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Extraction and Characterization of Vegetable Oils from Cherry Seed by Different Extraction Processes

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The aim of this work was to compare the content and composition of oil from cherry seeds using supercritical fluid extraction (SFE) using carbon dioxide as solvent, and Soxhlet extraction using diethyl ether. The compositions of free fatty acids and phytosterols, achieved with two extraction methods, were analyzed. Gas chromatographic analysis of unsaponifiables cherry seed oil samples, obtained with SFE and Soxhlet extraction, permitted to identify in particular two sterols, the β-sitosterol and campesterol. Brassicasterol or stigmasterol were not present in the extracts. The percentages of monitored components changed in function of extraction used procedure, allowing to assert that, with SFE, higher yield of phytosterols was provided. Preliminary data showed that there were significant differences also in the fatty acid composition of cherry seed oil obtained with the two extraction procedures (by means supercritical carbon dioxide or solvent). With the Soxhlet extraction, 48.6% of saturated fatty acids, 17.74% of unsaturated acids, 32.75% of polyunsaturated are obtained. On the contrary, with SFE decreased the percentage of saturated fatty acids (38.43%) in favor of larger amounts of unsaturated (19.93%) and polyunsaturated (36.04%). The supercritical fluid extraction procedure proved so effective in getting an oil of superior quality, without the possibility of degradation phenomena due to the solvent or heat. In both extraction procedures the cherry seed oil contained high percentages of arachidic acid (C20:0) and linoleic acid (C18:2, ω-6). An interesting result was also the presence of nervonic acid in remarkable amount, in all examined samples. Nervonic acid is a long-chain monounsaturated fatty acid (C24:1, ω-9), of marine origin, precursor of the neuronal cell membrane glycolipids, with a key role in the modulation of ion channels and membrane receptors, and therefore widely used as a neurotrophic factor in food supplements and nutraceuticals.

1. Introduction

Nuts oils, seed oil and oils of fruit and vegetables are receiving growing interest due to their high concentration of bioactive lipid components, such as polyunsaturated fatty acids and phytosterols, wich have shown various health benefits. Fats and oils, and their severals lipid components are extensively used in the food and also in cosmetics, pharmaceuticals, oleochemicals and other industries. Cherry seeds are waste product, currently used for the production of biomasses (Duman et al., 2011), but in future may constitute an important resource in the food and cosmetic fields. Vegetable lipids are usually obtained by solvent extraction and/or by pressing seeds and fruits of many plants, but supercritical fluid extraction (SFE) is currently becoming alternative method to conventional extraction

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with solvent (Sahena et al., 2009). In recent years, claim for natural and organic products has been growing, and new clean technologies for producing natural ingredients have been developed. In fact, present attention is paid to the real quality of products and their potency, reliability and naturalness. Owing to the increasing demand in bioactive compounds from plant origin, new extraction methods was used to obtain products with high quality and safety features. The conventional extraction methods are time consuming, laborious, have low selectivity and/or low extraction yields, and possible solvent contamination of final products. Supercritical fluid extraction allows to obtain extracts free of toxic residues that can be directly used, without any further treatment, and with excellent features and ultra-pure composition of final product.

2. Materials and methods

2.1 Samples

Sweet cherries (*Prunus avium*) were purchased from a local market in June 2011. The fruits are members of the cultivar "Black", a native production of Alto Casertano within the region of Campania (Italy). The cherries were selected by eliminating unripe and too ripe fruits, with lesions and without stalk. Prior to processing, the seeds of the fruit were manually separated from the pulp and were ground in a domestic grinder and sieved into several grades of particle size. The flour of seeds was dried in an oven at 60 °C for 24 h, then was packed in plastic tube, frozen, and kept at -20 °C until analyses.

