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Effects of Ingredients and Extrusion Parameters on Aquafeeds Containing DDGS and Potato Starch

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Abstract: Isocaloric (3.05 kcal g^{-1}) ingredient blends were factorially formulated using three 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 potato 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 process settings used produced viable extrudates, but some were of better quality than others. For example, increasing the DDGS levels from 20-30% (db), protein content from 30-35% (db), feed moisture content from 25-45% (db) and processing temperature from 100-150°C significantly decreased the PDI values by 7.5, 10.7, 4.0 and 16.8%, respectively, but in a curvilinear fashion. Increasing the feed moisture content from 25-45% (db) resulted in a curvilinear increase in SME values by 29.8%. On the other hand, increasing the screw speed from 100-200 rpm curvilinearly decreased the SME values by 8.5%. This study highlights the importance of experimentally determining the effects of feed ingredients and process variables when developing aquafeeds from novel materials.

Key words: Potato 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 is micro molecular degradation of starch, 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 or sinking feeds, because it acts as a binder and impacts product expansion. The minimum starch content needed for floating and sinking feed are generally 18-22% and 9-11%, respectively (Riaz, 1997).

Aquaculture is a rapidly growing sector of agriculture and is one of the most reliable growth markets for prepared feed (Riaz, 1997). Two of the 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 fat rations with acceptable pellet durability and immersion 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, hardness or softness, resiliency, buoyancy and chewiness (Kazamzadeh, 1989). Currently, the largest cost component in aquaculture production is feed, which constitutes between 30 and 60% of the total operational costs of the farm; protein is often

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the most expensive nutritional factor. Therefore, determination of low-priced alternate sources of protein which could provide better growth is beneficial for feed manufacturers and aquaculture producers alike (Lovell, 1988; Davis and Stickney, 1978; Keong, 2003).

Extrusion processing is an important food and feed processing operation (Harper, 1981; Paton and Spratt, 1984). High Temperature Short Time (HTST) extrusion cooking is used in many industries for the production of expanded snack foods, ready-to-eat cereals and pet 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). 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).

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).

Distiller's Dried Grains with Solubles (DDGS) is a valuable feed ingredient and is one of the three 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, fairly low phosphorous content and low cost compared to fish meal, there is mounting interest in using DDGS in aquaculture diets (US Grains Council, 2008). Research has shown that DDGS is an acceptable ingredient to aquaculture species like 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 are focused on processing aspects have been conducted with DDGS as a base material for aquaculture feeds. These have examined varying the levels 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 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 can be successfully incorporated up to 60% for the production of floating aquaculture feeds. Corn starch has a lesser amount of amylopectin (72%) (Fennema, 1985) compared to other starches, which may have an impact on expansion when incorporated with DDGS and other ingredients (Chevanan *et al*., 2007b-d).

Other starch sources, such as cassava (82-85%) and potato (79%), with considerably higher proportions of amylopectin (Fennema, 1985), could result in better expansion and functional properties. Therefore, the objectives of this study were to produce feeds for tilapia using DDGS as a protein source and potato starch as a binder and to investigate the effects of various levels of DDGS, protein content, feed moisture content, screw speed and extruder barrel temperatures on the resulting physical properties of 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 three proportions of DDGS (20, 25 and 30% db) and three moisture contents (25, 35 and 45% db), along with appropriate quantities of soybean meal, potato 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, Huron, SD. The materials were ground to a fine powder

Table 1: Ingredient components ($g100^{-1}$ g) in the feed blends used in the study, and their compositions (dry basis) †

	Protein levels (db%)					Composition (db%)			
Ingredients (db%)	30		32.5		35	Protein	Fat	Fiber	Ash
DDGS	20	30	25	20	30	28	11.4	6.8	4
Soybean meal	36.4	34.3	38	41.4	35.3	46.5	0.5	3.5	8
Potato starch	26.1	20.3	17.9	15.6	11	0.1	0.1	0.1	0.61
Fish meal	9.5	7.4	11.1	15	15.7	63	7	1	19
Whey	5	5	5	5	5	12	0.5	0	9.4
Vitamin mix	1	1	1	1	1				
Mineral mix	2	2	2	2	2				
Total	100	100	100	100	100				

†: Bold letters indicate the center point treatment; All blends were formulated on a dry basis

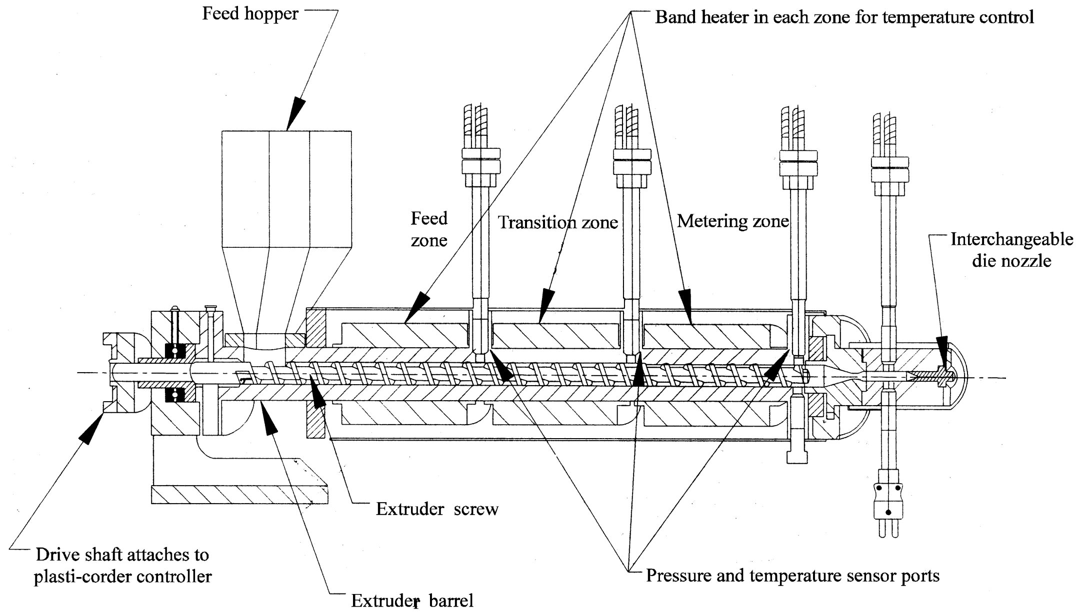

Fig. 1: Schematic of the single screw laboratory extruder used in the study (Kannadhason *et al*., 2008)

using a laboratory grinder (s500 disc mill, Genmills, Clifton, NJ) to an average particle size of ≈425 µm. Potato starch (PenPlus UM; 21% amylose, 79% amylopectin) was provided by Penford Food Ingredients, Centennial, CO. Whey was obtained from Bongards Creameries, Perham, MN; Menhaden fish meal from Consumers Supply Distribution Company, Sioux City, IA; 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 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 s^{-1}). After processing, the extrudates were allowed to cool and dry under ambient conditions for approximately 24 h.

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

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

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.

Unit Density (UD): The extrudates were cut into pieces ≈25.4 mm in length using a razor blade. Each piece was measured for its mass using an electronic balance (model A -250, Denver Instrument, Arvada, CO) and length 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). Distance travelled for the time taken gave the sinking velocity ($m\ s^{-1}$).

