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Effects of Amylose-To-Amylopectin Ratios on Binding Capacity of DDGS/Soy-Based Aquafeed Blends

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Abstract

Demands for seafood products are steadily increasing. Alternative protein sources are required to compensate for enormous amounts of fishmeal that is needed for global seafood production. Starch is a food polymer that can be added to fish feed formulations to enhance binding and expanding capabilities of extrudates. Floatability, a key factor for most aqua feeds, can be optimized by the addition of certain starch sources. Six ingredient blends with a similar protein content (~32.5%) containing two starch sources, Hylon VII (containing 70% amylose, 30% amylopectin) or Waxy I (containing 0% amylose, 100% amylopectin), 20% distillers dried grain with solubles (DDGS), and 15, 25, and 35% moisture content were used along with appropriate amounts of soybean meal, menhaden fishmeal, whey, vitamin and mineral mix to investigate nutritionally-balanced feeds for Nile tilapia (*Oreochromis niloticus* L.). The blends were processed using a laboratory single-screw extruder with varying temperature settings (90-90-90 ℃, 100-120-120 ℃, and 100-120-140 ℃), screw speeds (100, 120, and 140 rpm), and length/diameter ratio (3.4, 6.6, 9.2) of the die. Extensive analyses of expansion ratio (ER), unit density (UD), sinking velocity (SV), and pellet durability indices (PDI), water absorption (WAI) and water solubility indices (WSI) were conducted to evaluate the effects of the two starch sources on extrudate binding and floating capacity. By varying process conditions, significant differences ($P>0.05$) among the blends were detected for all extrudate physical properties. Significantly higher values for ER, UD, and PDI were achieved by using the Waxy I starch source, while values for SV and WAI decreased. For WSI no significant differences were detected. Increasing the moisture content from 15-35% resulted in a significant increase in ER, WAI, and PDI and a significant decrease in UD. WSI showed no clear pattern in changes. The impact of different amylopectin to amylose ratio, temperature and moisture content on extrudate stability, cohesion and physical properties was demonstrated in this study. All formulations yielded viable extrudates while the blends with the amylopectin as the sole source of starch resulted in higher quality extrudates.

Keywords: binding, corn, DDGS, expansion, extrusion, properties, soy, starch

1. Introduction

The world's hunger for seafood products has tremendously exploited and damaged global wild fish resources. Limited supply of seafood can only be regenerated by changing the global attitude towards utilizing nature's food resources and benefiting from aquaculture production. Moreover, aquaculture farming can take pressure of the dependency on wild fishery stocks by using alternative protein sources. In particular, carnivorous species require high amounts of fishmeal and fish oil in their diets. Furthermore, prices for fishmeal are so high that diets often represent 40-70% of aquaculture operating expenses (Thompson et al., 2008). Alternative protein sources are more cost effective and additionally, can support regional and local economies and can reduce environmental impact (Cheng and Hardy, 2004; Ayadi et al., 2009).

Numerous studies have examined alternative protein sources such as products from animal processing waste (e.g. meat and bone meal, poultry-by products, feather meal, etc.) and plant sources (e.g. soybean meal, corn, corn by-products, cottonseed meal, rapeseed meal, etc.). Soybean meal and corn are essential ingredients for fish feed formulations. Additional research is needed to investigate less expensive, more compatible, and sustainable sources that can replace fishmeal. Distillers dried grains with solubles (DDGS), a coproduct of grain fermentation from fuel ethanol or beverage alcohol production is another potential alternative to meet the protein requirements for fish. In contrast to the original grain, it contains about three times the amount of most nutrients due to the fermentation of starch to alcohol (Jacques et al., 2003; Klopfenstein et al., 2008). DDGS does not contain anti-nutritional factors that are commonly found in most plants (Lim et al., 2009). It has less phosphorus than fishmeal and may ultimately reduce the total phosphorus excreted into water and reduces water pollution (Cheng and Hardy, 2004). Changes in domestic energy policies and resulting growth of the fuel ethanol industry will subsequently increase quantities and availabilities of DDGS. DDGS is competitively priced and less expensive than other plant protein sources on a per unit protein basis (Bals et al., 2006; Lim et al., 2009), particularly when compared to soybean meal, which is the most commonly used substitute in aqua feeds.

Traditionally, DDGS has been fed to beef and dairy cattle (Klopfenstein et al., 2008; Schingoethe et al., 2009) and other livestock such as swine (Stein and Shurson, 2009) and poultry (Lumpkins et al., 2004). Since the late 1940s, DDGS has been integrated in fish feed at low inclusion levels (Thompson et al., 2008). In several studies, DDGS has been investigated for species such as Nile tilapia (Wu et al., 1996; Coyle et al., 2004; Lim et al., 2007), channel catfish (Webster et al., 1993; Robinson and Li, 2008; Lim et al., 2009), and rainbow trout (Cheng and Hardy, 2004) where 20-35%, 30-40%, and 22.5%, respectively, could be included without adverse effects on growth performance and weight gain. These studies indicated that quality of feed that contain DDGS as a protein source, plays a key role in fish diets. Several studies demonstrated that processing conditions of fish feed is crucial to extrudate properties from both single and twin screw extrusion, especially when innovative materials are being utilized (Kannadhason et al., 2009a; Rosentrater et al., 2009b). Processing conditions impact pressure and shear forces within the extruder. Moisture content and screw speed have significant effects on extruder throughput, extrudate durability, and color (Chevanan et al., 2008). Feed moisture content, barrel temperature, extruder screw speed, die geometry, protein, and DDGS content significantly affect expansion ratio, sinking velocity, pellet durability index, extrudate color, mass flow rate, pressure at the die, and apparent viscosity (Kannadhason et al., 2009b; Rosentrater et al., 2009a; Chevanan et al., 2007, 2010). These studies ascertained a successful incorporation of up to 40% and 60% DDGS, while DDGS levels between 20-30% are recommended for floating aquaculture feed (Kannadhason et al., 2009a). This is due to the moderately high fiber content of DDGS, which reduces the binding capacity of the blends (Webster et al., 1995). To assure a feed product with adequate floatability and binding capacities, binders such as cellulose or other less expensive starch sources can be added. Extrusion studies combining DDGS with different starch sources showed that cassava and tapioca starch, which have a high amylopectin proportion yielded the highest expansion ratios (Kannadhason et al., 2009a, 2009b; Rosentrater et al., 2009a, 2009b). Expansion, a key factor for floatability, is predominantly impacted by gelatinization of starch, which is governed by moisture content, temperature, pressure and shear forces in the extruder (Lai and Kokini, 1991). As an important food polymer, starch can be added to increase the viscosity, stability, and holding capacity of fat and water in fluids or semi-solid products (Hermansson and Svegmark, 1996). In aqua feeds, starch plays an important role for floatability based on its binding and expanding properties (Webster et al., 1995).

