

# Effect of Different Extrusion Processing Parameters on Physical Properties of Soy White Flakes and High Protein Distillers Dried Grains-Based Extruded Aquafeeds

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## Abstract

Nutritionally balanced ingredient blends for catla (*Catla catla*), belonging to the family Cyprinidae, were extruded using single screw extruder. The extrusion was carried out at five levels of soy white flakes content (21%, 29%, 40%, 52%, and 59% db), five levels of moisture content (15, 19, 25, 31, and 35% db) and five levels of barrel temperature (100, 110, 125, 140, and 150 °C) using three different die nozzles (having L/D ratios 3.33, 5.83, and 7.25). Blends with net protein content of 32.5% contains soy white flakes, along with high protein distillers dried grains (HP-DDG), corn flour, corn gluten meal, fish meal, vitamin, and mineral mix. A central composite rotatable design (CCRD) and response surface methodology (RSM) was used to investigate the significance of independent and interaction effects of the extrusion process variables on the extrudates physical properties namely pellet durability index, bulk density, water absorption and solubility indices and expansion ratio. Quadratic polynomial regression equations were developed to correlate the product responses and process variables as well as to obtain the response surfaces plots. The independent variables had significant ( $P < 0.05$ ) effects on physical properties of extrudates: (i) higher soy white flakes content increased the pellet durability index and water absorption index, but decreased the water solubility index, (ii) higher temperature decreased pellet durability index, bulk density and water solubility index, (iii) increased L/D ratio from 3.33 to 7.25 increased the pellet durability index, expansion ratio but decreased the bulk density of the extrudates.

**Keywords:** bulk density, die, extrusion, pellet durability index, unit density, soy white flakes

## 1. Introduction

In the food producing industries, aquaculture is one of the fastest growing sectors (FAO, 2012) and plays a pivotal role for the maintenance of commercial fishery markets (O'Mahoney et al., 2011). In aquaculture, diet is often the single largest operating cost item and can represent over 50% of the operating costs in intensive aquaculture (El-Sayed, 1999, 2004). Protein is the most important nutrient of the fish feed. The main protein source used in aquafeed production is primarily fish meal which is supplied through the consumption of wild fish stocks. Indubitably, with the increasing rate of farmed fish production (FAO, 2008) and consequently rising prices of fishmeal (Hardy, 2010), the continued use of fishmeal as the main protein source of the feed will no longer be ecologically and economically sustainable in the long run. Therefore, aquaculture industry now is focusing on alternative protein sources such as plant proteins as inexpensive source of protein to minimize production cost.

Soy white flakes and High Protein - Distiller Dried Grains (HP-DDG) contain significant amount of protein and are thus a possible alternative source of protein for aquaculture feeds (Chin et al., 1989; Wu et al., 1994, 1996). Use of soy products like full fatted soybean meal, defatted toasted soybean meal (SBM) and defatted untoasted soybean meal or soy white flakes is becoming common (Fallahi et al., 2012). Romarheim et al. (2005) found that extrusion of soy white flakes diet increased the digestibility of protein and all amino acids compared to the unextruded soy white flakes diet probably due to the reduction in trypsin inhibitor activity. Dersjant-Li, (2002) reported that soy protein isolate can be used to replace 40-100% fish meal without negative impact on growth performance of shrimp. Distillers Dried Grains (DDG) and Distillers Dried Grains with Solubles (DDGS), a co-product from corn-based dry grind fuel ethanol manufacturing, is also a viable protein source. Research

carried out by Wu et al. (1994, 1996a) indicated that tilapia fish can be grown with DDGS, and can improve the economic viability of aquaculture farms.

Extrusion cooking is widely used in the food and feed industries because of versatility during processing and the ability to produce various final textural properties (Mercier et al., 1989). Extruded aquafeed are designed to float or sink based on the fish species requirement. One of the important quality parameters for fish feed is floatability (Bandyopadhyay & Ranjan 2001; Rolfe et al., 2001) which depends on the unit density of extrudates. During extrusion cooking, the extent of expansion affects the unit density of the extrudates. Expansion can be monitored by changing the nature and type of ingredients used and the extruder process parameters. In the food industry, puffed products are often produced by using starch based ingredients, while texturized products are often produced by using protein based ingredients (Kokini et al., 1992a, 1992b). Extrudate properties of starch based products depend on the extent of gelatinization occurring inside the extruder barrel. The formation of elastic melt inside the barrel depends on the extent of gelatinization. (Case et al., 1992; Lin et al., 2000; Ilo et al., 1996; Ibanoglu et al., 1996; Sokhey et al., 1994). Expansion occurs due to the flashing of water vapor when the elastic melt exits through the die nozzle. (Lam & Flores, 2003; Alves et al., 1999). On the other hand, ingredients with higher protein content shows limited degree of expansion due to plastic melt formation and protein denaturation inside the extruder barrel. The material is in plastic and homogeneous state and when it exits through the die nozzle there is a sudden pressure drop resulting in the formation of voids. Due to this void formation the final product becomes more porous and fibrous textured (Singh et al., 1991; Gwiazda et al., 1987; Sandra & Jose, 1993). Depending on the type of species, aquaculture feed requires 26 to 50% protein content. (Lovell, 1988).

Extrusion process depends on many factors which includes the pressure developed inside the die and the degree to which the screw is filled. These variables in combination with the type and composition of raw ingredients used, affects operational capabilities (Mercier et al., 1989). Extruder die too have an impact on the processing conditions. For example, in case of circular dies, nozzle dimensions (i.e., nozzle diameter and length) will affect process conditions and performance (Chinnaswamy et al., 1987).

The objective of this study was to examine the effect of varying level of soy white flakes as the fish meal replacer, barrel temperature, die aspect ratio, and moisture content on physical properties of soy white flakes and HP-DDG based extrudates.

## **2. Materials and Methods**

### *2.1 Blends Preparation*

Five isocaloric (302 kcal/100g) different blends were adjusted to a target protein content of ~ 32.5% db and a target fat content of ~ 3.5%. The total energy content for each blend was determined based on the fraction of protein, lipid and carbohydrate contributing to the dietary energy. The total energy content was calculated based on the energy content of fractions namely, 4.5 kcal/g for protein, 9.1 kcal/g for lipid and 4.1 kcal/g for carbohydrate. The ingredient components of the feed blends are provided in Table 1. Soy white flakes were kindly donated by South Dakota Soybean Processors, Volga, SD. HP-DDG was obtained from the Dakota Ethanol LLC (Wentworth, SD). Corn gluten meal and fishmeal were purchased from Consumer Supply Distributing Co. (Sioux City, IA). Corn flour was from Cargill Dry Ingredients (Paris, IL). Vitamin and mineral premix was obtained from Lortscher Agri Service, Inc. (Bern, Kansas, USA). Soybean oil was provided from USDA (Brookings, SD). The different ingredients were mixed in a laboratory model Hobart mixer (Hobart Corporation, Troy, Ohio, USA) for 10 minutes; the moisture content of the ingredient mix was adjusted by adding required quantities of water during mixing. The resulting blends were then stored at ambient temperature overnight until processing.

Table 1. Ingredient composition of feed blends

Ingredients	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5
Soy white flakes	21	29	40	52	59
HP-DDG	39	32	20	9	1
Corn gluten meal	2	2	2	2	2
Corn flour	30	30	30	30	30
Fish meal	5	5	5	5	5
Soybean oil	1	1	1	1	1
Vitamin & mineral mix	2	2	2	2	2
Total	100	100	100	100	100

## 2.2 Extrusion Processing

Extrusion experiments were performed using a single screw extruder (BrabenderPlasti-Corder, Model PL 2000, South Hackensack, NJ), which was powered by a 7.5 hp motor with an operating range of screw speeds from 0-210 rpm. The extruder had a barrel length-to-diameter ratio of 20:1 and a barrel diameter of 19 mm. A uniform 19.05 mm pitch screw with compression ratio of 3:1 was used in the experiments (Figure 1). The screw had a variable flute depth, with a depth at the feed portion of 19.05 mm, and near the die of 3.81 mm. The raw materials were fed manually to the extruder in constant quantities. Experiments were conducted using five levels of soy white flakes (21, 29, 40, 52, and 59% db), five levels of temperature gradient in the barrel (45-100-100 °C, 45-110-110 °C, 45-125-125 °C, 45-140-140 °C, and 45-150-150 °C) hereafter referred as temperature of 100, 110, 125, 140, and 150 °C, and five levels of moisture content (15, 19, 25, 31, and 35% db), for three different die nozzles with various L/D ratios (3.33, 5.83 and 7.25) (Table 2). During the experiment the screw speed of extruder was maintained at 150 rpm.

