

Functional Properties of Cereal and Legume Based Extruded Snack Foods Fortified with By-Products from Herbs and Vegetables

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ABSTRACT

This study demonstrated the effect of extrusion processing on the functional properties of extruded snack foods developed from cereal and legumes, and the by-products from herbs and vegetables. The functional properties considered were antioxidant capacity, total phenolic compounds and resistant starch content. The results showed that Japanese green tea had the highest antioxidant capacity and phenolic content (68.31 mmol Trolox/g and 337.58 mg GAE/g, respectively) and egoma leaves had the second highest (8.35 mmol Trolox/g and 60.60 mg GAE/g, respectively). Red kidney beans had the highest resistant starch content (33.78 % w/w) and corn grits had the second highest content (13.67% w/w). The extrusion process slightly decreased the antioxidant capacity (3.61-13.07%) and phenolic content (4.54-29.75%), but substantially decreased the resistant starch content (89.17-96.33%) for all extruded products. The extrusion process was suitable to produce functional snack foods, while retaining their antioxidant capacity.

Key words: cereal-legume snacks, herbs, vegetable by-products, functional properties, extrusion

INTRODUCTION

Cereals and legumes, in general, play an important role in human nutrition. Recent studies have shown that cereals and beans contain constituents that have health benefits for humans, such as antioxidants and anti-disease factors (Ragaei *et al.*, 2006). Studies have also demonstrated that foods with high carbohydrate and dietary fiber content, such as resistant starch (Kutos *et al.*, 2003) derived from cereals, allowed withdrawal of oral hypoglycemic agents or a reduction in the insulin dose for diabetic subjects (Pathak *et al.*, 2000). Additionally, several health

claims on grain dietary components have been approved by the Food and Drug Agency in the USA.

Extrusion processing has become one of the major processes for producing convenience foods. Extruded foods range from breakfast cereals to snack foods containing modified starches and flour (Harper, 1981). Snack foods have become an integral part of the daily food intake of the majority of the world's population. Basically, they are prepared from natural ingredients or components according to predesigned plans to produce products with specified quality.

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Extruded snack products are predominantly made from rice flour or starch and tend to be low in protein and have a low biological value, as they have a low concentration of essential amino acids. A great deal of attention has been paid to fortifying the extruded food with cereals high in protein and lysine to improve the essential amino acids content. Generally, cereals and legumes, such as red kidney beans, soy and corn, have been used to make highly nutritious products (Baskaran and Bhattacharaya, 2004). Consumers have expressed concern about the functional properties, as well as the nutritional quality of products.

Several researchers have studied the effect of the extrusion process on antioxidant capacity. Camire *et al.* (2007) studied the functionality of fruit powders in extruded corn breakfast cereals. Dlamini *et al.* (2007) studied the effect of extrusion cooking on the antioxidant activity of sorghum-based products and compared it with raw grain. Kim *et al.* (2006) also studied the effect of extrusion processing on the resistant starch content.

Recently, the recovery and use of biomolecules from agricultural by-products has been gaining more attention. By-products from vegetables and herbs have been extracted and studied *in vitro* for their health benefits, but there has been little study on their use in convenience foods. In this study, by-products from the vegetables, yacon stem (*Smallanthus sonchifolius*) and carrot leaf (*Daucus carota*) and the herbs, garlic (*Allium sativum*), egoma (*Perilla flutescens* var. *flutescens*) and Japanese green tea (*Camellia sinensis*), were used to study their influence on the product quality and functional properties of extruded foods. Starch or flour from rice, red kidney beans, broad beans, corn grits, soybeans, black soybeans and green peas were also studied for their resistant starch content that has been reported to have positive health benefits (Goni *et al.*, 1995).

In the present study, functional products were developed by the extrusion process using by-products of selected vegetables and herbs. Further, the products were evaluated for their functional properties with reference to antioxidant activity, and total phenolic and resistant starch content.