2.2 Soxhlet extraction

The lipids of the seeds of cherries were extracted with the Soxhlet method according to the *Methods of analysis used for chemical control of food* of the National Institute of Health, 1996 (Reports ISTISAN 96/34, 1996). A sample of 20 g of seeds flour was extracted using diethyl ether as solvent, for 6 h. The solvent was removed with a rotary evaporator (Mod. Hei VAP Value, Heidolph). The residue was placed in a drier and weighed up to constant value.

2.3 Supercritical CO_2 extraction (SFE-CO_2)

The SFE experiments were performed with a Sped-SFE extractor (model 7010, Applied Separation, 930 Hamilton Street, Allentown, PA), equipment described by Ambrosino et al. (2004), and carbon dioxide was used as solvent (purity > 99.9955 %). With the Speed-SFE extractor was possible to work in static phase, with pressurized CO_2 in the extraction vessel for a specific period of time, and in dynamic phase where the flux of CO_2 was in continuous. A sample of about 20 g of flour of the seeds of cherries has been loaded into the extraction vessel (volume 50 mL) and placed in the oven of Speed-SFE extractor. All parameters of the extraction with supercritical CO_2 as temperature, pressure, flow of CO_2, and extraction time were investigated and optimized for the seeds of cherries (Bernardo-Gil et al., 2001; Temelli, 2009). The start of extraction was set to achieve temperature and pressure of work and after a period of stabilization parameters. In the static phase of supercritical extraction was observed the following conditions: pressure 45.7 MPa, vessel temperature 46 °C, valve temperature 65 °C, oven temperature 49 °C, extraction time 2 h. After the static extraction phase the inlet valve of CO_2 was closed and the valve of collection extract was opened, in the dynamic phase (extraction time 1 h) the continuous flow of CO_2 outbound (0.8 $mL \cdot min^{-1}$) has been gurgled in ethanol for trapping the extracted oil. Later was set a second extraction cycle with reduction extraction times. The second fraction of oil extracted from cherry seeds has been combined to the first and was taken to dryness under nitrogen. The oil was kept at 4 °C in a dark bottle.

2.4 Phytosterols preparation with saponification

The cherry seed oil samples obtained from the Soxhlet extraction and supercritical extraction were subjected to saponification. The process was perfomed according to Delgrado-Zamarreño et al. (2009). Approximately 1 g of the oil sample was mixed with 35 mL of 96 % ethanol, 5mL of aqueous 10 % ascorbic acid, 10 mL of aqueous 80 % KOH and 25 mL of distilled water. The mixture was stirred for 12 h at room temperature into a dark bottle. The unsaponificable fraction was extracted with *n*-hexane 95 % (2 X 25 mL). The hexane phase was washed with water (2 X 25 mL), then collected and dried

through anhydrous sodium sulfate. The solvent was evaporated in a rotary evaporator and the residue was dissolved in *n*-hexane, filtered and injected into gas chromatographic system.

2.5 Fatty acid methyl esters (FAMEs) preparation

The fatty acid profile was determined as fatty acid methyl esters (FAMEs) by gas chromatography. The methyl esters were prepared by according to method AOAC 996.06 (AOAC, 1997). The oil was transferred to a pyrex test tube with screw cap, and 2 mL of methanolic-HCl solution 3M were added. Sample was placed in a water bath at 80 °C for 60 min. The fatty acid methyl esters were extracted with *n*-hexane, after addition of distilled water. The solution was directly injected into the gas chromatograph (GC) for analysis.

