

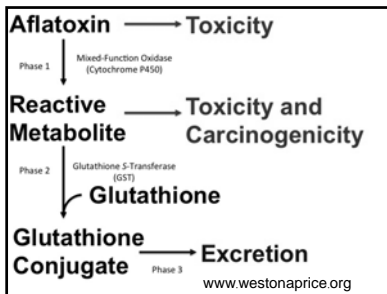
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Functional Foods & Dietary Supplements

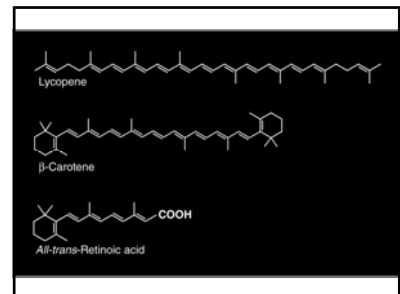
- อาหารฟังก์ชันและผลิตภัณฑ์เสริมอาหาร
- บางครั้งเรียกว่า "nutraceutical" = โภชนเภสัช หรือ ภัณฑาหารเป็นยา
- สารสกัดบริสุทธิ์ อาจออกฤทธิ์ได้ไม่เท่าเทียมกับ original food matrix
- อาหารฟังก์ชัน คือ อาหารที่มีสารออกฤทธิ์ทางชีวภาพ ทำให้มีประโยชน์ต่อสุขภาพเหนือจากคุณค่าทางอาหารพื้นฐาน
- ผลิตภัณฑ์เสริมอาหาร หมายถึง สารสกัดหรือทำให้เข้มข้นขึ้น เพื่อ "บำรุงสุขภาพ" แต่นักวิจัยจะหมายถึง "ป้องกันโรค" ด้วย

- การป้องกันโรค ไม่ได้หมายถึง การรักษาโรค บางครั้งการรักษาโรค ผู้ป่วยต้องปรึกษาแพทย์ ก่อนใช้ผลิตภัณฑ์เสริมอาหาร เพราะอาจยังมียาบางชนิดได้



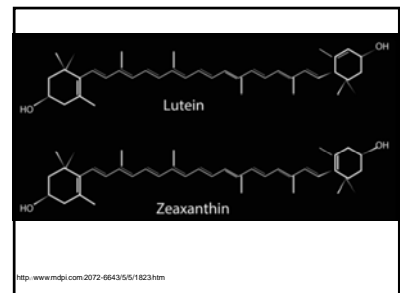
Carotenoids

- เป็นสารกลุ่ม tetraterpenes
- Precursors ของวิตามินเอ เช่น β -carotene
- ไม่ใช่ precursors ของวิตามินเอ เช่น lycopene, lutein และ zeaxanthin
- มีศักยภาพในการป้องกันโรคเรื้อรัง (chronic disease)



A comparison chart between Lycopene and Beta-Carotene. Lycopene is noted as not being produced by any vitamins, having no known side effects, and being associated with a lower risk of prostate cancer. Beta-Carotene is noted as being produced by vitamins A and C, having side effects like skin discoloration, and being associated with a higher risk of prostate cancer. Similarities include both being antioxidants, anti-inflammatory, and anticancer, and both protecting against cognitive decline and eye disease. Source: Dr. Axe, www.draxe.com.

A chart detailing the benefits and sources of Lycopene. It states that Lycopene lowers the risk of Prostate Cancer, Lung Cancer, Stomach Cancer, Cardiovascular Disease, and Stroke. It acts as an antioxidant and counters the oxidative effects of harmful free radicals. Sources listed include Tomatoes, Red Bell Peppers, Pink Grapefruit, Apricots, Red Carrots, Red Cabbage, Guava, Watermelon, and Papaya. Source: theufubaboutcancer.com/lycopene-benefits-prostate-cancer.





Fatty acids and sterols composition, and antioxidant activity of oils extracted from plant seeds



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ABSTRACT

This study determined and compared the contents of bioactive components in plant seed oils extracted with *n*-hexane (Soxhlet method) and chloroform/methanol (Folch method) from coriander, caraway, anise, nutmeg and white mustard seeds. Oleic acid dominated among unsaturated fatty acids in nutmeg and anise seed oils while petroselinic acid was present in coriander and caraway oils. Concerning sterols, β -sitosterol was the main component in seed oils extracted with both methods. The content of total phenolics in nutmeg, white mustard and coriander seed oils extracted with chloroform/methanol was higher than in their counterparts prepared with *n*-hexane. The seed oil samples extracted according to the Folch method exhibited a higher ability to scavenge DPPH radicals compared to the oil samples prepared with the Soxhlet method. DPPH values of the methanolic extracts derived from oils produced with the Folch method were also higher than in the oils extracted with *n*-hexane.

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1. Introduction

Lipids are major components of a man diet. Their high quantities may be found in plant seeds distributed in many regions of the world. They can provide oils with a high concentration of monounsaturated fatty acids, that prevent cardiovascular diseases by several mechanisms (López-Miranda et al., 2006). They are a good source of phenolic compounds that can scavenge the free radicals produced in the body. Polyphenols can also offer an indirect protection by activating endogenous defense systems and modulating the cellular signaling processes (Chen, Yu, Owuor, & Kong, 2000). The phenolic antioxidants present in spices and herbs used as food additives can prevent lipid oxidation and deterioration of colour, flavour and nutritional quality of food products (Kozłowska, Żbikowska, Gruczyńska, Żontała, & Póltorak, 2014). Sterols, which exhibit serum cholesterol-lowering properties and may be potent antioxidants, are other important constituents of plant seed oils. Their antioxidant activity was attributable to the formation of an allylic free radical, which undergoes isomerization to other relatively stable free radicals (Ramadan & Moersel, 2006). The most common plant sterols (phytosterols) are β -sitosterol, campesterol, stigmasterol and avenasterol. Among their saturated

forms called stanols, sitosterol is the most representative example. Biological properties of phytosterols, especially their capacity for reducing blood cholesterol level, are the main reason behind their use as food ingredients. Commercial food products enriched with phytosterols and phytosterols include spreads, milk, cheese or chocolate products. The content of phytosterols in these products ensures a daily consumption of 2–3 g of these components, which is sufficient for effective reduction of the total cholesterol and its LDL fraction in the human body (Kmieciak et al., 2011). Plant seed oils may also contain squalene which – as an antioxidant – is responsible for the protection against oxidative DNA damage of mammary epithelial cells in humans (Warleta et al., 2010).

