

# Fenugreek enriched extruded product: optimization of ingredients using response surface methodology

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### <u>Article history</u>

<u>Abstract</u>

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# Introduction

(DGPF), fenugreek seed powder (FSP) and fenugreek leave powder (FLP) on the physical and functional properties of extruded products. Results indicated that OF and FLP decreases lateral expansion (LE), water solubility index (WSI) and hardness (HD), while as OF had increased the bulk density (BD) and water absorption index (WAI). BD and HD were increased by DGPF. FSP had increased effect on LE and WAI, while decreased effect on WSI. OF had a positive (p < 0.05) effect on the "L" and "a" value and has negative effect on "b" value of extrudate. DGPF, FSP and FLP had negative (p < 0.0001) effect on "a" value and positive effect on "b" value of extrudate. Numerical optimization resulted in 21.72% OF, 8.62% DGPF, 1.78% FSP and 0.66% FLP to produce acceptable quality extrudates.

The study was carried out to investigate the effects of oat flour (OF), dried green pea flour

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Extrusion process produces a range of food products with different types of textures, shapes, colours, flavor and aromas from basic materials. A wide variety of products are produced using food extruder including snacks, pasta, breakfast cereals, pet foods, products for confectionery, modified starches used for various purposes, food for baby, instant foods, analogues of dal and rice, texturized vegetable proteins and beverage bases (Riaz, 2012). In spite of greater use of extrusion process technology, still extrusion process is a difficult process that has yet to be mastered. A little change in process parameters, extruder type and type of ingredients can affect the product quality (Desrumaux *et al.*, 1999).

Oats (*Avena sativa* L.) are good a source of functional ingredients such as  $\beta$ - glucan, with findings reveals beneficial effects of oat on health (Wani *et al.*, 2014). Oat possess mixed-linkage of (1, 3) and (1, 4) -  $\beta$ -D-glucan ( $\beta$  - glucan), arabinoxylans and cellulose (Skendi *et al.*, 2003). Arabinoxylans and  $\beta$ -glucans have impact on various food preparations. Both of them are known to decrease the symptoms of diseases like atherosclerosis, diabetes and colon cancer (Karppinen, 2000). Oats has been used for the development of products like bread, ready-to-eat breakfast cereals and snack bars (Decker *et al.*, 2014).

Peas (Pisum sativum L.) are cultivated throughout the world and are available easily. They represent an important source of proteins, dietary fibre and polysaccharides. So formulating foods using pea have high protein content and low glycaemic index (Ravindran et al., 2011). Lysine, an amino acid is high in pea and therefore it can complement cereals complying with the FAO reference pattern (FAO, 2007). Additionally, oat has been recently shown to reduce blood pressure (Jayalath et al., 2014) and possess polyphenols in good quantity indicating its good antioxidant properties (Azarpazhooh and Boye, 2012). Such health benefits may be partially due to the composition. Therefore, it is important to know the composition of the dried green pea flour, which has been used as one of the ingredient in the present study.

Fenugreek (*Trigonella foenum graecum*) is an annual herb widely grown in different parts of the world. Fenugreek seeds are used as spices and its leaves are edible and used as a vegetable in many parts of India. Fenugreek seed is reported to have antidiabetic (Karim *et al.*, 2011), antifertility, anticancer, antimicrobial, antiparasitic, lactation stimulant and hypocholesterolaemic effects (AlHabori and Raman, 1998). Fenugreek has been used for the development of extruded snack with low glycaemic index level. These findings suggest that the nutritional, functional

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and therapeutic characteristics of fenugreek can be used further in the development of healthy extruded products (Shirani and Ganesharanee, 2009; Wani and Kumar, 2015).

The aim of this work was to examine the effect of different proportions of oat flour (OF), dried green pea flour (DGPF), fenugreek seed powder (FSP) and fenugreek leave powder (FLP) on the physical and functional properties of extruded product. Overall target was to optimize the proportion of OF, DGPF, FSP and FLP for the production of quality extruded product.

#### **Materials and Methods**

The raw materials used in production of extruded snack products include: oats flour, dried green pea flour, fenugreek seed powder, fenugreek leave powder, rice and corn flour. All the flours were brought from local market, Sangrur, Punjab. Fenugreek leave powder was prepared by blanching leaves at 90°C for 4 min and then dried at 60°C for 12 h in tray drier. The dried leaves were then ground to powder and packed in polyethylene bags and kept at room temperature until use.

