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Characterization of Ethiopian Local Honey Varieties and Development of Honey Based Ready to Eat Snack

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Abstract: Ethiopia ranks first in Africa and tenth in the world in honey production. This study aimed at developing honey-based extruded ready-to-eat products and determining the physico-chemical properties of honey from three distinct areas of Ethiopia. Accordingly, honey samples obtained from Oromiya (yellow), Tigray (white) and Gonder (red) areas were analyzed. A blend of rice with wheat (60:40%) flour was first formulated, and thereafter, 5%, 10% and 15% of this composite flour was replaced by the same amount of honey for the preparation of extruded snack. Response Surface Methodology was used to investigate the effect of the response variables, lateral expansion, bulk density, color, water absorption and solubility index characteristics. The effect of each honey sample was studied separately and multiple regression equations were obtained to describe the effects of each variable on product responses. The results of the yellow honey analysis showed that it has 22%moisture, 0.273%ash, 4.1pH, 31.5%fructose, 33.9%glucose, 0.7%sucrose, 2.7%maltose, 7meq/kg free acidity, 0.52%water insoluble matters and 0.61µS/cm electrical conductivity; White honey contained 19%moisture, 0.155%ash, 4.1pH, 35.9%fructose, 31.5%glucose, 1.4%sucrose, 0.0%maltose, 5meq/kg free acidity, 0.56%water insoluble matters and 0.41µS/cm electrical conductivity. Red honey contained 19%moisture, 0.039%ash, 4.2pH, 35.4%fructose, 31.4%glucose, 3.3%sucrose, 4.6%maltose, 6meq/kg free acidity, 0.62%water insoluble matters and 0.22µS/cm electrical conductivity. For the purpose of optimization, the process parameters of 115 to 135°C BT, 270 to 350rpm SS and 5 to 15% honey were selected as independent variables. Results showed that increasing the barrel temperature resulted in extrudate with higher expansion, water absorption index, a* value and lower bulk density and L* and b* values. Increasing screw speed resulted in higher expansion, water solubility index, L value and lower bulk density whereas, increasing honey proportion of feed composition resulted in higher a* value, bulk density, water solubility index and lower expansion, water absorption index and L*value. The graphical optimization studies resulted in temperatures of 124.68, 125.12, 124.19°C, screw speed of 310.35, 309.88, 309.79rpm and amount of honey 9.69, 9.89, 9.66% as optimum variables for yellow, white and red honeys respectively. This is similar with the value 125°C BT, 310rpmSS and 10%honey obtained by panelists during sensory analysis for all types of samples. Accordingly white honey-based extrudate was found to be the best and addition of this honey upto 10% was selected as ideal for snack making.

Keywords: Extrusion, Extrudate, Honey, Response Surface Methodology

1. Introduction

Honey is the natural sweet substance produced by honeybees from the nectar and other parts of plants [1, 2]. The pleasant aroma and taste of this viscous liquid ranging in color from pale yellow to dark amber varies according to geographical and seasonal conditions [3, 4].

Honey contains energy giving carbohydrates, water soluble vitamins like thiamin, riboflavin, ascorbic acid, folic acid etc. The nutritional benefits of honey have been studied by many researchers [5]. It is also used for treating ulcers, kidney problems, asthma, wound healing etc. [3]. Antibiotic properties of honey have also been observed by some researchers [6].

The extrusion cooking process is high temperature short time process in which moist, soft grain is fed into the extruder where the desired temperature and pressure are obtained over the required period of residence time. For cooking of the product generally external heat is not supplied, heat for cooking is achieved through shear and friction in the extruder This technology has many distinct advantages like versatility, low cost, better product quality and no process effluents [7, 8].

Snacks contribute an important part of daily nutrient and calorie intake for many consumers. Cereals have been popular raw materials for extrusion for food uses mainly because of functional properties, low cost and ready availability. Owing to high protein content, pulses and oil seeds can be effectively utilized for nutritional improvement of cereal based extruded snack foods [9].

2. Materials and Methods

2.1. Raw Material Collection

Honey samples were collected from three distinct areas of Ethiopia. White honey was collected from Tigray region (South-eastern woreda, Atsibi), Red honey from Amhara (Gonder) and Yellow honey from Oromiya region (Sebetta), Ethiopia.

The rice (*Oryza sativa L.*) sample was collected from 'Wereta', Bahir Dar, Ethiopia. The wheat flour was obtained from Kebron Food Complex P. L. C., Addis Ababa.

2.2. Raw Material Preparation

2.2.1. Physico-chemical Analysis of Honey

Physical properties and chemical compositions of the honey samples were determined according to the Harmonized Methods of the International Honey Commission [10]. Those physico-chemical components of honey analyzed were moisture content, mineral content, pH and free acidity, reducing sugar, apparent sucrose, water insoluble matter and electrical conductivity.

2.2.2. Flour Preparation from Rice and Wheat

The flours of rice and wheat were prepared, sieved with 500μm sieve and weighed separately; thereafter sealed in polyethylene bags and stored at room temperature until the experiment was conducted. Ingredient sixty percent rice flour and forty percent wheat flour were first mixed. Of this, samples were taken and mixed with 5, 10, and 15% of honey. This mixture was then passed through a 2mm sieve to reduce the lumps formed due to differences in moisture content of honey and the composite flour. After mixing, samples were stored in polyethylene bags at room temperature for 24 h [11].

2.2.3. Extrusion Process

The moisture content of all the samples were measured before extrusion process and the required moisture content were calculated. The extrusion process was performed at Bahirdar University School of Chemical and Food Engineering by pilot scale co-rotating twin screw food extruder model (Clextral, BC-21N° 124 Firminy, France).

Table 1. *Coded levels of the variables for response surface methodology.*

Factors	Coded Levels		
	-1	0	1
BT (°C)	115	125	135
SS (rpm)	270	310	350
BR (%)	95RW:5H	90RW:10H	85RW:15H

Where BT- Barrel Temperature
SS - Screw Speed
BR - Blend Ratio
RW - Rice: Wheat
H – Honey
-1, 0 and 1 are the coded values for lower, middle and upper levels respectively.

