Effects of Extrusion Process Variables on Extractable Ginsenosides in Wheat−Ginseng Extrudates

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To evaluate effects of extrusion process variables on ginsenosides in wheat−ginseng extrudates, a wheat flour−ginseng powder blend (10% ginseng powder, 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 ginsenosides (Rb1, Rc, and Rd) extracted from the wheat−ginseng extrudates extruded at a zone 5 barrel temperature of ≥120 °C were significantly higher than those extracted from the nonextruded blend. New ginsenosides (Rg2 and Rg3), which were not present in the nonextruded blend, were found in certain wheat−ginseng extrudates, indicating that high temperature extrusion processing could lead to degradation of acidic malonyl ginsenosides (Rb1, Rc, and Rd) into neutral ginsenosides (Rb1, Rc, and Rd, respectively) as well as modification of protopanaxadiol-type and protopanaxatriol-type ginsenosides into Rg3 and Rg2, respectively.

KEYWORDS: Extrusion; wheat flour; ginseng powder; ginsenosides; RP-HPLC

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, and so on. This cooking processing, carried out using a high temperature and short time treatment, yields finished products with desirable qualities, such as high digestibility and nutritional value (*1*).

Wheat flour is a commonly used material in the extrusion industry, and the effects of extrusion process variables on the properties of wheat flour extrudates have been studied (*2−5*). In 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 (*6−9*).

Ginseng has been frequently used in Asian countries as a traditional medicine (*10*). Ginsenosides (ginseng saponins) have been known as the main active components in ginseng and have been typically used as marker compounds for quality properties (*11, 12*). Ginsenosides are dammarane-type triterpene glycosides and can be classified into three groups: the proto-panaxadiols, protopanaxatriols, and oleanolic acid (*13*). Four malonyl ginsenosides, including malonyl Rb1, malonyl Rb2,

malonyl Rc, and malonyl Rd, are also found in ginseng (*14*). The structures of some common ginsenosides are shown in **Figure 1**.

Raw ginseng has been processed into two kinds of ginseng, white ginseng and red ginseng, to improve its preservation and efficacy (*15, 16*). 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 h and then air-drying (*17*). Red ginseng is known to be more pharmaceutically active than white ginseng, as the steaming process causes changes in the chemical compositions of ginsenosides, thereby enhancing the biological activity of the ginseng (*18, 19*). 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 ginsenosides generated during the steaming process (*15, 18, 20*). In particular, Rg3 has been found to show various biological activities, including anticancer activity (*16, 19, 21*) and radical scavenging activity (*22*). Moreover, it has been reported that Rg3 inhibits the growth of *Helicobacter pylori* (*23*).

In spite of medical benefits of red ginseng and the processing efficiency (i.e., high temperature and short time) of extrusion cooking for making red ginseng products, Ha et al. (*17*), who studied changes in ginsenosides 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 making process. However, apparently no studies have reported the production of extruded wheat flour snack products

