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ORIGINAL ARTICLE

Optimization of extruder cooking conditions for the manufacture of fish feeds using response surface methodology

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Abstract

A composite blend consisting of sunflower cake, maize germ, wheat bran, fresh water shrimps and cassava flour was extruded using a single-screw extruder to produce expanded fish feed pellets. The effects of temperature (80–120 °C), die diameter (2–4 mm), and feed pre-conditioning time (50–150 s; steam 400 kPa) on properties of the pellets (expansion ratio, bulk density, floatability, durability, water absorption, water solubility, water stability, and in-vitro protein digestibility) were investigated using response surface methodology. Regression equations describing the effect of each variable on the product responses were obtained. The pellets extruded using a factor combination of 120 °C extruder barrel temperature, 2 mm die diameter, and 100 s of feed pre-conditioning time gave most desirable pellet floatability (100%), durability index (99%), expansion ratio (2.64), water absorption index (4.12), water solubility index (9.31), water stability (87%), bulk density (479 g/L), and in vitro protein digestibility (69.97%) with a composite desirability of 0.88.

Practical applications

Extrusion is a modern feed processing method whose use is fast gaining popularity among small feed processors in developing countries. However, extrusion is a process that involves many parameters that need to be optimized for desirable end properties. These findings guide fish feed manufacturers on the optimum conditions for single screw extruders for production of feeds with desirable properties especially for the fish types that are top feeders. In addition, the results offer important insights on how temperature, die diameter, and feed pre-conditioning, may be manipulated to influence properties of extruded aquafeed when using simple low-cost small-scale extruders.

1 | INTRODUCTION

Aquaculture contributes about 50% of the fish consumed by humans globally (Halden, Lindberg, & Masembe, 2014). About 80% of the world's aquaculture is practiced in developing countries mainly by small-scale farmers (FAO, 2016). For this reason, there is growing appreciation of the role that small-scale aquaculture can play in improving rural household nutrition and income security in these countries. However, access to affordable high-quality feeds is a challenge that limits smallholder participation, and threatens aquaculture profitability and sustainability (Munguti, Kim, & Ogello, 2014). Availability of ready-to-use or manufactured aquafeeds is not widespread (Ngugi, Bowman, & Omolo, 2007). Some small-scale farmers formulate low-cost feeds using locally available ingredients at farm level. The feeds fail to meet the needs of expanding semi-intensive aquaculture systems; they have inferior functional quality especially with regard to physico-chemical properties (Munguti et al., 2014; Ogello, Munguti, Sakakura, & Hagiwara, 2014). Thus, the search for alternative processing method with optimized operating conditions for the manufacture of high quality feeds has become imperative (Cocker, 2014).

Ideally, smallholder farmers mix locally sourced ingredients into a mash, which they sometimes moisten and press using simple mechanical tools to produce pellets. While this approach somewhat meets short-term needs, it is unable to produce consistently high quality

feeds in commercial quantities. A more versatile processing technique is extrusion cooking (Kazamzadeh, 1989). Small-scale feed manufacturers are increasingly adopting low-cost extruders to bridge the demand gap for good quality fish feed. The compounded mash is pushed through a heated closed barrel by means of screw(s) and pressed through a die at the end of barrel (Levic & Sredanovic, 2010). In the process, components of the mash, that is, carbohydrate, protein, fiber, and fat are subjected to heating, mixing, and shearing under high pressure resulting in cooked molten dough that is shaped into pellets as it leaves the die. Consequently, nutritional and functional properties of pellets are improved as a result of thermal and shear modification of the mash ingredients. Extrusion is also shown to destroy anti-nutritional factors such as trypsin inhibitor and lectins (Moscicki, 2011; Nikmaram, Kamani, & Ghalavand, 2015), increase the solubility of nitrogen and dietary fiber, reduce lipid oxidation by denaturing deteriorative enzymes (Alam, Kaur, Khaira, & Gupta, 2016; Filipovic et al., 2010; Levic & Sredanovic, 2010; Moscicki, 2011; Nikmaram et al., 2015; Sorensen, 2009) and destroy microbiological pathogens. It therefore provides the feed manufacturers with the means to improve quality of their products (Filipovic et al., 2010).

Extrusion parameters, among them feed composition, pre-conditioning, extruder temperatures, and die-diameter influence properties of extrudates (Sorensen, 2012). Starch-rich ingredients form a relatively elastic melt in the barrel and result in expanded products that have low bulk density (Alves, Grossmann, & Silva, 1999) whereas protein-rich ingredients form a plastic melt that extrudes into a porous product with less expansion (Singh, Nielsen, Chambers, Martinez-Serna, & Villota, 1991). Fat and fiber modify the viscosity and behavior of the melt in the extruder barrel (Ilo, Schoenlechner, & Berghofe, 2000). Feed pre-conditioning prepares the material for the actual extrusion. This may be achieved using steam, water or mechanical means (Levic & Sredanovic, 2010), but the use of steam is preferred because it pre-heats and plasticizes the raw materials resulting in lower extruder energy input, and improved physical quality of extrudates (Levic & Sredanovic, 2010; Rokey, Plattner, & Souza, 2010; Tumuluru, 2014). Temperature is usually a targeted value, and is attained through steam in the pre-conditioner, thermal energy dissipated by mechanical shearing and heat emanating from heated surfaces (Chiu & Solarek, 2009; Sorensen, 2009, 2012). Die-diameter controls the flow of the melt and contributes to pressure build-up through the extrusion barrel (Akdogan, 1999). Thus to achieve targeted product characteristics, understanding the relationships between ingredients and process parameters is necessary. The aim of this study was to establish the optimal conditions for production of floating pellets using a simple low-cost single screw extruder. A further aim was to investigate the effects of feed pre-conditioning, extruder temperature, and die diameter on properties of the extruded pellets processed from a fish feed blend containing locally sourced food ingredients.