The samples prepared in the above combination were fed in to the feeding hopper of the extruder. The barrel was provided with electric band heaters and water cooling jackets. It had smooth 300mm length and it consists of three modules each 100mm fitted with 25mm diameter screws. A temperature sensor was fitted on the front die plate which was connected to temperature control placed on the panel board. Each Zone temperature of the extruder was controlled by a Eurotherm controller (Eurroterm ItD, worthing, UK). The die plate of the die fixed by a screw nut was tightened by a special wrench provided. The automatic cutting knife is fixed on rotating shaft. The twin screw extruder will be kept on for 30 min to stabilize the set temperatures and samples were then poured in to feed hopper and the feed rate was adjusted (4kg/h) and die diameter 9mm was selected and while operating, water at ambient temperature was injected into the extruder via an inlet port by a positive displacement pump (DKM-Clextral, France) then the product was dried in the drying oven (DHG-9140) collected in an incubator and the samples were packed in polythene bags for further analysis [11].

2.3. Method of Analysis

Determination of proximate composition

Proximate composition and mineral content were determined as in AOAC [12] and calorific value of the samples and extruded products were determined according to James [13] and AOAC, [12] for energy value calculation.

2.3.1. Physical Properties of Extrudates

i. Lateral Expansion of Extrudates

The diameter ratio of extrudates and die were used to express the expansion of extrudates [11]. The diameter of the extrudates was measured, at 3 different positions along the length of samples, using a Vernier caliper from randomly selected samples among the extrudates from the same condition. Lateral expansion (LE,%) was then calculated using the mean of the measured diameters as follows:

$$LE=\frac{D_p-D_h}{D_h}*100. \quad (1)$$

Where
LE-lateral expansion
D_p-Diameter of the product
D_h-Diameter of the die hole

ii. Bulk Density, B_{den}

The bulk density of the extrudate samples was determined as in AOAC methods [12]. It is calculated as the ratio of weight of extrudates to the volume of extrudates assuming a cylindrical shape of extrudate

$$B_{den}(g/cm^3)=\frac{4*M}{\pi*De^2*Le} \quad (2)$$

Where
D_e= diameter of extrudate (cm),
L_e= length of extrudate (cm) and
M = mass of extrudate (g)

iii. Color of the Extrudate

The L*a*b color space was used for determining extruded product colors, Commission Internationale d'Eclairage (CIE) in 1976. Where L* is the luminance or lightness component, which ranges from 0 to 100, and parameters a* (from green to red) and b* (from blue to yellow) are the two chromatic components, which range from -120 to 120 [14, 15, 16]. The L*a*b* color space gives uniformity in color distribution and closeness to human perception [17]. The Images were captured by camera, processed in computer and analyzed using ImageJ 1.4 software and the mean value and standard deviation of color intensity in the image pixels was obtained.

The total color change, ΔE of the extrudate from the reference is:

$$\Delta E= [(L_0- L)^2 + (a_0 - a)^2 + (b_0 - b)^2]^{1/2} \quad (3)$$

Where:
L_0=100, a_0=0 and b_0=0

2.3.2. Functional Properties of the Extrudate

i. Water Absorption Index

Extrudates were milled to a mean particle size 180 – 250µm [18] and about 5g was placed in 40ml centrifuge tube and suspended in 15ml distilled water. The sample was incubated by using a shaker (New Brunswick scientific, Excella E24 Incubator shaker series) at 25°C for 30 minutes and was centrifuged at 3000g for 5minutes. The supernatant was decanted into an evaporating dish of known weight. The WAI was the weight of gel obtained after removal of the supernatant per unit weight of original dry solids. Mass of the sample was determined before and after the decantation of the clear supernatant of centrifugation [11].

$$WAI (g/g)=(W_g/W_o) \quad (4)$$

Where:
W_g - weight gain by gel (g) and
W_o -weight of sample (g)
WAI- water absorption Index

ii. Water Solubility Index

The clear supernatant of the centrifugation preserved from WAI measurement was transferred into pre-dried and weighed crucibles for the estimation of the WSI, and evaporated at 105°C for overnight in a drying oven. The WSI was the weight of dry solids in the supernatant expressed as a percentage of the original weight of sample [19].

$$WSI=(W_r/W_o)*100 \quad (5)$$

Where
W_r-Weight of residual after evaporation (g)
W_o- is weight of the initial sample (g) and
WSI-Water Solubility Index

2.3.3. Sensory Evaluation of Products

The extruded product samples were subjected to sensory evaluation with involvement of panelist, (20; 10 male and 10 female), using a nine point hedonic scale rated from 1 (dislike extremely) to 9 (like extremely) [20] and the results were then evaluated using statistical Soft Ware JMP in version 5.0.1.

2.4. Experimental Design

To obtain the optimum process variables for the honey based ready to extruded product, three-level-three factor Central Composite Face-centered Design (CCFD) with duplicate in each point was employed in this study requiring 20 experiments in single run with temperature 115- 135°C, screw speed 270- 350 rpm and feed proportion 5 to 15% as independent variables and the response variables were lateral expansion, bulk density, color, water absorption and solubility index characteristics.

The factors and the feed proportion levels were described in Table 1 with the lower middle and upper limits of the process variable.

The feed moisture content was first determined and to obtain the required moisture content, the following relation was used and accordingly the pump was adjusted to inject water to the extruder at a point close to the material feed port.

$$W_a=S_w*[\frac{m-mo}{100-m}] \quad (6)$$

Where
W_a – the amount of water added (g)
S_w-Sample flour (feed) weight (g)
m_o-original flour (feed) moisture content (%*wwb*)
m-required moisture content (%*wwb*)

2.5. Statistical Analysis

Response surface methodology was used to determine the combination of factors that yield a desired response and describe the response near the optimum. The data obtained from the laboratory analysis were analyzed using a software Design expert stat-Ease version 7.0, and the mean comparison of the process was performed using one-way analysis of variance (ANOVA) for all data at each processing stage. The significant terms in the models were identified by ANOVA for each response. Significance was judged by determining the probability level that the *F*-statistic calculated from the data was ($p<0.05$). All processing parameters were kept within range and lateral expansion, L* and water absorbability index were maximized while bulk density, a*, b* and water solubility index were minimized.

