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Characteristics of Biologically-Active Substances of Amaranth Oil Obtained by Various Techniques

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Amaranth seeds and their main product amaranth oil are a rich source of bioactive substances. The non-saponifiable substances which accompany lipids include: squalene, tocopherols, sterols and others. The aim of the study was to compare the content of squalene, tocopherols and phytosterols in amaranth oils obtained by various techniques. The oil was extracted from seeds (*Amaranthus cruentus*) with the use of supercritical fluid extraction (SFE), extraction with a chloroform/methanol mixture and expeller pressing. Contents of squalene and tocopherols were determined with high performance liquid chromatography (HPLC) method. The content of sterols in oils was determined by gas chromatography coupled with mass spectrometry (GC-MS).

The highest squalene content was found for the oil obtained as a result of supercritical CO_2 extraction (6.95 g/100 g of oil). A lower content of squalene was noted in the oil extracted with organic solvents and in cold-pressed oil – 6.00 and 5.74 g/100 g of oil, respectively. The amaranth oils were characterised by a significant content of tocopherols. The oil obtained as a result of fluid extraction was characterised by the highest content of tocopherols (131.7 mg/100 g of oil). A dominating homologue (40%) was β-tocopherol. Also the same sample was characterised by the highest content of sterols (2.49 g/100 g of oil). In all samples the predominating sterol was sum of α-spinasterol and sitosterol, which accounted for 45%, 56% and 53% of total analysed sterols for the oil obtained from SFE, from extraction with solvents and from cold pressing, respectively.

INTRODUCTION

Amaranth, also known as amaranthus and by ancient Aztecs and Incas as huautli, is one of the oldest crops in the world. It is one of few plants cultivated in prehistoric times which has survived until present-day [Black, 2006; Ceglińska & Cacak-Pietrzak, 1999]. Currently, amaranth is cultivated in many countries of both Americas, South East Asia and in Africa. Plantations are also located in many European states, including Poland since the early 1990s.

Amaranth seeds are a rich source of biologically-active compounds. Some of them are a part of the unsaponifiable substances accompanying oil [Barba de la Rosa *et al.*, 2009; Gorinstein *et al.*, 2007; León-Camacho *et al.*, 2001; Sun *et al.*, 1997]. There are many scientific reports describing the positive effects of bioactive components of unsaponifiable fractions of plant oils on human health. Among these components, the following are especially of note: squalene, tocopherols and sterols [Bartnikowska, 2009; Kelly, 1999; Martirosyan *et al.*, 2007; EFSA Report 2008; Lippi *et al.*, 2010].

Both consumers and farmers are becoming increasingly interested in this unconventional plant. The application of amaranth seeds is also growing, and they are currently used, *e.g.* as a source of lipids and to produce flour, flakes, expanded seeds, some types of bread [Januszewska-Jóźwiak & Synowiecki, 2008] and confectionery [Sindhuja *et al.*, 2005]. The content of crude fat in seeds of various species of amaranth varies from 4.8 to 10% [Januszewska-Jóźwiak & Synowiecki, 2008]. It is too low to ensure its cost-effective production without the rational use of by-products. It is necessary to develop a technology to enable obtaining oil from the material and using the by-product in order to obtain protein or starch [León-Camacho *et al.*, 2001; Gontarczyk, 1996]. Research has been carried out on the possibility of obtaining squalene [Martinez-Correa *et al.*, 2010; Westerman *et al.*, 2006, Sun *et al.*, 1997]. Products enriched with squalene from Amaranthus oil are a good squalene carrier in a human diet [Piotrowska *et al.,* 2007].

Cold-pressed oils are characterised by a higher content of native substances as compared to refined oils. During the refining process some nutrients and antioxidants are lost, such as carotenoids and tocopherols [Gunstone, 2002; Reichert, 2002]. Supercritical fluid extraction is an attractive alternative to traditional methods of oils separation, such as distillation or extraction with solvents. It was successfully used to fractionate active antioxidant components, *e.g.* from rosemary leaves, tea, various fruits and vegetables, as well as to isolate tocopherols from hibiscus and amaranth seeds [Pereira *et al*., 2010; Wang & Weller, 2006; Janiszewska & Witrowa-Rejchert, 2005]. In literature there are many scientif-

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ic reports describing different techniques applied in vegetable oils analysis [Gromadzka & Wardencki, 2011a, b]. The most widespread methods for both the qualitative and quantitative identification of sterols, tocopherols and squalene are high--pressure liquid chromatography and high-resolution gas chromatography.