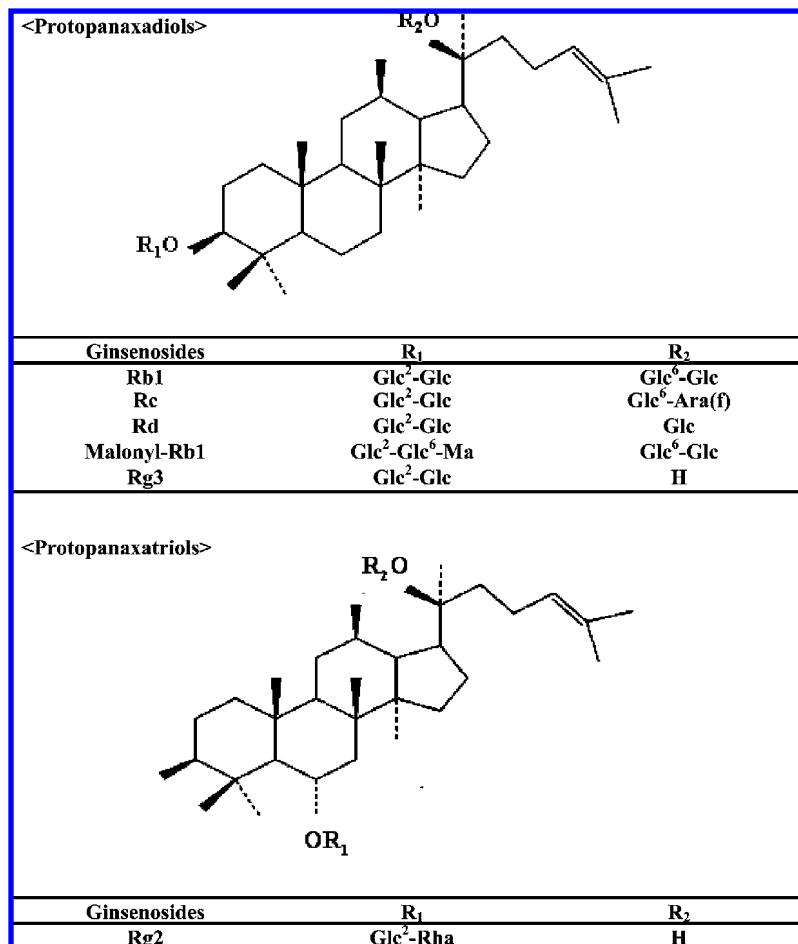* Corresponding author. Telephone: +1-517-355-8474ext. 111. Fax: +1-517-353-8963. E-mail: ngp@msu.edu.

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Figure 1. Structures of ginsenosides. Glc, glucose; Ara, arabinose; Rha, rhamnose; (f), furanosyl; Ma, HOOC−CH₂−COOH (modified from ref *19*).

Table 1. 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, 3 mm diameter single round type exit die.

with ginseng, nor have studies been found that investigate changes in ginsenosides in snack products extruded under different conditions. Extrusion cooking has been employed to make nutraceutical snack foods, and there is a possibility of using wheat flour in combination with ginseng to produce 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 ginsenosides in wheat−ginseng extrudates.

MATERIALS AND METHODS

Materials. Soft wheat flour was obtained from the Mennel Milling Co. (Fostoria, OH). 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 local ginseng market. Wheat flour and ginseng powder were analyzed for their moisture contents using AACCI Approved Method 44-15A (*24*), and the moisture contents of wheat flour and ginseng powder were 11.2% (as is) and 8.3% (as is), respectively. Standards for ginsenosides Rb1, Rc, Rd, Rg2, and Rg3 were provided by Ilhwa Co. (Kuri, Korea). Methanol (HPLC grade) was obtained from Sigma (St. Louis, MO).

Extrusion Cooking. Wheat flour and ginseng powder were mixed in a weight ratio of 9:1 using a model A-200 mixer (Hobart Co., Troy, OH) for 20 min. Extrusion cooking was done with a model MP 19T2-25 corotating twin screw extruder, with a barrel diameter of 19 mm and a barrel length to diameter (*L/D*) ratio of 25:1 (APV Baker, Grand Rapids, MI). 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 an E2 Metripump instrument (Brook Crompton, Huddersfield, England). Dry materials were fed using a K2 M twin-screw volumetric feeder (K-TRON, Pittman, NJ). Product temperature and die pressure were measured by using a model TB422J thermocouple (Dynisco, Sharon, MA) and a model ERP3-3 M pressure transducer (Dynisco, Sharon, MA), respectively, inserted before the die. Torque was measured as percent of the total motor power (2 kW).

A full factorial arrangement was used for the present study. Extrusion process variables were feed moisture (25, 30, and 35%), screw speed (200 and 300 rpm), and zone 5 barrel temperature (110, 120, 130, and 140 °C), for a total of 24 different combinations. Zones 1/2/3/4 barrel temperatures were constant (40/70/90/110 °C, respectively) for all the extrusion process conditions studied. The dry material feed rate was

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kept constant (2.0 kg/h), as was the screw configuration (**Table 1**). Extrusion cooking of the wheat flour−ginseng powder blend was done in duplicate.

