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Development and Optimization of Process Variables of Protein Enriched Rice Based RTE Food Products

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Abstract: The effect of barrel temperature (100, 120 and 140°C), feed moisture content (12, 14 and 16%) and screw speed (300, 350 and 400 rpm) on extruded product physical, functional and textural characteristics were investigated and optimized using response surface methodology (RSM) following Box-Behnken design. A standardized feed mix of Rice: Ragi: Defatted groundnut: Defatted soybean: Bengal gram (60:10:10:10:10) were used for the development of extruded food product. The extrusion conditions of 140°C temperature, 12.20% moisture content and 383.96 (384) rpm screw speed gave an optimized product with a desirability of 0.837. The respective optimized response values identified were Expansion ratio- 3.18, Bulk density- 0.658 g/cm^3, Water absorption index -5.61 g/g, Water solubility index- 30.24 %, Hardness -24.21 N, Fracturability -10.94 N and Crispness- 55.09 N respectively. The optimized extruded food has 14.31% protein, so that 100g extruded product developed at optimized condition will contribute 35.86% of recommended daily allowance for protein in case of boys, 34% for girls, 23.85% for adult men and 26.01% for adult women, respectively.

Keywords: Extrusion, Protein Enrichment, Texture, Optimization, Rice

Extrusion cooking is a well-known method for developing ready to eat (RTE) snack foods from cereals and plant protein food stuff, perceives evoked substantial curiosity and attraction over the last 30 years. In extrusion processing, pre-conditioned raw materials are allowed to pass under a set of processing conditions through a narrow-shaped hole (die) at a controlled rate to attain different products (Ajitha and Jha 2017). Extrusion is one of the commonly adopted processing techniques by agro-food industries which employ unit operations such as mixing, forming, texturing and cooking to develop a novel food product (Singh et al 2007). In modern food industries sensory evaluation of such RTE food products may be done by the application of fuzzy logic (Babu and Rajesh 2021).

Most of the extruded food products are prepared from starchy materials such as rice, corn and wheat flours but these are limited in essential amino acid content with inadequate nutritional value. Even though, the base component needs to be certain starch abundant materials which become gelatinized at the time of extrusion process. The extrudate physical properties such as expansion ratio and bulk density and textural properties such as hardness and crispness are largely affected by starch sources used in extrusion cooking. The extrudates based on rice starches comprising 20% and 30% amylose showed maximum expansion ratio (Pandiselvam et al 2018). The nutritional deficiency of snack foods can be corrected by using composite flour by selecting suitable raw materials containing high amount of carbohydrate, protein, fiber etc. Cereal based food products are ample in carbohydrates and energy but they are insufficient in terms of protein (Balasubramanian et al 2012).

Rice flour has turned an excellent component in the extrusion industries because of its specific attributes like bland taste, appealing white color, hypo allergenicity, and easiness for digestion (Kadan et al 2003). Addition of legume flour imparts a positive influence on levels of protein and dietary fiber of starch based extruded foods. For the manufacture of nutritious rich extruded snacks, proportion of starch fortification varied in accordance with the type of each material. Legumes have been characterized to impart excellent expansion and are considered as highly suitable for the manufacture of low-calorie and high-nutritional snacks. Finger millet also called ragi which is abundant in carbohydrate, protein, calcium, dietary fiber, iron, minerals and less fat. It is a fundamental food for those people having metabolic diseases such as obesity and diabetes (Mathanghi and Sudha 2012). Defatted groundnut cake flour extensively used in the preparation of variety of food products. It is also fortified with cereal flour to yield products with exceptional flavor, texture and color. Due to its unique nutritional properties it has been used in the diets of people suffering from cardio vascular disease, celiac disease and malnutrition. The fat present in soybean is removed and the remaining products is known as defatted soy flour, that can be used to develop high protein, low fat diet food and due to its unique nutritional profile it serve as promising protein source for the future (Singh et al 2008). Bengal gram or Chickpea has predominant amount of most of the essential amino acids except sulfur containing types and it also a very good source of vitamins such as riboflavin, niacin, thiamin and vitamin A precursor called β- carotene. The minerals such as calcium, magnesium, phosphorous and potassium also present in chickpea. Chickpea has numerous positive health effects and in mixing with other cereals and pulses, it might have beneficial impact on certain serious human diseases such as type 2 diabetes, digestive diseases, cardiovascular diseases and some cancer (Jukantil et al 2012). Protein energy malnutrition is a serious health problem especially in children because of changes in eating pattern and life style. The demand for extruded snack product is expanding at a phenomenal rate so that a protein enriched nutritionally balanced extruded snacks can be made by blending legumes such as Defatted groundnut, Defatted soybean and Bengal gram with rice and ragi. Because of this, the present study is concentrated on optimizing the extrusion process condition by using response surface methodology to achieve a desired extruded food product.