The aim of the study was to compare the content of squalene, tocopherols and phytosterols in amaranth oils extracted from seeds by the method of supercritical fluid extraction, extraction with a chloroform/methanol mixture and oil pressing.

MATERIALS AND METHODS

The research material consisted of oils obtained from amaranth seeds (*Amaranthus cruentus)* purchased from the "Szarłat" company in Łomża, Poland.

Amaranth seeds (25 g) were ground for 60 s in a mill and oil was extracted with the use of supercritical CO_2 extraction and extraction with a chloroform/methanol mixture. The whole seeds were used for expeller pressing.

Supercritical extraction (SFE) was performed with the use of an SFE-400 extractor (Supelco, USA). The process was conducted for 16 h using CO_2 under a working pressure of 306 atm at an extraction temperature of 50°C.

The extraction with a 2:1 (v/v) chloroform/methanol mixture was performed according to the modified method described by Folch *et al.* [1957], consisting in the extraction of a seeds sample mixed with 150 mL of a 2:1 (v/v) chloroform/methanol mixture, which was shaken in a 500 mL Erlenmeyer flask by a magnetic stirrer for 45 min. The mixture was then centrifuged and the solid phase was re-extracted two times more with the same volume of extractant. The liquid phases were combined in a separatory funnel. Thirty-five millilitres of 10% sodium chloride were added and the mixture was gently shaken. After phase separation, the chloroform phase was dried with anhydrous sodium sulfate and filtered again. Finally, the extractant was evaporated to dryness under an N_2 stream.

Oil pressing from seeds was performed with the use of an expeller produced by IBG Monforts & Reiners (Germany), of the Komet CA59G type, with a 4 mm nozzle. The pressing temperature did not exceed 40°C. The oil obtained was purified by centrifugation at 10,000 rpm in a Janetzki MLW K24D (Germany) centrifuge.

The content of squalene, tocopherols and phytosterols in the samples achieved was determined in triplicate.

The squalene content was determined with high performance liquid chromatography (HPLC), according to method described by Czaplicki *et al.* [2009] with modifications. Briefly, 10 mg of oil ($\pm$0.0001 g) were transferred into a 10 mL measuring flask and filled up with *n*-hexane. Next, 20 μL of the diluted oil sample were injected into the chromatographic system. Analyses were conducted on a 1200 series HPLC system, manufactured by Agilent Technologies (Palo Alto, CA, USA) and equipped with a photodiode detector (PDA). Chromatographic separations were conducted on a LiChrospher RP-18 column (Merck, Germany, 250 × 4.6 mm, 5 μm), at 30°C. A gradient mobile phase was used for elution: A, acetonitrile; B, isopropyl alcohol; C, hexane. The elution profile was 0–12 min, 20–22% B and 10–12% C in A (linear gradient),

12–15 min 22–25% B and 12–25% C in A (linear gradient), 15–20 min, 25% B and 25% C in A (isocratic), 20–25 min, 25–20% B and 25–10% C in A (linear gradient). The mobile phase flow rate was 1 mL/min. The analytical wavelength was set at 218 nm. An external calibration curve was used for quantitative analysis. The squalene standard was supplied by Sigma-Aldrich.

The analysis of tocopherols was carried out by HPLC method described by Peterson & Qureshi [1993]. Briefly, 0.1 g of oil (± 0.001 g) was diluted in *n*-hexane in a 10 mL measuring flask. After subsequent centrifugation (10 min at 16,000 rpm) the sample was transferred to a chromatographic vial. The analysis was carried out using a 1200 series HPLC system (Agilent Technologies), equipped with a fluorescent detector. Separations were performed on a LiChrospher Si60 column, 250 mm × 4 mm, 5 mm, manufactured by Merck (Germany), using an 0.7% isopropanol solution in hexane as a mobile phase. Peaks were identified on the basis of retention times determined for the of α-, β-, γ- and δ-tocopherol standards (Calbiochem, UK) separately, and their content was calculated using external calibration curves.