The responses for different experiment combinations were related to coded variables (x, i=1, 2 and 3) by second degree polynomial equation.

$$Y = \beta_o+\beta_1x_1+\beta_2x_2+\beta_3x_3+\beta_{11}x_1^2+\beta_{22}x_2^2+\beta_3 x_3^3+\beta_{12}x_1.x_2+\beta_{13} x_1.x_3 + \beta_{23} x_2.x_3 + \epsilon \quad (7)$$

Where x_1, x_2 and x_3 are coded values for temperature, screw speed, and feed proportion respectively. Y is the response function. The coefficients of the polynomial are represented by β_o (constant), β_1, β_2, β_3 (linear effect): β_{12}, β_{13}, β_{23} (interaction effect): β_{11}, β_{22}, β_{33} (quadratic) and ϵ Random error.

3. Results and Discussion

The physico chemical characteristics of honey samples were obtained as tabulated in the Table *2* and tested by honey quality standard according to the draft CL. 1998/12-Sof the codex Allimentarius and to the draft 96/0114 (CNC) of the EU. The parameters in these experiments met the entire honey quality standard specified in Codex Allimentarius except that the moisture content of Oromiya (yellow) honey (22%) exceeded the standard.

Table 2. *Results of physico-chemical analysis of honey.*

No	components		Types of honey samples			Standards	
			Yellow	White	Red	International	National*
1	Moisture content,% by mass		22.000	19.000	19.000	18-21	21 maximum.
2	Mineral (ash) content,%		0.273	0.155	0.039	≤ 0.60	0.60 maximum.
3	pH		4.100	4.100	4.200	3.4-6.0	----
4	Sugar values%	fructose	31.500	35.900	35.400	≥60%	65 minimum
		glucose	33.900	31.500	31.400		
		sucrose	0.700	1.400	3.300	≤5%	---
		maltose	2.700	0.000	4.600	---	---
5	Free acidity, meq, acid/kg		7.000	5.000	6.000	≤40meq/kg	40meq/kg
6	Water insoluble mater gm/100gm		0.520	0.560	0.620		
7	Electrical conductivity		0.610	0.410	0.220	3.4 - 4.4 μS/cm	

*Quality and standards authority of Ethiopia [21].

3.1. Proximate and Mineral Composition of the Rice Flour, Wheat Flour and Blend

The proximate and mineral components of the raw flours of rice, wheat and blend used in the production of honey based extruded product are shown in Table 3.

Protein, fat and ash of rice, protein content of wheat and protein and ash content of blends are almost the same as reported by Pitchaporn *et al.* [22] in substitution of rice flour with wheat flour and rice bran in flake products while others significantly differ. Milling of rice generally decreases the fiber contents of rice.

Table 3. *Proximate composition of raw rice and wheat flour.*

Sample	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	CHO (%)	Energy (kcal/100)
Rice flour	16.69	7.25	0.08	2.41	0.49	73.08	322.04
Wheat flour	11.11	10.20	0.33	1.55	0.75	76.06	348.01
Blend*	12.01	8.62	0.15	1.59	0.65	76.98	343.75

The values are triplicate means. *Rice: wheat (60:40)

Table 4. *Mineral content of rice, wheat flour and blend of rice and wheat.*

	Fe mg/100g	Zn mg/100g	Ca mg/100g	Na mg/100g	K mg/100g
Rice flour	4.565	1.019	11.758	19.877	119.261
Wheat flour	1.805	0.496	45.415	11.983	159.776
Blend RW	2.51	0.87	25.35	15.39	135.25

There is slight difference among the mineral contents compared with the results obtained by Peterson *et al.* [23] and Sotelo *et al*, [24].

3.2. Optimization of the Physical and Functional Properties of Extrudates with Yellow, White and Red Honey

3.2.1. Lateral Expansion of the Extrudates (LE%)

All statistical analysis including ANOVA test, post ANOVA statistics, Lack of Fit test, Normal plot of residuals, etc. are done in similar way. All the tests indicated that the model was statistically acceptable.

Lateral expansion of the extrudate ranged from 28.39-43.9, 28.34-43.5 and 27.15-43.2 with an average value of 35.93, 35.47 and 36.07% for yellow, white and red honey respectively. The maximum expansion at coded point (1,1,-1), were about 1.546, 1.534, and 1.591 times more than the minimums coded point at (-1,-1,1) *(*Table *5).*

Table 5. Physical and functional properties of the extrudate as the function of the independent variables for yellow, white and red honey.