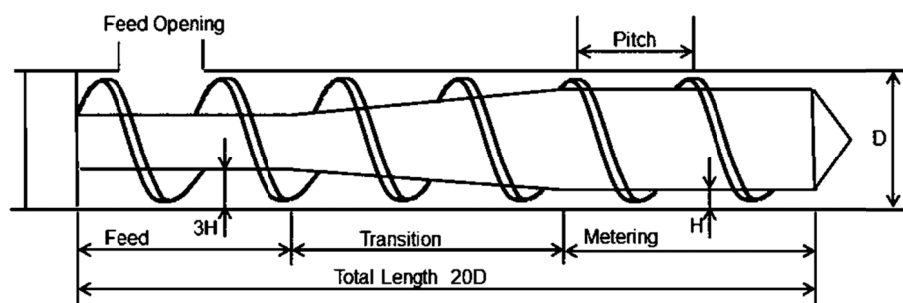


Figure 1. Schematic representation of screw in a single screw extruder

Table 2. Dimensions of die used in this study

Die No.	Diameter of nozzle (mm)	Length of nozzle (mm)	L/D ratio (-)
D1	6	20.0	3.33
D2	3	17.5	5.83
D3	2	14.5	7.25

## 2.3 Experimental Design and Statistical Analysis

In the present study, a central composite rotatable design (CCRD) was used to evaluate the effect of soy white flakes, moisture content, temperature and L/D of die nozzle on the physical properties of the extrudate. Pellet durability index, bulk density, water absorption and solubility indices and expansion ratio of the extrudates were measured as the response/dependent variables. The measurements were completed in triplicate, except for expansion ratio, which were measured with ten replications. The collected data were then analyzed with Proc GLM procedure to determine the treatment combination effects using SAS v9.3 (SAS Institute, Cary, NC). Then post hoc LSD tests were used to determine where the specific differences occurred. The experimental design was

developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN), which consisted of 3 numerical independent variables of soy white flakes ( $X_1$ ), moisture content ( $X_2$ ) and T ( $X_3$ ) each at five levels and one categorical variable of die nozzle configuration ( $X_4$ ) at three levels. The experimental design points (in coded and actual values) are shown in Table 3. Using Equation 1, the numerical independent variables in actual form ( $X_1, X_2$ ) were converted to their coded form ( $x_1, x_2$ ).

$$x_i = \frac{(X_i - X_0)}{\Delta X} \quad (1)$$

Where  $x_i$  is the dimensionless coded value of the  $i^{\text{th}}$  independent variable, and  $X_i, X_0$ , and  $\Delta X$  correspond to the actual value, actual value at the center point, and the step change of the  $i^{\text{th}}$  variable, respectively.

Table 3. Independent numerical and categorical variables and their levels

Numerical variables	Symbol	Coded variable levels				
		-1.682	-1	0	1	1.682
Soy white flakes (%)	$X_1$	21	29	40	52	59
Moisture content (% db)	$X_2$	15	19	25	31	35
Temperature ( $^{\circ}\text{C}$ )	$X_3$	100	110	125	140	150
<b>Categorical variable</b>		<b>D1</b>	<b>D2</b>	<b>D3</b>		
L/D (-)	$X_4/[1]$	1	0	-1		
	$X_4/[2]$	0	1	-1		

For each categorical variable, 20 experiments were performed in randomized order including six replications at the design center to obtain an accurate estimation of the experimental error (Table 4a and 4b). The pellet durability index ( $Y_{PDI}$ ), bulk density ( $Y_{BD}$ ), water absorption index ( $Y_{WAI}$ ), water solubility index ( $Y_{WSI}$ ) and expansion ratio ( $Y_{ER}$ ) were taken as the five responses of the designed experiments. The quadratic polynomial equation was used to describe the effect of the independent variables in terms of linear, quadratic and their interactions on the dependent variables as given by Equation 2.

$$Y_i = b_0 + \sum_{i=1}^4 b_i X_i + \sum_{i=1}^4 b_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 b_{ij} X_i X_j + \varepsilon \quad (2)$$

Where  $Y_i$  is the predicted response;  $b_0$  is the interception coefficient;  $b_i, b_{ii}$ , and  $b_{ij}$  are coefficients of the linear, quadratic, and interaction terms;  $\varepsilon$  is the random error; and  $X_i$  is the independent variables studied. The fitness of the model was evaluated and the interactions between the independent and dependent variables were identified by using an analysis of variance (ANOVA) presented in Tables 5 and 6. The goodness of fit of the second order equation was expressed by the coefficient of determination ( $R^2$ ) and its statistical significance was determined by  $F$ -test (Table 7). 3D response surfaces were used to visualize interactive effects of the independent variables.

## 2.4 Measurement of Physical Properties

### 2.4.1 Pellet Durability Index

Approximately 100 g of extrudates from each blend were manually sieved (U.S.A. standard testing, ASTM E-11 specification, Daigger, Vernon Hills, IL) to remove initial fines, and then tumbled in a pellet durability tester (Model PDT-110, Seedburo Equipment Company, Chicago, IL) for 10 min. Afterwards, the samples were again sieved, and then weighed on an electronic balance (Explorer Pro, Model: EP4102, Ohaus, Pine Brook, NJ) (ASAE, 2004). Pellet durability index was calculated following the Equation 3:

$$\text{Pellet durability index (\%)} = \left( \frac{M_a}{M_b} \times 100 \right) \quad (3)$$

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

### 2.4.2 Bulk Density

Bulk density was determined as the ratio of the mass of extrudates that they filled up to a given bulk volume and measured using a standard bushel tester (Seedburo Equipment Company, Chicago, IL) following the method recommended by USDA (2009).

### 2.4.3 Water Absorption Index and Water Solubility Index

Extrudates were ground to fine powders using a coffee grinder (Black & Decker ® Corporation, Towson, ML,

USA). The ground extrudates (2.5 g) was suspended in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminum cup and dried at 135 °C for 2 h (AACC, 2000). The weight of the gel remaining in the centrifuge tube was measured. The water absorption index and water solubility index were calculated by Equation 4 and 5, respectively:

$$\text{Water absorption index (unitless)} = \left( \frac{W_g}{W_{ds}} \right) \quad (4)$$

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

$$\text{Water solubility index (\%)} = \left( \frac{W_{ss}}{W_{ds}} \times 100 \right) \quad (5)$$

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

#### 2.4.4 Expansion Ratio

The radial expansion ratio of the extrudates was measured as the ratio of the diameter of the extrudates to the diameter of the die orifice.

Table 4a. Experimental design layout

Run	Coded variables					Actual variables			
	$x_1$	$x_2$	$x_3$	$x_4$		$X_1$	$X_2$	$X_3$	$X_4$
				$x_{4[1]}$	$x_{4[2]}$	(%)	(% db)	(°C)	(-)
1	0	0	-1.682	1	0	40	25	100	D1
2	0	0	0	0	1	40	25	125	D2
3	-1	-1	1	1	0	29	19	140	D1
4	1.682	0	0	1	0	59	25	125	D1
5	1	-1	-1	0	1	52	19	110	D2
6	0	0	0	1	0	40	25	125	D1
7	1	1	-1	0	1	52	31	110	D2
8	1	-1	1	0	1	52	19	140	D2
9	0	0	0	-1	-1	40	25	125	D3
10	1	-1	1	1	0	52	19	140	D1
11	-1	1	-1	0	1	29	31	110	D2
12	1	1	1	1	0	52	31	140	D1
13	0	0	1.682	0	1	40	25	150	D2
14	-1	-1	-1	0	1	29	19	110	D2
15	0	0	0	-1	-1	40	25	125	D3
16	1	-1	1	-1	-1	52	19	140	D3
17	-1	1	-1	1	0	29	31	110	D1
18	0	0	0	-1	-1	40	25	125	D3
19	-1.682	0	0	1	0	21	25	125	D1
20	-1	-1	-1	-1	-1	29	19	110	D3
21	0	0	0	1	0	40	25	125	D1
22	0	-1.682	0	0	1	40	15	125	D2
23	0	0	-1.682	-1	-1	40	25	100	D3
24	-1	-1	-1	1	0	29	19	110	D1
25	1.682	0	0	0	1	59	25	125	D2
26	0	0	0	-1	-1	40	25	125	D3
27	1	1	-1	1	0	52	31	110	D1
28	0	0	0	-1	-1	40	25	125	D3
29	-1.682	0	0	0	1	21	25	125	D2
30	0	0	0	0	1	40	25	125	D2