Water Absorption and Solubility Indices (WAI and WSI): WAI and WSI were determined as outlined by Anderson *et al*. (1969). About 2.5 g of finely ground sample (≈150 μm) was suspended in 30 mL of distilled water in a tarred 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 an aluminum dish, placed in the oven for 2 h at 135°C (AACC Method 44-19, 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 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 CM 2500d, Minolta Corporation, Ramsey, NJ), where:

L* = Refers to brightness/darkness
a* = Refers to redness/greenness
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. The 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 the extrudates at the exit of the die using plastic bags, sealed tightly, allowing the samples to cool under ambient conditions for 3 h and by following AACC Method 44-19 (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 dough viscosity in the extruder was calculated using Eq. 2:

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

Where :

n_{app} = The apparent dough 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: 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 (Rosentrater *et al.*, 2005):

$$\text{SME} = \left(\frac{T \times \omega \times 60}{M_{feed}} \right) \quad (3)$$

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} was calculated using Eq. 4:

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

Where :

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

M_{prod} = The dry mass flow rate of the extruded product (g min^{-1})

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.

Table 2: Experimental factors used in the study †

Feed blends			
DDGS levels (db%)	20	25	30
Protein levels (db%)	30	32.5	35
Moisture content (db%)	25	35	45
Extrusion conditions			
Temperature (°C)	100	125	150
Screw speed (rpm)	100	150	200

† The experimental design consisted of 2 (DDGS levels)×2 (protein levels)×2 (moisture contents)×2 (temperatures)×2 (screw speeds) + 1 center point (between all of these levels) = 33 total treatment combinations. Bold letters indicate the center point treatment

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 three 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 three screw speeds (100, 150 and 200 rpm) and three barrel temperatures (100, 125 and 150°C). Each of these factors used two levels (i.e., a high and a low setting) with a central composite point for all factors. 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 presents, the main treatment effects due to DDGS level, protein content, feed moisture content, processing temperature and screw speed on the physical properties of the resulting extrudates. Table 4 shows the interactions between the independent variables and Table 5 provides the treatment combination effects on each of the physical properties due to simultaneously changing the levels of all of the independent variables.

Moisture content: Extrudate moisture content is a very important parameter that influences many other extrudate

Table 3: Main treatment effects on extrudate physical properties †

Parameter	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	10.6b	847.1b	372.8b	1.27a	0.03c	3.27b	17.6a	89.1b	36.4b	4.02b	11.4ab
		(0.2)	(25.8)	(7.46)	(0.03)	(0.01)	(0.05)	(0.26)	(1.47)	(0.88)	(0.06)	(0.27)
	25	11.6a	969.9a	406.4a	1.21b	0.07a	3.44a	17.7a	96.4a	35.5a	3.61c	11.2b
		(0.04)	(48)	(3.25)	(0.02)	0	(0.01)	(0.13)	(0.17)	(0.2)	(0.01)	(0.04)
	30	10.5b	925.8ab	380.5b	1.13c	0.06b	3.10c	18.1a	82.4c	35.1c	4.44a	11.7a
		(0.15)	(18.9)	(9.48)	(0.02)	0	(0.05)	(0.21)	(2.17)	(0.65)	(0.09)	(0.28)
Protein content (db%)	30	10.4b	846.8b	373.1b	1.30a	0.03c	3.30b	17.2b	90.6b	35.4b	4.25a	11.4a
		(0.15)	(25)	(8.85)	(0.02)	0	(0.06)	(0.26)	(1.03)	(0.88)	(0.07)	(0.27)
	32.5	11.6a	969.9a	406.4a	1.21b	0.07a	3.44a	17.7ab	96.4a	38.5a	3.61b	11.2b
		(0.04)	(48)	(3.25)	(0.02)	0	(0.01)	(0.13)	(0.17)	(0.2)	(0.01)	(0.04)
	35	10.7b	926.1ab	380.1b	1.10c	0.06b	3.07c	18.5a	80.9c	36.2b	4.20a	11.7a
		(0.19)	(20)	(8.21)	(0.02)	0	(0.04)	(0.17)	(2.3)	(0.65)	(0.09)	(0.28)
Moisture content (db%)	25	9.93b	859.0b	386.7b	1.30a	0.03c	4.45a	18.6a	87.5b	39.2a	4.37a	12.5a
		(0.17)	(20.2)	(7.17)	(0.02)	0	(0.04)	(0.19)	(1.51)	(0.55)	(0.09)	(0.19)
	35	11.6a	969.9a	406.4a	1.21b	0.07a	3.44a	17.7b	96.4a	38.5a	3.61c	11.2b
		(0.04)	(48)	(3.25)	(0.02)	0	(0.01)	(0.13)	(0.17)	(0.2)	(0.01)	(0.04)
	45	11.2a	913.9ab	366.6c	1.10c	0.05b	2.92b	17.1b	84.0c	32.4b	4.08b	10.6c
		(0.11)	(25.5)	(9.51)	(0.02)	(0.01)	(0.03)	(0.24)	(2.22)	(0.64)	(0.07)	(0.27)
Temperature (°C)	100	11.0a	968.3a	403.8a	1.23a	0.05b	3.20b	18.1a	93.6b	33.0b	4.09b	10.4c
		(0.2)	(24.3)	(6.79)	(0.03)	(0.01)	(0.06)	(0.2)	(0.53)	(0.77)	(0.07)	(0.27)
	125	11.6a	969.9a	406.4a	1.21ab	0.07a	3.44a	17.7a	96.4a	38.5a	3.61c	11.2b
		(0.04)	(48)	(3.25)	(0.02)	0	(0.01)	(0.13)	(0.17)	(0.2)	(0.01)	(0.04)
	150	10.1b	804.6b	349.5b	1.17b	0.03c	3.18b	17.7a	77.9c	38.5a	4.36a	12.7a
		(0.11)	(14.8)	(8.29)	(0.02)	0	(0.04)	(0.27)	(2.11)	(0.54)	(0.09)	(0.15)
Screw speed (rpm)	100	10.6b	942.5a	399.5a	1.12c	0.05b	3.16b	18.0a	85.8b	35.1c	4.31a	11.4ab
		(0.15)	(26)	(8.42	(0.02	(0.01)	(0.06)	(0.22)	(1.95)	(0.78)	(0.09)	(0.29)
	150	11.6a	969.9a	406.4a	1.21b	0.07a	3.44a	17.7a	96.4a	38.5a	3.61c	11.2b
		(0.04)	(48)	(3.25	(0.02	0	(0.01)	(0.13)	(0.17)	(0.2)	(0.01)	(0.04)
	200	10.5b	830.4b	353.7b	1.28a	0.03c	3.21b	17.8a	85.6b	36.4b	4.14b	11.7a
		(0.2)	(16.8)	(7.29)	(0.02)	0	(0.05)	(0.26)	(1.89)	(0.76)	(0.08)	(0.26)