No	Coded and actual Independent variables Temperature (°C) Screw speed (rpm) Feed proportion						Actual dependent response variables with yellow, White and Red honey based extrudate respectively						
	X_1		X_2		X_3		LE	B_{den}	L*	a*	b*	WAI	WSI
	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded	(%)	(g/cc)	g/g	%	%	(g/g)	%
							33.09	0.46	58.97	1.462	19.1	5.05	4.96
1	-1	115	-1	270	-1	5	32.09	0.48	59.80	1.30	18.10	5.45	4.81
							32.12	0.431	57.80	1.47	18.50	5.35	4.88
							35.31	0.559	47.0	3.42	19.09	6.42	5.8
2	0	125	1	350	0	10	35.12	0.558	49.00	3.33	17.80	6.49	5.60
							36.00	0.52	46.90	3.51	18.30	6.20	5.61
							43.90	0.35	46.16	3.699	14.61	5.85	6.12
3	1	135	1	350	-1	5	43.50	0.34	46.20	3.71	13.52	6.21	5.99
							43.20	0.343	52.20	3.70	13.99	6.11	5.97
							36.67	0.42	63.1	2.69	17.45	5.5	5.99
4	-1	115	1	350	-1	5	35.12	0.41	64.01	2.59	15.42	6.05	5.78
							36.21	0.399	63.21	2.75	15.749	5.72	5.89
							39.10	0.449	45.25	3.517	15.31	5.4	5.1
5	1	135	-1	270	-1	5	38.54	0.52	45.90	3.24	14.36	5.98	4.51
							38.99	0.477	45.12	3.39	14.34	5.59	4.55
							35.96	0.53	49.4	3.31	20.46	6.13	6.4
6	0	125	0	310	0	10	35.32	0.56	50.60	3.40	19.89	6.37	6.01
							35.96	0.529	48.78	3.41	20.54	6.22	6.20
							41.14	0.551	46.42	4.44	18.3	5.65	6.7
7	1	135	0	310	0	10	41.65	0.59	45.99	4.42	17.52	6.12	6.00
							41.40	0.553	46.32	4.54	17.79	5.68	6.31
							36.06	0.523	47.8	3.391	21	5.98	6.67
8	0	125	0	310	0	10	35.01	0.54	49.21	3.45	19.87	6.35	6.12
							37.00	0.51	47.74	3.48	20.381	6.21	6.15
							34.65	0.582	49.57	4.11	21.21	5.61	9.8
9	0	125	0	310	1	15	33.99	0.62	48.42	3.84	20.99	6.01	9.74
							35.00	0.560	48.45	4.21	21.45	6.25	9.87
							34.56	0.563	56.74	2.653	20.18	5.21	6.025
10	-1	115	0	310	0	10	34.03	0.599	55.90	2.65	20.50	5.56	5.78
							34.90	0.563	55.99	2.74	19.99	5.25	6.30
							35.10	0.538	47.99	3.54	20.32	5.99	6.2
11	0	125	0	310	0	10	35.00	0.542	48.01	3.51	19.26	6.23	6.10
							36.14	0.522	47.29	3.49	20.02	6.23	6.20
							34.19	0.677	50.18	3.127	17.31	4.89	8.41
12	-1	115	1	350	1	15	32.90	0.678	51.23	3.21	16.32	5.42	8.12
							34.33	0.62	50.12	3.40	16.69	6.05	8.33
							31.50	0.601	44.5	2.75	21.9	5.9	5.3
13	0	125	-1	270	0	10	31.30	0.631	45.00	2.65	20.90	6.00	5.01
							32.25	0.587	43.25	2.89	21.00	5.88	5.20
							35.99	0.524	50.36	3.5	20.98	6.12	6.679
14	0	125	0	310	0	10	35.90	0.556	51.21	3.385	21.00	6.34	6.60
							36.52	0.512	49.13	3.53	20.56	6.23	6.59
							39.55	0.600	44.0	4.99	18.46	5.5	10.45
15	1	135	1	350	1	15	39.81	0.611	43.56	4.89	19.01	5.75	9.58
							40.54	0.58	43.81	5.12	18.65	5.40	9.60
							28.39	0.699	46.58	2.433	22.99	4.99	8.35
16	-1	115	-1	270	1	15	28.34	0.726	47.86	2.45	21.90	4.58	8.11
							27.15	0.698	45.50	2.50	21.99	4.75	8.19
							35.70	0.668	42.36	4.832	20.59	5.4	9.0
17	1	135	-1	270	1	15	36.01	0.721	43.26	5.01	19.65	5.68	8.80
							35.80	0.659	42.38	4.74	19.45	5.47	8.94
							38.59	0.356	56.96	2.99	17.79	6.0	6.68
18	0	125	0	310	-1	5	37.58	0.381	56.99	2.845	16.58	6.63	6.54
							38.10	0.354	55.94	3.04	17.06	6.65	6.42
							36.10	0.524	49.49	3.41	20.5	5.99	6.71
19	0	125	0	310	0	10	36.12	0.55	48.45	3.29	19.89	6.35	6.721
							35.89	0.535	48.99	3.51	20.24	6.12	6.80
							33.10	0.445	59.01	1.421	19.69	4.88	4.85
20	-1	115	-1	270	-1	5	32.12	0.491	60.35	1.45	18.32	5.16	4.81
							32.99	0.45	58.01	1.431	18.97	5.25	4.81

18% feed moisture is considered

The Model F-values 108.12, 119.52 and 83.99 imply that the models are significant ($P < 0.05$). Adequate precision values of 43.479, 43.916 and 38.053 indicates that the model can be used to navigate the design space as it is greater than 4.0 [26]. Values of "Prob > F" less than 0.050 indicate that model terms are significant. In this case X_1, X_2, X_3, X_1^2, X_2^2, X_3^2 are significant model and response model was selected for representing the variation of lateral expansion analysis are:

$$LE\% = 35.82+3.23* X_1+2.17 * X_2-1.90 * X_3-0.069 * X_1 * X_2-0.049 * X_1 * X_3+0.18 * X_2 * X_3+2.07 * X_1^2-2.38 * X_2^2+0.83 * X_3^2 \quad (8)$$

$$LE\% = 35.48+3.69 * X_1+2.00 * X_2-1.60 * X_3+0.17 * X_1 * X_2-9.887E-003 * X_1 * X_3+0.068 * X_2 * X_3+2.34 * X_1^2-2.29 * X_2^2+0.28* X_3^2 \quad (9)$$

$$LE\% = +36.31+3.41*X_1+2.29* X_2-1.69 * X_3-0.15* X_1 * X_2+0.26 * X_1 * X_3+0.59* X_2 *X_3+1.83 * X_1^2-2.19 * X_2^2+0.23 * X_3^2 \quad (10)$$

Where X_1, X_2 and X_3 are the coded values temperature, screw speed and feed proportion (rice flour + wheat flour): honey, respectively. And equation 8, 9 and 10 shows lateral expansion of yellow, white and red honey based extrudate respectively.

Therefore from the equations (8, 9 and 10), since the coefficients of X_1 and X_2 were positive and X_3 was negative, increasing the barrel tempareture and screw speed increases the lateral expanssion. An increase in the feed proportion decreases the expansion. The coefficient X_2 was negative this indicates that negative quadratic effect while X_1 and X_3 shows positive quadratic effect for all. As Singh et al. [25] reported in their result in extrusion of soyabean protien and corn starch that increasing the screw speed improve the expansion and reduce the bulk density of the extrudate, similar result was observed in extruding of honey based ready to eat extruded product.

As it is shown in Figure 1, an increase in feed proportion decreases the lateral expansion while an increase in temperature and screw speed increases the lateral expansion.

