Stability of Isoflavones during Extrusion Processing of Corn/Soy Mixture

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The influence of extrusion processing in the presence of corn on the quantity and quality of genistein, daidzein, and their respective β-glucoside, acetyl glucoside, and malonyl glucoside derivatives was evaluated. Products of 100% soy (textured) and a blend of 20% soy protein concentrate (SPC) and 80% corn meal (direct-expanded) were extruded, with evaluations before and after extrusion. In addition, a $3 \times (3 \times 3)$ split-plot factorial experiment investigated the influence of barrel temperature (110, 130, 150 °C), moisture content (22, 24, 26%), and relative residence time (1, 0.8, 0.6) on extruder response and isoflavone profile. The extrusion barrel temperature had the most influence on isoflavone profile, especially decarboxylation of the malonyl β-glucoside, followed by the moisture content. The amount of extractable isoflavones decreased after extrusion for both the SPC and SPC/corn meal blend when extracted with 80% aqueous methanol but remained approximately the same when first hydrated with water before extraction. However, initially hydrating with water produced enzymatic glycolysis in the unextruded samples, increasing the aglycons dramatically.

Keywords: *Extrusion; isoflavones; corn; soy; HPLC*

INTRODUCTION

Recent studies suggest that consumption of soy-containing foods is associated with lower blood cholesterol, protection against cardiovascular disease, and reduced risk of certain cancers (prostate and breast) and osteoporosis (Messina and Messina, 1991; Caragay, 1992; Anderson and Garner, 1997; Anderson et al., 1995; Potter et al., 1995). Countries with high intake of foods of soy origin, such as Japan, have lower breast cancer rates than in countries with lower intake of soy foods, such as the United States (Messina et al., 1994; Coward et al., 1993; Adlercreutz et al., 1992). It has also been reported that soy intake is good in relieving menopause symptoms (Broihier, 1997). The health benefits associated with high soy intake are derived in part from the isoflavone compounds present in soy. In particular, the isoflavone genistein has received considerable interest due to its ability to inhibit growth of a wide range of cancer cells including human breast carcinoma (Peterson and Barnes, 1991; Messina et al., 1994).

These findings have generated a substantial interest in the food industry to market soy-containing food products, whether traditional soy products such as tofu and soymilk, or foods containing soy ingredients, such as soy isolates, concentrates, and flours. However, the delivery of health benefits from soy will depend on the development of acceptable, mainstream products. The realization of health benefits may also depend on the stability of the isoflavones during processing as well as the specific profile of the isoflavone derivatives in the finished product. It has been reported that the profile of isoflavones depends on the temperature during processing (Wu, 1994; Kudou et al., 1991). Wu (1994) reported that during baking, heat degraded isoflavones and cleaved malonyl groups, acetyl groups, and glycosidic bonds. The effect of heat increased with an increase in heating temperature and time. During extraction of isoflavones at 80 °C some malonyldaidzin, malonylglycitin, and malonylgenistin were deesterified to daidzin, glycitin, and genistin, respectively (Coward et al., 1993; Kudou et al., 1991). These researchers reported that malonyl glucosides and acetyl glucosides were deesterified to their underivatized β-glucosides. Thus, they were able to account for the presence of mostly glycosides in soymilk, tofu, and soy molasses, which are heat-processed at temperatures around 100 °C (Coward et al., 1993). In the same reference, it is reported that the malonyl derivatives are easily decarboxylated to their corresponding acetyl derivatives, thus explaining the high content of acetyldaidzin and acetylgenistin in toasted defatted soyflakes (Farmakalidis and Murphy, 1985).

The presence of undenatured enzymes affects the profile of isoflavones. It has been reported that β-glucosidase specifically hydrolyzes soybean isoflavone glycosides, daidzin and genistin, converting them to daidzein and genistein, respectively (Matsuda et al., 1994; Matsamura et al., 1993). Thus, daidzein and genistein have been found to increase during the soaking of soybeans, the first step in soy milk manufacturing. The maximum production of these isoflavones occurred at 50 °C and at a pH of 6.0. However, the production of these aglycons is inhibited by glucono-δ-lactone which is a competitive inhibitor of β-glucosidase (Matsamura et al., 1993).

