

Effect of Starch Sources and Protein Content on Extruded Aquaculture Feed Containing DDGS

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Abstract A 3×3×3 completely randomized design was used to investigate extrusion cooking behavior and product characteristics of distillers dried grains with solubles (DDGS), protein levels, and various starch sources in a laboratory scale single screw extruder. Cassava, corn, and potato starches with varying levels of DDGS (20%, 30%, and 40% wet basis (wb)) were extruded with three different proportions of protein levels (28%, 30%, and 32% wb). The extrusion cooking was performed at a constant feed moisture content of 20% wb, barrel temperature of 120 °C, and a preset screw speed of 130 rpm (13.6 rad/s). Extrudate properties such as expansion ratio, unit density, sinking velocity, color, water absorption and solubility indices, and pellet durability index were determined to judge the suitability for various fish species. For all three starch bases, increasing the DDGS levels resulted in a significant increase in sinking velocity, redness (a^*), and blueness (b^*) and showed a decrease in whiteness (L^*). With the increase in DDGS and protein levels, a noticeable increase was observed for unit density and pellet durability indices for cassava and potato starch extrudates. The DDGS-based extrudates produced from cassava starch with lower proportions of DDGS (20%) and protein (28%) levels

exhibited better expansion and floatability. Also, the extrudates produced from corn starch with higher levels of DDGS (40%) and protein (32%) levels were more durable and possessed sinking characteristics. Overall, cassava and corn starch with lower and higher levels of DDGS could be more appropriate for the production of floating and sinking aquaculture feeds, respectively.

Keywords Aquaculture · DDGS · Protein · Physical properties · Single screw extruder · Starch

Introduction

Aquaculture is one of the fastest growing food production activities in the world and it plays a significant role in many countries by affording higher income, better nutrition, and better employment opportunities. Aqua feed manufacturers are experiencing a variety of technical developments such as enhanced pellet stability, usage of extrusion procedures for the improvement of digestibility, development of high energy feeds, and palatability additives. Advantages of extrusion cooking for aquaculture feed production include increased feed conversion, the control of pellet density, greater feed stability in water, better production efficiency, and versatility (Chang and Wang 1998). In general, fish possess better efficiency to convert feed into body weight when compared to chickens, pigs, beef cattle, or sheep (New 1986). In addition, fish are often fed higher percentages of protein in their diets in comparison with other land animals (Lovell 1991).

Starch plays a crucial role in determining the texture of many foods; texture is of great concern to both consumers and food manufacturers (Whistler and Paschall 1965). It consists of two chains of glucose molecules, namely

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amylose and amylopectin. Amylose is a linear molecule with a few branches, whereas amylopectin is a highly branched molecule (Tharanathan 1995). Extrusion processing of starch-based materials causes substantial changes in the physical constitution of the starch granule (Davidson et al. 1984). It also relates specific functional properties to the product. The ability of starch to swell and thereby produce a viscous paste when heated in water or treated with certain chemicals is its most important practical property (Knight 1969). Starch conversion during extrusion is also influenced by other components, especially proteins and fats (Lin et al. 1997). The expansion of starch mainly depends on its degree of gelatinization, and during the process of extrusion cooking up to 90% of the starch can be gelatinized, which promotes the molecular intertwining of the ingredients in the feed mixture (Chinnaswamy and Hanna 1988).

Extrusion cooking has been used for many years to produce several types of animal feeds and human foods, and its application in the production of aquaculture feeds has been more apparent (Chang and Wang 1998). It is a high temperature, short time (HTST) process with many present and possible future applications in the food industry, especially for cooking, forming, and expanding cereals, as well as for texturizing proteins. Processing and other related parameters (such as composition and moisture content) also have the ability to affect the degree of expansion, since they dictate the type and degree of physical and chemical modifications that take place during extrusion, which ultimately influence the extrudate expansion (Guy and Horne 1988; Arhaliass et al. 2008). Various reactions that take place during extrusion cooking include thermal treatment, gelatinization, protein denaturation, shearing, mixing, grinding, hydration, shaping, expanding, texture alteration, partial dehydration, and destruction of microorganisms and other toxic compounds. Not only does composition play a key role, but the residence time in the extruder is also important to final product quality (Nwabueze and Iwe 2008). More importantly, the gelatinization that takes place during extrusion cooking improves the durability of the feed rations and digestibility of the nutrients (Chang and Wang 1998).

Distillers dried grains with solubles (DDGS) is a valuable feed ingredient which is a coproduct of dry mill ethanol production from corn, and is increasingly available to domestic and international customers as an ingredient for livestock and poultry rations. It is high in protein and fiber, but low in starch content (Rosentrater and Muthukumarappan 2006). Due to its high proportions of protein, relatively lower phosphorous content, and cost in comparison with fish meal, there is a mounting global interest in incorporating DDGS in aquaculture rations (US Grains Council 2008). A few researchers have attempted to incorporate DDGS as a base protein material for the production of aquaculture feeds and

have investigated the effect of various feed and extruder parameters on resulting extrudate properties and processing parameters (Chevanan et al. 2007a, b, 2008a, b, c; Shukla et al. 2005; Kannadhasan et al. 2007). However, corn was the only starch source used by Chevanan et al. (2007a, b, 2008a, b, c) and Shukla et al. (2005). To date, the only study that compares the effect of different starch sources on the resulting physical properties of DDGS-based extrudates and extruder processing parameters was investigated by Kannadhasan et al. (2007). In their study, the varied parameters included DDGS levels (20% to 60% wet basis (wb)), feed moisture content (15% to 25% wb), and extruder barrel temperature (100 to 140 °C). Their outcomes proved that cassava and potato starch extrudates were more suitable for floating and sinking aquaculture feeds, respectively, whereas, corn starch extrudates were more durable. However, the authors did not consider varying the net protein content in the ingredient blends, which is also crucial in the formulation and production of aquaculture feeds. Therefore, this study, a continuation of work previously done by Kannadhasan et al. (2007) on extrusion of DDGS with other starch sources in a single screw extruder, was conducted with the following objectives:

1. To produce floating aquaculture feeds targeted at either tilapia or channel catfish (which generally require protein content in the range of 28% to 32%, depending upon age), using various starch sources (cassava, corn, and potato) by varying the proportions of DDGS and protein
2. To compare the effect of starch sources, DDGS levels, and protein content on the physical properties of the resulting extrudates

