Journal of Aquaculture Feed Science and Nutrition 1 (2): 44-60, 2009
ISSN: 2070-1667

Effects of Ingredients and Extrusion Parameters on Properties of Aquafeeds Containing DDGS and Corn Starch

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Abstract: Isocaloric (3.05 kcal g^{-1}) ingredient blends were factorially formulated using 3 levels each of DDGS (20, 25 and 30% db), protein (30, 32.5 and 35% db) and feed moisture content (25, 35 and 45% db), along with appropriate quantities of corn starch, soybean meal, fish meal, whey, vitamin and mineral mix to produce a balanced diet for tilapia feed. The ingredient blends were extruded using a laboratory-scale single screw extruder with varying screw speeds (100, 150 and 200 rpm) and extruder barrel temperatures (100, 125 and 150°C). The resulting extrudates were subjected to extensive analyses of physical properties, which included moisture content, unit density, bulk density, expansion ratio, sinking velocity, water absorption, water solubility, color (L*, a* and b*) and pellet durability indices. Several extruder parameters, including moisture content at the die, apparent viscosity, specific mechanical energy, mass flow rate, net torque and die pressure were measured to quantify the extruder behavior during processing. All the formulations and process settings used produced viable extrudates, but some were of better quality than others. For example, increasing the DDGS levels from 20-25% db, protein content from 30-32.5% db, feed moisture content from 25-35% db, processing temperature from 100-125°C and screw speed from 100-150 rpm significantly increased the PDI values by 28.1, 18.1, 31.8, 6.6 and 32.2%, respectively all of these curvilinearly decreased as these independent variables increased to their highest levels. Increasing the feed moisture content from 25-45% db resulted in a curvilinear increase in SME values by 69.3%. On the other hand, increasing the screw speed from 100-200 rpm curvilinearly decreased the SME values by 37.9%. This study highlights the importance of experimentally determining the effects of feed ingredients and process variables when developing aquafeeds from novel materials.

Key words: Corn starch, Distillers Dried Grains with Solubles (DDGS), extrusion, extruder parameters, physical properties

INTRODUCTION

Starch is a biopolymer, which is comprised of 2 types of macromolecules, namely amylose and amylopectin (Brouillet-Fourmann *et al*., 2003). It is well known that a key modification during processing of starch is micro molecular degradation, which affects both amylose and amylopectin components (Colonna and Mercier, 1983; Davidson *et al*., 1984). Starch, an important carbohydrate constituent, is best characterized in terms of loss of crystallinity and gelatinization during extrusion (Colonna *et al*., 1983; Gomez and Aguilera, 1984; Chinnaswamy *et al*., 1989). Starch plays a vital role in the production of floating versus sinking feeds, because it acts as a binder and impacts product expansion. The minimum starch content needed for floating and sinking feeds are generally 18-22 and 9-11%, respectively (Riaz, 1997).

Aquaculture is an intensely expanding sector of agriculture and is one of the most rapidly growing markets for manufactured feeds (Riaz, 1997). Two major challenges for profitable fish feed production include feed formulation and processing (Kazamzadeh, 1989). Aquafeed formulation plays a crucial role in product buoyancy, especially given the challenge of manufacturing high quality protein, high lipid rations with acceptable pellet durability and water stability (Rokey and Plattner, 2004). The major characteristics, which affect the quality of fish feed include color, pellet size, shape, bulk density, water absorption and solubility,

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hardness or softness, resiliency, buoyancy and chewiness (Kazamzadeh, 1989). The largest cost component in aquaculture production is feed, which constitutes between 30 and 60% of the total operational costs for an aquaculture farm; protein is often the most expensive nutritional factor. Therefore, using low-price alternate sources of protein, which could provide better fish growth is beneficial for feed manufacturers and aquaculture producers alike (Lovell, 1988; Davis and Stickney, 1978; Keong, 2003).

Extrusion processing is an important feed and food processing operation (Harper, 1981; Paton and Spratt, 1984) and is often used to manufacture aquafeeds. High Temperature Short Time (HTST) extrusion cooking is used in many industries for the production of expanded snack foods, ready-to-eat cereals and animal foods (Chinnaswamy and Hanna, 1988). During this type of process, a combination of moisture, pressure and mechanical shear can partially denature the protein and gelatinize the starch in the feed ingredients (Friesen *et al.*, 1992). Starch gelatinization during extrusion is crucial because it affects feed digestibility, expansion and contributes to water stability. Moreover, the amount of starch gelatinized during processing depends on the starch type, particle size and processing conditions (Rokey and Plattner, 2003). Extrusion can also increase the feed digestibility and palatability, inactivate anti nutritional factors, destroy pathogenic microorganisms in the feed and thus, provide feed manufacturers with the means to improve the quality of their products (Williams, 1991). The effects of extrusion cooking on starch have been comprehensively studied and reviewed by Harper (1981) and Linko *et al.* (1981). Additionally, extensive research has been conducted on the effect of moisture content on extrudate properties for several starches and protein-based feed materials (Colonna and Mercier, 1983; Kim *et al.*, 1989; Badrie and Mellowes, 1991; Sokhey *et al.*, 1994; Sriburi *et al.*, 1999; Lin *et al.*, 2000; Sriburi and Hill, 2000; Hashimoto and Grossman, 2003; Shukla *et al.*, 2005). Understanding the relationships between the ingredients, process parameters and equipment design and operation is necessary to achieve desired product quality targets and to develop new products (Aguilera and Stanley, 1999; Noel *et al.*, 1990).

Distiller's Dried Grains with Solubles (DDGS), a potential alternative protein source, is ⅓ co products produced in dry grind ethanol plants, along with fuel ethanol and carbon dioxide (Shurson, 2003; US Grains Council, 2008). Due to its reasonably high protein content and low cost compared to fish meal, there is growing interest in using DDGS in aquaculture diets (US Grains Council, 2008). Research has shown that DDGS is an acceptable ingredient for species such as tilapia (Wu *et al.*, 1996) and channel catfish (Tidwell *et al.*, 1990; Webster *et al.*, 1993) and can produce better, or at least comparable growth.

A key component to using DDGS as a protein source in aquaculture feeds is information on processing. Only a few studies that have focused on processing aspects have been conducted with DDGS in aquafeeds. These have examined varying factors such as the level of DDGS, feed moisture content, screw speed, barrel temperature and their impacts on the resulting products from both single and twin screw extruders (Shukla *et al.*, 2005; Chevanan *et al.*, 2007a-d, 2008; Kannadhason *et al.*, 2007a, b). For example, Chevanan *et al.* (2007b) examined the effect of die dimensions including nozzle diameter, length and L/D (Length-to-Diameter) ratio on extrusion processing parameters and properties of DDGS-based tilapia feeds. In addition, the effects of varying the levels of DDGS, feed moisture content and screw speed on the physical properties of the resulting extrudates were examined by Chevanan *et al.* (2007a, c) using a laboratory scale single screw extruder and an industrial-scale twin screw extruder. Their results indicated that DDGS could be successfully incorporated up to 60% of the feed blend and could still result in floating feed products.

To date, however, the influence of type of starch has not been examined in conjunction with DDGS and different protein levels. Corn starch has a lesser amount of amylopectin (72%) (Fennema, 1985) compared to other starches (such as tapioca or potato); this may have an impact on expansion when incorporated with DDGS and other ingredients (Chevanan *et al.*, 2007b-d).

The objectives of this study were to produce feeds for tilapia using DDGS as a protein source and corn starch as a binder and to investigate the effects of various levels of DDGS, protein content, feed moisture content, screw speed and extruder barrel temperature on the resulting physical properties of the extrudates and on various processing parameters.

MATERIALS AND METHODS

Sample preparation: Isocaloric (3.05 kcal g^{-1}) ingredient blends (Table 1) were formulated to 3 target protein levels (30, 32.5 and 35% db) using 3 proportions of DDGS (20, 25 and 30% db) and 3 moisture contents (25, 35 and 45% db), along with appropriate quantities of soybean meal, corn starch, fish meal, whey, vitamin and mineral mix, to produce balanced diets for tilapia. DDGS was provided by Dakota Ethanol LLC (Wentworth, SD); soybean meal was purchased from Dakotaland Feeds Inc.,

Table 1: Ingredient components (g/100 g) in the feed blends used in the study and their compositions (dry basis)

Ingredients (db%)	Blend protein levels (db%)					Ingredient composition (db%)			
	30	30	32.5[1]	35	35	Protein	Fat	Fiber	Ash
DDGS	20.0	30.0	25.0[1]	20.0	30.0	28.0	11.4	6.8	4.0
Soybean meal	36.4	34.3	38.0[1]	41.4	35.3	46.5	0.5	3.5	8.0
Corn starch	26.1	20.3	17.9[1]	15.6	11.0	7.9	4.1	2.2	1.2
Fish meal	9.5	7.4	11.1[1]	15.0	15.7	63.0	7.0	1.0	19.0
Whey	5.0	5.0	5.0[1]	5.0	5.0	12.0	0.5	0.0	9.4
Vitamin mix	1.0	1.0	1.0[1]	1.0	1.0	-	-	-	-
Mineral mix	2.0	2.0	2.0[1]	2.0	2.0	-	-	-	-
Total	100.0	100.0	100.0[1]	100.0	100.0	-	-	-	-