Therefore, the type and intensity of food-processing conditions appear to influence the amount and form of soy isoflavones in the final product. However, no infor-

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Table 1. Experimental Treatment Conditions and Extrusion Control Parameters for the Experiment Investigating the Influence of Extrusion Conditions on Isoflavone Profiles[a]

barrel temp (°C)	moisture (%)	feed rate (kg/h)	screw speed (rpm)	RRT
110	18	6.8	150	1.0
130	21	9.1	200	0.75
150	24	11.4	250	0.60

[a] The experiment was a 3 × (3 × 3) factorial with barrel temperature, moisture content, and relative residence time.

mation is available on the stability of isoflavones during extrusion of soy protein/corn meal blend, a primary ingredient of breakfast cereals and snack foods. Extruded cereal blends are a promising nutritious vehicle for incorporation of soy, with breakfast cereals and snack foods leading the category. Therefore, the major objective of this study was to examine how heating and shear conditions required during extrusion processing of a corn meal/soy blend affect the profile of the isoflavones.

MATERIALS AND METHODS

Materials. A blend of 80% corn meal and 20% soy protein concentrate was used in this study. Promax 70 soy protein concentrate (not less than 68% protein) was donated by Central Soya (Ft. Wayne, IN). Promax 70 was selected because of its high isoflavone content. Enriched degerminated corn meal, CC-260, was donated by the Lauhoff Grain Co. (Danville, IL). Initial moisture content of the blend was 12.3%.

Experimental Plan. *Experimental Design*. A 3 × (3 × 3) split-plot factorial design with two replicates was conducted to evaluate the influence of extrusion conditions on isoflavone profile. Three extrusion parameters were investigated at three levels of variation. The parameters studied were barrel temperature, feed moisture, and relative residence time (RRT). The RRT was controlled by increasing the screw speed and feed rate while maintaining a constant specific feeding load of 0.045 kg/h/rpm. The value for RRT was determined by assuming that the extruder volume degree-of-fill remains constant when specific feeding load (SFL) remains constant (Meuser and Wiedmann, 1989). The RRT was calculated as follows:

$$t = V/V^*$$

where t is the average residence time (h), V is the volume of extruder filled (m^3), and V^* is the volumetric flow rate (m^3/h). Then

$$\mathrm{RRT} = t_2/t_1 = (V_2/V_2^*)/(V_1/V_1^*) = V_1^*/V_2^*$$

assuming $V_1 = V_2$. Also assuming a constant extrudate density, RRT becomes the ratio of mass flow rates, M_1^*/M_2^*. Let t_1 correspond to the highest residence time (150 rpm, flow rate = 6.8 kg/h). Therefore,

$$\mathrm{RRT}_{150} = (6.8\ \mathrm{kg/h})/(6.8\ \mathrm{kg/h}) = 1.0$$

$$\mathrm{RRT}_{200} = (6.8\ \mathrm{kg/h})/(9.1\ \mathrm{kg/h}) = 0.8$$

$$\mathrm{RRT}_{250} = (6.8\ \mathrm{kg/h})/(11.4\ \mathrm{kg/h}) = 0.6$$

Table 1 shows the system variables studied. The design was split into barrel temperature as a whole plot and moisture and RRT as subplots within barrel temperature since it was not possible to randomly run all 27 resulting combinations at once. Randomization was used to select the barrel temperatures and to select the moisture and RRT combination within each barrel temperature level.

Table 2. Extruder Conditions for 100% Soy and 80/20 Corn Meal/Soy Blend for the Comparison of Pre- versus Postextrusion Experiment

extrusion parameter	100% soy	80/20 corn meal/soy blend
screw speed (rpm)	300	200
extruded moisture (%)	35	22
feed rate (kg/h)	6.8	13.6
product temperature (°C)	124	123
die pressure (kPa)	1520	2070
motor torque	24	52

Table 3. Screw Configuration in the Extruder Barrel

zone	screw element description flight angle type	no. of screw elements	length of one element (mm)
I	20°, FTLS[a]	1	10
(feed)	42°, FTLS undercut	5	21
II	42°, FTLS	3	42
III	28°, FTLS	14	28
IV	20°, FTLS	11	20
	45°, FKB[b]	2	28
V	20°, FTLS	5	20
	45°, RKB[c]	2	14
	20°, FTLS	5	20
	20°, FTLS	1	10

[a] FTLS, forwarding twin lead screw. [b] FKB, forwarding kneading block. [c] RKB, reversing kneading block. Note: Total screw length is 120 cm with an L/D of 40.

