MEDWELL PUBLISHING
Journal of Aquaculture Feed Science and Nutrition 1 (1): 6-21, 2009
ISSN: 2070-1667

Effects of Ingredients and Extrusion Parameters on Aquafeeds Containing DDGS and Tapioca 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 tapioca 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 7.50, 16.2, 17.2 and 16.6%, respectively. Increasing the feed moisture content from 25-45% db resulted in a substantial increase in SME values by 256.2%. On the other hand, increasing the screw speed from 100-200 rpm significantly decreased the SME values by 33.7%. This study highlights the importance of experimentally determining the effects of feed ingredients and process variables when developing aquafeeds from novel materials.

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

INTRODUCTION

Starch is a biopolymer which is comprised of two 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 the most expensive nutritional factor. Therefore,

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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).

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 40 and 60% for the production of floating aquaculture feeds, respectively. Corn starch, previously used in our extrusion studies, had a lesser amount of amylopectin (72%) (Fennema, 1985), which resulted in poor expansion when incorporated with DDGS and other ingredients (Chevanan *et al.*, 2007b-d).

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).

Using cassava or tapioca starch, which have a considerably higher proportion of amylopectin compared to other starch sources (Fennema, 1985), could result in better expansion and functional properties of aquafeeds. Therefore, the objectives of this study were to produce feeds for tilapia using DDGS as a protein source and tapioca 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 three 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, tapioca 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 using a laboratory grinder (s500 disc mill, Genmills, Clifton, NJ) to an average particle size of ~ 425 μm. Tapioca starch (Tistar pregelled tapioca starch; 17% amylose, 83% amylopectin) was provided by Multikem Corporation, Ridgefield, NJ. 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 (J g^{-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

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

Ingredients (% db)	Blend protein levels (% db)				
	30		32.5	35	
DDGS	20.0	30.0	25.00	20.0	30.00
Soybean meal	39.5	33.1	38.75	41.4	38.20
Tapioca starch	22.4	18.4	15.75	13.9	9.00
Fish meal	10.1	10.5	12.50	16.7	14.80
Whey	5.0	5.0	5.00	5.0	5.00
Vitamin mix	1.0	1.0	1.00	1.0	1.00
Mineral mix	2.0	2.0	2.00	2.0	2.00
Total	100.0	100.0	100.00	100.0	100.00

	Ingredient composition (% db)			
	Protein	Fat	Fiber	Ash
DDGS	28.0	11.4	6.8	4.0
Soybean meal	46.5	0.5	3.5	8.0
Tapioca starch	0.1	0.08	0.0	0.4
Fish meal	63.0	7.0	1.0	19.0
Whey	12.0	0.5	0.0	9.4

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

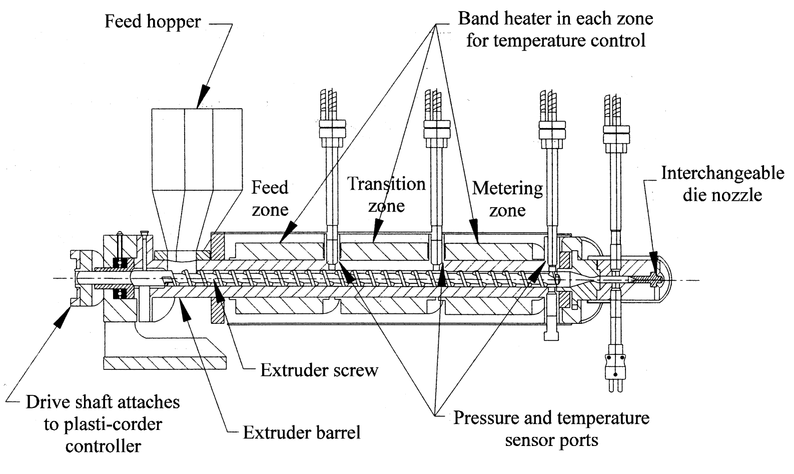

Fig. 1: Schematic of the single screw laboratory extruder used in the study

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). Total 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 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 two 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:

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

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:

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

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 (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 (Rosentrater *et al*., 2005):

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

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} 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.

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), net 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.

Table 2: Experimental factors used in the study[†]

Feed blends			
DDGS levels (% db)	20.0	25.0	30.0
Protein levels (% db)	30.0	32.5	35.0
Moisture content (% db)	25.0	35.0	45.0
Extrusion conditions			
Temperature (°C)	100	125	150
Screw speed (rpm)	100	150	200

[†]The experimental design consisted of 2 (DDGS levels) x2 (protein levels) x2 (moisture contents) x2 (temperatures) x2 (screw speeds) + 1 center point (between all of these levels) = 33 total treatment combinations. Bold letters indicate the center point treatment

RESULTS AND DISCUSSION

Extrudate properties

Moisture content: Extrudate moisture content is an important parameter that influences various properties such as water absorption, water solubility and pellet durability index (Rolfe *et al.*, 2001). The main treatment effects of each factor on moisture content of the extrudates are presented in Table 3. Increasing the feed moisture content from 25-45% (db) resulted in an increase in extrudate moisture content of 16.7%. Changing the other factors (DDGS, protein and screw speed) did not produce any significant differences in extrudate moisture content, although the interaction (Table 4) between DDGS, protein and temperature was significant (p = 0.0381). This was possibly due to the fact that increased feed moisture content dropped the barrel temperature, which created lower vapor pressure in the dough, resulting in less flashing of moisture upon die exit (Launay and Lisch, 1983). Our observations from the present study are in agreement with the findings reported by Badrie and Mellowes (1991) and Chevanan *et al.* (2007a). High temperatures, shear stresses and shear strains produced during extrusion processing affect the complex interactions between water and the other chemical constituents and thus alter the resulting internal cellular structures that occur during water evaporation upon die exit (Miller, 1985). Statistical analyses across all treatment combinations showed that protein, temperature and feed moisture content simultaneously significantly affected the moisture content of the extrudates at α = 0.05. The highest moisture content (17.3% db) was observed for the treatment combination 30% DDGS, 35% protein, 100°C, 200 rpm and 45% moisture (Table 5).

