

Effects of Extrusion on Dietary Fiber and Isoflavone Contents of Wheat Extrudates Enriched with Wet Okara

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ABSTRACT

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Okara is the residue left after soymilk or tofu production. In North America, okara is used either as animal feed, fertilizer, or landfill. The purpose of this study was to use wet okara to produce and enrich extruded cereal products and to study the effects of extrusion on the dietary fiber and isoflavone contents. Wet okara was combined with soft wheat flour to produce two different formulations (33.3 and 40% okara) and extruded using four combinations of two screw configurations and two temperature profiles. Various physicochemical properties, dietary fiber by enzymatic-gravimetric method, and isoflavone content by HPLC were

analyzed. The radial expansion ratio decreased as fiber content increased. On the other hand, both bulk density and breaking strength increased as fiber content increased. Combining okara with soft wheat flour resulted in increased protein, dietary fiber, and isoflavone contents compared with soft wheat flour alone. Extrusion of the formulations resulted in decreased insoluble fiber ($\leq 25.5\%$) and increased soluble fiber ($\leq 150\%$) contents of extrudates. Extrusion decreased the total detectable isoflavones ($\leq 20\%$) and altered the distribution of the six detected isoflavones.

Soybeans have grown in popularity in recent years due to their many attributes and versatility. The most notable attributes of soybeans are their oil and protein contents, however they are also a good source of dietary fiber and isoflavones.

Throughout their existence, soybeans have been used to make many food products. Soy foods are typically divided into two categories: nonfermented (soy flour, tofu, soymilk, and okara) and fermented (tempeh, miso, and soy sauce) (Golbitz 1995). Two popular products in the United States are tofu and soymilk. The production of each of these results in a by-product called okara (soy pulp). In the United States, okara is typically discarded but it may be used as animal feed or fertilizer. In Japan, however, it is used as a food source (e.g., okara tempeh). In any country, discarding okara as waste is potentially an environmental problem because okara is highly susceptible to putrefaction. Okara also has a high moisture content ($\approx 80\%$), making it difficult to handle and too expensive to dry by conventional means.

Okara contains $>20\%$ protein and $>50\%$ dietary fiber (Watanabe and Kishi 1984). Wang and Murphy (1996) reported that okara contains $\approx 10\%$ isoflavones, the much sought after phytochemicals present in raw soybeans. In light of these attributes, okara is a suitable candidate for nutritional enrichment for cereal-based products.

Finding convenient ways to incorporate okara into food could eliminate a possible source of pollution and add economic value to this currently valueless product. Extrusion could provide a convenient and relatively low cost way to incorporate the okara into food products. The extruder could serve as a cooker, drier, and sterilizer for okara-enriched products.

The purpose of this study was to use wet okara to produce extruded enriched cereal products and to determine the effects on dietary fiber and isoflavone contents of okara-enriched extruded cereal products due to extrusion.

MATERIALS AND METHODS

Wet okara, derived from the production of soymilk, was obtained from American Soy Products Inc. (Saline, MI). Soft wheat flour was obtained from the King Milling Co. (Lowell, MI). Both the okara and the flour were stored frozen (-10°C) until needed.

Wet okara (thawed) and soft wheat flour were combined to form two workable formulations designated A and B (Table I). The wet

okara and flour were weighed in the appropriate proportions and mixed for 3 min with a mixer (A-200, Hobart Mfg. Co., Troy, OH). The materials were mixed in order to develop a flow-like consistency that was imperative for delivering a consistent amount of material per unit time into the barrel of the extruder. Extrusion was performed using a corotating and intermeshing twin-screw extruder (model MP19TC-25, A PV Baker, Grand Rapids, MI), with a 19-mm barrel diameter and 25:1 length to diameter ratio. The materials were fed into the barrel of the extruder with a twin-screw volumetric feeder (K2M, K-Tron Corp., Pittman, NJ). The die used for this study had a single circular orifice with a 3-mm diameter opening.

Two screw configurations (Table II), low shear (LS) and high shear (HS), and two temperature profiles, low temperature (LT; 40, 60, 125, 135, and 145°C from feed port to die exit) and high temperature (HT; 40, 60, 130, 155, and 170°C), were combined to form four extrusion conditions (LS-LT, LS-HT, HS-LT, and HS-HT) for this study. Each formulation was extruded in two replicates at each of the four possible extrusion conditions. The replicates were run on different days. The screw speed was held constant at 100 rpm and the feed rates for samples A and B were 1.95 and 3.71 kg/hr, respectively. The extrusion conditions were chosen after a series of preliminary studies intended to produce extrudates similar to commercial extruded high-fiber breakfast cereal products. The extrusion process used for this study was unique in that no water was injected into the barrel of the extruder in contrast to traditional extrusion processes. The only source of water for the extrusion process was the existing water in the samples, derived mainly from the wet okara.

