

# Effects of Steam, Moisture, and Screw Speed on Physical Properties of DDGS-Based Extrudates

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## ABSTRACT

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A fractional factorial design with a replicated central composite point was used to investigate the effects of extrusion processing on physical properties of distillers dried grains with solubles (DDGS) based aquafeeds using a twin-screw extruder. Extrusion cooking trials were performed with a nutritionally balanced ingredient blend for Nile tilapia, with two levels of screw speed (350 and 450 rpm), two levels of extruder water (0.236 and 0.302 kg/min), and two levels of conditioner steam (0.1 and 0.15 kg/min). The central point was 400 rpm screw speed, 0.271 kg/min extruder water, and 0.12 kg/min conditioner steam. Effects of these processing conditions on extrudate characteristics were extensively analyzed and included moisture content, water activity, thermal properties, expansion ratio, unit density, bulk density, color, water stability, sinking velocity, water absorption and solubility indices, and pellet durability index. Increasing the extruder water and conditioner

steam resulted in a 5.3% decrease and nearly 8.6% rise in mass flow rate, respectively. As screw speed increased from 350 to 400 rpm, water stability and water activity increased by 13 and 58%, respectively. Increasing extruder water from 0.236 to 0.302 kg/min led to a significant increase in water stability by 12.5% and decreases in water absorption index, water activity, and expansion ratio by 13, 21, and 5.5%, respectively. As conditioner steam increased from 0.1 to 0.15 kg/min, sinking velocity and water absorption index decreased by 25 and 15%, respectively. Increasing conditioner steam from 0.1 to 0.12 kg/min resulted in 20, 5.5, 10, and 3% decreases in moisture content of the extrudates, brightness ( $L^*$ ), water stability, and expansion ratio, respectively. It also increased bulk density by 5.8% and unit density by 4.2%. Overall, all trials produced viable extrudates with properties appropriate for Nile tilapia feeding.

The aquaculture industry consumes a large amount of wild fish to raise other fish for harvest (human food). Aquaculture farms use a considerable amount of fishmeal and fish oil (made from wild fish) to maximize fish growth and enhance feed flavor. In fact, up to 60% of total costs of farm production can be accounted for by diet costs (Tan and Dominy 1997), of which protein is the most expensive ingredient. According to Naylor and Burke (2005), there are several viable protein substitutes, including protein made from grain and other livestock by-products (Ayadi et al 2012). One way of improving plant-based ingredient digestibility is to supplement with enzymes (Cain and Garling 1995; Rodehutsord and Pfeffer 1995; Storebakken et al 1998; Vielma et al 1998, 2000; Papatryphon et al 1999; Sugiura et al 2001; Cheng and Hardy 2002). Many studies have indicated that corn-based distillers dried grains with solubles (DDGS) (a coproduct from fuel ethanol manufacturing) could be a good protein source to replace portions of fish meal in fish diets (Cheng et al 2003; Ayadi et al 2010).

In recent years, interest in renewable energy has resulted from several factors, such as environmental pollution, the importance of clean air, the ban on methyl tertiary-butyl ether, and unstable oil supplies, all of which have led to tremendous growth in the biofuels industry. Although biological materials such as residue straw, corn stover, perennial grasses, and legumes can be used, corn starch is by far the most common substrate used for ethanol manufacture in the United States because of its economic viability (Rosentrater 2006). For example, in 1980, the annual production rate of ethanol fuel was about 175 million gallons in the United States. This amount increased to 900 million gallons, 1.630 billion gallons, and 13.23 billion gallons in 1990, 2000, and 2010, respectively (Renewable Fuels Association 2012). From such an increase in the production of ethanol, there has been a parallel increase in feed coproducts (primarily DDGS).

Ethanol can be produced commercially via two main methods: wet-mill processing and dry-mill processing (Belyea et al 2004). In the former method, grain is steeped in water, and then the slurry is ground and the components are mechanically separated. The products of this method are corn oil, corn gluten feed, gluten meal, starch, and ethanol. In the latter process, however, the entire corn kernel is ground into flour, and then the starch is converted to simple sugars with enzymes. Thereafter, the sugars are converted into ethanol using yeast. The products of dry milling include ethanol, DDGS, and CO<sub>2</sub>. Generally, for each 1 kg of corn consumed, 1/3 kg each of ethanol, DDGS, and CO<sub>2</sub> will be produced (Chevanan et al 2005; Rosentrater 2006; Rosentrater and Muthukumarappan 2006).

DDGS has become a widespread source of protein for livestock diets globally. DDGS is composed of the nonfermentable components of the original grain (i.e., proteins, lipids, fibers, and ash). Typically, DDGS contains 27–33% protein and 5–12% fiber (Belyea et al 2004; Rosentrater 2006; Rosentrater and Muthukumarappan 2006). The total production of DDGS increased nearly 200% between 2006 and 2010 in the United States, from 15.62 million to 32.44 million metric tons, and it is expected to reach 33.5 million metric tons by 2013 (Agricultural Marketing Resource Center 2011).

Considering the dramatic increase in the ethanol industry in recent years, developing alternative utilization opportunities for DDGS must be taken into consideration to avoid potential market saturation. Various potential alternatives have been proposed, including bioplastics (Schilling et al 2004; Cheesbrough et al 2008), biofillers (Tatara et al 2009), human food additives (Rosentrater 2006), and aquaculture feed ingredients (Naylor and Burke 2005; Chevanan et al 2005, 2007a, 2007b, 2007c, 2008, 2009, 2010). Generally, the majority of fish feed cost is accounted for by the cost of protein sources, because protein is the most expensive ingredient in animal diets. Belyea et al (1989) reported that variations in protein content of feeds can affect animal productivity.

Because of the moderately high protein content of DDGS, it has been used extensively in various animal feeds. Although DDGS can be a valuable source of protein, it has low levels of essential amino acids, particularly lysine and methionine (Lim et al 2009). This drawback can limit its use in aquafeeds. The average protein component of the fish body is 65–75%; thus, the basic nutrient structure of fish feed is protein, and balanced amino acid

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profiles are critical in fish feed formulation. To date, DDGS has generally been included in fish feed at low levels, and it is often incorporated with supplemental lysine and methionine. DDGS can be more palatable to fish and more cost efficient compared with other protein sources (Lim et al 2009).

Research by the National Research Council (1993) has determined that various species need different amounts of protein to grow well. According to Lovell (1989), the protein requirements for aquaculture feeds depend on the species and can vary from 26 to 50%. Metabolic demands of fish must be supplied by amino acids (Guimarães et al 2008). Lack of essential amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine can result in lower feed efficiency ratios, which will subsequently reduce fish growth performance (Anderson et al 1992). Abdel-Tawwab et al (2010) studied the dietary protein contents of fish feed for different species and determined that the optimum dietary protein to reach an optimum growth performance also depended on the hygiene and environmental conditions of the aquaculture facility.

Although DDGS is a good source of protein, its lack of essential amino acids has limited its inclusion in aquafeeds. In several studies, inclusion of DDGS as the main protein source in fish feeds for various species, with and without incorporation of supplemental essential amino acids, was investigated, and few ad-

verse effects on growth performance were observed: for example, juvenile channel catfish with 30–40% DDGS (Robinson and Li 2008; Lim et al 2009) and Nile tilapia with 20–35.5% DDGS (Wu et al 1996; Coyle et al 2004; Lim et al 2009).

Moreover, the effect of DDGS inclusion on disease resistance in catfish and tilapia has been evaluated by Lim et al (2009). They found that with 40% DDGS plus lysine, catfish species had good growth performance and demonstrated greater resistance to at least one major disease, whereas catfish raised on a diet without DDGS had fewer antibodies than those raised on the DDGS-based feed (AllAboutFeed 2012). Overall, when including plant protein sources in aquafeeds, factors such as protein quality, processing conditions, nutritional constraints, and economics should be taken into consideration (Tacon 1997).

Most aquafeeds are produced by extrusion processing. Extrusion is a process for shaping dough by forcing it through a narrow die restriction. The ingredients are cooked inside the extruder barrel chamber, and then the cooked dough is texturized and shaped in the die (Fedi-Soetaredjo et al 2003). An extruder is essentially a pump, simultaneously transferring, mixing, shearing, stretching, and shaping ingredients inside its barrel at an increasing rate of pressure during a short period. It is also a bioreactor, restructuring the chemical components within the ingredients (Riaz 2000). The extent of heating, mixing, and compressing inside the barrel of the extruder depends on the raw ingredient properties, processing conditions used, type of extruder, geometry, and operating conditions. In fact, screw speed (SS), screw configuration, die shape, barrel temperature, moisture content of feed, and the rate of feeding all influence the residence time within the extruder, thus material throughput, and mechanical and thermal energy inputs to the machine. All of these changes affect the rheological and physicochemical properties of the dough melt inside the barrel, as well as the properties of the extrudates upon die exit (Hurber 2000; Mercier et al 1989).

