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Extrusion Conditions Affect Chemical Composition and in Vitro Digestion of Select Food Ingredients

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An experiment was conducted to determine the effects of extrusion conditions on chemical composition and in vitro hydrolytic and fermentative digestion of barley grits, cornmeal, oat bran, soybean flour, soybean hulls, and wheat bran. Extrusion conditions altered crude protein, fiber, and starch concentrations of ingredients. Organic matter disappearance (OMD) increased for extruded versus unprocessed samples of barley grits, cornmeal, and soybean flour that had been hydrolytically digested. After 8 h of fermentative digestion, OMD decreased as extrusion conditions intensified for barley grits and cornmeal but increased for oat bran, soybean hulls, and wheat bran. Total short-chain fatty acid production decreased as extrusion conditions intensified for barley grits, soybean hulls, and soybean flour. These data suggest that the effects of extrusion conditions on ingredient composition and digestion are influenced by the unique chemical characteristics of individual substrates.

KEYWORDS: Extrusion; digestion; in vitro; short-chain fatty acids; chemical composition

INTRODUCTION

Extrusion is a processing technology used to produce foodstuffs such as cereals, snack foods, and pet foods. By controlling variables such as temperature, moisture, and retention time in the extruder, food ingredients undergo physical and chemical changes. Extrusion processing of ingredients can affect carbohydrate composition, specifically starch and fiber fractions.

A portion of starch, resistant starch (RS), displays fiber-like properties in that it is not accessible to pancreatic α-amylase. Processing affects the concentration of RS as a result of gelatinization of the starch granules present. The gelatinization process allows greater susceptibility of starch to enzymatic hydrolysis and, subsequently, higher starch digestibility (*1*).

Dietary fiber is categorized as soluble and insoluble. Soluble fibers form gels, increasing the viscosity of intestinal contents, which may affect the glycemic index. Soluble fibers are generally highly fermentable and affect the animal indirectly by production of gas, short-chain fatty acids (SCFA), and lactic acid as a result of fermentative digestion in the large intestine (*2*). Insoluble fibers contribute to fecal bulk and ease of laxation.

The proportion of soluble fiber affects SCFA production in the hindgut (*3*, *4*). Short-chain fatty acids are important fuels for the human colonocyte and promote intestinal cell turnover (*5*). Colonocytes receive up to 70% of their energy requirements from SCFA (*6*).

As a result of these outcomes associated with SCFA production in the hindgut, it is important to determine how carbohydrate profiles of ingredients affect SCFA production and how raw ingredients might be modified to effect changes in digestion responses. The objective of this study was to collect chemical composition data on select ingredients, to quantify the effects of extrusion on hydrolytic and fermentative digestion of these ingredients, and to determine if relationships exist between the two response criteria.

MATERIALS AND METHODS

Ingredients. Barley grits, cornmeal, oat bran, soybean flour, wheat bran (Bob's Red Mill Natural Foods, Inc. Milwaukie, OR), and soybean hulls (Central Soya Co., Inc., Fort Wayne, IN) were extruded under three different extrusion conditions at a pilot processing plant at Wenger Manufacturing Co. (Sabetha, KS) using a TX-57 single-screw extruder. The screw profile defined the three extrusion conditions: mild, moderate, and extreme. The mild-extruded ingredients were subjected to a screw profile of one reverse lobe at 80−90 °C, the moderate-extruded ingredients to a profile with three reverse lobes at 100−110 °C, and the extreme-extruded substrates to a profile with five reverse lobes at 120−130 °C. Increasing the number of reverse diameter lobes on the screw increased the specific mechanical energy within the extruder (75−329 kJ/kg for mild, 93−383 kJ/kg for moderate, and 145−613 kJ/kg for extreme). The length-to-diameter ratio of the screw was 19.5:1. Barley grits, cornmeal, and soybean flour were extruded with two open dies. Oat bran, soybean hulls, and wheat bran were extruded with one open die to decrease the amount of extrudate lost from high-pressure blowing. The extruder motor load ranged from 13 to 51% for mild-extruded, from 16 to 66% for moderate-extruded, and from 25 to 95% for extreme-extruded substrates. Addition of ingredient was held constant at 250 kg/h with the exception of wheat bran at 225 kg/h. Preconditioner speed was 350 rpm, steam flow in the preconditioner was 10 kg/h, and water flow to the preconditioner was 15 kg/h. The extruder shaft speed was held constant at 500 rpm.

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Samples were dried on a model 4800 belt drier (Wenger Manufacturing Co.). The temperatures of the three dryer zones were 90, 34, and 33 °C, respectively, with fan speed held constant at 1406 rpm. The period of time that samples were in the dryer and the number of passes through the dryer varied according to the moisture content of the sample.

Laboratory Analyses. Prior to their analysis, ingredients were ground through a 2-mm screen in a model 4 Wiley mill (Thomas-Wiley, Swedesboro, NJ). Soybean flour was ground with dry ice to avoid loss of oil. The samples then were stored until analyses could be performed. The following analyses were conducted on all samples: dry matter (DM; *7*), organic matter (OM; *7*) via determination of ash concentration (*7*), crude protein (*7*), neutral detergent fiber (NDF; *8*), acid detergent fiber (ADF; *8*), and acid detergent lignin (ADL; *8*). Insoluble hemicelluloses (IH) were calculated as the difference between NDF and ADF. Cellulose was determined by subtracting ADL from ADF. Additionally, total dietary fiber (TDF; *9*) and insoluble dietary fiber (IDF; *10*) were analyzed, with soluble dietary fiber (SDF) calculated as TDF minus IDF. Total starch (TS; *11*) and digestible starch (DS; *12*) were analyzed, with RS determined by difference. All ingredients were analyzed in duplicate with a 5% error allowed among duplicates for all components present at a concentration ≥10%. For substrates at a concentration of ≤10%, a 10% error between duplicates was accepted.

