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dissertation entitled

EFFECTS OF EXTRUSION AND BAKING PROCESSES ON GINSENOSIDES IN WHEAT FLOUR-GINSENG POWDER BLENDS

presented by

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has been accepted towards fulfillment
of the requirements for the

Doctoral degree in Food Science and Human Nutrition

Major Professor's Signature

May 7, 2008

Date

EFFECTS OF EXTRUSION AND BAKING PROCESSES ON GINSENOSIDES IN WHEAT FLOUR-GINSENG POWDER BLENDS

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOOSPHY

Department of Food Science and Human Nutrition

2008

ABSTRACT

EFFECTS OF EXTRUSION AND BAKING PROCESSES ON GINSENOSIDES IN WHEAT FLOUR-GINSENG POWDER BLENDS

By

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The specific objectives of the present study were: (1) to explore optimum extraction conditions of ginsenosides (G, ginseng saponins) from a blend of wheat flour (WF)-ginseng powder (GP), (2) to investigate effects of extrusion process variables on extractable G in wheat-ginseng extrudates (WGE), (3) to measure quality properties of WGE extruded under different conditions, and (4) to evaluate physicochemical properties of bread and cookies baked from hard wheat flour and soft wheat flour, respectively, blended with GP and effects of baking on their extractable G.

Through studies on optimization of extraction conditions for G from WF-GP, it was observed that interactions between a starch fraction and G as well as between a gluten fraction and G did occur in WF-GP heated at 90°C. It was also found that the interactions between WF components and G could be completely disrupted by increasing ultrasonic extraction time up to 90 min, for the maximum extraction.

To evaluate effects of extrusion process variables on the extractable G in and quality properties of WGE samples, WF-GP (10% GP, w/w) was extruded in a twin-screw extruder with full factorial combinations of feed moisture (25, 30, and 35%), screw speed (200 and 300 rpm), and zone 5 barrel temperature (110, 120, 130, and 140°C). The quantities of G (Rb1, Rc, and Rd) extracted from WGE samples extruded at a zone 5 barrel temperature of $\geq$ 120°C were significantly higher than those extracted

from the non-extruded WF-GP blend. New G compounds (Rg2 and Rg3), which were not present in raw non-extruded WF-GP, were found in certain WGE samples, indicating that high temperature extrusion cooking could lead to the chemical modification of thermolabile protopanaxatriol type G and protopanaxadiol type G into G Rg2 and G Rg3, respectively. The amounts of G (Rg2 and Rg3) were dependent on each of the three extrusion process variables studied. The physical (expansion ratio, water absorption index, and water solubility index), pasting, and thermal properties of WGE samples were significantly affected by changes in extrusion process variables studied.

From studies on effects of baking processes (bread-baking and cookie-baking) on G of WF-GP blends, it was found that the temperatures used for bread- and cookie-baking could increase the amounts of extractable G Rb1, G Rc, and G Rd in the hard wheat-ginseng bread and soft wheat-ginseng cookie samples. Ginsenosides Rg2 and Rg3 were present only in the hard wheat-ginseng bread sample, indicating that not only the higher baking temperature (215°C) but also the longer baking time (24 min) used for bread-baking could cause the production of ginsenosides Rg2 and Rg3, as compared to cookie-baking conditions (205°C and 11 min).

ACKNOWLEDGEMENTS

Many people have helped me in the completion of this dissertation, and I would like to acknowledge and thank them here. First of all, I would like to thank Dr. Perry K.W. Ng, my major advisor, for being a wonderful mentor and for his constant support throughout my study and research. His expert guidance and generosity with his time and ideas have enriched my graduate school experience and have contributed greatly to my personal and professional development over the years. I feel very fortunate to have had an advisor whose help, kindness, patience, and concern for my well being helped make my doctoral program an enjoyable and rewarding experience. I also would like to thank my committee members, Dr. James F. Steffe, Dr. Gale M. Strasburg, and Dr. Mark A. Uebersax, for the valuable contributions they have made to help improve this work and for the support and encouragement they have all given me along the way.

Thanks are extended to previous labmates, Ed, Hilda, and Ritu, and previous visiting scholars, Dr. Ahn, Dr. Kim, Dr. Koh, and Dr. Rhim. Furthermore, I would like to thank my current labmates, George and Kevser.

Finally, I am extremely grateful to my parents, parents-in-law, wife, and son for all of their love, support, encouragement, and sacrifice throughout the course of this study.

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CHAPTER 1

INTRODUCTION

Wheat is one of the most important cereal crops to humankind. Common wheat is divided into soft wheat and hard wheat varieties, depending on its kernel texture. In addition, hard wheat has higher protein content and stronger protein strength as compared to soft wheat (Atwell 2001; Posner 2000). Hard wheat flour (HWF) is used mainly for the production of bread products, while soft wheat flour (SWF) is usually used to produce snacks, cookies, cakes, pastries, crackers, and pretzels.

Extrusion cooking is a versatile, low cost, and very efficient technology in food processing, and has been used in the production of breakfast cereals, baby foods, snack foods, pasta products, pet foods, instant powders, modified starches, etc. (Ficarella et al 2006; Jin et al 1995; Singh and Singh 2004). During extrusion cooking, a variety of steps including feed transport, mixing, heating, forming, and drying occur within a short time (Harper 1998). The food components in the extruder barrel experience high temperature, shear, and pressure during extrusion cooking (Guy 2001; Harper 1981). The addition of diverse ingredients to extrusion materials has been done with the objective of improving the nutritional quality of final extruded products and imparting desirable functional properties (Jin et al 1995; Schmid et al 2005; Zazueta-Morales et al 2002).

Bread can be produced by many different ingredients and procedures depending on tradition, types of flour, and types of bread desired (Pyler 1988). Water and HWF are the most important ingredients in breadmaking, and yeast, salt, shortening, and sugar are also added according to manufacturer's choice. Bread can be produced by three basic processing steps: mixing or dough formation, fermentation, and baking (Hosney 1998). Recently, new ingredients such as various fibers, proteins, and vitamins have been introduced in bread formulations not only to improve the texture but

also to make nutritionally superior products (Dalgetty and Baik 2006; Filipovic et al 2007; Ranhotra et al 2000).

The formulation for making cookies basically includes SWF, sugar, shortening, and water. The addition of nutraceutical ingredients to the formulation of cookies could have advantages, including health benefits. Many recent studies have reported on nutraceutical cookies, such as high-protein cookies (Singh and Mohamed 2007) and high-dietary fiber cookies (Bilgicli et al 2007).

Ginseng has long been used in Asian countries as a traditional medicine (Fuzzati 2004). Ginsenosides (G, ginseng saponins) have been known as the main active components in ginseng root and have been typically used as marker compounds for quality properties (Chuang et al 1995; Lau et al 2004; Li et al 1996). It has been known that the extraction efficiency of G from raw plant materials, extracts, and commercial products is dependent on extraction conditions used, such as extraction solvents (pure methanol, aq. methanol, pure ethanol, or aq. ethanol), sample-to-solvent ratio, extraction time, extraction temperature, and extraction methods (heating or ultrasonication) (Court et al 1996; Fuzzati 2004; Lau et al 2004).

Raw ginseng is processed into two kinds of ginseng, white ginseng and red ginseng, to improve its preservation and efficacy (Kim et al 2000; Wang et al 2006). White ginseng is usually air-dried raw ginseng, while red ginseng is usually made by first steaming raw ginseng at 90-100°C for 2-3 hr and then air-drying (Ha et al 2005). Red ginseng is known to be more pharmaceutically active than white ginseng, because the steaming process causes changes in the chemical composition of G, thereby enhancing biological activities of ginseng (Ha et al 2007; Shibata 2001). The ginsenosides Rg3, Rg5, Rg6, Rh2, Rh3, Rh4, Rs3, Rk1, and F4 are not found in raw and

white ginseng, whereas red ginseng contains these new G generated during the steaming process (Ha et al 2007; Kim et al 2000; Kwon et al 2001). In particular, G Rg3 have been found to exhibit various biological activities, including anticancer activity (Shibata 2001; Wang et al 2006; Yun et al 2001) and radical scavenging activity (Kang et al 2006). In addition, it has been reported that G Rg3 inhibit the growth of *Helicobacter pylori* (Bae et al 2002).

Healthy foods, especially those with nutraceutical properties, are in great demand in our health-conscious society. Nutraceutical snacks, bread, and cookies could be good vehicles in this respect if a portion of the wheat flour (WF) were to be replaced with nutraceutical ingredients, such as ginseng. Extrusion cooking, bread-baking, and cookie-baking normally involve the use of high temperature to affect and alter the nature of many food constituents. Thus, there is a possibility of using WF in combination with ginseng to produce extruded snack foods, and baked bread and cookies that contain the ginsenosides found in red ginseng.

In order to pursue these possibilities, the present study was set up with the following objectives:

1. To explore optimum extraction conditions of G from a blend of WF-GP.
2. To investigate effects of extrusion process variables on extractable G in wheat-ginseng extrudates (WGE).
3. To evaluate quality properties of WGE extruded under different processing conditions.
4. To evaluate effects of adding GP on Farinograph, thermal, and pasting properties of HWF and SWF.
5. To study quality properties of hard wheat-ginseng bread and soft wheat-ginseng

cookies.

6. To investigate changes in G components in hard wheat-ginseng bread and soft wheat-ginseng cookies upon baking.

The information generated through the present study is aimed at providing information on a novel processing method of ginseng, and aiding production of new nutraceutical snack foods, bread, and cookies that incorporate ginseng components. The following dissertation entails: (1) Literature review, (2) Extraction of ginsenosides from a blend of wheat flour and ginseng powder, (3) Effects of extrusion process variables on extractable ginsenosides in wheat-ginseng extrudates (4) Effects of extrusion process variables on quality properties of wheat-ginseng extrudates, and (5) physicochemical properties of bread and cookies baked from wheat flour blended with ginseng powder. The chapters in this dissertation were written in the journal paper format and thus some of the information from chapter to chapter might appear repetitive.

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CHAPTER 2

LITERATURE REVIEW

2.1. Extrusion

A food extruder can be described as a device that transforms a variety of raw ingredients into intermediate and/or final extruded products. Extrusion processes involve operations of feed transport, mixing, heating, and forming at the same time (Anderson and Ng 2003; Camire et al 1990; Ding et al 2006; Harper 1979). The cooking temperature can be relatively high during extrusion cooking, but the residence time (time materials actually spend within the barrel) is usually relatively short, hence the extrusion cooking process is often referred to as a high temperature short time (HTST) process. Extrusion cooking can produce final extruded products with high quality, including high digestibility and nutritional value (Cauvain 2001), all characteristics with appeal.

In general, food extruders can be classified into two types: single-screw and twin-screw extruders (Harper 1979). Single-screw extruders are manufactured with and without grooved barrels and with either single-piece screws or individual slip-on screw elements of various designs (Van Zuilichem and Stolp 1984). Single-screw extruders are typically known to include three fundamental zones: a primary feed zone, a melt zone, and a pump zone. On the other hand, twin-screw extruders can be classified as co-rotating or counter-rotating and as intermeshing or non-intermeshing. The twin parallel screws fit into a horizontal barrel housing all the ingredients. The most popular type of twin-screw extruder is those with co-rotating and intermeshing screws, which have been found to be suitable for many extrusion processes due to the wider control of the residence time distribution, good mixing intensity, superior heat transfer, and self-wiping operation (Moore 1994; Van Zuilichem and Stolp 1984).

Via its heat treatment, extrusion cooking can affect and change the nature of

many food constituents, including starches and proteins, by changing physical, chemical and nutritional properties, such as starch gelatinization, protein denaturation, complex formation between amylose and lipids, and degradation reactions of vitamins, pigments, etc. (Camire et al 1990; Harper 1979; Iwe et al 2004; Sgaramella and Ames 1993). In addition, the moisture content in the final product largely determines the end product's physical state (glass, rubber, and crystalline).

In recent years, there has been an increasing interest in the production of extruded foods, such as texturized vegetable proteins, ready-to-eat (RTE) breakfast cereals, expanded snacks, baby foods, pet foods, instant powders, and pasta products (Anderson and Ng 2003; Ficarella et al 2006; Jin et al 1995; Singh and Singh 2004). An extruder essentially consists of one or two screws, mixing (kneading) paddles, an external barrel and an exit die (Ficarella et al 2006). During extrusion cooking, the ingredients are mixed, sheared and subjected to elevated temperatures and pressures in the barrel. The dough exhibits some degree of plasticity that enables the expansion of the final product upon exiting the die (Schmid et al 2005). In fact, the sudden drop in pressure at the die results in the release of gases entrapped in the product, determining the puffing of extrudates (Camire et al 1990).

With extrusion processing, a wide variety of foods can be produced using an economical, energy-efficient, rapid, and continuous system with numerous ingredients and processing conditions (Ficarella et al 2006). On the other hand, the extrusion process presents its own challenges as it is fundamentally extremely complex since there is a great deal of process variability. Not only process variables, but also product quality, can be influenced by small changes in processing conditions (Desrumaux et al 1999). Based on the extruder type, screw configuration and speed, feed moisture,

temperature profile in the barrels, feed rate, and die type, the final product quality can be changed remarkably (Unlu and Faller 2002).

2.1.1. Independent Variables

Extrusion process variables that directly control product quality properties are referred to as independent variables (Ryu and Ng 2001). The stability of an extrusion system and product quality is an optimization of extrusion process variables and feed ingredients. The major extrusion process variables, which include feed rate, feed moisture, screw designs and configurations, screw speed, die design and size, barrel temperatures, and the addition of other ingredients, can be directly controlled by the extruder operator (Harper 1981). Ryu and Ng (2001) evaluated the effects of extrusion process variables such as screw speed, feed moisture, and barrel temperature on the expansion ratio and mechanical properties of wheat flour and whole cornmeal extrudates. They observed that the expansion ratio, specific length, and breaking strength of these extrudates are significantly affected by changes in feed moisture and in barrel temperature.

The primary crucial extrusion process variable is the stable and consistent supply of feed ingredients into the extruder. Inconsistent introduction of feed ingredients is often the cause of inconsistent expansion ratios, poor shape, and varied textures of final extrudates (Moore 1994).

The screw is the key element of an extruder since its configuration and speed can influence the unit operation of the extruder. Screw speed directly affects the degree of the barrel fill, the residence time distribution, and the shear stress on the material being extruded. Moreover, die pressure during extrusion cooking by the

action of both single-screw and twin-screw extruders can be significantly affected by the configuration of the screw and its rotational speed (Harper 1981). Mechanical energy dissipation in the screws causes the temperature of the ingredients to increase rapidly, transforming the mass from a granular state into a continuous plasticized mass. The shear occurring during extrusion cooking is very high and causes mechanical modification of extruded ingredients (Ha et al 2005). In particular, damage to starch or protein molecules can reduce their ability to form an elastic matrix capable of expanding and retaining texture upon passing through the die (Harper 1998).

Most extruders have the ability to control temperature, and the degree of indirect heating or cooling depends on how the extruder is operated. Barrel heating generates conductive and convective heat in filled and partially-filled zones, and the temperatures of the barrel zones depend on the physical and rheological properties of the feed, as well as the chosen barrel temperature profile and screw speed (Moore 1994).

2.1.2. Dependent Variables

Dependent variables change as a result of changes made to the independent variables, and they include melt temperature, residence time in the extruder, pressure in the extruder, specific mechanical energy (SME) and quality properties of final extruded products (Ryu and Ng 2001). Melt temperature corresponds to the disappearance of native starch crystallinity and is a function of the feed material, mechanical energy input, and heat transfer (Harper 1981). The SME is a measure of the amount of work being input to the material during extrusion cooking and is affected by the feed rate, feed moisture, screw configuration, screw speed, barrel temperature profile, and rheological properties of the extruded material (Onwulata et al 2001). The SME can also control

target-dependent variables such as the expansion ratio, water absorption index (WAI) and water solubility index (WSI), density of extrudates, and many other measurable parameters (Ryu and Ng 2001).

The pressure at the final die of an extruder attains a value which balances the output of the extruder with the flow through the die (Harper 1981). The viscosity is considerably affected by composition and moisture of the feed materials, residence time, shear rate, and temperature. The rheological properties of a food dough in the extruder are quite complex and are known to be non-Newtonian (Hagenimana et al 2007).

Residence time is defined as the length of time which the process ingredients spend in the barrel of the extruder during extrusion cooking (Unlu and Faller 2002). This has commonly been used in evaluating the performance of an extruder. Analysis of residence time provides information about the degree of mixing, cooking, and shearing, all of which play significant roles in the quality of the final products. The residence time data are also used for scale-up and improving equipment design (Van Zuilichem and Stolp 1984).

The most important dependent variables are the quality properties, such as physicochemical properties [expansion ratio, density, water absorption index (WAI), water solubility index (WSI), texture, gelatinization, flavor, color, appearance, etc.], pasting properties (peak, trough, breakdown, final viscosity, and setback), and thermal properties [transition onset temperature (To), transition peak temperature (Tp), transition enthalpy (ΔH)] of the final extruded products. The following will review the effects of extrusion process variables on the major quality properties of extrudates.

2.1.2.1. Expansion Ratio and Bulk Density

Harper (1979) reported a couple of elements affecting the expansion ratio of an extrudate: dough viscosity and elasticity. Feed moisture, however, is known to be the main factor in controlling both extrudate density and expansion of final extruded products (Ding et al 2006; Fletcher et al 1985; Liu et al 2000). A reduction in gelatinization resulted in a decrease in the expansion ratio of and an increase in the bulk density of wheat flour extrudates (Ding et al 2006). In addition, Rinaldi et al (2000) showed that a decreasing trend in the expansion ratio of wheat flour extrudates enriched with wet okara, and an increasing trend in the bulk density of the extrudates were observed as the extrusion conditions became increasingly more severe (i.e., high shear screw configuration). They reported that the decreased expansion and increased bulk density may be because of the breakdown of starch granules into smaller particles, which may interfere with bubble expansion upon exiting the die.

The expansion ratio of an extrudate can also be affected by feed materials and addition of other ingredients. Anderson and Ng (2003) evaluated the effects of feed protein content levels on extrudate expansion of wheat flour. They reported that the expansion ratio of wheat flour extrudates was negatively affected by increasing feed protein content of raw feed material; therefore, higher protein content of raw materials may not be beneficial to expansion in the extrusion processing of wheat flour. According to Park et al (1993), increasing protein content levels in the feed for the extrusion of soy flour, corn starch, and raw beef blends reduced the expansion ratio of the extrudates and increased the density of the extrudates.

Calcium is one ingredient that has been reported to affect extrudate expansion ratio. In work done by Zazueta-Morales et al (2002), it was shown that the

addition of calcium hydroxide (0.0 to 0.2%, w/w) to blue maize flour decreased the expansion ratio of the extrudates from 3.4 to 1.4.

2.1.2.2. Water Absorption Index (WAI) and Water Solubility Index (WSI)

The WAI measures the amount of water absorbed, mainly by starch (Anderson et al 1969). According to Ding et al (2006), increasing feed moisture significantly increased values of WAI of wheat flour extrudates; however, an increase in barrel temperature was observed to cause a significant decrease in values of WAI of the extrudates. Hagenimana et al (2006) reported that high feed moisture during extrusion cooking of rice flour causes higher values of WAI of the extrudates due to reduced amounts of degradation of starch granules. On the other hand, values of WAI decreased with increasing barrel temperature and screw speed, because of an increase in starch degradation or fragmentation (Jin et al 1995).

The WSI measures the amount of soluble components released from the starch upon extrusion cooking (Kirby et al 1988) and has been used as an indicator of degradation of molecular components (Ding et al 2005). Hagenimana et al (2006) showed that values of WSI of rice flour extrudates increased with increasing both the severity of the screw profile in the extruder and the processing temperature. Tanhehco and Ng (2005) reported that decreasing extrusion feed moisture content or increasing extruder screw speed led to an increase in WSI values of wheat flour extrudates.

2.1.2.3. Pasting Properties

Extrusion cooking of starch leads to gelatinization, partial or complete destruction of the crystalline structure, and molecular fragmentation of starch

(McPherson et al 2000). The degree of gelatinization of starch granules and the extent of their molecular breakdown are known to affect the viscosity of final extruded products (E-Dash et al 1983).

McPherson et al (2000) investigated the pasting properties of extruded corn starch using a Rapid Visco Analyzer (RVA), and they reported that starches extruded at different conditions had differences in hot paste viscosity and final viscosity values. For instance, increasing feed moisture during extrusion cooking increased the starch viscosity, while increasing extrusion temperature and shear decreased the starch viscosity. High peak and final viscosity values were observed for the corn starch extrudates prepared at high moisture content and low screw speed (Blanche and Sun 2004); the researchers assumed that the degree of starch transformation was lower under these extrusion conditions.

2.1.2.4. Thermal Properties

Extrusion cooking causes physical and structural conversions of starches in wheat flour (Anderson and Ng 2001; Brent et al 1997). The phase transitions (including Tp and ΔH) of the extrudates have been studied using differential scanning calorimetry (DSC). Anderson and Ng (2001) reported that the value of Tp is particularly associated with the heat stability of non-extruded and extruded samples. Blanche and Sun (2004) found no gelatinization peak on DSC thermograms obtained for corn starch extrudates. They speculated that starch granules were completely gelatinized as a result of extrusion cooking or that starch molecules might be broken down or depolymerized due to heat and shear forces during extrusion cooking.

2.1.2.5. Breaking Strength

The breaking strength (hardness) is the maximum force required for a probe to snap an extrudate into two pieces. As already mentioned, feed moisture, screw speed, and barrel temperature are the main factors affecting the density and expansion of extrudates. Hardness is considerably influenced by the density and expansion of extrudates. Rinaldi et al (2000) observed that breaking strength increased as expansion ratio decreased and as bulk density increased; they postulated this was due to thickening of the cell walls and decreasing air cell size, which enhanced the resistance of the extrudates to fracturing. Onwulata et al (2001) reported that the addition of proteins to starches increases the sites available for cross-linking and affects texture properties of the extrudates. Ryu and Ng (2001) observed that breaking strength of wheat flour extrudates extruded at higher SME input was lower than those made at lower SME input, and they suggested that extrudates produced under higher SME conditions had a softer texture than those extruded under lower SME conditions.

2.1.3. Changes in Starch, Proteins, and Heat-Labile Components during Extrusion Cooking

2.1.3.1. Starch

It has been reported that starch is the predominant functional ingredient in extruded snacks, RTE cereals, and other extruded products (Mercier and Feillet 1975). Extrusion cooking of starch granules causes changes in their morphological and molecular structure depending on several factors including feed moisture, cooking temperature, and mechanical and thermal energy input (Barres et al 1990). Ozcan et al (2005) showed that extrusion cooking of corn starch at high temperature (165°C) and

low moisture content (8.72%) with moderate shear resulted in changing both gelatinized and melted starches into fully cooked amorphous extrudates whose starches crystallize after being cooled. Moreover, it has been known that increasing extrusion temperature causes a higher degree of gelatinization, while increasing screw speed and increasing die diameter each lead to a reduction in gelatinization (Blanche and Sun 2004; McPherson et al 2000).

2.1.3.2. Proteins

Heat and shear during extrusion cooking can induce the denaturation of proteins and weaken the forces that stabilize the tertiary and quaternary structures of proteins. The result is the formation of a new molecular aggregate structure (Guy 2001). According to Anderson and Ng (2003), during extrusion cooking of wheat flour, gluten is significantly involved in the microstructural and textural formation of the extrudates. Furthermore, intermolecular disulfide bonding is the main cause of protein aggregation in the extrusion process (Anderson and Ng 2000; Guy 2001).

During extrusion cooking, a reducing sugar such as glucose, fructose, lactose, or maltose may react with free amino groups in proteins following the so-called Maillard reaction (Harper 1979). This is a complex reaction that is often desired for the development of golden brown color and caramel aroma in extruded products, but it is also generally responsible for a reduction in the nutritional value (Harper 1979). According to Guy (2001), during extrusion cooking, the Maillard reaction was favored by conditions of high temperature (more than 180°C) and screw speed (more than 100 rpm) in combination with low feed moisture content (less than 15%).

2.1.3.3. Heat-Labile Components

A disadvantage of the extrusion process may be related to the relatively high temperature, shear, and pressure conditions. High temperatures in barrels during extrusion cooking can cause the loss of some heat-labile or unstable ingredients such as vitamins, antioxidants, and flavors (Camire et al 1990). Camire et al (2002) showed that the amounts of grape and blueberry anthocyanins in RTE breakfast cereals were significantly reduced during the extrusion process. According to Bhandari et al (2001), the addition of flavoring is sometimes required as a final step of extrusion due to the destruction of a great deal of flavor during the extrusion process. Schmid et al (2005) reported that vitamins were destroyed or decreased during high temperature extrusion cooking, and they reported that the loss of vitamins results from breakdown of chemical bonds because of shearing (mixing) or the reduction in vitamin stability during heating. After extrusion cooking, some extruded foods are fortified with vitamins by spraying onto the surface of the extrudate because of the critical loss of vitamins by high temperature extrusion cooking (Burns et al 2000).

According to Bhandari et al (2001), feed moisture is the most important factor affecting flavor loss during extrusion cooking, and they reported that increasing the feed moisture decreases the losses in flavor. Killeit (1994) showed that the reduction in thiamin loss at higher feed moisture contents was related to the lower dough viscosities, since the higher feed moisture extrusion conditions can lead to reductions in degree of shear and energy input on the extruded product.

Results from effects of different screw speeds on nutrient loss during extrusion cooking are still controversial. There have been two main opposing results: Guzman-Tello and Cheftel (1987) showed that elevating the screw speed caused more

thiamin loss at high temperatures, even though the extrusion residence time was shorter. Schmid et al (2005) also found similar results. They reported that increasing the screw speed resulted in more thiamin loss, indicating that shear effects played the more dominant role in thiamin degradation than did temperature effects. Conversely, in other studies, reduction in vitamin content was lessened with increasing screw speed, and was reported to be due to decreased residence time in the barrel (Killeit 1994).