Materials and Methods

Raw Ingredients and Sample Preparation

DDGS, provided by Dakota Ethanol LLC (Wentworth, SD, USA), was ground to a fine powder of approximately 425 µm size using a laboratory scale grinder (s500 disc mill, Genmills Inc, Clifton, NJ, USA) before it was added to the respective ingredient blends. Cassava and potato starch were obtained from American Key Food Products, Closter, NJ, USA, whereas corn starch was procured from Cargill Dry Ingredients, Paris, IL, USA. Soy flour was purchased from Cargill Soy Protein Solution (Cedar Rapids, IA, USA), menhaden fish meal from Consumer Supply Distribution Company, (MN, USA), and whey from Bongards Creameries (Perham, MN, USA). Vitamin and mineral mix (Vitapak) was from Land O' Lakes Feeds (St. Paul, MN, USA). The crude protein content of DDGS, cassava starch, corn starch, potato starch, soy flour, fish meal, and whey used in our experimental study was 28.9%, 1.1%, 6%, 0.1%,

52.3%, 62.7%, and 9% wb, respectively. Vitamin and mineral mix had a negligible amount of crude protein.

Appropriate quantities of all these ingredients were incorporated into individual blends on a wet basis to the targeted protein levels of 28%, 30%, and 32% wb and a total energy content of approximately 3.50 kcal/g (Table 1). Individual blends were prepared by mixing all these ingredients in appropriate quantities to achieve the targeted protein contents in a laboratory scale Hobart mixer (model D300, Hobart Corporation, Troy, OH, USA), at the lowest speed (82 rpm inner rotation and 36 rpm outer rotation) for 15 min. Water was added consistently to the mixture in order to achieve a target moisture content of 20% wb and stored overnight at room temperature for moisture stabilization.

Experimental Design and Statistical Analyses

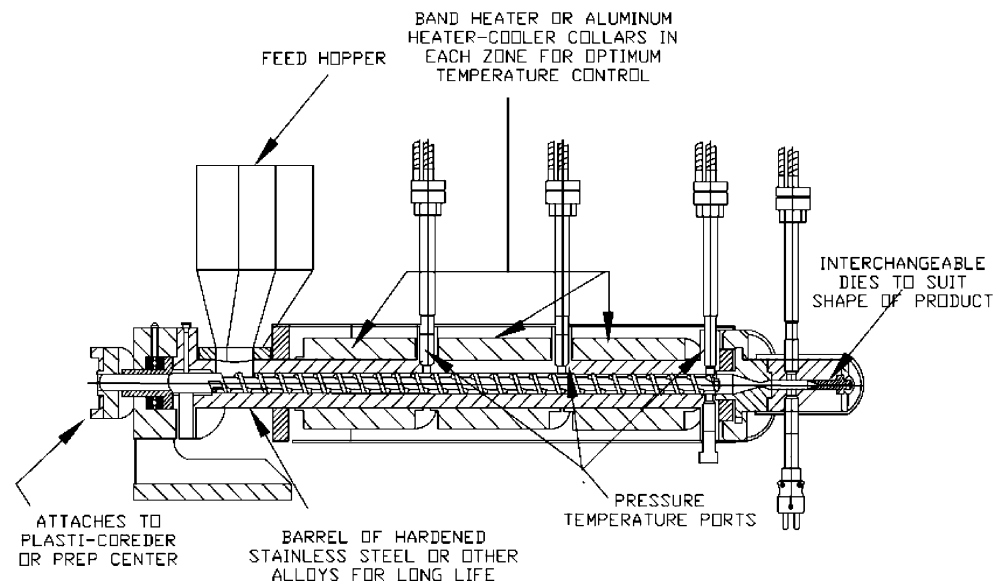
Extrusion cooking of the blends was performed using a laboratory scale Brabender single screw extruder (Brabender Plasti-corder extruder PL 2000, South Hackensack, NJ, USA), which is shown in Fig. 1. The extruder was powered by a 7.5-hp motor and had an operating range of screw speeds from 0 to 210 rpm (0 to 22 rad/s). The extruder screw used in

our study was single flight constructed from 4140 alloy, hardened, and chrome-plated. The screw had a compression ratio of 3:1, a uniform pitch of 9.05 mm that had a variable flute depth, with a depth of 9.05 mm at the feed portion and 3.81 mm at the die portion. The screw length to diameter ratio (L/D) and the length of the extruder barrel were 20:1 and 317.5 mm, respectively. The die assembly had an internal conical section and a length of 101.6 mm. The extruder barrel had five temperature zones, in which three active zones were used in our experimental study. The three active zones (feed zone, compression zone, and metering zone) required different processing temperatures depending on the characteristics of the final product. The extruder barrel was also equipped with thermocouples and pressure transducers, for monitoring the melt temperature profiles and pressure at the die, respectively, during the extrusion run. The extruder was operated at a preset screw speed of 130 rpm (13.6 rad/s) and the temperature profile maintained was 90–120–120 °C to obtain expanded product through a 3-mm diameter die. These processing conditions were based on previous work done by Kannadhasan et al. (2007). Raw material was introduced manually into the extruder, using a consistent methodology, through the feed hopper. The extruder was run

Table 1 Ingredient compositions (g/100 g) of the blends used in the study

Ingredients	Net protein content (% wb)								
	28			30			32		
Cassava starch									
DDGS	20.0	30.0	40.0	20.0	30.0	40.0	20.0	30.0	40.0
Soy flour	34.6	29.2	23.7	34.8	29.2	24.0	36.0	24.7	26.5
Cassava starch	31.4	26.8	22.2	31.1	26.6	22.0	22.2	22.9	15.8
Fish meal	6.00	6.00	6.00	9.00	9.20	9.00	9.60	14.4	9.00
Whey	5.00	5.00	5.00	2.00	2.00	2.00	9.15	5.00	5.68
Vitamin mix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Corn starch									
DDGS	20.0	30.0	40.0	20.0	30.0	40.0	20.0	30.0	40.0
Soy flour	34.6	29.2	32.7	35.5	30.2	25.5	33.0	30.0	23.2
Corn starch	34.7	29.7	24.6	31.2	28.7	22.1	25.0	20.2	12.9
Fish meal	6.00	6.00	6.00	6.00	6.00	6.00	13.8	12.2	4.85
Whey	5.00	5.00	5.00	4.25	2.00	5.40	5.20	5.00	4.85
Vitamin mix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Potato starch									
DDGS	20.0	30.0	40.0	20.0	30.0	40.0	20.0	30.0	40.0
Soy flour	35.2	29.7	24.2	35.4	29.7	24.4	34.0	26.2	25.5
Potato starch	30.8	26.5	21.8	30.6	26.1	21.6	23.9	23.6	15.2
Fish meal	6.00	6.00	6.00	9.02	9.10	9.00	13.4	12.1	11.5
Whey	5.00	5.00	5.00	4.25	2.00	5.40	5.20	5.00	4.85
Vitamin mix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Fig. 1 Cross section of the single screw extruder used in the study



to stabilization using approximately 5 kg of DDGS before the actual experimental runs commenced.