The wheat−ginseng extrudate samples produced at the different extrusion conditions were collected and dried in an air oven (45 °C) overnight. After drying, portions of each wheat−ginseng extrudate sample were ground to a particle size of ≤330 µm (54 mesh screen) using a coffee grinder (Braun Inc., Woburn, MA). After grinding, moisture contents of ground wheat−ginseng extrudate samples were measured by AACCI Approved Method 44-15A (*24*). Both ground and unground wheat−ginseng extrudate samples were placed in ziplock bags, sealed, and stored at −20 °C until analysis.

Specific Mechanical Energy. The specific mechanical energy (SME) in watt h/kg was calculated according to the equation of Brent et al. (*25*):

$$SME = [\text{actual speed rpm/rated screw rpm}] \times [\%\,\text{torque}/100] \times [\text{motor power in watt/feed rate in kg/h}]$$

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

Optimization of Ultrasonic Extraction Time for Ginsenosides from Wheat−Ginseng Extrudates. Four mL of 70% (v/v) aq methanol was added to ginseng powder (0.1 g). Fourteen mL of 70% (v/v) aq methanol was added to wheat flour (0.9 g), a blend of wheat flour−ginseng powder (0.9 g of wheat flour + 0.1 g of ginseng powder), or wheat−ginseng extrudate samples (1.0 g) extruded under different conditions. Each mixture was ultrasonically extracted for 60, 90, 120, or 150 min using a model FS 14H ultrasonicator (Fisher Scientific, Pittsburgh, PA) with a power of 155 W at room temperature and then centrifuged at 11 200*g* for 20 min using a model J2-21 M centrifuge (Beckman Instruments Inc., Fullerton, CA) at 25 °C. The supernatant was evaporated using a model 421-4000 microrotary evaporator (Labconco, Kansas City, MO) at 50 °C. Prior to reversed-phase high-performance liquid chromatography (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 on each extract.

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

The solvents were Milli-Q-Plus distilled water (A) and HPLC-grade acetonitrile (B). 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 (*26*), and the detector output was transmitted simultaneously to the computer for data storage and graphic representation. Individual ginsenosides (Rb1, Rc, Rd, Rg2, and Rg3) were identified by the comparison of their retention times with those obtained from the chromatograms of each standard solution. Calibration curves for each ginsenoside were constructed from the measured peak areas, and the amounts (mg/100 mg ginseng powder) of ginsenosides in each sample were calculated using the standard curves.

Statistical Analysis. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC). Analysis of variance was performed using the general linear models procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference 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.

Table 2. Effects of Ultrasonic Extraction Time of Ginseng Powder (0.1 g), Wheat Flour (0.9 g), and a Wheat Flour−Ginseng Powder Blend (0.9 g of Wheat Flour + 0.1 g of Ginseng Powder) on Extractable Ginsenosides (Rb1, Rc, and Rd)[a]

sample	ultrasonic extraction time (min)	ginsenosides (mg/100 mg ginseng powder)		
		Rb1	Rc	Rd
ginseng powder	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
wheat flour	60	ND	ND	ND
	90	ND	ND	ND
	120	ND	ND	ND
	150	ND	ND	ND
wheat flour−ginseng powder blend	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

