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A Thesis for the Degree of Doctor of Philosophy

Biofunctional characterization of puffed red ginseng

膨化紅參的生理性質研究

by

Sang Jun Lee

February, 2017

Department of Agricultural Biotechnology

College of Agriculture and Life Sciences

Seoul National University
A Thesis for the Degree of Doctor of Philosophy

Biofunctional characterization of

puffed red ginseng

彭化홍삼의 생리기능성 구명

by

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February, 2017

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Abstract

Ginseng (Panax ginseng C.A. Meyer) has been cultivated and consumed as a medicinal herb in East Asia for a long time. Ginseng has a lot of bioactive components including ginsenosides, polyacetylenes, polysaccharides, and phenolic compounds. Among them, ginsenosides have been regarded as major active components of ginseng and used as index component for the quality control. Many researches have been conducted to develop methods for increasing the pharmacological effect of ginseng by conversion of the dammarane-based saponin by high temperature and high pressure thermal processing. However, it is complicated and time-consuming to extract the active components of ginseng because of its dense texture. Thus, researchers have conducted the studies on the production of expanded ginseng using an extruder and explosive puffing process.

This study was designed to examine the effect of puffing process on the biofunctional property of red ginseng. Red ginseng was puffed using a rotary puffing machine at 0.30 MPa. After puffing, the changes in physicochemical properties, antioxidant activity and volatile components in puffed red ginseng were investigated. Puffing process increased the total ginsenoside content including ginsenoside Rg3 with anticancer activity. Extraction yields (16.7-42.2%) from puffed red ginseng were higher than those from non-puffed red
ginseng (9.0-32.7%) at all extraction times. When comparing the free sugars and amino acids, the contents of maltose and arginine drastically decreased because puffing process accelerated the reaction of maltose and arginine to produce maltulosyl arginine.

Effects of explosive puffing on the changes of volatiles in red ginseng were investigated using headspace-solid phase microextraction (HS-SPME)-gas chromatograph (GC) with a mass selective detector (MS). Formation of porous structures and smaller pieces were clearly observed on the surface of puffed red ginseng by scanning electron microscopy. Total volatiles in puffed red ginseng increased by 87% compared with those in red ginseng. Hexanal, Δ-selinene, and β-panasinsene were major volatiles in red ginseng, whereas α-gurjunene, β-panasinsene, and calarenne were main volatiles in puffed red ginseng. Puffing process decreased volatiles from lipid oxidation including aldehydes, ketones, and 2-pentylfuran and increased terpenoids in red ginseng. Selective ion monitoring (SIM) mode for GC/MS results showed that 2-furanmethanol and maltol were present at the concentrations of 0.20 and 0.24%, respectively, in red ginseng and 5.86 and 3.99%, respectively, in puffed red ginseng. Explosive puffing process increased 2-furanmethanol and maltol in puffed red ginseng significantly ($p<0.05$) with the changes of microstructure.

The antioxidant properties of extracts of red ginseng and puffed red ginseng were determined in bulk oil and oil-in-water (O/W) emulsions. 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-picrylhydrazyl (DPPH), 2,2’-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP), total phenolic content (TPC), and total flavonoid content (TFC), were also performed. The total ginsenoside contents of extract from red ginseng and puffed red ginseng were 42.33 and 49.22 mg/g, respectively. All results from these *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 antioxidant properties in riboflavin photosensitized O/W emulsions. However, in bulk oil systems, extracts of puffed red and red ginseng inhibited or accelerated rate of lipid oxidation, depending on the treatment temperature and the type of assay used.

These results suggest that the puffing process can provide us with an alternative means to produce functional red ginseng products with the additional advantage of reduced processing time.

**Keywords:** puffed red ginseng, volatile component, ginsenoside, antioxidant property, bulk oil, oil-in-water emulsion, radical scavenging activity.
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Chapter 1.

Introduction
1.1. Background

Ginseng occupies a prominent position in the list of best-selling natural products in the world (Qi et al., 2011). Ginsenosides have been regarded as major active components of ginseng and used as index components for the quality control (Nam et al., 1998). Therefore, the researches has been conducted to develop the methods for increasing the pharmaceutical effect of ginseng.

However, it is complicated and time-consuming to extract the active components of ginseng because of its dense texture (Gui and Ryu, 2014). Thus, researchers have conducted the studies on the production of expanded ginseng using an extruder and explosive puffing process (An et al., 2011).

There have been a few studies on the extrusion and puffing process applied to ginseng. Ha and Ryu (2005) conducted the studies using the extrusion to improve the physical and chemical properties of ginseng sample. They reported that acidic polysaccharide content increased by 2-3% and ginsenoside Rg3 was detected in extruded red ginseng after extrusion cooking. Han et al. (2006) reported that α-amylase susceptibility of extruded ginseng has been found to be higher than that of traditionally dried ginseng. Like this, although extrusion is an efficient and widely used industrial technology for the production of expanded product from ginseng, it has disadvantages that ginseng sample has to be ground and there are a
lot of parameters to be controlled.

In comparison with extrusion, puffing process is simple and low cost industrial technology which small company can operate. Han et al. (2007) studied the effect of puffing treatment on ginsenosides, acidic polysaccharide, and pepsin digestibility of dried red ginseng tail root. They reported that acidic polysaccharide content was slightly decreased and pepsin digestibility was increased by puffing treatment. Kim et al. (2008) elucidated the elevated production of ginsenoside Rg3 with some changes in the chemical structure of major ginsenosides after puffing.

So far, while most of researches of puffing and extrusion process have been focused on the change of the bioactive constituents of ginseng, such as ginsenosides, little attention has been given to the research of the change of physicochemical properties, antioxidant activity and volatile components after puffing process. Therefore, this study was conducted to investigate the effects of puffing process on the biofunctional properties.
1.2. Botanical species of ginseng

Panax ginseng has been used for medicinal purposes for centuries. The term ginseng is derived from Chinese “jen-shen”, which means “images of man” (Hosettmann and Marston, 1995). Ginseng roots physically resemble the human body. Russian botanist Carl Anton von Meyer botanically named the ginseng as Panax ginseng C.A. Meyer in 1843 (Court, 2000). In Greek, “pan” means all and “axos” means cure. As a whole, Panax indicates “all-heal” (Jia and Zhao, 2009). Ginseng has been used as a traditional medicine in Asian countries for more than 2000 years.

Traditionally, ginseng refers to the root of Panax ginseng, and the other parts of ginseng, such as the leaves and berries, are rarely used (Baek et al., 2012). According to the report (Ahn et al., 2008), the contents of each ginsenoside reveal significant differences between the epidermal part and inner part of the ginseng root. Some experimental results reveal that the concentration of protopanaxadiol ginsenosides is shown to be high in the epithermal parts but is low inside the body part. Protopanaxatriol ginsenosides are evenly distributed in all parts of red ginseng relatively (Lee et al., 2015).

Ginseng is also named after its native habitat, such as Korean, Chinese, American, and Japanese ginseng. Of these, ginseng (Korea) is the most well-
known ginseng with potent pharmacological efficacies (Keifer et al., 2003). Chinese ginseng is *P. notoginseng* (Burk.) F. H. Chen native to China. American ginseng is *P. quinquefolius* L. native to North America, including Canada and United States. These 3 species are widely used as a functional food and in traditional medicine. In addition, *P. japonicus* Meyer (Japanese ginseng), *P. pseudoginseng* subspecies *Himalacius* (Himalayan ginseng), and *P. trifolius* (dwarf ginseng) are lesser known *Panax* species (Angelova et al., 2008).
1.2.1. Types of ginseng preparations

*P. ginseng* cultivated in Korea is harvested after 4 to 6 years of cultivation, and it is classified into three types depending on how it is processed. Fresh ginseng refers to immediate harvest without any additional processing. It is easily deteriorated at room temperature. Therefore, fresh ginseng is normally processed in the forms (white and red ginseng) with lower water content and longer shelf-life compared to fresh ginseng (Park et al., 2001).

White ginseng is produced by drying fresh ginseng, while red ginseng is produced by multiple steps of steaming and then drying. Red ginseng exhibits greater bioactivities than white ginseng due to their much more ginsenoside contents (Wang et al., 2007) and is commonly used as herbal medicines in South Korea.

The basic process of red ginseng production from fresh ginseng simply consists of three steps of washing, steaming, and drying. Fig. 1-1 is a diagram for manufacturing red ginseng in the major companies of red ginseng in Republic of Korea. Most manufacturers are producing red ginseng by using the traditional production processes which can be summarized as follows (Lee et al., 2015). The fresh ginseng grown for 6 years is selected by size and shape, the dirt is shaken off and then the root is washed with clean water. Subsequently, washed fresh ginseng is steamed for 1-3h at 90-98℃. Then the steamed ginseng is dried by hot air and laid in the sun until its moisture contents drops to 15% and 18%.
Fig. 1-1. Manufacturing process of red ginseng from fresh ginseng (Lee et al., 2015).

- **Fresh Ginseng**: The fresh ginseng grown for six years is elected by size and shape, shaken off the dirt.

- **Washing**: The fresh ginseng is washed with spraying water to remove foreign matter such as soils.

- **Steaming**: The fresh ginseng is steamed at 90-98°C, 1-3h, for proper gelatinization of ginseng starch.

- **Drying**: Moisture content will be 15-18% in mechanical drying 45-55°C and sundrying successively.

- **Packaging**: Red ginseng sealed from oxygen can be stored for up to 10 years.
Red ginseng is not skinned before it is steamed or otherwise heated and subsequently dried. During the steaming process, ginseng starch is gelatinized, causing an increase in saponin content (Lee et al., 2013; Lee, 2014).

Black ginseng is prepared by repeated cycles of atmospheric steaming and sun-drying (Yun et al., 2010). Taekuksam is the fresh ginseng blanched in the water and dried (Baeg and So, 2013). Other types of processed ginseng have been developed using modified steaming, explosive puffing, or fermentation with specific microorganisms, such as intestinal microbial flora. Puffed ginseng is prepared by applying an optimized puffing pressure and residual moisture content in ginseng (Han et al., 2008).

Fermentation with intestinal microbial flora leads to structural modification of the ginsenosides. Compound K, the intestinal metabolite of ginsenoside, is a representative bioengineered ginsenoside that shows potent anti-cancer effects (Choi et al., 2009). New types of processed ginseng preparations will be developed in the future.
1.2.2. Puffing process

The puffing phenomenon results from the sudden expansion of water vapour (steam) in the internal structure of the granule. Puffing process involves the release of gas within a product either to expand or rupture an existing structure (Dutta et al., 2015). The puffing processes can be divided into two types, atmospheric pressure procedures and pressure-drop processes (Luh, 1991). Atmospheric pressure procedures rely on the sudden application of heat to obtain the necessary rapid vaporization of water. Pressure-drop procedures involve sudden transferring of superheated water vapour into a space at lower pressure. In this case, the pressure drop may be achieved by releasing the seal on a vessel containing a product that has been equilibrated with high-temperature steam. The treatment of heating the sample for puffing is generally done either with hot sand, hot air or hot oil as well as by gun-puffing where the grains at high temperature and pressure are suddenly released to atmospheric condition (Chandrashekhar and Chattopadhyay, 1990).

Commercial puffing is largely conducted by two processes, gun-puffing and oven-puffing (Kim et al., 2008). The former process is much more widely used. Gun-puffing may result in an increase of apparent volume (bulk density decrease) of six- to eightfold. Oven puffing causes a lesser increase about three- to four fold. Oven-puffing is performed using high-temperature, short-time (HTST) treatment with heated sand. In either case, the sample is expanded by the dehydration
resulting from the rapid diffusion of the water vapour out of it. Puffing process is accompanied by some chemical reactions including dehydration, gelatinization of carbohydrates, increase of the product volume, and textural changes (Hoke et al., 2007). As a result of that, the changes of the nutritional and sensory quality of foods can be induced (Lee et al., 2009; Mariotti et al., 2006).

Although ginseng shows various nutraceutical effects without appropriate processing, the bioavailability tends to be very low because the structure of the cell walls is tough and rigid. Puffing process in ginseng factory has been tried as an efficient method to reduce the extraction time and improve the extraction yield (An et al., 2011).

A lot of studies revealed the change of physicochemical properties and moisture absorption of puffed red ginseng. Kim et al. (2009) examined the changes in the chemical components of red and white ginseng after puffing. Explosive puffed red ginseng had more 2-furanmethanol and maltol and higher porous structures than non-puffed red ginseng (Lee et al., 2010). An et al (2011) reported that puffed red ginseng showed higher crude saponin contents (201.0-219.0 mg/g extract) than non-puffed one (161.7-189.0 mg/g extract). Kim et al (2008) observed in HPLC analysis that amounts of measured major ginsenosides (Rb1, Rb2, Re, Rd, Re, and Rg1) decreased with increasing puffing pressure, and ginsenoside Rg3 was produced after puffing. This fact means that chemical structure of some ginsenosides might be altered during the puffing process.
1.2.3. Ginsenosides

Saponin was first mentioned as an active ingredient of ginseng in 1957 by Brekhman of Russia, and its structure was elucidated and named as ginsenoside by the Shibata research group of Japan (Shibata et al., 1963; Cho et al., 2013). Ginsenoside is one of the derivatives of triterpenoid dammarane consisting of thirty carbon atoms (Lee et al., 2007). Ginsenosides are different from general saponins, since they have very mild effects and show much less toxicity even at high doses (Nah, 1997). Ginsenoside has been used to establish the quality specifications of ginseng (Cho et al., 2013).

Shibata et al. (1966) separated saponin components of ginseng using thin layer chromatography and named ginsenoside Rx (x=o, a, b1, b2, c, d, e, f, g1, g2, g3, h1, h2) with the increasing order of Rf value. Rg1 was first identified, and recently the chemical structure of many ginsenoside Rx has been determined (Jee et al., 2014). As shown in Fig. 1-2, ginsenosides are divided into three types by the differences in the aglycone. They are protopanaxadiol ginsenoside, protopanaxtriol ginsenoside, and oleanan-type saponin. As four-ring structure triterpenoid dammarane-type saponin, there are protopanaxadiol (Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rg5, Rh2) and protopanaxatriol (Re, Rf, Rg1, Rg2, Rh1). Oleanan-type saponin (Ro) has five-ring structure and is not a dammarane group glycoside (Cho et al., 2014). The chemical structures of ginsenosides are different from each other in the linkage position and kind of sugar (Anoja et al., 1999).
Fig. 1-2. Structures of ginsenosides based on chemical structure, there are two major groups: protopanaxadiol (A) and protopanaxatriol (B), ginsenoside Ro, a nonsteroidal saponin, is shown in (C).
Each type of ginsenoside has at least three side chains called R1, R2, and R3 and these three side chains are free or connected with sugars containing a monomer, dimmer, or trimer. With the development of modern technology, more than 150 ginsenosides have been isolated from *Panax* species (Christensen, 2009; Shi et al., 2010). In the aspect of quantity in ginseng, the main ginsenosides of raw ginseng are the ginsenoside Rb1, Rb2, Rc, Rd, Re, Rg1 and Rf (Lu et al., 2009). Park (2004) reported that the major ginsenosides of *Panax* ginseng were Rb1, Rc, Rg1, Re, Rb2, Rd in the order of their prevalence and they made up about 90% of the total amount of ginsenosides.