Laboratory Procedures for in Vitro Digestion Experiment. Prior to the in vitro digestion experiment, ingredients were ground through a 1-mm screen in a model 4 Wiley mill (Thomas-Wiley). Soybean flour was ground with dry ice to avoid loss of oil. Unprocessed beet pulp, an ingredient on which our laboratory has a large database, was included as a standard in the experiment.

Donors. Three healthy, growing pigs (~50 kg) served as fecal donors from which microbial inoculum was prepared. The donors were given ad libitum access to water and a standard antibiotic-free corn−soybean meal-based diet formulated for growing pigs for at least 10 days prior to collection of a single fecal sample. The pigs originated from an antibiotic-free herd and were housed in a climate-controlled room in the animal care facility in the Edward R. Madigan Laboratory at the University of Illinois. Animals used in this study had no contact with other pigs or exposure to antibiotics for the duration of this study. The University of Illinois Institutional Animal Care and Use Committee approved all experimental procedures prior to experiment initiation.

Hydrolytic Digestion/Fermentation Procedures. Samples of 0.5 g of ground ingredients were weighed in triplicate into 50-mL polypropylene tubes. A set of blank tubes was processed through all procedures to account for organic matter disappearance (OMD) and SCFA appearance not originating from the ingredients. Samples were subjected to pepsin/hydrochloric acid, amyloglucosidase, α-amylase, and pancreatin to simulate hydrolytic digestion for 24 h according to the method of Boisen (*13*). The material remaining after enzymatic digestion was used as substrate for in vitro fermentation according to the method of Bourquin et al. (*14*). Aliquots (26 mL) of medium were aseptically transferred into the 50-mL tubes containing all substrate remaining from enzymatic digestion. The composition of the medium used for the fermentation portion of the experiment is presented in **Table 1**. All components except for vitamin solutions were mixed before autoclave sterilization of the medium. Filter-sterilized vitamin solutions were added just before the medium, which was maintained under anaerobic conditions at all times after preparation, was dispensed. To maintain anaerobic conditions, tubes were flushed with CO_2 and sealed with rubber stoppers equipped with one-way gas release valves (Nalge Nunc International, Rochester, NY).

The inoculum used in the in vitro fermentation assay was prepared from porcine feces following the method of Campbell and Fahey (*15*). The freshly voided (within 15 min of defecation) porcine fecal samples were placed in a plastic bag that was sealed after excess air had been removed. The samples were diluted 1:10 (w/v) in a 39 °C anaerobic dilution solution (*16*) and blended for 10 s in a Waring blender. Blended, diluted feces were filtered through four layers of cheesecloth, and the filtrate was sealed in 125-mL serum bottles under a stream of CO_2. A 4-mL portion of inoculum was injected aseptically through the rubber stopper using sterile 18-gauge needles into the 26 mL of medium plus substrate remaining from enzymatic digestion. For the blank tubes containing no substrate, 4 mL of inoculum was added to 26 mL of medium. The tubes were placed in a forced-air incubator at 39 °C with periodic mixing for fermentation periods of 0, 4, 8, and 12 h. After these time periods, appropriate tubes were removed from the incubator and processed immediately. A 2-mL aliquot was removed from each tube for SCFA analysis. The remaining 28 mL was combined with 112 mL of 95% ethanol and kept at room temperature for 1 h in order to precipitate the water-soluble polysaccharide fractions. Samples were filtered through Whatman 541 filter paper and washed successively with 78% ethanol, 95% ethanol, and acetone to recover the unfermented residue. Dry matter and OM contents of the residues then were determined according to an AOAC method (*7*) in order to calculate OMD. The percentage OMD was calculated as

$$\{1 - [(\text{sample residue wt} - \text{sample ash wt}) - (\text{blank residue wt} - \text{blank ash wt})]/(\text{original sample wt (DMB)} \times \%\ \text{OM})\} \times 100$$

where sample residue weight is the residue recovered after 4, 8, or 12 h of fermentation and blank residue weight from appropriate blank tubes containing medium and inoculum but no substrate. The denominator is the original OM added to the tube. Data were expressed on an OM basis due to inherent analytical difficulties with using DM for the assay. These difficulties relate to various ingredients becoming hydrated during the assay, resulting in a residue weight higher than the initial sample weight. Oven-drying methodologies are unable to remove the water from the substrates; thus, the sample is ashed to remove the hydration effect. Dry matter is influenced by hydration, whereas OM is not.

The 2-mL aliquot removed for SCFA analysis was mixed immediately with 0.5 mL of 25% metaphosphoric acid, precipitated for 30 min, and centrifuged at 20000*g* for 20 min. The supernatant was decanted and frozen at −20 °C in microfuge tubes. After freezing, the supernatant was thawed and centrifuged in microfuge tubes at 10000*g* for 10 min. Acetate, propionate, and butyrate concentrations in the supernatant were determined using a Hewlett-Packard 5890A series II gas−liquid chromatograph and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80:100 mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Individual SCFA concentrations were corrected for by subtracting the amount of SCFA produced in the blank tubes.