Unit density: Unit density is an important quality parameter, which determines whether the extrudates will float or sink (Chevanan *et al*., 2007c). Table 3 summarizes the main treatment effects of changing the feed and extruder parameters on the unit density of the extrudates. We noticed that increasing the protein content of the feed blend from 30-32.5% (db) resulted in a 7.65% increase, whereas no significant difference could be observed for changes in DDGS levels or screw speeds. Increasing the feed moisture content from 25-45% (db) and barrel temperature from 100-150°C resulted in a 9.47% increase and 26.4% decrease, in the unit density values, respectively. Many of the interactions between the independent variables (Table 4) were significant as well. Our observations with respect to temperature change are in agreement with the results discussed by Kannadhason *et al.* (2007a). Results show that temperature has an inverse relationship with the apparent viscosity of the ingredient melt inside the barrel and die (Harper *et al*., 1981; Bhattacharya and Hanna, 1986) and hence higher temperatures result in lower apparent viscosity of the melt. Therefore, as the ingredient melt with lower viscosities exits through the die, the extrudates exhibit greater expansion and thus reduced unit density. Results show that the treatment combination effects were significant (α = 0.05) and affected the extrudate unit density (Table 5). The highest unit density (1241.9 kg m^{-3}) was observed for the treatment combination 20% DDGS, 35% protein, 100°C, 200 rpm and 45% moisture, which indicates that these extrudates would be more suited for species of fish that prefer sinking feeds. The lowest unit density (628.4 kg m^{-3}) for the treatment combination 20% DDGS, 30% protein, 100 rpm, 150°C and 25% moisture (Table 5), which suggests that these extrudates possess the tendency to float.

Bulk density: Bulk density is a property which describes the weight of an ingredient per unit volume and thus affects transportation and storage costs. It is an important factor to consider when determining the storage volume of transport vehicles, vessels and containers (US Grains Council, 2008).The main effects on the bulk density of the extrudates are shown in Table 3. Changing the DDGS levels from 20-25% (db), protein content from 30-32.5% (db), feed moisture content from 25-35% (db), barrel temperature from 100-125°C and screw speed from 100-150 rpm decreased the bulk density values by 10.5, 13.0, 12.5, 18.1 and 12.0%, respectively. Statistical analysis across all treatment combinations simultaneously showed significant differences at α = 0.05 (Table 5);

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	10.5a	941.3a	394.0b	1.16a	0.04b	3.26a	18.6a	90.2b	33.80b	3.75b	10.40b
		(0.15)	(31.40)	(8.09)	(0.02)	(0.01)	(0.06)	(0.43)	(1.32)	(0.94)	(0.07)	(0.32)
	25	11.7a	930.7a	352.5c	1.14a	0.05a	3.29a	18.1a	95.8a	34.90a	3.42c	10.20b
		(0.18)	(5.63)	(5.67)	(0.01)	(0.01)	(0.02)	(0.19)	(0.10)	(0.23)	(0.05)	(0.03)
	30	10.9a	991.0a	407.9a	1.07b	0.05a	3.06b	18.7a	84.1c	35.70a	4.38a	11.70a
		(0.38)	(23.20)	(7.20)	(0.02)	(0.01)	(0.04)	(0.24)	(2.05)	(0.87)	(0.11)	(0.33)
Protein content (% db)	30	10.3a	930.5b	405.4a	1.18a	0.03c	3.27a	18.2a	92.7b	34.20a	4.04a	10.80b
		(0.16)	(30.20)	(8.66)	(0.02)	(0.01)	(0.06)	(0.42)	(0.59)	(1.06)	(0.08)	(0.34)
	32.5	11.7a	930.7b	352.5c	1.14b	0.05b	3.29a	18.1a	95.8a	34.90ab	3.42b	10.20c
		(0.18)	(5.63)	(5.68)	(0.01)	(0.01)	(0.02)	(0.19)	(0.10)	(0.23)	(0.05)	(0.03)
	35	11.0a	1001.7a	396.5b	1.05c	0.05a	3.05b	19.0a	81.6c	35.30a	4.08a	11.40a
		(0.37)	(24.10)	(6.58)	(0.02)	(0.01)	(0.04)	(0.24)	(2.16)	(0.73)	(0.13)	(0.33)
Moisture content (% db)	25	9.9b	922.4b	402.8a	1.19a	0.03c	3.19ab	19.9a	90.6b	36.70a	4.26a	11.70a
		(0.09)	(21.10)	(5.57)	(0.02)	(0.01)	(0.06)	(0.26)	(1.36)	(0.56)	(0.96)	(0.19)
	35	11.7a	930.7b	352.5b	1.14b	0.05b	3.29a	18.1b	95.8a	34.90b	3.42c	10.20b
		(0.18)	(5.63)	(5.68)	(0.01)	(0.01)	(0.02)	(0.19)	(0.10)	(0.23)	(0.05)	(0.03)
	45	11.5a	1009.8a	399.1a	1.04c	0.06a	3.13b	17.4b	83.7c	32.80c	3.86b	10.40b
		(0.36)	(32.00)	(9.38)	(0.02)	(0.01)	(0.05)	(0.33)	(1.99)	(1.09)	(0.10)	(0.42)
Temperature (°C)	100	11.3ab	1113.2a	430.3a	1.13a	0.06a	3.19ab	17.7b	93.4b	30.80c	3.85b	9.60c
		(0.36)	(19.80)	(6.34)	(0.02)	(0.01)	(0.06)	(0.33)	(0.63)	(0.87)	(0.10)	(0.35)
	125	11.7a	930.7b	352.5c	1.14a	0.05b	3.29a	18.1b	95.8a	34.90b	3.42c	10.20b
		(0.18)	(5.63)	(5.68)	(0.01)	(0.01)	(0.02)	(0.19)	(0.10)	(0.23)	(0.05)	(0.03)
	150	10.1b	819.1c	371.6b	1.10a	0.03c	3.13b	19.5a	80.9c	38.60a	4.27a	12.60a
		(0.14)	(15.50)	(6.52)	(0.03)	(0.01)	(0.05)	(0.31)	(2.07)	(0.51)	(0.10)	(0.12)
Screw speed (rpm)	100	10.5a	980.7a	400.6a	1.08b	0.04b	3.10b	18.7a	85.5c	33.10c	4.10a	10.60b
		(0.13)	(29.10)	(8.93)	(0.02)	(0.01)	(0.06)	(0.40)	(2.06)	(0.79)	(0.10)	(0.32)
	150	11.7a	930.7a	352.5b	1.14a	0.05a	3.29a	18.1a	95.8a	34.90b	3.42b	10.20c
		(0.18)	(5.63)	(5.68)	(0.01)	(0.01)	(0.02)	(0.19)	(0.10)	(0.23)	(0.05)	(0.03)
	200	10.9a	951.5a	401.3a	1.15a	0.05a	3.22ab	18.6a	88.8b	36.40a	4.03a	11.50a
		(0.38)	(26.30)	(6.28)	(0.28)	(0.01)	(0.04)	(0.28)	(1.40)	(0.96)	(0.11)	(0.35)