[1] Bold letters indicate the center point treatment. All blends were formulated on a dry basis

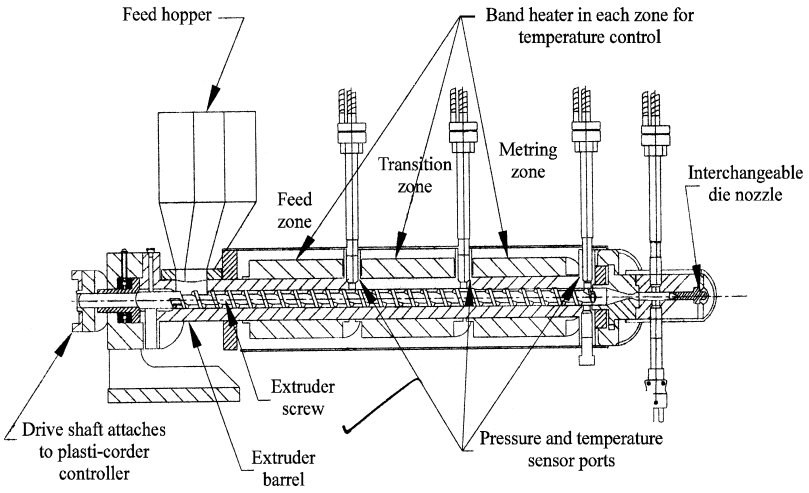

Fig. 1: Schematic of the single screw laboratory extruder used in the study (based on Kannadhason *et al*., 2008)

Huron, SD. Whole corn was obtained from a local market. These materials were ground to a fine powder using a laboratory grinder (s 500 disc mill, Genmills, Clifton, NJ) to an average particle size of ≈425 μm. Menhaden fish meal was purchased from Consumers Supply Distribution Company, Sioux City, IA; whey was obtained from Bongards Creameries, Perham, MN; vitamin and mineral mix (Vitapak) from Land O' Lakes Feed, St. Paul, MN. All these ingredients were mixed using a laboratory-scale mixer (N50, Hobart Corporation, Troy, OH) for a period of 10 min. The moisture content of the feed blends were then corrected to the target moisture content by adding appropriate quantities of water during the process of mixing. The resulting feed blends were then stored overnight at room temperature (25±1°C) for moisture stabilization prior to processing.

Extrusion processing: Extrusion cooking was performed using a single-screw extruder (Fig. 1; Brabender Plasti Corder, model PL 2000, South Hackensack, NJ), which had a compression ratio of 3:1, screw Length-to-Diameter (L/D) ratio of 20:1 and a barrel length of 317.5 mm. The die had a diameter of 2.90 mm, with a length of 9.25 mm, which resulted in a die L/D of 3.19. The temperature inside the barrel and speed of the screw were controlled by a computer that was connected to the extruder. The extruder (Fig. 2) was connected to a 7.5 HP motor and the speed of the screw was capable of adjusting from 0-210 rpm (0-22 rad sec^{-1}). After processing, the extrudates were allowed to cool and dry under ambient conditions for approximately 24 h.

Processing conditions monitored during extrusion included moisture content at the die (db%), apparent viscosity (Pa-s), specific mechanical energy (J g^{-1}), mass flow rate (g min^{-1}), die pressure (Pa) and net torque (N-m).

Measurement of extrudate properties: The resultant products were subjected to extensive physical property testing, including moisture content (db%), unit density (kg m^{-3}), bulk density (kg m^{-3}), expansion ratio (-), sinking velocity (m sec^{-1}), water absorption (-), water solubility (%), color (L*, a*, b*) and pellet durability (%) indices.

Moisture Content (MC): MC of the extrudates was determined following AACC method 44-19 (2000), using a laboratory oven (Thelco Precision, Jovan Inc., Wincester, VA) at 135°C for 2 h.

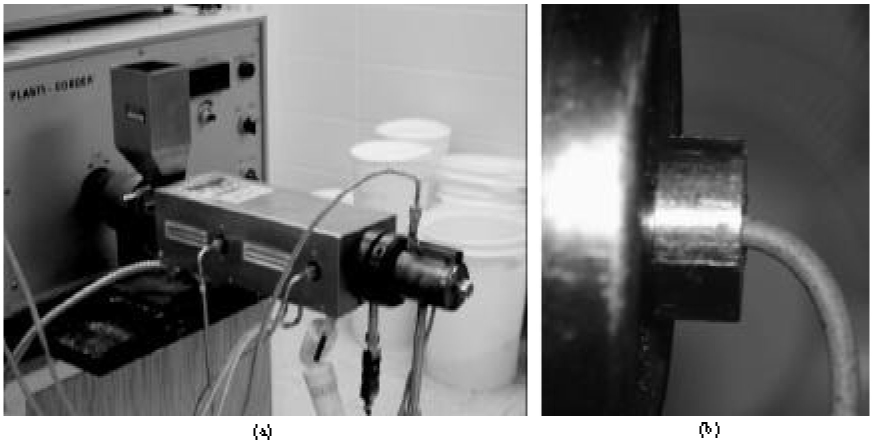

Fig. 2: a): View of the single screw laboratory extruder used in the study, b): Extrudate exiting the extruder die

Unit Density (UD): The extrudates were cut into pieces ≈25.4 mm in length using a razor blade. Each piece's mass was measured using an electronic balance (model A-250, Denver Instrument, Arvada, CO) and its corresponding length was measured using a digital caliper (Digimatic Series No. 293, Mitutoyo, Tokyo, Japan). UD was determined as the ratio of mass to the volume of each piece, by assuming cylindrical shapes for each extrudate, following Jamin and Flores (1998) and Rosentrater *et al*. (2005).

Bulk Density (BD): BD was measured using a standard bushel tester (Seedburo Equipment Co., Chicago, IL) following the method described by USDA (1999).

Expansion Ratio (ER): ER was determined as described by Conway and Anderson (1973) the diameter of the extrudates for each treatment was measured with a digital caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan) and then divided by the diameter of the die nozzle (2.90 mm).

Sinking Velocity (SV): SV was measured using the method developed by Himadri *et al*. (1993) and was determined by monitoring the time taken for an extrudate of length ≈25.4 mm to reach the bottom of a 2000 mL measuring cylinder filled with distilled water (total distance = 0.415 m). The ratio of distance travelled to sinking time gave the sinking velocity (m sec^{-1}).

Water Absorption and Solubility Indices (WAI and WSI): WAI and WSI were determined as outlined by Anderson *et al*. (1969): 2.5 g of finely ground sample (≈150 μm) was suspended in 30 mL of distilled water in a tared 50 mL centrifuge tube. The centrifuge tube was placed in a laboratory oven (Thelco precision, Jovan Inc., Wincester, VA) at 30°C, stirred intermittently for a period of 30 min and centrifuged at 3000 rpm for 10 min. The supernatant liquid was transferred into a tared aluminum dish, placed in an oven for 2 h at 135°C (AACC, 2000) and then desiccated for 20 min before weighing the dry solids of the supernatant. The mass of the remaining gel was weighed and WAI (-) was calculated as the ratio of gel mass to the original sample mass. WSI (%), on the other hand, was determined as the ratio of the mass of dry solids in the extract to the original sample mass.

Color: Color (L*, a* and b*) of the extrudates was determined using a spectrophotometer (Portable model CM2500d, Minolta Corporation, Ramsey, NJ), where, L* refers to brightness/darkness, a* refers to redness/greenness and b* refers to yellowness/blueness of the extrudates.

Pellet Durability Index (PDI): PDI was determined using method S269.4 (ASAE, 2004). Approximately, 200 g of extrudates were broken into pieces of ≈25.4 mm in length and were divided into 2 batches of 100 g each. Each batch was placed in a pellet durability tester (model PDT-110, Seedburo Equipment Company, Chicago, IL) for tumbling over a period of 10 min. Each sample was sieved with a no. 6 sieve (3.35 mm) before and after tumbling and measured for the quantity of sample retained on the screen. The pellet durability index was then calculated using Eq. 1:

$$PDI = \left(\frac{M_{at}}{M_{bt}}\right) \times 100 \qquad (1)$$

Where:

PDI = The pellet durability index (%)
M_{at} = The mass of the pellets after tumbling (g)
M_{bt} = The mass of the pellets before tumbling (g)

Measurement of extrusion processing parameters

Moisture content at the die: Moisture content at the die was determined by collecting extrudates at the exit of the die using plastic bags, sealing tightly, allowing the samples to cool under ambient conditions for 3 h and then following AACC (2000), using a laboratory (Thelco precision, Jovan Inc., Wincester, VA) oven at 135°C for 2 h.