There are two main types of extruders: single screw and twin screw, each of which can be further classified based on different geometric and operational characteristics. In general, twin-screw extruders offer more flexibility, can process a wider variety of ingredients, and provide better mixing of steam and water compared with single-screw extruders. More specific details regarding extruders are reported by Riaz (2000). Both chemical and physical changes happen during the travel of the dough from the feeding zone to the die exit. Chemical changes can be explained by structural modifications of starch and protein macromolecules as a result of interactions between water, starch, protein, and heat. Physical changes are a result of extrudate expansion because of water evaporation during die exit (Mercier et al 1989). Thus, the role of water is highly important in both chemical and physical phenomena. According to Friesen et al (1992), protein denaturation and starch gelatinization are the result of water content,

**TABLE I**  
Ingredient Components of the Feed Blend Used (% wet basis) and Resulting Chemical Composition (% dry basis)

Components	% Wet Basis
Distillers dried grains with solubles <sup>1</sup>	20
Soybean meal <sup>u</sup>	50
Corn flour <sup>v</sup>	10
Whey <sup>w</sup>	5
Fishmeal, menhaden <sup>x</sup>	10
Vitamin premix <sup>y</sup>	3
Soybean oil <sup>z</sup>	2
Total	100
Chemical Composition	% Dry Basis
Crude protein	38.25
Crude fat	5.76
Crude fiber	8.53
Ash	10.66
Carbohydrate (net)	36.80

<sup>1</sup> Dakota Ethanol, Wentworth, SD, U.S.A.

<sup>u</sup> 46.5% protein, solvent extracted, Dakotaland Feeds, Huron, SD, U.S.A.

<sup>v</sup> 505 yellow corn flour, Cargill Dry Corn Ingredients, Paris, IL, U.S.A.

<sup>w</sup> Deproteinized whey, Bongards' Creamery, Perham, MN, U.S.A.

<sup>x</sup> Special Select, Omega Protein, Houston, TX, U.S.A.

<sup>y</sup> A-D-E & K vitamin premix, CSD Nutrition, Sioux City, IA, U.S.A.

<sup>z</sup> OF1870E soybean oil, Consumers Supply Distributing, Sioux City, IA, U.S.A.

**TABLE II**  
Experimental Design<sup>z</sup>

Treatment	Coded Variables			Actual Variables		
	Screw Speed	Extruder Water	Conditioner Steam	Screw Speed (rpm)	Extruder Water (kg/min)	Conditioner Steam (kg/min)
1	-1	0	0	350	0.271	0.12
2	1	0	0	450	0.271	0.12
3	0	1	0	400	0.302	0.12
4	0	-1	0	400	0.236	0.12
5	0	0	1	400	0.271	0.15
6	0	0	-1	400	0.271	0.1
7 (CP)	0	0	0	400	0.271	0.12
8 (CP)	0	0	0	400	0.271	0.12
9 (CP)	0	0	0	400	0.271	0.12
10 (CP)	0	0	0	400	0.271	0.12

<sup>z</sup> The central composite experimental design consisted of two screw speed levels, two extruder water levels, two conditioner steam levels, and one center point (between all of these levels, denoted as CP, replicated four times), for 10 total treatment combinations.

heat, pressure, and shear during the evaporation process. Starch gelatinization affects many extrudate properties, such as water stability, digestibility, and expansion ratio. However, the extent of starch gelatinization by itself depends on starch type, particle size, and conditions of the extrusion process (Rokey and Plattner 2003). The various effects of extrusion cooking on starch have been further discussed by Harper (1981) and Linko et al (1981).

Use of DDGS as a protein component of aquaculture feed products has been extensively studied recently. For example, the effects of different levels of DDGS, moisture content, SS, and die dimension on extrudate properties have been examined by Shukla et al (2005), Chevanan et al (2007b, 2007c, 2008), and Kan-nadhasan et al (2007a, 2007b, 2010). But information about the effects of processing parameters on the production of feed products that use DDGS is still limited. Therefore, the objectives of this study were to investigate the effects of three extrusion processing parameters (conditioner steam [CS], extruder water [EW],

and SS) on physical properties of DDGS-based extrudates produced by twin-screw extrusion.

## MATERIALS AND METHODS

### Sample Preparation

An experimental diet containing 20% DDGS (by weight), in combination with appropriate quantities of corn flour, soybean meal, menhaden fish meal, whey, vitamin mix, and soybean oil, was formulated to contain a net protein content of 20% db (Table I). DDGS was provided by Dakota Ethanol (Wentworth, SD, U.S.A.) and was ground to a particle size of approximately 100  $\mu\text{m}$  with a laboratory-scale grinder (S500 disc mill, Glenmills, Clifton, NJ, U.S.A.). Menhaden fish meal was obtained from Omega Protein (Houston, TX, U.S.A.). Corn flour, soybean meal, and soybean oil were provided by Cargill Dry Corn Ingredients (Paris, IL, U.S.A.), Dakotaland Feeds (Huron, SD, U.S.A.), and Consumers Supply Distributing (Sioux City, IA, U.S.A.), respectively. Deproteinized whey was from Bongards' Creamery (Perham, MN, U.S.A.); A-D-E & K vitamin premix was from CSD Nutrition (Sioux City, IA, U.S.A.). The ingredients were premixed with a laboratory-scale mixer to disperse the soybean oil in the mixture thoroughly. Then, the entire blend was mixed with a twin-shell dry blender (Patterson-Kelly, East Stroudsburg, PA, U.S.A.) for 10 min to produce a homogenous blend. The resulting blend was then stored at ambient temperature overnight until processing.

### Extrusion Processing

Experimental extrusions were carried out with an industrial-scale twin-screw extruder (Wenger TX-52, Sabetha, KS, U.S.A.).

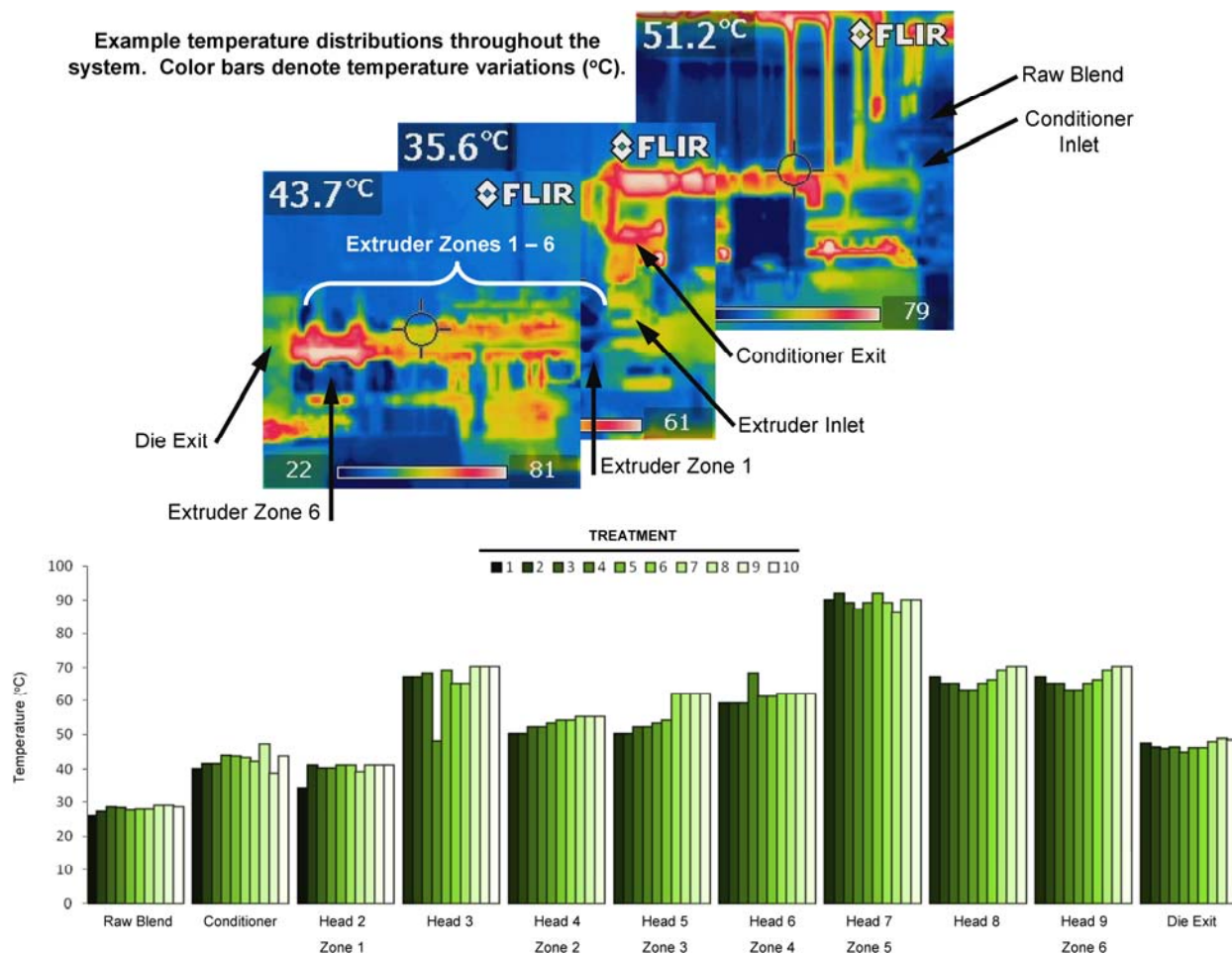


Fig. 1. Temperature distribution throughout the extrusion process for each treatment (1 through 10).

