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Research article

Oxidative stability of extracts from red ginseng and puffed red ginseng in bulk oil or oil-in-water emulsion matrix

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ABSTRACT

Background: Explosive puffing can induce changes in the chemical, nutritional, and sensory quality of red ginseng. The antioxidant properties of ethanolic extracts of red ginseng and puffed red ginseng were determined in bulk oil and oil-in-water (O/W) emulsions.
Methods: Bulk oils were heated at 60°C and 100°C and O/W emulsions were treated under riboflavin photosensitization. *In vitro* antioxidant assays, including 2,2-diphenyl-1-picrylhudrazyl, 2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid, ferric reducing antioxidant power, total phenolic content, and total flavonoid content, were also performed.
Results: The total ginsenoside contents of ethanolic extract from red ginseng and puffed red ginseng were 42.33 mg/g and 49.22 mg/g, respectively. All results from above *in vitro* antioxidant assays revealed that extracts of puffed red ginseng had significantly higher antioxidant capacities than those of red ginseng ($p < 0.05$). Generally, extracts of puffed red and red ginseng had high antioxidant properties in riboflavin photosensitized O/W emulsions. However, in bulk oil systems, extracts of puffed red and red ginseng inhibited or accelerated rates of lipid oxidation, depending on treatment temperature and the type of assay used.
Conclusion: Although ethanolic extracts of puffed red ginseng showed stronger antioxidant capacities than those of red ginseng when *in vitro* assays were used, more pro-oxidant properties were observed in bulk oils and O/W emulsions.

1. Introduction

Red ginseng (*Panax ginseng* Meyer) has been consumed as a medicinal herb and a functional food ingredient in some parts of Asia due to its beneficial physiological effects. Red ginseng is produced via a repeated process of steaming and drying fresh ginseng [1]. Explosive puffing can induce changes in the chemical, nutritional, and sensory quality of foods [2–4]. Explosively puffed products undergo some chemical changes including dehydration, gelatinization of carbohydrates, increased product volume, and textural changes due to the explosive release of water vapor pressure from the foods [2,5].

An explosive puffing process has been introduced for the tail roots of dried red ginseng [6] and red ginseng [7] to produce new types of ginseng products. Han et al [6] determined the changes in saponins, total sugars, acidic polysaccharides, phenolic compounds, microstructures, and pepsin digestibility of the tail roots of dried red ginseng processed using the puffing process. Also, volatile changes in puffed tail roots of red ginseng were reported using a simultaneous steam distillation process [8]. Explosively puffed red ginseng was found to have more 2-furanmethanol and maltol and higher porous structures than non-puffed red ginseng [7]. The crude saponin content and minor ginsenosides, including Rg3, F2, Rk1, and Rg5, were found to be increased in puffed red ginseng [2].

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The antioxidant capacities of chemical compounds are influenced by the concentration and polarity of compounds and the environmental conditions under which compounds are located [9–11]. Generally, hydrophilic compounds show better antioxidant capacities in nonpolar media, such as bulk oil systems, while lipophilic compounds inhibit the rates of lipid oxidation more efficiently in more polar media, such as oil-in-water (O/W) emulsions and liposomes; this finding is referred to as the antioxidant polar paradox. Recently, the theory of the antioxidant polar paradox has been re-evaluated and a modification has been suggested [12–14]. It is strongly recommended that the antioxidant capacities of compounds, mixtures, or extracts be tested in real food systems. For example, curcumin [15] and extracts of roasted hulled barley [16] have different antioxidant properties depending on the food matrices, including bulk oil or O/W emulsions.

Although the physicochemical properties, *in vitro* antioxidant capacities, and volatile changes in explosively puffed red ginseng have been reported [2,7,8], studies on the antioxidant capacities in real food matrices have not been reported in the literature.

The objective of this study was to determine the antioxidant properties of ethanolic extracts of red ginseng (ERG) and ethanolic extracts of puffed red ginseng (EPRG) in the different matrices including in corn oil and O/W emulsion and *in vitro* assays.

2. Materials and methods

2.1. Materials

Red ginseng was kindly provided by a local ginseng supplier (Icheon, Gyinggido, Korea). Ginseng was a 6-year-old Korean ginseng cultivated at Geumsan (Chungcheongnam-do, Korea) in 2014. Aluminum chloride, potassium acetate, and 2,2-diphenyl-1-picrylhudrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Folin–Denis' reagent, and 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were purchased from Fluka (Buchs, Switzerland). Isooctane was purchased from Junsei Chemical Co. (Tokyo, Japan) and *p*-anisidine was purchased from Kanto Chemical Co. (Tokyo, Japan). Other reagent grade chemicals were purchased from Daejung Chemical Co. (Seoul, Korea).