2.2. Wheat Flour

Wheat is one of the most important cereal crops in the world. It has been used for human food since it has a higher nutritive profile and is relatively easily harvested, stored, transported and processed, as compared to other grains (Dobraszczyk 2001; Posner 2000). There are three main wheat species: *Triticum aestivum,* which is also called common wheat, *T. durum*, or durum wheat, and *T. compactum*, or club wheat (Pyler 1988; Yamazaki et al 1981).

Wheat flour consists mainly of starch (about 70-75%), water (about 12-14%), protein (about 8-16%), and minor constituents such as non-starch polysaccharides (about 2-3%), lipids (about 2%), and ash (about 1%). Quality of wheat flour depends on the quantity and characteristics of its constituents that are different from other wheat cultivars.

2.2.1. Main Components of Wheat Flour: Starch and Protein

Starch, the most important storage polysaccharide and the most abundant component of many plant seeds and tubers, is a major component of wheat flour. Wheat starch, in particular, is used in the food industry to provide the desired rheological properties or to improve the physical stability of different products, such as salad dressings, sauces, soups, extruded products, and baked goods (Dobraszczyk 2001).

The major components of starch are the glucose polymers, amylose and amylopectin (Heinemann et al 2003; Tester et al 2004). Amylose is a fundamentally linear molecule, comprised of α-1,4 linked glucose polymers. In general, a single amylose molecule has 1500-6000 glucose units (Atwell 2001) and a molecular weight of about 1×10^5 to 1×10^6 (Tester et al 2004). In recent years, it has also been reported

that a fraction of the amylose molecules has a low degree of branching by α-1,6 linkages (Bail et al 2005). In contrast, amylopectin is a very large and highly branched chain molecule, consisting of both α-1,6 and α-1,4 linked glucose polymers. In general, a single amylopectin molecule has 3×10^5 to 3×10^6 glucose units (Atwell 2001) and a molecular weight of about 1×10^7 to 1×10^9 (Tester et al 2004). The amylose/amylopectin ratio differs among starches, but typical levels of amylose and amylopectin are 25-28% and 72-75%, respectively (Atwell 2001; Tester et al 2004).

According to Osborne (1907), wheat proteins can be classified into albumins, globulins, gliadins, and glutenins. Albumins are extractable in water, globulins in salt solutions, gliadins in aqueous alcohol, and glutenins in dilute acid or alkali. Among these four groups of proteins, the gluten proteins, which include the gliadins and glutenins, are the major storage proteins of wheat.

The gluten proteins are known to make up 80 to 85% of total wheat proteins (Atwell 2001). Gluten proteins can be easily separated from other components including starch and water-soluble constituents in wheat flour because they are insoluble in water. Therefore, gluten can also be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and water-soluble constituents (Wieser 2007). Gliadins represent a highly polymorphic group of monomeric gluten proteins with molecular weights about 40,000 and are very sticky when hydrated (Lindsay and Skerritt 1999). Gliadins are known to be associated with the cohesiveness of wheat dough (Hoseney 1998). Gliadins can be divided into the α, β, γ, and ω-type in terms of their different primary structures (Wieser 2007).

In contrast, glutenins are a heterogeneous mixture of polymers with molecular weights varying from about 100,000 to several millions (Atwell 2001).

Generally speaking, glutenins comprise 55-70% of the gluten, and glutenin molecules are larger than gliadin molecules. Glutenins appear to be responsible for the resistance of wheat dough to extension (Hoseney 1998). It is known that glutenin is built of glutenin subunits that are linked via disulfide bonds (Wieser 2007). These glutenin subunits can be separated by reduction of these disulfide bonds with reducing agents such as β-mercaptoethanol or dithiothreitol. Four different groups of glutenin subunits can be distinguished: high molecular weight glutenin subunits (HMW-GS) with molecular weights between 65,000 and 90,000, and B-, C-, and D-type low molecular weight glutenin subunits (LMW-GS) with molecular weights between 30,000 and 60,000 (Hou et al 1996; Lindsay and Skerritt 1999).

2.2.2. Baked Products

Common wheat is divided into soft wheat and hard wheat, based on its kernel texture. In particular, soft wheat has lower protein content and weaker protein strength as compared to hard wheat (Posner 2000). Hard wheat flour is used mainly for the production of bread products, while soft wheat flour is usually used to produce chemically-leavened products such as cakes, cookies, crackers, and pretzels (Pyler 1988).

A visco-elastic dough can be made when wheat flour is combined with water. It is known that the dough can retain the gas produced during fermentation, thus yielding a leavened product, especially bread (Cauvain 2001). In general, the major ingredient for making bread is hard wheat flour with other ingredients being yeast, salt, and water (Hoseney 1998), whereas the major ingredient for baking cookies is soft wheat flour. In particular, the cookie formulation has higher sugar, higher shortening, and lower water contents as compared to the bread formulation (Hoseney 1998). In addition,

other ingredients such as non-fat dry milk, salt, sodium bicarbonate, high fructose corn syrup, and ammonium bicarbonate are added to make cookies (Townsend 2001; Yamazaki 1962).

2.2.3. Cereal-Based Snack Foods from Extruders

Snack foods contribute a significant portion to many consumers' daily food and caloric intake. They consist essentially of a cereal blend extruded with a certain amount of water. Wheat flour and corn meal have usually been used as ingredients in snack foods (Matz 1993). Wheat flour has been widely used in the extrusion industry and the effects of process variables on wheat extrudate properties have been studied (Ali et al 1996; Anderson and Ng 2001; Ding et al 2006; Harper 1979; Mercier and Feillet 1975; Ryu and Ng 2001; Schmid et al 2005).

Today's consumers are expecting a wide variety of selection of snack foods. Extrusion has imparted one manufacturing method for producing novel snack products and has modernized many traditional snack manufacturing processes (Huber and Rokey 1990). Currently, there is an expanding number of extrusion equipment types and suppliers available in the market for snack producers. Although this may challenge the snack producers in their choice of equipment, it should also encourage a wider use of extrusion cooking and provide the possibility of producing low cost and functional snack foods (Huber 2001; Huber and Rockey 1990).

During the extrusion cooking of snack foods, flour and other ingredients are combined and cooked simultaneously in barrels. The resulting mass is forced through an extrusion die. Upon exiting the die, moisture in the extrudate flashes off, leading to expansion and cooling of the product (Killeit 1994). The shaped products are cut into

individual pieces, and the newly produced snack food is then ready for packaging and distribution.

A snack food can be made of many diverse ingredients including those from plant, animal, and chemical sources. A usual formula for making snack foods is composed fundamentally of carbohydrates (starch), plus smaller amounts of proteins, fats and seasonings (Moore 1994). Starch is a main food constituent, present in most extruded snack food products at about a 70% level, and it functions in extruded products as a binding agent, viscosity builder, and emulsifier. In addition, it enhances the textural characteristics of foods, such as the expansion ratio, bulk density, mouth feel, and the aesthetic characteristics, such as color (Guy 2001).

2.3. Ginseng

Ginseng is one of the most popular medicinal plants in the world, and has long been conventionally consumed for strengthening immunity and relieving fatigue (Kim et al 2005). Many current researchers have confirmed the pharmaceutical effects of ginseng (Bae et al 2002; Kang et al 2006; Shibata 2001; Yun et al 2001). Ginseng is referred to as being in a group of "adaptogenic" herbs in most of the alternative medicine literature, because ginseng has many beneficial effects, including increased resistance to physical, chemical, and biological stresses and improved general vitality (Fuzzati et al 1999; Fuzzati 2004). In recent years, ginseng products have become increasingly popular and readily available in pharmacies and health stores around the world.

Its name, ginseng, originated in the Chinese language from the similarity of its root shape to the shape of the human body (Kwon et al 1990). The genus name *Panax* means "cure all" in Greek (Cho et al 1995; Ha et al 2005). The most widely used *Panax* species are *Panax ginseng* (Korean or Asian ginseng), *Panax quinquefolius* (American ginseng), *Panax notoginseng* (Tienchi or Sanchi), *Panax vietnamensis* (Vietnamese ginseng) and *Panax japonicus* (Japanese ginseng). Korean ginseng has been studied frequently by many researchers, and is known as a tonic and a panacea in Asia (Fuzzati 2004).

2.3.1. Processing Methods of Ginseng

In general, 4-6 year old Korean ginseng plants are used for medicinal purposes. "Susam", which is freshly harvested ginseng root, is also called fresh or raw ginseng because it is not yet dried. It contains about 70-75% water and easily spoils, so that it is very hard to store Susam for more than one week. Raw ginseng root is processed to make white and red ginseng. White ginseng is another name for dried ginseng (about 12-16% moisture content,

wb), and has a much longer storage stability than Susam (Cho et al 1995). Sun or hot-air drying is required to produce white ginseng. The color of white ginseng is either milky white or light yellow, depending on the variety (Ha et al 2005). Red ginseng is a unique ginseng product produced by the steaming and then drying of raw ginseng. It preserves the original root shape but the color changes to a light reddish brown or deep maroon and it can be stored for a long period of time (Cho et al 1995).

The traditional processing of red ginseng is depicted in Figure 2.1. During the heating or steaming process to produce red ginseng, the ginsenosides (G, ginseng saponins), polyacetylene, acidic polysaccharides, and amino acids go through chemical changes and are transformed to active substances that do not exist in raw ginseng or white ginseng (Ha et al 2005; Kwon et al 2001). Table 2.1 shows the compositional differences between white and red ginseng.

2.3.2. Major Components of Ginseng

In general, herbal medicines include very intricate chemical compositions which may differ significantly based on factors that include growing location, climate, and species used (Ji et al 2001; Lau et al 2004; Li et al 1996). Ginseng is one of the most popular herbal medicines in the world and it has particularly complex compositions since many products from different genera and species can be described as 'ginseng' (Ji et al 2001).

Traditionally, the dried roots of the plants belonging to the genus *Panax* are called ginseng. The dried root contains various pharmaceutical components, including ginsenosides (G, ginseng saponins), polyacetylenes, polyphenolic compounds, and acidic polysaccharides (Kim et al 2005). The various *Panax* species are used slightly

differently for traditional medicines and contain different components, but all species contain G, which are the most widely studied chemical components of ginseng. In particular, they are believed to be a main active constituent (Attele et al 1999), and are typically used as marker compounds for quality properties (Chuang et al 1995; Lau et al 2004; Li et al 1996). About 30 different G [Ra1, Ra2, Ra3, Rb1, Rb2, Rb3, Rc, Rd, 20(S)-Rg3, 20(R)-Rg3, Rh2, Rs1, Rs2, Quinquenoside-R1, Notoginsenoside-R4, malonyl Rb1, malonyl Rb2, malonyl Rc, malonyl Rd, Re, Rf, Rg1, Rg2, Rh1, 20Glc-Rf, Notoginsenoside-R1, 20(R)-Rg2, 20(R)-Rh1, Rh4, and Ro] have so far been separated from various ginseng species, including five major G (G Rb1, G Rb2, G Rc, G Re, and G Rg1) that constitute more than 80% of the total G content (Kim et al 2005).

Aglycons of G are of the dammarane-type triterpenes. The characteristic dammarane G can be divided into three major groups, depending on their characteristic aglycon: the protopanaxadiol, protopanaxatriol, and oleanoic acid (Li et al 2006). Figure 2.2 shows the structures of the three main types of aglycon moieties of G. The only oleanolic acid-type G is ginsenoside Ro. Four malonyl derivatives (malonyl G Rb1, malonyl G Rb2, malonyl G Rc, and malonyl G Rd) of G Rb1, G Rb2, G Rc, and G Rd, respectively, have also been isolated and characterized (Fuzzati et al 1999). In addition, the G Ro and the malonyl G are regarded as "acidic" G; however, the others are typically called "neutral" G (Fuzzati 2004). The protopanaxadiols include the G Rb1, G Rb2, G Rc, G Rd, and G Rg3 and the protopanaxatriols include G Re, G Rf, G Rg1, G Rg2, and G Rh (Kwon et al 1990). G (Rg3, Rg5, Rg6, Rh2, Rh3, Rh4, Rs3, Rk1, and F4) are known to be unique to red ginseng, while malonyl G Rb1, malonyl G Rb2, malonyl G Rc, and malonyl G Rd are known to be unique to white ginseng (Cho et al 1995; Ha et al 2007).

2.3.3. Detection of Ginsenosides

Many studies have been done since the 1970's with the purpose of developing analytical methods for the extraction, identification, quantification and quality control of G in raw plant materials (Corbit et al 2005; Court et al 1996; Fuzzati et al 1999; Ji et al 2001; Li et al 1996; Wang et al 2006), extracts (Choi et al 1982; Ko et al 2005), and marketed products (Choi et al 1984; Harkey et al 2001). Those researchers attempted to differentiate the G composition among the various *Panax* species. Furthermore, studies regarding changes in G composition due to different traditional processing of white and red ginseng have been undertaken (Fuzzati 2004; Kim et al 2000; Kwon et al 2001; Lau et al 2003; Lau et al 2004).

There are many methods for the analysis of G, including thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), GC-mass spectrometry (MS), HPLC-MS and most recently HPLC-MS/MS. HPLC has been used for the identification and fractionation of ginsenosides in raw ginseng or ginseng products, but generally does not offer structural information. The advantage of GC-MS is to identify the structural information, but on the other hand, the broad fractionation that occurs during GC-MS could make identification of specific G challenging. Since HPLC-MS and HPLC-MS/MS procedures are somewhat expensive, they can be impractical for typical commercial analysis (Ji et al 2001).

2.3.4. Medicinal Effects of Ginseng

G Rg3, one of the most important components of the protopanaxadiols and

major components of red ginseng, have been found to show various biological activities including anticancer activities (Shibata 2001; Yun et al 2001), and radical scavenging activities (Kang et al 2006). Furthermore, it has been reported that G Rg3 inhibit the growth of *Helicobacter pylori* (Bae et al 2002). G Rb1 and G Rd (the protopanaxadiols) are reported to influence the overall activity of the CNS (central nervous system, Cho et al 1995). G Rg1 and G Rg2 (the protopanaxatriols) are known to possess properties of CNS excitation and anti-fatigue (Cho et al 1995; Li et al 2005). In addition to G, ginseng also contains polyacetylenes, which have anticancer activities (Matsunaga et al 1989), phenolic compounds, which have antioxidant activities (Suh et al 1996), and acidic polysaccharides, which have immune-system-controlling activities (Jie et al 1984). Na et al (2004) reported that ginseng polysaccharides also have antitumor, anticomplementary, antiulcer, and immunopotentiating effects even though G have been regarded as the major component for explanation of ginseng's medicinal effects.

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Table 2.1.

Comparison between White Ginseng and Red Ginseng (from Cho et al 1995)

	White Ginseng	Red Ginseng
Processing Methods	Sun or air drying	Steaming and drying
Color	Milky white or light yellow	Light reddish brown or deep maroon
Unique Ginsenosides	Malony ginsenosides (Rb1, Rb2, Rc, and Rd)	Ginsenosides Rg3, Rg5, Rg6, Rh2, Rh3, Rh4, Rs3, Rk1, and F4
Acidic Polysaccharides	2-3%	7-8%
Enzymes	Mostly remain active (Amylase, invertase)	All inactivated
Starch	Intact	Gelatinized
Polyacetylene Compounds	0.1-0.2 mg/g	0.6-1.0 mg/g
Pigments	Natural pigments	Melanoidin pigments

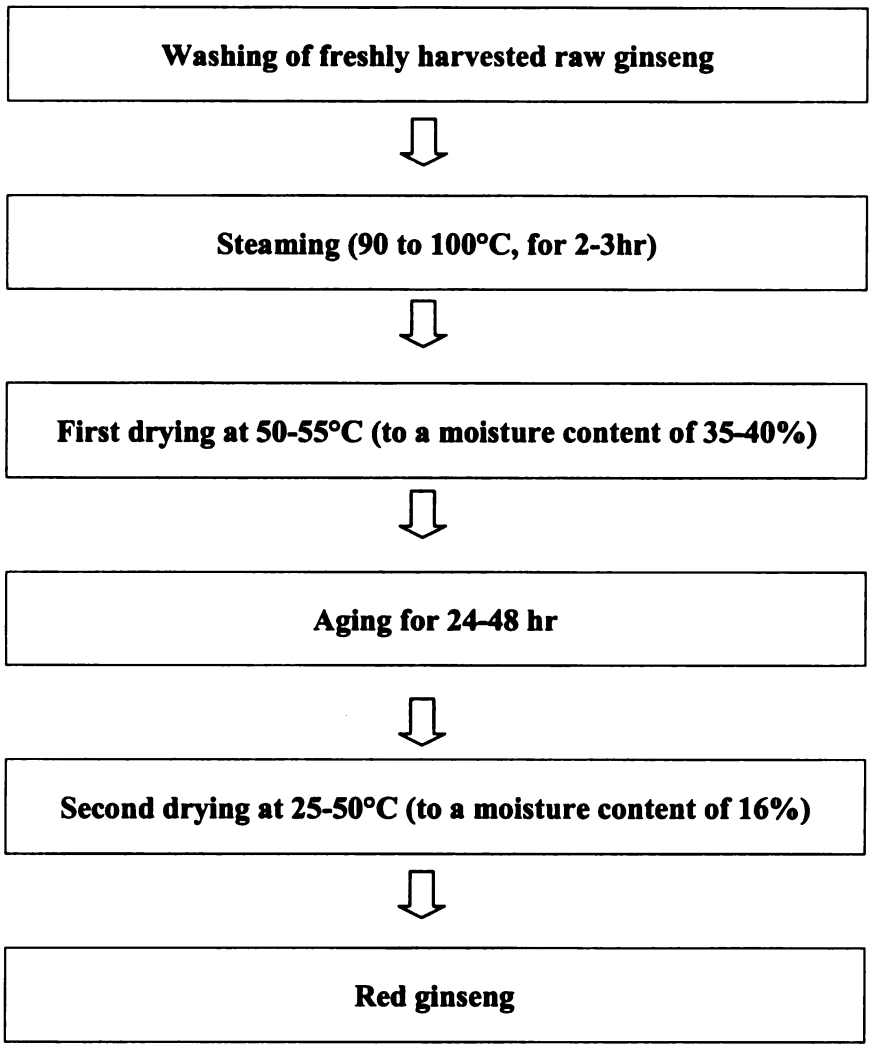

Fig. 2.1. A traditional processing method for making red ginseng.

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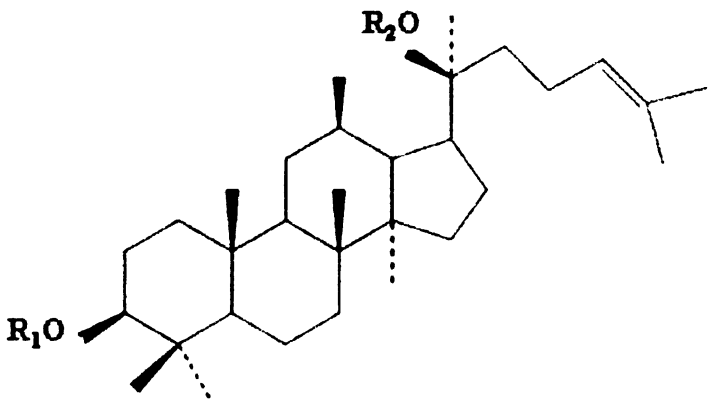

Ginsenosides	R_1	R_2
Rb1	Glc^2-Glc	Glc^6-Glc
Rb2	Glc^2-Glc	Glc^6-Ara(p)
Rc	Glc^2-Glc	Glc^6-Ara(f)
Rd	Glc^2-Glc	Glc
Malonyl-Rb1	Glc^2-Glc^6-Ma	Glc^6-Glc
Malonyl-Rb2	Glc^2-Glc^6-Ma	Glc^6-Ara(p)
Malonyl-Rc	Glc^2-Glc^6-Ma	Glc^6-Ara(f)
Malonyl-Rd	Glc^2-Glc^6-Ma	Glc
Rg3	Glc^2-Glc	H
Rh2	Glc	H

(p): pyranosyl; (f): furanosyl; Ma: $HOOC-CH_2-COOH$.

Fig. 2.2. Structures of the three main types of aglycon moieties of ginsenosides (from Shibata 2001).

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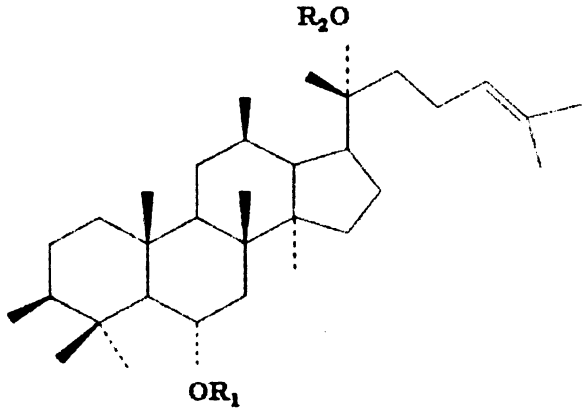
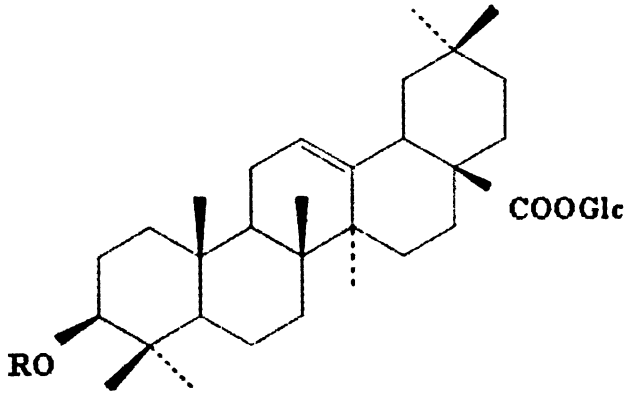


Ginsenosides	R_1	R_2
Rg2	Glc^2-Rha	H
Re	Glc^2-Rha	Glc
Rh1	Glc	H
Rf	Glc^2-Glc	H

<Oleanolic acid>



Ginsenosides	R
Ro	Glc^2-Glc

Fig. 2.2. (Continued).

CHAPTER 3

Extraction of Ginsenosides from a Blend of Wheat Flour and Ginseng Powder

3.1. ABSTRACT

This study explored conditions for maximum extraction of ginsenosides (G, ginseng saponins) from a blend of wheat flour (WF)-ginseng powder (GP). The weight of each sample (WF: 0.9 g, GP: 0.1 g, or WF-GP: 0.9 g WF + 0.1 g GP) was fixed and different amounts of 70% (v/v) aq. methanol (1-14 mL) were added to each sample. Samples were then ultrasonically extracted for G for 30 min. Individual ginsenosides Rb1, Rc, and Rd were fractionated and identified by RP-HPLC. The maximum extraction of the G were obtained by the sample-to-solvent ratio of 0.025:1 (mg/μL) for GP alone and the sample-to-solvent ratio of 0.071 (mg/μL) for WF-GP. To investigate temperature effects on extractable G in a WF-GP blend, WF (0.9 g), GP (0.1 g), or WF-GP (0.9 g WF + 0.1 g GP) was mixed with distilled water (4.5, 0.5, or 5.0 mL, respectively) and heated at temperatures from 25 to 90°C prior to extraction. The quantities of G extracted from GP were constant at all the temperatures studied, while the quantities of G extracted from WF-GP decreased with increasing heating temperature. Results suggested that interactions between WF components and G (Rb1, Rc, and Rd) existed, and that the interactions increased with increasing temperature. WF was fractionated into two components: a starch fraction (SF) and a gluten fraction (GF) to examine the interactions between WF components and G in a heated blend of WF-GP. Extractable G (Rb1, Rc, and Rd) decreased with increasing heating temperature for both the SF-GP blend and the GF-GP blend. These findings confirmed that interactions occurred not only between the SF and G but also between the GF and G in a blend of WF-GP at the higher temperature (90°C). In addition, the quantities of extractable G ultrasonically extracted for 30 or 60 min from GF-GP heated at 90°C were significantly higher than those of extractable G ultrasonically extracted for 30 min from

SF-GP heated at 90°C, indicating that the SF has more interactions with G than did the GF. To disrupt the interactions, ultrasonic extraction (UE) time was increased to 60, 90, or 120 min for each sample. The quantities of G extracted from GP were not affected by increasing UE time. However, the quantities of G extracted from WF-GP increased with increasing UE time, and similar quantities of Rb1, Rc, and Rd (0.87, 0.53, and 0.26 mg per 100 mg of GP, respectively) were obtained after 90 min of UE as were extracted from pure GP at 30 min. Thus, the interactions between WF components and G could be disrupted by increasing UE time to 90 min for maximum extraction.

3.2. INTRODUCTION

Saponins are known as glycosides with distinctive foaming and emulsifying characteristics and are composed of steroid or triterpenoid aglycons and various sugar moieties (Ikedo et al 1996). Saponins have been found in many plant species, including ginseng, spinach, asparagus, sugarbeets, oats, and legumes. Ginsenosides (G, ginseng saponins) are the major active components of ginseng, one of the most popular herbal medicines worldwide, contributing to pharmaceutical activity. Their basic structure contains triterpenoid aglycon and various sugar moieties (Fuzzati 2004; Shibata, 2001). Over 30 G have been identified; among these, Rb1, Rb2, Rc, Rd, and Re are the most abundant (Corbit et al 2005; Kim et al 2005).

Numerous studies have reported that proteins interact with relatively small molecules, such as saponins and flavor compounds (Heng et al 2004; Ikedo et al 1996; Potter et al 1993; Sarnthein-Graf and Mesa 2004). Potter et al (1993) studied the interactions of isolated soy proteins and caseins with quillaja saponin using gel electrophoresis. They noted that the saponins interacted with soy proteins much more quickly than with caseins. In addition, Ikedo et al (1996) reported that soyasaponin interacts with bovine serum albumin (BSA). They hypothesized that the interactions between BSA and soyasaponin include electrostatic and hydrophobic interactions.

It has been shown that starch interacts with various components of food systems, such as lipids, proteins, iodine, organic alcohols or acids, and flavor compounds, including terpenes, aldehydes, and lactones (Arvisenet et al 2002; Derycke et al 2005; Heinemann et al 2001; Jouquand et al 2006; Le Bail et al 2005; Raphaelides and Georgiadis 2006; Toro-Vazquez et al 2003). Arvisenet et al (2002) reported that

the interactions of gelatinized starch with its interaction partners occur during heating process of a starch-based food system.

