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Effect of Soy–Jackfruit Flour Blend on the Properties of Developed Meat Analogues Using Response Surface Methodology

Thokachichu Sadhana, Akash Pare[*], S. Bhuvana and R. Jagan Mohan

Department of Academics and HRD, Indian Institute of Food Processing Technology, Pudukottai Road, Thanjavur-613005, Tamil Nadu, India

*Corresponding Author E-mail: akashpare@iifpt.edu.in

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ABSTRACT

In the current study two independent variables, defatted soya flour (SF) and raw jackfruit flour (JF) extracts were used for the development of meat analogues and their effect on properties of product were studied. Preliminary runs were conducted using a wide range of extrusion parameters and the best possible extrusion parameters within the ranges were optimized. Different compositions of raw materials used in preliminary study helped in fixing the proportions. The effect of independent variables was studied using response surface methodology (RSM) of central composite design. The evaluated physical properties were the Oil Holding Capacity (OHC), Water Holding Capacity (WHC), Hardness, Springiness, Fracturability, Swelling Index (S.I) and Colour value of the meat analogues. To calculate the variation occurred in WHC, OHC, Hardness, Springiness, Fracturability, S.I and Colour value, the high R^2 values of second order regression model were considered. From the results, the optimum formula that was predicted for development of meat analogues was, defatted soy flour at 36% and raw jackfruit flour extracts at 12%. The validation of this optimum formula is shown by experimental results and observed to be not dissimilar statistically on 5% level. Thus, response surface methodology can be utilized to optimize the formulation and can be successfully employed for development of unconventional meat analogues.

Key words: *Meat analogues. Extrusion parameters RSM Physicochemical analysis*

INTRODUCTION

Animal protein is very valued nutritious food. It is important resource of all essential micronutrients and high biological value protein. However, these helpful attributes have frequently been followed by some negative attributes, this taps into people shifting to vegetarian or vegan diets. World Health Organization classified consuming processed meat as “carcinogenic to humans”. And the red meat which contains high saturated fat can also be a threat[1].

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Therefore, studies were going on to find the alternative healthier food to meat which could give same mouth feel with great nutritional values from plant sources by modifying some of the functional components[2,3]. In current years, the need for alternative animal proteins are growing demand which increased the interest in prospective of soya products as a basis of consumable vegetable protein because of its high protein content with distinguishing functional properties[4]. The low energy consumption is advantages in extrusion cooking of soya protein which also improves its texture and producing little waste compared to the fibre spinning process even versatility in product shapes are possible[5,6].

To increase its physiological and nutritional characteristics there is a need for new intervention which increases its properties, in that aspect Jackfruit, is which alike to meat in its texture, characteristics and often used by vegans as meat replacer and those trying to alter their diet[7]. The unripe jackfruit is used as meat analogue because of its texture mimics the pulled pork. Containing soluble dietary fibre helps to remove cholesterol from your body and improve the sensory as well as psychochemical properties[8,9]. Mathematical and statistical technique used for testing linear, quadratic and interaction effects of various process variables is Response surface methodology (RSM). Mainly used to determine the interaction between responses and factors where optimum condition for processing can be predicted[10].

Objective of this study was to evaluate the combined effect of defatted soy flour (SF) and raw jackfruit flour (JF) concentration on Water Holding Capacity (WHC), Oil Holding Capacity (OHC), Hardness, Springiness, Fracturability, Swelling Index and Colour value of soya jackfruit meat analogues by using RSM. To the best of our knowledge, this is the earliest report on development of soya jackfruit based meat analogues.

MATERIALS AND METHOD

Materials

Defatted soya flour purchased from Pune, Maharashtra. The proximate composition in flour is found to be 62.73% protein, 0.79% fat and particle size 0.5mm, Raw jack fruit, Calcium chloride (1%), sodium alginate (2%), water (14%) with pH 7-8, corn flour (11-14%), wheat flour (10%) blend help in developing texturized protein[11], plant oil (4%), guar gum (1%), flavouring (7%) were procured from local market. Jackfruit flour is grounded to particle size of 0.8-1mm latter sieved to 0.5mm.