The content of sterols in oils was determined by gas chromatography coupled with mass spectrometry (GC--MS-QP2010 PLUS Shimadzu, Japan) according to method described by Vlahakis & Hazebroek [2000]. The unsaponifiables were extracted with diethyl ether, which was subsequently evaporated under nitrogen conditions. The dry residues were re-dissolved in 1.5 mL of *n*-hexane and 0.2 mL of 5α-cholestane internal standard solution was used. The extract was transferred into a vial and evaporated with a nitrogen stream. The residues were re-dissolved in 100 μL of pyridine and 100 μL BSTFA (N,O-bis (trimethylsilyl) trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) and left in the dark for 24 h to complete derivatization. Then, 1 mL of hexane was added and 1 μL of the resultant mixture used for GC-MS analysis.

A DB-5ms capillary column (Agilent JW Scientific, USA) was used for separations of phytosterols with helium as a carrier gas at a flow rate of 0.9 mL/min. The injector temperature was 230°C, and the column temperature was programmed as follows: 50°C for 2 min, a subsequent increase to 230°C at the rate of 15°C/min, and to 310°C at the rate of 3°C/min 10 min hold. The interface temperature of GC--MS was 240°C. The temperature of the ion source was 220°C and the electron energy was 70 eV. The total ion current (TIC) mode was used for quantification (100–600 m/z range). The measurements were carried out using the internal standard method.

RESULTS AND DISCUSSION

Squalene is present in practically all plant oils, although in the majority of them it is found in minute or trace amounts. Its high concentrations may be found in the oil obtained from shark liver [Bakes & Nichols, 1995]. However, due to the concern for the protection of sea animals, researchers have started to look for new sources of squalene. Investigations have been conducted with seeds of unconventional plants, *e.g.* evening primrose, borage, sea buckthorn and am-
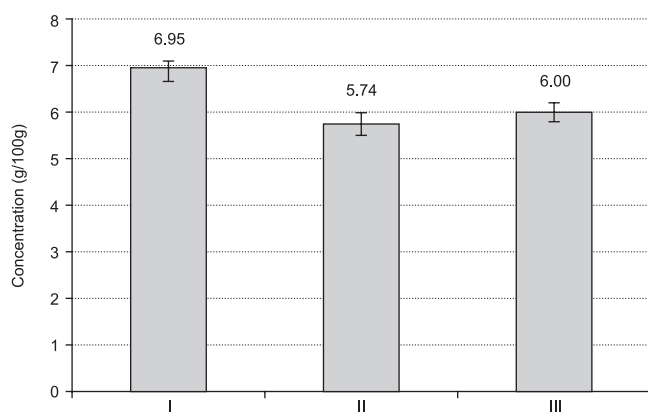
FIGURE 1. Squalene content in the amaranth oils obtained by I - supercritical fluid extraction (SFE), II - cold-pressing and III - extraction with chloroform/methanol (2:1, v/v).

TABLE 1. The content of tocopherols in amaranth oils obtained by supercritical fluid extraction (SFE), cold-pressing and extraction with chloroform/methanol (2:1, v/v) (mg/100 g of oil).

Tocopherol	SFE	Cold-pressing	Solvent extraction
α-Tocopherol	22.8 ± 0.06	23.9 ± 0.02	23.0 ± 0.40
β-Tocopherol	53.0 ± 0.01	34.0 ± 0.47	49.0 ± 0.54
γ-Tocopherol	14.8 ± 0.03	11.2 ± 0.28	15.6 ± 0.29
δ-Tocopherol	41.1 ± 0.03	31.8 ± 0.11	40.3 ± 0.46
Total	131.7 ± 0.04	101.0 ± 0.62	127.9 ± 1.47

aranth [Czaplicki *et al.*, 2011]. It was established that from the listed group of plants, only amaranth seeds provided a rich source of squalene. Figure 1 presents squalene content in the amaranth oils under analysis. The highest squalene content was found for the oil obtained as a result of supercritical CO_2 extraction (6.95 g/100 g of oil). A lower content of squalene occurred in the oil obtained by extraction with organic solvents (6.00 g/100 g of oil), while the lowest one in cold-pressed oil (5.74 g/100 g of oil). The results achieved correspond to the reports of other authors. For instance, Bodroža-Solarov *et al.* [2007] determined squalene content in seeds of amaranth at the level of 517.1 mg/100 g. Gamel *et al.* [2007] determined squalene amount at the levels of 4.8% and 4.9% in oil extracted using the Soxhlet's method from seeds of *Amaranthus cruentus* and *Amaranthus caudatus*, respectively. Berganza *et al.* [2003] obtained similar values, between 2.26% and 5.90%, for the oil extracted with *n*-hexane from different varieties of *Amaranthus cruentus* seeds. A slightly higher content of squalene (7.2%) was noted by Sun *et al.* [1995] in oil extracted with the same method from seeds of *Amaranthus hypochondriacus*. There is little information concerning the squalene content in the oil obtained as a result of supercritical fluid extraction; however, it is known that some attempts have been made to use it in order to optimise the production of plant oils, including amaranth oil [Temelli, 2009; Westerman *et al.*, 2006].

