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Original Article

Sterol composition of black cumin (*Nigella sativa* L.) and Aleppo pine (*Pinus halepensis* Mill.) seed oils

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Abstract

Oils extracted from Tunisian and Iranian *Nigella* seeds (TNS and INS, respectively) and Tunisian Aleppo pine seeds were analysed for sterol composition. These components could have many health benefits, especially in lowering LDL-cholesterol and preventing heart disease. The highest level of total unsaponifiable matter was found in *Pinus* seed oil (17.23 g/kg oil, compared to 15.58 oil and 14.82 g/kg oil in TNS and INS seed oils, respectively). The total sterol content in the three studied oils represented 18.03%, 17.41% and 42.66% of the unsaponifiable matter, respectively. β-Sitosterol was the major sterol in all oils with 44%, 54% and 74% of the total sterols in Tunisian and Iranian *Nigella* seed oils and *Pinus* seed oil, respectively. The next major sterol was stigmasterol in both *Nigella* seed oils studied (16.57–20.92% of total sterols) and campesterol in *Pinus* seed oil (11.42% of total sterols). TMS 484, Δ7-stigmasterol, Δ7-avenasterol and cholesterol were detected at lower levels. The high level of β-sitosterol in *Pinus* seed oil could make it the most suitable and effective for lowering blood cholesterol and preventing coronary heart disease.

Keywords: *Nigella* seed oil; *Pinus* seed oil; Oleaginous seeds; Phytosterol composition; Gas chromatography–mass spectrometry; Tunisian seeds; Iranian seeds; Black cumin; Aleppo pine nut

1. Introduction

Sterols are an important part of the unsaponifiable matter of fats and oils and therefore present in human diet (Apparich and Ulberth, 2005; Cunha et al., 2006; Lagarda et al., 2006). These ''minor'' lipids attract the interest of food chemists because they are of great importance for food labelling and nutritional purposes. They are also characteristic of the genuineness of vegetable oils (Crane et al., 2005). On the other hand, the qualitative analysis of sterols is very helpful when adulteration is suspected (Lognay et al., 1993). By virtue of their unsaturated character, they are vulnerable towards free-radical mediated oxidation (Savage et al., 2002). Plant sterols, called phytosterols, resemble cholesterol in function and structure (Kritchevsky and Shirley, 2005). They are mainly present in nuts, vegetable oils, seeds, cereals and beans (de Jong et al., 2003). More than 100 types of phytosterols have been reported in plant species, but the most abundant are sitosterol, stigmasterol and campesterol (Moreau et al., 2002; Berger et al., 2004, Kritchevsky and Shirley, 2005). Other relevant phytosterols that can be found in plants in minor amounts are brassicasterol, Δ5-avenasterol, sitostanol and campestanol (Phillips et al., 2002). Ergosterol occurs in corn, cottonseed, peanut and linseed oils (Kritchevsky and Shirley, 2005). Clinical studies have demonstrated that the dietary intake of phytosterols (as part of normal diet or as a supplement) may decrease blood cholesterol levels (de Jong et al., 2003; Ostlund, 2004; Ostlund et al., 2002a, b) resulting in significant reduction in the risk of heart disease (Li et al.,

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2007). Phytosterols play major roles in several areas namely in pharmaceuticals (production of therapeutic steroids), nutrition (anticancer properties) and cosmetics (creams, lipstick). Additionally, it has been suggested that they have anti-inflammatory, antibacterial, antifungal, anti-ulcerative, antioxidant and antitumoral activities (Stuchlík and Žàk, 2002; Awad et al., 2000).

Sterols can occur in vegetable oils either in free form or esterified with fatty acids (Stuchlík and Žàk, 2002; Cunha et al., 2006). Since both fractions have different compositions, the combined determination of these two classes of compounds provides a more informative approach to check vegetable oils authenticity (Mariani et al., 1999). Separation of sterol classes is extremely useful in the monitoring of the refining process, which decreases free and increases esterified sterol contents (Cunha et al., 2006).