Experimental Design

Response surface central composite design was used to conduct the experiments. Two independent variables including soya flour (S.F %), raw jackfruit flour (J.F %), were explored as shown Table 1. For each factor an experimental range was given based on the preliminary study results. Using central composite design (CCD), quadratic model, 7 responses are chosen including WHC, OHC, swelling capacity, colour and different textural as indicators of product quality. CCD data were analyzed by multiple regressions to fit as shown in the equation 2.1.

$$Y = \beta o + \sum_{i=1}^{k} \beta ii xi^2 + \sum_{i=1}^{k-1} \sum_{j>i}^{k} \beta ij \times xi \times xj + \epsilon \qquad \text{Eq- (2.1)}$$

Where y is the predicted response, e the error, i and j the coded factors of system and k, the number of the variables considered. b_i the coefficients of the linear effects, b_0 the model constant, b_{ij} the coefficients of interaction between the factors, b_{ii} the coefficients of the quadratic effects, x_j and x_i the coded independent variables, by using regression analysis and their significance the coefficients were calculated and verified using analysis of variance.

Sample preparation

Raw JF flour was prepared by drying the cut pieces in tray drier at 60°C, grinding and sieving to 0.5mm. Percentage of SF and JF were taken according to runs given by RSM, remaining ingredients Calcium chloride (1%), sodium alginate (2%), water (18%), wheat flour (10%) which blend help in developing texturized protein, corn flour(10%), plant oil (4%), guar gum(1%), flavouring (4%) are taken according on a weight by weight basis. All ingredients are mixed for 20 min with 12-15% moisture which helps in getting texturized protein structure through extrusion[12].

Extrusion cooking

Extrusion was done with an counter-current twin screw extruder of 'Dacheng Electrical Machinery model YS7124' with 13.5:1 L/D extruder barrel and die hole of 4.5 mm diameter. Temperature for extrusion cooking was set to 30°C, 60°C, 110°C and 130°C for the four barrel temperature zones respectively, decided based on preliminary runs. Screw speed and cutter speed was set at 210 rpm and 520 rpm respectively[13]. Extrusion was followed by drying at 75°C for 20 minutes and products allowed to cool at room temperature.

Textural measurements

Texture was objectively analyzed with a TA. HD. Plus texture analyzer (Stable Micro Systems) of cell size 30 kg. Five bladed Kramer shear cell is used for measuring fracturability (kg) by observing the series of force peaks and 35mm cylindrical probe (p/35) used to measure hardness (kg) and springiness (%) of extrudate by calculating force in compression.

Physicochemical analysis

Proximate analysis, texture, oil holding capacity, water holding capacity, swelling Index, colour and solubility indexes of the extrudates were calculated and determined as indicators of product quality.

Water Holding Capacity (WHC) and Oil Holding Capacity (OHC):

The *WHC* & *OHC* was measured using the technique of Traynham *et al*., 2007[14]. Extruded sample (5g) was placed in distilled water for WHC and in oil for OHC and kept a side for 30 min by gentle stirring at normal room temperature. After hydration, the samples were subjected to centrifuge at 3000rpm for 20 min; weight of the sample was noted after removal of the supernatant. WHC & OHC was determined in triplicate for each sample and noted as g/g of sample.

Colour evaluation:

The sample colour and the standard colour (referral commercial sample) values should be measured. The colour differences between the sample and standard are calculated by Commission of International de l'Eclairage (CIE), L*a*b* Coordinates and calculating ΔE value[15].

$$\Delta E=\sqrt{(L2-L1)2+(a2-a1)2+(b2-b1)2} \qquad \text{Eq- (2.2)}$$

Swelling Index (SI) and Water Solubility Index (WSI):

SI and WSI were calculated according to the methods followed by Gunaratne A *et al*., 2011[16]. Sample (2g) was taken into beaker having 10mL of distilled water. These were incubated in water bath for 30 minutes at 85±2°C with regular mixing. At room temperature the beakers were cooled and centrifuged for 10 minutes at 2500 rpm, the supernatant was collected separately and residual left in the tube was weighed (W_s). To a constant weight (W_1) the supernatant was dried in a hot air oven at 100±2°C. Then sample's swelling Index is calculated as follows.

$$\text{SI} = \frac{sample\ weight\ (g)}{[0.1\times(100\%-WSI)]g}$$

Where,

$$\text{WSI} = \frac{\text{w1}}{0.1}\times 100 \qquad \text{Eq- (2.3)}$$

Proximate analyses:

Proximate composition of extruded soya jackfruit meat analogues and control was calculated in triplicate according to the procedures given by AOAC, 2003. Moisture, ash, Fat - 950.46B, 938.08A and 983.23A respectively and protein content using kjeldahl method with 6.25 as conversion factor was used for determining the samples. Carbohydrates were calculated by difference of total nutrients.

