Effect of Extrusion on Isoflavone Content and Antiproliferative Bioactivity of Soy/Corn Mixtures

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The present studies were conducted to determine changes in the quantities of select isoflavones and in the bioactivity (ability to inhibit proliferation of human breast cancer cell lines) of extracts from blends of soy protein and cornmeal during extrusion processing. The extrusion of samples resulted in an average 24% decrease in the concentration of total isoflavones for all samples. Although the amounts of specific genistein-derived and daidzein-derived forms changed following extrusion, the content of the aglycones genistein and daidzein per g sample generally did not change. The extrusion of samples generally resulted in decreased antiproliferative action toward breast cancer cells, although antiproliferative activity was not eliminated. Therefore, extrusion of soy protein/cornmeal-containing foods are likely to retain a considerable portion of their isoflavone content and some of the health benefits associated with soy.

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

INTRODUCTION

Soy-containing foods have been shown to reduce risks of breast and other cancers and to protect against cardiovascular disease and osteoporosis (Messina and Messina, 1991; Caragay, 1992; Potter, 1995; Anderson and Garner, 1997). Compared to countries with a high intake of foods of soy origin, the consumption of soy foods in the U. S. is very limited. Because of the potential health benefits of soy protein and soy phytochemicals, there is an increasing interest in incorporating soy products into consumer foods. As the marketing of soybean-containing food products expands, there is a critical need for scientific data on the stability of soybean constituents during a variety of food processing techniques and for a better understanding of these processing effects on the biological activity of these soy constituents. The focus of this paper is to determine how extrusion processing, in particular, influences the content and the anticancer bioactivity of soy isoflavones.

It has been reported that a substantial portion of the health benefits of soy-containing foods is due to its content of isoflavones, which include genistein, daidzein, glycetein, and their derivatives as glucosides, 6″-O-acetyl-glucosides, and 6″-O-malonylglucosides. There is some evidence that changes in stability and form of these bioactive isoflavones occur under the conditions of routine food processing (Anderson and Wolf, 1995). For example, it was reported that the extraction of isoflavones at 80 °C causes decarboxylation and deesterification. In the former reaction, the malonyl derivatives are easily decarboxylated to their corresponding acetyl derivatives, whereas in the latter, the aglycones are formed (Kudou et al., 1991; Coward et al., 1993; Farmakalidis and Murphy, 1985). Barnes et al. (1994) reported that 100 °C heating leads to mostly glucosides. In fermented soybean products, Fukutake et al. (1996) reported that the level of genistein was higher than in soybeans and soybean products such as soy milk and tofu. It was suggested that the glycosidic bond of genistein was cleaved to produce genistein by microbes during fermentation. In soy milk manufacturing, the quantity of daidzein and genistein have been found to increase due to the activity of glucosidase. This process is inhibited by this enzyme's competitive inhibitor, glucono-lactone (Matsuura et al., 1989; Matsuda, 1994). Soaking soy in boiling 0.25% $NaHCO_3$ is another means of inhibiting the formation of daidzein and genistein (Ha et al., 1992). It is also known that a protein concentrate of soybean made by aqueous alcohol extraction is very low in isoflavones because the isoflavones are soluble in aqueous alcohol and are, thus, largely removed during processing (Anderson and Wolf, 1995). Taken together, these studies indicate that the type and intensity of food processing conditions will influence the stability and form of soybean isoflavones in the final products. However, the changes in isoflavone quantity, quality, and bioactivity during extrusion processing of soybeans, especially in combination with other food ingredients such as cornmeal, are not known. Soy protein and cornmeal have potential uses as primary ingredients of breakfast cereals and snack foods. Therefore, the effect of extrusion processing on the isoflavone content of combinations of soy protein and cornmeal was chosen for evaluation in our study.

In addition, the effect of extrusion on the ability of extracts of soy protein/cornmeal blends to inhibit neoplastic (transformed or cancerous) and preneoplastic (nontransformed or noncancerous) human breast cancer cell proliferation was examined. Although numerous studies have indicated that the soy isoflavones, genistein

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and daidzein, can inhibit the proliferation of many cancer cell lines (Barnes, 1995), human breast cell lines were utilized here. This is because breast cancer is a leading cause of cancer-related death in American women and because international epidemiological studies and animal experiments point to a potential breast cancer protective benefit of increased soy consumption (Barnes et al., 1990; Coward et al., 1993).

