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Characterization of seed oils from fresh Bokbunja (*Rubus coreanus* Miq.) and wine processing waste

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Abstract

The physicochemical characteristics, fatty acid (FA) profile, and triacylglyceride (TAG) composition of seed oils from fresh Bokbunja (*Rubus coreanus* Miq.) fruits and traditional Bokbunja wine processing waste were determined in this study. Oil contents of the fresh seeds and the seeds from wine processing waste were similar, accounting for about 18% of dry weight. The free fatty acid (FFA) content between the two seed oils was significantly different (0.50% for fresh seed oil and 73.14% for wine seed oil). Iodine, conjugated diene, saponification values, and unsaponifiable matter were very similar in the oil samples, but the specific extinction coefficients at 232 and 270 nm of wine seed oil were higher than those of fresh seed oil. Linoleic (C18:2, 50.45–53.18%, L) and linolenic (C18:3, 29.36–33.25%, Ln) acids were the dominant FAs in the two seed oils, whereas oleic (C18:1, 7.32–8.04%, O), palmitic (C16:0, 1.55–1.65%, P), and stearic (C18:0, 0.65–0.68%, S) acids were the minor FAs. LLL, OLL, LLLn, OOL, LLnLn, and OOO were the abundant TAGs, representing >90% of the oils.

Keywords: Bokbunja; Wine processing waste; Seed oil; Physicochemical characteristics; Fatty acid profile; Triacylglyceride

1. Introduction

Rubus coreanus Miq. is a deciduous broadleaf shrub in the family Rosaceae. Its growth is limited to the south-east Asian countries (especially southern parts of the Korean peninsula, China and Japan) (Choi et al., 2006). The unripe fruits of *Rubus coreanus* Miq., more popularly known as Bokbunja in South Korea, have been mostly used as folk medicines for treating spermatorrhea, enuresis, asthma, and allergic diseases (Shin et al., 2002). Phenolic acids, organic acids, triterpenosides, flavonoids, gallotannin and ellagitannin are the reported constituents in Bokbunja fruits (Yoon et al., 2003). The growth area for Bokbunja in Jeonbuk province in South Korea has increased year by year. The harvest quantity rose from about 1510 tons in 2001 to 2733 tons in 2005. Bokbunja is mainly used to produce traditional wine by fermenting and maturing the fully ripened fruits harvested in June and July. Thus, we focused especially on the utilization of wine processing wastes. In order to prepare the traditional wine, seeds must be removed from Bokbunja fruits in the production line. The resulting seed wastes correspond to amounts large enough to cause serious problems in the related industry. Generally, seed oils have a wide range of use as foods or lubricants, fuel for paraffin lamps, additives for paint formulations, and as a soup ingredient. Some seed oils may have a high potential in food and medicinal applications. Seed oils can potentially be developed for nutritional, pharmaceutical, and oleochemical industries. However, to date, there is no information on the physicochemical properties, the FA profile, and the TAG structure of Bokbunja seed oil, despite its importance in nutritional and biochemical applications. Thus, we evaluated the Bokbunja seed oil composition for its effective application. To this end, we used seeds from fresh ripe fruits and the waste seeds

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discarded from wine processing. Hereafter, for simplicity, the two seeds are denoted as fresh Bokbunja seeds (FBS) and wine Bokbunja seeds (WBS).

2. Methods

2.1. Seeds and extraction of seed oils

Fresh fruits of Bokbunja and waste seeds discarded from wine processing were provided by Go-In-Dol Bokbunja Wine Company, Gochang, Korea. WBS was obtained as follows: (1) Fermentation of a mixture including Bokbunja fruit, sugar and yeast at 28–30 °C for 45–60 days, (2) Filtration with a membrane filter, and (3) WBS removal with a seed separator. The seeds in the fresh fruits were separated using a blender. The seeds were then washed with water to remove fibrous substances and pigments on the surface and dried in a convection oven at 60 °C for 24 h. To extract the seed oil, the two hard seeds were powdered by a roll-mill. The seed oils were prepared by Soxhlet extraction. The seed oils were then treated with anhydrous sodium sulfate and stored at −20 °C until use. Before use, the remaining *n*-hexane in the seed oil was released in a vacuum dryer at room temperature for 24 h.

