J Ginseng Res xxx (2017) 1–6

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Technical note

Quality and characteristics of fermented ginseng seed oil based on bacterial strain and extraction method

Myung-Hee Lee, Young-Kyoung Rhee, Sang-Yoon Choi, Chang-Won Cho, Hee-Do Hong, Kyung-Tack Kim*

Ginseng Research Team, Division of Strategic Food Research, Korea Food Research Institute, Gyeonggi, South Korea

ARTICLE INFO

Article history:
Received 6 April 2016
Received in Revised form
31 January 2017
Accepted 15 March 2017
Available online xxx

Keywords:
extraction method
fermented ginseng seed oil
Korean Red Ginseng
phenolic compounds
phytosterol

ABSTRACT

Background: In this study, the fermentation of ginseng seeds was hypothesized to produce useful physiologically-active substances, similar to that observed for fermented ginseng root. Ginseng seed was fermented using *Bacillus, Pediococcus, and Lactobacillus* strains to extract ginseng seed oil, and the extraction yield, color, and quantity of phenolic compounds, fatty acids, and phytosterol were then analyzed.

Methods: The ginseng seed was fermented inoculating 1% of each strain on sterilized ginseng seeds and incubating the seeds at 30°C for 24 h. Oil was extracted from the fermented ginseng seeds using compression extraction, solvent extraction, and supercritical fluid extraction.

Results and Conclusion: The color of the fermented ginseng seed oil did not differ greatly according to the fermentation or extraction method. The highest phenolic compound content recovered with the use of supercritical fluid extraction combined with fermentation using the *Bacillus subtilis* Korea Food Research Institute (KFRI) 1127 strain. The fatty acid composition did not differ greatly according to fermentation strain and extraction method. The phytosterol content of ginseng seed oil fermented with *Bacillus subtilis* KFRI 1127 and extracted using the supercritical fluid method was highest at 983.58 mg/100 g. Therefore, our results suggested that the ginseng seed oil fermented with *Bacillus subtilis* KFRI 1127 and extracted using the supercritical fluid method can yield a higher content of bioactive ingredients, such as phenolics, and phytosterols, without impacting the color or fatty acid composition of the product.

1. Introduction

The incidence of chronic diseases including hyperlipidemia, heart disease, cancer, diabetes, and obesity are rising due to imbalances caused by dietary lifestyle changes. Ginseng is an important herb that has been used as a medicinal plant to remedy such imbalances for thousands of years in Asia and eastern North America. Korean ginseng (*Panax ginseng* Meyer) root has long been used as an oriental medicine, and demand has been rising with the accumulation of scientific evidence for its pharmacological efficacy. Ginseng byproducts, such as leaf, stem, and flower extracts, have been added to cosmetics and soaps, and the plant body is used in animal feed [1–3].

Studies have employed fermentation by lactic acid bacteria to increase the yield of active compounds recovered in extracts of natural substances [4–7]. Particularly, fermentation methods have also been used to improve the bioactivity and sensory qualities of plant products including ginseng [8–10]. However, such studies have been limited to ginseng root, with fermentation of ginseng fruits and seeds seldom considered.

Phytosterols are natural constituents of plants and perform critical roles in plant cells. β-Sitosterol, campesterol, and stigmasterol are integral natural components of plant cell membranes that are abundant in vegetable oils, nuts, seeds, and grains [11,12]. Moreover phytosterols have important bioactive properties, such as cancer prevention [13,14], lowering of plasma total cholesterol levels [15,16], and other nutritive properties. Most plants contain polyphenolic compounds, which are present as free, esterified, or combined forms depending on the species. Phenolic acids are divided into benzoic acids and cinnamic acids, which are responsible for the flavor and aroma of fruits and vegetables, and have specific physiological roles [17–20]. In this study, the fermentation

* Corresponding author. Ginseng Research Team, Division of Strategic Food Research, Korea Food Research Institute, Gyeonggi 13539, South Korea.
E-mail address: tack@kfri.re.kr (K.-T. Kim).

http://dx.doi.org/10.1016/j.jgr.2017.03.003
p1226-8453 e2093-4947/$ – see front matter

Please cite this article in press as: Lee M-H, et al., Quality and characteristics of fermented ginseng seed oil based on bacterial strain and extraction method, Journal of Ginseng Research (2017), http://dx.doi.org/10.1016/j.jgr.2017.03.003

of ginseng seeds was hypothesized to produce useful physiologically-active substances, similar to that observed for fermented ginseng root. Ginseng seeds were fermented using *Bacillus subtilis, Pediococcus pentosaceus*, and *Lactobacillus gasseri* strains, and the resultant oil quality characteristics, fatty acid contents, phenolic compounds, and phytosterols were analyzed and evaluated.

2. Materials and methods

2.1. Materials

The ginseng seeds used in this study were from 4-yr-old ginseng plants grown in 2012 and obtained from the Geumsan Ginseng Market in Chungcheongnam-do (Geumsan, Korea). The ginseng seeds were dried after removing the skin and the endosperm was used for compression extraction and supercritical fluid extraction. Maltol, coumaric acid, cinnamic acid, salicylic acid, vanillic acid, syringic acid, ferulic acid, gentisic acid, β-sitosterol, campesterol, and stigmasterol were purchased from Sigma Co. (St. Louis, MO, USA). Hydroxyl benzoic acid was purchased from Junsei Co. (Tokyo, Japan).