† Means followed by similar letters within each parameter are not significantly different (a = 0.05, LSD). Values in parentheses are standard error. MC is Moisture Content; UD is Unit Density; BD is Bulk Density; ER is Expansion Ratio; SV is Sinking Velocity; WAI is Water Absorption Index; WSI is Water Solubility Index; PDI is Pellet Durability Index

properties, including durability, WAI and WSI (Rolfe *et al*., 2001). No clear pattern was observed for moisture content due to increases in DDGS level, protein content, feed moisture content, processing temperature, or screw speed. However, increasing the feed moisture content from 25-45% (db) and processing temperature from 100-150°C, resulted in a significant increase and decrease in the moisture content of the resulting extrudates by 12.8 and 8.2%, respectively. Interactions between the independent variables were significant as well (Table 4). Additionally, results show that the treatment combination effects were significant (Table 5). The lowest and the highest extrudate moisture content were found to be 8.78 and 12.5% (db) for the ingredient moisture content of 25 and 45% (db). Also, from Table 5, we observe that the highest unit and bulk density values occurred for the highest ingredient moisture content (45% db), which indicates that higher the moisture, the more dense the extrudate. Similar findings were reported by Badrie and Mellowes (1991). Furthermore, Chevanan *et al*. (2008) also pointed out similar changes in extrudate moisture content with respect to processing effects.

In extrusion processing, the micropore structure in extrudates results from the expansion, which occurs due to flashing of water because of the sudden drop in pressure at the die exit and as such, expansion, texture and final moisture content will depend on the ability of the matrix to stretch and to retain the evaporating water. Changes in the extrudate moisture content are influenced by the high temperatures, shear stresses and shear strains produced during extrusion processing that affect the interactions between water and the other chemical constituents, which influence the resulting internal cellular structures that occur during water evaporation upon the die exit (Miller, 1985). Our extrudate moisture contents were found to be lower compared to the raw ingredient moisture content, not only due to steam flashing at the die exit, but also because the extrudates were allowed to cool and dry at room temperature for 24 h, which resulted in reduction in the amount of water in the extrudates.

Unit density: The unit density of extrudates is directly related to the degree of expansion obtained during processing (Colonna *et al*., 1984). Floatability of extrudates is impacted by the unit density. The unit densities of the extrudates are summarized in Table 3. From our results, no clear effects on the unit density of

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	0.0365	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0295	<0.0001	<0.0001	<0.0001	<0.0001
Protein	0.1159	<0.0001	0.0169	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0249	0.0002
SS	0.2449	<0.0001	<0.0001	<0.0001	<0.0001	0.0035	0.2221	0.7418	<0.0001	<0.0001	<0.0001
Temp	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1961	0.0134	<0.0001	<0.0001	<0.0001	<0.0001
MC	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DDGS*Protein	0.9949	0.0209	<0.0001	0.1154	0.0003	0.0308	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DDGS*SS	0.3006	<0.0001	0.0244	<0.0001	0.0728	0.1484	0.0087	0.1599	<0.0001	0.0141	<0.0001
DDGS*Temp	0.0309	0.2275	0.0459	0.5256	<0.0001	<0.0001	0.1765	<0.0001	<0.0001	<0.0001	<0.0001
DDGS*MC	0.1357	0.0045	<0.0001	0.0102	<0.0001	0.0004	0.0005	0.0212	0.0002	<0.0001	0.3680
Protein*SS	0.3481	0.2994	0.6905	0.5862	<0.0001	0.0013	0.1026	<0.0001	0.0482	<0.0001	0.0023
Protein*Temp	0.2541	0.0036	<0.0001	0.0009	<0.0001	<0.0001	0.1618	<0.0001	<0.0001	<0.0001	<0.0001
Protein*MC	0.031	0.7126	0.270	0.0427	0.4925	0.0023	<0.0001	<0.0001	0.0023	<0.0001	<0.0001
SS*Temp	0.0676	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SS*MC	0.4537	0.0943	0.0004	0.0705	<0.0001	0.0215	0.0651	<0.0001	0.5544	<0.0001	0.0726
Temp*MC	0.0504	<0.0001	<0.0001	0.0115	<0.0001	<0.0001	0.0070	<0.0001	<0.0001	<0.0001	<0.0001
DDGS*Protein*SS	0.5234	0.2178	0.5766	0.0033	<0.0001	0.0023	0.1436	<0.0001	<0.0001	0.5490	<0.0001
DDGS*Protein*Temp	0.0004	0.2043	<0.0001	0.1809	<0.0001	<0.0001	0.9206	0.0004	0.2193	0.0002	0.0074
DDGS*Protein*MC	0.6001	0.0050	0.0004	0.0071	<0.0001	<0.0001	0.0598	<0.0001	0.0001	<0.0001	<0.0001
DDGS*SS*Temp	0.951	0.0027	0.0344	0.0037	0.0014	<0.0001	<0.0001	0.4852	0.7042	<0.0001	0.1854
DDGS*SS*MC	0.6078	0.2837	0.6794	0.1120	<0.0001	<0.0001	0.1527	0.2774	<0.0001	0.0006	<0.0001
DDGS*Temp*MC	0.1686	0.3979	<0.0001	0.0524	<0.0001	<0.0001	0.0299	<0.0001	<0.0001	0.0077	0.0367
Protein*SS*Temp	0.0052	0.0010	0.4255	<0.0001	<0.0001	<0.0001	0.0113	<0.0001	0.0013	0.0002	<0.0001
Protein*SS*MC	0.2112	0.0971	0.0794	0.1579	<0.0001	0.4709	0.9900	0.2036	0.0004	<0.0001	<0.0001
Protein*Temp*MC	0.2583	0.9357	0.2862	0.7853	0.0368	<0.0001	<0.0001	<0.0001	0.7005	<0.0001	<0.0001
SS*Temp*MC	0.1423	0.3408	<0.0001	0.0013	<0.0001	0.5486	0.8223	<0.0001	<0.0001	0.1189	<0.0001
DDGS*Protein*SS*Temp	0.5743	0.0279	0.3519	0.4776	<0.0001	0.0001	0.8317	<0.0001	0.0001	0.7216	0.0198
DDGS*Protein*SS*MC	0.7914	0.6860	<0.0001	0.1623	<0.0001	0.0821	0.0186	0.8940	<0.0001	0.0035	<0.0001
DDGS*Protein*Temp*MC	0.0046	0.6133	<0.0001	0.5555	0.1427	<0.0001	0.7061	<0.0001	0.0990	<0.0001	<0.0001
DDGS*SS*Temp*MC	0.0263	0.1982	0.0006	0.3053	<0.0001	0.0006	0.2095	0.0004	0.4971	<0.0001	0.0008
Protein*SS*Temp*MC	0.2236	0.9391	0.001	0.2649	0.0011	0.0002	0.1306	0.0678	0.0005	0.8551	0.0006
DDGS*Protein*SS*Temp*MC	0.2310	0.1493	0.7131	0.0215	<0.0001	0.0152	0.2703	0.3865	0.0011	0.0192	0.0011