Samples (2 kg) were collected and dried in an air oven (45°C) overnight. After drying, 1 kg of extrudate was ground using a cyclone mill (Udy Corp., Fort Collins, CO) with a 1.00-mm mesh screen. The remaining 1 kg was left unground. Both ground and unground samples were stored frozen (-10°C) until analyzed.

Measurement of Physicochemical Properties

Proximate analyses were performed using AACC Approved Methods (AACC 1995), with the exception of the total fat analysis. Total fat analysis was based on the procedure described by Foster and Gonzales (1992) using an extraction unit (Soxtec HT 1043, Tecator, Hoganas, Sweden) with modifications. The samples were not weighed into the extraction thimbles and mixed with sand. They were weighed onto 11.0-cm diameter Whatman No. 1 filter papers and placed into the extraction thimbles.

Radial expansion ratio (Harper 1981), bulk density (Park et al 1993), and breaking strength were analyzed for all unground extrudate samples. Breaking strength was determined with a texture analyzer (TA-XT2, Stable Micro Systems, Surry, England) with a

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1.27-cm diameter stainless steel sphere as the crushing implement. Extrudate pieces from a sample were placed into a cylinder with a 1.5-cm diameter opening and a 10-cm depth. Sample weight was recorded, and the sample was then crushed a distance of 1.0 cm at a rate of 1 mm/sec.

Dietary fiber was determined using the enzymatic-gravimetric AOAC method 991.43 (1990). The amounts of total dietary fiber (TDF) and insoluble dietary fiber (IDF) were directly determined by analysis, while the soluble dietary fiber (SDF) was indirectly determined by the difference (TDF – IDF = SDF).

Isoflavone analysis was based on the procedure described by Wang and Murphy (1994), with the exception of the use of methanol as the mobile phase in place of acetonitrile. Sample sizes were adjusted such that the samples A and B contained 1.000 g of okara on a dry basis. Therefore, all isoflavone results are based on 1.000 g of dry okara.

The isoflavone extracts were analyzed by HPLC with an AGP-1 gradient pump and EDM-2 eluent degas module (Dionex Corp., Sunnyvale, CA), a tunable absorbance detector (486b, Waters Corp., Milford, MA), and a Microsorb MV column (5 µm, 100 Å, C₁₈) (Rainin Instrument Co., Woburn, MA). Samples were injected onto a 20-µL loop with an autoinjector (AS 3000, Spectrasystem, Fremont, CA). The isoflavones were detected at 260 nm. A linear gradient (Table III) of two solvents was used.

Statistical Analysis

All statistical analyses were performed with a statistical analysis system (SAS Institute, Cary, NC) using the general linear model procedure. One-way and two-way analyses of variance (ANOVA)

TABLE I
Wet Okara and Soft Wheat Flour Used for Samples A and B

Sample ^a	Okara (%)	Soft Wheat Flour (%)
A	33.33	66.67
B	40.00	60.00

^a A = 11.0% okara (db), 33.0% water, and 56.0% flour (db); B = 14.0% okara (db), 37.5% water, and 48.5% flour (db).

TABLE II
Screw Elements^a Used for Low Shear (LS) and High Shear (HS) Configurations

LS	8 D twin lead screws (TLS), 7 × 30° forward kneading elements (FKE), 8 D TLS, 3 × 60° FKE, 3 × 30° reverse KE (RKE), 2 D single LS (SLS), 4 × 60° FKE, 3 × 30° RKE, 2 D SLS
HS	8 D TLS, 7 × 30° FKE, 8 D TLS, 4 × 60° FKE, 4 × 30° RKE, 2 D TLS, 6 × 60° FKE, 4 × 30° RKE, 1 D SLS, 7 × 90° KE, 2 D SLS

^a One kneading element = 0.25 D (1 D = 19 mm).

TABLE III
Linear Gradient Program Used for HPLC Analysis of Isoflavones

Time (min)	Solvent A (%) ^a	Solvent B (%) ^b
0.0	80	20
2.0	70	30
28.0	30	70
30.0	15	85
35.0	80	20
48.0	80	20

^a 89.9% water, 10% methanol, 0.1% acetic acid.