[†] Means followed by similar letters within each parameter are not significantly different at $\alpha = 0.05$, 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

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	0.2622	0.0010	<0.0001	<0.0001	<0.0001	<0.0001	0.5990	<0.0001	<0.0001	<0.0001	<0.0001
Protein	0.0299	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	0.1611	<0.0001
SS	0.1557	0.0285	0.6421	<0.0001	<0.0001	<0.0001	0.5695	<0.0001	<0.0001	0.0060	<0.0001
Temp	0.0002	<0.0001	<0.0001	0.0005	<0.0001	0.0356	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MC	<0.0001	<0.0001	0.0265	<0.0001	<0.0001	0.0419	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DDGS*Protein	0.9515	0.1090	<0.0001	0.1904	<0.0001	0.1377	0.1091	<0.0001	<0.0001	<0.0001	<0.0001
DDGS*SS	0.8471	0.3977	0.1031	<0.0001	0.0149	0.0038	0.0061	0.2560	<0.0001	<0.0001	<0.0001
DDGS*Temp	0.2178	0.0047	<0.0001	0.0153	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0054	<0.0001
DDGS*MC	0.5301	0.0180	<0.0001	<0.0001	0.2918	0.0004	0.0015	0.0076	<0.0001	0.7047	<0.0001
Protein*SS	0.1233	0.9937	0.0004	0.0115	<0.0001	0.6268	0.1463	<0.0001	<0.0001	<0.0001	<0.0001
Protein*Temp	0.1690	0.0292	<0.0001	0.6433	<0.0001	0.1676	0.0047	<0.0001	<0.0001	<0.0001	<0.0001
Protein*MC	0.8014	<0.0001	<0.0001	0.6048	<0.0001	0.0015	<0.0001	<0.0001	<0.0001	0.0182	<0.0001
SS*Temp	0.1728	<0.0001	0.0107	0.3416	<0.0001	<0.0001	0.0002	<0.0001	0.0036	<0.0001	<0.0001
SS*MC	0.0652	0.0051	0.0017	0.7782	<0.0001	0.4138	0.0444	<0.0001	0.0345	0.8402	0.5662
Temp*MC	0.1152	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3351	0.0004	<0.0001	0.0027	<0.0001
DDGS*Protein*SS	0.1489	0.8207	0.0054	<0.0001	<0.0001	0.6231	0.1721	0.9157	0.0044	<0.0001	<0.0001
DDGS*Protein*Temp	0.0381	0.7233	0.2359	0.1034	<0.0001	0.0017	0.2894	<0.0001	<0.0001	0.0041	<0.0001
DDGS*Protein*MC	0.9934	0.0455	<0.0001	0.0020	<0.0001	0.6634	0.2499	<0.0001	<0.0001	<0.0001	<0.0001
DDGS*SS*Temp	0.9831	0.7442	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2708	<0.0001	<0.0001	0.3464
DDGS*SS*MC	0.1902	0.6353	0.0290	0.0001	0.0369	0.5141	0.4136	0.5495	<0.0001	<0.0001	<0.0001
DDGS*Temp*MC	0.3530	0.7673	<0.0001	0.0153	<0.0001	0.0007	0.7656	0.0172	<0.0001	<0.0001	<0.0001
Protein*SS*Temp	0.0822	0.4903	<0.0001	0.5194	<0.0001	0.9447	0.4046	0.0151	<0.0001	0.0050	<0.0001
Protein*SS*MC	0.1176	0.1593	0.5955	0.4736	<0.0001	0.9293	0.7525	0.2019	<0.0001	<0.0001	<0.0001
Protein*Temp*MC	0.5392	0.5810	0.8012	0.0014	<0.0001	0.0425	0.7192	0.0045	<0.0001	<0.0001	<0.0001
SS*Temp*MC	0.2673	0.0002	0.0233	<0.0001	<0.0001	0.2250	0.9072	0.0006	<0.0001	<0.0001	0.0047
DDGS*Protein*SS*Temp	0.0926	0.6005	<0.0001	<0.0001	<0.0001	0.9697	0.9693	0.1418	<0.0001	<0.0001	<0.0001
DDGS*Protein*SS*MC	0.1560	0.0964	<0.0001	<0.0001	0.0188	0.2453	0.0038	0.1051	<0.0001	<0.0001	<0.0001
DDGS*Protein*Temp*MC	0.1297	0.0278	<0.0001	<0.0001	<0.0001	0.2737	0.3182	<0.0001	0.5507	<0.0001	<0.0001
DDGS*SS*Temp*MC	0.9553	0.0644	<0.0001	0.0342	<0.0001	0.1245	0.3240	0.0233	0.0037	0.8909	<0.0001
Protein*SS*Temp*MC	0.0663	0.9515	0.0002	0.0025	<0.0001	0.4555	0.5333	0.0369	0.2598	<0.0001	0.0001
DDGS*Protein*SS*Temp*MC	0.3101	0.9864	0.2039	<0.0001	<0.0001	0.0077	0.9481	0.2141	<0.0001	<0.0001	<0.0001

†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

interaction effects between independent variables were also significant (Table 4). The lowest bulk density was found for the treatment combination of 20% DDGS, 30% protein, 150°C, 100 rpm and 25% moisture.