Wheat flour (WF) primarily consists of proteins and starch. Depending on the wheat variety, the contents of protein and starch in flour can vary from 7-15% and 63-72%, respectively, at a 14% moisture basis (Atwell 2001). Therefore, in the present study, it was hypothesized that interactions between WF components and G would take place during the heating process required to make a cereal product, if ginseng is to be used as one of the ingredients. It was also hypothesized that the extraction efficiency of G from the cereal product would be related to the degree of interaction between WF components and the G. For more than 30 years, numerous studies on the extraction of G have been performed on raw plant materials (Corbit et al 2005; Court et al 1996; Fuzzati et al 1999; Ji et al 2001; Kwon et al 2001; Li et al 1996; Park et al 1996; Wang et al 2006), extracts (Choi et al 1982; Ko et al 2005), and commercial products (Choi et al 1984; Harkey et al 2001). Studies have employed various extraction solvents (pure methanol, aq. methanol, pure ethanol, or aq. ethanol) and extraction methods (heating or ultrasonication) to facilitate the extraction of G from various sources. However, the optimum extraction conditions, such as solvent, time, temperature, and sample-to-solvent ratio, for G that have interacted with other components (such as proteins and starch) in a food system have not been reported. Therefore, the objective of the present study was to explore optimum extraction conditions of G (Rb1, Rc, and Rd) from a blend of WF and ginseng powder (GP).

3.3. MATERIALS AND METHODS

3.3.1. Materials

Soft WF was obtained from the Mennel Milling Co. in Fostoria, OH, USA. The raw ginseng root (*Panax ginseng* C. A. Meyer), harvested after four years growth and milled into powder (particle size of ≤ 167 μm), was cultivated in Geumsan, Korea, and purchased at a ginseng market. Standards for ginsenosides Rb1, Rc, and Rd were provided by ILHWA Co. (Kuri, Korea).

3.3.2. Fractionation of Wheat Flour

Wheat flour was fractionated into two components: a starch fraction (SF) and a gluten fraction (GF) using the procedure described by MacRitchie (1985) with some modifications. WF (100 g) was mixed into dough with an amount of water according to its Farinograph water absorption; the dough was mixed for 30 sec, just enough to give a cohesive mass that was easy to handle. The SF was isolated by hand kneading this dough in small aliquots of distilled water (total of 800 mL), which were collected, and the GF was obtained as the residual viscoelastic mass. The distilled water-starchy slurry was centrifuged at 5,000 x g for 10 min using a Sorvall RC-5B centrifuge (Dupont, Wilmington, DE, USA) and the supernatant was decanted. The SF (sediment) was spread in aluminum pans and allowed to air-dry at room temperature for 72 hr. The wet GF was divided into six parts, frozen, and freeze-dried for 48 hr. The air-dried SF and freeze-dried GF were initially each ground using a mortar and pestle and then finally ground to a particle size of ≤ 330 μm (54 mesh screen) using a coffee grinder (Braun Inc., Woburn, MA, USA). All ground fraction samples were placed in ziplock bags, sealed, and stored at -20°C until needed.

3.3.3. Chemical Analysis

WF, GP, SF, and GF samples were analyzed for their moisture and protein contents using AACCI Approved Methods 44-15A and 46-13 (AACCI, 2000), respectively. Protein contents were calculated by multiplying the nitrogen content by the conversion factor 5.7, and reported on a 14% moisture basis.

3.3.4. Optimization of Sample-to-Solvent Ratio for Extraction of Ginsenosides

Different amounts of extraction solvent (70%, v/v, aq. methanol) were added to each sample (GP, WF, or a blend of WF-GP): 1, 2, 3, 4, 5, 6, 8, or 10 mL of 70% (v/v) aq. methanol was added to GP (0.1 g); 14 mL of 70% (v/v) aq. methanol to WF (0.9 g); and 12 or 14 mL of 70% (v/v) aq. methanol to WF-GP (0.9 g WF + 0.1 g GP). These tested amounts of extraction solvents gave sample-to-solvent ratios that ranged from 0.07:1 to 0.10:1 (mg/μL). The suspension was ultrasonically extracted for 30 min using an Ultrasonicator (Model FS 14H, Fisher Scientific, Pittsburgh, PA, USA, Power: 155W) at room temperature and then centrifuged at 11,200 x g for 20 min using a centrifuge (Model J2-21M, Beckman Instruments Inc., Fullerton, CA, USA) at 25°C. The supernatant was evaporated using a Micro Rotary Evaporator (Model 421-4000, Labconco, Kansas City, MO, USA) at 50°C. Prior to RP-HPLC analysis for G, the residue was dissolved in 2 mL of 70% (v/v) aq. methanol and filtered through a 0.45 μm nylon filter membrane (Millipore, Ireland). Each sample was extracted in duplicate and two chromatographic runs were performed for each extract.

3.3.5. Temperature Effects on Extractable Ginsenosides

WF (0.9 g), GP (0.1 g), or WF-GP (0.9 g WF + 0.1 g GP) was mixed with distilled water (4.5, 0.5, or 5.0 mL, respectively) and heated at 25, 50, 70, or 90°C using a water bath for 30 min. The SF (0.9 g), GF (0.9 g), a blend of SF-GP (0.9 g SF + 0.1 g GP), or a blend of GF-GP (0.9 g GF + 0.1 g GP) was mixed with distilled water (4.5, 4.5, 5.0, or 5.0 mL, respectively) and heated at 25 or 90°C using a water bath for 30 min. The mixture was frozen and dried in a freeze-dryer for 24 hr. To prevent the loss of G from the freeze-dried samples, the samples were not ground. An aliquot of 14, 4, or 14 mL of 70% (v/v) aq. methanol was added to the prepared freeze-dried WF sample, GP sample, or WF-GP sample, respectively. Fourteen mL of 70% (v/v) aq. methanol were added to each sample of freeze-dried SF, GF, SF-GP, or GF-GP. The suspension was ultrasonically extracted for G for 30 min, then centrifuged, evaporated, dissolved, and filtered as described in 3.3.4. Each sample was extracted in duplicate and two chromatographic runs were performed for each extract.

3.3.6. Optimization of Ultrasonic Extraction Time for Ginsenosides from Heat-Treated Samples

WF (0.9 g), GP (0.1 g), or WF-GP (0.9 g WF + 0.1 g GP) was mixed with distilled water (4.5, 0.5, or 5.0 mL, respectively) and heated at 90°C using a water bath for 30 min. SF (0.9 g), GF (0.9 g), SF-GP (0.9 g SF + 0.1 g GP), or GF-GP (0.9 g GF + 0.1 g GP) was mixed with distilled water (4.5, 4.5, 5.0, or 5.0 mL, respectively) and heated at 90°C using a water bath for 30 min. Each mixture was frozen and dried in a freeze dryer for 24 hr. To prevent the loss of G from the freeze-dried samples, the samples were not ground. Fourteen, 4, or 14 mL of 70% (v/v) aq. methanol were added to the prepared sample of freeze-dried WF, GP, or WF-GP, respectively. Fourteen mL of 70% (v/v) aq. methanol were added

to each prepared sample of freeze-dried SF, GF, SF-GP, or GF-GP. The suspension was ultrasonically extracted for G for 30, 60, 90, or 120 min, then centrifuged, evaporated, dissolved, and filtered as described in 3.3.4. Each sample was extracted in duplicate and two chromatographic runs were performed for each extract.

3.3.7. Fractionation and Identification of Ginsenosides by RP-HPLC

RP-HPLC was carried out on a Millenium 2010 HPLC Workstation, consisting of a Waters 600E multi-solvent delivery system (Waters, Milford, MA, USA), a temperature control module, and a 996 photodiode array detector. Separation of G was performed at 30°C on a Phenomenex 300 RP, Jupiter C-18 column (4.6 x 250 mm, 5 μm particle diameter; Phenomenex, Torrance, CA, USA). A Phenomenex security guard with the same packing material served as the guard column.

The solvents were A: water (distilled water filtered through Milli-Q-Plus, Millipore, Bedford, MA, USA), and B: HPLC-grade acetonitrile. Separation was achieved using the following gradient: 0-5 min, 20-30% B; 5-25 min, 30-40% B; 25-30 min, 40-20% B. The solvent flow rate was 1 mL/min and the injection volume was 20 μL. The solvents were purged with helium at the rate of 20 mL/min. The column was equilibrated for 10 min with 20% acetonitrile prior to injection. The UV detection wavelength was set at 203 nm (Lau et al 2004), and the detector output was transmitted simultaneously to the computer for data storage and graphic representation.

3.3.8. Statistical Analysis

All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed using

the general linear models (GLM) procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level.

3.4. RESULTS AND DISCUSSION

3.4.1. Chemical Analysis

Moisture and protein contents of WF, GP, SF, and GF samples are reported in Table 3.1. The moisture contents of the samples ranged from 1.9 to 11.2% (wb). Only a very low protein content (2.1%) in the SF was obtained, indicating that good fractionation had been achieved. The protein content of the WF (8.3%) represents a typical soft wheat variety.

3.4.2. Optimization of Sample-to-Solvent Ratio for Extraction of Ginsenosides

The representative RP-HPLC chromatograms of G ultrasonically extracted for 30 min from pure GP and a blend of WF-GP are illustrated in Figure 3.1. The RP-HPLC chromatograms of G extracted from WF-GP were nearly identical to the chromatograms of G extracted from pure GP. This result indicated that adding WF to GP led to no significant impact on the extractable chemical profile of GP. Rb1, Rc, and Rd were the most abundant among all the G and, based on information in the literature, they are associated with the pharmaceutical activity (Ji et al 2001). Therefore, these three G were fractionated and identified in the present study. Ginsenosides Rb1, Rc, and Rd were eluted in that order during RP-HPLC fractionation. This finding was in good agreement with reports in the literature (Ji et al 2001; Lau et al 2003; Lau et al 2004; Li et al 1996).

Previous studies by others have shown that different solvents extract different amounts of individual G and the amounts of G change depending on the amounts of the solvents used (Court et al 1996; Fuzzati 2004; Lau et al 2003; Lau et al 2004). In addition, Corbit et al (2005) noted that the quantities of G are affected by the physical

and chemical extraction procedures and extraction time used. Fuzzati (2004) reported that most extraction methods of G employ pure methanol or aq. methanol. Lau et al (2003) showed that the amounts of G (R1, Rb1, Rc, Rd, Re, and Rg1) extracted from raw *Panax notoginseng* in 70% (v/v) aq. methanol are higher than those of the G extracted in 100% methanol. Therefore, they concluded that 70% (v/v) aq. methanol is the optimum solvent for the extraction and quantification of the G. Corbit et al (2005) reported similar results from the study using *Panax quinquefolius*, i.e., that 100% methanol was less effective than 70% (v/v) aq. methanol when each solvent was used in combination with the ultrasonic extraction (UE) method. Court et al (1996) compared UE and the Soxhlet method for evaluating the quantities of G (Rb1, Rb2, Rc, Rd, Re, and Rg1) extracted from *Panax quinquefolius*. They found that UE yields higher quantities of the G than the Soxhlet method. Furthermore, Lau et al (2003) noted that UE is a rapid and efficient extraction method for G, as compared to the Soxhlet method. Based on the studies mentioned above, 70% (v/v) aq. methanol (extraction solvent) and the UE method were chosen for extraction of ginsenosides in the present study.

A preliminary study was performed to identify the most appropriate range of sample-to-solvent ratios for the extraction of G from WF, GP, and WF-GP. The weight of each sample (WF: 0.9 g, GP: 0.1g, or WF-GP: 0.9 g WF + 0.1 g GP) was fixed and different amounts of 70% (v/v) aq. methanol were added to each sample. Solvent functions as a medium to solubilize target compounds, such as G; thus, the sample-to-solvent ratio is one of several critical parameters to be considered. Table 3.2 lists the quantities of G (Rb1, Rc, and Rd; on a mg per 100 mg GP basis) ultrasonically extracted for 30 min from WF, GP, and WF-GP with different sample-to-solvent ratios. The quantities of G (Rb1, Rc, and Rd) extracted from pure GP increased with decreases in

the sample-to-solvent ratio from 0.100:1 to 0.025:1 (mg/μL), but did not increase significantly with further decreases in the ratio. This observation established that the maximum extraction of G Rb1, G Rc, and G Rd from pure GP could be obtained by the sample-to-solvent ratio of 0.025:1 (mg/μL). The quantities of G (Rb1, Rc, and Rd) extracted from WF-GP with the sample-to-solvent ratio of 0.071:1 (mg/μL) were not significantly different from the quantities of the G extracted from pure GP with the sample-to-solvent ratios of less than 0.025:1 (mg/μL). However, no measurable G (Rb1, Rc, and Rd) in pure WF were found. Based on these results, the sample-to-solvent ratio of 0.025:1 (mg/μL) for pure GP and the sample-to-solvent ratio of 0.071:1 (mg/μL) for WF-GP were chosen as one of the UE conditions for subsequent studies.

3.4.3. Temperature Effects on Extractable Ginsenosides

Effects of heating of pure GP and a blend of WF-GP on subsequent extractable G (Rb1, Rc, and Rd) are shown in Figure 3.2. Extractable G (Rb1, Rc, and Rd) decreased with increasing heating temperature for the WF-GP-water blend, but remained essentially the same for pure GP. No measurable G (Rb1, Rc, and Rd) in pure WF, with distilled water, were found at any of the temperatures studied (data not shown). These findings together indicate that when blends of WF-GP-water were heated, increased interactions between WF components and G took place at the higher temperatures (70-90°C), causing more difficulty in the extraction of G from these blends. The decreased amounts of extractable G in the heated blends can be related to the interactions of G with WF components. Furthermore, the interactions between WF components and G increased with increasing heating temperature. It is possible that the G are interacting with wheat proteins or with starch during the heating process.

To examine the interactions between WF components and G in a heated blend of WF-GP, WF was fractionated into a SF and a GF. A blend of SF-GP or a blend of GF-GP was mixed with distilled water and heated at 25 or 90°C for 30 min. Figure 3.3 illustrates effects of heating these SF-GP and GF-GP samples on subsequent extractable G (Rb1, Rc, and Rd). Extractable G (Rb1, Rc, and Rd) decreased with increasing heating temperature for both the SF-GP-water blend and the GF-GP-water blend. No measurable G (Rb1, Rc, and Rd) in pure SF and GF, with distilled water, were found at any of the temperatures studied (data not shown). These findings confirmed that interactions between the SF and G and between the GF and G in a blend of WF-GP-water heated at 90°C did exist. Furthermore, the quantities of extractable G ultrasonically extracted for 30 or 60 min from GF-GP heated at 90°C were significantly higher (on a g/g basis) than those of extractable G ultrasonically extracted for 30 min from SF-GP heated at 90°C (Table 3.3). This result could indicate that the SF had a higher degree of interactions with G than did the GF, and/or that interactions between the SF and G were stronger than those between the GF and G, thereby resulting in more difficulty in the extraction of G from SF-GP under the given extraction conditions.

As mentioned earlier, G have a complex chemical structure consisting of triterpenoid aglycon and various sugar moieties. Because the aglycon is highly hydrophobic and the sugar is hydrophilic, G can be considered as amphiphilic molecules (Kim et al 1985). According to Ikedo et al (1996), saponins show excellent emulsifying characteristics due to the presence of the hydrophilic and hydrophobic groups. These groups can participate in interactions with proteins. Shimoramada et al (2000) observed interactions between soybean saponin and whey proteins. Potter et al (1993) reported that interactions between quillaja saponin and casein occur via the sugar

moieties of the saponin with the free amino groups of the proteins (e.g., Maillard reaction). In the present study, it is suggested that interactions between hydrophilic groups (sugar moieties) of G and the free amino groups of wheat proteins could take place during the heating process of the WF-GP-water blend. Moreover, upon unfolding of wheat protein molecules during the heating process, the previously buried hydrophobic groups in wheat proteins become accessible for interactions with hydrophobic groups (aglycons) in G via hydrophobic interactions.

In general, starch undergoes gelatinization upon heating of its granules in the presence of water. During gelatinization, amylose leaches out of the starch granules and forms a helix. According to Conde-Petit et al (2006), the outside surface of the helix is hydrophilic due to the oxygen and hydrogen atoms, while the inner surface of the helix is hydrophobic because of the carbon atoms. They also reported that the helix can interact with hydrophilic and hydrophobic molecules. According to Heinemann et al (2005), various compounds in food system such as terpenes, alcohols, aldehydes, and lactones can form complexes with amylose during the heating process, and these complexes display a V type pattern as assessed by X-ray diffraction. When amylose is not forming a complex with other molecules, it is in the form of a double helix (A and B type, Zobel et al 1988). Conde-Petit et al (2006) reported that higher temperatures are necessary to plasticize amylose in order to enable the formation of complexes with its interaction partners. Heinemann et al (2003) evaluated the structural features of potato starch and lactone complexes and they explained interactions between amylopectin and lactone, as well as interactions between amylose and lactone. Because the external branches of amylopectin have the same structure as amylose, amylopectin can also interact in the same way with lactone. In the present study, it is assumed that the outer

and inner groups of gelatinized amylose helix could interact with the hydrophilic and hydrophobic groups, respectively, of G during the heating process. Furthermore, the leaching of amylose from starch granules could be increased at higher temperatures, improving the interactions between gelatinized amylose helix and G.

3.4.4. Validation of Interactions between Wheat Flour Components and Ginsenosides in a Blend of Wheat Flour and Ginseng Powder

The amounts of G Rb1 ultrasonically extracted for 30 min from GP and blends of WF-GP, SF-GP and GF-GP, each heated at 90°C for 30 min, were used to validate the interactions between WF components and G Rb1 in the WF-GP blend. The amounts of G Rb1 ultrasonically extracted for 30 min from pure GP (100 mg) heated at 90°C and a blend of WF (900 mg)-GP (100 mg) heated at 90°C were 0.88 mg/100 mg GP and 0.72 mg/100 mg GP, respectively (Table 3.4). The amounts of G Rb1 ultrasonically extracted for 30 min from a blend of SF (900 mg)-GP (100 mg) heated at 90°C and a blend of GF (900 mg)-GP (100 mg) heated at 90°C were 0.71 mg/100 mg GP and 0.77 mg/100 mg GP, respectively (Table 3.3).

Based on the amounts of G Rb1 ultrasonically extracted from heated GP and WF-GP, the amount of G Rb1 which could interact with WF components in the heated WF-GP blend was calculated as:

0.88 mg/100 mg GP (the amount of G Rb1 extracted from heated GP) – 0.72 mg/100 mg GP (the amount of G Rb1 extracted from heated WF-GP) = 0.16 mg/100mg GP (the amount of G Rb1 which could interact with WF components in heated WF-GP)

The amounts of G Rb1 which could interact with the SF or with the GF in each blend were calculated in the same way; the amounts of G Rb1 calculated were 0.17

mg/100 mg GP for the SF and 0.11 mg/100 mg GP for the GF. For purposes of comparison, it was assumed that WF (based on dry weight) grossly consists of about 85% SF, 10% GF, and 5% nonstarch polysaccharides and lipids. Based on this assumption, the following WF composition was obtained for a 900 mg sample:

900 mg (WF) = 765 mg (SF) + 90 mg (GF) + 45 mg (nonstarch polysaccharides and lipids)

Based on results in the present study of the amounts of G Rb1 which could interact with the SF (900 mg) or the GF (900 mg), calculated values were determined for the total amounts of G Rb1 which could interact with the SF (765mg) and the GF (90 mg) in a blend of WF (900 mg)-GP (100 mg) heated at 90°C. The amounts of G Rb1 calculated were 0.14 mg/100 mg GP for the SF and 0.01 mg/100 mg GP for the GF. The sum (0.15 mg/100 mg GP) of those two calculated values is close to the measured amount (0.16 mg/100 mg GP) of G Rb1 which appeared to interact with WF components in the WF-GP blend heated at 90°C. Moreover, G Rc and G Rd showed the same trends with their respective calculated values (data not shown). Therefore, based on the data obtained and the calculations, the degree of interactions of G (Rb1, Rc, and Rd) with the SF in a heated blend of SF-GP and the G with the GF in a heated blend of GF-GP could explain the total interactions between WF components and the G in a heated blend of WF-GP. Furthermore, it is postulated that, quantitatively, higher amounts of G are interacting with the SF than with the GF in a blend of WF-GP heated at 90°C.

3.4.5. Optimization of Ultrasonic Extraction Time for Ginsenosides from Heat-Treated Samples

Effects of UE time of heated (90°C) samples of WF, GP, SF, GF, and blends of WF-GP, SF-GP, and GF-GP on extractable G (Rb1, Rc, and Rd) are listed in Tables 3.3 and 3.4. No measurable G (Rb1, Rc, and Rd) in pure WF, SF, or GF, with distilled water, were found at any of the UE times studied. The quantities of G (Rb1, Rc, and Rd) extracted from pure GP were not affected by increasing UE time. However, the quantities of G (Rb1, Rc, and Rd) extracted from WF-GP increased with increasing UE time, and amounts of the three individual G obtained after 90 min of UE were similar to their counterpart amounts extracted from pure GP at 30 min. Thus, the interactions between WF components and G could be disrupted by increasing UE time up to 90 min, for the maximum extraction.

The quantities of G (Rb1, Rc, and Rd) extracted from the blend of SF-GP increased with increasing UE time, and similar amounts of G Rb1, G Rc, and G Rd were obtained after 90 min of UE time as were extracted from pure GP at 30 min. However, similar amounts of G (Rb1, Rc, and Rd) extracted from GF-GP were obtained after 60 min of UE time as were extracted from pure GP at 30 min. Thus, the interactions between the SF and G as well as between the GF and G could be disrupted by increasing UE time up to 90 min and 60 min, respectively, for the maximum extractions, indicating a difference in the strength of the interactions of ginsenosides with starch and protein.

According to Onofre and Hettiarachchy (2007), ultrasonication can increase the extraction efficiency of targeted compounds. They also reported that the UE efficiency is associated with the rate of diffusivity of the compounds from the solid to the solvent, and is significantly affected by the UE conditions such as UE temperature and UE time. Ultrasound breaks down the cell walls and matrix of a sample mechanically, exposing targeted compounds to the solvent and subsequently enhancing

the rate of mass transfer of compounds to the extractrant (Capelo and Mota 2005; Onofre and Hettiarachchy 2007). It is plausible in the present study that the number of interactions disrupted increases with increasing UE time, facilitating the access of the solvent (70% aq. methanol) to the G, resulting in an increase in the quantities of individual G until achieving the maximum extraction of the G. Based on the results obtained in the present study, optimization of UE time appears to be an important parameter for accomplishing the maximum extraction of G from a cereal product containing ginseng.

3.5. SUMMARY

It is necessary to quantify G in a cereal product if ginseng is to be used as one of the ingredients, and to be able to do so to an accurate degree despite any interactions with components of the ingredients. In the present study, optimum extraction conditions of G (Rb1, Rc, and Rd) from a blend of WF-GP was investigated. The amounts of extractable G (Rb1, Rc, and Rd) decreased with increasing heating temperature of the blends of WF-GP, SF-GP, or GF-GP, indicating interactions between the SF and G as well as between the GF and G in a blend of WF-GP heated at 90°C. In addition, it was revealed that G had more interactions with the SF than with the GF in the heated WF-GP, due to proportionally greater amounts of starch in the WF. The interactions between WF components and G in WF-GP blend heated at 90°C were increasingly disrupted with increasing UE time, up to 90 min for the maximum extraction of G. Based on the findings in the present study, optimized extraction conditions for G from a WF-GP blend were a sample-to-solvent ratio of 0.071:1 (mg/μL) followed by ultrasonication for 90 min for as maximum an extraction of G as from pure GP.

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Table 3.1.

Moisture and Protein Contents of Wheat Flour (WF), Ginseng Powder (GP), Starch Fraction (SF), and Gluten Fraction (GF) Samples

Sample	Moisture Content (%, wb)	Protein Content (%)[a]
WF	11.2	8.3
GP	8.3	11.2
SF	1.9	2.1
GF	2.6	62.3

[a]Protein percentages are on a 14% moisture basis.

Table 3.2.

Quantities of Ginsenosides (Rb1, Rc, and Rd) Ultrasonically Extracted for 30 min at Room Temperature from Wheat Flour (WF, 0.9 g), Ginseng Powder (GP, 0.1 g), and a Blend of WF-GP (0.9 g WF + 0.1 g GP) with Different Sample-to-Solvent[a] Ratios

Sample	Solvent (μL)	Sample-to-Solvent Ratio (mg/μL)	Ginsenosides (mg/100 mg GP)		
			Rb1	Rc	Rd
GP	1000	0.100:1	0.53d	0.39d	0.18d
	2000	0.050:1	0.70c	0.47c	0.21c
	3000	0.030:1	0.83b	0.49b	0.23b
	4000	0.025:1	0.89a	0.52a	0.25a
	5000	0.020:1	0.88a	0.53a	0.25a
	6000	0.017:1	0.88a	0.52a	0.25a
	8000	0.013:1	0.88a	0.52a	0.25a
	10000	0.010:1	0.89a	0.52a	0.25a
WF	14000	0.064:1	ND	ND	ND
WF-GP	12000	0.083:1	0.82b	0.50b	0.23b
	14000	0.071:1	0.88a	0.53a	0.25a

Values with different letters within the same column differ significantly ($P<0.05$).
ND: not detectable.
[a]70% (v/v) aq. methanol

Table 3.3.

Effects of Ultrasonic Extraction (UE) Time on Extractability of Ginsenosides (Rb1, Rc, and Rd) from a Heated (90°C) Starch Fraction (SF), a Gluten Fraction (GF), a Blend of SF-Ginseng Powder (GP), and a Blend of GF-GP

Sample	UE Time (min)	Ginsenosides (mg/100 mg GP)		
		Rb1	Rc	Rd
SF	30	ND	ND	ND
	60	ND	ND	ND
	90	ND	ND	ND
	120	ND	ND	ND
SF-GP	30	0.71d	0.46c	0.20d
	60	0.82b	0.50b	0.24b
	90	0.87a	0.54a	0.27a
	120	0.88a	0.54a	0.26a
GF	30	ND	ND	ND
	60	ND	ND	ND
	90	ND	ND	ND
	120	ND	ND	ND
GF-GP	30	0.77c	0.49b	0.23c
	60	0.88a	0.54a	0.27a
	90	0.87a	0.53a	0.27a
	120	0.88a	0.54a	0.27a

Values with different letters within the same column differ significantly ($P<0.05$).
ND: not detectable.