[a] Values with different letters within the same column differ significantly ($P < 0.05$). ND = not detectable.
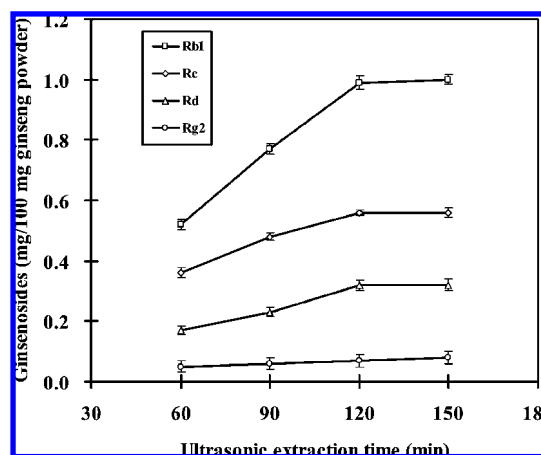
Figure 2. Effects of ultrasonic extraction time of wheat−ginseng extrudates extruded at 30% feed moisture, 200 rpm screw speed, and 120 °C zone 5 barrel temperature on extractable ginsenosides (Rb1, Rc, Rd, and Rg2).

RESULTS AND DISCUSSION

Optimization of Ultrasonic Extraction Time for Ginsenosides from Wheat−Ginseng Extrudates. Effects of ultrasonic extraction time of wheat flour, ginseng powder, and a blend of wheat flour−ginseng powder on extractable ginsenosides Rb1, Rc, and Rd are presented in **Table 2**. No measurable Rb1, Rc, and Rd in pure wheat flour were found at any of the ultrasonic extraction times studied. The quantities of Rb1, Rc, and Rd extracted from both pure ginseng powder and the blend were not significantly different within the range of ultrasonic extraction times (60−150 min) studied. However, the quantities of ginsenosides extracted from wheat−ginseng extrudate samples extruded at 30% feed moisture, 200 rpm screw speed, and 120 °C zone 5 barrel temperature increased with increasing ultrasonic extraction time, and finally reached a plateau after an ultrasonic extraction time of 120 min (**Figure 2**). All the studied wheat−ginseng extrudate samples showed a similar trend (data not shown). The findings established that the maximum extraction of extractable ginsenosides from wheat−ginseng extrudate samples produced under each of the different extrusion process conditions could be achieved by ultrasonication for 120 min or longer.

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Table 3. Quantities of Ginsenosides (Rb1, Rc, Rd, Rg2, and Rg3) Ultrasonically Extracted for 150 min from a Nonextruded Blend of Wheat Flour—Ginseng Powder (10% Ginseng Powder, w/w) and Wheat—Ginseng Extrudates[a]

extrusion conditions	ginsenosides (mg/100 mg ginseng powder)				
M/S/T[b]	Rb1	Rc	Rd	Rg2	Rg3
nonextruded blend	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

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

Changes in Ginsenosides Rb1, Rc, and Rd during Extrusion Cooking. The quantities of Rb1, Rc, and Rd ultrasonically extracted for 150 min from a blend of wheat flour—ginseng powder and wheat—ginseng extrudate samples extruded at different conditions are listed in **Table 3**. As compared with those in the nonextruded blend sample (control), the quantities of Rb1 and Rc extracted from wheat—ginseng extrudate samples extruded at 110 °C zone 5 barrel temperature were not changed upon extrusion cooking. However, the quantities of Rd extracted from the same wheat—ginseng extrudate samples were significantly increased upon extrusion cooking. The quantities of each of Rb1, Rc, and Rd extracted from wheat—ginseng extrudate samples extruded at a zone 5 barrel temperature of more than 120 °C were significantly higher than those of their counterpart ginsenosides extracted from the nonextruded blend. This finding was consistent with Ha et al. (*17*) who showed significant increases in the quantities of Rb1, Rb2, Rc, and Rd after an extrusion processing of raw *Panax ginseng*.