Table 4b. Experimental design layout

Run	Coded variables					Actual variables			
	$x_1$	$x_2$	$x_3$	$x_4$		$X_1$	$X_2$	$X_3$	$X_4$
				$x_{4[1]}$	$x_{4[2]}$	(%)	(% db)	(°C)	(-)
31	0	0	0	0	1	40	25	125	D2
32	-1	1	1	0	1	29	31	140	D2
33	1	-1	-1	1	0	52	19	110	D1
34	0	0	0	-1	-1	40	25	125	D3
35	1	1	-1	-1	-1	52	31	110	D3
36	0	0	-1.682	0	1	40	25	100	D2
37	0	1.682	0	1	0	40	35	125	D1
38	0	-1.682	0	1	0	40	15	125	D1
39	0	0	0	1	0	40	25	125	D1
40	-1	1	1	1	0	29	31	140	D1
41	0	0	0	0	1	40	25	125	D2
42	0	1.682	0	-1	-1	40	35	125	D3
43	1.682	0	0	-1	-1	59	25	125	D3
44	-1	-1	1	0	1	29	19	140	D2
45	0	0	0	0	1	40	25	125	D2
46	0	0	0	0	1	40	25	125	D2
47	0	0	0	1	0	40	25	125	D1
48	1	1	1	0	1	52	31	140	D2
49	0	0	0	1	0	40	25	125	D1
50	-1	1	-1	-1	-1	29	31	110	D3
51	-1.682	0	0	-1	-1	21	25	125	D3
52	0	0	1.682	-1	-1	40	25	150	D3
53	0	1.682	0	0	1	40	35	125	D2
54	0	-1.682	0	-1	-1	40	15	125	D3
55	1	1	1	-1	-1	52	31	140	D3
56	0	0	0	1	0	40	25	125	D1
57	1	-1	-1	-1	-1	52	19	110	D3
58	-1	-1	1	-1	-1	29	19	140	D3
59	-1	1	1	-1	-1	29	31	140	D3
60	0	0	1.682	1	0	40	25	150	D1

Table 5. Analysis of Variance (ANOVA) for pellet durability index and bulk density

Source	df	Pellet durability index				Bulk density			
		SS	MS	F value	P-value	SS	MS	F value	P-value
Model	17	702.94	41.35	6.41	< 0.0001	$1.93 \times 10^{-1}$	$1.13 \times 10^{-2}$	16.54	< 0.0001
$X_1$	1	77.83	77.83	12.06	0.0012	$7.18 \times 10^{-4}$	$7.18 \times 10^{-4}$	1.05	0.3117
$X_2$	1	17.18	17.18	2.66	0.1102	$9.79 \times 10^{-3}$	$9.79 \times 10^{-3}$	14.30	0.0005
$X_3$	1	203.67	203.67	31.56	< 0.0001	$2.61 \times 10^{-2}$	$2.61 \times 10^{-2}$	38.06	< 0.0001
$X_4$	2	198.72	99.36	15.40	< 0.0001	$1.24 \times 10^{-1}$	$6.19 \times 10^{-2}$	90.37	< 0.0001
$X_1^2$	1	0.58	0.58	0.09	0.7663	$5.95 \times 10^{-6}$	$5.95 \times 10^{-6}$	0.01	0.9262
$X_2^2$	1	105.47	105.47	16.35	0.0002	$1.28 \times 10^{-2}$	$1.28 \times 10^{-2}$	18.66	< 0.0001
$X_3^2$	1	5.40	5.40	0.84	0.3654	$4.65 \times 10^{-3}$	$4.65 \times 10^{-3}$	0.07	0.7956
$X_1X_2$	1	10.60	10.60	1.64	0.2071	$5.00 \times 10^{-4}$	$5.00 \times 10^{-4}$	0.73	0.3979
$X_1X_3$	1	1.64	1.64	0.25	0.6169	$1.23 \times 10^{-3}$	$1.23 \times 10^{-3}$	0.02	0.8939
$X_1X_4$	2	32.82	16.41	2.54	0.0907	$5.14 \times 10^{-3}$	$2.57 \times 10^{-3}$	3.75	0.0316
$X_2X_3$	1	1.55	1.55	0.24	0.6270	$2.96 \times 10^{-4}$	$2.96 \times 10^{-4}$	0.43	0.5146
$X_2X_4$	2	7.67	3.84	0.59	0.5565	$6.50 \times 10^{-4}$	$3.25 \times 10^{-4}$	0.47	0.6254
$X_3X_4$	2	40.41	20.20	3.13	0.0540	$1.27 \times 10^{-2}$	$6.36 \times 10^{-3}$	9.29	0.0005
Residual	42	271.01	6.45	-	-	$2.88 \times 10^{-2}$	$6.85 \times 10^{-4}$	-	-
Lack of fit	27	202.96	7.52	1.66	0.1536	$2.37 \times 10^{-2}$	$8.76 \times 10^{-4}$	2.58	0.0291
Pure error	15	68.05	4.54	-	-	$5.10 \times 10^{-3}$	$3.40 \times 10^{-4}$	-	-

<sup>+</sup>df – degree of freedom, SS – Sum of squares, MS – Mean square.

Table 6. Analysis of Variance (ANOVA) for water solubility index and expansion ratio

Source	df	Water solubility index				Expansion ratio			
		SS	MS	F value	P-value	SS	MS	F value	P-value
Model	17	22.25	1.31	2.77	0.0037	$4.20 \times 10^{-1}$	$2.50 \times 10^{-2}$	13.22	< 0.0001
$X_1$	1	2.91	2.91	6.15	0.0172	$3.38 \times 10^{-3}$	$3.38 \times 10^{-3}$	1.82	0.1842
$X_2$	1	3.64	3.64	7.71	0.0082	$2.20 \times 10^{-2}$	$2.20 \times 10^{-2}$	11.65	0.0014
$X_3$	1	6.22	6.22	13.17	0.0008	$2.50 \times 10^{-2}$	$2.50 \times 10^{-2}$	13.71	0.0006
$X_4$	2	0.25	0.12	0.26	0.7700	$2.30 \times 10^{-1}$	$1.10 \times 10^{-1}$	61.92	< 0.0001
$X_1^2$	1	6.12	6.12	12.95	0.0008	$5.14 \times 10^{-3}$	$5.14 \times 10^{-3}$	2.77	0.1035
$X_2^2$	1	1.27	1.27	2.68	0.1090	$3.10 \times 10^{-2}$	$3.10 \times 10^{-2}$	16.76	0.0002
$X_3^2$	1	0.19	0.19	0.41	0.5277	$2.20 \times 10^{-2}$	$2.20 \times 10^{-2}$	11.94	0.0013
$X_1X_2$	1	0.21	0.21	0.45	0.5055	$5.12 \times 10^{-3}$	$5.12 \times 10^{-3}$	2.76	0.1039
$X_1X_3$	1	0.61	0.61	1.30	0.2604	$8.62 \times 10^{-4}$	$8.62 \times 10^{-4}$	0.46	0.4991
$X_1X_4$	2	0.05	0.03	0.06	0.9452	$1.10 \times 10^{-2}$	$5.74 \times 10^{-3}$	3.10	0.0557
$X_2X_3$	1	0.00	0.00	0.00	0.9927	$5.55 \times 10^{-4}$	$5.55 \times 10^{-4}$	0.30	0.5871
$X_2X_4$	2	1.35	0.68	1.43	0.2501	$3.13 \times 10^{-3}$	$1.57 \times 10^{-3}$	0.84	0.4371
$X_3X_4$	2	0.08	0.04	0.09	0.9160	$6.50 \times 10^{-2}$	$3.30 \times 10^{-2}$	17.58	< 0.0001
Residual	42	19.84	0.47	-	-	$7.80 \times 10^{-2}$	$1.85 \times 10^{-3}$	-	-
Lack of fit	27	15.87	0.59	2.22	0.0541	$6.60 \times 10^{-2}$	$2.45 \times 10^{-3}$	3.16	0.0114
Pure error	15	3.97	0.26	-	-	$1.20 \times 10^{-2}$	$7.76 \times 10^{-4}$	-	-

<sup>+</sup>df – degree of freedom, SS – Sum of squares, MS – Mean square.