The amounts of some major protopanaxadiol ginsenosides in red ginseng such as ginsenosides Rb1, Rc, Rb2, and Rd are higher than the amounts of those in fresh ginseng (Lee et al., 2015). The reason is that protopanaxadiol ginsenosides are contained as malonyl ginsenosides in fresh ginseng and the malonyl ginsenosides are demalonylated by heat and inner acidity in processing of red ginseng (Kitagawa et al., 1983).
1.2.4. Potential health effects of ginsenosides

Ginsenoside has also been determined to be the active ingredient that confers a wide range of beneficial health effects of ginseng. The cytotoxic and antiproliferative effects of ginsenosides toward human and animal cancer cell lines have been demonstrated in numerous investigations (Yoon et al., 2010).

Wang et al. (2007) tested the cytotoxicity of 10 ginsenosides isolated from the fruits of *P. ginseng*, toward several human cancer cell lines, including breast cancer cell lines (e.g. MCF-7 cells). Among the ginsenosides tested, ginsenoside 20(S)-PPD, Rh2 showed substantial activity in all cell lines and were clearly the most effective inhibitors of cancer cell growth and proliferation.

As immunomodulatory effect, Ginsenosides of *P. notoginseng* and *P. ginseng*, such as Rb1, Rb2, and Rg1, have also shown to strongly suppress the production of TNF-α in macrophages treated with LPS (Cho et al., 2001).

Allergic diseases of type 1, such as asthma, allergic rhinitis, atopic dermatitis, and food allergy afflict up to 20% of the human population in many countries (Park et al., 2003). Ginsenosides Rb1, Rc, Rd, F2, and Rh1 have been shown to inhibit histamine and/or leukotriene release from peritoneal mast cells (Choo et al., 2003).

Many studies have shown that ginseng has a protective effect on the development of atherosclerosis that may lead to myocardial infarction and other cardiovascular diseases (Kim, 2012). Ginsenoside Rb1, Re, and Rg1 cause
endothelium-dependent vascular relaxation through increased NO production (Chen, 1996).

According to the World Health Organization (WHO), more than 180 million people suffer from diabetes and more than 90% of these have type 2 diabetes (T2D) and this number is likely to be doubled by 2030 due to the increasing prevalence of obesity (Wild et al., 2004). Animal experiments have demonstrated that ginseng and ginsenosides are able to lower blood glucose. Ginsenoside Rh2 has been shown to increase insulin secretion and to lower plasma glucose in Wistar rats (Lee et al., 2006).
1.3. Volatile compounds of ginseng

In recent, ginseng is used in diverse types of culinary dishes (e.g., salad, soup, stew, steamed dishes, tea, and other beverages) as well as processed food due to its distinct flavor characteristics (Cho, 2015). Volatile compounds in ginseng are very important as the main contributor to its characteristic aroma properties affecting consumer acceptability. While there are extensive studies on the bioactive constituents of ginseng, such as ginsenosides, little attention has been given to the volatile components of ginseng. In particular, the research on the change of volatile components after diverse manufacturing process is rare.

1.3.1. Volatile compounds of fresh ginseng

Since Takahashi and Yoshikura (1966) isolated panaxynol from the ether extract of P. ginseng, a few studies have been conducted to investigate the volatile components of ginseng. Yoshihara and Hirose (1975) also identified 15 sesquiterpene hydrocarbons extracted from P. ginseng, including apanasinsene, β-panasinsene, α-neoclovene, and β-neoclovene. Another study found thirteen pyrazines in the basic fraction of P. ginseng, and that 3-sec-butyl-2-methoxy-5-methyl pyrazine was the main contributor to its characteristic aroma properties (Iwabuchi et al., 1984). More sesquiterpene hydrocarbons compounds (e.g.,
ginsenosol, panasinsenol A, panasinsenol B, (+)-spathulenol) and sesquiterpenoids were additionally identified in the neutral fraction of P. ginseng (Iwabuchi et al., 1987, 1989, 1990). Sesquiterpenes are a class of terpenes that consist of three isoprene units, with the molecular formula C\textsubscript{15}H\textsubscript{24}. In general, sesquiterpenes, together with monoterpenes (C\textsubscript{10}H\textsubscript{16}), are strongly associated with the aroma characteristics of plants (Reineccius, 2007).

1.3.2. Volatile compounds of red ginseng

Generally, red ginseng is produced by steaming the root followed by drying (Nam, 2005). Many characteristic volatile compounds of fresh ginseng might disappear, while some new compounds could be produced through processing steps during the heat treatment. In reality, red ginseng exhibited different profile of volatile compounds as compared with that of white ginseng (Cho, 2015). Ko et al. (1996) profiled and compared the volatiles of white and red P. ginseng. As shown in Fig. 1-3, the volatiles of red ginseng were primarily of the following compounds: \(\beta\)-caryophyllene, spathulenol, \(\beta\)-panasinsene, bicyclogermacrene, aneoclovene, selina-4,11-diene, and \(\alpha\)-panasinsene. Sohn et al. (1997) focused on the ratios of \(\beta\)-panasinsene and c-muurolene as contributors of the discrimination.
Adapted from Cho (2015)

Fig. 1-3. Representative sesquiterpene hydrocarbons and sesquiterpene alcohols of ginseng.
between Korean and Chinese white and red ginseng. Abd El-Aty et al. (2008) compared the volatile profiles from fresh, white, and red *P. ginseng*. They reported that fresh ginseng was characterized by a high proportion of 3-actyl-1-(3,4-dimethoxyphenyl)-5-ethyl-4,5-dihydro-7,8-dimethoxy-4-methylene-3H-2,3-benzodiazepine and 23,24-dinor-3-oxolean-4,12-dien-28-oic acid. In addition, 2-furanmethanol and 3-hydroxy-2-methyl-4H-pyran-4-one were main compounds of white ginseng, while the major compounds of red ginseng were 1,2-benzenedicarboxylic acid dibutyl ester and 2-furanmethanol.

1.3.3. Volatile compounds of processed red ginseng

Han et al. (2008) evaluated changes in concentrations of volatile compounds contained in red ginseng tail roots after puffing treatment. They showed that 59 out of 63 volatile compounds were detected from the puffed red ginseng tail roots. While most of alcoholic, aldehyde and acid compounds are decreased, while terpene and furan compounds were increased through puffing treatment. Terpene compounds content accounted for 70% of the 63 volatile components in the puffed red ginseng tail roots. Park et al. (1999) identified 29 volatile compounds from red ginseng marc roasted at 200 °C for twenty minute. Of them 7 pyrazines and maltol are thought to be compounds which have characteristic odor such as roasted odor and scorched-rice odor in the roasted red ginseng marc. Lee et al.
(2010) reported that total volatiles in puffed red ginseng increased by 87% compared to those in red ginseng. Hexanal, \( \Delta \)-selinene, and \( \beta \)-panasinsene were major volatiles in red ginseng, whereas \( \alpha \)-gurjunene, \( \beta \)-panasinsene, and calarene were main volatiles in puffed red ginseng.

As other research of volatile components, a few researches have been tried to identify the ginseng origin. Actually, similar researches have been conducted to distinguish olive oil from different cultivars. Vichi et al. (2006) developed the headspace SPME method coupled to GC mass spectrometry (GC/MS) to determine the composition of mono- and sesquiterpenic hydrocarbon in virgin olive oils of different olive origin. They reported that the monoterpenes and, particularly, the sesquiterpene composition of olive oil may be used to distinguish samples from different cultivar and geographical areas. In recent, Lee et al. (2012) attempted GC analysis equipped with solid-phase microextraction (SPME) apparatus to identify the volatile compounds of ginseng cultivated by 3 different cultivating methods.
1.4. Antioxidant activity of ginseng

Oxidative stress contributes to the development of a wide range of diseases: neurodegenerative disorders, cardiovascular diseases, diabetes, cancer, and chronic fatigue syndrome (Giustarini et al., 2009; Heistad et al., 2009). The cause of oxidative stress is reactive oxygen species (ROS), which are formed by incomplete reduction of molecular oxygen. They include superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl ($\cdot$OH) radical, and singlet oxygen ($^1O_2$). Generally, they are toxic and induce an oxidative cell damage through lipid peroxidation and alteration of protein structure (Baud and Ardaillou, 1986). Ameliorating oxidative stress with antioxidants might be an effective strategy for treating various diseases. Therefore, many researchers have tried to find safe and effective scavengers of reactive oxygen species for prevention and treatment of oxidative stress-related diseases.

Ginseng is one of the most widely used medicinal plants in the Orient. Many studies have revealed that ginseng has a lot of bioactive components, which are the major sources of antioxidant activity. Chae et al. (2010) evaluated the antioxidant activities of ginsenosides on the intracellular reactive oxygen species (ROS). Ginsenoside Rb2 and Rc showed the strongest antioxidant activity, followed by (in decreasing order) Rg2, Rh2, Rh1, Rf, Rg3, Rg1, Rb1, Re and Rd. The presence of arabinose linked at the glucopyranosyl group may have enhanced
the antioxidant activity. This means that antioxidant activity of ginsenosides was influenced by the types of dammarane, as well as the number of sugar moieties, and substitutive groups.

A lot of studies have mainly been focused on ginsenosides, and other constituents of ginseng have been studied in less detail in terms of antioxidant activity. Kang et al. (2006) elucidated that the phenolic compounds and Maillard reaction products were more active free radical scavenging components than ginsenoside from the study of sun ginseng (steamed ginseng at 120°C). They suggested that phenolic compounds such as maltol, salicylic acid, vanillic acid and p-coumaric acid are principal antioxidant components of ginseng.

Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity (Cai et al., 2004). Maltol, one of the Maillard reaction products (MRPs), was reported to be an antioxidant component of red ginseng.
1.5. Research objectives

Many researchers have been studying the diverse methods to produce new types of ginseng products. Recently, the number of studies on the high temperature and pressure treatment in ginseng have been on the rise. Among them, the researches on how puffing process affects the biofunctional properties of red ginseng are still rare. In particular, there are a lot of unrevealed facts regarding the change of physicochemical properties, bioactive, and volatile components after puffing process.

Until now, although many researchers have investigated the change of major ginsenosides, no study has reported the changes of minor ginsenosides with pharmacological activities after puffing. Also, there is a need to investigate the antioxidant activity in food matrix model with in vitro assay.

The objectives of this study were to elucidate the change of effective components including ginsenosides after puffing, to determine the antioxidant activity of extracts of red ginseng and puffed red ginseng, and to identify characteristic volatile compounds in puffed red ginseng.
1.6. References


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Chapter 2.
The physicochemical properties of red ginseng
by puffing process
2.1. Introduction

Ginseng (Panax ginseng C. A. Meyer) is a flowering plant, which belongs to the Araliaceae family (Choi et al., 2014). The root of ginseng has been widely used as a traditional herbal medicine in Asia for its pharmacological effects over thousands of years (Gui and Ryu, 2014). Pharmacological effects of ginseng include anti-tumor, anti-inflammatory, anti-diabetic, and antioxidant activity (Bachran et al., 2008; Wang et al., 2011; Xie et al., 2005; Cho et al., 2008). The major bioactive components of ginseng are ginsenosides, polyaetylenes, phenolic compounds, alkaloids, acidic polysaccharides, and amino acids (Attele et al., 1999).

Ginseng deteriorates easily within a few days after harvest. So, the process for decreasing the moisture content of ginseng is necessary. Drying is the process, which is commonly used to maintain their desirable qualities and extend their shelf life. Currently, ginseng products are sold in the three types such as fresh ginseng, white ginseng, and red ginseng. White ginseng is produced by simple drying process of fresh ginseng, but red ginseng is manufactured by steaming and drying process of fresh ginseng (Jeong et al., 2015).

It has been reported that red ginseng has more powerful pharmacological activities than white ginseng (Nam, 2005). The difference in the biological activities of red and white ginseng may result from a change in the chemical
constituents that occur during the steaming process (Ha et al., 2007). Several investigators have reported new ginsenosides from red ginseng, which are not usually found in fresh ginseng. These ginsenosides are Rg3, Rg5, Rg6, Rh2, Rh4, Rs3, and Rf (Park, 1996).

Since it has been elucidated that red ginseng has a lot of bioactive components and numerous pharmaceutical activities, many manufacturers of ginseng have tried to develop the diverse functional food containing bioactive components of red ginseng. For utilization of ginseng as ingredient of functional food, it is necessary to extract the pharmacological components in the root of ginseng. But extraction process is difficult and time consuming work because ginseng has dense texture. Thus, researchers have investigated the production of expanded ginseng using extruder and explosive puffing process (Yoon et al., 2010).

Extrusion, classified as a high-temperature short-time process, is a versatile, low cost, efficient, and widely used industrial technology for the continuous production of expanded product from cereals (Gui and Ryu, 2014). Recently, few studies have been conducted to improve the physical and chemical properties of extruded ginseng samples (Son et al., 2009).

Explosive puffing is a fast and inexpensive method for drying of fruits and vegetables (Kozempel et al., 1989). Moisture in raw materials is converted to high temperature vapor through a continuous heating of the raw materials in a rotator cylinder. Sudden release of expanded water vapor pressure leads to structural
changes of raw materials such as corn, rice, and soybeans (Kim et al., 2007; Lee and Lee, 2009). According to the literature, potatoes, carrots, and apples have been successfully processed in puffing system (Du et al., 2013).

Recently, explosive puffing process on the tail roots of dried red ginseng have been introduced to produce new types of ginseng products. Yang et al. (2006) analyzed the contents of total phenolic compounds and ginsenosides in red ginseng treated with high temperature and pressure treatment. Han et al. (2007) determined the changes of saponins, total sugars, acidic polysaccharides, phenolic compounds, microstructures and pepsin digestibility of the tail roots of dried red ginseng by puffing process. Yoon et al. (2010) predicted the optimum conditions of explosive puffing process for ginseng using response surface methodology.

Although explosion puffing is an excellent processing method, there is no literature concerning the change of contents of free fatty acids, free sugars and free amino acids in puffed ginseng and red ginseng. To achieve a better explosion puffing technology and an increased consumption of puffed red ginseng, it is critical to fully understand various health-promoting components and their concentrations. The objective of this study was to evaluate the effect of explosive puffing process on the physicochemical properties of red ginseng. Therefore, the contents of the important bioactive compounds of red ginseng and puffed red ginseng were determined and compared.
2.2. Materials and Methods

2.2.1. Materials

Ginseng was kindly provided by a local manufacturer (Icheon, Gyinggido, Korea). Standards of amino acids and sugars were purchased from Sigma- Aldrich (St. Louis, MO, USA). 22 standards of ginsenosides Rg1, Re, Rf, Rh1(S), Rg2(S), Rg2(R), Rh1(R), Rb1, Rc, F1, Rb2, Rb3, Rd, F2, Rg3(S), Rg3(R), PPT(S), PPT(R), compound K, Rh2(S), Rh2(R) and PPD (Felton Natural Products, Chengdu, P.R. China) were used. All solvents were chromatographic or HPLC grade (Merk). Other reagents including ethanol and acetic acid were ACS reagent grade.