Table 1. Composition of Medium Used for Fermentation of Residues Obtained from Hydrolytic Digestion of Unprocessed and Extruded Ingredients

component	concn in medium
solution A[a]	330 mL
solution B[b]	330 mL
distilled water	296 mL
water soluble vitamin solution[c]	20 mL
trace mineral solution[d]	10 mL
folate/biotin solution [e]	5 mL
riboflavin solution[f]	5 mL
hemin solution[g]	5 mL
resazurin solution[h]	1 mL
short-chain fatty acid mix[i]	0.4 mL
sodium carbonate	4.0 g/L
yeast extract	0.5 g/L
trypticase	0.5 g/L
cysteine HCl monohydrate	0.5 g/L

[a] Composition (g/L): NaCl, 5.4; KH_2PO_4, 2.7; $CaCl_2 \cdot 2H_2O$, 0.18; $MgCl_2 \cdot 6H_2O$, 0.12; $MnCl_2 \cdot 4H_2O$, 0.06; $CoCl_2 \cdot 6H_2O$, 0.06; $(NH_4)_2SO_4$, 5.4. [b] Composition: K_2HPO_4, 2.7 g/ L. [c] Composition (mg/L): thiamin hydrochloride, 100; *d*-pantothenic acid, 10; niacin, 100; pyridoxine, 100; *p*-aminobenzoic acid, 5; vitamin B_{12}, 10 mL (0.0025 g/100 mL of distilled H_2O). [d] Composition (mg/L): ethylenediaminetetraacetic acid (disodium salt), 500; $FeSO_4 \cdot 7H_2O$, 200; $ZnSO_4 \cdot 7H_2O$, 10; $MnCl_2 \cdot 4H_2O$, 3; H_3PO_4, 30; $CoCl_2 \cdot 6H_2O$, 20; $CuCl_2 \cdot 2H_2O$, 1; $NiCl_2 \cdot 6H_2O$, 2; $Na_2MoO_4 \cdot 2H_2O$, 3. [e] Composition (mg/L): folic acid, 10; *d*-biotin, 2; NH_4HCO_3, 100. [f] Composition (mg/L): riboflavin, 10; HEPES, 1300. [g] Composition (mg/L): hemin, 500; NaOH, 400. [h] Composition: resazurin, 1 g/L in distilled H_2O. [i] Composition: 250 mL/L each of *n*-valerate, isovalerate, isobutyrate, and DL-α-methylbutyrate.

Table 2. Chemical Composition (Percent) of Unprocessed and Extruded Ingredients

substrate/extrusion condition	dry matter	organic matter[a]	crude protein[a]
barley grits			
unprocessed	90.0	98.2	11.0
mild	88.7	98.4	11.0
moderate	89.4	98.2	11.0
extreme	88.7	98.4	8.5
cornmeal			
unprocessed	88.7	98.8	11.0
mild	89.5	98.7	8.9
moderate	91.3	98.6	9.2
extreme	93.8	98.7	8.9
oat bran			
unprocessed	90.9	97.3	16.0
mild	91.3	97.4	16.2
moderate	91.7	97.5	15.5
extreme	90.3	97.0	17.1
soybean flour			
unprocessed	91.9	94.8	37.6
mild	92.6	94.8	38.1
moderate	92.0	94.8	37.5
extreme	86.7	94.7	38.9
soybean hulls			
unprocessed	91.0	94.5	10.9
mild	89.7	94.6	11.2
moderate	88.1	94.5	11.2
extreme	89.6	94.4	13.7
wheat bran			
unprocessed	89.7	93.9	18.9
mild	91.1	94.1	18.8
moderate	92.1	94.1	18.3
extreme	89.8	94.1	18.3

[a] Expressed as a percentage of dry matter.

Table 3. Detergent Fiber Concentrations (Percent) of Unprocessed and Extruded Ingredients[a]

substrate/extrusion condition	NDF[b]	ADF[b]	ADL[b]	IH[c]	cellulose[d]
barley grits					
unprocessed	28.8	3.7	1.6	25.1	2.1
mild	18.8	3.2	1.5	15.6	1.7
moderate	17.6	3.1	1.5	14.5	1.6
extreme	18.3	3.2	1.5	15.1	1.7
cornmeal					
unprocessed	23.0	2.8	1.6	20.2	1.2
mild	17.4	1.6	1.5	15.8	0.1
moderate	12.4	2.0	1.3	10.4	0.7
extreme	10.8	2.4	1.7	8.4	0.7
oat bran					
unprocessed	15.9	4.5	4.1	11.4	0.4
mild	15.5	3.8	3.8	11.7	0.0
moderate	13.9	4.2	4.2	9.7	0.0
extreme	14.0	4.5	3.5	9.5	1.0
soybean flour					
unprocessed	13.0	6.5	1.0	6.5	5.5
mild	17.1	5.3	0.5	11.8	4.8
moderate	15.9	5.3	0.5	10.6	4.8
extreme	17.6	5.2	0.5	12.4	4.7
soybean hulls					
unprocessed	67.0	49.3	2.3	17.7	47.0
mild	66.0	49.3	3.1	16.7	46.2
moderate	67.6	49.4	3.0	18.2	46.4
extreme	57.7	45.9	2.8	11.8	43.1
wheat bran					
unprocessed	49.0	15.8	5.3	33.2	10.5
mild	45.5	14.8	4.6	30.7	10.2
moderate	45.3	12.6	3.8	32.7	8.8
extreme	44.5	14.3	4.8	30.2	9.5

[a] Percentages are reported on a dry matter basis. [b] NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin. [c] Insoluble hemicelluloses calculated as NDF minus ADF. [d] Cellulose calculated as ADF minus ADL.