2.6 Phytosterols and FAMEs Gas chromatographic analysis

The apparatus for gas chromatographic analyses was a Trace GC Ultra, (Termoscientific, Milan, Italy) equipped with a flame ionization detector and an autosampler AS 3000 (Thermoscientific). Helium was used as carrier gas with a constant flow rate of 1.5 $mL \cdot min^{-1}$. The unsaponifiable oil was analyzed to identify the most significant phytosterols using a TRX-5 (30 m x 0.25 mm x 0.25 µm; Restek Bellefonte, PA) capillary column. Sample was introduced by injection system split-splitless in split mode (ratio 1:10). The initial oven temperature was 200 °C for 2 min, and then it increased at a rate of 20 °C/min. The temperatures of injector and detector were set at 250 °C and 260 °C, respectively (Bernardo-Gil et al., 2001). Plant Sterol Mix (beta-sitosterol, campesterol, brassicasterol, stigmasterol), beta-sitosterol and campesterol were used as external standards for identification of phytosterols in unsaponifiable samples. The fatty acid content of cherry seed oil were determined using a SP-2560 (100 m x 0.25 mm x 0.20 µm, Supelco) capillary column. Sample was introduced by injection system split-splitless in split mode (ratio 1:100). The operating conditions were conforming to Bernardo-Gil et al. (2001), with some modifications. The oven temperature program was initiated at 140 °C (held for 5 min), linearly increased to 260 °C (4 °C/min) and kept at this temperature for the remaining time of analysis. Fatty acids composition of cherry seed oil was obtained by comparison with retention times of the standard mixture FAMEs (Fatty Acid Methyl Esters) and was expressed as percentage. Data were recorded and processed by the ChromQuest 5.0 software (Thermo, Rodano, Italy).

3. Result and discussion

Gas chromatographic analysis allowed the identification of two phytosterols in unsaponifiables cherry seed oil samples, β-sitosterol and campesterol (Table 1). Brassicasterol and stigmasterol are not present in the extracts. Vegetable oils are mainly composed of triacylglicerols (95-98%) and complex mixtures of minor compounds (2-5 %). Phytosterols are minor components naturally present in the unsaponifiable fraction of vegetable oils and butters. These are structurally similar to cholesterol and are derived by biosynthesis of squalene. Potential uses and benefits of phytosterols are extensively examined, in human nutrition (Ostlund, 2002; Moghadasian, 2000; Piironen et al., 2000; Ling et al., 1995) for lowering cholesterol levels in human and animal blood (Brufau et al., 2008), anti-bacterial (Zhao et al., 2005), anti cancer (Awad et al., 2000; Ifere et al., 2009), anti-inflammatory and reparative effects (Henrotin et al. 2003; Lippiello et al. 2008; Richette 2011). Antioxidant properties of the unsaponifiables from tomato seeds, oats and wheat germ, were compared with the efficacy of potent antioxidants such as butylated hydroxyanisole (Malecka, 2002) and dermatological and cosmetic applications go back to studies of Thiers (1955-1958).

Table 1: Phytosterols content (g/Kg) in cherry seeds

Phytosterols	Soxhlet	SFE
Beta-sitosterol	0.569 ±0.038	0.725 ±0.047
Campesterol	0.025 ±0.001	0.083 ±0.004

*Results are expressed as mean value ± standard deviation.

Table 2: Fatty acid composition (%) in cherry oil

Fatty acid		Soxhlet	SFE
Undecanoic	(C11:0)	0.33 ±0.09	0.06 ±0.01
Lauric	(C12:0)	0.14 ±0.01	0.28 ±0.02
Tridecanoic	(C13:0)	0.28 ±0.03	0.22 ±0.02
Palmitoleic	(C16:0)	8.42 ±0.72	11.20 ±0.98
Eptadecanoic	(C17:0)	0.29 ±0.04	0.27 ±0.03
Oleic	(C18:1)	2.96 ±0.53	3.90 ±0.89
Linoleic	(C18:2)	32.25 ±1.52	35.85 ±1.24
Linolenic	(C18:3)	0.50 ±0.03	-
Arachidic	(C20:0)	41.19 ±2.72	37.57 ±1.61
Cis-11,14 eicosadienoic	(C20:2)	-	0.10 ±0.01
Cis-5,8,11,14,17 eicosapentenoic	(C20:5)	-	0.09 ±0.02
Nervonic	(C24:1)	6.36 ±0.78	4.83 ±0.95

*Results are expressed as mean value ± standard deviation.

