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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.

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# 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, Tween 20, riboflavin, and 2,2-diphenyl-1-picrylhudrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Folin–Denis' reagent, and 2,2'-azinobis-3-ethyl-benzothiazoline-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 34.47 Mpa, 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  $60^{\circ}$ C for 20 d and  $100^{\circ}$ 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<sub>18</sub> short-length column (2 mm internal diameter  $\times$  50 mm long, 2  $\mu$ m) and a LaChromUltra C<sub>18</sub> middlelength column (2 mm internal diameter  $\times$  100 mm long, 2  $\mu$ m) 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

collected and mixed with 2.8 mL methanol/1-butanol (2:1, v:v). Thiocyanate/Fe<sup>2+</sup> solution (30  $\mu$ L) was added to the mixture and the mixture was vortex-mixed for 10 s. The samples were incubated for 30 min at room temperature and the absorbance at 510 nm was measured using an UV/VIS-spectrometer (Model UV-1650PC; Shimadzu, Kyoto, Japan). The concentration of lipid hydroperoxide was calculated using a cumene hydroperoxide standard curve.

# 2.9. Conjugated dienoic acid and p-anisidine value analysis in bulk oil

The conjugated dienoic acid (CDA) of samples was measured according to American Oil Chemists' Society method Ti 1a-64 [22] and the *p*-anisidine value (*p*-AV) of oxidized samples was determined according to American Oil Chemists' Society method Cd 18-90 [22] with minor modifications.

### 2.10. Statistical analysis

Data of headspace oxygen content, CDA, and *p*-AV were analyzed statistically via analysis of variance and Duncan's multiple range test using SPSS software version 22 (SPSS Inc., Chicago, II, USA). Data of antioxidant assays and ginsenoside contents were analyzed by Student's *t* test using the same software. A *p* value < 0.05 was considered significant.

# 3. Results and discussion

3.1. Antioxidant activities and ginsenoside profiles in ethanolic extracts of red ginseng and puffed red ginseng determined using in vitro assays

The *in vitro* antioxidant properties of ERG and EPRG analyzed by DPPH, ABTS, FRAP, TPC, and TFC are shown in Fig. 1. The free-radical scavenging activity of ERG and EPRG determined by DPPH assays were 51.0% and 86.2%, respectively, whereas those determined by ABTS assays were 28.7% and 72.2%, respectively. EPRG showed significantly higher free radical scavenging activity than ERG (p < 0.05).

The FRAP in ERG and EPRG were 1.04  $\mu$ g ascorbic acid equivalents/g and 2.34  $\mu$ g ascorbic acid equivalents/g, respectively. The ferric ion reducing ability in EPRG was significantly higher than that of ERG (p < 0.05).

The TPC in ERG and EPRG was 1.32 µg tannic acid equivalent/g and 6.72 µg tannic acid equivalent/g, respectively, while the TFC was 0.27 µg quercetin acid equivalent/g and 1.35 µg quercetin acid equivalent/g, respectively. Therefore, the amount of phenolic compounds including flavonoids in EPRG were significantly higher than those in ERG (p < 0.05). Based on the results of these *in vitro* assays, EPRG demonstrated to have significantly higher *in vitro* antioxidant properties than ERG (p < 0.05).



**Fig. 1.** *In vitro* antioxidant properties of ethanolic extract of red ginseng and puffed red ginseng by (A) DPPH, (B) ABTS, (C) FRAP, (D) TPC, and (E) TFC. Normal and Puffing were ethanolic extracts of red ginseng and puffed red ginseng, respectively. Bars with \* and \*\*\* were significantly different from each other at p = 0.05 and p = 0.001, respectively, and conducted by Student *t* test. ABTS, 2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhudrazyl; FRAP, ferric reducing antioxidant power; TFC, total ferric content; TPC, total phenolic content.

This enhancement of the *in vitro* antioxidant properties could be due to the extra puffing process, which may help to convert the bound forms of phenolics into free forms or to generate stronger antioxidants from the less active forms in ginsengs. Kang et al [23] reported that heat-treated ginseng, including red ginseng and ginseng steamed at 120°C, showed better superoxide anion ( $\cdot$ O<sub>2</sub>), peroxynitrite (ONOO<sup>-</sup>) and hydroxyl radical scavenging activities than white ginseng. Extrusion cooking of red ginseng was shown to increase DPPH radical scavenging activity and reducing power [24]. Red ginseng extracts completely eliminated DPPH radicals at 2 mg/ mL [25].