Starch is the reserve carbohydrate of plants and exists as water-insoluble heterogeneous granules with both amorphous and crystalline regions (Keetels et al., 1996a; Yu and Christie, 2005). It consists of the two major polysaccharides, amylose and amylopectin, of which amylopectin commonly makes up 70-80%. The physical and biological properties of amylopectin are affected by a multiple branched structure formed by inter-chain linkage of every 20-25 glucose monomer. On the contrary, amylose content represents 20-30% of the content of most starches and forms longer linear glucose chains that are only lightly branched (Manners, 1989). Amylose and amylopectin have different functionalities and can be altered to desired property by plant breeding. Due to its crystalline order, amylopectin has stabilizing properties and shows higher storage stability whereas amylose tends to form gels and complexes. Waxy corn is a natural mutant without an amylose-producing enzyme that contains 100% amylopectin (Hermansson and Svegmark, 1996). The amylose portion and the branching points of amylopectin form the amorphous region in the starch granules (Keetels et al., 1996; Yu and Christie, 2005). Hylon VII is a commercial available corn hybrid with high amylose content (70%) that is used in the confectionary industry as a gelling and film-forming agent such as for jelly gum and batter coating (NSFI, 2008).

Gelatinization is not only determined by the molecular structure and chemical composition of starch granules, but heat treatment and available water also highly affect the processing of starch. During gelatinization, the granules swell and form a gel as the amylose and the amylopectin solubilize. This process generally occurs in presence of water at temperatures between 60-70 ℃ (Hermansson and Svegmark, 1996). The swelling of the starch granules involves the separation of amylose and amylopectin (i.e., the leaching of amylose out of the granules) (Keetels et al., 1996b) and the destruction of the crystalline structure of the amylopectin (Yu and

Christie, 2005).

Chinnaswamy and Hanna (1988) determined that expansion properties of starches were lowest at 70% and highest at 50% amylose content, when investigating in starch sources with 0, 25, 50, and 70% amylose proportions and were additionally influenced by temperature. Different starch blends expanded best at uniform moisture contents between 13-14%.

Thermal processing of starch leads to degradation (debranching) and is affected by the moisture content. First, during heating at lower temperatures, long chains are cleaved followed by decomposition of the glucose rings. The second scission is affected by the moisture content: the higher the moisture content, the lower the temperature that is required to decompose the glucose ring (Liu et al., 2009).

Extrusion is a continuous process of short time cooking and forming of materials at relatively high pressure, heat, mechanical shear forces, and moisture. This involves order-disorder transition at different temperatures that impact the starch molecules size and shape, which includes starch gelatinization (under excess moisture) and protein denaturation (Lai and Kokini, 1991). Extrusion processing has been widely applied to produce digestible, palatable, durable, water stable, floating feed with high intake for fish.

Whey is the byproduct of the cheese making production. In studies with DDGS-based aquaculture feed, whey improved binding properties of extrudates and increased the pellet durability indices (Chevanan et al., 2009).

This study continued the research previously done by Kannadhason et al. (2009a, 2009b) and Rosentrater et al. (2009a, 2009b) to examine the effect of starch as a binder. Thus, the objectives of this study were: 1) to produce viable extruded feed for juvenile Nile tilapia using DDGS/soy as an alternative protein source in combination with two different starch sources, and 2) to examine the effects of different amylose/amylopectin ratios, various levels of feed moisture content, extruder die temperature, screw speed, and length-to-diameter (L/D) ratio, on the resulting physical properties of the extrudates and on various processing parameters.

2. Materials and Methods

2.1 Feed Blend Preparation

Six ingredient blends suitable for Nile tilapia fish were formulated to a target protein level of 32.5% db using 20% DDGS and 48% soybean meal, and 15% corn [either Hylon VII (containing 70% amylose, 30% amylopectin) or Waxy I (containing 0% amylose, 100% amylopectin)] as the starch source, and three moisture contents (15, 25, and 35%), along with appropriate quantities of soybean meal, menhaden fishmeal, whey, vitamin and mineral mix (Table 1) to prepare nutritionally-balanced diets for Nile tilapia. Hylon VII corn was obtained from National Starch Food Innovation (Bridgewater, NJ), while Waxy No. 1 corn was from Tate and Lyle (Decatur, IL). DDGS was provided by Dakota Ethanol LLC (Wentworth, SD) and soybean meal was from Dakotaland Feeds Inc., LLC (Huron, SD). All were ground with a Wiley Mill (Model 4, Thomas Scientific, Swedesboro, NJ) to a powder with an average particle size of approximately 500 µm. Menhaden fishmeal was purchased from Consumers Supply Distributing Co. (North Sioux City, SD); vitamin C was from DSM Nutritional Products France SAS (Village-Neuf, France); whey, vitamin mix and mineral mix were obtained from Lortscher Agri Service, Inc. (Bern, KS). The ingredients were mixed in a laboratory-scale mixer (N50 mixer, Hobart Co., Troy, OH) for 10 min and adjusted with adequate amounts of water to the target moisture content.

Table 1. Ingredient components (g/100 g, dry basis) in the feed blends used in the study.

	Dry weight of ingredients (g/100g)
DDGS[1]	20
Soybean meal[2]	48
Corn[3]	15
Menhaden fish meal[4]	9
Whey[5]	5
Vitamin/mineral premix[6]	2
Vitamin C mix[7]	1
Total	100

1 Dakota Ethanol Plant (Wentworth, SD)

2 Dakotaland Feeds Inc., LLC (Huron, SD)

3 Hylon VII: National Starch Food Innovation (Bridgewater, NJ); Waxy No. 1: Tate and Lyle (Decatur, IL)

4 Consumers Supply Distribution Co. (North Sioux City, SD)

5 Animal feed dried whey, Midor Ltd (Elroy, WI)

6 Lortscher Agri Service, Inc. (Bern, KS)

7 DSM Nutritional Products France SAS (Village-Neuf, France)

2.2 Extrusion Processing

All blends were processed using a single screw extruder (Brabender Plasti-Corder, Model PL 2000, South Hackensack, NJ) which had a compression ratio of 3:1, with a screw length-to-diameter ratio of 20:1, and a barrel length of 317.5 mm. The center of the die assembly was conical, and tapered from an initial diameter of 6.0 mm to the die diameters of 1.8, 2.6, and 2.8 mm (length-to-diameter ratios of 9.2, 6.6 and 3.4 mm, respectively) at the exiting of the extruder. The screw speed and the barrel temperature were monitored by a computer that was attached to the extruder. The temperature of the feed, transition and die zone were adjusted with external band heaters to three temperature profiles (feed-transition-die sections) in the barrel (90-90-90 ℃, 100-120-120 ℃, and 100-120-140 ℃, respectively). A 7.5 HP (5.5 kW) motor was connected to the extruder to control the screw speed from 0 to 210 rpm (22 rad/s).