Sensory assessment:

The hedonic scale test for soya jackfruit meat analogue and commercial control sample was estimated according to the method described by Edelstein *et al*., 2014[17]. Total of 15 panellists conducted sensory testing at IIFPT, Thanjavur. The samples were steam cooked (15 min, 180°C) and then fried (10 min) before subjected to testing. The session was conducted at room temperature and day light, to avoid inheritance factors. The panellists were insisted to rate for fibrous structure of the samples (visual examination by sample tearing), hardness (nibbling the sample completely), chewiness (by masticating completely), appearance (observation of colour, structure) and flavour (taste, odour) using 9-point hedonic scale.

Structural analysis (SEM):

Meat analogues with jackfruit and control without jackfruit were cut approximately 10.5×10.5×10.5 mm, dried below 5% moisture for visualisation under SEM, The samples were placed using tape onto an aluminium stub then were layered with 100 nm of gold approximately for conduction and viewed under SEM (VEGA3 TESCAN) at 500× magnification, wd: 25.93mm with 7 kV accelerating voltage and view field of 415µm.

Statistical analysis:

The fitted model quality was assured by values of Lack of fit, R^2 and Adjusted R^2 from design expert software. Using an F-test significance of its statistics was determined. 0.05% of statistical significance was set.

RESULTS AND DISCUSSION

Effects of different concentrations of soya flour and jackfruit flour:

The values obtained from each experimental running studied in the response variables are shown in Table 1. Extrusion resulted in production of low moisture product with 5±0.3% and expansion ratio of 1.3±0.2. The highest hardness with good WHC and OHC was noticed on run number 10. This may be due to the proteins present in soya are molten / plasticized by combined effect of hydration, cooking and mechanical restructuring inside the barrel resulted in better product. And ingredients such as sodium alginate, Calcium chloride may also helped increase in swelling capacity WHC, OHC, and textural integrity of extruded protein product[18].

Experimental design

RSM of central composite design helped to illustrate the factor interactions and influence of percentage of soya flour and jackfruit flour on extruded samples shown in Fig.1. The table 2 explain the factors, predicted and experimental values of the response from the design software. The regression coefficient and second order polynomial models of responses of developed extruded meat analogue is shown in Table 3. The obtained experimental values for the chosen responses are almost near to the predicted standard values signifying that the model is appropriate. No significant difference is shown in lack of fit in table 3. The responses of WHC (Y1), OHC (Y2), Hardness (Y3), Springiness (Y4), Fracturability (Y5), Swelling Index (Y6) and colour (Y7) and independent variables (X1&X2) are mentioned in final equations in terms of actual factors in table 4. The predictions about the response for given levels of each factor can be made in terms of actual factors equation.

On the basis of responses obtained for restructured product is shown in Table 1, the response surface analysis showed that the soya flour and jackfruit flour concentrations significantly affected the responses at $p<0.05$ and fitted to second-order model. Equations Y1, Y2 and Y5 showed a positive quadratic effect on WHC, OHC and swelling capacity of the determined factors. That mean by increasing the concentration of soya flour and jackfruit flour (with neutral effect) will increase the WHC, OHC and swelling capacity this may be due to these responses are related to Protein–water interactions[19], therefore, the protein content within the flour blends may influence the WHC,OHC and swelling capacity, similar results were quoted in[14].

Increasing J.F flour into a formula of soya jackfruit meat analogues will increase crude fibre of the end products by 26% which did not negatively affect any of the parameters. Significant increase in extrudates hardness is observed with increasing the S.F ratio in the mixture ($P < 0.05$). It means that increase in protein concentration could result in increase of hardness in the product[20]. Whereas the jackfruit flour did not show any negative effect on hardness up to certain increase in its concentration. But extrudates springiness showed no significant differences of different SF& JF ratios ($P < 0.05$). Higher J.F concentration led to a significant decrease in fracturability ($P > 0.05$), this could be happened due to the disturbance caused by fibres of J.F to protein cross linking[21]. And colour (ΔE) value ($p<0.05$) increased with increase in Jackfruit flour this might be due to the darker colour of the flour which resulted in increase in the difference value with the control.

Both of the independent variables, soy flour and jack fruit flour, showed quadratic effect on WHC, OHC, hardness, fracturability, swelling Index and colour of meat analogues with R^2 value or the coefficient of determination greater than 87%, Higher R^2 value, nearly 1 is mostly preferable[22]. This is structurally verified by SEM analysis fig no.4. Therefore the developed RSM is appropriate for this study.