In addition to the factorial experiment above, a 2 × 2 factorial replicated experiment to compare the isoflavone profiles before and after extrusion in the presence and absence of corn meal was conducted. Extruder conditions for this experiment are given in Table 2.

Extrusion Processing. Extrusion was performed using a corotating twin-screw extruder, model ZSK-30 (Krupp Werner & Pfleiderer, Ramsey, NJ), with the screw profile shown in Table 3. For the experiment investigating extrusion environment, the temperature profile in the first three barrel zones from the feeder toward the die plate was 40, 90, and 110 °C, respectively. For the first three zones, this profile was kept constant while changing the last two zones to the desired temperature according to the experimental plan: 110, 130, or 150 °C. A dual orifice die with a 4-mm diameter was used. Product temperature, motor torque, and die pressure were measured just prior to the die plate using a j-type thermocouple and a Dynisco model PT411-3M-9 pressure transducer (Dynisco, Sharon, MA). Extruded samples were dried for 20 min at 90 ± 2 °C in a forced circulation air dryer (Proctor & Schwartz, Inc., Philadelphia, PA). Residual moisture content after drying was measured according to AOAC method 925.10 (AOAC, 1990). For the experiment investigating pre- versus postextrusion, the barrel temperature profile was 40, 90, 120, 120, and 140 °C from feed to die, respectively.

Isoflavone Analysis. *Sample Preparation for the Experiment Evaluating the Influence of Extrusion Environment*. Sample extracts were concentrated prior to HPLC analysis since the products contained only 20% soy protein concentrate. Samples of approximately 2 g of ground soy product were weighed on an analytical balance. The samples were hydrated with 5 mL of HPLC grade water for 4 h at room temperature (25 °C) to soften the starch matrix. Afterward, 20 mL of analytical grade methanol was added, and samples were placed in a water bath shaker with controlled temperature at 30 °C for 2 h. The samples were prefiltered through a Whatman#42 filter paper (Micron Separation, Inc., Westborough, MA), and the residue was washed with 80% methanol. The insoluble residue was discarded, and the filtered portion was dried by first removing the methanol using a water bath with agitation at 35 °C for approximately 5 h and subsequently removing the water by freeze-drying.

The freeze-dried samples were redissolved in 5 mL of 80% aqueous methanol solution and stored at refrigeration temperature (12 °C) until HPLC analysis. Before injection, the

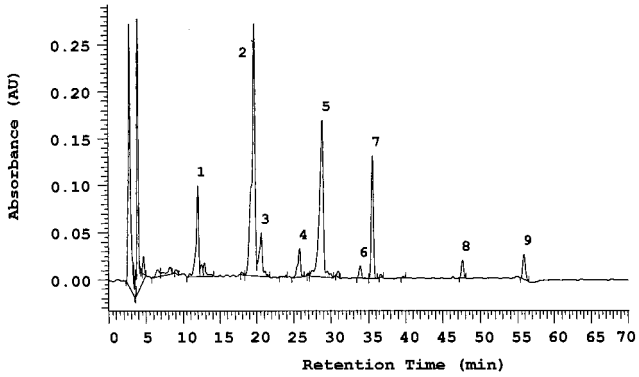

Figure 1. Isoflavone profile of the soy/corn meal blend after extrusion (barrel temperature = 110 °C, feed moisture = 26%, relative residence time = 0.8); absorbance at 260 nm. Peak identifications: (1) daidzin, (2) genistin, (3) malonyldaidzin, (4) acetyldaidzin, (5) malonylgenistin, (6) daidzein, (7) acetylgenistin, (8) genistein, (9) flourescein (internal standard).

samples were filtered through a 0.45-μm filter (Alltech Associates, Inc., Deerfield, IL). One extraction was prepared per sample with one HPLC injection per extraction.

Sample Preparation for the Experiment Evaluating Pre- versus Postextrusion Effects. Samples were prepared in essentially the same manner as described above, except that the samples were not initially hydrated with water followed by extraction with methanol. Instead, the samples were extracted with 80% aqueous methanol. The extraction proceeded for 3 h at room temperature using a magnetic stirrer. The samples were then prefiltered through Whatman #42 filter paper, the residue washed with 80% methanol, and the combined filtrate dried using a rotary evaporator at 30 °C. Subsequently, the samples were handled as described above. Two extractions were prepared for each sample with three injections per extraction.

