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쓴메밀의 서로 다른 부위에서 압출성형이 식이섬유 및 생리활성물질의 함량에 미치는 영향

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Influence of Extrusion on Dietary Fiber Profile and Bioactive Compound in Different Parts of Tatary Buckwheat (*Fagopyrum tataricum*)

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ABSTRACT : The aim of this investigation was to examine the influence of extrusion on dietary fiber profile and the content of bioactive compounds, rutin and quercetin in young sprout, whole seed, and matured stem of Tartary buckwheat. WSI(water soluble index) is increased by a function of both screw profile and process temperature, compared to control in different parts of Buckwheat. Also, WSI of ME is increased more than 5.2 times in grain, compared to that of control. The effect of precooking by extrusion on the dietary fiber profile of buckwheat flour was evaluated. Precooking by extrusion significantly increased SDF in flour, although in most cases extrusion decrease in TDF a little. The thermo-mechanical treatment undergone by the buckwheat flour during extrusion led to redistribute part IDF fraction to SDF, leading to an increase in the latter. The content of rutin was increased about two fold in extruded flour of sprout, compared to in control. This increase maybe why these compounds are released from cell wall by high shear processing under high temperature.

Key Words **:** Extrusion, Dietary fiber, Tatary Buckwheat, Rutin

INTRODUCTION

Several recent studies have focused on the development of buckwheat (*Fagopyrum spp.*) as a potential “functional food” material, particularly with respect to its seed and sprout (Kim *et al.*, 2006), Both seed and seed sprout of buckwheat are rich in nutrients and phenolic compounds, and show a good balance of amino acids and minerals (Kim *et al.*, 2004; Pomeranz and Robbins, 1972; Steadman *et al.*, 2001). Tian *et al.*, (2002) identified four main flavonolglycosides in methanol extracts, rutin, quercetin, kaempferol-3-rutinoside and a trace quantity of a flavonol triglycoside. Rutin (quercetin-3-beta-D-rutinoside) is an important therapeutic substance that favorably influences the increase of blood vessel elasticity, the treatment of circulatory disorders, atherosclerosis and the reduction of blood pressure and stimulates the organism to utilize vitamin C (Yildzogleari *et al.* 1991). Rutin is a secondary plant metabolite that antagonizes the increase of capillary fragility associated with haemorrhagic disease, reduces high blood pressure (Abeywardena and Head, 2001), decreases the permeability of the blood vessels has an anti-oedema effect,

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reduces the risk of arteriosclerosis (Wojcicki *et al*., 1995), and has as antioxidant activity (Baumgertel *et al*., 2003). The most popular derivative food is buckwheat noodles made from buckwheat flour of seed and sprout water dough by extrusion, which are very popular in Japan, China and Korea. The presence of rutin in buckwheat is one of the main reasons for the production of different kinds of buckwheat foods (Kreft *et al*., 2006). Thus the aboveground parts of buckwheat such as sprout are rich source of flavonoids, predominantly of rutin which is used for pharmaceutical purpose (Hwang *et al*., 2006).

The two main species of buckwheat, common and tartary buckwheats (*Fagopyrum tataricum* Gaertn.), are consumed all around the world. Especially, *F. tataricum* is a valuable medicinal plant which can be used as a raw material of oriental medicine or health food, because rutin is retained in flower, leaf, stem and root as well as seed. Even though seed rutin is contained mostly in bran, bran is removed during milling. Bran as well as stem and sprout composed mainly of fiber are the water insoluble dietary fiber and can not be digested by human, when milled using the current technologies, such as flint-stone, pin or Hammer type mill (Bonafaccia *et al*., 2003a; Bonafaccia *et al*., 2003b).

In recent years, dietary fiber has received increased amounts of attention. Several studies have related consumption of dietary fiber and whole grains with reduction in chronic ailments like high serum cholesterol, cardiovascular disease (Gordon, 1999; Jones, 2006), certain forms of cancer (Decker *et al*., 2002) and constipation (Lue *et al*., 1991; Kantor *et al*., 2001). Dietary fiber can be divided into insoluble and soluble dietary fiber (SDF). Insoluble dietary fiber (IDF), primarily consisting of cellulose, hemicellulose, and lignin, is the fraction of dietary fiber that is not soluble in water; SDF, primarily consisting of pectin and gums, is the water-soluble fraction (Qian and Ding, 1996). Both SDF and IDF are associated with several health benefits. Soluble fibers are known to be effective in reducing total blood cholesterol and promoting satiety, and insoluble fibers give help in treating constipation and reduce the risk of colon cancer and diverticular disease (Seiz, 2006).