The profiles of ginsenosides in ERG and EPRG are shown in Table 1. The total ginsenoside contents in ERG and EPRG were 42.33 mg/g and 49.22 mg/g, respectively. The content of Rb1 was the highest followed by Re and Rg1 in both ERG and EPRG (Table 1). Generally, the puffing process increased ginsenoside content significantly, including total contents, Rg3, Rb1, Re, and Rf (p < 0.05). In particular, ginsenoside Rg3(S) was substantially increased from 0.23 mg/g in red ginseng to 0.46 mg/g in puffed red ginseng, which is about a two fold increase. Ginsenoside Rg3 has various functional activities, including being tumor-suppressive, hepatoprotective [26], immunostimulating, antifatigue [27], and anti-inflammatory [28]. An et al [2] reported that the puffing process increased minor ginsenosides, including Rg3, F2, Rk1, and Rg5, while decreasing major ginsenosides, including Rb1, Rb2, Rc, Rd, Re, and Rg1. However, in this study, the amounts of Rb2 and Rb3 decreased and those of other ginsenosides increased after the puffing process (Table 1). Differences in extraction procedures or thermal instability of ginsenosides may induce changes in the profiles of ginsenosides [29]. The enhanced in vitro antioxidant activities in puffed red ginseng extracts could be partly due to increases in ginsenosides or to the generation of a specific ginsenoside such as Rg3(S).

# 3.2. Oxidative stability of red ginseng and puffed red ginseng extracts in O/W emulsions

The effects of ERG (A) and EPRG (B) (0-1.0% w/w) on headspace oxygen in O/W emulsions under riboflavin photosensitization are shown in Fig. 2. After 12 h of treatment, samples containing 1.0% ERG and EPRG showed the highest headspace oxygen content, which implied that the 1.0% ginseng extracts acted as antioxidants.

Table 1									
Profiles of ginse	nosides in ethan	olic extracts	of red	ginseng	and p	ouffed	red	ginser	ıg

	Ginsenoside content in red ginseng (mg/g)	Ginsenoside content in puffed red ginseng (mg/g)
Rg1 Re	$\begin{array}{c} 6.02 \pm 0.09 \\ 7.11 \pm 0.29 \end{array}$	$\begin{array}{c} 7.86 \pm 0.42^{***} \\ 8.15 \pm 0.31^{**} \end{array}$
Rf	$1.71\pm0.09$	$2.15 \pm 0.07^{***}$
Rb1	$11.72\pm0.19$	$14.04 \pm 0.08^{***}$
Rc	$5.37 \pm 0.12$	$5.76 \pm 0.10^{**}$
Rb2	$5.99\pm0.18^*$	$5.74 \pm 0.08$
Rb3	$\textbf{0.90} \pm \textbf{0.07}$	$\textbf{0.85} \pm \textbf{0.04}$
Rd	$\textbf{1.29}\pm\textbf{0.09}$	$1.52 \pm 0.02^{**}$
Rg2(S)	$\textbf{0.94} \pm \textbf{0.03}$	$1.18 \pm 0.03^{***}$
Rg3(S)	$\textbf{0.23} \pm \textbf{0.01}$	$0.46 \pm 0.01^{***}$
Rg3(R)	$\textbf{0.42} \pm \textbf{0.06}$	$\textbf{0.59} \pm \textbf{0.07}^{*}$
Rh1(S)	$\textbf{0.14} \pm \textbf{0.02}$	$0.25 \pm 0.03^{***}$
Total ginsenoside contents	$42.33\pm0.92$	$49.22 \pm 0.68^{***}$

Data are presented as mean  $\pm$  standard deviation (n = 3)

\*, \*\*, and \*\*\* were significantly different between red ginseng and puffed red ginseng at *p* = 0.05, *p* = 0.01, and *p* = 0.001, respectively



**Fig. 2.** Effects of ethanolic extract of (A) red ginseng and (B) puffed red ginseng (0– 1.0% w/w) on the headspace oxygen in oil-in-water emulsions under riboflavin photosensitization at room temperature. Different letters indicate significant differences at the same time point at p < 0.05.