2.2. Sample preparation

Puffed red ginseng was prepared according to Lee et al [7]. ERG and EPRG were prepared based on the previous report with some modifications [16]. Briefly, 50 g red ginseng or puffed red ginseng was placed into a 2-L Erlenmeyer flask and 1 L 70% aqueous ethanol was added. The mixture was refluxed for 16 h at 70°C. The mixtures of red ginseng or puffed red ginseng and 70% ethanol were filtered through Whatman #2 filter paper and the filtrate was recovered. The solvent was reduced using a vacuum evaporator and lyophilized using a freeze-drier (Ilshinbiobase Co. Ltd., Gyeonggi, Korea). The yields of ethanolic extracts from red ginseng and puffed red ginseng after lyophilization were 23.1% and 30.0%, respectively (data not shown).

2.3. Sample preparation of O/W emulsion containing ethanolic ginseng extract

O/W emulsions were prepared according to the previous method [17]. Deionized water mixed with 0.25% (w/w) Tween 20 was combined with 2.5% (w/w) corn oil. The mixture was treated using a DE/T 25 homogenizer (IKA Werke, Staufen, Germany) for 3 min and then passed three times using a Nano disperser (ISA – NLM100, Ilshinautoclave Co. Ltd., Daejoen, Korea) at 5,000 psi, which is an O/W emulsion. Riboflavin was added to the emulsion at 0.13mM with overnight mixing. Ginseng extracts were added to the O/W emulsions containing riboflavin at 0.25%, 0.5%, and 1.0% (w/v). Two milliliters of emulsion was put in a 10-mL vial and sealed air tight. Sample vials were placed in a fluorescent light box with 1333 lux light intensity at room temperature and analyzed at 0, 12 h, 24 h, and 36 h. Sample vials were prepared in triplicate at each point. Samples without added ginseng extracts served as controls.

2.4. Sample preparation of corn oil containing ethanolic extracts of ginsengs

Ethanolic extracts of ginsengs dissolved in methanol were added to corn oil at 0.25%, 0.5%, and 1.0% (w/w). The solvent in the mixture was removed under nitrogen gas flushing. The 0.5 g corn oil containing ginseng extracts was put in 10-mL vials and sealed air tight. Sample vials were stored at 60°C for 20 d and 100°C for 27 h in a drying oven (HYSC Co. Ltd., Seoul, Korea). Controls were samples without added extracts of ginsengs. Samples were prepared in triplicate at each sample point.

2.5. In vitro antioxidant assays

The free-radical scavenging ability and radical cation scavenging activity of the ginseng extracts was determined using DPPH and ABTS, respectively according to previous reports with modification [16,17]. The ferric reducing antioxidant power (FRAP) was performed, with some modifications using the method reported by Benzie and Strain [18] and Ka et al [17]. Total phenolic content (TPC) and total flavonoid content (TFC) in the extracts of ginseng was determined according to the previous method [19].

2.6. Analysis of ginsenosides in ethanolic ginseng extracts

The ginsenosides in samples were analyzed using an ultra-HPLC equipped with an autoinjection system and an ultraviolet detector at 203 nm (Hitachi, Tokyo, Japan). Two different columns including a LaChromUltra C_{18} short-length column (2 mm internal diameter × 50 mm long, 2 μm) and a LaChromUltra C_{18} middle-length column (2 mm internal diameter × 100 mm long, 2 μm) were used. Mobile phase was a mixture of 20% acetonitrile (Solvent A) and 80% acetonitrile (Solvent B). The gradient profile was 100% A–0%B (0 min) for 10 min, changed to 25% B in 30 min, 70% B in 10 min, 100% B in 30 min, and returned to 0% B in 5 min, which was then maintained for 5 min. The flow rate for the short-length and for the middle-length column was 0.2 mL/min and 0.3 mL/min, respectively. The temperature of the analytical column was 30°C. Ethanolic extract of ginsengs were dissolved in 20% aqueous acetonitrile solution, filtered through a 0.20-μm polytetrafluoroethylene membrane and 5 μL of the solution was then analyzed. The concentrations of ginsenosides were calculated based on calibration curves prepared using each standard compound [20].

2.7. Headspace oxygen analysis

The degree of oxidation was determined by the depletion of headspace oxygen in air-tight samples of corn oil or O/W emulsions using gas chromatography with a thermal conductivity detector according to the method of Kim et al [21].

2.8. Lipid hydroperoxides in O/W emulsion

Concentration of lipid hydroperoxides were determined using a modified method of Yi et al [15]. Sample (0.3 mL) was mixed with 1.5 mL isooctane/2-propanol (3:2, v:v), vortex-mixed three times, and centrifuged for 3 min at 2000 g. The upper layer of 0.2 mL was

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