Apparent viscosity: Following Rosentrater *et al.* (2005) the apparent viscosity of the dough in the extruder was calculated using Eq. 2:

$$n_{app} = \left(\frac{C_{ss}}{C_{sr}}\right) * \left(\frac{T}{\omega}\right) \tag{2}$$

Where:

n_{app} = The dough apparent viscosity (Pa-s)

C_{ss} = A screw-dependent empirical correction factor {6157.57 m^{-3} for the specific screw barrel configuration used in our study (Rosentrater *et al.*, 2005)}

C_{sr} = A barrel-dependent empirical geometric correction factor {7.63 for the extruder used in our study (Rosentrater *et al.*, 2005)}

T = The net torque (N-m) exerted on the screw drive shaft

ω = The screw speed (rpm)

Specific Mechanical Energy (SME): Specific Mechanical Energy (SME), defined as the total mechanical energy input to obtain 1 g of extrudate (J g^{-1}), was determined using Eq. 3 (based on Rosentrater *et al.*, 2005):

$$SME = \left(\frac{T * \omega * 60}{M_{feed}}\right) \tag{3}$$

Where:

SME = The specific mechanical energy consumption (J g^{-1})

T = The net torque exerted on the extruder drive (N-m)

ω = The screw speed (rpm)

M_{feed} = The mass flow rate of the dry feed material (g min^{-1})

M_{feed} = Calculated using Eq. 4

$$M_{feed} = M_{prod} * \left(\frac{1 - MC_f}{1 - MC_i}\right) \tag{4}$$

Where:

M_{feed} = The dry mass flow rate of the raw material (g min^{-1})

Table 2: Experimental design used in the study[1]

	Feed properties			Extrusion conditions	
Treatments	DDGS level (db%)	Protein level (db%)	Moisture level (db%)	Temperature (°C)	Screw speed (rpm)
1	20	30	25	100	100
2	-	-	-		200
3	-	-	-	150	100
4	-	-	-		200
5	-	-	45	100	100
6	-	-	-		200
7	-	-	-	150	100
8	-	-	-		200
9	-	35	25	100	100
10	-	-	-		200
11	-	-	-	150	100
12	-	-	-		200
13	-	-	45	100	100
14	-	-	-		200
15	-	-	-	150	100
16	-	-	-		200
17	30	30	25	100	100
18	-	-	-		200
19	-	-	-	150	100
20	-	-	-		200
21	-	-	45	100	100
22	-	-	-		200
23	-	-	-	150	100
24	-	-	-		200
25	-	35	25	100	100
26	-	-	-		200
27	-	-	-	150	100
28	-	-	-		200
29	-	-	45	100	100
30	-	-	-		200
31	-	-	-	150	100
32	-	-	-		200
33 (center point)	25	32.5	35	125	150

[1] The experimental design consisted of 2 (DDGS levels) x 2 (protein levels) x 2 (moisture contents) x 2 (temperatures) x 2 (screw speeds) + 1 center point (between all of these levels) = 33 total treatment combinations

M_{prod} = The dry mass flow rate of the extruded product (g min^{-1}) (i.e., exiting the extruder die)

Mc_f = The moisture content of the collected extrudate samples (wb %)

Mc_i = The moisture content of the raw feed ingredient blend before entering the extruder (wb %)

Mass Flow Rate (MFR): Product MFR was determined by collecting extrudate samples at 30 sec intervals and then weighing using an electronic balance (model A-250, Denver Instrument, Arvada, CO). The dry mass flow rate was determined by measuring the moisture content of the extrudates immediately upon exit from the die.

Pressure at the die and torque: The temperature and absolute pressure in the die were simultaneously recorded with a combined thermocouple/pressure transducer (GP50, New York Ltd., Grand Island, NY) with a sensing range of 0-68.9 Mpa. The torque was measured with a torque transducer (Measurement Specialists, Huntsville, AL) with a sensing range of 0-390 N-m.

Statistical analysis: Feed blends were prepared with 3 levels each of DDGS (20, 25 and 30% db), protein content (30, 32.5 and 35% db) and feed moisture content (25, 35 and 45% db) and were extruded in a Brabender laboratory scale single screw extruder at 3 screw speeds (100, 150 and 200 rpm) and 3 barrel temperatures (100, 125 and 150°C). Each of these factors used 2 levels (i.e., a high and a low setting) with a central composite point for all factors simultaneously. This resulted in 33 total treatment combinations (i.e., 2×2×2×2×2 = 32, plus 1 center point for all factors). The experimental design used for this study is shown in Table 2.

Triplicates (n = 3) were measured for most properties (i.e., dependent variables) for each treatment combination, except for mass flow rate and specific mechanical energy, where duplicates (n = 2) were measured instead; additionally, net torque and die pressure were determined using 10 replications (n = 10) for each treatment combination. The collected data were analyzed with the Proc GLM procedure to determine the main, interaction and treatment combination effects using SAS v. 9 (SAS Institute, Cary, NC) using a type I error rate (α) of 0.05.

RESULTS AND DISCUSSION

Extrudate properties: Table 3 shows the main treatment effects of all the parameters studied on the properties of the resulting extrudates.

Moisture content: As shown in Table 3, increasing the DDGS level, protein content and feed moisture content increased the moisture content of the resulting extrudates, whereas, increasing the processing temperature from 100-150°C resulted in decreased moisture content of the extrudates. Changing the screw speed from 100-200 rpm appeared not to have any significant effect on the moisture content of the extrudates, however, increasing the DDGS levels from 20-30% db and feed moisture content from 25-45% db resulted in an increased moisture content of the extrudates by 23.4 and 25.4%, respectively, while, increasing the processing temperature from 100-150°C resulted in a 10.3% decrease in moisture content values (Table 3). Several interactions between independent variables were observed as well (Table 4), including DDGS x protein x temperature x moisture content. The highest moisture content of the extrudates (16.2% db) was observed for the treatment combination of 30% DDGS, 35% protein, 100°C, 200 rpm and 45%

Table 3: Main treatment effects on extrudate physical properties†

Parameters	Levels	MC (db%)	UD (kg m^{-3})	BD (kg m^{-3})	ER (-)	SV (m s^{-1})	WAI (-)	WSI (%)	PDI (%)	L* (-)	a* (-)	b* (-)
DDGS (db%)	20	9.97b	1026.0a	421.4a	1.00b	0.07b	2.88a	18.3b	70.9b	37.4b	4.32c	12.6b
		(0.22)	(20.2)	(5.93)	(0.02)	(0.00)	(0.02)	(0.13)	(2.40)	(0.58)	(0.08)	(0.21)
	25	10.5b	1007.8a	318.7b	1.10a	0.10a	2.75c	19.7a	90.8a	39.3a	4.44b	12.6b
		(0.3)	(42.2)	(6.54)	(0.03)	(0.00)	(0.02)	(0.42)	(0.48)	(0.29)	(0.02)	(0.08)
	30	12.3a	1022.0a	421.4a	1.03b	0.09a	2.82b	18.9ab	69.7b	37.1b	4.81a	12.9a
		(0.33)	(17.8)	(5.28)	(0.01)	(0.00)	(0.03)	(0.20)	(3.70)	(0.65)	(0.08)	(0.23)
Protein content (db%)	30	10.7ab	1031.2a	418.6a	1.04b	0.07b	2.91a	18.5b	76.9b	37.7b	4.68a	12.7a
		(0.33)	(21.7)	(5.56)	(0.01)	(0.00)	(0.02)	(0.21)	(2.38)	(0.71)	(0.09)	(0.25)
	32.5	10.5b	1007.8a	318.7b	1.10a	0.10a	2.75b	19.7a	90.8a	39.3a	4.44b	12.6a
		(0.30)	(42.2)	(6.54)	(0.03)	(0.00)	(0.02)	(0.42)	(0.48)	(0.29)	(0.02)	(0.08)
	35	11.5a	1016.9a	424.2a	0.99b	0.09a	2.78b	18.7b	63.7c	36.7c	4.45b	12.7a
		(0.32)	(16.0)	(5.64)	(0.01)	(0.00)	(0.02)	(0.13)	(3.45)	(0.50)	(0.09)	(0.20)
Moisture content(db%)	25	9.89b	945.2c	429.7a	1.08a	0.08b	2.86a	18.9b	68.9b	40.4a	4.59a	13.8a
		(0.21)	(7.67)	(3.855)	(0.01)	(0.004)	(0.02)	(0.17)	(2.96)	(0.49)	(0.09)	(0.16)
	35	10.5b	1007.8b	318.7c	1.10a	0.10a	2.75b	19.7a	90.8a	39.3b	4.44b	12.6b
		(0.30)	(42.2)	(6.54)	(0.03)	(0.002)	(0.02)	(0.42)	(0.48)	(0.29)	(0.02)	(0.08)
	45	12.4a	1102.9a	413.1b	0.94b	0.08b	2.84a	18.3b	71.7b	34.1c	4.54ab	11.7c
		(0.33)	(20.2)	(6.73)	(0.01)	(0.005)	(0.02)	(0.16)	(3.26)	(0.31)	(0.08)	(0.17)
Temperature (°C)	100	11.7a	1093.1a	429.9a	1.05b	0.09a	2.79b	18.8b	85.2b	37.0b	4.25c	12.0c
		(0.31)	(17.4)	(4.20)	(0.01)	(0.00)	(0.02)	(0.17)	(0.96)	(0.75)	(0.08)	(0.23)
	125	10.5b	1007.8b	318.7c	1.10a	0.10a	2.75b	19.7a	90.8a	39.3a	4.44b	12.6b
		(0.30)	(42.2)	(6.54)	(0.03)	(0.00)	(0.02)	(0.42)	(0.48)	(0.29)	(0.02)	(0.08)
	150	10.5b	955.0b	412.9b	0.98c	0.06b	2.90a	18.5b	55.4c	37.4b	4.88a	13.5a
		(0.33)	(14.9)	(6.50)	(0.02)	(0.00)	(0.03)	(0.17)	(3.01)	(0.45)	(0.07)	(0.15)
Screw speed (rpm)	100	11.2a	1011.1a	417.9a	1.00b	0.08b	2.80b	18.8b	68.7b	36.3c	4.60a	12.6b
		(0.29)	(18.8)	(5.80)	(0.02)	(0.00)	(0.02)	(0.13)	(2.37)	(0.57)	(0.09)	(0.23)
	150	10.5a	1007.8a	318.7b	1.10a	0.10a	2.75c	19.7a	90.8a	39.3a	4.44b	12.6b
		(0.30)	(42.2)	(6.54)	(0.03)	(0.00)	(0.02)	(0.42)	(0.48)	(0.29)	(0.02)	(0.08)
	200	11.0a	1037.1a	424.9a	1.03b	0.08b	2.90a	18.5b	71.9b	38.1b	4.53ab	12.9a
		(0.37)	(19.2)	(5.38)	(0.01)	(0.00)	(0.02)	(0.21)	(3.70)	(0.64)	(0.09)	(0.22)