Calculations. Due to processing limitations, the in vitro experiment was repeated on four nonconsecutive days. To compensate for any error among days in which the experiments were conducted, a correction factor using the values for beet pulp (standard) was calculated and used. Beet pulp OMD and SCFA production for each in vitro run were calculated for each time of fermentation. Mean values per fermentation time per in vitro run were divided by the mean value for beet pulp per fermentation time for all in vitro runs collectively. This resulted in a correction factor that was applied to the appropriate fermentation time and in vitro run by multiplying each value for the unprocessed ingredients by the correction factor for that particular fermentation time. The final correction factors were 0.9988 $\pm$ 0.0843 for OMD, 1.0792 $\pm$ 0.4383 for acetate production, 1.0158 $\pm$ 0.1893 for propionate production, and 2.0082 $\pm$ 1.3894 for butyrate production (values for acetate, propionate, and butyrate were summed to obtain a total SCFA production value; values for individual SCFA are not reported).

Statistical Analyses. In vitro data were analyzed as a randomized complete block design with fecal donor serving as block. Treatments were factorially arranged within each block, with 6 substrates $\times$ 4 extrusion conditions $\times$ 3 fermentation times. Data from each fermentation period were analyzed separately, and only the results from the 8-h fermentation period are presented. Effects of donor were found not to be significant. Therefore, the model statement included substrate, extrusion condition, and substrate $\times$ extrusion condition interactions. All analyses were performed according to the General Linear Models procedure of SAS (SAS Institute Inc., Cary, NC). Least-squares means are reported for each combination of substrate and extrusion condition. Nonorthogonal contrasts including linear, quadratic, and unprocessed versus extruded substrate comparisons were conducted to determine the effects of fermentation properties due to extrusion conditions on outcome variables.

RESULTS AND DISCUSSION

Dry matter, OM, and crude protein concentrations of ingredients are presented in **Table 2**. Dry matter values varied little between extruded ingredients and their unprocessed counterparts. Extrusion conditions changed the DM concentration by only 6.4% at most (soybean flour−mild vs soybean flour−extreme). Organic matter concentrations of the ingredients also did not change as a result of extrusion conditions.

Crude protein concentration varied somewhat more, with a difference of 25% between barley grits−unprocessed, −mild, and −moderate and barley grits−extreme and between cornmeal−unprocessed and cornmeal−mild, −moderate, and −extreme. Perhaps the extrusion temperatures used to prepare these treatments volatilized a portion of the nitrogenous compounds present, resulting in lower concentrations of crude protein, or the amino acids in these ingredients were modified to the point that their nitrogen moiety was not detectable by Kjeldahl N analysis. Rebello and Schaich (*17*) reported that extrusion of wheat flour reduced total protein values due to alteration of the gliadin fraction.

Results of detergent fiber analyses are reported in **Table 3**. Neutral detergent fiber (NDF) is insoluble cell wall material including cellulose, insoluble hemicelluloses, and lignin. For certain ingredients, the NDF fraction is contaminated with protein and starch, resulting in an artificially inflated NDF value. For barley grits, cornmeal, and soybean hulls, NDF values were at least 10 percentage units higher for unprocessed substrates compared to substrates processed at extreme temperatures, indicating a conversion from insoluble fiber to soluble fiber as a result of extrusion processing. Oat bran and wheat bran did not exhibit dramatic changes in NDF as a result of extreme extrusion temperatures. Soybean flour actually increased in NDF content as a result of extrusion processing. This may be due to

Table 4. Total Dietary Fiber, Insoluble Dietary Fiber, and Soluble Dietary Fiber Concentrations (Percent) of Unprocessed and Extruded Ingredients[a]

substrate/extrusion condition	TDF[b]	IDF[b]	SDF[c]	I:S[d]
barley grits				
unprocessed	18.9	11.0	7.9	1.4
mild	18.8	6.3	12.5	0.5
moderate	18.3	9.9	8.4	1.2
extreme	15.7	8.9	6.8	1.3
cornmeal				
unprocessed	15.0	11.7	3.3	3.5
mild	16.2	11.8	4.4	2.7
moderate	15.1	10.7	4.4	2.4
extreme	14.2	7.4	6.8	1.1
oat bran				
unprocessed	21.7	12.1	9.6	1.3
mild	22.5	9.4	13.1	0.7
moderate	21.7	9.3	12.4	0.8
extreme	20.0	9.0	11.0	0.8
soybean flour				
unprocessed	25.1	21.6	3.5	6.2
mild	28.9	18.1	10.8	1.7
moderate	26.6	18.6	8.0	2.3
extreme	24.5	19.5	5.0	3.9
soybean hulls				
unprocessed	83.3	69.5	13.8	5.0
mild	81.2	68.4	12.8	5.3
moderate	80.5	67.2	13.3	5.1
extreme	85.1	66.5	18.6	3.6
wheat bran				
unprocessed	52.4	47.0	5.4	8.7
mild	55.7	45.4	10.3	4.4
moderate	55.1	45.5	9.6	4.7
extreme	53.0	45.4	7.6	6.0