The raw blends were funneled into the extruder manually in constant quantities to avoid jamming at the opening to the barrel. The mass flow rate (MFR) was determined by collecting extrudate samples at 30 s intervals during extrusion, and then weighing the collected amount on an electronic balance (PB 5001, Mettler Toledo, Switzerland).

2.3 Extrudate Properties

After processing, the extrudates were dried for 24 h at room temperature (20 ±1 ℃) and triplicates (n=3) were then subjected to an extensive physical property analysis of expansion ratio (-), unit density (kg/m^3), water absorption index (-), water solubility index (%), and pellet durability index (%).

Expansion ratio (ER)

Radial expansion ratio has been determined as the ratio of the diameter of the dry extrudate that was measured with a digital calliper (Digimatic calliper, Model No: CD-6’’C, Mitutoyo Corp., Tokyo, Japan), to the diameter of the die nozzle (1.8, 2.6, and 2.8 mm, respectively). The results were displayed as the mean of ten (n=10) measurements.

Unit density (UD)

Extrudate at approximately sizes of 25.4 mm were weighed on an analytical balance (Adventurer™, Item No: AR 1140, Ohaus Corp. Pine Brook, NJ), and then measured with a digital calliper (Digimatic calliper, 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:

$$\mathrm{UD} = \frac{M}{V} \tag{1}$$

Sinking velocity (SV)

Sinking velocity was determined following Himadri et al. (1993) by measuring the time an extrudate of approximately 25.4 mm length needed to reach the bottom of a 2 L measuring cylinder filled with distilled water. The total distance (0.415 m) required for the time yielded the sinking viscosity (m/s).

Water absorption and water solubility index (WAI and WSI)

The procedure described by Anderson et al. (1969) was used to measure water absorption index (WAI) and water solubility index. These parameters were used to quantify binding capacity of the extrudates. Approximately 2.5 g of finely ground extrudate sample (150 µm) was suspended in 30 mL of distilled water in a tarred centrifuge tube of 50 mL and placed in a laboratory oven (Thelco precision, Jovan Inc., Wincester, VA) at 30 ℃. At intervals, it was then stirred intermittently for a period of 30 min. Subsequently, the centrifuge tube was centrifuged and the supernatant was decanted into an aluminum dish, which was placed in the oven for 2 h at 135 ℃ (AACC method 44-19, 2000) and then desiccated for 20 min. The dry solids and the gel mass were weighed. WAI (-) was determined as the ratio of gel mass to the original sample mass (2.5 g ground sample). WSI (%) was calculated as the ratio of the mass of the dry solids (recovered from evaporation of the supernatant from the WAI test) to the original sample mass. WAI is the gel weight received per gram of dry sample, whereas WSI quantifies the starch portion that remains in the water phase when exposed to water. The better the binding capacity, the lower the leaching losses to water.

Pellet durability (PDI)

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

$$\mathrm{PDI} = \left(\frac{M_a}{M_b}\right) \times 100 \tag{2}$$

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

2.4 Experimental Design

Six blends were prepared with 20% DDGS, one starch sources (Waxy I or Hylon VII), three levels of moisture content (15, 25, and 35%), with a constant protein content (32.5%). During processing, three temperature profiles were adjusted in the barrel (90-90-90 ℃, 100-120-120 ℃, and 100-120-140 ℃, respectively), and were referred to as temperatures of 100, 120, and 140 ℃, respectively, at three screw speeds (100, 130, and 160 rpm, respectively), and three levels of die geometry with various nozzle length-to-diameter (L/D) ratios (9.2, 6.6 and 3.4 mm, respectively).

This resulted in 3x3x3x2=162 total treatment combinations (Table 2). Triplicates (n=3) were measured for most physical properties (i.e., dependent variables) for each treatment combination, except for pellet durability index, which was only measured in duplicate.

The data were then analyzed with Proc GLM to determine the main, interaction and treatment combination effects using SAS software (SAS Institute, Cary, NC) with a Type I error rate (α) of 0.05.

Table 2. Experimental design.*

Treat-ment	Starch type	Moisture content	Tdie	Screw speed	Die L/D	Treat-ment	Starch type	Moisture content	Tdie	Screw speed	Die L/D	Treat-ment	Starch type	Moisture content	Tdie	Screw speed	Die L/D
		(% db)	(℃)	(rpm)	(-)			(% db)	(℃)	(rpm)	(-)			(% db)	(℃)	(rpm)	(-)
1	Hylon VII	15	100	100	3.4	55		35	100	100	3.4	109		25	100	100	3.4
2					6.6	56					6.6	110					6.6
3					9.2	57					9.2	111					9.2
4				130	3.4	58				130	3.4	112				130	3.4
5					6.6	59					6.6	113					6.6
6					9.2	60					9.2	114					9.2
7				160	3.4	61				160	3.4	115				160	3.4
8					6.6	62					6.6	116					6.6
9					9.2	63					9.2	117					9.2
10			120	100	3.4	64			120	100	3.4	118			120	100	3.4
11					6.6	65					6.6	119					6.6
12					9.2	66					9.2	120					9.2
13				130	3.4	67				130	3.4	121				130	3.4
14					6.6	68					6.6	122					6.6
15					9.2	69					9.2	123					9.2
16				160	3.4	70				160	3.4	124				160	3.4
17					6.6	71					6.6	125					6.6
18					9.2	72					9.2	126					9.2
19			140	100	3.4	73			140	100	3.4	127			140	100	3.4
20					6.6	74					6.6	128					6.6
21					9.2	75					9.2	129					9.2
22				130	3.4	76				130	3.4	130				130	3.4
23					6.6	77					6.6	131					6.6
24					9.2	78					9.2	132					9.2
25				160	3.4	79				160	3.4	133				160	3.4
26					6.6	80					6.6	134					6.6
27					9.2	81					9.2	135					9.2
28		25	100	100	3.4	82	Waxy I	15	100	100	3.4	136		35	100	100	3.4
29					6.6	83					6.6	137					6.6
30					9.2	84					9.2	138					9.2
31				130	3.4	85				130	3.4	139				130	3.4
32					6.6	86					6.6	140					6.6
33					9.2	87					9.2	141					9.2
34				160	3.4	88				160	3.4	142				160	3.4
35					6.6	89					6.6	143					6.6
36					9.2	90					9.2	144					9.2
37			120	100	3.4	91			120	100	3.4	145			120	100	3.4
38					6.6	92					6.6	146					6.6
39					9.2	93					9.2	147					9.2
40				130	3.4	94				130	3.4	148				130	3.4
41					6.6	95					6.6	149					6.6
42					9.2	96					9.2	150					9.2
43				160	3.4	97				160	3.4	151				160	3.4
44					6.6	98					6.6	152					6.6
45					9.2	99					9.2	153					9.2
46			140	100	3.4	100			140	100	3.4	154			140	100	3.4
47					6.6	101					6.6	155					6.6
48					9.2	102					9.2	156					9.2
49				130	3.4	103				130	3.4	157				130	3.4
50					6.6	104					6.6	158					6.6
51					9.2	105					9.2	159					9.2
52				160	3.4	106				160	3.4	160				160	3.4
53					6.6	107					6.6	161					6.6
54					9.2	108					9.2	162					9.2

