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Modeling Single-Screw Extrusion Processing Parameters and Resulting Extrudate Properties of DDGS-Based Nile Tilapia (*Oreochromis niloticus*) Feeds

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Abstract

A single-screw laboratory extruder was used to conduct an L_{18} ($2^2 \times 3^6$) Taguchi fractional factorial study of aquafeed processing. The ingredients were based on a formulation for nutritionally-balanced Nile tilapia diets containing distillers dried grains with solubles (DDGS) and soybean meal as the main protein sources, in addition to constant amounts of corn flour, whey, and fishmeal. The effects of three levels of DDGS (20, 30 and 40%), soybean meal (30, 40 and 50%), ingredient moisture content (20, 30 and 40% db), screw speed (100, 150 and 200 rpm), die dimension (L/D ratios of 5, 9 and 13), barrel temperature (80-100-100°C, 80-120-120°C and 80-140-140°C) and two levels of screw configuration (compression ratios of 2:1 and 3:1) on extrudate physical properties (moisture content, water activity, bulk density, unit density, expansion ratio, pellet durability index, water absorption and solubility indices, water stability, color) and extruder processing parameters (resulting temperatures, die pressure, extruder torque, mass flow rate, apparent viscosity, and specific mechanical energy) were determined. Data from raw materials, processing conditions, and extrudate properties were used to develop surface response curves and equations. However, predominantly low R^2 values (< 0.5) only permitted linear relationships between some independent parameters and response variables. Regarding main effects, die pressure significantly decreased with higher DDGS levels, moisture content, temperature, lower die L/D, and higher screw compression. Expansion ratio decreased significantly with higher moisture content and lower die L/D. Significant differences in color were caused by changes in DDGS levels and moisture content. In summary, DDGS, moisture content, die dimension, and extrusion conditions had the biggest impact on most of the extrudate physical properties and processing conditions. Different combinations of these independent factors can be used to achieve desired extrudate physical properties and processing conditions.

Keywords: alternative protein, aquaculture, extrusion, modeling, physical properties

Abbreviations: DDGS: Distillers dried grains with solubles

1. Introduction

Depletion of wild fisheries, combined with rising demands for seafood products for human foods, has led to increased aquaculture production during the last several decades. Depending upon the species and maturity, fish have high dietary protein demands of up to 55% (NRC, 1993). Fish meal is one of the main protein sources used in aquatic feeds; because of good amino acid balance, high palatability, and growth performance, continually increasing demand for the limited supply of wild fish has steadily increased fish meal prices. For example prices for fish meal for July 2010 were above $1370 per ton, compared to $600 per ton for July 2005 (USDA, 2010). For certain fish species, feed costs alone can represent up to 70% of the total production cost for an aquaculture operation (Webster & Lim, 2002; Metts et al., 2007). Protein accounts for the major feed cost. Studies have shown that less expensive alternative protein sources can, at least partially, replace fish meal, satisfy protein demands, and result in good growth performance. These alternatives include various animal and plant sources. Ayadi et al. (2010) provided a comprehensive review of many of these feed ingredients. For instance, meat and bone meal has been used in salmonids feeds (Bureau et al., 2000). Poultry by-product meal has been used in commercial diets for sunshine bass and hybrid striped bass (Rawles et al., 2009; Rawles et al., 2010). Soybean meal (SBM) is one of the most studied and widely used plant protein sources in commercial aquatic feeds for many species, such as tilapia, hybrid striped bass, rainbow trout, Atlantic salmon (*Salmo salar*) and sunshine bass (*Morone chrysops* × *M. saxatilis*) (Steffens, 1994; Thompson et al., 2008; Furuya et al., 2004; Rawles et al., 2009). Distillers dried grains with solubles (DDGS) is another ingredient. It is the major nonfermentable coproduct of fuel ethanol production, and is mostly made from corn grain. Compared to other protein sources, such as SBM, DDGS is very competitive on a cost per unit protein basis, highly palatable to fish (Lim et al., 2009), and does not contain anti-nutritional factors that are present in most pulses. In numerous studies, DDGS has been examined as a potential protein ingredient in fish feed for species such as Nile tilapia, channel catfish, and rainbow trout (Webster et al., 1993; Wu et al., 1996; Cheng & Hardy, 2004; Lim et al., 2007).

Nile tilapia (*Oreochromis niloticus*) originated in Africa, and is one of the most important cultured fish species worldwide. Global tilapia production has increased exponentially within the last 30 years. For example, in 1998, 0.7 million metric tonnes (t) of Nile tilapia were produced, compared to 2.3 million t in 2008, of which Asia has become the major producer (FAO, 2010). For the US market, tilapia production increased from 15,521 t in 1998 to 81,130 t in 2008 (FAO, 2010). It is the fifth most popular seafood consumed in the US (ATA, 2010). Tilapia has relatively fast growth, undemanding feed conditions, and physical hardiness (Fitzsimmons, 2006). Even though Nile tilapia has been classified as herbivorous, it has been reported that Nile Tilapia can also feed on insects, algae, and potentially other fish (Njiru et al., 2004). Protein requirements depend on maturity; they can be up to 45% for Nile tilapia fry (El-Sayed & Teshima, 1992; Hafedh, 1999), whereas bigger fish can require down to 30% protein or less (Hafedh, 1999; Bahnasawy, 2009).

Dietary components are only one aspect of fish feeding, however. The other is feed production. High quality aquatic feeds are commonly produced by extrusion processing, which can produce floating or sinking feeds and improve nutrient digestibility (Pezzato, 1999). Extrusion processing has become very popular in the feed and food industries due to high versatility, productivity, and product quality. Previous research by our group has focused on several processing aspects of DDGS-based feeds. Single-screw and twin-screw extrusion have been used to produce feeds for tilapia, channel catfish, yellow perch, and rainbow trout. The effects of various levels of DDGS inclusion, ingredient moisture content, protein content screw speed, barrel temperature, and die dimension, on resulting extruder processing conditions and extrudate properties have been examined (Chevanan et al., 2007a, 2007b, 2007c, 2008, 2009, 2010; Kannadhason et al., 2009a, 2009b, 2010; Rosentrater et al., 2009a, 2009b; Ayadi et al., 2011a, 2011b, 2011c). Additionally, we have used these extruded feeds in feeding trials to test their efficacy (Schaeffer et al., 2009, 2010). Most of these studies, however, were empirical and deterministic in nature. Follow-up modeling studies on extrusion can help to predict output parameters (e.g. extrudate properties) based on extruder processing settings and/or formulations of the raw blends.

Extrusion cooking involves many complex processes that can be difficult to control due to interactions between mass, energy, and momentum transfer phenomena. Physicochemical changes impact extrudate properties, and can be difficult to predict (Wang et al., 2001). Some research has been conducted on modeling of extrusion processes and resulting product quality. For example, Meng et al. (2010) used second-order polynomial regression to model twin-screw extruder system parameters (feed moisture content, screw speed, and barrel temperature) and physical properties of chickpea flour-based snacks. A similar modeling study was accomplished by Ding et al. (2005) for rice-based expanded snacks. Chevanan et al. (2007c) developed neural network and regression models of single-screw extrusion of aquaculture feeds containing DDGS; data collected from different trials were combined to predict extrudate properties and extrusion processing parameters based on die dimensions, ingredient moisture content, barrel temperature, and screw speed. Wang et al. (2001) modeled twin-screw extrusion to control extrudate quality attributes. Multiple regression models were developed by Ganjal et al. (2004) to relate the radial expansion of extrudates to die nozzle dimensions and back pressure at the die for acetylated starch in a twin-screw extruder. Ali et al. (1996) developed a regression model to study the effects of temperature and screw speed on the radial, axial, and overall expansion, as well as bulk density of extruded corn grits in a single-screw extruder.

In this study, the goal was to model the effects of various levels of DDGS, soybean meal, ingredient moisture content, screw speed, screw compression ratio, die dimension, and barrel temperature on resulting extrudate physical properties (e.g., moisture content, water activity, bulk density, unit density, expansion ratio, pellet durability index, water absorption and water solubility indices, water stability, and color) and on resulting extruder processing conditions (barrel temperature, die pressure, extruder torque, mass flow rate, apparent viscosity, and specific mechanical energy).

2. Materials and Methods

2.1 Feed Blend Preparation

DDGS was provided by Dakota Ethanol, LLC (Wentworth, SD) and soybean meal was obtained from Dakotaland Feeds, LLC (Huron, SD). Low temperature menhaden fish meal was purchased from Consumers Supply Distributing Co. (Huron, SD); corn flour was from Cargill Dry Corn Ingredients, Inc. (Paris, IL); dried whey was from Midor Ltd. (Elroy, WI).