Extrusion of samples was done using a twin-screw extruder (Basic Technology Pvt. Ltd., Kolkata, India). The die diameter used in this study was selected at 4 mm as recommended by the manufacturer for such type of products and recommended by Stojceska *et al.* (2008). In this study the temperature of the extruder barrel and the moisture of the feed material were kept constant at 110°C and 12% wet basis, respectively. The extruded products were cut by a knife by hand (approx. 10 cm long) as they come out from the die. Extrudates were then left for cooling at normal ambient temperature for about 30 min, packaged in plastic bags and stored until analyzed.

# Experimental design and data analysis

Response surface methodology (RSM) was adopted in the design of experimental combinations. The central composite rotatable design for four independent variables of OF, DGPF, FSP and FLP was selected. The independent variables and variation levels were (OF, 10-50%; DGPF, 5-25%; FSP, 0.5-2.5%; FLP, 0.5-2.5%). Each independent variable was used according to preliminary trials and literature. Composite flour (rice flour and corn flour of the ratio 80:20) as a base material was used according to the preparatory trials and literature details for suitable extrusion cooking. In the present study composite flour was replaced by OF, DGPF, FSP and FLP. Dependent variables were product responses like lateral expansion, bulk density, water absorption index and water solubility index and hardness.

# Lateral expansion (LE)

Lateral expansion was determined by using vernier caliper as per methods described by Meng *et al.* (2010). The basic formula used for calculation of lateral expansion is

$$LE = \frac{(\text{diameter of extrudate - diameter of die opening})}{\text{diameter of die opening}} \times 100 \dots Eqn. (1)$$

# Bulk density (BD)

Bulk density was calculated according to the method of Alvarez-Martinez *et al.* (1988) using the formula below

$$BD = \frac{4m}{\pi d^2 L} \qquad \dots Eqn. (2)$$

Where m is mass (g), of a length L (cm) of extrudate with diameter d (cm).

# *Water absorption index (WAI) and water solubility index (WSI)*

The method described by Anderson (1982) was used to determine WSI and WAI. The basic formulas used for calculation of WAI and WSI are given in Eqn. 3 and 4.

WAI 
$$(g/g) = (Weight of sediment) / (weight of dry solids) ... Eqn. (3)$$

# Hardness (HD)

Instrumental hardness (N), maximum peak force of the extruded puffs was measured in triplicate using Texture analyzer (TA-X2Ti). Extrudate of about 10 cm long was compressed with a probe three point bend ring with a target mode distance of 3 mm and load cell of 50 kg.

# Color

All the analysis of color were carried out using a Hunter lab colorimeter and  $L^*$ ,  $a^*$ , and  $b^*$  readings were recorded. The letters  $L^*$ ,  $a^*$  and  $b^*$  represents the whiteness–blackness, redness–greenness and yellowness–blueness of the sample, respectively. Calibration was done according to standard white reference tile. Every sample were measured in triplicate and values then averaged.

## Statistical analysis

Statistical analysis was conducted using a commercial statistical package, Design-Expert version 6.0.10 (Stat-Ease Inc., Minneapolis, USA).