†Means followed by similar letters within each independent variable are not significantly different at $\alpha = 0.05$, LSD, for that dependent variable. Values in parentheses are standard error. MC = Moisture Content, UD = Unit Density, BD = Bulk Density, ER = Expansion Ratio, SV = Sinking Velocity, WAI = Water Absorption Index, WSI = Water Solubility Index, PDI = Pellet Durability Index

Table 4: Interaction results for DDGS, protein, Screw Speed (SS), Temperature (Temp) and Moisture Content (MC) on extrudate physical properties (p-values)†

Interactions	MC (db%)	UD (kg m^{-3})	BD (kg m^{-3})	ER (-)	SV (m s^{-1})	WAI (-)	WSI (%)	PDI (%)	L* (-)	a* (-)	b* (-)
DDGS	<0.0001	0.8346	<0.0001	0.0002	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Protein	<0.0001	0.2167	0.0531	<0.0001	<0.0001	<0.0001	0.2020	<0.0001	<0.0001	<0.0001	0.8180
SS	0.1334	0.0265	0.0160	0.0008	0.0210	<0.0001	0.0713	<0.0001	<0.0001	0.0127	<0.0001
Temp	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0287	<0.0001	0.0078	<0.0001	<0.0001
MC	<0.0001	<0.0001	<0.0001	<0.0001	0.0103	0.0964	0.0003	0.0001	<0.0001	0.0481	<0.0001
DDGS x Protein	0.7787	0.8032	0.8449	0.4426	<0.0001	0.3758	<0.0001	<0.0001	<0.0001	0.0097	<0.0001
DDGS x SS	0.3035	0.0351	0.0008	0.0664	0.0010	0.0279	0.6266	<0.0001	0.1039	<0.0001	0.0014
DDGS x Temp	0.4855	0.6070	<0.0001	0.0051	0.9857	<0.0001	0.0017	<0.0001	0.0565	<0.0001	<0.0001
DDGS x MC	<0.0001	0.4065	0.0102	0.7853	0.0148	<0.0001	0.7430	0.0033	0.0004	<0.0001	0.4486
Protein x SS	0.0076	0.2616	0.0541	0.7853	0.8222	<0.0001	0.2800	<0.0001	<0.0001	0.0047	<0.0001
Protein x Temp	0.0442	0.5052	<0.0001	<0.0001	0.2448	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.8962
Protein x MC	0.8667	0.0222	0.7049	0.2921	0.1284	0.0001	0.2888	<0.0001	<0.0001	<0.0001	0.9755
SS x Temp	0.0965	0.0086	0.0014	0.0265	0.3583	0.0025	<0.0001	<0.0001	0.0225	0.0001	0.9572
SS x MC	0.0056	0.0035	0.9840	0.9234	0.2957	0.3965	0.7324	0.9654	0.0001	<0.0001	<0.0001
Temp x MC	0.0360	<0.0001	<0.0001	0.8475	0.0008	<0.0001	0.0008	<0.0001	<0.0001	<0.0001	<0.0001
DDGS x protein x SS	0.0929	0.0415	0.2921	0.4057	0.0006	0.9276	0.9234	<0.0001	0.5790	0.0337	0.4486
DDGS x protein x temp	0.6143	0.0196	<0.0001	0.5430	0.4489	0.0045	0.0144	<0.0001	<0.0001	<0.0001	<0.0001
DDGS x protein x MC	0.0219	0.8195	0.6112	0.2574	0.0495	0.1525	0.4613	0.4310	<0.0001	0.0223	0.0054
DDGS x SS x temp	0.0034	0.0016	0.0027	0.2921	0.8758	0.0001	0.7588	<0.0001	<0.0001	<0.0001	<0.0001
DDGS x SS x MC	0.5787	0.5337	<0.0001	0.2197	0.9474	<0.0001	0.9515	<0.0001	0.3840	0.0001	0.0114
DDGS x temp x MC	0.0371	0.8105	0.0378	0.8349	0.0012	0.0054	0.2637	0.0721	<0.0001	<0.0001	<0.0001
Protein x SS x temp	0.0922	0.1633	0.0033	0.4715	0.0676	<0.0001	0.1961	<0.0001	0.0533	0.0037	0.0157
Protein x SS x MC	0.0416	0.5655	0.1037	0.3221	<0.0001	<0.0001	<0.0001	0.1755	<0.0001	0.0001	<0.0001
Protein x temp x MC	0.8212	0.7371	0.1444	0.1055	<0.0001	0.0058	0.8196	0.0728	0.2364	0.0145	0.1420
SS x temp x MC	<0.0001	0.1867	0.0527	0.1523	0.0151	0.0618	0.0081	<0.0001	0.0162	<0.0001	0.0044
DDGS x protein x SS x temp	0.9150	0.0067	0.0010	0.0578	0.0191	<0.0001	0.0991	0.0001	<0.0001	0.8177	<0.0001
DDGS x protein x SS x MC	0.1216	0.1175	0.0004	0.0598	0.1363	0.0385	0.0247	0.3701	0.0002	<0.0001	0.6733
DDGS x protein x temp x MC	0.0063	0.0194	0.0031	0.2574	<0.0001	0.0018	0.5847	0.0033	<0.0001	<0.0001	<0.0001
DDGS x SS x temp x MC	0.0471	0.0195	0.4178	0.5972	0.1183	<0.0001	0.1370	<0.0001	<0.0001	0.0896	<0.0001
Protein x SS x temp x MC	0.4681	0.1555	0.0094	0.2508	0.5144	0.0248	0.0685	0.0686	0.2014	<0.0001	<0.0001
DDGS x protein x SS x temp x MC	0.0789	0.0400	0.0117	0.0360	<0.0001	0.0002	0.4980	0.1801	<0.0001	<0.0001	<0.0001

†MC = Moisture Content, UD = Unit Density, BD = Bulk Density, ER = Expansion Ratio, SV = Sinking Velocity, WAI = Water Absorption Index, WSI = Water Solubility Index, PDI = Pellet Durability Index

moisture, whereas, the lowest value (6.27%) was found for the treatment combination of 20% DDGS, 30% protein, 150°C, 200 rpm and 25% moisture (Table 5). An increase in the extrudate moisture content due to an increase in feed moisture content was anticipated a priori. Our observations for changes in extrudate moisture content were consistent with the results of Badrie and Mellowes (1991) and Chevanan *et al.* (2007a). According to Faubion *et al.* (1982), due to the high temperatures and pressures involved, extrusion processing has a drying effect on materials and often up to 8 percentage points of a raw ingredient's moisture can evaporate due to the sudden change to ambient conditions upon exiting the die. A maximum moisture content of ≈12% is often recommended for feed products, because this level minimizes transportation costs and is microbiologically stable (Beauchat, 1981). The extrudate moisture content of our samples ranged from 6.27-16.2% (db).