Extrusion experiments were conducted with three starch sources (cassava, corn, and potato), three levels of DDGS (20%, 30%, and 40% wb), and protein (28%, 30%, and 32% wb) as shown in Table 2. The moisture content of the ingredients was adjusted to 20% (wb) and the temperature profile maintained in the extruder was 90 °C in the feeding zone and 120 °C in both compression and metering zones. A completely randomized, three-level experimental design, with three replications was used; with starch source, DDGS, and protein levels being the independent variables (Table 2). This resulted in $3 \times 3 \times 3 = 27$ extrusion runs, where each treatment was extruded once. The resulting products were subjected to analyses of physical properties, which included expansion ratio (ER), unit density (UD), color (L^* , a^* , and b^*), sinking velocity (SV), water absorption (WAI), water solubility (WSI), and pellet durability (PDI) indices. Statistical analyses on the collected data were performed using SAS 9.1 (SAS 1990, User's Guide) using type I error rate at $\alpha=0.05$, which included main treatment, interaction, and treatment combination effects, Duncan's multiple range test for the comparison of means, and linear correlation analysis.

Analysis of Extrudate Physical Properties

The physical properties of the extrudates were measured as outlined in Kannadhasan et al. (2007).

Expansion Ratio

The diameter of the extrudates were measured with a digital caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan), averaged, and expressed as the ratio of diameter of the extrudate over the diameter of the die.

Table 2 Experimental design used in the study

Treatment	Starch type	DDGS level (% wb)	Protein level (% wb)
1	Cassava	20	28
2			30
3			32
4		30	28
5			30
6			32
7		40	28
8			30
9			32
10	Corn	20	28
11			30
12			32
13		30	28
14			30
15			32
16		40	28
17			30
18			32
19	Potato	20	28
20			30
21			32
22		30	28
23			30
24			32
25		40	28
26			30
27			32

(3 starch sources) \times (3 DDGS levels) \times (3 protein levels) = 27 total treatment combinations

Unit Density

The sample extrudates were cut into pieces of length approximately 25.4 mm; each piece was weighed using a balance (PM 2500, Mettler Instrument Corporation, Hightstown, NJ, USA); height and diameter were measured using a vernier caliper (500-321, Digimatic, Mitutoyo Corporation, Japan). By considering the extrudates to be right circular cylinders, the unit density was calculated using the formula:

$$UD = \frac{M}{V} \quad (1)$$

where UD is the unit density of extrudates (kg/m^3), M is mass (kg), and V is the volume (m^3).

Sinking Velocity

The extrudates were cut to a length of approximately 25.4 mm, then dropped into a 2,000-mL graduated cylinder filled with distilled water, and the time taken for each piece to reach the bottom was recorded. The height of the water column (0.415 m) was noted and the sinking velocity was determined using:

$$SV = \frac{h}{t} \quad (2)$$

where SV is the sinking velocity (m/s), h is the height of the water column (m), and t is the time taken by the extrudates to reach the bottom of the container(s).

Color (L^* , a^* , b^*)

The color of the extrudates was determined according to CIE Lab standards using a Minolta Chromameter (model CM 2500d, Minolta, Japan). Color values L^* , a^* , and b^* correspond to the whiteness, redness–greenness, and yellowness–blueness of the extrudates, respectively. The instrument was appropriately calibrated before the sample extrudates were tested. The extrudates were ground to a fine powder of particle size approximately 150 μm before the corresponding L^* , a^* , and b^* values were measured.

Water Absorption and Water Solubility Indices

The extrudates were ground to fine powder of particle size approximately 150 μm using a laboratory mill (Smart Grind, Black & Decker Corporation, Towson, MD, USA). Finely ground 2.5 g sample was suspended in 30 mL of distilled water in a 50-mL centrifuge tube, stirred intermittently, placed in an oven at 30 °C for a period of 30 min, and then centrifuged at 1,000 \times g for 10 min. The supernatant

liquid was transferred into an aluminum cup, which was then oven-dried for 2 h at 135 °C (AACC method 44-19, 1995). The gel remaining in the centrifuge tube was weighed and WAI(–) was calculated as:

$$WAI = \frac{W_g}{W_{ds}} \quad (3)$$

where W_g is the weight of the gel (g), and W_{ds} is the weight of the dry sample (g).

WSI (%) was calculated as:

$$WSI = \left(\frac{W_{ss}}{W_{ds}} \right) \times 100 \quad (4)$$

where W_{ss} is the weight of dry solids of supernatant (g), and W_{ds} is the weight of the dry sample (g).

Pellet Durability Index

PDI was determined using method S269.4 (ASAE 2004). About 200 g of extrudates was broken into pieces of lengths approximately 25.4 mm and then divided into two batches of 100 g each. Each batch was placed in a pellet durability tester (model PDT-110 Seedburo Equipment Company, Chicago, IL, USA) and tumbled for 10 min. The sample was sieved through sieve no. 6 (3.36 mm) before and after tumbling, and then weighed for its underflow and overflow. The PDI (%) was calculated as:

$$PDI = \left(\frac{M_{at}}{M_{bt}} \right) \times 100 \quad (5)$$

where M_{at} is the mass of the pellets after tumbling (g), and M_{bt} is the mass of the pellets before tumbling (g).

Results and Discussion

The main treatment, treatment combination, and interaction effects of changing the levels of DDGS and net protein content for the different starch sources on the resulting extrudate properties are summarized in Tables 3, 4, and 5, respectively. Changing the levels of DDGS from 20% to 40% wb did have a significant effect on all the extrudate properties studied at $\alpha=0.05$. Also, for all the three starch bases, changing the levels of DDGS from 20% to 40% wb resulted in decreased ER and L^* values of extrudates, whereas SV, a^* , and b^* values of extrudates were found to increase. Moreover, for cassava and potato starch extrudates, changing the proportions of protein in the blends from 28% to 32% wb resulted in decreased ER values and increased UD, SV, and PDI values. However, changes in the levels of DDGS did not affect UD and a^* values significantly for corn starch extrudates (Table 5).