Expansion ratio: The degree of radial expansion achieved during extrusion processing affects the floatability of extrudates (Oliveira *et al*., 1992). The degree of expansion of an extrudate is closely related to the size, number and distribution of air cells surrounded by the cooked material (Lue *et al*., 1990). Table 3 presents the main treatment effects for all the processing conditions on the expansion ratio of the extrudates. Changing the protein content from 30-32.5% (db) and feed moisture content from 25-45% (db) resulted in a 11.0 and 12.5% decrease in the expansion ratio values, respectively. Our results for changes in feed moisture content are consistent with the findings of Chinnaswamy and Hanna (1988), Gomez and Aguilera (1984), Hashimoto and Grossman (2003), Chang and Wang (1998) and Chang *et al*. (1999). This decrease in expansion values could be accounted for by the low moisture content, which may restrict the material flow inside the extruder barrel, increasing the shear rate and residence time, which would perhaps alter the extent of starch gelatinization and hence, the extrudate expansion (Colonna *et al*., 1984). Increasing the DDGS levels from 20-30% (db) and screw speed from 100-200 rpm resulted in a significant decrease of 7.96% and an increase of 6.92%, respectively. Our findings for changes in DDGS corroborate the results reported by Chevanan *et al*. (2007a) and Shukla *et al*. (2005). Interaction effects between independent variables, not only the lower-order interactions but also the higher-order interactions, were also significant (Table 4). Treatment combination effects were also significant ($\alpha = 0.05$; Table 5). The highest expansion ratio (1.52) was observed for the treatment combination of 20% DDGS, 30% protein, 150°C, 200 rpm, 25% moisture, while the lowest expansion ratio was found for the treatment combination of 30% DDGS, 30% protein, 150°C, 200 rpm, 25% moisture. From our results, we observed that the higher the addition of DDGS in the feed blends, the lower the expansion of the extrudates; this was probably due to the lower starch content in the DDGS.

Sinking velocity: For aquaculture feeds, sinking velocity is an important extrudate property. This determines the stability of the extrudates in water and is closely related to

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

Protein level (% db)		30							
Temperature °C		100				150			
Screw speed (rpm)		100		200		100		200	
Moisture content (% db)		25	45	25	45	25	45	25	45
Property	**DDGS level (% db)**								
MC (% db)	20	9.5c-d	11.1b-d	10.0b-d	12.1b-d	9.2d	10.0b-d	9.2d	10.0b-d
	30	10.1b-d	12.5b-c	9.6c-d	10.9b-d	9.7c-d	10.6b-d	9.3d	11.5b-d
UD (kgm^{-3})	20	1060.3d-f	1187.2a-b	807.1i-l	1257.5a	628.4n	734.6l-n	682.8m-n	803.1i-l
	30	1071.3c-e	1238.5a	917.9g-i	1197.2a-b	847.1h-l	793.2j-m	858.2h-k	804.5i-l
BD (kgm^{-3})	20	434.4e-f	520.2a	403.5h-k	498.1b	326.1q	331.9p-q	375.2m	344.0o-p
	30	431.2e-f	477.9c	389.5k-l	455.2d	413.7g-h	329.1q	398.5i-k	358.5n
ER (-)	20	1.28b-d	1.13f-g	1.47a	1.12f-g	1.11f-g	0.98i-l	1.52a	1.30b-c
	30	1.21d-e	1.02i-k	1.34b	1.04h-j	1.27c-d	1.12f-g	0.91m	1.18e-f
SV (m s^{-1})	20	0.01h	0.13a	0.01h	0.12a	0.01h	0.01h	0.01h	0.02g
	30	0.01h	0.01h	0.01h	0.12a	0.01h	0.06d	0.06d	0.05d-e
WAI (-)	20	3.78a	3.69a-b	3.79a	3.54a-d	2.57l	2.91i-j	2.93i-j	3.56a-d
	30	3.02g-j	2.82j-l	3.01h-j	2.95i-j	3.32d-f	3.50b-e	3.61a-c	3.35c-f
WSI (%)	20	16.7g-i	14.1j	19.0b-e	14.3j	23.5a	20.3b	22.4a	15.9h-j
	30	19.0b-f	15.5i-j	20.0b-c	16.9f-i	19.6b-d	15.9h-j	20.2b-c	17.3e-i
PDI (%)	20	96.2a-c	96.1a-c	94.3a-e	98.3a	87.8h-i	91.4e-h	90.9e-h	94.8a-e
	30	96.9a-b	92.2c-f	92.9b-f	89.9f-h	94.2a-f	81.5j	93.4b-f	91.6d-g
L* (-)	20	31.10i	22.20o	35.30g	22.60n-o	42.70b	39.10c-d	41.80b	38.40d-e
	30	32.00i	24.60m	35.70g	23.60m-n	35.80g	35.30g	41.80b	44.50a
a* (-)	20	4.14h-i	3.26o-p	4.35f-h	3.54l-n	3.88j	4.48e-f	3.53l-n	3.42m-o
	30	4.37f-h	3.70j-l	4.79d	3.63k-l	5.08c	4.66d-e	4.12i	3.84j-k
b* (-)	20	10.20k	6.42p	11.70g-i	6.69o-p	12.10e-h	13.00c	11.70g-i	11.60h-i
	30	10.40k	7.59m-n	12.20e-g	7.12n-o	12.30e-f	12.40d-e	12.80c-d	13.60b

Protein level (% db)		35							
Temperature °C		100				150			
Screw speed (rpm)		100		200		100		200	
Moisture content (% db)		25	45	25	45	25	45	25	45
Property	**DDGS level (% db)**								
MC (% db)	20	10.4b-d	11.0b-d	10.4b-d	12.7b	9.6c-d	11.5b-d	9.9b-d	11.1b-d
	30	10.9b-d	11.1b-d	10.7b-d	17.3a	10.0b-d	9.8b-d	9.2d	10.4b-d
UD (kgm^{-3})	20	1110.2b-d	1229.0a-b	965.6e-h	1241.9a	852.4h-l	800.5i-l	947.8f-h	752.2k-m
	30	1145.8a-d	1205.2a-b	996.7e-g	1179.7a-c	931.8g-h	856.1h-l	936.2g-h	876.7g-j
BD (kgm^{-3})	20	423.1f-g	394.7j-l	409.6g-i	408.8h-j	382.1l-m	326.2q	384.4l-m	343.1o-p
	30	339.9o-q	434.7e-f	406.0h-j	458.4d	489.9b-c	354.2n-o	437.6e	351.7n-o
ER (-)	20	1.16e-g	0.98i-l	1.26c-d	0.99i-l	1.12f-g	0.97j-m	1.17e-f	1.13f-g
	30	1.09g-h	0.96k-m	1.17e-f	0.98i-m	1.03h-k	0.93l-m	1.04h-i	0.92l-m
SV (m s^{-1})	20	0.09b	0.04f	0.01h	0.12a	0.01h	0.01h	0.08c	0.02g
	30	0.12a	0.10b	0.01h	0.10b	0.04e-f	0.05d-e	0.05d-f	0.06d
WAI (-)	20	3.55a-d	3.30d-f	3.43b-f	3.29d-g	2.61k-l	2.82j-l	3.24e-h	3.18f-i
	30	2.87j-k	2.61k-l	2.87j-k	2.63k-l	3.24e-h	3.01h-j	3.29d-g	2.98h-j
WSI (%)	20	18.2c-g	15.7i-j	18.9b-f	16.8g-i	22.5a	20.2b	20.0b-c	17.8d-g
	30	19.1b-e	20.3b	19.9b-d	18.6b-g	19.0b-e	18.9b-g	19.7b-d	18.6b-g
PDI (%)	20	97.2a-b	88.1g-i	96.4a-c	90.5e-h	87.9h-i	61.2m	93.1b-f	78.3j
	30	96.6a-b	85.7i	96.9a-b	85.4i	65.4l	49.2n	69.1k	64.8l-m
L* (-)	20	28.50j-k	27.70k	37.30e-f	26.10l	39.90c	35.10g-h	37.60e	35.20g
	30	35.60g	29.60j	39.40c-d	41.60b	35.70g	33.90h	36.30f-g	44.50a
a* (-)	20	3.55l-n	3.03q	3.65k-m	3.10p-q	4.28f-i	4.24g-i	4.21h-i	3.35n-o
	30	3.43m-o	3.37n-o	3.89j	5.93a	5.76a	4.45f-g	5.30b	3.84j-k
b* (-)	20	8.46l	7.84m	11.20j	7.50m-n	12.80c-d	11.90e-h	12.10e-h	11.20i-j
	30	10.30k	8.57l	11.80f-h	15.40a	13.80b	12.00e-h	13.60b	13.60b