2.2.2. Free amino acids

Two hundred milligrams of each sample was extracted by sonification in 10 mL of 70% ethanol for 1 h using a Branson 2210 sonicator (Danbury, CT, USA). Fifty microliters of internal standard (100 μmole/mL L-allylglycine) was added. The extracts were centrifuged at 6000xg for 20 min. The supernatant was pooled and evaporated under reduced pressure to dryness. To this, 1 mL of deionized water was added, respectively. The sample were analyzed by HPLC system, Dionex Ultimate 3000 (Dionex, Sunnyvale, CA) coupled with fluorescence
detector (Emission 450 nm, Excitation 340 nm) and UV detector (338 nm). Separations were carried out on an VDSpher 100 C18-E (4.6 mm x 150 mm, 3.5 μm, VDS optilab, Germany). The liquid chromatography system was run in binary gradient mode, buffer A (40 mM Na₂HPO₄, pH 7.0) and buffer B (3DW : Acetonitrile : Methanol = 10 : 45 : 45 % (v/v)). The analytes were eluted via stepwise gradient mode at 40°C with buffer A/buffer B 95:5 (3 min), 45:55 (21 min), 20:80 (7 min), 95:5 (4 min) at a flow rate of 1.5 mL/min.

2.2.3. Free sugar

One g of each sample was extracted by sonification in 20 mL of distilled water for 30 min at 50°C. The extracts were filtered through 0.45 μm syringe filter and then analyzed using HPLC. A HPLC system, Dionex Ultimate 3000 (Dionex) equipped with a pump system, a refractive index detector (RI-101) was used for sugar analysis. Sugars in the non-puffed red ginseng and puffed red ginseng were analyzed onto a Sugar-pak column (300×6.5 mm) (Waters) and kept at 70°C. The analytical conditions used were as follows: flow rate 0.5 mL/min, eluent 3-D.W., injection volume 10 μL.

2.2.4. Puffing
Panax ginseng C.A. Meyer was washed and steamed in an autoclave at 100°C for 2 h, and then dried at 70°C until its moisture content was down to 14%. The steamed and dried ginseng (red ginseng) was used as a control sample. Dried red ginseng tail root (3 kg) was put in the cylindrical chamber of rotary puffing machine with the maximum pressure 1.47 MPa. The cylindrical chamber was heated with propane gas burner during rotation at the speed of 20 rpm. When the inner pressure of chamber reached 0.30 MPa, the door of cylindrical chamber was opened to release the high vapor pressure. The puffed red ginseng was recovered for further analysis.

2.2.5. Extraction yield

Briefly, 50 g of red ginseng and puffed red ginseng was placed into a 2 L round flask and 1 L of 70% aqueous ethanol was added. The mixture was refluxed for 20 h at 70°C. Three mL of the extracts of red ginseng or puffed red ginseng were filtered through Whatman #2 filter paper and the filtrate was transferred to a weighing bottle for moisture measurement. The weighing bottle was dried at 105°C, cooled in a desiccator, and monitored until it reached the constant weight. When it reached the constant weight, the weight was compared to the weight of the empty bottle. The weight difference corresponded to the amount of the soluble solids of the sample. Extraction yield was calculated as follows:
Extraction yield (g solid extract/100 g sample) = gram of solid extract/100 g of red ginseng or puffed red ginseng.

2.2.6. Color analysis

The color of the sample was determined using a Minolta Chromo Meter CR-400 (Minolta, Tokyo, Japan). Powdered samples were placed on a white standard plate to measure Hunter L, a, and b values. The color values were recorded in Hunter units: \( L = 0 \) (black or darkness) to \( L = 100 \) (white or brightness); \( a = -80 \) (greenness) to \( a = 100 \) (redness); and \( b = -80 \) (blueness) to \( b = 70 \) (yellowness).

2.2.7. Ginsenoside analysis

The ginsenosides of red ginseng and puffed red ginseng were analyzed according to the method of Ha et al. (2013). Ultra-high performance liquid chromatograph (u-HPLC) equipped with an autoinjection system using a fixed injection volume of 5 μL and an ultraviolet detector set to detect at 203 nm (Hitachi, Tokyo, Japan) was used. A LaChromUltra C18 short-length column (2 mm i.d. x 50 mm L., 2 μm) and a LaChromUltra C18 middle-length column (2 mm i.d. x 100 mm L., 2 μm) were used to analyzed the sample according to the method of Ha et al. (2013). The binary gradient elution solvents were prepared by
mixing 20% acetonitrile (solvent A) and 80% acetonitrile (solvent B). The gradient profile for the separation of the ginsenosides by u-HPLC was 100% A-0% B (0 min), which maintained for 10 min. The gradient profile was subsequently changed linearly 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 in the u-HPLC was 0.2 mL/min for the short-length column and 0.3 mL/min for the middle-length column. The temperature of the analytical column was maintained at 30°C. Ethanol extract of ginsengs was dissolved in 20% aqueous acetonitrile solution, filtered through a 0.20 μm PTFE membrane and 5 μL of solution were analyzed. The concentrations of ginsenosides were calculated based on calibration curves for each standard compound.

2.2.8. Fatty acid analysis by gas chromatography with a flame ionization detector (FID)

Fatty acids were derivatized to fatty acid methyl esters (FAME) using BF₃/MeOH (14% boron trifluoride) with some modification of AOAC 969.33 (2000). Red ginseng and puffed red ginseng was pulverized and 15 g of fine powder was put in the porous thimble to extract the lipid. After extraction for 16 h, triundecanoin (C11:0), an internal standard, was dissolved in n-hexane and added to the extracted lipid to the concentration of 1,000 ppm (w/v) and solvent was
removed under nitrogen gas flow. Two mL of BF₃ and 1mL of toluene was mixed in glass methylation tube and maintained for 45 min at 100°C. After cooling, 1 mL of hexane and 5 mL of distilled water was added to the vial. Fatty acid methyl esters were moved in hexane phase. The upper hexane layer was removed and concentrated under nitrogen gas. The residue was re-dissolved in 200 μL hexane, subsequently subjected to GC analysis. FAME was analyzed by Hewlett-Packard 6890 gas chromatograph (Agilent Technologies) with a FID, and a DB-23 column (60 m x 0.32 mm i.d., 0.25 mm film thickness) from J&W Scientific (Folsom, CA, USA). The oven temperature started at 100°C for 1 min, increased to 195°C at 15°C/min, to 210°C at 1°C/min, and to 240°C at 5°C/min and held at 240°C for 7.5 min. The temperatures of both injector and detector were 260°C. The flow rate of helium carrier gas was 1.1 mL/min, the injection volume was 1 mL, and the split ratio was 1:50. Peaks of GC chromatograms were identified comparing the retention times of a mixture of standard fatty acid methyl esters (Sigma-Aldrich). Each peak of fatty acid was quantified using an equivalent of the concentration of the internal standard. Samples were separately analyzed in triplicate.

2.2.9. Crude fat analysis

Crude fat in samples was determined using the Soxhlet method. The gram of ground sample was placed in a porous thimble. Solvent was diethylether and
extraction was continued for 8 h.

2.2.10. Statistical analysis

The Data were analyzed statistically by independent-paired t-test using SPSS software program (SPSS Inc., Chicago, IL, USA). Significant differences were defined at $p < 0.05$. 
2.3. Results and Discussion

2.3.1. The change of extraction yield

The extraction yield of non-puffed red ginseng and puffed red ginseng was shown in Table 2-1. The extraction yields (16.7-42.2%) from puffed red ginseng showed higher than those from non-puffed red ginseng (9.0-32.7%) at all extraction times. The tendency of increase in the extraction yield after puffing was well agree with the study by An (2011), who reported that extraction yield increased from 29.7% at 4 h to 45.7% at 24 h after puffing red ginseng. Kim et al. (2008) obtained the highest extraction yield (61.9%) from the puffed red ginseng, which is puffed at 10 kgf/cm². Yoon et al. (2010) also reported that extraction yields in explosively puffed ginseng at 98, 294, and 490 kPa were 53.93%, 55.87%, and 57.00%, respectively. This means that the puffing pressure has an effect on the extraction yield. Mariotti et al. (2006) elucidated that the new organization of the outer layers with the high porosity of the matrix was responsible for the rapid hydration of the puffed material and the predominance of capillary water absorption. Therefore, the increase of extraction yield in puffed red ginseng could be explained by the fact that explosive puffing process softened the rigid cell wall structure and induced expanded and porous structure to make the solvent access easy (An et al., 2011). As shown in Table 2-1, extraction yield from both puffed
and non-puffed red ginsengs gradually increased with extraction time but yields did not increase drastically at times greater than 16 h. This suggests that extraction for 16 h economically provides optimal yield in terms of time and energy.
Table 2-1. Changes in extraction yield (%) of puffed and non-puffed red ginseng

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extraction time (h)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Non-puffed red ginseng</td>
<td>9.0±0.2</td>
<td>14.8±0.4</td>
<td>23.4±0.2</td>
<td>29.7±1.8</td>
<td>32.7±0.7</td>
</tr>
<tr>
<td>Puffed red ginseng</td>
<td>16.7±0.4*</td>
<td>26.1±0.3*</td>
<td>33.5±0.5*</td>
<td>40.7±0.4*</td>
<td>42.2±0.2*</td>
</tr>
</tbody>
</table>

*mean±standard deviation (n=3)

In the same column, data with ‘*’ were significantly different from each other at α=0.05
2.3.2. Ginsenoside analysis

The amount and composition of ginsenoside are presented in Table 2-2 and Table 2-3. The ginsenoside content of non-puffed red ginseng and puffed red ginseng was 11.98 mg/g and 13.65 mg/g, respectively. This result is similar to the study of Kim et al. (2008), which reported that the total content of major ginsenoside (Rb1, Rb2, Rc, Rd, Re and Rg1) in puffed red ginseng was higher than that of non-puffed ginseng. In that report, the total content of major ginsenoside (Rb1, Rb2, Rc, Rd, Re and Rg1) was 7.23 mg/g in comparison to non-puffed ginseng, 5.40 mg/g. Yoon et al. (2010) reported that puffing treatment of dried raw ginseng roots at 294 kPa induced an increase in the content of main ginsenoside (Rb1, Rb2, Rc, Rd, Re, Rg1 and Rg3) compared to red ginseng from 6.90 mg/g to 7.82 mg/g. There is another study that shows different result with this study. An et al. (2011) elucidated that the major ginsenosides (Rb1, Rb2, Rc, Rd, Re and Rg1) of red ginseng decreased from 13.32 mg/g to 9.39 mg/g through puffing at 686 kPa, while the minor ginsenosides (Rg3, F2, Rk1 and Rg5) increased from 0.5 mg/g to 7.39 mg/g. In this study, the content of major ginsenosides increased from 11.54 mg/g to 12.96 mg/g and the content of minor ginsenosides (Rg2, Rg3, Rh1) increased from 0.44 mg/g to 0.70 mg/g after puffing process. Among minor ginsenosides, Rg3(S) increased by 25 times, from 0.08 mg/g to 0.20 mg/g. Experimental studies have demonstrated that ginsenoside Rg3 could inhibit cancer cell growth by promoting the apoptosis of cancer cells.
and also prevent an angiogenetic formation in prostate, breast, ovarian, colorectal, gastric, liver and lung cancer (Nam et al., 2014). According to Ha et al. (2007), ginsenoside Rg3 is typically considered as a unique ginsenoside which only exists in red ginseng products. Rg3 was naturally absent in white ginseng but was produced by a thermal process like steaming white ginseng at 98 to 100°C for 2 to 3 h. Kang et al. (2013) reported that the concentrations of less-polar ginsenosides (20(S)-Rg3, 20(R)-Rg3) in P. ginseng were significantly increased in a heat-processing temperature-dependent manner. They also observed that the formation of Rg3 was associated with the breakdown of the more abundant ginsenoside Rc. Ginsenoside Rg3, which contains two glucose residues bound to C-3, is formed through the thermal decomposition of glucoses linked at the C-20 residue in ginsenosides of the 20 (S)-protopanaxadiol (PPD) type (Rb2, Rc, and Rd) (Nam, 2005).

The puffed red ginseng is produced by releasing high pressure in the cylindrical chamber. This process can reduce processing time (within 10 min) as compared to the conventional ginseng process. So, puffing process could be an alternative method to produce ginseng with increased amount of bioactive ginsenoside Rg3.
### Table 2-2. Comparison of ginsenosides belonging to main group in non-puffed red ginseng and puffed red ginseng

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ginsenoside content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rb1</td>
</tr>
<tr>
<td>Red ginseng</td>
<td>3.13±0.11</td>
</tr>
<tr>
<td>Puffed red ginseng</td>
<td>4.35±0.19*</td>
</tr>
</tbody>
</table>

a mean±standard deviation (n=3)

b In the same column, data with ‘*’ was significantly different from each other at α=0.05
Table 2-3. Comparison of ginsenosides belonging to minor group in non-puffed red ginseng and puffed red ginseng

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ginsenoside content (mg/g)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rg2(S)</td>
<td>Rg2(R)</td>
<td>Rg3(S)</td>
<td>Rg3(R)</td>
<td>Rh1(S)</td>
<td>Rh1(R)</td>
<td>Total</td>
</tr>
<tr>
<td>Red ginseng</td>
<td>0.20±0.00</td>
<td>0.05±0.01</td>
<td>0.08±0.01</td>
<td>0.05±0.01</td>
<td>0.04±0.01</td>
<td>0.02±0.00</td>
<td>0.44±0.14</td>
</tr>
<tr>
<td>Puffed red</td>
<td>0.26±0.02*</td>
<td>0.06±0.01</td>
<td>0.20±0.01*</td>
<td>0.09±0.01</td>
<td>0.06±0.00</td>
<td>0.03±0.00</td>
<td>0.70±0.09</td>
</tr>
</tbody>
</table>
ginseng          |                            |    |    |    |    |    |    |

*a* mean±standard deviation (n=3)

*b* In the same column, data with ‘*’ were significantly different from each other at $\alpha=0.05$
2.3.3. Free amino acids

Table 2-4 summarizes the contents of twenty free amino acids including GABA in non-puffed red ginseng and puffed red ginseng. Total content of free amino acids, 12.7 mg/g in non-puffed red ginseng was reduced to 7.1 mg/g in puffed red ginseng. Cho et al. (2008) compared the total content of free amino acids in white ginseng, red ginseng (steamed at 100°C) and steamed ginseng (steamed at 120°C). They reported that the total content of free amino acids in white ginseng (17.9 mg/g) was reduced to 12.2 mg/g in red ginseng (steamed at 100°C) and 2.79 mg/g in steamed ginseng (steamed at 120°C). The content of most of the amino acids except for histidine, tyrosine and valine, decreased after puffing process. The major amino acids were arginine (4.39 mg/g), asparagine (0.60 mg/g), GABA (0.31 mg/g), aspartic acid (0.28 mg/g), and alanine (0.46 mg/g) after puffing process while the predominant amino acids in red ginseng were arginine (8.6 mg/g), asparagine (0.79 mg/g), GABA (0.72 mg/g), aspartic acid (0.53 mg/g), alanine (0.46 mg/g). The reduction of amino acid with the intensity of the steam treatment means that instability at high temperature and amino carbonyl reaction is one of the reasons. There is report that some of amino acids increased after heat treatment. In the experiment about the effect of steaming on the free amino acid contents, aspartic acid increased when white ginseng was changed to red ginseng (Cho et al., 2008).
By the report of Nam (2005), arginine is main amino acid and takes 60% of total free amino acids in the ginseng. In this study, the content of arginine (4.39 mg/g) was decreased greatly after puffing process, compared to that of red ginseng (8.62 mg/g). This result agrees well with the study of Cho et al. (2008) in which the content of arginine was abruptly reduced after steaming process. But the decrease of arginine content induced by steaming was larger than that by puffing process. This difference presumably results from accelerated Maillard reaction, which is in line with the report that high temperature, high relative humidity, and alkaline conditions all promote browning reaction when working with reducing sugars found in food. (Nam, 2005; Turkmen et al., 2006). In other words, puffing process has relatively little adverse effect on the nutritional characteristics such as the loss of amino acids. Like this, although puffing process causes the loss of amino acids, it also produces the brown color substance that has antioxidant activity in food (Suzuki et al., 2004). Therefore, it is more important to maintain the puffing condition not to deteriorate the quality of puffed red ginseng.
Table 2-4. The contents of free amino acids (mg/g of dry matter) in non-puffed red ginseng and puffed red ginseng