Table 3 Main treatment effects on extrudate properties due to cassava, corn, and potato starches and DDGS and protein levels

Starch source	Parameters (% wb)	Levels	ER (-)	UD (kg/m ³)	SV (m/s)	L* (-)	a* (-)	b* (-)	WAI (-)	WSI (-)	PDI (-)
Cassava	DDGS	20	1.49a (0.04)	780.0b (26.6)	0.029c (0.01)	61.2a (1.07)	6.10c (0.14)	30.1c (0.36)	4.10a (0.07)	15.6a (0.39)	81.6c (0.27)
		30	1.32b (0.02)	879.1a (14.8)	0.055b (0.00)	61.6a (0.40)	6.72b (0.09)	31.3b (0.40)	3.82a (0.04)	13.9a (1.23)	84.2b (0.59)
	Protein	40	1.28c (0.02)	863.5a (5.33)	0.061a (0.01)	59.1b (0.82)	7.62a (0.09)	33.4a (0.32)	3.47b (0.19)	10.7b (1.64)	86.1a (0.23)
		28	1.46a (0.05)	799.1b (29.1)	0.028c (0.01)	61.9a (0.31)	6.85a (0.20)	32.0a (0.66)	4.05a (0.67)	15.0a (0.52)	83.4b (0.66)
Corn	DDGS	30	1.35b (0.03)	856.0a (19.6)	0.047b (0.00)	61.4a (1.08)	6.80a (0.32)	31.8ab (0.67)	3.65b (0.23)	11.5b (1.83)	83.8ab (1.04)
		32	1.28c (0.02)	867.5a (9.92)	0.071a (0.00)	58.5b (0.58)	6.78a (0.21)	31.0b (0.38)	3.70b (0.41)	13.6a (1.21)	84.7a (0.92)
	Protein	20	1.17a (0.02)	899.3a (23.4)	0.093b (0.00)	64.3a (0.68)	5.68b (0.05)	28.3b (0.26)	3.16b (0.07)	15.8b (0.29)	85.3a (0.94)
		30	1.11b (0.01)	935.0a (6.33)	0.099a (0.00)	63.0b (0.61)	6.70a (0.14)	31.1a (0.47)	3.25a (0.01)	16.0a (0.07)	75.7b (4.36)
Potato	DDGS	40	1.10b (0.01)	910.9a (20.6)	0.098a (0.00)	63.3b (0.86)	6.69a (0.29)	30.6a (0.53)	3.21ab (0.03)	15.8b (0.25)	62.7c (2.43)
		28	1.14a (0.03)	915.7a (24.6)	0.095a (0.00)	61.7c (0.36)	6.50a (0.21)	29.8a (0.43)	3.09b (0.05)	16.7a (0.11)	80.1a (4.24)
	Protein	30	1.14a (0.01)	906.3a (11.8)	0.095a (0.00)	62.9b (0.45)	6.47a (0.25)	30.1a (0.65)	3.27a (0.02)	15.4c (0.14)	75.6b (3.24)
		32	1.10a (0.01)	923.1a (18.2)	0.099a (0.00)	66.0a (0.40)	6.11b (0.27)	30.0a (0.73)	3.26a (0.02)	15.6b (0.10)	67.9c (5.82)
Potato	DDGS	20	1.46a (0.04)	792.0b (29.0)	0.040c (0.01)	61.2a (0.63)	6.14c (0.16)	29.1c (0.43)	3.95a (0.16)	16.6a (0.62)	81.8c (0.62)
		30	1.31b (0.01)	879.7a (9.75)	0.071b (0.00)	57.7b (0.48)	6.96b (0.06)	30.9b (0.35)	3.94a (0.02)	15.4b (0.10)	85.3b (0.34)
	Protein	40	1.24c (0.01)	896.6a (5.39)	0.080a (0.00)	56.6c (0.79)	7.44a (0.18)	32.2a (0.22)	3.78b (0.01)	14.7c (0.11)	87.4a (0.43)
		28	1.42a (0.05)	810.5b (32.0)	0.063b (0.00)	58.1b (1.10)	6.96a (0.20)	31.0a (0.52)	3.73b (0.11)	16.4a (0.67)	84.0b (1.04)
Protein	30	1.32b (0.03)	872.4a (16.7)	0.058c (0.01)	57.0c (0.48)	7.09a (0.22)	30.8a (0.52)	3.99a (0.08)	15.1b (0.22)	84.3b (1.17)	
	32	1.27c (0.02)	885.3a (7.12)	0.070a (0.00)	60.3a (0.72)	6.49b (0.24)	60.4a (0.62)	3.95a (0.06)	15.2b (0.07)	86.1a (0.96)	

Means followed by similar letters for a given parameter are not significantly different at $\alpha=0.05$, LSD, for a given main effect (protein level or DDGS level for a given starch). Values in parentheses are standard error

ER expansion ratio, UD unit density, L* brightness/luminosity, a* redness/greenness, b* blueness/yellowness, SV sinking velocity, WAI water absorption index, WSI water solubility index, PDI pellet durability index

Table 4 Treatment combination effects on extrudate properties due to cassava, corn, and potato starches and DDGS and protein levels