2 | MATERIALS AND METHODS

2.1 | Extruder

A low cost single screw extruder powered by a 15-HP motor (Model: DOLLY, Unitech, New Delhi, India) with a processing capacity of 50–80 kg/hr was used. The extruder comprised feed pre-conditioning system and extruding system (Figure 1). The feed pre-conditioning system comprised a boiler supplying steam to the pre-conditioning chamber at a pressure of 400 kPa. The extruding system comprised a single screw with three differentiated channel-width zones (feeding zone: 30 mm; transition zone: 12 mm; metering zone: 6 mm) having uniform flight width (1 mm) and uniform channel depth (1 mm). The length and diameter of the screw was 55 and 6 cm, respectively (L/D 9:1), and the screw rotating speed was maintained at 200 rpm, as set by the manufacturer. The extruder barrel was equipped with band heaters, and temperature was controlled at the metering zone. The

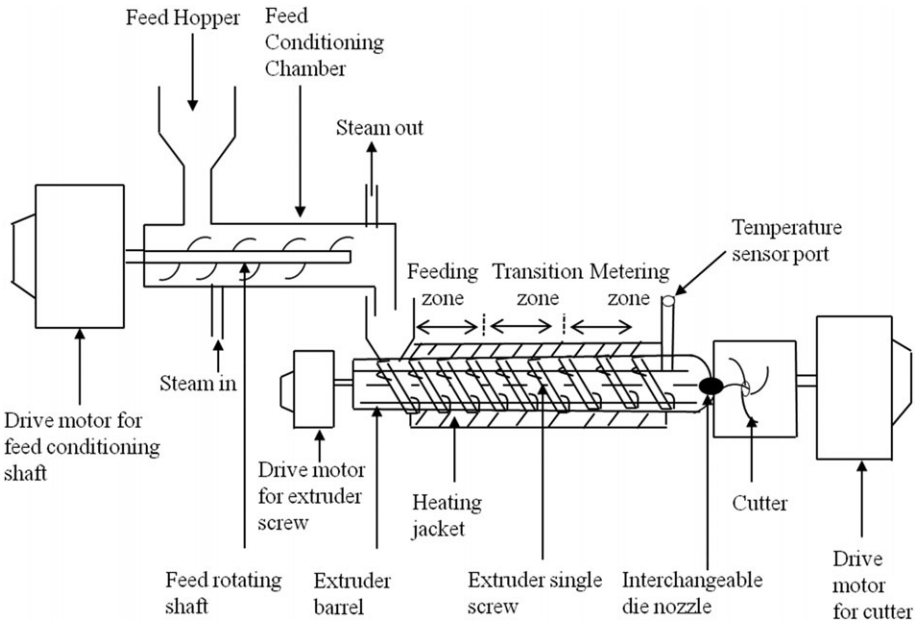

FIGURE 1 Schematic representation of single screw extruder

die assembly comprised of interchangeable discs, each with equal-size holes drilled concentrically close to the edge of the disc. The dies had conical centers with initial diameters of 6, 9, and 12 mm and exit diameters of 2, 3, and 4 mm, respectively.

2.2 | Composition of feed blend

The formulation used in this study was based on the minimum nutritional requirement for tilapia fish (*Oreochromis niloticus*), which is commonly farmed in small-scale aquaculture systems in developing countries. Protein is the key nutrient in fish diets and therefore the priority was to achieve the minimum protein content requirement when mixing the various ingredients. We selected to process a product that would be suitable for adult tilapia (FAO, 2017). Sunflower cake, maize germ, wheat pollard, fresh water shrimps, and cassava flour were purchased locally from an approved agro dealer shop. Each of the ingredients was separately milled into a fine powder using a Thomas Model 4 Wiley® laboratory mill (Thomas Scientific Inc., Swedesboro, NJ) and sieved through a 1.0 mm aperture sieve. The proximate compositions of the various ingredients were determined using AOAC (2000) methods. Crude protein was determined by AOAC Method 984.13, crude fat by AOAC Method 920.39 and crude ash by AOAC Method 942.05. Crude fiber was determined according to ISO 6865.2000 (ISO, 2000), while carbohydrate content was determined by difference. Based on the proximate compositions of ingredients, the quantities required to formulate 5 kg blends containing 27% protein recommended for tilapia fish (FAO, 2017), were calculated. These were proportionately weighed using digital weighing scale into 20 L buckets. The amount of water needed to raise the moisture content of the mash to 20% was added and the ingredients were mixed manually by hand. This moistened mash was then transferred to a multivane paddle mixer (Unitech, New Delhi, India) for further mixing for 2 min at moderate speed.

2.3 | Experimental design

A response surface methodology using Box–Behnken design with three independent variables: extrusion temperature (factor A), die-diameter (factor B), and feed pre-conditioning time (factor C) was used. The design was generated using Minitab 14.12.0 (Minitab Inc, State College, PA), and comprised 30 trials (Table 1). The response variables were expansion ratio, bulk density, floatability, durability index, water absorption index, water solubility index, water stability, and in vitro protein digestibility of the extruded pellets.

2.4 | Extrusion process

The blended ingredients were introduced into the steam conditioning chamber (with steam inlet pressure set at 400 kPa) through a manual hopper at a rate of 60 kg/hr. Residence time in the conditioning chamber was 50 s per cycle. The conditioning step was varied to have 1–3 cycles equivalent to conditioning times of 50, 100, or 150 s, after which the mash was channeled into the extrusion barrel. The extrusion temperature was set at 80, 100, or 120 °C, as measured at the end of the metering zone of the extruder barrel. Extrudates exited through a die of 2, 3, or 4 mm diameter. The choice of feed pre-conditioning times, extrusion temperatures, and die diameters was based on range of values that have been recommended by others (Vijayagopal, 2004) for the production of floating fish feeds. Screw speed was maintained at 200 rpm. Each extruded sample was collected into a separate 20 L bucket and then dried in a solar tent to constant weight. The design of the solar tent consisted of a transparent plastic polythene sheet stretched over a wooden box (0.6 m wide × 1.2 m long × 0.2 m high) placed on a slanting metal frame constructed to a height of 1 m on the air inlet end and 1.2 m on air exit end. The inside of the box was lined with a black polythene sheet and the air entry and exit ends were drilled with closely spaced holes of 1 cm diameter. The temperature and relative humidity in the solar tent before introducing the samples ranged between 50–60 °C and 15.5–24.5%, respectively, as determined using an EI-USB-1 data logger (Lascar electronics Inc., Pennsylvania). Duplicate samples of the dried pellets, each weighing 500 g, were taken and packed into zip-lock bags for analyses.

TABLE 1 Experimental arrangement of Box–Behnken design and the treatment combinations

	Coded variables			Actual variables		
Run	A	B	C	A	B	C
1	−1	0	−1	80	3	50
2	1	0	−1	120	3	50
3	1	0	1	120	3	150
4	0	0	0	100	3	100
5	1	1	0	120	4	100
6	−1	1	0	80	4	100
7	0	−1	−1	100	2	50
8	−1	−1	0	80	2	100
9	−1	−1	0	80	2	100
10	1	−1	0	120	2	100
11	0	0	0	100	3	100
12	0	1	1	100	4	150
13	0	1	−1	100	4	50
14	1	0	1	120	3	150
15	0	0	0	100	3	100
16	0	0	0	100	3	100
17	0	−1	−1	100	2	50
18	0	0	0	100	3	100
19	1	1	0	120	4	100
20	0	1	1	100	4	150
21	−1	0	−1	80	3	50
22	1	0	−1	120	3	50
23	−1	0	1	80	3	150
24	1	−1	0	120	2	100
25	−1	0	1	80	3	150
26	0	−1	1	100	2	150
27	−1	0	+1	80	3	150
28	+1	0	+1	120	3	150
29	0	−1	+1	100	2	150
30	0	-1	+1	100	2	150

A = Temperature (°C); B = Die diameter (mm); C = Feed pre-conditioning time (s).