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

the extrudates due to increases in the DDGS level, protein content, or feed moisture content were observed. But, increasing the DDGS levels from 20-30% (db) and protein content from 30-35% (db) resulted in a significant increase in unit density values by 9.3 and 9.4%, respectively. In contrast, the unit densities of the extrudates fell progressively by 16.9 and 11.9% for an increase in processing temperature from 100-150°C and screw speed from 100-200 rpm, respectively. Interactions between the independent variables were significant as well (Table 4). Additionally, results show that the treatment combination effects were significant (Table 5). The lowest unit density (600.7 kg m^{-3}) and the highest expansion ratio (1.79) were found for the same treatment combination (20% DDGS, 30% protein, 100°C, 200 rpm and 25% moisture). From our observations, the highest (1232.2 kg m^{-3}) and the lowest unit density values (600.7 kg m^{-3}) were observed for the same DDGS level (20% db), protein content (30% db) and processing temperature (100°C), but they differed by treatment combinations (i.e., 100 rpm and 45% moisture content vs. 200 rpm and 20% moisture content, respectively). Good agreement existed between our results and the findings of Kannadhason *et al.* (2007a, b) and Chevanan *et al.* (2007a). Additionally, Harper *et al.* (1981), Bhattacharya and Hanna (1986) and Chevanan *et al.* (2007b) reported that an increase in temperature resulted in a significant decrease in extrudate unit density, which is reflective of our results. Research conducted by Harper *et al.* (1981) and Bhattacharya and Hanna (1986) pointed out that higher temperatures typically result in lower melt apparent viscosities. Hence, at lower viscosities, when the extrudates exit through the die, they tend to expand more, which results in reduced unit density.

Bulk density: Bulk density is a very important variable because it impacts container fill and thus storage and transport (Mercier *et al.*, 1989). It depends on the size, shape and the extent of expansion during extrusion. The main treatment effects due to changing the DDGS level, protein content, feed moisture content, processing temperature and screw speed on the unit density values are presented in Table 3. Increasing the DDGS level from 20-30% (db), or the protein content from 30-35% (db), did not show any clear pattern on the unit density values, respectively. However, an increase in feed moisture

Table 5: Treatment combination effects on extrudate physical properties †

		Protein levels (db%) 30							
		100 (°C)				150 (°C)			
		Screw speed (rpm)							
		100		200		100		200	
		Moisture content (db%)							
Property	DDGS level (db%)	25	45	25	45	25	45	25	45
MC (% db)	20	10.3e-m	12.0a-c	9.45i-n	11.4a-f	9.54h-n	10.2e-n	9.92g-n	11.5a-e
	30	10.6d-k	11.8a-d	9.42j-n	11.6a-e	8.78n	10.4d-l	9.09l-n	11.2a-g
UD (kg m^{-3})	20	1027.6c-d	1232.2a	600.7o	823.9I-m	690.4n-o	690.6n-o	727.1m-n	780.4k-n
	30	1003.5c-f	1075.3b-c	899.2f-j	911.00e-i	764.1l-n	688.9n-o	859.7g-l	774.2l-n
BD (kg m^{-3})	20	446.3b-c	468.5a-b	345.9i-j	386.9f-g	389.5e-g	307.6l-m	343.4i-j	315.4k-l
	30	430.7c-d	475.0a	378.2g-h	391.9e-g	342.9i-j	312.8k-m	376.3g-h	259.7n
ER (-)	20	1.32e-g	1.11m-o	1.79a	1.37c-f	1.42c-d	1.17k-n	1.51b	1.34d-g
	30	1.27g-j	1.09m-p	1.38c-e	1.23h-k	1.30e-h	1.11m-o	1.29e-i	1.17k-n
SV (m s^{-1})	20	0.00m	0.13a	0.00m	0.00m	0.00m	0.00m	0.00m	0.00m
	30	0.08d	0.06e-g	0.00m	0.06f-g	0.06g	0.04i-j	0.00m	0.03k-l
WAI (-)	20	4.27a	3.03h-i	3.66b-c	3.13g-h	2.89i-k	3.04h-i	3.39e	3.60c
	30	3.55c-d	2.74k-l	3.78b	2.69l	3.57c-d	2.94i-j	3.69b-c	2.96i-j
WSI (%)	20	16.8h-k	16.9h-k	19.4b-d	16.3i-k	21.3a	16.8h-k	18.2d-h	13.1l
	30	17.5f-i	16.8h-k	17.4f-j	17.6e-i	18.1d-h	15.7k	18.0d-h	15.9j-k
PDI (%)	20	96.4a-c	96.2a-c	86.6h-i	95.9a-d	90.2f-h	88.5g-i	85.6i	94.97a-e
	30	95.3a-d	94.2b-f	90.9e-g	96.1a-c	85.8i	67.7k	92.6c-g	92.05d-g
L*(-)	20	32.4m	25.6p	39.9d-f	28.5o	44.04b	37.9g-h	46.3a	40.7c-d
	30	34.6a	26.4p	36.9h-i	28.1o	41.32c-d	34.8k-l	32.7m	36.5h-i
a* (-)	20	4.63e-f	3.89l-m	4.69e-f	4.21i-j	3.94k-m	4.54e-g	3.54o-p	3.55o-p
	30	4.73d-e	3.94k-m	4.95c	4.34g-i	4.71d-f	4.90c-d	3.31q	4.27i-j
b* (-)	20	10.9i	8.15n	13.4b-c	9.44k	13.1c-d	12.9d-f	12.7d-f	12.4f-g
	30	11.8h	8.36n	12.8d-f	9.27l-m	13.6b	12.6d-f	9.46k	12.5f-g
		Protein levels (db%) 35							
MC (% db)	20	10.9b-i	12.5a	12.2a	11.5a-e	9.90g-n	10.0f-n	8.85m-n	10.44d-l
	30	9.83g-n	12.1a-c	9.17k-n	11.7a-d	10.7c-j	11.0b-h	10.1e-n	10.2e-m
UD (kgm^{-3})	20	1019.7c-e	1159.8a-b	739.9m-n	886.2g-k	886.7g-k	769.5l-n	724.8m-n	795.0j-n
	30	1068.0b-c	1149.1a-b	854.9h-l	1041.8c-d	941.3d-h	913.3e-i	936.5d-h	931.9d-h
BD (kgm^{-3})	20	430.7c-d	443.4b-c	373.3g-h	328.2j-l	314.5k-m	391.5e-g	356.3h-i	323.9j-l
	30	415.0d-e	425.1c-d	314.5k-m	408.1d-f	464.8a-b	334.9i-k	465.2a-b	293.2m
ER (-)	20	1.19j-m	1.02p-q	1.43c	1.18k-m	1.10m-p	0.99q	1.29f-i	1.12l-n
	30	1.12m-o	0.99q	1.28g-i	1.03o-q	1.00q	0.831r	1.14k-m	0.98q
SV (m/s)	20	0.00m	0.11b	0.00m	0.05h	0.031	0.03j-k	0.04h-i	0.05f-g
	30	0.11b-c	0.10b-c	0.06f-g	0.08d	0.07e-f	0.00m	0.06e-g	0.09c
WAI (-)	20	3.55c-d	2.94i-j	3.34e-f	2.84j-l	3.59c-d	2.81j-l	3.401e	2.96i-j
	30	2.95i-j	2.77k-l	3.24f-g	2.75k-l	3.23f-g	2.78k-l	3.20f-g	2.83j-l
WSI (%)	20	18.1d-h	17.0g-k	19.2b-e	18.3d-h	18.8c-f	18.2d-h	17.7e-i	16.2i-k
	30	20.3a-b	19.2b-e	20.0a-c	18.5c-g	17.5f-i	18.7c-f	19.3b-d	18.9b-f
PDI (%)	20	96.2a-c	98.6a	89.9g-h	97.6a-b	88.8g-i	60.64l	88.5g-i	70.5j-k
	30	94.8a-e	90.7f-g	91.2e-g	86.6h-i	72.6j	56.1m-n	53.9n	57.7l-m
L* (-)	20	35.5i-l	28.3o	40.1d-e	28.8o	42.0c	36.1h-k	41.0c-d	36.3h-j
	30	40.1d-e	30.7n	41.9c	31.0n	38.8e-g	34.3l	39.2e-f	34.9j-l
a* (-)	20	3.64n-o	3.39p-q	4.13i-k	3.45o-q	4.06j-l	4.50f-h	4.30h-i	3.88l-m
	30	3.81m-n	3.60n-p	4.51e-g	3.67n-o	5.70a	5.05c	5.38b	4.18i-j
b* (-)	20	10.4j	8.29n	12.5f-g	8.58m-n	13.0c-e	12.5f-g	13.1c-d	11.8h
	30	11.8h	8.91l-m	13.5b-c	9.18k-l	14.3a	12.6e-g	14.2a	12.1g-h