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Non-puffed red ginseng</th>
<th>Puffed red ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.526</td>
<td>0.005</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.048</td>
<td>0.004</td>
</tr>
<tr>
<td>asparagine</td>
<td>0.785</td>
<td>0.005</td>
</tr>
<tr>
<td>Serine</td>
<td>0.128</td>
<td>0.008</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.574</td>
<td>0.002</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.077</td>
<td>0.003</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.017</td>
<td>0.006</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.117</td>
<td>0.008</td>
</tr>
<tr>
<td>Arginine</td>
<td>8.636</td>
<td>0.012</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.463</td>
<td>0.004</td>
</tr>
<tr>
<td>GABA</td>
<td>0.724</td>
<td>0.006</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.072</td>
<td>0.005</td>
</tr>
<tr>
<td>Valine</td>
<td>0.070</td>
<td>0.002</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.015</td>
<td>0.008</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>0.063</td>
<td>0.005</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.079</td>
<td>0.007</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.070</td>
<td>0.003</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.083</td>
<td>0.004</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.064</td>
<td>0.005</td>
</tr>
<tr>
<td>proline</td>
<td>0.039</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Total content</strong></td>
<td><strong>12.650</strong></td>
<td><strong>0.081</strong></td>
</tr>
</tbody>
</table>

\(^a\text{GABA: \(\gamma\)-aminobutyric acid}\)

\(^b\text{In the same row, data with ‘*’ were significantly different from each other at \(\alpha=0.05\)}\)
2.3.4. Free sugars

Four of free sugars, maltose, glucose, mannose and fructose were determined as sugar components in red ginseng and puffed red ginseng. Maltose is present in the largest amounts for red ginseng (233.83 mg/g) and puffed red ginseng (130.46 mg/g), account for about 97.9% and 90.6% of total sugar content in red ginseng and puffed red ginseng, respectively. Total amounts of sugar were 238.78 mg/g and 143.92 mg/g in red ginseng and puffed red ginseng, respectively (Table 2-5). The decrease of free sugar contents in the puffed red ginseng is attributed to heat decomposition of disaccharide at high temperature and participation in the Maillard reaction. Lee et al. (2006) reported that among sugar tested, maltose resulted in the greatest acceleration of browning followed in decreasing order by glucose and lactose, whereas pentose, fructose, sucrose and raffinose had negligible effect.

The Maillard, or nonenzymatic browning reaction between carbonyl and amino groups is a common reaction in foods which undergo thermal processing. As is well known, browning reactions are very complex and a lot of different products
may be formed from sugars, depending on the conditions used. Generally, the Maillard reaction is a desirable consequence of many industrial and domestic processes and is responsible for the attractive flavor and brown color of some cooked foods. On the other hand, undesirable consequence of the Maillard reaction in foods is the destruction of some essential amino acids, such as lysine. In addition to this, cytotoxicity, mutagenicity, and immunochemical aspects of selected Maillard reaction products have also been examined (O’Brien and Morrissey, 1989).

In the process of steaming to make red ginseng, maltose reacts with amino acid like arginine and produces 4-O-α-D-glucosyl-1-deoxy-2,3-diketosaccharide. Since this compound is unstable, 2 ketone group and C-6-hydroxyl group condensate to be glycoside B. After that, glycoside B takes further hydrolysis of glucose and rearrangement to be maltol (Li, 1992). Therefore, it was considered that puffing process may accelerate the reaction of maltose and amino acids in red ginseng to produce maltol.

After puffing process, the contents of fructose, glucose and mannose increased
1.96-fold, 1.72-fold, and 13.2-fold, respectively. Ko et al. (1996) reported that the content of glucose and fructose increased 27.5% and 15%, respectively as white ginseng was changed into red ginseng by steaming process. The contents of free sugars showed a tendency to increase as heating temperatures were increased (Kwak et al., 2008).
Table 2-5. The contents of free sugars (mg/g of dry matter) in non-puffed red ginseng and puffed red ginseng

<table>
<thead>
<tr>
<th>Free sugar</th>
<th>Non-puffed red ginseng</th>
<th>Puffed red ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Maltose</td>
<td>233.83</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.87</td>
<td>0.03</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.61</td>
<td>0.02</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.46</td>
<td>0.01</td>
</tr>
<tr>
<td>Total content</td>
<td>238.78</td>
<td></td>
</tr>
</tbody>
</table>

*In the same row, data with ‘*’ were significantly different from each other at α=0.05.
2.3.5. Color measurement

The color analysis results of the puffed red ginseng and red ginseng are shown in Table 2-6. The three Hunter color parameters measured, *L* (lightness), *a* (greenness-redness), and *b* (blueness-yellowness), were significantly affected (*p* < 0.05) by the puffing process. The *L* value (76.72) of red ginseng was higher than that of puffed red ginseng (62.90). It means that the puffed red ginseng is darker than red ginseng. Park et al. (1993) reported in the study of roast ginseng that the color distribution of roast ginseng demonstrated lower *L* values and higher *a* values were observed according to higher temperature, while *b* values were almost not changed. Yoon et al. (2010) studied the effect of the explosive puffing process on the color of ginseng and noted that the puffed red ginseng at higher pressure was darker than that of red ginseng. This result may come from the formation of some Maillard reaction products, which cause darkening of the ginseng.

An increase in Hunter *a* value, indicating increase in redness, was observed in the puffed red ginseng as compared to red ginseng. The Hunter *a* values of red ginseng and puffed red ginseng were 2.68 and 7.35, respectively. The result
showed that the color of puffed red ginseng turned to be red but the difference was little. This result may be due to the increase in the formation of Maillard reaction products. Generally, the formation of such products increases temperature dependently. It has been reported that Maillard reaction products are formed from the reaction of the free amino group of amino acids with the carbonyl group of a reducing sugar during thermal treatment of raw ginseng below 100°C and in the drying period after steaming (Suzuki et al., 2004). The Hunter b value represents the change from blue to yellow. The Hunter b values were obtained as 20.09 in red ginseng and 20.95 in puffed red ginseng. It indicates that the puffed red ginseng has a tendency to be yellow. When people choose the food, the selection point is its various appearance characteristics, such as its color, surface structure, and shape. Color of ginseng, in particular, is an important sensory attribute to determine the consumer acceptability. As the result of color measurement, the difference of L between the puffed red ginseng and non-puffed red ginseng was not degree to affect to consumer acceptability.
Table 2-6. Comparison of Hunter color values of red ginseng and puffed red ginseng.

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red ginseng</td>
<td>76.72±0.35</td>
<td>2.68±0.05</td>
<td>20.09±0.29</td>
</tr>
<tr>
<td>Puffed red ginseng</td>
<td>62.90±0.62*</td>
<td>7.35±0.38*</td>
<td>20.95±0.43*</td>
</tr>
</tbody>
</table>

* a mean±standard deviation (n=3)

*b In the same column, data with ‘*’ were significantly different from each other at α=0.05
2.3.6. Analysis of crude lipid and fatty acid

The effect of puffing process on the lipid content and fatty acid composition of red ginseng were investigated. The content of crude lipid was decreased from 1385 mg/100 g solid to 1058 mg/100 g solid after puffing. This result is due to the oxidation of fat by the high heat treatment in the puffing process of red ginseng. Ko et al. (1996) reported that the content of crude lipid in white ginseng and red ginseng was 1180 mg/g and 1140 mg/g, respectively. Six free fatty acids including palmitic acid were isolated and identified from red ginseng and puffed red ginseng. As shown in Table 2-7, linoleic acid showed the highest content among six fatty acids identified. The contents were 68.7% of the total free fatty acids in red ginseng and 68.9% in puffed red ginseng. Zhang et al. (2013) reported linoleic acid (C18:2 n-6), palmitic acid (C16:0) and oleic acid (C18:1 n-9) were main fatty acids in *Panax ginseng* C.A. Meyer. There was the change of composition of free fatty acids after puffing process. Palmitoleic acid was detected in the puffed red ginseng and the ratio of unsaturated fatty acids in the puffed red ginseng was higher than that of red ginseng. The unsaturated fatty acids, including monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), are health-promoting components. This means fatty acids in red ginseng might partly contribute to the whole beneficial effects of red ginseng together with ginsenosides and polysaccharides. Also, the free fatty acid profile
has been suggested as an indicator for determining the authenticity of red ginseng (Zhang et al., 2013).
Table 2-7. Fatty acid composition of non-puffed red ginseng and puffed red ginseng (mg%)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Non puffed red ginseng</th>
<th>Puffed red ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>280.96±0.96</td>
<td>185.11±0.91*</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1)</td>
<td>0.00</td>
<td>16.49±0.06*</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>23.96±0.14</td>
<td>17.60±0.06*</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>71.71±0.72</td>
<td>67.66±1.07*</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>951.71±1.31</td>
<td>728.74±0.48*</td>
</tr>
<tr>
<td>Linolenic acid (C18:3)</td>
<td>57.41±0.21</td>
<td>42.79±0.23*</td>
</tr>
<tr>
<td>Total fatty acid</td>
<td>1385</td>
<td>1058</td>
</tr>
<tr>
<td>SFA(%)</td>
<td>22.00</td>
<td>19.15</td>
</tr>
<tr>
<td>USFA(%)</td>
<td>78.00</td>
<td>80.85</td>
</tr>
<tr>
<td>SFA/UFA</td>
<td>0.28</td>
<td>0.24</td>
</tr>
<tr>
<td>Crude fat(mg/100g)</td>
<td>1385</td>
<td>1058</td>
</tr>
</tbody>
</table>

\(^a\) mean±standard deviation \((n=3)\)

\(^b\) In the same row, data with ‘*’ were significantly different from each other at \(\alpha=0.05\)
2.4. Conclusions

The effects of puffing process on the extraction yield, ginsenoside content and the change of physicochemical properties of ginseng were investigated. Puffed red ginseng showed higher extraction yields than those of non-puffed red ginseng at all extraction times. As the result of analyzing the amount of ginsenoside after puffing, the contents of major and minor ginsenosides were both increased. Among minor ginsenosides, Rg3(S) with anti-cancer activity was increased by 25 times in comparison to red ginseng. Total content of free amino acids decreased after puffing due to the instability at high temperature and amino carbonyl reaction. In particular, the content of arginine was greatly reduced after puffing process. Puffing influenced the content of free sugars, as revealed by the decrease of maltose, glucose, mannose, and fructose. Puffing process induced a color change by browning reaction. Puffed red ginseng showed lower $L$ value (darker) and higher $a$ value than that of red ginseng. There was the change of composition of free fatty acids after puffing process. Palmitoleic acid was detected in the puffed red ginseng. Based on these results, puffing process could be an alternative technology to produce biofunctional red ginseng products with the advantage of short extraction time.
2.5. References


Ko, S. R., Choi, K.J., and Han, K.W. 1996. Comparison of proximate composition,


Chapter 3.

Increases of 2-furanmethanol and maltol in Korean red ginseng during explosive puffing process
3.1. Introduction

Ginseng (*Panax ginseng* C.A. Meyer) has been cultivated and consumed as a medicinal herb in East Asia for a long time. Red ginseng is produced by steaming fresh ginseng first, and then drying the steamed ginseng while white ginseng is made of fresh ginseng by drying process only (Cho et al., 1995). Sensory evaluation has shown that fresh ginseng has strong fresh, earthy, herbaceous, and floral flavor notes, while steamed ginseng has only moderate earthy flavor notes. Red ginseng has strong fragrant, sweet, and roast flavor notes (Lee et al. 2005). Major volatile compounds identified in ginsengs are monoterpenes, esters, ethers, and sesquiterpenoids such as α-gurjunene, α-guaiene, β-patchoulene, (-)aromadendrene, and β-elemene (Park et al., 1985; Richter et al., 2005). Iwabuchi et al. (1989) reported that 3-sec-butyl-2-methoxy-5-methyl pyrazine from ether extracts of white ginseng was the characteristic flavor of white ginseng, and sesquiterpene alcohols with a mass of 220 or 222 including ginsenol, panasinsenol A, panasinsenol B, (+)-spathulenol, (-)-4β,10α-aromadendrandiol, and (-)-
neointermedeol were also identified in ether extracts of white ginseng. Lee et al. (2005) elucidated the characteristic aroma component of red ginseng as 3-hydroxy-2-methyl-pyran-4-one or maltol through a combination of GC/MS and nuclear magnetic resonance (NMR) spectrometry.

Puffing has been used to alter the structural characteristics of foods such as puffed rice and to improve the rehydration characteristics of air-dried fruits and vegetables (Payne et al., 1989). Explosive puffing heats raw material with superheated steam, holds it under pressure for some time, and then releases the pressure suddenly to expand the product. In the course of puffing process using high temperature and high pressure, diverse chemical reactions including browning reaction can be occurred and there is possibility that characteristic volatile compounds can be produced. Recently, Korean ginseng has been puffed using a rotary gun puffing machine to produce new types of ginseng products (An et al., 2011). Han et al. (2008) puffed the tail roots of red ginseng and reported volatile changes using a simultaneous steam distillation and extraction method. Although explosive puffing process could provide different physicochemical
properties in red ginseng, studies on the changes of volatile distribution and microstructure in puffed red ginseng are rare in the literature.

Headspace-solid phase microextraction (HS-SPME) is a rapid, solvent-free, and simple method for volatile analysis and has been widely applied to many types of foods including fruit juices (Foley et al., 2002), fats and oils (Lee et al., 2007), and dairy products (Lee et al., 2003). Previous studies for volatile analysis of ginseng used steam distillation, solvent extraction (Sohn et al., 2000; Lee et al., 2005), or solvent-free solid injector vaporization (Abd El-Aty et al., 2008). HS-SPME study on the profiles changes of volatiles from red ginseng has been reported (Ryu et al., 2002) while to my knowledge, no literature is available on the volatile profiles from puffed red ginseng by HS-SPME.

The objectives of this study were to analyze the volatiles from puffed red ginseng using HS-SPME method and to identify characteristic volatiles in puffed red ginseng.
3.2. Materials and Method

3.2.1. Materials

*Panax ginseng* C.A. Meyer was purchased at a wholesale market in Keumsan, Korea in autumn of 2005. 2-Furanmethanol and maltol were obtained from Wako Chem. Co. (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO, USA), respectively. Standard volatile compounds and *n*-paraffin were purchased from Sigma-Aldrich, Teflon-coated rubber septa, 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB), aluminum caps, serum bottles, and a SPME fiber assembly holder were purchased from Supelco, Inc. (Bellefonte, PA, USA).