TABLE 2 Proximate composition of ingredients and gross composition of formulated mash

	Composition (g/100 g dry matter basis)					
Ingredient/formulated mash	Crude protein	Crude fat	Crude fiber	Crude ash	Available carbohydrate	Inclusion level (%)
Sunflower cake	20.60 ± 0.67	21.29 ± 0.51	31.89 ± 0.74	4.78 ± 0.97	21.44 ± 1.31	19.0
Maize germ	13.81 ± 0.16	9.87 ± 0.05	16.17 ± 1.20	5.89 ± 0.14	54.26 ± 0.09	19.0
Wheat pollard	16.01 ± 0.33	8.50 ± 0.49	11.49 ± 1.34	3.44 ± 0.03	60.56 ± 0.43	28.0
Fresh water shrimps	53.98 ± 1.52	10.53 ± 1.44	4.18 ± 0.23	22.34 ± 2.02	11.97 ± 1.26	29.0
Cassava flour	1.96 ± 1.12	0.26 ± 0.20	1.9 ± 0.03	2.34 ± 0.02	93.54 ± 0.25	5.0
Formulated mash	26.77	11.37	13.66	9.59	39.49	

2.5 | Determination of expansion ratio

Expansion ratio (ER) was determined as outlined by Tumuluru (2013). For each sample, the diameter (D) of 10 randomly selected pellets were measured using a digital Vernier caliper, and their average value recorded. Expansion ratio was calculated using the expression D^2/D_i^2 where D_i is the die diameter.

2.6 | Determination of bulk density

Extruded pellets were milled using a laboratory-scale grinder and passed through a 1 mm aperture sieve. A 50 ml graduated measuring cylinder was tarred, and gently filled with 50 g of the powder. The bottom of the cylinder was repeatedly tapped gently until there was no further reduction in sample volume. Bulk density (BD) was calculated as weight of the sample divided by the respective volume (g/L).

2.7 | Determination of floatability

For each sample, 10 randomly selected pellets were put into 250 ml beakers containing 200 ml of distilled water at room temperature. This was done in three replicates and average number of pellets that were found floating after 20 min was recorded. Floatability was calculated as the number of floating pellets after 20 min divided by the total number of pellets introduced in the water multiplied by 100 (Umar, Kamarudin, & Ramezani-Fard, 2013).

2.8 | Determination of pellet durability index

The method described by Umar et al. (2013) was used. About 15 g of each sample was sieved on a 2.36 mm sieve in triplicate. The pellets that were retained on the sieve were weighed (W_i) and placed in a flask mounted on a Lab-line shaker (Lab-Line Instruments, Inc, Illinois), which was then shaken for 20 min at 260 oscillations per min. The

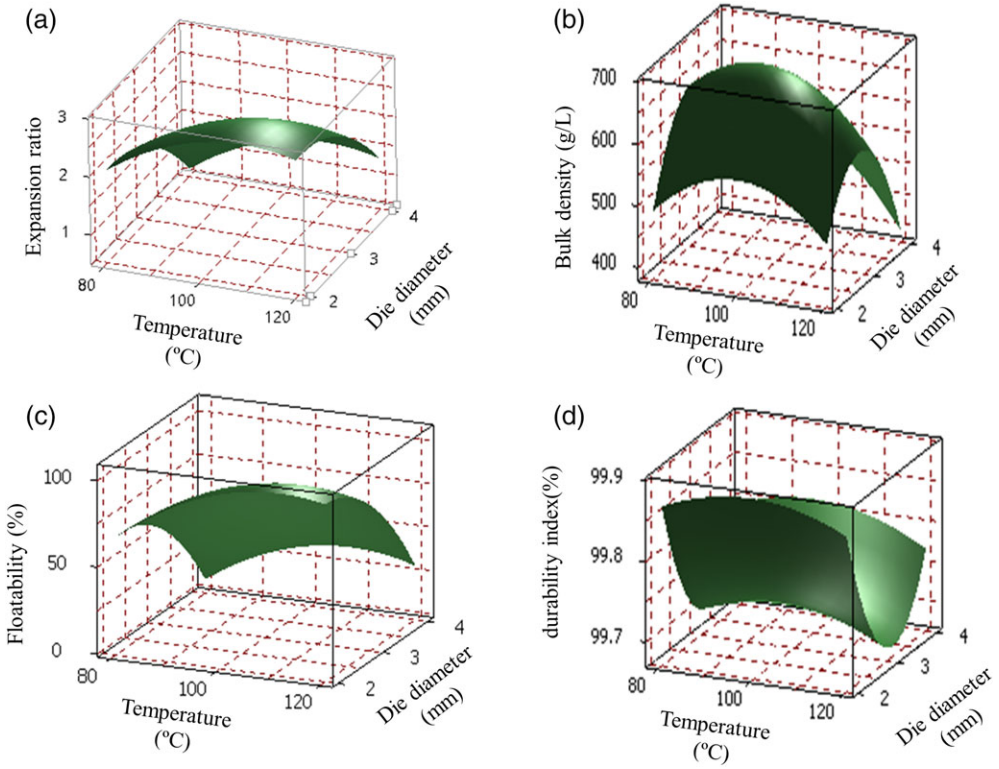

FIGURE 2 Surface plots showing the effects of temperature and die diameter at constant feed conditioning time of 100 s on expansion ratio (a), bulk density (b), floatability (c), and durability index (d)