Table 7. Final equation in terms of coded factors after excluding the insignificant terms for pellet durability index, bulk density, water solubility index and expansion ratio

Coded model equations	$R^2$	Adj $R^2$	Pred $R^2$	Adeq. precision
$Y_{PDI} = +89.91 + 1.38x_1 - 2.23x_3 - 1.86x_4[I] - 0.61x_4[2] + 1.56x_2^2$	0.72	0.61	0.36	9.98
$Y_{BD} = +0.42 - 0.015x_2 - 0.025x_3 + 0.058x_4[I] - 4.89 \times 10^{-3}x_4[2]$ $+ 1.92 \times 10^{-3}x_1x_4[I] + 0.013x_1x_4[2] + 0.02x_3x_4[I]$ $+ 2.87 \times 10^{-3}x_3x_4[2] + 0.017x_2^2$	0.87	0.82	0.69	19.03
$Y_{WSI} = +14.07 - 0.27x_1 - 0.30x_2 - 0.39x_3 + 0.38x_1^2$	0.53	0.34	-0.09	6.52
$Y_{ER} = +1.19 - 0.023x_2 + 0.025x_3 - 0.081x_4[I] + 0.013x_4[2]$ $- 0.056x_3x_4[I] + 0.023x_3x_4[2] - 0.027x_2^2 - 0.023x_3^2$	0.84	0.78	0.62	14.85

### 3. Results and Discussion

Response surface methodology (RSM) was used to analyze the relationship between the dependent and independent variables. The predictive models in coded terms (i.e.,  $Y_{PDI}$ ,  $Y_{BD}$ ,  $Y_{WSI}$  and  $Y_{ER}$ ) are presented in Table 7. On the contrary, the final equations in actual form are defined for each type of categorical factor separately. The final equations in actual form obtained for pellet durability index ( $Y_{PDI}$ ), bulk density ( $Y_{BD}$ ), water solubility index ( $Y_{WSI}$ ) and expansion ratio ( $Y_{ER}$ ) for each level of categorical variable (D1, D2 and D3) are given in Table 8.

Table 8. Best-fit response surface models for extrudate physical properties

L/D	Response Surface Model	$R^2$	Std. Deviation	F Statistic
D1	$Y_{PDI} = +155.64 + 0.10X_1 - 3.14X_2 - 0.46X_3 + 0.01X_1X_2 - 1.51 \times 10^{-3}X_1X_3 + 2.94 \times 10^{-3}X_2X_3 - 8.74 \times 10^{-4}X_1^2 + 0.05X_2^2 + 1.57 \times 10^{-3}X_3^2$	0.72	2.54	6.41
	$Y_{BD} = +0.81 + 1.51 \times 10^{-3}X_1 - 0.02X_2 - 6.48 \times 10^{-4}X_3 - 6.90 \times 10^{-5}X_1X_2 + 4.15 \times 10^{-6}X_1X_3 - 4.07 \times 10^{-5}X_2X_3 + 2.80 \times 10^{-6}X_1^2 + 5.20 \times 10^{-4}X_2^2 + 4.61 \times 10^{-6}X_3^2$	0.87	0.026	16.54
	$Y_{WSI} = +30.07 - 0.17X_1 - 0.41X_2 - 0.07X_3 + 1.42 \times 10^{-3}X_1X_2 - 9.28 \times 10^{-4}X_1X_3 - 1.50 \times 10^{-5}X_2X_3 + 2.84 \times 10^{-3}X_1^2 + 5.18 \times 10^{-3}X_2^2 + 2.96 \times 10^{-4}X_3^2$	0.53	0.69	2.77
	$Y_{ER} = -0.97 + 0.02X_1 + 0.04X_2 + 0.02X_3 - 2.21 \times 10^{-4}X_1X_2 - 3.47 \times 10^{-5}X_1X_3 + 5.58 \times 10^{-5}X_2X_3 - 8.24 \times 10^{-5}X_1^2 - 8.11 \times 10^{-4}X_2^2 - 1.01 \times 10^{-4}X_3^2$	0.84	0.043	13.22
D2	$Y_{PDI} = +172.09 + 0.23X_1 - 3.29X_2 - 0.59X_3 + 0.01X_1X_2 - 1.51 \times 10^{-3}X_1X_3 + 2.94 \times 10^{-3}X_2X_3 - 8.74 \times 10^{-4}X_1^2 + 0.05X_2^2 + 1.57 \times 10^{-3}X_3^2$	0.72	2.54	6.41
	$Y_{BD} = +0.82 + 2.45 \times 10^{-3}X_1 - 0.02X_2 - 1.80 \times 10^{-3}X_3 - 6.90 \times 10^{-5}X_1X_2 + 4.15 \times 10^{-6}X_1X_3 - 4.07 \times 10^{-5}X_2X_3 + 2.80 \times 10^{-6}X_1^2 + 5.20 \times 10^{-4}X_2^2 + 4.61 \times 10^{-6}X_3^2$	0.87	0.026	16.54
	$Y_{WSI} = +27.68 - 0.17X_1 - 0.33X_2 - 0.06X_3 + 1.42 \times 10^{-3}X_1X_2 - 9.28 \times 10^{-4}X_1X_3 - 1.50 \times 10^{-5}X_2X_3 + 2.84 \times 10^{-3}X_1^2 + 5.18 \times 10^{-3}X_2^2 + 2.96 \times 10^{-4}X_3^2$	0.53	0.69	2.77
	$Y_{ER} = -1.56 + 0.02X_1 + 0.04X_2 + 0.03X_3 - 2.21 \times 10^{-4}X_1X_2 - 3.47 \times 10^{-5}X_1X_3 + 5.58 \times 10^{-5}X_2X_3 - 8.24 \times 10^{-5}X_1^2 - 8.11 \times 10^{-4}X_2^2 - 1.01 \times 10^{-4}X_3^2$	0.84	0.043	13.22
D3	$Y_{PDI} = +184.83 + 0.05X_1 - 3.31X_2 - 0.61X_3 + 0.01X_1X_2 - 1.51 \times 10^{-3}X_1X_3 + 2.94 \times 10^{-3}X_2X_3 - 8.74 \times 10^{-4}X_1^2 + 0.05X_2^2 + 1.57 \times 10^{-3}X_3^2$	0.72	2.54	6.41
	$Y_{BD} = +1.09 + 7.74 \times 10^{-5}X_1 - 0.02X_2 - 3.51 \times 10^{-3}X_3 - 6.90 \times 10^{-5}X_1X_2 + 4.15 \times 10^{-6}X_1X_3 - 4.07 \times 10^{-5}X_2X_3 + 2.80 \times 10^{-6}X_1^2 + 5.20 \times 10^{-4}X_2^2 + 4.61 \times 10^{-6}X_3^2$	0.87	0.026	16.54
	$Y_{WSI} = +28.31 - 0.17X_1 - 0.36X_2 - 0.06X_3 + 1.42 \times 10^{-3}X_1X_2 - 9.28 \times 10^{-4}X_1X_3 - 1.50 \times 10^{-5}X_2X_3 + 2.84 \times 10^{-3}X_1^2 + 5.18 \times 10^{-3}X_2^2 + 2.96 \times 10^{-4}X_3^2$	0.53	0.69	2.77
	$Y_{ER} = -1.62 + 0.02X_1 + 0.04X_2 + 0.03X_3 - 2.21 \times 10^{-4}X_1X_2 - 3.47 \times 10^{-5}X_1X_3 + 5.58 \times 10^{-5}X_2X_3 - 8.24 \times 10^{-5}X_1^2 - 8.11 \times 10^{-4}X_2^2 - 1.01 \times 10^{-4}X_3^2$	0.84	0.043	13.22



Overall, changing the level of temperature content significantly affected ( $P < 0.05$ ) all the resulting physical properties. Changing the level of moisture content significantly affected ( $P < 0.05$ ) all the resulting physical properties except pellet durability index. Whereas changing soy white flakes content significantly affected ( $P < 0.05$ ) pellet durability index, water absorption and solubility indices (Table 5 and 6). The behavior observed for the treatment combinations were produced due to the various competing interaction effects (Table 9).

### 3.1 Pellet Durability Index

The response surface plot presented (Figure 2) showed that for all the L/D ratio, the pellet durability index of extrudates increased on increasing soy white flakes content and decreasing temperature. ANOVA showed that moisture content had no significant effect on pellet durability index and hence response surface plots of interaction effect involving moisture content are not shown. Lack of fit was not significant relative to the pure error, which meant the model was well fitted. The regression equation for pellet durability index in coded and actual form is shown in Table 7 and 8, respectively.