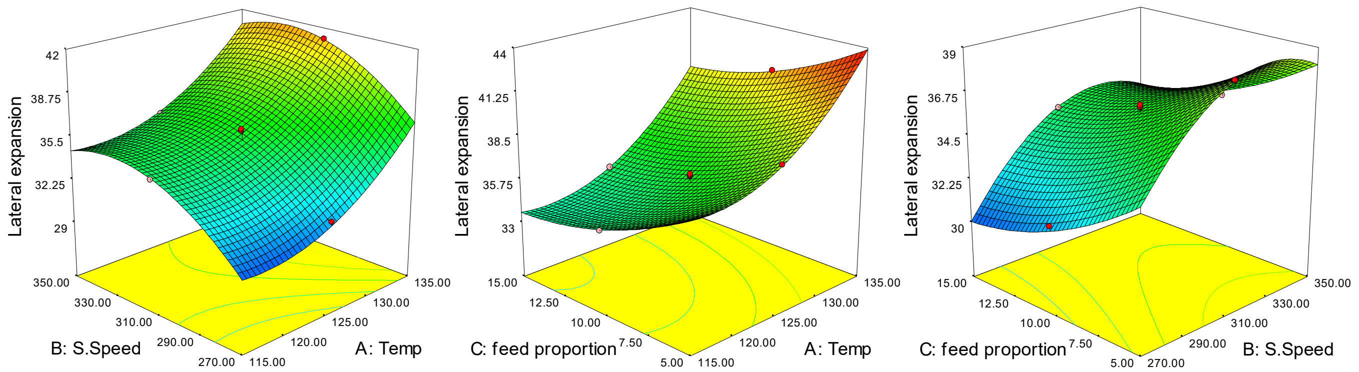

Figure 1. *Response surface plots for the expansion ratio as a function of (a) temperature and speed (b) temperature and feed proportion (c) speed and feed proportion for yellow honey extrudate.*

3.2.2. Bulk Density, B_{den}

Among the response variables, bulk density is the one which significantly affected response variable in the processing of extruded snacks, ready-to-eat cereals and puffed products.

Bulk density of the extrudate ranged from (0.335- 0.699, 0.34-0.726 and 0.343-0.698) with an average values of 0.53, 0.56 and 0.52 for yellow, white and red in g/cm^3 respectively. The maximum max density at coded point (-1,-1, 1) was about 2.08654, 2.1356, and 2.0349 times more than the minimum coded point at (1, 1,-1) (Table *5*).

Adequate precision values of 41.819, 45.909 and 32.893 indicates that the models can be used to navigate the design space as it is greater than 4.0 (Montgomery, [26]). The Model F-values of 150.66, 166.11 and 85.96 implies the models are significant and these model terms are X_1, X_2, X_3, X_1X_2, X_1^2, X_2^2, X_3^2 for yellow honey and temprature, screw speed, feed proportion, quadratic effect of temprature, screw speed and feed proportion and interaction effect of temprature and screw speed affect the process for both white and red honey based extrudates in the process.

The response model was selected for representing the variation of bulk density analysis in coded factors.

$$\text{Bulk density} = +0.53-0.021 * X_1-0.028 * X_2+0.12 * X_3-0.016 * X_1 * X_2-2.560E-003 * X_1 * X_3+6.940E-003 * X_2 * X_3+0.024 * X_1^2+0.047 * X_2^2-0.064*X_3^2 \quad (11)$$

$$\text{Bulk density} = +0.55-0.012 * X_1-0.048 * X_2+0.12 * X_3-0.022 * X_1 * X_2-5.801E-003 * X_1 * X_3+0.013 * X_2 * X_3+0.035 * X_1^2+0.035 * X_2^2-0.059 * X_3^2 \quad (12)$$

$$\text{Bulk density} = +0.52-0.010 * X_1-0.039 * X_2+0.11 * X_3-0.012 * X_1 * X_2-8.142E-003* X_1 * X_3+1.608E-003 * X_2 * X_3+0.037 * X_1^2+0.032 * X_2^2-0.064 * X_32 \quad (13)$$

Where X_1, X_2 and X_3 are the coded values temperature, screw speed and feed proportion (rice flour + wheat flour): honey, *respectively. And equation 11, 12 and 13 shows bulk density of yellow, white and red honey based extrudate respectively.*

From the equations (11, 12 and 13), it can be observed that an increase in screw speed decreases the bulk density to some extent since the coeffiecient of X_2 is negative. Whereas an increase in the feed proportion increases the bulk density, but when barrel temprature increases, bulk density decreases to some extent and then increases as it is shown in Figure 2. The coefficient of X_1^2 and X_2^2 are positive therefore they show positive quadratic effect while X_3^2 shows negative quadratic effect on the bulk density for all types of honey based extrudate. This result is similar with that described by Praneeth *et al.* [11] except that they concluded that the temperature and screw speed had only negative effect on the bulk density. According to Frame, [27] and Jose [28], at higher temperature the vapor pressure of the free moisture is greater causing an increased rate of moisture flashing and puffing up on exit from the die, and this results in decreased bulk density. As the barrel temperature increases from 115°C to 125°C, the density decreases from 0.6075g/cm^3 to 0.601g/cm^3.

For screw speed from 270 rpm to 310 rpm, the density decreases from 0.6075 g/cm^3 to 0.563 g/cm^3. Praneeth *et al.* [11] also stated that density will decreases with increase in the barrel temperature due to starch gelatinization.

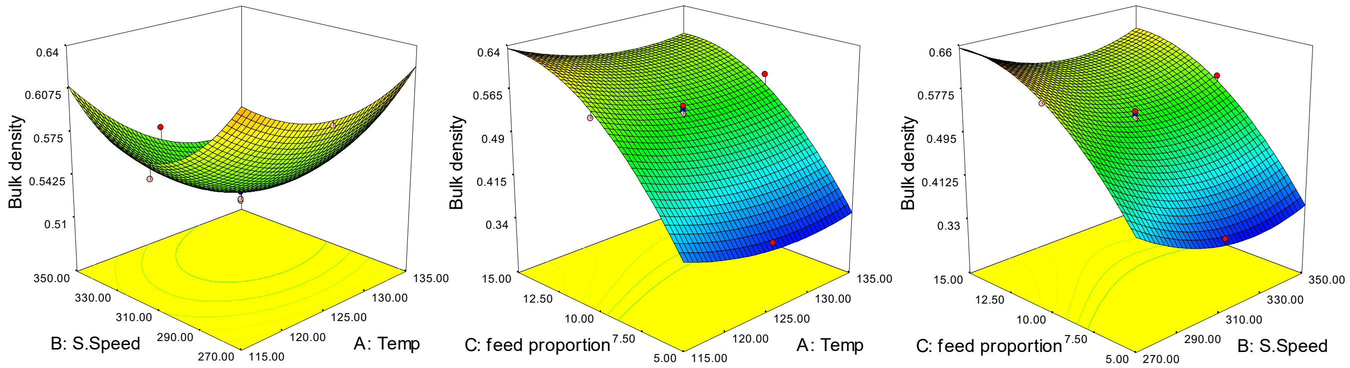

Figure 2. *Response surface plots for the bulk density as a function of (a) temperature and speed (b) temperature and feed proportion (c) speed and feed proportion for yellow honey extrudate.*

3.2.3. Colour

i. L* Value

The 'L*'value of the extrudate ranged from (42.54 - 61.94, 42.26-64.01 and 42.38-63.21) with an average values of 50.38, 50.55 and 49.50. The maximum L*value at coded points (-1,1,-1) were about 1.456, 1.479 and 1.4915 times more than the minimum coded points at (1,-1,1) for yellow, white and red honey based extrudate respectively (Table 5*)*.