content from 25-45% (db), processing temperature from 100-150°C, or screw speed from 100-200 rpm resulted in decreased unit density values of 5.2, 13.4 and 11.5%, respectively. Interactions between the independent variables were significant as well (Table 4). Thus, our results show that the treatment combination effects were significant (Table 5). From our results, the bulk density values of the extrudates ranged from 259.6-475.0 kg m^{-3}, depending on the treatment combination used. The highest bulk density (475.0 kg m^{-3}) was observed for the treatment combination of 30% DDGS, 30% protein, 100°C, 100 rpm and 45% moisture. According to Badrie and Mellowes (1991) and Chevanan *et al*. (2007a), increases in feed moisture content resulted in corresponding increases in the bulk density values. But results from our study were found to be somewhat different, which may be due to differences in the ingredient compositions used. On the other hand, in the research conducted by Badrie and Mellowes (1991) and Chevanan *et al.* (2007a), the authors found that extruder screw speed and bulk density were inversely proportional to each other, a behavior, which was consistent with our observations. Also, Chevanan *et al.* (2007b) postulated that increases in the processing temperature led to a decrease in bulk density of the extrudates, which was also reflective of our results.

Expansion ratio: The degree of expansion is an important factor in aquafeeds, as it impacts the unit density, bulk density, fragility and hardness of extruded products. The expansion ratios observed in our extrudates are provided in Table 3. Increasing the DDGS levels from 20-30% (db), protein content from 30-35% (db), feed moisture content from 25-45% (db) and processing temperature from 100-150°C significantly reduced the expansion ratio values by 11.2, 15.4, 15.4 and 4.9%, respectively. Interactions between the independent variables were significant as well (Table 4). Additionally, results show that the treatment combination effects were significant (Table 5). Decreases in expansion ratio with increases in these variables can be partially attributed to the increase in fiber and protein content and thus the dilution of the starch content in the feed blends, resulting in an increase in the mass viscosity, thereby restricting the expansion ability. Also, the presence of fiber in the DDGS probably ruptured cell walls and thus prevented bubbles from expanding to their full potential when the steam flashed at the die exit (Guy and Horne, 1988; Lue *et al*., 1990). In contrast, the expansion ratio of the extrudates substantially increased by 14.3% with an increase in screw speed from 100-200 rpm, which contradicts the findings of Chinnaswamy and Hanna (1988) and Hashimoto and Grossmann (2003). This was due to differences in chemical composition.

DDGS is by its very nature low in starch and high in fiber, which resulted in a reduced expansion ratio. Also, the maximum degree of expansion is related to starch content. Furthermore, Chevanan *et al.* (2007a, b) and Shukla *et al.* (2005) found decreased expansion ratio values with an increase in DDGS levels, which is reflected by our findings.

Additionally, our findings for expansion ratio as impacted by moisture content are supported by Gomez and Aguilera (1984), Hashimoto and Grossmann (2003), Kokini *et al.* (1992), Paton and Spratt (1984), Mercier and Feillet (1975), Seiler *et al.* (1980), Antila *et al.* (1983), El-Dash *et al.* (1984), Batistuti *at al.* (1991), Oliveira *et al.* (1992), Pan *et al.* (1992), Tomas *et al.* (1994) and Chang and Wang (1998). The decrease in expansion ratio values with respect to feed moisture content was partially due to the drop in the barrel temperature, which created lower vapor pressure in the dough at the die, resulting in less flashing of moisture at the die exit and hence reduced expansion (Badrie and Mellowes, 1991). Our findings regarding the influence of temperature on the expansion of our extrudates is supported by the findings of Hashimoto and Grossmann (2003) and Chang and Wang (1998). It is also interesting to note that the highest expansion ratio (1.79) and the lowest unit density (600.7 kg m^{-3}) of our extrudates were found for the same treatment combination, which is quite logical, as they are inversely related. The statistical analyses on expansion ratio values showed that statistical differences did exist among the treatment combinations. The highest expansion ratio (1.79) was found for the treatment combination of 20% DDGS, 30% protein, 100°C, 200 rpm, 25% moisture, whereas the lowest value (0.83) was found for the treatment combination of 30% DDGS, 35% protein, 150°C, 100 rpm, 45% moisture.

Sinking velocity: Sinking velocity depends on the extent of extrudate expansion and thus the biochemical and biomechanical changes which occur inside the extruder barrel and die (Chevanan *et al*., 2007b). Table 3 summarizes the main treatment effects due to the independent variables. Increasing the DDGS levels from 20-30% (db), protein content from 30-35% (db) and feed moisture content from 25-45% (db) resulted in a substantial increase in sinking velocity values by nearly 100, 100 and 67%, respectively. Interactions between the independent variables were significant as well (Table 4). Additionally, results show that the treatment combination effects were significant (Table 5). The increase in sinking velocity values could be attributed to the decrease in expansion ratio with the increase in DDGS levels, protein content and feed moisture content, all of which resulted in extrudates that were denser and had a higher potential to sink. On the other hand, the sinking velocity values of the extrudates progressively fell by 40 and 40% with an increase in processing temperature from 100-150°C and screw speed from 100-200 rpm, respectively. We also observed that expansion ratio increased with screw speed, which produced extrudates that were less dense, which in turn reduced the sinking velocity of the extrudates. Kannadhason *et al.* (2007a, b) found increased sinking velocity values for corresponding increases in DDGS levels, which were supported by our observations.