The extruder was self-wiping, with two corotating, fully intermeshing screws, a feed hopper, and a preconditioner. The feed blends were manually transferred to the feed hopper and conveyed automatically into the preconditioner, where steam was injected at the rate of 0.1–0.15 kg/min (Table II). Being adjusted to the desired moisture content and temperature inside the preconditioner (Table III), which was equipped with steam and water injectors, the conditioned blends were transferred into the extruder. The barrel of the extruder had a length-to-diameter ratio of 25.5:1, and its twin screws each had a diameter of 52 mm. The screws used in this experiment each had 25 individual sections, and their configurations, from the feeding to the die sections, were composed of four conveying screws, three shear locks, one conveying screw, one conveying screw backward, three conveying screws, one conveying screw backward, four conveying screws, one shear lock, one interrupted flight conveying screw, one conveying screw, one interrupted flight conveying screw, one conveying screw, one interrupted flight conveying screw, one shear lock, and finally a screw with a cone-shaped end point (Chevanan et al 2007b). Moreover, the barrel was composed of six temperature zones, each of which was set between 25 and 90°C, (Fig. 1). The actual temperature profile of the barrel varied, however, depending on the dough's properties and frictional heating that developed during processing. Temperatures (°C) of the blend inside the hopper, in the preconditioner, and of the exiting extrudates were all monitored by a portable infrared thermometer (model 42540, Extech Instruments, Waltham, MA, U.S.A.). The amount of water added to the extruder was maintained at 0.236–0.302 kg/min (Table II). The extruder had two die nozzles, each of which had a circular opening of 3 mm. The exiting extrudates were cut to desired lengths with a three-blade rotary cutter.

### Experimental Design and Statistical Analyses

Extrusion experiments were performed with a 2 × 2 × 2 factorial design with a central composite point (with four replicate runs), for a total of 10 treatment combinations (Table II). The experimental design consisted of two SSs (350 and 450 rpm), two amounts of CS (0.1 and 0.15 kg/min), and two levels of water injected into the extruder (0.236 and 0.302 kg/min), in addition to the one center point between all of these levels. The diet blend was kept constant throughout (Table I). Each of the 10 treatments was extruded once, except the center-point treatment, which was replicated four times. When the extrusion cooking process

reached steady state (≈5 min), temperatures at the die, at the feed hopper, and inside the conditioner were monitored with 10 replications for each treatment combination; approximately 1 kg of the blend and the extrudates were collected after the conditioner and at the die exit, with three replicates, to investigate the extrudates' properties and process parameters for each treatment combination. Mass flow rate (MFR) of the extrudates was measured for each treatment combination in triplicate. All extrudate properties were measured in triplicate, except sinking velocity, expansion ratio, and unit density, which were measured with 10 replicates each.

All collected data were analyzed with Microsoft Excel 2007 and SAS version 9.0 software (SAS Institute, Cary, NC, U.S.A.), with a type I error rate ( $\alpha$ ) of 0.05, by analysis of variance (ANOVA) to find if there were significant differences between treatments. If found, then post hoc LSD tests were used to determine where the specific differences occurred. Additionally, response surface regressions were used to investigate relationships among the variables.

### Measurement of Raw Ingredient Properties

The raw blend was analyzed for several properties, including moisture content, water activity, thermal properties, and color (Table III). These properties were measured with the same techniques and equipment that were used to measure the properties of the extrudates (discussed subsequently).

### Measurement of Extrusion Processing Parameters

Several parameters were measured during processing. These included temperature, moisture content, and MFR. Temperature ( $T$ ) of the raw material in the hopper, at the conditioner exit, and at the die exit were all monitored with a portable infrared thermometer (model 42540, Extech Instruments). Moisture contents of the blend at the conditioner exit (MCC) and of the extrudate at the die exit (MCD) were measured according to AACC International Approved Method 44-19.01 with a laboratory-scale oven (Thelco Precision, Jovan, Winchester, VA, U.S.A.) at 135°C for 2 hr. Therefore, dry-matter mass balance could be determined for the extrusion process. In other words, steam evaporation at the die could be explained. MFR was determined during the extrusion process by collecting extruded samples at 30 sec intervals. Using an electronic balance (Defender 3000 series, Ohaus, Pine Brook, NJ, U.S.A.), the mass of the collected extrudates was weighed, and MFR was reported as grams per minute.

TABLE IV  
Main Effects of Screw Speed (SS), Extruder Water (EW), and Conditioner Steam (CS) on Processing Properties<sup>z</sup>

Property	Level	RT (°C)	CT (°C)	DT (°C)	MCC (% db)	MCD (% db)	MFR (kg/min)
SS (rpm)	350	25.70b (0.01)	40.00a–c (0.14)	46.65bc (0.21)	29.62a (0.07)	44.65bc (0.02)	1.9ab (0.01)
	400	27.91a (0.67)	42.93bc (2.35)	46.73ab (1.47)	28.28ab (1.65)	40.81ab (3.98)	1.8ab (0.19)
	450	28.40a (1.39)	40.53ab (0.90)	46.77a–c (1.36)	26.55b (0.19)	37.94a–c (1.45)	1.85a (0.35)
EW (kg/min)	0.236	27.76ab (0.93)	43.38ab (0.48)	45.7ab (1.04)	27.53b (0.34)	36.43c (0.74)	1.9b (0.14)
	0.271	27.84a–c (1.01)	42.21bc (2.73)	47.09b (1.28)	27.97b (1.57)	40.48b (3.22)	1.81ab (0.21)
	0.302	27.63bc (0.06)	43.64a–c (0.21)	45.03a (0.47)	30.62a (0.40)	47.42a (0.78)	1.8ab (0.00)
CS (kg/min)	0.1	28.70bc (0.10)	38.74c (0.40)	48.73b (0.55)	25.81c (0.32)	36.83bc (0.50)	1.75a–c (0.35)
	0.12	27.65a–c (0.93)	42.37b (1.73)	46.37a (1.24)	28.25b (1.44)	41.27a–c (4.09)	1.81ab (0.19)
	0.15	28.17ab (1.01)	47.07a (0.49)	47.52ab (0.95)	30.18a (0.17)	40.97ab (0.71)	1.90bc (0.14)

<sup>z</sup> Means followed by similar letters for a given dependent variable are not significantly different for that independent variable at  $P < 0.05$ ; values in parentheses are  $\pm 1$  standard deviation. RT = raw material temperature; CT = conditioner temperature; DT = extruder die section temperature; MCC = moisture content at conditioner exit; MCD = moisture content at die exit; and MFR = mass flow rate exiting the die.

## Measurement of Extrudate Physical Properties

The extrudates were cooled for 72 hr at ambient temperature ( $24 \pm 1^\circ\text{C}$ ) and then dried in an oven (TAH-500, Grieve Corporation, Round Lake, IL, U.S.A.) for 24 hr at  $45^\circ\text{C}$ . The extrudates were then subjected to extensive physical properties analyses, including moisture content, water activity ( $a_w$ ), thermal conductivity ( $k$ ), thermal resistivity ( $R$ ), thermal diffusivity ( $\alpha$ ), expansion ratio, unit density, bulk density,  $L^*$  (brightness/darkness),  $a^*$  (redness/greenness),  $b^*$  (yellowness/blueness), water stability, sinking velocity, water absorption index (WAI), water solubility index (WSI), and pellet durability index (PDI).

**Moisture Content.** Moisture content of the extruded samples was determined according to AACCI Approved Method 44-19.01 with a laboratory-scale oven at  $135^\circ\text{C}$  for 2 hr.