MATERIALS & METHODS

Experimental Plan and Statistical Analysis. Samples were prepared according to a 2 × 2 × 2 factorial plan with two soy protein types, with and without extrusion, along with the presence or absence of blending with corn meal. The samples with corn meal contained 80% corn and 20% soy protein concentrate by weight. Two extrusions were performed on each sample. The experiments to assay antiproliferative activity were organized as a 3 × (2 × 2 × 2) split-plot factorial design with three breast cell types as the whole plots and the samples as the sub-plot. The cell types were BT-20, MCF-7, and MCF-10F. The isoflavone contents of the samples were statistically analyzed as a full factorial design using PROC GLM in SAS with LSD means separation. The cell proliferation results (IC_{50} values) were analyzed as a split-plot factorial design to determine the significance, main effects, and interactions among soy protein type, presence of cornmeal, and extrusion treatment using PROC MIXED in SAS. In addition, LSD means separations were conducted within a cell type by simply analyzing each cell type individually in the same manner as for the isoflavone analysis. All of the IC_{50} analyses were conducted on log-transformed means to equalize the variance across samples and cell types.

Materials. Promax 70 water-washed soy protein concentrate and Procon 2000 alcohol precipitated soy protein concentrate were donated by Central Soya (Ft. Wayne, IN). Promax 70 was selected because of its high isoflavone content. Procon 2000 was selected because of its low isoflavone content. Enriched degerminated corn meal, CC-260, was donated by the Lauhoff Grain Company (Danville, Illinois). MCF-7 and BT-20 cells were obtained from the American Type Culture Collection (Bethesda, MD) and MCF-10F cells from the Michigan Cancer Foundation (Detroit, MI). MCF-7 and BT-20 cells are transformed or neoplastic human breast cells. MCF-10F cells are nontransformed but immortalized human mammary epithelial cells, which were included for the MTT assays as cells representing a preneoplastic stage of breast carcinogenesis (Stampfer et al., 1997).

Extrusion Processing. Extrusion was performed using a co-rotating twin-screw extruder, Model ZSK-30 (Krupp Werner & Pfleiderer, Ramsey, NJ). The soy samples were extruded at 300 rpm screw speed, and the corn/soy blend was extruded at 200 rpm. Extrusion conditions are given in Table 1. The feed rate/screw speed combination selected for the pure protein samples represents the maximum feed rate achievable due to the protein's powder flow properties. The higher feed rates and lower screw speeds for the blended products are necessary for stable operation of the extruder due to improved powder flow properties. The pure protein samples also required greater moisture content because of their tremendous water-binding capacity. The extruded samples were dried for 20 min at 200 °F in a forced-circulation air-dryer (Proctor & Schwartz, Inc., Philadelphia, PA). The residual moisture content after drying was measured according to AOAC methods 925.10 (AOAC, 1990). The barrel temperature profile was 40, 90, 120, 120, and 140 °C from feed to die, respectively. The extruder screw profile is given in Table 2.

Table 1. Conditions and Parameters Used in Extrusion Processing

protein	corn	moisture content (%)	feed rate (kg/hr)	motor torque (%)	die pressure (kPa)	product temp (°C)
Procon 2000	no	35	6.8	22	1060	120
Procon 2000	yes	22	13.6	47	1820	123
Promax 70	no	35	6.8	24	1520	124
Promax 70	yes	22	13.6	52	2070	123

Table 2. Extruder Screw Profile[a]

element type[b]	number of elements	barrel zone
20/10 FTLS	1	I (Feed)
42/21 FTLS undercut	4	
42/21 FTLS undercut-normal	1	
42/42 FTLS	3	II
28/28 FTLS	14	III
20/20 FTLS	11	IV
45/28 FKB	2	
20/20 FTLS	5	V
45/14 RKB	2	
20/20 FTLS	5	
20/10 FTLS	1	

[a] note: total screw length is 115 cm with screw length/diameter of 37. [b] Krupp Werner Pfleiderer, ZSK-30 screw element type: pitch/length (mm) FTLS = forwarding twin lead screw; FKB = forwarding kneading block; RKB = reversing kneading block.