Seeds of coriander (*Coriandrum sativum* L.) and caraway (*Carum carvi* L.) are important sources of monounsaturated fatty acids, especially petroselinic acid, which can be oxidatively cleaved to produce lauric and adipic acids with their promising application in the chemical industry. Caraway seeds are used for the extraction of carvone, which inhibits sprouting in stored potatoes and onions (Raal, Arak, & Orav, 2012). In addition, caraway essential oil is important in pharmaceutical applications and in human medicine. Anise seeds (*Pimpinella anisum* L.) contain 1.5–5% of essential oil and 8–11% of lipids rich in fatty acids, such as palmitic and oleic acids. In the food industry, they are used as flavour-enhancing and aromatic agents in ice creams, sweets and gums. In turn, mustard seeds have many applications including frying oil, condiments

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and preservatives for pickles. Nutmeg (*Myristica fragrans*) oil is used in the flavouring of meat products, pastry, cola drinks and perfumery products. Most of these plant seeds are tested for their nutritional and medicinal properties. They may be considered as a non-traditional source of oils rich in fatty acids and other phytochemicals such as phenolic compounds, sterols and squalene. Additionally, they may be used in the production and stabilization of structured lipids with increased thermo-oxidative stability.

Therefore, the aim of this investigation was to assess and compare the content of these components in oils extracted from coriander, caraway, anise, nutmeg and mustard seeds using *n*-hexane (Soxhlet method) or a mixture of solvents – chloroform/methanol (Folch method). The antioxidant activity of methanolic extracts prepared from extracted oils and of seed oil samples measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay were also determined and compared. The results derived from these studies expand our knowledge with regard to the content of fatty acids, sterols and phenolic compounds in the studied oils and may be applied to develop new foods.

2. Materials and methods

2.1. Materials and reagents

Anise (*Pimpinella anisum* L.), coriander (*Coriandrum sativum* L.), caraway (*Carum carvi* L.), white mustard (*Sinapis alba*) and nutmeg (*Myristica fragrans*) seeds were obtained from the store with organic products and healthy food in Warsaw, Poland. All chemicals and solvents were of analytical grade and used without further purification. Chloroform, ethanol, methanol, *n*-hexane, anhydrous magnesium sulphate, sodium carbonate and potassium hydroxide were purchased from Avantor Performance Materials (Gliwice, Poland). Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent and gallic acid (GA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). A certified fatty acids methyl ester (FAME) reference standard mixture (37 fatty acids from C4 to C24) and Sylon BTZ were from Supelco (Bellefonte, PA, USA). Anhydrous pyridine, 1 M methanolic KOH, and sterol standards campesterol (~65%), stigmasterol (~95%), β-sitosterol (≥97%) and sitostanol (≥95%) as well as the internal standard 5α-cholestane (≥95.0%) were purchased from Sigma-Aldrich.

2.2. Seed oil extraction

2.2.1. *n*-Hexane extraction

Plant seeds were crushed and ground in an electric coffee grinder into a fine powder. Thirty grams of a powdered sample from each plant seeds were extracted with *n*-hexane (300 mL) using a Soxhlet extractor for 8 h at 70 °C. After extraction, the hexane-oil mixture was passed through a layer of anhydrous magnesium sulphate placed over a filter paper in a funnel and then the solvent was removed using a rotary evaporator, Rotavapor R-215 (Büchi Labortechnik, Switzerland) at 40 °C. The oil samples were weighed, closed under a nitrogen stream and stored at –20 °C until further analysis.

2.2.2. Chloroform/methanol extraction

Thirty grams of ground powdered plant seeds were extracted with 300 mL of a chloroform/methanol (2:1, v/v) solution at room temperature under shaking for 2 h (Folch, Lees, & Stanley, 1957). Then, the mixture was filtered through Whatman No. 1 paper filter into a separatory funnel and a 1 M KCl solution (70 mL) was added. After gentle shaking, the mixture was left overnight for separation into two phases. The lower phase was collected and solvents were

evaporated under reduced pressure at 40 °C (Rotavapor R-215, Büchi Labortechnik, Switzerland). The extracted oil was weighed and flushed with nitrogen, and stored at –20 °C until further analysis.

2.3. Fatty acid analysis

Fatty acid composition was analyzed by gas chromatography (GC) after derivatization to fatty acid methyl esters with a 2 M methanolic solution of potassium hydroxide according to ISO 12966-2 (2011). A Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector and a BPX capillary column (30 m × 0.22 mm; film thickness 0.25 μm) was used. The analysis was performed with nitrogen (1.0 mL/min) as a carrier gas at the following temperature program: 60 °C held for 1 min, after which the temperature was increased to 170 °C at a rate of 10 °C/min and from 170 to 230 °C at a rate of 3 °C/min. The temperature was kept at 230 °C for the subsequent 15 min. The injector and detector temperatures were set at 225 °C and 250 °C, respectively. Individual fatty acids were identified by comparing their retention times with a certified fatty acid methyl esters (FAME) mix and quantified as a percentage of the total fatty acids.