**Water Activity.** Water activity of the extrudates was measured with a water activity meter (AW Sprint TH-500, Novasina, Pfäffikon, Switzerland). Before measurement, the system was calibrated according to the specified procedure of the manufacturer.

**Thermal Properties.** Thermal conductivity ( $k$ ), thermal diffusivity ( $\alpha$ ), and thermal resistivity ( $R$ ) were determined with a thermal properties analyzer (KD2, Decagon Devices, Pullman, WA, U.S.A.).

**Expansion Ratio.** Expansion ratio was expressed as the diametral expansion of the extrudate, which was determined as the ratio of the extrudate diameter to the diameter of the die nozzle, measured with a digital caliper (Digimatic Series 293, Mitutoyo, Tokyo, Japan), following Conway and Anderson (1973) and Van Zuilichem et al (1975).

**Unit Density.** Assuming cylindrical shapes for the extruded samples, unit density was determined as the ratio of the mass to volume for 10 randomly chosen extrudates, following Rosentrater et al (2005). The mass of each of the extrudates was measured with an analytical balance (Adventurer AR 1140, Ohaus), and the diameter of each extrudate was measured with a digital caliper (Digimatic Series 293, Mitutoyo); both were used to calculate the unit density:

$$\text{unit density} = \frac{M}{V} \text{ (kg/m}^3\text{)} \quad (1)$$

where  $M$  is the extrudate mass and  $V$  is the extrudate volume.

**Bulk Density.** Bulk density was measured with a standard bushel tester (Seedburo Equipment, Chicago, IL, U.S.A.). Bulk density ( $\text{g/cm}^3$ ) was defined as the ratio of the mass of the extrudates ( $\text{g}$ ) occupying a given bulk volume to the volume of the

bulk ( $\text{cm}^3$ ), according to the method recommended by USDA (1999):

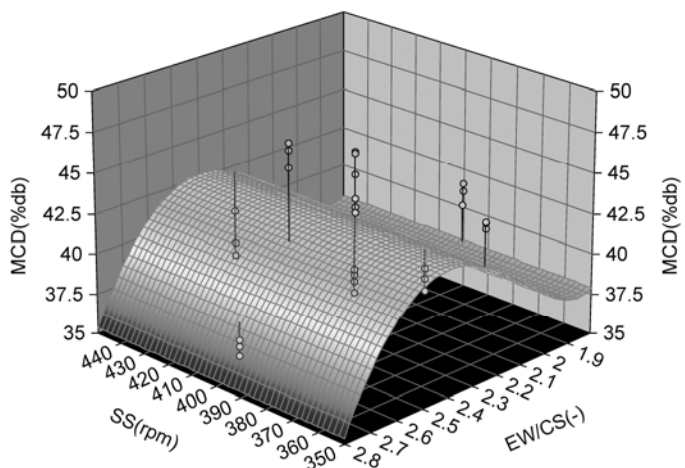
$$\text{bulk density} = \frac{m}{V} \quad (2)$$

where  $m$  is the mass of extrudates ( $\text{g}$ ) and  $V$  is the container's given volume.

**Color.** Color included  $L^*$  (brightness/darkness),  $a^*$  (redness/greenness), and  $b^*$  (yellowness/blueness) and was measured with a spectrophotometer (Lab Scan XE, Hunter Lab, Reston, VA, U.S.A.).

**Water Stability.** Water stability of the extrudates was defined as the time taken for an extrudate to start breaking (i.e., disintegrating) in water. Water stability was monitored by placing extrudates in water and then stirring to simulate real aquacultural conditions. Approximately 1 g of extrudate from each extrusion run was placed in 200 mL of distilled water, which was placed over a magnetic stirrer (PMC 524C, Barnstead International, Dubuque, IA, U.S.A.) until physical breakage of the pellets commenced.

**Sinking Velocity.** Following the method used by Chevanan et al (2007a, 2007b, 2007c), sinking velocity was measured as the time taken for an extrudate to travel the distance between the



**Fig. 2.** Surface response relationship between the screw speed (SS) and ratio of the extruder water to conditioner steam (EW/CS) and moisture content exiting the die (MCD).

**TABLE V**  
Interaction Effects of Screw Speed (SS), Extruder Water (EW), and Conditioner Steam (CS) on Processing Properties ( $P$  Values)<sup>z</sup>

Interactions	RT ( $^\circ\text{C}$ )	CT ( $^\circ\text{C}$ )	DT ( $^\circ\text{C}$ )	MCC (% db)	MCD (% db)	MFR (kg/min)
SS	0.0009	0.0018	0.9932	0.0012	0.0214	0.8063
EW	0.6944	0.4454	0.0071	0.0001	<0.0001	0.7831
SS $\times$ EW	0.0066	0.2867	0.0700	0.0085	0.0002	0.6667
CS	0.1404	<0.0001	0.0311	<0.0001	0.0327	0.7101
SS $\times$ CS	0.0009	0.0001	0.0375	0.0005	0.1127	0.6414
EW $\times$ CS	0.4381	0.0001	0.0024	0.0001	0.0004	0.7006
SS $\times$ EW $\times$ CS	0.0066	0.0001	0.0149	0.0001	0.0001	0.6565

<sup>z</sup> RT = raw material temperature; CT = conditioner temperature; DT = extruder die section temperature; MCC = moisture content at conditioner exit; MCD = moisture content at die exit; and MFR = mass flow rate exiting the die.

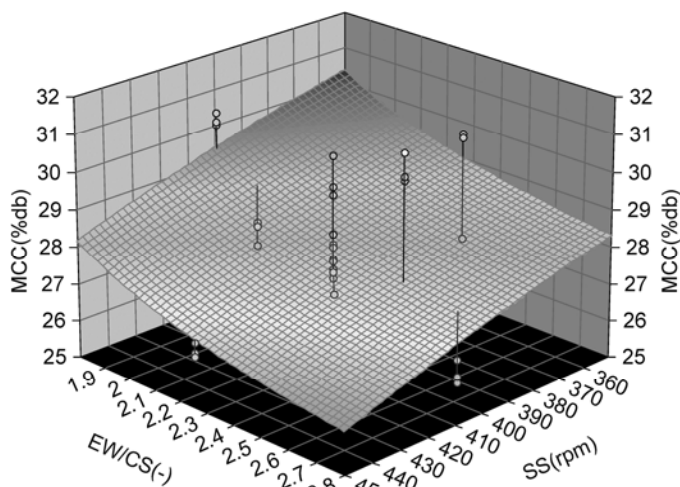
**TABLE VI**  
Best-Fit Response Surface Models for Extrusion Processing Properties<sup>z</sup>

Equation	Response Surface Model	$R^2$	Standard Error	$F$ Statistic	Figure
5	$\text{MCD} = (421.71) + (-10.88)(\text{EW/CS})^{2.5} + (-1854.75)\ln(\text{EW/CS})/(\text{EW/CS})^2$	0.22	3.54	3.76	2
6	$\text{MCC} = (27.55) + (-4.55)(\text{SS})^3 + (2.80)/\ln(\text{EW/CS})$	0.20	1.51	3.38	3

<sup>z</sup> MCD (% db) = moisture content at die exit; SS (rpm) = screw speed; EW/CS (-) = the ratio of extruder water to conditioner steam; CS (kg/min) = conditioner steam; and MCC (% db) = moisture content at conditioner exit.

surface of the water and the bottom of a filled 2,000 L graduated cylinder.

**WAI and WSI.** These two physical characteristics were determined following the methods of Anderson et al (1969) and



**Fig. 3.** Surface response relationship between screw speed (SS) and ratio of the extruder water to conditioner steam (EW/CS) and moisture content exiting the conditioner (MCC).

Jones et al (2000). Using a laboratory-scale grinder (Chemical Rubber Co., Cleveland, OH, U.S.A.), extrudate samples were ground to approximately 150  $\mu\text{m}$  particle size, and 2.5 g of the finely ground sample was placed in a tared 50 mL centrifuge tube; 30 mL of distilled water at 30°C was added to the tube, and a suspension was obtained. After the tube was stirred intermittently over 30 min, the suspension was centrifuged at 3,000  $\times g$  for 15 min with a laboratory-scale centrifuge (Fischer accuSpin 400, Thermo Electron, New Castle, DE, U.S.A.). Thereafter, the supernatant phase was transferred into tared aluminum dishes and placed in a laboratory oven at 135°C for 2 hr. WAI was calculated as the mass ratio of the remaining gel in the centrifuge tube to the original mass of the sample:

$$\text{WAI} (-) = \frac{\text{mass of gel}}{\text{mass of sample}} \quad (3)$$

Subsequently, WSI (%) was calculated as the mass ratio of the extracted dry solid to the original sample mass, following AACCI Approved Method 44-19.01.