Six ingredient blends (Table 1) were adjusted to a target protein content of ~ 30% db, a target fat content of ~ 17% db, and three different moisture contents of 20, 30, and 40% db. With increasing DDGS levels (20, 30, 40% db), and thus decreasing soybean meal levels (50, 40, 30%), but constant levels of fish meal (approximately), corn flour, and whey, these ingredients were used to prepare nutritionally-balanced diets for Nile tilapia (Schaeffer et al., 2010; Chevanan et al., 2007b). DDGS and soybean meal were ground with a laboratory mill (Model 4, Thomas Scientific, Swedesboro, NJ) to a flour with an average particle size of approximately 500 micrometer (µm). The whey was sieved in manually (Sieve No. 14, ASTM E-11, Daigger, Vernon Hills, IL) to prevent coagulation within the blends. The components were then blended in a rotary mixer for 10 min (Kushlan Products, Inc., Goldendale, WA). After all ingredients were thoroughly combined, each blend was adjusted to the desired moisture content of 20, 30, and 40%, respectively, by adding adequate amounts of water, and then thoroughly mixed using a laboratory-scale mixer (Professional 6, KitchenAid, St. Joseph, MI).

Table 1. Ingredient components (for each diet) used in the study

	Dry weight of ingredients (g/kg)		
	Diet1	Diet2	Diet3
DDGS	20	30	40
Soybean meal	50	40	30
Corn flour	15	15	15
Whey	5	5	5
Fishmeal	10	10	10
TOTAL	100	100	100

2.2 Experimental Design and Extrusion Processing

A L_{18} ($2^2 \times 3^6$) Taguchi fractional factorial design (Table 2) was used for the study. The treatment combinations consisted of 18 uniquetrials, which consisted of different combinations of 2 levels of screw compression ratio (2:1, 3:1), 2 levels of fishmeal (9.99% and 9.98%), 3 levels of DDGS (20, 30 and 40%), 3 levels of soybean meal (30, 40 and 50%), 3 levels of raw blend moisture content (20, 30 and 40% db), 3 levels of screw speed (100, 150 and 200 rpm), 3 levels of die dimension (L/D ratios of 5, 9 and 13), and 3 levels of barrel temperature profile (80-100-100, 80-120-120 and 80-140-140°C).

A single-screw extruder (Model PL 2000, Brabender Plasti-Corder, South Hackensack, NJ), with a barrel length of 317.5 mm, was used to extrude each blend. Three different dies were used, with length to diameter (L/D) ratios of 5, 9 and 13. The center of the die assembly was conical, and tapered from an initial diameter of 6.0 mm to an exit diameter of 2.0, 3.2 or 6.0 mm, respectively, at the discharge opening. A 7.5 HP (5.5 kW) motor was connected to the extruder drive shaft. During extrusion, the screw speed was adjusted to 100, 150 and 200 rpm, respectively. For all runs, the temperature of the feed zone was controlled and maintained at 80ºC, that of the transition zone at 100, 120, or 140ºC, respectively, and that of the die zone at 100, 120, or 140ºC, respectively. The raw blends were manually funneled into the extruder in constant quantities to avoid jamming at the opening of the barrel and to provide a continuous feed. All processing data were collected every 60 s, and the average of eight (n = 8) recordings were used for statistical analyses, except for mass flow rate where three samples (n = 3) were used.

2.3 Raw Ingredient Properties

Each raw blend was analyzed for moisture content, water activity, and color (Hunter L, a, b values). Methods used will be discussed subsequently.

Table 2. Experimental design used in the study

	Ingredient Properties				Extruder Properties			
Treatment	Fishmeal (g/kg)	DDGS (g/kg)	SBM (g/kg)	MC_{raw} (g/kg)	Screw Speed (rpm)	Screw compression ratio	Die L/D	Temperature profile (°C)
1	9.98	20	30	20	100	2:1	5	80-100-100
2	9.98	40	40	30	150	2:1	9	80-120-120
3	9.98	30	50	40	200	2:1	13	80-140-140
4	9.98	30	40	20	200	3:1	9	80-100-100
5	9.98	20	50	30	100	3:1	13	80-120-120
6	9.98	40	30	40	150	3:1	5	80-140-140
7	9.99	20	40	40	150	2:1	13	80-100-100
8	9.99	40	50	40	100	2:1	9	80-100-100
9	9.99	30	30	20	150	2:1	13	80-120-120
10	9.99	40	50	20	200	2:1	5	80-120-120
11	9.99	30	30	30	100	2:1	9	80-140-140
12	9.99	20	40	30	200	2:1	5	80-140-140
13	9.99	30	50	30	150	3:1	5	80-100-100
14	9.99	40	30	30	200	3:1	13	80-100-100
15	9.99	30	40	40	100	3:1	5	80-120-120
16	9.99	20	30	40	200	3:1	9	80-120-120
17	9.99	40	40	20	100	3:1	13	80-140-140
18	9.99	20	50	20	150	3:1	9	80-140-140

* The experimental design consisted of an L_{18} Taguchi fractional factorial design with 18 total treatment combinations. MC_{raw} is raw blend moisture content, die L/D is length to diameter ratio of the die, SBM is soybean meal

2.4 Extrusion Processing Parameters

2.4.1 Temperature Profile, Die Pressure and Torque

The absolute pressure at the die zone and the actual temperature profile at the feed, metering, and die zones were simultaneously monitored every minute for eight (n=8) recordings using a combined thermocouple/pressure transducer (GP50, New York Ltd., Grand Island, NY). Likewise, the net torque exerted on the screw drive shaft was recorded with a torque transducer (Measurement Specialists, Huntsville, AL) at a sensing range of 0-390 N.m every minute for eight (n=8) recordings.

2.4.2 Mass Flow Rate (MFR)

Extrudate samples exiting the die were collected at 30 s intervals, dried, and weighed using an electronic balance (PB 5001, Mettler Toledo, Switzerland) to quantify the mass flow rate.

2.4.3 Apparent Viscosity (η_{app})

The extruder was approximated as a coaxial cylinder-shaped viscometer, where the screw and barrel were considered as an inner and an outer cylinder, respectively (Rogers, 1970; Lu et al., 1992; Rosentrater et al., 2005; Chevanan et al., 2007a). The apparent viscosity of the dough was calculated using:

$$\eta_{app} = \left(\frac{C_{ss}}{C_{sr}}\right) \times \left(\frac{\tau}{\omega}\right) \tag{1}$$

where η_{app} is the apparent viscosity of the dough (Pa•s), τ is the net torque exerted on the screw shaft (N•m), ω is the screw speed (rpm), Css is an empirical correction factor for the shear rate which relates to the screw configuration, and Csr is an empirical correction factor for the shear rate which relates to the barrel size, where:

$$C_{ss} = \frac{1}{2\pi \cdot L_s \cdot r_{corr}^{2}} \tag{2}$$

$$C_{sr} = \frac{2r_b^2}{r_b^2 - r_{corr}^2} \tag{3}$$

$$r_{Corr} = \sqrt{\left(r_{eff_1}^2 + r_{eff_1} \cdot r_{eff_2} + r_{eff_2}^2\right)/3} \tag{4}$$

where r_{corr} is the radius correction factor due to the frustum geometry of the screw (m), r_b is the barrel radius (m), L_S is the screw length in the axial direction (m), and r_{eff} is the effective radius of the screw obtained from the sum of the screw root and half of the flight height (m). Specific values for these parameters have been discussed elsewhere (Rosentrater et al., 2005).

2.4.4 Specific Mechanical Energy (SME)

Specific mechanical energy (J/g) was calculated using equation (5), following Harper (1981):

$$SME = \frac{\tau \cdot \omega \cdot 60}{m_{feed}} \tag{5}$$

where τ is the net torque exerted on the screw shaft (N.m), ω is the screw speed (rpm), and m_{feed} is the mass flow rate of the input dry feed (g/min), calculated using the following equation:

$$m_{feed} = MFR \cdot \left(\frac{1 - MC_f}{1 - MC_e}\right) \tag{6}$$

where MC_f is the moisture content of the raw feed blend (% wb) and MC_e is the moisture content of the extrudate at the die (% wb).

2.4.5 Extrudate Physical Properties

After the prepared blends were cooked in the extruder and dried for 72 h at room temperature (25±1ºC), they were then analyzed for moisture content (% db), water activity, bulk density (kg/m^3), unit density (kg/m^3), expansion ratio, pellet durability index (%), water absorption and water solubility indices (%), water stability (min), and color. For all treatment runs, three samples (n=3) were used to determine the physical properties.

2.4.6 Moisture Content (MC)

According to AACC method 44-19 (2000), the moisture content of the raw material and extrudate samples for each blend were determined using a laboratory oven (Thelco Precision, Jovan, Wincester, VA) at 135ºC for 2 h.

2.4.7 Water Activity (a_w)

Water activity was measured for the raw material and extrudate samples from each treatment with a water activity meter (aw Sprint TH-500, Novasina, Pfäffikon, Switzerland). The sample bowl was filled with each sample and then placed into the measuring chamber of the pre-calibrated instrument.

2.4.8 Bulk Density (BD)

Bulk density (BD) was determined as the ratio of the mass of extrudates in a given bulk volume. A standard bushel tester (Seedburo Equipment Company, Chicago, IL) was used following the method described by USDA (1999).