Water absorption and solubility indices: The main treatment effects of each factor on the WAI and WSI values are presented in Table 3. From our results, we found that increasing the DDGS level from 20-30% (db), protein content from 30-35% (db) and feed moisture content from 25-45%, significantly decreased the WAI values by 5.2, 7.0 and 34.4%, respectively. However, any increase in processing temperature and screw speed did not show any clear patterns on WAI values. Additionally, increasing the DDGS levels from 20-30% (db), processing temperature from 100-150°C and screw speed from 100-200 rpm did not have any significant effects on WSI values. On the other hand, an increase in feed moisture content from 25-45% (db) resulted in a significant decrease of 8.1% in WSI values. Interactions between the independent variables were significant as well (Table 4). Additionally, results show that the treatment combination effects were significant (Table 5). The highest WAI value (4.27) was observed for the treatment combination of 20% DDGS, 30% protein, 100°C, 100 rpm, 25% moisture. The lowest WSI value (15.7%) was observed at the treatment combination of 30% DDGS, 30% protein, 150°C, 100 rpm, 45% moisture.

Pellet durability index: Pellet durability essentially refers to quality; it is a measure of the physical integrity of finished feed pellets during handling and transport, with a goal of minimal fines generation and broken pellets (Dozier, 2001). Also, durable pellets alleviate the problem of dust formation. The main effects on PDI due to different processing conditions are presented in Table 3. As shown, we observed no clear patterns due to changes in screw speed from 100-200 rpm on the resulting PDI values. However, increasing the DDGS level from 20-30% (db), protein content from 30-35% (db), feed moisture content from 25-45% (db) and processing temperature from 100-150°C significantly decreased the PDI values by 7.5, 10.7, 4.0 and 16.8%, respectively. Interactions between the independent variables were significant as well (Table 4). Additionally, results show that the treatment combination effects were significant (Table 5). Ingredients such as fiber, minerals and fat can have a great influence on pellet durability. Other researchers (Chevanan *et al*., 2007a, c; Kannadhason *et al*., 2007a) have reported decreased pellet durability for an increase in the DDGS levels, which were strongly supported by our results. This could be attributed to the increase in fat and fiber and the concomitant decrease in starch content in the raw ingredient blends with an increase in DDGS level. These compositional changes can reduce the friction between the feed and die during processing. The same researchers (Chevanan *et al*., 2007a, c; Kannadhason *et al*., 2007a) have also reported that moisture content and PDI are directly proportional to each other, which compares favorably to the results obtained in our study. According to Cheftel *et al*. (1985), proteins possess binding properties, which can also increase the PDI. From our study, we observed that the mean PDI values ranged from 53.9-98.6%; the highest value (98.6%) was found for the treatment combination of 20% DDGS, 35% protein, 100°C, 100 rpm and 45% moisture content. Such a high PDI indicates that these extrudates were highly resistive to the destructive forces commonly encountered by feed materials during handling and storage. The lower PDI values might be due to the presence of highly altered materials that do not easily interact, or processing conditions, which do not lead to highly-bonded extrudates and thus poor durability (Colonna *et al*., 1984).

Color: Color is one of the physical properties that is often used by feed customers and manufacturers to qualitatively assess the quality of feed materials (Turner, 1995). Processing conditions during extrusion often favor non-enzymatic browning by Maillard reactions between proteins and reducing sugars (Berset, 1989). The main treatment effects due to changing DDGS level, protein content, feed moisture content, processing temperature and screw speed on the color values (L*, a* and b*) are shown in Table 3. We observed that increasing the feed moisture content from 25-45% (db) significantly decreased the L*, a* and b* values by 17.3, 6.6 and 15.2%, respectively. In contrast, an increase in the processing temperature from 100-150°C resulted in significant increases in color values (L*, a* and b*). Furthermore, increasing the DDGS levels from 20-30% (db) and screw speed from 100-200 rpm resulted in decreased (by 3.6%) and increased L* values, respectively. The DDGS used in our study was relatively dark in comparison with the other ingredients used (i.e., soybean meal, starch, whey). Therefore, as the DDGS content was increased, the feed brightness (L*) decreased as well. Increasing the DDGS levels from 20-30% (db) resulted in increased extrudate yellowness (b*) by 2.6%, which could be attributed to the yellowish color of DDGS. On the other hand, an increase in DDGS levels and screw speed significantly increased (by 10.4%) and decreased (by 3.9%) a* values of the extrudates, respectively. The color values were also affected by the differences in the raw ingredients used, interactions between them and the extruder processing parameters used. Interactions between the independent variables were significant as well (Table 4). Additionally, results show that the treatment combination effects were significant (Table 5). For example, the L* values ranged from 25.6 to 46.3; the highest value (46.3) was observed for the treatment combination of 20% DDGS, 30% protein 150°C, 200 rpm and 25% moisture.

Table 6: Main treatment effects on 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	20	26.9a	4214.8b	769.4b	101.8b	5.43a	68.7b
		(1.19)	(436.7)	(75.3)	(5.81)	(42.8)	(2.77)
	25	26.4a	2269.9c	418.1c	116.8a	4.73b	44.1c
		(0.47)	(41.4)	(3.64)	(2.01)	(5.25)	(0.89)
	30	26.2a	4408.0a	902.0a	94.1c	5.30a	72.7a
		(1.24)	(434.5)	(82.1)	(6.06)	(41.2)	(2.87)
Protein content (db%)	30	26.5a	4882.4a	936.6a	96.8b	5.74a	77.2a
		(1.2)	(458.1)	(83.1)	(6.22)	(43.5)	(2.67)
	32.5	26.4a	2269.9c	418.1c	116.8a	4.73b	44.1c
		(0.47)	(41.4)	(3.64)	(2.01)	(5.25)	(0.89)
	35	26.6a	3740.4b	734.8c	99.13b	4.99b	64.0b
		(1.23)	(395.2)	(71.7)	(5.71)	(40.1)	(2.89)
Moisture content (db%)	25	18.7c	4326.0a	727.3b	114.6a	7.91a	70.9a
		(0.32)	(443.1)	(69.5)	(6.05)	(40.2)	(2.79)
	35	26.4b	2269.9b	418.1c	116.8a	4.73b	44. 1 b
		(0.47)	(41.4)	(3.64)	(2.01)	(5.25)	(0.89)
		34.4a	4296.8a	944.1a	81.3b	2.80c	70.5a
		(0.49)	(428.4)	(84.3)	(4.11)	(12.7)	(2.87)
Temperature (°C)	100	28.6a	4532.6a	891.3a	114.6a	7.52a	75.5a
		(1.29)	(434.9)	(80.2)	(5.04)	(44.5)	(2.86)
	125	26.4b	2269.9c	418.1c	116.8a	4.73b	44.1c
		(0.47)	(41.4)	(3.64)	(2.01)	(5.25)	(0.89)
	150	24.5c	4090.2b	780.1b	81.3b	3.25c	66.0b
		(1.06)	(434.3)	(77.9)	(6.51)	(19.1)	(2.74)
Screw speed (rpm)	100	26.5a	6393.1a	872.6a	75.4b	5.11b	82.9a
		(1.19)	(406.4)	(72.5)	(2.91)	(40.3)	(2.83)
	150	26.4a	2269.9b	418.1b	116.8a	4.73c	44.1c
		(0.47)	(41.4)	(3.64)	(2.01)	(5.25)	(0.89)
	200	26.5a	2229.7b	798.8a	120.5a	5.63a	58.3b
		(1.24)	(174.1)	(85.8)	(5.49)	(43.5)	(2.46)