The Model F-value are 79.70, 64.37 and 110.02 (g/g), implies the model is significant. In this case X_1, X_2, X_3, X_1X_3, X_1^2, X_2^2, X_3^2 for yellow X_1, X_2, X_3, X_1X_2, X_1X_3, X_2^2, X_3^2 for white and X_1, X_2, X_3, X_1X_2, X_1X_3, X_1^2, X_2^2, X_3^2 fore red honey are significant model terms and the response model selected for representing the variation of L*value analysis in coded factors are.

$$L^* = 49.51 - 5.12 * X_1 + 1.29 * X_2 - 3.76 * X_3 - 0.67 * X_1 * X_2 + 2.51 * X_1 * X_3 + 7.715E\text{-}003 * X_2 * X_3 + 1.45 * X_1^2 - 4.38 * X_2^2 + 3.13 * X_3^2 \quad (14)$$

$$L^* = +49.73 - 5.42 * X_1 + 1.19 * X_2 - 3.89 * X_3 - 0.83 * X_1 * X_2 + 2.47 * X_1 * X_3 - 0.065 * X_2 * X_3 + 0.93 * X_1^2 - 3.01 * X_2^2 + 2.69 * X_3^2 \quad (15)$$

$$L^* = +48.80 - 4.97 * X_1 + 1.52 * X_2 - 3.70 * X_3 - 1.07 * X_1 * X_2 + 2.65 * X_1 * X_3 + 0.064 * X_2 * X_3 + 1.84 * X_1^2 - 4.24 * X_2^2 + 2.88 * X_3^2 \quad (16)$$

Where X_1, X_2 and X_3 are the coded values temperature, screw speed and feed proportion (rice flour + wheat flour): honey, respectively. And equation 14, 15 and 16 shows L Value of yellow, white and red honey based extrudate respectively.*

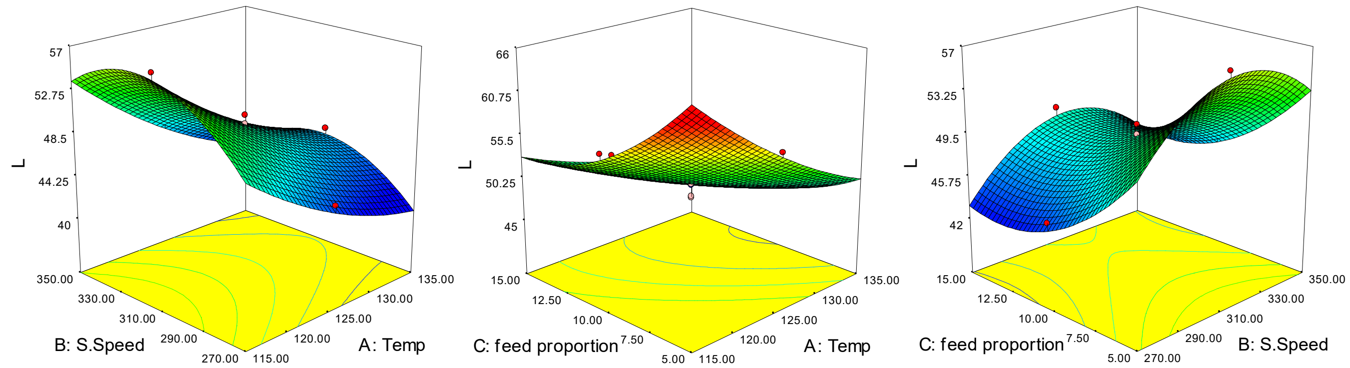

Figure 3. *Response surface plots for the L* as a function of (a) temperature and speed (b) temperature and feed proportion (c) speed and feed proportion.*

From the equations (14, 15 and 16), the conclusion can be drawn that increase in barrel temprature and honey content decreases the L*value since the coeffiecient of X_1 and X_3 were negative and increase in screw speed increase the L* value (Figure 3) and the coefficient of $X_1$2 and$X_3$2 are positive therefore they show positive quadratic effect while $X_2$2 shows negative quadratic effect on the L*value for all varieties of honey.

ii. a*% Value

The 'a*' value of the extrudate ranged from (1.421 - 4.99, 1.3-5.01 and 1.43-5.12) with an average value of 3.28, 3.23 and 3.34%, for yellow, white and red honey based extrudate respectively. The maximum a* value at coded point (1,1,1) was about 3.51162, 3.853 and 3.5779 times more than the minimum coded point at (-1,-1,-1) (Table 5).

The Model F-value are 224.17, 224.97 and 346.42 (g/g), implies the models are significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case X_1, X_2, X_3, X_1X_3, X_1X_2, X_2X_3, X_1^2, X_2^2, X_3^2 for yellow honey, X_1, X_2, X_3, X_1X_3, X_1X_2, X_2X_3, X_1^2, X_2^2 for white honey and X_1, X_2, X_3, X_1X_3, X_1X_2, X_1^2, X_2^2 for red honey are significant model terms.