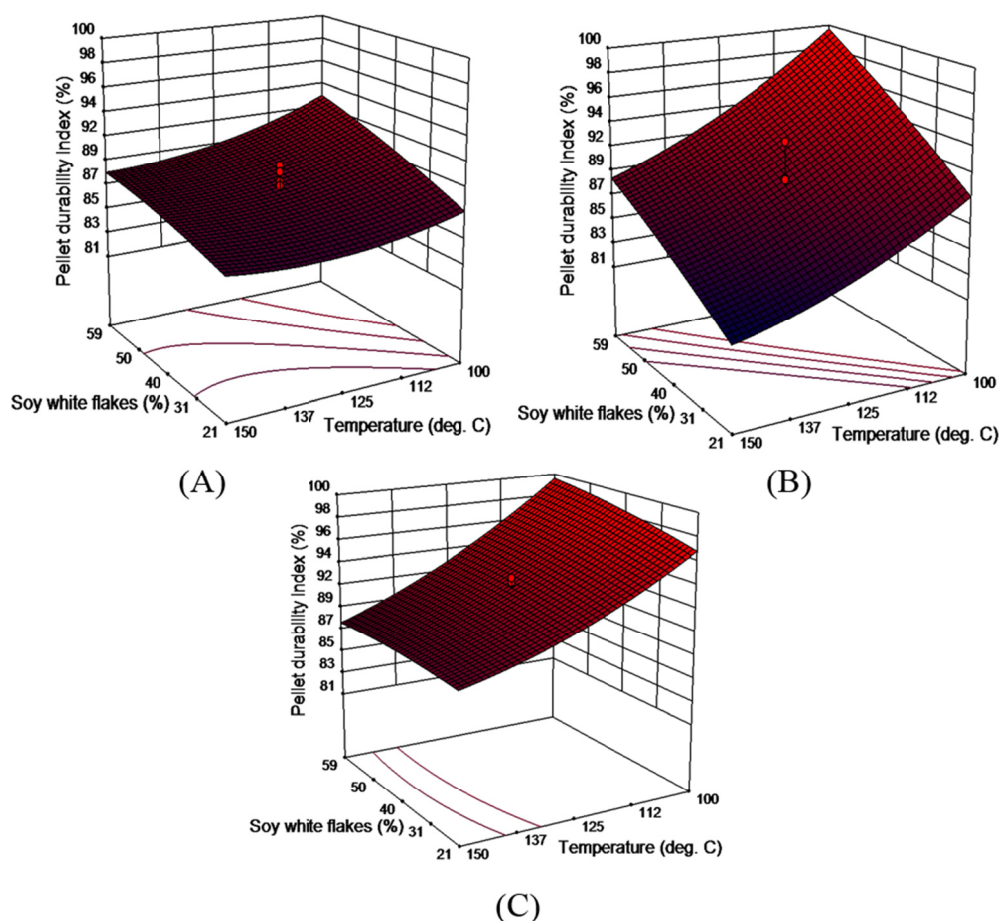


Figure 2. Response surface plots of pellet durability index for the effect of soy white flakes content and temperature at 25 % db moisture content at different die aspect ratio (L/D), (A) 3.33, (B) 5.83, and (C) 7.25

The values of pellet durability index of extruded products under experimental conditions are presented in Table 9. The maximum and minimum pellet durability index values were related to the treatments with 40% soy white flakes, 25% moisture content, 125°C barrel temperature, 3.33 L/D and 21% soy white flakes, 25% moisture content, 125°C barrel temperature, 5.83 L/D ratio, respectively (Table 9).

### 3.2 Bulk Density

The response surface plot presented (Figure 3) showed that for all the L/D ratio, the bulk density of extrudates decreased with an increasing of moisture content and temperature. A significant decrease in bulk density was observed when length to diameter ratio of die was increased from 3.33 to 7.25. ANOVA showed that soy white

flakes had no significant effect on bulk density and hence response surface plots of interaction effect involving soy white flakes are not shown. The regression equation for bulk density in coded and actual form is shown in Table 7 and 8, respectively.

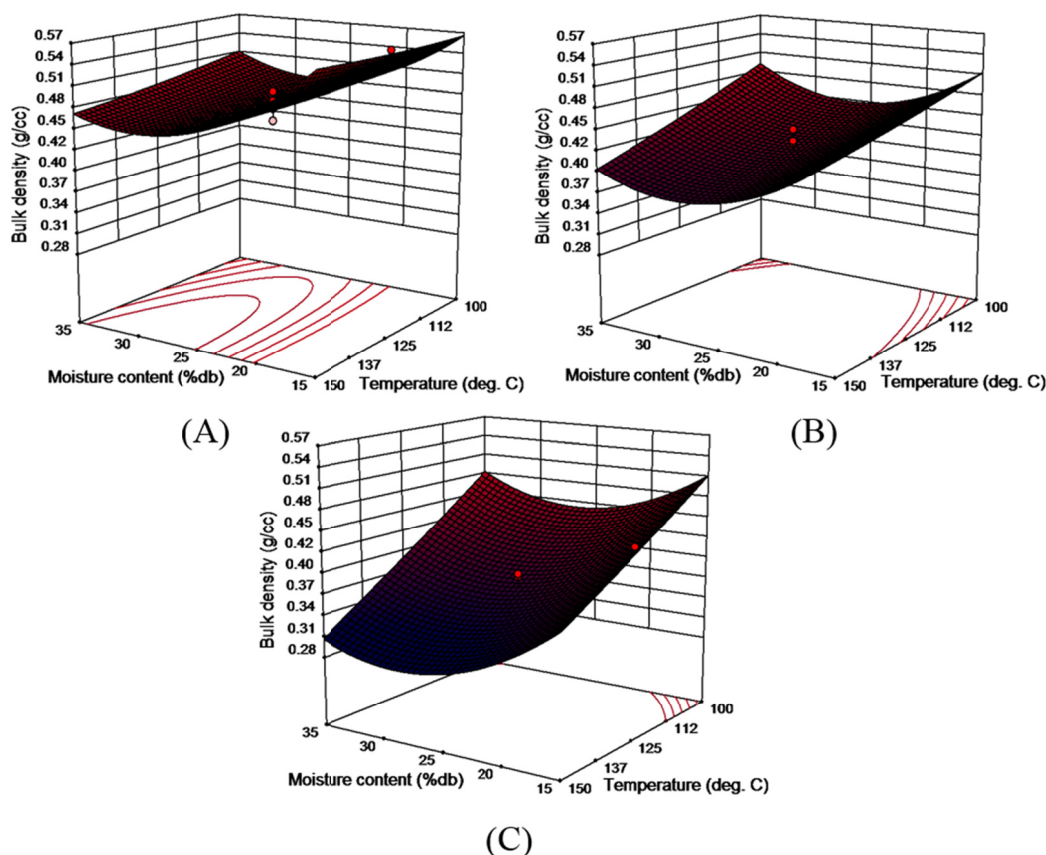


Figure 3. Response surface plots of bulk density for the effect of temperature and moisture content at 40% db soy white flakes at different die aspect ratio (L/D), (A) 3.33, (B) 5.83, and (C) 7.25

The treatment combination effects of soy white flakes, moisture content, temperature and L/D ratio on bulk density of extrudates are presented in Table 9. The lowest bulk density,  $0.32 \text{ g/cm}^3$ , was recorded at 29% soy white flakes, temperature of  $140^\circ\text{C}$ , moisture content of 31%, L/D of 7.25 and at 59% soy white flakes, temperature of  $125^\circ\text{C}$ , moisture content of 25%, L/D ratio of 7.25; and the highest bulk density of  $0.57 \text{ g/cm}^3$ , was obtained at 40% soy white flakes, temperature of  $125^\circ\text{C}$ , moisture content of 15%, and L/D of 3.33 (Table 9).

### 3.3 Water Absorption Index

Soy white flakes, moisture content and temperature significantly affected the water absorption index through a linear model (response surface plots were prepared but not shown due to linear model and to reduce the manuscript size). ANOVA analysis demonstrated that the linear model was significant ( $P < 0.05$ ). Increasing the soy white flakes content from 21% to 52%, there was a significant increase of in water absorption index. Water absorption index was found to increase when temperature was raised from  $100^\circ\text{C}$  to  $150^\circ\text{C}$ . With extruded corn grits, a similar trend was observed by Anderson et al. (1969). Furthermore, water absorption index also increased when moisture content level of the feed was increased from 15% to 35%. This was in agreement with Williams et al. (1977) who observed that higher temperature and drier conditions could result in higher dextrinization, which could lead to a decreased extrudate water absorption index and higher extrudate water solubility index. According to Mason and Hosney (1986), water absorption index indicates the part of the starch that was not affected by the extrusion cooking and maintained its internal structure. The experimental values of water absorption index of extrudates under different designed extrusion conditions are presented in Table 9.