2.2. Strains

The strains used to ferment the ginseng seeds were Gram-positive *L. gasseri* KCTC 3162, *P. pentosaceus* LY011, *B. subtilis* KFRI 1124, and *B. subtilis* KFRI 1127 obtained from Korean Collection for Type Cultures maintained by the Korea Research Institute of Bioscience and Biotechnology (KRIBB) and the Korea Food Research Institute (KFRI). The *Bacillus* strains were inoculated in TS broth, and the *Lactobacillus* and *Pediococcus* strains were inoculated in MRS broth and incubated at 30°C for 24 h.

2.3. Fermentation

The sterilized ginseng seeds (500 g) were fermented by inoculating 1% of each strain and then incubating at 30°C for 24 h. Independent fermentations were carried out in triplicates, and fermented ginseng seeds were combined and freeze-dried for analysis.

2.4. Extraction

The fermented ginseng seed oil was extracted by compression extraction, solvent extraction, or supercritical fluid extraction. Fermented ginseng seed endosperm was pressed using a screw-type oil sampler (Hyeondae Green Industry, Seoul, Korea) for compression extraction and then centrifuged at 8,224g for 20 min to eliminate impurities and obtain the fermented ginseng seed oil. For solvent extraction, fermented ginseng seeds were extracted twice with *n*-hexane in a vacuum evaporator for 3 h per extraction and vacuum filtered. The solvent in the filtrate was eliminated using a vacuum rotary evaporator (N-1001S; EYELA, Tokyo, JAPAN). Supercritical fluid extraction (Greentek21 Co., Anyang, Korea) was conducted at 150 bar and 65°C.

2.5. Color measurements

After each extraction, fermented ginseng seed oil color was determined using lightness (L), redness (a), and yellowness (b) values with a Minolta CR-200 colorimeter (Tokyo, Japan). All samples were measured five times to obtain an average value.

2.6. Phenolic compound analysis

The phenolic compounds in the ginseng seed oil were analyzed by high-performance liquid chromatography (PU-980; Jasco, Tokyo, Japan) under the following analytical conditions: Waters C-18 column (5.0μm, 4.6mm × 250mm; Milford, MA, USA), the mobile consisted of 2% acetic acid in water (Solvent A) and 50% acetonitrile with 0.5% acetic acid (Solvent B) utilizing the following gradient over a total run time of 80 min: 45% A for 70 min, 0% A for 73 min, 100% A for 78 min, and 100% A until completion of the run. The flow rate of the mobile phase was 0.8 mL/min. The sample was detected at 280 nm. Each 2-g sample was dissolved in 10 mL *n*-hexane, and 20 mL of 80% methanol was added to extract the phenolic compounds. Finally, 10 mL *n*-hexane was added to the extract to eliminate the remaining lipid constituents, and solvent in the 80% methanol layer was evaporated completely using a vacuum evaporator. The concentrated extract was dissolved in LC grade methanol (Merck, USA) to 10 mg/mL and filtered through a 0.45-μm syringe filter (Whatman, Maidstone, England).

2.7. Fatty acid analysis

Fatty acid analysis of the ginseng seed oil was performed by gas chromatography (GC) (Agilent 6890; Agilent Technologies, Santa Clara, CA, USA) according to an Association of Official Analytical Chemists (AOAC) official method [21]. The GC column was an HP-FFAP (polyethylene glycol–terephthalic acid; 25m× 0.32mm × 0.5μm). Column temperature was maintained at 150°C for 1 min, which was increased at 4°C/min up to 230°C, and maintained for 10 min. The injection temperature was 230°C, with the detector temperature at 250°C. The carrier gases were He at a flow rate of 1.5 mL/min, H_2 at a flow rate of 30 mL/min, and air at a flow rate of 300 mL/min. The samples were treated with a methanol–sodium hydroxide solution to form an alkaline salt, and trifluoroborane–methanol was added and heated for esterification. The fatty acid esters were dissolved in isooctane to obtain samples for the experiment. The samples (1 μL) were injected and analyzed using a flame ionization detector. The standard material for fatty acid identification was the Supelco 37 component fatty acid methyl ester mix C4–C24 (Supelco, Belfonte, PA, USA), and samples were identified by comparing retention times.

2.8. Phytosterol analysis

The samples were pretreated for the phytosterol analysis according to the plant sterol test solution preparation method (4.3.38. phytosterol) in the Health Functional Food Code, and phytosterols were analyzed by GC (M600D; Youngling, Seoul, Korea). The standard materials used for analysis were 70% β-sitosterol and 5α-cholestane. Each standard was dissolved in the internal standard solution (1–5 mg/mL dihydrocholesterol in chloroform) for analysis. The GC column was an HP-ultra-2 crosslinked 5% PHME siloxane (25m × 0.25mm × 0.33μm), and the column temperature was 285°C. The injection and detector temperatures were 300°C, and the carrier gas was N_2 (1.0 mL/min). The samples (2 μL) were analyzed using an flame ionization detector.

2.9. Statistical analysis

All expressed values are means ± standard deviations of triplicate determinations. All statistical analyses were performed using SAS Version 9.3 [22]. Differences were detected using Duncan's multiple range tests and one-way analysis of variance. A p value < 0.05 was considered significant.

Please cite this article in press as: Lee M-H, et al., Quality and characteristics of fermented ginseng seed oil based on bacterial strain and extraction method, Journal of Ginseng Research (2017), http://dx.doi.org/10.1016/j.jgr.2017.03.003