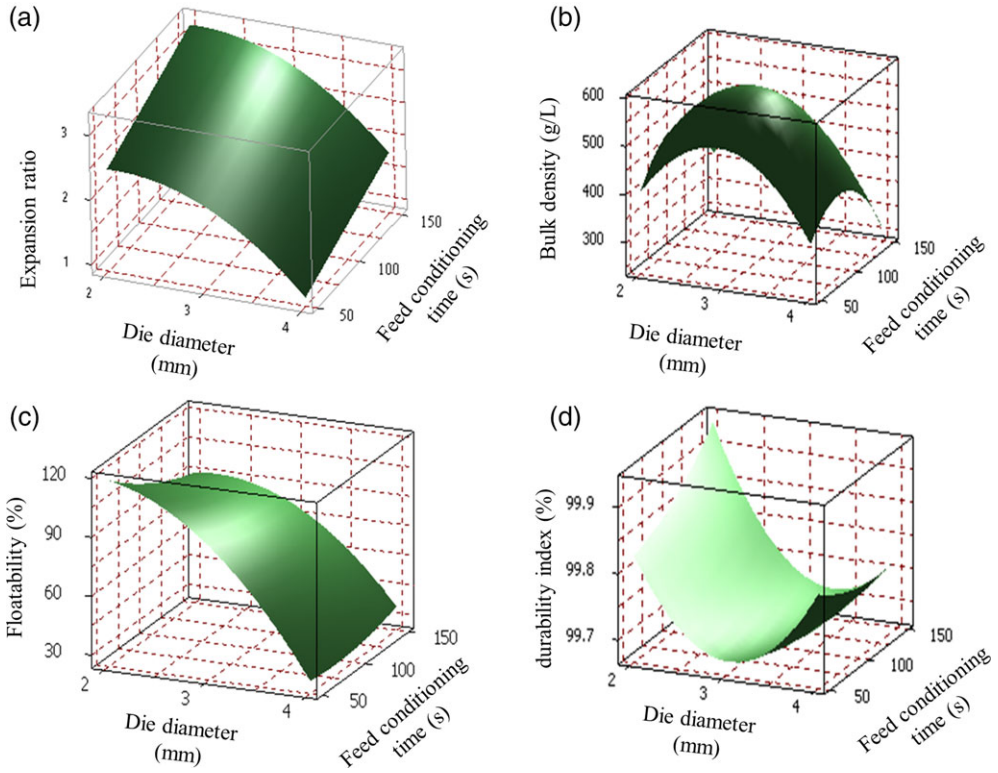

FIGURE 3 Surface plots showing the effects of die diameter and feed conditioning time at constant barrel temperature of 120 °C on expansion ratio (a), bulk density (b), floatability (c), and durability index (d)

pellets were then sieved and re-weighed (W_r) and the pellet durability index (PDI) calculated as $(W_r/W_i) \times 100$.

2.9 | Determination of water absorption index and water solubility index

Water absorption index (WAI) and water solubility index (WSI) were determined as described by Rosentrater, Muthukumarappan, and Kannadhason (2009). About 0.625 g of each sample (W_i) was ground and suspended in 8 ml distilled water in a tarred 12 mL centrifuge tube. The contents were shaken vigorously for 3 min and then centrifuged (using a bench-top DSC-200T centrifuge (Digisystem Laboratory Instruments Inc, Taipei, Taiwan) at 2,500 rpm for 10 min. The supernatant was decanted and transferred into a tarred aluminum dish and placed in a hot air oven (DAIHAN Scientific, Gangwon-do, Korea) maintained at 135 °C for 2 hr. The dish and its contents was cooled in a desiccator and re-weighed on a sensitive weighing scale (Shinko Denshi, Tokyo, Japan), and the difference in weight (W_s) obtained. The mass of the gel remaining in the centrifuge tube (W_g) was obtained as well. The WAI and WSI were calculated using the expressions (W_g/W_i) and $(W_s/W_i) \times 100$, respectively.

2.10 | Determination of water stability

The procedure described by Umar et al. (2013) was used. About 4 g of each sample was weighed and put on a 0.5 mm wire mesh screen in three replicates. The screen with the sample was immersed into a 250 ml beaker containing 200 ml distilled water for 20 min. The pellets retained on the wire mesh were then dried in a hot-air oven (105 °C) for 24 hr. The percent ratio of weight of pellets retained on the wire mesh to the initial weight gave the water stability (WS).

2.11 | Determination of in vitro protein digestibility

The procedures outlined by March and Hickling (1982) and Fenerci and Şener (2005) were used with modifications. About 0.2 g of ground sample was weighed into a 50 mL centrifuge tube. Exactly 15 mL of 0.1 mol/L hydrochloric acid solution containing 0.02% sodium azide and 1.5 mg pepsin from porcine gastric mucosa (P7000; activity ≥250 units/mg solid, Sigma-Aldrich, Schnelldorf, Germany) was added, and the tube incubated in a shaking water bath (Model; WSB-30, Witeg Labortechnink GmbH, Wertheim, Germany) maintained at 15 °C for 3 hr. A control preparation was incubated without a sample. Samples and the control treatments were then centrifuged at 5,000*g* for 20 min at room temperature, and the supernatant carefully separated from the sediment. Nitrogen contents of the supernatants of the sample (N_s) and control (N_o) treatments were determined by the Kjeldahl method according to the AOAC Method 984.13, using a DK 20 digester and UDK 129 distillation unit (VELP Scientifica, Usmate, Italy). Nitrogen content of the undigested sample (N) was also determined. in vitro protein digestibility (IVPD) was determined by the expression $(N_s - N_o)/N \times 100$.

2.12 | Statistical analysis

Data on expansion ratio, bulk density, floatability, pellet durability index, water absorption index, water solubility index, water stability, and in vitro protein digestibility were analyzed using multiple regression analysis on Minitab 14.12.0. Each response variable was fitted to

TABLE 3 Properties of extrudates processed under treatment combinations of the various runs

	Response variables							
Run	Y_1 (%)	Y_2 (–)	Y_3 (g/L)	Y_4 (%)	Y_5 (–)	Y_6 (%)	Y_7 (%)	Y_8 (%)
1	27.50	1.72	489.38	99.76	3.88	11.62	75.11	65.22
2	87.50	2.50	494.94	99.68	4.06	11.92	82.52	65.77
3	85.00	1.71	430.40	99.71	4.22	7.71	81.33	65.55
4	80.00	1.16	761.64	99.73	4.20	8.75	75.99	63.57
5	22.50	2.14	409.32	99.77	3.86	11.95	82.07	60.93
6	10.00	1.15	500.03	99.79	3.98	8.03	81.56	61.82
7	100.00	2.64	431.92	99.88	4.00	10.31	83.62	73.28
8	80.00	2.54	470.98	99.87	3.80	13.29	82.03	63.89
9	65.00	2.12	477.54	99.86	3.79	13.22	79.87	63.99
10	97.50	1.62	499.96	99.86	4.11	9.07	86.75	64.22
11	77.50	2.44	643.11	99.69	4.19	9.26	74.72	62.17
12	57.50	1.24	413.29	99.79	4.15	6.68	84.28	66.97
13	10.00	1.18	451.05	99.88	3.91	7.65	80.45	64.93
14	85.00	1.29	464.00	99.73	4.16	8.91	82.93	65.56
15	85.00	1.97	634.82	99.73	4.17	7.89	76.61	61.34
16	79.50	1.59	676.16	99.71	4.19	8.79	78.09	64.46
17	100.00	2.13	461.99	99.87	3.98	9.50	82.31	70.26
18	97.50	1.79	712.48	99.73	4.17	8.95	75.73	63.43
19	22.50	1.67	409.32	99.77	3.86	11.95	82.07	60.94
20	52.50	1.95	426.11	99.79	4.13	7.59	84.31	68.18
21	27.50	1.17	474.23	99.72	3.91	11.98	76.51	65.29
22	100.00	2.58	491.00	99.69	4.07	11.29	84.69	65.58
23	75.00	1.74	655.98	99.70	4.07	11.09	83.83	58.62
24	100.00	1.17	477.33	99.86	4.14	7.52	85.78	63.04
25	80.00	2.68	599.69	99.69	4.02	11.15	84.18	57.89
26	100.00	1.37	477.89	99.96	4.06	6.66	86.09	57.67
27	97.50	1.25	486.30	99.93	4.06	6.91	85.24	57.98
28	5.00	2.50	499.06	99.80	3.94	7.74	83.33	62.25
29	77.50	2.76	687.68	99.73	4.13	8.95	77.80	63.28
30	35.00	2.78	424.41	99.85	3.87	8.07	80.00	64.49