MATERIAL AND METHODS

Raw materials: The raw materials utilized for the production of RTE extruded food products are rice (Jaya variety), ragi, defatted groundnut, defatted soybean and Bengal gram. Jaya variety of rice was obtained from Regional Agriculture Research Station (RARS), Pattambi and other raw materials were procured from the local markets in Kuttippuram, Kerala. All the flours then sieved manually using ISS 85 mesh in order to get uniform particle size.

Blending of flours: The standardization of the feed composition of RTE foods were done during the preliminary evaluation in order to develop extrudate with superior expansion ratio and lesser bulk density. Ten feed compositions were prepared by mixing these raw materials in different proportions and extruded at 120°C barrel temperature, 14% feed moisture content (wet basis) and 350 rpm screw speed. The extrudates developed from the feed composition Rice: Ragi: Defatted groundnut: Defatted soybean: Bengal gram in the ratio 60:10:10:10:10 showed superior expansion ratio and lesser bulk density. Hence it was selected for further experiments.

Extruder and processing conditions: A co-rotating Twin screw extruder (Basic Technology Private, Ltd., Kolkata, India) with an L/D ratio 16:1 and die diameter of 3mm were used for the development protein enriched extruded foods. Feed moisture content ranging from 12 to16% (wet basis) with three variation levels 12, 14 and 16%, barrel temperature ranging between 120 to 140°C with three variation levels 120,130 and 140°C and screw speed ranging from 300 to 400 rpm with three variation levels 300,350 and 400 rpm were selected based on the literature (Hagenimana et al 2006, Garber et al 2006) and preliminary studies. The products obtained were dried for one hour in a mechanical dryer at 40°C temperature and then packed in poly ethylene pouches, sealed and stored for further studies.

Proximate composition: The proximate composition of standardized feed mix before extrusion and optimized extruded product was determined by standard method of the AOAC (2005)- moisture, water activity (Aqua lab water activity meter -Aqua lab, Decagon device Inc., Pullman (Wa), USA), crude protein (AOAC, 2005, 920.86), fat (AOAC 2005, 920.85) and the carbohydrate were determined. The carbohydrate content was analyzed by the Anthrone method (Hedge and Hofreiter 1962), dietary fiber was determined by the method of Ranganna (1986) and the energy content was determined by the formula given by Ekanayake et al (1999).

Effect of Extrusion Condition on Extrudate Properties

Expansion ratio: The expansion ratio (ER) of extrudate is the ratio of diameter of extrudate to the diameter of die hole (3 mm). Ten extruded samples were randomly selected and mean diameter was measured with a vernier caliper (Fan et al 1996).

$$\text{Expansion ratio} = \frac{\text{Diameter of extruded product (mm)}}{\text{Diameter of die hole (mm)}} \quad (1)$$

Bulk density: The bulk density (BD) of the protein enriched extrudates was determined using the method described by Chinnaswamy and Bhattacharya (1986).

$$\text{Bulk density (g/cm}^3) = \frac{4\text{m}}{\pi d^2 L} \quad (2)$$

Where, m is the mass (g) of the extruded sample, L is the length (cm) of extrudate and d is diameter (cm) of the extruded sample.