Predicted optimization with its verification:

On design expert demand criteria to obtain optimization variables the range is given as per reference to the commercial sample, maximum WHC, OHC and swelling Index, hardness and fracturability is in the range, maximum springiness, minimum colour value were chosen. The optimum formulation required for developing soy jackfruit meat analogues was as follows: soy flour of concentration 36% w/w and jackfruit flour concentration 12% w/w shown in Fig.2. Which resulted in producing meat analogues with WHC 1.80g, OHC 0.44g, Hardness 12.48 Kg, Springiness 21.099%, Fracturability 19.65 Kg, S.I 3.95%, Colour (ΔE) value 5.16 respectively and the highest desirability of '1'. The attained desirability means 100% affect by the independent variables responses and noises affect 0%. To evaluate that the experimental results were not biased to the predicted value a verification measure was conducted, with the objective that every response produce maximum result arriving at a conclusion by reasoning from evidence of optimal condition. Three replications experiments were conducted to recheck. The results showed that WHC, OHC, Hardness, Springiness, Fracturability, S.I, Colour (ΔE) value are 1.82±0.1, 0.42±0.2, 12.37±0.1, 21.00±0.1, 19.52±0.2, 3.81±0.1, 5.12±0.1 respectively. The model was confirmed to be fit with less than 2% differences in responses between the repeated experiments and predicted results.

Proximate analysis:

Fig 3 shows the proximate composition for the developed product at optimised composition and also the control sample. Fortification of soya flour with jackfruit at 36:12 ratios substantially increased the crude fibre, moisture and mineral content compared to unfortified meat analogues due to the presence of jackfruit flour having high fibre content of 28.9±0.01, whereas there was slight reduction in protein content i.e., 47.35g and carbohydrates 11.04g in product compared to control might be due to high percentage of defatted soya flour in control sample compared to developed product which tentatively increased slight amount of protein. Almost similar values of moisture and fat content were obtained in two samples. The processing method most likely influenced the protein and fibre content.

Sensory evaluation:

To analyse the differences in sensory properties, 9 point hedonic scale test was performed. Two samples control and the meat analogues were taken for test. The results of sensory attributes like hardness, fibrous structure, chewiness, flavour and also appearance of meat analogues and control are shown in Fig.4. The fibrous structure observes the fibrous network, increased scores were observed in the developed sample than the control sample. This might be due high fibre content in the developed sample. The force required to deform a substance between teeth is hardness[23] which is higher for control sample because of higher protein content which is correlated with hardness. The energy required to masticate food is chewiness which almost similar with slight variation. The flavour score are high for the meat analogue than the control due to the fruit flavour of jackfruit increased the acceptance whereas appearance scored low for the meat analogue due to darker colour compared to the control.

Structural analysis (SEM) analysis:

The microstructure of control (S1) showed discontinuous loose network with a diffuse structure and wide pores, well connected small pores distributed uniformly and dense network can be clearly observed in optimized sample (S2). A fine-stranded network with 'tighter' in appearance was visible in sample, shown in Fig.5. Thus soya jackfruit in combination promoted more homogeneous structure by formation of fine-stranded networks with desirable physical values. Firmer product was obtained, for the samples especially with 36% soya and 12% jackfruit. This suggests that intervention of jackfruit flour in soya meat analogues preparation may offer a means to increase the structural and physiological properties.

Table.1 The experimental design, Independent variables and their levels

Independent variables	Factor levels				
	-1.414	-1	0	+1	1.414
Soya flour	17.893	21	28.5	36	39.106
Jackfruit flour	-3.106	0	7.5	15	18.106

Table 2 Central composite design (CCD) for each experimental running and their physical properties

	Factor 1	Factor 2	Response-1	Response- 2	Respons-3	Response-4	Response-5	Response-6	Response-7
Run	A:soya flour	B:Jack fruit	WHC g/g	OHC g/g	Hardness kg	Springiness %	fracturability kg	swelling Index %	colour Δvalue
1	28.5	7.5	2.00	0.40	11.20	23.90	17.35	3.98	4.90
2	28.5	7.5	1.98	0.39	10.80	33.47	17.37	3.91	4.89
3	21.0	15.0	1.23	0.18	8.21	10.00	12.98	2.88	6.89
4	28.5	18.1	1.21	0.20	10.80	45.38	12.11	2.12	7.23
5	21.0	0	2.21	0.38	7.99	15.64	19.98	3.83	2.21
6	28.5	7.5	1.99	0.40	12.10	18.01	17.29	3.89	4.89
7	28.5	0	2.46	0.48	13.90	57.78	20.49	3.96	2.23
8	28.5	7.5	2.02	0.40	10.90	31.39	17.10	3.99	4.68
9	17.8	7.5	1.75	0.24	11.07	16.38	15.32	3.14	5.23
10	39.1	7.5	2.22	0.52	14.54	34.38	21.98	4.20	2.89
11	36.0	0	2.48	0.61	14.41	36.30	24.55	4.63	2.03
12	36.0	15.0	1.52	0.45	11.00	9.31	20.01	3.98	6.02
13	28.5	7.5	2.00	0.41	9.98	9.45	16.90	3.90	4.20