Today phytosterols are widely utilized as surfactant, co-emulsifiers, thickeners and functional ingredients in cosmetic formulations (Folmer, 2003). Several underutilized tropical seeds were exploited to obtain the unsaponifiable fraction (Esuoso et al., 2000). As confirmed by the literature (Bernardo-Gil et al., 2001) cherry seed oil contains severals phytosterols as campestanol, sigmasterol, clesrosterol, Δ5-avenasterol, Δ5,24-stigmadienol, and β-sitosterol constitutes the largest share going up to 90 % of the total. Unsaponifiable fraction of cherry seed oil can be used as a resource of phytosterols to be included in functional foods and cosmetic products.

Table 2 shows the composition of the fatty acid of oil, analyzed with gas chromatography. There are significant differences in the fatty acid composition of cherry seed oil obtained with the two extraction procedures. With the Soxhlet extraction 48.6 % of saturated, 17.74 % of unsaturated, and 32.75 % of polyunsaturated fatty acids were obtained. On the contrary, the percentage of saturated fatty acids decreased (38.43 %) with SFE-CO_2, in favor of larger amounts of unsaturated (19.93%) and polyunsaturated (36.04 %) fatty acid. SFE-CO_2 produced an oil of superior quality, without degradation products as off flavours presence (Cravotto et al., 2011) due to solvent or high temperature (Raventos et al., 2002). In both cases arachidic (C20:0) and linoleic acid (C18:2, ω-6) are the two major component of total fatty acids, while the oleic acid percentage is lower than other seed oils (Ryan et al., 2007). An interesting data is the presence of a high percentage of nervonic acid, found in all oil samples. This is a long-chain monounsaturated fatty acid (C24:1, ω-9), of marine origin, precursor of the neuronal cell membrane glycolipids, with a key role in the modulation of ion channels (Wu et al., 2000) and membrane receptors, and therefore widely used as a neurotrophic factor (Lauritzen et al., 2000) in food supplements and nutraceuticals. Currently there are not literature data to confirm the presence of this important fatty acid in the oil of the seeds of cherries, but that is present in *Tropaeolum speciosum*, plant native to Chile (Bettger et al., 2001) and seeds of *Lunaria Annua* (Fehling and Mukhrjee, 1990). This data could be interesting for future perspectives in drug therapy of neuromuscular diseases. In fact, an attempt is to obtain large amounts of acid nervonic through genetic engineering (Tailor et al., 2009; Guo et al., 2009). The yield of cherry seed oil with Soxhlet extraction was higher (6.5 % ±0.5) than with SFE (5.0 % ±1.1), but, in recovering bioactive compounds, the extraction with supercritical carbon dioxide was quantitatively better than the traditional method with solvent. The cherry seed oil has been called a specialty oil, wich is small volume but high value (Temelli et al., 2005).

4. Conclusions

In the past decades Soxhlet extraction was optimized in order to shorten the time of separation with use of auxiliary forms of energy and automation of extraction (Luque de Castro and Priego-Capote, 2010; Luque de Castro and Garcia-Ayuso, 1998). This was the traditional method for lipid extraction from different matrices, but involves a series processes of separation and refining with use of

hazardous organic solvents and often high temperatures, which create many economic, environmental and security. The SFE, instead, has been developed for analytical applications in the mid-1980's to reduce the use of organic solvents, and is becoming a standard method for oil extraction, fractionation, refining and deodorization of fats or oils (Sahena et al., 2009), and is currently included among the methods recommended by the Association Official Analytical Chemistry (AOAC) for the extraction of oils from seeds (AOAC, 1997). Also in the extraction of cherry seed oil, the supercritical carbon dioxide showed an efficient, selective and safe solvent and the SFE is a viable alternative to classical procedures (Taylor et al., 1993). Moreover the quality of cherry oil seed obtained with SFE was better than of Soxhlet extraction oil. The use of supercritical carbon dioxide is an green method to obtain cosmetic product, functional and nutraceutical food, pure and free solvents for natural products and environmentally friendly.

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