HPLC Analysis. A Hitachi high-performance liquid chromatograph (Tokyo, Japan) equipped with a L-6200 Intelligent pump and AS-2000 autosampler in combination with a Hitachi L-4500 diode array detector (DAD) was used to quantify the isoflavone content. Separation of isoflavones was achieved using a YMC-pack ODS-AM C_{18} (S-5, 120A, 250 × 4.6 mm) column (YMC, Inc., Wilmington, NC). A YMC S-5, 120A ODS-AM guard column was used. The photodiode detector was monitored from 200 to 350 nm, and the eluting components were detected from their absorbance at 260 nm. The quantitative analysis of the isoflavones was carried out according to the method of Wang and Murphy (1994) with modifications. The mobile phase consisted of 0.1% acetic acid in water (v/v) (solvent A) and 0.1% acetic acid in acetonitrile (solvent B).

For the experiment evaluating influence of extrusion environment, after a 50-mL sample was injected, solvent B was increased from 15 to 35% in 50 min and the flow rate was maintained at 1 mL/min. A typical HPLC chromatogram showing the isoflavone order is shown in Figure 1 (Wang and Murphy, 1994; Coward et al., 1993; Eldrige, 1982). For the experiments evaluating pre- versus postextrusion effects, the HPLC analysis was carried out according to the method of Murphy et al. (1997). After injection of a 20-μL sample, the system was maintained at 15% B for 5 min, then increased to 29% in 31 min, and then to 35% in 8 min. The system was recycled to 15% B at the end of 45 min. The flow rate was 1.0 mL/min for the first 5 min, then increased to 1.5 mL/min for the next 40 min, and returned to 1.0 mL/min for recycle.

Concentration of isoflavones on a dry protein basis was calculated by comparison of the retention time, UV absorption patterns, and area responses with authentic isoflavone standards or with values given in the literature (Wang and Murphy, 1994). Authentic standards for genistein, daidzein, daidzin, and genistin were obtained from the Indofine Chemical Co. (Somerville, NJ). The concentrations of malonyl and acetyl glucosides were calculated from the curves for the corresponding β-glucoside, corrected for molecular weight differences since the molar extinction coefficient of the esterified isoflavone approximates that of the β-glucoside (Barnes et al., 1994).

Table 4. Response of Product Temperature, Motor Torque, and Die Pressure to the Barrel Temperature[a]

barrel temp (°C)	product temp (°C)	motor torque (%)	die pressure (kPa)
110	110a	64.9a	2270a
130	123ab	47.7ab	2170a
150	134b	42.2b	1920a

[a] Means with different superscripts are different at $\alpha = 0.05$.

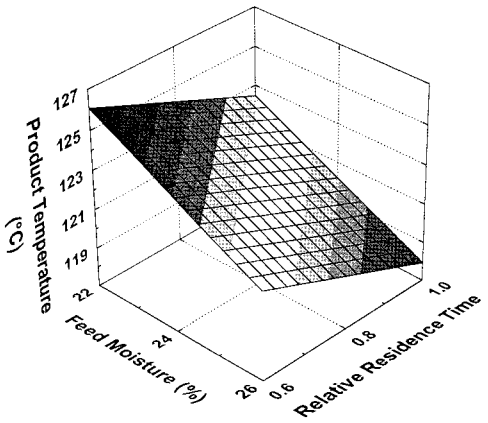

Figure 2. Product temperature response to feed moisture and relative residence time (barrel temperature = 130 °C) for extrusion of an 80/20 corn/soy blend.

Statistical Analysis. The experimental results were analyzed using the General Linear Models (GLM) procedure in SAS, version 6.11 (SAS Institute, Inc., Cary, NC), for both experiments. The three-dimensional plots for the extruder response were made using the Statistica software package, release 5.1 (Statsoft, Inc., Tulsa, OK), from models obtained from the REG procedure with backward elimination model selection in SAS.

RESULTS AND DISCUSSION

Extruder Response. Results from this study indicate that the design parameters (RRT, barrel temperature, and feed moisture content) selected for extrusion processing did influence the chemical reaction environment of the product, as determined by product temperature, die pressure, and motor torque (Table 4; Figures 1−3). Product temperature increased when residence time decreased (Figure 2). This is opposite to what might have been expected since the product remains in the heated barrel for a shorter time. However, a higher screw speed at the low residence time will promote heat generation by shearing. Increasing moisture content decreased product temperature by reducing viscosity and viscous heat dissipation. Product temperature increased as barrel temperature increased (Table 4).