Table 3 Predicted and experimental values of two independent variables by Central composite design

S.No	Soya Flour	Jackfruit Flour	Responses	WHC (g/g)	OHC (g/g)	Hardness (Kg)	Springiness (%)	Fracturability (Kg)	S.I (%)	Colour (ΔE)
1	36.00	12.00	Predicted values	1.80	0.44	12.48	21.09	19.65	3.95	5.16
			Experimental values [a]	1.82±0.1	0.42±0.2	12.37±0.1	21.00±0.1	19.52±0.2	3.81±0.1	5.12±0.1
2	35.34	12.00	Predicted values	1.79	0.43	12.33	21.56	19.19	3.91	5.16
			Experimental values [a]	1.72±0.1	0.3±0.1	12.12±0.2	21.52±0.4	20.11±0.2	3.81±.1	5.00±0.4
3	36.00	11.67	Predicted values	1.824	0.450	12.33	21.56	19.19	3.99	5.035
			Experimental values [a]	1.79±0.2	0.4±0.3	11.83±0.1	20.86±0.1	19.20±0.2	3.89±.2	5.02±.1

a Mean values ± standard deviation.

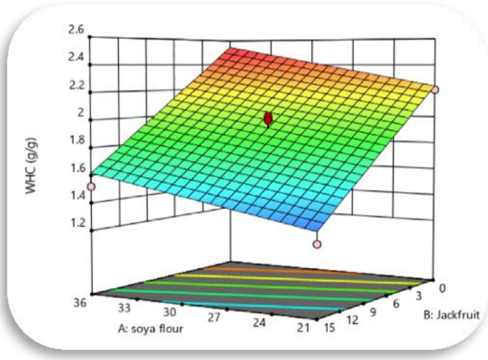
Table 4 Responses predicted second-order polynomial models and Regression coefficients

Term	Product Responses						
Factor	WHC g/g	OHC g/g	Hardness Kg	Springiness %	fracturability Kg	swelling Index %	colour Δvalue
Model F value	128.88	96.9	8.67	1.59	23.64	10.25	71.31
Lack of fit	2.7*	1.8*	4.61*	3.04*	3.21*	2.68*	3.68*
C V (%)	4.46	7.63	12.04	51.40	5.96	7.9	10.78
R^2	0.9627	0.9510	0.6343	0.5315	0.9441	0.8798	0.9345
Adjusted R^2	0.9552	0.9411	0.5612	0.1968	0.9041	0.7940	0.9214
Predicted R^2	0.9310	0.8965	0.2501	-1.5389	0.6086	0.1553	0.8735
Adequate precision	31.7338	28.8824	8.3179	4.6996	15.2767	9.8844	23.9576

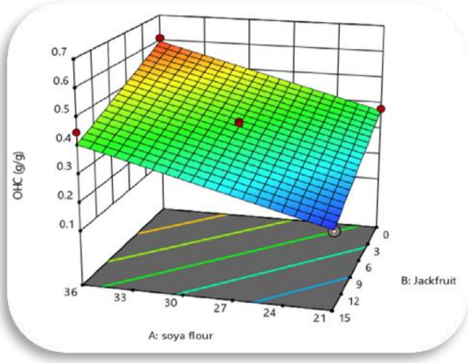
* Non significant

Table 5 Equations of actual factors of given responses

S.No.	**Responses**	**Final Equation in Terms of the Actual Factors**
1	WHC	$Y1 = 1.81 + 0.02X_1 - 0.06X_2$
2	OHC	$Y2 = 0.05 + 0.01X_1 - 0.01X_2$
3	Hardness	$Y3 = 5.57 + 0.23X_1 - 0.12X_2$
4	Springiness	$Y4 = -62.53 + 5.62X_1 - 0.53X_2 - 0.09X1X_2 - 0.07X_1{}^\wedge 2 + 0.X_2{}^\wedge 2$
5	Fracturability	$Y5 = 29.87 - 0.94X_1 - 0.70X_2 + 0.X_2 + 0.02X_1{}^\wedge 2 + 0.0004X_2{}^\wedge 2$
6	Swelling Index	$Y6 = 2.61 + 0.05X_1 - 0.02X_2 + 0.001X_1X_2 - 0.0002X_1{}^\wedge 2 - 0.005X_2{}^\wedge 2$
7	colour	$Y7 = 4.58732 - 0.072X_1 + 0.26X_2$