2.4. Sterols and squalene analysis

The content and composition of the sterols and squalene were determined by GC following the procedure described by AOCS (1997) Official Method Ch 6–91. Each seed oil (50 mg) was saponified with 1 M KOH in methanol for 18 h at room temperature, then water was added and the unsaponifiables were extracted three times with *n*-hexane/methyl *tert*-butyl ether (1:1, v/v). The solvent was evaporated under a stream of nitrogen. Dry residues were dissolved in 0.2 mL pyridine and silylated with 0.8 mL of Sylon BTZ (Supelco, Bellefonte, PA, USA). Sterol derivatives were separated on a Trace GC Ultra (Thermo Electron, Rodano, Italy) equipped with DB-35MS capillary column (25 m × 0.20 mm × 0.33 μm; J&W Scientific, Folsom, CA, USA). A sample of 1.0 μL was injected in a splitless mode with an injection time of 5 min. The column temperature was held at 100 °C for 5 min, then increased to 250 °C at a rate of 25 °C/min, held for 1 min, then further increased to 290 °C at a rate of 3 °C/min and held for 20 min. The detector temperature was set at 300 °C. Hydrogen was used as a carrier gas at a flow rate of 1.5 mL/min. An internal standard, 5α-cholestane, was used for sterols quantification. Sterols were identified by comparing their retention times (relative to 5α-cholestane) with those of commercially available standards and results were expressed as mg/kg of oil.

2.5. Methanolic extract of seed oil preparation

A sample of oil (1 g) was dissolved with 5 mL of *n*-hexane and then 5 mL of methanol was added. The mixture was vigorously stirred by vortexing for 10 s and centrifuged at 6170g for 5 min (Biofuge Stratos, Thermo Fisher Scientific, Waltham, MA, USA). The methanolic phase was separated from the lipid phase using Pasteur pipette and the residue was extracted twice with a new portion of methanol (2 × 5 mL). The methanol phases were combined and concentrated to a volume of 5 mL under reduced pressure using a rotary evaporator, Rotavapor R-215 (Büchi Labortechnik, Flawil, Switzerland) at 40 °C.

2.6. Total phenolic content determination

Total phenolic content in the methanolic extracts of seed oils was determined using the Folin-Ciocalteu's reagent (Singleton & Rossi, 1965) with some modifications. The methanolic extract

(0.5 mL) was diluted with water (4 mL) and then the Folin-Ciocalteu's reagent (0.5 mL) was added. After 3 min, 1 mL of a sodium carbonate solution (1.9 M) was added and filled up to 10 mL with water. The samples were left to stand in darkness for 60 min and then the absorbance was measured at 760 nm using a UV/Vis spectrophotometer (Model 8500, Techcomp, HongKong). Total phenolics content in each sample was determined using a standard curve prepared for gallic acid. The results were expressed as milligrams of gallic acid per gram of oil.

2.7. Measurement of antioxidant activity – DPPH assay

The antioxidant activity of the methanolic extracts of seed oils and seed oil samples was determined using DPPH radicals as described by Kiralan, Bayrak, and Özkaya (2009), with some modifications. 0.5 mL of each methanolic extract of seed oils was diluted with 3.25 mL of methanol and then 0.25 mL of 1 mM methanolic solution of DPPH was added. The mixture was vigorously mixed for 10 s in a vortex apparatus and left in darkness for 10 min. The absorbance was measured at 515 nm against pure methanol using a UV/Vis spectrophotometer (Model 8500, Techcomp, HongKong). The radical scavenging activity was expressed as Trolox equivalent antioxidant capacity (TEAC) using a Trolox calibration curve ($\mu\text{mol TEAC/g}$ of oil).

In order to determine antiradical activity in seed oil samples, 50 mg of each oil was dissolved in 3.0 mL of ethyl acetate. Then, 1 mL of an oil solution was diluted with ethyl acetate (2.75 mL) and 0.25 mL of a freshly prepared DPPH solution (1 mM) was added. The samples were vigorously mixed for 10 s in a vortex and after 20 min the absorbance was measured at 515 nm using a UV/Vis spectrophotometer (Model 8500, Techcomp, HongKong). The results were expressed as Trolox equivalent antioxidant capacity using a Trolox calibration curve ($\mu\text{mol TEAC/g}$ of oil).

2.8. Statistical analysis

The analysis of the studied samples was performed in triplicate. The results were presented as the mean \pm standard deviation (SD). The statistical analysis was carried out with SPSS Statistical Software version 16. One-way analysis of variance (ANOVA) was used to compare sterols composition between the studied seed oils. For total phenolic compounds and antioxidant activity determination, a two-way analysis of variance was applied. Difference between samples were examined using a Tukey's test and were considered significant at $P < 0.05$. The Pearson's test was used to find the correlation between total polyphenol content, antioxidant capacity and total sterols content in the studied oils.

3. Results and discussion

3.1. Oil extraction yield

The oils used in this study were extracted from selected plant seeds with *n*-hexane in Soxhlet apparatus and with the mixture of chloroform/methanol using the Folch method. Comparing these two lipid extraction processes, there was no significant difference regarding oil extraction yield, with the exception of oil extracted from white mustard (Table 1). The percentage value of oil extraction yield was higher for white mustard extracted with the Folch method compared to that obtained by Soxhlet extraction. Oils extracted using polar solvents such as a combination of chloroform and methanol may cause extraction of polar materials (phospholipids) besides of neutral triacylglycerols and thus can affect a higher oil yield. In many cases, the effect of extraction time and temperature can also be significant for oil yield. White mustard

Table 1
The oil extraction yield and the fatty acids composition of the plant seed oils extracted from seeds using *n*-hexane and chloroform/methanol mixture of solvents.