^b 99.9% methanol, 0.1% acetic acid.

TABLE IV
Proximate Analysis Data (% db) for Nonextruded Samples

Sample	Moisture	Protein	Ash	Fat
Okara	77.65	24.21	4.06	9.83
Flour	10.57	8.54	0.56	1.03
Sample A	33.00	9.75	0.96	1.59
Sample B	37.50	10.44	1.07	1.80

were performed and Fisher's least significant difference (LSD) was used for multiple means comparison.

RESULTS AND DISCUSSION

The proximate analysis data (Table IV) for samples of the two formulations reflect the combination of okara and flour in different ratios. The increased protein content of each of the samples compared with flour alone demonstrates that okara can be used to enrich the protein content of soft wheat flour.

TABLE V
Radial Expansion Ratio, Bulk Density, and Breaking Strength for Extruded Samples

Extrusion Condition ^a	Radial Expansion Ratio	Bulk Density (g/100 mL)	Breaking Strength (N/g)
Sample A			
LS-LT	1.25a ^b	51.15f	38.46c
LS-HT	1.07b	55.65e	39.61bc
HS-LT	1.00c	58.30d	42.54a–c
HS-HT	0.98de	59.40c	45.15ab
Sample B			
LS-LT	1.00cd	55.60e	40.09bc
LS-HT	0.96ef	57.40d	44.28ab
HS-LT	0.95fg	61.30b	45.86a
HS-HT	0.93g	63.10a	47.24a

^a Extrusion conditions used (screw configuration-temperature profile): LS-LT = low shear-low temperature; LS-HT = low shear-high temperature; HS-LT = high shear-low temperature; HS-HT = high shear-high temperature.

^b Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

TABLE VI
Insoluble, Soluble, and Total Dietary Fiber Contents (% db) for Nonextruded and Extruded^a Samples A and B

Sample	% Insoluble	% Soluble	% Total
Okara	43.27	9.98	53.25
Flour	2.22	1.71	3.93
Sample A			
Nonextruded	7.66a ^b	2.08b	9.74a
LS-LT	6.42b	2.82ba	9.24b
LS-HT	6.63b	3.16a	9.79a
HS-LT	5.81c	3.69a	9.50ab
HS-HT	5.71c	3.72a	9.43ab
LSD ^c	0.56	0.98	0.48
Sample B			
Nonextruded	8.84a	1.46d	10.30b
LS-LT	8.50a	2.22c	10.72b
LS-HT	7.31b	3.19ab	10.50b
HS-LT	8.31a	3.09b	11.40a
HS-HT	6.72c	3.64a	10.36b
LSD	0.56	0.48	0.43

^a Extrusion conditions used (screw configuration-temperature profile): LS-LT = low shear-low temperature; LS-HT = low shear-high temperature; HS-LT = high shear-low temperature; HS-HT = high shear-high temperature.

^b Values followed by the same letter in the same column for Samples A or B are not significantly different ($P < 0.05$).

^c Least significant difference.

TABLE VII
Individual and Total Detected Isoflavone Contents (µg/g of dried okara) of Nonextruded Samples

Compound	Okara	Sample A	Sample B
Daidzin	108	83	90
Genistin	141	65	45
Malonyl daidzin	399	360	360
Malonyl genistin	489	465	447
Acetyl genistin	80	142	147
Genistein	24	103	116
Total detected	1,241	1,219	1,205

Radial Expansion Ratio, Bulk Density, and Breaking Strength

Radial expansion ratio, bulk density, and breaking strength data are listed in Table V. Increasing the content of certain ingredients has affected the radial expansion of extruded products (Moore et al 1990, Lue et al 1991, Jin et al 1995). Radial expansion ratio decreased in the present study as fiber content was increased (sample A vs. sample B). Jin et al (1995) suggested that increasing fiber content caused thickening of the cell walls and decreased air cell size in the microstructure of the extrudate, resulting in decreased radial expansion. A decreasing trend was observed in the radial expansion ratio as the extrusion conditions became increasingly more severe for both samples A and B. This may be due to the breaking down of components into smaller particles, which may interfere with bubble expansion, reducing the extensibility of the cell walls and causing premature rupture of steam cells in the extrudate microstructure resulting in decreased radial expansion (Guy 1985).