There are many processes to manufacture processed products as well as to produce raw materials for processing food and medicines. Generally, extrusion is a process that raw materials are continuously crushed, heated, sterilized, reacted, and puffed for short time in an extruder. Strong shearing force originated from high temperature, high pressure, and high-speed rotary of screw for short time breaks off plant cell wall and resultantly increases amount of water soluble dietary fiber. Extruded materials are known to be higher in quality (functionality, appealability etc.) than other kinds of products produced by other heat treatment process with lower expense, which are used to manufacture a number of food products. The hot melt extrusion (HME) has been studied to make water insoluble dietary fiber of natural resources soluble in water. Extrusion, using high temperature, high pressure and high shear strength, can change the physical, chemical, nutritional and physiological characteristics of natural resource (Vasanthan *et al*., 2002). Also, if water insoluble dietary fiber is extruded, much part is changed into the water soluble, and the physiologically active components in fiber can be extracted more by disrupting the cell wall structure (Hwang *et al*., 1994). If natural resource is milled using the ultra fine mill after extruding, some of water insoluble resources can be changed into the water soluble through the breakdown of cell wall structure (Kang, 2004).

In this paper, the individual parts of the buckwheat plant were investigated in terms of rutin content and dietary fiber profiling after extrusion processing to verify the possibility of using buckwheat as a functional component in extruded products.

MATERIALS AND METHODS

1. Flour processing

The seed, stem and sprout (9 days-old) of buckwheat were donated from the plant gene laboratory in Kangwon National University, and dried in a convective dry oven (VS-1202D3H, Vision Scientific Co. LTD, Korea) at 105℃ for 24 hours. The materials were cooled in a desiccator and weighed. The water contents of seed, stem and sprout were 14.31, 7.38 and 7.35%, respectively.

The different parts of buckwheat flour were processed in a co-rotating intermeshing type twin-screw extruder (STS HANKOOK E.M Co. Korea) with barrel temperatures of 90, 100, 120, 150, and 180℃ and screw speed of 400 rpm. The extruder has self wiping screws (32 ㎜ diameter and 878 ㎜ processing length; length to diameter ratio of 32.0) in a steel barrel with eight zones. Each zone was heated by resistive electric heaters and cooled by tap water circulating in the jackets. Temperature of each zone can be controlled independently. The screw configuration used in extrusion experiments consisted of forward conveying elements (48 ㎜, 33 ㎜, 24 ㎜, 11 ㎜, forward pitch), 24 ㎜ (90°, 45°; forward, 45°; backward) of kneading elements and reverse elements.

The effect of extrusion process parameters (barrel temperature and screw speed) on the efficacy of the extruding treatment was studied in the previously study (Kim *et al*., 2005). The extruder screw profile and barrel temperature zones are shown in Fig. 1. Feed moisture content was maintained at 20% (wet basis) for all treatments. The target moisture was achieved by adding the flour with water in a Metering pump (HANKOOK E.M. Co. Korea), taking the initial moisture of the flour into account. The hydrated flour was stored overnight at 4℃ for equilibration before extrusion. The average feed rate and specific mechanical energy (SME) input were 10 ㎏/h and 315.43 ± 0.95 kJ/㎏, respectively. The extruded product was dried using convective dry oven at 50℃ for 3 hour (VS-1202D3H, Vision Scientific Co. LTD, Korea). The moisture contents of dried extrudate ranged between 5.1% and 7.6%(wet basis). The dried product was ground to pass through a 10 ㎛ sieve using a HKP-05 ultra fine mill (Korea Energy Technology Co. Ltd, Korea). For control studies, uncooked different part of flour was ground to pass through 10 ㎛ sieve, using the same mill.