Table 3.4.

Effects of Ultrasonic Extraction (UE) Time on Extractability of Ginsenosides (Rb1, Rc, and Rd) from Heated (90°C) Wheat Flour (WF), Ginseng Powder (GP), and a WF-GP Blend

Sample	UE Time (min)	Ginsenosides (mg/100 mg GP)		
		Rb1	Rc	Rd
GP	30	0.88a	0.54a	0.27a
	60	0.86a	0.53a	0.26a
	90	0.87a	0.53a	0.26a
	120	0.87a	0.53a	0.26a
WF	30	ND	ND	ND
	60	ND	ND	ND
	90	ND	ND	ND
	120	ND	ND	ND
WF-GP	30	0.72c	0.47c	0.21c
	60	0.82b	0.51b	0.24b
	90	0.87a	0.53a	0.26a
	120	0.87a	0.54a	0.26a

Values with different letters within the same column differ significantly ($P<0.05$).
ND: not detectable.

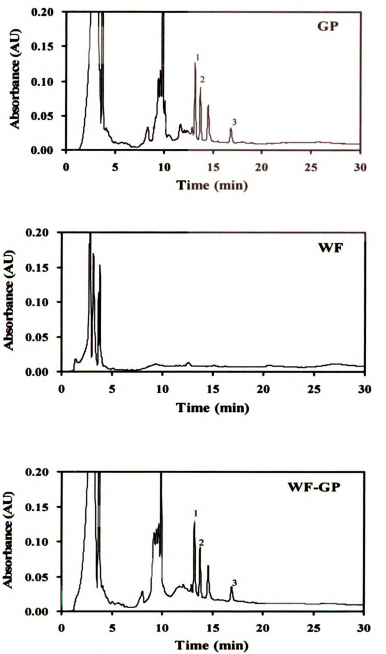


Fig. 3.1. RP-HPLC chromatograms of ginsenosides ultrasonically extracted for 30 min from pure ginseng powder (GP), wheat flour (WF), and a blend of WF-GP in 70% (v/v) aq. methanol. 1: Rb1; 2: Rc; 3: Rd.

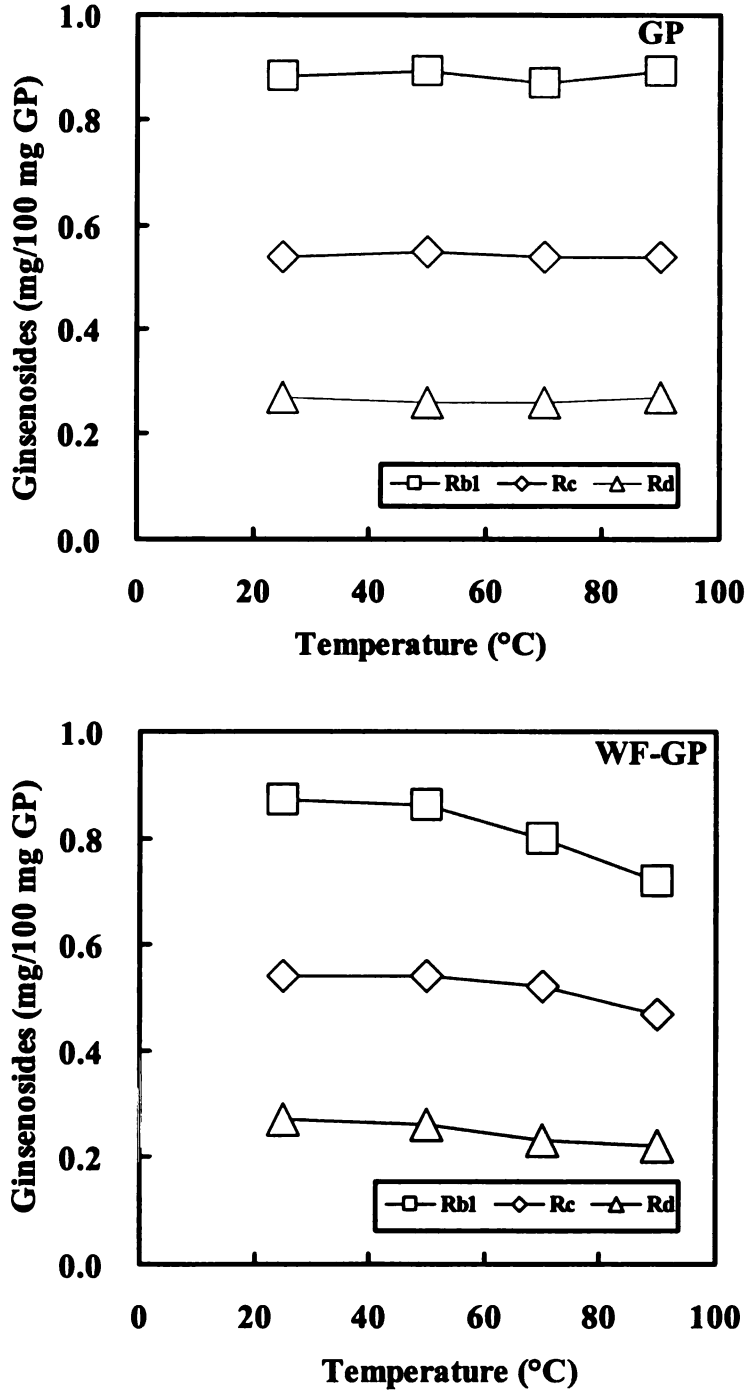
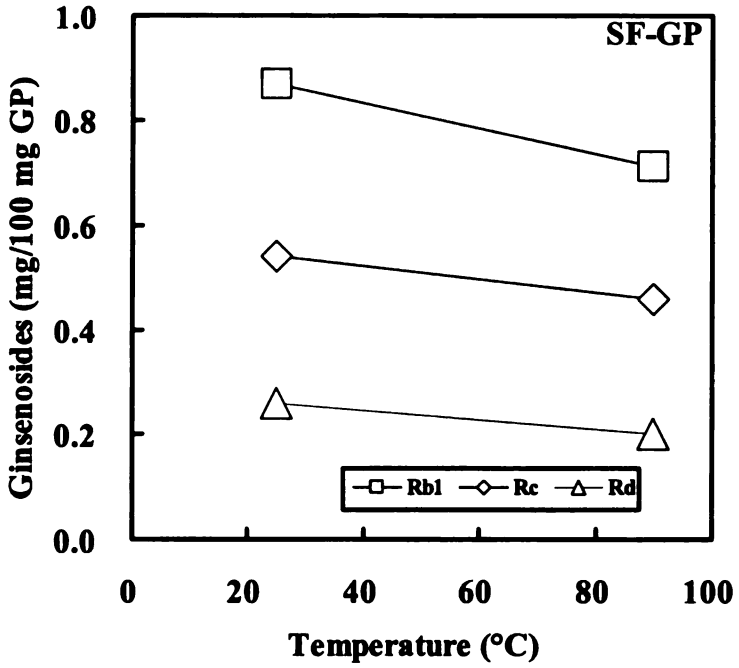


Fig. 3.2. Effects of sample heating of pure ginseng powder (GP) and a wheat flour (WF)-GP blend on subsequent extractable ginsenosides (Rb1, Rc, and Rd).



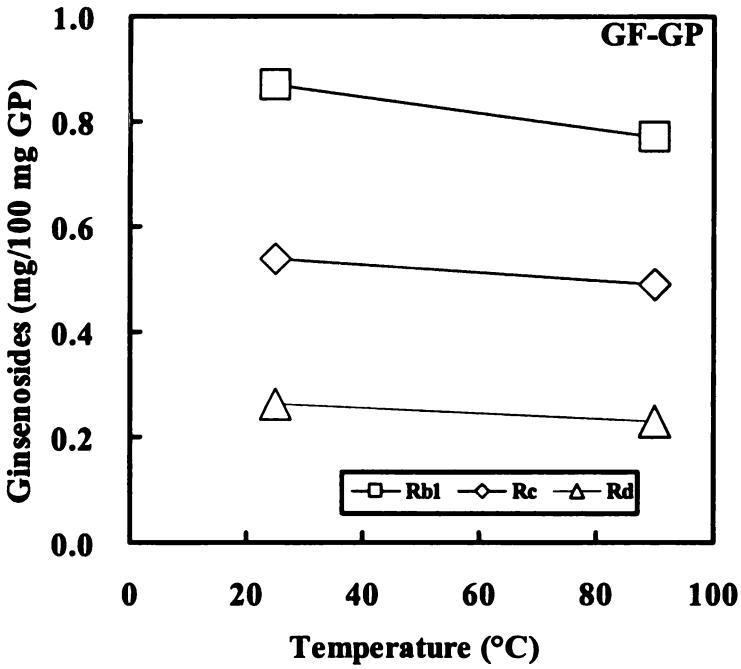


Fig. 3.3. Effects of sample heating of a starch fraction (SF)-ginseng powder (GP) blend and a gluten fraction (GF)-GP blend on subsequent extractable ginsenosides (Rb1, Rc, and Rd).

CHAPTER 4

Effects of Extrusion Process Variables on Extratable Ginsenosides in Wheat-Ginseng Extrudates

4.1. ABSTRACT

Effects of extrusion process variables (feed moisture, screw speed, and barrel temperature) on extractable ginsenosides (G, ginseng saponins) in wheat-ginseng extrudates (WGE) produced under different extrusion conditions were investigated. A wheat flour (WF)-ginseng powder (GP) blend (10% GP, w/w) was extruded in a twin-screw extruder (L/D ratio of 25:1) with full factorial combinations of feed moisture (25, 30, and 35%), screw speed (200 and 300 rpm), and zone 5 barrel temperature (110, 120, 130, and 140°C). Samples of WF, GP, WF-GP, and WGE extruded at different conditions were ultrasonically extracted for G from 60 to 150 min. Individual G (Rb1, Rc, Rd, Rg2, and Rg3) were fractionated and identified by RP-HPLC from each sample. The quantities of G (Rb1, Rc, and Rd) extracted from WGE samples extruded at a zone 5 barrel temperature of more than 120°C were significantly higher than those extracted from the control blend, explaining the conversion of malonyl G (Rb1, Rc, and Rd) into G (Rb1, Rc, and Rd, respectively) during extrusion cooking. Neither G Rg2 nor G Rg3 were found in pure WF, GP, nor in the WF-GP blend. G Rg2 were found in all the WGE samples studied. Increasing feed moisture caused a decrease in the quantities of G Rg2 in WGE samples. In contrast, increasing screw speed and barrel temperature each led to an increase in the quantities of G Rg2 in WGE samples. G Rg3 were present in WGE samples produced under only the following three extrusion conditions: (1) 25% feed moisture, 300 rpm screw speed, and 130°C zone 5 barrel temperature, (2) 25% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature, and (3) 30% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature. Thus, it was suggested that the production of new G Rg3 was dependent upon the extrusion process conditions used.

4.2. INTRODUCTION

Extrusion cooking is a widely used technology to process cereals or starches into food products, such as snack foods, ready-to-eat breakfast cereals, infant formulas, etc. (Harper 1979). This cooking processing, carried out using a high temperature and short time (HTST) treatment, yields finished products with desirable qualities, such as high digestibility and nutritional value (Harper 1998).

Wheat flour (WF) is a commonly used material in the extrusion industry and the effects of extrusion process variables on the properties of WF extrudates have been studied (Ali et al 1996; Anderson and Ng 2001, 2003; Ding et al 2006; Harper 1979; Mercier and Feillet 1975; Ryu and Ng 2001; Schmid et al 2005). Based on these studies, extrusion process variables (including screw configuration, screw speed, temperature profile of the barrel, residence time, feed rate, feed moisture, diameter of exit die, and others) have been found to alter the physical and chemical properties of final products. The addition of diverse ingredients to extrusion materials has been done with the objective of improving the nutritional quality of final extruded products and imparting desirable functional properties (Jin et al 1995; Rinald et al 2000; Schmid et al 2005; Singh & Singh 2004; Zazueta-Morales et al 2002).

Ginseng has been frequently used in Asian countries as a traditional medicine (Fuzzati 2004). Ginsenosides (G, ginseng saponins) have been known as the main active components and have been typically used as marker compounds for quality properties (Chuang et al 1995; Lau et al 2004; Li et al 1996). G are dammarane-type triterpene glycosides, and can be classified into three groups: the protopanaxadiols, protopanaxatriols, and oleanolic acid (Li et al 2006). Four malonyl G, including

malonyl G Rb1, malonyl G Rb2, malonyl G Rc, and malonyl G Rd, are also found in ginseng (Fuzzati et al 1999).

Raw ginseng has been processed into two kinds of ginseng, white ginseng and red ginseng, to improve its preservation and efficacy (Kim et al 2000; Wang et al 2006). White ginseng is usually air-dried raw ginseng, while red ginseng is usually made by first steaming raw ginseng at 90-100°C for 2-3 hr and then air-drying (Ha et al 2005). Red ginseng is known to be more pharmaceutically active than white ginseng, because the steaming process causes changes in the chemical compositions of G, thereby enhancing the biological activity of the ginseng (Ha et al 2007; Shibata 2001). The ginsenosides Rg3, Rg5, Rg6, Rh2, Rh3, Rh4, Rs3, Rk1, and F4 are not found in raw and white ginseng, whereas red ginseng contains these new G generated during the steaming process (Ha et al 2007; Kim et al 2000; Kwon et al 2001). In particular, G Rg3 have been found to show various biological activities, including anticancer activity (Shibata 2001; Wang et al 2006; Yun et al 2001) and radical scavenging activity (Kang et al 2006). Moreover, it has been reported that G Rg3 inhibit the growth of *Helicobacter pylori* (Bae et al 2002).

In spite of medical benefits of red ginseng and the processing efficiency (e.g., high temperature and short time) of extrusion cooking for making red ginseng products, Ha et al (2005), who studied changes in G in final extruded *Panax ginseng*, only applied the extrusion processing technique to produce pure red ginseng products. They reported that the application of extrusion cooking to produce red ginseng can considerably reduce the processing time, as compared to the traditional red ginseng process. However, no studies have apparently reported the production of extruded WF snack products with ginseng, nor have studies been found that investigate changes in G

in snack products extruded under different conditions. Extrusion cooking has been employed to make nutraceutical snack foods and there is a possibility of using WF in combination with ginseng to provide highly nutritious snack food products. Thus, the objective of this study was to investigate the effects of extrusion process variables (feed moisture, screw speed, and barrel temperature) on extractable G in wheat-ginseng extrudates (WGE).

4.3. MATERIALS AND METHODS

4.3.1. Materials

Soft WF was obtained from the Mennel Milling Co. in Fostoria, OH, USA. The raw ginseng root (*Panax ginseng* C. A. Meyer), harvested after four years' growth and milled into powder (particle size of $\leq$ 167 μm), was cultivated in Geumsan, Korea, and purchased at a ginseng market. WF and GP were analyzed for their moisture contents using AACCI Approved Method 44-15A (AACCI, 2000) and the moisture contents of WF and GP were 11.2% (wb) and 8.3% (wb), respectively. Standards for ginsenosides Rb1, Rc, Rd, Rg2, and Rg3 were provided by ILHWA Co. (Kuri, Korea).

4.3.2. Extrusion Cooking

WF and ginseng powder (GP) were mixed in a weight ratio of 9:1 using a mixer (Model A-200, Hobart MFG. Co., Troy, OH, USA) for 20 min; hereafter, this blend is referred to as WF-GP. Extrusion cooking was done with an APV co-rotating, twin screw extruder, with a barrel diameter of 19 mm and a barrel length to diameter (L/D) ratio of 25:1 (Model MP 19T2-25, APV Baker, Grand Rapids, MI, USA). Heating of the barrel was controlled by electric heating elements jacketing the barrel and thermal probes. Heating was divided into five zones along the length of the barrel, with zone 1 nearest the feed section and zone 5 nearest the exit die. Feed moisture was adjusted to the predetermined dough moisture content during extrusion cooking by injection of water into the barrel using a Brook Crompton E2 Metripump (Hudders Field, England). Dry materials were fed using a K-TRON K2M twin-screw volumetric feeder (K-TRON, Pittman, NJ, USA). Product temperature and Die pressure was measured by a thermocouple (Model TB422J, Dynisco, Sharon, MA, USA) and by a pressure

transducer (Model ERP3-3M, Dynisco, Sharon, MA, USA), respectively, inserted before the die. Torque was measured as % of the total motor power (2000W).

A full factorial arrangement was used for the present study. Extrusion process variables were: (1) feed moisture (25, 30, and 35%), (2) screw speed (200 and 300 rpm), and (3) zone 5 barrel temperature (110, 120, 130, and 140°C), for a total of 24 different combinations (Table 4.1). Zone 1/2/3/4 barrel temperatures were constant (40/70/90/110°C, respectively) for all the extrusion process conditions studied (Table 4.1). The dry material feed rate was kept constant (2.0 kg/hr) along with the screw configuration (Table 4.2). Extrusion cooking of WF-GP was repeated on a different day.

The WGE samples produced at different extrusion conditions were collected and dried in an air oven (45°C) overnight. After drying, portions of each WGE sample were ground to a particle size of $\leq$ 330 μm (54 mesh screen) using a coffee grinder (Braun Inc., Woburn, MA, USA). After grinding, moisture contents of ground WGE samples were measured by AACCI Approved Method 44-15A (AACCI, 2000). Both ground and unground WGE samples were placed in ziplock bags, sealed, and stored at -20°C until analysis.

4.3.3. Specific Mechanical Energy

The specific mechanical energy (SME) in Watt hours/kg was calculated according to the equation of Brent et al (1997):

SME = [actual screw speed rpm / rated screw rpm] x [% torque / 100] x [motor power in W / feed rate in kg/hr]

Rated screw speed = 500 rpm; motor power = 2000 W

4.3.4. Optimization of Ultrasonic Extraction Time for Ginsenosides from Wheat-Ginseng Extrudates

Four mL of 70% (v/v) aq. methanol were added to GP (0.1 g). Fourteen mL of 70% (v/v) aq. methanol were added to WF (0.9 g), WF-GP (0.9 g WF + 0.1 g GP), or WGE samples (1.0 g) extruded under different conditions. Each mixture was ultrasonically extracted for 60, 90, 120, or 150 min using an Ultrasonicator (Model FS 14H, Fisher Scientific, Pittsburgh, PA, USA, Power: 155W) at room temperature, and then centrifuged at 11,200 x g for 20 min using a centrifuge (Model J2-21M, Beckman Instruments Inc., Fullerton, CA, USA) at 25°C. The supernatant was evaporated using a Micro Rotary Evaporator (Model 421-4000, Labconco, Kansas City, MO, USA) at 50°C. Prior to RP-HPLC analysis, the residue was dissolved in 2 mL of 70% (v/v) aq. methanol and filtered through a 0.45 μm nylon filter membrane (Millipore, Ireland). Each sample was extracted in duplicate and two chromatographic runs were performed for each extract.

4.3.5. Fractionation and Identification of Ginsenosides by RP-HPLC

RP-HPLC was carried out on a Millenium 2010 HPLC Workstation, consisting of a Waters 600E multi-solvent delivery system (Waters, Milford, MA, USA), a temperature control module, and a 996 photodiode array detector. Separation was performed at 30°C on a Phenomenex 300 RP, Jupiter C-18 column (4.6 x 250 mm, 5 μm particle diameter; Phenomenex, Torrance, CA, USA). A Phenomenex security guard with the same packing material served as the guard column.

The solvents were A: water (distilled water filtered through Milli-Q-Plus,

Millipore), and B: HPLC grade acetonitrile. Separation was achieved using the following gradient: 0-5 min, 20-30% B; 5-25 min, 30-40% B; 25-30 min, 40-20% B. The solvent flow rate was 1 mL/min and the injection volume was 20 μL. The solvents were purged with helium at the rate of 20 mL/min. The column was equilibrated for 10 min with 20% acetonitrile prior to injection. The UV detection wavelength was set at 203 nm (Lau et al 2004), and the detector output was transmitted simultaneously to the computer for data storage and graphic representation.

4.3.6. Statistical Analysis

All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level. In addition, Pearson correlation coefficients were determined among the samples. The CORR procedure was used to obtain correlation coefficients.

4.4. RESULTS AND DISCUSSION

4.4.1. Optimization of Ultrasonic Extraction Time for Ginsenosides from Wheat-Ginseng Extrudates

Effects of ultrasonic extraction (UE) time of WF, GP, and WF-GP on extractable G (Rb1, Rc, and Rd) are presented in Table 4.3. No measurable G (Rb1, Rc, and Rd) in pure WF were found at any of the UE times studied. The quantities of G Rb1, G Rc, and G Rd extracted from both pure GP and WF-GP were not significantly different at all the ranges of UE time (60-150 min) studied. However, the quantities of G extracted from WGE samples extruded at 30% feed moisture, 200 rpm screw speed, and 120°C zone 5 barrel temperature increased with increasing UE time, and finally reached a plateau after UE time of 120 min (Figure 4.1). All the studied WGE samples showed the same trend (Appendix 3). The findings established that the maximum extraction of extractable G from WGE samples produced under each of the different extrusion process conditions could be achieved by ultrasonication for 120 min or longer.

4.4.2. Changes in Ginsenosides during Extrusion Cooking

The quantities of G (Rb1, Rc, and Rd) ultrasonically extracted for 150 min from WF-GP and WGE samples extruded at different conditions are listed in Table 4.4. The quantities of G Rb1 and G Rc extracted from WGE samples extruded at 110°C zone 5 barrel temperature were not changed upon extrusion cooking. However, the quantities of G Rd extracted from the same WGE samples were significantly increased upon extrusion cooking. The quantities of each of G Rb1, G Rc, and G Rd extracted from WGE samples extruded at a zone 5 barrel temperature of more than 120°C were significantly higher than those of their counterpart G extracted from the control WF-GP.

This finding was consistent with Ha et al (2005) who showed significant increases in the quantities of G Rb1, G Rb2, G Rc, and G Rd after an extrusion process of raw *Panax ginseng*.

Some studies have been conducted to compare G compositions of white and red ginseng (Ha et al 2005; Kim et al 2000; Kwon et al 2001; Lau et al 2003; Lau et al 2004). The G composition of red ginseng was obviously different from that of white ginseng, because of the loss of malonyl G during the steaming process used to produce red ginseng (Fuzzati 2004). Shibata (2001) noted that white ginseng includes the malonyl esters of G Rb1, G Rc, and G Rd, and that the malonyl group, which is originally attached at the 6″-position of the glycosyl moieties of G Rb1, G Rc, and G Rd, is easily lost during the steaming process when making red ginseng. Ren and Chen (1999) reported that malonyl G are thermally very unstable; therefore, they are readily demalonylated by heating. According to Fuzzati (2004), the acidic malonyl G (malonyl G Rb1, malonyl G Rc, and malonyl G Rd) degraded or converted into the corresponding neutral G (G Rb1, G Rc, and G Rd, respectively) during the steaming process. Therefore, it is postulated in the present study that extrusion cooking under a zone 5 barrel temperature of $\geq$ 120°C could stimulate the loss of malonyl acid from glycosyl moieties of malonyl G Rb1, malonyl G Rc, and malonyl G Rd, subsequently inducing an increase in the quantities of G Rb1, G Rc, and G Rd, respectively, in WGE samples.

RP-HPLC chromatograms of G ultrasonically extracted for 150 min from non-extruded WF-GP and WGE samples extruded at 25% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature are shown in Figure 4.2. The RP-HPLC chromatograms of the WF-GP and the WGE samples were found to be distinctively

different, indicating that when WF-GP was extruded, the HPLC profile of G was changed. In particular, G Rg2 (peak 3) and G Rg3 (peak 5), which were not present in WF-GP, were found in the WGE sample. Furthermore, the quantities of G Rg2 and G Rg3 were dependent on the extrusion process conditions studied (Table 4.4); increases in the quantities of G Rg2 and G Rg3 were observed when feed moisture was decreased, and screw speed and barrel temperature each increased.

Wang et al (2006) studied the changes in G in berries of *Panax quinquefolius* after steaming treatment (100-120°C for 1 hr). They found that the amounts of G (Rg2, Rg3, Rh1, and Rh2) in the steamed samples were increased as compared to those in raw samples. They reported that these G can be used as marker compounds to distinguish red ginseng from white ginseng. Kim et al (2000) evaluated the effect of steaming *Panax ginseng*, at 100, 110, and 120°C for 2 hr using an autoclave, on its chemical components. They observed that G (F4, Rg3, and Rg5) were not found in the raw ginseng, but in the steamed ginseng samples. According to Lau et al (2003), when *Panax notoginseng* was steamed at 120°C for 1, 2, 3, and 9 hr, G (Rb1, Rd, and Re) significantly decreased and four new major peaks appeared in the RP-HPLC fractionation which had not been present in chromatograms of the raw ginseng sample. They reported that the new peaks were associated with the unique G which are present only in red ginseng products, and that the quantities of the new G in the steamed samples increased with increasing duration of the steaming process. According to Ha et al (2007), less polar G, including G (Rg3, Rg5, Rk1, and F4), are typically considered as G unique to red ginseng products.

Ha et al (2005) employed an extrusion process to produce red ginseng products using raw *Panax ginseng*. The extrusion conditions used were feed moisture

of 15 or 22% (wb), screw speed of 200 rpm, and barrel temperatures of 110/110/60/40°C (the barrel zones of 1/2/3/4, respectively). They found an increase in the G Rg group (G Rg1, G Rg2, and G Rg3) in the final extruded samples. Shibata (2001) reported that the glycosyl moieties at the C-20 position of the protopanaxadiol type G, including G Rb1, G Rb2, G Rc, and G Rd, are partly degraded to produce G Rg3 during the steaming process to make red ginseng. Popovich and Kitts (2004) showed that G Rg3 are naturally absent in *Panax quinquefolius*, but are produced by a thermal process. They also reported that the formation of G Rg3 is associated with the breakdown of the more abundant G Rb1 and G Rc. On the other hand, G Rg2 can be produced by the protopanaxatriol type G, such as G Re, in the same way (Kim et al 2000). Thus, it is suggested in the present study that the presence of G Rg3 and G Rg2 in WGE samples is attributed to the chemical degradation and/or conversion of the thermolabile protopanaxadiol type G and protopanaxatriol type G, respectively, during high temperature extrusion cooking. Furthermore, it is suggested that, utilizing raw ginseng as one of the ingredients, extruded nutraceutical WF snack products can be produced by extrusion cooking and yield red ginseng components in the extrudates.