MATERIALS AND METHODS

Vegetable and herb samples

Yacon stem (*Smallanthus sonchifolius*) was supplied from a local farm (Tsukuba-yacon-no-kai, Japan). Carrot leaf (*Daucus carota*) was obtained from the Miyazaki Prefecture Foods Development Center (Miyazaki, Japan). Garlic (*Allium sativum*) and Japanese green tea (*Camellia sinensis*) were purchased from a local supermarket (Japan). Egoma (*Perilla flutescens* var. *flutescens*) was supplied by the Fukushima Prefecture Agricultural College (Fukushima, Japan).

Cereal and legume grains

Rice (*Oryza sativa*) starch and soy beans (*Glycine max*) were purchased from a local supermarket (Japan). Red kidney beans (*Phaseolus vulgaris*), broad beans (*Vicia faba*), black soy beans (*Glycine max*) and green peas (*Pisum sativum*) were supplied by Fujicco (Kobe, Japan). Corn grit (*Zea mays*) was obtained from Nisshin Foods (Japan).

Chemicals

1,1- Diphenyl -2- picryl- hydrazyl (DPPH), methanol, 6- hydroxyl- 2,5,7,8 – tetramethyl-chroman- 2- carboxylic acid (Trolox), Folin-Ciocalteau reagent, sodium carbonate, gallic acid monohydrate, ethanol, potassium hydroxide, sodium acetate, acetic acid, hydrochloric acid, sodium hydroxide, food grade calcium carbonate and D(+)-glucose were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). The resistant starch assay kit was purchased from Biocon Japan Ltd (Nagoya, Japan).

Sample preparation

The vegetables and herbs used in this study were dehydrated in a freeze dryer (model TF 20-85 ATNNS; Takara Seisakusho Co. Ltd., Tokyo, Japan), ground (Vita-Mix model Absolute; Osaka Chemical Co. Ltd., Osaka, Japan) and sieved through a 50-mesh sieve (British standard). The powder from each sample was packed separately in an aluminum bag and stored at -20°C until further use. Cereal and legume grains (except for rice and corn grits) were ground by a hammer mill (model 1018-S-3; Yoshida Seisakusho Co. Ltd., Tokyo, Japan) using a 0.5 mm screen, packed in plastic bags and stored at 4°C until further use.

For each sample, 1 g was extracted with 80% methanol (10 mL) on an ultrasonic machine for 15 min and centrifuged at 5,000 rpm for 10 min. The precipitate was rinsed with 80% methanol (10 mL) twice. The supernatant was used for analyses of antioxidant activity and total phenolic content after being diluted to proper concentration with 80% methanol.

Antioxidant capacity: DPPH radical-scavenging activity

The antioxidant capacity of samples before and after the extrusion process was determined with a stable radical, DPPH, as described by Tachibana *et al.* (2001), with some modifications. In brief, antioxidant activity was determined by reacting the methanolic extract of a sample (3 mL) with 200 µM DPPH (3 mL). Absorbance of the samples was measured at 515 nm after 40 min incubation at room temperature in darkness. A calibration curve was constructed using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a synthetic, hydrophilic vitamin E analogue, as an external standard with a range of concentrations from 0 to 100 µM. Results were expressed as Trolox equivalents (Sensoy *et al.*, 2005).

Total phenolic content

The total phenolic content of initial samples and extruded products was determined by the Folin-Ciocalteu method, as described by Li *et al.* (2007) with some modifications. One mL of Folin-Ciocalteu reagent (1:10 diluted) was added to 0.2 mL of methanolic extract sample. After 4 min, 0.8 mL of saturated Na₂CO₃ solution was added. After 30 min of incubation at room temperature, the sample was centrifuged for 10 min at 5,000 rpm. Absorbance of supernatant was measured at 765 nm. Gallic acid was used to calibrate the standard curve. The results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of sample.