2.4.9 Unit Density (UD)

The extrudates were cut to a length of 25.4 mm, weighed on an analytical balance (Adventurer™, Item No. AR 1140, Ohaus Corp. Pine Brook, NJ), and then measured with a digital caliper (Model No. CD-6''C, Mitutoyo Corp., Tokyo, Japan) to determine their diameter. According to Rosentrater et al. (2005) the unit density (UD, kg/m^3) was calculated as the ratio of the mass m (kg) to the volume V (m^3) of each measured and weighed extrudate sample, assuming a cylindrical shape for each extrudate:

$$\text{UD} = m / V \tag{7}$$

2.4.10 Expansion Ratio (ER)

The diameter of the extrudates was measured with a digital caliper (Digimatic caliper, Model No: CD-6''C, Mitutoyo Corp., Tokyo, Japan), and then the ratio at that diameter to the diameter of the die nozzle (2.0, 3.2, or 6.0 mm) was used to quantify the radial expansion ratio.

2.4.11 Pellet Durability Index (PDI)

Pellet durability index was determined following Method S269.4 (ASAE, 2004). Approximately 100 g of extrudates from each blend were manually sieved (ASTM E-11, Daigger, Vernon Hills, IL) for about 10 s to remove initial fines, and then tumbled in a pellet durability tester (Model PDT-110, Seedburo Equipment Company, Chicago, IL) for 10 min. Afterwards, the samples were again hand sieved for about 10 s, and then weighed on an electronic balance (Explorer Pro, Model. EP4102, Ohaus, Pine Brook, NJ). PDI was calculated as:

$$\text{PDI (\%)} = (Ma / Mb) \times 100 \tag{8}$$

where M_a is the mass (g) after tumbling and M_b is the sample mass (g) before tumbling.

2.4.12 Water Absorption and Water Solubility Index

Water absorption index (WAI) and water solubility index (WSI) were measured according to the method of Anderson et al. (1969) and Jones et al. (2000). Extrudate sample of each treatment combination were ground with a cyclone mill (Cyclone Sample Mill, Model 3010-830, UDY Corporation, Fort Collins, CO) to an average particle size of about 500 µm. Approximately 2.5 g of the extrudate powder was suspended in 30 mL of water in a tarred 50 mL centrifuge tube. The tube was placed in a laboratory oven (Thelco Precision, Jovan, Wincester, VA) at 30°C and stirred periodically every 10 min for 30 min. Afterwards, the water-extrudate suspension was centrifuged for 15 min at 3000 rpm in a laboratory-scale centrifuge (Durafuge 100, Precision, Winchester, VA). The supernatant was decanted into tarred aluminum dishes and dried for 2 h at 135°C in the laboratory oven. The ratio of the remaining gel mass in the centrifuge tube to the original sample mass (approximately 2.5 g) was used to determine the water absorption index:

$$WAI = Wg / Ws \tag{9}$$

where W_g is gel weight (g) and W_s is the original sample weight (g).

WSI was calculated as the ratio of the dry solids (remaining from evaporation of the supernatant from the WAI test) to the original sample mass, following AACC Method 44-19 (2000).

$$WSI\ (\%) = (Wds / Ws) \times 100 \tag{10}$$

where W_{ds} is the dry weight of the supernatant (g) and W_s is original weight of the sample (g).

2.4.13 Water Stability

Water stability is defined as the amount of time that it takes for an extrudate to begin to break apart after it has been placed in water. For extrudates of each blend, a 1-g sample was placed in 200 mL of distilled water and gently stirred using a magnet stirrer (PMC No. 524C, Barnstead International, Dubuque, IA) until the extrudates began to visibly dissolve, and the time was then recorded.

2.4.14 Color

A spectrophotometer (LabScan XE, HunterLab, Reston, VA) was used to determine color, where L quantified brightness/darkness, a redness/greenness, and b yellowness/blueness of the samples.

2.5 Statistical Analysis

Each blend was extruded once. For each treatment combination, three replicates (n=3) were determined for all physical properties. All collected data were analyzed with Microsoft Excel v.2007 and SAS v.9 (SAS Institute, Cary, NC). The Proc GLM (general linear models) procedure was used to identify the main effects (i.e., individual effects due to each independent variable) and the treatment (simultaneous) combination effects using a Type I error rate (α) of 0.05. Then, post-hoc LSD tests were used to determine where the specific differences occurred. TableCurve 3D v.4.0.01 (SYSTAT Software, Inc., San Joes, CA) was also used for response surface modeling.

3. Results

3.1 Extrusion Processing Parameters

3.1.1 Die Pressure

The barrel of the extruder essentially acts as a pressure cooker, where steam and pressure are released at the die opening (Harper, 1981). The design of the die can impact pressure release as well as result in additional pressure. Die pressure and temperature highly affect expansion and mass flow of extrudate. At lower temperatures and lower pressures, less water evaporates which results in less expansion. Hence, moisture content and screw speed are important factors that affect die pressure and extrudate expansion as well.

With changes in screw speed, no significant differences were detected for the die pressure for the main effects (Table 4). This may be related to the high standard deviations. Generally, all standard deviations for the recorded parameters for processing conditions (SME, torque, viscosity) were relatively high. The highest value for die pressure was recorded at 1603.00 MPa (Run 4), while the lowest value was at 42.50 MPa (Run 15) (Table 5). As expected, the die pressure decreased with higher moisture content. This is in agreement with other extrusion studies (Lin et al., 2002; Meng et al., 2010; Singh et al., 2007). At high moisture water can act as a lubricant, and will reduce friction of the extruded dough, which in turn decreases die pressure (Lin et al., 2002). This is reflected in the values for the a_w, which increased with higher blend moisture content. The data for raw blend $a_w$varied between 0.62-0.66, 0.74-0.77, and 0.78-0.80 for 20, 30, and 40% moisture content, respectively (Table 3). Raising the processing temperature from 100 to 140°C resulted in a significant decrease in die pressure of 43.2% (Table 4). Similar results were reported by other investigators (Fletcher et al., 1985; Kirby et al., 1988; Singh et al., 2007). Furthermore, the die pressure showed a significant decrease with a larger die diameter. The die pressure dropped by 46.0% by decreasing the die L/D from 13 to 5 (Table 4). This is in agreement with other observations (Sokhey et al., 1997), and was expected due to an increasing die area, and thus less resistance to flow. Increasing the DDGS level from 20 to 40% yielded a drop in pressure by 31.4%. Likewise, a similar trend was observed by Chevanan et al. (2010). Changes due to increasing DDGS, MC, temperature, and die diameter resulted in significantly lower die pressure values. Examining the treatment combination effects (Table 5) reveals that many treatments were significantly different from each other, which resulted from simultaneous changes of the combined independent variables.

Table 3. Physical properties of the raw feed blends*

Property	Treatment 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
a_w	0.64g	0.74e	0.80a	0.66f	0.77d	0.78c	0.80ab	0.80ab	0.62h	0.62h	0.74e	0.74e	0.74e	0.74e	0.79b	0.80ab	0.62h	0.62h
(-)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.02)
L	62.43a	55.05de	54.04ef	56.76cd	51.57g	53.18fg	53.99ef	52.94fg	59.16b	58.23bc	57.30c	59.11b	57.30c	55.05de	54.04ef	53.99ef	58.23bc	62.43a
(-)	(0.82)	(1.37)	(1.33)	(0.93)	(1.11)	(0.84)	(0.61)	(0.46)	(2.08)	(1.19)	(0.55)	(0.92)	(0.55)	(1.37)	(1.33)	(0.61)	(1.19)	(0.82)
a	3.63g	5.63b	5.57b	4.54ef	5.38bc	6.24a	4.98d	6.45a	4.34f	5.13cd	4.86de	4.38f	4.86de	5.63b	5.57b	4.98d	5.13cd	3.63g
(-)	(0.18)	(0.10)	(0.35)	(0.31)	(0.33)	(0.18)	(0.21)	(0.19)	(0.16)	(0.07)	(0.13)	(0.07)	(0.13)	(0.10)	(0.35)	(0.21)	(0.07)	(0.18)
b	18.59g	20.71abc	20.49bcd	19.15efg	18.82fg	21.28ab	19.74de	21.43a	19.24efg	20.01cde	19.91cde	19.63def	19.91cde	20.71abc	20.49bcd	19.74de	20.01cde	18.59g
(-)	(0.47)	(0.45)	(0.72)	(0.91)	(0.97)	(0.42)	(0.33)	(0.32)	(0.59)	(0.36)	(0.32)	(0.22)	(0.32)	(0.45)	(0.72)	(0.33)	(0.36)	(0.47)

* Means followed by similar letters for a given dependent variable are not significantly different among treatments at $P < 0.05$, LSD. Values in parentheses are standard deviation. a_w is water activity, L is brightness, a is redness/greenness, b is yellowness/blueness.

3.1.2 Torque

Torque quantifies the force that is required to rotate the extruder screw. Thus, it is affected by the viscosity of the dough, moisture content, temperature, and screw speed (Akdogan, 1996). High torque requires more energy and can lead to wear of the extruder. Optimal torque values can save energy and reduce stress on the equipment.