To investigate the effects of extrusion process variables on changes in the quantities of G Rg2 and G Rg3 in WGE samples, WF-GP were extruded at 24 different extrusion process conditions. The quantities of G Rg2 ultrasonically extracted for 150 min from non-extruded WF-GP samples and from WGE samples extruded under different conditions are listed in Table 4.4. The SME values obtained from different extrusion process conditions studied are presented in Table 4.5. G Rg2 were found in all the WGE samples studied, and the quantities of G Rg2 were significantly affected by extrusion process variables studied.

First of all, increasing feed moisture from 25 to 35%, at constant zone 5 barrel temperature and screw speed (130°C and 300 rpm, respectively), resulted in a significant decrease in the quantities of G Rg2 in WGE samples, from 0.15 to 0.08 mg per 100 mg GP, and significantly reduced SME values from 268.5 to 243.9 Wh/kg. The finding (a decrease in SME values with increasing feed moisture) was consistent with observations by Ryu and Ng (2001), who reported that lower feed moisture induces higher viscosity of the melt during extrusion cooking and this can lead to an increase in the degree of mechanical energy input on the extruded products. Therefore, it is suggested that in the present study, the increased mechanical energy input caused by the lower feed moisture extrusion cooking condition could facilitate the loss of the glycosyl moieties at the C-20 position of the protopanaxatriol type G, subsequently causing an increase in the quantities of G Rg2 in WGE samples.

Secondly, elevating screw speed from 200 to 300 rpm, at constant zone 5 barrel temperature and feed moisture (140°C and 25%, respectively), significantly increased the quantities of G Rg2 in WGE samples from 0.09 to 0.20 mg per 100 mg GP, and led to a significant increase in SME values from 131.8 to 262.8 Wh/kg. According to Frame (1994), the degree of fill in the barrel and the viscosity of the dough can be diminished with increasing the screw speed during extrusion cooking. However, in the present study, the increase in screw speed was large enough to overcome a slight reduction in the % torque from the motor, resulting in a higher SME. Thus, it appears in the present study that more protopanaxatriol type G could be readily converted to G Rg2 at the higher screw speed extrusion process conditions, due to higher mechanical energy input occurring during high screw speed extrusion cooking conditions.

Finally, increasing zone 5 barrel temperature from 110 to 140°C, at constant

feed moisture and screw speed (25% and 300 rpm, respectively), caused a significant increase in the quantities of G Rg2 in WGE samples, from 0.09 to 0.20 mg per 100 mg GP. As already mentioned, the traditional processing to make red ginseng includes steaming raw ginseng at 90-100°C for 2-3 hr. Therefore, it was hypothesized in the present study that temperature higher than these could improve the production of red ginseng components and shorten the processing time. The finding indicated that higher barrel temperature during extrusion cooking is indeed one of the important factors that increases the quantities of G Rg2 in WGE samples.

As reported earlier, increasing SME values (i.e., the lower feed moisture or higher screw speed extrusion cooking conditions) were associated with production of greater quantities of G Rg2 in WGE samples. However, the increase in barrel temperature from 110 to 140°C at these extrusion process conditions caused an increase in the quantities of G Rg2 in WGE samples, despite significantly decreased SME values (from.282.9 to 262.8 Wh/kg). The trend of decreasing SME values with increasing barrel temperature is generally consistent with other published results (Altan et al 2008; Guha et al 1997; Ilo et al 1999; Ryu and Ng 2001; Tanhehco and Ng 2005). Lower SME values are a result of the lower viscosity of the melt at higher temperatures, thereby leading to lower mechanical energy input on the extruded products (Altan et al 2008; Ryu and Ng 2001).

Based on all of the results (on effects of extrusion process variables on the increase in G Rg2 in WGE samples) obtained in the present study, it was suggested that increasing barrel temperature played the more dominant role in the conversion of protopanaxatriol type G into G Rg2 than did mechanical energy input during extrusion cooking of WF-GP.

The quantities of G Rg3 ultrasonically extracted for 150 min from WF-GP and WGE samples extruded under different conditions are listed in Table 4.4. In particular, G Rg3 are valued for their pharmaceutical activities. Numerous studies have focused on G Rg3 to evaluate the effects of heating processes on producing red ginseng products (Ha et al 2005; Kim et al 2000; Popovich and Kitts 2004; Wang et al 2005). Kim et al (2000) observed that during a steaming process of raw *Panax ginseng*, increasing steaming temperature from 100 to 120°C resulted in an increase in the quantities of G Rg3. In work done by Wang et al (2006), when berries of *Panax quinquefolius* were steamed at 120°C, the quantities of G Rg3 were significantly higher than for those steamed at 100°C.

In the present study, unlike G Rg2 which were found in all the WGE samples produced, G Rg3 were present in WGE samples produced at only the following three extrusion process conditions: (1) 25% feed moisture, 300 rpm screw speed, and 130°C zone 5 barrel temperature, (2) 25% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature, (3) 30% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature. Of these, extrusion process condition (2) produced the highest quantities of G Rg3, and is the extrusion condition that also produced the highest quantities of G Rg2. In particular, the extrusion condition (2) had the highest zone 5 barrel temperature (140°C), the highest screw speed (300 rpm), and the lowest feed moisture (25%), indicating that an increase in severity of the extrusion conditions could improve the conversion of protopanaxadiol type G to G Rg3 in the WGE samples.

4.4.3. Correlations among Ginsenosides and Product Temperature

Pearson's coefficients of correlation (r) among G (Rb1, Rc, Rd, and Rg2) and product temperature are reported in Table 4.6. The quantities of G Rb1 were found to be positively associated with the amounts of G Rc ($r = 0.97$, $p \leq 0.001$), G Rd ($r = 0.90$, $p \leq 0.001$), and G Rg2 ($r = 0.47$, $p \leq 0.05$). Product temperature represents the actual temperature of the dough at the exit die during extrusion cooking. The quantities of G Rb1, Rc, Rd, and Rg2 were positively correlated ($r = 0.90$, $p \leq 0.001$; $r = 0.86$, $p \leq 0.001$; $r = 0.76$, $p \leq 0.001$; $r = 0.61$, $p \leq 0.01$, respectively) with the product temperature, indicating that increasing product temperature not only could increase the chemical loss of malonyl acid from glycosyl moieties of the malonyl G (Rb1, Rc, and Rd), but also could improve the conversion efficiency of protopanaxatriol type G to G Rg2.

4.5. SUMMARY

The present study focused on changes in chemical compositions of G after extrusion cooking of a blend of WF-GP. Extrusion cooking led not only to an increase in the quantities of G Rb1, G Rc, and G Rd in WGE samples, but also to the production of new G Rg2 and G Rg3 in certain WGE samples, indicating that changes in chemical composition of G can take place during high temperature extrusion cooking. The quantities of G Rg2 and G Rg3 in WGE samples were significantly affected by the extrusion process variables (feed moisture, screw speed, and barrel temperature) studied. The optimal extrusion process condition in the present study, producing the maximum quantities of G Rg2 and G Rg3 in WGE samples, was 25% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature. Moreover, the presence of G Rg2 and G Rg3 in WGE samples offered the possibility of the production of extruded nutraceutical WF snack foods containing red ginseng components.

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Table 4.1.

Extrusion Conditions for a Wheat Flour-Ginseng Powder (GP) Blend

(10% GP, w/w)

Feed Moisture (%, wb)	Screw Speed (rpm)	Barrel Temperature Zones 1-5 (°C)[a]
25	200	40/70/90/110/110
30	200	40/70/90/110/110
35	200	40/70/90/110/110
25	300	40/70/90/110/110
30	300	40/70/90/110/110
35	300	40/70/90/110/110
25	200	40/70/90/110/120
30	200	40/70/90/110/120
35	200	40/70/90/110/120
25	300	40/70/90/110/120
30	300	40/70/90/110/120
35	300	40/70/90/110/120
25	200	40/70/90/110/130
30	200	40/70/90/110/130
35	200	40/70/90/110/130
25	300	40/70/90/110/130
30	300	40/70/90/110/130
35	300	40/70/90/110/130
25	200	40/70/90/110/140
30	200	40/70/90/110/140
35	200	40/70/90/110/140
25	300	40/70/90/110/140
30	300	40/70/90/110/140
35	300	40/70/90/110/140

[a] Zone 5 = nearest to exit die.
Feed rate = 2.0 kg/hr, db

Table 4.2.

Screw Configuration for Twin-Screw Extruder

Zone	Type[a]	Flight Angle (°)
I	5D Twin	–
II	3D Twin	–
	7 FP	30
III	5D Twin	–
	3D Twin	–
IV	3 FP	60
	3 RP	30
	2D Single	–
	4 FP	60
V	3 RP	30
	2D Single	–
	Die	–

[a] D: screw diameter (19 mm); P: kneading paddle (0.25 D); FP: forwarding paddle; RP: reversing paddle; Twin: twin lead screw; Single: single lead screw; Die: 3mm single round type exit die.

Table 4.3.

Effects of Ultrasonic Extraction (UE) Time of Wheat Flour (WF, 0.9 g), Ginseng Powder (GP, 0.1 g), and a WF-GP (0.9 g WF + 0.1 g GP) Blend on Extractable Ginsenosides (Rb1, Rc, and Rd)

Sample	UE time (min)	Ginsenosides (mg/100 mg GP)		
		Rb1	Rc	Rd
GP	60	0.86a	0.51a	0.24a
	90	0.85a	0.50a	0.25a
	120	0.86a	0.52a	0.25a
	150	0.86a	0.51a	0.24a
WF	60	ND	ND	ND
	90	ND	ND	ND
	120	ND	ND	ND
	150	ND	ND	ND
WF-GP	60	0.86a	0.52a	0.25a
	90	0.85a	0.51a	0.24a
	120	0.86a	0.51a	0.25a
	150	0.86a	0.51a	0.25a

Values with different letters within the same column differ significantly ($P<0.05$).
ND: not detectable.

Table 4.4.

Quantities of Ginsenosides (Rb1, Rc, Rd, Rg2, and Rg3) Ultrasonically Extracted for 150 min from a Wheat Flour (WF)-Ginseng Powder (GP) Blend (10% GP, w/w) and Wheat-Ginseng Extrudates

Extrusion Conditions	Ginsenosides (mg/100 mg GP)				
M/S/T[a]	Rb1	Rc	Rd	Rg2	Rg3
WF-GP (non-extruded)	0.86d	0.51d	0.25e	ND	ND
25/200/110	0.86d	0.51d	0.28cd	0.06h	ND
30/200/110	0.87d	0.51d	0.29c	0.06h	ND
35/200/110	0.87d	0.51d	0.26de	0.05h	ND
25/300/110	0.86d	0.50d	0.28cd	0.09e	ND
30/300/110	0.85d	0.50d	0.27cd	0.06h	ND
35/300/110	0.85d	0.51d	0.27cd	0.06h	ND
25/200/120	1.03ab	0.61a	0.32ab	0.08f	ND
30/200/120	1.00b	0.56c	0.32ab	0.08f	ND
35/200/120	0.96c	0.55c	0.32ab	0.07g	ND
25/300/120	1.01b	0.62a	0.33ab	0.11d	ND
30/300/120	0.96c	0.58b	0.32ab	0.08f	ND
35/300/120	0.96c	0.58b	0.31b	0.08f	ND
25/200/130	1.04a	0.61a	0.32ab	0.08f	ND
30/200/130	1.04a	0.62a	0.34a	0.07g	ND
35/200/130	1.04a	0.63a	0.33ab	0.07g	ND
25/300/130	1.04a	0.62a	0.33ab	0.15b	0.05b
30/300/130	1.05a	0.61a	0.32ab	0.09e	ND
35/300/130	1.05a	0.62a	0.32ab	0.08f	ND
25/200/140	1.06a	0.61a	0.32ab	0.09e	ND
30/200/140	1.04a	0.61a	0.31ab	0.09e	ND
35/200/140	1.05a	0.62a	0.32ab	0.07g	ND
25/300/140	1.05a	0.61a	0.33ab	0.20a	0.07a
30/300/140	1.05a	0.61a	0.33ab	0.12c	0.05b
35/300/140	1.04a	0.62a	0.32ab	0.09ef	ND

Values with different letters within the same column differ significantly ($P<0.05$).
[a]M: Feed moisture (%, wb); S: screw speed (rpm); T: zone 5 barrel temperature (°C).
ND: not detectable.

Table 4.5.

System Variables Related to Different Extrusion Process Variables of Wheat-Ginseng Extrudates

Extrusion Process Variables			System Variables	
Feed Moisture (%, wb)	Screw Speed (rpm)	Temperature[a] (°C)	SME[b] (Wh/kg)	PT[c] (°C)
25	200	110	157.8h	117jkl
30	200	110	154.0hi	116kl
35	200	110	146.6hi	115l
25	300	110	282.9a	119j
30	300	110	259.8cde	118jk
35	300	110	252.0defg	117jkl
25	200	120	155.0hi	130h
30	200	120	153.2hi	127i
35	200	120	133.8j	126i
25	300	120	274.8ab	132h
30	300	120	253.5def	131h
35	300	120	249.3efg	130h
25	200	130	146.0i	145fg
30	200	130	148.0hi	145efg
35	200	130	129.2j	144f
25	300	130	268.5bc	147e
30	300	130	261.9cd	147ef
35	300	130	243.9fg	146efg
25	200	140	131.8j	156bc
30	200	140	130.6j	154cd
35	200	140	117.2k	153d
25	300	140	262.8cd	163a
30	300	140	256.8de	157b
35	300	140	241.8g	152d

Values with different letters within the same column differ significantly ($P<0.05$).

[a]Temperature: zone 5 barrel temperature.

[b]SME: specific mechanical energy.

[c]PT: product temperature.

Table 4.6.

Pearson's Coefficients of Correlation (r) among Ginsenosides (Rb1, Rc, Rd, and Rg2) and PT[a]

	Rb1	Rc	Rd	Rg2	PT
Rb1		**0.97*** **	**0.90*** **	**0.47***	**0.90*** **
Rc			**0.90*** **	**0.46***	**0.86*** **
Rd				**0.49***	**0.76*** **
Rg2					**0.61****
PT					

*, **, ***, Significantly different at the 5%, 1%, and 0.1% levels, respectively.
[a]PT: product temperature.

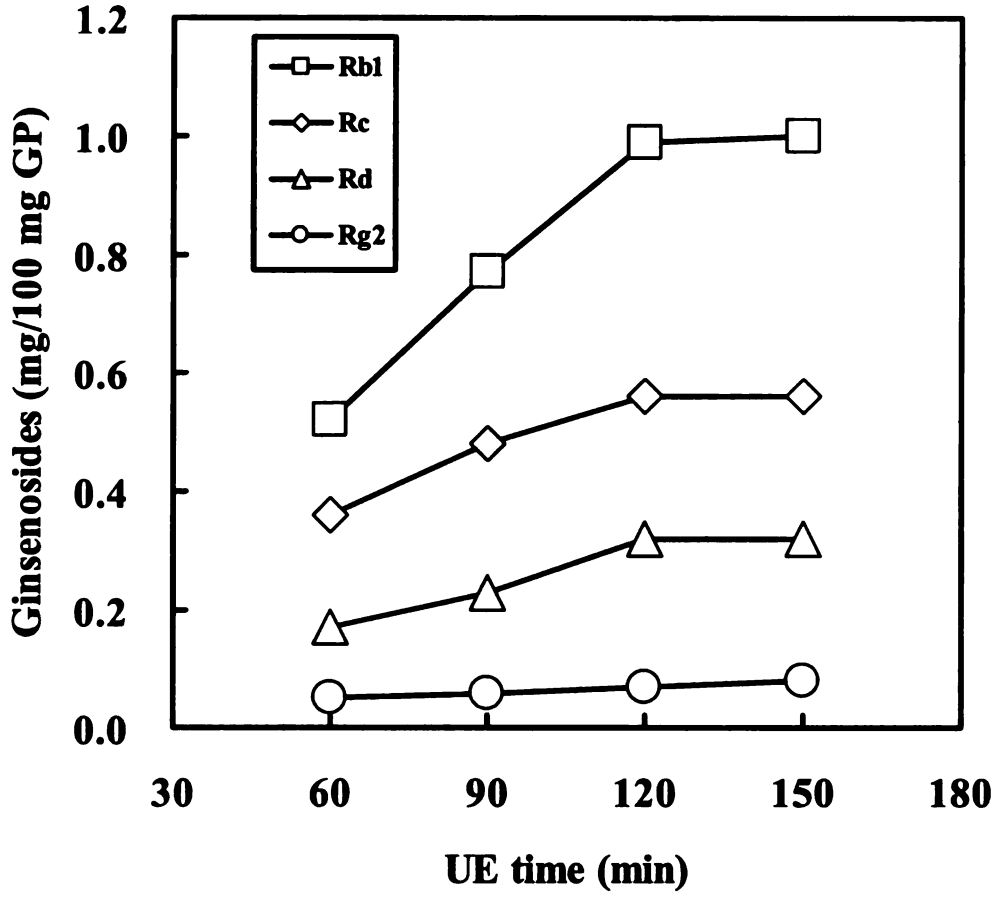

Fig. 4.1. Effects of ultrasonic extraction (UE) time of wheat-ginseng extrudates extruded at 30% feed moisture, 200 rpm screw speed, and 120°C zone 5 barrel temperature on extractable ginsenosides (Rb1, Rc, Rd, and Rg2). GP: ginseng powder.

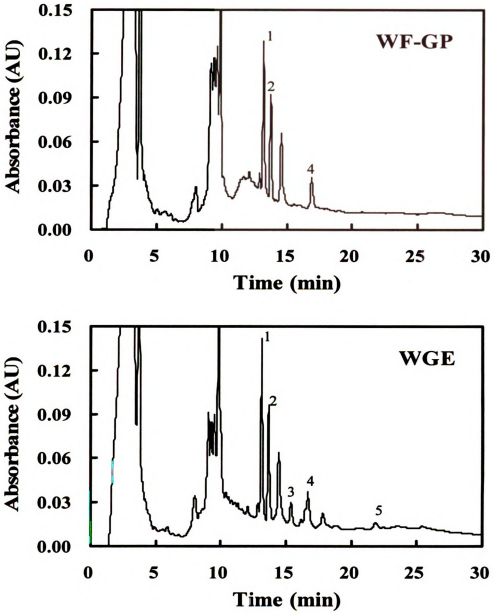

Fig. 4.2. RP-HPLC chromatograms of ginsenosides ultrasonically extracted for 150 min from a non-extruded blend of wheat flour (WF)-ginseng powder (GP) and a wheat-ginseng extrudate (WGE) extruded at 25% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature. 1: Rb1; 2: Rc; 3: Rg2; 4: Rd; 5: Rg3.

CHAPTER 5

Effects of Extrusion Process Variables on Quality Properties of Wheat-Ginseng Extrudates

5.1. ABSTRACT

The present study was conducted to investigate the effects of extrusion process variables (feed moisture, screw speed, and barrel temperature) on the physical [expansion ratio, water absorption index (WAI), and water solubility index (WSI)], pasting, and thermal properties of wheat-ginseng extrudates (WGE). A wheat flour-ginseng powder (GP) blend (10% GP, w/w) was extruded in a twin-screw extruder (L/D ratio of 25:1) with full factorial combinations of feed moisture (25, 30, and 35%), screw speed (200 and 300 rpm), and zone 5 barrel temperature (110, 120, 130, and 140°C). The expansion ratios of WGE samples were significantly increased with decreasing feed moisture, decreasing screw speed, and increasing barrel temperature. Increasing feed moisture significantly increased WAI values of WGE samples and significantly decreased WSI values of WGE samples. However, an increase in either of screw speed or barrel temperature caused a significant decrease in WAI values of WGE samples and a significant increase in WSI values of WGE samples. Rapid Visco Analyzer peak viscosity values of WGE samples were significantly affected by changes in extrusion process variables studied, indicating that the degree of starch degradation and/or gelatinization in WGE samples is a very important factor associated with their peak viscosity. WAI values of WGE samples were positively correlated ($r = 0.88$, $p \leq 0.001$) with peak viscosity values of WGE samples, whereas WSI values of WGE samples were negatively correlated ($r = -0.82$, $p \leq 0.001$). Increasing feed moisture resulted in an increase in values of transition peak temperature (Tp) of WGE samples, whereas increasing screw speed and barrel temperature each led to a decrease in Tp values of WGE samples, determined by differential scanning calorimetry.

5.2. INTRODUCTION

The quality properties of final extruded products are considerably affected by extrusion process variables, such as feed rate, feed moisture, screw configuration, screw speed, diameter of exit die, barrel temperature, and the addition of other materials (Anderson and Ng 2001, 2003; Ding et al 2006; Hagenimana et al 2006; Ryu and Ng 2001; Singh and Singh 2004). These variables can be optimized during the extrusion process to improve the quality properties of final extruded products, including physical [expansion ratio, color, bulk density, water absorption index (WAI), and water solubility index (WSI)], pasting, and thermal characteristics (Anderson and Ng 2001; Ding et al 2005; Ding et al 2006; Guha et al 1998; Kaletunc and Breslauer 1993; McPherson et al 2000; Ozcan and Jackson 2005; Ryu and Ng 2001).

It has been reported that texture is one of the most important factors in the quality properties of extruded snack products, and that texture is associated with an extrudate's expansion ratio (Ali et al 1996). Ryu and Ng (2001) observed that the expansion ratio of wheat flour (WF) extrudates or cornmeal extrudates significantly increased with decreasing feed moisture, and Hagenimana et al (2006) reported that rice flour extrudates extruded at higher feed moisture levels are harder after cooling than those with lower feed moistures.

Pasting properties of extrudates have been studied using a Rapid Visco Analyzer (RVA). Ozcan and Jackson (2005) reported that extruded corn starch shows lower RVA viscosity profiles than raw corn starch because of starch degradation during extrusion cooking. Bhattacharya et al (1999) evaluated the pasting properties of a potato and WF blend extruded in a twin-screw extruder at different moisture contents (16-21%) and screw speeds (200-400 rpm). They noted that the degree of starch

gelatinization that occurred during extrusion cooking affected the viscosity of the extrudates.

Differential scanning calorimetry (DSC) has been applied to investigate the thermal properties of extruded corn starch (Blanche and Sun 2004; McPherson et al 2000; Ozcan and Jackson 2005), corn flour (Kaletunc and Breslauer 1993), and WF (Anderson and Ng 2001). Anderson and Ng (2001) investigated the transition peak temperature (Tp), and transition enthalpy (ΔH) of non-extruded and extruded WF samples and they reported that the value of Tp is particularly associated with the heat stability of the non-extruded and extruded samples.

Ginseng is one of the most widely used herbal medicines in the world. It has been known that ginseng exhibits various pharmaceutical effects, including antioxidation, antistress, immunostimulation, and anticancer effects (Bae et al 2002; Kang et al 2006; Shibata 2001; Yun et al 2001).

In recent years, many consumers have become interested in nutraceutical foods. Ginseng can be a health supplement for consumers. Ginseng is usually consumed in the forms of raw plant materials, dried plant materials, extracts, and commercial products such as capsules, tablets, drinks, and jellies in the world. The addition of ginseng to common cereal flours (wheat, corn, and rice) for producing nutraceutical extruded products (snacks or instant powder) could be of interest to consumers. The extrusion process is fundamentally extremely complicated due to its high degree of process variability, thus the product quality of any final extrudates can be considerably influenced by even small changes in extrusion processing conditions. However, no studies have been found evaluating the effects of extrusion process variables on the product quality of final extruded nutraceutical WF products containing

ginseng root powder. Therefore, the present study was designed to investigate the quality properties [physical (expansion ratio, WAI, and WSI), pasting, and thermal characteristics] of wheat-ginseng extrudates (WGE) produced with a twin-screw extruder at different extrusion process conditions.

5.3. MATERIALS AND METHODS

5.3.1. Materials

Soft WF was obtained from the Mennel Milling Co. in Fostoria, OH, USA. The raw ginseng root (*Panax ginseng* C. A. Meyer), harvested after four years growth and milled into powder (particle size of ≤ 167 μm), was cultivated in Geumsan, Korea, and purchased at a ginseng market. WF and GP were analyzed for their moisture contents using AACCI Approved Method 44-15A (AACCI, 2000), and the moisture contents of WF and GP were 11.2% (wb) and 8.3% (wb), respectively.

5.3.2. Extrusion Cooking

Extrusion cooking was described in Chapter 4 of this dissertation.

5.3.3. Specific Mechanical Energy

Specific mechanical energy (SME) was described in Chapter 4 of this dissertation.

5.3.4. Expansion Ratio

After drying WGE samples extruded under different conditions in an air oven (45°C) overnight, a digital caliper (Model CD-6"CS, Mitutoyo Corp., Japan) was used to measure their diameters. To calculate the expansion ratio, the average measurement of 10 WGE diameters was divided by the die diameter of 3 mm.

5.3.5. Water Absorption Index (WAI) and Water Solubility Index (WSI)

WAI and WSI of WGE samples extruded under different conditions were measured according to the procedure of Jin et al (1995) with some modifications. Two g of ground WGE samples were combined with 20 mL of distilled water in 30 mL round bottom centrifuge tubes. The tubes were allowed to sit for 10 min and were inverted 3 times each at 5 and 10 min. After 10 min, the suspensions were centrifuged (Model J2-21M, Beckman Instruments Inc., Fullerton, CA, USA) at 1000 x g for 25 min. The supernatant was collected and WAI was calculated as:

WAI = weight of pellet/sample weight (db)

WSI was measured by drying the supernatant in an air oven overnight at 60°C. WSI was calculated as:

WSI = weight of dried supernatant/sample weight (db)

5.3.6. Pasting Properties

Pasting properties (values of peak, trough, breakdown, final viscosity, and setback) of a non-extruded WF-ginseng powder (GP) blend (3.15 g WF + 0.35 g GP) and ground WGE samples extruded under different conditions were analyzed. Pasting properties were determined using a RVA (Model 4, Newport Scientific Inc., Warriewood, Australia). For each analysis, 3.5 g of a sample (14% wb) were mixed with 25 mL of distilled water. The profile for analysis was Standard Method 1 according to AACCI Approved Method 76-21 (AACCI, 2000) and data from the RVA were processed by Thermocline version 1.2 software (Newport Scientific Inc., Warriewood, Australia).