Resistant starch content

The resistant starch (RS) content of the starch/flour for rice, red kidney beans, broad beans, corn grits, soy beans, black soy beans and green peas, and the extrudates were measured according to the procedure supplied with the resistant starch assay kit. In brief, to each 100 mg of sample, 4 mL of mixture containing pancreatic α-amylase (10 mg/mL) and amyloglucosidase (3 U/mL) was added and incubated in a shaking water bath at 37°C for 16 h. After incubation, 4 mL of ethanol (99%) was added to stop the reaction, the suspension was stirred vigorously and centrifuged at 1,500g for 10 min. The supernatant was removed and 8 mL of 50% (v/v) ethanol was added to the residue and stirred. The extraction procedure was repeated three times. The 50% ethanol-washing step was repeated once more. Then, 2 M KOH (2 mL) was added to the residue, with gentle stirring in an ice water bath to dissolve the residue for 20 min, after which 1.2 M sodium acetate buffer (8 mL, pH 3.8) and amyloglucosidase (0.1 mL, 3300 U/mL) were added. Samples were incubated in a water bath at 50°C for 30 min and centrifuged at 1,500g for 10 min. To the supernatant (0.1 mL), 3 mL of glucose oxidase-peroxidase-aminoantipyrine (GOPOD, >12,000 U/l glucose

oxidase; >650 U/l peroxidase; 0.4 mM 4-aminoantipyrin) was added and the mixture was incubated in the water bath at 50°C for 20 min. Absorbance was measured using a spectrophotometer at 510 nm. Sodium acetate buffer (0.1 M, pH 4.5) and glucose (1 mg/mL in 0.2% benzoic acid) were used as a blank and glucose standard, respectively (Kim *et al.*, 2006). Each analysis was performed in triplicate. The % resistant starch was calculated using Equations 1 and 2:

$$\text{Resistant starch (g/100 g sample)} = \Delta E \times F/W \times 90 \quad (\text{samples containing} > 10\% \text{ RS}) \quad (1)$$

$$= \Delta E \times F/W \times 9.27 \quad (\text{samples containing} < 10\% \text{ RS}) \quad (2)$$

where, DE = absorbance (reaction) read against the reagent blank

F = conversion from absorbance to micrograms

W = dry weight of sample analyzed

Effect of extrusion process on functional properties of extruded products

Extrusion was performed in a co-rotating, twin-screw extruder (model TEX 30FC-18.5 PW-V; The Japan Steel Works Co. Ltd., Hiroshima, Japan) consisting of four barrels and a maximum screw speed of 439 rpm. For the study, the screw speed was set at 200 rpm and the moisture content of the mixed raw materials was 16% and vegetables or herbs content was 3%. After stable conditions were established, the extrudates were collected and dried in a hot-air oven at 60°C for 15 min. The extruded products were stored at -20°C in aluminum bags until further analysis. All experiments were conducted in triplicate.

The composition of the ingredients used for the process of extruded products is given in Table 1. The temperature profile of the extruder was: barrel 1 = 70°C, barrel 2 = 90°C, barrel 3 = 140°C, barrel 4 = 120°C and die temperature = 150°C.

Table 1 Composition of ingredients used to produce extruded foods.

| Ingredient | % |
|----------------------------|------|
| Rice starch | 29.5 |
| Red kidney bean flour | 20.0 |
| Broad bean flour | 15.0 |
| Corn grit | 10.0 |
| Sugar | 6.0 |
| Soy flour | 5.0 |
| Black soy flour | 5.0 |
| Green pea flour | 5.0 |
| Vegetable or herbal powder | 3.0 |
| Calcium carbonate | 1.5 |

Statistical analysis

All experiments were performed in triplicate and results were expressed as means \pm standard deviation and analyzed by the SAS statistical software, version 9.1. Multiple comparison of the means was carried out by a least significant difference (LSD) test at $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

Antioxidant capacity

Radical-scavenging activity, employing DPPH, has been extensively used in the field of food processing for screening the antioxidant capacity of agricultural produce (Robards *et al.*, 1999; Sanchez-Moreno, 2002). In the current study, DPPH was expressed as mmol Trolox/ g dry sample. The antioxidant capacity of the by-products of vegetables, herbs and raw materials of cereals and legumes evaluated is shown in Table 2. The methanolic extract of vegetables, herbs and raw materials (starch or flour) exhibited obviously different antioxidant capacities. These values ranged from 1.21 to 3.64 mmol Trolox/ g (vegetables), 0.04 to 68.31 mmol Trolox/ g (herbs) and 0.01 to 0.87 mmol Trolox/ g (cereals and legumes). Among the samples analyzed, Japanese green tea showed the highest antioxidant capacity, with 68.31 mmol Trolox/ g, followed by egoma and carrot leaves, with 8.35 and 3.64 mmol Trolox/ g,

respectively. This result was in agreement with other studies (Azuma *et al.*, 1999; Iris *et al.*, 1999) and demonstrated that green tea and egoma leaves were rich in phenols that exhibited high antioxidant properties. The lowest antioxidant capacity was obtained from rice starch with 0.01 mmol Trolox/ g.