Independent variables											
OF	DGPF	FSP	FLP	LE	BD	WA	WSI	Hardness	1	~	b
(%)	(%)	(%)	(%)	(%)	(gcm <sup>-3</sup> )	(%)	(%)	(N)	L	а	D
20	10	1	1	190	0.156	1.58	13.2	17.93	68.85	-0.87	27.06
40	10	1	1	192	0.153	1.60	15.0	17.23	68.95	-0.69	29.23
20	20	1	1	215	0.111	1.59	11.6	20.29	69.75	-0.95	27.66
40	20	1	1	184	0.16	1.61	11.8	18.58	68.48	-0.57	29.62
20	10	2	1	186	0.16	1.58	17.6	23.60	64.81	-0.10	29.20
40	10	2 2 2 2	1	161	0.19	1.6	17.6	17.39	68.89	-0.30	28.50
20	20	2	1	209.7	0.16	1.59	18.6	18.63	65.53	-0.59	29.53
40	20	2	1	196.8	0.15	1.6	15.6	15.26	66.75	-0.64	28.50
20	10	1	2	195	0.16	1.57	12.8	16.15	66.99	-0.74	28.80
40	10	1	2	190	0.155	1.61	13.6	14.10	69.25	-0.33	28.36
20	20	1	2	211	0.143	1.57	12.6	22.93	68.76	-0.48	29.45
40	20	1	2	176	0.2	1.65	14.6	21.50	66.94	0.09	29.95
20	10	2	2 2 2	204.3	0.125	1.58	13.8	22.80	66.70	-0.96	31.41
40	10	2 2 2 2	2	170	0.153	1.58	11.6	15.24	68.02	-1.02	28.61
20	20	2	2	200.6	0.15	1.57	11.6	21.83	65.68	-1.18	31.20
40	20		2	199.7	0.15	1.58	12.8	14.64	68.38	-0.99	28.74
10	15	1.5	1.5	195	0.146	1.53	14.6	18.52	66.19	-0.70	29.49
50	15	1.5	1.5	186	0.15	1.6	12.0	12.69	67.67	-0.41	28.79
30	5	1.5	1.5	184	0.149	1.53	16.2	14.99	66.54	-0.16	29.42
30	25	1.5	1.5	189	0.14	1.54	18.2	17.97	66.25	-0.30	30.06
30	15	0.5	1.5	192	0.146	1.55	13.0	18.60	70.93	-0.50	27.34
30	15	2.5	1.5	181.1	0.147	1.56	11.4	17.00	65.68	-0.89	28.12
30	15	1.5	0.5	198	0.143	1.56	12.0	19.10	68.28	-0.99	28.35
30	15	1.5	2.5	190	0.14	1.53	13.5	20.41	66.88	-1.20	30.92
30	15	1.5	1.5	190	0.156	1.58	20.0	20.37	66.83	-0.59	29.48
30	15	1.5	1.5	192	0.153	1.60	21.0	21.34	65.83	-0.57	28.09
30	15	1.5	1.5	215	0.111	1.59	19.3	19.90	66.00	-0.57	28.49
30	15	1.5	1.5	184	0.16	1.61	17.2	20.24	64.83	-0.59	28.46
30	15	1.5	1.5	186	0.16	1.58	20.0	19.02	66.20	-0.61	28.99
30	15	1.5	1.5	161	0.19	1.6	21.5	20.20	66.54	-0.59	29.01

Table1. Effect of independent variables on physical and functional properties of extruded product

Statistical significance of the terms was examined by analysis of variance (ANOVA) for each response. The experimental analysis was conducting in triplicates.

# **Results and Discussion**

#### Lateral expansion

Expansion characteristics of extruded products have an important role in the acceptability of the final product. The range of lateral expansion was between 165.37 and 234.37% as shown in Table 1. The regression analysis (Table 2) showed that OF and DGPF had significant (p < 0.0001) negative linear effect on lateral expansion of extruded products, the quadratic effect of FLP has also shown significant (p < 0.05) negative effect. There was a positive (p < 0.001) interactive effect of OF and FLP. The interactive effect of DGPF and FLP, FLP and FSP has significant (p < 0.05) positive effect, where as DGPF and FSP had negative (p < 0.05) interactive effect. It was observed from the response plot (Figure 1a) that with the increase in the content of all four ingredients, a decrease in the lateral expansion of extruded product was observed. This may be attributed to the high protein and high dietary fiber contents in OF, DGPF, FLP and FSP. Because protein affect the lateral expansion by their ability to influence the distribution of water in the matrix and by means of their macromolecular structure and conformation that affects the extensional properties of the extruded melts (Moraru and Kokini, 2003). Martinez-Serna et al. (1990) and Onwulata et al. (2001) observed the effects of whey protein concentrate and isolate on the extruded product of corn and rice starch and they found that a decrease in expansion at higher concentrations of protein. It can be due to modification in the viscoelastic properties of the dough as a result of competition for the water available between the protein and starch portion which results in the delay of gelatinization of starch and leads to lower expansion in the extrudate.