P1



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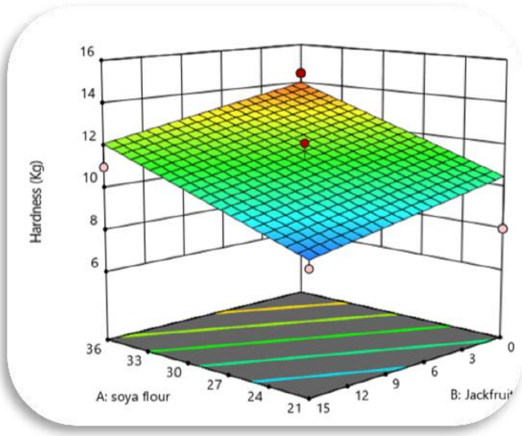

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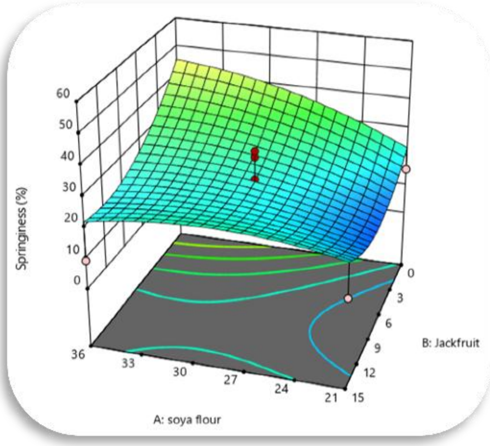

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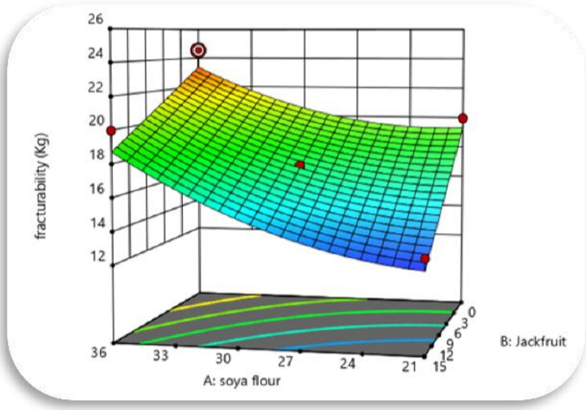

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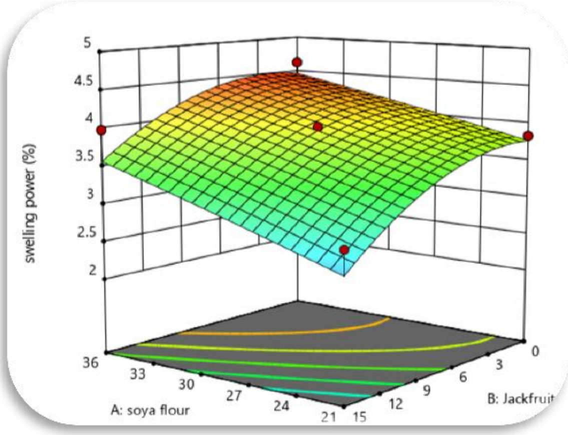