Sample Extraction. Four-gram samples were stirred for 3 h with 20 mL of 80% aqueous methanol and filtered through Whatman #42 filter paper (Micron Separation, Inc., Westborough, MA); the residue washed with 80% methanol. The resulting insoluble residue was discarded, the filtrates were combined, and the total 80% methanol fraction was dried in a rotary evaporator at 30 °C. The dried samples were redissolved in 10 mL of 80% aqueous methanol solution and stored at 4 °C until HPLC analysis. Two separate extractions were prepared for each of two separate extrusions. Each extraction was analyzed by HPLC in triplicate.

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, 120 A, 250 × 4, 6 mm) column (YMC, Inc., Wilmington, NC) with a YMC S-5, 120 A ODS-AM guard column. The photodiode detector was monitored from 200 to 350 nm, and the eluting compounds were detected from their absorbance at 260 nm. The quantitative analysis of the isoflavones was carried out according to the methods 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 acetronitrile (solvent B). 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 increased 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 during recycling.

The concentration of isoflavones on a dry-weight basis was calculated by comparison of retention time, UV absorption patterns, and area responses with authentic isoflavone standards for genistein, daidzein, genistin, and daidzin (Indofine Chemical Company, Somerville, NJ). The concentration of malonyl and acetyl glucosides were calculated from the curves for the corresponding glucoside and corrected for molecular weight differences, because the molar extinction coefficient of the esterified isoflavone approximates that of the glucoside (Wang and Murphy, 1994).

MTT Proliferation Assay. The breast cancer antiproliferative action of each sample was determined by a modification of the MTT assay (Sobottka and Berger, 1992), a procedure that determines the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by the mitochondrial dehydrogenases of viable cells. One mL aliquots from the 80% aqueous methanol extract (prepared from the extruded and nonextruded samples) were dried in vacuo and dissolved in 4 mL assay media. Serial 2-fold dilutions of this were plated along with the breast cells into 96-well tissue culture plates (200 μL medium/well) at cell densities of 2×10^3 cells/well (MCF-7), 5×10^2 cells/well (MCF-10F) and 2×10^3 cells/well (BT-20). After plating, the cells were allowed to attach for 2 d, and the fresh medium containing sample was added. Incubation with the sample was continued for 4 d, at which time reduction of MTT was determined by measuring the optical density at 500 nm. The absorbance at 690 nm was also measured to compensate for the interfering effects of cell debris and the plate itself. The percent survival was determined by comparing the absorbance for extract-treated cells to that obtained for solvent control cells. The results are the average of two different batches of samples from independent treatments. IC_{50} values were assigned by determining the volume of the 80% aqueous methanol extract needed to decrease cell proliferation by 50% compared to controls.