2.2. Physicochemical properties

^{1}H NMR spectra of seed oils were obtained in chloroform-d_1 using a JEOL JNM-EX 400 spectrometer (Japan). The FA composition was quantified by means of ^{1}H NMR integration. The iodine value (IV) and conjugated diene (CD) levels for the two seed oils were calculated from the FA composition and the signal coupling relationships.

$E^{1\%}_{1\mathrm{cm}}$, the extinction coefficient of 1% solution, of the oil samples in *n*-hexane was evaluated by UV absorbance at 232 and 270 nm using a Hewlett Packard 8452A UV/Vis spectrophotometer (USA). The AV was calculated by the standard method given by the Association of Official Analytical Chemists (AOAC, 1984).

To determine the saponification value, seed oil (500 mg) was mixed with 20 mL of 0.5 N methanolic potassium hydroxide. Each mixture was saponified at 70 °C for 1 h under reflux. Excess potassium hydroxide in the reactant was titrated with 0.5 N hydrochloric acid using phenolphthalein indicator.

To determine unsaponifiable matter, seed oil (1 g) was mixed with deionized water (100 μL) and 0.7 M methanolic potassium hydroxide (20 mL). The mixture was saponified at 70 °C for 1 h under reflux. The reactants were transferred into a separatory funnel containing 20 mL of saturated sodium chloride. The unsaponifiable matters were extracted with diethyl ether. The ether layer was washed with 0.5 M aqueous potassium hydroxide and deionized water to remove FFAs and any residual potassium hydroxide, respectively. Ether was evaporated at 40 °C. To quantitate unsaponifiable matters, the remaining FFAs in the ether extract were quantitatively analyzed by GC. Palmitic and oleic acids were used as standard FAs. Unsaponifiable matters $(\%) = \{(W_\mathrm{r} - W_\mathrm{f})/W_\mathrm{o}\} \times 100$, where W_r is the mass of the residue in g, $\mathrm{W_f}$ is the mass in g of the FFA determined by GC, and W_o is the mass of the seed oil in g.

2.3. Free fatty acid and sterol contents

The original seed oils and the extracted unsaponifiable matters were dissolved in anhydrous pyridine and silylated with N,O-bis(trimethylsilyl)-triflouroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) as a TMS reagent at 50 °C for 30 min. The TMS-derivatives were analyzed with a gas chromatograph-mass spectrometer (GC–MS) and a GC. One microliter of each sample was injected into a GC (GC17A, Shimadzu, Japan) equipped with a flame ionization detector (FID). The peak identification was performed with a GC–MS (QP5050, Shimadzu, Japan). Separation of the compounds was achieved using an Rtx-1 silica fused capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness). The oven temperature was elevated from 200 to 300 °C at a rate of 3 °C/min and the final temperature was maintained at 300 °C for 20 min. The flow rate of helium gas was 1.0 mL/min. The split ratio was adjusted to 20. The MS was operated in electron ionization (EI) mode at 70 eV. The interface temperature was 230 °C. The unknown sterols and unsaturated FAs contents, such as oleic, linoleic and linolenic acids, were evaluated by the peak area ratio of cholesterol, palmitic and stearic acids to cholestane (internal standard).

2.4. Fatty acid composition

Seed oil (30 mg) was added to a PTFE screw-capped vial and mixed with 2 mL of 0.5 M methanolic potassium hydroxide. The mixture reacted in a heating block at 62 °C for 1 h. After saponification, 2 mL of 1 M methanolic sulfuric acid was added to the vial and then reacted at 62 °C for 15 min. Deionized water (2 mL) and *n*-hexane (5 mL) were added to the vial to extract the transesterified FAs. The transesterified FAs were analyzed by GC. One microliter of each sample was injected into the GC equipped with a FID. Separation of the compounds was achieved using a Supelcowax$^{\mathrm{TM}}$-10 silica fused capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness). The oven temperature was elevated from 180 to 250 °C at a rate of 3 °C/min and maintained at 250 °C for 15 min. The helium gas flow rate was 1 mL/min. The split ratio was adjusted to 10. FID and injection port temperatures were 250 and 230 °C, respectively. Resolved derivatives were quantitatively determined using myristic (C14:0, internal standard), palmitic, and stearic acids.

2.5. Triacylglyceride composition

Seed oils (5 μL) were mixed with 495 μL of chloroform and again diluted ten times with chloroform. 2,5-Dihydroxybenzoic acid (DHB, 10 mg) matrix was dissolved in