Black cumin (*Nigella sativa* L.), belonging to *Ranunculaceae* family, and Aleppo pine (*Pinus halepensis* Mill.) from *Pinaceae* family, produce highly nutritional oleaginous seeds. They have been used as a supplement to help maintain good health and well-being. Black cumin seeds, on account of their aromatic nature, are used as a spice in cooking, as a carminative and diuretic by oriental people (Hedrick, 1972). Whereas, Aleppo pine seeds still traditionally used throughout all Islamic countries, for preparing a sweet pudding of ground pine seeds, and they have recently been employed as an ingredient in ice-creams and candies. Some scientists have studied chemical characteristics and fatty acid composition of black cumin and Aleppo pine seed oils (Burits and Bucar, 2000; D'Antuono et al., 2002; Ramadan and Mörsel, 2003; Nasri and Triki, 2004). In previous papers, we reported that the lipid fractions of black cumin and Aleppo pine seeds were rich in linoleic acid, which has a beneficial effect on blood lipids, lowering blood pressure and serum cholesterol (Cheikh Rouhou et al., 2006, 2007). Their unique fatty acid composition, relatively high polyphenol content and quality and hence high protection against oxidative stress, relatively good shelf life, and other desirable physicochemical characteristics lead to more diverse and novel applications of black cumin and Aleppo pine seed oils in the food, pharmaceutical, cosmetic and other non-food industries (Cheikh Rouhou et al., 2006, 2007). Sterol profile of black cumin lipid fraction was widely investigated for Turkish and German origins (Nergiz and Ötles, 1993; Ramadan and Mörsel, 2004). Nevertheless, no work was undertaken about the sterol content of Tunisian variety. Moreover, no published study has reported the sterol composition of Aleppo pine seed oil. In fact, the sterol composition and content of vegetable oils is affected by geographical growing area, difference in species or processing, and the ripening stage of the fruit or the seed (Rivera del Alamo et al., 2004; Phillips et al., 2005a). It may also be due to other agronomical and technological factors such as cultivar, climate, soil type, and extraction and conservation procedures (Cunha et al., 2006). This variation makes interesting the study of the sterol fraction of Aleppo pine, Tunisian and Iranian *Nigella* seed oils.

The aim of this present work was to study Tunisian and Iranian *Nigella* seed oils and Tunisian *Pinus* seed oil by investigating their sterol profile which has not yet been investigated. The results could help to improve economic and health utilization of these seeds as new sources of edible oils.

2. Materials and methods

2.1. Samples

Two varieties of mature black cumin (*Nigella sativa* L.) seeds from Tunisia (TNS) (20 kg) and Iran (INS) (20 kg) were purchased from a spice market of Menzel Temim (Tunisia). Twenty kilograms of Aleppo pine seed (*Pinus halepensis* Mill.) samples were purchased from Bizerta (Tunisia), where they were harvested. The samples were directly stored at 15 °C for maximum 3 days. Then, they were soaked in water, washed and air-dried. The seed samples were separately milled in a heavy-duty grinder for 2 min, to pass 1–2 mm screens and then were preserved in hermetic bags at −20 °C.

2.2. Lipid extraction

Fifty grams of black cumin and Aleppo pine seeds were placed in a dark flask (capacity = 1 l) and homogenized with 250 ml of hexane. After mixing for 4 h in a shaker (Selecta, Spain) at a rate of 180 U/min, the mixture was centrifuged for 15 min at $1000 \times g$ at ambient temperature (20 °C). The supernatant was then filtered through a filtering paper (Whattman no. 2). The extraction procedure was repeated twice and the collected solvent was removed using a rotary evaporator at 40 °C. The seed oils obtained finally were drained under a stream of nitrogen and then stored in a freezer (−20 °C) until analysed.

2.3. Extraction and gas chromatography–mass spectrometric (GC–MS) analysis of sterols

2.3.1. Saponification and extraction

Nigella sativa and *Pinus halepensis* seed oils (samples of 1 g), with added betulin as an internal standard (1 mg betulin/g oil), were saponified with 50 ml methanolic potassium hydroxide (2 mol/l) for 1 h (under reflux), and the unsaponifiables were then extracted three times with 100 ml diethyl ether. The pooled extracts were then washed three times with 50 ml of deionised water and the solvent was subsequently removed under reduced pressure with a rotary evaporator at 35 °C. The unsaponifiable was diluted in pure chloroform (1:10, v/v) and then submitted to thin layer chromatography (TLC) on silica gel G60 (Merck, Darmstadt, Germany), 0.5-mm-thick plates previously activated at 103 °C over a 30-min period. We precise that 50 µl of the samples were deposed in TLC plates.