RESULTS

Effect of Extrusion on Soy Isoflavone Content. The first objective of our study was to measure the effect of extrusion processing on the amount of four major forms of genistein (genistin, malonylgenistin, acetylgenistin, and genistein) and of daidzein (daidzin, acetyldaidzin, malonyldaidzin, and daidzein) in samples containing varying blends of soy protein and cornmeal. The data for isoflavone content of Promax 70 has been reported previously as μmol/g soy protein (Mahungu et al., 1999). This Promax 70 data on isoflavone content was included in Tables 3 to 5 (below) for comparison with that of Procon 2000. However, in the present study, the values are expressed as μmol/g sample. As expected, nonextruded samples prepared with Promax 70 contained substantially more total isoflavones than those containing Procon 2000 (Table 3). Extrusion processing resulted in decreases in total isoflavone content for all of the samples examined (Table 3). Samples of Procon 2000 (less isoflavones) and Promax 70 (more isoflavones) without cornmeal exhibited 29% and 9% decreases, respectively, in total isoflavone content compared to nonextruded samples. However, the 9% decrease was not statistically significant. Likewise, extruded cornmeal-supplemented samples Procon 2000 and Promax 70 showed a 24% and 38% reduction, respectively, in total isoflavone content compared to nonextruded samples. The decreases in total isoflavones observed in the extruded samples were similar in magnitude to the decreases in total daidzein-derived and total genistein-derived forms (Table 3). However, for six of the eight extruded samples, there was no change in concentration of the aglycones genistein and daidzein compared to nonextruded ones (Tables4 and 5). With only two exceptions, the most obvious response to extrusion processing in both the corn-supplemented and non-supplemented samples was a decrease in malonyl forms and an increase in acetyl forms. This is consistent with a heat-induced decarboxylation of the malonyl group found by Mahungu et al. (1999) and Barnes et al. (1994). The malonyl forms decreased an average of 50.2%, whereas the acetyl forms increased an average of 26.3%. Another major trend was that the content of simple glucosides was reduced by extrusion in every case, although not all decreases were significant. A de-esterification of the glucoside to yield aglycones is not indicated because the aglycones did not increase with extrusion. Changes in the malonyl glucosides for Procon

Table 3. Content of Total Daidzein-derived, Total Genistein-derived, and Total Isoflavones (μmol/g Sample) in Extruded and Nonextruded Samples[a]

extrusion	corn	soy	ToTD	ToTG	TotIso
no	no	Procon 2000	0.566^{b}	0.552^{b}	1.12^{c}
yes	no	Procon 2000	0.384^{c}	0.413^{e}	0.797^{d}
no	yes	Procon 2000	0.198^{d}	0.296^{f}	0.494^{e}
yes	yes	Procon 2000	0.130^{e}	0.247^{g}	0.377^{f}
no	no	Promax 70	2.04^{a}	1.28^{a}	3.32^{a}
yes	no	Promax 70	1.83^{a}	1.21^{a}	3.03^{a}
no	yes	Promax 70	0.649^{b}	0.703^{b}	1.35^{b}
yes	yes	Promax 70	0.367^{c}	0.469^{d}	0.836^{d}

[a] ToTD = total daidzein; ToTG = total genistein; TotIso = total isoflavones. Means within a column with different superscripts are statistically different ($p < 0.05$). Pure corn samples have no isoflavone content.

Table 4. Content of Genistein Derivatives (μmol/g Sample) in Extruded and nonextruded samples[a]

extrusion	corn	soy	Gten	Gtin	Agtin	Mgtin
no	no	Procon 2000	0.009^{c}	0.291^{b,c}	0.099^{e}	0.153^{c}
yes	no	Procon 2000	0.005^{c}	0.207^{d}	0.107^{e}	0.093^{d}
no	yes	Procon 2000	0.000^{e}	0.125^{e}	0.081^{f}	0.090^{d}
yes	yes	Procon 2000	0.000^{e}	0.097^{e}	0.080^{f}	0.070^{d}
no	no	Promax 70	0.048^{a}	0.533^{a}	0.246^{b}	0.458^{a}
yes	no	Promax 70	0.053^{a}	0.504^{a}	0.379^{a}	0.270^{b}
no	yes	Promax 70	0.012^{b}	0.365^{b}	0.122^{d}	0.205^{b,c}
yes	yes	Promax 70	0.009^{b}	0.228^{c,d}	0.144^{c}	0.089^{d}

[a] Gten = genistein; Gtin = genistin; Agtin = acetylgenistin; Mgtin = malonylgenistin. Means within a column with different superscripts are statistically different ($p < 0.05$).