[a] Percentages are reported on a dry matter basis. [b] TDF, total dietary fiber; IDF, insoluble dietary fiber. [c] SDF calculated as TDF minus IDF. [d] Ratio of insoluble to soluble dietary fiber.

the high protein content of soybean flour and the potential ability of the lysine component to react with reducing sugars to form a Maillard browning product. In the NDF assay, no correction is made for proteins that may be associated with NDF, thus inflating the concentration value.

Acid detergent fiber (ADF) consists of cellulose and lignin, with ADL consisting solely of lignin. Processing had a lesser effect on ADF and ADL and, thus, cellulose concentrations. However, insoluble hemicellulose concentrations for barley grits and cornmeal were markedly affected by extrusion processing. Smaller differences were noted for oat bran and wheat bran. Soybean flour had greater concentrations of IH that resulted from extrusion processing, but this higher value may have resulted from contamination of the sample with nitrogen and (or) Maillard product production. For soybean hulls, only extreme temperature extrusion processing affected IH content.

Results of total dietary fiber (TDF) analyses are reported in **Table 4**. TDF measures the total amount of fiber (insoluble and soluble) in an ingredient and includes its RS content. Concentrations of TDF did not change greatly as a result of extrusion processing. Values for IDF were lower for extruded ingredients compared to their unprocessed counterparts, whereas SDF concentrations were higher. When the ratios of insoluble to soluble fiber (I:S) were compared, a decrease due to extrusion processing was noted for all substrates except soybean hulls subjected to mild and moderate extrusion conditions. Decreases in I:S probably are due to decreases in concentrations of IH with subsequent conversion to soluble fiber. Björck et al. (*18*) examined extrusion and its effects on dietary fiber components. Unprocessed and extruded wheat flours contained similar amounts of TDF, but the I:S changed in favor of increased soluble fiber concentration when extruded. Also, in the same study, soluble arabinose and xylose concentrations increased as a result of extrusion, whereas their insoluble counterparts decreased.

In this study, ingredients were analyzed using both the detergent fiber methods and the TDF method. The TDF assay is traditionally used to analyze for all components of dietary fiber, including RS, whereas the detergent assays measure only insoluble dietary fibers. In using both assays, it was determined that although TDF concentrations were not greatly affected by processing, the IDF and SDF fractions were modified for barley grits, cornmeal, oat bran, soybean flour, and wheat bran (less IDF after extrusion, with greater SDF concentrations). These changes appeared to be due to the reduction in IH noted for all above-named substrates except for soybean flour, the IH value of which is no doubt confounded by nitrogen contamination.

Another explanation for the changes in IDF concentrations as a result of extrusion of high-starch ingredients is the direct relationship between the decrease in IDF and RS concentrations. The TDF analysis includes RS, and RS analyzes as IDF; therefore, TDF values could be inflated due to the presence of RS. A means of assessing this is by summing values for crude protein, TDF, and starch. If the resulting value for OM is higher than the analyzed value, then RS is being assayed as fiber, resulting in an overestimation of the actual cell wall concentration of the ingredient. The calculated OM concentration for the high-starch ingredients was greater than the analyzed OM. Therefore, RS likely influenced the TDF values. The high-fiber substrates (soybean hulls and wheat bran) generally were similar when the summed values for crude protein, TDF, and starch were compared to the analyzed OM value. Soybean flour had a lower calculated OM value, but this probably was due to the fat content of soybeans being ~20% (the concentration of fat in whole soybeans). If fat concentrations had been included, the summed value would have been closer to the analyzed OM value.

Total starch concentrations varied widely among ingredients, but processing conditions did not greatly affect concentrations (**Table 5**). Digestible starch concentrations of barley grits, cornmeal, and wheat bran increased when unprocessed substrates were compared to extruded substrates. The greatest difference was observed when unprocessed barley grits (27.7% DS) were compared to barley grits subjected to moderate and extreme extrusion conditions (50.3−50.4% DS). Oat bran and soybean flour DS concentrations generally were unaffected by extrusion processing. Soybean hulls contained too little starch to fractionate.

An inverse relationship was noted when RS concentrations were examined. Resistant starch decreased substantially for barley grits, cornmeal, and wheat bran as a result of extrusion processing. Due to experimental variation, slight negative values occurred for wheat bran RS, but these values would be considered not different from zero. Starch in wheat is considered to be highly digestible. In an in vitro study by Björck et al. (*1*), starch digestibilities of 100% for both unprocessed and extruded forms of wheat starch were reported.