Y_1 = Floatability, Y_2 = Expansion ratio; Y_3 = Bulk density; Y_4 = Pellet durability index, Y_5 = Water absorption index; Y_6 = Water solubility index; Y_7 = Water stability; Y_8 = In-vitro protein digestibility.

a second order model expressed with the coded variables (*A*, *B*, and *C*) with the following equation;

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \varepsilon;$$

where, Y is the estimated response, β_0 is the constant term, β_i are the linear terms, β_{ii} represents the quadratic terms for a single variable, β_{ij} are the interaction terms (i = 1–3 and j = 1–3), and ε is the random error. Response surfaces were plotted as a function of two independent variables while keeping the other independent variable at the optimal value.

3 | RESULTS AND DISCUSSION

3.1 | Composition of formulated feed blend

Proximate compositions of the various raw ingredients are presented in Table 2. Gross composition of the formulated blend comprised 26.77% protein, 11.37% fat, 13.66% fiber, 9.59% ash, and 39.49% carbohydrate. This composition corresponded well with requirements for tilapia fish (FAO, 2017).

3.2 | Effects on expansion ratio, bulk density, floatability, and durability of pellets

Expansion ratio is a measure of how extrudates puff at the die exit. Expansion affects bulk density and floatability and to some extent the durability of pellets. Figures 2 and 3 show surface plots for pellet expansion ratio, bulk density, floatability and durability as influenced by temperature, die diameter, and feed conditioning time. The measured values of response variables are presented in Table 3, and analyses of variances given in Table 4. The surface plot models, after excluding the insignificant terms, are presented in Table 6. All the models were highly significant ($p < 0.01$). Increasing extrusion temperature resulted in higher extrudate expansion whereas increasing die diameter decreased rate of expansion at constant feed conditioning time of 100 s (Figure 2a). Likewise, increasing feed conditioning time

TABLE 4 Analysis of variance for floatability, expansion ratio, bulk density and durability index

		Expansion ratio		Bulk density		Floatability		Durability index	
Source of variation	Df	Ms	*F* value	Ms	*F* value	Ms	*F* value	Ms	*F* value
Regression	9	0.958	28.90***	30,896.9	33.49***	3,121.39	50.68***	0.02	111.64***
Linear	3	0.237	7.15**	60,990.2	66.11***	987.7	16.04***	0.03	143.08***
Square	3	0.288	8.68**	76,786	83.23***	1,295.67	21.04***	0.05	257.68***
Interaction	3	0.213	6.41**	8,426.3	9.13**	786.2	12.77**	0	26.04***
Lack of fit	3	0.072	2.72^{ns}	1,332.5	1.57^{ns}	129.69	2.62^{ns}	0	0.02^{ns}
Pure error	17	0.026		850.3		49.57		0	
Total	29								
R^2			0.93		0.94		0.96		0.98
R^2-Adj			0.90		0.91		0.94		0.97
s			0.18		30.37		7.85		0.01

ms = mean square; s = standard error of the regression; *** significant at $p < 0.001$; ** significant at $p < 0.01$; *significant at $p < 0.05$; ^{ns} not significant at $p < 0.05$.

resulted in higher expansion (Figure 3a). The expansion of extrudates starts to occur at approximately 100 °C when starch has gelatinized and viscosity of the melt has considerably decreased (Majumdar & Singh, 2014; Rokey et al., 2010; Rosentrater et al., 2009). Furthermore, higher barrel temperature results in high pressure at the die, which leads to greater extrudate expansion (Meng, Threinen, Hansen, & Driedger, 2010). But as the temperature is increased further, viscosity of the melt continues to decrease and the material tends to expands more longitudinally while cross-sectional expansion decreases (Singh, Majumdar, & Venkateshwarlu, 2014), which explains tendency of expansion ratio to decrease. Increasing dextrinization as well as weakening of the dough structure could also lower expansion as processing temperature is increased further (Rosentrater et al., 2009). Our findings agree with those of other studies that reported an increase in expansion ratio with increasing barrel temperature (Badrie & Mellowes, 1991; Jozinovic et al., 2013; Peluola-Adeyemi, Idowu, Sanni, & Bodunde, 2014; Tumuluru, 2013). The increase in expansion ratio with longer conditioning times is due to moistening of the feed, which promotes starch gelatinization (Adeparusi & Famurewa, 2011; Rokey et al., 2010), whereas the decrease in expansion with increasing die diameter can be attributed to reduced pressure within the extrusion barrel (Singh & Muthukumarappan, 2014) as well as increase in melt viscosity (Akdogan, 1999). The two phenomena cause less puffing effect as the extrudate exits the die.

Increasing barrel temperature, die diameter, and feed pre-conditioning time had curvilinear effects on bulk density; higher bulk density was recorded close to the mid-levels of these factors (Figures 2b and 3b; Table 4). Bulk density accounts for expansion of the product in all directions and a low bulk density is desirable for the extrudates to float in water. Generally, bulk density would decrease with