Property	DDGS (% wb)	Cassava			Corn			Potato		
		Protein content (% wb)								
		28	30	32	28	30	32	28	30	32
ER (-)	20	1.64a	1.49b	1.35e	1.21i-j	1.19j	1.12k	1.60a	1.43b-c	1.34e-f
	30	1.42c-d	1.28g-h	1.28f-h	1.13k	1.12k	1.10k	1.37d-e	1.29f-h	1.28g-h
	40	1.33e-g	1.29f-h	1.21i-j	1.09k	1.12k	1.10k	1.29f-h	1.25h-i	1.19i-j
UD (kg/m ³)	20	687.1g	790.6f	862.4b-e	874.0a-e	875.3a-e	948.5a	688.3g	815.6e-f	872.0b-e
	30	831.1d-f	913.1a-c	893.0a-d	935.3a-b	936.1a-b	933.5a-b	846.9c-f	905.6a-d	886.6a-e
	40	879.2a-e	864.3b-e	847.0c-f	937.9a-b	907.4a-c	887.4a-e	896.3a-d	896.1a-d	897.4a-d
SV (m/s)	20	0.00n	0.03m	0.053j-k	0.09c-e	0.09a-c	0.10a	0.04l	0.00n	0.08f-g
	30	0.05k-l	0.05k-l	0.07g-h	0.10a	0.10a	0.10a-b	0.07h-i	0.08d-f	0.06h-i
	40	0.03m	0.06i-j	0.09b-d	0.10a	0.09a-c	0.10a	0.08e-f	0.09b-d	0.07g-h
L* (-)	20	61.0e-f	64.9b	57.6i-j	62.2d-e	64.4b-c	66.4a	61.6d-f	58.9h-i	63.1c-d
	30	62.5d-e	61.2d-f	60.6f-g	61.2e-f	62.6d-e	65.2a-b	58.6h-i	56.1j-k	58.4h-i
	40	62.3d-e	57.5i-k	57.5i-k	61.8d-f	61.6d-f	66.4a	54.2l	56.0k	59.5g-h
a* (-)	20	6.27i	5.75j	6.27i	5.76j	5.70j	5.57j	6.43h-i	6.44h-i	5.57j
	30	6.86e-h	6.74f-i	6.57g-i	6.51g-i	6.44h-i	7.17c-f	6.79e-i	7.00d-g	7.10c-f
	40	7.44a-d	7.86a	7.56a-c	7.26b-f	7.28b-e	5.57j	7.68a-b	7.83a	6.81e-h
b* (-)	20	30.3f-i	29.5i-l	30.6e-i	28.5k-m	27.7m	28.6j-m	29.9h-k	29.6i-k	28.0l-m
	30	31.6c-g	32.1c-e	30.2f-j	30.1e-i	30.6e-i	32.9a-c	29.9h-k	30.1g-j	31.7c-f
	40	34.2a	33.7a-b	32.3b-d	31.1d-h	32.0c-e	28.6j-m	32.4b-d	32.7b-c	31.6c-g
WAI (-)	20	4.29a	4.20a-b	3.83c-e	2.90i	3.28g-i	3.29g-i	3.40f-h	4.30a	4.16a-c
	30	3.98a-d	3.76d-e	3.73d-f	3.27g-i	3.25g-i	3.22g-i	4.02a-d	3.89b-e	3.93b-d
	40	3.87b-e	3.00i	3.55e-g	3.08h-i	3.29g-i	3.27g-i	3.78d-e	3.79c-e	3.77d-e
WSI (%)	20	15.5b-d	15.1b-d	16.1b-c	17.0a-b	15.3b-d	15.2b-d	18.8a	15.7b-d	15.3b-d
	30	16.0b-c	15.3b-d	10.3e	16.3b-c	15.9b-c	15.9b-d	15.5b-d	15.4b-d	15.2b-d
	40	13.6d	4.22f	14.3c-d	16.7b	15.0b-d	15.7b-d	14.8b-d	14.4c-d	15.0b-d
PDI (%)	20	81.1h	80.8h	82.1f-h	87.3a-b	82.9e-h	85.6b-e	81.1h	80.8h	83.4d-h
	30	82.8e-h	83.9c-g	85.9a-d	86.3a-c	77.6i	63.2k	84.7b-g	84.8b-f	86.3a-c
	40	85.5b-e	86.5a-c	86.4a-c	66.8j	66.2j	54.9l	86.4a-c	87.2a-b	88.7a

Means followed by similar letters for a given parameter are not significantly different at $\alpha=0.05$, LSD, among treatment combinations ER expansion ratio, UD unit density, L* brightness/luminosity, a* redness/greenness, b* blueness/yellowness, SV sinking velocity, WAI water absorption index, WSI water solubility index, PDI pellet durability index

Table 5 Interaction effects of extrudate properties due to cassava, corn, and potato starches and DDGS and protein levels (p values)

Starch source	Interactions	Extrudate properties								
		ER (-)	UD (kg/m ³)	SV (m/s)	L* (-)	a* (-)	b* (-)	WAI (-)	WSI (%)	PDI (%)
Cassava	DDGS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0011	0.0001	<0.0001
	Protein	<0.0001	<0.0001	<0.0001	<0.0001	0.8745	0.0736	0.0216	0.0038	0.0004
	DDGS × protein	<0.0001	<0.0001	<0.0001	<0.0001	0.0995	0.0681	0.0953	<0.0001	0.0006
Corn	DDGS	0.0156	0.4153	0.0160	0.0410	<0.0001	<0.0001	0.0237	0.0011	<0.0001
	Protein	0.2044	0.8215	0.1342	<0.0001	0.0030	0.6254	<0.0001	<0.0001	<0.0001
	DDGS × protein	0.5471	0.4102	0.0056	0.1914	<0.0001	<0.0001	<0.0001	<0.0001	0.0002
Potato	DDGS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0369	<0.0001	<0.0001
	Protein	<0.0001	<0.0001	0.0006	<0.0001	0.0003	0.4016	0.0028	0.0003	0.0006
	DDGS × protein	<0.0001	<0.0001	<0.0001	<0.0001	0.0026	0.0343	<0.0001	0.0002	0.6324

p values<0.05 are significantly different; p values>0.05 are not significantly different using $\alpha=0.05$, LSD ER expansion ratio, UD unit density, L* brightness/luminosity, a* redness/greenness, b* blueness/yellowness, SV sinking velocity, WAI water absorption index, WSI water solubility index, PDI pellet durability index