† Means followed by similar letters within each parameter are not significantly different (a = 0.05, LSD). Values in parenthesis are standard error. MC is Moisture Content; SME is Specific Mechanical Energy; MFR is Mass Flow Rate

Extrusion processing parameters

Apparent viscosity: The main treatment effects due to changing the DDGS level, protein content, feed moisture content, processing temperature and screw speed on the resulting apparent viscosity values are shown in Table 6. From this study, we found that increasing the screw speed from 100-200 rpm significantly reduced the viscosity values by 65.1%. A factor that reduced the apparent viscosity was structural damage caused by an increasing amount of shear (due to the increase in the extruder screw speed), known rheologically as being a pseudoplastic material, an effect, which was more evident at low moisture. This observation was in agreement with the findings reported by Chevanan *et al.* (2008) and Gonzalez *et al.* (2005). Also, an increase in DDGS levels from 20-30% (db) significantly increased the apparent viscosity values by 4.58%. One way to explain this increase is that the DDGS contained less starch and higher fiber compared to the other ingredients (such as starch, soybean meal, fish meal) in the ingredient mix. Hence, increasing the DDGS content in the blends changed the chemical composition and the potential functionality of the ingredients in the dough, which contributed to higher apparent viscosity values. Observations from this study are comparable with those reported by Chevanan *et al.* (2008). Furthermore, increasing the protein content, feed moisture content and processing temperature to the mid-level setting of each parameter resulted in markedly decreased viscosity values, but a further increase in each of the parameters (i.e., to their highest level) resulted in a substantial increase in viscosity values. Thus, the behavior of each response was curvilinear. The decrease in viscosity values with an increase in feed moisture content can be attributed to water acting as a plasticizer in the extruder channel, which results in a decrease in shear stress and thus apparent viscosity. Similar reports have been reported 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 study, we observed that the apparent viscosity values ranged from 1013.4-9489.2 Pa-s and the highest value (9489.2 Pa-s) was observed for the treatment combination of 20% DDGS, 35% protein, 100°C, 100 rpm, 45% moisture.

Specific mechanical energy: Specific mechanical energy is a dependent variable that amalgamates extrusion responses, required torque, screw speed, extrudate mass flow rates (Onwulata *et al.*, 2001) and quantifies energy

Table 7: Interaction results for DDGS, protein, Screw Speed (SS), Temperature (Temp) and Moisture Content (MC) on 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	0.0085	<0.0001	<0.0001	<0.0001	<0.0001	0.0044
Protein	0.6625	<0.0001	<0.0001	0.0568	<0.0001	<0.0001
SS	0.9679	<0.0001	0.0009	<0.0001	<0.0001	<0.0001
Temp	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MC	<0.0001	0.2862	<0.0001	<0.0001	0.3683	<0.0001
DDGS*Protein	0.102	<0.0001	<0.0001	<0.0001	<0.0001	0.0045
DDGS*SS	0.2066	0.0752	<0.0001	<0.0001	<0.0001	0.0003
DDGS*Temp	0.9628	<0.0001	0.0034	<0.0001	<0.0001	0.1843
DDGS*MC	0.15	<0.0001	0.0016	0.0043	<0.0001	0.1005
Protein*SS	0.0498	<0.0001	<0.0001	0.0007	<0.0001	<0.0001
Protein*Temp	0.3773	<0.0001	<0.0001	0.0003	<0.0001	0.8623
Protein*MC	0.173	<0.0001	<0.0001	0.0008	<0.0001	<0.0001
SS*Temp	<0.0001	0.0051	<0.0001	<0.0001	<0.0001	0.0008
SS*MC	0.0006	0.0215	0.0004	0.0934	0.986	0.1832
Temp*MC	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DDGS*Protein*SS	0.6053	<0.0001	0.1971	<0.0001	0.0012	<0.0001
DDGS*Protein*Temp	0.0714	<0.0001	<0.0001	0.0001	<0.0001	0.1114
DDGS*Protein*MC	0.7389	<0.0001	<0.0001	0.0373	<0.0001	0.7312
DDGS*SS*Temp	0.0354	<0.0001	<0.0001	<0.0001	<0.0001	0.0067
DDGS*SS*MC	0.641	<0.0001	<0.0001	0.0001	<0.0001	0.0014
DDGS*Temp*MC	0.4838	<0.0001	0.3934	<0.0001	<0.0001	0.8665
Protein*SS*Temp	0.1287	<0.0001	<0.0001	<0.0001	<0.0001	0.8037
Protein*SS*MC	0.0019	0.9118	0.0022	0.3916	<0.0001	0.0012
Protein*Temp*MC	0.534	<0.0001	0.0077	<0.0001	<0.0001	0.0064
SS*Temp*MC	0.2607	<0.0001	<0.0001	<0.0001	<0.0001	0.4623
DDGS*Protein*SS*Temp	0.7276	<0.0001	<0.0001	0.001	<0.0001	0.0467
DDGS*Protein*SS*MC	0.5471	<0.0001	<0.0001	0.0746	<0.0001	0.1042
DDGS*Protein*Temp*MC	0.012	<0.0001	<0.0001	0.0005	<0.0001	<0.0001
DDGS*SS*Temp*MC	0.001	<0.0001	0.0003	0.0009	<0.0001	<0.0001
Protein*SS*Temp*MC	0.6501	<0.0001	<0.0001	<0.0001	<0.0001	0.4309
DDGS*Protein*SS*Temp*MC	0.0025	<0.0001	0.0015	<0.0001	<0.0001	0.1954

† MC is Moisture Content; SME is Specific Mechanical Energy; MFR is Mass Flow Rate

consumption required to process each unit of mass, which passes through the extruder. Table 6 summarizes the main treatment effects due to changing each of the parameters studied on the SME values of the extrudates. From our results, we observed that increasing the DDGS level from 20-30% (db) and feed moisture content from 25-45% (db) significantly increased the SME consumption by 17.2 and 29.8%, respectively, while SME values fell by 21.5 and 12.5% with an increase in protein content from 30-35% (db) and processing temperature from 100-150°C, respectively. All of these changes, however, were curvilinear in nature. Interactions between the independent variables were significant as well (Table 7). Additionally, results show that the treatment combination effects were significant (Table 8). The highest SME value was 1759.7 J g^{-1} for the treatment combination of 30% DDGS, 30% protein, 100°C, 200 rpm and 45% moisture.

Mass flow rate: The main effects on extrusion mass flow rate due to each of the processing variables is presented in Table 6. As shown, increasing the DDGS levels from 20-30% (db), feed moisture content from 25-45% (db) and processing temperature from 100-150°C significantly decreased the mass flow rates of the extrudates, but in a curvilinear fashion. As expected, an increase in the extruder screw speed from 100-200 rpm resulted in a significant increase in mass flow rate values; in fact, they increased by 59.8%. This is logical because as the screw speed increases, the higher the ability of the screw to convey the material through the extruder barrel. Similar results have been reported by Chevanan *et al*. (2007b) in their studies of fish feed processing using DDGS. No clear pattern emerged in mass flow rate values with an increase in protein content from 30-35% (db), however, but the behavior was also curvilinear. From our results, the mass flow rate ranged from 53.2 and 167.3 g min^{-1}. Interactions between the independent variables were significant as well (Table 7). Additionally, results show that the treatment combination effects were significant (Table 8).

