SUPPLEMENTARY MATERIAL

Sterols and squalene in apricot (*Prunus armeniaca* L.) kernel oils: The variety as a key factor

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Abstract

The profile of sterols and squalene content in oils recovered from the kernels of fifteen apricot (*Prunus armeniaca* L.) varieties were investigated. Nine sterols (campesterol, β-sitosterol, Δ5-avenasterol, 24-methylene-cycloartanol, cholesterol, gramisterol, Δ7-stigmasterol, Δ7-avenasterol and citrostadienol) were identified in apricot kernel oils. The β-sitosterol was the predominant sterol in each cultivar and consisted of 76–86% of the total detected sterols. The content of total sterols and squalene were significantly affected by the variety and ranged between 215.7–973.6 and 12.6–43.9 mg/100 g of oil, respectively.

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Keywords: *Sterols, Squalene, Apricot (Prunus armeniaca L.) kernel oil, Variety.*

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Experimental

Reagents

The Sylon BTZ was received from Supelco (Bellefonte, PA, USA). Methanol, *tert*-butyl methyl ether, *n*-hexane (HPLC grade), 5α-cholestane (≥97%, GC) and KOH (analytical grade) were purchased from Sigma-Aldrich (Steinheim, Germany).

Plant material

Apricot (*Prunus armeniaca* L.) kernels were obtained from mature fruits of fifteen varieties (HL PSŠ 5, Apguldes4, 'Edelveis', 'Veselka', 'Aisberg', 110107, 'Rasa', 5064, Apguldes3, 'Daiga', 'Ilga', Apguldes1, 'Dzintars', 'Spicā' and 'Pogremok'). Apricots were collected during July 2013 in the Latvia State Institute of Fruit-Growing, Dobele, Latvia, GPS location: N: 56° 36' 39" E: 23° 17' 50". The present study was conducted as a part of an experiment designed to evaluate suitability for growing different apricot varieties in Latvia. Detailed information about plant origin and specification has been reported previously (Górnaś et al. 2015). The kernels were recovered from fruit pits (by removing the outer shells), frozen and subsequently freeze dried using a FreeZone freeze-dry system (Labconco, Kansas City, MO, USA) at a temperature of –51 ± 1 °C under vacuum of 0.055–0.065 mBar for 48 h. Undamaged kernels were selected (~20 g) and milled with a Knifetec^{TM} 1095 universal laboratory mill (Foss, Höganäs, Sweden) to pass through a sieve of 0.75 mm mesh size to finally obtain a powder. Dry weight basis (dw) in the studied samples was measured gravimetrically.

Oil extraction

Oil was extracted according to an earlier introduced method (Górnaś et al. 2014). In brief, ground fruit kernels (5 g) were supplemented with 25 mL of *n*-hexane (Sigma–Aldrich, Steinheim, Germany) in a centrifuge tube and mixed on a Vortex REAX top (Heidolph, Schwabach, Germany) at 2500 rpm (1 min). Samples were subjected to ultrasound treatment in the Sonorex RK 510 H ultrasonic bath (Bandelin electronic, Berlin, Germany) (5 min, 35 °C) and centrifuged on a Centrifuge 5804 R (Eppendorf, Hamburg, Germany) (10000 × g, 5 min, 21 °C). The supernatant was collected in a round bottom flask and the remaining solid residue was re-extracted (twice) as described above. The combined supernatants were evaporated in a Laborota 4000 vacuum rotary evaporator (Heidolph, Schwabach, Germany) at 40 °C until constant weight. The oil content was expressed in % (w/w) dw (dry weight basis – measured gravimetrically) of kernels.

Contents of sterols and squalene

Contents of plant sterols and squalene were determined according to AOCS (1997). In brief, the oil sample (50 mg) was saponified with 1M KOH in methanol for 18 hrs at room temperature, and then unsaponifiables were extracted thrice with *n*-hexane/*tert*-butyl methyl ether (1:1, v/v). After silylation using a Sylon BTZ, sterols were separated on a HP 6890 gas chromatograph equipped with a DB-35MS capillary column (25 m × 0.20 mm × 0.33 μm; J&W Scientific, Folsom, CA). Samples of 0.5 μL were injected in a splitless mode. Column temperature was held at 100 °C for 5 min, then increased to 250 °C at 25 °C/min, held for 1 min, then increased to 290 °C at 3 °C/min and held for 20 min. 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 sterol quantifications. Sterols and squalene were identified by comparing retention data of standards previously verified by mass spectrometry.

Statistical Analysis

The results were presented as means ± standard deviation (n=3) from three batches of ground kernels. One-way analysis of variance (ANOVA) and the Bonferroni post-hoc test was used to denote statistically significant values at $p \leq 0.05$ with the assistance of Statistica 10.0 (StatSoft, Tulsa, OK, USA) software.

References

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