The bulk density values in the present study increased as radial expansion decreased (Table V). This is in agreement with the findings of other researchers (Hsieh et al 1991, Lue et al 1991, Jin et al 1995). The increase in bulk density is due to an increase in the density of the microstructure of the extrudate as the radial expansion decreases.

The present study revealed that the breaking strength increased as radial expansion ratios decreased (Table V). The increase in breaking strength is presumed to be due to the thickening of the cell walls and the decrease in air cell size. This may provide increased strength to the extrudate microstructure and increased resistance to fracture.

Dietary Fiber Contents in Nonextruded and Extruded Samples

The total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) contents for the nonextruded samples are listed in Table VI. As a result of the combination of okara and flour, the TDF contents for the nonextruded samples A and B increased 147 and 162%, respectively, compared with the TDF content of flour alone, demonstrating that okara can be used to increase the TDF content of soft wheat flour.

Sample A extruded at LS-LT conditions was the only extruded sample to significantly differ (lower) ($P < 0.05$) from the nonextruded sample A in TDF content (Table VI). Sample B extruded at HS-LT conditions was the only extruded sample to significantly differ (higher) ($P < 0.05$) from the nonextruded sample B in TDF content. The increased TDF content for this sample B may be due to the formation of enzyme-resistant starch. Englyst et al (1995) suggested the increase in TDF contents of starchy foods, such as those containing white flour, after heat processing is due to the formation of enzyme-resistant starch during the cooling of the product.

The IDF contents of the extruded products of both samples A

and B decreased for all extrusion conditions when compared with their respective nonextruded samples. These results were in agreement with Wang et al (1993), Qian and Ding (1996), and Ralet et al (1990). Extrudates from sample A had significantly ($P < 0.05$) decreased IDF contents at all conditions. However, of sample B extrudates, only those extruded at LS-LT and HS-HT conditions had significantly decreased ($P < 0.05$) IDF contents. A significant ($P < 0.001$) effect on IDF contents was observed when the temperature profile was changed from low to high for sample B. On the other hand, changing the screw configuration from low to high resulted in a significant ($P < 0.01$) effect for sample A. It appears that the extrusion conditions required to affect the IDF contents are formulation-dependent.

The SDF contents for the extruded samples A and B increased significantly ($P < 0.05$) for all extrusion conditions when compared with their respective nonextruded samples, with the exception of sample A extruded at LS-LT. These observations were in general agreement with Bjorck et al (1984), Caprez et al (1986), Siljestrom et al (1986), Ralet et al (1990), Wang et al (1993), and Qian and Ding (1996). No one parameter had a significant effect on the SDF content for extruded A samples. However, both temperature and screw configuration had independent but significant effects ($P < 0.01$) on the SDF contents of sample B. In general, extrusion resulted in decreased IDF and increased SDF contents for both samples A and B.

Isoflavone Contents in Nonextruded and Extruded Samples

There were no detectable isoflavones in flour, based on spectroscopy at 260 nm. Six isoflavone compounds were identified in okara and in the nonextruded products and extruded samples A and B. Increasing soy and soy isoflavone consumption by non-Asians appears to be highly desirable given the potential for soy and soy isoflavones to mitigate onset of chronic disease (Messina et al 1998). The six isoflavones identified were (in order of elution) daidzin, genistin, malonyl daidzin, malonyl genistin, acetyl genistin, and genistein. The levels of detected isoflavones for nonextruded samples A and B are listed in Table VII.

The glucoside, malonyl, and acetyl forms of daidzin and genistin combined represent 90–98% of the total detectable isoflavones in okara and the nonextruded samples A and B. Wang and Murphy (1994) and Anderson and Wolf (1995) also found $\geq 90\%$ of the total detectable isoflavones to be a combination of the glucoside, malonyl, and acetyl isoflavones in soy products. Malonyl daidzin and malonyl genistin were the most abundant of the isoflavone compounds in okara and the nonextruded samples A and B. Soybeans typically contain 50% more malonyl glycosides than the simple glucosidic forms of the isoflavones. On the other hand, soy milk contains more glucosidic than malonyl forms of the isoflavones. The ratio of glucosidic

TABLE VIII
Individual and Total Detected Isoflavone Contents ($\mu\text{g/g}$ of dried okara) for Nonextruded and Extruded^a Samples A and B