Table 5. Content of Daidzein Derivatives (μmol/g Sample) in Extruded and nonextruded samples[a]

extrusion	corn	soy	Dzen	Dzin	Adzin	Mdzin
no	no	Procon 2000	0.008^{c}	0.357^{b}	0.068^{c,d}	0.133^{b}
yes	no	Procon 2000	0.004^{d}	0.240^{c}	0.082^{c}	0.059^{c}
no	yes	Procon 2000	0.004^{d}	0.105^{d}	0.039^{d}	0.051^{c}
yes	yes	Procon 2000	0.002^{d}	0.065^{e}	0.041^{d}	0.022^{d}
no	no	Promax 70	0.044^{a}	1.13^{a}	0.250^{b}	0.610^{a}
yes	no	Promax 70	0.044^{a}	1.06^{a}	0.493^{a}	0.228^{b}
no	yes	Promax 70	0.014^{b}	0.380^{b}	0.100^{c}	0.155^{b}
yes	yes	Promax 70	0.009^{c}	0.201^{c}	0.112^{c}	0.044^{c,d}

[a] Dzen = daidzein; Dzin = daidzin; Adzin = acetyldaidzin; Mdzin = malonyldaidzin. Means within a column with different superscripts are statistically different ($p < 0.05$).

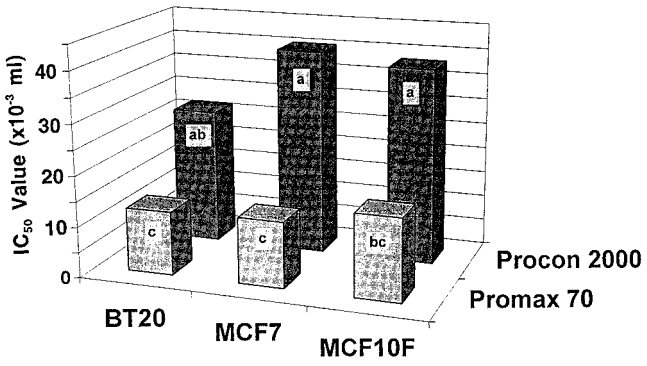

Figure 1. Interactive effect between breast cell type and soy protein type on antiproliferative activity. Mean values represent the relative amounts of 80% aqueous methanol extracted from equivalent quantities of samples that were needed to obtain an IC_{50} value. Values are averages (from split-plot ANOVA) across both corn and extrusion treatments. Bars sharing different letters are statistically different at $p < 0.05$.

2000 samples without corn compared to Promax 70 samples without corn tended to be smaller. This may have resulted from less severe extrusion conditions as witnessed by lower motor torque, die pressure, and product temperature (Table 1).

Effect of Extrusion on the Antiproliferative Activities of Soy Isoflavones. The second objective of these studies was to determine the antiproliferative effect of extrusion processing of the soy protein- and cornmeal-containing samples toward three different human breast cell lines, BT-20 (an ER-negative human breast cancer cell), MCF-7 (an ER-positive human breast cancer cell), and MCF-10F (a nonneoplastic or nontransformed human mammary epithelial cell). As expected, the samples with higher isoflavone and genistein content (Promax 70) exhibited higher inhibition (lower IC_{50}) of the growth of these three cell lines. For example, Promax 70, containing 3- to 4-fold more isoflavones per gram sample compared to Procon 2000, exhibited average IC_{50} values 28% of the IC_{50} values for Procon 2000-containing samples in all three cell lines (Figure 1). The same trend applied to the samples after extrusion compared to the samples before extrusion (as discussed below). In other words, the extruded samples had lower isoflavone content compared to nonextruded samples, and also exhibited higher IC_{50} values compared to the nonextruded samples.

The effect of adding cornmeal to the blend depended on the cell line and the treatment conditions tested. In general, for all three cell lines, supplementation of samples with cornmeal decreased the IC_{50} values compared to samples without corn (Table 6), with the exception of the extruded Promax 70 samples. The magnitude of the decreases in IC_{50} values ranged from 33% to 83%, with the largest decreases being observed in nonextruded Procon 2000 samples. For BT-20 cells, the IC_{50} values of samples decreased in those supplemented with cornmeal only under conditions in which no extrusion was performed. For MCF-7 cells, the IC_{50} values of cornmeal-containing samples, both extruded and nonextruded, decreased significantly compared to those without cornmeal. For the MCF-10F cells, only the blend containing cornmeal and Procon 2000 exhibited a significant decrease in IC_{50} value compared to the Procon 2000 sample without cornmeal.