**PDI.** PDI was determined following ASAE standard method S269.4 (ASAE 1996). Extruded sample (200 g) was tumbled inside a PDI tester (PDT-110, Seedburo Equipment, Chicago, IL, U.S.A.) for 10 min and then sieved manually via a no. 6 screen. Thereafter, PDI was calculated following equation 4, where  $M_a$

**TABLE VII**  
Main Effects of Screw Speed (SS), Conditioner Steam (CS), and Extruder Water (EW) on Extrudate Physical Properties<sup>z</sup>

Property	Level	MC (% db)	$a_w$ (-)	$k$ (W/m°C)	$R$ (m°C/W)	$\alpha$ (mm <sup>2</sup> /sec)	ER (-)	UD (kg/m <sup>3</sup> )	BD (kg/m <sup>3</sup> )
SS (rpm)	350	3.37ab (0.07)	0.12b (0.01)	0.05a (0.01)	20.27bc (0.64)	0.18a (0.01)	1.09b (0.03)	1.02a (0.03)	529.37a (0.67)
	400	3.46a (0.31)	0.19a (0.03)	0.05a (0.01)	20.71a (1.22)	0.19a (0.01)	1.07b (0.04)	0.99a (0.05)	529.69a (15.98)
	450	3.08b (0.17)	0.11b (0.02)	0.05a (0.01)	20.53ab (0.45)	0.18a (0.01)	1.13a (0.03)	0.92b (0.04)	465.01b (1.35)
EW (kg/min)	0.236	3.61bc (0.15)	0.19a-c (0.01)	0.05a (0.01)	20.27bc (0.99)	0.18bc (0.02)	1.10a (0.04)	1.00ab (0.11)	525.42ab (0.93)
	0.271	3.41ab (0.32)	0.17ab (0.04)	0.05a (0.01)	20.70a-c (1.14)	0.19ab (0.01)	1.08a (0.04)	0.98bc (0.05)	519.54a (25.22)
	0.302	3.29a-c (0.17)	0.15bc (0.01)	0.05a (0.01)	20.57ab (1.40)	0.19a-c (0.02)	1.04b (0.03)	1.02a-c (0.03)	550.19b (1.53)
CS (kg/min)	0.1	4.14a (0.05)	0.24b (0.01)	0.05b (0.01)	20.97ab (1.65)	0.19ab (0.02)	1.11 (0.03)	0.95bc (0.05)	498.15bc (1.92)
	0.12	3.31b (0.18)	0.16a (0.03)	0.05a (0.01)	20.38a (0.90)	0.18a (0.01)	1.08 (0.04)	0.99ab (0.06)	527.24a-c (25.29)
	0.15	3.52b (0.02)	0.19ab (0.02)	0.04b (0.01)	22.47b (0.45)	0.20b (0.01)	1.05 (0.03)	0.99a-c (0.06)	515.83ab (0.83)
		$L^*$ (-)	$a^*$ (-)	$b^*$ (-)	WS (min)	SV (m/sec)	WAI (-)	WSI (%)	PDI (%)
SS (rpm)	350	39.81b (0.32)	5.06b (0.10)	14.03b (0.15)	26.0b (5.29)	0.08a (0.01)	3.64ab (0.17)	16.85ab (0.46)	97.70a (0.31)
	400	40.61ab (1.13)	5.35b (0.32)	14.40b (0.47)	29.38a (1.53)	0.07b (0.01)	3.43b (0.26)	16.89a-c (0.80)	97.28a (0.66)
	450	41.73a (0.50)	5.86a (0.03)	15.46a (0.14)	18.83c (5.01)	0.07ab (0.02)	3.85a (0.06)	16.67bc (0.15)	94.35b (0.19)
EW (kg/min)	0.236	40.48ab (0.05)	5.87a (0.08)	14.75a-c (0.05)	26.67a-c (2.89)	0.08b (0.01)	3.73a (0.07)	17.18ab (0.23)	97.06ab (0.23)
	0.271	40.84a (1.10)	5.28b (0.31)	14.48ab (0.57)	27.90ab (4.35)	0.07ab (0.01)	3.49ab (0.28)	16.78b (0.80)	96.84b (1.08)
	0.302	39.15b (0.33)	5.61ab (0.17)	14.08bc (0.55)	30.00bc (0.01)	0.07ab (0.01)	3.24b (0.07)	17.18ab (0.10)	98.53a (0.01)
CS (kg/min)	0.1	42.63a (0.24)	5.19ab (0.03)	15.15b (0.07)	30.00a-c (0.01)	0.08a (0.01)	3.91a (0.03)	16.67ab (0.12)	96.28bc (0.02)
	0.12	40.33b (0.96)	5.42bc (0.37)	14.39a (0.56)	27.60bc (4.40)	0.07ab (0.01)	3.46b (0.26)	16.78a (0.75)	97.06ab (1.18)
	0.15	41.07b (0.04)	5.12a-c (0.06)	14.35ab (0.10)	29.00ab (1.73)	0.06b (0.01)	3.31b (0.07)	17.70b (0.22)	97.54a-c (0.08)

<sup>z</sup> Parentheses indicate  $\pm 1$  standard deviation; Means followed by similar letters for a given dependent variable are not significantly different for that independent variable at  $P < 0.05$ . MC = moisture content;  $a_w$  = water activity;  $k$  = thermal conductivity;  $R$  = thermal resistivity;  $\alpha$  = thermal diffusivity; ER = expansion ratio; UD = unit density; BD = bulk density;  $L^*$  = brightness/darkness;  $a^*$  = redness/greenness;  $b^*$  = yellowness/blueness; WS = water stability; SV = sinking velocity; WAI = water absorption index; WSI = water solubility index; and PDI = pellet durability index.

and  $M_b$  are the mass of the extrudates after tumbling and before tumbling, respectively:

$$PDI (\%) = \frac{M_a}{M_b} \times 100 \quad (4)$$

## RESULTS AND DISCUSSION

### Extrusion Processing Parameters

**Temperatures During Processing.** Temperatures at different points of the process were monitored (Fig. 1); temperature increased proportionately, because of heat addition but primarily because of frictional heating. As depicted in Table IV, a significant increase in die temperature was observed by increasing SS from 350 to 450 rpm, and increasing EW from 0.236 to 0.271 kg/min resulted in a 3% increase in die temperature, whereas die temperature decreased 4% by increasing the EW from 0.271 to 0.302 kg/min. Increasing CS from 0.1 to 0.12 kg/min resulted in a 4.8% decrease in die temperature. On the other hand, an increase in CS from 0.12 to 0.15 kg/min had no significant effect on die temperature. CS changed the conditioner temperature significantly. This change was expected, because of the direct steam injection into the conditioner chamber. No significant change in conditioner temperature was observed with increasing SS and EW levels, which was as expected, because the extruder did not impact the conditioner. Interactions (Table V) could only be estimated for the main effects, because of lack of replication at the treatment levels.

**Moisture Contents.** MCC and MCD were determined, and the main effects on MCD and MCC are presented in Table IV. As SS increased from 350 to 450 rpm, MCD decreased by 15%. Using response surface modeling, the simultaneous effects of various levels of SS, EW, and CS on MCD and MCC were also examined. The best-fit regression equations fit the data with a very low  $R^2$  value of 0.20 (equations 5 and 6, Table VI). As shown in Table VI, the effect of SS on MCC was cubic, whereas it did not impact MCD. The effects owing to changing EW and CS levels simultaneously can be assessed with the EW/CS ratio, and the impact of this ratio on MCD and MCC is shown in Figures 2 and 3, respectively. Increasing EW/CS ratio and SS decreased MCD curvilinearly, whereas decreasing EW/CS ratio and increasing SS led to a 7% decrease in extrudate MCC.

**MFR.** The main effects of SS, EW, and CS on MFR of the extrudate exiting the die are shown in Table IV. MFR varied from 1.75 to 1.9 kg/min. SS, EW, and CS had no significant effects on MFR. This observation was confirmed in Table V. Increasing the

SS from 350 to 400 rpm resulted in a decrease in MFR by 5.3%, whereas increasing the SS from 400 to 450 rpm led to a 2.8% increase in MFR. Increasing the EW from 0.236 to 0.271 kg/min resulted in a negligible decrease in MFR, and an increase from 0.271 to 0.302 kg/min did not change the MFR either. The highest increase in MFR was obtained by increasing the CS from 0.1 to 0.15 kg/min, which was 8.6%.