Water absorption index (WAI) and water solubility index (WSI): WAI and WSI were calculated by the method explained by Anderson (1982). The extrudates were milled and sieved in ISS 90 mesh in order to get uniform particle size. 1g of sample was weighed and shifted into a centrifuge tube and 10ml distilled water was added. Using a test tube shaker the centrifuge tube with sample was shaken for 15 minutes. After that the samples was centrifuged at 4000 rpm for 15 minutes. The supernatant was transferred into petri dish for finding its solid content by keeping the petri dish in an oven at 100°C for 2-3 hr and final weight of the petri dish was noted. The weight of the wet sediment was also recorded and

WAI and WSI were determined by using the following equations;

$$\text{Water absorption index (g/g)} = \frac{\text{Weight of wet sediment}}{\text{Intial weight of dry solids taken}} \quad (3)$$

$$\text{Water solubility index (\%)} = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Weight of dry solids}} \times 100 \quad (4)$$

Texture analysis: Textural properties of RTE expanded products were analyzed using a Texture Analyzer (TA.XT texture analyzer, Stable micro systems Ltd). The textural properties such as hardness (HD) and fracturability (FR) were determined by using penetration test. Crispiness (CR) was determined by using shear test. In the penetration test, the 5 mm cylinder probe was used to pierce into the extrudate test sample and the force required attaining a specific piercing depth or the depth of piercing in a particular time, under definite conditions, was estimated and indicated as hardness. The TA setting for penetration test includes mode: Measure Force in Compression, Pre Text speed: 1mm/s, Option: Return to Start, Distance (compression): 4 mm, Post Test speed: 10 mm/s, Data Acquisition Rate: 400 pps and Test speed: 1 mm/s. Kramer shear cell five-blade probe was used, with test speed of 1 mm/s for determining crispness. Adequate amount of extruded products was used to cover the bottom of the cell, without overlapping, and the test was performed until the probe had completed its travel. The peak force obtained (in newton's) was noted.

Optimization of process parameters: The process parameters such as barrel temperature (°C), feed moisture content (% w.b) and screw speed (rpm) were optimized using Box-Behnken design using Response Surface Methodology.

RESULTS AND DISCUSSION

Experimental design: The effect of process parameters such as barrel temperature (°C), feed moisture content (% wb) and screw speed (rpm) on physical, functional and textural characteristics of extrudates were investigated. The experiments were designed based on Box-Behnken design using Response Surface Methodology with three factors at three levels (-1, 0 and +1). The number of experiments (N) or runs in the Box-Behnken design is obtained from the equation N= 2 k (k-1) +C_0 (where k is the number of factors and C_0 is the number of central points). In the present investigation there were 18 experiments with 6 central points. The coded (±1 and 0) and natural value of the independent variable with design matrix is illustrated in Table 1. A second order polynomial was used for three factor design which is given as

$$Y=b_0+b_1A+b_2B+b_3C+b_{11}A^2+b_{22}B^2+b_{33}C^2+b_{12}AB+b_{13}AC+b_{23}BC \quad (5)$$

Where, Y is the predicted response, b_0, b_1, b_2 and b_3 are linear terms, b_{11}, b_{22} and b_{33} represent square terms, b_{12}, b_{13} and b_{23} are interaction terms and A, B and C are the process parameters. The responses evaluated were ER, BD, WAI, WSI, HD, FR and CR. The ANOVA was performed by using Design Expert 7.0.0 (State-Ease, Inc., Minneapolis) to identify the significance at 0.01%, 0.1%, 1%, and 5% for the linear, interaction, and quadratic effects of the independent parameters and goodness of fit. The regression coefficients estimated for the actual values are indicated in Table 2. The quality parameters such as ER and BD are one of the important characteristics of expanded RTE food products. Low BD and high ER are most desirable factors for consumer acceptance (Ajitha and Jha 2017). Apart from these properties, low HD and FR and high CR are also suitable for an extruded food product. Considering these characteristics as prime importance, the optimization of the extrusion process parameters was conducted and recorded.