## Bulk density

Akubor and Obiegbuna (1999) reported that bulk density of a sample could be used in determining its packaging requirements. The density of extrudate varies between 0.111 to 0.2 g.cm<sup>-3</sup> as shown in Table 1. Our findings are similar to those reported for chickpea flour snack product (0.130 and 0.275 g.cm<sup>-</sup> <sup>3</sup>) (Meng et al., 2010) and maize grits extrudates (0.09–0.32 g.cm<sup>-3</sup>) (Ilo et al., 1996). The results of regression analysis (Table 2) showed that DGPF have significant (p < 0.05) positive linear effect, whereas OF had positive (p < 0.05) quadratic effect on bulk density of extruded products, the interactive effect of OF and FLP was significantly (p < 0.001) negative on BD of extrudate. From the response surface plot (Figure 1b) it was observed that with the increase in the content of OF and DGPF to the mixture there was an increase in bulk density. This could be due to higher protein content of both OF and DGPF, because protein affect the water distribution in the matrix. The proteins may interact with other components like water by various types of interactions such as polar, hydrophilic interactions, hydrogen bonding which resulted in increased bulk density. The increase

	PRODUCT RESPONSES									
PARAMETERS	LE	BD	WAI	WSI	HD	L	а	b		
βο	189.01	0.14	1.54	17.91	20.178	66.038	-0.589	28.75		
OF (X1)	-8.31***	0.009***	0.016***	0.13	-1.74***	0.481*	0.083***	-0.17*		
DGPF (X <sub>2</sub> )	-6.77***	0.008	-0.0004	0.73*	0.63*	-0.115	-0.024***	0.197*		
FSP (X <sub>3</sub> )	-0.32	-0.0001	0	0.13	0.120	-0.170	-0.055***	0.51***		
FLP (X <sub>4</sub> )	-1.61	0.001	0.007	1.32	-0.105	-0.987***	-0.084***	0.29*		
OF (X12)	1.23	0.007***	0.017	-1.02	-0.93**	0.284*	0.008*	0.10		
DGPF (X <sub>2</sub> <sup>2</sup> )	-0.35	-0.0009	0.01*	-0.05**	-0.72*	0.150	0.089***	0.25*		
FSP (X32)	2.9*	0.001	0.008***	-1.3**	0.097	0.446*	-0.12***	0.22*		
FLP (X <sub>4</sub> <sup>2</sup> )	0.48	0.001	0.005*	-1.03***	-0.391	0.628**	-0.026***	-0.24*		
$OF \times DGPF(X_1X_2)$	2.21	0.001	0.001*	-0.02**	0.176	-0.43*	0.047***	0.046		
OF ×FLP (X1X4)	4.78**	-0.012***	0.001	-0.02	-1.15**	0.628*	-0.10***	-0.69***		
OF ×FSP(X <sub>1</sub> X <sub>3</sub> )	-1.14	0.001	-0.0018	0.2	-0.39	0.020	0.049***	-0.47***		
DGPF × FLP $(X_2X_4)$	4.5**	-0.007	0.002	0.14	-1.66***	-0.123	-0.108***	-0.18*		
DGPF × FSP(X <sub>2</sub> X <sub>3</sub> )	-3.32*	0.001	-0.0018	0.37	0.99*	-0.013	0.079***	0.051		
FLP & FSP (X <sub>4</sub> X <sub>3</sub> )	6.07***	0.0007	0	-0.87*	-0.063	0.430*	-0.258***	0.078		

Table 2. Regression coefficient of different responses of extruded product

\* Significant at  $\bar{p} < 0.05$ 

\*\* Significant at p < 0.001

\*\*\* Significant at p < 0.0001

may be due to the increased fiber, the fiber tended to fracture the cell walls before the bubbles of gas had expanded to their full potential (Lue *et al.*, 1991). Same effect of fiber was found for extruded products made from corn meal and soy fiber, yellow corn with wheat and oat fiber, salt and sugar, jatoba flour and cassava starch blends and corn starch with pectin and wheat fiber (Lue *et al.*, 1991; Jin *et al.*, 1994; Chang *et al.*, 1998; Yanniotis *et al.*, 2007).

#### *Water absorption index*

WAI measures the amount of water absorbed by starch that can be used as an index of gelatinization. However as polysaccharides are the main responsible ingredients for the water absorption, the increase in polysaccharides content will increase WAI. In this experiment the WAI ranged from 1.53 to 1.65% (Table 1). The regression analysis report for WAI showed that OF (p < 0.0001) and FSP (p < 0.05) have significant positive linear effect. The quadratic term of OF has positive (p < 0.0001) effect on WAI of extruded products. Positive (p < 0.05) quadratic effect had also shown by DGPF and FLP (Table 2). On observing the response surface plot (Figure 2a) it was found that with the increase in the content of OF and FSP there was an increase in the value of WAI of extruded product. The increased WAI values observed in the extruded products supplemented with OF, DGPF, FLP and FSP could be related to the high water absorption capacity of OF and FSP, because

the absorption of water is the characteristic feature of fiber supplemented flours as reported by several researchers (Dreese and Hoseney, 1982; Haridas Rao and Hemamalini, 1991; Chaplin, 2003). High dietary fiber in oats, FSP and FLP may interact with water by means of various types of interactions such as polar, hydrophobic interactions, hydrogen bonding. Greater WAI results are similar to those obtained by Roberston and Eastwood (1981) they have found that lyophilized potato fiber absorbed a large amount of water. In the same way Larrea *et al.* (2005) also found that higher water absorption index in the extruded biscuits supplemented by fibrous orange pulp.