The headspace oxygen contents in samples containing 0, 0.25%, 0.50%, and 1.0% ERG after 36 h were 17.0%, 17.5%, 18.3%, and 19.3%, respectively, indicating that ERG inhibited the consumption of headspace oxygen in a concentration-dependent manner (Fig. 2A). However, EPRF showed a different pattern of headspace oxygen consumption after 24 h and 36 h of treatment compared to equivalently treated ERG. After 36-h treatment, all the samples containing EPRG showed lower headspace oxygen contents than controls, which implied that EPRG accelerated the consumption of headspace oxygen molecules (Fig. 2B). The oxygen molecules may have been consumed by unsaturated fat in corn oils and/or oxidation of phenolic compounds. Because EPRG had significantly higher levels of phenolic consumption by phenolics could not be ruled

out. Therefore, excess phenolics in EPRG could be targets for oxygen consumption.

Changes in lipid hydroperoxides in O/W emulsions under riboflavin photosensitization containing ERG (A) and EPRG (B) are shown in Fig. 3. The antioxidant properties of ERG in O/W emulsions were influenced by the extract concentration and the puffing process. The 0.25% ERG did not show antioxidant properties while 0.50% and 1.0% ERG acted as antioxidants based on lipid hydroperoxides (Fig. 3A). Lipid hydroperoxides in O/W emulsion containing 1.0% EPRG were significantly lower than those of controls after 36 h (p < 0.05) (Fig. 3B). However, 0.25% and 0.5% EPRG did not inhibit the formation of lipid hydroperoxides over 24 h (p > 0.05; Fig. 3B).



**Fig. 3.** Changes of lipid hydroperoxides in oil-in-water emulsions under riboflavin photosensitization containing ethanolic extract of (A) red ginseng and (B) puffed red ginseng -at room temperature. Different letters indicate significant differences at the same time point at p < 0.05.

# 3.3. Oxidative stability of ethanolic extracts of ginseng in bulk oil

Changes in headspace oxygen, CDA, and *p*-AV in corn oil with addition of ERG and EPRG (0–1.0% w/w) at 60°C and 100°C are shown in Tables 2 and 3, respectively. Headspace oxygen content in bulk oil containing EPRG was found to be increased in a concentration-dependent manner (0.25%, 0.50%, and 1.0%) compared to in the control after 20 d. CDA and *p*-AV in bulk oil containing EPRG were lower than those of controls, which agrees with the results of our headspace oxygen assay. Therefore, EPRG had antioxidant properties in bulk oil at 60°C when the concentration was > 1.0% (Table 2). However, ERG showed different patterns. Based on the results of the headspace oxygen content assay after 20 d storage, 0.25% and 0.50% ERG acted as pro-oxidants, while 1.0% ERG acted as an antioxidant. Results from CDA and *p*-AV assays showed no significant differences among samples (p > 0.05; Table 2).

The headspace oxygen content in bulk oil containing 1.0% EPRG was significantly lower than that of a control at 100°C after 27 h, while samples containing 1.0% ERG had significantly higher headspace oxygen content than controls (p < 0.05; Table 3). These results indicate that EPRG and ERG accelerated or retarded the consumption of headspace oxygen molecules, respectively. Samples containing 0.25% and 0.50% ERG and EPRG were lower than controls. This trend was also observed in both CDA and p-AV assays. EPRG acted as a pro-oxidant when present at concentrations ranging from 0.25% to 1.0%, while 0.25% and 0.50% ERG showed prooxidant properties and 1.0% ERG acted as an antioxidant based on the results of CDA and *p*-AV assays after 27 h of treatment at 100°C (Table 3). CDA is a typical assay for primary oxidation products whereas *p*-AV can detect alkenals, which are secondary oxidation products [3]. The oxidative stability in oils containing EPRG was greatly influenced by the thermal temperature. Relatively high temperatures like 100°C may change the thermal stability of phenolics and enhance the reaction rates of chemical reactions relative to samples incubated at 60°C.