Proximate analysis: The proximate composition of standardized feed mix before extrusion and optimized extruded product were determined. The protein content of the extruded product is lower than that of the raw feed mix (Table 3). It may be due to hot extrusion process. The reason for this decrease may be due to the denaturation of protein at higher temperature. Low amount of protein was also due to the loss of nitrogen during extrusion due to the development of isopeptide bonds with instantaneous discharge of ammonia (Kasprzak and Rzedzicki 2008, Jhoe et al 2009). Fat content also reduced after extrusion. The possible reason may be due to the burning of fat at high extrusion process temperatures. This variation in fat content during extrusion was occurred by the development of starch-lipid and protein-lipid networks (Singh et al 2007). Carbohydrate was also decreased during extrusion. This possibly owing to the degradation of starch at higher temperatures. The dietary fiber content is increased after extrusion. It may due to increment in soluble dietary fiber due to disturbance in covalent and non-covalent bonds presented in the carbohydrate and protein moieties causing smaller and more number of soluble molecular fragments in addition to the development of resistant starch and 'enzyme-resistant indigestible glucans' created by transglycosidation. (Rashid et al 2015).

Extrudate Properties

Expansion ratio: The effects of process parameters on ER of the extrudates are interpreted in Table 1. The regression coefficients of dependent variables are given in Table 2. ER varied between 2.42 to 3.21. Figure 1(a) and (b) showed the effect of process parameters on expansion ratio. The reason for expansion at higher barrel temperature can be attributed to the starch gelatinization and strengthening of structure (Ainsworth et al 2007). The ER decreased from 3.21 to 2.42

Table 1. Box –behnken experimental design with independent variables and dependent variables of protein enriched extruded food

Run	Independent variables			Dependent variables						
	Temperature (°C)	Moisture content (%w.b)	Screw speed (rpm)	ER	BD (g/cm^3)	WAI (g/g)	WSI (%)	HR (N)	FR (N)	CR (N)
1	130	14	350	2.77	0.775	4.32	23.12	26.57	13.52	54.12
2	140	14	300	3.06	0.681	5.52	27.67	25.15	12.77	53.17
3	120	12	350	2.61	0.821	3.75	20.38	30.16	17.08	48.51
4	140	14	400	3.10	0.673	5.37	29.14	24.77	11.82	54.82
5	130	16	400	2.69	0.752	4.12	25.43	28.15	15.00	51.95
6	140	16	350	2.98	0.663	5.40	28.02	24.31	11.24	54.22
7	140	12	350	3.21	0.652	5.61	30.25	23.52	10.30	55.72
8	130	14	350	2.78	0.776	4.22	23.22	27.85	14.82	53.18
9	130	12	300	2.72	0.762	4.85	24.15	28.45	15.98	51.13
10	130	14	350	2.79	0.769	4.25	23.24	27.52	14.53	53.42
11	130	12	400	2.82	0.745	4.58	26.75	27.12	14.12	52.48
12	120	14	400	2.59	0.835	3.40	19.52	31.20	18.62	47.45
13	130	14	350	2.78	0.765	4.39	22.35	27.32	14.31	53.67
14	130	14	350	2.79	0.764	4.36	22.24	27.41	14.44	53.53
15	120	14	300	2.52	0.841	3.58	18.30	32.99	19.43	46.37
16	130	14	350	2.87	0.765	4.20	22.21	26.44	13.41	54.29
17	120	16	350	2.42	0.858	3.62	15.68	33.86	20.54	45.23
18	130	16	300	2.60	0.783	4.70	21.52	29.12	16.41	50.18

ER- Expansion ratio, BD- Bulk density, WAI- Water absorption index, WSAI- Water solubility index, HD- Hardness, FR- Fracturability, CR- Crispness

Table 2. Regression coefficients of the fitted second order polynomial for dependent variables