[†] Means followed by similar letters within each parameter are not significantly different at $\alpha = 0.05$, LSD. 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 absorption of water during feeding at the water surface (Chevanan *et al*., 2007b). The main treatment effects of each factor on sinking velocity of the extrudates are presented in Table 3. We observed that changing the DDGS levels from 20-30% db, protein levels from 30-35% db and feed moisture content from 25-45% db increased the sinking velocity of the extrudates by 30, 55.1 and 127.7%, respectively. Our results for changes in DDGS and protein levels from this study are in agreement with the findings reported by Kannadhason *et al.* (2007b). Also, increasing the extruder barrel temperature from 100-150°C resulted in a 47.1% decrease in sinking velocity values and this observation parallels the findings of Kannadhason *et al.* (2007a). Most of the interactions between independent variables were significant (Table 4). Statistical analyses across all treatment combinations simultaneously showed that DDGS levels, protein levels, feed moisture content, barrel temperature and screw speed were all found to be significant at $\alpha = 0.05$ (Table 5). The highest sinking velocity (0.125 m s^{-1}) was found for the treatment combination of 20% DDGS, 30% protein, 100°C, 100 rpm and 45% moisture, which signifies that these extrudates could possibly be more suitable to the fishes that prefer sinking feed.

Water absorption and solubility indices: The WAI and WSI characterize how extruded products will interact with water and are often important in predicting how the extruded materials may behave if further processed. Also, the degree of conversion of starch from granule form during processing can be assessed via WAI and WSI (Sriburi *et al.*, 1999). Table 3 summarizes the main treatment effects of DDGS level, protein level, feed moisture content, barrel temperature and screw speed on WAI values of the extrudates. We found that WAI values decreased by 5.94, 6.53, 1.92 and 1.99% with an increase in DDGS level from 20-30% db, net protein level from 30-35% db, moisture content from 25-45% db and temperature from 100-150°C, respectively, whereas it was found to increase by 4.05% with the increase in screw speed from 100-200 rpm. Similar findings were discussed by Badrie and Mellowes (1991) and Hashimoto and Grossmann (2003) regarding temperature effects on WAI values and Kannadhason *et al.* (2007a) on the effects of DDGS levels on tapioca starch-based extrudates. This decrease in WAI values with an increase in temperature might be due to an increase in starch degradation (Colonna and Mercier, 1983; Badrie and Mellowes, 1991). Most of the interactions were significant for the lower-order interactions, but few were significant for the higher-order interactions (Table 4). Statistical analyses across all the treatment combinations (i.e., treatment combination effects) showed that statistical differences between treatment combinations did exist at $\alpha = 0.05$ (Table 5). The highest (3.78) and lowest WAI values (2.57) were found for the treatment combinations 20% DDGS, 30% protein, 100°C, 100 rpm, 25% moisture and 20% DDGS, 30% protein, 150°C, 100 rpm, 25% moisture.

Water solubility index also depends on the quantity of soluble materials, which can increase due to starch degradation (Guha *et al.*, 1997). The main treatment effects of all the parameters on WSI values of the extrudates are shown in Table 3. From our results, we observed that the WSI values decreased by 12.7% with the increase in moisture content from 25-45% (db). Similar reports were reported by Gomez and Aguilera (1984), Badrie and Mellowes (1991), Chang and Wang (1998), Chang *et al.* (1999) and Kannadhason *et al.* (2007a). Changes in DDGS levels and screw speed, on the other hand, did not have significant effects on WSI values for tapioca starch extrudates ($p<0.05$). WSI values were found to increase by 10.0% with the increase in barrel temperature from 100-150°C. Treatment combination effects are provided in Table 5. The highest (23.5%) and the lowest WSI (14.1%) values were observed for the treatment combination of 20% DDGS, 30% protein, 150°C, 100 rpm, 25% moisture and 20% DDGS, 30% protein, 100°C, 100 rpm, 45% moisture, respectively. Extrudates that exhibited high water solubility index values signified that the starch had more fully undergone conversion during extrusion cooking (Sriburi *et al.*, 1999).

Pellet durability index: Pellet durability index is a direct measurement of a pellet's ability to withstand breakage and disintegration (Chang and Wang, 1998; Chang *et al.*, 1999). It is also an indirect measure of mechanical strength, which is a very important quality of feed materials (Rosentrater *et al.*, 2005). The main treatment effects of individually changing DDGS level, protein level, feed moisture content, barrel temperature and screw speed on pellet durability index has been summarized in Table 3. Statistical analyses of all parameters showed significant differences at $p<0.05$. Increasing the DDGS levels from 20-30% db, net protein content from 30-32.5% db, feed moisture content from 25-45% db and barrel temperature from 100-150°C decreased the pellet durability index values by 6.71, 11.9, 7.61 and 13.3%, respectively (Table 3). Our observations are in agreement with the findings discussed by Chevanan *et al.* (2007c) and Kannadhason *et al.* (2007a). This decrease in PDI might be due to the fact that as the DDGS content in the blend increased, the quantity of starch decreased, which resulted in poorer starch gelatinization, reduced cohesion and thus durability (Chevanan *et al.*, 2007c). Moreover, ingredients such as fat can decrease the pellet quality and durability (Scheideler, 1995) and as the DDGS levels increased in the ingredient blends, so did the fat content, which thus resulted in poor pellet durability values. In contrast, increasing the screw speed from 100-200 rpm increased the pellet durability index values by 3.88%.

