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Analytical Methods

Extraction of ginsenosides from a blend of wheat flour and ginseng powder

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ABSTRACT

This study explored conditions for maximum extraction of ginsenosides (G) from a blend of wheat flour (WF) and ginseng powder (GP). 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. Individual G (Rb1, Rc, and Rd) were fractionated and identified by RP-HPLC. Interactions between WF components and G, including interactions between the wheat starch fraction (SF) and G and between the gluten fraction (GF) and G, were observed in WF–GP heated at 90 °C. The degree of interactions between the SF and G was greater than that between the GF and G. The interactions between WF components and G decreased the amounts of G extractable from the heated WF–GP. The interactions between WF components and G could be disrupted by increasing ultrasonic extraction time to 90 min for maximum extraction.

1. 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, Shimoyamada, & Watanabe, 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 its pharmaceutical activity. Their basic structure contains triterpenoid aglycon and various sugar moieties (Fuzzati, 2004). Over 30 G have been identified; amongst these, Rb1, Rb2, Rc, Rd, and Re are the most abundant (Corbit, Ferreira, Ebbs, & Murphy, 2005).

Numerous studies have reported that proteins interact with relatively small molecules, such as saponins and flavour compounds (Heng et al., 2004; Ikedo et al., 1996; Potter, Jimenez-Flores, Pollack, Lone, & Berber-Jimenez, 1993; Sarnthein-Graf & La Mesa, 2004; Shimoyamada, Ootsubo, Naruse, & Watanabe, 2000). Potter et al. (1993) observed the interactions of isolated soy proteins and caseins with quillaja saponins using gel electrophoresis. In addition, Ikedo et al. (1996) reported that soyasaponin interacts with bovine serum albumin (BSA). They hypothesised that the interactions between BSA and soyasaponin include electrostatic and hydrophobic interactions.

It has been shown that starch interacts with various components in food systems, such as lipids, proteins, iodine, organic alcohols or acids, and flavour compounds, including terpenes, aldehydes, and lactones (Arvisenet, Le Bail, Voilley, & Cayot, 2002; Conde-Petit, Escher, & Nuessli, 2006; Heinemann, Escher, & Conde-Petit, 2003; Heinemann, Zinsli, Renggli, Escher, & Conde-Petit, 2005; Le Bail, Rondeau, & Buleon, 2005; Raphaelides & Georgiadis, 2006). Arvisenet et al. (2002) reported that the interactions of gelatinized starch with its interaction partners occur during the 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% to 15% and 63% to 72%, respectively, at a 14% moisture basis (Atwell, 2001). Therefore, in the present study, it was hypothesised 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.

There is no report in the literature, as far as the authors are aware of, on the bioavailability of G when complexed with other food components. However, it was assumed in the present study that any interactions between WF components and G could affect the amounts of G readily available for human absorption in the gut upon consumption of WF products that include ginseng powder (GP), such as cereal-based snacks, breads, and noodles, and furthermore, that the higher the degree of interaction, the lower the bioavailability of G would be. Hence, an optimum extraction procedure to quantify G in cereal products containing ginseng was needed to characterise the degree of interactions, if any, between G and major food constituents, such as starch and protein, in a cereal product. It was hypothesised that the extraction efficiency of G from the cereal product would be related to the degree of interaction between WF components and the G. Numerous studies on the

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extraction of G have been performed on raw plant materials (Corbit et al., 2005; Court, Hendel, & Elmi,1996; Ji et al., 2001; Kwon et al., 2001; Lau, Woo, & Koh, 2003; Li, Mazza, Cottrell, & Gao, 1996; Wang, Wu, McEntee, & Yuan, 2006), on extracts (Ko et al., 2005), and on commercial products (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 solvent-to-sample 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).

2. Materials and methods

2.1. Materials

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

2.2. 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 s, 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 5000xg for 10 min using a Sorvall RC-5B centrifuge (Dupont, Wilmington, DE, USA) and the supernatant was decanted. The SF (sediment) was spread in aluminium pans and allowed to air-dry at room temperature for 72 h. The wet GF was divided into six parts, frozen, and freeze-dried for 48 h. The air-dried SF and freeze-dried GF were initially each ground using a mortar and pestle and then finely ground to a particle size of $\leqslant$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.

2.3. Chemical analysis

WF, GP, SF, and GF samples were analysed 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.

2.4. Optimisation of solvent-to-sample 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 solvent-to-sample ratios that ranged from 1:0.070 to 1:0.100 (μl/mg). Each suspension was ultrasonically extracted for 30 min using an Ultrasonicator (Model FS 14H, Fisher Scientific, Pittsburgh, PA, USA, Power: 155 W) at room temperature and then centrifuged at 11,200xg for 20 min using a centrifuge (Model J2-21 M, 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 reversed-phase high-performance liquid chromatography (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 on each extract.