5.3.7. Thermal Properties

Thermal properties of a non-extruded WF-GP blend (9 mg WF + 1 mg GP) and ground WGE samples extruded under different conditions were analyzed using a DSC (Model TA 2910, TA Instruments, Newcastle, DE, USA). Ten mg of a sample were weighed into a DSC aluminum pan (Model 0319-1525, PerkinElmer Inc., Shelton, CT, USA) and 20 μL of distilled water were added using a micro-syringe. The sample pan was hermetically sealed and allowed to equilibrate overnight at room temperature. Samples were heated from 30 to 200°C at the heating rate of 10°C/min. A sealed empty pan was used as a reference. Tp and ΔH were recorded and analyzed using TA Universal Analysis Software (version 3.6, TA Instruments, Newcastle, DE, USA).

5.3.8. Statistical Analysis

All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level. In addition, Pearson correlation coefficients were determined among the samples. The CORR procedure was used to obtain correlation coefficients.

5.4. RESULTS AND DISCUSSION

5.4.1. Expansion Ratio

The effects of extrusion process variables (feed moisture, screw speed, barrel temperature) on the expansion ratio of WGE samples are reported in Table 5.1. Increasing feed moisture from 25 to 35%, at constant screw speed and zone 5 barrel temperature (200 rpm and 140°C, respectively), caused a significant decrease in the expansion ratios of WGE samples from 2.81 to 2.13. The trend of decreasing expansion ratio of extrudates with increasing feed moisture has also been noted by Ding et al (2005), who observed that increasing feed moisture in the extrusion cooking of rice flour induces a significant decrease in the expansion ratio of the rice-based extrudates. Ryu and Ng (2001) noted that higher feed moisture for the extrusion cooking of WF or whole cornmeal decreases the expansion ratios of those extrudates. They reported that not only bubble growth but also bubble shrinkage during extrusion cooking can be influenced by the viscosity of the melt in a barrel (Ryu and Ng 2001). More specifically, the lower viscosity of a melt at higher feed moisture extrusion process conditions can cause an increase in the shrinkage and collapse of bubbles at the exit die, resulting in a reduction in the expansion ratio of the extrudates. In work conducted by Ding et al (2006), it was shown that feed moisture was found to be the main factor affecting the expansion ratio of WF extrudates. They reported that the expansion ratio of extrudates is attributed to the elasticity properties of the melt. They also reported that the elasticity of the melt at higher feed moisture extrusion conditions decreases due to plasticization of the melt, thus the SME is reduced and starch gelatinization is also considerably reduced, subsequently causing a decrease in the expansion ratio of the extrudates.

An increase in screw speed from 200 to 300 rpm, at constant feed moisture and zone 5 barrel temperature (25% and 140°C, respectively), led to the significant reduction in the expansion ratios of WGE samples from 2.81 to 1.99. A change in screw speed can affect residence time, the network of starch and proteins, and the melt viscosity, thereby affecting the expansion ratio of extrudates (Colonna et al 1998). Blanche and Sun (2004) reported that melt viscosity decreased with increasing screw speed from 300 to 400 rpm during extrusion cooking of corn starch, causing a decrease in pressure, subsequently lessening expansion ratio of the extrudates. In addition, according to Anderson and Ng (2003), the trend of decreasing expansion ratio at higher screw speed extrusion conditions is a result of breakdown of the feed materials into smaller molecules, which may prevent bubble formation.

The expansion ratios of WGE samples significantly increased from 2.23 to 2.81 with increasing zone 5 barrel temperature from 110 to 140°C, at constant feed moisture and screw speed (25% and 200 rpm, respectively). This finding was consistent with Ding et al (2005), who reported that higher barrel temperatures led to increased expansion ratios of rice flour extrudates. In the present study, the increasingly higher barrel temperature extrusion cooking could have caused increasingly greater flashing-off of moisture upon die exit, inducing an increase in the expansion ratio of WGE samples. This occurrence is mainly due to a great difference between the exit die temperature and room temperature, causing an instant expansion of the extrudates upon exiting the die due to the very high water vaporization rate.

5.4.2. Water Absorption Index (WAI) and Water Solubility Index (WSI)

The effects of extrusion process variables on WAI and WSI of WGE samples are listed in Table 5.1. WAI values of WGE samples produced under different extrusion process conditions ranged from 6.53 to 8.73 g/g, and WSI values of WGE samples ranged from 0.06 to 0.26 g/g.

The WAI of extrudates measures the amount of water held by the starch after dispersion of starch in excess water and may be related to the degree of starch damage due to gelatinization and fragmentation of starch during high temperature and shear extrusion cooking (Anderson and Ng 2003). The WSI measures the amount of soluble components released from starch upon extrusion and is considered an indicator of degradation of molecular components (Kirby et al 1988).

In the present study, increasing feed moisture from 25 to 35%, at constant screw speed and zone 5 barrel temperature (200 rpm and 140°C, respectively), significantly increased WAI values of WGE samples from 6.70 to 7.68 g/g, whereas it significantly decreased WSI values of WGE samples from 0.12 to 0.07 g/g. Higher WAI values and lower WSI values of WGE samples with increasing feed moisture may be associated with a decrease in the degradation of starch granules during the higher feed moisture extrusion cooking. During extrusion cooking at higher feed moisture levels, more water acts as a plasticizer, decreasing the degree of degradation of starch granules, leading to an increase in WAI values of WGE samples and a decrease in WSI values of WGE samples (Hagenimana et al 2006).

Secondly, an increase in screw speed from 200 to 300 rpm, at constant feed moisture and zone 5 barrel temperature (25% and 110°C, respectively), resulted in a significant decrease in WAI values of WGE samples from 8.04 to 7.28 g/g, but caused a significant increase in WSI values of WGE samples from 0.09 to 0.13 g/g. Anderson

and Ng (2003) noted that WAI values of WF extrudates are significantly decreased with increasing screw speed, because increasing screw speed leads to more damaged polymer chains, therefore decreasing the ability of starch molecules to bind more water molecules. This is also in agreement with Jin et al (1995) who reported that higher screw speeds result in lower WAI values of corn meal extrudates using a twin screw extruder.

Finally, WAI values of WGE samples were significantly reduced from 8.04 to 6.70 g/g, and WSI values of WGE samples were significantly increased from 0.09 to 0.12 g/g when zone 5 barrel temperature was increased from 110 to 140°C, at a constant feed moisture and screw speed (25% and 200 rpm, respectively). Ding et al (2006) also observed the same result in the extrusion cooking of WF extrudates. They reported that lower WAI values and higher WSI values of the extrudates are the result of higher degree of starch gelatinization occurring during higher temperature extrusion cooking. It is suggested in the present study that extrusion cooking under higher zone 5 barrel temperature conditions could increase the degree of wheat starch gelatinization, which causes lower WAI values and higher WSI values of WGE samples. Furthermore, it is plausible that at higher barrel temperatures, the combination of thermal and mechanical energies could have fully cooked the starch, subsequently increasing the degree of starch gelatinization and/or degradation.

5.4.3. Pasting Properties

RVA pasting curves of a non-extruded blend of WF-GP and a ground WGE sample, extruded at 30% feed moisture, 200 rpm screw speed, and 110°C zone 5 barrel temperature, are illustrated in Figure 5.1. During the initial 4 min, RVA viscosity of the WGE sample was higher than that of WF-GP, indicating that the gelatinized starch

can hydrate faster than unmodified starch in the non-extruded blend. However, RVA viscosity of WGE samples remained low throughout the heating cycle (50-95°C), in contrast to that of WF-GP which had substantial increases. The result can be related to the degradation and/or gelatinization of starch granules occurring during extrusion cooking. This reduced the swelling capacity of amylose and amylopectin in extruded starches.

RVA peak viscosity values of ground WGE samples produced under different extrusion process conditions ranged from 36.3 to 206.1 RVU (Table 5.2). Increasing feed moisture from 25 to 35%, at a constant screw speed and zone 5 barrel temperature (200 rpm and 120°C, respectively), significantly increased the peak viscosity values of WGE samples from 113.9 to 176.5 RVU. However, peak viscosity values of WGE samples were significantly decreased from 78.2 to 36.3 RVU with increasing screw speed from 200 to 300 rpm, at a constant feed moisture and zone 5 barrel temperature (25% and 140°C, respectively). Increasing zone 5 barrel temperature from 110 to 140°C, at constant feed moisture and screw speed (25% and 200 rpm, respectively), significantly decreased peak viscosity values of WGE samples from 118.9 to 78.2 RVU. The effects of extrusion process variables on peak viscosity values of WGE samples were generally consistent with other previous published results (Blanche and Sun 2004; Guha et al 1998).

In the present study, it is speculated that a decrease in the peak viscosity values of WGE samples resulted from an increase in severity of extrusion process conditions. It is possible that extrusion cooking at the higher screw speed led to more starch degradation, subsequently producing WGE samples with much lower pasting peak viscosity values. With increasing barrel temperature, the starches were likely

gelatinized to a greater extent, resulting in reduced peak viscosity values of the ground WGE samples. Blanche and Sun (2004) observed that higher feed moisture and lower screw speed each cause an increase in the peak viscosity values of corn starch extrudates. They reported that the increased peak viscosity of the corn starch extrudates is due to the presence of starch granules remaining ungelatinized upon extrusion cooking. According to Guha et al (1998), on the other hand, who showed that the peak viscosity values of rice flour extrudates decrease with increasing barrel temperature and screw speed, the degree of breakdown of a starch granule is dependent on the type of starch, mechanical shear, temperature and chemical agents present during the gelatinization of starch.

5.4.4. Thermal Properties

DSC thermograms of a non-extruded blend of WF-GP and a WGE sample, extruded under 30% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature, are shown in Figure 5.2. Tp and ΔH values of WGE samples extruded under different conditions are listed in Table 5.2. A gelatinization peak was observed on the DSC thermogram obtained for WF-GP, and Tp and ΔH values of WF-GP were 69.4°C and 3.36 J/g, respectively. However, WGE samples did not exhibit a gelatinization peak on the DSC thermogram. Tp and ΔH values of WGE samples extruded under different conditions were observed in the range from 153.7 to 172.3°C and in the range from 1.75 to 6.60 J/g, respectively.

Ozcan and Jackson (2005) suggested that a gelatinization peak was not found on the DSC thermogram obtained for corn starch extrudates, since the starch granules were already melted and gelatinized during extrusion cooking. Blanche and Sun

(2004) noted that high shear and pressure extrusion cooking can produce damaged and depolymerized starch molecules, so that no gelatinization peak is found on the DSC thermograms of these corn starch extrudates. Therefore, it seems consistent in the present study that no gelatinization peak was found on the DSC thermograms for the ground WGE samples due to the gelatinization and/or breakdown of starch granules during extrusion cooking.

In the present study, increasing feed moisture from 25 to 35%, at constant screw speed and zone 5 barrel temperature (300 rpm and 140°C, respectively), resulted in a significant increase in Tp values of WGE samples from 153.7 to 159.7°C. On the other hand, Tp values of WGE samples were significantly decreased from 161.5 to 153.7°C with increasing screw speed from 200 to 300 rpm, at constant feed moisture and zone 5 barrel temperature (25% and 140°C, respectively). Increasing zone 5 barrel temperature from 110 to 140°C, at constant feed moisture and screw speed (30% and 200 rpm, respectively), significantly reduced Tp values of WGE samples from 170.3 to 162.6°C. Anderson and Ng (2001) evaluated the effects of twin-screw extrusion processing on the thermal properties of WF extrudates. They observed that increasing screw speed from 240 to 320 rpm and die temperature from 120 to 160°C each decreased Tp values of WF extrudates, noting that extrusion cooking under lower screw speed or lower die temperature improved heat stability of the extrudates. In general, heat stability of an extrudate describes the resistance to breakdown of molecular aggregate structures formed during extrusion cooking by gelatinized starch and/or denatured proteins. It was hypothesized in the present study that higher Tp values of WGE samples would be associated with a higher melting temperature of WGE samples, subsequently increasing the heat stability of WGE samples. Based on the results

obtained in the present study, it is suggested that lower feed moisture, higher screw speed, and higher barrel temperature extrusion conditions each could diminish the heat stability of WGE samples.

5.4.5. Correlations among Extrudate Quality Properties and System Variables

Pearson's coefficients of correlation (r) among quality properties and system variables (SME and product temperature) are reported in Table 5.3. Product temperature represents the actual temperature of the dough at the exit die during extrusion cooking. For the WGE samples studied, product temperature of WGE samples was found to be negatively associated ($r = -0.71$, $p \leq 0.001$) with WAI values and positively associated ($r = -0.45$, $p \leq 0.05$) with WSI values. SME values were negatively related ($r = -0.45$, $p \leq 0.05$) to WAI values and negatively related ($r = -0.79$, $p \leq 0.001$) to peak viscosity values of WGE samples. In contrast, SME values were positively related ($r = 0.69$, $p \leq 0.01$) to WSI values of WGE samples. Tanhehco and Ng (2005) also found a negative relationship between SME values and WAI values of ground WF extrudates as well as a positive correlation between SME values and WSI values of ground WF extrudates. They attributed their findings to the fact that SME values reflect degree of starch fragmentation.

As expected, WAI values of WGE samples were negatively correlated ($r = -0.76$, $p \leq 0.001$) with the respective WSI values. WAI values were positively correlated ($r = 0.88$, $p \leq 0.001$) with peak viscosity values, whereas WSI values were negatively correlated ($r = -0.82$, $p \leq 0.001$) with peak viscosity values for the WGE samples in the present study. The positive relationship between WAI and peak viscosity of the extrudates can be probably explained by the fact that both WAI and peak

viscosity values describe the degree of starch degradation and/or gelatinization occurring during extrusion cooking (Anderson and Ng 2003; Guha et al 1998). For example, increasing barrel temperature increased the starch gelatinization during extrusion cooking, subsequently leading to a reduction in both WAI and peak viscosity values of WGE samples (Ding et al 2006; Guha et al 1998).

Tp values of WGE samples were positively correlated ($r = 0.88$, $p \leq 0.001$) with their respective WAI values as well as positively correlated ($r = 0.86$, $p \leq 0.001$) with peak viscosity values. Based on these results, it is suggested that heat stability of WGE samples could be increased if the degree of starch gelatinization and/or degradation were decreased during extrusion cooking.

5.5. SUMMARY

Effects of extrusion process variables (feed moisture, screw speed, and barrel temperature) on the quality properties (physical, pasting, and thermal) of WGE samples produced under different extrusion conditions were investigated. During extrusion cooking, the melt viscosity, bubble growth or shrinkage, and the degree of gelatinization and/or degradation could influence the final quality of WGE samples. The data on quality properties obtained in the present study also suggested that the expansion ratio, WAI, WSI, peak viscosity, and Tp of WGE samples were affected by each of the extrusion process variables tested in the present study. Data obtained from the present extrusion study may be helpful in predicting the expected performance of extruded materials in investigations into the potential use of WF mixed with ginseng, or other nutraceutical materials, for the improvement of nutritional quality of final extruded products.

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Table 5.1.

Effects of Extrusion Process Variables on the Expansion Ratio (ER), Water Absorption Index (WAI), and Water Solubility Index (WSI) of Wheat-Ginseng Extrudates

Extrusion Process Variables			ER	WAI (g/g)	WSI (g/g)
Feed Moisture (%, wb)	Screw Speed (rpm)	Temperature[a] (°C)			
25	200	110	2.23e	8.04cd	0.09ijk
30	200	110	2.11f	8.47b	0.08kl
35	200	110	1.99g	8.73a	0.06m
25	300	110	1.92ij	7.28gh	0.13e
30	300	110	1.89j	7.67f	0.12g
35	300	110	1.85k	7.86de	0.09ijk
25	200	120	2.45c	7.31gh	0.11gh
30	200	120	2.25e	7.92d	0.09ijk
35	200	120	1.97gh	8.21c	0.08kl
25	300	120	1.99g	6.91i	0.19c
30	300	120	1.99g	7.17h	0.13ef
35	300	120	1.94hi	7.38g	0.10hi
25	200	130	2.51b	6.70jk	0.12g
30	200	130	2.24e	7.31gh	0.09ij
35	200	130	1.98g	7.92d	0.07lm
25	300	130	1.98g	6.53k	0.23b
30	300	130	1.97gh	7.22gh	0.18d
35	300	130	1.97gh	7.21gh	0.11g
25	200	140	2.81a	6.70jk	0.12fg
30	200	140	2.30d	7.28gh	0.09jkl
35	200	140	2.13f	7.68ef	0.07lm
25	300	140	1.99g	6.56k	0.26a
30	300	140	1.99g	6.81ij	0.19cd
35	300	140	1.96gh	7.14h	0.13ef

Values with different letters within the same column differ significantly ($P<0.05$).
[a]Temperature: zone 5 (nearest exit die) barrel temperature.

Table 5.2.

Effects of Extrusion Process Variables on Peak Viscosity (PV), Transition Peak Temperature (Tp), and Transition Enthalpy (ΔH) of Wheat-Ginseng Extrudates

Extrusion Process Variables			PV (RVU[b])	Tp (°C)	ΔH (J/g)
Feed Moisture (%, wb)	Screw Speed (rpm)	Temperature[a] (°C)			
25	200	110	118.9ef	167.8cd	2.65ijk
30	200	110	138.0d	170.3b	3.70fgh
35	200	110	206.1a	172.3a	1.75jk
25	300	110	56.3kl	158kl	6.00ab
30	300	110	62.8k	163.4ef	5.04cde
35	300	110	111.8fgh	164.0e	2.72ij
25	200	120	113.9efg	166.1d	3.94fgh
30	200	120	121.3e	167.9cd	4.56def
35	200	120	176.5b	168.7bc	3.43ghij
25	300	120	44.7mn	160.5hij	3.98fgh
30	300	120	55.6kl	161.8fgh	5.89abc
35	300	120	97.1i	162.3efg	3.90fgh
25	200	130	112.5fg	162.4efg	3.76fgh
30	200	130	110.1gh	162.9efg	3.78fgh
35	200	130	159.4c	163.9e	3.81fgh
25	300	130	38.3no	156.2m	2.57jk
30	300	130	55.9kl	159.9j	5.39bcd
35	300	130	83.7j	160.2ij	3.53ghi
25	200	140	78.2j	161.5ghi	5.41bcd
30	200	140	103.9hi	162.6efg	3.49ghij
35	200	140	138.4d	163.6ef	4.37efg
25	300	140	36.3o	153.7n	3.42hij
30	300	140	49.0lm	156.2lm	6.60a
35	300	140	62.8k	159.7jk	4.55def

Values with different letters within the same column differ significantly ($P<0.05$).
[a]Temperature: zone 5 (nearest exit die) barrel temperature.
[b]RVU: Rapid Visco Units.

Table 5.3.

Pearson's Coefficients of Correlation (r) among Quality Properties [Expansion Ratio (ER), Water Absorption Index (WAI), Water Solubility Index (WSI), Peak Viscosity (PV), Transition Peak Temperature (Tp), and Transition Enthalpy (ΔH)] of Wheat-Ginseng Extrudates and System Variables [Specific Mechanical Energy (SME) and Product Temperature (PT)]

	ER	WAI	WSI	PV	Tp	ΔH	SME	PT
ER		- 0.22	- 0.18	0.13	0.17	0.05	- 0.63***	0.27
WAI			- 0.76***	0.82***	0.88***	- 0.38	- 0.45*	- 0.71***
WSI				- 0.82***	- 0.81***	0.23	0.69**	0.47*
PV					0.86***	- 0.52**	- 0.79***	- 0.39
Tp						- 0.42*	- 0.67**	- 0.67**
ΔH							0.31	0.24
SME								- 0.02
PT								

*, **, ***, Significantly different at the 5%, 1%, and 0.1% levels, respectively.

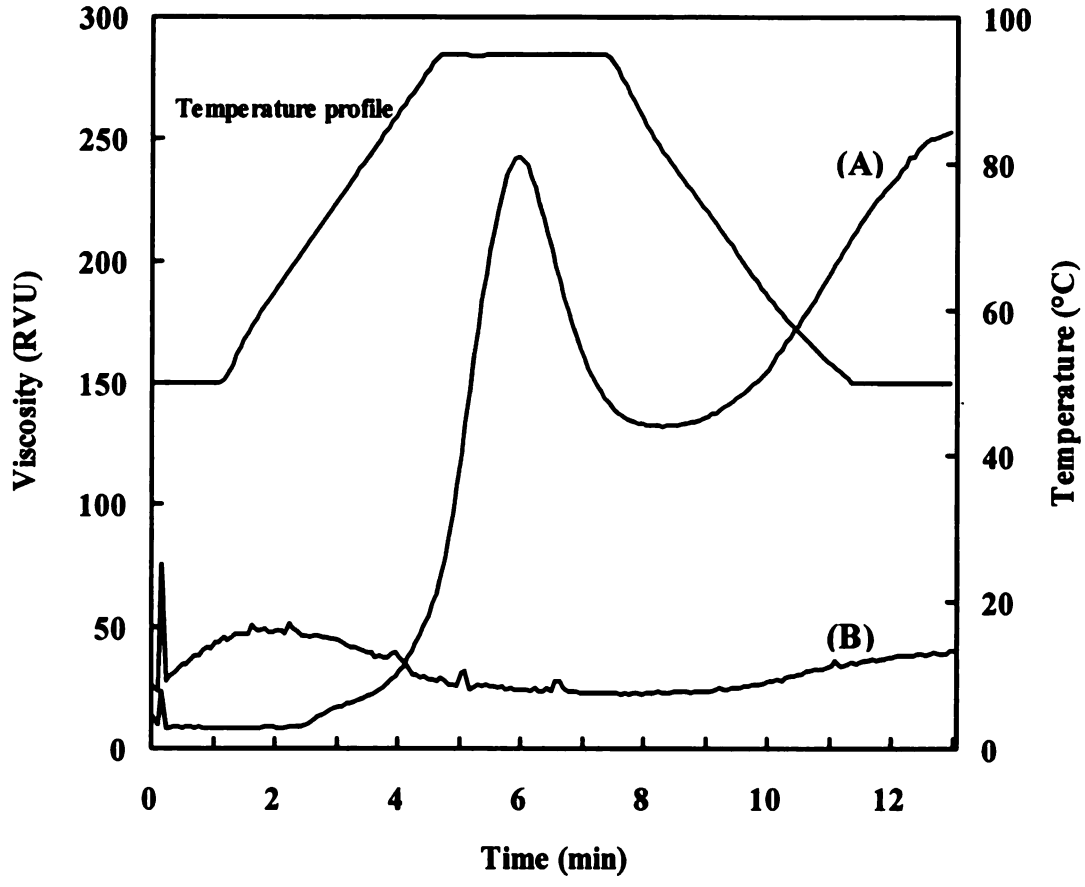

Fig. 5.1. Rapid Visco Analyzer pasting curves of a non-extruded wheat flour (WF)-ginseng powder (GP) blend (10% GP, w/w), and a wheat-ginseng extrudate (WGE) extruded at 30% feed moisture, 200 rpm screw speed, and 110°C zone 5 barrel temperature. Viscosity reported in Rapid Visco Units (RVU).

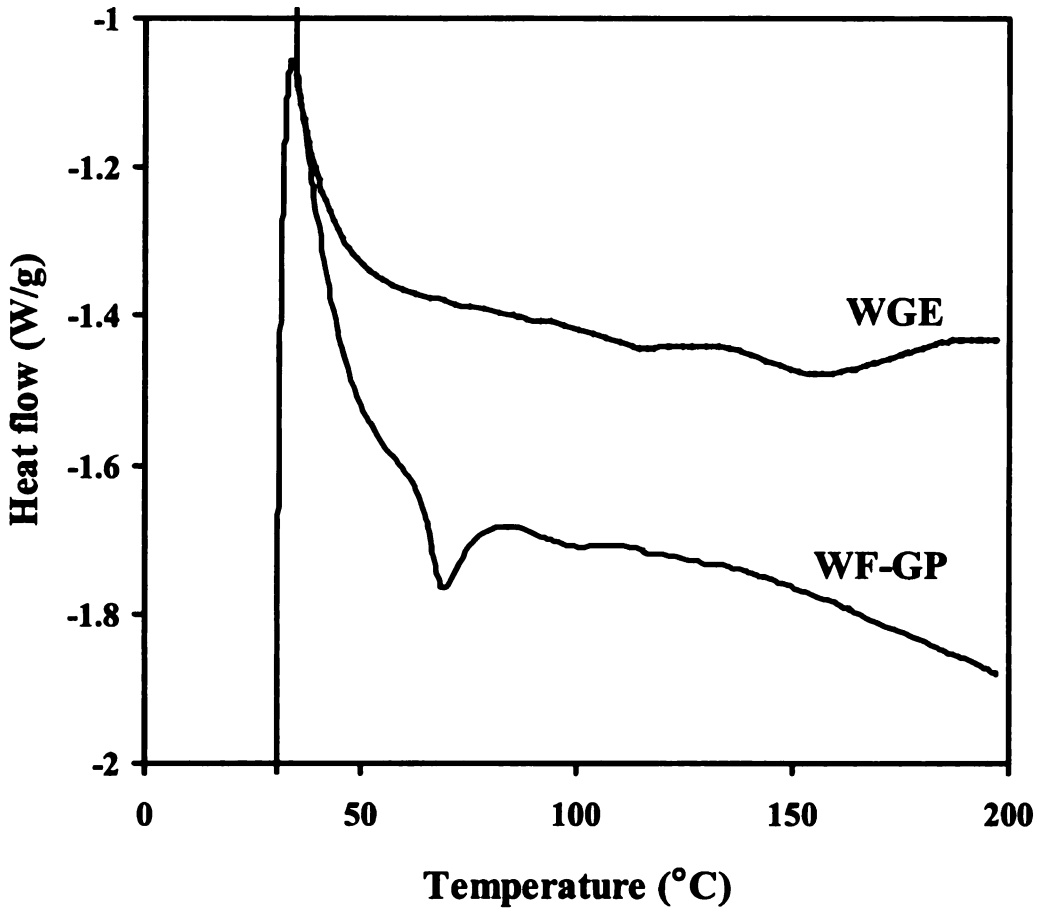

Fig. 5.2. Differential scanning thermograms of a wheat-ginseng extrudate (WGE) extruded at 30% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature, and a non-extruded wheat flour (WF) and ginseng powder (GP) blend (10% GP, w/w).