In vitro assays of extracts of natural resources or standard chemical compounds with high antioxidant capacities may not show similar antioxidant properties in a food matrix [15-17]. Radical scavenging ability (DPPH or ABTS assays) or ferric ion reducing capacity (FRAP assay) can provide information on the chemical potential of compounds based on their structural characteristics. However, polarity and the concentration of compounds are important factors in real food matrices like bulk oil and O/W emulsions [10,12,14]. For example, curcumin, which inhibited lipid oxidation in O/W emulsions, did not act as an antioxidant nor as a pro-oxidant in corn oil [15]. Ka et al [17] showed that amino acid cysteine had the highest antioxidant properties followed by tryptophan and tyrosine using in vitro assays, whereas tyrosine inhibited lipid oxidation whereas tryptophan acted as a prooxidant in O/W emulsion under riboflavin photosensitization. Aqueous extracts of hulled barley (Hordeum vulgare L.) had antioxidant or pro-oxidant properties in bulk oil, while they showed antioxidant properties in riboflavin-photosensitized O/W emulsions [16]. Phenolic compounds with proper polarity showed higher antioxidant capacities in O/W emulsions than those with lower or higher polarity, which was tested using phenolipids such as rosmarinate esters or chlorogenate esters [10,12]. Additionally, Shahidi and Zhong [14] proposed that the concentration of phenolic compounds plays an important role in determining the antioxidant properties of these compounds in bulk oils.

The interface of lipid and water in association colloids where antioxidant or pro-oxidant compounds could be located can be critical places for the regulation of the rates of lipid oxidation [30]. Puffing process significantly increased TPC and TFC in the extracts

Table 2
Changes of headspace oxygen, CDA, and <i>p</i> -anisidine values in corn oil with addition of ethanolic extract of red ginseng and puffed red ginseng (0–1.0% w/w) at 60°C treatment

	Time (d)	Red ginseng				Puffed red ginseng				
		0 %	0.25 %	0.5 %	1.0 %	0 %	0.25 %	0.5 %	1.0 %	
Headspace	0	$20.80\pm0.01^{ab}$	$20.80\pm0.01^a$	$20.80\pm0.01^a$	$20.80\pm0.01^a$	$20.80\pm0.01^a$	$20.80\pm0.01^a$	$\textbf{20.80} \pm \textbf{0.01}^{a}$	$20.80\pm0.01^a$	
oxygen (%)	10	$19.73\pm0.05^{c}$	$19.77\pm0.09^{c}$	$19.91\pm0.06^{b}$	$20.11\pm0.01^a$	$19.73\pm0.05^{a}$	$\textbf{18.97} \pm \textbf{1.42}$	$19.92\pm0.19^a$	$20.27 \pm 0.02^a$	
	15	$17.62\pm0.15^{ab}$	$17.03\pm0.12^{b}$	$16.83 \pm 0.83$	$18.08\pm0.28^a$	$17.62\pm0.15^a$	$15.78\pm1.77^{\mathrm{b}}$	$17.74\pm0.57^a$	$18.65\pm0.05^a$	
	20	$13.73\pm0.58^{ab}$	$12.63\pm0.90^{b}$	$11.80 \pm 1.90^{b}$	$15.14\pm0.01~^a$	$13.73\pm0.58^{d}$	$14.35\pm0.14^{c}$	$15.14\pm0.03^{b}$	$16.51\pm0.11^a$	
CDA (%)	0	$0.21\pm0.01^{ab}$	$0.21\pm0.01^{ab}$	$0.20\pm0.01^{b}$	$0.21\pm0.01^a$	$0.21\pm0.01^{ab}$	$0.21\pm0.01^{ab}$	$0.20\pm0.01^{\rm b}$	$0.21\pm0.01^a$	
	10	$\textbf{0.39} \pm \textbf{0.01}$	$0.39\pm0.01^a$	$\textbf{0.37} \pm \textbf{0.01}^{b}$	$0.34\pm0.01^{c}$	$\textbf{0.39}\pm\textbf{0.01}^{a}$	$\textbf{0.40} \pm \textbf{0.02}^{a}$	$\textbf{0.38} \pm \textbf{0.02}$	$\textbf{0.30} \pm \textbf{0.01}$	
	15	$1.18\pm0.06^{bc}$	$\textbf{1.33} \pm \textbf{0.06}$	$1.38\pm0.16^a$	$1.07\pm0.02$	$1.18\pm0.06^{b}$	$1.57\pm0.33^a$	$1.10\pm0.04^{b}$	$0.87\pm0.02^{b}$	
	20	$2.27\pm0.55^a$	$\textbf{2.26} \pm \textbf{0.20}^{a}$	$\textbf{2.37} \pm \textbf{0.28}^{a}$	$2.15\pm0.65^a$	$\textbf{2.27} \pm \textbf{0.55}^{a}$	$1.89\pm0.03^{ab}$	$1.71 \pm 0.01$	$1.56\pm0.27^{b}$	
p-Anisidine	0	$11.98\pm0.33~^a$	$12.37\pm0.33^a$	$11.42\pm0.63^a$	$12.22\pm0.70^a$	$11.98\pm0.33^{a}$	$12.37\pm0.33^a$	$11.42\pm0.63^a$	$12.22\pm0.70^a$	
	10	$12.65\pm0.30^a$	$10.20\pm0.01^c$	$9.67\pm0.55^c$	$11.48\pm0.85^{\mathrm{b}}$	$12.65\pm0.30^{ab}$	$13.08 \pm 1.23^{a}$	$12.20 \pm 0.46^{a\ b}$	$11.33\pm0.72$	
	15	$12.42\pm0.49^{\mathrm{b}}$	$13.23\pm0.43^a$	$13.95\pm0.51^a$	$13.25\pm0.17^a$	$12.42\pm0.49^{b}$	$15.15\pm1.82^{a}$	$13.08\pm0.53^a$	$13.13\pm0.49^a$	
	20	$\textbf{18.83} \pm \textbf{2.98}^{a}$	$19.75\pm2.67^a$	$21.40\pm3.75^a$	$21.37\pm8.43^a$	$\textbf{18.83} \pm \textbf{2.98}^a$	$15.10\pm0.30^{ab}$	$13.73\pm0.29^{b}$	$15.83\pm4.04^{ab}$	