Expansion Ratio

Extrudate expansion is an event that generally occurs as a result of high temperature and low moisture extrusion cooking, which is an outcome of several operations combined together, which includes biopolymer structural transformation, phase transitions, nucleation, extrudate swell, bubble growth, and bubble collapse (Chang 1992). From Table 3, we observe that for all three starch sources (cassava, corn, and potato) increasing the DDGS levels from 20% to 40% resulted in a marked decrease in expansion ratio values by 14.1%, 5.98%, and 15.1%, respectively. This is directly attributed to the decrease in starch content in the ingredient blends with a corresponding increase in the DDGS levels (Table 1). The amylopectin component present in starch is a branched chain of molecules and is primarily responsible for the expansion of extruded products. The branched locations in the amylopectin chains are the most susceptible to rupture during processing at elevated temperatures (Davidson et al. 1984). Higher amylopectin content often results in light, elastic, and homogeneous texture with a smooth and sticky surface; in contrast, the higher the proportion of amylose, the harder and less expanded the product (Mercier and Feillet 1975). The increase in the expansion ratio of corn starch extrudates was lower (5.98%) in comparison to the other starch sources used (cassava and potato), which could be attributed to the lower amylopectin content (72%), whereas the increase in ER of cassava (83%) and potato (79%) starch extrudates were considerably higher, probably due to the higher proportion of amylopectin. In addition, the lower expansion of corn starch extrudates might be because the starch could have degraded at the elevated processing temperatures. Since starch plays a vital role in the production of expanded products, as the proportion of starch in the ingredient blend decreased, gelatinization of starch decreased, which resulted in extrudates with poor expansion. Similar results were observed by Chevanan et al. (2007b, 2008a) and Kannadhasan et al. (2007) in their studies with DDGS as a base material. Similarly, the expansion ratio of cassava and potato starch was found to decrease by 12.3% and 10.6%, respectively, with the change in net protein content from 28% to 32% wb (Table 3). Protein is found to have a negative impact on the expansion of the extruded products and therefore as the protein content in the respective blends increased, a marginal decrease in expansion ratio could be observed. Due to the simultaneous action of heating at elevated temperatures and shearing in the extruder, alteration of protein takes place, which involves denaturation and rearrangement (i.e., disruption of the three-dimensional structure and the formation of a network structure). As protein content increases vis-à-vis the other components, the high shearing action within the extruder

barrel can disrupt the interactions with other ingredient components and limit the extensibility of the dough during expansion at the die exit, and thus hinder the degree of expansion (Guy 1994). Changes in the net protein content appeared to be insignificant on the expansion ratio of corn starch extrudates, however (Tables 4 and 5).

The highest (1.64) and the lowest (1.09) ER values were found with cassava and corn starch extrudates, respectively. Among the three starch sources used, cassava and corn starch had the highest and the lowest proportion of amylopectin (responsible for expansion), which resulted in the highest and the lowest expansion ratio values, respectively. The highest ER and the lowest UD values were found with cassava starch extrudates for the same treatment combination (20% DDGS and 28% protein). It is quite obvious that the higher the expansion, the lower the unit density of the resulting extrudates.

Unit Density

Unit density of the extrudates is directly related to the degree of expansion that occurs during extrusion processing (Colonna et al. 1989). Floatability of the feed, especially for the top feeding species, results in reduced feed waste and is dependent upon expansion and unit densities (Chang and Wang 1998). For cassava and potato starch extrudates, increasing the DDGS levels from 20% to 40% wb resulted in increased unit density values by 10.7% and 13.2%, respectively. Results from our study parallel the findings of Chevanan et al. (2008a) and Kannadhasan et al. (2007). Similarly, increasing the net protein content from 28% to 32% wb resulted in 8.56% and 9.23% increased unit density values (Table 3).

The unit density values of cassava, corn, and potato starch extrudates ranged from 687.1 to 913.1 kg/m³, 874 to 948.5 kg/m³, and 688.3 to 905.6 kg/m³, respectively. The lowest unit density (687.1 kg/m³) and the highest expansion ratio (1.64) values were observed with cassava starch extrudates for the same treatment combination of 20% DDGS and 28% protein (Table 4). This finding is quite logical because expansion and unit density are inversely related to each other and hence the higher the expansion, the lower the unit density. Changes in DDGS levels and net protein content in the ingredient blends were found to be insignificant for unit density values of corn starch extrudates (Table 5). From our study, we observe that cassava and corn starch extrudates, which possessed the lowest and the highest unit density values, could be more appropriate for floating and sinking aquaculture feeds, respectively. The higher unit density values and lower expansion ratios of corn starch extrudates might be due to the lower levels of gelatinization of starch, and also due to the molecular degradation of the starch (Owusu-Ansah et al. 1983). Moreover, the moisture content of the starch prior to

extrusion affected the expansion greatly compared to temperature and screw speed (Owusu-Ansah et al. 1984).

Color

Color is an important physical property that is often used by feed customers to predict pellet quality (Turner 1995). L^* refers to the luminosity or brightness, a^* refers to the redness or greenness, and b^* refers to the blueness or yellowness. Our study revealed that for all three starch bases, with an increase in DDGS levels from 20% to 40% wb, L^* values were found to decrease. However, an increase in DDGS levels in the ingredient blend resulted in increased a^* and b^* values for all the three starch extrudates (Table 3). Similar results were reported by Kannadhasan et al. (2007) for L^* , a^* , and b^* values in their study with DDGS as a base material. The decrease in L^* values might be due to the darker color of DDGS in comparison with other ingredients, namely soy flour and starch (cassava, corn, and potato), and thus as the percent of DDGS increased, brightness or luminosity values decreased. Moreover, the changes in color values could be due to the non-enzymatic browning by Maillard reaction between proteins and reducing sugars that occurs due to the elevated temperature conditions in the extruder (Berset 1989). Apart from the changes in color of the extrudates, Maillard reaction also has some nutritional implications due to the loss of lysine available. Also, the increase in b^* values with DDGS levels might be due to the yellow-colored nature of the DDGS. The changes in yellowness (b^*) values during extrusion cooking might have been due to the effects of two different reactions, namely non-enzymatic browning and pigment destruction (Ilo and Berghofer 1999). Increasing the protein content from 28% to 32% wb resulted in decreased L^* values for cassava starch extrudates, whereas for corn and potato starch extrudates, it was found to increase. No regular pattern emerged for a^* and b^* values for all the three starch bases with changes in protein content, however. The highest L^* , a^* , and b^* values were 66.4 (corn starch extrudates), 7.86 (cassava starch extrudates), and 34.2 (cassava starch extrudates) and the lowest L^* , a^* , and b^* values were 54.2 (potato starch extrudates), 5.57 (potato and corn starch extrudates), and 27.7 (potato starch extrudates; Table 4). Statistical analyses across all the collected data confirmed that changes in net protein content did not result in statistically significant differences in b^* values of all three starch bases at $\alpha=0.05$ (Table 5).