Coefficients	Regression coefficients						
	ER	BD (g/cm^3)	WAI (g/g)	WSI (%)	HD (N)	FR (N)	CR (N)
Intercept	2.80	0.77	4.29	22.73	27.19	14.17	53.71
A	0.28****	-0.086****	0.94****	5.15****	-3.86****	-3.69****	3.80****
B	-0.084***	9.500E-003*	-0.12*	-1.36**	0.72**	0.71*	-0.78**
C	0.039*	-7.750E-003*	-0.15**	1.15**	-0.56*	-0.63*	0.73**
AB	-9.250E-003	-6.500E-003	-0.020	0.62	-0.63	-0.63	0.45
AC	-7.500E-003	-5.000E-004	7.500E-003	0.062	0.35	-0.035	0.14
BC	-2.500E-003	-3.500E-003	-0.078	0.33	0.090	0.11	0.11
A^2	0.060*	-0.012	0.11	0.024	0.60	0.45	-1.88***
B^2	-0.050*	-8.750E-003	0.20**	0.83	0.28	0.17	-0.90*
C^2	-0.037	2.500E-004*	0.072	0.90	0.74*	1.04*	-1.37**

Significance at ****(P < .0001), ***(P < .001), **(P < .01), *(P < .05)

Table 3. Proximate composition of standardized feed mix and optimized extrudate

Material	Moisture content (%)	Water activity	Protein (%)	Carbohydrate (%)	Fat (%)	Dietary fibre (%)	Energy content (kJ/100 g)
Standardized feed mix before extrusion	8.95	0.562	16.01	71.54	3.51	8.61	1594.41
Optimized extruded product	4.39	0.430	14.31	68.60	2.20	9.49	1467.54

as the moisture content of feed increased from 12 to 16% (w.b). The increase in feed moisture content can decline the elasticity of the mass by plasticizing the melt and, consequently, lowering gelatinization, decline the expansion and raises the density (Korkerd et al 2016). The process parameter screw speed also showed a positive influence on expansion ratio of the extrudates. Elevated screw speeds may be likely to reduce melt viscosity of the mixture enhancing the elasticity of the dough, causing increased expansion and depletion in the density of the extrudate (Ding et al 2006).

Bulk density: The BD of extrudates decreased from 0.858 to 0.652 g/cm^3 as the barrel temperature increased from 120 to 140°C (Table 1). The 3D graphs (Figure 1(c) and (d)) representing the response surface are also indicates the effect of process parameters on BD. The viscosity of plasticized mass inside the extruder decreases with increase in barrel temperature and it would enhance the bubble enlargement throughout extrusion cooking and thus lowering the density. The higher screw speeds may reduce the melt viscosity of the mix which causes an increase in elasticity of the dough and thus producing extrudates with lower density.

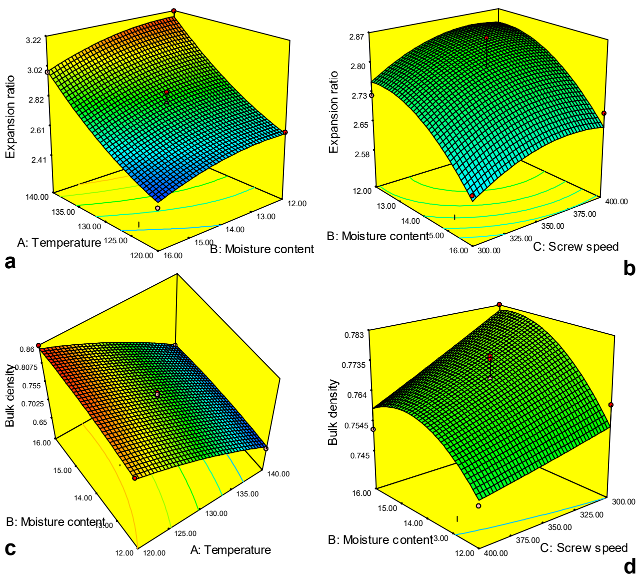

Fig. 1. Response surface diagram illustrating the effect of process parameters on Expansion ratio (a and b) and Bulk density (c and d)