### 3.4 Water Solubility Index

Changing the level of soy white flakes, moisture content of ingredient mix and barrel temperature had significant effect ( $P < 0.05$ ) on water solubility index (Table 6). Williams et al. (1977) reported that there was an inverse relationship between the water absorption index and water solubility index values of the extrudates. Similarly, in this study the water solubility index at 15% moisture content was higher compared to the water solubility index at 35% moisture content. Increasing the barrel temperature from 100 to 150 °C resulted in decrease in water solubility index. In another study, Chevanan et al. (2007) reported that there was no significant change in water solubility index of the DDGS-based extrudates due to the change in extruder barrel temperature. Increasing the soy white flakes content initially led to a significant decrease in water solubility index and then further increase in soy white flakes level resulted in slight increase in water solubility index. An inverse relationship between the water solubility index and water absorption index values were observed. This observation was in agreement with what Anderson et al. (1969), Williams et al. (1977), and Fallahi et al. (2012, 2013) reported.

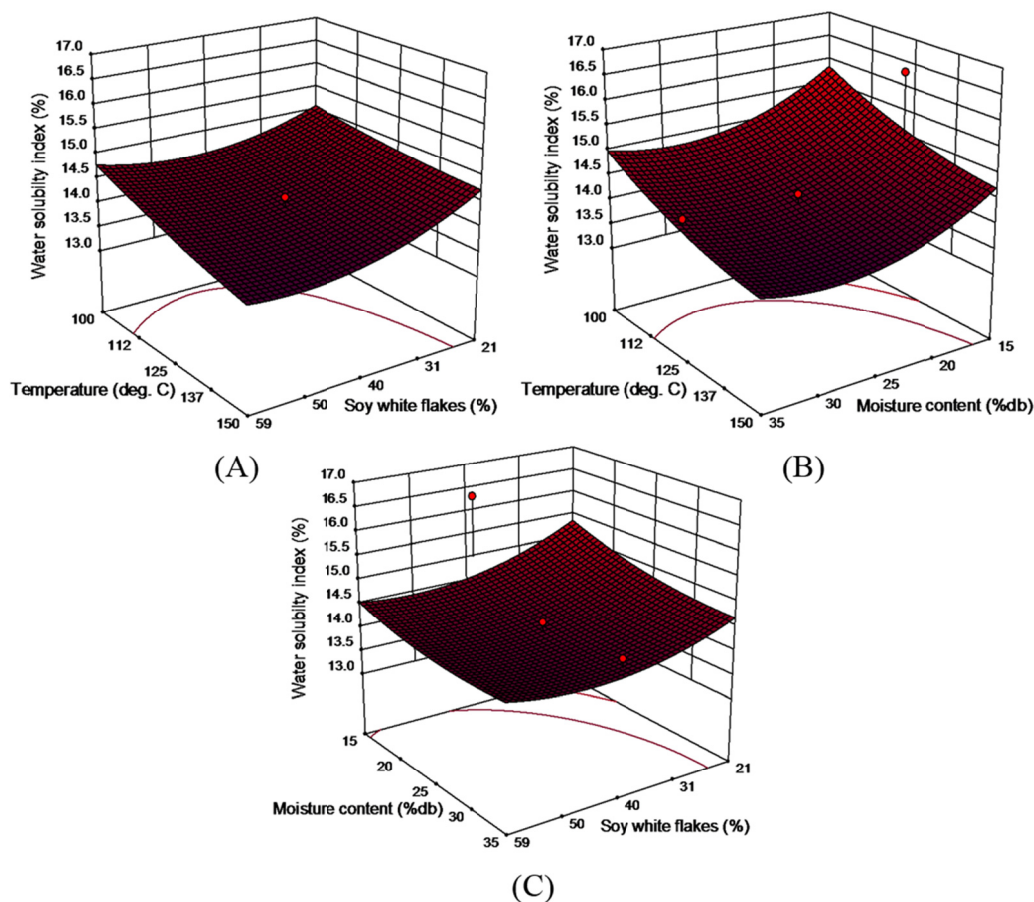


Figure 4. Response surface plots of water solubility index at die aspect ratio (L/D) of 7.25 for the effect of (A) temperature and soy white flakes content, (B) temperature and moisture content and (C) moisture and soy white flakes content

ANOVA analysis showed that quadratic effect of soy white flakes significantly affected water solubility index and all linear effects including soy white flakes, moisture content and temperature had significant effect on water solubility index. The interaction effect of soy white flakes, moisture content and temperature at L/D ratio at 7.25 on water solubility index was maximum and since L/D ratio does not significantly affect the water solubility index, the response surface plots of water solubility index at L/D ratio 7.25 are shown (Figure 4). Water solubility index is found to increase when temperature and soy white flakes content, temperature and moisture content, and moisture content and soy white flakes content are decreased (Figure 4). Lack of fit was not significant relative to the pure error, which meant the model was well fitted. The regression equation for the

empirical relationship between water solubility index and the independent extrusion processing variables for each L/D ratio is shown in Table 8.

### 3.5 Expansion Ratio

The extent of puffing of extruded products is indicated by the expansion ratio. The response surface plot presented in Figure 5 showed that for all the L/D ratio, the expansion ratio of extrudates increased with an increasing moisture content and after reaching a maximum the expansion ratio decreased with further increasing in moisture content. The expansion ratio of extrudates increased for L/D ratio 5.83 and 7.25 but decreased for L/D ratio 3.33 with increase in temperature. ANOVA showed that soy white flakes had no significant effect on expansion ratio and hence response surface plots of interaction effect involving soy white flakes are not shown. The regression equation for the relationship between expansion ratio and independent variables in terms of coded and actual form for each L/D ratio is shown in Table 7 and 8, respectively. Increasing the level of moisture content of ingredient mix and temperature had a significant effect on expansion ratio of extrudates.

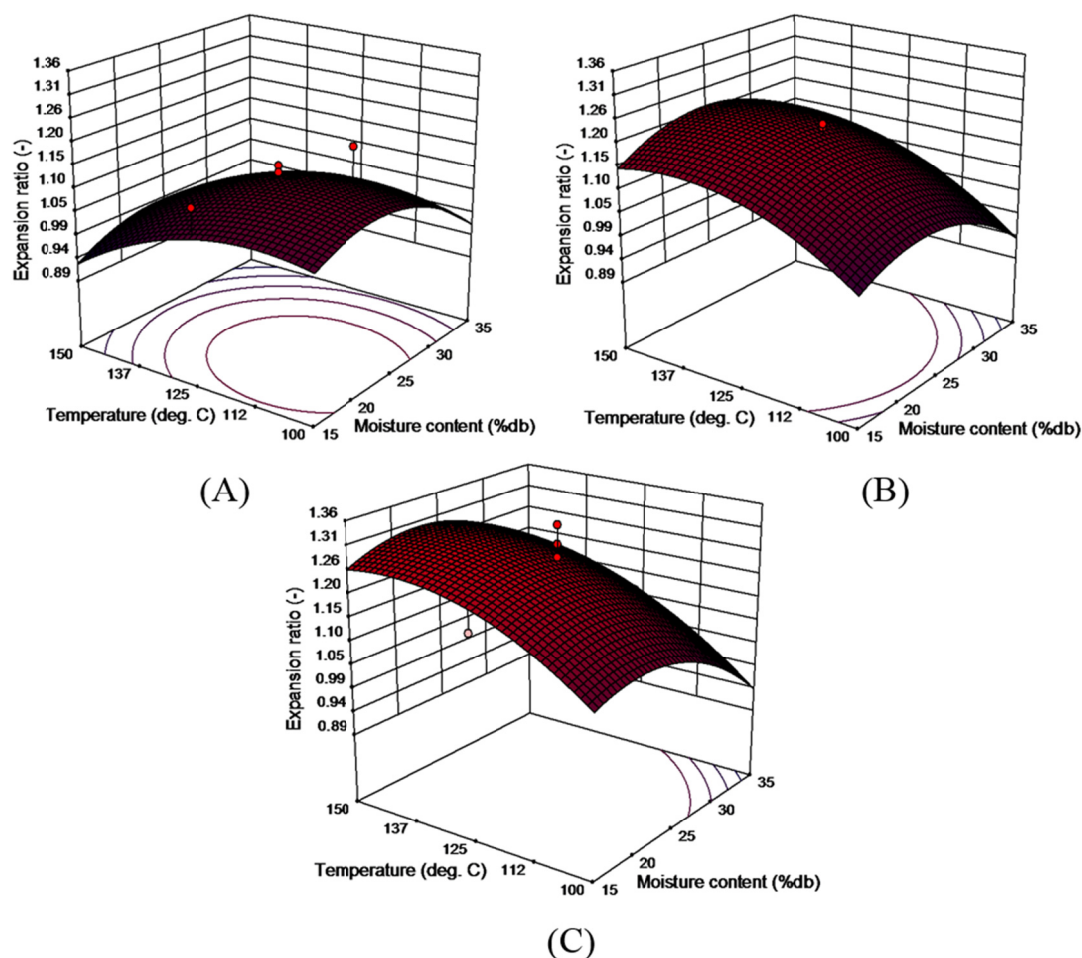


Figure 5. Response surface plots of expansion ratio for the effect of temperature and moisture content at 40% db soy white flakes at different die aspect ratio (L/D), (A) 3.33, (B) 5.83, and (C) 7.25.