With regard to extrusion, in every case an increase in IC_{50} values (less antiproliferative effect) in extruded samples compared to nonextruded samples was found, although in some cases the differences were not statistically significant. For BT-20 cells, IC_{50} values from soy samples without corn increased 46%−70% after extrusion, although the values using Promax 70 were not significant. In the samples containing the soy protein/cornmeal blends, IC_{50} values with BT-20 increased ~2.1-fold after extrusion. For MCF-7 cells, the soy protein samples without corn exhibited an increase in IC_{50} values of 38% to 92% after extrusion, although the values for Promax 70 were not significant. Using the soy protein/cornmeal blends, MCF-7 IC_{50} values increased 2.8- to 3.6-fold after extrusion. Using MCF-10F cells, the soy protein samples without added cornmeal exhibited an increase in IC_{50} values after extrusion, although the differences were not significant. For the samples containing the soy protein/cornmeal blends, the MCF-10F IC_{50} values increased 2.7- to 3.0-fold after extrusion. Thus, each cell line responded to treated samples in a similar manner. Figure 2 depicts the interaction of cornmeal supplementation and extrusion on IC_{50} values for all cell lines considered together because split-plot analysis of variance indicated no significant differences due to cell line. Although adding cornmeal to the nonextruded samples enhanced the antiproliferative action of the extracts, those samples containing cornmeal lost a relatively greater portion of their antiproliferative activity following extrusion, compared to samples without cornmeal added.

Table 6. Effect of Extrusion Processing on Proliferation of BT-20, MCF-7, and MCF-10F Breast Cells in Soy Samples with or without Corn Supplementation[a]

cell type	soy sample	corn	extrusion	IC_{50}
BT-20	Procon 2000	yes	yes	$23.3^{a,b}$
BT-20	Procon 2000	no	yes	34.8^{a}
BT-20	Procon 2000	yes	no	$7.3^{e,f}$
BT-20	Procon 2000	no	no	$20.5^{b,c}$
BT-20	Promax 70	yes	yes	18.8^{c}
BT-20	Promax 70	no	yes	$15.0^{c,d}$
BT-20	Promax 70	yes	no	6.0^{f}
BT-20	Promax 70	no	no	$10.3^{d,e}$
MCF-7	Procon 2000	yes	yes	45.3^{b}
MCF-7	Procon 2000	no	yes	71.0^{a}
MCF-7	Procon 2000	yes	no	9.8^{d}
MCF-7	Procon 2000	no	no	44.0^{b}
MCF-7	Promax 70	yes	yes	22.8^{c}
MCF-7	Promax 70	no	yes	18.8^{d}
MCF-7	Promax 70	yes	no	6.0^{e}
MCF-7	Promax 70	no	no	9.8^{d}
MCF-10F	Procon 2000	yes	yes	35.3^{b}
MCF-10F	Procon 2000	no	yes	67.3^{a}
MCF-10F	Procon 2000	yes	no	8.8^{e}
MCF-10F	Procon 2000	no	no	$50.3^{a,b}$
MCF-10F	Promax 70	yes	yes	$27.5^{d,e}$
MCF-10F	Promax 70	no	yes	$18.0^{c,d}$
MCF-10F	Promax 70	yes	no	7.5^{e}
MCF-10F	Promax 70	no	no	$11.8^{d,e}$

[a] IC_{50} values are expressed as means $\times 10^{-3}$ mL. Means among treatment groups (within each cell type only) sharing different superscripts are statistically different at $p < 0.05$. IC_{50} values increase as antiproliferative effect decreases.

DISCUSSION

Our studies were designed to determine whether extrusion processing of soy protein/cornmeal blends affects the content of isoflavones and their antiproliferative bioactivity. Our results indicate that, under the extrusion conditions used in this experiment, extrusion processing did influence the content of isoflavones in