Fatty acids (%)	Sample of seed oils					After chloroform/methanol extraction				
	After <i>n</i> -hexane extraction					After chloroform/methanol extraction				
	Nutmeg	White mustard	Anise	Coriander	Caraway	Nutmeg	White mustard	Anise	Coriander	Caraway
C16:0	17.53 \pm 0.01 ^d	3.07 \pm 0.01 ^h	25.11 \pm 0.04 ^a	3.49 \pm 0.03 ^g	4.17 \pm 0.03 ^f	18.29 \pm 0.04 ^c	3.07 \pm 0.02 ^h	22.23 \pm 0.04 ^b	3.01 \pm 0.02 ⁱ	4.46 \pm 0.03 ^e
C16:1	5.01 \pm 0.02 ^b	0.14 \pm 0.00 ^f	–	0.28 \pm 0.02 ^d	0.05 \pm 0.01 ^g	6.58 \pm 0.03 ^a	0.19 \pm 0.00 ^e	–	0.33 \pm 0.00 ^c	–
C18:0	1.93 \pm 0.00 ^d	1.14 \pm 0.01 ^g	5.83 \pm 0.03 ^a	0.84 \pm 0.02 ⁱ	1.53 \pm 0.02 ^f	2.44 \pm 0.03 ^c	1.05 \pm 0.01 ^h	5.06 \pm 0.03 ^b	0.70 \pm 0.01 ^j	1.72 \pm 0.02 ^e
C18:1 <i>n</i> -12	–	–	–	73.41 \pm 0.05 ^a	33.37 \pm 0.04 ^d	–	–	–	73.84 \pm 0.05 ^b	33.57 \pm 0.04 ^c
C18:1 <i>n</i> -9	59.44 \pm 0.03 ^a	23.53 \pm 0.03 ^f	36.63 \pm 0.05 ^c	6.03 \pm 0.03 ^h	29.12 \pm 0.03 ^d	56.48 \pm 0.05 ^b	22.94 \pm 0.03 ^g	36.69 \pm 0.04 ^c	5.97 \pm 0.02 ⁱ	28.97 \pm 0.04 ^e
C18:2 <i>n</i> -6	13.83 \pm 0.02 ^g	8.83 \pm 0.02 ^j	31.34 \pm 0.04 ^b	15.35 \pm 0.03 ^f	31.00 \pm 0.04 ^c	13.56 \pm 0.04 ^h	9.11 \pm 0.02 ⁱ	34.51 \pm 0.04 ^a	15.65 \pm 0.02 ^e	29.64 \pm 0.03 ^d
C18:3 <i>n</i> -3	1.94 \pm 0.01 ^b	8.14 \pm 0.02 ^a	0.71 \pm 0.01 ^e	0.22 \pm 0.01 ^h	0.37 \pm 0.02 ^g	1.79 \pm 0.03 ^c	8.14 \pm 0.02 ^d	1.22 \pm 0.01 ^d	0.18 \pm 0.00 ^j	0.62 \pm 0.02 ^f
C20:0	0.14 \pm 0.01 ^g	0.81 \pm 0.01 ^a	0.39 \pm 0.01 ^d	0.09 \pm 0.00 ^h	0.13 \pm 0.01 ^g	0.33 \pm 0.01 ^e	0.73 \pm 0.01 ^b	0.29 \pm 0.00 ^f	0.10 \pm 0.00 ^k	0.60 \pm 0.02 ^c
C22:1 <i>n</i> -9	0.18 \pm 0.00 ^g	10.88 \pm 0.03 ^a	–	0.22 \pm 0.00 ⁱ	0.24 \pm 0.00 ^e	0.53 \pm 0.01 ^c	9.70 \pm 0.02 ^b	–	0.22 \pm 0.01 ^f	0.42 \pm 0.01 ^d
C22:1 <i>n</i> -9	–	43.46 \pm 0.04 ^b	–	–	–	–	45.07 \pm 0.04 ^a	–	–	–
SFA	19.60 \pm 0.02 ^d	5.02 \pm 0.03 ^g	31.33 \pm 0.05 ^a	4.42 \pm 0.04 ^j	5.83 \pm 0.05 ^f	20.86 \pm 0.05 ^c	4.85 \pm 0.04 ^h	27.58 \pm 0.05 ^b	3.81 \pm 0.03 ⁱ	6.78 \pm 0.03 ^e
MUFA	64.63 \pm 0.04 ^d	78.01 \pm 0.06 ^c	35.63 \pm 0.05 ^b	79.94 \pm 0.07 ^b	62.78 \pm 0.07 ^g	63.59 \pm 0.07 ^e	77.90 \pm 0.06 ^c	36.69 \pm 0.05 ^b	80.36 \pm 0.08 ^a	62.96 \pm 0.06 ^f
PUFA	15.77 \pm 0.04 ^g	16.97 \pm 0.04 ^f	32.05 \pm 0.05 ^b	15.57 \pm 0.04 ^h	31.37 \pm 0.05 ^c	15.35 \pm 0.05 ^f	17.25 \pm 0.03 ^e	35.73 \pm 0.05 ^a	15.83 \pm 0.04 ^g	30.26 \pm 0.04 ^d
PUFA/SFA ratio	0.80 \pm 0.01 ^h	3.38 \pm 0.03 ^e	1.02 \pm 0.03 ^g	3.52 \pm 0.03 ^d	5.38 \pm 0.04 ^h	0.74 \pm 0.03 ⁱ	3.56 \pm 0.05 ^d	1.30 \pm 0.03 ^j	4.15 \pm 0.05 ^c	4.46 \pm 0.04 ^b
Oil extraction yield (%)	7.43 \pm 2.25 ^d	25.30 \pm 1.24 ^b	7.40 \pm 3.03 ^d	20.00 \pm 3.25 ^c	20.13 \pm 2.27 ^c	8.50 \pm 2.13 ^d	29.80 \pm 2.95 ^a	5.40 \pm 2.28 ^d	22.10 \pm 4.25 ^c	18.90 \pm 2.95 ^c

Data represents means \pm SD (standard deviation) ($n = 3$). –; not identified. SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids. Values with different superscript letters (a–j) within each row are significantly different at $P < 0.05$.