3.2.2. Sample preparation

*Panax ginseng* C.A. Meyer was washed and steamed in an autoclave at 100°C for 2 h, and then dried at 70°C until its moisture content was down to 14%. The steamed and dried ginseng or red ginseng was used as a control sample. Explosive puffing facilities were composed of a cylindrical drum, heating unit with propane...
gas and a motor for rotating the drum (JSP021, Jinsung, Seoul, Korea). Red ginseng was put in the cylindrical drum, which was rotated at the speed of 20 rpm. The temperature and time of the cylindrical drum were maintained at 120~150°C and for 30 min, respectively. When the inner pressure of the drum reached to 1.47 MPa, the door of cylindrical drum was opened to release the high vapor pressure. The puffed red ginseng was recovered for further analysis.

3.2.3. Scanning electron microscopy (SEM)

A scanning electron microscope (JSM 5410LV; JEOL, Tokyo, Japan) was used to observe the microstructure of red and puffed red ginsengs. About 3 g of dried, finely ground sample was placed on double-sided tape, mounted on an aluminum specimen holder, coated with a thin film of gold, and examined at 20 kV.

3.2.4. HS-SPME analysis of volatile compounds

One gram of samples was put in 10 mL bottles and left in the dark at room temperature for 30 min for the equilibrium of volatiles in the headspace of the
bottles. Sample bottles were prepared in triplicate. The volatile compounds in samples were isolated by 65 µm PDMS/DVB solid phase at 30°C for 30 min in a water bath. The isolated volatile compounds were analyzed in a gas chromatograph equipped with a mass selective detector (MS).

GC/MS conditions for volatile analysis were adapted from Lee et al. (2009) with slight modification. Briefly, a Hewlett-Packard 6890 GC-5975B mass selective detector (MS) (Agilent Technology 5973, Palo Alto, CA, USA) equipped with a 30 m × 0.25 mm i.d., 0.25 µm film thickness, HP-5ms column was used. The oven temperature was programmed starting at 40°C for 2 min and increased from 40 to 160°C at 6°C/min and from 160 to 220°C at 10°C/min and held for 3 min. Helium carrier gas flow rate was at 0.6 mL/min (10.3 kPa). The isolated volatile compounds in the solid phase of SPME were desorbed at 250°C for 2 min in a GC injector. All mass spectra were obtained at 70 eV and 230°C ion source temperature. Identification of compounds was tentatively made by the combination of NIST Mass Spectra, linear retention indices (RI) of each compounds using n-paraffin (C 5, 6, 7, 8, 10, 12, 14, and 16) as external
references, and gas chromatographic retention times of some standard compounds.

Selective ion monitoring (SIM) analysis was conducted to detect target compounds in the chromatograms of GC/MS. A mass to charge ratio of 98 and 126, which are molecular weight of 2-furanmethanol and maltol, respectively, were used to detect these volatile peaks in mass spectra from red and puffed red ginsengs.

3.2.5. Statistical analysis

The results of SIM analysis for detecting 2-furanmethanol and maltol were analyzed statistically by ANOVA and Duncan’s multiple range test using SPSS software program (SPSS Inc., Chicago, IL, USA). A $p$ value of 0.05 or less was considered significant.
3.3. Results and Discussion

3.3.1. Puffing effects on the structures of red ginseng

SEM photographs of red and puffed red ginsengs are shown in Fig. 3-1. Large changes in the surface structures caused by explosive puffing were clearly observed in both scanning electron micrographs magnified by 1000 and 2000 times (Fig. 3-1). Puffed red ginseng had a more porous structure and smaller pieces than red ginseng. Surfaces of observed structures in red ginseng were relatively smooth, while those in puffed red ginseng were coarse and scattered. The high pressure of vapor during sudden explosive puffing may lead to swelling of the matrix and rupturing of inner structures to outside.

Puffing process has been introduced to various grain products to modify textural properties and sensory qualities. Mariotti et al. (2006) puffed cereal grains and reported that size of puffed grains was enlarged and ultrastructure, especially the distribution of porosity, was radically changed. As expected, explosive puffing induced substantial changes in the microstructure of red ginseng.
Fig. 3-1. SEM photographs of red and puffed red ginsengs. Pictures of: a) red ginseng x 1000 magnification, b) puffed red ginseng x 1000 magnification, c) red ginseng x 2000 magnification, and d) puffed red ginseng x 2000 magnification.
3.3.2. Distribution of volatiles in red and puffed red ginsengs

Distributions of major volatiles in red and puffed red ginsengs are shown in Table 3-1. The number of identified volatile peaks from red and puffed red ginsengs was 20 and 17, respectively. Red ginseng had 4 alcohols, 4 aldehydes, 2 acids, 5 terpenoids, 2 ketones and 3 other volatiles, while puffed red ginseng had 3 alcohols, 13 terpenoids, and 1 acid. Total peak areas of volatiles from red and puffed red ginsengs were $3.85 \times 10^8$ and $7.02 \times 10^8$ ion counts, respectively. Puffed red ginseng possessed 87% more total peak areas than red ginseng. Hexanal, $\Delta$-selinene, and $\beta$-panasinsene were major volatiles in red ginseng, and $\alpha$-gurjunene, $\beta$-panasinsene, and calarene were identified as main volatiles in puffed red ginseng. Hexanal, heptanal, 2-pentyl furan, 1-octen-3-ol, and octanal in red ginseng are typical volatiles generated from lipid oxidation (Frankel, 1985; Lee et al., 2009). Generally, red ginseng is produced through first steam treatment and then drying process, which may accelerate the lipid oxidation in red ginseng. $\alpha$-Gurjunene, $\beta$-panasinsene, calarene structurally belong to sesquiterpenes. They could be generated from $\Delta$-selinene, which was drastically decreased after
puffing process through internal shifts of electron pairs and hydride shift along with ring opening. Volatiles from lipid oxidation are relatively low molecular weight compounds and these compounds may be evaporated easily into the atmosphere with high moisture vapor pressure during explosive puffing process. Therefore, remained major volatiles in puffed red ginseng are terpenoids with relatively high molecular weight and low volatility.

Two peaks with the retention time of 7.60 and 14.53 min, were greatly increased in puffed red ginseng compared with those in red ginseng. These two peaks were identified as 2-furanmethanol and maltol later. Also, they were constantly detected and reported as major compound for characteristic fragrant in red ginseng by the studies of other researchers (Lee et al., 2005; El-Aty et al., 2008). The production via Maillard reaction in ginseng was elucidated. In addition, considering the antioxidative activity of maltol and 2-furanmethanol (Wei et al., 2001), they could be used as biomarkers to discriminate puffed red ginseng from other types of ginseng without puffing process.

The changes of volatiles in ginseng have been studied through diverse analysis
methods and processing conditions. Han et al. (2008) puffed the tail roots of red
ginseng and reported the increases of terpenes and furans and the decrease of
alcohols, aldehydes, and acids in puffed red ginseng. Lee et al. (2005) used gas
chromatograph equipped with an electric nose unit with metal oxide sensors to
differentiate profiles of volatiles from fresh, steamed and red ginsengs. Sohn et al.
(1997) used sensory evaluation and headspace volatile analysis for the comparison
of red ginseng from Korea and China. Red ginseng from Korea had sweet and
pleasant odor, while China-originated red ginseng possessed earthy, woody and
hay notes. In this study, explosive puffing process clearly increased the total peak
areas of volatiles and changed the profiles of volatiles from red ginseng. Red
ginseng possessed more volatile compounds from lipid oxidation, while puffed
red ginseng had more terpenoids including aristolene, β-panasinsene, and calarene
(Table 3-1). However, the profiles of volatiles from ginseng samples by HS-
SPME analysis may not be compared directly with those by electronic nose
method or by solvent extraction because the distribution of volatiles depends on
the volatile extraction and concentration methods greatly.
<table>
<thead>
<tr>
<th>RT</th>
<th>RI</th>
<th>Volatile compounds</th>
<th>Aroma description</th>
<th>Relative percent (%)</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Red ginseng</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Puffed red ginseng</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.11</td>
<td>-</td>
<td>Ethanol</td>
<td>ethanol-like, pungent, sweet</td>
<td>8.92</td>
<td>3.08</td>
</tr>
<tr>
<td>3.06</td>
<td>616</td>
<td>Acetic acid</td>
<td>sour, vinegar, pungent</td>
<td>ND&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.97</td>
</tr>
<tr>
<td>5.39</td>
<td>748</td>
<td>Toluene</td>
<td>pungent, caramel, solvent-like</td>
<td>3.32</td>
<td>ND</td>
</tr>
<tr>
<td>6.17</td>
<td>784</td>
<td>Hexanal</td>
<td>green, fruity, fishy, grassy</td>
<td>19.42</td>
<td>ND</td>
</tr>
<tr>
<td>7.60</td>
<td>836</td>
<td>2-Furanmethanol</td>
<td>weak, creamy, burnt sugar</td>
<td>0.20</td>
<td>5.86</td>
</tr>
<tr>
<td>7.91</td>
<td>847</td>
<td>Xylene</td>
<td>geranium, oily, fatty, pungent</td>
<td>2.23</td>
<td>ND</td>
</tr>
<tr>
<td>8.76</td>
<td>879</td>
<td>Heptanal</td>
<td>green, rancid, pesticide, solvent</td>
<td>3.75</td>
<td>ND</td>
</tr>
<tr>
<td>10.40</td>
<td>939</td>
<td>Benzaldehyde</td>
<td>burnt sugar, almond</td>
<td>2.08</td>
<td>ND</td>
</tr>
<tr>
<td>10.88</td>
<td>956</td>
<td>1-Octen-3-ol</td>
<td>mushroom, soap, plastic</td>
<td>1.89</td>
<td>ND</td>
</tr>
<tr>
<td>11.22</td>
<td>969</td>
<td>2-Pentyl furan</td>
<td>buttery, green bean-like</td>
<td>4.29</td>
<td>ND</td>
</tr>
<tr>
<td>11.42</td>
<td>976</td>
<td>Hexanoic acid</td>
<td>sweaty, pungent, goat-like, rancid</td>
<td>4.42</td>
<td>ND</td>
</tr>
<tr>
<td>11.53</td>
<td>980</td>
<td>Octanal</td>
<td>sweaty, boiled meat, rancid, soapy</td>
<td>7.59</td>
<td>ND</td>
</tr>
<tr>
<td>12.50</td>
<td>1017</td>
<td>3-Octene-2-one</td>
<td>-</td>
<td>3.45</td>
<td>ND</td>
</tr>
<tr>
<td>13.34</td>
<td>1050</td>
<td>3,4-Octadiene-2-one</td>
<td>-</td>
<td>1.85</td>
<td>ND</td>
</tr>
<tr>
<td>14.53</td>
<td>1096</td>
<td>Maltol</td>
<td>caramel-like</td>
<td>0.24</td>
<td>3.99</td>
</tr>
<tr>
<td>16.51</td>
<td>1174</td>
<td>Octanoic acid</td>
<td>fatty acid, cheese, fresh, moss</td>
<td>2.19</td>
<td>ND</td>
</tr>
<tr>
<td>19.94</td>
<td>1319</td>
<td>Bicycloelemene</td>
<td>-</td>
<td>ND</td>
<td>0.86</td>
</tr>
<tr>
<td>20.48</td>
<td>1343</td>
<td>Longiborn-8-nen</td>
<td>-</td>
<td>ND</td>
<td>0.90</td>
</tr>
<tr>
<td>21.04</td>
<td>1366</td>
<td>β-Panasinsene</td>
<td>-</td>
<td>9.88</td>
<td>11.82</td>
</tr>
<tr>
<td>21.18</td>
<td>1372</td>
<td>β-Elemene</td>
<td>waxy, herbaceous</td>
<td>4.19</td>
<td>5.91</td>
</tr>
</tbody>
</table>

Table 3-1. Total peak areas and major volatiles identified in red and puffed red ginsengs
(continued)

<table>
<thead>
<tr>
<th>Retention time (RT)</th>
<th>Linear retention index (RI)</th>
<th>Compound</th>
<th>Description</th>
<th>Retention</th>
<th>Linear Retention</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.84</td>
<td></td>
<td>Caryophyllene</td>
<td>oily, fruity, woody</td>
<td>ND</td>
<td>2.43</td>
<td>MS/RS</td>
</tr>
<tr>
<td>22.14</td>
<td></td>
<td>Calarene</td>
<td>-</td>
<td>ND</td>
<td>5.21</td>
<td>MS</td>
</tr>
<tr>
<td>22.26</td>
<td></td>
<td>Aromadendrene</td>
<td>-</td>
<td>ND</td>
<td>2.88</td>
<td>MS/RS</td>
</tr>
<tr>
<td>22.55</td>
<td></td>
<td>Δ-Selinene</td>
<td>-</td>
<td>ND</td>
<td>10.81</td>
<td>MS/RI</td>
</tr>
<tr>
<td>22.71</td>
<td></td>
<td>α-Gurjunene</td>
<td>Earthy, Mango-like</td>
<td>ND</td>
<td>18.64</td>
<td>MS/RI</td>
</tr>
<tr>
<td>22.86</td>
<td></td>
<td>trans-Caryophyllene</td>
<td>woody, terpene, fruity, sweet</td>
<td>ND</td>
<td>3.79</td>
<td>MS/RI/RS</td>
</tr>
<tr>
<td>22.99</td>
<td></td>
<td>β-Neoclovene</td>
<td>-</td>
<td>ND</td>
<td>2.23</td>
<td>MS/RI</td>
</tr>
<tr>
<td>23.19</td>
<td></td>
<td>β-Selinene</td>
<td>herbaceous</td>
<td>ND</td>
<td>1.62</td>
<td>MS/RI</td>
</tr>
<tr>
<td>23.37</td>
<td></td>
<td>Bicyclogermacrene</td>
<td>spicy, mushroom-note, dry-wood</td>
<td>ND</td>
<td>6.90</td>
<td>MS/RI</td>
</tr>
<tr>
<td>24.76</td>
<td></td>
<td>Spathulenol</td>
<td>fruity, herbaceous, weak herbal</td>
<td>ND</td>
<td>0.90</td>
<td>MS/RI</td>
</tr>
</tbody>
</table>

| Total peak areas of volatiles (x 10^8 ion counts) | 3.85 | 7.20 |

1 Retention times (RT) of each volatile in minute.
2 Linear retention indices (RI) were determined using n-paraffin (C 5, 6, 7, 8, 10, 12, 14, and 16) as external references and compared with those in flavornet (http://flavornet.org.; Accessed at Apr. 30, 2009).
4 Volatile identification was performed through a combination of NIST Mass spectra library (MS), linear retention index (RI), and retention times of standard compounds (RS).
5 Not detected.
3.3.3. Increase of 2-furanmethanol and maltol in puffed red ginseng

Mass spectra of peaks from 7.60 and 14.53 min and those of standard compounds of 2-furanmethanol and maltol are shown in Fig. 3-2. Two peaks from puffed red ginseng were identified as 2-furanmethanol and maltol using a combination of standard compounds, linear retention indices (RI), and the library from the GC/MS. Selective ion monitoring (SIM) analysis on 2-furanmethanol and maltol in red and puffed red ginsens are displayed in Fig. 3-2. 2-Furanmethanol and maltol increased significantly during the puffing process ($p<0.05$). SIM mode for GC/MS results showed that total ion counts for 2-furanmethanol and maltol were $7.81 \times 10^5$ (0.20% of total ion counts) and $9.14 \times 10^5$ (0.24% of total ion counts), respectively, in red ginseng, and $4.22 \times 10^7$ (5.86% of total ion counts) and $2.87 \times 10^7$ (3.99% of total ion counts), respectively, in puffed red ginseng (Fig. 3-2). The puffing process increased 2-furanmethanol and maltol more than 54 and 30 times, respectively, in ion counts.