* The experimental design consisted of 2 (starch sources) x 3 (moisture contents) x 3 (die temperatures) x 3 (screw speeds) x 3 (die L/D) = 162 total treatment combinations. Tdie is temperature of the die, die L/D is length-to-diameter ratio of the die.

3. Results and Discussion

Tables 3, 4, and 5 summarize the main treatment effects and interaction effects of the two different amylose/amylopectin ratios, different moisture contents, temperature profiles, screw speeds, and L/D ratios of the die. The different starch sources had significant effects on all extrudate properties at α=0.05. Likewise, the moisture contents (15, 25, and 25%) significantly affected all tested parameters, except for WSI, as well as increasing the screw speed from 100 to 130 and 160 rpm resulted in significant differences, except for UD and PDI. Table 3 provides main effects resulting from considering both starches simultaneously; Table 4 also provides the main effects, but type of starch is used as a blocking factor. It can readily be seen that the behaviours of the dependent variables, when considering each starch separately, were similar to the behaviours when the starches were considered together (i.e., Figures 1 and 2).

Table 3. Main effects due to starch source, moisture content, die temperature, screw speed, and die L/D on resulting extrudate physical properties (n=3, α=0.05).*

Parameter	Levels	ER (-)	UD (kg/m^3)	SV (m/s)	WAI (-)	WSI (-)	PDI (%)
Starch source	Hylon VII	1.17a	0.97a	0.076a	3.04a	18.17a	92.85a
		(0.06)	(0.08)	(0.012)	(0.29)	(2.56)	(3.53)
	Waxy I	1.20b	0.99b	0.068b	2.83b	18.27a	95.98b
		(0.09)	(0.10)	(0.024)	(0.30)	(2.27)	(1.64)
Moisture content (% db)	15	1.22a	1.02a	0.081a	2.86a	18.42a	93.98a
		(0.06)	(0.05)	(0.009)	(0.29)	(2.09)	(4.59)
	25	1.20b	0.94b	0.065c	2.93b	17.67b	93.17b
		(0.08)	(0.11)	(0.026)	(0.30)	(3.07)	(2.22)
	35	1.14c	0.97c	0.069b	3.02c	18.55a	95.54c
		(0.07)	(0.10)	(0.018)	(0.31)	(2.23)	(2.05)
Temperature of die (℃)	100	1.17a	1.04a	0.082a	2.88a	18.12a	95.13a
		(0.07)	(0.08)	(0.009)	(0.26)	(2.33)	(2.64)
	120	1.19b	0.98b	0.072b	2.89a	18.61b	94.65b
		(0.08)	(0.07)	(0.015)	(0.30)	(2.57)	(1.81)
	140	1.19b	0.92c	0.061c	3.04b	17.93a	92.98c
		(0.08)	(0.09)	(0.026)	(0.34)	(2.60)	(4.53)
Screw speed (rpm)	100	1.17a	0.99b	0.073a	2.94a	18.61a	93.78a
		(0.07)	(0.10)	(0.016)	(0.32)	(2.83)	(4.45)
	130	1.19b	0.98ab	0.074b	2.89b	18.35b	94.58b
		(0.07)	(0.09)	(0.018)	(0.30)	(2.55)	(2.53)
	160	1.20c	0.97a	0.067c	2.98c	17.67c	94.67b
		(0.09)	(0.09)	(0.025)	(0.30)	(2.00)	(1.93)
Die L/D (-)	3.4	1.16a	0.97a	0.073a	2.85a	19.55a	94.36a
		(0.06)	(0.10)	(0.022)	(0.28)	(2.72)	(3.52)
	6.6	1.16a	0.97a	0.077b	2.98b	17.86b	94.23a
		(0.06)	(0.08)	(0.020)	(0.30)	(2.47)	(2.03)
	9.2	1.24b	1.00b	0.065c	2.96b	17.52c	-
		(0.08)	(0.10)	(0.015)	(0.33)	(1.93)	-

* Means followed by similar letters within each dependent variable are not significantly different (P>0.05, LSD). Values in parentheses are standard deviation. ER is expansion ratio, UD is unit density, WAI is water absorption index, WSI is water solubility index, PDI is pellet durability index, die L/D is length-to-diameter ratio of the die.

Table 4. Main effects on extrudate physical properties using starch source as a blocking variable (n=3, α=0.05).*