seeds yielded the highest amount of oil; at 29.80% with chloroform/methanol and 25.30% with *n*-hexane, whereas aniseed had the lowest oil yield, at 5.40% with chloroform/methanol and 7.40% with *n*-hexane. The results obtained for mustard seeds oil after *n*-hexane extraction were lower than these presented in works by Sengupta and Bhattacharyya (1996) or Lohani, Fallahi, and Muthukumarappan (2015). They reported the following values of mustard oil extraction yield: 35.10 and 28.80%. These differences may be mainly attributed to the geographic origin and the genera of mustard seeds. In our study, a low yield of oil extraction was also observed for nutmeg seeds using both methods – Soxhlet and Folch. From coriander seeds, oils were extracted with the yield of 22.10% with the Folch method and 20.00% with the Soxhlet method. From caraway seeds, oils were extracted with yield of 18.90% using chloroform/methanol and 20.13% using *n*-hexane. These results were similar to those reported by Sriti et al. (2010) who found that oil from coriander seeds extracted with *n*-hexane constituted 22.6% on dry matter basis. On the other hand, our values were lower than those presented by Ramadan and Mörsel (2002), who determined the amount of total lipid in coriander seeds extracted with chloroform/methanol as 28.4% of seed weight. These differences may be primarily related to the degree of ripeness of coriander fruit-seeds used in the study.

3.2. Fatty acid composition

The fatty acid composition of the seed oils studied is summarized in Table 1. The anise oils were characterized by the highest contribution of PUFAs and SFAs, whereas the MUFAs content was the lowest among all oil samples studied. The contents of PUFAs and SFAs were 32.05 and 31.33%, respectively, in anise oil extracted from seeds with *n*-hexane, while the oil extracted using chloroform/methanol contained 35.73% of PUFAs and 27.58% of SFAs. In turn, contents of MUFAs found in anise oils accounted for 35.63 and 36.69% in the oil extracted with the Soxhlet and Folch methods, respectively. Caraway seed oils were also rich in PUFAs. Moreover, the amount of saturated fatty acids in these oils was considerably low, 5.83% using the Soxhlet method and 6.78% using the Folch method. The contribution of PUFAs in the other oils extracted from seeds with *n*-hexane was 15.77% in nutmeg oil, 16.97% in white mustard oil and 15.57% in coriander oil. Similar values were also determined in these oils after chloroform/methanol extraction. Furthermore, white mustard and coriander seed oils were characterized by a relatively low content of SFAs (5.02 and 4.42% using Soxhlet method, respectively) and simultaneously a high contribution of MUFAs (78.01 and 79.94%, respectively). Among MUFAs, petroselinic acid (C18:1, *n*-12) was the most prevalent in coriander oils and was determined at a high level of 73% regardless of extraction method. The level of petroselinic acid obtained in our investigations is quite similar to that reported in the literature (Ramadan & Mörsel, 2002). This acid was also determined in caraway oil with the content exceeding 33%. Caraway oil also contained oleic (29.12% – *n*-hexane extraction) and linoleic (31% – *n*-hexane extraction) acids. The contribution of oleic acid was 5 times higher and that of linoleic acid was twice higher compared to coriander oil. Similar results were reported by Laribi, Kouki, Mougou, and Marzouk (2010), who demonstrated that in caraway oil extracted from three varieties of caraway seed ecotypes, the content of petroselinic acid ranged from 31.53 to 38.36%, whereas linoleic acid exceeded 30% and linolenic acid contributed less than 0.5%.

As expected, white mustard oil was the only one containing erucic acid (C22:1, *n*-9) which accounted for 43.46% (Soxhlet method) and 45.07% (Folch method). Other fatty acids found in white mustard oil in considerable amounts included oleic (C18:1, *n*-9), gondoic (C20:1, *n*-9), linoleic (C18:2, *n*-6) and linolenic

(C18:3, *n*-3) acids. The contribution of gondoic acid was the highest among all oils analyzed, and the values were 10.88% (*n*-hexane seeds extraction) and 9.70% (chloroform/methanol seeds extraction). Among all oils tested, white mustard oil was also characterized by the highest level of linolenic acid (8.14%). An and Choe (2012) also found erucic, oleic, gondoic, linoleic and linolenic acids as the most represented fatty acids of mustard oil, however they reported that erucic acid percentage was 13.96%, whereas our results showed its contribution to be three times higher. The high content of erucic acid in mustard oil was also proven by Sengupta and Ghosh (2011) or Parti, Deep, and Gupta (2003), with respective values at 48.55 and 49.43%. Our results are also partially in agreement with those of Zheljazkov, Vick, Ebelhar, Buehring, and Astatkie (2012) and Vaidya and Choe (2011) who noticed that erucic, oleic, gondoic, linoleic and linolenic acids accounted for the main components of mustard oil, but they reported 2.4 times higher linoleic acid content and twice lower erucic acid contribution compared to our results.

In nutmeg oil, oleic acid was determined as predominant regardless of extraction method and was followed by palmitic and linoleic acids. These acids were also found as major in oils from aniseeds extracted with the Soxhlet and Folch methods. The fatty acid profile for African nutmeg oil (*Monodora miristica*) in which linoleic and oleic acids were also present, was different from the fatty acid profile of nutmeg oil analyzed in our study. The amount of linoleic acid was three times higher and oleic acid was 1.5 times lower compared to our study (Yeboah, Mitei, Ngila, Wessjohann, & Schmidt, 2011).

