

2. Material and methods

2.1. Plant material

Strawberry seeds were separated from industrial press cake of strawberry juice production. The juice production was carried in three consecutive harvest seasons at ALPEX (Łęczeszyce, Poland). Batches of 20 kg of fresh press cake were collected from the production line and dried in convection dryer (1.6 kW KC-100/200, WAMiE, Warsaw, Poland), at 65–70 °C for 8 h. Dried press cake c.a. 9 kg were manually sieved on screens with screen sizes 3 mm and 0.6 mm, respectively. Next c.a. 4 kg of 0.6–3 mm fraction rich in seeds were again sieved on 0.6, 0.8, 1.0 and 1.2 mm screens by the use of Analysette 3 (Fritsch, Idar-Obersten, Germany) siever. C.a. 1.5 kg of seeds was obtained in the fraction 0.8–1.2 mm, with not more than 5% of dried flesh. Part of seeds fraction was defatted by supercritical carbon dioxide at experimental installation at Fertilizers Research Institute, Extraction Department at Puławy, Poland. To do this the seed portions (approximately 350 g) were crushed in roller crusher in CO_2 atmosphere to the particle size of 0.4–0.6 mm and they were immediately placed in supercritical extractor. Extraction process was carried out on the laboratory plant for extraction of plant raw materials. The plant was composed of the extractor with 1 L volume, 2 separators S_1 and S_2 with separation capacity up to 50 MPa –S_1 and up to 10 mPa – S_2, circulating pump and condenser and CO_2 tank. The following process conditions were applied: extraction pressure: 25 MPa, I grade separation level pressure 5.3 MPa, temperature at the inlet to the reactor $T_w = 40$ °C, mass of raw material loaded into the reactor 300.0 g. Flow rate Q $co_2 = 6.0–7.0$ kg (one cycle duration 180–210 min).

From each seedless fraction an average laboratory sample was prepared, from which quantities of 100 g of material were taken and then ground in liquid nitrogen in IKA A11 basic mill (IKA-Analytical Mill, Staufen, Germany). The ground seed was stored at 4 °C before analyses.

2.2. Methods

2.2.1. Protein, fat, ash and total dietary fibre

Double measurements of protein content in samples were made according to AOAC official method 920.152 (AOAC, 2005c), Kjeldahl method. The results are given as g/kg d.m. of protein in the seed.

Fat was determined using Soxhlet method with petroleum ether according to AOAC official method 930.09 (AOAC, 2005d). All the seed samples were analysed in duplicates. The results are expressed as g/kg d.m. of fat per seed mass.

Ash was determined according to AOAC official method 940.26 (AOAC, 2005a). The results are expressed as g/kg d.m. of ash per seed mass.

Total dietary fibre (TDF) was determined by the enzymatic weight method according to AOAC official method 985.29 (AOAC, 2005b). The results are expressed as g/kg d.m. per seed mass.

2.2.2. Minerals

The contents of elements were determined as previously described (Sójka, Kołodziejczyk, & Milala, 2013). The samples were calcined in a furnace at 550 °C for 16 h. The ash was then solubilized in 1 mol/L HNO_3 and minerals were analysed by atomic absorption spectroscopy (AAS) method by the use of SOLAAR 969 AAS spectrometer (Unicam, UK).

2.2.3. Saccharides and water activity

Saccharides were determined by HPLC. Ground seeds (2.5 g) were suspended in 25 mL of water with 1 g of $CaCO_3$. The suspension was heated to the boiling point and maintained for 5 min. Next, the solution was cooled, transferred quantitatively with water to a 50 mL volumetric flask, filled up to the graduation mark, kept at room temperature for 15 min and filtered. The filtrate was cleaned on SPE column containing cation exchanger resin Amberlite IR120 and anion exchanger resin Amberlite IRA420 (both Sigma-Aldrich Sp. z.o.o., Poznań, Poland) at 1:2 volumetric ratio in non-retention mechanism. The samples were injected in Bio-Rad Aminex HPX87C column, 300 × 7.8 mm (Phenomenex, Torrance, CA, USA) Elution conditions were: mobile phase: water, isocratic flow: 0.5 mL/min, at 85 °C. RI detector (Knauer, Berlin, Germany) and a Knauer integrating system were used. The results are expressed as g of individual sugar per 100 g of dry substance. Water activity was determined by the use of HygroPalm-23-AW with probe HC2-AW (Rotronic, Basserdorf, Germany).

2.2.4. Determination of ellagitannins, flavonols and anthocyanins

Ellagitannins and other polyphenols were extracted in three steps by the use of aqueous acetone: (acetone: water 700: 300 mL:mL). Free ellagic acid and agrimoniin as well as other polyphenols (anthocyanins and flavonols) were determined in acetone solutions. C.a. 0.5 g of material was weighted with accuracy 0.0001 g in stopped PET 7 mL tubes. Aqueous acetone (4 mL) was added to each tube, carefully stirred and sonicated for 15 min. Tubes were next centrifuged (4.800× g) for 5 min at 20 °C. The residue was re-extracted twice with 3 mL of extracting solution and poured into 10 mL volumetric flask. The solution was analysed by HPLC.

To measure total ellagic acid the ellagitannins were hydrolysed. Seeds powder (50 mg) was weighted into 2 mL tube, 0.5 mL of aqueous glycerol (glycerol: water 700: 300 mL: mL) and 0.075 mL of trifluoracetic acid were added. The hydrolysis was carried at 95± 1 °C for 18 h. The solutions after hydrolysis were extracted in three steps with respectively: 1 mL, 1.5 mL and 1.5 mL of methanol. Resulting extract was transferred to 5 mL volumetric flask and filled up to graduation mark with methanol. The extracts were analysed by HPLC in the same conditions as for free ellagic acid.

Sum of ellagitannins as galloyl-bis-HHDP-glucose monomer was calculated by multiplication of bound ellagic acid (total minus free ellagic acid) by 1.55 factor. 1.55 factor arises from the molar share of two ellagic acids molecules in galloyl-bis-HHDP-glucose (936/604 = 1.55).

A Dionex chromatograph with UV-DAD detector was used (Dionex, Sunnyvale, CA, USA). The separation was carried out on a Gemini 5μ C18 110A (250 × 4.60 mm; 5 μm) column (Phenomenex, Torrance, CA, USA), at 25 °C. Eluents: A – 0.5 mL/L phosphoric acid in water, B – 0.5 mL/L phosphoric acid in acetonitrile. Flow rate was 1.25 mL/min. Gradient program: 0–5 min 4% B; 5–12.5 min 4–15%