Most of the interactions between the independent variables were significant (Table 4). Treatment combination effects are provided in Table 5. The highest pellet durability index value (98.4%) was observed for the treatment combination of 20% DDGS, 30% protein, 100°C, 200 rpm, 45% moisture, which signifies that these extrudates could better resist mechanical damage during transportation and storage.

Color: Color is one of the physical properties that are often used by feed customers and manufacturers to assess the quality of feed materials (Turner, 1995). Processing conditions during extrusion favor non-enzymatic browning by Maillard reactions between proteins and reducing sugars (Berset, 1989). Table 3 presents the main treatment effects, for all conditions studied, on the color values of the extrudates. As shown, Hunter L* value, a measure of brightness or luminosity/darkness (100 = white, 0 = black), significantly decreased (by 10.6%) with an increase in the feed moisture content from 25-45%, while they noticeably increased by 25.2 and 9.99% with an increase in processing temperature from 100-150°C and screw speed from 100-200 rpm, respectively. Hunter a* and b* values, chromaticity measures of redness/greenness and yellowness/blueness, did not follow a clear pattern as the processing conditions were changed (Table 3). Increasing the screw speed from 100-150 rpm led to a significant increase and decrease in L* and a* values, respectively. Most of the interactions (Table 4) were significant. Furthermore, the simultaneous effects of changing all conditions (i.e., treatment combinations) on color values (L*, a * and b*) were more difficult to interpret (Table 5). An increase in screw speed reduced the residence time and thus browning; on the other hand, the increased screw speed increased the shear and temperature and could lead to more browning, the final effect being the result of the two opposite trends (Mercier *et al*., 1989).

Extrusion processing parameters

Apparent viscosity: The effect of each factor (i.e., independent variable) on the apparent viscosity values are presented in Table 6. An increase in DDGS levels, protein levels, processing temperature, or screw speed resulted in decreased viscosity values. As the processing temperature was increased from 100-150°C, the viscosity was found to decrease by 26.8%. Similar findings were reported by Chen *et al.* (1979), Chevanan *et al.* (2008) and Kokini *et al.* (1992). These behaviors occur due to the structural changes in the dough in the extruder barrel

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 (% db)	20	28.6b	3603.3a	686.5a	114.9a	5.23a	57.9a
		(1.20)	(451.8)	(104.9)	(8.26)	(41.60)	(3.07)
	25	30.0a	2645.3c	485.9b	111.7a	3.66c	51.6b
		(0.80)	(67.3)	(20.1)	(1.59)	(5.36)	(0.60)
	30	28.3b	2904.8b	592.8a	110.4a	4.48b	49.4c
		(1.24)	(370.9)	(86.3)	(7.34)	(36.30)	(2.77)
Protein content (% db)	30	28.8ab	3486.7a	664.1a	113.9a	5.28a	58.4a
		(1.25)	(430.5)	(96.5)	(7.73)	(42.15)	(3.11)
	32.5	30.0a	2645.3c	485.9b	111.7a	3.66c	51.6b
		(0.80)	(67.3)	(20.1)	(1.59)	(5.36)	(0.60)
	35	28.1b	3021.5b	615.2a	111.3a	4.42b	48.9c
		(1.20)	(399.1)	(96.1)	(7.92)	(35.60)	(2.71)
Moisture content (% db)	25	20.7c	1917.3c	280.4c	135.7a	7.33a	35.2c
		(0.33)	(255.3)	(37.1)	(7.21)	(37.10)	(2.14)
	35	30.0b	2645.3b	485.9b	111.7b	3.66b	51.6b
		(0.80)	(67.3)	(20.1)	(1.59)	(5.36)	(0.60)
	45	36.2a	4590.9a	998.9a	89.7c	2.38c	72.0a
		(0.60)	(453.5)	(94.3)	(6.05)	(9.61)	(2.91)
Temperature (°C)	100	30.9a	3758.5a	670.9a	109.1a	6.64a	63.4a
		(1.26)	(443.6)	(85.3)	(6.91)	(43.10)	(3.12)
	125	30.0a	2645.3b	485.9b	111.7a	3.66b	51.6b
		(0.80)	(67.3)	(20.1)	(1.59)	(5.36)	(0.60)
	150	26.0b	2749.7b	608.5a	116.2a	3.07c	43.9c
		(1.07)	(373.2)	(106.1)	(8.60)	(19.60)	(2.53)
Screw speed (rpm)	100	27.7b	4653.0a	769.6a	78.6c	4.38b	60.0a
		(1.17)	(485.1)	(117.9)	(3.99)	(36.20)	(3.36)
	150	30.0a	2645.3b	485.9b	111.7b	3.66c	51.6b
		(0.80)	(67.3)	(20.1)	(1.59)	(5.36)	(0.60)
	200	29.2a	1855.2c	509.8b	146.7a	5.33a	47.3c
		(1.27)	(168.3)	(59.9)	(5.63)	(41.40)	(2.35)

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

(i.e., unfolding of molecules and material structures) during processing (Rosentrater *et al*., 2005). Increasing the screw speed from 100-200 rpm decreased the viscosity values substantially (by 60.1%); this decrease might be due to the fact that the material in the extruder barrel was pseudoplastic, which exhibits a decrease in apparent viscosity when experiencing an increase in shear rate (Mercier *et al*., 1989). Furthermore, viscosity values were found to increase with respect to the feed moisture content (from 25-45% db) and this finding from our current study corroborates the results reported by Chevanan *et al*. (2008) and Gonzalez *et al*. (2005). From our results, we observed that as the DDGS level in the ingredient blend was increased from 20-30% db; there was a reduction in viscosity values by 19.4%. Moreover, most of the interactions between independent variables were significant (Table 7). Statistical analyses across all the parameters simultaneously (i.e., treatment combinations) showed that significant differences did exist, as shown in Table 8.