3.3. Sterols content

The tested oil samples were characterized by the presence of the following sterols: cholesterol, brassicasterol, campesterol, Δ^5 -stigmasterol, clerosterol, β -sitosterol, Δ^5 -avenasterol, cycloartenol, Δ^7 -avenasterol, 24-methylenecycloartenol as well as two stanols: campestanol and sitostanol. The data is listed in Table 2. Campestanol was identified only in white mustard oils at a level of 324 mg/kg of oil, whilst clerosterol was present only in caraway oils. The oil extracted from caraway seeds using the chloroform/methanol mixture contained 2.6 times higher amount of clerosterol than the one extracted with *n*-hexane. However, campesterol was present in the highest amount in white mustard oil, regardless of extraction method. On the other hand, white mustard oil was the only one that did not contain Δ^5 -stigmasterol. The highest content of this compound (2799.9 mg/kg of oil) was determined in caraway oil extracted with *n*-hexane. Compared to our study, Hassanien et al. (2014) reported 10 times lower content of Δ^5 -stigmasterol (243.00 mg/kg of oil) in black cumin (*Nigella sativa*) seeds oil. Brassicasterol occurred in white mustard oils and the amounts were 514.56 and 489.68 mg/kg of oil after seeds extraction with *n*-hexane and chloroform/methanol, respectively. This phytosterol was also found in nutmeg oil, however its content reached only 48.97 and 51.20 mg/kg of oil extracted using *n*-hexane and chloroform/methanol, respectively. The content of β -sitosterol, the most prevalent sterol, ranged from 997.97 in coriander oil extracted with *n*-hexane to 6264.84 mg/kg of oil in anise oil extracted with chloroform/methanol, which accounted for 28.7 and 73.7% of total sterols, respectively. The comparable amount of β -sitosterol in Tunisian coriander seeds was reported by Sriti et al. (2010). They also found significant content of stigmasterol in Tunisian coriander seeds, which is consistent with results of our research. However, Ramadan and Mörsel (2002) reported slightly higher results concerning coriander seeds oil. Contents of β -sitosterol and stigmasterol in their samples were 1464 and 1548 μ g/g of oil, respectively. In our study, the highest content of Δ^5 -avenasterol was found in caraway oil regardless of extraction method.

Table 2
Sterol composition of the plant seed oils extracted from seeds using *n*-hexane and chloroform/methanol mixture solvents.

Sterols (mg/kg oil)	Sample of seed oils					After chloroform/methanol extraction				
	After <i>n</i> -hexane extraction					After chloroform/methanol extraction				
	Nutmeg	White mustard	Anise	Coriander	Caraway	Nutmeg	White mustard	Anise	Coriander	Caraway
Cholesterol	287.46 ± 1.39 ^b	240.40 ± 7.55 ^c	-	-	-	302.76 ± 10.21 ^a	231.13 ± 6.73 ^c	-	-	-
Brassicasterol	48.97 ± 0.54 ^d	514.56 ± 5.81 ^a	-	-	-	51.20 ± 0.99 ^c	489.68 ± 1.61 ^b	-	-	-
Campesterol	361.19 ± 2.38 ^f	2289.57 ± 10.05 ^a	775.74 ± 15.07 ^d	385.08 ± 12.48 ^e	299.87 ± 1.10 ^h	380.09 ± 1.44 ^e	2208.74 ± 4.90 ^b	911.60 ± 4.05 ^c	366.31 ± 2.48 ^f	317.10 ± 0.46 ^g
Campestanol	-	324.06 ± 5.13 ^a	-	-	-	-	324.79 ± 0.24 ^a	-	-	-
Δ ⁵ -Stigmasterol	279.37 ± 1.68 ^h	-	354.92 ± 8.62 ^f	1095.60 ± 1.12 ^d	2799.91 ± 5.98 ^a	301.94 ± 1.27 ^g	-	679.29 ± 22.27 ^e	1180.32 ± 19.47 ^c	2454.26 ± 20.32 ^b
Clerosterol	-	-	-	-	129.22 ± 2.23 ^b	-	-	-	-	338.61 ± 5.80 ^a
β-Sitosterol	1203.19 ± 1.67 ^h	4455.18 ± 14.01 ^b	4034.53 ± 3.21 ^d	997.97 ± 8.07 ⁱ	2231.28 ± 7.11 ^f	1314.33 ± 8.42 ^g	4298.99 ± 28.68 ^c	6264.84 ± 26.45 ^a	1009.08 ± 6.44 ⁱ	2449.38 ± 27.90 ^e
Sitosterol	53.49 ± 2.04 ^e	76.98 ± 2.45 ^c	-	268.77 ± 8.09 ^b	400.82 ± 8.05 ^a	50.62 ± 0.85 ^d	76.63 ± 0.24 ^e	-	279.72 ± 3.29 ^b	393.58 ± 2.02 ^a
Δ ⁵ -Avenasterol	233.64 ± 3.66 ^g	736.53 ± 10.36 ^b	-	516.79 ± 4.72 ^e	859.00 ± 12.73 ^a	253.78 ± 0.91 ^f	719.12 ± 3.08 ^c	-	544.66 ± 5.43 ^d	871.81 ± 10.79 ^a
Cycloartenol	169.11 ± 0.57 ^d	-	369.12 ± 9.19 ^b	68.18 ± 4.36 ^g	123.04 ± 5.53 ^e	186.91 ± 2.68 ^c	-	643.08 ± 1.07 ^a	51.29 ± 5.46 ^h	102.92 ± 0.71 ^f
Δ ⁷ -Avenasterol	83.29 ± 1.40 ^e	-	-	142.47 ± 1.07 ^d	260.11 ± 7.86 ^a	86.44 ± 1.97 ^e	-	-	152.27 ± 3.96 ^c	237.02 ± 2.21 ^b
24-Methylene cycloartenol	-	-	153.72 ± 0.81 ^c	-	170.37 ± 4.05 ^b	176.01 ± 6.39 ^b	-	-	-	229.49 ± 3.05 ^a
Total sterols	2836.39 ± 15.04 ⁱ	8597.90 ± 95.86 ^a	5688.03 ± 16.90 ^f	3474.83 ± 5.53 ^h	7273.59 ± 52.43 ^e	3104.84 ± 19.58 ⁱ	8349.07 ± 25.82 ^c	8498.80 ± 0.94 ^b	3583.59 ± 41.57 ^g	7394.15 ± 16.63 ^d
Squalene content (mg/kg oil)	538.29 ± 10.54 ^d	-	-	-	1072.41 ± 14.89 ^a	567.68 ± 11.17 ^c	-	-	-	777.48 ± 19.68 ^h

Values are means ± SD (standard deviation) (*n* = 3). -: not identified. Values with different superscript letters (^{a-i}) within each row are significantly different at *P* < 0.05.