#### *Water solubility index*

WSI determines the amount of polysaccharides which are soluble and released from the starch granules after excess of water has been added and is often used as an indicator of degradation of molecular components (Van den Einde *et al.*, 2003). In this experiment the WSI ranged from 11.4 to 21.5% (Table 1). From the regression analysis report, it was found that DGPF had positive (p < 0.05) linear effect on WSI of extruded product. The quadratic effect of OF, FLP and FSP had negative (p < 0.001) effect on WSI of the extruded product. The interactive terms of FLP and FSP has negative (p < 0.05) effect as shown in Table 2. From response surface plot of WSI (Figure 2b), it was observed that with the increase in the content of OF, the WSI value increased at first upto

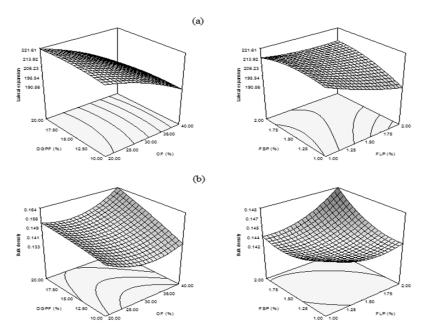


Figure 1. Response surface plot for (a) lateral expansion (b) bulk density as affected by OF, DGPF, FSP and FLP

the addition of 30%, thereafter it decreases down on further addition, on the other hand little effect was observed in WSI as the content of DGPF increased. Further, it was observed from the graph that with the increases in the content of FLP there was increase in the value of WSI. The peculiar behaviour of oat grits perhaps could be attributed to the amount of oil and dietary fiber present in OF, FSP and FLP. Same results were found by Shirani and Ganesharanee (2009) that WSI of the extrudates decreased (p < 0.05) as compared to control chickpea-rice blend, when inclusion level of the fenugreek and fenugreek polysaccharide increased.

#### Hardness

The hardness of extruded product is a sensory perception of the humans and is associated with expansion and cell structure of the product. Hardness is the peak force required for a probe to penetrate the extrudate. The range of hardness found to be 12.69 to 23.60 N (Table 1). For hardness the regression analysis report showed the significant (p < 0.0001) negative linear effect on extrudate with increase in the content of OF, whereas DGPF has positive (p < p0.05) linear effect on extrudates, OF showed negative (p < 0.001) quadratic effect on extrudates and the interactive term of OF and FLP showed negative (p < 0.001) effect and DGPF and FLP have negative (p < 0.0001) interactive effect on extruded products as shown in the Table 2. As revealed from the response surface plot (Figure 2c) that with the increase in content of DGPF there was an increase in the

hardness of extrudate at lower levels of OF, whereas with the increase in the content of OF a decrease in the hardness was observed at lower values of DGPF. The interactive effect of OF and FLP showed decrease in the value of hardness as shown from response surface plot. Low hardness, which is also a favourable property of extrudate, was observed at lower levels of DGPF. This could be attributed to the lower expansion of products lead to increased hardness as observed from expansion values, because of high protein in DGPF. Because proteins affect the lateral expansion through their ability to influence water distribution in the matrix and through their macromolecular structure and conformation that affects the extensional properties of the extruded melts (Moraru and Kokini, 2003).