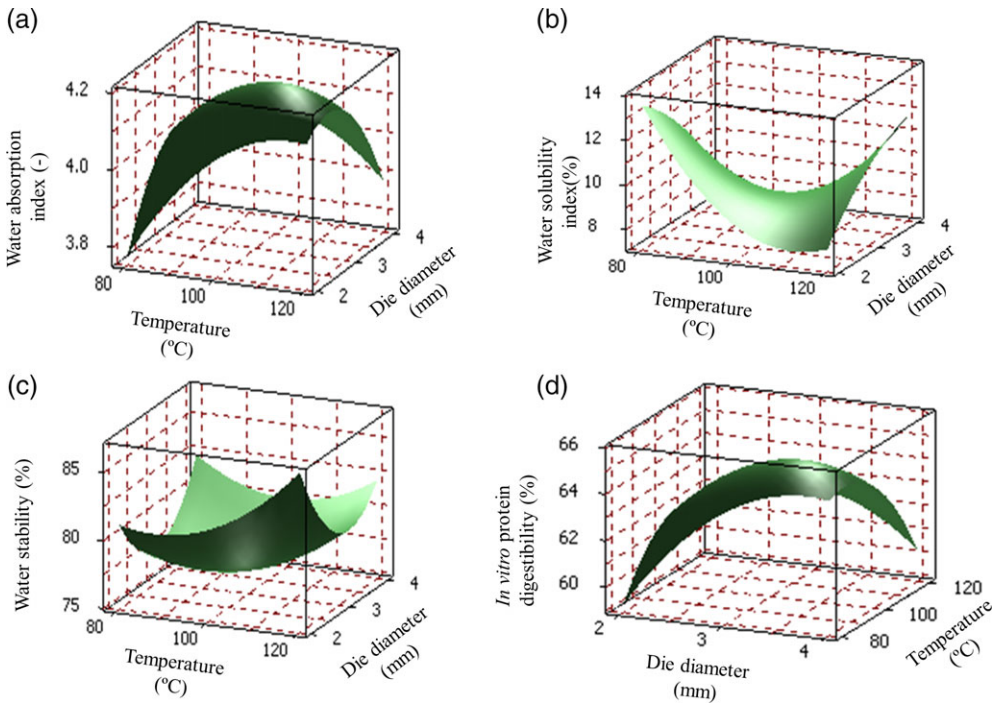

FIGURE 4 Surface plots for the effects of temperature and die diameter at constant feed conditioning time of 100 s on water absorption index (a), water solubility index (b), water stability (c), and in-vitro protein digestibility (d)

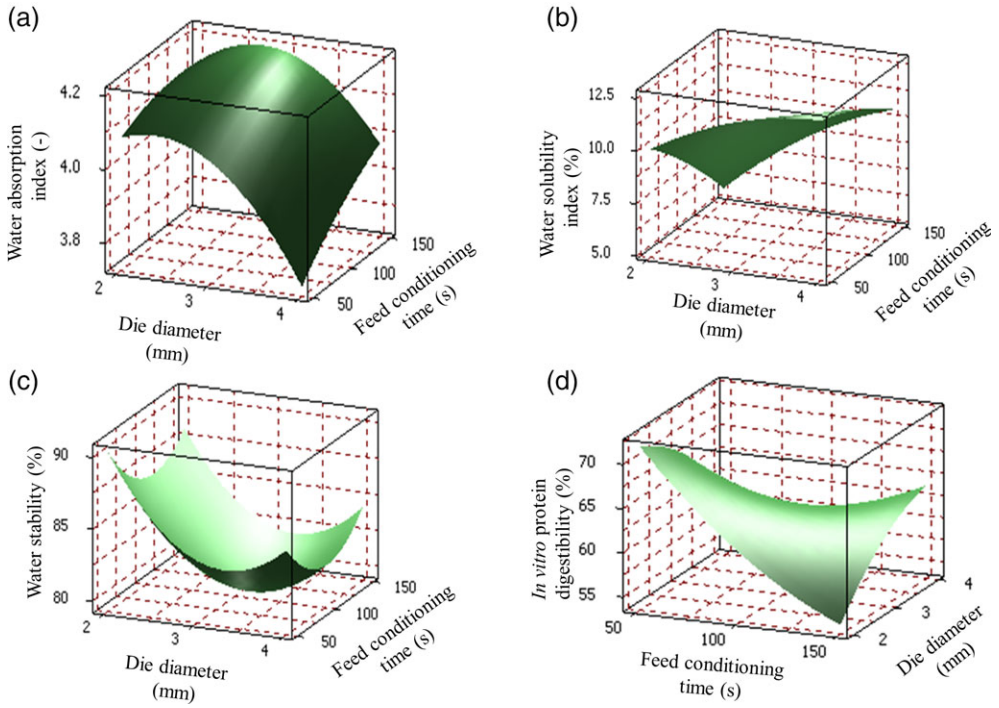

FIGURE 5 Surface plots for the effects of die diameter and feed conditioning time (s) at constant barrel temperature of 120 °C on water absorption index (a), water solubility index (b), water stability (c), and in-vitro protein digestibility (d)

increase in temperature due to decreased melt viscosity that encourages bubble growth and greater expansion of the product (Giri & Bandyopadhyay, 2000; Meng et al., 2010; Singh & Muthukumarappan, 2016). However, some other studies reported an increase in bulk density where extrusion conditions favor longitudinal expansion as opposed to cross-section expansion as already mentioned (Singh et al., 2014).

Increasing extrusion temperature increased floatability whereas increasing die-diameter decreased it (Figure 2c). Increasing the feed conditioning time increased floatability only marginally (Figure 3c). Depending on composition, it has been found that extrudates do not typically expand until temperature approaches approximately 100 °C (Rosentrater et al., 2009). As with expansion, floatability of extrudates increased as the extruding temperature increased (Foley & Rosentrater, 2013). Adeparusi and Famurewa (2011) also found a positive correlation between floatability and temperature and feed conditioning time. Saalah, Shapawi, Othman, and Bono (2010) have, however, reported a trend where temperature did not affect floatability of fish feed, an observation that was probably due to the overall composition of the feed used in their study. With respect to die diameter, a small die diameter restricts extrudate exit, hence higher pressure is developed within the extrusion barrel resulting in greater expansion and therefore higher floatability (Vijayagopal, 2004).

Pellet durability index (PDI) is a measure of how strong the pellets can withstand mechanical handling during transportation, storage

TABLE 5 Analysis of variance for water absorption index, water solubility index, water stability, and in-vitro protein digestibility

		Water absorption		Water solubility		Water stability		Protein digestibility	
Source of variation	*df*	ms	*F* value	ms	*F* value	ms	*F* value	ms	*F* value
Regression	9	0.05	80.70***	12.18	46.04***	38.0	39.29***	32.84***	12.95
Linear	3	0.09	136.49***	19.11	72.27***	17.13	17.70***	57.79***	22.79
Square	3	0.07	102.13***	13.53	51.15***	63.92	66.06***	12.2**	4.82
Interaction	3	0.04	55.40***	15.67	59.25***	21.29	22.00***	55.09***	21.72
Lack of fit	3	0.00	2.41^{ns}	0.37	1.52^{ns}	0.24	0.22^{ns}	12.68**	17
Pure error	17	0.00		0.25		1.09		0.75	
Total	29								
R^2			0.97		0.95		0.95	0.85	
R^2-Adj			0.96		0.93		0.92	0.79	
S			0.03		0.51		0.98	1.59	

ms = mean square; s = standard error of the regression; *** significant at $p < 0.001$; ** significant at $p < 0.01$; *significant at $p < 0.05$; ^{ns} not significant at $p < 0.05$.