The response model selected for representing the variation of a*value analysis in coded factors are.

$$a^*\% = +3.43+0.92 * X_1+0.30 * X_2+0.52 * X_3-0.20 * X_1 * X_2+0.14 * X_1 * X_3-0.076 * X_2 * X_3+0.12 * X_1^2-0.34 * X_2^2+0.13 * X_3^2 \quad (17)$$

$$a^*\% = +3.36+0.90* X_1+0.30 * X_2+0.56* X_3-0.20* X_1* X_2+0.16 * X_1* X_3-0.13 * X_2 * X_3+0.22 * X_1^2-0.32 * X_2^2+0.031* X_3^2 \quad (18)$$

$$a\% = +3.50+0.87* X_1+0.35* X_2+0.57 * X_3-0.19 * X_1 * X_2+0.13 * X_1 * X_3-0.045 * X_2 * X_3+0.11* X_1^2-0.33 * X_2^2+0.096 * X_3^2 \quad (19)$$

Where X_1, X_2 and X_3 are the coded values temperature, screw speed and feed proportion (rice flour + wheat flour): honey, respectively. And equation 17, 18 and 19 shows a Value of yellow, white and red honey based extrudate respectively.*

From the equations (17, 18 and 19), the conclusion can be drawn that increase in barrel temperature screw speed and honey content increase the a*value since the coefficient of X_1, X_2 and X_3 were positive (Figure 4) and the coefficient of X_1^2 and X_3^2 are positive therefore they show positive quadratic effect while X_2^2 shows negative quadratic effect on the a*value for all types of extrudate.

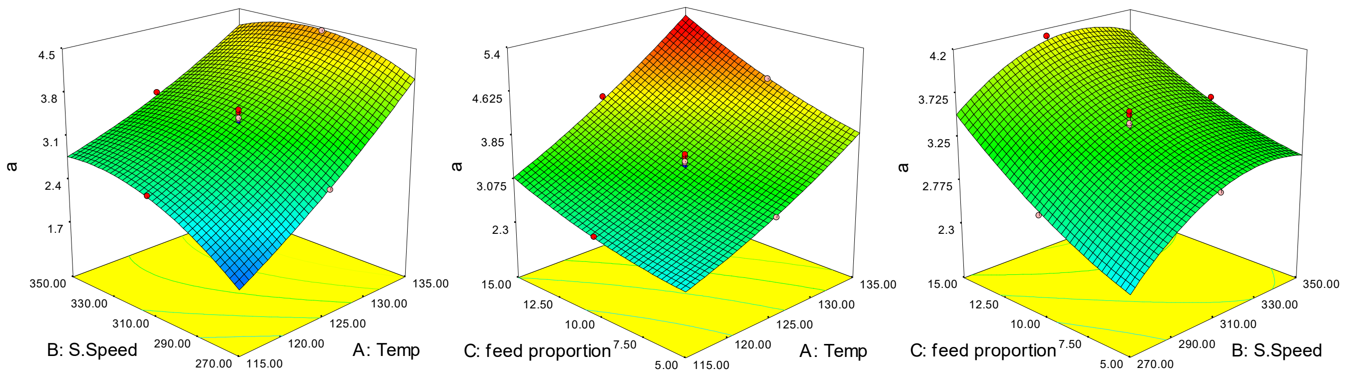

***Figure 4.** Response surface plots for the a* as a function of (a) temperature and speed (b) temperature and feed proportion (c) speed and feed proportion.*

iii. 'b*'% Value

The 'b*' value of the extrudate ranged from (14.61-22.99, 13.52-21.91 and13.99-21.99) with an average value of 19.36, 18.54 and18.78. The maximum b* values at coded point (-1,-1,1) were about 1.571, 1.6198 and 1.5186 times more than the minimum coded point at (1,1,-1) (Table 5. The Model F-value are 68.04, 45.84, and 82.13, implies the model are significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case X_1, X_2, X_3, X_1X_3, X_1X_2, X_2X_3, $X_1$2, $X_3$2 for yellow X_1, X_2, X_3, X_1X_2, X_1X_3, $X_1$2, $X_3$2 for white varieties and X_1, X_2, X_3, X_1X_3, X_1X_2, X_2X_3, $X_1$2, X_2^2 and $X_3$2 for red are significant model terms.

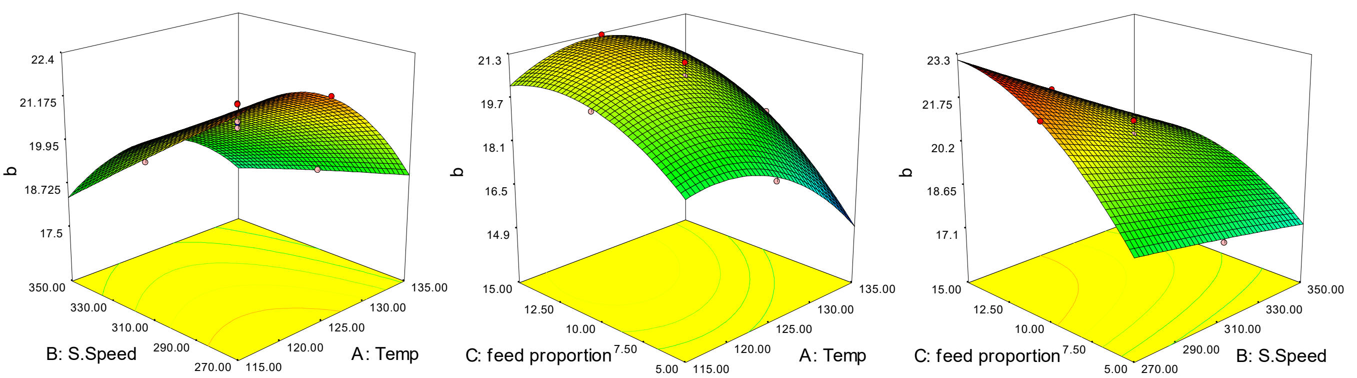

***Figure 5.** Response surface plots for the b* as a function of (a) temperature and speed (b) temperature and feed proportion (c) speed and feed proportion*

From this, the following response model was selected for representing the variation of b*value analysis in coded factors.

$$b^* = 20.60 - 0.99 * X_1 - 1.31 * X_2 + 1.61 * X_3 + 0.58 * X_1 * X_2 + 0.69 * X_1 * X_3 - 0.66 * X_2 * X_3 - 1.29 * X_1^2 - 0.040 * X_2^2 - 1.03 * X_3^2 \quad (20)$$

$$b^* = +19.97 - 0.80 * X_1 - 1.26 * X_2 + 2.01 * X_3 + 0.82 * X_1 * X_2 + 0.73 * X_1 * X_3 - 0.36 * X_2 * X_3 - 0.94 * X_1^2 - 0.60 * X_2^2 - 1.17 * X_3^2 \quad (21)$$

$$b^* = +20.29 - 0.88 * X_1 - 1.20 * X_2 + 1.85 * X_3 + 0.88 * X_1 * X_2 + 0.68 * X_1 * X_3 - 0.36 * X_2 * X_3 - 1.33 * X_1^2 - 0.57 * X_2^2 - 0.97 * X_3^2 \quad (22)$$