Specific mechanical energy: Monitoring the Specific Mechanical Energy (SME) is for a common way to quantify the fragmentation of starch molecules (Gomez and Aguilera, 1984; Davidson *et al*., 1984; Van Lengerich, 1990; Wen *et al*., 1990; Politz *et al*., 1994) during processing. Specific mechanical energy integrates extrusion responses, such as net torque, screw speed and the product mass flow rates (Onwulata *et al*., 2001). Table 6 illustrates the main treatment effects for all the parameters considered on specific mechanical energy values. No clear effects on SME consumption due to changes in processing temperature, DDGS, or protein levels emerged from this analysis, except for the results due to the changes in feed moisture content and the screw speed. Increasing the feed moisture content from 25-45% db resulted in a substantial increase in SME values (by 256.2%); similar results were discussed by Chevanan *et al*. (2008) in their extrusion studies with DDGS as a base material. Increasing the screw speed from 100-200 rpm significantly decreased the SME values by 33.7% (Table 6). Often, as screw speed increases, SME generally increases as well, due to the fact that changes in energy input to the screw can be of a greater order of magnitude than the decrease in torque associated with decrease in viscosity due to the shear thinning behavior of the non-Newtonian material (Mercier *et al*., 1989). Most of the interactions between independent variables were significant (Table 7) as well. Statistical analyses on all collected data confirmed that significant differences across all experimental treatment combinations did exist at $\alpha = 0.05$ (Table 8).

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	0.0925	<0.0001	<0.0001	0.2373	<0.0001	<0.0001
Protein	0.0062	<0.0001	0.0214	0.3311	<0.0001	<0.0001
SS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Temp	<0.0001	<0.0001	0.0041	0.0111	<0.0001	<0.0001
MC	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DDGS*Protein	0.0598	<0.0001	<0.0001	0.0413	<0.0001	<0.0001
DDGS*SS	0.1699	<0.0001	<0.0001	0.0015	<0.0001	0.7403
DDGS*Temp	0.0007	<0.0001	<0.0001	0.1102	<0.0001	0.4377
DDGS*MC	0.0052	0.0003	0.0002	0.3564	<0.0001	<0.0001
Protein*SS	0.0872	0.3975	0.0115	0.3579	<0.0001	<0.0001
Protein*Temp	0.2792	<0.0001	<0.0001	0.3587	<0.0001	0.9644
Protein*MC	0.3587	<0.0001	0.0597	0.3566	<0.0001	<0.0001
SS*Temp	<0.0001	0.0195	<0.0001	0.0741	<0.0001	<0.0001
SS*MC	<0.0001	<0.0001	<0.0001	0.1462	<0.0001	<0.0001
Temp*MC	<0.0001	0.0078	0.7772	0.0022	<0.0001	<0.0001
DDGS*Protein*SS	0.9864	<0.0001	0.5801	0.8101	0.0004	0.6496
DDGS*Protein*Temp	0.5083	<0.0001	<0.0001	0.0127	<0.0001	0.0036
DDGS*Protein*MC	0.1472	<0.0001	<0.0001	0.6022	<0.0001	0.2014
DDGS*SS*Temp	<0.0001	<0.0001	0.0807	0.6379	<0.0001	<0.0001
DDGS*SS*MC	0.3769	0.0091	0.1905	0.5369	<0.0001	0.3097
DDGS*Temp*MC	0.0926	<0.0001	0.0135	0.0085	<0.0001	0.2218
Protein*SS*Temp	0.6422	0.0599	0.9442	0.3761	<0.0001	<0.0001
Protein*SS*MC	0.0007	<0.0001	0.0006	0.5557	<0.0001	<0.0001
Protein*Temp*MC	0.0321	<0.0001	<0.0001	0.3690	<0.0001	0.0023
SS*Temp*MC	0.1678	<0.0001	<0.0001	0.4207	<0.0001	0.0349
DDGS*Protein*SS*Temp	0.1245	<0.0001	<0.0001	0.1633	<0.0001	0.0598
DDGS*Protein*SS*MC	0.3993	<0.0001	0.0013	0.6060	<0.0001	0.6903
DDGS*Protein*Temp*MC	0.4465	<0.0001	<0.0001	0.1024	<0.0001	0.2107
DDGS*SS*Temp*MC	0.0005	0.0036	0.0163	0.0044	0.2160	0.2060
Protein*SS*Temp*MC	0.6481	<0.0001	0.0005	0.2618	<0.0001	0.1121
DDGS*Protein*SS*Temp*MC	0.1083	<0.0001	0.0325	0.0312	<0.0001	0.1930

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

Table 8: Treatment combination effects on processing parameters†

Protein level (% db)		30							
Temperature °C		100				150			
Screw speed (rpm)		100		200		100		200	
Moisture content (% db)		25	45	25	45	25	45	25	45
Property	**DDGS level (% db)**								
MC at die (% db)	20	23.8i	42.3a	24.1j	39.2b-c	19.0m-o	28.9i	20.9k-n	35.3e-f
	30	21.1k-m	38.6b-c	21.0k-m	40.9a-b	18.5n-o	34.2e-f	19.3m-o	33.6f-g
Viscosity (Pa-s)	20	2038.4l	8963.7b	2846.6i	2500.2k	814.4q-r	7101.7e	565.5r-s	521.8s
	30	1542.6m-n	9552.3a	1241.0o-p	4365.4g	1722.5m	7240.7e	3396.0i	1374.8n-o
SME (J g^{-1})	20	178.8i-l	1207.2d	532.7f	838.7e	89.5l	1753.7a	155.5i-l	162.2i-l
	30	133.4j-l	1443.0b-c	322.6g-j	1278.0c-d	156.4i-l	1173.2d	712.2e	488.6f-g
MFR (gmin^{-1})	20	91.5j	63.8k	184.9a-b	116.9e-i	97.0g-j	50.5k	165.2b-c	137.7d-e
	30	102.3g-j	62.0k	132.4d-f	119.7e-g	115.9e-j	65.6k	196.5a	121.4e-g
Pressure (MPa)	20	10.80b	3.01l	12.60a	3.52i-k	5.16g	1.62q-r	7.09e	2.22o-p
	30	9.33c	2.92l-m	11.10b	3.18k-l	3.26j-l	1.14s	5.59f	1.87p-q
Torque (N-m)	20	25.1n	115.1b	73.6h	63.6i	10.5r	92.9e	12.9q-r	13.7q
	30	21.0o	121.7a	35.3l	112.5b	25.0n	93.4e	84.2f	33.8l

