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Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars

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ABSTRACT

The objective of this study was to determine the antioxidant properties, and provide characteristics, of the oil obtained from the seeds of 12 pumpkin varieties belonging to the species *Cucurbita maxima* Duch. and *Cucurbita pepo* L. Another objective was to establish which of the two extracting agents, ethanol or methanol, is more effective. The seeds of the pumpkin varieties examined differ in chemical composition and antioxidant activity. The seeds of the cultivars belonging to the species *C. maxima* are characterised by a higher content of fatty acids than are the cultivars of the species *C. pepo*. In the seed oil, unsaturated acids are dominant (oleic and linoleic), and their proportion depends on the pumpkin variety. The highest content of unsaturated acids has been measured in the oil extracted from the seeds of the cultivar, Jet F1 (*C. pepo*). Antioxidant activity analysis has produced the following findings. The seeds of the pumpkin varieties that belong to the species *C. pepo* exhibit better antioxidant properties, regardless of the extraction solvent used. 50% ethanol is more efficient than 80% methanol when used as an extracting agent. The antioxidant activity values obtained with 50% ethanol are higher than those achieved with 80% methanol. Owing to the considerable differences in composition among the fatty acids examined, it is possible to choose the desired pumpkin variety for the intended use.

1. Introduction

These days, when the environment is heavily polluted with a variety of chemically and biologically active substances such as steroid sex hormones, phytohormones (Dudziak & Bodzek, 2009) or the ubiquitous free radicals, considerable attention is being focussed on compounds and substances with antioxidant properties, which are believed to reduce the risks and hazards inherent in the pollutants mentioned. Forming as a result of many reactions, oxygen free radicals induce oxidative changes in fat, protein and nucleic acid fractions, which may become an underlying cause of pathological changes in human organisms. In order to protect itself against the reactive forms of oxygen, an organism utilises its own enzymatic system and endogenous antioxidants, e.g. bilirubin (Horubała, 1999).

Natural antioxidants are found in many compounds classified as secondary plant metabolites, e.g. in polyphenols (phenolic acids, flavonoids) and terpenoids (carotenoids), and the consumption of foods which contain these compounds in large quantities seems to play an important role in prophylaxis against many diseases. Epidemiological studies have revealed that the incidence of some cardio-vascular and cancer diseases is less frequent when fruit and vegetables are consumed regularly (Horubała, 1999), which should be attributed to the large quantities of the phenolics present. Phenolic compounds belong to a numerous group of antioxidants and act *via* different modes, e.g. by 'scavenging' free radicals, by a direct reaction with them, by enhancing the dissimilation of free radicals to compounds of a substantially lower reactivity, by chelating pro-oxidant metals, and by inhibiting or enhancing the activity of some enzymes. Phenolic compounds can also enhance the activity of other antioxidants, for example that of fat-soluble vitamins (Drużyńska, Strzecha, Wołosiak, & Worobiej, 2008).

Owing to their composition and, more importantly, to a specific spectrum of fats present, pumpkin seed kernels are regarded as a valuable dietary component. In some countries they are served as a snack, mostly after being salted and roasted. Pumpkin seed kernels are also used as additives to confectionery and bakery products. Pumpkin seed oil (especially that obtained from hull-less seeds) is utilised by both food and pharmaceutical industries (in food industry generally as salad oil). For many years pumpkin seed

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kernels have been used in complementary medicine – primarily as vermifuge. They are part of a minor group of plants and herbs containing fatty acids and phytosterols that are administered at the early stage of prostatic hyperplasia therapy (Benzie & Strain, 1996; Murkovic et al.,1996).

With oil as their main component (which in many instances accounts for 50% of the total content), pumpkin seeds are regarded as a valuable source of protein and fat. Pumpkin seed oil includes fatty acids: palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1) and linoleic (C 18:2) (Kulaitiene, Jariene, Danilcenko, Kita, & Venskutoniene, 2007). Their content, however, differs among the pumpkin varieties, and was found to dependent on the climate and cultivation conditions. It is therefore advisable to assess the composition of the fatty fraction in the seed oil before determining the range of potential uses for a new pumpkin variety.

Among the biologically active substances which are found in pumpkin seeds, tocopherols and phytosterols occur in the largest quantities. With β + γ-tocopherol as the dominant substance, the content of tocopherols (16 mg/100 g) noticeably exceeds that of α-tocopherol. Phytosterols occur in considerably lower quantities compared to those found in other oily seeds and differ in composition. Even β-sitosterol, which is a dominant compound, accounts for scarcely 10% of all the phytosterols contained in pumpkin seeds. It has also been reported that pumpkin seeds contain squale (89 mg/100 g), a biosynthetic precursor to all steroids both in plant and animal cells (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007). Another beneficial property of pumpkin seeds is the presence of phenolic compounds, which largely contributes to their antioxidant activity (Parry, Cheng, Moore, & Yu, 2008).