Data are presented as mean  $\pm$  standard deviation (n = 3)

<sup>a,b,c</sup> In the same row, different letters are significantly different at p = 0.05

CDA, conjugated dienoic acid

Table 3

Changes of headspace oxygen, CDA, and p-anisidine values in corn oil with addition of ethanolic extract of red ginseng and puffed red ginseng (0–1.0% w/w) at 100°C treatment
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	Time (h)	Red ginseng				Puffed red ginseng			
		0 %	0.25 %	0.5 %	1.0 %	0 %	0.25 %	0.5 %	1.0 %
Headspace	0	$20.80\pm0.01^{ab}$	$20.80\pm0.01^a$	$20.80\pm0.01^a$	$20.80 \pm 0.01^a$	$20.80 \pm 0.01^a$	$20.80\pm0.01^a$	$20.80\pm0.01^a$	$20.80\pm0.01^a$
oxygen (%)	9	$20.17 \pm 0.04^{b}$	$20.22 \pm 0.04^{b}$	$20.27\pm0.08^{a\ b}$	$20.39\pm0.13^a$	$20.17 \pm 0.04^{b}$	$20.23 \pm 0.05^{b}$	$20.24 \pm 0.03^{b}$	$20.45 \pm 0.03^a$
	18	$18.63\pm0.20^{b}$	$18.08\pm0.26^{c}$	$18.70\pm0.26^{b}$	$19.35\pm0.18^a$	$18.63\pm0.20^{b}$	$18.01\pm0.25^{c}$	$18.55\pm0.15^{b}$	$19.19\pm0.11^a$
	27	$13.91\pm0.70^{b}$	$11.23\pm0.27^{c}$	$13.40\pm0.36^b$	$15.04\pm0.12^a$	$13.91\pm0.70^a$	$10.0\pm0.7^{b}$	$10.14\pm1.39^{b}$	$11.57 \pm 1.04^{\text{b}}$
CDA (%)	0	$0.21\pm0.01^{ab}$	$0.21\pm0.01^{ab}$	$0.20\pm0.01^{b}$	$0.21\pm0.01^a$	$0.21\pm0.01^{ab}$	$0.21\pm0.01^{ab}$	$0.20\pm0.01^{b}$	$0.21\pm0.01^a$
	9	$0.37\pm0.01^a$	$0.36\pm0.01^{ab}$	$0.35\pm0.01^{b}$	$0.31\pm0.01^{c}$	$0.37\pm0.01^a$	$\textbf{0.36} \pm \textbf{0.01}^a$	$\textbf{0.36} \pm \textbf{0.01}^{a}$	$0.30\pm0.01^{b}$
	18	$0.90\pm0.02^{b}$	$1.03\pm0.09^a$	$0.90\pm0.04^{b}$	$\textbf{0.77} \pm \textbf{0.07}^c$	$0.90\pm0.02^{b}$	$\textbf{1.07} \pm \textbf{0.05}$	$0.96\pm0.01^{b}$	$\textbf{0.76} \pm \textbf{0.03^c}$
	27	$1.82\pm0.02^{b}$	$\textbf{2.12} \pm \textbf{0.06}^{a}$	$1.90\pm0.05^{b}$	$1.69\pm0.04^{c}$	$1.82\pm0.02^{b}$	$\textbf{2.26} \pm \textbf{0.17}^{a}$	$\textbf{2.20} \pm \textbf{0.03}^{a}$	$2.01\pm0.11^{b}$
p-Anisidine value	0	$11.98\pm0.33^a$	$12.37\pm0.33^a$	$11.42\pm0.63^a$	$12.22\pm0.70^a$	$11.98\pm0.33^a$	$12.37\pm0.33^a$	$11.42\pm0.63^a$	$12.22\pm0.70^a$
	9	$9.42\pm0.21^{b}$	$\textbf{8.62}\pm\textbf{0.16}^{b}$	$8.12\pm0.46^{\rm b}$	$16.65 \pm 1.31^{a}$	$9.42\pm0.21^a$	$\textbf{9.48} \pm \textbf{0.36}^{a}$	$9.43\pm0.52^a$	$\textbf{9.08} \pm \textbf{0.16}^{a}$
	18	$11.18\pm0.40^{b}$	$12.92\pm0.98^a$	$12.08\pm0.03^{ab}$	$12.17 \pm 0.99^{ab}$	$11.18\pm0.40^{c}$	$12.87\pm0.68^{ab}$	$13.05\pm0.30^a$	$11.60 \pm 1.13$