2. Water solubility index (WSI)

Water solubility index (WSI) was determined in triplicate using the method of Anderson *et al*. (1969). One gram of ultra fine milled sample was suspended in 20 ㎖ of distilled water at room temperature for 30 min, gently stirring during this period, and then centrifuged at 2000 g (14520 rpm) for 10 min. The supernatant was decanted into an evaporating dish of known weight. WSI is the weight of dry solids in the supernatant expressed as a percentage of the original weight of sample.

3. Dietary fiber analysis

Total, soluble and insoluble dietary fiber contents of seed, stem and sprout which were milled into ultra fine flour after drying (control) or extruding (ME) were determined using the AACC Approved Method 32-21 (AACC 2000). One gram of sample and 40 ㎖ of 0.05 M MES-TRIS buffer solution was prepared by dissolving 19.52 g 2(*N*-morpholino) ethanesulfonic acid (MES) (Sigma, M 8250) and 14.2 g tris(hydroxymethyl)aminomethane (TRIS) (Sigma, T1503) in 1.7 L deionised water, adjusting pH to 8.2 with 6.0 N NaOH, and then diluting to 2 L with water. The sample was digested enzymatically by adding 50 L of heat-stable-amylase in a water bath at 95℃ for 35 min, shaking continuously. After removing from water bath and adding 100 L of protease, the sample was incubated in the water bath at 60℃ for 30 min, stirring continuously. The sample was removed from water bath and 5 ㎖ of 0.56 N HCl was

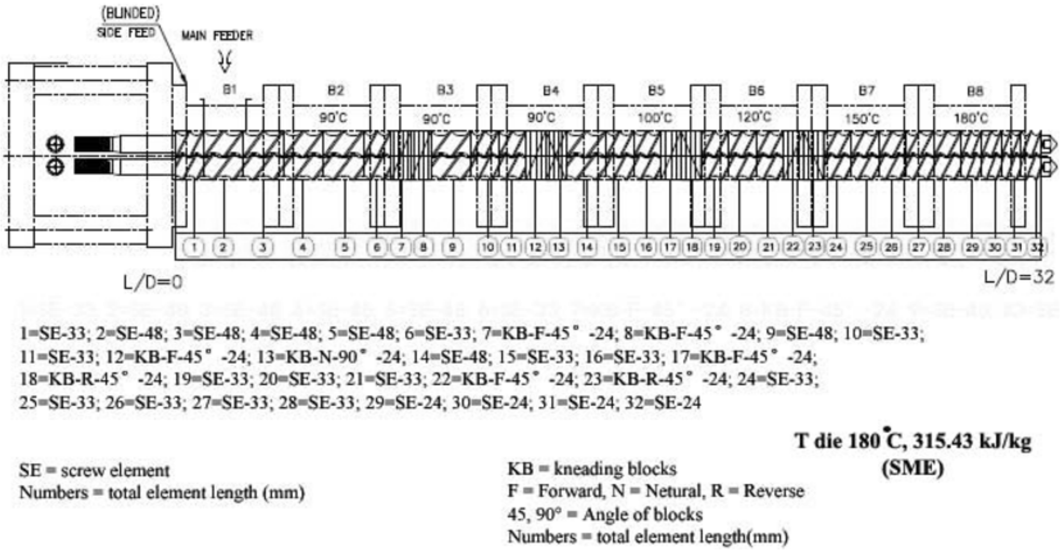


Fig. 1. Schematic of twin screw extruder barrel section and screw configuratio.

Table 1. Instrument and working conditions for rutin(quercetin 3-O-β-D-rutinoside) and quercetin analysis by high performance liquid chromatography.