		Hylon VII	Waxy I	Hylon VII	Waxy I	Hylon VII	Waxy I	Hylon VII	Waxy I	Hylon VII	Waxy I	Hylon VII	Waxy I
Parameter	Levels	ER (-)		UD (kg/m^3)		SV (m/s)		WAI (-)		WSI (-)		PDI (%)	
Moisture content (% db)	15	1.21ax	1.24ay	1.02ax	1.03ax	0.081ax	0.080ax	2.99ax	2.73ay	18.59ax	18.25ax	91.81ax	95.95ay
		(0.05)	(0.06)	(0.05)	(0.06)	(0.01)	(0.01)	(0.26)	(0.27)	(1.93)	(2.23)	(5.88)	(1.13)
	25	1.17bx	1.22by	0.94cx	0.94cx	0.075bx	0.055by	3.05bx	2.79by	18.03bx	17.28bx	91.92ax	94.80by
		(0.04)	(0.09)	(0.08)	(0.13)	(0.01)	(0.03)	(0.27)	(0.29)	(3.12)	(2.99)	(1.40)	(2.04)
	35	1.12cx	1.15cy	0.96bx	0.99bx	0.071cx	0.067cx	3.07bx	2.97cy	17.88bx	19.25cy	94.36bx	96.91cy
		(0.04)	(0.08)	(0.10)	(0.10)	(0.01)	(0.02)	(0.33)	(0.28)	(2.49)	(1.68)	(2.00)	(0.98)
Temperature die (°C)	100	1.18ax	1.17ax	1.01ax	1.07ay	0.083ax	0.081ax	2.97ax	2.78ay	18.26ax	17.96acx	93.75ax	96.90ay
		(0.06)	(0.08)	(0.08)	(0.07)	(0.01)	(0.01)	(0.22)	(0.26)	(2.23)	(2.44)	(2.56)	(1.42)
	120	1.17bx	1.22by	0.98bx	0.98bx	0.076bx	0.070by	3.03bx	2.75ay	18.46ax	18.77bx	93.79ax	95.81by
		(0.05)	(0.09)	(0.08)	(0.07)	(0.01)	(0.02)	(0.30)	(0.24)	(2.55)	(2.59)	(1.54)	(1.49)
	140	1.16cx	1.22by	0.93cx	0.91cx	0.070cx	0.052cy	3.11cx	2.96by	17.79bx	18.08acx	89.89bx	95.29cy
		(0.05)	(0.08)	(0.07)	(0.10)	(0.02)	(0.03)	(0.32)	(0.34)	(2.84)	(2.33)	(5.32)	(1.63)
Screw speed (rpm)	100	1.16ax	1.19ay	0.97abcx	1.00ax	0.075ax	0.071ax	3.06ax	2.82ay	18.36ax	18.86ax	91.70ax	96.43ay
		(0.05)	(0.08)	(0.09)	(0.10)	(0.01)	(0.02)	(0.30)	(0.30)	(3.01)	(2.63)	(4.94)	(1.31)
	130	1.17bx	1.21by	0.97abx	0.99ax	0.077bx	0.072ax	2.94bx	2.84ax	18.35ax	18.34abx	93.21bx	96.06by
		(0.06)	(0.08)	(0.08)	(0.10)	(0.01)	(0.02)	(0.28)	(0.32)	(2.79)	(2.24)	(2.39)	(1.74)
	160	1.17bx	1.22cy	0.98acx	0.96bx	0.075ax	0.060by	3.13cx	2.84ay	17.75bx	17.60cx	93.89cx	95.45cy
		(0.06)	(0.10)	(0.08)	(0.11)	(0.02)	(0.03)	(0.25)	(0.28)	(1.53)	(2.28)	(1.81)	(1.74)
Die L/D (-)	3.4	1.14ax	1.18ay	0.96ax	0.97ax	0.077ax	0.070ax	2.81ax	2.89ax	19.46ax	19.67ax	92.71ax	96.01ay
		(0.05)	(0.07)	(0.09)	(0.11)	(0.02)	(0.03)	(0.26)	(0.30)	(2.89)	(2.54)	(4.04)	(1.77)
	6.6	1.15bx	1.17ax	0.95ax	0.98ay	0.083bx	0.072ay	3.14bx	2.82by	17.59bx	18.13bx	93.19bx	95.81ay
		(0.05)	(0.06)	(0.07)	(0.10)	(0.01)	(0.03)	(0.27)	(1.60)	(2.50)	(2.42)	(1.79)	(1.05)
	9.2	1.21cx	1.27by	1.01bx	1.00bx	0.068cx	0.062by	3.13bx	2.80by	17.66bx	17.39cx	.	.
		(0.06)	(0.09)	(0.09)	(0.10)	(0.01)		(0.21)	(0.34)	(1.86)	(2.01)	.	.

* Means within a column followed by similar letters (a, b, c) for a given dependent variable are not significantly different (P>0.05) for that independent variable. Means within a row followed by similar letters (x or y) for a given dependent variable are not significantly different (P>0.05) due to starch source. Values in parentheses are standard deviation. ER is expansion ratio, UD is unit density, WAI is water absorption index, WSI is water solubility index, PDI is pellet durability index, L/D is length-to-diameter ratio of the die.

Table 5. Interaction effects due to starch source, moisture content, die temperature, screw speed and die L/D on extrudate physical properties (p values).*

Independent variables and interactions	ER (-)	UD (kg/m^3)	SV (m/s)	WAI (-)	WSI (%)	PDI (%)
Starch	<.0001	0.0003	<.0001	<.0001	0.7628	<.0001
MC	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Tdie	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Speed	<.0001	0.1904	<.0001	0.0037	<.0001	<.0001
L/D die	<.0001	<.0001	<.0001	<.0001	<.0001	0.1173
Starch*MC	0.0011	0.0709	<.0001	<.0001	<.0001	<.0001
Starch*Tdie	<.0001	<.0001	<.0001	0.0041	<.0001	<.0001
Starch*Speed	0.1294	<.0001	<.0001	<.0001	<.0001	<.0001
Starch*L/D die	<.0001	0.0051	<.0001	<.0001	0.0015	0.0002
MC*Tdie	<.0001	<.0001	<.0001	0.0025	<.0001	<.0001
MC*Speed	<.0001	0.0015	<.0001	0.0041	0.0003	<.0001
MC*L/D die	0.0061	<.0001	<.0001	<.0001	0.0002	<.0001
Tdie*Speed	0.9351	0.5716	<.0001	0.0001	<.0001	<.0001
Tdie*L/D die	<.0001	<.0001	0.3124	<.0001	<.0001	0.4163
Speed*L/D die	0.5090	0.3242	<.0001	0.0004	<.0001	0.0007
Starch*MC*Tdie	<.0001	<.0001	<.0001	<.0001	0.0194	<.0001
Starch*MC*Speed	0.0006	0.2487	<.0001	<.0001	<.0001	<.0001
Starch*MC*L/D die	<.0001	0.0011	<.0001	0.0012	<.0001	.
Starch*Tdie*Speed	0.3710	0.0013	<.0001	0.0661	<.0001	<.0001
Starch*Tdie*L/D die	0.0008	0.0346	<.0001	0.5730	<.0001	.
Starch*Speed*L/D die	0.1755	<.0001	<.0001	0.0158	<.0001	.
MC*Tdie*Speed	0.1539	0.0302	<.0001	<.0001	<.0001	<.0001
MC*Tdie*L/D die	0.0553	<.0001	<.0001	<.0001	<.0001	<.0001
MC*Speed*L/D die	0.0003	0.2030	<.0001	0.0263	<.0001	0.8807
Tdie*Speed*L/D die	0.2703	0.0472	<.0001	0.0009	<.0001	0.0013
Starch*MC*Tdie*Speed	0.1026	0.0401	<.0001	0.0038	<.0001	<.0001
Starch*MC*Tdie*L/D die	0.0156	<.0001	<.0001	<.0001	<.0001	.
Starch*MC*Speed*L/D die	0.0446	0.0003	<.0001	<.0001	<.0001	.
Starch*Tdie*Speed*L/D die	0.0413	0.0205	<.0001	0.0012	<.0001	.
MC*Tdie*Speed*L/D die	0.1373	0.0002	<.0001	<.0001	<.0001	0.4805
Starch*MC*Tdie*Speed*L/D die	0.0036	<.0001	<.0001	<.0001	<.0001	.