Regarding the main treatment effects (Table 4), no clear pattern of changes in torque could be observed for several independent variables, which again might be related to the high standard deviations. The highest torque value was recorded for run 13 at 51.73 N.m, whereas the lowest was recorded for run 8 at 8.26 N.m (Table 5). Some significant differences in torque were detected with interactive changes across the treatment combinations (Table 5). The torque decreased with higher levels of DDGS, and with higher moisture content. Conversely, torque increased with higher levels of SBM. Changes in screw speed yielded no significant differences in torque for the main effects, which was related to the high standard deviations. But the dough did exhibit shear thinning behavior: the apparent viscosity decreased significantly by 42.2% when increasing the screw speed from 100 to 200 rpm (Table 4). The different screw configurations also had a significant effect on torque. This was as expected, due to the changes in flight height of the screw which increased compression and thus torque for the 2:1 compression ratio screw versus the 3:1 compression ratio. And, as die L/D increased, the torque increased due to greater resistance to flow, which resulted in a higher pressure.

3.1.3 Mass Flow Rate

A common way to examine the productivity of an extruder is to measure its output. Previous studies have shown that the amount of extrudate produced per unit time is impacted by screw speed, die geometry, shear rate, diet formulation (such as DDGS level), moisture content, and the viscosity of the dough melt (Chevanan et al., 2008; Kannadhason et al., 2010). In this study, except for screw speed, none of the independent variables had significant effects on MFR for the main effects, which again was related to the high standard deviations observed in the data.

For the main effects, with increasing screw speed from 100 to 150 rpm, MFR increased significantly by 109.8% (Table 4). The highest MFR was detected for run 3 at 225.60 g/min, while the lowest was for run 8 at 67.13 g/min (Table 5). Treatment combination effects were also studied and response surface generated for MFR, SS, and DDGS level (Figure 1). As shown in Figure 1, the higher screw speed and lower levels of DDGS substitution resulted in decreased flow rate. Overall, changing in DDGS levels in diet did not have considerable impact on extrudate output.

Table 4. Main effects of DDGS, SBM, moisture content of raw material, screw speed, screw compression ratio, die L/D, and extruder temperature profile on raw blends, measured processing properties, and extrudate physical properties*

	Raw Materials				Processing conditions				
	a_{wRaw}	L_{Raw}	a_{Raw}	b_{Raw}	P at Die	Torque	MFR	η_{app}	SME
Variable					(Mpa)	(N m)	(g/min)	(Pa s)	(J/g)
DDGS (% db)									
20	0.73a	57.25a	4.50c	19.19c	932.04a	29.14a	115.32a	1880.83a	860.90a
	(0.07)	(4.48)	(0.72)	(0.70)	(484.21)	(11.98)	(31.43)	(1094.42)	(367.52)
30	0.73a	56.43a	4.96b	19.86b	790.56ab	30.41a	129.67a	1637.52a	716.52ab
	(0.07)	(2.17)	(0.53)	(0.76)	(580.72)	(17.13)	(56.00)	(783.61)	(423.89)
40	0.72a	55.45a	5.70c	20.69a	639.02b	18.43b	111.58a	1067.25b	513.44b
	(0.08)	(2.38)	(0.53)	(0.65)	(302.58)	(11.10)	(51.14)	(648.75)	(210.87)
SBM (% db)									
30	0.72a	56.85a	4.95a	19.91a	713.02a	22.08b	102.92a	1405.04b	606.10a
	(0.07)	(3.44)	(0.88)	(0.98)	(523.26)	(10.16)	(34.69)	(898.54)	(220.73)
40	0.73a	56.20a	5.04a	19.96a	787.58a	24.54b	132.61a	1365.37b	673.92a
	(0.07)	(2.24)	(0.52)	(0.71)	(576.56)	(13.36)	(49.13)	(624.69)	(360.37)
50	0.72a	56.09a	5.17a	19.88a	861.02a	31.36a	121.04a	1815.18a	810.84a
	(0.08)	(3.86)	(0.89)	(1.10)	(299.51)	(17.89)	(53.30)	(1124.53)	(471.29)
MC_{Raw} (% db)									
20	0.63c	59.54a	4.40c	19.27c	1220.75a	28.17a	106.14a	1686.17a	624.98b
	(0.02)	(2.46)	(0.65)	(0.76)	(346.90)	(10.49)	(35.13)	(721.03)	(186.87)
30	0.75b	55.90b	5.12b	19.95b	602.00b	32.29a	133.67a	1948.87a	929.56a
	(0.01)	(2.61)	(0.49)	(0.80)	(312.52)	(16.94)	(44.99)	(1125.72)	(505.40)
40	0.79a	53.70c	5.63a	20.53a	538.88b	17.52b	116.76a	950.55b	536.33b
	(0.01)	(0.91)	(0.62)	(0.80)	(446.25)	(11.55)	(57.15)	(504.72)	(208.11)
SS (rpm)									
100	-	-	-	-	718.88a	23.18a	76.31c	1922.54a	612.02a
					(474.17)	(14.08)	(7.68)	(1129.17)	(344.64)
150	-	-	-	-	884.60a	27.97a	120.18b	1551.38b	710.81a
					(481.08)	(15.72)	(41.49)	(812.55)	(397.06)
200	-	-	-	-	758.15a	26.83a	160.08a	1111.67c	768.04a
					(485.30)	(13.81)	(37.87)	(563.07)	(366.22)
Screw comp.									
2:1	-	-	-	-	950.83a	32.05a	135.01a	1964.79a	769.13a
					(521.87)	(14.26)	(66.32)	(1085.34)	(269.57)
3:1	-	-	-	-	705.40b	22.97b	110.78a	1310.41b	660.87a
					(441.37)	(13.88)	(32.09)	(744.16)	(408.05)
Die L/D									
5	-	-	-	-	544.81c	22.06b	103.08a	1345.70b	729.19a
					(508.60)	(17.97)	(34.12)	(1115.82)	(531.36)
9	-	-	-	-	807.42b	25.43ab	127.83a	1397.26b	601.21a
	-	-	-	-	(466.37)	(13.41)	(47.60)	(654.04)	(223.36)
13	-	-	-	-	1009.40a	30.50a	125.66a	1842.63a	760.47a
					(346.56)	(10.44)	(55.72)	(873.81)	(276.47)
T (°C)									
100	-	-	-	-	1002.50a	31.77a	121.83a	1774.88a	796.78a
					(463.65)	(15.90)	(40.82)	(860.92)	(399.40)
120	-	-	-	-	789.63b	22.90b	111.63a	1431.02ab	627.75a
					(486.22)	(13.93)	(44.18)	(1105.86)	(326.42)
140	-	-	-	-	569.50c	23.31b	123.10a	1379.70b	666.34a
					(399.99)	(12.28)	(56.84)	(728.32)	(375.58)