CHAPTER 6

Physicochemical Properties of Bread and Cookies Baked from Wheat Flour Blended with Ginseng Powder

6.1. ABSTRACT

This study investigated (1) the effects of adding ginseng powder (GP) on Farinogrgh, thermal, and pasting properties of hard wheat flour (HWF) and soft wheat flour (SWF), (2) the quality properties of hard wheat-ginseng bread (10% GP, w/w) and soft wheat-ginseng cookies (10% GP, w/w), and (3) the changes in ginsenosides (G, ginseng saponins) in hard wheat-ginseng bread and soft wheat-ginseng cookies upon baking. Farinograph data showed that water absorption values of HWF-GP and SWF-GP were significantly higher than those of the respective HWF and SWF. The dough made from HWF-GP was weaker and less stable than that made from HWF alone. Differential scanning calorimetry data showed that HWF-GP and SWF-GP have higher transition onset temperature values than do their respective HWF and SWF, whereas HWF and SWF show larger transition enthalpy values for gelatinization than do their respective blends. Rapid Visco Analyzer data showed that the addition of GP to either HWF or SWF significantly decreased the peak viscosity of the samples. The bread loaf weight increased and the bread loaf volume decreased when GP was added to HWF bread formulations. The width of the cookie sample baked from SWF-GP was significantly smaller than that of the cookie sample baked from SWF. Individual G (Rb1, Rc, Rd, Rg2, and Rg3) were fractionated and identified by RP-HPLC from GP, ground bread crust and ground bread crumb of samples baked from HWF and HWF-GP, and ground cookie samples baked from SWF and SWF-GP. The quantities of G (Rb1, Rc, and Rd) extracted from the bread crust and bread crumb samples baked from HWF-GP, and the cookie samples baked from SWF-GP were significantly higher than those of the G extracted from pure GP, weight per original weight GP, indicating the conversion of malonyl G (Rb1, Rc, and Rd) into G (Rb1, Rc, and Rd, respectively) during bread-

and cookie-baking. G Rg2 were found in the bread crust and bread crumb of samples baked from HWF-GP, whereas G Rg3 were present only in the bread crust samples. Ginsenosides Rg2 and Rg3 were not present in cookie samples baked from SWF-GP.

6.2. INTRODUCTION

Common wheat is divided into soft wheat and hard wheat varieties, depending on its kernel texture (Hoseney 1998). In particular, soft wheat has lower protein content and weaker protein strength as compared to hard wheat (Atwell 2001; Posner 2000). Hard wheat flour (HWF) is used mainly for the production of bread products, while soft wheat flour (SWF) is usually used to produce chemically-leavened products, such as cookies, cakes, pastries, crackers, pie dough, and pretzels.

Bread, a critical staple food, is consumed in some form or another all over the world. In general, the major ingredient for making bread is HWF, with other ingredients being yeast, salt, and water. Over the years, many different food ingredients, such as barley and soy flours (Basman et al 2003), fiber (Dalgetty and Baik 2006; Filipovic et al 2007; Mohamed et al 2008; Park et al 1997; Pomeranz et al 1977), folic acid (Osseyi et al 2001), immature wheat meal (Mujoo and Ng 2003), polysaccharides (Fan et al 2006), vitamin E (Ranhotra et al 2000), and whole waxy wheat flour (Hung et al 2007), have been included in bread formulations to improve the nutritional value.

Cookies are one of the most popular bakery products because of their convenience, ready-to-eat nature, and long shelf-life (Singh and Mohamed 2007). Recently, the addition of fiber to cookie formulations has been studied to make nutraceutical cookie products (Bilgicli et al 2007; Larrea et al 2005; Uysal et al 2007).

Ginseng has long been used in Asian countries as a traditional medicine (Fuzzati 2004). Ginsenosides (G, ginseng saponins) are the main active components of ginseng, one of the most popular herbal medicines worldwide (Ren and Chen 1999). Over 30 G have been identified; among these, Rb1, Rb2, Rc, Rd, and Re are the most

abundant (Corbit et al 2005; Kim et al 2005). On the other hand, G compound Rg3 is only present in ginseng after special treatment of the ginseng root, which yields so-called red ginseng (Bae et al 2002; Kim et al 2000; Popovich and Kitts 2004; Shibata 2001; Wang et al 2006). In terms of health benefits, this red ginseng is considered vastly superior to untreated ginseng, or so-called white ginseng (Ha et al 2005; Ha et al 2007; Kim et al 2000; Kwon et al 2001; Lau et al 2004; Popovich and Kitts 2004; Wang et al 2006).

So-called healthy foods, especially those with nutraceutical properties, are in great demand in our health conscientious society. Bread and cookies are good candidates in this respect, for a portion of their wheat flour to be replaced with nutraceutical ingredients, such as ginseng. However, no studies have reported evaluating the product quality of and ginsenosides content in baked HWF bread and SWF cookies that incorporate ginseng root powder (GP). Thus, the objectives of this study were to investigate (1) the effects of adding GP on the Farinograph, thermal, and pasting properties of HWF and SWF, (2) the quality properties of hard wheat-ginseng bread and soft wheat-ginseng cookies, and (3) the changes in G in hard wheat-ginseng bread and soft wheat-ginseng cookies upon baking, relative to their respective unbaked doughs.

6.3. MATERIALS AND METHODS

6.3.1. Materials

HWF and SWF were obtained from the Mennel Milling Co. in Fostoria, OH, USA. The raw ginseng root (*Panax ginseng* C. A. Meyer), harvested after four years growth and milled into powder (particle size of ≤ 167 μm), was cultivated in Geumsan, Korea, and purchased at a ginseng market. Standards for ginsenosides Rb1, Rc, Rd, Rg2, and Rg3 were provided by ILHWA Co. (Kuri, Korea).

6.3.2. Chemical Analysis

HWF, SWF, and GP samples were analyzed for their moisture and protein contents using AACCI Approved Methods 44-15A and 46-13 (AACCI, 2000), respectively (Table 6.1). Protein contents were calculated by multiplying the nitrogen content by the conversion factor 5.7, and reported on a 14% moisture basis.

6.3.3. Farinograph Properties

Farinograph properties of HWF (50 g), SWF (50 g), a blend of HWF-GP (45 g HWF + 5 g GP), and a blend of SWF-GP (45 g SWF + 5 g GP) were determined using AACCI Approved Method 54-21 (AACCI 2000). Optimal water absorption, dough development time, stability, and mixing tolerance index were determined.

6.3.4. Thermal Properties

A differential scanning calorimeter (DSC Model TA 2910, TA Instruments, Newcastle, DE, USA) was used to measure the thermal properties of HWF (10 mg), SWF (10 mg), GP (10 mg), HWF-GP (9 mg HWF + 1 mg GP), and SWF-GP (9 mg

SWF + 1 mg GP). Each sample was weighed into a DSC aluminum pan (Model 0319-1525, PerkinElmer Inc., Shelton, CT, USA), and 20 μL of distilled water were added using a micro-syringe. The sample pan was hermetically sealed and allowed to equilibrate overnight at room temperature. Samples were heated from 30 to 200°C at the heating rate of 10°C/min. A sealed empty pan was used as a reference. Transition onset temperature (To), transition peak temperature (Tp), and transition enthalpy (ΔH) were recorded and analyzed using the TA Universal Analysis Software (version 3.6, TA Instruments, Newcastle, DE, USA).

6.3.5. Pasting Properties

The pasting properties of HWF (3.5 g), SWF (3.5 g), GP (3.5 g), HWF-GP (3.15 g HWF + 0.35 g GP), and SWF-GP (3.15 g SWF + 0.35 g GP) were analyzed using a Rapid Visco Analyzer (RVA Model 4, Newport Scientific Inc., Warriewood, Australia). The pasting properties included the following parameters: peak viscosity – highest viscosity during heating; trough viscosity – lowest viscosity following peak viscosity; breakdown viscosity – peak viscosity minus trough; final viscosity – the viscosity at the completion of the heating cycle; setback – final viscosity minus peak viscosity. Each sample (14%, wb) was mixed with 25 mL of distilled water. The profile of the analysis was Standard Method 1 according to AACCI Approved Method 76-21 (AACCI, 2000) and data obtained from the RVA were processed by Thermocline version 1.2 software (Newport Scientific Inc., Warriewood, Australia).

6.3.6. Breadmaking

Breads were prepared from HWF (100 g) and from HWF-GP (90 g HWF + 10 g

GP) according to the optimized straight-dough breadmaking method (AACCI Approved Method 10-10B; AACCI 2000) with some modifications. The formulation of bread dough also included sugar (6.0 g), salt (1.5 g), shortening (3.0 g), yeast (5.3 g, Safmex, Milwaukee, WI, USA), ascorbic acid (5.0 mg), and water based on optimum water absorption from the Farinograph. Breads were baked at 215°C for 24 min in a rotary oven (National MFG Co., Lincoln, NE, USA), removed from their pans, and then cooled at room temperature for 2 hr prior to quality analysis. The bread-baking procedures were performed in duplicate for each sample.

6.3.7. Bread Quality

The cooled loaves were weighed and loaf volumes were determined by the rapeseed displacement procedure in a volume-measuring apparatus. After loaf volumes were measured, loaves were placed into individual ziplock bags, sealed, and stored for 24 hr at room temperature prior to bread firmness analysis. All subsequent bread firmness determinations were performed on the central bread crumb. Two slices (each 12.5 mm thick) of bread were cut from the center of loaves using a cutter (Model FF-3199, Hobart MFG. Co., Troy, OH, USA). Bread firmness was determined by compression using a texture analyzer (Model TA-XT2, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 25 kg load cell. The force (N) required to compress 25% (6.25 mm) of 2 slices was determined with an acryl probe (38 mm diameter) at a compression rate of 1.7 mm/s.

6.3.8. Cookiemaking

Wire-cut cookies were prepared according to AACCI Approved Method 10-54 (AACCI 2000). Cookies were prepared from SWF (40 g) and from SWF-GP (36 g SWF + 4

g GP). In addition, the formulation of cookie dough also included sucrose (12.8 g), granulated brown sugar (4 g), non-fat dry milk (0.4 g), salt (0.5 g), sodium bicarbonate (0.4 g), shortening (16 g), high fructose corn syrup (0.6 g), ammonium bicarbonate (0.2 g), and water (8.7 g). Cookies were baked at 205°C for 11 min in a rotary oven (National MFG Co., Lincoln, NE, USA) and then put on racks to cool to room temperature for 30 min prior to quality analysis. The cookie-baking procedures were performed in duplicate for each sample.

6.3.9. Cookie Quality

Weight, width, and thickness of the cookies were evaluated after the cookies were cooled. Two cookies which were made in the same batch were placed together in a ziplock bag, sealed, and stored for 24 hr at room temperature prior to measuring breaking strength (hardness). Hardness of cookies was determined from the peak of the displacement plot of a cookie during shearing in a texture analyzer (Model TA-XT2, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 25 kg load cell. The cookie was cut into two pieces by a blade (3 mm thickness) at a compression rate of 2.0 mm/s, and the maximum force (N) was recorded.

6.3.10. Sample Preparation for RP-HPLC

Crust portions of bread samples were obtained from the top 1 cm of a bread slice (12.5 mm thickness) which had been cut from the central section of a bread loaf. Central crumb portions (2cm x 2 cm) of breads were cut from the center of the same bread slice (12.5 mm thickness). Samples of bread crust, bread crumb, and cookies were frozen and dried in a freeze dryer for 24 hr. After drying, portions (about 10 g) of each sample were ground to a particle size of ≤ 330 μm (54 mesh screen) using a coffee

grinder (Braun Inc., Woburn, MA, USA). After grinding, moisture contents of ground samples were determined according to AACCI Approved Method 44-15A (AACCI, 2000). Ground samples were placed in ziplock bags, sealed, and stored at -20°C until analysis.

6.3.11. Extraction of Ginsenosides

Four mL of 70% (v/v) aq. methanol were added to GP (0.1 g). Fourteen mL of 70% (v/v) aq. methanol were added to ground bread crust and to ground bread crumb samples (1.11 g, based on 0.1 g GP) baked from HWF and HWF-GP, and to ground cookie samples (1.72 g, based on 0.1 g GP) baked from SWF and SWF-GP. Each mixture was ultrasonically extracted for 150 min using an Ultrasonicator (Model FS 14H, Fisher Scientific, Pittsburgh, PA, USA, Power: 155W) at room temperature and then centrifuged at 11,200 x g for 20 min using a centrifuge (Model J2-21M, Beckman Instruments Inc., Fullerton, CA, USA) at 25°C. The supernatant was evaporated using a Micro Rotary Evaporator (Model 421-4000, Labconco, Kansas City, MO, USA) at 50°C. Prior to RP-HPLC analysis, the residue was dissolved in 2 mL of 70% (v/v) aq. methanol and filtered through a 0.45 μm nylon filter membrane (Millipore, Ireland). Each sample was extracted in duplicate and two chromatographic runs were performed for each extract.

6.3.12. Fractionation and Identification of Ginsenosides by RP-HPLC

RP-HPLC was carried out on a Millenium 2010 HPLC Workstation, consisting of a Waters 600E multi-solvent delivery system (Waters, Milford, MA, USA), a temperature control module, and a 996 photodiode array detector. Separation was

performed at 30°C on a Phenomenex 300 RP, Jupiter C-18 column (4.6 x 250 mm, 5 μm particle diameter; Phenomenex, Torrance, CA, USA). A Phenomenex security guard with the same packing material served as the guard column.

The solvents were A: water (distilled water filtered through Milli-Q-Plus, Millipore), and B: HPLC grade acetonitrile. Separation was achieved using the following gradient: 0-5 min, 20-30% B; 5-25 min, 30-40% B; 25-30 min, 40-20% B. The solvent flow rate was 1 mL/min and the injection volume was 20 μL. The solvents were purged with helium at the rate of 20 mL/min. The column was equilibrated for 10 min with 20% acetonitrile prior to injection. The UV detection wavelength was set at 203 nm (Lau et al 2004), and the detector output was transmitted simultaneously to the computer for data storage and graphic representations.

6.3.13. Statistical Analysis

All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level.

6.4. RESULTS AND DISCUSSION

6.4.1. Farinograph Properties

Farinograph properties of HWF, HWF-GP, SWF, and SWF-GP are shown in Table 6.2. The addition of GP to either HWF or SWF significantly increased water absorption values, indicating that water absorption capacity of GP is higher than that of either HWF or SWF alone. The dough development time and stability value of HWF-GP were significantly lower than those of the HWF, whereas the mixing tolerance index value was significantly higher when GP was added to HWF. In the present study, the findings indicated that the dough made from HWF-GP is weaker and less stable during mixing than that made from HWF alone. The deterioration in each of the Farinograph properties could be related to the lower amounts of gluten proteins in HWF-GP, due to a diluting effect with the addition of GP, as compared to HWF. On the other hand, the dough development time, stability, and mixing tolerance index values of SWF-GP were not significantly different from those of the SWF. Since the SWF already has very weak dough properties, the addition of GP did not significantly further weaken the SWF dough samples, though there was a trend of slight decreases in the measured Farinograph properties.

6.4.2. Thermal Properties

Thermal properties of HWF, SWF, GP, HWF-GP, and SWF-GP are presented in Table 6.3. To and Tp values of both HWF-GP and SWF-GP were significantly higher than those of HWF and SWF, respectively. On the other hand, ΔH values of the blends were significantly lower than those of the respective HWF and SWF samples. Pure GP did not exhibit an endothermic peak.

Nashed et al (2003) observed that values of To and Tp of wheat starch increased with the addition of glycerol, while increasing the water content resulted in decreases in the values of To and Tp. They reported that adding glycerol delays the gelatinization process of wheat starch rather than assisting it. However, water behaves as a plasticizer, leading to a decrease in the values of To and Tp. Adding gluten protein (Mohamed and Rayas-Duarte 2003), soy protein concentrate (Li et al 2007), and sugars (Baek et al 2004) to wheat starch, corn starch, and corn starch, respectively, has been shown to increase values of To and Tp of the starches. In the present study, because GP demonstrated no endothermic peak, it can be assumed that wheat starch plays a major role in the thermal behavior of the blends. The observation (an increase in values of To with the addition of GP to either HWF or SWF) indicated that adding GP to either HWF or SWF can make wheat starch gelatinization more difficult due to the competition for water between wheat starch and GP components. This absorption competition limits the water available for gelatinization of wheat starch, resulting in increased To of the blends. This speculation is confirmed by the Farinograph water absorption data (Table 6.2). On the other hand, a decrease in ΔH with the addition of GP to either HWF or SWF could be due to the proportionate reduction in the amounts of HWF or SWF in the blends. In other words, the quantity of wheat starch (g starch/g sample) in pure HWF or in SWF is greater than in their respective blends, increasing energy requirements to gelatinize these amounts of starch, resulting in increased values of ΔH.

6.4.3. Pasting Properties

Effects of addition of GP on the pasting properties of HWF and SWF were studied via the RVA. The pasting properties (peak, trough, breakdown, final viscosity, and setback) of HWF, SWF, GP, HWF-GP, and SWF-GP are reported in Table 6.4. SWF had higher values for peak, trough, breakdown, final viscosity, and setback than did HWF. This finding was good agreement with Ragaee and Abdel-Aal (2006), who reported that higher RVA viscosity values (peak, trough, breakdown, final viscosity, and setback) of SWF, as compared to HWF, are attributed to the presence of more starch granules within SWF and this results in increased swelling capacity of the sample. All RVA values for HWF and SWF were significantly higher than those of pure GP and of their respective blends, except for the trough viscosity for HWF and its blend, which were similar. Pure GP had very low viscosity values for all of the RVA parameters studied, indicating that pure GP has very low swelling capacity. Therefore, it is suggested in the present study that a decrease in peak viscosity of each blend relative to its parent flour was probably a result of the lower wheat starch content within the blends (see end of section 6.4.2) as well as a low swelling capacity of the GP.

6.4.4. Bread Quality Properties

Quality properties of bread samples baked from HWF and HWF-GP are listed in Table 6.5. A small but significant increase in the loaf weight of the baked bread sample was noted when GP was added to HWF. As was noted earlier, GP had a higher water absorption capacity than did HWF. Thus, it is plausible that HWF-GP could absorb more water than did HWF during bread-baking, subsequently increasing the bread loaf weight.

The addition of GP to the HWF bread formulation led to a significant decrease in the loaf volume of the bread sample. The result in the present study was consistent with the studies on bread baked from HWF and fiber (Dalgetty and Baik 2006; Hung et al 2007; Mujoo and Ng 2003; Pomeranz et al 1977). Pomeranz et al (1977) reported that the addition of different fibers (cellulose, oat hulls, and wheat bran) to the HWF bread formulation decreased the loaf volume of the bread samples mainly due to decreased gas retention. Dalgetty and Baik (2006) reported that the loaf weight of the bread sample increases and loaf volume of the bread sample decreases when hulls and soluble fibers (isolated from peas, lentils, and chickpeas) are added to HWF. Filipovic et al (2007) observed that the loaf volume of a bread sample made from a blend of HWF and sugar-beet fiber is lower than that of a bread sample made from HWF alone. Mujoo and Ng (2003) studied the physicochemical properties of bread baked from HWF blended with immature wheat meal. They reported that the loaf volume of the bread sample baked from a blend of HWF-immature wheat meal is lower than that of the bread sample baked from HWF, and suggested that the reduced loaf volume of the bread sample results from reduced gas retention. In the present study, a decrease in the loaf volume of the bread sample baked from the HWF-GP blend could be explained by lower amounts of wheat gluten in the bread formulation, resulting in a weaker gluten matrix with the reduced ability to retain gases created during fermentation, and hence a decrease in the loaf volume of the bread sample baked from the blend.

No effect on the crumb firmness of the bread sample was observed when GP was added to HWF. It had been expected that the firmness of the bread samples baked from HWF-GP would be increased due to the lower loaf volume that compacts the crumb structure. In the present study, however, lack of change in the firmness of the

bread samples baked from the blend could be associated with not only increased water content in the formula, but also higher moisture retention capacity during baking.

6.4.5. Cookie Quality Properties

Quality properties of cookie samples baked from SWF and SWF-GP are shown in Table 6.6. Weight, thickness, and hardness of the cookie samples baked from the blend were not significantly different from those of the cookie samples baked from SWF. However, a significant decrease in width of the cookie samples was noted when GP was added to SWF.

Uysal et al (2007) studied the effect of incorporation of dietary fiber (from apples, lemons, wheat, and wheat bran) on the spread ratio of wire-cut cookies. They reported that adding fiber to the SWF cookie formulation reduced the spread ratio of cookie samples. Miller et al (1997) reported that cookie spread is dependent on the water absorption properties of the flour sample. They suggested that flours with higher water absorption capacity decrease the cookie spread since this higher absorption decreases the amount of water available to dissolve the sugar in the cookie formulation. According to Yamazaki (1962), flours which have a low water retention capacity are considered good quality flours in terms of cookie production, producing large spread cookies. In the present study, it was confirmed that the higher water absorption capacity of the blend is associated with a decrease in the width of the cookie samples.

6.4.6. Changes in Ginsenosides during Bread-Baking and Cookie-Baking

The quantities of G (Rb1, Rc, Rd, Rg2, and Rg3) ultrasonically extracted for 150 min from GP, bread crusts and bread crumbs from HWF and HWF-GP bread

samples, and cookies baked from SWF and SWF-GP are shown in Table 6.7. The quantities of G Rb1, G Rc, and G Rd extracted from the bread crust and crumb samples baked from the blend and the cookie samples baked from the blend were significantly higher than their respective quantities extracted from GP. In addition, G Rg2 were found in the bread crust and crumb samples baked from HWF-GP. Interestingly, the amounts of G Rg2 extracted from the bread crust sample baked from HWF-GP were significantly higher than the amounts extracted from the bread crumb of the same sample. G Rg3 were only present in the bread crust sample baked from the blend. However, no G (Rb1, Rc, Rd, Rg2, and Rg3) in the bread crust and bread crumb of samples baked from HWF and the cookie samples baked from SWF were found.

The types of G extracted from ginseng are quite different depending on the processing methods used, such as in the production of white and red ginseng (Ha et al 2005; Ko et al 2005; Shibata 2001). Ha et al (2005) showed a significant increase in the quantities of G Rb1, G Rb2, G Rc, and G Rd after extrusion processing of raw *Panax ginseng*. Shibata (2001) noted that white ginseng includes the malonyl esters of G Rb1, G Rb2, G Rc, and G Rd, while the malonyl group, which is originally attached at the 6″-position of the glycosyl moieties of G Rb1, G Rb2, G Rc, and G Rd, is easily lost during the steaming process used to make red ginseng. Fuzzati (2004) reported that the acidic malonyl G (malonyl G Rb1, malonyl G Rb2, malonyl G Rc, and malonyl G Rd) are thermally very unstable; they degraded or converted into the corresponding neutral G (G Rb1, G Rb2, G Rc, and G Rd, respectively) during the steaming process in red ginseng production.

Based on results in the present study, it is proposed that the high temperature bread- and cookie-baking processes could lead to the loss of malonyl acid from the

glycosyl moieties of malonyl G Rb1, malonyl G Rc, and malonyl G Rd, thereby leading to increases in the amounts of G Rb1, G Rc, and G Rd in the bread crust and bread crumb of samples baked from HWF-GP and in the cookie samples baked from SWF-GP. Furthermore, the amounts of G Rb1, G Rc, and G Rd extracted from the bread crust and bread crumb samples baked from HWF-GP were higher than those extracted from the cookie samples baked from SWF-GP. It is speculated that not only the higher baking temperature (215°C) but also the longer baking time (24 min) used for bread-baking could cause an increase in the amounts of extracted G, as compared to cookie-baking conditions (205°C and 11 min).

Wang et al (2006) noted that the amounts of G Rg2, G Rg3, G Rh1, and G Rh2 increase after steaming process of raw *Panax quinquefolius* and they reported that these G can be used as marker compounds to distinguish red ginseng from white ginseng. Ha et al (2005) also found an increase in the G Rg group (G Rg1, G Rg2, and G Rg3) in the final extruded *Panax ginseng* samples. Shibata (2001) reported that the glycosyl moiety at the C-20 position of the protopanaxadiol type G, including G Rb1, G Rb2, G Rc, and G Rd, is partly degraded to produce G Rg3 during the steaming process to make red ginseng. Popovich and Kitts (2004) showed that G Rg3 are naturally absent in *Panax quinquefolius*, but are produced by a thermal process. On the other hand, G Rg2 can be produced by the protopanaxatriol type G, such as G Re, in the same way (Kim et al 2000). In the present study, results indicated that the bread-baking conditions used could lead to the chemical degradation and/or conversion of the thermolabile protopanaxadiol type G and protopanaxatriol type G into G Rg3 and G Rg2, respectively.

Kim et al (2000) observed that during steaming processing of raw *Panax ginseng*, increasing the steaming temperature from 100 to 120°C resulted in an increase

in the quantities of G Rg2 and G Rg3. In work conducted by Wang et al (2006), when berries of *Panax quinquefolius* were steamed at 120°C, the quantities of G Rg2 and G Rg3 increased significantly compared with those steamed at 100°C. In the present study, it was hypothesized that higher temperatures could chemically stimulate an increase in the conversions of protopanaxatriol type G and protopanaxadiol type G to G Rg2 and G Rg3, respectively. It is suggested in the present study that the greater amounts of G Rg2, as well as the presence of G Rg3, in the crusts of bread samples baked from the HWF-GP blend, as compared to the bread crumb sample baked from the same blend, could be due to a difference in the actual temperature between the outside crust and the inside crumb during bread-baking.