### Extrudate Physical Properties

**Moisture Content.** As depicted in Tables VII and VIII, SS and CS had significant effects on moisture content of the extrudates, as did EW. Increasing the SS from 350 to 400 rpm increased the extrudate moisture content by 2.7%, and increasing the SS from 400 to 450 rpm decreased the moisture content by 11%, which probably resulted from greater heating. Changing the CS from 0.1 to 0.12 kg/min led to a 20% decrease in moisture content, and increasing the CS from 0.12 to 0.15 kg/min increased the moisture content by 6.3%. Increasing EW from 0.236 to 0.302 kg/min resulted in a nearly 9% decrease in the extrudate moisture content. The combination treatment of 400 rpm SS along with 0.271 kg/min EW and 0.1 kg/min CS resulted in the highest moisture content. The lowest moisture content was obtained at a SS of 450 rpm, EW of 0.271 kg/min, and CS of 0.12 kg/min.

The response surface generated for extrudate moisture content as a function of SS and the EW/CS ratio is shown in Figure 4. The regression equation is given in Table IX. This surface indicated that increasing the SS and increasing the EW/CS ratio had a curvilinear effect on the extrudate moisture content.

**Water Activity.** Water activity is an important factor, as it affects the shelf life, activity of enzymes, vitamins, and color of food products over time. Water activity is a measure of the existing free water in a material. It might be the most important factor in controlling spoilage, because it shows the lowest limit of available water for microbial growth. The lower the water activity, the lower the chance of spoilage. Lowe and Kershaw (1995) found that water activity of less than 0.6 can guarantee a longer shelf life for most food products. Also, water activity is temperature dependent, because of changes in water binding and solubility in food and feed matrices.

The main effects on the water activity of the extrudates are summarized in Table VII. SS changed the water activity of the products significantly. As SS increased from 350 to 400 rpm, water activity increased by 58%, whereas increasing SS from 400 to 450 rpm decreased water activity by 42%. Increasing EW from 0.236 to 0.302 kg/min had a significant effect water activity and led to a 21% decrease. Increasing CS from 0.1 to 0.15 kg/min

TABLE VIII  
Interaction Effects of Screw Speed (SS), Extruder Water (EW), and Conditioner Steam (CS) on Extrudate Physical Properties ( $P$  Values)<sup>a</sup>

Interactions	MC (% db)	$a_w$ (-)	$k$ (W/m°C)	$R$ (m°C/W)	$\alpha$ (mm <sup>2</sup> /sec)	ER (-)	UD (kg/m <sup>3</sup> )	BD (kg/m <sup>3</sup> )
SS	0.0001	0.0001	0.8444	0.7662	0.934	0.0001	0.0001	0.0001
EW	0.0074	0.001	0.7554	0.6813	0.7201	0.0001	0.0351	0.0001
SS × EW	0.1841	0.0001	0.9626	0.9087	0.9541	0.0001	0.0001	0.0001
CS	0.0001	0.0001	0.0026	0.0138	0.0403	0.0006	0.1481	0.0001
SS × CS	0.0001	0.0001	0.0082	0.0327	0.1182	0.0001	0.0001	0.0001
EW × CS	0.0001	0.0072	0.0082	0.0322	0.0975	0.0001	0.0464	0.1169
SS × EW × CS	0.0001	0.0001	0.0363	0.1152	0.2687	0.0001	0.0001	0.0001
	$L^*$ (-)	$a^*$ (-)	$b^*$ (-)	WS (min)	SV (m/sec)	WAI (-)	WSI (%)	PDI (%)
SS	0.0063	0.0005	0.0001	0.0001	0.0234	0.0001	0.8785	0.0001
EW	0.0016	0.0001	0.0533	0.1336	0.0404	0.0001	0.5217	0.0001
SS × EW	0.0171	0.0001	0.0015	0.0001	0.0260	0.0043	0.8446	0.0001
CS	0.0001	0.6731	0.0006	0.8457	0.0366	0.0001	0.0714	0.0001
SS × CS	0.0001	0.0147	0.0001	0.0001	0.0286	0.0001	0.3475	0.0001
EW × CS	0.0004	0.0207	0.1056	0.6818	0.0953	0.0024	0.1358	0.0628
SS × EW × CS	0.0001	0.0001	0.0001	0.0001	0.0070	0.0001	0.3158	0.0001

<sup>a</sup> MC = moisture content;  $a_w$  = water activity;  $k$  = thermal conductivity;  $R$  = thermal resistivity;  $\alpha$  = thermal diffusivity; ER = expansion ratio; UD = unit density; BD = bulk density;  $L^*$  = brightness/darkness;  $a^*$  = redness/greenness;  $b^*$  = yellowness/blueness; WS = water stability; SV = sinking velocity; WAI = water absorption index; WSI = water solubility index; and PDI = pellet durability index.

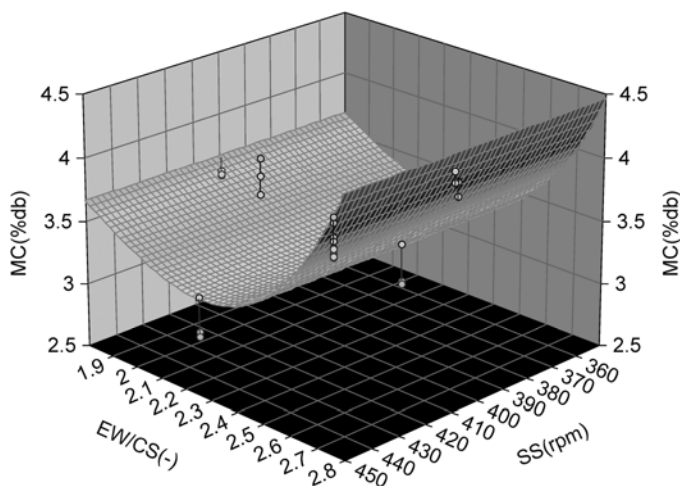
decreased the water activity significantly in a curvilinear trend. The lowest water activity was obtained at the treatment combination of SS, EW, and CS of 400 rpm, 0.271 kg/min, and 0.12 kg/min, respectively.

The response surface relationship between SS and the EW/CS ratio is shown in Figure 5. It is clear that increasing SS affected the water activity curvilinearly, whereas increasing the EW/CS ratio had no effect on water activity of the extrudates for a given SS.

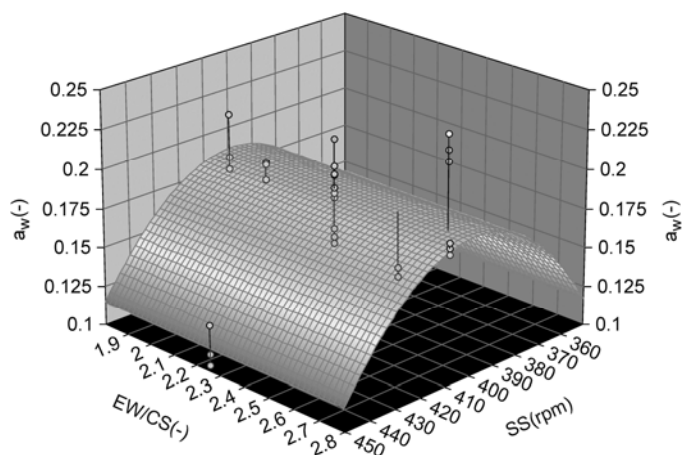
**Thermal Properties.** Thermal properties of a material can be explained by three physical characteristics: thermal conductivity ( $k$ ), which indicates a material's potential for transferring heat through itself by conduction; thermal resistivity ( $R$ ), which is the ability of a material to prevent heat transfer through that material and is the reciprocal of thermal conductivity; and thermal diffusivity ( $\alpha$ ), which indicates the material's capability for heat storage versus transfer (Kawasaki and Kawai 2006) and is defined as the ratio of thermal conductivity to the volumetric heat capacity and mass density. According to Arámbula-Villa et al (2007), substances with lower thermal diffusivity need a longer heating time to conduct a given heat flow. As shown in Tables VII and VIII, none of the independent variables (SS, EW, or CS) had significant impacts on the thermal properties of the extrudates.