Four methods are available for the determination of antioxidant properties, DPPH, ORAC, FRAP and ABTS, and each of them produces different values. Preference is given to two or three of these methods. They are often used simultaneously, in order to enable comparisons of the results obtained. But the value of the antioxidant property obtained also depends on the type of the solvent used. Therefore, it seems worthwhile to determine not only the antioxidant properties of the new pumpkin variety, but also to assess the influence on these parameters exerted by the solvents that are to be used for extraction (Drużyńska et al., 2008; Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005).

In the literature there are many reports on the composition and properties of pumpkin seeds (El-Adawy & Taha, 2001; Kulaitiene et al., 2007; Murkovic, Hillebrand, Winkler, Leitner, & Pfannhauser, 1996; Parry et al., 2008; Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007; Stevenson et al., 2007; Younis, Ghirmay, & Al-Shihry, 2000), which concern mainly non-specific varieties. In the present study Polish varieties of pumpkin were used for comparison of each species to those popular in the world.

The primary objective of the study reported on in this paper was to determine the antioxidant properties, and provide characteristics of the oil obtained from the seeds of 12 pumpkin varieties belonging to the species *Cucurbita maxima* and *Cucurbita pepo*. Another major objective was to establish which of the two extracting agents, ethanol or methanol, is more effective.

2. Materials and methods

2.1. Chemicals

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH); 6-ydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Folin–Ciocalteu reagent were obtained from Sigma (Germany). Tocopherols (-T) standards used are: alpha-(a-T), beta-(b-T), gamma-(g-T), delta-(d-T), with a purity of 95% (Calbiochem). Acetonitrile (gradient grade for HPLC) and formic acid (98.0–100%) were from Sigma (Germany). Phenolic acid standards were purchased from Extrasynthese (France). All reagents were of analytical grade.

2.2. Pumpkin cultivation

The seeds of the varieties of the following *Cucurbita* species were analysed: *C. maxima:* 1-'Amazonka', 2-'Ambar', 3-'Bambino', 4-'Karowita', 5-'Melonowa Żółta', 6-'Uchiki Kiuri' cvs, and *C. pepo*: 7-'Danka', 8-'Junona', 9-'Miranda', 10-'Pyza', 11- 'Makaronowa Warszawska', 12-'Jet F1' cvs. Both the species were grown in the Experimental Station belonging to the Department of Horticulture, Wroclaw University of Environmental and Life Sciences. Four-week-old transplants produced in greenhouses were planted on nitrogen-fertilised plots (200 kg N/ha) in the second decade of May, and fruits were harvested in mid-September 2007.

Pumpkin seedes were purified from the remaining pulp and dried at room temperature. In all varieties, except Junona and Miranda, the dry seed coat was removed.

2.3. Chemical analysis of pumpkin seeds

Dry matter content and protein content were determined according to AOAC standards (1995). Fat content was measured by the Soxhlet method. Fat was extracted using a Büchi B-811 Universal Extraction System (Büchi Labortechnik AG, Flawil, Switzerland). Two grammes of sample were extracted for 180 min using diethyl ether as a solvent. Fatty acid methyl esters were prepared with BF3 in methanol as the methylating agent. Fatty acid composition was determined by gas chromatography (GC) using an RTX-2330 capillary column with a length of 105 m, an internal diameter of 0.25 mm, and a film thickness of 0.20 mm. Detector (FID) temperature and injection temperature was 260 °C. The temperature of the column was 160 °C (30 min) – 3 °C/min – 180 °C (17 min) – 5 °C/min – 210 °C (45 min). Helium was used as the carrier gas.

Tocopherols were determined using a liquid chromatograph HPLC with detectors (UV–VIS PAD, refractometric, fluorescence, electrochemical) from Varian, according to Katsanidis and Addis (1999).

2.4. Extraction of polyphenol compounds for antioxidant activity analysis

Seeds obtained from pumpkin fruit were peeled and ground. Samples for the analysis of polyphenol, ABTS and FRAP were prepared as follows. Approximately 5 g of each pumpkin seed powder was weighed into a test tube for antioxidant property analysis. A total of 25 ml of 50% aqueous ethanol was added, and the suspension was stirred slightly. The sample was sonicated for 15 min, and left at 4 °C. After 24 h the extract was centrifuged at 12,500 rpm for 5 min, and the supernatants were recovered.

To assess the antioxidant power (DPPH) of the pumpkin seed, two types of extraction, hydrophilic and lipophilic, were conducted. Hydrophilic extraction was performed with different amounts (0.4–0.5 g) of homogenised pumpkin seeds, which were dissolved with 6 ml methanol and water (80% v/v). The samples were made subject to sonication for 15 min, followed by centrifugation at 12,500 rpm for 5 min. The supernatant and precipitate of each sample were recovered. Lipophilic extraction was done on a pellet by adding acetone (5 ml), and then the samples were sonicated for 15 min, and centrifuged at 12,500 rpm for 5 min (Scalzo et al., 2005).

2.4.1. Analysis of polyphenols

Total polyphenols were determined by the Folin–Ciocalteu (Slinkart & Singleton, 1977) method using gallic acid (GA) as a