Protein level (% db)		35							
Temperature °C		100				150			
Screw speed (rpm)		100		200		100		200	
Moisture content (% db)		25	45	25	45	25	45	25	45
Property	**DDGS level (% db)**								
MC at die (% db)	20	22.8j-k	38.2c-d	22.0j-l	39.3b-c	17.6o	28.3i	19.6l-o	35.4e-f
	30	23.2j-k	36.0d-f	21.1k-m	39.9b-c	18.7m-o	31.6g-h	18.5n-o	36.2d-e
Viscosity (Pa-s)	20	8088.7d	8354.9c	936.1q	3943.7h	857.3q	7358.3e	1495.9m-o	1266.8n-p
	30	960.5q	2064.6l	1368.8n-o	1368.8n-o	1366.2n-o	6421.9f	1436.5n-o	1055.3p-q
SME (J g^{-1})	20	740.0e	1381.7c	194.5i-l	1202.1d	91.5l	1761.5a	291.6h-k	403.1f-h
	30	85.6l	410.1f-h	328.4g-i	456.8f-h	129.5k-l	1578.0b	344.9f-i	444.6f-h
MFR (gmin^{-1})	20	92.6i-j	58.9k	169.4b-c	130.3e-f	100.0g-j	56.0k	192.8a	131.3e-f
	30	94.3h-j	54.0k	154.5c-d	118.4e-h	103.2g-j	49.3k	177.8a-b	99.0g-j
Pressure (MPa)	20	9.29c	3.33n-o	10.80b	3.76h-i	4.03h	1.76q-r	4.05h	1.43r-s
	30	8.55d	2.23o-p	9.37c	3.28j-l	2.62m-n	1.92p-q	3.46i-k	1.83p-q
Torque (N-m)	20	103.7d	107.6c	25.1n	102.0d	12.0q-r	94.3e	39.8k	34.7l
	30	12.4q-r	25.9n	35.6l	35.0l	18.0	81.4g	29.8m	25.5n

†Means followed by similar letters within each parameter are not significantly different at $\alpha = 0.05$, LSD. MC is Moisture Content; SME is Specific Mechanical Energy; MFR is Mass Flow Rate

Mass flow rate: Mass flow rate in a single screw extruder is dependent on the drag flow developed by the rotation of the screw and the pressure developed due to the restriction of the die (Mercier *et al.*, 1989). The main treatment effect due to each factor on mass flow rate is summarized in Table 6. From our results, we observed that increasing the DDGS levels, protein levels and processing temperatures did not affect the mass flow rate values. However, changes in the feed moisture content and screw speed did exhibit a significant effects on mass flow rate values. Increasing the feed moisture content from 25-45% db resulted in decreased mass flow rate values by 33.9%. Chevanan *et al.* (2007b) found similar mass flow rate results with respect to changes in feed moisture content. As anticipated, screw speed did have a significant effect on mass flow rate values as well. Increasing the screw speed from 100-200 rpm resulted in an 86.7% increase in mass flow rate values. Similar results were reported by Chevanan *et al.* (2007c) and Gonzalez *et al.* (2005). Higher screw speeds typically produce higher mass flow rates due to the increased capability of the extruder screw to convey material through the extruder barrel (Bouvier *et al.*, 1987; Mercier *et al.*, 1989). Most of the interactions between independent variables, however, were not significant (Table 7). These patterns were also observed when examining the treatment combination effects (Table 8).

Die pressure: The pressure that is developed within the die depends on various parameters such as rheological properties of the ingredient blend, pumping characteristics of the material down the length of the extruder and the dimensions (i.e., length/diameter ratio) of the die used in the extruder (Chevanan *et al.*, 2007b). Table 6 shows the main treatment effects due to DDGS level, protein level, feed moisture content, processing temperature and screw speed on die pressure values. As shown in Table 6, die pressure values markedly decreased for any change in feed moisture content and processing temperature. Increasing the feed moisture content from 25-45% db and processing temperature from 100-150°C resulted in decreased die pressure values by 67.5 and 53.8%, respectively. Similar findings were reported by Chevanan *et al.* (2007b). No clear patterns on die pressure values due to changes in levels of DDGS, protein, or screw speed could be observed. Many of the interactions between independent variables were significant (Table 7), especially for the lower-order interactions. Most of the higher-order interactions, however, were not significant. The statistical analyses across all the parameters studied simultaneously (i.e., treatment combinations) showed that there existed significant differences at $\alpha = 0.05$ (Table 8) as well.

Torque: The net torque required to run the extruder screw is related to its speed, degree of fill and the viscosity of the feed material in the screw channel. Consequently, torque is a dependent variable that provides insight into the operation of the extruder (Mercier *et al.*, 1989) during processing. Resulting net torque results due to the various processing conditions are shown in Table 6. All parameters studied (DDGS level, protein level, feed moisture content, processing temperature and screw speed) did have a statistically significant main effects ($\alpha = 0.05$). Increases in DDGS level, protein level, processing temperature and screw speed (i.e., low settings to high settings) led to significant decreases in torque values by 14.7, 16.2, 30.8 and 21.1%, respectively. The torque values fell progressively by 30.8% as the processing temperature was increased from 100-150°C (Table 6). Additionally, as the temperature was increased, the viscosity was found to decrease substantially and consequently so did the torque values. As shown in Table 6, net torque values significantly increased (104.3%) with an increase in the feed moisture content from 25-45% db. Chevanan *et al.* (2007b, 2008) showed similar results for net torque values due to the changes in feed moisture content, processing temperature and screw speed. Moreover, most of the interactions between independent variables were significant (Table 7). The treatment combination effects on all the extruder and processing conditions studied were observed to have significant effects on the extruder torque at $\alpha = 0.05$ (Table 8) as well.

CONCLUSION

This investigation was conducted with a broad intention of enhancing the value of DDGS and 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 parameters (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. Increasing the DDGS levels, protein content, feed moisture content and processing temperature significantly decreased the PDI values. 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 effect of DDGS in large-scale single and twin screw extrusion will provide a broader understanding of these feed and extruder variables studied.

ACKNOWLEDGEMENT

The authors 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 and Tista Foods for supplying DDGS and tapioca starch, respectively. 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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