	Extrudate Properties											
	MC_{Ext}	a_{wExt}	BD	UD	ER	PDI	WAI	WSI	WS	L_{Ext}	a_{Ext}	b_{Ext}
Variable	(% db)		(kg/m^3)	(kg/m^3)		(%)			(min)			
DDGS (% db)												
20	9.74a	0.41a	217.52a	9.74a	0.90a	94.00a	3.37a	19.20a	20.73a	44.09a	3.94b	12.76b
	(1.82)	(0.04)	(27.80)	(1.82)	(0.08)	(4.04)	(0.26)	(0.96)	(7.76)	(5.05)	(0.49)	(2.27)
30	8.88b	0.41a	218.29a	8.88b	0.8a	94.94a	3.24ab	19.61a	20.39a	42.27a	4.77a	14.55a
	(0.85)	(0.04)	(29.38)	(0.85)	(0.18)	(4.71)	(0.35)	(1.60)	(10.19)	(4.31)	(0.73)	(1.94)
40	9.00ab	0.39a	221.61a	9.00ab	0.89a	94.85a	3.05b	18.20b	22.22a	41.91a	5.17a	14.43a
	(0.83)	(0.04)	(18.46)	(0.83)	(0.16)	(4.36)	(0.31)	(0.79)	(6.33)	(3.62)	(1.01)	(1.83)
SBM (% db)												
30	9.31a	0.41a	214.33a	9.31a	0.86b	94.53a	3.28a	19.50a	21.22ab	42.22a	4.68ab	13.93a
	(1.57)	(0.04)	(16.14)	(1.57)	(0.16)	(4.73)	(0.39)	(1.77)	(9.94)	(3.65)	(1.01)	(2.52)
40	9.22a	0.40a	228.11a	9.22a	0.86b	95.20a	3.35a	18.55b	17.34b	42.33a	4.94a	14.32a
	(1.44)	(0.04)	(31.62)	(1.44)	(0.17)	(3.07)	(0.25)	(0.92)	(6.74)	(3.90)	(1.06)	(2.24)
50	9.10a	0.40a	214.97a	9.10a	0.95a	94.07a	3.02b	18.95ab	24.78a	43.71a	4.26b	13.50a
	(0.78)	(0.04)	(24.46)	(0.78)	(0.07)	(5.06)	(0.26)	(0.88)	(5.71)	(5.48)	(0.49)	(1.64)
MC_{Raw} (% db)												
20	8.79b	0.41a	231.54a	8.79b	0.99a	91.96b	3.17a	18.85ab	17.44b	45.39a	5.06a	15.54a
	(1.23)	(0.05)	(26.40)	(1.23)	(0.06)	(5.00)	(0.27)	(0.97)	(6.67)	(3.25)	(1.09)	(1.16)
30	8.89b	0.40a	228.40a	8.89b	0.92b	94.34b	3.31a	19.51a	18.42b	44.62a	4.61ab	14.47b
	(0.99)	(0.04)	(16.86)	(0.99)	(0.09)	(3.66)	(0.38)	(1.73)	(7.91)	(3.53)	(0.70)	(1.42)
40	9.93a	0.40a	197.47b	9.93a	0.77c	97.49a	3.18a	18.65b	27.47a	38.27b	4.22b	11.73c
	(1.36)	(0.03)	(15.96)	(1.36)	(0.17)	(1.84)	(0.34)	(0.93)	(5.87)	(2.17)	(0.76)	(1.70)
SS (rpm)												
100	8.36b	0.41a	221.90ab	8.36b	0.84b	94.78a	3.37a	19.29a	22.33a	42.30a	4.97a	14.22a
	(0.81)	(0.04)	(11.70)	(0.81)	(0.16)	(3.98)	(0.39)	(1.84)	(9.07)	(3.52)	(1.03)	(1.51)
150	9.50a	0.40a	206.41b	9.5a	0.89b	94.16a	3.09b	19.17a	20.08a	43.28a	4.47a	13.62a
	(1.09)	(0.04)	(19.77)	(1.09)	(0.16)	(4.47)	0.29	(0.83)	8.13	(4.84)	(0.74)	(2.13)
200	9.76a	0.40a	229.10a	9.76a	0.95a	94.85a	3.19ab	18.55a	20.92a	42.69a	4.45a	13.90a
	(1.47)	(0.05)	(34.27)	(1.47)	(0.10)	(4.68)	(0.27)	(0.91)	(7.43)	(4.86)	(0.92)	(2.72)
Screw comp.												
2:1	8.06b	0.46a	222.61a	8.06b	0.88a	95.91a	3.07b	18.68a	24.56a	42.30a	4.41	13.54a
	(0.56)	(0.01)	(29.52)	(0.56)	(0.15)	(3.85)	(0.24)	(0.83)	(6.26)	(4.47)	(0.67)	(1.98)
3:1	9.78a	0.37b	217.40a	9.78a	0.90a	93.94a	3.29a	19.17a	19.39b	42.98a	4.74	14.10a
	(1.16)	(0.02)	(23.07)	(1.16)	(0.14)	(4.44)	(0.35)	(1.46)	(8.48)	(4.39)	(1.01)	(2.23)
Die L/D												
5	9.25a	0.40a	233.02a	9.25a	0.82c	91.35b	3.04b	19.53a	24.98a	45.76a	4.28b	14.38a
	(1.13)	(0.04)	(21.60)	(1.13)	(0.22)	(4.39)	(0.27)	(0.59)	(7.81)	(4.58)	(0.47)	(1.41)
9	9.10a	0.41a	224.97ab	9.10a	0.90b	96.23a	3.24ab	18.92ab	19.94ab	42.69b	4.57ab	13.56a
	(1.49)	(0.04)	(22.20)	(1.49)	(0.06)	(1.89)	(0.39)	(1.97)	(8.38)	(3.41)	(0.89)	(2.40)
13	9.26a	0.40a	199.42b	9.26a	0.96a	96.21a	3.37a	18.56b	18.42a	39.82c	5.03a	13.80a
	(1.29)	(0.04)	(19.37)	(1.29)	(0.05)	(4.30)	(0.25)	(0.71)	(7.07)	(2.96)	(1.15)	(2.51)
T (^oC)												
100	9.03a	0.40a	221.36a	1160.89a	0.95a	94.47a	3.05b	18.95ab	23.42	42.91a	4.68a	14.24a
	(1.42)	(0.05)	(30.00)	(142.38)	(0.06)	(4.86)	(0.26)	(1.09)	(7.08)	(5.09)	(0.74)	(2.10)
120	9.64a	0.41a	219.75a	1185.14a	0.88b	94.59a	3.29a	18.59b	21.78a	42.83a	4.42a	13.48a
	(1.46)	(0.04)	(19.24)	(155.36)	(0.18)	(5.05)	(0.26)	(0.88)	(7.57)	(2.98)	(0.86)	(2.40)
140	8.94a	0.40a	216.30a	1096.63b	0.85b	94.73a	3.31a	19.47a	18.14a	42.52a	4.79a	14.02a
	(0.85)	(0.04)	(26.49)	(163.00)	(0.16)	(2.99)	(0.41)	(1.69)	(9.13)	(5.02)	(1.13)	(1.98)

* Means within a column (a given dependent variable) followed by similar letters for a given independent variable are not significantly different at $P < 0.05$, LSD. Values in parentheses are standard deviation. SBM is soybean meal; MC_{Raw} is raw blend moisture content; SS is screw speed; Screw Comp. is screw compression ratio; die L/D is length-to-diameter ratio of the die; T (°C) is temperature profile (100 is 80-100-100°C, 120 is 80-120-120, 140 is 80-140-140°C); $a_{w\,Raw}$ is water activity of raw blend; L_{Raw} is brightness/darkness of the raw blend; a_{Raw} is redness/greenness of the raw blend; b_{Raw} is yellowness/blueness of the raw blend; P is die pressure; MFR is mass flow rate; η_{app} is dough apparent viscosity; SME is specific mechanical energy; MC_{Ext} is extrudate moisture content; a_{wExt} is extrudate water activity; BD is extrudate bulk density; UD is extrudate unit density; ER is extrudate expansion ratio; PDI is extrudate pellet durability index; WAI is water adsorption index; WSI is water solubility index; L_{Ext} is extrudate brightness/darkness; a_{Ext} is extrudate redness/greenness; b_{Ext} is extrudate yellowness/blueness.

Table 5. Treatment effects on the measured extrusion processing parameters*

Property	Treatment 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
P at Die	9.32b	5.03ef	5.15ef	11.05a	7.92c	0.87kl	9.12b	4.10fg	9.90b	6.77d	2.55ij	1.53jk	3.77gh	4.11fg	0.29l	2.76hi	5.56e	7.91c
(Mpa)	(2.06)	(0.97)	(1.71)	(1.52)	(0.77)	(0.28)	(0.59)	(0.08)	(0.50)	(1.63)	(0.38)	(0.87)	(0.45)	(0.66)	(0.07)	(0.76)	(1.50)	(1.60)
Torque	31.67bcd	26.81c-f	35.21b	44.05b	46.26a	8.26h	24.94def	8.59h	22.93ef	13.24gh	20.20fg	19.08fg	51.73a	29.66b-e	8.38h	19.76fg	23.99def	33.14bc
(Nm)	(2.27)	(12.61)	(9.46)	(1.76)	(4.62)	(1.66)	(3.53)	(0.31)	(4.76)	(4.39)	(9.08)	(14.93)	(16.60)	(10.04)	(0.79)	(8.90)	(5.08)	(5.08)
MFR	74.90a	186.80b	225.60a	172.60bc	78.07g	72.07g	124.53de	67.13g	88.00fg	105.73ef	85.67fg	159.67c	130.60d	161.20c	75.53g	135.67d	76.53g	119.09de
(g min^{-1})	(4.95)	(50.95)	(7.88)	(2.03)	(0.64)	(5.16)	(3.29)	(2.19)	(9.10)	(4.01)	(2.04)	(9.47)	(4.86)	(0.53)	(2.23)	(3.83)	(14.45)	(4.09)
η_{app}	2877.54b	1623.54cd	1599.64cd	1697.67cd	3565.88a	424.44h	1510.01cde	762.06fgh	1388.15de	601.36gh	1835.30c	866.54fg	2658.80b	1143.18ef	645.54gh	761.64fgh	1848.94c	1703.35cd
(Pa·s)	(205.99)	(763.30)	(429.84)	(67.92)	(356.36)	(85.74)	(213.96)	(88.28)	(288.26)	(199.37)	(824.80)	(678.28)	(853.39)	(386.99)	(61.07)	(343.18)	(391.46)	(261.15)
SME	755.96bcd	582.70c-f	784.55bcd	830.24bc	1250.33a	411.01def	615.51c-f	297.70f	473.70c-f	350.50ef	408.10def	1055.00ab	1449.40a	831.80bc	353.20ef	756.00bcd	606.90c-f	732.50b-e
(J g^{-1})	(10.71)	(36.24)	(66.08)	(18.24)	(88.99)	(75.12)	(59.62)	(3.35)	(46.00)	(14.80)	(192.87)	(809.03)	(469.37)	(252.57)	(42.07)	(138.74)	(96.15)	(175.40)
T1	80.75	78.63	81.25	79.38	80.75	78.80	79.25	79.75	80.25	80.00	80.00	82.13	82.13	81.00	80.63	79.63	79.75	79.63
(°C)	(1.39)	(3.34)	(2.55)	(0.52)	(1.83)	(1.98)	(0.71)	(0.46)	(0.71)	(0.53)	(0.76)	(1.96)	(2.10)	(2.51)	(0.74)	(0.52)	(0.71)	(0.52)
T2	101.38	118.88	139.13	102.75	121.00	140.25	99.75	100.00	122.50	119.38	140.00	133.00	100.88	96.88	122.38	119.63	139.88	138.00
(°C)	(0.91)	(0.35)	(0.83)	(4.50)	(0.53)	(0.46)	(0.46)	(0.00)	(1.19)	(0.92)	(1.07)	(8.64)	(0.64)	(3.87)	(3.85)	(0.74)	(0.35)	(1.07)
T3	103.00	119.00	141.38	102.75	123.13	143.50	99.50	100.00	121.50	119.75	140.00	142.00	99.88	103.25	121.88	120.00	139.88	140.63
(°C)	(2.20)	(0.76)	(0.74)	(3.10)	(1.73)	(1.41)	(0.53)	(0.00)	(1.31)	(0.46)	(0.00)	(4.41)	(0.35)	(2.05)	(2.53)	(0.00)	(0.35)	(1.85)