The experimental values of expansion ratio of extrudates under different designed extrusion conditions are shown in Table 9. The maximum and minimum expansion ratio were achieved at 52% soy white flakes, 19% moisture content, 140 °C, and 7.25 L/D ratio and 40% soy white flakes, 25% moisture content, 150 °C temperature, and 3.33 L/D ratio, respectively

Table 9a. Treatment combination effects for soy white flakes, moisture content of raw material, temperature and die on extrudate physical properties

Treatment	PDI (%)	BD (g/cc)	WAI (unitless)	WSI (%)	ER (unitless)
1	90.09±0.18 <sup>16-18</sup>	0.46±0.01 <sup>12-14</sup>	3.62±0.05 <sup>23-25</sup>	14.97±0.11 <sup>4-8</sup>	1.06±0.02 <sup>27-29</sup>
2	87.49±0.02 <sup>23</sup>	0.39±0.00 <sup>21-22</sup>	4.70±0.11 <sup>5</sup>	13.50±0.39 <sup>18-23</sup>	1.22±0.01 <sup>7-11</sup>
3	85.30±0.10 <sup>26-27</sup>	0.49±0.01 <sup>4-6</sup>	3.72±0.10 <sup>21-25</sup>	14.83±0.41 <sup>4-11</sup>	1.06±0.01 <sup>27-29</sup>
4	85.93±0.79 <sup>25</sup>	0.47±0.01 <sup>9-11</sup>	5.14±0.11 <sup>3-4</sup>	13.56±0.20 <sup>17-22</sup>	1.12±0.01 <sup>18-23</sup>
5	97.03±0.13 <sup>2-3</sup>	0.48±0.01 <sup>6-9</sup>	4.34±0.12 <sup>7-9</sup>	14.48±0.25 <sup>7-14</sup>	1.131±0.00 <sup>8-22</sup>
6	89.75±0.28 <sup>17-18</sup>	0.48±0.00 <sup>8-10</sup>	4.81±0.10 <sup>5</sup>	13.15±0.21 <sup>22-24</sup>	1.09±0.02 <sup>23-27</sup>
7	95.61±0.47 <sup>4-5</sup>	0.44±0.01 <sup>14-16</sup>	4.64±0.14 <sup>5-6</sup>	14.29±0.40 <sup>10-16</sup>	1.07±0.02 <sup>26-28</sup>
8	92.01±0.16 <sup>14</sup>	0.49±0.01 <sup>4-5</sup>	5.39±0.06 <sup>2</sup>	13.31±0.18 <sup>21-24</sup>	1.16±0.02 <sup>15-18</sup>
9	90.94±0.14 <sup>15</sup>	0.33±0.00 <sup>25</sup>	4.15±0.12 <sup>10-15</sup>	13.85±0.38 <sup>15-21</sup>	1.32±0.01 <sup>2</sup>
10	88.00±0.30 <sup>22-23</sup>	0.50±0.02 <sup>4</sup>	4.23±0.07 <sup>8-12</sup>	14.43±0.18 <sup>7-16</sup>	1.03±0.01 <sup>30-31</sup>
11	90.51±0.63 <sup>15-16</sup>	0.45±0.01 <sup>13-15</sup>	3.54±0.14 <sup>25-26</sup>	14.88±0.32 <sup>4-10</sup>	1.08±0.02 <sup>26-28</sup>
12	92.36±0.11 <sup>13-14</sup>	0.49±0.01 <sup>4-7</sup>	5.16±0.08 <sup>3-4</sup>	13.40±0.32 <sup>19-24</sup>	0.96±0.01 <sup>32</sup>
13	81.75±0.15 <sup>30</sup>	0.33±0.01 <sup>25</sup>	5.30±0.05 <sup>2-3</sup>	12.83±0.23 <sup>24</sup>	1.29±0.02 <sup>2-3</sup>
14	90.80±0.17 <sup>15</sup>	0.38±0.01 <sup>22</sup>	4.29±0.14 <sup>7-11</sup>	13.83±0.26 <sup>16-21</sup>	1.10±0.01 <sup>21-26</sup>
15	92.01±0.21 <sup>14</sup>	0.34±0.01 <sup>24-25</sup>	5.04±0.14 <sup>4</sup>	12.94±0.34 <sup>23-24</sup>	1.28±0.02 <sup>3-5</sup>
16	90.21±0.22 <sup>16-17</sup>	0.34±0.01 <sup>24-25</sup>	5.59±0.05 <sup>1</sup>	12.91±0.11 <sup>23-24</sup>	1.41±0.02 <sup>1</sup>
17	90.17±0.42 <sup>16-17</sup>	0.47±0.00 <sup>8-11</sup>	3.71±0.08 <sup>22-25</sup>	14.57±0.25 <sup>7-13</sup>	1.05±0.00 <sup>28-31</sup>
18	93.13±0.25 <sup>10-12</sup>	0.36±0.01 <sup>23</sup>	4.47±0.01 <sup>6-7</sup>	13.262±0.25 <sup>1-24</sup>	1.28±0.02 <sup>3-4</sup>
19	84.29±0.41 <sup>28</sup>	0.46±0.00 <sup>11-13</sup>	3.10±0.13 <sup>28-29</sup>	15.19±0.36 <sup>3-6</sup>	1.08±0.00 <sup>25-27</sup>
20	97.54±0.19 <sup>2</sup>	0.47±0.01 <sup>10-12</sup>	3.15±0.07 <sup>28-29</sup>	15.27±0.28 <sup>3-5</sup>	1.15±0.01 <sup>16-19</sup>
21	90.16±0.22 <sup>16-17</sup>	0.50±0.01 <sup>4</sup>	3.93±0.14 <sup>16-20</sup>	14.25±0.18 <sup>11-16</sup>	1.10±0.01 <sup>21-26</sup>
22	96.71±0.14 <sup>3</sup>	0.49±0.00 <sup>4-6</sup>	3.74±0.07 <sup>20-23</sup>	14.99±0.07 <sup>4-7</sup>	1.14±0.01 <sup>17-20</sup>
23	98.11±0.37 <sup>1</sup>	0.44±0.01 <sup>14-16</sup>	3.37±0.09 <sup>26-27</sup>	15.01±0.08 <sup>4-7</sup>	1.13±0.01 <sup>18-21</sup>
24	88.91±0.38 <sup>20-21</sup>	0.54±0.00 <sup>2</sup>	3.03±0.02 <sup>29</sup>	15.70±0.12 <sup>2-3</sup>	1.09±0.01 <sup>23-27</sup>
25	92.25±0.18 <sup>14</sup>	0.44±0.00 <sup>16-18</sup>	4.04±0.12 <sup>13-18</sup>	15.77±0.33 <sup>1-3</sup>	1.19±0.02 <sup>11-14</sup>
26	93.29±0.05 <sup>10-12</sup>	0.38±0.01 <sup>21-22</sup>	4.12±0.05 <sup>11-15</sup>	14.15±0.25 <sup>12-17</sup>	1.25±0.01 <sup>4-7</sup>
27	94.84±0.13 <sup>6</sup>	0.52±0.00 <sup>3</sup>	4.03±0.09 <sup>14-18</sup>	14.46±0.12 <sup>7-15</sup>	1.02±0.01 <sup>31</sup>
28	92.87±0.31 <sup>11-13</sup>	0.38±0.00 <sup>22</sup>	4.34±0.15 <sup>7-10</sup>	14.35±0.18 <sup>9-16</sup>	1.23±0.02 <sup>6-9</sup>
29	83.23±0.18 <sup>29</sup>	0.41±0.01 <sup>19-20</sup>	3.07±0.09 <sup>29</sup>	16.26±0.18 <sup>1-2</sup>	1.19±0.02 <sup>11-14</sup>
30	89.92±0.36 <sup>17-18</sup>	0.43±0.01 <sup>18</sup>	4.11±0.12 <sup>11-16</sup>	14.04±0.35 <sup>13-18</sup>	1.19±0.01 <sup>12-15</sup>

+The values with the same superscript for a given property are not significantly different ( $P < 0.05$ ). PDI – Pellet durability index, BD – Bulk density, WAI – Water absorption index, WSI – Water solubility index, ER – Expansion ratio.