TABLE 6 Explanatory equations for various pellet properties after excluding the insignificant terms

Model equation	R^2 (%)	R^2-Adj (%)	s
$Y_1 = 549.7 + 9.98A + 55.1B + 1.144C - 0.039A^2 - 17.46B^2 - 0.015AC + 0.169BC$	95.3	93.8	7.93
$Y_2 = -8.57 + 0.1905A + 1.234B - 0.01508C - 0.0008A^2 - 0.01246B^2 - 0.01055AB + 0.0002AC$	92.86	90.59	0.17
$Y_3 = 686.0 - 30.66A - 76.1A^2 - 141.9B^2 - 97.4C^2 - 26.2AB - 48.0AC$	90.8	88.4	34.42
$Y_4 = +99.72 - 0.041B - 0.02813A^2 + 0.131B^2 + 0.01813C^2 + 0.0200AC - 0.03625BC$	97.4	96.7	0.01
$Y_5 = -1.54438 + 0.0723A + 1.23875B + 0.00044C - 0.00026A^2 - 0.13438B^2 - 0.00001C^2 - 0.00538AB + 0.00090BC$	97.3	96.2	0.03
$Y_6 = +95.00 - 1.399A - 9.40B + 0.0099C + 0.0055A^2 - 0.585B^2 + 0.11281AB - 0.0007AC + 0.0120BC$	95.0	93.1	0.52
$Y_7 = +128.2 - 0.613A - 18.766B + 0.065C + 0.006A^2 + 4.171B^2 + 0.001C^2 - 0.071AB - 0.002AC$	94.4	92.2	0.98
$Y_8 = +62.809 - 2.275C + 1.768C^2 + 1.720AC + 4.203BC$	78.3	74.8	1.73

Y_1 = Floatability; Y_2 = Expansion ratio; Y_3 = Bulk density; Y_4 = Pellet durability index, Y_5 = Water absorption index; Y_6 = Water solubility index; Y_7 = Water stability; Y_8 = in-vitro protein digestibility; R^2 = Co-efficient of determination; R^2-Adj = Adjusted co-efficient of determination; s = Standard error of the regression; A = Extrusion temperature; B = Die diameter; C = Feed conditioning time.

and subsequent use. A high PDI is desirable to both manufacturers and farmers (Ayadi, Muthukumarappan, Rosentrater, & Brown, 2011). Figures 2d and 3d show surface plots for PDI as influenced by temperature, die diameter and feed conditioning time. The effect of temperature was curvilinear. Highest pellet durability was achieved at extrusion temperature of 100 °C, while temperatures of 80 and 120 °C resulted in lower pellet durability. Increasing the feed conditioning time resulted in lower PDI (Figure 3d) whereas a die diameter of 3 mm gave the lowest PDI. Pellet durability is a function of starch gelatinization whereby the molten polymer binds the other ingredients upon setting (Chevanan, Muthukumarappan, & Rosentrater, 2009). Extrusion temperature of 100 °C is close to the peak gelatinization temperature of most starches (Chiu & Solarek, 2009). The decrease in PDI with increased time of feed conditioning is unclear because increased moisture should encourage gelatinization of starch. However, it can be argued that too high moisture results in lower shearing effect within the extruder barrel as a result of excessive plasticization. Sitaula (2012) also reported a decrease in PDI with the inclusion of steam conditioning. Nonetheless all pellets obtained in this study had PDI higher than 99%, which could be attributed to the high carbohydrate content in the feed blend (Chiu & Solarek, 2009) and the addition of cassava starch as binder. According to Tumuluru, Conner, and Hoover (2016), binders added at about 4% inclusion results into PDI of over 98%.

3.3 | Effects on water absorption, water solubility, and water stability

Figures 4 and 5 show surface plots for water absorption index, water solubility index, and water stability at the various levels of temperature, die diameter, and feed conditioning time. The analyses of variances are given in Table 5 and the surface plot models are given in Table 6. Water absorption increased in a curvilinear manner as extrusion temperature and feed conditioning time were increased (Figures 4a and 5a) suggesting effects of enhanced starch gelatinization and probably other structural modifications involving fiber or protein during extrusion. These modifications, seemingly, became less intense with increasing die diameter (Figures 4a and 5a). Water solubility decreased with increasing temperature as well as with increasing feed conditioning time (Figures 4b and 5b) but increased as the size of the die was made bigger (Figures 4b and 5b). The findings point to an inverse relationship between WAI and WSI which is also reported by other researchers (Chevanan, Muthukumarappan, Rosentrater, & Julson, 2007; Fallahi, Muthukumarappan, & Rosentrater, 2012; Gui, Gil, & Ryu, 2012; Rosentrater et al., 2009; Singh & Muthukumarappan, 2014).

TABLE 7 Pearson's correlation coefficients of response variables (n = 30)

	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8
Y_1	0.814[ab]	0.311	0.039	0.572[a]	−0.085	0.218	0.092
Y_2		0.194	0.265	0.575[a]	−0.334	0.177	0.096
Y_3			−0.484[a]	0.511[a]	−0.052	−0.535[a]	−0.327
Y_4				−0.374[b]	−0.275	0.457[b]	0.049
Y_5					−0.582[a]	−0.031	0.019
Y_6						−0.168	0.042
Y_7							−0.067

Y_1 = Floatability, Y_2 = Expansion ratio; Y_3 = Bulk density; Y_4 = Pellet durability index, Y_5 = Water absorption index; Y_6 = Water solubility index; Y_7 = Water stability; Y_8 = in-vitro protein digestibility.
[a] Correlation is significant at the 0.01 level.
[b] Correlation is significant at the 0.05 level.

Water stability is a measure of how strongly the extruded pellets will resist disintegration and thus disallow leaching of nutrients when placed in water (Ayadi et al., 2011). Water stability increased with increasing temperature (Figure 4c). Curvilinear relationships between water stability and die diameter (Figures 4c and 5c) as well as pre-conditioning time (Figure 5c) were observed. Generally, pellets produced under the experimental conditions of the present study exhibited high water stability ranging from 75–87% (Table 3), which is the consequence of strong starch–protein matrix formation from the interaction of the gelatinized starch and denatured protein (Tumuluru, 2013). Increasing extrusion temperature enhanced the formation of such matrix (Vijayagopal, 2004; Bandyopadhyay & Rout, 2001). The curvilinear effects observed with respect to die diameter and conditioning time may be related to the interaction effects of temperature, pre-conditioning, and pressure within the barrel, which affect modification of the polymers, and the viscosity and integrity of the melt as it pushed through the die. Water stability of extruded pellets might decrease when die diameter is increased, partly because melt viscosity increases (Akdogan, 1999). Water stability may also increase with increased conditioning but decrease if the conditioning results in moisture level that diminishes cohesive strength of polymers due to excessive plasticization. The high water stability of pellets reported in this study implies that the products would exhibit minimum nutrient loss and environmental problems in fish ponds.