Unit density: The main treatment effects of each independent variable on the unit density values of the extrudates are presented in Table 3. Increasing the feed moisture content from 25-45% db and processing temperature from 100-150°C resulted in a significant increase and decrease in unit density values by 16.7 and 12.6%, respectively. Kannadhason *et al.* (2007b) found that changes in DDGS and protein levels did not have any significant effects and our results were similar. DDGS level, protein content and screw speed did not exhibit a significant effect on the unit density values of the resulting extrudates. Several interactions between independent variables were observed (Table 4) as well, including DDGS x protein x speed x temperature x moisture. The extrudates with highest unit density (1279.1 kg m^{-3}) were found at the treatment combination 30% DDGS, 30% protein, 100°C, 200 rpm and 45% moisture; the lowest value (866.7 kg m^{-3}) was for the treatment combination of 20% DDGS, 30% protein, 150°C, 100 rpm and 45% moisture (Table 5). Generally, expansion of the extrudates decreases when the moisture content of the feed ingredient blend increases (Seiler *et al.*, 1980; Faubion and Hoseney, 1982a; Antila *et al.*, 1983;

Table 5: Treatment combination effects on extrudate physical properties[†]

		Protein levels (db%)															
		30								35							
		Temperature								Temperature							
		100				150				100				150			
		Screw speed (rpm)								Screw speed (rpm)							
		100		200		100		200		100		200		100		200	
	DDGS	25	45	25	45	25	45	25	45	25	45	25	45	25	45	25	45
Property	level (db%)	Moisture content (db%)															
MC (db%)	20	9.88f-i	11.3d-g	9.32i	10.5e-i	9.24i	10.3e-i	6.27j	9.8g-i	10.1f-i	11.1d-h	9.93f-i	11.8d-e	9.92f-i	9.54g-i	9.381i	10.9d-i
	30	10.5e-i	15.9a-b	10.6d-i	14.1c	10.1e-i	11.5d-f	9.97f-i	12.3d	11.3d-g	16.1a	10.8d-i	14.5b-c	11.1d-h	12.3d	9.67g-i	16.2a
UD (kg m^{-3})	20	997.2c-d	1225.8a-b	984.2c-f	1179.8a-b	873.6f-g	866.7g	928.2d-g	1221.5a-b	1002.1c-d	1203.1a-b	980.7c-f	1211.4a-b	917.4d-g	919.4d-g	932.4d-g	973.0c-g
	30	979.5c-f	1230.3a-b	956.8c-g	1279.1a	910.0d-g	1010.3c-d	881.7e-g	979.5c-g	989.9c-e	1136.9b	949.0c-g	1183.5a-b	926.6d-g	987.4c-e	913.7d-g	1043.5c
BD (kg m^{-3})	20	431.7c-g	453.6a-d	413.0g-i	450.5a-c	432.2c-g	341.3l-m	447.1b-e	382.9j-k	428.7d-h	447.9b-e	418.3f-i	475.2a	440.4b-f	327.6m	457.1a-c	396.2i-k
	30	404.9h-j	446.7b-e	411.8g-i	464.4a-b	447.3b-e	385.6j-k	432.6c-g	353.6l	377.1k	458.3a-c	412.3g-i	384.2j-k	447.0b-e	416.5f-i	474.6a	430.0e-h
ER (-)	20	1.11a-b	0.98e-g	1.14a	1.00e	1.10a-b	0.84i	1.08a-d	0.98e-f	1.12a-b	0.97e-h	1.13a	0.98e-h	0.90h-i	0.83i	1.02d-e	0.90g-i
	30	1.11a-b	1.00e	1.12a-b	0.97e-h	1.08a-d	0.96e-h	1.16a	1.00d-e	1.11a-b	0.99e	1.15a	0.99e	1.04b-e	0.90h-i	1.02c-e	0.90f-i
SV (m sec^{-1})	20	0.11a-d	0.00j	0.09b-f	0.08d-h	0.00j	0.06h	0.02i-j	0.07f-h	0.09c-g	0.13a	0.10b-f	0.10b-e	0.07g-h	0.06h	0.10b-e	0.07g-h
	30	0.11a-c	0.11a-b	0.09b-g	0.10b-e	0.07g-h	0.08d-h	0.04i	0.09b-f	0.09b-f	0.10b-e	0.09b-f	0.11a-b	0.07g-h	0.08e-h	0.09b-f	0.06g-h
WAI (-)	20	2.78h-k	2.96d-e	2.99b-d	2.92d-f	2.91d-g	2.98b-d	3.13a	2.88e-h	2.85f-i		2.94d-f	2.97c-e	2.80h-k	2.60m-n	2.99b-d	2.74j-l
	30	2.59m-n	2.76i-k	2.67l-m	2.80h-k	3.10a	3.08a-b	3.07a-c	3.07a-c	2.73j-l	2.76i-k	2.72j-l	2.60m-n	2.72j-l	2.56n	2.81g-j	3.11a
WSI (%)	20	18.2g-l	17.1	18.5d-l	17.6j-l	18.4e-l	18.0g-l	17.2l	17.6-l	19.1d-i	18.0g-l	19.1d-j	18.5d-l	19.4c-g	20.0a-d	19.1-j	17.8i-l
	30	20.8-b	18.4-l	20.6-c	21.0a	19.0d-k	18.9d-k	17.3l	18.1g-l	19.3c-h	17.9h-l	19.5b-g	17.5k-l	18.2g-l	19.9-e	19.41-h	17.3
PDI (%)	20	73.1h	88.5b-c	88.6b-c	90.2b	61.16i	53.5j	52.7j	78.6g-h	73.5h	85.1b-f	83.1c-g	85.7b-e	79.1f-h	53.2j	36.9l	52.0j
	30	78.8g-h	89.2b-c	89.9b	99.3a	49.1j-k	59.4i	97.2a	81.5d-g	80.4e-g	84.0b-d	86.5b-e	84.8b-g	43.5k	44.7k	29.1m	14.3n
L* (-)	20	33.4m-n	31.6p	43.6b-c	33.7l-n	4.94d	34.9k-l	42.3c	46.3i-j	43.9a-b	34.5k-m	43.1b-c	34.9k-l	37.7f-h	33.9k-n	37.3g-i	36.4h-j
	30	44.2a-b	30.8p	44.2a-b	32.0o-p	36.8g-i	35.3j-k	45.1a	38.2e-g	38.8e-f	33.0n-o	39.4e	31.0p	37.5f-i	33.7l-n	38.1e-g	34.7k-m
a* (-)	20	3.81l-m	4.16j-k	3.72m	4.52g-h	3.76l-m	4.88e-f	4.86e-f	4.08j-k	3.78l-m	3.69m	3.74m	3.65m	5.22b-d	4.79f	4.87e-f	4.15j-k
	30	5.11c-d	4.81f	5.05d-e	5.33b-c	5.24b-d	5.70a	5.25b-d	4.73f	4.13j-k	4.43h-i	4.25i-j	3.96k-l	5.42b	4.68f-g	5.28b-c	5.16c-d
b* (-)	20	11.1k	10.1n	13.1f-g	10.9k-m	14.7b-c	12.7g-h	14.5b-c	12.2i-j	13.6e-f	10.5m-n	13.3f	10.6l-n	14.3c-d	12.7g-h	13.9d-e	12.5h-i
	30	14.8b	10.1n	14.7b-c	11.0k-l	11.8j	13.4f	15.4a	13.1f-g	13.2f-g	11.2k	13.4f	10.3n	14.3c-d	12.6h-i	14.4b-c	13.3f

[†] Means followed by similar letters within each treatment combination are not significantly different at $\alpha = 0.05$, LSD, for that dependent variable. MC = Moisture Content, UD = Unit Density, BD = Bulk Density, ER = Expansion Ratio, SV = Sinking Velocity, WAI = Water Absorption Index, WSI = Water Solubility Index, PDI = Pellet Durability Index

Guy and Horne, 1988). Also, we can infer that an increase in the processing temperature resulted in decreased unit density values, which was reflected in a decrease in expansion (Case *et al*., 1992).

Bulk density: Bulk density is an important factor, as it impacts the storage volume of transport vehicles, vessels, containers, totes and bags (US Grains Council, 2008). The main effects on the bulk densities of the extrudates are summarized in Table 3. As shown, no clear pattern emerged on the bulk density of the extrudates due to the changes in DDGS levels, protein content, feed moisture content, processing temperature, or screw speed, although in appears that the behavior may be somewhat curvilinear in nature. Increasing the DDGS level from 20-25% db, protein content from 30-32.5% db, feed moisture content from 25-35% db, processing temperature from 100-125°C and screw speed from 100-150 rpm resulted in a significant decrease of bulk density values by 24.3, 23.8, 25.8, 25.8 and 23.7%, respectively. Further increasing the DDGS from 25-30% db, protein content from 32.5-35% db, feed moisture content from 35-45% db, processing temperature from 125-150°C and screw speed from 150-200 rpm led to a significant increase in bulk density values by 32.2, 33.1, 29.6, 29.5 and 33.3%, respectively (Table 3). Many interactions between independent variables were observed as well (Table 4). Furthermore, statistical analyses on all collected data confirmed that differences between experimental treatments did exist (Table 5). The highest bulk density value (475.2 kg m^{-3}) was observed for the treatment combination of 20% DDGS, 35% protein, 100°C, 200 rpm and 45% moisture. A higher bulk density lowers trucking costs, reduces shrinkage (because less material is lost as dust) and improves handling in feeding equipment (Dozier, 2001), compared to lower bulk densities. The lowest bulk density was 327.6 kg m^{-3}.