Table 9b. Treatment combination effects for soy white flakes, moisture content of raw material, temperature and die on extrudate physical properties

Treatment	PDI (%)	BD (g/cc)	WAI (unitless)	WSI (%)	ER (unitless)
31	85.77±0.18 <sup>25-26</sup>	0.43±0.01 <sup>18</sup>	3.91±0.10 <sup>17-21</sup>	14.34±0.16 <sup>9-16</sup>	1.23±0.02 <sup>6-10</sup>
32	85.12±0.43 <sup>27</sup>	0.40±0.01 <sup>20-21</sup>	4.15±0.14 <sup>10-15</sup>	14.31±0.33 <sup>9-16</sup>	1.21±0.02 <sup>8-12</sup>
33	93.55±0.07 <sup>9-10</sup>	0.53±0.01 <sup>2</sup>	3.36±0.08 <sup>26-27</sup>	15.68±0.07 <sup>2-3</sup>	1.09±0.01 <sup>23-27</sup>
34	92.81±0.50 <sup>12-13</sup>	0.35±0.01 <sup>24</sup>	4.03±0.04 <sup>14-18</sup>	14.34±0.16 <sup>9-16</sup>	1.24±0.01 <sup>5-8</sup>
35	96.72±0.47 <sup>3</sup>	0.44±0.01 <sup>15-17</sup>	4.16±0.08 <sup>9-14</sup>	14.37±0.40 <sup>8-16</sup>	1.12±0.02 <sup>19-24</sup>
36	96.03±0.11 <sup>4</sup>	0.46±0.00 <sup>11-13</sup>	3.42±0.01 <sup>26-27</sup>	15.79±0.21 <sup>1-3</sup>	1.09±0.01 <sup>22-27</sup>
37	86.19±0.96 <sup>24-25</sup>	0.47±0.01 <sup>10-12</sup>	4.36±0.19 <sup>7-8</sup>	13.84±0.22 <sup>16-21</sup>	1.09±0.01 <sup>23-27</sup>
38	94.59±0.35 <sup>6-7</sup>	0.57±0.01 <sup>1</sup>	3.43±0.07 <sup>26-27</sup>	15.35±0.25 <sup>3-4</sup>	1.12±0.01 <sup>18-23</sup>
39	88.43±0.06 <sup>21-22</sup>	0.48±0.02 <sup>5-8</sup>	3.90±0.06 <sup>17-22</sup>	14.35±0.17 <sup>9-16</sup>	1.12±0.01 <sup>19-25</sup>
40	92.02±0.16 <sup>14</sup>	0.47±0.00 <sup>9-11</sup>	4.07±0.04 <sup>12-18</sup>	13.34±0.35 <sup>20-24</sup>	1.03±0.01 <sup>29-31</sup>
41	93.06±0.18 <sup>10-12</sup>	0.45±0.01 <sup>14-16</sup>	3.55±0.06 <sup>24-26</sup>	14.73±0.27 <sup>5-12</sup>	1.16±0.00 <sup>15-18</sup>
42	93.91±0.29 <sup>8-9</sup>	0.37±0.00 <sup>23</sup>	4.08±0.15 <sup>12-17</sup>	14.41±0.18 <sup>7-16</sup>	1.12±0.01 <sup>20-25</sup>
43	93.50±0.09 <sup>9-10</sup>	0.32±0.01 <sup>26</sup>	4.11±0.17 <sup>11-16</sup>	15.34±0.23 <sup>3-5</sup>	1.26±0.01 <sup>3-6</sup>
44	90.18±0.20 <sup>16-17</sup>	0.40±0.00 <sup>20-21</sup>	3.38±0.02 <sup>26-27</sup>	15.62±0.44 <sup>3</sup>	1.17±0.00 <sup>14-17</sup>
45	87.75±0.47 <sup>23</sup>	0.41±0.01 <sup>19</sup>	4.05±0.14 <sup>12-18</sup>	14.05±0.28 <sup>13-18</sup>	1.19±0.02 <sup>10-14</sup>
46	89.81±0.25 <sup>17-18</sup>	0.43±0.01 <sup>17-18</sup>	3.97±0.03 <sup>15-19</sup>	14.13±0.19 <sup>12-17</sup>	1.18±0.02 <sup>13-17</sup>
47	83.37±0.66 <sup>29</sup>	0.47±0.00 <sup>8-11</sup>	3.63±0.05 <sup>23-25</sup>	14.59±0.21 <sup>6-13</sup>	1.13±0.01 <sup>18-22</sup>
48	92.09±0.06 <sup>14</sup>	0.39±0.01 <sup>21-22</sup>	4.46±0.04 <sup>6-7</sup>	14.01±0.34 <sup>13-18</sup>	1.17±0.01 <sup>13-17</sup>
49	89.61±0.25 <sup>18-19</sup>	0.48±0.01 <sup>7-10</sup>	3.53±0.03 <sup>25-26</sup>	14.48±0.14 <sup>7-14</sup>	1.08±0.02 <sup>26-28</sup>
50	94.88±0.57 <sup>6</sup>	0.44±0.01 <sup>16-18</sup>	3.39±0.06 <sup>26-27</sup>	15.35±0.43 <sup>3-4</sup>	1.06±0.01 <sup>27-30</sup>
51	94.58±0.10 <sup>6-7</sup>	0.40±0.01 <sup>19-20</sup>	3.01±0.08 <sup>29</sup>	16.26±0.21 <sup>1-2</sup>	1.17±0.02 <sup>13-17</sup>
52	86.59±0.27 <sup>24</sup>	0.33±0.01 <sup>25</sup>	3.89±0.04 <sup>18-22</sup>	14.57±0.21 <sup>7-13</sup>	1.28±0.02 <sup>3-4</sup>
53	93.40±0.22 <sup>9-11</sup>	0.44±0.00 <sup>15-16</sup>	4.39±0.01 <sup>7-8</sup>	13.98±0.26 <sup>13-19</sup>	1.03±0.01 <sup>29-31</sup>
54	97.46±0.18 <sup>2</sup>	0.44±0.00 <sup>14-16</sup>	3.26±0.04 <sup>27-28</sup>	16.33±0.38 <sup>1</sup>	1.18±0.01 <sup>13-17</sup>
55	95.11±0.31 <sup>5-6</sup>	0.33±0.01 <sup>25</sup>	5.02±0.03 <sup>4</sup>	14.36±0.35 <sup>8-16</sup>	1.18±0.02 <sup>12-16</sup>
56	88.81±0.24 <sup>20-21</sup>	0.46±0.00 <sup>12-14</sup>	3.92±0.04 <sup>17-20</sup>	14.41±0.30 <sup>7-16</sup>	1.09±0.02 <sup>24-27</sup>
57	98.37±0.61 <sup>1</sup>	0.44±0.00 <sup>14-16</sup>	3.74±0.02 <sup>20-24</sup>	15.32±0.21 <sup>3-5</sup>	1.20±0.01 <sup>9-13</sup>
58	94.24±0.19 <sup>7-8</sup>	0.34±0.01 <sup>25</sup>	3.78±0.02 <sup>19-23</sup>	14.92±0.37 <sup>4-9</sup>	1.24±0.01 <sup>6-9</sup>
59	85.88±0.22 <sup>25</sup>	0.32±0.00 <sup>26</sup>	4.23±0.09 <sup>8-13</sup>	14.22±0.07 <sup>12-16</sup>	1.25±0.02 <sup>4-7</sup>
60	89.17±0.43 <sup>19-20</sup>	0.48±0.00 <sup>8-10</sup>	4.37±0.04 <sup>7-8</sup>	13.95±0.18 <sup>14-20</sup>	0.90±0.01 <sup>33</sup>

+The values with the same superscript for a given property are not significantly different ( $P < 0.05$ ). PDI – Pellet durability index, BD – Bulk density, WAI – Water absorption index, WSI – Water solubility index, ER – Expansion ratio.

#### 4. Conclusions

This experimental study was conducted to investigate the effect of various extrusion processing conditions on the soy white flakes and HP-DDG based extrudates. Overall, it can be concluded that increasing the level of soy white flakes from 21% to 59%, resulted in increase of pellet durability and water absorption index. Increasing L/D ratio from 3.33 to 7.25 resulted in increase in pellet durability index, expansion ratio, but a decrease in bulk density of the extrudates. The increase in pellet durability indicates that the aquaculture feed could resist

mechanical damage during transportation and storage. Significant decrease in bulk density ( $P < 0.001$ ) due to increase in L/D ratio is desirable for storage purpose. Further studies should aim for the production of aquaculture feed with incorporation of soy white flakes levels between 20% and 60% db at different screw speeds and should optimize processing conditions.

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