The extruder motor torque is an indicator of viscosity, and it also indicates the amount of mechanical energy used in cooking the product. Motor torque decreased as moisture increased (Figure 3). With respect to RRT, the torque tended to decrease as residence time decreased (high screw speed and high feed rate), being more noticeable at the low-moisture condition. The torque also decreased with an increase in barrel temperature (Table 4). This is to be expected since an increase in temperature will decrease the viscosity of the material inside the barrel. Temperature effects could also explain the

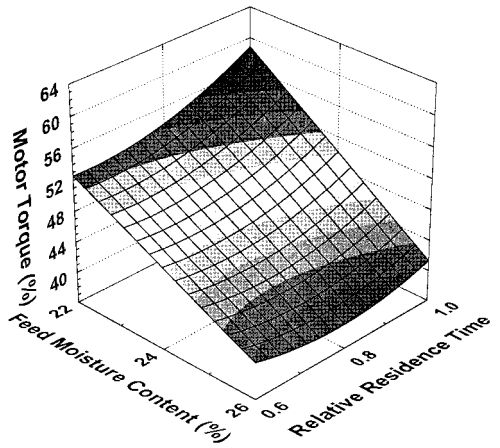

Figure 3. Motor torque response to feed moisture and relative residence time (barrel temperature = 130 °C) for extrusion of an 80/20 corn/soy blend.

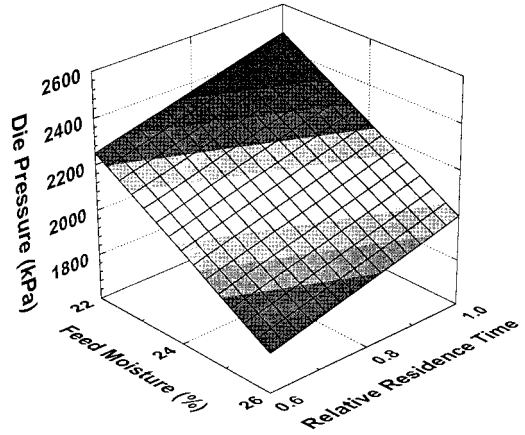

Figure 4. Die pressure response to feed moisture and relative residence time (barrel temperature = 130 °C) for extrusion of an 80/20 corn/soy blend.

motor torque decrease with decreasing residence time since product temperature increased with decreasing RRT.

Die pressure builds as a response to the restrictiveness of the die. Moisture and relative residence time were the most significant variables affecting the die pressure response. When moisture increased the die pressure decreased due to viscosity reduction (Figure 4). With respect to residence time, there was a significant decrease in the die pressure as screw speed and feed rate increased. In general, a higher feed rate leads to higher die pressure. The pressure drop must be due to viscosity reduction, possibly due to a decrease in starch molecular weight because of high shear at the high screw speed. Also, the increase in temperature at the higher screw speed/feed rate condition will contribute to viscosity reduction. Decreasing barrel temperature was accompanied by an increase in die pressure (Table 4) due to an increase in viscosity, although this was not significant statistically.

Influence of Barrel Temperature, Feed Moisture, and Residence Time on Isoflavone Profile. Extrusion had a large influence on the isoflavone profile, while the total amount was little changed. The total levels of genestein and its derivatives tended to decrease, while daidzein and its derivatives tended to increase. Only barrel temperature and feed moisture significantly affected the amount of isoflavones. The