* MC is moisture content, Tdie is temperature of the die, ER is expansion ratio, UD is unit density, WAI is water absorption index, WSI is water solubility index, PDI is pellet durability index, L/D is length-to-diameter ratio of the die.

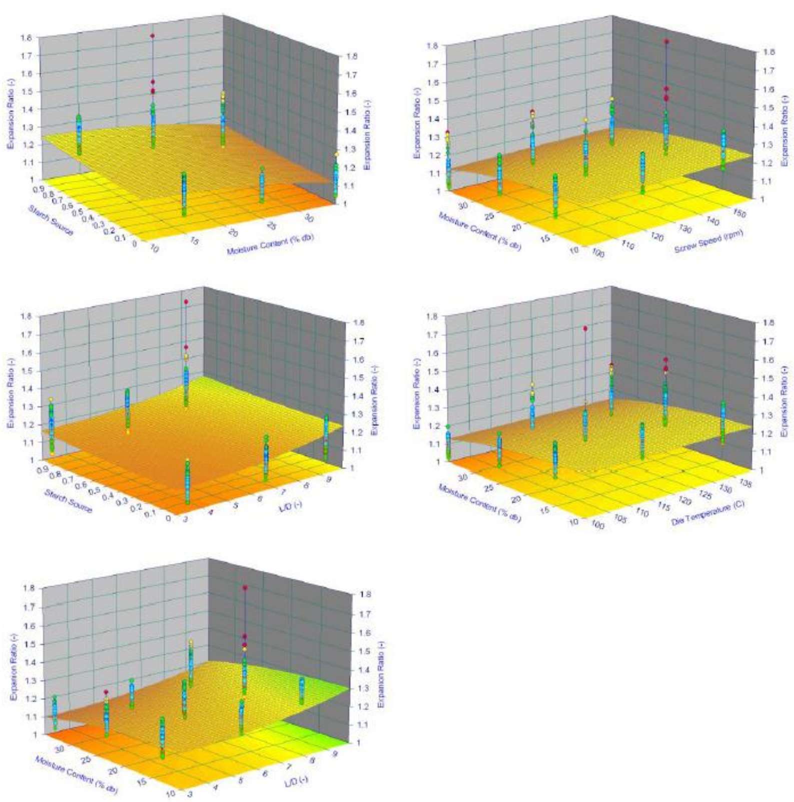

Figure 1. Treatment combination effects on expansion ratio. (Starch source 0 = 0% amylopectin, 1 = 100% amylopectin).

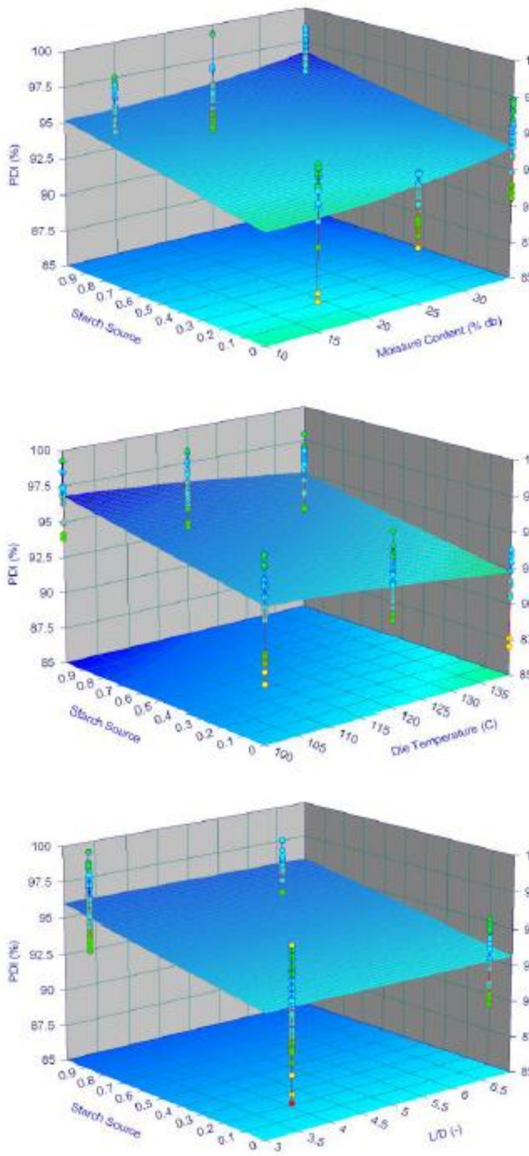

Figure 2. Treatment combination effects on pellet durability index (PDI). (Starch source 0 = 0% amylopectin, 1 = 100% amylopectin).

3.1 Extrudate Properties

Expansion ratio (ER)

Expansion ratio is a decisive factor for aqua feeds. The degree of expansion upon exiting the die impacts the unit density, and thus the floatability of the feed. Fragility and hardness of extrudates are also affected by ER (Kannadhason et al., 2009a, Rosentrater et al., 2009a).

The proportion of amylopectin in starch and the feed moisture content mainly affect expansion. Studies on the effects of different starch sources on DDGS-based extrudates showed increasing expansion for blends with higher amylopectin proportions of the starch (Kannadhason et al., 2009a). Amylopectin is considered to have swelling properties whereas amylose acts as a diluent (Tester and Morrison, 1990). Regarding the main treatment effects (Table 3), the impact of higher amylopectin portion was detected by a 2.5% increase of ER when using the blend with the 100% amylopectin proportion compared to the one with 30%. Investigating different starch sources, the highest expansion ratio was observed at 1.65 when using cassava starch (83% amylopectin) in combination with 20% DDGS, whereas the lowest ER at 1.09 had been recorded for the 72% amylopectin corn starch in combination with 40% DDGS (Kannadhason et al., 2009a).

Feed moisture is a key factor in extrusion processing which highly impacts expansion and density. Due to changes in the molecular structure of amylopectin, moisture reduces the melt elasticity and expansion decreases whereas density increases (Ding et al., 2005). This could be observed in the main treatment effects (Table 3) where an increase in moisture content from 15 to 35% yielded a 6.6% decrease in ER from 1.22 to 1.14. The blends with the Hylon VII as the starch source reached significantly higher ER than the Waxy I (100% amylopectin) blends at the same MC level (Table 4).