The presence of maltol or 3-hydroxy-2-methyl-pyran-4-one has been reported in red ginseng by Lee et al. (2005) and Matsuura et al. (1984). The researchers
showed that maltol was one of the major compounds for the characteristic fragrant and sweet aroma in red ginseng. El-Aty et al. (2008) identified a total of 47 compounds from fresh, white, and red ginseng using a solvent free solid injection. The researchers reported that 3-acetyl-1-(3,4-dimethoxyphenyl)-5-ethyl-4,5-dihydro-7,8-dimethoxy-4-methylene-3H-2,3-benzodiazepine was the most abundant volatile comprising 64.24% of total volatiles in fresh ginseng. Also, maltol was detected at 17.59% of total volatiles in white ginseng but not in red ginseng (El-Aty et al., 2008). Difference of red ginseng preparation and extraction methods for volatiles may give this discrepancy on the distribution of maltol between the results from El-Aty et al. (2008) and from current study.

Maltol has been marketed as a food flavor enhancing agent for the products of breads and cakes (E number E636). It is tasteless at the recommend concentration at the levels ranging from 50 to 200 mg/kg and up to 2 mg/kg per day was acceptable concentration for human beings (Sanfeliu Alonso et al., 2001). Maltol is formed by thermal degradation of starch or by pyrolysis of sucrose (Bjeldanes et al., 1979). The increases of maltol may play a role on the sensory
changes of puffed red ginseng as a food flavoring enhancer.

Limited studies are found on the detection of 2-furanmethanol or furfuryl alcohol in ginseng. El-Aty et al. (2008) reported that 2-furanmethanol in fresh, white, and red ginseng was detected about 0.12, 20.26, and 13.82% of total volatiles, respectively. White ginseng is processed by drying fresh ginseng and applied thermal energy may enhance the formation of 2-furanmethanol in white and red ginsengs. 2-Furanmethanol has been frequently found in Maillard browning model systems containing amino acids and sugars (Ames et al., 2001). The low moisture condition and high temperature during explosive puffing may accelerate the formation rate of 2-furanmethanol and maltol in puffed red ginseng.

Sensory properties of 2-furanmethanol are weak, creamy, and burnt sugar while those of maltol are caramel-like (Table 3-1). The increases of 2-furanmethanol and maltol may provide different characteristic sensory attributes to puffed red ginseng although sensory evaluation of red and puff red ginsengs were not performed in this study.

It has been reported that red ginseng has better nutraceutical activities than
white ginseng, which may be due to the changes of chemical constituents through additional steaming process (Matsuura et al., 1984; Park et al., 2005). Explosive puffing process for red ginseng induced the profile changes in volatiles such as the increase of maltol and 2-furanmethanol. Considering the antioxidative activity of maltol and 2-furanmethanol (Wei et al., 2001), puffed red ginseng may have different biological activities compared to red ginseng or white ginseng, which needs further studies.
Fig. 3-2. GC mass spectra of a) standard compound of 2-furanmethanol, b) peak of 7.60 min in puffed red ginseng.
Fig. 3-3. GC mass spectra of a) standard compound of maltol, and b) peak of 13.53 min in puffed red ginseng.
Fig. 3-4. SIM analysis on 2-furanmethanol and maltol in red and puffed red ginsengs. Bars with '***' were significantly different from each other at $\alpha=0.001$.
3.4. Conclusions

Explosive puffing process of red ginseng, which induced changes in microstructure such as formation of pores and swelling, changed the distribution of volatile compounds greatly. Generally, volatiles from lipid oxidation decreased and terpenoid compounds increased during explosive puffing. 2-Furanmethanol and maltol were significantly increased during explosive puffing and these compounds could be useful markers to distinguish puffed red ginseng with other types of ginseng without puffing process. Explosive puffing process may provide different biological functionality and sensory properties to red ginseng products, which could meet the consumers’ expectation. More studies on the biological activities and sensory properties of puffed red ginseng are needed and various factors for puffing process including temperature, time, and moisture contents in red ginseng should be optimized.
3.5. References


Chapter 4.

Oxidative stability of extracts from red ginseng and puffed red ginseng in bulk oil or oil-in-water (O/W) emulsion matrix
4.1. Introduction

Ginseng is a perennial plant of the *Panax* genus that has been consumed as a medicinal plant and a functional food ingredient in some parts of Asia due to its beneficial physiological effects. From a long time ago, fresh ginseng was either dried or steamed to preserve for an extended period of time (Gui et al., 2012). Red ginseng is produced via a repeated process of steaming and drying fresh ginseng (Cho et al., 1995). This steaming process causes a color change of ginseng and improves the biofunctional properties of ginseng by chemical conversion of bioactive components such as ginsenosides (An et al., 2011). In recent, methods which can enhance the yield of these red ginseng specific ginsenosides by high temperature steaming, extrusion, and explosive puffing have been developed (Kang et al., 2006).

Explosive puffing has been used to give food material a porous structure by using a combination of high temperature and high pressure (Hui et al., 2004). Explosive puffing process involves the use of a puffing gun in which the material to be puffed is heated at the condition of high temperature and pressure. The puffing gun is then suddenly opened and the material discharged to atmosphere, resulting in the explosive puffing of the cereal material (Sullivan and Craig, 1984). Since red ginseng has dense texture and low moisture content, explosive puffing
process can be used to produce bioactive ginsenosides and antioxidant substances. Explosive puffing process has been introduced to develop the new types of ginseng products (Han et al., 2007; Lee et al., 2010; An et al., 2011). Han et al. (2007) investigated the effect of puffing on quality characteristics of red ginseng tail root. They reported that crude saponin content of puffing red ginseng tail root was increased 26.5% compared to non-puffing and total phenolic compounds was increased from 7.86% to 9.94% by puffing. Lee et al. (2010) elucidated that the explosive puffing process increased maltol with antioxidant activity. An et al. (2011) found that explosive puffing influenced the ginsenoside composition, as revealed by the generation of ginsenosides Rg5 and Rk1.

Accumulated researches on ginseng have pointed out that its medicinal efficacy is closely related to its protective effects against free radical attack. It has been reported that ginseng extract has scavenging activity to hydroxyl (·OH), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, superoxide anion (O₂⁻), and peroxynitrite (ONOO⁻) (Kang et al., 2007). On the other hand, the antioxidant capacities of chemical compounds are influenced by the concentration and polarity of compounds and the environmental conditions under which compounds are located (Chaiyasit et al., 2007; Lee et al., 2013; Kim et al., 2015). Generally, hydrophilic compounds show better antioxidant capacities in non-polar 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 (Laguerre et al., 2010, 2015; Kim et al., 2012; Shahidi et al., 2011). It is strongly recommended that the antioxidant capacities of compounds, mixtures or extracts be tested in real food systems. For example, curcumin (Yi et al., 2015) and extracts of roasted hulled barley (Oh et al., 2015) had different antioxidant properties depending on the food matrices, including bulk oil or oil-in-water emulsions.

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

The objectives of this study were to utilize in vitro assays to determine the antioxidant properties of extracts of red ginseng and puffed red ginseng and to test these extracts in the different matrices, including in corn oil and O/W emulsion.
4.2. Materials and Methods

4.2.1. Materials

Red ginseng was kindly provided by a local ginseng supplier (Icheon, Gyeonggi, Korea). Aluminum chloride, potassium acetate, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Folin-Denis’ reagent, and 2,2’-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) were purchased from Fluka (Buchs, Switzerland). Tannic acid was obtained from Riedel-deHaen (Seelze, Germany) and potassium phosphate was purchased from Wako (Tokyo, Japan). 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).

4.4.2. Sample preparation

*Panax ginseng* C.A. Meyer was washed and steamed in an autoclave at 100°C for 2 h, and then dried at 70°C until its moisture content was down to 14%. The steamed and dried ginseng (red ginseng) was used as a control sample. Dried red ginseng (3 kg) was put in the cylindrical chamber of rotary puffing machine with the maximum pressure 1.47 MPa. The cylindrical chamber was heated with
propane gas burner during rotation at the speed of 20 rpm. When the inner pressure of chamber reached 0.30 MPa, the door of cylindrical chamber was opened to release the high vapor pressure.

4.2.3 Ethanol extract of puffed red ginseng and non-puffed red ginseng

Briefly, 50 g of red ginseng or puffed red ginseng was placed into a 2 L Erlenmeyer flask and 1 L of 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, lyophilized using a freeze-drier (Ilshinbiobase Co, Ltd., Gyeonggi, Korea) and used for further studies. The yields from red ginseng and puffed red ginseng after lyophilization were 23.1% and 30.0%, respectively.

4.2.4. Sample preparation of O/W emulsion containing ginseng extract

O/W emulsions were prepared according to the method of Ka et al. (2016). Tween 20 was added in deionized water at a concentration of 0.25% (w/w) and then combined with 2.5% (w/w) corn oil in deionized water. A coarse emulsion
was made by homogenizing the mixture for 3 min using a DE/T 25 homogenizer (Ika®werke, Staufen, Germany). This coarse emulsion was then passed three times through a Nano disperser (ISA – NLM100, Ilshinautoclave Co., Ltd., Daejoen, Korea) at 5000 psi. After the O/W emulsion was prepared, riboflavin was added to the emulsion at 0.13 mM and the solution was mixed overnight. Ginseng extracts were added to the O/W emulsions containing riboflavin at concentrations of 0.25, 0.5, and 1.0% (w/v). Two milliliters of each emulsion containing riboflavin and the extracts was put in a 10 mL vial and sealed air-tight with a rubber septa and an aluminum cap. Sample vials were stored in a light box with 1333 lux light intensity under fluorescent light and analyzed at 0, 12, 24, and 36 h. Sample vials were prepared in triplicate at each sampling. Samples without added ginseng extracts served as controls.

4.2.5. Sample preparation of corn oil containing ginseng extract

Extracts of ginsengs were dissolved in methanol and added to corn oil to achieve final concentrations of 0.25, 0.5, and 1.0% (w/w). The solvent in the mixture was removed under nitrogen gas flushing. The 0.5 g of corn oil containing ginseng extracts was put in 10 mL vials and sealed air-tight with Teflon coated rubber septa and aluminum caps. Sample vials were stored at 60°C for 20 days and 100°C for 27 h in a drying oven (HYSC Co, Ltd.). Samples
without added extracts of ginsengs were prepared as controls. Samples were prepared in triplicate at each sample point.

4.2.6. *In vitro* antioxidant assays

**DPPH free radical scavenging activity**

The free radical scavenging ability of the ginseng extracts was determined based on methods used in a previous report with a slight modification (Oh et al., 2015; Ka et al., 2016). Briefly, 0.75 mL of 0.1 mM DPPH in methanol and 0.25 mL of 1,000 ppm (w/v) ginseng extract were mixed in a 1.5 mL tube in triplicate. The absorbance of samples was measured at 517 nm using a Shimadzu UV-2101 PC spectrophotometer (Shimadzu, Kyoto, Japan) after a 30 min period of storage in the dark. Samples were prepared in triplicate and free radical scavenging activity was calculated according to the following equation:

DPPH radical scavenging activity (%) = \([\left(\frac{A_0 - A_1}{A_0}\right) \times 100]\),

where \(A_0\) was the absorbance of the blank and \(A_1\) the absorbance in the presence of the test compound.

**ABTS radical cation scavenging activity**

The radical cation scavenging activity of samples was determined using the
ABTS method according the method in a previous report (Oh et al., 2015; Ka et al., 2016). Briefly, a mixture of 7 mM aqueous ABTS solution and 2.45 mM potassium persulfate was diluted with ethanol to an absorbance at 734 nm of 0.700 ± 0.050. A volume of 1.9 mL of diluted solution was mixed with 50 μL of 1,000 ppm (w/v) sample, and the absorbance of the sample mixture was determined at 734 nm using a spectrophotometer (Shimadzu) after 6 min of incubation. Samples were prepared in triplicate. Data was expressed using the following equation:

\[
\text{ABTS radical cation scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100,
\]

where \(A_0\) was the absorbance of the blank and \(A_1\) the absorbance in the presence of the test compound.

The ferric reducing antioxidant power (FRAP) method

The FRAP assay was performed, with some modifications, using the method reported by Benzie and Strain (1996) and Ka et al. (2016). The stock solutions were 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl₃ solution. The freshly prepared FRAP working solution was prepared by mixing the above stock solutions at a ratio of 10:1:1 (v/v/v) and maintained at 37°C prior to use. 50 μL of 1,000 ppm (w/v) samples were allowed to react with 1.5 mL of the FRAP solution for 30 min in the dark. The amount of colored product (ferrous tripyridyltriazine complex) was
determined at 593 nm using a spectrophotometer (Shimadzu). The results are expressed in μg ascorbic acid equivalent /g.

**Total phenolic content**

Total phenolic content (TPC) was determined according to the method of Riedl et al. (2007). Briefly, 0.25 mL of 5,000 ppm (w/v) samples were mixed with 4 mL water and 0.25 mL Folin-Denis’ reagent (previously diluted with water at the ratio of 1:1, v/v). After a 5 min incubation period, 0.5 mL saturated sodium carbonate was added to the sample mixture. The absorbance of the mixture was measured at 725 nm using a spectrophotometer (Shimadzu, Kyoto, Japan) after a 30 min incubation. Data are expressed as tannic acid equivalents (μg). Samples were prepared in triplicate.

**Total flavonoid content**

First, 0.5 mL samples were mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was then incubated for 30 min at room temperature and the absorbance of the mixture was measured at 415 nm using a spectrophotometer (Shimadzu). Samples were prepared in triplicate and the data are expressed using quercetin equivalents (μg).
4.2.7. Analysis of ginsenosides in ginseng extracts

The ginsenosides in ginseng extracts were analyzed according to the method of Ha et al. (2013). Ultra-high performance liquid chromatograph (u-HPLC) equipped with an autoinjection system using a fixed injection volume of 5 μL and an ultraviolet detector set to detect at 203 nm (Hitachi, Tokyo, Japan) was used. Two different columns were used to analyze ginsenosides: a LaChromUltra C18 short-length column (2 mm i.d. x 50 mm L., 2 μm) and a LaChromUltra C18 middle-length column (2 mm i.d. x 100 mm L., 2 μm). The gradient was prepared by mixing 20% acetonitrile (solvent A) and 80% acetonitrile (solvent B). The gradient profile for the separation of the ginsenosides using u-HPLC was 100% A-0% B (0 min), which was maintained for 10 min. The gradient profile was subsequently changed linearly 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 in the u-HPLC was 0.2 mL/min for the short-length column and 0.3 mL/min for the middle-length column. The temperature of the analytical column was maintained at 30°C. Ethanol extract of ginsengs were dissolved in 20% aqueous acetonitrile solution, filtered through a 0.20-μm PTFE 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.