Expansion ratio: The main treatment effects of all the parameters on the expansion ratio of the resulting extrudates are shown in Table 3. Increasing the DDGS level, protein content and screw speed showed a curvilinear increase in expansion ratio values. A significant decrease of 14.5 and 10.9% were observed when the feed moisture content and processing temperature were increased from 35-45% db and 125-150°C, respectively (Table 3). A few interactions between independent variables were also present (Table 4). The highest expansion ratio (1.14) was found for the treatment combination of 20% DDGS, 30% protein, 100°C, 200 rpm and 25% moisture, whereas, the lowest expansion ratio (0.83) was found for the treatment combination of 20% DDGS, 35% protein, 150°C, 100 rpm and 45% moisture (Table 5). Depending upon composition, it has been found that extrudates typically do not start to expand until a temperature of ~100°C has been reached (Gomez and Aguilera, 1984; Paton and Spratt, 1984). Studies done by Gomez and Aguilera (1984) and Paton and Spratt (1984) found an increase in expansion ratio with increasing temperature, but this behavior depends on composition. Our results support the findings of Badrie and Mellowes (1991) and Chang and Wang (1998). The decrease in expansion ratio values with an increase in processing temperature found in this study may be attributed to the increased dextrinization and weakening of structure (Launay and Lisch, 1983). Generally, expansion ratio decreases when the moisture content increases (Chinnaswamy and Hanna, 1988; Gomez and Aguilera, 1984; Seiler *et al*., 1980; Faubion and Hoseney, 1982a; Antila *et al*., 1983; Guy and Horne, 1988) and our findings support these outcomes. In addition, it has been shown that particle size of the ingredient blend, amylose content, degree of gelatinization and lipid level also have effects on the expansion ratio values (Chauhan and Bains, 1985; Mercier and Feillet, 1975). Moreover, degree of expansion of extrudates is closely related to the size, number and distribution of air cells in the cooked material (Lue *et al*., 1990).

Sinking velocity: The main effects of the independent variables upon sinking velocity of the extrudates are shown in Table 3. From our results, no clear patterns emerged on sinking velocity values due to changes in DDGS level, protein content, feed moisture content, processing temperature, or screw speed, although curvilinear behavior may be present. Increasing the DDGS levels from 20-30% db and protein content from 30-35% db resulted in a significant increase in sinking velocity values by 28.6 and 28.6%, respectively. Kannadhason *et al*. (2007a, b) also found an increasing trend of sinking velocity values as DDGS level increased. In contrast, sinking velocity values fell by 33.3% for an increase in processing temperature from 100-150°C, which was in agreement with the findings reported by Kannadhason *et al*. (2007a). A few interactions existed between independent variables (Table 4). Additionally, the statistical analyses on all the collected data proved that significant differences did exist between experimental treatments (Table 5).

Water absorption and solubility indices: The Water Absorption Index (WAI) is the weight of gel obtained g^{-1} of dry sample; Water Solubility Index (WSI) is the percent of dry matter recovered after the supernatant is evaporated from the WAI determination (Anderson *et al*., 1969). Table 3 summarizes the main treatment effects of all the parameters studied on the WAI and WSI values for the extrudates. Once again, the main effects appeared to be curvilinear in nature. Increasing the DDGS level from 20-30% db and protein content from 30-35% db significantly decreased the WAI values, whereas, an increase in the processing temperature from 100-150°C and screw speed from 100-200 rpm resulted in significant increases in WAI values (Table 3). The change in WAI values with the increase in screw speed might be due to the degradation of starch, but the further increase at higher shear conditions was probably due to structural modifications of the fiber (Badrie and Mellowes, 1991). Studies done by Colonna and Mercier (1983) and Badrie and Mellowes (1991) indicated that an increase in processing temperature decreases WAI of the extrudates. Our results were due to different ingredient compositions used. Also, the increase in WAI values with an increase in processing temperature and screw speed could be accounted for by structural modifications involving fiber and the starch in the feed ingredient blends, which may have reduced the solubility (Hashimoto and Grossman, 2003). Other explanations for the increased WAI of the extruded products could be protein denaturation, starch gelatinization and swelling of the fiber, all of which take place during extrusion.

WSI, on the other hand, significantly increased by 7.6, 6.5, 4.2, 4.8 and 4.8% for an increase in DDGS level from 20-25% db, protein content from 30-32.5% db, feed moisture content from 25-35% db, processing temperature from 100-125°C and screw speed from 100-200 rpm, respectively (Table 3). Once again, these changes appeared to be curvilinear. Kannadhason *et al.* (2007a) also found a similar trend in WSI values as DDGS levels increased. Increasing the shear rate or screw speed (from 100-200 rpm) increased the WSI, even though residence time was reduced. Starch and/or fiber degradation was sufficient to increase the solubility (Hashimoto and Grossman, 2003). In addition, the water solubility of starch increases with expansion and the results from our study reflect this. Also, the stickiness of extruded starches is related to increased solubility.

For both WAI and WSI, a few interactions between independent variables were observed (Table 4). The highest WAI value (3.11) was observed for the treatment combination 30% DDGS, 35% protein, 150°C, 200 rpm and 45% moisture, whereas, the highest WSI (21.0%) was found for the treatment combination of 30% DDGS, 30% protein, 100°C, 200 rpm and 45% moisture (Table 5). The values of the WAI for the extrudates are reflective of undamaged polymer chains and the availability of hydrophilic groups, which can bind water molecules (Gomez and Aguilera, 1984).

Pellet durability index: The mechanical strength of extrudates is often assessed via durability, which is an important quality of feed materials (Rosentrater *et al.*, 2005). Durability is governed by the extent of heat treatment and the relative degree of starch transformation during processing. The effects of each factor on pellet durability index are presented in Table 3. Curvilinear effects emerged for pellet durability index values due to changes in DDGS level, protein, feed moisture content, processing temperature and screw speed. Increasing the protein content from 30-35% db and processing temperature from 100-150°C, significantly decreased PDI by 17.2 and 35.0%, respectively.

Faubion and Hoseney (1982b) showed that an increase in protein, gluten, final moisture and hydrogen ion concentration can strengthen extrudates, depending on composition. On the other hand, our observations reflect the findings of Chevanan *et al.* (2007a, c) and Kannadhason *et al.* (2007a), whose studies were based on DDGS. Many interactions between independent variables were observed (Table 4). The highest pellet durability value was 99.3% for the treatment combination of 30% DDGS, 30% protein, 100°C, 200 rpm and 45% moisture (Table 5) and were thus, able to successfully withstand mechanical damage during storage and transportation. The lowest PDI, on the other hand, was 14.3%, which was extremely low and indicated very poor pellet quality.

Color: Color is a physical property often used by feed customers to access the quality of pellets (Turner, 1995). The main treatment effects due to changing the DDGS level, protein content, feed moisture content, processing temperature and screw speed are shown in Table 3. Treatment combinations effects are shown in Table 5. Hunter L* value, a measure of brightness or luminosity, ranged from 30.8-46.3; Hunter a* value, a measure of redness/greenness, ranged from 3.65-5.70; Hunter b* value, a measure of blueness/yellowness, ranged from 10.1-15.4. Overall, increasing the DDGS level in the ingredient blend from 20-30% db significantly increased a* values by 11.3%. On the other hand, no clear effects were found for L* or b* values for changes in DDGS levels. An increase in feed moisture content from 25-45% db resulted in decreased L* and b* values by 15.6 and 15.2%, respectively. Furthermore, as the processing temperature was increased from 100-150°C, a significant increase in a* and b* values were found of 14.8 and 12.5%, respectively. Often the main reason for color changes during extrusion are Maillard reactions that occur between the reducing ends of carbohydrates and proteins and/or protein denaturation (Goedeken, 1991).