Important components of the amaranth lipid fraction are tocopherols. Due to their high antiradical activity, tocopherols and tocotrienols protect phospholipids present in cell membranes, strengthen the walls of blood vessels, reduce the risk of inflammatory conditions and are one of the reasons for the hypocholesterolemic effects of food products containing amaranth seeds [Januszewska-Jóźwiak & Synowiecki, 2008]. Table 1 presents tocopherol content in the examined oils. The oil obtained as a result of supercritical CO_2 extraction was characterised by the highest content of tocopherols (131.7 mg/100 g of oil). A dominating homologue (40%) was β-tocopherol, the content of which amounted to 53.0 mg/100 g of oil. Δ-Tocopherol accounted for a lower share (31%, 41.1 mg/100 g of oil). The lowest content (11%) was established for γ-tocopherol. Bruni *et al.* [2002], characterised tocopherols of *Amaranthus cau-*

datus seeds using supercritical fluid extraction with different working pressure, and obtained the following results: α-tocopherol – 26.37–34.81 mg/kg of seeds, β-tocopherol – 33.18–43.86 mg/kg of seeds, γ-tocopherol – 1.42–1.81 mg/kg of seeds, and δ-tocopherol – 39.36–48.79 mg/kg of seeds. Assuming that fat content in the material analysed in the current experiment amounted to 8.3%, similar values were obtained for α-tocopherol, β-tocopherol and δ-tocopherol, but completely different values in the case of γ-tocopherol. These differences could result from features of individual species and from the application of various parameters of pressure and temperature of the extraction process. The above-mentioned authors also used extraction of oil with an organic solvent (methanol) [Bruni *et al.,* 2002]. The content of tocopherols in the samples obtained with this method was lower in comparison to supercritical fluid extraction. This trend is consistent with the results presented in the current study (Table 1). Oil extracted with the mixture of chloroform:methanol contained: α-tocopherol – 23.0 mg/100 g of oil, β-tocopherol – 49.0 mg/100 g of oil, γ-tocopherol – 15.6 mg/100 g of oil, and δ-tocopherol – 40.3 mg/100 g of oil. A similar content in the case of α-tocopherol (24.8 mg/100 g of oil) and β-tocopherol (54.6 mg/100 g of oil) was obtained by León-Camacho *et al.* [2001], but they did not observe any presence of γ-tocopherol.

The lowest content of tocopherols was found in the sample obtained as a result of cold pressing (Table 1). Dominating homologous compounds were also β-tocopherol and δ-tocopherol, the amount of which accounted for 34 and 31 % of total forms of tocopherols under analysis.

The analysed material also revealed a high content of sterols (Table 2). Many studies confirm that regular consumption of phytosterols and/or phytostanols in the amounts of 1–3 g/day could result in the reduction of cholesterol level in blood serum by 10 to 15% [EFSA Report, 2008]. The content of phytosterols analysed in this study was determined with gas chromatography coupled with a mass spectrometer. On the basis of available standards (Sigma-Aldrich, Poland), the following sterols were analysed: stigmasterol and campesterol, while other sterols were identified on the basis of the literature data [Berger *et al.*, 2003; Takatsuto *et al.*, 1999]. The highest content of sterols was characteristic for the oil obtained with the method of supercritical CO_2 extraction (2.49 g/100 g of oil), a lower content of sterol was found in the fat extracted with organic solvents (2.14 g/100 g of oil), while the lowest in cold-pressed oil (1.93 g/100 g of oil). In all samples un-

TABLE 2. The content of sterols in amaranth oils obtained by supercritical fluid extraction (SFE), cold-pressing and extraction with chloroform/methanol (2:1, v/v) (mg/100 g of oil).