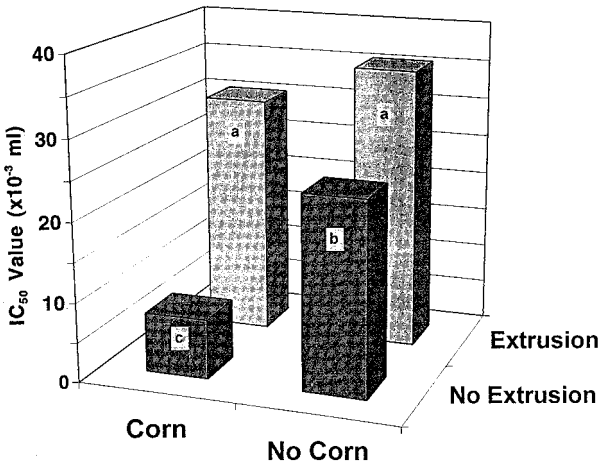

Figure 2. Interactive effect between cornmeal and extrusion treatments on IC_{50} values. Mean values represent the amounts of 80% aqueous methanol extract from equivalent quantities of sample necessary to obtain an IC_{50} value. Values are averages (from split-plot ANOVA) across breast cell type and soy protein type. Bars sharing different letters are statistically different at $p < 0.05$.

samples of the final products. We observed that after the extrusion of all soy/corn blends, the amount of total isoflavones per unit of sample significantly decreased. The magnitude of these decreases was modest, ranging from 9% to 38%, and was reflective of similar decreases in genistein-derived and daidzein-derived forms. Although clear patterns of change in specific isoflavone forms due to extrusion processing were not apparent, quantities of the aglycones genistein and daidzein were generally not significantly changed by the extrusion treatment. These aglycones are perceived to be important constituents contributing to soy's health benefits (Barnes, 1995). These results are consistent with a previous study showing that extrusion does not significantly change the isoflavone profile in soybean extracts (Mahungu et al., 1999). In contrast, others have reported that the quality and quantity of isoflavones can be altered by specific food-processing conditions (Farmakalidis and Murphy, 1985; Kudou et al., 1991; Coward et al., 1993). Further study is needed to characterize the food-processing conditions that will minimize the alterations in isoflavone quantities and forms.

The results of our study also indicate that extrusion processing in general reduced the antiproliferative effect of extracts prepared from soy/corn blends toward several human breast cell lines. The magnitude of the decrease in antiproliferative action of soy samples without cornmeal was approximately 34% to 92%, which is similar to the magnitude of the decrease in total isoflavones quantified in the samples. Although, as previously mentioned, the quantities of the aglycones genistein and daidzein did not change in most extruded samples, compared to nonextruded samples, there was nonetheless an extrusion-associated decrease in antiproliferative efficacy. This suggests that other isoflavones may also be involved in soy's antiproliferative and anticancer health benefit, and that these may be sensitive to the extrusion conditions. Nonetheless, it should be pointed out that the antiproliferative effect of soy was not eliminated by extrusion processing. This suggests that appreciable quantities of isoflavones, including genistein and daidzein, do remain after extrusion, and that some bioactivity remains in the extruded product.

Of additional interest with regard to the blends was the added antiproliferative action of cornmeal constituents. We used samples containing cornmeal because corn is widely used as a component in snack and breakfast cereals. Although nonextruded samples supplemented with cornmeal contained lower contents of isoflavones, they nonetheless did exhibit considerable effectiveness in inhibiting cell proliferation. However, a large portion of this antiproliferative bioactivity was lost following extrusion, suggesting sensitivity of the active constituents to this form of food processing. The specific phytochemical(s) responsible for this cornmeal-associated bioactivity are not known, although carotenoids and xanthophylls from corn and other similar crop products have received attention as possible cancer prevention agents (Gerster, 1993; Tarantilis et al., 1994; Rock et al., 1995; Gross et al., 1997).

In summary, the data indicate that extrusion processing does alter the isoflavone content and antiproliferative biological activities of extracts of samples prepared from blends of soy protein and cornmeal. Although the total isoflavone content of extruded samples decreased modestly compared to nonextruded samples, there was, in general, no significant change in the contents of the bioactive genistein and daidzein. Also, extrusion processing did reduce the antiproliferative efficacy of extracts prepared from the soy/corn blends, yet did not eliminate bioactivity of samples. Thus extrusion processing of similar soybean-containing foods is likely to allow for some of the beneficial health properties associated with soy constituents to be retained.

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