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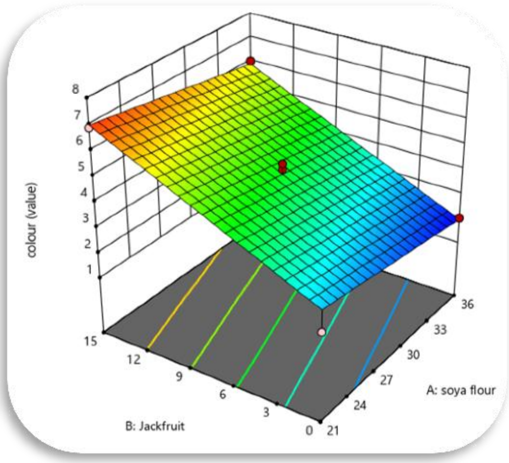
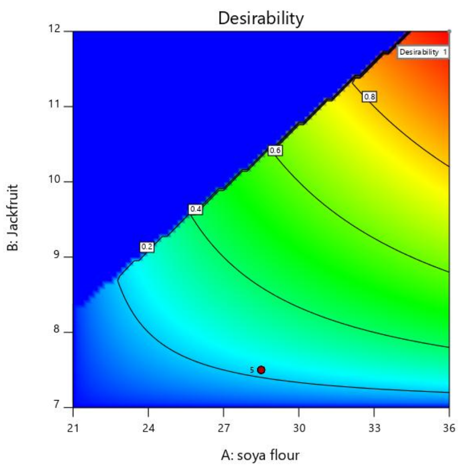
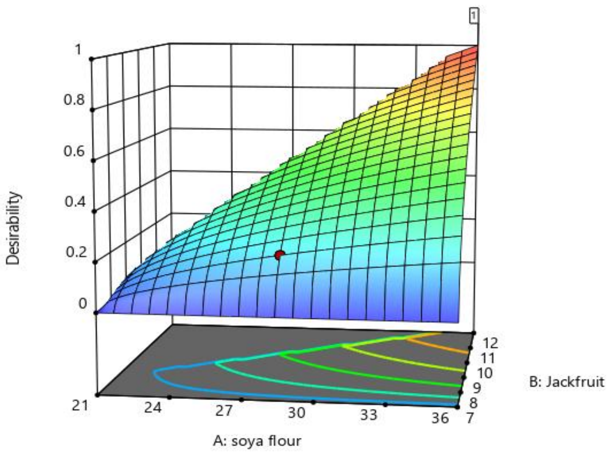


P7

Fig. 1: Response surface design plots of WHC(P1), OHC(P2), Hardness(P3), Springiness(P4), Fracturability(P5), Swelling capacity (P6), Colour (P7) of extruded soy jackfruit meat analogues



C1



C2

Fig. 2: Contour and response surface design plots of desirability (C1, C2) quadratic effect of interactions between soy flour and jack fruit flour concentration for the optimum predicted results

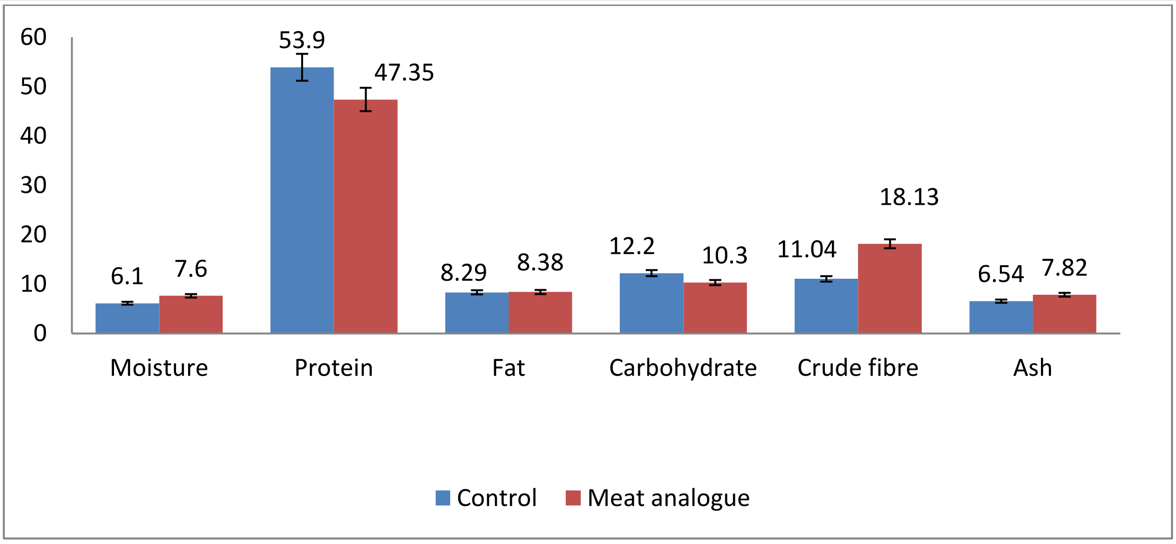


Fig. 3: Graphical representation of proximate composition (g/100 g), vertical error bars represent standard deviation of 5%