Total phenolic content

The content of total phenolic compounds has been used for determining antioxidant capacity (Wang *et al.*, 2008). In this study, the phenolic content of the by-products of vegetables, herbs and raw starch and flour materials was expressed as mg GAE/g dry sample. The content of phenolics of the samples is presented in Table 2. The methanolic extracts of samples showed obviously different amounts of total phenolics. The level of phenolic compounds ranged from 8.59 to 22.80

mg GAE/g (vegetables), 7.72 to 337.58 mg GAE/g (herbs) and 0.20 to 5.11 mg GAE/g (cereals and legumes), respectively. As was the case with antioxidant activity, Japanese green tea showed the highest content of phenolics, with 337.58 mg GAE/g, followed by egoma and carrot leaves, with 60.60 and 22.80 mg GAE/g, respectively. The lowest phenolic content was obtained from rice starch with 0.20 mg GAE/g.

The comparative data showed that Japanese green tea had significantly ($p < 0.05$) higher phenolic content and antioxidant capacity than other samples. Since, the Japanese green tea exhibited higher antioxidant capacity with a higher phenolic content, it could be used as a good source of antioxidants in food formulation (Richelle *et al.*, 2001). Products including ice cream, bakery items and beverages, have been fortified with

Table 2 Antioxidant capacity, total phenolic and resistant starch content of vegetables, herbs and raw materials evaluated by DPPH radical-scavenging activity, Folin-Ciocalteu method and resistant starch assay kit.

| Sample | Antioxidant capacity (mmol Trolox /g sample) [†] | Total phenolic content (mg GAE / g sample) [†] | Resistant starch content (% w/w) [†] |
|--|--|--|--|
| Vegetables | | | |
| Yacon stem | 1.21 ± 0.04 ^d | 8.59 ± 0.92 ^d | Not detected |
| Carrot leaf | 3.64 ± 0.04 ^c | 22.80 ± 0.28 ^c | Not detected |
| Herbs | | | |
| Japanese green tea | 68.31 ± 1.23 ^a | 337.58 ± 10.12 ^a | Not detected |
| Egoma leaf | 8.35 ± 0.22 ^b | 60.60 ± 1.47 ^b | Not detected |
| Garlic | 0.04 ± 0.00 ^f | 7.72 ± 0.68 ^{de} | Not detected |
| Raw materials (cereal/legume) | | | |
| Rice starch | 0.01 ± 0.00 ^f | 0.20 ± 0.07 ^h | 0.11 ± 0.02 ^e |
| Red kidney bean flour | 0.87 ± 0.03 ^e | 5.11 ± 0.17 ^{ef} | 33.78 ± 0.67 ^a |
| Broad bean flour | 0.28 ± 0.01 ^f | 1.80 ± 0.06 ^{gh} | 1.40 ± 0.05 ^c |
| Corn grit | 0.03 ± 0.00 ^f | 0.56 ± 0.07 ^h | 13.67 ± 0.11 ^b |
| Soy flour | 0.17 ± 0.01 ^f | 4.21 ± 0.10 ^{fg} | 0.08 ± 0.01 ^e |
| Black soy flour | 0.13 ± 0.01 ^f | 4.05 ± 0.04 ^{fg} | 0.06 ± 0.01 ^e |
| Green pea flour | 0.03 ± 0.00 ^f | 0.32 ± 0.04 ^h | 0.63 ± 0.09 ^d |

[†] Mean ± standard deviation of triplicate analysis. Values with different letters within the same column are significantly different at $p < 0.05$.