Sterol	SFE	Cold-pressing	Solvent extraction
Campesterol	45.7 ± 0.9	37.9 ± 1.1	42.0 ± 0.1
5,24-Stigmastadienol	47.8 ± 3.1	39.4 ± 5.6	31.0 ± 2.6
24-Metylenecholesterol	40.8 ± 2.4	29.7 ± 2.4	30.2 ± 0.2
Δ7-Stigmastenol	373.9 ± 1.6	279.6 ± 14.7	251.2 ± 22.0
α-Spinasterol + sitosterol	1119 ± 444	1028 ± 38	1205 ± 31
Stigmasterol	31.9 ± 1.4	20.9 ± 1.3	31.9 ± 7.0
Sitostanol	29.4 ± 1.9	26.1 ± 5.1	23.4 ± 0.7
Δ5-Avenasterol	19.8 ± 1.8	14.3 ± 1.0	7.4 ± 0.3
Δ7-Ergosterol	430.4 ± 78.5	315.2 ± 4.6	345.0 ± 17.8
Δ7-Avenasterol	213.2 ± 21.5	140.3 ± 16.6	173.1 ± 11.4
Cycloartenol	56.4 ± 2.8	0.0 ± 0.0	0.0 ± 0.0
Citrostadienol	82.3 ± 4.1	0.0 ± 0.0	0.0 ± 0.0
Total	2490 ± 147	1931 ± 91	2140 ± 94

der analysis, dominating sterols were the sum of α-spinasterol and sitosterol, which accounted for 45%, 56% and 53% of total analysed sterols for the oil obtained from SFE, extraction with solvents and cold pressing, respectively. Those results are consistent with findings of Grajeta [1997], who reported a spinasterol content amounting to 46–54% in amaranth seeds sterols. In turn, Δ7-sterols, *i.e.*: Δ7-ergosterol, Δ7-stigmastenol and Δ7-avenasterol, were also present in significant amounts. The content of Δ7-ergosterol amounted to 17% of total sterols in oil obtained by SFE and 16% of total sterols in samples obtained by extraction with organic solvents and the cold pressing method. Furthermore, Δ7-stigmastenol was found in the oil obtained by SFE (373.3 mg/100 g of oil), extracted with solvents (251.2 mg/100 g of oil) and cold-pressed (279.6 mg/100 g of oil). The presence of Δ7-avenasterol was at a lower level, as compared to the above-presented sterols, and amounted to 7–9% of the level of the analysed sterols. The other phytosterols, which occurred in the amount of 1–3% of total sterols, included: 24-methylenecholesterol, Δ5-avenasterol, citrostadienol, cycloartenol, campesterol, sitostanol, stigmasterol, and 5,24–stigmastadienol. Cycloartenol (56.4 mg/100 g of oil) and citrostadienol (82.3 mg/100 g of oil) were present only in the oil obtained by SFE. The results achieved correspond with the data provided in the literature. In the experiment conducted by Berger *et al.* [2003], the total content of phytosterols in crude oil (extracted with hexane) was determined at the level of 2.73 g/100 g, whereas in refined oil it was 2.59 g/100 g. The total content of sterols in the oil obtained as a result of extraction in Soxhlet's apparatus (solvent: petroleum ether), from amaranth seeds reported by Martirosyan *et al.* [2007] was 2 g/100 g.

While comparing the results of research concerning the presence of phytosterols in amaranth seeds with the application of the methods used so far, the observation arises that the identification of sterols is questionable. For example, León-Camacho *et al.* [2001] found that the main phytosterol occurring in the amaranth oil was clerosterol, but they did not observe any presence of α-spinasterol. On the other hand, Marcone *et al.* [2004] reported that they observed the presence of only β-sitosterol, stigmasterol and campesterol in different varieties of amaranth seeds.

To summarise, the oil with the highest amount of biologically-active substances was that obtained as a result of supercritical fluid extraction. The other oils revealed a lower content of the examined compounds, but these amounts were also significant for the diet of a potential consumer. While applying CO_2 extraction, much higher squalene content was obtained in comparison to the other techniques applied. The advantages of this method include, first of all, fractioning of extracted substances during their separation, conducting the process in low temperature and without air admission [Janiszewska & Witrowa-Rejchert, 2005], which is particularly important in laboratory practice. The possibility of complete separation of the solvent from the extract and application of non-toxic solvents favours the application of SFE instead of extraction with organic solvent in research laboratories.