Instrument	Shimadzu LC-20AT HPLC system	
Column	YMC AM303, 4.6 ㎜×250 ㎜	
Detector	UV-VIS detector (355 ㎚)	
Solvent A	45% Acetonitrile containing 2% acetic acid	
Solvent B	H_2O containing 2% acetic acid	
Flow rate	1 ㎖/min	
Gradient elution system		
Time(min)	%A	%B
Initial	50	50
18m	100	0
20m	50	50
22m	50	50
Injection volume : 20 ㎕		

added to adjust the pH to 4.1. After adding 200 L amyloglucosidase solution, the sample was incubated in the water bath at 60℃ for 30 min, stirring continuously. The solution incubated by enzyme was filtered through a celite-containing crucible and the residue was washed twice with 10 ㎖ of water at 70℃. The residue was then washed with 10 ㎖ of 95% ethanol, acetone and dried for overnight in 103℃ oven. The weight of the dried residue gave the insoluble dietary fiber (IDF) content. Filtrate from the crucible and water washings were precipitated with four volumes of 95% ethanol at 60℃ for soluble dietary fiber (SDF) determination. The precipitate was filtered, washed with 78% and 95% ethanol and acetone, and then dried over night. The weight of this dried residue gave the SDF content. The total dietary fiber (TDF) content of the sample was obtained from the sum of IDF and SDF values (Berglund *et al.*, 1994).

4. HPLC analysis

One gram of the different parts of powdered buckwheat sample was weighed and put into a bottle. Twenty milliliters of distilled water was added to the bottle. The sample was extracted in a water bath at 95℃ for 3 hours with stirring to get water extract. The supernatant and the sediment were filtered. The extraction solution was dried by vacuum-evaporator. The dried extract was weighed to calculate yield, based on the weight of buckwheat. HPLC (high performance liquid chromatography) was used for quantitative analysis. Working conditions were shown in Table 1.

For calibration curve of rutin and quercetin the typical concentrations of 5, 10, 20 and 40 ㎍/㎖ were prepared. Each solution was injected and chromatogram was recorded. The peak areas of rutin and quercetin were calculated and respective calibration curves were plotted against concentration.

RESULTS AND DISCUSSION

1. Water solubility index (WSI)

WSI is increased in different part of buckwheat by a function of both screw profile and process temperature, compared to in control. Also, WSI of ME is increased more than 5.2 times in seed, compared to that of control. The difference of WSI is the highest in seed, followed by stem and sprout. Generally, solubility is increased as increased the amount of degraded starch granules were increased by extrusion, and so water-soluble products are increased. The extrusion of starchy foods results in gelatinization, partial or complete destruction of the crystalline structure and molecular fragmentation of starch polymers as well as protein denaturation, and formation of complexes between starch and lipids and between protein and lipids (Colonna and Mercier, 1983; Ho and Izzo, 1992; Mercier and Feillet, 1975; Mercier *et al.*, 1980).This phenomenon is caused by greater shear fragmentation of the starch during extrusion. The content of starch is higher in seed, compared to that of stem and sprout, and the crystalline regions of starch is irreversibly broken and disintegrated to form gel by the thermal degradation of extrusion process. If starch is thermally degraded at high temperature, starch is easily dissolved even in cold water by the increase of water solubility of starch. Also, if the cell wall structure of fiber is disrupted by extruding, more water soluble components and hydroxyl groups in cell wall are exposed by the disintegration of the cell wall layers. If milled after extruding, some part of water insoluble fiber can be milled into the colloidal particle, which is easily dissolved in water. By above reasons WSI of ME may be the highest in seed.

2. Effect of extrusion processing on dietary fiber content

The thermomechanical nature of extrusion cooking has the added potential of causing a redistribution of soluble and insoluble components of fiber in favor of the former. The total, insoluble and soluble dietary fiber content of seed, stem and sprout of buckwheat are shown in Table 2 and Fig. 3. In seed, the insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) content of control were 2.9 and 2.7%, respectively. When seed of buckwheat was pulverized into ultra fine powder after extruding, SDF is increased into 3.5%, but IDF is decreased into 1.9%. Also, extrusion processing increases SDF from 5.4% to 12.0% and decreased IDF from 12.6% to 5.6% in stem and SDF from 9.0% to 12.4% and decreases IDF from 15.3% to 10.5% in sprout. These results indicate that the thermomechanical treatment undergone by the buckwheat flour during extrusion changes part of the IDF fraction into SDF, leading to an increase in the latter. This would tend to improve the hypocholesterolemic properties of fiber, and therefore, enhance or improve the dietary fiber profile. The content of insoluble, soluble and total dietary fiber of buckwheat groats were 2.32, 3.79 and 6.11%, respectively on a dry matter basis (Kim *et al.*, 1993).