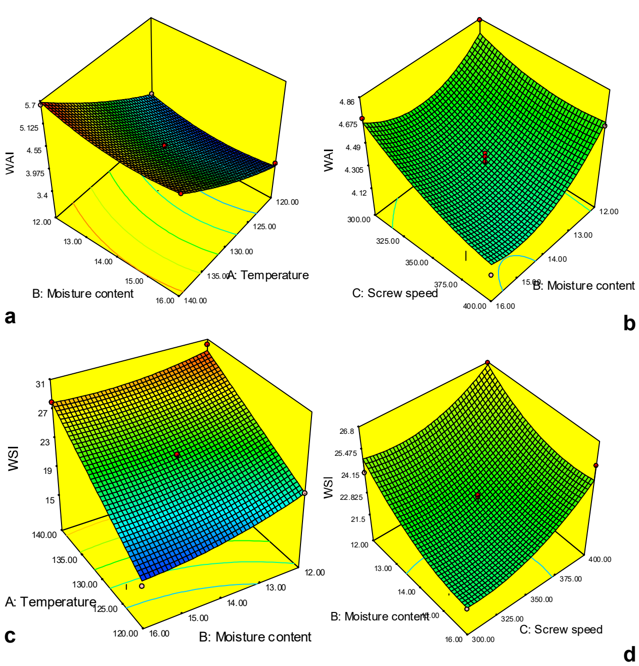

Fig. 2. Response surface diagram illustrating the effect of process parameters on WAI (a and b) and WSI (c and d)

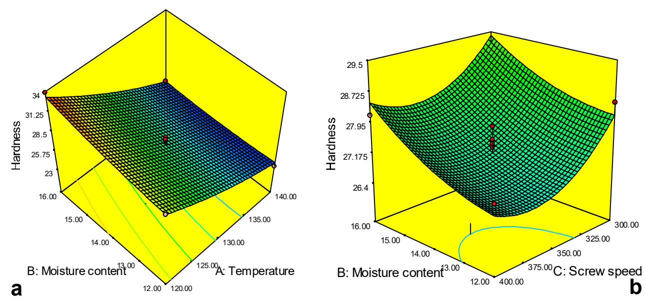

Fig. 3. Response surface diagram illustrating the effect of process parameters on HR

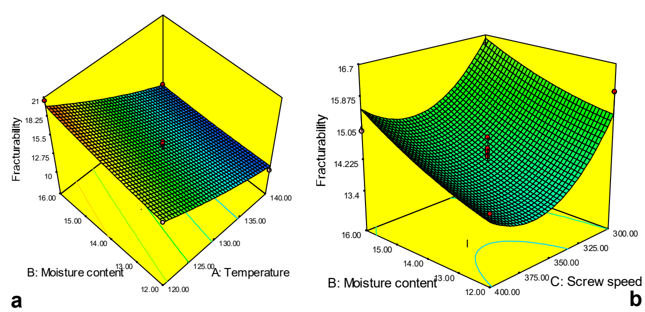

Fig. 4. Response surface diagram illustrating the effect of process parameters on FR

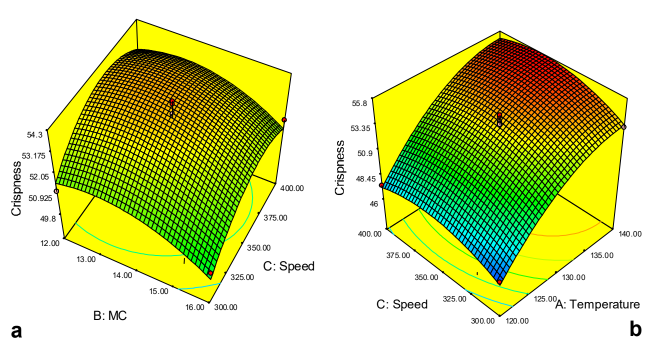

Fig. 5. Response surface diagram illustrating the effect of process parameters on CR

The process parameter moisture content had a negative influence on BD. An increase feed moisture content during extrusion process may lower the elasticity of the dough through plasticization of the melt, resulting in decreased specific mechanical energy and thereby lesser gelatinization, reducing the expansion and an increase in the density of extrudate (Ding et al 2006).