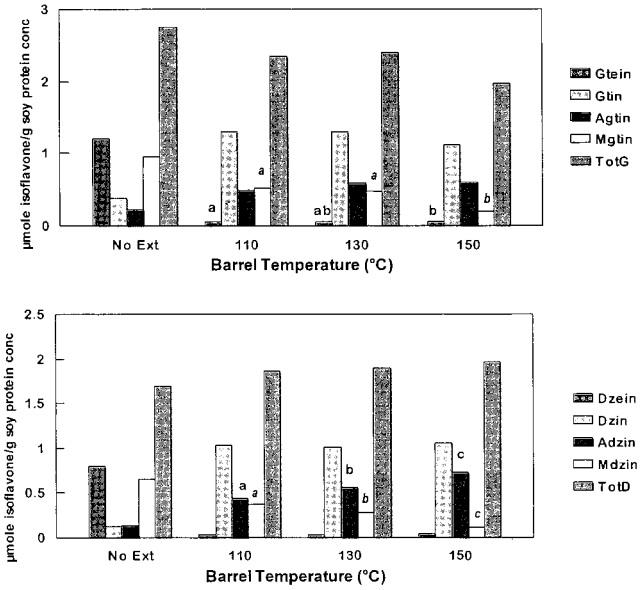

Figure 5. Response of genistein, daidzein, and their glucosides to extruder barrel temperature. Isoflavone concentrations are reported per gram of soy protein concentrate (SPC). Extrudates contained 20% SPC and 80% corn. Data are averaged across feed moisture and RRT. Letters indicate isoflavone derivative concentrations are significantly different at $\alpha = 0.05$.

RRT was not associated with any significant changes in the amount of isoflavones, indicating that equilibrium conditions are achieved quickly. The high concentration of aglycons in the unextruded sample is due to hydrolysis of the simple glucosides by β-glucosidase during extraction (Matsuda et al., 1994; Matsamura et al., 1993) since these samples were first hydrated in water at room temperature for 4 h before extracting with methanol. The β-glucosidase was inactivated by the extrusion process in extruded samples. Heat-induced decarboxylation of the malonyl to acetyl derivatives is also indicated. The heat-induced decarboxylation of malonyldaidzin and malonylgenistin to acetyldaidzin and acetylgenistin has been reported previously (Barnes et al., 1994; Coward et al., 1993; Wang and Murphy, 1996; Wang et al., 1998).

The effect of barrel temperature on genistein and daidzein derivatives is illustrated in Figure 5. The acetyl derivatives of genistin and daidzin increased with an increase in barrel temperature while the malonyl analogues decreased proportionally. This inverse relationship indicates that increased heat treatment produces greater decarboxylation. High temperature causes malonyl conjugates to undergo heat-induced decarboxylation and deesterification. The limited water environment and heat deactivation of the enzyme β-glucosidase during extrusion favored decarboxylation over hydrolysis leading to a greater increase of the acetyl derivative compared to the respective aglycon.

The effect of feed moisture on genistein and daidzein derivatives is illustrated in Figure 6. Neither the aglycons nor the glucosides responded to the moisture content. Significant differences between moisture contents, however, were found for the malonyl and acetyl glucosides. As with the barrel temperature response, a decrease in moisture content caused an increase in acetyl glucoside which was accompanied by a decrease in malonyl glucoside. A decrease in moisture is correlated to an increase in product temperature, and

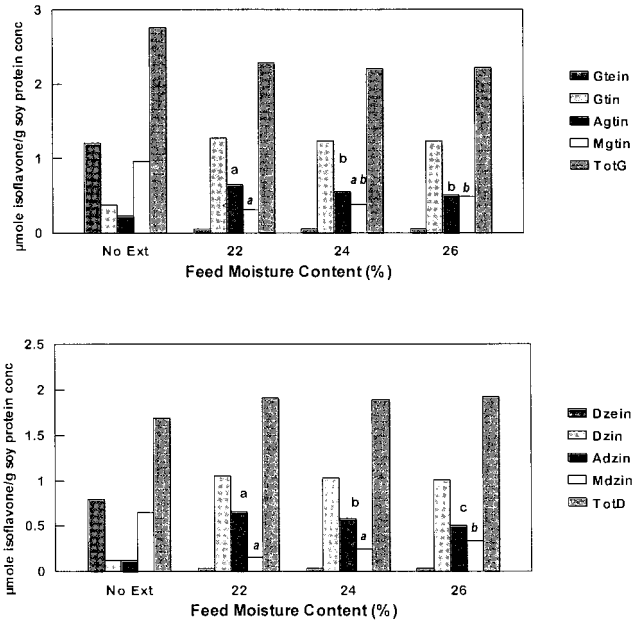

Figure 6. Response of genistein, daidzein, and their glucosides to extruder feed moisture content. Isoflavone concentrations are reported per gram of soy protein concentrate (SPC). Extrudates contained 20% SPC and 80% corn. Data are averaged across barrel temperature and RRT. Letters indicate isoflavone derivative concentrations are significantly different at $\alpha = 0.05$.