2.5. 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 h. 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 millilitres of 70% (v/v) aq. methanol were added to each sample of freeze-dried SF, GF, SF–GP, or GF–GP. The suspension was vortexed for two minutes and then ultrasonically extracted for G for 30 min, then centrifuged, evaporated, dissolved, and filtered as described in 2.4. Each sample was extracted in duplicate and two chromatographic runs were performed on each extract.

2.6. Optimisation 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 h. 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 millilitres 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 2.4. Each sample was extracted in duplicate and two chromatographic runs were performed for each extract.

2.7. 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 × 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, Seo, Woo, & Koh, 2004), and the detector output was transmitted simultaneously to the computer for data storage and graphic representation.

2.8. 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 amongst the samples. Means were compared by using Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level.

3. Results and discussion

3.1. Chemical analysis

Moisture and protein contents of WF, GP, SF, and GF samples are reported in Table 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.

3.2. Optimisation of solvent-to-sample 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 Fig. 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 amongst 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 (Fig. 1, Peaks 1, 2, and 3, respectively) 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 their 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 solvent-to-sample ratios for the extraction of G from WF, GP, and WF–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 (ranging from 1 to 14 ml) were added to each sample. Solvent functions as a medium to solubilise

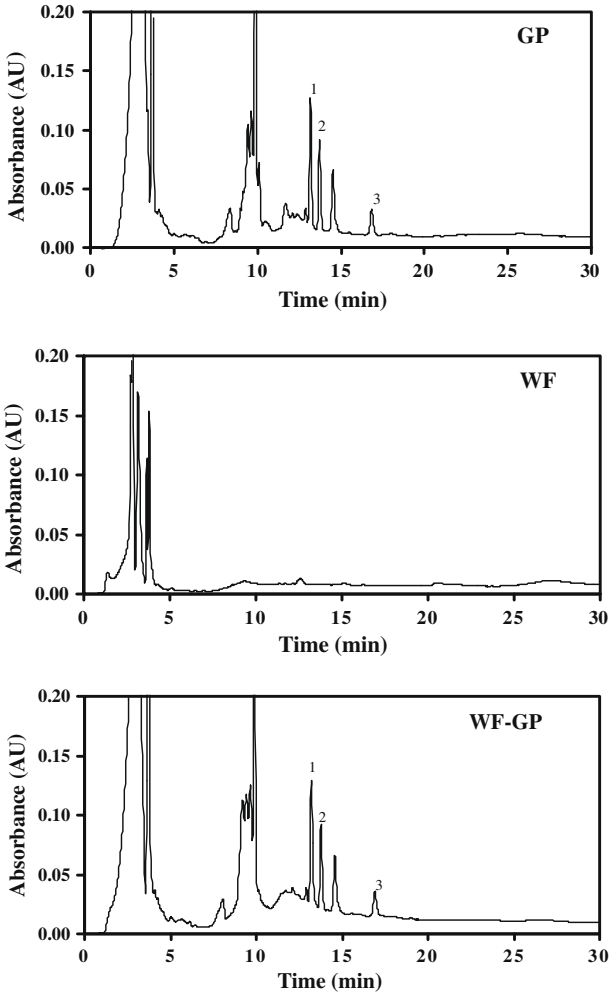

Fig. 1. RP-HPLC chromatograms of ginsenosides ultrasonically extracted for 30 min from pure ginseng powder (GP), wheat flour (WF), and a blend of WF–GP in 70% (v/v) aq. methanol. 1: Rb1; 2: Rc; 3: Rd.

Table 1
Moisture and protein contents of wheat flour (WF), ginseng powder (GP), wheat starch fraction (SF), and wheat 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 2
Quantities of ginsenosides (Rb1, Rc, and Rd) ultrasonically extracted for 30 min at room temperature from ginseng powder (GP, 0.1 g), wheat flour (WF, 0.9 g), and a blend of WF–GP (0.9 g WF + 0.1 g GP) with different solvent[a]-to-sample ratios.