	27	$\textbf{32.67} \pm \textbf{1.11}^{c}$	$50.47\pm3.68^a$	$\textbf{38.15} \pm \textbf{0.15}^{b}$	$30.57 \pm 0.90^c$	$\textbf{32.67} \pm \textbf{1.11}$	$\textbf{73.32} \pm \textbf{14.48}^a$	$61.27\pm6.7^{ab}$	$51.83 \pm 8.06^{b}$

Data are presented as the mean  $\pm$  standard deviation (n = 3)

 $^{a,b,c}$  In the same row, different letters are significantly different at p = 0.05

CDA, conjugated dienoic acid

compared to unpuffed red ginseng (Fig. 1) and those phenolic compounds could be located on the surface of association colloids in O/W emulsions and show enhanced antioxidant capacity (Figs. 2 and 3). However, extracts did not have antioxidant activity in bulk oils, especially at 100°C treatment. In the case of bulk oil systems, heat treatment can induce other chemical reactions, like the degradation of phenolic compounds, in addition to lipid oxidation. Depending on the assays used to for determine the degree of lipid oxidation, slightly different oxidative properties of the extracts were observed. The results on the consumption of headspace oxygen and of CDA in bulk oil matched each other at 100°C, while those of 2-alkenals were different, particularly at a concentration of 1.0% (Table 3). It is well known that the results of measuring antioxidant capacities differ depending on the types of assays used due to the differences in the principles and limitation of each assay. Therefore, it is advisable to use a combination of assays that cover the detection of both primary and secondary lipid oxidation products, including oxygen molecules, conjugated dienes, anisidine values, and volatiles [15,31].

#### 4. Conclusion

Using *in vitro* assays, we demonstrated that EPRG had higher antioxidant activity than ERG. The puffing process increased the total ginsenoside content and the content of Rg3. In riboflavinphotosensitized O/W emulsions, ERG showed concentrationdependent high antioxidant activity. However, in bulk oil systems, ERG and EPRG had antioxidant or pro-oxidant properties depending on the treatment temperature, concentration of the extracts, and types of assays used. Therefore, the antioxidant or pro-oxidant properties of red ginseng should be tested in food systems as well as in *in vitro* assays.

# **Conflicts of interest**

All authors have no conflicts of interest to declare.

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# Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgr.2017.04.002.

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