Two of the three high-starch ingredients (barley grits and cornmeal) used in this study exhibited dramatically decreased RS concentrations as a result of extrusion processing. Extrusion cooking gelatinized the starch granules and increased enzymatically accessible DS. Granule size, moisture, temperature, and the native crystalline form of the starch can affect its degree of gelatinization. Murray et al. (*19*) analyzed high-starch ingredi-

Table 5. Starch Concentrations (Percent) of Unprocessed and Extruded Ingredients[a]

substrate/extrusion condition	total starch	digestible starch	resistant starch[b]
barley grits			
unprocessed	78.4	27.7	50.7
mild	78.4	47.5	23.1
moderate	79.8	50.3	29.4
extreme	81.2	50.4	20.4
cornmeal			
unprocessed	80.2	36.7	43.5
mild	82.8	45.6	37.2
moderate	80.9	49.8	31.1
extreme	83.0	58.9	24.1
oat bran			
unprocessed	67.2	42.7	24.5
mild	65.1	39.9	25.1
moderate	67.2	41.5	25.7
extreme	66.4	37.6	28.8
soybean flour			
unprocessed	4.9	5.9	–1.0
mild	5.0	6.7	–1.7
moderate	5.2	5.7	–0.5
extreme	4.6	4.2	0.4
soybean hulls			
unprocessed	0.3	N/A[c]	N/A
mild	1.0	N/A	N/A
moderate	0.4	N/A	N/A
extreme	0.8	N/A	N/A
wheat bran			
unprocessed	17.9	17.7	0.2
mild	19.0	25.1	–6.1
moderate	19.2	27.7	–8.5
extreme	21.4	26.7	–5.2

[a] Percentages are reported on a dry matter basis. [b] Resistant starch calculated as total starch minus digestible starch. [c] Insufficient starch for analysis.

ents extruded at low (79−93 °C) and high (124−140 °C) temperatures and compared them to unprocessed sources. Resistant starch values decreased dramatically after extrusion of all samples studied. Björck et al. (*1*) conducted a study quantifying in vivo starch digestibility by rats. Digestibility of starch increased as a result of more extreme extrusion conditions. Wheat flours extruded at high temperatures, when fed to rats, resulted in the fastest uptake of glucose and insulin into the blood. The authors concluded that disruption of the starch granule was responsible for the increase in enzymatic hydrolysis and subsequent increase in the rate of starch absorption in vivo.

Organic Matter Disappearance. Although data were collected after 4, 8, and 12 h of fermentation, only data collected after hydrolytic digestion and after 8 h of fermentation are reported. Results at this hour were similar to the 12-h results and assessed to be the most relevant physiologically.

OMD due to hydrolytic digestion (**Table 6**) was increased ($P < 0.01$) for extruded versus unprocessed samples of barley grits, cornmeal, and soybean flour. The average OMD for the three extruded soybean hull treatments was slightly lower ($P < 0.05$) than for the unprocessed sample. Disappearance of OM from barley grits and soybean hulls was affected in quadratic fashion ($P < 0.01$), lower for moderately extruded samples than for those subjected to mild or extreme extrusion conditions. Disappearance of OM from cornmeal also was affected in a quadratic manner ($P < 0.01$), increasing when subjected to moderate extrusion conditions, then leveling off, with little increase in OMD as a result of extreme extrusion conditions. Disappearance of OM from soybean flour was affected in a quadratic manner ($P < 0.03$), lower for the unprocessed sample and slightly higher for each extrusion condition imposed. There

Table 6. Organic Matter Disappearance (OMD) after Hydrolytic Digestion of Unprocessed and Extruded Ingredients

			contrast *P* values[c]		
substrate/extrusion condition	OMD[a] (%)	SEM[b]	linear	quadratic	unprocessed vs extruded
barley grits		0.84	0.01	0.01	0.01
unprocessed	27.5				
mild	64.1				
moderate	56.9				
extreme	73.5				
cornmeal		0.35	0.01	0.01	0.01
unprocessed	41.9				
mild	67.0				
moderate	82.3				
extreme	81.9				
oat bran		1.72	0.28	0.20	0.22
unprocessed	31.4				
mild	33.5				
moderate	37.1				
extreme	34.5				
soybean flour		0.37	0.01	0.03	0.01
unprocessed	52.5				
mild	56.4				
moderate	57.4				
extreme	57.5				
soybean hulls		0.21	0.11	0.01	0.05
unprocessed	10.0				
mild	8.1				
moderate	7.0				
extreme	11.4				
wheat bran		0.57	0.95	0.49	0.67
unprocessed	34.2				
mild	35.0				
moderate	35.2				
extreme	34.3				

[a] OMD = {1 − [(sample residue wt − sample ash wt) − (blank residue wt − blank ash wt)]/(original sample wt (DMB) × % OM)} × 100. [b] Standard error of the mean for samples within substrate. [c] Contrast with *P* value for each comparison: linear = unprocessed vs mild vs moderate vs extreme extrusion conditions; quadratic = each extrusion condition vs the other; unprocessed vs extruded = unprocessed substrate vs all extrusion conditions.

was no significant effect on disappearance of OM from oat bran or wheat bran due to extrusion. The OMD of barley grits and cornmeal generally increased with increasing severity of extrusion conditions, which paralleled the increase in DS concentration.

Soybean hulls and wheat bran were expected to have somewhat lower OMD during the hydrolytic phase of digestion. The OMD that occurs is likely the starch and (or) protein components of the substrate. The OMD data for soybean flour may be attributed to its high protein and moderate fiber concentrations, which would allow for greater hydrolytic digestion and a higher OMD. Soybean hulls had high TDF concentrations (>80%) that correlate with the pattern noted for OMD after enzymatic digestion, with moderately extruded soybean hulls having a lower OMD than samples processed under mild or extreme conditions. Extruded oat bran and wheat bran as compared to their unprocessed counterparts did not exhibit significant OMD differences after enzymatic digestion, probably due to the few changes found in their chemical composition after extrusion.