However, Δ⁷-avenasterol was detected in nutmeg, coriander and caraway oils. The other sterols found in the studied oils included cycloartenol and 24-methylenecycloartenol. The highest content of cycloartenol was determined in anise oil at 643.08 mg/kg of oil, whereas the lowest one in coriander oil at 51.29 mg/kg of oil. This sterol was not detected in white mustard oil. In turn, 24-methylenecycloartenol was identified in caraway and nutmeg oils extracted with both methods – Soxhlet and Folch. It was also detected in anise oil samples but only in these extracted with *n*-hexane.

The contribution of total sterols reached the highest level in white mustard oil (8597.90 mg/kg of oil) extracted with the Soxhlet method and in anise oil (8498.80 mg/kg of oil) as assessed after using chloroform/methanol mixture. Interestingly, in anise oil extracted with the Folch method (8498.80 mg/kg of oil), the total sterols content was 1.5 times higher as compared with the Soxhlet method (5688.03 mg/kg of oil). In the other oils, significant differences were also noticed between the total sterols content in oils extracted from seeds with *n*-hexane and chloroform/methanol.

Cholesterol, untypical sterol of plant lipids, was only detected in nutmeg and white mustard oils in the amounts exceeding 230 mg/kg of oil. Hassanien et al. (2014) identified cholesterol in tomato seed oil, whereas Ciftci, Przybylski, Rudzinska, and Acharya (2011) detected confirmed its presence in fenugreek seeds which are used as a spice to enhance the sensory quality of food. Vegetable oils from the Brassicaceae, such as mustard and rapeseed, may also contain modest amounts of cholesterol. Among vegetable oils, camelina oil is characterized by an unusually high content of cholesterol (Shukla, Dutta, & Artz, 2002). On the other hand, cholesterol was not detected in coriander seeds (Sriti et al., 2012), which is in agreement with the results of our study. Nutmeg and caraway oils were considerable sources of squalene and significant differences were noticed between oils obtained with Soxhlet and Folch methods. This compound was not detected in flaxseed, grape seed and soybean oils, but was quite prominent in peanut, pumpkin and olive oils (Amarowicz, 2009).

3.4. Total phenolic content

The total phenolic contents in plant seeds oils were evaluated as phenolic compounds are important indicators of oil quality. They protect lipids from peroxidation and are responsible for their free radical scavenging capacity. As reported in Table 3, the content of phenolics in plant oils varied depending on the method of oil extraction. Oils extracted using the Folch method showed significantly a higher total phenolic content (*P* < 0.05) than those extracted with *n*-hexane. This is attributed to the presence of a polar solvent (methanol) which extracts the polar phenolics from the seeds (Khatab, Rempel, Suh, & Thiyam, 2012). The content of total phenolic compounds is known to depend on the type and polarity of the extraction solvent and other factors, such as plant cultivar, degree of seeds maturation, climate and location. In the present study, the highest contents of these components were detected in nutmeg and aniseed oils with 3.21 and 2.52 mg gallic acid (GA) per g of oil, respectively; the lowest amounts were recorded in coriander and caraway oils (0.20 and 0.78 mg GA per g of oil). These levels were greater than those detected in oils extracted with *n*-hexane. In this case, the highest total phenolics content was determined in nutmeg oil followed by aniseed, coriander, caraway and white mustard oil. Janu et al. (2014) reported that the content of phenolic compounds in unrefined mustard oil was 0.56 mg GAE/100 g oil. In turn, Ramadan and Mörsel (2004) analyzed various crude seed oils and determined the total phenolics content in coriander oil at 11 mg caffeic acid equivalents per kg oil equivalents. In another study, Ramadan (2013) reported the total phenolics content in coriander cold-pressed oil as

Table 3

Total phenolics and antioxidant activity determined by the DPPH method in seed oil samples and their methanolic extracts.

	Sample of seed oils after extraction	Nutmeg	White mustard	Anise	Coriander	Caraway
Total phenolics [*]	<i>n</i> -Hexane	1.19 ± 0.12 ^{bA}	0.04 ± 0.01 ^{bE}	0.42 ± 0.04 ^{bB}	0.17 ± 0.15 ^{bC}	0.07 ± 0.01 ^{bD}
	Chloroform/methanol	3.21 ± 0.27 ^{aA}	1.50 ± 0.15 ^{aC}	2.52 ± 0.20 ^{aB}	0.20 ± 0.02 ^{aE}	0.78 ± 0.09 ^{aD}
DPPH ^{**} methanolic extract	<i>n</i> -Hexane	8.04 ± 0.44 ^{bA}	1.34 ± 0.11 ^{bC}	1.71 ± 0.15 ^{bB}	1.26 ± 0.11 ^{bD}	1.78 ± 0.16 ^{bB}
	Chloroform/methanol	13.98 ± 0.69 ^{aA}	1.94 ± 0.17 ^{aE}	8.33 ± 0.45 ^{aB}	3.45 ± 0.31 ^{aC}	2.91 ± 0.22 ^{aD}
DPPH ^{**} seed oil sample	<i>n</i> -Hexane	16.46 ± 0.77 ^{bA}	4.21 ± 0.18 ^{bC}	3.44 ± 0.29 ^{bD}	2.24 ± 0.21 ^{bE}	6.32 ± 0.25 ^{bB}
	Chloroform/methanol	31.69 ± 1.27 ^{aA}	7.39 ± 0.29 ^{aD}	12.52 ± 0.66 ^{aB}	4.96 ± 0.22 ^{aE}	8.81 ± 0.41 ^{aC}

Values are means ± SD (standard deviation) ($n = 3$). Values with different uppercase letters (^{A–E}) within each row are significantly different at $P < 0.05$. Values with different lowercase letters (^{a–b}) within each column are significantly different at $P < 0.05$.