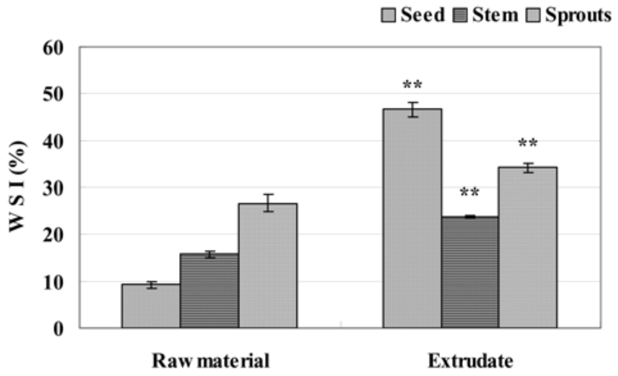


Fig. 2. Water solubility index of raw materials, and extrudates in different parts of buckwheat. **P < 0.01 versus raw materials of tatary buckwheat.

Lee *et al.*, (1995) analyzed indigestive and soluble polysaccharides of fractions, which were isolated from the supernatant and residue after enzyme treatment of raw, roasted and steamed buckwheat. Total ingestible polysaccharides (TIPs) content of raw, roasted and steamed buckwheat groats were 10.3, 10.0 and 14.7%, respectively.

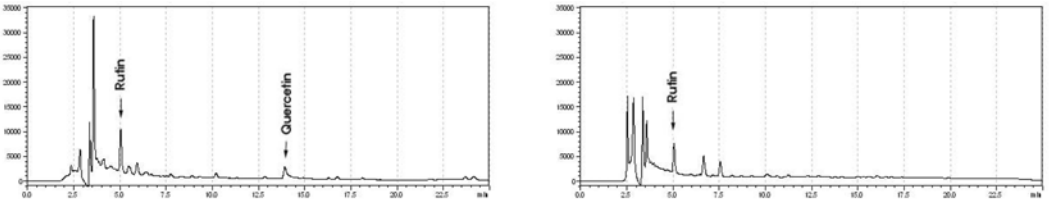
Similar changes in the dietary fiber profile of various extruded products have been reported by numerous studies. SDF increased in extrusion wheat flour (Siljestrom *et al.*, 1986; Wang and Klopfenstein, 1993), barley (Berglund *et al.*, 1994), sugar beet fiber with corn meal (Lue *et al.*, 1991), wheat bran (Caprez *et al.*, 1986; Aoe *et al.*, 1989; Ralet *et al.*, 1990; Wang *et al.*, 1993; Gualberto *et al.*, 1997), soy fiber (Qian and Ding, 1996), and potato peels (Camire and Flint, 1991; Camire *et al.*, 1997). The increase in SDF was usually at the expense of IDF due to the fragmentation or other type of thermomechanical decomposition of cellulose and lignin that are major components of insoluble fiber. The TDF of product after extruding processing was lower, compared to that of control. This implies that a portion of both SDF and IDF are probably converted into sugars due to extrusion treatment, leading to a decrease in TDF. The swelling power values below indicate that buckwheat starch has a greater toward swelling compared to wheat and rye starches. These above-mentioned

Table 2. Dietary fiber content[†] of control and ME in buckwehat different part of flour.

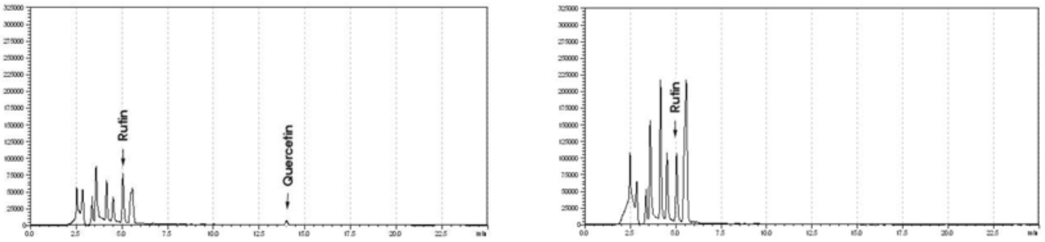
	Seed (%)			Stem			Sprout		
	SDF[‡] (%)	IDF[§] (%)	TDF[¶] (%)	SDF[‡] (%)	IDF[§] (%)	TDF[¶] (%)	SDF[‡] (%)	IDF[§] (%)	TDF[¶] (%)
Raw material	2.9±0.4	2.7±0.6	5.6±0.5	5.4±0.8	12.6±0.4	18.0±0.5	9.0±0.4	15.3±0.5	24.3±0.7
Extrudate	3.6±0.5[*]	1.9±0.6[*]	5.5±0.5	12.0±0.7[**]	5.6±0.4[**]	17.6±0.3	12.4±0.7[**]	10.5±0.8[**]	22.9±0.7