Die pressure: Table 6 shows the main treatment effects of each of the variables studied on the die pressure values. It was observed that increasing the processing temperature from 100-150°C and feed moisture content from 25-45% (db) resulted in a significant decrease in die pressure values by 64.6 and 56.8%, respectively. The decrease in die pressure values with corresponding increase in extruder barrel temperature may be attributed to a decrease in the apparent viscosity of the ingredient melt, which may have contributed to the decreased

Table 8: Treatment combination effects on extruder processing parameters †

		Protein levels (db%) 30							
		100 (°C)				150 (°C)			
		Screw speed (rpm)							
		100		200		100		200	
		Moisture content (db%)							
Property	DDGS level (db%)	25	45	25	45	25	45	25	45
MC at die (db%)	20	22.8j	36.6b-c	19.1l-n	37.5b	16.8o-p	32.63e-f	19.2l-m	31.8e-g
	30	20.0k-l	39.6a	18.4l-p	35.3c-d	16.6o-p	28.7h	17.3m-p	31.4f-g
Viscosity (Pa-s)	20	8782.5b	8076.6d	2016.2m	1581.7n-o	8129.8d	7850.5e	1695.2n	3855.4g
	30	3224.0i	8404.8c	1556.0n-o	3972.8f-g	8721.2b	8203.5c-d	1013.4p	1036.1p
SME (J g^{-1})	20	854.4h	1158.2d-f	582.5i-j	535.9i-l	1007.1f-h	1303.4c-d	386.4k-o	1506.0b
	30	309.6l-o	1152.9d-f	1186.2d-f	1759.7a	1027.2f-h	1516.6b	257.3o	441.9i-o
MFR (g min^{-1})	20	89.4h-j	63.8k	140.6c-d	110.1e-f	91.3h-j	63.0k	167.3a	111.5e-f
	30	89.3h-j	61.5k	54.3k	88.31h-j	91.9h-j	61.5k	166.9a	98.9g-h
Die Pressure (MPa)	20	10.5c-d	3.55h-i	13.8a	4.73f	4.63f	1.77j-k	5.98e	2.28j
	30	11.4b	3.58h-i	11.0b-c	4.36f-g	4.93f	1.77j-k	5.83e	1.63j-k
Torque (N-m)	20	112.3b-d	104.9e	52.21i	40.0l	104.3e-f	103.5e-f	44.9j	99.2g
	30	41.8k-l	110.2d	44.0j-k	102.4f	114.4b	105.9e	31.0o	25.1p
		Protein levels (db%) 35							
MC at die (db%)	20	21.7j-k	37.4b	18.7l-o	38.1a-b	16.9n-p	29.3h	18.5-o	33.6d-e
	30	21.7j-k	37.4b	17.1m-p	36.4b-c	16.2p	30.1g-h	17.6m-p	34.7c-d
Viscosity (Pa-s)	20	2522.3k	9489.2a	4079.7f-g	887.4p	2537.7k	2787.0j	1753.0n	1392.7o
	30	8830.2b	3270.4i	4186.3f	1642.5n	8769.9b	2690.6j-k	1399.3o	3608.4h
SME (J g^{-1})	20	256.0o	1451.7b-c	1160.4d-f	345.3l-o	295.7n-o	492.7i-m	467.0i-n	508.3i-l
	30	934.3g-h	532.2i-l	1417.7b-c	624.1i	1108.2e-g	561.2i-k	386.6k-o	1216.4d-e
MFR (g min^{-1})	20	87.3i-j	57.8k	139.1d	96.14g-i	97.9g-i	62.1k	149.3b-c	103.5f-g
	30	84.8j	55.6k	133.9d	97.77g-i	96.6g-i	53.2k	154.0b	116.9e
Die Pressure (MPa)	20	10.2	3.53h-i	10.8b-d	3.66h-i	4.39f-g	1.53k	3.86g-h	2.15k
	30	10.8b-d	3.11i	10.5c-d	3.62h-i	3.73g-i	2.08j-k	3.86g-h	312.5j-k
Torque (N-m)	20	35.5n	122.9a	104.2e-f	23.6p	34.5m-n	36.8m	45.3j	36.7m
	30	113.1b-c	42.0k-l	112.0c-d	41.8k-l	111.2c-d	34.7m-n	35.4m-n	92.4h

pressure inside the die. Lam and Flores (2003) and Chevanan *et al*. (2007b) observed similar trends during extrusion of fish feeds. Also, Chevanan *et al*. (2007b) showed an inverse relation between die pressure and feed moisture content, which is supported by our findings. Increasing DDGS levels did not show a clear pattern on the die pressure values, however. On the other hand, we could observe that an increase in the extruder screw speed from 100-200 rpm resulted in increased die pressure values by 10.2%. Bhattacharya and Hanna (1986) proposed that die pressure is directly related to screw speed, which was in a good agreement with our observations. This increase in die pressure was anticipated because as the extruder screw speed increases, the mass flow rate is also seen to increase and consequently so does die pressure. Interactions between the independent variables were significant as well (Table 7). Additionally, results show that the treatment combination effects were significant (Table 8).

Torque: The torque required to turn the extruder screw is related to its speed, fill and the viscosity of the material in the screw channel. Consequently, torque is a dependent variable that provides insight, albeit a composite one, into the operation of the extruder (Mercier *et al*., 1989). The effects of each factor on torque values of the extrudates is illustrated in Table 6. From our results, it could be observed that increasing the DDGS level, protein content and feed moisture content to the mid level of each factor markedly decreased the torque values; but a further increase in these parameters increased the torque values substantially. Thus, their behaviors were curvilinear in nature. Also, the torque required to rotate the screw decreased by 12.6 and 26.7% due to increasing the processing temperature from 100-150°C and extruder screw speed from 100-200 rpm, respectively, which reflects the findings reported by Chevanan *et al*. (2008). Interactions between the independent variables were significant as well (Table 7). Additionally, results show that the treatment combination effects were significant (Table 8). In our study, the torque values ranged from 23.6-122.9 N-m; the highest value (122.9 N-m) was observed for the treatment combination of 20% DDGS, 35% protein, 100°C, 100 rpm and 45% moisture.

CONCLUSION

This study was conducted with the broad intention of enhancing the value of DDGS and investigating its suitability for incorporation into aquaculture feeds. This was accomplished by varying the feed properties (DDGS level, protein content and feed moisture content) and extruder parameters (processing temperature and extruder screw speed) and determining their effects on the resulting extrudate properties. Changing the levels of these parameters significantly affected expansion ratio, sinking velocity, color (L*, a* and b*) and pressure at the die. Increasing the DDGS level, protein content, feed moisture content and processing temperature significantly decreased the PDI values, although these behaviors were curvilinear in nature. Increasing the feed moisture content resulted in a significant decrease in SME values and increasing the screw speed decreased the SME values; all of these behaviors were curvilinear in nature as well. Further studies on the effect of DDGS in large-scale single and twin screw extrusion will provide a broader understanding of these feed and extruder variables studied.

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