* Means followed by similar letters for a given dependent variable (row) are not significantly different at $P < 0.05$, LSD. Values in parentheses are standard deviation. P is die pressure, MFR is mass flow rate, η_{app} is apparent viscosity, SME is specific mechanical energy, T1 is extruder feed zone temperature, T2 is extruder metering zone temperature, T3 is extruder die zone temperature.

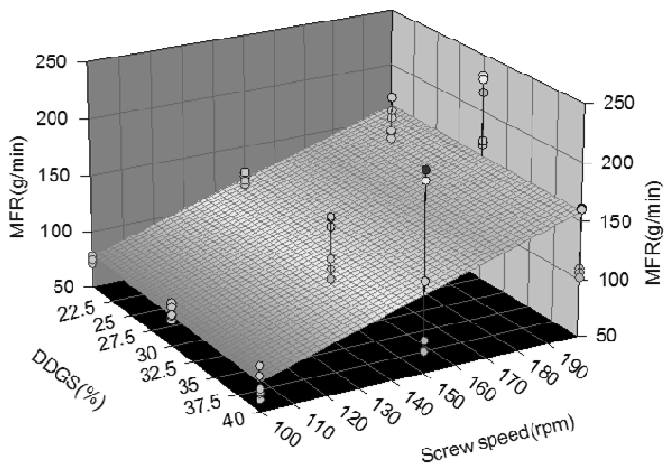

Figure 1. Treatment combination effects on mass flow rate

MFR (g/min) = -1.19 – 0.19*DDGS (g/kg) + 0.84*SS (rpm);(R^2 = 0.54, F = 29.58).

3.1.4 Apparent Viscosity

All independent variables had significant effects on the apparent viscosity, as shown by the main and treatment effects (Tables 4 and 5). Standard deviations for the apparent viscosity were somewhat high; values for apparent viscosity ranged between 424.44 (Run 6) and 3565.88 (Run 5) Pa.s. For the main effects (Table 4), with increasing DDGS content, temperature, screw speed, and screw compression ratio, decreases in apparent viscosity were observed. Raising the DDGS level from 20 to 40% yielded a decrease in apparent viscosity of 43.3%. Similar results were observed by Kannadhason et al. (2009b). Compared to soybean meal, DDGS has higher fiber content and less protein. Increasing the DDGS content in the blends, while reducing the amount of SBM, changed the chemical composition and the potential functionality of the ingredients in the dough, thus affecting the apparent viscosity (Chevanan et al., 2010). A decrease in apparent viscosity by 22.3% was observed as the die zone temperature was raised from 100 to 140°C; this result is supported by findings reported by Kannadhason et al. (2009b) and Chevanan et al. (2010). Likewise, previous studies reported that increasing the temperature resulted in a decrease in viscosity (Launay & Lisch, 1983; Senouci & Smith, 1988). The reduced viscosity can be related to starch gelatinization, protein denaturation, chemical and structural transformations, respectively. Changes due to higher moisture content were curvilinear and showed a significant difference only when increasing MC from 30 to 40%, specifically a reduction of 51.2%. Similar findings were made by Chevanan et al. (2007a, 2010), who concluded that this behavior was caused by competing interactions between moisture content and other independent variables. Increasing the screw speed from 100 to 150 and 200 rpm had significant effects on all recorded values for the apparent viscosity, and yielded an overall decrease by 42.3%. These recordings are similar to observations made by other researchers (Kannadhason et al., 2009b), as the dough was pseudoplastic. On the other hand, an increase in viscosity of 36.9% was observed when increasing the die L/D ratio from 5 to 13.

Chevanan et al. (2007a) made similar observations as well. With changes in die L/D ratio, only the die diameter changed, whereas the length stayed the same. With a smaller die diameter, pressure and shear increased, which led to an increase in viscosity. Increasing the screw compression led to a decrease in apparent viscosity by 33.3%, and was pseudoplastic behavior.

3.1.5 Specific Mechanical Energy

Specific mechanical energy consumption quantifies the net energy that is required to convey the material through the extruder per unit rate of mass flow. For the main effects, no clear pattern of changes could be observed with varying levels of the independent variables, which again was related to the high standard deviations. The highest SME was detected at 1449.40 J/g (Run 13) and the lowest at 297.70 J/g (Run 8; Table 5). With increasing DDGS level, SME showed a significant decrease (Table 4). With increasing SBM, however, the SME increased as well. Increasing the screw speed and compression ratio of the screw yielded an increase and decrease in SME by 25.4% and 14%, respectively. As temperature profile and L/D ratio increased, SME exhibited curvilinear behavior.

3.1.6 Temperature

Temperature settings were adjusted in the beginning of each extrusion run to the desired value. However, throughout processing, temperatures within the different zones increased due to friction (Table 5) and were adjusted by using external air when temperature increased more than 5°C. These temperature effects were expected, due to frictional heating and shear forces in the barrel during extrusion processing. This was due to the design of the extruder (i.e. the conveying mechanism of the flighted screw, the viscous properties of the raw material, and the grooved walls that reduce slip and cause friction) (Harper, 1981).

3.2 Extrudate Physical Properties

3.2.1 Moisture Content

The moisture content of the raw blends had one of the most important impacts on almost all extrudate physical properties and their cohesiveness (Table 4). Previous studies have shown that extrudate MC increased with higher DDGS levels (Ayadi et al., 2011b; Kannadhason et al., 2010) as well as the MC of the raw blends (Kannadhason et al., 2009b; Rosentrater, 2009b). In this study, extrudate MC decreased significantly (by 8.8%) when DDGS content of the blends increased from 20 to 30%, whereas MC did not show significant effects when increasing DDGS level from 30 to 40%. Increases with higher initial MC were as expected due to the higher water content of the raw blends. The difference between initial and final MC can be caused by greater flashing of moisture during exiting the die. The highest value for extrudate MC was 12.01% (Run 16) and the lowest was 7.24% (Run 1) (Table 6). Regarding the other independent variables, some differences occurred for the extrudate MC, in terms of main effect or treatment combination effects (Table 6). Also, using the 3:1 screw (with a higher compression ratio) yielded higher extrudate MC, by 21.3%.