[†] Mean, n = 3; means with same superscript in the same column are significantly different. All percentages are on dry weight basis. ([*] $P < 0.05$, [**] $P < 0.05$ versus raw materials of tatary buckwheat).

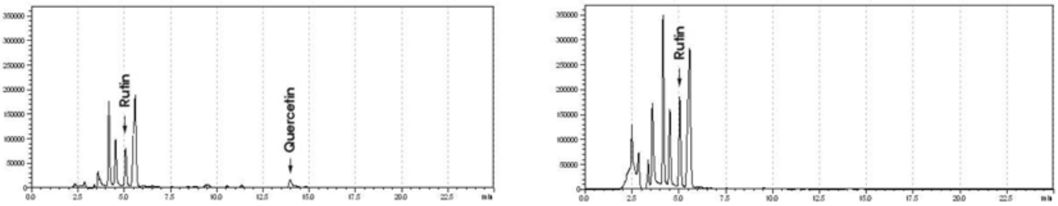
[‡] SDF = soluble dietary fiber; [§] IDF = insoluble dietary fiber; [¶] TDF = total dietary fiber.



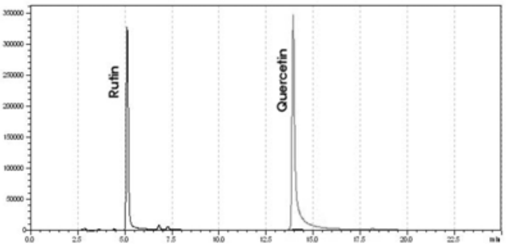
(A) Seed-Control (B) Seed-ME



(C) Stem-Control (D) Stem-ME



(E) Sprout-Control (F) Sprout-ME



(G) STD

Fig. 4. Comparison of HPLC profiles of rutin (quercetin 3-O-β-D-rutinoside, RT 5.10 min) and quercetin (RT 13.9 min) in hot water extract of control and ME in buckwehat different part of flour.

studies have reported conflicting results with regard to TDF, which either remained unchanged (Siljestrom *et al.,* 1986), decreased (Lue *et al*., 1991), or even increased (Camire and Flint, 1991; Camire *et al*., 1997) in some cases.

3. Quantatification of Rutin and Quercetin by HPLC

Although the extrusion processing has the added potential of causing a redistribution of soluble and insoluble components of fiber in favor of the former, the effect on health beneficial properties of other components also needs to be considered in medicinal plant such as buckwheat. HPLC assay results (Fig. 4) shows that rutin and quercetin can be determined by HPLC under the chromatography conditions. The rutin character peak was appeared earlier than quercetin character peak, and two peaks can be separated easily (Fig. 4-G), the contents of rutin is not uniform in its different parts of buckwheat. Analytical determinations show that seed has the lowest content of rutin among the parts, but sprout has one hundred-fold amount of rutin. In the extruded grain buckwheat flour HPLC analysis, rutin increased 69.6 μg/g, but quercetin character does not appear, indicates that the main exist form of flavonoids in extruded flour is rutin. Rutin peak appears and the amount is 286.8 μg/gDW much higher than that of control by 45.71 μg/gDW in stem after extrusion (Table 3). The content of rutin is increased about two fold in

Table 3. Content of rutin(quercetin 3-O-β-D-rutinoside) and quercetin in water extract of control and ME in buckwheat different part of flour.