6.5. SUMMARY

The addition of GP to either HWF or SWF induced an increase in water absorption values, increased To and Tp values, and decreased the RVA viscosity values during the heating process. It can be concluded that the higher water absorption capacity of GP interferes with the moisture uptake of wheat starch in the blends, and hence diminishes the effective moisture contents of the starch available to aid gelatinization. The addition of GP to the HWF bread formulations and to the SWF cookie formulations led to decreases in the loaf volumes of the bread and in the widths of the cookies. It is presumed that the temperatures used for bread- and cookie-baking caused increases in the amounts of G (Rb1, Rc, and Rd) in the samples. New G compounds (Rg2 and Rg3), and highly desirable ones pharmaceutically, were found only in bread samples baked from the HWF-GP blend, with G Rg3 appearing in the crust but not the crumb of bread samples. Although the quality properties of the bread samples baked from HWF-GP and the cookie samples baked from SWF-GP were slightly lower than those of their counterpart samples without GP, the slight decreases in quality seem outweighed by the presence of nutraceutical components, G, in the products baked from the blends. Therefore, the production of bread and cookies which incorporate ginseng powder can broaden the utilization of ginseng, and the products can be regarded as possible health-promoting nutraceutical foods.

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Table 6.1.

Moisture and Protein Contents of Hard Wheat Flour (HWF), Soft Wheat Flour (SWF), and Ginseng Powder (GP) Samples

Sample	Moisture Content (%, wb)	Protein Content (%)[a]
HWF	13.2	12.7
SWF	11.2	8.3
GP	8.3	11.2

[a]Protein percentages are on a 14% moisture basis.

Table 6.2.

Farinograph Properties of Hard Wheat Flour (HWF), a Blend of HWF-Ginseng Powder (GP), Soft Wheat Flour (SWF), and SWF-GP[a]

Sample	Water Absorption (%)	Dough Development Time (min)	Stability (min)	Mixing Tolerance Index (BU)[b]
HWF	63.0a	6.5a	4.5a	20c
HWF-GP	65.0b	4.5b	3.5b	60b
SWF	49.2d	1.5c	2.0c	95a
SWF-GP	51.6c	1.0c	1.0c	100a

Values with different letters within the same column differ significantly ($P<0.05$).
[a]Flour-GP blends were a ratio of 9:1 w/w.
[b]BU: Brabender Units.

Table 6.3.

Thermal Properties[a] of Hard Wheat Flour (HWF), Ginseng Powder (GP), a Blend of HWF-GP, Soft Wheat Flour (SWF), and SWF-GP[b]

Sample	To (°C)	Tp (°C)	ΔH (J/g)
HWF	60.0d	67.4c	4.27c
HWF-GP	61.7c	69.0a	3.36d
SWF	62.5b	68.2b	7.13a
SWF-GP	63.7a	69.4a	6.73b
GP	ND	ND	ND

Values with different letters within the same column differ significantly ($P<0.05$).
ND: not detectable.
[a]To: transition onset temperature; Tp: transition peak temperature; ΔH: transition enthalpy.
[b]Flour-GP blends were a ratio of 9:1 w/w.

Table 6.4.

Pasting Properties of Hard Wheat Flour (HWF), Ginseng Powder (GP), a Blend of HWF-GP, Soft Wheat Flour (SWF), and SWF-GP[a]

Sample	Peak (RVU)	Trough (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Setback (RVU)
HWF	102.7c	29.8c	72.9c	79.4c	49.6c
HWF-GP	97.6d	28.8c	68.8d	71.9d	43.1d
SWF	287.3a	167.0a	120.2a	296.0a	128.6a
SWF-GP	242.8b	132.0b	110.7b	252.5b	120.4b
GP	25.2e	21.8d	3.4e	30.1e	8.3e

Values with different letters within the same column differ significantly ($P<0.05$).
RVU: Rapid Visco Units.
[a]Flour-GP blends were a ratio of 9:1 w/w.

Table 6.5.

Quality Properties of Breads Baked from Hard Wheat Flour (HWF) and a Blend of HWF-Ginseng Powder (GP)[a]

Sample	Weight (g)	Volume (cm^3)	Firmness (N)
HWF	148.6b	725a	2.20a
HWF-GP	153.2a	650b	2.56a

Values with different letters within the same column differ significantly ($P<0.05$).
[a]HWF-GP blend was a ratio of 9:1 w/w.

Table 6.6.

Quality Properties of Cookies Baked from Soft Wheat Flour (SWF) and a Blend of SWF-Ginseng Powder (GP)[a]

Sample	Weight (g)	Width (cm)	Thickness (cm)	Hardness (N)
SWF	21.1a	16.1a	1.75a	42.5a
SWF-GP	20.5a	15.0b	1.68a	40.2a

Values with different letters within the same column differ significantly ($P<0.05$).
[a]SWF-GP blend was a ratio of 9:1 w/w.

Table 6.7.

Quantities of Ginsenosides (Rb1, Rc, Rd, Rg2, and Rg3) Ultrasonically Extracted for 150 min from Ginseng Powder (GP), Bread Crusts and Bread Crumbs Baked from Hard Wheat Flour (HWF) and a Blend of HWF-GP, and Cookies Baked from Soft Wheat Flour (SWF) and a Blend of SWF-GP[a]

Sample	Ginsenosides (mg/100 mg GP)				
	Rb1	Rc	Rd	Rg2	Rg3
GP	0.86c	0.51c	0.25b	ND	ND
Bread Crust					
HWF	ND	ND	ND	ND	ND
HWF-GP	0.96a	0.59a	0.33a	0.17a	0.07
Bread Crumb					
HWF	ND	ND	ND	ND	ND
HWF-GP	0.98a	0.59a	0.31a	0.06b	ND
Cookie					
SWF	ND	ND	ND	ND	ND
SWF-GP	0.89b	0.56b	0.33a	ND	ND

Values with different letters within the same column differ significantly ($P<0.05$).
ND: not detectable.
[a]Flour-GP blends were a ratio of 9:1 w/w.

CHAPTER 7

GENERAL CONCLUSIONS

It has been known that ginseng exhibits various pharmaceutical effects, including antioxidation, antistress, immunostimulation, and anticancer effects. Though ginseng has been consumed in the form of raw plant materials, dried plant materials, extracts, and commercial products such as capsules, tablets, drinks, and jellies in the world, the production of nutraceutical wheat flour (WF) snack foods, bread, and cookies that incorporate ginseng has not been reported. The present study investigated (1) the optimum extraction conditions of ginsenosides (G, ginseng saponins) from a blend of WF-ginseng powder (GP), (2) the effects of extrusion process variables on quantities and types of extractable G in wheat-ginseng extrudates (WGE), (3) the quality properties of WGE extruded under different conditions, and (4) physicochemical properties of bread and cookies baked from WF blended with GP, and quantities and types of extractable G in these products.

While studying the optimum extraction conditions of G from WF-GP, interactions between WF components and G were observed in WF-GP heated at 90°C. The interactions were found to occur between the wheat starch fraction (SF) and G as well as between the gluten fraction (GF) and G. Furthermore, the degree of interactions between the SF and G was greater than that between the GF and G in the heated WF-GP. The interactions between WF components and G significantly decreased the amounts of G extractable from the heated WF-GP samples. These interactions were disruptable, and the degree of disruption increased with increasing ultrasonic extraction time (i.e., total energy input). Ultrasonic extraction may have facilitated the access of the solvent [70% (v/v) aq. methanol] to the G, resulting in an increase in the extractable quantities of individual G. Based on the results obtained in the present study, the optimum extraction conditions to achieve the maximum extraction

of G from WF-GP were a sample-to-solvent ratio of 0.071:1 (mg/μL) and an ultrasonication time of 90 min.

Effects of extrusion process variables (feed moisture, screw speed, and barrel temperature) on extractable G in WGE were investigated. It was revealed that an increase in the quantities of ginsenosides Rb1, Rc, and Rd in WGE samples is a result of the degradation and/or conversion of malonyl ginsenosides Rb1, Rc, and Rd into ginsenosides Rb1, Rc, and Rd, respectively, and that the presence of the new ginsenosides Rg2 and Rg3 is due to the chemical modification of thermolabile protopanaxatriol type G and protopanaxadiol type G into G Rg2 and G Rg3, respectively. It was observed that the presence and quantities of G Rg2 and G Rg3 are significantly affected by extrusion process variables studied. In particular, increasing zone 5 barrel temperature played the more dominant role in the conversion of protopanaxatriol type G into G Rg2 than did other processing variables (e.g., screw speed and feed moisture). The optimal extrusion process condition in the present study, producing the maximum quantities of G Rg2 and G Rg3 in WGE samples, was 25% feed moisture, 300 rpm screw speed, and 140°C zone 5 barrel temperature.

The physical [expansion ratio, water absorption index (WAI), and water solubility index (WSI)], pasting, and thermal properties of WGE samples extruded under different conditions were determined. It was found that expansion ratio, WAI, WSI, peak viscosity and transition peak temperature of WGE samples were dependent on extrusion process variables studied, indicating that the final quality properties of WGE samples can be controlled by the combination of extrusion process variables tested in the present study.

Farinograph, thermal, and pasting properties of hard wheat flour (HWF), soft

wheat flour (SWF), and blends of HWF-GP and SWF-GP were analyzed. Farinograph data revealed that the dough made from HWF-GP was weaker and less stable than that made from HWF alone. Differential scanning calorimetry data indicated that adding GP to either HWF or SWF can inhibit wheat starch gelatinization due to the competition for water between wheat starch and GP components. Rapid Visco Analyzer data showed that a decrease in peak viscosity of HWF-GP or SWF-GP relative to HWF or SWF, respectively, is probably a result of the lower wheat starch content in the blends as well as a low swelling capacity of the GP.

Bread samples were prepared from HWF and HWF-GP, and cookie samples were prepared from SWF and SWF-GP. A significant decrease in the loaf volume of the bread and in the width of the cookie was observed when GP was added to the bread formulation and the cookie formulation. It was found that bread- and cookie-baking can cause an increase in the amounts of G Rb1, G Rc, and G Rd in bread samples baked from HWF-GP and cookie samples baked from SWF-GP. Although new G Rg2 were found in both bread crumb and bread crust samples baked from HWF-GP, new G Rg3 were present only in the bread crust sample.

Based on the overall findings obtained in the above studies, interactions between WF components and G, including interactions between the SF and G and between the GF and G, did occur in a blend of WF-GP heated at 90°C. G had a higher degree of and/or stronger interactions with the SF than with the GF in WF-GP. These interactions could be disrupted, given the proper extraction conditions. An increase in the amounts of G (Rb1, Rc, and Rd) in WGE, HWF-GP bread, and SWF-GP cookie samples explained the chemical degradation of acidic malonyl G (Rb1, Rc, and Rd) to neutral G (Rb1, Rc, and Rd, respectively) during the extrusion and baking processes.

The presence of new ginsenosides (Rg2 and Rg3) in WGE and HWF-GP bread samples indicated that both extrusion and bread-baking processes can produce the more desirable ginsenosides. A higher zone 5 barrel temperature (≥ 130°C) during extrusion cooking and higher actual bread-baking temperature (i.e., bread crust) improved the conversion efficiency of thermolabile protopanaxatriol type G and protopanaxadiol type G into G Rg2 and G Rg3, respectively. A healthy extruded snack product with nutraceutical properties and acceptable quality properties can be optimized by the combinations of extrusion process variables. Furthermore, the production of WF snacks, bread, and cookies containing ginseng can broaden the utilization of ginseng and the products can be considered as possible nutraceutical foods.

CHAPTER 8

FUTURE RECOMMENDATIONS

The following are recommendations for further research:

(1) To better understand the types of interactions (i.e., physical and chemical interactions) between wheat flour (WF) components and ginsenosides (G, ginseng saponins) in a heated blend of WF-ginseng powder (GP), other analytical tools, such as confocal laser microscopy, X-ray diffractometry, and nuclear magnetic resonance spectroscopy, should be used.

(2) To improve not only the chemical degradation of malonyl G Rb1, malonyl G Rc, and malonyl G Rd into G Rb1, G Rc, and G Rd, respectively, but also the conversion efficiency of protopanaxadiol type G and protopanaxatriol type G into G Rg3 and G Rg2, respectively, in wheat-ginseng extrudates, more severe extrusion process conditions should be considered: e.g., high shear screw configurations, zone 5 barrel temperature $\geq$ 150°C, screw speed $\geq$ 400 rpm, and feed moisture $\leq$ 20%.

(3) To improve the loaf volume of the bread sample baked from a blend of hard WF-GP, effects of adding dough enhancers such as vital gluten and ascorbic acid to the bread formulation should be determined.

APPENDIX 1.

Effects of Sample Heating of Wheat Flour (WF, 0.9 g), Ginseng Powder (GP, 0.1 g), and a Blend of WF-GP (0.9 g WF + 0.1 g GP) on Subsequent Extractable Ginsenosides (Rb1, Rc, and Rd)

Sample	Temperature (°C)	Ginsenosides (mg/100 mg GP)		
		Rb1	Rc	Rd
GP	25	0.87	0.54	0.26
	50	0.89	0.55	0.26
	70	0.87	0.53	0.26
	90	0.89	0.54	0.27
WF	25	ND	ND	ND
	50	ND	ND	ND
	70	ND	ND	ND
	90	ND	ND	ND
WF-GP	25	0.87	0.54	0.26
	50	0.86	0.53	0.26
	70	0.79	0.51	0.23
	90	0.72	0.47	0.21

ND: not detectable.

APPENDIX 2.

Moisture Contents of Wheat-Ginseng Extrudates Extruded at Different Conditions

Extrusion Process Variables			Moisture Content[b] (%, wb)
Feed Moisture (%, wb)	Screw Speed (rpm)	Temperature[a] (°C)	
25	200	110	8.2
30	200	110	8.3
35	200	110	8.2
25	300	110	7.2
30	300	110	8.1
35	300	110	7.1
25	200	120	8.0
30	200	120	9.3
35	200	120	8.2
25	300	120	7.2
30	300	120	6.1
35	300	120	5.0
25	200	130	7.8
30	200	130	6.6
35	200	130	5.9
25	300	130	5.5
30	300	130	5.5
35	300	130	5.4
25	200	140	6.6
30	200	140	6.5
35	200	140	5.9
25	300	140	5.3
30	300	140	5.4
35	300	140	5.6

[a]Temperature: zone 5 (nearest exit die) barrel temperature.
[b]Moisture content was determined immediately after drying at 45°C overnight.

APPENDIX 3.

Effects of Ultrasonic Extraction (UE) Time on Extractability of Ginsenosides (Rb1, Rc, Rd, Rg2, and Rg3) from Wheat-Ginseng Extrudates Extruded at Different Conditions

Extrusion Conditions M/S/T[a]	UE Time (min)	Ginsenosides (mg/100 mg Ginseng Powder)				
		Rb1	Rc	Rd	Rg2	Rg3
25/200/110	60	0.64	0.43	0.21	0.05	ND
	90	0.78	0.48	0.26	0.05	ND
	120	0.87	0.52	0.28	0.06	ND
	150	0.87	0.51	0.28	0.06	ND
30/200/110	60	0.64	0.42	0.20	0.05	ND
	90	0.80	0.46	0.25	0.05	ND
	120	0.86	0.52	0.28	0.06	ND
	150	0.87	0.51	0.29	0.06	ND
35/200/110	60	0.63	0.41	0.20	0.04	ND
	90	0.78	0.45	0.22	0.04	ND
	120	0.86	0.52	0.26	0.05	ND
	150	0.87	0.51	0.26	0.05	ND
25/300/110	60	0.60	0.41	0.19	0.05	ND
	90	0.80	0.47	0.23	0.07	ND
	120	0.85	0.51	0.28	0.09	ND
	150	0.86	0.50	0.28	0.09	ND
30/300/110	60	0.56	0.36	0.19	0.05	ND
	90	0.76	0.46	0.23	0.05	ND
	120	0.85	0.50	0.27	0.06	ND
	150	0.85	0.50	0.27	0.06	ND
35/300/110	60	0.62	0.41	0.21	0.05	ND
	90	0.82	0.47	0.23	0.05	ND
	120	0.85	0.51	0.27	0.06	ND
	150	0.85	0.51	0.27	0.06	ND

[a]M: feed moisture (%, wb); S: screw speed (rpm); T: zone 5 barrel temperature (°C).
ND: not detectable.

APPENDIX 3.

Continued

Extrusion Conditions M/S/T[a]	UE Time (min)	Ginsenosides (mg/100 mg Ginseng Powder)				
		Rb1	Rc	Rd	Rg2	Rg3
25/200/120	60	0.69	0.44	0.21	0.05	ND
	90	0.79	0.48	0.25	0.05	ND
	120	1.02	0.61	0.31	0.08	ND
	150	1.03	0.61	0.32	0.08	ND
30/200/120	60	0.53	0.37	0.19	0.05	ND
	90	0.79	0.49	0.23	0.06	ND
	120	0.99	0.57	0.31	0.07	ND
	150	1.00	0.56	0.32	0.07	ND
35/200/120	60	0.60	0.41	0.19	0.05	ND
	90	0.79	0.46	0.23	0.06	ND
	120	0.96	0.55	0.32	0.07	ND
	150	0.96	0.55	0.32	0.07	ND
25/300/120	60	0.66	0.43	0.21	0.06	ND
	90	0.80	0.48	0.23	0.08	ND
	120	1.01	0.62	0.33	0.11	ND
	150	1.01	0.62	0.33	0.11	ND
30/300/120	60	0.55	0.38	0.19	0.05	ND
	90	0.79	0.49	0.24	0.06	ND
	120	0.96	0.58	0.32	0.08	ND
	150	0.96	0.58	0.32	0.08	ND
35/300/120	60	0.64	0.41	0.19	0.06	ND
	90	0.84	0.49	0.24	0.07	ND
	120	0.96	0.58	0.31	0.08	ND
	150	0.96	0.58	0.31	0.08	ND

[a]M: feed moisture (%, wb); S: screw speed (rpm); T: zone 5 barrel temperature (°C).
ND: not detectable.

APPENDIX 3.

Continued

Extrusion Conditions M/S/T[a]	UE Time (min)	Ginsenosides (mg/100 mg Ginseng Powder)				
		Rb1	Rc	Rd	Rg2	Rg3
25/200/130	60	0.71	0.45	0.22	0.06	ND
	90	0.81	0.48	0.24	0.07	ND
	120	1.04	0.62	0.33	0.08	ND
	150	1.04	0.61	0.32	0.08	ND
30/200/130	60	0.71	0.44	0.22	0.05	ND
	90	0.82	0.48	0.24	0.06	ND
	120	1.05	0.62	0.33	0.07	ND
	150	1.04	0.62	0.33	0.07	ND
35/200/130	60	0.68	0.44	0.22	0.05	ND
	90	0.81	0.49	0.23	0.05	ND
	120	1.04	0.62	0.32	0.07	ND
	150	1.04	0.63	0.33	0.07	ND
25/300/130	60	0.69	0.45	0.22	0.08	0.04
	90	0.80	0.49	0.24	0.11	0.05
	120	1.04	0.62	0.32	0.14	0.06
	150	1.04	0.62	0.32	0.15	0.06
30/300/130	60	0.71	0.43	0.22	0.06	ND
	90	0.80	0.49	0.24	0.07	ND
	120	1.04	0.61	0.32	0.09	ND
	150	1.05	0.61	0.32	0.09	ND
35/300/130	60	0.68	0.45	0.23	0.05	ND
	90	0.78	0.48	0.22	0.07	ND
	120	1.04	0.62	0.32	0.08	ND
	150	1.05	0.62	0.32	0.08	ND

[a]M: feed moisture (%, wb); S: screw speed (rpm); T: zone 5 barrel temperature (°C).
ND: not detectable.

APPENDIX 3.

Continued

Extrusion Conditions M/S/T[a]	UE Time (min)	Ginsenosides (mg/100 mg Ginseng Powder)				
		Rb1	Rc	Rd	Rg2	Rg3
25/200/140	60	0.70	0.43	0.21	0.07	ND
	90	0.78	0.48	0.22	0.08	ND
	120	1.06	0.61	0.32	0.09	ND
	150	1.06	0.61	0.32	0.09	ND
30/200/140	60	0.66	0.44	0.21	0.06	ND
	90	0.80	0.48	0.23	0.08	ND
	120	1.04	0.62	0.31	0.09	ND
	150	1.04	0.61	0.31	0.09	ND
35/200/140	60	0.65	0.43	0.21	0.05	ND
	90	0.79	0.49	0.23	0.06	ND
	120	1.06	0.62	0.32	0.07	ND
	150	1.05	0.62	0.32	0.07	ND
25/300/140	60	0.66	0.44	0.21	0.14	0.06
	90	0.79	0.48	0.23	0.16	0.06
	120	1.06	0.61	0.32	0.19	0.07
	150	1.05	0.61	0.33	0.30	0.07
30/300/140	60	0.64	0.43	0.21	0.09	0.04
	90	0.79	0.49	0.24	0.10	0.05
	120	1.04	0.61	0.32	0.11	0.05
	150	1.05	0.61	0.32	0.12	0.05
35/300/140	60	0.63	0.43	0.21	0.06	ND
	90	0.78	0.49	0.23	0.08	ND
	120	1.03	0.62	0.32	0.09	ND
	150	1.04	0.62	0.32	0.09	ND

[a]M: feed moisture (%, wb); S: screw speed (rpm); T: zone 5 barrel temperature (°C).
ND: not detectable.

APPENDIX 4.

Die Pressure Related to Different Extrusion Process Variables of Wheat-Ginseng Extrudates

Extrusion Process Variables			Die Pressure (psi)
Feed Moisture (%, wb)	Screw Speed (rpm)	Temperature[a] (°C)	
25	200	110	705
30	200	110	585
35	200	110	325
25	300	110	415
30	300	110	375
35	300	110	230
25	200	120	600
30	200	120	500
35	200	120	275
25	300	120	295
30	300	120	315
35	300	120	190
25	200	130	510
30	200	130	220
35	200	130	100
25	300	130	130
30	300	130	70
35	300	130	40
25	200	140	350
30	200	140	220
35	200	140	120
25	300	140	100
30	300	140	90
35	300	140	40

[a]Temperature: zone 5 (nearest exit die) barrel temperature.

APPENDIX 5.

Residence Time Related to Different Extrusion Screw Speeds of Wheat-Ginseng Extrudates

Extrusion Process Variables			Residence Time (sec)
Feed Moisture (%, wb)	Temperature[a] (°C)	Screw Speed (rpm)	
25	120	200	67
25	120	300	63

[a]Temperature: zone 5 (nearest exit die) barrel temperature.

APPENDIX 6.

Pasting Properties of Wheat-Ginseng Extrudates Extruded at Different Conditions

Extrusion Conditions M/S/T[a]	Trough (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Setback (RVU)
25/200/110	64.3	54.7	106.0	41.8
30/200/110	66.3	71.7	104.3	38.0
35/200/110	27.5	178.6	49.9	22.4
25/300/110	16.6	39.7	31.0	14.5
30/300/110	14.2	48.6	25.1	10.9
35/300/110	23.2	88.6	41.5	18.3
25/200/120	64.8	49.1	105.9	41.1
30/200/120	47.9	73.4	83.4	35.5
35/200/120	42.0	134.5	80.7	38.7
25/300/120	12.4	32.3	24.5	12.1
30/300/120	17.0	38.6	32.0	15.0
35/300/120	20.5	76.7	35.6	16.1
25/200/130	61.7	50.3	100.1	37.9
30/200/130	28.6	81.5	54.2	25.6
35/200/130	17.6	141.8	36.7	19.1
25/300/130	8.1	30.2	17.4	8.4
30/300/130	15.5	40.4	30.3	14.8
35/300/130	10.5	73.3	20.5	10.0
25/200/140	33.4	44.8	57.6	24.3
30/200/140	28.3	75.6	53.4	25.1
35/200/140	19.4	119.0	41.8	22.4
25/300/140	7.8	28.5	22.5	14.7
30/300/140	10.7	38.3	21.0	10.0
35/300/140	10.1	52.8	19.6	9.5

[a]M: feed moisture (%, wb); S: screw speed (rpm); T: zone 5 barrel temperature (°C).
RVU: Rapid Visco Units.

APPENDIX 7.

Thermal Properties[a] of Hard Wheat Flour (HWF), Ginseng Powder (GP), Blends of HWF-GP (10, 20, and 30% GP, w/w), Soft Wheat Flour (SWF), and Blends of SWF-GP (10, 20, and 30% GP, w/w)

Sample	To (°C)	Tp (°C)	ΔH (J/g)
HWF	60.0	67.4	4.27
GP	ND	ND	ND
90% HWF-10% GP	61.7	68.9	3.36
80% HWF-20% GP	62.4	69.4	2.89
70% HWF-30% GP	63.3	69.8	2.29
SWF	62.5	68.2	7.13
90% SWF-10% GP	63.7	69.4	6.73
80% SWF-20% GP	64.7	69.9	4.67
70% SWF-30% GP	65.6	70.7	3.81

[a]To: transition onset temperature; Tp: transition peak temperature; ΔH: transition enthalpy. ND: not detectable.

APPENDIX 8.

Pasting Properties of Hard Wheat Flour (HWF), Ginseng Powder (GP), Blends of HWF-GP (10, 20, and 30% GP, w/w), Soft Wheat Flour (SWF), and Blends of SWF-GP (10, 20, and 30% GP, w/w)

Sample	Peak (RVU)	Trough (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Setback (RVU)
HWF	102.7	29.8	72.9	79.4	49.6
GP	25.2	21.8	3.4	30.1	8.3
90% HWF-10% GP	97.5	28.8	68.8	71.8	43.0
80% HWF-20% GP	86.1	26.3	59.8	59.3	33.0
70% HWF-30% GP	79.7	25.6	54.1	56.1	30.0
SWF	287.3	167.0	120.2	296.0	128.6
90% SWF-10% GP	242.8	132.0	110.7	252.5	120.4
80% SWF-20% GP	199.6	103.8	95.8	199.2	95.4
70% SWF-30% GP	163.1	81.6	81.7	153.0	71.3

RVU: Rapid Visco Units.