After 8 h of fermentation (**Table 7**), OMD was lower ($P < 0.01$) for residues from extruded versus unprocessed barley grits that had been hydrolytically digested and increased ($P < 0.01$) for residues from extruded versus unprocessed oat bran, soybean hulls, and wheat bran that had been hydrolytically digested. Disappearance of OM from barley grits was affected in a quadratic manner ($P < 0.02$), higher for samples subjected to

Table 7. Organic Matter Disappearance (OMD) after 8 h of Fermentation with Porcine Fecal Microflora of Unprocessed and Extruded Residues Remaining after Hydrolytic Digestion

substrate/extrusion condition	OMD[a] (%)	SEM[b]	contrast *P* values[c] linear	quadratic	unprocessed vs extruded
barley grits		0.97	0.01	0.02	0.01
unprocessed	20.6				
mild	4.4				
moderate	9.0				
extreme	3.1				
cornmeal		0.56	0.01	0.41	0.32
unprocessed	4.6				
mild	5.6				
moderate	0.02				
extreme	–0.80				
oat bran		1.66	0.01	0.44	0.01
unprocessed	–3.1				
mild	7.3				
moderate	6.7				
extreme	11.9				
soybean flour		0.72	0.12	0.73	0.15
unprocessed	22.6				
mild	21.0				
moderate	20.1				
extreme	19.6				
soybean hulls		0.25	0.02	0.07	0.01
unprocessed	9.7				
mild	12.4				
moderate	11.2				
extreme	12.1				
wheat bran		0.42	0.01	0.08	0.01
unprocessed	11.1				
mild	14.5				
moderate	17.1				
extreme	17.4				

[a] Data are 0 h corrected. OMD = {1 – [(sample residue wt – sample ash wt) – (blank residue wt – blank ash wt)]/(original sample wt (DMB) × % OM)} × 100. [b] Standard error of the mean for samples within substrate. [c] Contrast with *P* value for each comparison: linear = unprocessed vs mild vs moderate vs extreme extrusion conditions; quadratic = each extrusion condition vs the other; unprocessed vs extruded = unprocessed substrate vs all extrusion conditions.

Table 8. Total SCFA Production after 8 h of Fermentation with Porcine Fecal Microflora of Unprocessed and Extruded Residues Remaining after Hydrolytic Digestion

substrate/extrusion condition	total SCFA (μmol/g of original OM)	SEM[a]	contrast *P* values[b] linear	quadratic	unprocessed vs extruded
barley grits		56.86	0.01	0.85	0.01
unprocessed	1311.2				
mild	1094.0				
moderate	964.8				
extreme	790.2				
cornmeal		46.24	0.81	0.12	0.27
unprocessed	502.1				
mild	335.6				
moderate	335.1				
extreme	469.2				
oat bran		62.67	0.17	0.20	0.58
unprocessed	353.3				
mild	369.2				
moderate	295.3				
extreme	638.2				
soybean flour		24.45	0.10	0.05	0.80
unprocessed	230.8				
mild	362.9				
moderate	219.1				
extreme	153.6				
soybean hulls		21.96	0.06	0.22	0.03
unprocessed	301.8				
mild	210.9				
moderate	168.9				
extreme	188.4				
wheat bran		49.07	0.14	0.42	0.63
unprocessed	318.0				
mild	406.2				
moderate	228.8				
extreme	157.4				

[a] Standard error of the mean for samples within substrate. [b] Contrast with *P* value for each comparison: linear = unprocessed vs mild vs moderate vs extreme extrusion conditions; quadratic = each extrusion condition vs the other; unprocessed vs extruded = unprocessed substrate vs all extrusion conditions.

moderate extrusion versus substrates subjected to mild or extreme extrusion conditions. Disappearance of OM from cornmeal was affected in a linear manner ($P < 0.01$), highest for unprocessed cornmeal and lowest for extreme-extruded cornmeal. Disappearance of OM from oat bran and wheat bran increased in a linear manner ($P < 0.01$), lowest for unprocessed material and highest for extreme-extruded material. Disappearance of OM from soybean hulls increased in a linear manner ($P < 0.02$), lowest for unprocessed and highest for extruded samples. Soybean flour OMD as a result of fermentation was unaffected by treatment.

The high-starch substrates (barley grits, cornmeal, and oat bran) varied in digestion characteristics as extrusion conditions intensified. Barley grits and cornmeal had generally lower OMD values after fermentative digestion, likely due to a decrease in RS and an increase in DS concentrations with increasing extrusion intensity. Thus, more substrate would have been digested during hydrolytic digestion, leaving little substrate available for fermentation. As much as a 17% difference in OMD between unprocessed and extruded barley grits was noted. The unprocessed barley grits had higher concentrations of RS and IDF than the extruded barley grits. The RS was apparently available for fermentation, thus the increase in OMD.

Oat bran increased in RS concentration as extrusion conditions intensified. OMD correlates with this result in that this response criterion increased as extrusion conditions intensified. Bourquin et al. (*20*) and Sunvold et al. (*3*) found similar OMDs for oat bran (5.8 and 7.8%, respectively) as occurred in the present study.