Table 5. Mole Fractional Isoflavone Profiles for Genistein and Daidzein Derivatives[a]

extrusion	corn	Gtein	Gtin	AGtin	MGtin
no	no	0.0373b	0.415b	0.191c	0.356a
no	yes	0.0164c	0.521a	0.173d	0.290b
yes	no	0.0443a	0.418b	0.314a	0.223c
yes	yes	0.0183c	0.486a	0.307b	0.189c
extrusion	corn	Dzein	Dzin	ADzin	MDzin
no	no	0.0217c	0.564a	0.121b	0.294a
no	yes	0.0219bc	0.589a	0.153b	0.236b
yes	no	0.0241ab	0.583a	0.268a	0.124c
yes	yes	0.0252a	0.550a	0.304a	0.121c

[a] Letters following means indicate significant difference at $\alpha = 0.05$. Dzein, daidzein; Dzin, daidzin; ADzin, acetyldaidzin; MDzin, malonyldaidzin; Gtein, genistein; Gtin, genistin; AGtin, acetylgenistin; MGtin, malonylgenistin.

therefore, an increase in heat-induced decarboxylation is suspected.

Comparison of Isoflavone Profile Before and After Extrusion. For both the daidzein and genistein derivatives, extrusion increased the percentage of acetyl derivatives at the expense of malonyl derivatives (Table 5) due to heat-induced decarboxylation. This is the same result as found by Wang and Murphy (1996) during cooking of soybeans for manufacture of tofu and tempeh. However, heat-induced deesterification to the simple glucosides as described by Coward et al. (1994) and Barnes et al. (1994) was not found along with only small changes in the aglycons. The presence of corn had less impact on the profiles. Little changes were seen due to the presence of corn in the daidzein derivatives, but the genistein derivatives showed an increase in genistin with corresponding decreases in all three other derivatives. As this was seen in both the extruded and unextruded samples, the result may be due to the preferential difference in extractability of the different compounds.

Table 6. Comparison of Total Isoflavone Contents before and after Extrusion, with and without Corn[a]

		total		
extrusion	corn	daidzein series	genistein series	isoflavones
no	no	2.04b	1.28c	3.32c
no	yes	3.24a	3.52a	6.76a
yes	no	1.83c	1.21c	3.03c
yes	yes	1.83c	2.35b	4.18b

[a] Total daidzein and genistein derivatives and the overall totals of isoflavones are all in μmol/g of soy protein concentrate. Letters following means indicate significant difference at $\alpha = 0.05$.

Generally, the postextruded soy protein sample had lower isoflavone levels than the preextruded sample (Table 6). This could be due to the destruction of isoflavones or to a difference in extractability of the compounds from the matrix. The presence of corn yielded higher isoflavone levels as well; however, it is difficult to assign a cause. Perhaps the lower total amounts of isoflavones in the corn-containing samples were extracted more efficiently since a greater amount of solvent in relative terms was used.

CONCLUSIONS

Extrusion processing of soy-containing ingredients can affect the quantity and quality of the soy isoflavone profile of the finished product. These variations in relative amounts of each soy isoflavone as a result of extrusion need to be considered in the design of clinical studies in which bioavailability and metabolism of isoflavones are being evaluated. Some studies have suggested that the aglycons, especially genistein, show the greatest biological activity (Messina et al., 1994; Anderson and Garner, 1997). In the present study, the malonyl forms of the isoflavones were found to be the most susceptible to the extrusion conditions evaluated. The isoflavones were found to be lower after extrusion than before extrusion for both the soy protein and the corn meal/soy protein blend. Thus, the extruded samples had a lower content of isoflavones per gram of dry weight soy protein than the unextruded samples. These results underscore the need to examine the impact of extrusion on other soy blends in order to characterize changes in isoflavone profiles. Information from the present study in combination with data on the bioavailability and biological activity of isoflavone forms should assist in evaluation of the health impact of soy-containing processed foods.

From a dietary perspective, these findings suggest the food industry may be able to include soy concentrates in a great variety of processed foods, including those requiring extrusion, without large losses in total isoflavones. However, the rapid conversion by β-glucosidase of the glucosidic forms to the aglycons during the water extraction process suggests this same conversion will occur during consumption. Since extrusion alters the relative percentages of each form, the ability of enzymes to convert each form in vivo may have significant health impacts.

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