Table 6. Treatment effects on extrudate physical properties*

	Treatment																	
Property	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
MC	7.24k	8.10i	8.62gh	7.67j	7.88ij	8.84fg	11.43b	9.27e	10.12d	10.28cd	8.22hi	10.54c	9.19ef	9.41e	9.43e	12.01a	8.11i	9.33e
(g kg $^{-1}$)	(0.09)	(0.05)	(0.10)	(0.06)	(0.11)	(0.04)	(0.04)	(0.32)	(0.21)	(0.32)	(0.05)	(0.23)	(0.29)	(0.58)	(0.31)	(0.45)	(0.12)	(0.13)
a_w	0.47a	0.45c	0.44c	0.47a	0.46b	0.45c	0.38f	0.37gh	0.38fg	0.37fgh	0.39e	0.37gh	0.37gh	0.35i	0.38fg	0.41d	0.37fg	0.36h
(-)	(0.00)	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)	(0.00)
BD	241.22d	230.81e	177.10l	267.39a	215.98g	203.16j	175.04l	209.17hi	196.62k	254.73c	228.93ef	259.34b	228.85ef	206.49ij	210.82h	209.55hi	225.29f	204.00j
(kg m^{-3})	(2.08)	(2.10)	(4.78)	(1.42)	(2.19)	(1.41)	(2.56)	(3.11)	(3.36)	(0.66)	(2.48)	(1.24)	(0.74)	(1.40)	(2.76)	(0.28)	(0.40)	(1.89)
UD	1012.01ij	1132.85eh	1254.34bcd	1109.81ei	1329.95ab	1170.72def	1389.22a	1257.15bcd	1182.74cde	1023.55i	925.42j	1081.64fi	1045.62i	1153.98efg	1164.84dg	1279.89bc	1080.48fi	1068.86ghi
(kg m^{-3})	(58.16)	(51.00)	(49.20)	(50.00)	(200.92)	(167.36)	(65.73)	(62.34)	(55.12)	(27.95)	(148.21)	(43.24)	(52.83)	(75.80)	(198.15)	(80.01)	(224.16)	(46.30)
ER	0.94de	0.91e	0.94de	0.99bc	0.94de	0.56g	0.90e	0.85f	1.02ab	1.05a	0.82f	0.84f	0.99bc	0.99bc	0.53g	0.85f	0.97cd	0.94de
(-)	(0.04)	(0.02)	(0.02)	(0.03)	(0.02)	(0.03)	(0.01)	(0.02)	(0.03)	(0.02)	(0.02)	(0.15)	(0.01)	(0.03)	(0.07)	(0.03)	(0.06)	(0.05)
PDI	87.79gh	96.07a-e	98.99a	97.61abc	97.95abc	97.07a-d	96.35a-e	98.44ab	92.93ef	86.49h	94.38b-f	91.01fg	88.70gh	97.94abc	97.04a-e	97.07a-d	93.11def	93.83c-f
(%)	(0.20)	(0.11)	(0.17)	(0.08)	(0.01)	(0.05)	(4.60)	(0.06)	(8.71)	(0.30)	(2.29)	(0.53)	(0.76)	(0.30)	(0.06)	(0.23)	(2.97)	(0.27)
WAI	3.00i	3.04ghi	3.03hi	3.05gh	3.55d	2.77k	3.55d	2.81k	3.33f	2.90j	3.90a	3.48e	2.81k	3.08g	3.30f	3.60c	3.67b	3.04ghi
(-)	(0.01)	(0.04)	(0.02)	(0.02)	(0.01)	(0.02)	(0.01)	(0.02)	(0.00)	(0.01)	(0.02)	(0.06)	(0.00)	(0.06)	(0.01)	(0.01)	(0.01)	(0.01)
WSI	20.10b	17.72gh	18.56efg	17.98fgh	18.64def	19.05cde	19.51bc	17.73gh	19.06cde	19.10cde	22.72a	19.58bc	20.24b	18.16fgh	19.10cde	17.93fgh	17.43h	19.45bcd
(%)	(0.15)	(0.40)	(0.22)	(0.05)	(0.08)	(0.50)	(0.18)	(0.55)	(0.03)	(0.71)	(0.09)	(0.11)	(0.17)	(0.45)	(0.22)	(1.72)	(0.16)	(0.45)
WS	>30.00a	19.67c	>30.00a	15.33de	22.33b	>30.00a	14.83de	>30.00a	9.17f	19.50c	7.83f	10.37f	>30.00a	20.33b	>30.00a	>30.00a	13.83e	16.83d
(min)	(0.00)	(4.73)	(0.00)	(0.58)	(2.52)	(0.00)	(2.25)	(0.00)	(1.04)	(0.87)	(1.53)	(1.10)	(0.00)	(1.53)	(0.00)	(0.00)	(1.26)	(1.26)
L	48.96a	43.94b	35.55g	43.53b	42.61bc	39.20de	36.53fg	38.42ef	42.44bc	48.10a	42.44bc	48.64a	49.40a	40.66cd	40.25de	39.64de	41.11cd	48.18a
(-)	(0.35)	(1.19)	(0.92)	(0.19)	(1.42)	(3.18)	(0.37)	(0.92)	(0.54)	(0.55)	(1.94)	(1.19)	(0.23)	(1.04)	(0.10)	(1.56)	(1.11)	(0.37)
a	3.96e	4.54cde	3.87ef	5.39b	4.15e	4.53cde	4.04e	5.05bcd	5.61b	4.40de	5.15bc	4.20e	4.01e	5.60b	4.58cde	3.21f	6.92a	4.06e
(-)	(0.16)	(0.80)	(0.19)	(0.07)	(0.21)	(0.89)	(0.11)	(0.25)	(0.05)	(0.39)	(0.39)	(0.16)	(0.48)	(0.20)	(0.40)	(0.88)	(0.24)	(0.38)
b	14.84bcd	13.63de	11.26fg	16.47ab	12.59ef	12.46ef	10.58gh	12.98e	16.21ab	15.33abc	14.85bcd	15.15a-d	14.99a-d	15.60ab	13.52de	9.60h	16.56a	13.83cde
(-)	(0.47)	(1.85)	(0.44)	(0.11)	(0.45)	(1.72)	(0.22)	(0.57)	(0.06)	(1.03)	(1.09)	(0.08)	(1.51)	(0.64)	(0.75)	(1.88)	(0.61)	(1.02)

* Means followed by similar letters for a given dependent variable (row) are not significantly different at P<0.05, LSD. Values in parentheses are standard deviation. MC is moisture content, a_w is water activity, BD is bulk density, UD is unit density, ER is expansion ratio, PDI is pellet durability index, WAI is water absorption index, WSI is water solubility index, WS is water stability; L is brightness/darkness of extrudate; a is redness/greenness of extrudate; b is yellowness/ blueness of extrudate.

3.2.2 Water Activity

Water activity measures the free water that is unbound in a material and can be available for microorganisms such as bacteria, molds, and yeast. A critical aw exists for every microorganism, below which growth is inhibited. In contrast to bacteria, yeasts and molds can reproduce at lower a_w. Generally, a_w below 0.60 sufficiently restricts microbial growth and reduces the risk of deterioration (Chirife & Del Pilar Buera, 1994; Lowe & Kershaw, 1995).

Regarding main effects, standard deviations for the raw blends were relatively low and significant differences occurred only due to higher moisture content levels. Water activity increased by 25.4% when raising the moisture content from 20 to 40%. Water activity of the extrudate did not exhibit any significant differences due to processing conditions or ingredient composition, except for different screw configuration. Significant differences for the extrudates occurred only due to screw configuration: aw decreased by 19.6% from 0.46 to 0.37 when increasing the compression ratio of the screw (Table 4). Water activity for the raw material ranged between 0.62 and 0.80 (Table 3) and between 0.35 and 0.47 for the final product (Table 6).

3.2.3 Bulk Density

Bulk density is an important parameter for the design of storage vessels. It determines the required storage space for the processing plant or shipping (Guy, 2001). High values for BD imply a higher capacity of extrudates which can be stored in a container. Bulk density varied between 175.04 and 267.39 kg/m^3 for the extrudates (Table 6). Concerning the main effects (Table 4), some significant differences were detected due to varying moisture content, screw speed, and die L/D ratio. A significant decrease for BD, by 13.5%, was detected when increasing MC from 30 to 40%. Raising the screw speed from 100 to 150 rpm resulted first in a decrease in BD by 7.0%, and then in an increase by 11.0% when increasing the screw speed from 150 to 200 rpm. Bulk density decreased by 14.4% with a higher die L/D ratio; a significant difference was observed between the highest and lowest die L/D ratio (Table 6).

3.2.4 Unit Density

Unit density ranged from 925.42 to 1389.22 kg/m^3 (Table 6). The main effects of each independent variable on the unit density of the extrudates are presented in Table 4. For some of the response variables, significant differences were detected. Increasing SBM content of the blend, screw speed and screw compression ratio had no significant impacts on the unit density values of the extrudates. Increasing DDGS from 20 to 30% and temperature profile from 120 to 140°C decreased the UD by 6.7% and 7.5%, respectively. As the L/D of the die was increased from 9 to 13, unit density increased by 9.1%. With increases in moisture content from 20 to 40%, unit density of the extrudates increased by 16.0%, while expansion ratio decreased significantly. As reported by other studies, unit density is related to expansion ratio, which in turn is affected by the moisture content of the feed blend (Ding et al., 2005; Fang & Hanna, 2000), which was supported by our results.

Unit density quantifies the density of a single extrudate. In aquafeeds, unit density plays a key role in the floatability of the feeds. For many fish species, such as Nile tilapia, floatability is recommended since they tend to feed close to the water surface. Extrudates that sink to the bottom of the tank may not be eaten, and present potential feed loss and, overtime, contamination of the water. Additionally, floating feed can show how much feed is consumed by fish and indicate changes in feeding behavior.

3.2.5 Expansion Ratio

Generally, expansion ratio is inversely related to the unit density. In this study, expansion ratio only related to the radial expansion (neglecting longitudinal and volumetric expansion), whereas unit density includes expansion in all directions. Expansion ratio varied between 0.53 and 1.05 within the treatment effects (Table 6). Significant differences were detected for most of the response variables. Expansion ratio significantly decreased by 22.2% by increasing moisture content from 20 to 40%, and by 10.5% by increasing temperature from 100 to 140°C (Table 4). This conforms to the changes for the unit density, which increased with higher moisture content and higher temperature settings. Temperature impacts the rheological characteristics of the dough inside the extruder and thus, expansion ratio (Meng et al., 2010). Higher moisture reduces the viscosity of the melt and can act as a plasticizer, decreasing expansion ratio. With increasing screw speed (100 to 200 rpm), expansion ratio increased by 13.1%, and with higher die L/D ratio (5 to 13), ER increased significantly by 17.1%. Expansion ratio increased with lower die diameter. These results are in agreement with Sokhey et al. (1997), who determined that extrudate radial expansion significantly decreased with increasing die diameter when extruding yellow corn grits and Meng et al. (2010) who observed that increased screw speed led to increased expansion ratio.