The high-fiber substrates (wheat bran and soybean hulls) had varying OMD results with increasing severity of extrusion conditions. Wheat bran had higher OMD as extrusion conditions intensified, perhaps due to the shift in I:S. Bourquin et al. (*20*) reported a somewhat higher OMD (25.4%) than was noted in the present study, likely due to the longer fermentation period of 24 h (vs 8 h used in the present study). Soybean hull OMD varied as severity of extrusion increased. Its high concentration of TDF (>60%) and the negligible shift in IDF and SDF as a result of extrusion may have limited the amount of substrate available for fermentation.

Short-Chain Fatty Acid Production. Total SCFA production (**Table 8**) differed between unprocessed and extruded barley grits ($P < 0.01$) and soybean hulls ($P < 0.03$). Extrusion of barley grits linearly decreased ($P < 0.01$) total SCFA production as extrusion conditions intensified. Soybean hulls tended ($P < 0.06$) to decrease linearly in total SCFA production as extrusion conditions intensified. Total SCFA production as a result of soybean flour fermentation was affected in a quadratic manner ($P < 0.05$), higher for mild and lower for unprocessed and moderate- and extreme-extruded samples. Total SCFA production from cornmeal, oat bran, and wheat bran was not affected by extrusion condition.

Total SCFA production from high-starch substrates (barley grits, cornmeal, and oat bran) paralleled OMD data. Barley grits

and cornmeal fermentation resulted in less total SCFA production as extrusion conditions intensified. As the RS concentration decreased with extrusion, the decrease in SCFA production would be expected as there would be less RS available for fermentation and, thus, SCFA production, with more DS being available for hydrolytic digestion.

The extrusion of oat bran increased the amount of RS in the substrate, resulting in an increase in total SCFA production for sample processed under extreme extrusion conditions. Total SCFA production values from oat bran fermentation were intermediate to values reported by Bourquin et al. (*20*) and Sunvold et al. (*3*).

High-fiber substrates can have different fermentability characteristics based on their concentrations of IDF and SDF. The lower the I:S, the greater the capacity for fermentation. Typically, IDF has lower fermentability unless retained in the fermentation compartment for extended periods of time. Soluble fiber, on the other hand, is typically highly fermentable, even when the time allowed for fermentation is relatively short.

The high-fiber substrates, soybean hulls and wheat bran, had generally lower SCFA production as extrusion conditions intensified. Soybean hulls exhibited an increase in soluble fiber with a decrease in I:S, but total SCFA production decreased as a result of extrusion. Previous in vitro studies utilizing soybean fiber (*20*, *21*) found total SCFA production values of 6.14 and 5.75 mmol/g of DM, respectively, after 24 h of fermentation. Less total SCFA production from wheat bran occurred with increasing severity of extrusion conditions. The I:S decreased with extrusion, but SCFA production was not well correlated with I:S. Bourquin et al. (*20*) found total SCFA production after 24 h of fermentation of wheat bran to be 1.99 mmol/g of OM. It is possible that the soluble fiber made available by extrusion may not be as fermentable as the soluble fiber present in unprocessed soybean hulls or wheat bran.

Soybean flour, a high-protein, moderate fiber ingredient, may be more susceptible to hydrolytic digestion, leaving little substrate for fermentation. However, the residue remaining after enzymatic digestion must have consisted of some fermentable fiber as there was some SCFA production.

Overall, two of the three high-starch ingredients (barley grits and cornmeal) were 40% higher in OMD between unprocessed and extreme-extruded samples after hydrolytic digestion. Extrusion of these ingredients apparently enabled greater availability of starch for hydrolytic digestion. The higher OMD as a result of hydrolytic digestion resulted in less substrate being available for fermentation. This led to lower OMD and total SCFA production values after 8 h of fermentation. However, barley grits and cornmeal had high total SCFA production values, which is likely a result of the substrates containing ~30% fermentable material (RS and SDF). Extrusion of oat bran, the other high-starch substrate, did not alter the DS:RS ratio; therefore, OMD after enzymatic digestion was similar for unprocessed and extruded oat bran.

For the high-fiber ingredients (wheat bran and soybean hulls), there was no clear relationship between chemical composition and OMD or total SCFA production. Wheat bran had ~34% OMD after enzymatic digestion, perhaps due to its DS content. After 8 h of fermentation, wheat bran had a higher OMD with increasing intensity of extrusion. Total SCFA production responses varied, however. Perhaps the protein component was bound during extrusion, making it unavailable for enzymatic digestion, but later, during fermentation, microbes were able to access the protein, fermenting it and resulting in some OMD and SCFA production.

As a result of extruding high-fiber and high-starch ingredients, the chemical composition as analyzed by current methodology, can be altered. Altering an ingredient to contain more RS and SDF would have the effect of increasing SCFA production, compounds known to be important for optimal colonic health. Resistant starch may serve as a proxy for traditional dietary fibers, whereas SDF has recognized effects of attenuating the glycemic response and reducing concentrations of blood cholesterol. Greater knowledge of extrusion and its effects on starch- and fiber-containing ingredients would be of potential benefit as consideration is given to the manufacture of functional foods for improvement of colonic health, glycemic control, and (or) blood lipid attenuation.

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