**Expansion Ratio.** The main effects on expansion ratio are provided in Table VII, and the interactions are in Table VIII. Contrary to what Martin-Cabrejas et al (1999) and Chevanan et al (2007c) observed in their studies, increasing the SS in our study affected the expansion ratio of the extrudates. Our observations agreed with Rosentrater and Tulbek (2010). Increasing the SS from 350 to 450 rpm increased the expansion ratio curvilinearly. This increase could result from high shear rate developed inside the extruder. Increasing EW from 0.236 to 0.302 kg/min and CS from 0.1 to 0.15 kg/min decreased the expansion ratio by 5.5 and 5.4%, respectively. As shown in Table VIII, SS, EW, and CS all had

significant effects on expansion ratio. The maximum and minimum values for expansion ratio were 1.13 and 1.04, respectively. In the previous study done by Rosentrater and Tulbek (2010), expansion ratio of the extrudates increased with increasing EW, and the products exhibited good floatability. The low expansion ratio value of the extrudates obtained in this study could be because of the high protein and low starch contents of the raw feed blends. Also, starch content of the DDGS can vary between 4.7 and 5.9% (Rosentrater and Muthukumarappan 2006). Most response surfaces for the relationships between SS, EW/CS, and expansion ratio resulted in low correlations and thus are not shown. The expansion obtained during extrusion processing is an important factor in aquafeeds, because expansion ratio affects floatability of extrudates (Oliveria et al 1992; Rosentrater et al 2009a). Extrudate expansion is defined by the ratio between extrudate diameter and die diameter (Conway and Anderson 1973; Van Zuilichem et al 1975). According to Launay and Lisch (1983), it is important to consider both longitudinal and diametral expansion. Fan et al (1994) suggested that the molten dough expansion at the die occurs as two phases: an expansion phase followed by a shrinkage phase. As the melted dough exits the die, the expansion phenomenon occurs because of the immediate change in water state from liquid into vapor, which arises from the sudden pressure drop from high pressure inside the extruder to atmospheric pressure outside of the die. Therefore, internal water flashes off and forms bubbles in the extruded dough. The internal structure of the melt is affected by radial expansion during die exit and results in extrudates with different textures (Arhaliass et al 2003). Several studies have shown that the degree of expansion is dependent on various factors, such as composition of the melt (Bouzaza et al 1996), temperature and rheological behavior of the molten dough, MFR, residence time (Fan et al 1994; Mitchell et al 1994), and die design (Bouzaza et al 1996). High levels of temperature, shear stress, and shear strain during extrusion processing



**Fig. 4.** Surface response relationship between the screw speed (SS) and ratio of the extruder water to conditioner steam (EW/CS) and the final moisture content (MC) of the extrudates.



**Fig. 5.** Surface response relationship between the screw speed (SS) and ratio of the extruder water to conditioner steam (EW/CS) and water activity ( $a_w$ ) of the extrudates.

**TABLE IX**  
Best-Fit Response Surface Models for Extrudate Physical Properties<sup>z</sup>

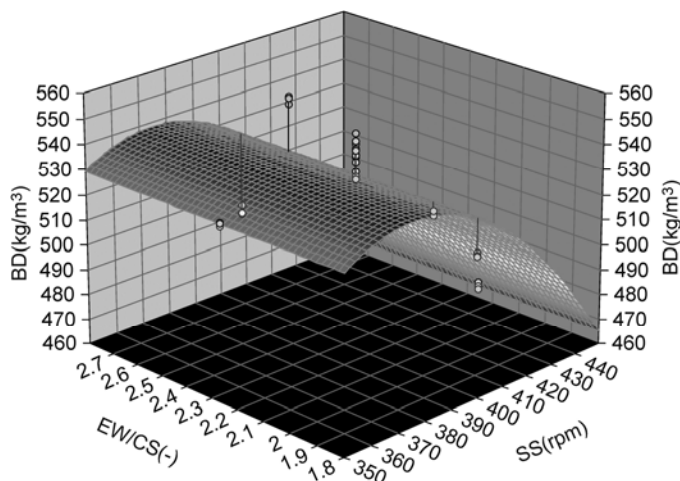
Equation	Response Surface Model	R <sup>2</sup>	Standard Error	F Statistic	Figure
7	MC = (0.33) + (-9.30)(EW/CS) <sup>2</sup> ln(EW/CS) + (3.60)(EW/CS) <sup>3</sup>	0.73	0.16	35.85	4
8	$a_w = (-13.18) + (-0.17)(SS) + (1.20)(SS)/\ln(SS)$	0.57	0.026	17.91	5
9	BD = (-5420.14) + (-78.31)(SS) + (558.30)(SS)/ln(SS)	0.66	14.75	26.00	6
10	$b^* = (75.14) + (0.85)(SS) + (-6.0)(SS)/\ln(SS)$	0.42	0.43	9.65	7
11	WS = (-1271.10) + (-16.71)(SS) + (119.54)(SS)/ln(SS)	0.66	2.43	26.00	8
12	PDI = (-127.93) + (-3.02)(SS) + (21.46)(SS)/ln(SS)	0.71	0.62	31.80	9

<sup>z</sup> MC (% db) = moisture content;  $a_w$  = water activity; BD (kg/m<sup>3</sup>) = bulk density;  $b^*$  (-) = yellowness/blueness; WS (min) = water stability; PDI (%) = pellet durability index; SS (rpm) = screw speed; EW/CS (-) = the ratio of extruder water to conditioner steam; and CS (kg/min) = conditioner steam.

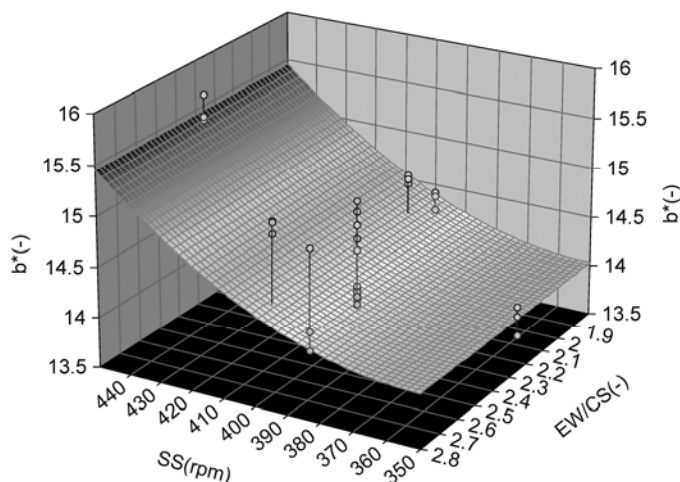


affect the interactions between the water molecules and other chemical components of the dough, alter the internal cellular structure during the evaporation process, and thus impact the expansion ratio of the extrudates (Miller 1985; Chevanan et al 2007c; Moore et al 1990). According to Nielsen (1976), materials with higher starch content tend to exhibit higher expansion because of starch gelatinization and formation of an elastic melt inside the barrel; those with higher protein content, on the other hand, show a limited degree of expansion resulting from forming of a plastic melt and protein denaturation. Martin-Cabrejas et al (1999) and Ding et al (2005) showed that in twin-screw extrusion, SS had no significant effect on expansion ratio of the extruded products. Launay and Lisch (1983) believed that viscosity and elastic properties of the melted starch were the main reasons for volumetric expansion phenomena during extrusion processing.

**Unit Density.** Unit density is inversely related to the expansion ratio (Colonna et al 1989; Bhatnagar and Hanna 1996). In aquafeeds, unit density is considered a key parameter related to the floatability of the feeds. The main treatment effects of each independent variable on the unit density of the extruded products are shown in Table VII. Increasing SS from 350 to 450 rpm decreased unit density by 9.8%. Increasing the SS led to a curvilinear increase in expansion ratio, whereas it resulted in a decrease in unit density. The interaction effects of the independent variables, pre-



**Fig. 6.** Surface response relationship between the screw speed (SS) and ratio of the extruder water to conditioner steam (EW/CS) and bulk density of the extrudates (BD).



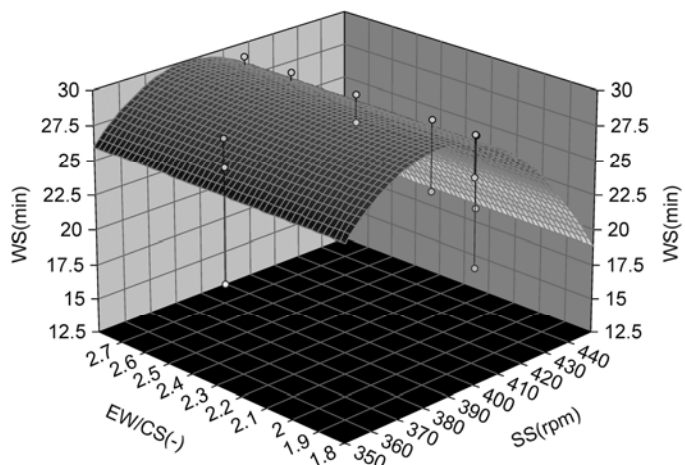
**Fig. 7.** Surface response relationship between the screw speed (SS) and ratio of the extruder water to conditioner steam (EW/CS) and yellowness index of the extrudates ( $b^*$ ).

sented in Table VIII, show that CS had no significant effects on the unit density of the extrudates.