CONCLUSIONS

The methods of extraction used in the experiments – with supercritical carbon dioxide, with a chloroform/methanol mixture and expeller pressing – made it possible to obtain oils that were rich in squalene, tocopherols and sterols. The highest amounts of those components were isolated while extruding the oil by supercritical fluid extraction and slightly lower after the extraction with a chloroform/methanol mixture. Both extraction methods resulted in obtaining oils with a higher content of bioactive components than in the case of the cold-pressed oil.

REFERENCES

1. Barba de la Rosa A.P., Fomsgaard I.S., Laursen B, Mortensen A.G., Olvera–Marínez L., Silva-Sánchez C., Mendoza-Herrera A., Gonzalez-Castaneda J., De Leon-Rodriguez A., Amaranth (*Amaranthus hypochondriacus*) as an alternative crop for sustainable food production: Phenolic acids and flavonoids with potential impact on its nutraceutical quality. J. Cereal Sci., 2009, 49, 117–121.

2. Bakes M.J., Nichols P.D., Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters. Comp. Biochem. Physiol., 1995, 110, 267–275.

3. Bartnikowska E., Biological activities of phytosterols with particular attention to their effects on lipid metabolism. Pol. J. Food Nutr. Sci., 2009, 59, 105–112.

4. Berganza B.E., Moran A.W, Rodríguez G.M., Coto N.M., Santa-maría M., Bressani R., Effect of variety and location on the total fat, fatty acids and squalene content of amaranth. Plant Foods Hum. Nutr., 2003, 58, 1–6.

5. Berger A., Monnard I., Dionisi F., Gumy D., Hayes K.C., Lambelet P., Cholesterol-lowering properties of amaranth flakes, crude and refined oils in hamsters. Food Chem., 2003, 81, 119–124.

6. Black M., Bewley J.D., Halmer P., The Encyclopedia of Seeds, Science, Technology and Use. 2006, CABI, p. 336.

7. Bodroža-Solarov M., Filipčev B., Kevrešan Ž., Mandić A., Šimurina O., Quality of bread supplemented with popped *Amaranthus cruentus* grain. J. Food Proc. Eng., 2007, 31, 602–618.

8. Bruni R., Guerrini A., Scalia S., Romagnoli C., Sacchetti G., Rapid Techniques for the Extraction of Vitamin E Isomers from *Amaranthus caudatus* Seeds: Ultrasonic and Supercritical Fluid Extraction. Phytochem. Anal., 2002, 13, 257–261.

9. Ceglińska A., Cacak-Pietrzak A., Mity a Nauka. Magiczne właściwości dzikich zbóż św. Hildegardy. Orkisz, szarłat, komosa ryżowa, 1999, Wrocławskie Wydawnictwo Naukowe, 93–142.

10. Czaplicki S., Ogrodowska D., Derewiaka D., Tańska M., Zadernowski R., Bioactive compounds in unsaponifiable fraction of oils from unconventional sources. Eur. J. Lipid Sci. Technol., 2011, 113, 1456–1464.

11. Czaplicki S., Zadernowski R., Ogrodowska D. Triacylglycerols from viper bugloss (*Echium vulgare* L.) seeds bio-oil. Eur. J. Lipid Sci. Technol, 2009, 111, 1266–1269.

12. EFSA Report, Consumption of food and beverages with added plant sterols in the European Union. The EFSA Journal, 2008, 133, 1–21.

13. Folch J., Lees M., Stanley S.G.H., A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem., 1957, 226, 497–509.

14. Gamel T.H., Mesallam A.S., Damir A.A., Shekib L.A., Linssen J.P., Characterization of amaranth seed oils. J. Food Lipids, 2007, 14, 323–334.

15. Gontarczyk M., Szarłat uprawny – *Amaranthus ssp*. Nowe rośliny uprawne na cele spożywcze, przemysłowe i jako odnawialne źródła energii. 1996, Wyd. SGGW, Warszawa, 21–43.

16. Gorinstein S., Medina Vergas O.J., Jaramillo N.O., Salas I.A., Martinez Ayala A.L., Arancibia-Avila P., Toledo F., Katrich E., Trakhtenberg S., The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. Eur. Food Res. Technol., 2007, 225, 321–328.

17. Grajeta H., Nutritive value and utilization of amaranth. Bromat. Chem. Toksykol. 1997, 30, 17–23 (in Polish).

18. Gromadzka J., Wardencki W., Trends in edible vegetable oils analysis. Part A. Determination of different components of edible oils – a review. Pol. J. Food Nutr. Sci., 2011, 61, 33–43.