Sinking Velocity

Sinking velocity is an important property that dictates the stability of the extrudates in water and is closely related to the absorption of water during the floating of the feed on

the surface of the water (Chevanan et al. 2008b). Increasing the proportion of DDGS in the ingredient blends resulted in pronounced increases in the sinking velocity values of cassava and potato starch extrudates by 110.3% and 100%, respectively (Table 3). On the contrary, the increase in sinking velocity values of corn starch extrudates was lower (5.37%). Our findings with respect to potato starch extrudates were in agreement with the findings of Kannadhasan et al. (2007). The highest sinking velocity value (0.100 m/s) was observed for corn starch extrudates. This finding was quite reasonable because corn starch extrudates possessed the lowest expansion ratio and the highest unit density, which thus resulted in the highest sinking velocity value. The lowest value (0.000 m/s) could be observed for cassava and potato starch extrudates (Table 4). For all the three starch bases, the increase in sinking velocity values could be accounted for the decrease in expansion ratio with the corresponding increase in DDGS levels, which resulted in denser products and hence higher sinking velocities. In addition, an increase in the net protein content from 28% to 32% wb resulted in a 153.6% increase in sinking velocity values of cassava starch extrudates. However, the increase in the sinking velocity values of potato starch extrudates were very little (11.1%) in comparison with cassava starch extrudates (Table 3). The sinking velocity values of cassava, corn, and potato starch extrudates ranged from 0.000 to 0.090 (m/s), 0.086 to 0.100 (m/s), and 0.000 to 0.090 (m/s), respectively. Also, changes in protein content appeared to be insignificant on sinking velocity values of corn starch extrudates (Table 5).

Water Absorption and Water Solubility Indices

Water absorption and solubility indices are often used as indicators of volume of swollen gelled particles which maintain their integrity in aqueous dispersion (Mason and Hosney 1986), and also degradation of molecular components (Kirby et al. 1988). The water solubility index also depends on the quantity of soluble matter which increases due to the degradation of starch (Guha et al. 1997).

Increasing the DDGS levels from 20% to 40% resulted to decreased WAI values by 15.4% and 4.30% for cassava and potato starch extrudates, respectively (Table 3). Our findings are in agreement with those of Chevanan et al. (2008b) and Kannadhasan et al. (2007). Moreover, with an increase in protein content from 28% to 32%, a marginal increase in WAI values by of 5.50% and 5.90% was observed with corn and potato starch extrudates, respectively. The WAI values ranged from 3.4 to 4.29 for cassava and potato starch extrudates and from 2.92 to 3.29 for corn starch extrudates. The highest WAI value (4.29) was obtained for cassava and potato starch blends and the

Table 6 Pearson linear correlation coefficients for extrudate properties

Starch	ER	UD	SV	L^*	a^*	b^*	WAI	WSI	PDI
ER	Cassava 1.000 (–)								
	Corn 1.000 (–)								
	Potato 1.000 (–)								
UD	Cassava –0.887 (<0.0001)	1.000 (–)							
	Corn –0.818 (<0.0001)	1.000 (–)							
	Potato –0.938 (<0.0001)	1.000 (–)							
SV	Cassava –0.865 (<0.001)	0.645 (0.000)	1.000 (–)						
	Corn –0.630 (0.000)	0.485 (0.010)	1.000 (–)						
	Potato –0.603 (0.000)	0.575 (0.002)	1.000 (–)						
L^*	Cassava 0.444 (0.020)	–0.201 (0.315)	–0.543 (0.003)	1.000 (–)					
	Corn 0.015 (0.941)	–0.162 (0.419)	0.177 (0.376)	1.000 (–)					
	Potato 0.457 (0.017)	–0.496 (0.008)	–0.318 (0.106)	1.000 (–)					
a^*	Cassava –0.544 (0.003)	0.328 (0.095)	0.457 (0.017)	–0.523 (0.005)	1.000 (–)				
	Corn –0.346 (0.077)	0.216 (0.279)	0.174 (0.386)	–0.502 (0.008)	1.000 (–)				
	Potato –0.426 (0.027)	0.363 (0.062)	0.388 (0.045)	–0.844 (<0.0001)	1.000 (–)				
b^*	Cassava –0.432 (0.024)	0.338 (0.084)	0.205 (0.304)	–0.298 (0.131)	0.904 (<0.0001)	1.000 (–)			
	Corn –0.393 (0.042)	0.250 (0.209)	0.186 (0.353)	–0.262 (0.187)	0.914 (<0.0001)	1.000 (–)			
	Potato –0.466 (0.014)	0.337 (0.085)	0.347 (0.076)	–0.628 (0.000)	0.895 (<0.0001)	1.000 (–)			
WAI	Cassava 0.601 (0.000)	–0.468 (0.014)	–0.549 (0.003)	0.579 (0.001)	–0.660 (0.000)	–0.519 (0.006)	1.000 (–)		
	Corn –0.298 (0.131)	0.147 (0.465)	0.481 (0.011)	0.332 (0.090)	0.001 (0.996)	0.085 (0.674)	1.000 (–)		
	Potato –0.213 (0.284)	0.353 (0.071)	–0.260 (0.189)	0.071 (0.724)	–0.241 (0.225)	–0.181 (0.367)	1.000 (–)		
WSI	Cassava 0.332 (0.091)	–0.233 (0.241)	–0.289 (0.144)	0.348 (0.075)	–0.462 (0.015)	–0.331 (0.092)	0.607 (0.000)	1.000 (–)	
	Corn 0.183 (0.360)	–0.056 (0.780)	–0.147 (0.464)	–0.425 (0.027)	0.100 (0.616)	0.008(0.966)	–0.772 (<0.0001)	1.000 (–)	
	Potato 0.841 (<0.0001)	–0.851 (<0.0001)	–0.427 (0.026)	0.462 (0.015)	–0.424 (0.028)	–0.464 (0.015)	–0.579 (0.001)	1.000 (–)	
PDI	Cassava –0.746 (0.000)	0.511 (0.030)	0.611 (0.007)	–0.473 (0.047)	0.778 (0.000)	0.583 (0.011)	–0.612 (0.007)	–0.648 (0.004)	1.000 (–)
	Corn 0.525 (0.025)	0.030 (0.906)	–0.317 (0.199)	–0.287 (0.248)	–0.427 (0.077)	–0.531 (0.023)	–0.205 (0.414)	0.176 (0.485)	1.000 (–)
	Potato –0.868 (<0.0001)	0.704 (0.001)	0.679 (0.002)	–0.451 (0.060)	0.538 (0.021)	0.636 (0.004)	–0.113 (0.655)	–0.568 (0.014)	1.000 (–)

Values in parentheses are corresponding p values for each correlation

ER expansion ratio, UD unit density, L^* brightness/luminosity, a^* redness/greenness, b^* blueness/yellowness, SV sinking velocity, WAI water absorption index, WSI water solubility index, PDI pellet durability index

lowest WAI value (2.92) for corn starch extrudates (Table 4).