Sample	Solvent (μl)	Solvent-to-sample ratio (μl/mg)	Ginsenosides (mg/100 mg GP)		
			Rb1	Rc	Rd
GP	1000	1:0.100	0.53d	0.39d	0.18d
	2000	1:0.500	0.70c	0.47c	0.21c
	3000	1:0.030	0.83b	0.49b	0.23b
	4000	1:0.025	0.89a	0.52a	0.25a
	5000	1:0.020	0.88a	0.53a	0.25a
	6000	1:0.017	0.88a	0.52a	0.25a
	8000	1:0.013	0.88a	0.52a	0.25a
	10000	1:0.010	0.89a	0.52a	0.25a
WF	14000	1:0.064	ND	ND	ND
WF–GP	12000	1:0.083	0.82b	0.50b	0.23b
	14000	1:0.071	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.

target compounds, such as G; thus, the solvent-to-sample ratio is one of several critical parameters to be considered. Table 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 solvent-to-sample ratios. The quantities of G (Rb1, Rc, and Rd) extracted from pure GP increased with decreases in the solvent-to-sample ratio from 1:0.100 to 1:0.025 (μL/mg), 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 solvent-to-sample ratio of 1:0.025 (μl/mg). The quantities of G (Rb1, Rc, and Rd) extracted from WF–GP with the solvent-to-sample ratio of 1:0.071 (μl/mg) were not significantly different from the quantities of the G extracted from pure GP with the solvent-to-sample ratios of less than 1:0.025 (μl/mg). However, no measurable G (Rb1, Rc, and Rd) in pure WF were found. Based on these results, the solvent-to-sample ratio of 1:0.025 (μl/mg) for pure GP and the solvent-to-sample ratio of 1:0.071 (μl/mg) for WF–GP were chosen as one of the UE conditions for subsequent studies.

3.3. 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 Fig. 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), resulting in subsequently lower amounts of G extracted 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 and/or 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. Fig. 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,

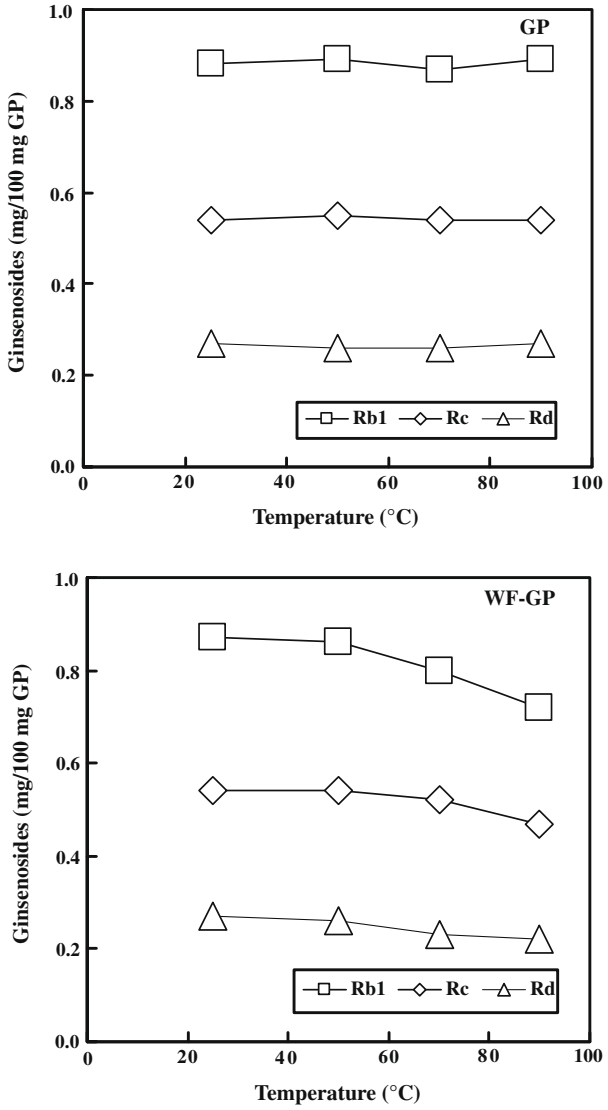

Fig. 2. Effects of sample heating of pure ginseng powder (GP) and a wheat flour (WF)–GP blend on subsequent extractable ginsenosides (Rb1, Rc, and Rd).

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). 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 lower rates of extraction of G from SF–GP under the given extraction conditions.

Ginseng saponins have a complex chemical structure, as mentioned earlier, 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, Lee, & Joo, 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. Shimoyamada et al. (2000) observed interactions between soybean saponin and whey proteins. Potter