During gelatinization, the crystalline structure of the starch granules is destroyed and the amylopectin turns into an amorphous state. The starch granules form a gel that is more expanded than their initial state. At the same time the amylose (if present) leaches out of the amylopectin and forms a continuous gel phase outside the granules (Hermansson and Svegmark, 1996; Yu and Christie, 2005). Shear forces and temperature affect the gel formation. However, different starch sources show different optimum temperatures for expansion (Chinnaswamy and Hanna, 1988). For the main treatment effects, a small but significant increase in ER from 1.17 to 1.20 was observed when increasing the screw speed from 100 to 160 rpm (Table 3). Increasing the temperature from 100 to 120 ℃ yielded also a small but significant increase in ER from 1.17 to 1.19. The blends with Hylon VII resulted in significantly lower ER with increasing temperature whereas the Waxy I (100% amylopectin) blends increased in ER with increasing temperature only from 100 to 120 ℃. The Waxy I blends reached significantly higher ER than the Hylon VII blends. For both blends ER increased significantly with increasing screw speed. Raising the screw speed from 130 to 160 resulted in no significant difference in ER for the Hylon VII blends (Table 4). The highest ER for Hylon VII and Waxy I were found at 1.31 and 1.43 for the treatment combination at 15% MC, 120 ℃, 160 rpm and 9.2 L/D ratio of the die and 25% MC, 140 ℃, 160 rpm, and 6.6 L/D ratio of the die, respectively.

Radial expansion was calculated as the ratio between the diameter of the extrudate and the die, after extrusion and drying. Longitudinal and volumetric expansion were neglected. For all blends, ER did not change when the die L/D ratio increased from 3.4 to 6.6, whereas expansion ratio significantly increased by 6.9% with an increase in die L/D ratio (decrease in die diameter) from 6.6 to 9.2 (Table 3). Again, ER for the Waxy 1 blends was significantly higher than the Hylon VII blends (Table 4). Several interactions existed between independent variables (Table 4). Regarding the treatment combination effects, ER decreased with higher moisture content in combination with lower amylopectin content, lower screw speed, lower temperature setting, and lower L/D ratio, respectively. Figure 1 shows these behaviours, and indicates non-linear relationships between these variables. The highest ER was found at 1.43 with the 100% amylopectin starch, containing 35% feed moisture content, at highest temperature setting (140 ℃), highest screw speed and highest L/D ratio of the die. The lowest ER was detected at 1.05 for the same blend but with 25% feed moisture content, lowest temperature setting (100 ℃), lowest screw speed (100 rpm) and lowest L/D ratio of the die (3.4). Also, ER significantly increased with higher screw speed. This is in line with the assumption that increased shear leads to higher expansion due to increased gelatinization unless a certain shear degree is reached. Both, die nozzle diameter and length determine an increase or decrease in back pressure, residence time and shear as the dough passes the die section. This produces a sudden drop from high pressure to atmospheric pressure impacting the strength of water flashing-off at the die and applying enormous tensile forces while the melt expands. Forces and temperature drop lead to changes in the material states. For fully expansion an optimum amount of pressure is required which is achieved by an optimum die L/D ratio (Ganjyal and Hanna, 2007).

Unit density (UD)

Unit density determines the density for a single extrudate. Generally, it is inversely related to the expansion ratio. Unit density can predict the floatability of extrudates which is mandatory for top feeding fish, as Nile tilapia, to ensure food supply and reduce feed loss and water pollution.

All main treatment effects significantly affected the unit density, except for screw speed and die L/D ratio where at least two treatments did not show significant differences. Changes due to the starch source were small but significant. The Hylon VII blends had mostly lower UD values than the Waxy I (100% amylopectin) blends. Initially, UD declined from 1.02-0.94 kg/m^3 when increasing MC from 15-25%, but then increased significantly when enhancing the MC from 25-35% (Table 3 and Table 4). These differences do not conform with the changes in ER what may be related to the higher standard deviation of UD. Increasing the temperature from 100-140 ℃ yielded a significant reduction in UD by 11.5% (Table 3). This agrees with Ding et al. (2005) findings that feed moisture and temperature significantly affect extrudate density.

The highest UD was recorded at 1.18 kg/m^3 for the treatment combination with Waxy I, at highest MC (35%), lowest temperature setting (100 ℃), highest die L/D ratio of the die (9.2) and medium screw speed (130 rpm). The lowest UD was also observed for the high amylopectin blends at 0.76 kg/m^3 at medium MC (25%), highest temperature (140 ℃), highest screw speed (160 rpm) and highest L/D ratio of the die (9.2) setting. For the same treatment combination as the lowest UD was determined, the highest expansion ratio was observed. Blends with high amylopectin content starch sources in combination with appropriate moisture content, temperature, and screw speed, resulted in increased gel formation and thus higher expansion. As anticipated, the significance of higher temperature resulting in lower unit density was evident in this study. These findings are in complete agreement with the results of Kannadhason et al. (2009b) and Rosentrater et al. (2009a). An inverse relationship between UD and ER was not clearly detected which could be ascribed to the relatively high standard deviation of the UD data.

Sinking velocity (SV)

The sinking velocity relates to the stability and floatability of extrudates. It can conclude about the porosity of an extrudate and how fast it absorbs water and sinks. Therefore, sinking velocity is related to the unit density, expansion ratio and the biochemical and biomechanical changes that occur inside the extruder barrel. All main treatment effects showed significant differences in SV (Table 3). Regarding the main effects, the blend containing Waxy I as a starch source had 10.5% significantly lower SV than the Hylon VII blends. No clear pattern could be observed on changes in SV due to moisture content, screw speed, and die L/D ratio. Increasing the processing temperature from 100 to 140 ℃ yielded a significant decrease in SV by 25.6%, which is in line with the findings of Kannadhason et al. (2009b) and Rosentrater et al. (2009a, 2009b). Changes in SV are only related to changes in UD for moisture content and temperature. Increasing the screw speed from 130 to 160 rpm resulted in a 9.5% significant increase in SV, whereas the increase in screw speed from 100 to 130 rpm yielded a decrease of 1.4%. The slowest SV value was observed at 0.002 m/s for the Waxy I diet containing at 25% moisture content, highest temperature (140 ℃), highest screw speed setting (160 rpm) and highest L/D ratio of the die (9.2). This setting complies with the treatment combination, which had the highest expansion and evidently leading to higher floatability. The same observation was made by Kannadhason et al. (2009a) and Rosentrater et al. (2009a). Unexpectedly, the fastest SV at 0.102 m/s could also be observed at the settings as for the fastest SV but at medium screw speed (130 rpm), and lowest die L/D ratio (3.4). Interaction effects among all independent variables were observed, except for temperature and L/D. This showed the big impact of screw speed on SV.