^{*} Results are expressed as milligram gallic acid per gram of oil (mg GA/g oil).

^{**} Results are expressed as μmol Trolox equivalent antioxidant capacity per gram of oil (μmol TEAC/g oil).

Table 4

Correlation (Pearson) coefficients between total phenolics, total sterols and antioxidant activity determined by DPPH method in seed oil samples and their methanolic extracts.

	Total phenolics	DPPH methanolic extracts	DPPH seed oil samples	Total sterols
Total phenolics	1.000	0.880 [*]	0.860 [*]	−0.070
DPPH methanolic extracts		1.000	0.960 [*]	−0.400
DPPH seed oil samples			1.000	−0.370
Total sterols				1.000

^{*} Significant at the ($P < 0.05$).

4.3 mg gallic acid equivalents per gram of oil. Yu, Zhou, and Parry (2005) found that total phenolic content of a cold-pressed black caraway seed oil was 3.53 mg GAE/g oil. However, phenolics content in the six cold-pressed black cumin seed oils varied from 1.02 to 1.40 mg GAE/g oil (Lutterodt et al., 2010) and could partially contribute to oil stability under the accelerated oxidative conditions. To the best of our knowledge, our work is the first that reports on the comparison of total phenolic compounds of plant seed oils extracted using two alternative methods – Folch and Soxhlet.

3.5. Antioxidant activity – DPPH assay

The antioxidant activity of the studied plant seed oils, as well as their methanolic extracts, was evaluated using the DPPH assay and the results obtained were expressed as Trolox equivalent antioxidant capacity. All the seed oil samples exhibited DPPH radical scavenging capacity which varied from 2.24 up to 31.69 μmol TE/g of oil, as shown in Table 3. Oils extracted with *n*-hexane showed lower DPPH values than these extracted using chloroform/methanol. Regardless the extraction method, nutmeg oil was the most effective as a DPPH radical scavenger. When it was extracted using the Folch method, its significantly higher effect on the DPPH radical ($P < 0.5$) was followed by aniseed, caraway, white mustard and coriander. Whereas, the order of effectiveness of oils extracted from seeds with *n*-hexane in scavenging of DPPH radicals was as follows: nutmeg > caraway > white mustard > aniseed > coriander. A relatively similar sequence of the antioxidant activity compared to the seed oil samples was observed for the methanolic extracts of oils obtained with the Folch method. In the case of methanolic extracts derived from oils extracted using *n*-hexane, nutmeg oil had the highest ability to scavenge DPPH radicals. DPPH values of the methanolic extracts from other oils were lower but close to one another. Nutmeg's methanolic extract had also the strongest scavenging ability against free radicals based on DPPH and FRAP tests in a study by Assa, Widjanarko, Kusnadi, and Berhimpon (2014). This high antioxidant capacity was due to the relatively high contents of tannins, flavonoids and terpenoids. The best antioxidant activity was also found for methanolic extracts obtained from hemp and pumpkin oils (Siger, Nogala-Kalucka, & Lampart-Szczapa, 2008). These oils contained the highest amount

of total phenolic compounds. However, it is difficult to compare the DPPH values estimated for methanolic extracts with those of seed oils samples because the measurements in oils were carried out using another solvent (ethyl acetate). Solvents may influence the antioxidant activity of samples because they may affect the hydrogen-donating ability of antioxidants. Moreover, according to the “polar paradox” theory, polar antioxidants are more effective in the lipophilic media, while nonpolar antioxidants are more active in the polar media (Ramadan & Moersel, 2006).

The Pearson correlation test was used to determine the correlation between total polyphenols, antioxidant capacity and sterols (Table 4). Especially, the correlation between total phenolics and antioxidant activity was widely studied in different types of food-stuffs such as fruit and vegetables (Kedage, Tilak, Dixit, Devasagavam, & Mhatre, 2007) or seeds (Del Carlo et al., 2004). In our study, a good correlation was observed between total polyphenols and radical scavenging activity of both methanolic extracts ($r = 0.88$, $P < 0.05$) and seed oil samples ($r = 0.86$, $P < 0.05$). It is known that the presence of phenolics and polar lipids in oils as the bioactive components and their possible interaction with other constituents of oils may contribute to their stronger activity in scavenging free radicals. The radical scavenging activity of methanolic extracts was also positively correlated with the radical scavenging activity in seed oil samples ($r = 0.96$, $P < 0.05$). Our results are in agreement with those reported by Kiralan et al. (2009). However, a negative correlation was found between sterols content of the oils and antioxidant capacity of the methanolic extracts and seed oil samples. This trend was also observed by Maruyama et al. (2014).

4. Conclusions

In the present study, oils from coriander, caraway, nutmeg, anise and white mustard seeds were extracted using *n*-hexane (Soxhlet method) and chloroform/methanol (Folch method) solvents. Comparing these two methods, there was no significant difference in oil extraction yield, with the exception of oil extracted from white mustard. The fatty acid composition indicated that palmitic, oleic and linoleic acids were the predominant fatty acids regardless of the extraction method. Additionally, coriander and caraway oils were found rich source of petroselinic acid and white

mustard contained linolenic acid. The studied plant oils may be considered good sources of phytosterols with β -sitosterol being the major component in all the oils extracted with both methods. Considering the total phenols content in plant oil samples, it was found that oils extracted using chloroform/methanol were richer in phenolic compounds than their *n*-hexane counterparts. Similar observation was also made regarding the antioxidant activity determined with the DPPH assay of seed oil samples and their methanolic extracts. The correlation analysis revealed a positive correlation between total phenolics and antioxidant activity measured with the DPPH test in methanolic extracts and seed oil samples and a negative correlation with total sterols. The oils studied may be considered as important components in the synthesis of structured lipids.

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