4.2.8. Headspace oxygen analysis
The degree of oxidation was determined by the depletion of headspace oxygen in air-tight samples containing corn oil or O/W emulsions. The headspace oxygen in air-tight sample bottles was analyzed according to methods of Kim et al. (2014). First, 20 μL of headspace gas was removed from the sample bottle using an air-tight syringe and the oxygen content was determined using a Hewlett-Packard 7890 GC gas chromatograph (Agilent Technologies, Inc., Santa Clara, Ca., USA) equipped with a 60/80 packed column (3.0 m × 2 mm i.d., Restek Ltd., Bellefonte, Pa., USA) and a thermal conductivity detector (TCD). The flow rate of helium gas was 30 mL/min. The temperatures of the oven, injector, and thermal conductivity detector were 60, 180, and 180°C, respectively.

4.2.9. Lipid hydroperoxides in O/W emulsion

Concentration of lipid hydroperoxides was determined using a modified method of Yi et al. (2015). 0.3 mL of sample was mixed with 1.5 mL of iso-octane/2-propanol (3:2, v:v), vortex-mixed three times for 10 s each, and centrifuged for 3 min at 2000 g. The upper layer of 0.2 mL was collected and mixed with 2.8 mL of methanol/1-butanol (2:1, v:v). 30 μL of thiocyanate/Fe²⁺ solution was added to the mixture and the mixture was vortex-mixed for 10 s. The thiocyanate/Fe²⁺ solution was made by mixing equal volumes of 3.94 M thiocyanate solution with 0.072 M Fe²⁺ solution (obtained from the supernatant of a mixture of one part of 0.144 M FeSO₄ and one part of 0.132 M BaCl₂ in 0.4 M
HCl). 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.

4.2.10. Conjugated dienoic acid and \( p \)-anisidine value analyses in bulk oil

The CDA of samples was measured according to AOCS method Ti 1a-64 (2006) and the \( p \)-AV of oxidized samples was determined according to AOCS method Cd 18-90 (2006) with minor modifications.

4.2.11. Statistical analysis

Data from the \textit{in vitro} assays, headspace oxygen content, CDA, \( p \)-AV, and lipid hydroperoxides were analyzed statistically via ANOVA and Duncan’s multiple range test using SPSS software program (SPSS Inc., Chicago, IL., USA). The other data were analyzed statistically by independent-paired t-test using SPSS software program (SPSS Inc., Chicago, IL., USA). A \( p \) value <0.05 was considered significant.
4.3. Results and Discussion

4.3.1. Antioxidant activities and ginsenoside profiles in extracts of red ginseng and puffed red ginseng determined using \textit{in vitro} assays

It has been known that ginsenosides and phenolic compounds including flavonoids are main antioxidant substances in ginseng (Kim et al., 2008). Recent literature indicates that reactive oxygen species (ROS) and metal ions such as ferric ion play critical roles in oxidative damage (Forbes et al., 2008). It is necessary to measure the radical scavenging activity, ferrous ion chelating activity, and ferric reducing antioxidant power to determine the antioxidant activity. The \textit{in vitro} antioxidant properties of the extracts of red ginseng and puffed red ginseng were analyzed by DPPH, ABTS, FRAP, TPC, and TFC and their results are shown in Fig. 4.1-4.5. The free radical scavenging activity of the extracts of red ginseng and puffed red ginseng determined by DPPH assays were 51.0% and 86.2%, respectively, whereas those determined by ABTS assays were 28.7% and 72.2%, respectively. The extract of puffed red ginseng showed significantly higher free radical scavenging activity than that of red ginseng \((p<0.05)\).
The FRAP of red ginseng and puffed red ginseng extracts were 1.04 and 2.34 μg ascorbic acid equivalents/g, respectively. The ferric ion reducing ability of puffed red ginseng extracts was significantly higher than that of red ginseng extract (p<0.05).

The TPC of red ginseng and puffed red ginseng extracts was 1.32 and 6.72 μg tannic acid equivalent/g, respectively, while the TFC was 0.27 and 1.35 μg quercetin acid equivalent/g, respectively. Therefore, the amount of phenolic compounds including flavonoids in the extract of puffed red ginseng were significantly higher than those of red ginseng (p<0.05).

Based on the results of these in vitro assays, extracts of puffed red ginseng were demonstrated to have significantly higher in vitro antioxidant properties than the extracts of red ginseng (p<0.05). 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. (2006) reported that heat-treated ginseng, including red ginseng and ginseng steamed at 120°C, showed better superoxide anion (O₂⁻), peroxynitrite (ONOO⁻) and hydroxyl radical scavenging activities than white ginseng. Extrusion cooking of red ginseng was shown to increase DPPH radical scavenging activity and reducing power (Gui et al., 2014). Red ginseng extracts completely eliminated DPPH radicals at 2 mg/mL (Kim et al., 2002).
Fig. 4-1. *In vitro* antioxidant properties of extract of red ginseng and puffed red ginseng by DPPH. Bars with '*' and '***' were significantly different from each other at $\alpha=0.05$ and 0.001, respectively. 'Normal' and 'Puffing' were extracts of red ginseng and puffed red ginseng, respectively.
Fig. 4-2. *In vitro* antioxidant properties of extract of red ginseng and puffed red ginseng by ABTS.

Bars with '*' and '***' were significantly different from each other at $\alpha=0.05$ and 0.001, respectively. 'Normal' and 'Puffing' were extracts of red ginseng and puffed red ginseng, respectively.
Fig. 4-3. *In vitro* antioxidant properties of extract of red ginseng and puffed red ginseng by FRAP. Bars with '*' and '***' were significantly different from each other at $\alpha=0.05$ and 0.001, respectively. 'Normal' and 'Puffing' were extracts of red ginseng and puffed red ginseng, respectively.
Fig. 4-4. *In vitro* antioxidant properties of extract of red ginseng and puffed red ginseng by TPC. Bars with ‘*’ and ‘***’ were significantly different from each other at $\alpha=0.05$ and 0.001, respectively. 'Normal' and 'Puffing' were extracts of red ginseng and puffed red ginseng, respectively.
Fig. 4-5. *In vitro* antioxidant properties of extract of red ginseng and puffed red ginseng by TFC. Bars with '*' and '***' were significantly different from each other at $\alpha=0.05$ and 0.001, respectively. 'Normal' and 'Puffing' were extracts of red ginseng and puffed red ginseng, respectively.
The profiles of ginsenosides in extracts of red ginseng and puffed red ginseng are shown in Table 4-1. The total ginsenoside contents in red ginseng and puffed red ginseng were 42.33 and 49.22 mg/g, respectively. Puffed red ginseng possessed significantly higher ginsenoside content than red ginseng \((p<0.05)\). Rb1 was the most detected ginsenoside, followed by Re and Rg1 in both red ginseng and puffed red ginseng (Table 4-1). Generally, the puffing process increased ginsenoside content, including Rg3, Rb1, Re, and Rg1. In particular, ginsenoside Rg3 was substantially increased from 0.23 mg/g in red ginseng to 0.46 mg/g in puffed red ginseng, which is about 2.0-fold increase. Ginsenoside Rg3(S) has various functional activities, including being tumor-suppressive, hepatoprotective (Zhang et al., 2012), immune-stimulating, antifatigue (Tang et al., 2008), and anti-inflammatory (Yoo et al., 2012). An et al. (2011) 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 decreased and those of other ginsenosides increased after the puffing process (Table 4-1). Differences in extraction procedures or thermal instability of ginsenosides (Yoon et al., 2005) may induce changes in the profiles of ginsenosides. The enhanced \textit{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. 20(S) and 20(R) are stereoisomers of each other that depend on the position of the C-20 hydroxyl in
ginsenosides. The hydroxyl radical scavenging activity of 20(S)-Rg3 is higher than that of 20(R)-Rg3 (Lee et al., 2008c).
Table 4-1. Profiles of ginsenosides belonging to protopanaxadiol group (PPD) in extracts of red ginseng and puffed red ginseng

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ginsenoside content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rb1</td>
</tr>
<tr>
<td>Red ginseng</td>
<td>11.72±0.19</td>
</tr>
<tr>
<td>Puffed red ginseng</td>
<td>14.04±0.08*</td>
</tr>
</tbody>
</table>

*a mean±standard deviation (n=3)*

*bIn the same column, data with ‘*’ were significantly different from each other at α=0.05.*
Table 4-2. Profiles of ginsenosides belonging to protopanaxatriol group (PPT) in extracts of red ginseng and puffed red ginseng

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ginsenoside content (mg/g)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Re</td>
<td>Rf</td>
<td>Rg1</td>
<td>Rg2(S)</td>
<td>Rg2(R)</td>
<td>Rh1(S)</td>
<td>Rh1(R)</td>
<td></td>
</tr>
<tr>
<td>Red ginseng</td>
<td>7.11±0.29</td>
<td>1.71±0.09</td>
<td>6.02±0.09</td>
<td>0.94±0.03</td>
<td>0.15±0.02</td>
<td>0.14±0.02</td>
<td>0.06±0.00</td>
<td>16.14±0.48</td>
</tr>
<tr>
<td>Puffed red ginseng</td>
<td>8.15±0.31*</td>
<td>2.15±0.07*</td>
<td>7.86±0.42*</td>
<td>1.18±0.03*</td>
<td>0.13±0.02</td>
<td>0.25±0.03*</td>
<td>0.06±0.01</td>
<td>19.78±1.28</td>
</tr>
</tbody>
</table>

*a mean±standard deviation (n=3)

*bIn the same column, data with ‘*’ were significantly different from each other at α=0.05
4.3.2. Oxidative stability of red ginseng and puffed red ginseng extracts in O/W emulsions

The effects of red ginseng and puffed red ginseng extract (0-1.0% w/w) on headspace oxygen in oil-in-water emulsions under riboflavin photosensitization are shown in Fig. 4-6 and Fig. 4-7. After 12 h of treatment, samples containing 1.0% red and puffed red ginseng extracts showed the highest headspace oxygen content, which implies that the 1.0% ginseng extracts acted as antioxidants. The headspace oxygen contents in samples containing 0, 0.25, 0.50, and 1.0% red ginseng extract after 36 h were 17.0, 17.5, 18.3, and 19.3%, respectively, indicating that the extract of red ginseng inhibited the consumption of headspace oxygen in a concentration dependent manner (Fig. 4-6). However, puffed red ginseng extracts showed a different pattern of headspace oxygen consumption after 24 and 36 h of treatment compared to equivalently treated red ginseng. After 36 h treatment, all the samples containing extracts of puffed red ginseng showed lower headspace oxygen contents than control samples, which implies that extracts of puffed red ginseng accelerated the consumption of headspace oxygen molecules. The oxygen molecules may have been consumed by unsaturated fat in corn oils and/or oxidation of phenolic compounds. Because extracts of puffed red ginseng had significantly higher levels of phenolic compounds than extracts of red ginseng, the possibility of oxygen consumption by phenolic compounds cannot be
ruled out. Therefore, excess phenolic compounds in puffed red ginseng extract could be targets for oxygen consumption.
Fig. 4-6. Effects of extract of red ginseng (0-1.0% w/w) on the headspace oxygen in oil-in-water emulsions under riboflavin photosensitization. Different letters were significantly different at the same time at 0.05. Symbols without letters were not significantly different at $\alpha=0.05$. 
Fig. 4-7. Effects of extract of puffed red ginseng (0-1.0% w/w) on the headspace oxygen in oil-in-water emulsions under riboflavin photosensitization. Different letters were significantly different at the same time at α=0.05. Symbols without letters were not significantly different at α=0.05.
Changes in lipid hydroperoxides in O/W emulsions under riboflavin photosensitization containing extract of red ginseng and puffed red ginseng are shown in Fig. 4-8 and Fig. 4-9. The antioxidant properties of extracts of red ginseng in O/W emulsions were influenced by the extract concentration and the puffing process. The 0.25% red ginseng extracts did not show antioxidant properties while 0.50% and 1.0% red ginseng extracts acted as antioxidants based on lipid hydroperoxides (Fig. 4-8). Lipid hydroperoxides in O/W emulsion containing 1.0% extracts of puffed red ginseng were significantly lower than those of controls after 36 h (p<0.05) (Fig. 4-9). However, 0.25 and 0.5% extracts of puffed red ginseng did not inhibit the formation of lipid hydroperoxides over 24 h (p>0.05) (Fig. 4-9).
Fig. 4-8. Changes of lipid hydroperoxides in oil-in-water emulsions under riboflavin photosensitization containing red ginseng extract. Different letters were significantly different at the same time at $\alpha=0.05$. 
Fig. 4-9. Changes of lipid hydroperoxides in oil-in-water emulsions under riboflavin photosensitization containing extract of puffed red ginseng.

Different letters were significantly different at the same time at $\alpha=0.05$. 

Lipid hydroperoxides (mmol/kg oil) vs. Time (h)
4.3.3. Oxidative stability of red ginseng and puffed red ginseng extracts in bulk oil

Changes in headspace oxygen, CDA, and \( p \)-anisidine values in corn oil with addition of extract of red ginseng and puffed red ginseng (0-1.0% w/w) at 60°C and 100°C are shown in Tables 4-3 and 4-4, respectively. Headspace oxygen content in bulk oil containing extracts of puffed red ginseng was found to be increased in a concentration-dependent manner (0.25%, 0.50%, and 1.0%) compared to in the control after 20 days. CDA and \( p \)-AV in bulk oil containing puffed red ginseng extracts were lower than those of controls, which agrees with the results of our headspace oxygen assay. Therefore, puffed red ginseng extract had antioxidant properties in bulk oil at 60°C when the concentration was greater than 1.0% (Table 4-3). However, red ginseng extract showed different patterns. Based on the results of the headspace oxygen content assay after 20 days of storage, 0.25% and 0.50% red ginseng extracts acted as prooxidants, while 1.0% red ginseng extract acted as an antioxidant. Results from CDA and \( p \)-AV assays showed no significant differences among samples \((p>0.05)\) (Table 4-3).