# Color

Color is an important quality factor directly related to the acceptability of food products, and is an important physical property to report for extrudate products. According to the regression results (Table 2), OF had a significant positive (p < 0.05) linear effect on the "L" value, significant positive (p < 0.0001) linear effect on "a" value and has negative linear effect on "b" value of extruded product, whereas FLP had negative (p < 0.0001) linear effect on "L" value of extruded product. OF and FSP had a positive (p < 0.05) quadratic effect on "L" value of extrudates, FLP had positive (p < 0.001) quadratic effect on FLP, FLP and FSP on "L" value had positive (p < 0.001) for the set of the

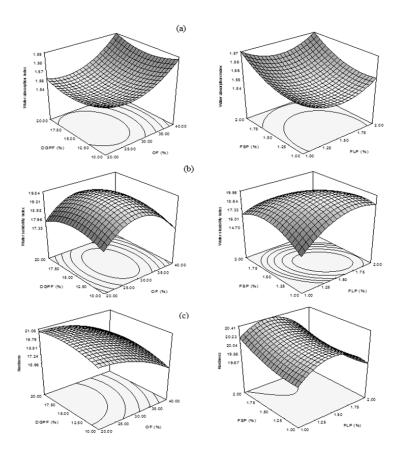


Figure 2. Response surface plot for (a) water absorption index and (b) water solubility index and (c) hardness as affected by DGPF, OF, FSP and FLP

(p < 0.05) effect. DGPF, FSP, and FLP had negative (p < 0.0001) linear effect on "a" value of extruded products. OF (p < 0.05) and DGPF (p < 0.0001) had positive quadratic effect on "a" value of extrudate. FSP and FLP had negative linear (p < 0.0001) effect on "a" value of extrudate. The interactive effect of OF and DGPF, OF and FSP, DGPF and FSP had positive (p < 0.0001) effect on "a" value of extrudate, while OF and FLP, DGPF and FLP, FLP and FSP had negative (p < 0.0001) interactive effect on extrudate. Report on "b" value of extrudate showed that OF has negative (p < 0.05) linear effect, DGPF and FLP had positive (p < 0.05) linear effect on "b" value of extrudate, FSP had also positive linear (p < 0.0001) effect on extrudate. The quadratic terms of extrudate showed that DGPF and FSP had positive (p < 0.05) effect on extruded product. The interactive effect of OF and FLP, OF and FSP (p < 0.0001), DGPF and FLP also has negative (p < 0.05) effect on the "b" value of extrudate. Response surface plots of color are shown in Figure 3.

The reduction in lightness with increasing FLP level may be due to darker color of leaves of fenugreek. Decrease in the value of "a" was observed with the addition of DGPF, FLP and FSP it can be due

to the green color of raw material like fenugreek leave powder and dried green pea flour. The decrease in the value of "b" indicated that yellowness of product was observed due to the addition of FSP, it can be due to the yellowish color of FSP.

#### Optimization

numerical multi-response А optimization technique of RSM was applied to determine the optimum combination of OF, DGPF, FSP and FLP for the production of extrudates using base material of composite flour of rice and corn with a twin-screw extruder. The main criteria of optimization were lateral expansion (high expansion), bulk density (low bulk density), WAI, WSI, HD (low hardness) and color (high "L" value, low "a" value and "b" value in range). From the numerical response analysis, it was found that optimum values were 21.72% OF, 8.62% DGPF, 1.78% FSP and 0.66% FLP with 0.875 desirability. The product response variables resulted in the identification of a combination that satisfied all constraints. Finally the optimized solution provided the ranges of variables which could be considered as the optimum range for best product quality in terms of lateral expansion, bulk density, WAI, WSI,

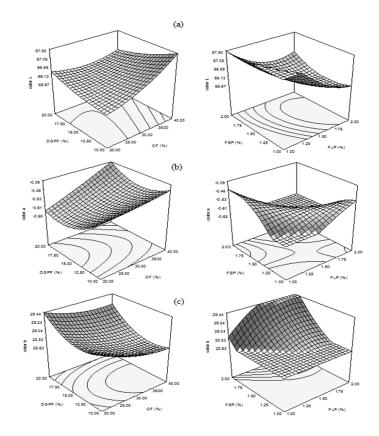


Figure 3. Response surface plot for color (a) L (b) a (c) b as a affected by DGPF, OF, FSP and FLP

hardness and color. These optimum conditions can be used to produce quality extrudate.

#### Conclusion

The product responses including LE, BD, WSI, WAI, HD and color were affected by all the ingredients OF, DGPF, FSP and FLP. The present study revealed that a blend of 21.72% OF, 8.62% DGPF, 1.78% FSP and 0.66% FLP had higher preference levels for parameters of physical, functional and color and could be extruded with acceptable quality characteristics. These results suggest that the physical and functional characteristics of extruded products of OF, DGPF, FSP and FLP can be exploited further in the development of nutritious and healthy extruded products.

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