Table 6: Main treatment effects on extruder processing parameters†

Parameter	Levels	MC at the die (db%)	Viscosity (Pa-s)	SME (J g^{-1})	MFR (g min^{-1})	Die pressure (MPa)	Torque (N-m)
DDGS (db%)	20	26.1a	2745.9a	549.2a	118.1a	5.18a	45.8a
		(1.19)	(409.7)	(91.9)	(7.86)	(39.4)	(3.23)
	25	27.9a	785.8b	146.9b	120.0a	4.27c	12.0c
		(0.32)	(58.1)	(9.38)	(9.41)	(8.20)	(0.80)
	30	27.4a	2753.9a	470.7a	111.8a	4.87b	44.10b
		(1.18)	(396.06)	(69.6)	(8.28)	(34.8)	(2.90)
Protein content (db%)	30	27.0a	3218.0a	528.3a	118.0a	5.51a	49.7a
		(1.12)	(451.15)	(83.0)	(6.92)	(39.2)	(3.16)
	32.5	27.9a	785.8c	146.9b	120.0a	4.27c	12.0c
		(0.32)	(58.1)	(9.38)	(9.41)	(8.20)	(0.80)
	35	26.5a	2281.8b	491.7a	111.9a	4.54b	40.2b
		(1.25)	(334.5)	(80.5)	(9.09)	(34.2)	(2.93)
Moisture content (db%)	25	19.6c	2229.7b	378.7b	138.0a	6.61a	42.8b
		(0.41)	(301.6)	(57.2)	(7.82)	(40.6)	(3.06)
	35	27.9b	785.8c	146.9c	120.0b	4.27b	12.0c
		(0.32)	(58.1)	(9.38)	(9.41)	(8.20)	(0.80)
	45	33.9a	3270.0a	641.3a	91.98c	3.44c	47.0a
		(0.70)	(471.4)	(94.9)	(5.98)	(21.4)	(3.07)
Temperature (°C)	100	28.8a	3282.0a	534.9a	113.8a	7.17a	52.6a
		(1.30)	(463.2)	(81.1)	(7.95)	(38.1)	(3.45)
	125	27.9a	785.8c	146.9b	120.0a	4.27b	12.0c
		(0.32)	(58.1)	(9.38)	(9.41)	(8.20)	(0.80)
	150	24.68b	2217.7b	485.1a	116.1a	2.88c	37.3b
		(0.97)	(313.2)	(82.4)	(8.23)	(10.1)	(2.49)
Screw speed (rpm)	100	26.8a	4054.1a	629.0a	79.53c	4.72b	52.6a
		(1.14)	(463.7)	(99.1)	(4.05)	(35.8)	(3.27)
	150	27.9a	785.8c	146.9c	120.0b	4.27c	12.0c
		(0.32)	(58.1)	(9.38)	(9.41)	(8.20)	(0.80)
	200	26.7a	1445.7b	390.9b	150.4a	5.33a	37.3b
		(1.23)	(193.1)	(51.5)	(5.79)	(38.2)	(2.73)

†Means followed by similar letters within each independent variable are not significantly different at $\alpha = 0.05$, LSD, for that dependent variable. Values in parenthesis are standard error. MC = Moisture Content, SME = Specific Mechanical Energy, MFR = Mass Flow Rate

Extrusion processing parameters

Apparent viscosity: The main treatment effects of all the parameters studied on the apparent viscosity values are shown in Table 6. Curvilinear patterns emerged in apparent viscosity for changes in DDGS level, protein content, feed moisture content, processing temperature and screw speed. Increasing the levels of each of the independent variable from the low level to the center point led to significantly decreased apparent viscosity values. Increases in each from the center point value to the high value resulted in increased viscosity values, for all independent variables. Increasing the DDGS levels from 25-30% db, protein content from 32.5-35% db, feed moisture content from 35-45% db, processing temperature from 125-150°C and screw speed from 150-200 rpm resulted in a substantial increase in apparent viscosity values by 250.4, 190.4, 316.1, 182.2 and 84.0%, respectively and thus, are curvilinear. According to Chevanan *et al.* (2008) and Gonzalez *et al.* (2005), viscosity values often decrease with respective increases in screw speed, because feed doughs are often pseudoplastic in nature. Our findings are consistent with their observations. Viscosity is also related to the level of moisture. At lower moisture levels, the viscosity undergoes less change than at higher moisture. From our results, the viscosity values were found to be proportional to the feed moisture content and DDGS levels. Similar findings have been shown by Lam and Flores (2003). Interactions between the independent variables were significant as well (Table 7). Additionally, results show that the treatment combination effects were significant (Table 8). From our results, we observed that the apparent viscosity values ranged from 382.2-9156.4 Pa-s.

Specific mechanical energy: The effects of changing each of the parameters studied on specific mechanical energy values are shown in Table 6. Many interactions were present between the independent variables (Table 7). Statistical analyses on all the collected data indicated that there were significant differences among all the treatment combinations as well (Table 8). Results showed that the SME values ranged from 94.1 and 1703.2 J g^{-1}. Increasing the DDGS level from 20-25% db, protein content from 30-32.5% db and processing temperature from 100-125°C, resulted in significant decreases in SME values by 73.3, 72.2 and 72.5%, respectively. SME depends on the composition of the product being extruded (Maga and Fapojuwo, 1986) and thus, upon starch content

Table 7: Interaction results for DDGS, protein, Screw Speed (SS), Temperature (Temp) and Moisture Content (MC) on extruder processing parameters (p-values)†

Interactions	MC at the die (db%)	Viscosity (Pa-s)	SME ($J\ g^{-1}$)	MFR ($g\ min^{-1}$)	Torque (N-m)	Die pressure (Mpa)
DDGS	0.0164	<0.0001	<0.0001	0.0137	<0.0001	<0.0001
Protein	0.3460	<0.0001	0.0369	0.0068	<0.0001	<0.0001
SS	0.8872	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Temp.	<0.0001	<0.0001	0.0057	0.2757	<0.0001	<0.0001
MC	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DDGS x protein	0.9347	<0.0001	0.0066	0.6816	<0.0001	<0.0001
DDGS x SS	0.0244	<0.0001	0.0077	0.9894	<0.0001	0.0047
DDGS x temp	0.2120	<0.0001	<0.0001	0.0343	<0.0001	<0.0001
DDGS x MC	0.2675	<0.0001	<0.0001	0.4039	<0.0001	<0.0001
Protein x SS	0.0434	<0.0001	<0.0001	0.0054	<0.0001	<0.0001
Protein x temp	0.5810	<0.0001	0.0320	0.0036	<0.0001	<0.0001
Protein x MC	0.5483	<0.0001	0.0124	0.0001	<0.0001	0.0922
SS x temp	0.4679	<0.0001	0.2473	0.0338	<0.0001	<0.0001
SS x MC	0.5840	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Temp x MC	<0.0001	<0.0001	<0.0001	0.7946	<0.0001	<0.0001
DDGS x protein x SS	0.0111	<0.0001	0.5657	0.0368	<0.0001	<0.0001
DDGS x protein x temp	0.3063	<0.0001	<0.0001	0.0706	<0.0001	<0.0001
DDGS x protein x MC	0.2955	<0.0001	0.2448	0.5300	<0.0001	0.0034
DDGS x SS x temp	0.0002	<0.0001	<0.0001	0.5886	<0.0001	<0.0001
DDGS x SS x MC	0.2905	<0.0001	<0.0001	0.0004	<0.0001	<0.0001
DDGS x temp x MC	0.0113	<0.0001	0.0023	0.0003	<0.0001	<0.0001
Protein x SS x Temp	0.3674	<0.0001	0.0394	0.0044	<0.0001	<0.0001
Protein x SS x MC	0.0206	<0.0001	<0.0001	0.0003	<0.0001	<0.0001
Protein x temp x MC	0.0014	<0.0001	0.0010	0.7523	<0.0001	0.0009
SS x temp x MC	0.5856	0.0033	0.0188	<0.0001	0.1624	<0.0001
DDGS x protein x SS x temp	0.0282	<0.0001	<0.0001	0.6641	<0.0001	<0.0001
DDGS x protein x SS x MC	0.0848	<0.0001	0.0544	0.5045	<0.0001	0.1102
DDGS x protein x temp x MC	0.0075	<0.0001	<0.0001	0.0053	<0.0001	0.8568
DDGS x SS x temp x MC	0.0176	<0.0001	<0.0001	0.8988	<0.0001	<0.0001
Protein x SS x temp x MC	0.0441	<0.0001	<0.0001	0.2942	<0.0001	0.5367
DDGS x protein x SS x temp x MC	0.0048	<0.0001	<0.0001	0.4708	<0.0001	0.1702

†MC = Moisture Content, SME = Specific Mechanical Energy, MFR = Mass Flow Rate

(Meuser *et al*., 1987; Meuser and Wiedmann, 1989). The highest SME value was found for the treatment combination that had 20% DDGS and thus, had the highest starch content. However, further increasing the DDGS levels from 25-30% db, protein content from 32.5-35% db and processing temperature from 125-150°C resulted in increases in SME values by 220.4, 234.7 and 230.2%, respectively. Thus, the main effects were curvilinear in nature. Chevanan *et al*. (2008) also found similar results for changes in DDGS levels in extrusion of tilapia feeds. Increasing the feed moisture content from 25-35% db decreased the SME values significantly because increases in water can reduce the viscosity of the extrudates, which is reflected in decreased shear. Our observations were consistent with the findings of Chevanan *et al*. (2007b), Harmann and Harper (1973), Bruin *et al*. (1978), Sahagu and Harper (1979), Van Zullichem *et al*. (1988), Maga and Fapojuwo (1986) and Lam and Flores (2003). It is also, interesting to point out that the SME values decreased significantly as the screw speed was increased from 100-200 rpm. Rosentrater *et al*. (2005) observed that SME was directly proportional to extruder screw speed, because feed doughs are rheologically pseudoplastic and increases in shear lead to decreases in viscosity. The highest SME value observed was 1703.2 $J\ g^{-1}$ for the treatment combination of 20% DDGS, 35% protein, 100°C, 100 rpm and 45% moisture (Table 8). The lowest SME value was 94.1 $J\ g^{-1}$. Furthermore, Robutti *et al*. (2002) postulated that, when corn-based ingredients are extruded, harder endosperm is converted to a melt more rapidly than softer endosperm and would thus, require less energy during the passage through the extruder barrel. This means that the dough melt can be subjected to shear for a longer time, thus, undergoing a higher degree of structural changes (and a higher degree of cooking). Consequently, type of starch will influence processing conditions and final product characteristics.