Compound	Nonextruded	LS-LT	LS-HT	HS-LT	HS-HT	LSD ^b
Sample A						
Daidzin	83d ^c	171bc	198b	155c	261a	34.11
Genistin	66c	177b	202b	163b	298a	86.37
Malonyl daidzin	360a	302b	145c	263b	131c	47.36
Malonyl genistin	466a	327b	150d	267c	146d	56.77
Acetyl genistin	142b	121b	238a	119b	212a	36.85
Genistein	104a	36b	36b	32b	29b	17.59
Total detected	1,219a	1,133ab	963c	1,002b	1,072ab	151.49
Sample B						
Daidzin	90b	153a	201a	192a	198a	47.83
Genistin	45b	188a	180a	214a	226a	116.86
Malonyl daidzin	361a	335a	155c	273b	138c	63.50
Malonyl genistin	448a	358b	181c	313b	155c	57.71
Acetyl genistin	147a	104b	181a	104b	180a	41.81
Genistein	116a	36b	35b	32b	40b	27.40
Total detected	1,205a	1,175a	935b	1,133a	967b	84.04

^a Extrusion conditions used (screw configuration/temperature profile) as in Table V.

^b Least significant difference.

^c Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

sides to malonyl isoflavones in okara suggests that extraction of soy protein to produce soy milk preferentially extracts the glucosidic forms of daidzein and genistein, leaving more of the malonyl forms in the okara. There was little difference in total isoflavone contents among the samples. Nonextruded samples of A and B had decreased levels of daidzin, genistin, malonyl daidzin, and malonyl genistin, and increased acetyl genistin and genistein levels when compared with the okara sample. A possible explanation for the redistribution of isoflavones in the two samples may be an interaction between okara and flour and fermentation of the samples during mixing that may have caused the conversion of the glucoside and malonyl isoflavones into the acetyl and aglycone isoflavones through a hydrolysis reaction with glucosidases already in the samples. Wang and Murphy (1994) observed similar distributional changes in fermented soy products.

Table VIII lists the levels of the six detectable isoflavones for the nonextruded and extruded samples A and B. Total isoflavone contents of the extruded samples were 3–22% lower ($P < 0.05$) than those of the nonextruded samples. The decrease may be due to the degradation of the isoflavone compounds as a result of the heat and shear generated by the extrusion process.

The combination of the glucoside, malonyl, and acetyl isoflavones in extruded samples A and B represented 96–98% of the total detected isoflavones. Extruding samples A and B resulted in distributional changes among the detected isoflavones. The glucosides, daidzin and genistin, were significantly increased ($P < 0.05$) for both samples after extrusion regardless of the extrusion conditions. Wang and Murphy (1994) also observed increased glucoside isoflavone contents in soy protein isolate when compared with intact soybeans from the same source. A possible cause for this change could be the loss of the malonyl group, which may have been cleaved by heat and/or shear generated by the extrusion process, resulting in the respective glucoside. Extrusion decreased the malonyl daidzin and malonyl genistin contents for both samples A and B. Significant decreases ($P < 0.05$) were observed for all extrusion conditions for sample A, and all but the sample extruded at LS-LT for sample B. The high temperature conditions resulted in the largest decreases in the malonyl daidzin and genistin contents for both samples. The high temperature extrusion conditions also resulted in significantly increased ($P < 0.05$) acetyl genistin contents for sample A and nonsignificant increases for sample B. Farmakalidis and Murphy (1985) reported that decreased malonyl isoflavone contents were offset by increased acetyl isoflavone contents after toasting defatted soy flakes. Wang and Murphy (1984) suggested that malonyl isoflavones may be converted into their respective acetyl isoflavones by the decarboxylation of the heat-labile malonyl isoflavones.

The aglycone, genistein, was significantly decreased ($P \leq 0.05$) for both samples A and B at all extrusion conditions. This observation was in general agreement with a recent report by Mahungu et al (1999), who also noted a decrease in aglycone in soy during an extrusion process. It is presumed that genistein is either degraded or converted to forms of isoflavones that are nondetectable at 260 nm.

SUMMARY

The overall results indicate that by using a novel twin-screw extrusion process to cook, sterilize, and remove the major portion of water, wet okara can successfully be used to make and enrich extruded products. Increasing the fiber content in this manner resulted in decreased radial expansion, and increased bulk density and breaking strength of extrudates. Incorporating wet okara with soft wheat flour increased the protein, dietary fiber, and isoflavone contents. Extrusion resulted in decreased insoluble dietary fiber and increased soluble dietary fiber contents. Extrusion also resulted in decreased total detectable isoflavone contents and altered the distribution of the six detectable isoflavones.

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