3.4 | Effects on in-vitro protein digestibility

Protein is the most important nutrient for growth of fish, and the digestibility is influenced by processing conditions (Aksnes, Hjertnes, & Opstvedt, 1996). Extrusion improves IVPD of feeds in comparison to feeds produced through pelletization (Fenerci & Şener, 2005). In the present study, in vitro protein digestibility of pellets ranged between 59 and 73% (Table 3). These results correspond well with those reported by Fenerci and Şener (2005). Surface plots showing the effects of die diameter, temperature and feed conditioning are shown in Figures 4d and 5d. The linear, square and interaction components of the various factors were significant (Table 5). Increasing die diameter from 2 to 4 mm resulted in increasing IVPD (Figure 4d). Increasing temperature from 80 to approximately 100 °C also resulted in increasing IVPD but further increase to 120 °C resulted in decreased IVPD (Figure 4d). The initial increase in IVPD with increasing extrusion temperature could be attributed to protein denaturation which exposes more polypeptide bonds to enzymes (Opstvedt et al., 2003) and probably also the degradation of enzyme-specific inhibitors. On the other hand, the decrease in IVPD as temperature was increased from 100 to 120 °C could be linked to formation of complexes that resist hydrolysis such as those involving covalent binding of proteins and polyphenolic compounds present in the feed ingredients (Opstvedt et al., 2003) or the formation of disulfide linkages within the matrix (Kinyuru, Kenji, Njoroge, & Ayieko, 2010). However, the dynamics of these events may depend on the intrinsic forces contributing to conformational stability of proteins from different sources. Increasing the feed conditioning time decreased IVPD (Figure 5d) which could be due to the ability of high amount of steam to promote protein–polyphenol complexes at high extrusion temperatures (Onyango, Noetzold, Bley, & Henle, 2004).

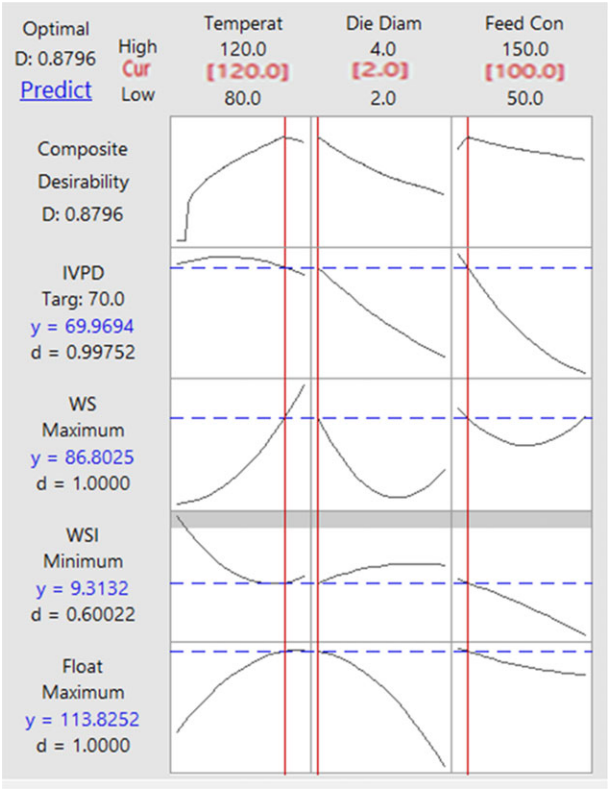

FIGURE 6 Optimization plot for in vitro protein digestibility (IVPD), water stability (WS), water solubility index (WSI), and floatability with respect to temperature (mm) and feed conditioning time (s)

3.5 | Correlation between response variables

Table 7 shows the correlation between the various measured variables. As expected there was significant direct correlation between expansion ratio and floatability (Umar et al., 2013; Vijayagopal, 2004). From this observation, an inverse relationship between expansion ratio and bulk density would be expected (Majumdar & Singh, 2014) but this was not observed in the present case probably because of effects of the ingredients used. Nonetheless, there was significant inverse relationship between bulk density and pellet durability as well as water stability, which is explained by the fact that extrudates with high bulk density are linked to lesser thermal or shear modification of constituent polymers and so have weaker binding of the ingredients. Furthermore, the significant direct correlation between pellet durability and water absorption index can be linked to more intense polymer modifications involving starch gelatinization, protein denaturation or even swelling of fibers. Water solubility was inversely related to water absorption as also reported by others (Fallahi et al., 2012; Singh & Muthukumarappan, 2014). This relationship is related to polymer modifications that increase the capacity to hold water while at the same time entangling soluble molecular components and stabilizing them within the molten mass. Water soluble ingredients weaken pellet structure in water allowing components to separate thereby affecting feed utilization. Moreover decomposition of ingredients not

eaten by the fish may increase nitrogenous waste in the water which is toxic to fish.

3.6 | Optimum conditions for production of floating pellets

Optimal conditions for processing good quality floating fish pellets were established using the graphical method of response surface methodology with the aim of obtaining extrudates with most desirable properties. The main criteria involved maximizing water stability and floatability, minimizing water solubility index and targeting IVPD of about 70%. Figure 6 shows the final optimized plots for the response optimization of extrusion conditions (temperature, die diameter and feed conditioning time) in making floating fish feeds. The optimized responses for IVPD, water stability, water solubility index, and floatability gave higher desirability values of 0.9972, 1.0000, 0.6002, and 1.0000, respectively. The optimum extrusion conditions of temperature, die diameter, and feed conditioning were identified as 120 °C, 2 mm, and 100 s, respectively. The optimum values had a composite desirability of 0.8796 which is acceptable though it implies that other than the study parameters, there are other factors such as feed composition and rate of feeding (not within the scope of this study) that probably also affected the physico-chemical properties of extruded fish feeds.

4 | CONCLUSION

Physical properties that relate to floating, stability in water, and stability during handling are especially critical for aquafeeds. This study was conducted to optimize extruder conditions for the manufacture of fish feeds with desirable physico-chemical properties. The target study variables were temperature, die diameter and feed conditioning time, while the response parameters were floatability, expansion ratio, bulk density, pellet durability index, water absorption index, water solubility index, water stability, and IVPD. Response surface methodology gave optimum treatment combinations of temperature, die diameter, and feed conditioning time of 120 °C, 2 mm, and 100 s, respectively. One limitation of extrusion is that it may lead to loss of vitamins particularly the water soluble and heat sensitive ones where about 15–20% of the vitamins in the raw materials are lost during extrusion. Further studies should explore ways of addressing this limitation.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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