As the DDGS level was increased from 20% to 40% wb, a substantial decrease could be observed in WSI values by 31.4% and 11.4% for cassava and potato starch extrudates, respectively. In contrary, corn starch extrudates did not show significant differences for WSI values at $\alpha=0.05$ (Table 3). Increasing the protein content from 28% to 32% resulted in decreased WSI values by 6.58% and 7.31% for corn and potato starch extrudates, respectively, but no significant effect was observed for cassava starch extrudates (Table 3). The highest WSI value (18.8%) and the lowest WSI value (4.22%) were observed for potato and cassava starch extrudates (Table 4). The higher WSI values for potato starch extrudates might be due to the interactions with protein in the mixtures, which may have imparted rigidity to them, may have contributed to limiting the leaching of the starch in the sample mixture. Moreover, the changes in WAI and WSI values may be interpreted on the extent of starch–water interactions that dictate the solid-phase structure of the processed starch (Colonna et al. 1989).

Pellet Durability Index

PDI is a direct measurement of a pellet's ability to withstand breakage and disintegration (Chang and Wang 1998). The effect of changing the proportion of DDGS and protein content on the physical properties of the extrudates is shown in Table 3. With an increase in DDGS levels from 20% to 40%, pellet durability values increased by 5.51% for cassava starch blends and 6.84% for potato starch blends. In contrast, PDI values of corn starch extrudates were found to decrease substantially by 26.5%, which is in agreement with the observations of Chevanan et al. (2007a, 2008b) and Kannadhasan et al. (2007). The decrease in PDI values for corn starch extrudates with an increase in DDGS content could be attributed to the decrease in the starch content, but also the interaction of the starch with the proteins, which resulted in poor starch gelatinization, reduced cohesion, and hence the durability of the extrudates (Chevanan et al. 2008a). However, the findings with respect to PDI of cassava and potato starch extrudates might be due to the difference in the starches themselves and thus their interactions. An increase in protein levels from 28% to 32% resulted in increased PDI values by 1.55% and 2.49% for cassava and potato starch blends, respectively. According to Cheftel et al. (1985), protein possesses binding properties and hence as the proportion of protein in the ingredient blend increased, so did the PDI values (Cheftel et al. 1985). In contrast, the PDI values of corn starch extrudates resulted in a 15.2% decrease for a protein change from 28% to 32% in the ingredient blend (Table 3). PDI values

ranged from 80.8% to 86.5% for cassava starch extrudates, whereas 54.9% to 87.3% and 80.8% to 88.7% for corn and potato starch extrudates, respectively. The highest PDI value (88.7%) was obtained for potato starch extrudates, while the lowest value (55.0%) for corn starch extrudates for the same treatment combination of 40% DDGS and 32% protein levels (Table 4). The higher PDI values of corn starch extrudates indicate that these extrudates could be more resistant to mechanical damage during transportation and storage. Analyses across all collected data confirmed that there existed statistically significant differences at $\alpha=0.05$ for PDI values (Table 5) due to interaction effects.

Correlation Analysis

Linear correlation analysis on the collected multivariate data was conducted to examine and thereby deepen the understanding of strength of the relationships that existed between all measured physical properties of the extrudates studied under various extrusion conditions. Table 6 summarizes the Pearson correlation coefficients (absolute r values shown) of all dependent variables of cassava, corn, and potato starch extrudates. The values in the parenthesis represent the corresponding p values, which show the existence of statistical significant differences ($\alpha=0.05$) among the response variables targeted. Results from our study revealed that ten among 36 possible correlations were insignificant ($p>0.05$) for both cassava and potato starch extrudates; 22 among 36 correlations studied were insignificant ($p>0.05$) for corn starch extrudates (Table 6). As anticipated, expansion ratio and unit density of the extrudates possessed a strong negative correlation. This was quite logical because expansion and density are inversely proportional to each other. Cassava and corn starch extrudates possessed absolute r values >0.80 for the correlation between expansion ratio and unit density, whereas potato starch extrudates had r values >0.90 . Also, a strong negative correlation existed between expansion ratio and sinking velocity for all the three starch extrudates (cassava, corn, and potato). In addition, b^* (blueness or yellowness) were very strongly and positively correlated with a^* (redness or greenness) having absolute r values of nearly 0.90 for all the three starch extrudates considered in our study.

Conclusions

This experimental study was conducted with an objective of determining the effect of starch sources, DDGS levels, and protein content on the physical properties of the resulting extrudates. Changing the DDGS levels from 20% to 40% and protein levels from 28% to 32% had a significant effect

on expansion ratio, unit density, sinking velocity, L^* , water absorption, water solubility, and pellet durability indices for cassava starch extrudates, whereas the same had a significant effect on all the extrudate properties studied, with an exception of b^* for potato starch extrudates. Moreover, the change in DDGS and protein levels had a significant effect on very few properties studied (L^* , water absorption, water solubility, and pellet durability indices) for corn starch extrudates. The extrudates produced from cassava starch with lower proportions of DDGS (20%) and protein (28%) levels considered in our study showed better expansion, and hence floatability. Also, the extrudates produced from corn starch with higher DDGS (40%) and protein (32%) levels were more durable. It was evident that cassava and corn starch, incorporated with lower and higher levels of DDGS, could be more appropriate for the production of floating and sinking aquaculture feeds, respectively. As anticipated, correlation analysis results revealed that the expansion ratio possessed a strong negative correlation with unit density and sinking velocity of all the three starch extrudates. From our results, it appears that the inclusion of DDGS for the production of floating aquaculture feed lies between 20% and 30% db, whereas inclusion of DDGS at levels higher than 30% db could be more suited for the production of sinking aquaculture feeds, at least for the extrusion processing conditions employed. Follow-up studies should target the production of floating aquaculture feed with incorporation of DDGS levels between 20% and 30% db, which would be more appropriate for tilapia, and should aim to optimize processing conditions.

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