Studies have been conducted to compare ginsenoside compositions of white and red ginseng (*11, 15, 17, 20, 26*). The ginsenoside composition of red ginseng was different from that of white ginseng mainly due to the loss of malonyl ginsenosides during the steaming process used to produce red ginseng (*10*). Shibata (*19*) noted that white ginseng contains the malonyl esters of Rb1, Rc, and Rd, and that the malonyl group, which is originally attached at the 6″-position of the glycosyl moieties of Rb1, Rc, and Rd, is easily lost during the steaming process when making red ginseng. Ren and Chen (*27*) reported that malonyl ginsenosides are thermally very unstable, and therefore, they are readily demalonylated by heating. According to Fuzzati (*10*), the acidic malonyl ginsenosides (malonyl Rb1, malonyl Rc, and malonyl Rd) degraded or converted into the corre-
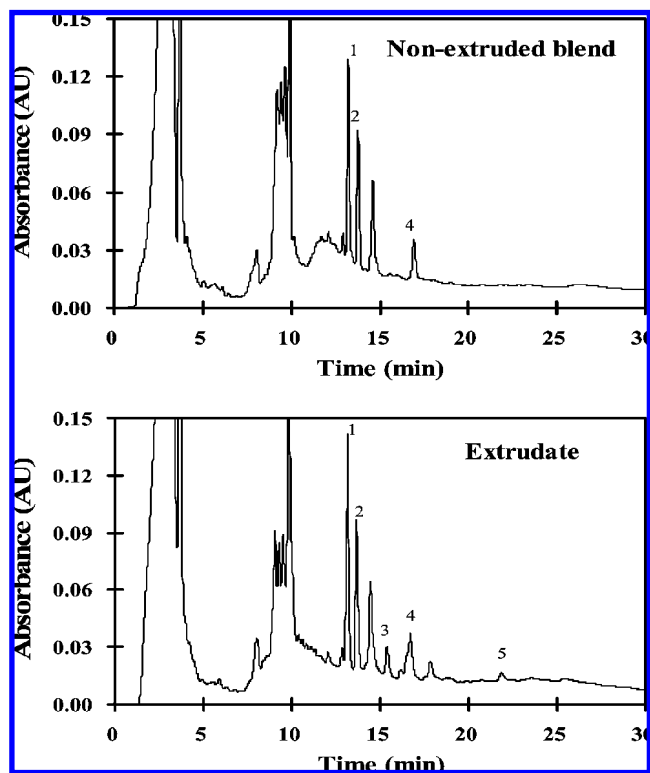
Figure 3. RP-HPLC chromatograms of ginsenosides ultrasonically extracted for 150 min from a nonextruded blend of wheat flour—ginseng powder and a wheat—ginseng extrudate extruded at 25% feed moisture, 300 rpm screw speed, and 140 °C zone 5 barrel temperature. (1) Rb1, (2) Rc, (3) Rg2, (4) Rd, (5) Rg3.

sponding neutral ginsenosides (Rb1, Rc, and Rd, respectively) during the steaming process. Therefore, it is postulated in the present study that extrusion cooking under a zone 5 barrel temperature of ≥120 °C could stimulate the loss of malonyl acid from glycosyl moieties of malonyl Rb1, malonyl Rc, and malonyl Rd, subsequently inducing an increase in the quantities of Rb1, Rc, and Rd, respectively, in wheat—ginseng extrudate samples.

Presence of New Ginsenosides Rg2 and Rg3 during Extrusion Cooking. RP-HPLC chromatograms of ginsenosides ultrasonically extracted for 150 min from a nonextruded blend of wheat flour—ginseng powder and wheat—ginseng extrudate samples extruded at 25% feed moisture, 300 rpm screw speed, and 140 °C zone 5 barrel temperature are shown in **Figure 3**. The RP-HPLC chromatograms of the blend and the wheat—ginseng extrudate samples were found to be distinctively different, indicating that when the blend was extruded, the HPLC profile of ginsenosides was changed. In particular, Rg2 (peak 3) and Rg3 (peak 5), which were not present in the blend, were found in the wheat—ginseng extrudate sample. Furthermore, the quantities of Rg2 and Rg3 were dependent on the extrusion process conditions studied (**Table 3**); increases in the quantities of Rg2 and Rg3 were observed when feed moisture was decreased, and screw speed and barrel temperature each increased.