**Bulk Density.** From a commercial point of view, bulk density is an important property of the final products. It is defined as the ratio of the mass of extrudates to the apparent volume of a specific container. Thus, it influences the required storage space either at the processing plant or during shipping (Guy 2001). Therefore, the higher the bulk density, the lower the packaging, storage, and transportation costs. Several parameters such as die design and extent of expansion affect the bulk density of the extrudates. As expansion impacts the bulk density, temperature, pressure, and feed compositions can affect this property. In this study, all independent variables (i.e., SS, EW, and CS) affected the bulk densities of the products significantly. The main effects on bulk densities of the extrudates are summarized in Table VII, and the interactions are in Table VIII. As shown, increasing the SS levels from 350 to 450 rpm decreased the bulk density by 12%, because of increased expansion ratio. Increasing the EW from 0.236 to 0.302 kg/min and CS from 0.1 to 0.15 kg/min increased the bulk density curvilinearly, because of decreased expansion ratio. These increasing effects probably resulted from the increase in moisture content of the dough. However, increasing EW and CS had no significant impact on expansion ratio.

Surface response plots were generated to study the relationships between SS and the EW/CS ratio on extrudate bulk density. As Figure 6 depicts, increasing SS decreased the bulk density curvilinearly, whereas an increase in EW/CS had little impact on this property. The highest (550.20 kg/m<sup>3</sup>) bulk density was found for the treatment combination of 400 rpm SS, 0.302 kg/min EW, and 0.12 kg/min CS, and the lowest (465.01 kg/m<sup>3</sup>) was found for the treatment combination of 450 rpm SS, 0.271 kg/min EW, and 0.12 kg/min CS.

**Color ( $L^*$ ,  $a^*$ ,  $b^*$ ).** Color is a physical property that can be changed during the extrusion cooking process. According to Rosentrater et al (2009b) and Bjorck et al (1983), Maillard reactions and protein denaturing play main roles in color alterations during the cooking process resulting from high temperatures (Goedecken 1991). Reactions occur between the reducing end of carbohydrates and proteins, resulting in a darker product with lower nutritional value. These nonenzymatic browning reactions can destroy the amino acid chains of the protein molecules (Dahl and Villota 1991; Rosentrater et al 2005). Changes in color of the extruded products may also be a sign of lysine loss or alteration (Chevanan et al 2007a, 2007b, 2007c). The effects of each factor on color of the extrudates are presented in Table VII. Increasing SS resulted in a slight increase in  $L^*$  (brightness/darkness) and  $a^*$



**Fig. 8.** Surface response relationship between the screw speed (SS) and ratio of the extruder water to the conditioner steam (EW/CS) and water stability of the extrudates (WS).

(redness/greenness). Increasing SS raised the  $b^*$  (yellowness/blueness) value of the extrudates by 10%. Increasing EW and CS had little impact on the  $L^*$  and  $a^*$  values. Changing EW from 0.236 to 0.302 kg/min decreased the  $b^*$  value curvilinearly. To examine how the SS, EW, and CS affected the physical properties of the extrudates, the best-fit regression models for the relationship between them were studied (Table IX). The best regression equation for the relationship between the SS, EW/CS, and  $b^*$  of the extrudate (equation 10 in Table IX) had a relatively low  $R^2$  value of 0.42, indicating that SS affected  $b^*$  linearly. As SS increased, the resulting  $b^*$  value for all extrudates increased (Fig. 7). No significant change in  $b^*$  value was observed with changing EW/CS (Fig. 8).

**Water Stability.** The main effects of the independent variables on the water stability of the extrudates are shown in Table VII. Only SS changed the water stability significantly. As SS increased from 350 to 400 rpm and from 400 to 450 rpm, water stability of the extruded fish feeds increased by 13% and decreased by 36%, respectively. In our observations, the highest water stability was 30.00 min and the lowest was 18.83 min. Overall, all the extrudates were found to be sufficiently stable. The best-fit regression model for the relationship between the SS, EW/CS, and water stability fit the data with a moderately high  $R^2$  value of 0.66 (equation 11 in Table IX). As is shown in Figure 8, increasing SS decreased the water stability curvilinearly, whereas changing EW/CS did not impact the water stability. Water stability indicates the period of time that extrudates can remain in water without being dissolved, which is important to feeding of the fish.

**Sinking Velocity.** The main effects of all parameters upon sinking velocity of the extrudates are presented in Table VII; the interactions are in Table VIII. From our observations, no significant effects were found on the sinking velocity because of changes in SS, EW, or CS.

**WAI and WSI.** All independent variables exhibited significant effects on the WAI of the extrudates, whereas none of them impacted the WSI significantly. Increasing SS from 350 to 400 rpm decreased WAI, and increasing SS from 400 to 450 rpm increased WAI. WAI decreased by 13% as the EW increased from 0.236 to 0.302 kg/min. Increasing the CS from 0.1 to 0.15 kg/min led to a 15.3% decrease in WAI. WAI can be defined as the amount of occupied volume (resulting from starch content of the material) after swelling up in water. In other words, WAI indicates the part of the starch that was not affected by the extrusion cooking and maintained its internal structure (Mason and Hosney 1986). Thus, changing WAI with increasing SS could be explained by structural modifications of the feed compositions, such as starch

gelatinization and protein denaturation (Badrie and Mellowes 1991; Rosentrater et al 2009a, 2009b). WSI, on the other hand, can be defined as the portion of starch that was converted during the extrusion cooking process. Ng et al (1999) suggested that WAI was inversely related to WSI. In our study, an inverse relationship between the WAI and WSI was observed, although WSI did not exhibit significant alteration with changing of the independent variables.

**PDI.** PDI is another important physical property of extrudates. It indicates the mechanical strength of the extruded products. The higher the PDI, the more stable the extrudates will be during storage and handling processes. It is believed that the extent of heat treatment, along with the level of starch transformation and water content, influence the PDI quality of the extrudates (Rosentrater et al 2009a, 2009b). The effects of each independent variable on PDI are presented in Table VII. All the processing parameters exhibited significant effects on PDI of the extrudates. Increasing SS from 350 to 450 rpm significantly decreased PDI. As EW increased, PDI increased curvilinearly. Increasing CS from 0.1 to 0.15 kg/min also raised PDI values. All extrudates had high PDI, ranging from 94.35 to 98.53%. In a similar study done by Rosentrater and Tulbek (2010), all products exhibited high PDI, with increasing effects because of EW and CS as well. To investigate the interaction effects between the SS, EW, and CS, a response surface plot was generated (Fig. 9). As shown, increasing the EW/CS ratio did not change the PDI value, whereas SS had a drastic effect on PDI.

## CONCLUSIONS

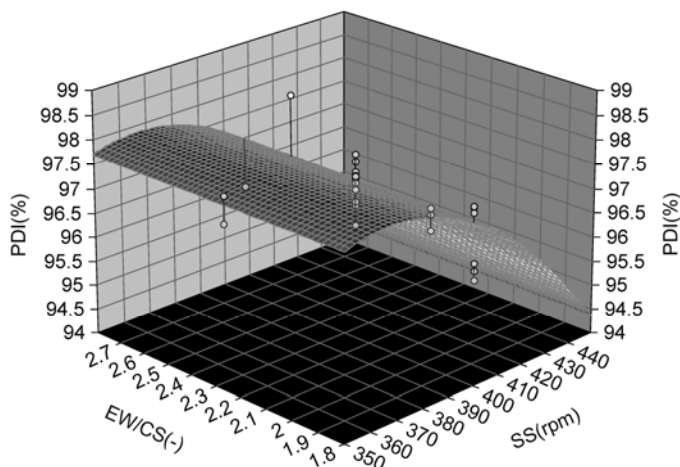
The aim of this study was to investigate the effects of extrusion cooking process parameters including SS, EW, and CS on the quality of DDGS-based aquafeeds for Nile tilapia. SS significantly affected extrudate moisture content, water activity, expansion ratio, unit and bulk densities,  $b^*$ , water stability, WAI, WSI, and PDI. EW had significant impacts on expansion ratio, bulk density,  $a^*$ , WAI, and PDI; it also impacted MCD. CS had significant effects on moisture content, bulk density, WAI, and PDI. All the pellets showed high PDI, which is important to retaining their physical structure during transportation and storage. Moisture content of all pellets was low, and water activity of all the products was lower than 0.25, which indicated that the extrudates all had long shelf life. All of the extruded pellets had good water stability, WAI, and WSI and would be available for fish for a proper time without losing nutrients by dissolving in water. All trials produced viable extrudates, with properties appropriate for Nile tilapia. Results from this experiment indicated that the ranges of SS, EW, and CS used did not change the overall quality of extrudates compared with previous research. These ranges appeared to be close to optimum for this specific formulation. Future studies should evaluate the effects of processing parameters on changes in protein and other nutrients of these DDGS-based Nile tilapia diets.

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**Fig. 9.** Surface response relationship between the screw speed (SS) and ratio of the extruder water to conditioner steam (EW/CS) and pellet durability index of the extrudates (PDI).

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