19. Gromadzka J., Wardencki W., Trends in edible vegetable oils analysis. Part B. Application of different analytical techniques. Pol. J. Food Nutr. Sci., 2011, 61, 89–99.

20. Gunstone F., Production and trade of vegetable oil. 2002, *in:* Vegetable Oils in Food Technology: Composition, Properties and Uses. Blackwell Publishing, pp. 1–4.

21. Janiszewska E., Witrowa-Rejchert D., Supercritical extraction in food industry. Żywność. Nauka. Technologia. Jakość, 2005, 4, 5–16 (in Polish).

22. Januszewska-Jóźwiak K., Synowiecki J., Characteristics and suitability of amaranth components in food biotechnology. Biotechnology, 2008, 3, 89–102.

23. Kelly G.S., Squalene and its potential clinical uses. Altern. Med. Rev., 1999, 4, 29–36.

24. León-Camacho M., Garcia-González D.L., Aparicio R., A detailed and comprehensive study of amaranth (*Amaranthus cruentus* L.) oil fatty profile. Eur. Food Res. Technol., 2001, 213, 349–355.

25. Lippi G., Targher G., Franchini M., Vaccination, squalene and anti-squalene antibodies: Facts or fiction?, Eur. J. Intern. Med., 2010, 21, 70–73.

26. Marcone M.F., Kakadu Y., Yada R.Y., Amaranth as a rich dietary source of β-sitosterol and other phytosterols. Plant Foods Hum. Nutr., 2004, 58, 207–211.

27. Martinez-Correa H.A., Gomes D.C.A., Kanehisa S.L., Cabral F.A., Measurements and thermodynamic modeling of the solubility of squalene in supercritical carbon dioxide. J. Food Eng., 2010, 96, 43–50.

28. Martirosyan D.M., Miroshnichenko L.A., Kulakova S.N., Pogojeva A.V., Zoloedov V.I., Amaranth oil application for coronary heart disease and hypertension. Lipids Health Dis., 2007, 6, 1–12.

29. Pereira C.G., Meireles M.A.A., Supercritical fluid extraction of bioactive compounds: Fundamentals, applications and economic perspectives. Food Bioprocess Technol., 2010, 3, 340–372.

30. Peterson D.M., Qureshi A.A., Genotype and environment effects on tocols of barley and oats. Cereal Chem., 1993, 70, 157–162.

31. Piotrowska A., Żebrowska M., Waszkiewicz-Robak B., Małecka K., Sensory quality of yoghurts enriched with squalene from amaranthus oil. Pol. J. Food Nutr. Sci., 2007, 57(4B), 437–444.

32. Reichert R.D., Oilseed medicinals: In natural drugs, dietary supplements and in new functional foods. Trends Food Sci. Tech., 2002, 13, 353–360.

33. Sindhuja A., Sudha M.L., Rahim A., Effect of incorporation of amaranth flour on the quality of cookies. Eur. Food Res. Technol., 2005, 221, 597–601.

34. Sun H., Wiesenborn D., Rayas-Duarte P., Mohamed A., Hagen K., Bench-scale processing of amaranth seed for oil. JAOCS, 1995, 72, 1551–1555.

35. Sun H., Wiesenborn D., Tostenson K., Gillespie J., Rayas--Duarte P., Fractionation of squalene from amaranth seed oil. JAOCS, 1997, 74, 413–418.

36. Takatsuto S., Kosuga N., Abe B., Noguchi T., Occurrence of potential brassinosteroid precursor steroids in seeds of wheat and foxtail millet. J. Plant Res., 1999, 112, 27–33.

37. Temelli F., Perspectives on supercritical fluid processing of fats and oils. J. Supercritical Fluids, 2009, 47, 583–590.

38. Vlahakis C., Hazebroek J., Phytosterol accumulation in canola, sunflower, and soybean oils: effects of genetics, planting location, and temperature. JAOCS., 2000, 77, 49–53.

39. Wang L., Weller C.L., Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Tech., 2006, 17, 300–312.

40. Westerman D., Santos R.C.D., Bosley J.A., Rogers J.S., Al-Duri B., Extraction of Amaranth seed oil by supercritical carbon dioxide. J. Supercritical Fluids, 2006, 37, 38–52.