	Seed			Stem			Sprout		
	Yield (%)	Rutin (μg/gDW)	Quercetin (μg/gDW)	Yield (%)	Rutin (μg/gDW)	Quercetin (μg/gDW)	Yield (%)	Rutin (μg/gDW)	Quercetin (μg /gDW)
Control	4.87±0.1	20.9±0.3	4.7±0.5	11.3±0.1	45.71±1.8	6.5±0.5	14.4±0.2	470.0±2.0	3.1±0.2
ME	22.8±1.1	69.6±0.2	–	12.0±0.1	286.84±1.9	0.5±0.1	23.6±0.3	996.4±2.6	0.4±0.1

extruded flour of sprout compared to control.

According to Lee and Ryu (2006), the rutin content was significantly influenced by screw speed and barrel temperature, rutin content decreased during extrusion process because there was an inactivated and changed of molecular structure by complex action of screw speed and barrel temperature. Also, it is known that extensive heat treatment has been known caused to degradation of flavonoid (Dietrych-Szostak and Oleszek, 1999). Several researchers have studied antioxidant activities of several grains and obtained interesting results.

It has been known that mechanical energy supplied by extruder can have influence on formation of some complexes between the flour components and, on degradation of larger molecules such as starch. But, increasing the specific mechanical energy of extrusion from 270 to 475 kJ/kg did not show any change in the chromatographic profile of dark buckwheat flour phenolic compounds (Sensoy *et al.*, 2006). The result obtained by Sensoy *et al.* (2006) indicated that roasting (200℃, 10 min) did not affect the phenolic content of buckwheat flour significantly using Folin-Ciocalteu assay. In addition, according to Gumul et al. (2007) the effect of grains extrusion of three rye cultivars decreased total polyphenols content by about 40%, with exception for extrudates prepared at the 14% initial moisture and temperature 180℃. In this case total polyphenols content was about 6% higher. Similar results were obtained by Zielinski and Troszynska (2000) extracting the samples with 80% methanol, because TPC in rye extrudates was decreased by about 40% at extrusion temperature 120℃, and at 200℃ an increase was observed in content of these compounds by about 11% in comparison to raw material. In our results, an increased of rutin in plant material after extrusion in comparison to raw control material could explained by the release of these compound from cell wall, the possible reason of high content rutin in extruded flour was that starch gelatinization and protein denaturation happen partially during specific extrusion processing by 180 of high extursion temperature and high level of SME (315.43 kJ/kg) that could shorten boiled time and keeping rutin in product.

Although extrusion is a high temperature process that transforms raw ingredient into modified intermediate or finished product, the processing time at high is very short. When seed, stem and sprout were extracted with water, the rutin content is much lower in control and ME, compared to in solvent extracted ones. But the rutin content is significantly higher in ME than in control, which keeps intact cell wall structure, even though rutin is destroyed by high temperature in ME. This result shows that if rutin, which is water insoluble, is extracted in water, cell wall structure is disintegrated by a process as extrusion using high temperature and high shear.

Some investigators have studied the effects of extrusion conditions on the properties of flour. Previously studies show the effect of extrusion conditions, including the feed rate, feed moisture content, screw speed, and barrel temperature on the physicochemical properties (density, expansion, water absorption index [WAI]), and water solubility index (WSI) and sensory characteristics (hardness and crispness) in grain of cereal plants.

However, we found few published paper concerning the effect of extrusion on the functional properties and bioactive compound in aboveground of buckwheat such as stem and sprout. Therefore, the aim of this investigation was not only to determine the effect of extrusion on dietary fiber profile and rutin contents of different part of tatary buckwheat. It was very interesting to know how the extrusion conditions influence the bioactive compounds including rutin and the dietary fiber profile of the different part of buckwheat. We found a good correlation between the result of extrusion process and dietary fiber profile and rutin content. So the best condition of extrusion process was registered in sprout with high content of rutin and increased SDF. Also, it can be concluded that the content of rutin differs in different parts of the buckwheat plant in ascending order from seed, stem and sprout. The highest content of rutin can be found in buckwheat sprout of control and extrusion.

We suggest that sprout of buckwheat has been used for isolating rutin for pharmaceutical purposes and for the preparation of herbal tea mixes by extrusion. The idea of a wider use of buckwheat sprout in the food industry leads to the construction of an original formula of a functional food product.

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