Wang et al. (*16*) studied the changes in ginsenosides in berries of *Panax quinquefolius* after steaming treatment (100—120 °C for 1 h). They found that the amounts of Rg2, Rg3, Rh1, and Rh2 in the steamed samples were increased as compared to those in raw samples. They reported that these ginsenosides can be used as marker compounds to distinguish red ginseng from white ginseng. Kim et al. (*15*) evaluated the effect of steaming *Panax ginseng*, at 100, 110, and 120 °C for 2 h using an autoclave, on

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its chemical components. They observed that F4, Rg3, and Rg5 were not found in the raw ginseng but in the steamed ginseng samples. According to Lau et al. (*26*), when *Panax notoginseng* was steamed at 120 °C for 1, 2, 3, and 9 h, Rb1, Rd, and Re each 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 ginsenosides which are present only in red ginseng products, and that the quantities of the new ginsenosides in the steamed samples increased with increasing duration of the steaming process. According to Ha et al. (*18*), less polar ginsenosides, including Rg3, Rg5, Rk1, and F4, are typically considered as ginsenosides unique to red ginseng products.

Ha et al. (*17*) employed an extrusion process to produce red ginseng products using raw *Panax ginseng.* The extrusion conditions used were feed moisture of 15 or 22%, screw speed of 200 rpm, and barrel temperature of 110 °C. They found increases in the Rg groups (Rg1, Rg2, and Rg3) in the final extruded samples. Shibata (*19*) reported that the glycosyl moieties at the C-20 position of the protopanaxadiol-type ginsenosides, including Rb1, Rb2, Rc, and Rd, are partly degraded to produce Rg3 during the steaming process to make red ginseng. Popovich and Kitts (*28*) reported that Rg3 are naturally absent in *Panax quinquefolius* but are produced by a thermal process. They also reported that the formation of Rg3 is associated with the breakdown of the more abundant Rb1 and Rc. On the other hand, Rg2 can be produced by the protopanaxatriol-type ginsenosides, such as Re, in the same way (*15*). Thus, it is suggested in the present study that the presence of Rg3 and Rg2 in wheat−ginseng extrudate samples could be attributed to the chemical degradation and/or conversion of the thermolabile protopanaxadiol-type ginsenosides and protopanaxatriol-type ginsenosides, respectively, during high temperature extrusion cooking. Furthermore, it is suggested that, utilizing raw ginseng as one of the ingredients, extruded nutraceutical wheat flour snack products can be produced by extrusion cooking and yield red ginseng components in the extrudates.

In the present study, 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 Rg2 in wheat−ginseng extrudate samples, from 0.15 to 0.08 mg/100 mg ginseng powder, and significantly reduced SME values from 268.5 to 243.9 W h/kg. The finding (a decrease in SME values with increasing feed moisture) was consistent with observations by Ryu and Ng (*5*), 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 appears that 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 ginsenosides, subsequently causing an increase in the quantities of Rg2 in wheat−ginseng extrudate samples in the present study.

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 Rg2 in wheat−ginseng extrudate samples from 0.09 to 0.20 mg per 100 mg ginseng powder, and led to a significant increase in SME values from 131.8 to 262.8 W h/kg (**Table 4**). According to Frame (*29*), the degree of fill in the barrel and the viscosity of the dough can each be diminished with increasing the screw

Table 4. System Variables Related to Different Extrusion Process Variables of Wheat−Ginseng Extrudates[a]

extrusion process variables			system variables	
feed moisture (%, wet basis)	screw speed (rpm)	temp (°C)[b]	SME (W h/kg)[c]	PT (°C)[d]
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	262.8 cd	163a
30	300	140	256.8de	157b
35	300	140	241.8g	152d

[a] Values with different letters within the same column differ significantly (*P* < 0.05). [b] Temperature: zone 5 barrel temperature. [c] SME: specific mechanical energy. [d] PT: product temperature at exit die.

speed during extrusion cooking. However, in the present study, the increase in screw speed was large enough to overcome a slight reduction in the percent of torque from the motor, resulting in a higher SME. Thus, it appears in the present study that more protopanaxatriol-type ginsenosides could be readily converted to Rg2 at the higher screw speed extrusion process conditions, due to higher mechanical energy input occurring during high screw speed extrusion cooking conditions.