Water absorption index: The water absorption index (WAI) of extrudates samples changed from 3.40 to 5.61 g/g. WAI of extrudates increased significantly with an increase in barrel temperature (Table 1, Fig. 2a). As temperature rises, the starch got damaged because of gelatinization and extrusion process. The elevated temperature produced dextrinization, which create additional hydrophilic space and therefore increased WAI. WAI showed a decreasing pattern with moisture content (Figure 2b)). It may due to the fact that at higher moisture situations cause lesser shear degradation of starch throughout extrusion (Anastase et al 2006). Screw speed also found to have a negative effect on WAI of the extrudates (Fig. 2 b). This is due to the fact that high input of thermal energy due to high residence time i.e., at low screw speeds may cause enhanced level of starch degradation and increased WAI (Yagcı et al 2008).

Water solubility index: The increasing trend was noticed in WSI with temperature (Table 1, Fig. 2c and d). It may be due to the fact that elevated temperature imparts additional soluble constituents leads to increment in WSI (Ding et al 2005). Screw speed also showed an increasing trend in WSI. The increase in screw speed causes increment in specific mechanical energy which resulting the breakdown of macromolecules and thus causing rise in soluble constituents after extrusion cooking. The higher mechanical shear enhanced breakdown of macromolecules to micro molecules with elevated solubility (Dogan and Karwe 2003). But the process parameter moisture content had an inverse effect on WSI. This trend was created by lesser shear disintegration of the starch at the time of extrusion at elevated moisture contents causing lower WSI (Pardhi et al 2017).

Textural properties: For extruded snacks, low values for hardness, factorability and high value of crispness are desirable. The 3D graphs representing the response surface are depicted in Figure 3, 4 and 5. The regression coefficients of dependent variables are given in Table 2. Both HR and FR showed a negative relationship with extrusion temperature but CR had a positive effect. An increase in temperature increased the degree of superheating of water in the extruder. This enhances the bubble development and also decreased melt viscosity, which in turn results reduction in density and hardness of extrudate and produce crispy extrudates. Screw speed also showed a negative trend with both HR and FR but positive trend with CR. It may be related to higher expansion and lower density of extrudates at higher screw speed (Ding et al 2005, Sharma et al 2016). But the process parameter feed moisture content had a positive effect on both HD and FR and negative effect on CR. The reason may be due to plasticizing characteristics of starch containing ingredients causing viscosity reduction and the mechanical energy liberation in the extruder and thus producing the product somewhat denser and suppress the bubble development (Chiu et al 2013).

Optimization of extrusion process variables: The independent variables were optimized numerically by using statistical software Design Expert- (Ver- 7.7.0. -Stat-Ease Inc.). For the optimization of process parameters ER, WAI, WSI and CR values were kept maximum and simultaneously BD, HR and FR values were kept minimum. From the numerical optimization process, it was observed that 140°C temperature, 12.20% moisture content and 383.96 (384) rpm screw speed gave an optimized product with a desirability of 0.837. The resultant optimized response values identified were ER- 3.18, BD- 0.658 g/cm^3, WAI -5.61 g/g, WSI- 30.24 %, HD -24.21 N, FR -10.94 N and CR- 55.09 N. The extrusion process was conducted based on the optimized conditions and the proximate composition of optimized product were recorded (Table 3).

CONCLUSION

Designed experiments applying Box-Behnken method effectively showed the impact of independent variables such as barrel temperature, feed moisture content and screw speed on the response variables including physical, functional and textural properties of extrudates developed from protein enriched formulation. The optimized extrusion process conditions were found as 140°C temperature, 12.20% moisture content and 383.96 (384) rpm screw speed, respectively. The optimized extruded product has 14.31% protein, so that 100 g extruded product developed at optimized condition will contribute 35.86, 34, 23.85 and 26.01% of RDA of protein in boys, girls, adult men and women, respectively.

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