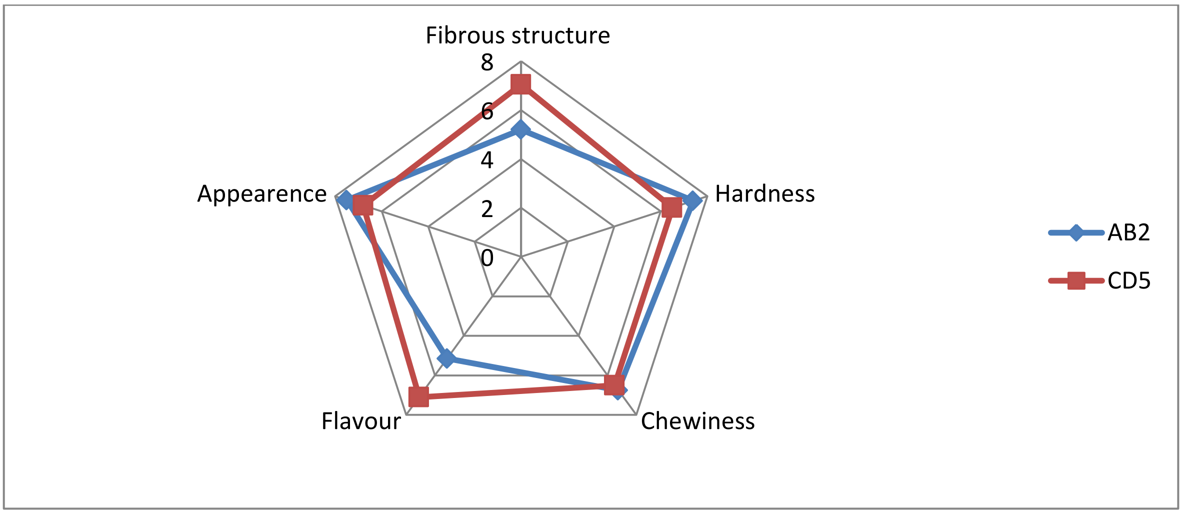


Fig. 4: Graphical representation of sensory attributes AB2 as control and CD5 as the meat analogue

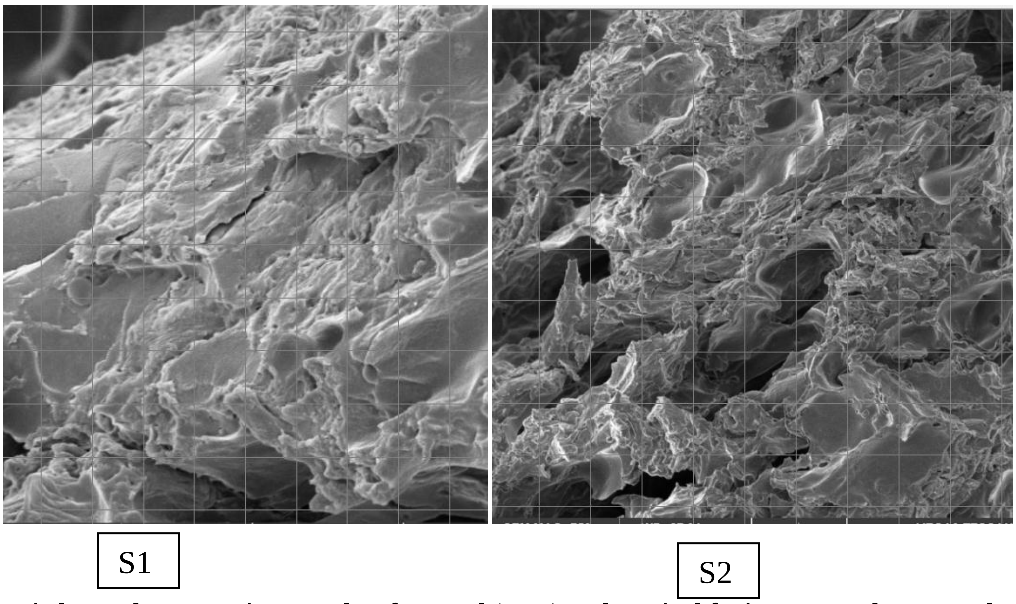


Fig. 5: Dried sample SEM micrographs of control (soya) and soy jackfruit meat analogue. Both control (S1), Meat analogue with 36% soya and 12% of jackfruit meat analogue (S2) are shown with magnification 1.00k x and view field of 208 μm

CONCLUSION

Soya jackfruit based meat analogues are developed with proper formulation using RSM. The WHC, OHC, Hardness, Springiness, Fracturability, S.I and Colour value of the reformulated meat analogues showed differences in results by adding the different concentration of soya flour and jackfruit flour. The structural analysis and sensory also proved the structural improvement and acceptance of product. The predicted values and experimental values were in concurrence and found statistically not different more than 2 % level. The desirable physical qualities of meat analogues by addition of soya flour and jackfruit flour can be estimated by the models suggested in this study. The optimized formula predicted by the model is soya flour of 36% w/w and jack fruit flour of 12% w/w with desirability 1.

In future prospects, improvement of texture and physical quantities of the product with different conditions of enzymatic treatment are going to be studied.

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