Water absorption and water solubility index (WAI and WSI)

Materials based on starch absorb water due to the abundance of hydroxyl group forming hydrogen bonds with water. Gelatinization involves the destruction of the crystalline-like structure of starch (Pizzoferrato et al., 1999) and expands the water absorption and water solubility properties (Colonna and Mercier, 1983).

The water absorption index reflects the weight of gel that is retained per unit weight of dry sample, whereas the water solubility index describes the percent of dry sample of the supernatant (Anderson et al., 1969). Both properties depend on the amylose-to-amylopectin ratio, moisture content, screw speed, and die geometry (Colonna and Mercier, 1983), which was observed in this study. However, the main treatment effects showed a significant increase of 6.9% in WAI when raising the amylopectin ratio from 30 to 100%, whereas the WSI yielded no significant difference with changes in starch source. Similar results were reported by Mani and Bhattacharya for WAI (1998). The increase in WAI with higher amylopectin ratio can be ascribed to the escalating gelatinization and debranching of the amylopectin structure yielding an expanded matrix capable of holding more water. Other than the starch source, feed moisture, and temperature had significant effects on WAI, which increased with higher moisture content and temperature. This can be explained by the gel-forming capacity of macromolecules and the water-binding capacity of hydrophilic groups for gelatinized corn starch (Gomez and Aguilera, 1983). Regarding the main treatment effects due to the starch source, Hylon VII blends reached significantly higher values than Waxy I (100% amylopectin) blends (Table 4).

The WAI of the blends decreased with lower temperatures indicating a more dense structure of the extrudate with a lower water holding capacity. The lower WAI values with reducing moisture content can be ascribed to the starch dextrinization that occurs at low-moisture contents below 20% during high-shear extrusion cooking, whereas gelatinization predominates at moisture contents higher than 20% (Gomez and Aguilera, 1984). The highest WAI was detected at 3.6 for the treatment combination of Waxy I blends, at 35% moisture content, 140 ℃, 130 rpm and L/D ratio of the die of 3.4. The lowest WAI was observed at 2.3 for the treatment combination of the Hylon VII blends at 35% moisture content, 120 ℃, 130 rpm and L/D ratio of the die of 3.4.

WSI is a measure of starch dextrinization (Bhatnagar and Hanna, 1994) and depends on available solubles that increase with starch degradation (Jin et al., 1995). In contrast to WAI, the starch source showed no significant effect on WSI and with higher screw speeds from 100 to 160 rpm WSI significantly decreased by 5.1% (Table 3). These unexpected results are also contradictory to other findings where WSI increased with higher screw speed starting at 150 and 180 rpm, respectively (Gomez and Aguilera, 1983; Jin et al., 1995; Iwe, 1998) and can be ascribed to relatively high standard deviations in this study. They same may apply for changes in L/D of the die from 3.4 to 9.2 where a significant decrease of WSI by 10.4% occurred. The findings for highest and lowest WSI confirm the assumption that relatively high standard deviations may have impacted the results. The treatment combination with the 100% amylopectin starch at 25% moisture content, 120 °C, 130 rpm and L/D of 3.4 showed the highest WSI at 24.2%, whereas the lowest WSI was found at 10.75% for 30% amylose starch at 35% moisture content, 120 °C, 130 rpm and L/D ratio of 3.4.

Pellet durability index (PDI)

Resistance against destructive and abrasive forces during transportation, handling, and storage is a highly desired property of extrudates in order to maintain their value and quality (Rosentrater et al., 2005). Fines, such as dust, will not be consumed by fish and have to be minimized to avoid economic losses and minimize water pollution (Sørensen et al., 2010).

Durability is tested by simulating the mechanical handling of extrudates during tumbling test and is defined by possible fines produced. It is not only dependent on the gelatinization of starch but also on the heat treatment and moisture content of the blends. This is reflected in the main treatment effects of each independent factor on pellet durability index (Table 3). A significant increase in PDI from 92.85 to 95.98% was observed between Hylon VII and Waxy I (100% amylopectin) blends. Decreasing the temperature setting from 140 to 100 °C, resulted in an increase of PDI from 92.98 to 95.13% for all blends. There were no significant differences detected when changing the L/D of the die from 3.4 to 6.4. The treatment combination effects illustrated that PDI steadily increased with higher moisture content and higher amylopectin portion. As shown in Figure 2, the graphs show an increase in PDI with lower temperature and using Waxy I starch (100% amylopectin). Figure 2 shows these behaviours, and indicates non-linear relationships between these variables. Changes in PDI in combination with starch source and L/D ratio, demonstrated an increase only when the amylopectin content was raised, but not with changes in L/D ratio only. This was also reflected in the main treatment effects. The highest PDI was found at 99.3% when Waxy I blends were used containing 25% moisture content, using a L/D ratio of 3.4 at 100 °C and 130 rpm screw speed. The lowest PDI was detected with the Hylon VII starch (30% amylopectin), with 15% MC, at 140 °C, 100 rpm, and L/D ratio of 3.4. Overall, all extrudates yielded good pellet durability indices that were significantly affected by the starch source, moisture content, and temperature settings. These observations are in accordance with findings done by Kannadhason et al. (2009a, 2009b) and confirm better stabilizing properties with higher amylopectin portions of starch. Heat treatment and available water also highly affected the gelatinization of starch and thus the cohesion and stability of the extrudates.

4. Conclusions

The incorporation of either of two starch sources with varying amylopectin portions in combination with distillers dried grains with solubles, soy, and other ingredients was investigated to determine their effects on resulting binding capacity. Feed ingredients (moisture content and amylopectin-amylose ratio) and processing conditions (die temperature, L/D, and screw speed) were modified to examine their effects on the properties of the resulting extrudates. Altering the amylopectin portions and the starch sources, respectively, had significant effects on all measured parameters. Moisture content, temperature setting, and screw speed, significantly affected most of the extrudate properties, whereas L/D ratio of the die showed only some effects on the resulting extrudates. Unexpectedly, expansion ratio was low. As anticipated, ER increased with higher amylopectin portion and higher screw speed, whereas it declined with higher moisture content. Highest PDI values were achieved by using the starch source with the highest amylopectin portion, highest moisture content, and lowest temperature setting. The starch source with higher amylopectin in combination with adequate moisture content, temperature, and screw speed demonstrated to be the best choice for better quality extrudates in terms of durability and binding capacity.

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