3.2.6 Pellet Durability Index

Extruded feed should be of high enough quality to survive transportation and storage without breaking or major crumbling. Pellet durability index is typically used to assess an extrudates' ability to withstand destructive forces. Pellet durability index ranged from 86.49 to 98.99% (Table 6). Regarding the main effects, a significant difference was observed when increasing blend MC level from 30 to 40%; PDI increased by 3.3%. Increasing the moisture content from 20 to 40%, on the other hand, yielded an increase in PDI by 6.0% (Table 4). This behavior was affected by the composition of the blends, which were relatively low in starch and high in protein. Similar observations due to moisture were likewise made in previous studies (Chevanan et al., 2008, 2009). Protein plasticizes under heat and will act as a binder when exposed to shear forces, heat, and moisture. No significant changes occurred with increasing DDGS levels, however, as observed in previous studies (Ayadi et al., 2011a; 2011b). Significantly reduced PDI was observed at the lowest die L/D ratio. This could be ascribed to nearly no expansion of the extrudate for this die geometry.

3.2.7 Water Absorption Index

Water absorption index (WAI) represents the hydrophilic aspect of blend formulation, while WSI is considered a measure of hydrophobic behavior (Ravindran et al., 2011). Starch-based materials have the ability to absorb water when the starch granules are damaged (Colonna et al., 1989). In this case, formulations were primarily protein-based; the blends used in these studies had low starch contents, which is reflected in the low WAI values, ranging between 2.77 and 3.90% (Table 6). The low starch content of the blends also prevented drastic changes in WAI due to the independent variables. Increasing the DDGS content from 20 to 40% and SBM from 30 to 50%, yielded a decrease in WAI by 9.5% and 7.9%, respectively, while an increase in temperature from 100 to 140°C yielded an increase of 8.5%. The increase in WAI by higher temperatures can be related to the destruction of the crystalline structure of the starch, which allows it to absorb more water. Other researchers made similar observations for WAI with increasing temperature and DDGS content, respectively, when extruding DDGS-based feeds (Chevanan et al., 2007a, 2007b; Kannadhason et al., 2009b; Shukla et al., 2005). Increases in DDGS and SBM reduced the WAI of the blends due to less available starch in the blend. The die geometry also had some effect on WAI: a higher L/D ratio (from 5 to 13) resulted in significant increase in WAI by 10.9%. This might be due to the increased expansion and thus destruction of starch granules. Increasing the screw compression from 2:1 to 3:1 resulted in a significant increase in WAI by 7.2% (Table 4). This was related to a higher compression, which increased shear and frictional forces, and resulted in a greater starch granule destruction.

3.2.8 Water Solubility Index

Referring to Kirby et al. (1988), WSI is related to the macromolecular degradation of starch. It is a measure of soluble polysaccharides that are cleaved by degradation of the starch granules (Ding et al., 2005). Water solubility index ranged from 17.43 to 22.72% (Table 6). Regarding the main effects, only a few significant differences were detected (Table 4). Increasing the DDGS level from 20 to 40%, SBM level from 30 to 50%, and die L/D ratio from 5 to 13, yielded a decrease in WSI by 5.2%, 2.8% and 5.0%, respectively. Similar results for WSI with increases in DDGS level were observed in previous studies (Chevanan et al., 2007a; Kannadhason et al., 2010). Extrusion cooking denatures proteins and releases hydrophobic amino acids that reduce solubility in water (Camire, 1991). With increasing moisture content (20 to 40%) and temperature settings (100 to 140°C), WSI showed a curvilinear behavior. It increased by 3.5% when raising MC from 20 to 30%, but it decreased significantly by 4.4% when MC increased from 30 to 30%; similar observations with increasing MC were made by Rosentrater et al. (2009b).

3.2.9 Water Stability

The length of time an extrudate will float without dissolving in water will dictate availability of feed for fish, loss of nutrients, and potential water pollution. Maintaining cohesive extrudates, once they are placed in water, is crucial. Water stability varied between 7.83 min and 30 min for all treatment combinations (Table 6). Only some significant differences were detected for the main effects (Table 4). This can be ascribed to the high standard deviations. An increase in MC from 20 to 40% yielded an increase in WS by 57.5%. As for PDI, this behavior can be related to better binding which was achieved with higher MC. Blends with lower MC were more expanded and absorbed water faster. With a higher die L/D ratio (from 5 to 13), WS decreased significantly, by 26.3%.

3.2.10 Color

The values for brightness (Hunter L) of the extrudates varied from 35.55 to 49.40 (Table 6), whereas the brightness of the raw materials varied from 51.57 to 62.43 (Table III). For the main effects, significant differences for the raw blends occurred only with increasing moisture content; brightness decreased by 9.8% when increasing the moisture content from 20 to 40%. The level of DDGS or SBM did not affect the brightness of the raw ingredients. For the extruded material, brightness decreased significantly by 13.0% with a higher die L/D ratio, but it was not really affected by any other factor.

For redness (Hunter a), values ranged between 3.63 and 6.45 for the raw material, and between 3.21 and 6.92 for the extrudates. Regarding the main effects, redness of the unprocessed blends showed significant differences only with higher DDGS levels and higher moisture content. Redness increased by 26.7% when raising the DDGS content from 20 to 30%, and by 28.0% when increasing the moisture content from 20 to 30%. Similar changes were observed for yellowness (Hunter b).The values for yellowness ranged between 18.59 and 21.43 for the raw blends, and between 9.60 and 16.56 for the extrudates (Table 3 and 6). Increasing the DDGS amounts of the raw blends from 20 to 40%, yielded a significant increase of 7.8% for yellowness. Increasing the moisture content from 20 to 40% yielded an increase of 6.5% (Table 4). Treatment combination effects on extrudate brightness are shown Figure 2. The generated response surface indicated a downward trend for extrudate brightness value with increase in both raw blend moisture content and DDGS level. As shown in Figure 2, raw blend moisture content exerted a greater effect on extrudate brightness than DDGS level.

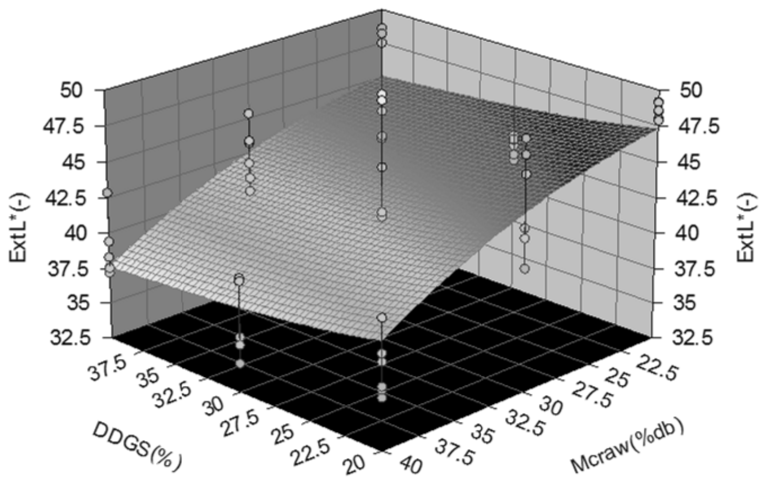

Figure 2. Treatment combination effects on extrudate brightness (Hunter L)

$L_{ext} = 43.88 + 90.59/DDGS$ (g/kg) – $1.33*10^{-4}*MC_{raw}^3$ (g/kg); ($R^2 = 0.55$, F = 31.76).

4. Conclusions

This study was conducted to examine the effects of various levels of raw blend properties (such as DDGS, SBM, moisture), andprocessing conditions (screw speed, screw configuration ratio, die L/D ratio, and temperature profile) on extrusion processing conditions and extrudate physical properties. Raw moisture content and die dimensions significantly affected most of the extrudate physical properties. The pressure at the die decreased significantly with higher processing temperature, lower die L/D ratio, and higher screw compression, whereas the specific mechanical energy showed significant decrease only with higher DDGS content. The mass flow rate exhibited a significant increase with higher screw speed. Color and $a_w$of the raw materials were only affected by raw moisture content and by DDGS level. Overall, it can be concluded that all independent variables had significant effects on certain dependent variables, while others did not. Changes of particular extruder and die settings, moisture content, and DDGS levels had significant effects on certain processing conditions and extrudate physical properties, and can be modified using various combinations thereof. Quantifying these relationships on a small scale is instructive, but this work needs to be done on either pilot or large scale processing equipment to truly understand these behaviors for commercial production of these types of diets.

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