Japanese green tea and developed by various processing techniques.

Resistant starch content

Resistant starch has been recognized as a functional fiber (prebiotic), performing an important role in the digestive physiology of the colon. Similar to oligosaccharides, especially fructo-oligosaccharides, it bypasses digestion and provides fermentable carbohydrates for colonic bacteria. Resistant starch has also been shown to provide benefits, such as the production of desirable metabolites, including short-chain fatty acids in the colon. In addition to its therapeutic effects, resistant starch provides a better appearance, texture and feeling in the mouth than conventional fibers (Martinez *et al.*, 1998). In the current study, resistant starch was measured according to the procedure described in the resistant starch assay kit and expressed as % (w/w). The resistant starch content of the raw starch and flour materials is shown in Table 2. The values ranged from 0.06 to 33.78% (w/w). Among the materials screened, the red kidney beans contained the highest content of resistant starch with 33.78% (w/w), followed by the corn grits and broad beans, with 13.67 and 1.40% (w/w), respectively. The lowest resistant starch content was obtained from the black soy beans with 0.06% (w/w).

Effect of extrusion process on functional properties of extruded products

The effects of the extrusion process on antioxidant capacity, and the phenolic and resistant

starch content of the raw materials used in this study are shown in Figure 1. The % loss in the above parameters is given in Table 3. The results showed that the loss of antioxidant capacity in the carrot leaf snack was the highest with 13.07%, followed by the Japanese green tea snack, with 11.83%. The egoma leaf snack showed the lowest loss of 3.61%. These results were in agreement with the report of Anton *et al.* (2009), who found a significant decrease in the antioxidant capacity of extruded snacks containing common bean (*Phaseolus vulgaris* L.) flour.

As was the case with antioxidant capacity, the change in the phenolic content also was expressed as % loss. The effect of the extrusion process on the reduction in phenolics was highest (29.75%) in the carrot leaf snack, followed by the garlic snack, with 27.64%. The yacon stem snack showed the lowest loss with 4.54%. Sensoy *et al.* (2005) concluded that extrusion caused changes in polar compounds. It has been known that the mechanical energy supplied by the extruder can influence the nature of some complexes formed between the flour components and influence the degradation of larger molecules, such as starch. In the current study, the results showed a minimum loss in the phenolic content of the samples before and after extrusion.

After extrusion, the reduction in the resistant starch content was high for all the snack foods, with values for snacks made from yacon stem, carrot leaf, Japanese green tea, egoma leaf and garlic of 96.26, 89.17, 91.57, 89.98 and

Table 3 Percentage loss of functional properties after extrusion process.

| Sample | % Loss | | |
|-------------------|----------------------|------------------|--------------------------|
| | Antioxidant capacity | phenolic content | Resistant starch content |
| Yacon snack | 4.63 | 4.54 | 96.26 |
| Carrot leaf snack | 13.07 | 29.75 | 89.17 |
| Green tea snack | 11.83 | 13.11 | 91.57 |
| Egoma leaf snack | 3.61 | 8.85 | 89.98 |
| Garlic snack | 4.48 | 27.64 | 96.33 |

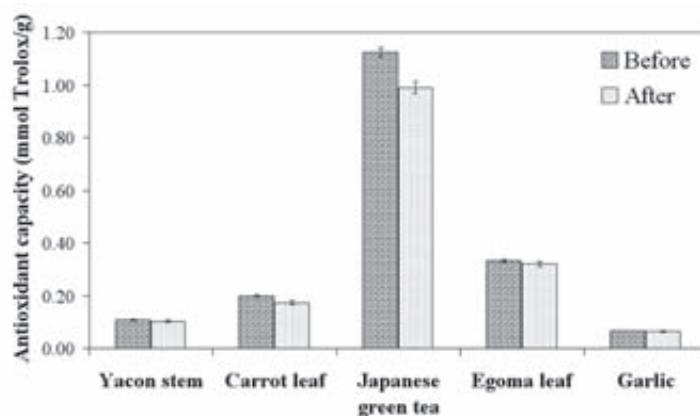


Figure 1 (A)