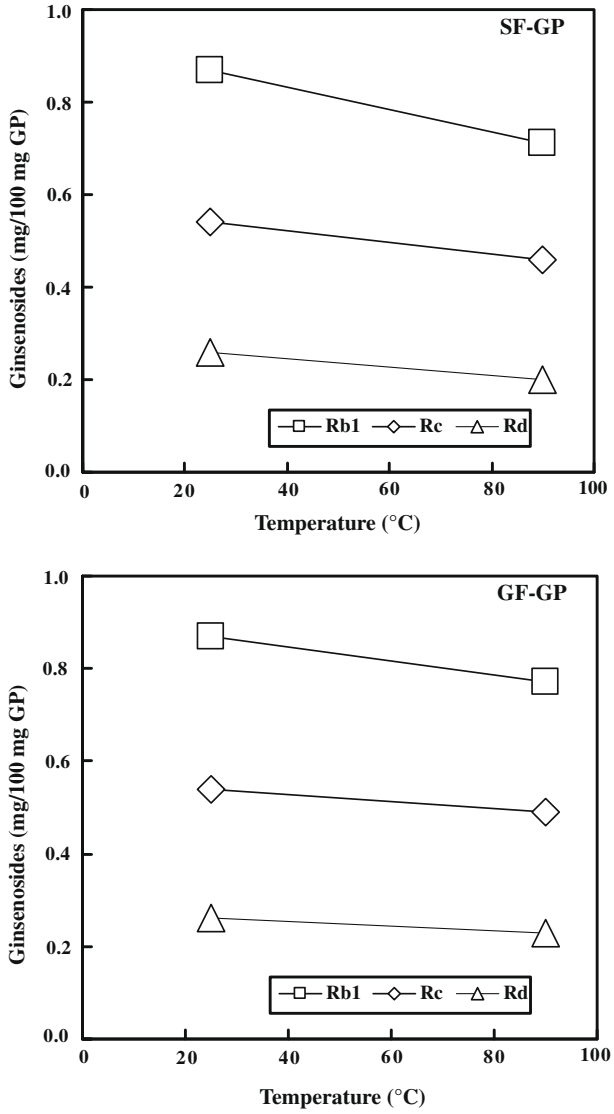

Fig. 3. Effects of sample heating of a starch fraction (SF)–ginseng powder (GP) blend and a gluten fraction (GF)–GP blend on subsequent extractable ginsenosides (Rb1, Rc, and Rd).

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, whilst 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 systems, 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

Table 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.

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, Young, & Rocca, 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. 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 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).

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

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/100 mg GP (the amount of G Rb1 which was available to interact with WF components in heated WF–GP, i.e., not extractable).

The amounts of G Rb1 which were available to interact with the SF or with the GF (i.e. not extractable) in each blend were

Table 4
Effects of ultrasonic extraction (UE) time on extractability of ginsenosides (Rb1, Rc, and Rd) from heated (90 °C) ginseng powder (GP), wheat flour (WF), 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.

calculated in the same way; the amounts of G Rb1 calculated were 0.17 mg/100 mg GP for the SF (0.88–0.71) and 0.11 mg/100 mg GP for the GF (0.88–0.77). For purposes of comparison, it was assumed that WF (based on dry weight) grossly consisted of about 85% SF, 10% GF, and 5% nonstarch polysaccharides and lipids, yielding the following composition for a 900 mg WF sample:

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 were available to interact with the SF (900 mg) or the GF (900 mg), calculated values were determined for the total amounts of G Rb1 which were available to interact with the SF (765 mg) 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 plus 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, the findings suggest that, quantitatively, higher amounts of G interacted with the SF than with the GF in a blend of WF–GP heated at 90 °C.

3.5. Optimisation 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 and 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, SF–GP, and GF–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 and 60 min, respectively, for the maximum extractions, indicating a difference in the strength of the interactions of ginsenosides with starch and with protein. Based on the results obtained in the present study, optimisation of UE time appears to be an important parameter for accomplishing the maximum extraction of G from a cereal product containing ginseng.

4. Conclusions

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 were 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 had occurred 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 a greater degree of interactions with the SF than with the GF in the heated WF–GP, due to not only proportionally greater amounts of starch in the WF but also the greater propensity of G (on a g/g basis) to interact with the SF than with the GF. Moreover, the interactions between the SF and G appeared to be stronger (less disruptable) than those between the GF and G, based on the extraction conditions used. The interactions between WF components and G in a 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, optimised extraction conditions for G from the WF–GP blend were a solvent-to-sample ratio of 1:0.071 (μL/mg) followed by ultrasonication for 90 min for as maximum an extraction of G as from pure GP.

In the present study, a physical method, i.e., ultrasonic extraction, was used to disrupt interactions between WF components and G. However, the results obtained from the present study can not be applied directly to explain the possibility of *in vivo* metabolizing of WF–G complexes, since the human body has different conditions to break down food components in its digestive system [e.g., presence of salivary amylase in the mouth, highly acidic environment (pH 1–3) in the stomach, various proteases in the intestines]. Thus, studies are needed to compare means of disruption, physical and biological, of interactions between G and food constituents in cereal products and effects of these interactions on the bioavailability of G in humans.

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