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 Rg2 in wheat−ginseng extrudate samples, from 0.09 to 0.20 mg/100 mg ginseng powder (**Table 3**). As already mentioned, the traditional processing to make red ginseng includes steaming raw ginseng at 90−100 °C for 2−3 h. Therefore, it was hypothesized in the present study that temperatures higher than these could improve the production of red ginseng components and shorten the processing time. The findings indicated that a higher barrel temperature during extrusion cooking is indeed one of the important factors that increases the quantities of Rg2 in wheat−ginseng extrudate 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 Rg2 in wheat−ginseng extrudate samples. However, the increase in barrel temperature from 110 to 140 °C at these extrusion process conditions caused an increase in the quantities of Rg2 in wheat−ginseng extrudate samples, despite significantly decreased SME values (from 282.9 to 262.8 W h/kg). The trend of decreasing SME values with increasing barrel temperature is generally consistent with other published results (*5, 30, 31*). 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 (*5, 30*).

Effects of Extrusion on Ginseng

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On the basis of all of the results obtained in the present study (on effects of extrusion process variables on the increase in Rg2 in wheat−ginseng extrudate samples), it was suggested that increasing barrel temperature played the more dominant role in the conversion of protopanaxatriol-type ginsenosides into Rg2 than did mechanical energy input during extrusion cooking of a blend of wheat flour−ginseng powder.

Rg3 is valued for its pharmaceutical activities, and numerous studies have focused on Rg3 to evaluate the effects of heating processes on producing red ginseng products (*15−17, 28*). Kim et al. (*15*) observed that, during a steaming process of raw *Panax ginseng*, increasing the steaming temperature from 100 to 120 °C resulted in an increase in the quantities of Rg3. In work done by Wang et al. (*16*), when berries of *Panax quinquefolius* were steamed at 120 °C, the quantities of Rg3 were significantly higher than those for berries steamed at 100 °C.

In the present study, unlike Rg2 which was found in all the wheat−ginseng extrudate samples produced, Rg3 was present in wheat−ginseng extrudate samples produced at only the following three extrusion process conditions (**Table 3**): 25% feed moisture, 300 rpm screw speed, and 130 °C zone 5 barrel temperature; 25% feed moisture, 300 rpm screw speed, and 140 °C zone 5 barrel temperature; and 30% feed moisture, 300 rpm screw speed, and 140 °C zone 5 barrel temperature. Of these extrusion process conditions, the second produced the highest quantities of Rg3 and was the extrusion condition that also produced the highest quantities of Rg2. In particular, this extrusion condition 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 ginsenosides to Rg3 in the wheat−ginseng extrudate samples.

Correlations among Ginsenosides and Product Temperature. The quantities of Rb1 were found to be positively associated with the amounts of Rc ($r = 0.97$, $p \leq 0.001$), Rd ($r = 0.90$, $p \leq 0.001$), and 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 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 ginsenosides (Rb1, Rc, and Rd), but also could improve the conversion efficiency of protopanaxatriol-type ginsenosides to Rg2.

In conclusion, extrusion cooking led not only to an increase in the quantities of ginsenosides Rb1, Rc, and Rd in wheat−ginseng extrudate samples, but also to the production of new Rg2 and Rg3 in certain wheat−ginseng extrudate samples, indicating that changes in chemical composition of ginsenosides can take place during high temperature extrusion cooking. The quantities of Rg2 and Rg3 in wheat−ginseng extrudate 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 Rg2 and Rg3 in wheat−ginseng extrudate samples, was 25% feed moisture, 300 rpm screw speed, and 140 °C zone 5 barrel temperature. Moreover, the presence of Rg2 and Rg3 in wheat−ginseng extrudate samples offers the possibility of producing extruded nutraceutical wheat flour snack foods containing red ginseng components.

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