Mass flow rate: Mass flow rate in a single-screw extruder depends on the drag flow developed by screw rotation and the pressure developed due to the constriction at the die (Mercier *et al*., 1989). The main effects on the mass flow rate are presented in Table 6. Increasing the feed moisture content from 25-45% db resulted in a significant decrease of mass flow rate values by 33.3%, whereas, an increase in the screw speed from 100-200 rpm significantly increased the mass flow rate values by 89.1% (Table 6). This behavior was expected, because drag flow in extruders has been shown to be proportional to screw

Table 8: Treatment combination effects on extruder processing parameters[†]

		Protein levels (db%)															
		30								35							
		Temperature								Temperature							
		100				150				100				150			
		Screw speed (rpm)								Screw speed (rpm)							
		100		200		100		200		100		200		100		200	
	DDGS level	25	45	25	45	25	45	25	45	25	45	25	45	25	45	25	45
Property	(db%)	Moisture content (db%)															
MC at die (db%)	20	20.1g-h	35.5a-c	20.9g-h	36.8a-b	18.1g-h	29.4e-f	17.0h	32.5c-e	19.4g-h	38.2a-b	18.0g-h	38.93	20.0g-h	32.5c-e	20.1g-h	20.0g-h
	30	22.0g	37.9a-b	21.2g-h	37.2a-b	18.4g-h	30.7d-f	19.7g-h	34.0b-d	20.5g-h	38.7a	20.3g-h	35.9a-c	17.5h	29.4e-f	20.3g-h	34.7a-d
Viscosity (Pa-s)	20	1955.4k	7950.7c	3517.3h	1096.8l-n	871.7n-p	7286.6d	526.6r	631.4p-r	852.0n-p	9156.4a	4452.7g	1052.4l-n	1889.5k	1161.4l-m	601.0q-r	932.5n-o
	30	8646.0b	7990.3c	408.9r	382.2r	852.8n-p	5883.5e	2983.8i	504.5r	2452.2j	1211.8l	886.0n-o	502.5r	1100.6l-n	5605.2f	3679.8h	973.1l-o
SME (J g^{-1})	20	183.0j-m	1047.5d	807.4e-f	317.3h-k	99.1m	1553.8b	157.2k-m	180.1j-m	103.7m	1703.2a	1044.2d	381.4h-i	237.5i-m	227.5i-m	168.2k-m	577.4g
	30	813.4e-f	886.8e	94.10m	125.4m	95.3m	1199.5c	748.6e-f	144.9l-m	400.6h	172.7j-m	287.8h-l	190.0j-m	118.6m	1222.8c	701.0f-g	330.8h-j
MFR (g min^{-1})	20	110.2e-g	76.3i-j	171.1b	135.6c-d	101.0f-h	57.3k	168.5b	146.3c	101.5f-h	54.6k	198.8a	106.6f-g	102.4f-h	58.4j-k	168.9b	133.2c-d
	30	103.0f-h	84.3h-i	167.9b	113.1e-g	98.93g-h	54.8k	171.5b	128.8c-e	58.61j-k	57.0k	181.4b	101.0f-h	103.2f-h	50.74k	201.2a	113.3e-g
Die Pressure (MPa)	20	10.5c	3.95i-k	11.8a	5.67g	3.68j-n	2.08r	3.55k-o	2.47q-r	9.86d-e	2.85p-q	11.2b	3.81i-m	3.34m-o	2.21r	3.83i-l	2.07r
	30	10.2c-d	9.11f	9.70e	4.56h	3.13o-p	1.60s	3.88i-l	2.27r	3.60j-o	3.40l-o	10.5c	4.05i-j	3.20n-p	2.80p-q	3.85i-l	2.08r
Torque (N-m)	20	26.0k-l	102.1e	92.7g	27.0k	12.2q-r	94.2g	14.9o-p	14.2p-q	10.7r-s	122.0a	114.2b	26.4k	23.3m-n	14.5o-p	15.0o-p	23.7m-n
	30	111.9c	104.0d	10.8r-s	9.50s	11.8r	73.5i	76.2h	14.3p-q	32.0j	16.5o	22.0n	14.2p-q	15.0o-p	72.3i	96.8f	24.4l-m

[†]Means followed by similar letters within each treatment combination are not significantly different at $\alpha = 0.05$, LSD, for that dependent variable. MC = Moisture Content, SME = Specific Mechanical Energy, MFR = Mass Flow Rate

speed (Harper, 1981) and hence, higher screw speeds result in higher mass flow rates, due to a greater ability to convey the material along the extruder barrel. No significant differences existed due to changes in DDGS level, protein content, or processing temperature (Table 6). Interaction effects were significant as well (Table 7). MFR values ranged from 50.7-201.2 g min^{-1} (Table 8). The highest MFR value (201.2 g min^{-1}) was found for the treatment combination of 30% DDGS, 35% protein, 150°C, 200 rpm and 25% moisture.

Die pressure: The pressure developed inside an extruder die depends on parameters such as composition and rheological properties of the ingredient blend, melting and pumping characteristics and the die geometry of the extruder. The main treatment effects of each factor on the die pressure values are summarized in Table 6. From our results, we observe that increasing the feed moisture content from 25-45% db and processing temperature from 100-150°C significantly decreased the die pressure values by 48.0 and 59.8%, respectively, in approximately a linear fashion. Lam and Flores (2003) observed a similar trend during the extrusion of fish feed. Increases in DDGS levels and protein content produced a curvilinear decrease in the die pressure values, however. On the other hand, we found that increases in the extruder screw speed from 100-200 rpm resulted in a curvilinear increase in die pressure values by 13.0%, which was in agreement with the findings of Bhattacharya and Hanna (1986), who proposed that die pressure is related to screw speed. This increase in die pressure was anticipated, because as the extruder screw speed increased, the mass flow rate was also seen to increase and consequently so did die pressure. Many interaction effects between independent variables were significant (Table 7).

Statistical analyses on all collected data confirmed that statistical differences did exist among all the treatment combinations (Table 8). The die pressure values ranged from 1.6-11.8 MPa.

Torque: Torque is a dependent variable that provides some insight, albeit a composite one, into the operation of an extruder (Mercier *et al*., 1989). Table 6 summarizes the main treatment effects of all the parameters studied on the resulting torque values. Curvilinear decreases emerged for increases in DDGS level, protein content, processing temperature and screw speed. However, a curvilinear increase (by 9.8%) was observed for an increase in the feed moisture content from 25-45% db. Chevanan *et al.* (2008) also observed a similar trend for the torque values with changes in feed moisture content. Overall, the torque required to rotate the screw decreased with an increase in screw speed and this was due, at least in part, to the fact that viscosity decreases with an increase in screw speed, because the ingredient blend was pseudoplastic in nature. Reduced viscosity at higher shear rates affects the mass flow rate as well; mass flow rate also affects the torque requirement, in turn. Similar findings were reported by Chevanan *et al.* (2007b). Most independent variables had significant interactions (Table 7).

CONCLUSION

This investigation was conducted with the broad intention of enhancing the value of DDGS and examining its suitability for incorporation into aquaculture feeds. This was accomplished by varying the feed properties (DDGS levels, protein content and feed moisture content) and extruder conditions (processing temperature and extruder screw speed) and determining their effects on resulting extrudate properties. Changing the DDGS levels, protein content, feed moisture content, extruder barrel temperature and extruder screw speed significantly affected expansion ratio, sinking velocity, color (L*, a* and b*) and pressure at the die, in a curvilinear fashion. And increasing the DDGS levels, protein content, feed moisture content and processing temperature curvilinearly changed the PDI values as well. Increasing the feed moisture content resulted in a substantial increase in SME values; whereas increasing the screw speed significantly decreased the SME values. Further studies on the effects of using DDGS in aquafeeds containing various starch sources using large-scale single and twin screw extruders will provide a broader understanding of these feed and extruder variables.

ACKNOWLEDGEMENTS

The researchers gratefully acknowledge the financial support provided by the Agricultural Experiment Station, South Dakota State University, Brookings, SD and the North Central Agricultural Research Laboratory, USDA ARS, Brookings, SD to carry out this research project. The authors' appreciation extends to Dakota Ethanol, Wentworth, SD, for supplying DDGS. Also, the cooperation and assistance of Vykundeshwari Ganesan, Rumela Bhadra, Jenna Carsrud, Sharon Nichols and Lloyd Francisco in performing the experimental work is greatly appreciated.

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