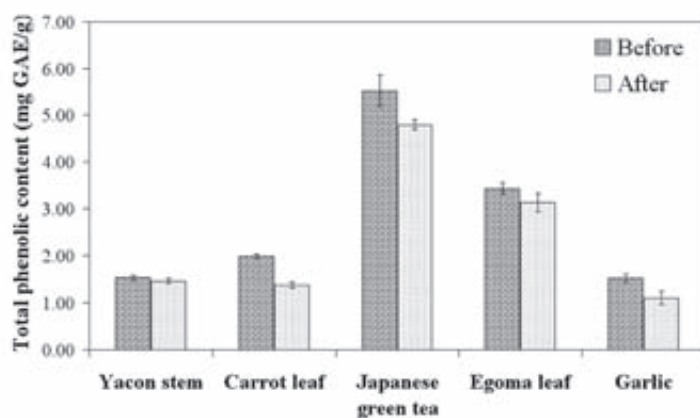


Figure 1 (B)

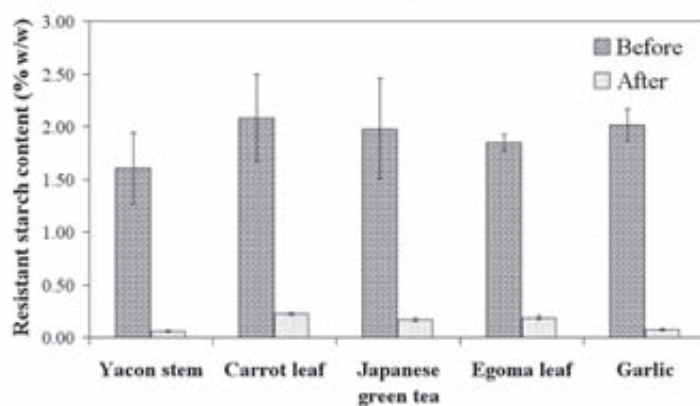


Figure 1 (C)

Figure 1 Effect of extrusion process (before and after) on functional properties of extruded products: (A) antioxidant capacity; (B) total phenolic content; and (C) resistant starch content. Each value is presented as mean \pm standard deviation. The vertical bars indicate standard errors.

96.33%, respectively. The resistant starch may have been digested as a result of the high temperature and pressure during processing, thus changing from insoluble to soluble starch, while the total content of starch was not changed. These results were quite similar to the study by Faraj *et al.* (2004), who reported that the amount of resistant starch in native flour was generally found to be higher than in extruded starch or flour samples. Parchure and Kulkarni (1997) also reported a lower content of resistant starch after extrusion cooking of rice and amaranth starch compared to pressure cooking. Ostergard *et al.* (1989) did not observe any resistant starch formation during extrusion of barley flour. Siljestrom *et al.* (1986) observed a decrease in the resistant starch content in extruded wheat flour and reported that there were no changes in dietary fiber when wheat flour was extruded.

The literature on resistant starch formation in extruded cereal flours shows contrasting information. A few reports reported the formation of resistant starch during the extrusion of cereal flour in a mixed system. Huth *et al.* (2000) observed that up to 6 % resistant starch formed during extrusion of barley flour, followed by frozen storage at -18°C for 3-7 days. Unlu and Faller (1998) reported the formation of resistant starch during extrusion of corn meal blended with high amylase maize starch or in the presence of citric acid.

CONCLUSION

Japanese green tea had the highest antioxidant capacity and phenolic content (68.31mmol Trolox/g and 337.58 mg GAE/g, respectively) and egoma leaves had the second highest (8.35 mmol Trolox/g and 60.60 mg GAE/g, respectively). The raw material with the highest resistant starch content was red kidney beans (33.78% w/w), followed by corn grits (13.67% w/w). Slight changes in the functional properties

were observed in the extruded snacks, with a slight decrease in antioxidant capacity, but a greater amount of both phenolic and resistant starch content. Further studies are in progress to evaluate the effect of the extrusion process on the physical, chemical and functional properties of the products, to optimize the conditions for selecting suitable extruded products for sensory evaluation.

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