The headspace oxygen content in bulk oil containing 1.0% puffed red ginseng extract was significantly lower than that of a control at 100°C after 27 h, while samples containing 1.0% red ginseng extract had significantly higher headspace oxygen content than controls \((p<0.05)\) (Table 4-4). These results indicate that extracts of puffed red and red ginseng accelerated or retarded the consumption of
headspace oxygen molecules, respectively. Samples containing 0.25 and 0.50% red ginseng or puffed red ginseng extracts were lower than controls. This trend can also be observed in both CDA and \( p\)-AV assays. The puffed red ginseng extracts acted as prooxidants when present at concentrations ranging from 0.25 to 1.0%, while 0.25% and 0.50% red ginseng extracts showed prooxidant properties and 1.0% red ginseng extract acted as an antioxidant based on the results of CDA and \( p\)-AV assays after 27 h of treatment at 100°C (Table 4-4). CDA is a typical assay for primary oxidation products whereas \( p\)-AV can detect alkenals, which are secondary oxidation products (Kim et al., 2014). The oxidative stability in oils containing extracts of puffed or red ginseng 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 (Yi et al., 2015; Oh et al., 2015; Ka et al., 2016). 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 (Laguerre et al., 2010, 2015; Lee et al., 2013; Shahidi et al., 2011). For example, curcumin, which inhibited lipid oxidation in O/W emulsions, did not act as an antioxidant nor as a prooxidant in corn oil (Yi et al., 2015). Ka et al. (2016) 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 RF photosensitization. Aqueous extracts of hulled barley (Hordeum vulgare L.) had antioxidant or prooxidant properties in bulk oil while showed antioxidant properties in RF photosensitized O/W emulsions (Oh et al., 2015). 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 (Laguerre et al., 2010; Lee et al., 2013). Additionally, Shahidi et al. (2011) 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 prooxidant compounds could be located can be critical places for the regulation of the rates of lipid oxidation (Laguerre et al., 2010, 2015). Puffing process significantly increased TPC and TFC in the extracts compared to non-puffed red ginseng (Fig. 4-4 and Fig. 4-5) and those phenolic compounds could be located on the surface of association colloids in O/W emulsions and show enhanced antioxidant capacity (Fig. 4-6 and Fig. 4-7). However, extracts did not have antioxidant activity in bulk oils, especially at 100°C temperature 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 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 a somewhat different, particularly at a concentration of 1.0% (Table 4-4). 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 (Alamed et al., 2009; Decker et al., 2005; Kim et al., 2014; Yi et al., 2015).
Table 4-3. Changes of headspace oxygen, CDA, and $p$-anisidine values in corn oil with addition of extract of red ginseng and puffed red ginseng (0-1.0% w/w) at 60°C treatment

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Red ginseng</th>
<th>Puffed red ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 %</td>
<td>0.25 %</td>
</tr>
<tr>
<td>Headspace oxygen (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20.80±0.01a</td>
<td>20.80±0.01a</td>
</tr>
<tr>
<td>10</td>
<td>19.73±0.05c</td>
<td>19.77±0.09c</td>
</tr>
<tr>
<td>15</td>
<td>17.62±0.15ab</td>
<td>17.03±0.12b</td>
</tr>
<tr>
<td>20</td>
<td>13.73±0.58ab</td>
<td>12.63±0.90b</td>
</tr>
<tr>
<td></td>
<td>CDA (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.21±0.01ab</td>
<td>0.21±0.01ab</td>
</tr>
<tr>
<td>10</td>
<td>0.39±0.01a</td>
<td>0.39±0.01a</td>
</tr>
<tr>
<td>15</td>
<td>1.18±0.06bc</td>
<td>1.33±0.06ab</td>
</tr>
<tr>
<td>20</td>
<td>2.27±0.55a</td>
<td>2.26±0.20a</td>
</tr>
<tr>
<td></td>
<td>$p$-Anisidine</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11.98±0.33a</td>
<td>12.37±0.33a</td>
</tr>
<tr>
<td>10</td>
<td>12.65±0.30a</td>
<td>10.20±0.01c</td>
</tr>
<tr>
<td>15</td>
<td>12.42±0.49b</td>
<td>13.23±0.43a</td>
</tr>
<tr>
<td>20</td>
<td>18.83±2.98a</td>
<td>19.75±2.67a</td>
</tr>
</tbody>
</table>

*a mean±standard deviation (n=3)  

b In the same row, different letters are significantly different at $\alpha=0.05$.  

Table 4-4. Changes of headspace oxygen, CDA, and $p$-anisidine values in corn oil with addition of extract of red ginseng and puffed red ginseng (0-1.0% w/w) at 100°C treatment

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Headspace oxygen (%)</th>
<th>Red ginseng</th>
<th>Puffed red ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20.80±0.01%a</td>
<td>20.80±0.01a</td>
<td>20.80±0.01a</td>
</tr>
<tr>
<td>9</td>
<td>20.17±0.04b</td>
<td>20.22±0.04b</td>
<td>20.27±0.08ab</td>
</tr>
<tr>
<td>18</td>
<td>18.63±0.20b</td>
<td>18.08±0.26c</td>
<td>18.70±0.26b</td>
</tr>
<tr>
<td>27</td>
<td>13.91±0.70b</td>
<td>11.23±0.27c</td>
<td>13.40±0.36b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>CDA (%)</th>
<th>Red ginseng</th>
<th>Puffed red ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.21±0.01ab</td>
<td>0.21±0.01ab</td>
<td>0.20±0.01b</td>
</tr>
<tr>
<td>9</td>
<td>0.37±0.01a</td>
<td>0.36±0.01ab</td>
<td>0.35±0.01b</td>
</tr>
<tr>
<td>18</td>
<td>0.90±0.02b</td>
<td>1.03±0.09a</td>
<td>0.90±0.04b</td>
</tr>
<tr>
<td>27</td>
<td>1.82±0.02b</td>
<td>2.12±0.06a</td>
<td>1.90±0.05b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>$p$-Anisidine value</th>
<th>Red ginseng</th>
<th>Puffed red ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11.98±0.33a</td>
<td>12.37±0.33a</td>
<td>11.42±0.63a</td>
</tr>
<tr>
<td>9</td>
<td>9.42±0.21b</td>
<td>8.62±0.16b</td>
<td>8.12±0.46b</td>
</tr>
<tr>
<td>18</td>
<td>11.18±0.40b</td>
<td>12.92±0.98a</td>
<td>12.08±0.03ab</td>
</tr>
<tr>
<td>27</td>
<td>32.67±1.11c</td>
<td>50.47±3.68a</td>
<td>38.15±0.15b</td>
</tr>
</tbody>
</table>

*a mean±standard deviation (n=3)*

*b In the same row, different letters are significantly different at $\alpha=0.05$. 
4. 4. Conclusions

Using *in vitro* assays, it was elucidated that extracts of puffed red ginseng had higher antioxidant activity than those of red ginseng. The puffing process increased the total ginsenoside content and especially the content of Rg3. In riboflavin photosensitized O/W emulsions, extracts of red ginseng showed concentration-dependent high antioxidant activity. However, in bulk oil systems, extracts of puffed red and red ginseng had antioxidant or prooxidant properties depending on the treatment temperature, concentration of the extracts, and types of assays used. Therefore, the antioxidant or prooxidant properties of samples should be tested in food systems as well as *in vitro* assays.
4.5. References


Chaiyasit, W., Elias, R. J., McClements, D. J., and Decker, E. A. 2007. Role of physical structures in bulk oils on lipid oxidation. Critical Reviews in Food


Overall discussion
Red ginseng was puffed to possess a more porous structure and to reduce the extraction time of bioactive compounds in ginseng compared with traditional red ginseng extraction. Puffing process changed biofuctional properties of red ginseng including ginsenoside composition, antioxidant activity, and volatile components profile.

The puffing process applied to red ginseng changed the total ginsenoside content. The content of major ginsenosides increased from 11.54 mg/g to 12.96 mg/g and the content of minor ginsenosides (Rg2, Rg3, Rh1) increased from 0.44 mg/g to 0.70 mg/g after puffing process. Among minor ginsenosides, Rg3(S) increased by 25 times, from 0.08 mg/g to 0.20 mg/g. Experimental studies have demonstrated that ginsenoside Rg3 can inhibit cancer cell growth by promoting the apoptosis of cancer cells. Although extraction yield of puffed red ginseng gradually increased with extraction time but yields did not increase drastically at any time beyond 16 h. This suggests that extraction for 16 h economically provides optimal yield in terms of time and energy. Comparing the extraction
yields of puffed red ginseng and non-puffed red ginseng at 16 h, the extraction yield of puffed red ginseng was 37.0% higher than that of red ginseng. The tendency of increase in the extraction yield after puffing was in agreement with the studies of other researchers. The increase of extraction yield in puffed red ginseng could be explained by the fact that explosive puffing process softened the rigid cell wall structure and induced expanded and porous structure to make the solvent access easy. Arginine among amino acids was abruptly reduced in the process of making puffed red ginseng because it reacted with maltose and produced maltulosyl arginine through Maillard reaction. When comparing the free sugars in puffed red ginseng and red ginseng, maltose was decreased drastically. Presumably, puffing process accelerated the reaction of maltose and amino acids in red ginseng to produce maltol.

Also, explosive puffing affected the profile of volatile compounds in red ginseng. Large changes in the surface structures caused by explosive puffing were clearly observed in both scanning electron micrographs magnified by 1000 and
2000 times (Fig. 3-1). Puffed red ginseng had a more porous structure and smaller pieces than red ginseng. These changes in the microstructure of red ginseng led to the change of volatile compounds. Red ginseng had 4 alcohols, 4 aldehydes, 2 acids, 5 terpenoids, 2 ketones and 3 other volatiles, while puffed red ginseng had 3 alcohols, 13 terpenoids, and 1 acid. While major volatiles in red ginseng were hexanal, $\Delta$-selinene, and $\beta$-panasinsene, main volatiles in puffed red ginseng were $\alpha$-gurjunene, $\beta$-panasinsene, and calarene. Volatiles from lipid oxidation decreased and terpenoid compounds increased during explosive puffing. Two peaks with the retention time 7.60 and 14.53 min were greatly increased in puffed red ginseng compared with those in red ginseng. These two peaks were identified as 2-furanmethanol and maltol later. Considering the antioxidative activity of maltol and 2-furanmethanol, they could be used as biomarkers to discriminate puffed red ginseng from other types of ginseng without puffing process.

Lastly, the antioxidant properties of extracts of red ginseng and puffed red ginseng were determined by \textit{in vitro} assay and food matrix model including bulk
oil and oil-in-water emulsions. The extract of puffed red ginseng showed significantly higher free radical scavenging activity than that of red ginseng ($p<0.05$). 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 ginseng. In riboflavin photosensitized O/W emulsions, extracts of red ginseng showed concentration-dependent high antioxidant activity. However, in bulk oil systems, extracts of puffed red and red ginseng had antioxidant or prooxidant properties depending on the treatment temperature, the concentration of the extracts, and the types of assays used.

In conclusion, puffing process could induce the changes in physicochemical properties, antioxidant activity, and volatile compounds by altering the microstructure such as formation of pores and swelling. Therefore, puffing process is an efficient alternative means to produce functional red ginseng products with the additional advantage of reduced processing time.
국문 초록

인삼( *Panax ginseng* C. A. Meyer)의 주요 약리활성 성분으로는 ginsenoside, 페놀화합물, 다당체 등이 있다. 이러한 성분 중 일부는 원료 수삼을 증자 또는 건조하여 홍삼과 백삼을 만드는 과정 중 화학구조가 변환되어 새로운 성분이 생성되기도 하고 일부 생리활성 성분의 함량이 증가되기도 한다. 지금까지 많은 연구가들이 인삼과 홍삼에서 생리활성 성분의 종류, 기능, 구조를 밝혀왔지만, 단단한 뿌리 조직에서 생리활성 성분들을 효율적으로 추출하기 위한 방법들에 관한 연구는 상대적으로 많지 않다. 최근에 extrusion 과 팽화 공정을 도입하여 공정 최적조건, 다양 ginsenoside profile 변화, 산화 방지 활성, 휘발성 성분에 대한 연구가 일부 보고되고 있는 정도로 산업적 이용을 위해서는 보다 많은 연구가 필요한 실정이다.

따라서 본 연구는 산업적 이용을 위한 기초자료를 제공하고자 홍삼을 팽화하였을 때 일어나는 생리기능성을 구명하기 위해 수행되었다. 6 년근 인삼을 증자 건조하여 홍삼을 만든 후 고온고압 조건에서
팽화하여 홍삼의 미세구조, 취발성 성분, ginsenoside 를 포함한 유효성분의 변화를 조사하였고, 팽화홍삼 에탄올 추출물의 산화방지 활성을 in vitro 방법과 oil in water 에멀션 모형을 통해 비교하였다.

팽화 후 20 시간 동안 추출하면서 4 시간 간격으로 추출 수율을 측정한 결과 팽화홍삼의 추출 수율은 16.7%에서 42.2%로 증가한 반면, 홍삼의 추출 수율은 9.0%에서 32.7%로 증가했다. 팽화홍삼의 ginsenoside 함량은 13.65 mg/g 으로 홍삼보다 높았으며, 홍삼에 다량 존재하는 ginsenoside (Rb1, Rb2, Rc, Rd, Re and Rg1)의 함량은 7.23 mg/g 으로 팽화하지 않은 홍삼(5.40 mg/g)보다 높았다. 또한 미량으로 존재하는 ginsenosides (Rg2, Rg3, Rh1)의 함량도 0.44 mg/g 에서 0.70 mg/g 으로 증가했다. 미량 ginsenoside 중 Rg3 는 0.08 mg/g 에서 0.20 mg/g 으로, 팽화하지 않은 홍삼보다 25 배 증가했다. 유리아미노산의 총 함량이 12.7 mg/g 에서 7.1 mg/g 으로 감소했고, 홍삼에 존재하는 유리아미노산 중 arginine 과 유리당 중 맥아당 함량이 팽화 후 크게 줄어든 것은 맥아당과 arginine 이 반응하여 갈변물질을 생성하기
때문이다. 색차계로 색도 변화를 측정한 결과 L 값은 팽화 후 감소했고, a 값은 증가한 것으로 보아, 팽화 후 홍삼은 색이 더 어두워지고, 붉은 색이 증가했다. 유리 지방산의 변화를 측정한 결과, 팽화 후 홍삼에 없었던 palmitoleic acid 가 새로 생성되었고, 불포화지방산의 비율이 증가했다.

팽화 후 전자현미경으로 관찰한 결과 홍삼의 조직은 다공성의 미세입자로 변해있었고, 팽화홍삼의 휘발성 성분의 홍삼보다 87% 증가했다는 것이 HS-SPME를 이용한 GC-MS 분석으로 밝혀졌다. 홍삼의 주요 휘발성 성분은 hexanal, Δ-selinene, and β-panasinsene 인 반면, 팽화홍삼에는 α-gurjunene, β-panasinsene, calarene 이 주요 성분으로 측정되었다. 홍삼에는 2-furanmethanol 과 maltol 이 각각 0.20%와 0.24% 존재했었는데, 팽화 후에는 그 함량이 5.86%와 3.99%로 증가했다.

홍삼과 팽화홍삼 에탄올 추출물의 산화 방지 활성을 in vitro 실험과 oil-in-water 에멀션 모형에서 측정한 결과, DPPH, ABTS, FRAP 방법에서는 팽화홍삼의 산화 방지 활성이 홍삼보다 높게 나타났다.
이것은 팽화홍삼 추출액에 홍삼보다 ginsenoside 가 더 많이 존재하고 있고, 산화 방지 활성을 나타내는 폴리페놀이 함량이 높았기 때문이다. 실제 식품과 유사한 환경을 만들어 산화 방지 활성을 측정하기 위한 본 연구의 모델시스템인 riboflavin photosensitized O/W emulsions 에서 반응 온도 및 팽화홍삼 추출액의 농도에 따라 지방질 산화 속도가 조절되었다.

결론적으로 홍삼을 팽화하면 고온고압에 의해 ginsenoside 구조가 변화되면서 기존에 없던 ginsenoside 가 생성되고, 홍삼이 다공성 조직으로 팽창되어 폴리페놀성 화합물이 많이 추출되어 산화 방지 활성이 증가함을 확인하였다. 또한 식품산업에 응용할 경우 짧은 시간에 많은 생리활성 성분을 추출할 수 있어 원가 절감에 기여할 수 있으리라 생각된다.

주요어: 인삼, 홍삼, 팽화, 산화 방지 활성, 훈발성 성분, 에멀션 모형, 폴리페놀성 화합물

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