Where X_1, X_2 and X_3 are the coded values temperature, screw speed and feed proportion (rice flour + wheat flour): honey, respectively. And equation 20, 21 and 22 shows b Value of yellow, white and red honey based extrudate respectively.*

From the equations (20, 21 and 22), the conclusion can be drawn that increase in barrel temperature and screw speed decrease the b*value since the coefficient of X_1, X_2 were negative (Figure 5) and the coefficient of X_1^2, X_2^2 and X_3^2 are negative therefore they show negative quadratic effect on the process unlike the result observed by Praneeth *et al*, [11] for all varieties of honey and also the increase in honey proportion in feed composition resulted in decrease in L* value of extrudate and increase in a* and b* value of extruded. This was due to occurrence of browning reaction, which may be due to caramellization of sugars present in honey.

iv. The total color change, ΔE of the extrudate from the reference is:

$$\Delta E = [(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2]^{1/2}$$

***Table 6.** The total color change, ΔE of the extrudate.*

	yellow	white	Red
L	50.38	50.55	49.5
a*	3.28	3.25	3.34
b*	19.36	18.54	18.78
ΔE	53.36	52.911	53.98

Where: mean values of L, a* and b* where taken for yellow white and red honey varieties and $L_0=100$, $a_0=0$ and $b_0=0$

From the Table 6, the total color change, ΔE, red honey based extrudate has highest color, (53.98), change compared to others.

3.2.4. Water Absorption Index

The water absorption index of extrudates varied in the range of (4.88 - 6.42 for yellow, 4.58-6.63 for white and 4.75-6.65g/g for red honey extrudate with an average value of 5.65, 5.605 and 5.7g/g respectively. The maximum water absorption index at coded point (0,1,0) was about 1.3156, 1.446 and 1.4001 times more than the minimum water absorption index at the coded point of (-1,-1,-1) (Table *5*). The Model F-value are 48.56, 73.30 and 94.62 signifies that the model was significant ($P < 0.05$). In these case X_1, X_2, X_3, X_2X_3, X_1^2, X_3^2 for yellow X_1, X_2, X_3, X_1X_2, X_1X_3, X_1^2, X_2^2 for white and X_1, X_2, X_3, X_2X_3, X_1^2, X_1, $^2X_3^2$ for red are significant models terms where X_1, X_2 and X_3 are barrel temprature, screw speed and feed proportion respectively. The response models selected for representing the variation of water absorption index for further analysis are.

$$WAI = 6.04 + 0.22 * X_1 + 0.14 * X_2 - 0.14 * X_3 + 0.025 * X_1 * X_2 + 0.040 * X_1 * X_3 - 0.11 * X_2 * X_3 - 0.61 * X_1^2 + 0.12 * X_2^2 - 0.23 * X_3^2 \quad (23)$$

$$WAI = +6.35 + 0.28 * X_1 + 0.24 * X_2 - 0.27 * X_3 - 0.16 * X_1 * X_2 + 0.076 * X_1 * X_3 - 6.909E-003 * X_2 * X_3 - 0.53 * X_1^2 - 0.13 * X_2^2 - 0.055 * X_3^2 \quad (24)$$

$$WAI = +6.22 + 0.21 * X_1 + 0.15 * X_2 - 0.25 * X_3 - 0.029 * X_1 * X_2 + 0.053 * X_1 * X_3 - 0.084 * X_2 * X_3 - 0.78 * X_1^2 - 0.21 * X_2^2 + 0.20 * X_3^2 \quad (25)$$

Where X_1, X_2 and X_3 are the coded values temperature, screw speed and feed proportion (rice flour + wheat flour): honey, respectively. And equation 23, 24 and 25 shows water absorption index of yellow, white and red honey based extrudate respectively.

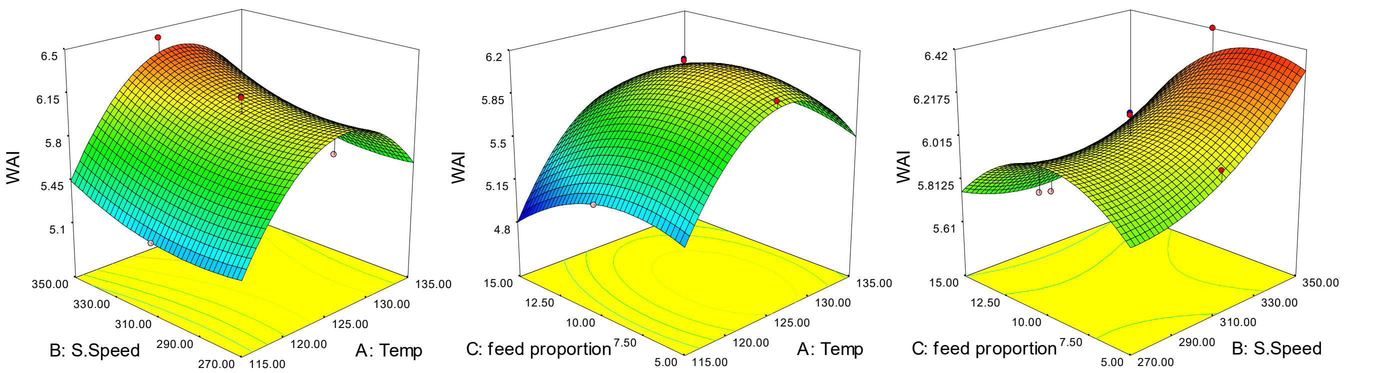

***Figure 6.** Response surface plots for the WAI* as a function of (a) temperature and speed (b) temperature and feed proportion (c) speed and feed proportion.*

It is observed that from the equations (23, 24 and 25) and Figure 6 that the coefficients of X_1 and X_2 were positive, but that of X_3 is negative; therefore increase in temperature and screw speed may increase the water absorption index, whereas increase in feed proportion may decrease the water absorption index of all varieties of products. The coefficients of x_1^2, and x_3^2 were negative therefore they will show negative quadratic effect on water absorption index.

3.2.5. Water Solubility Index

Water solubility index was used as an indicator of degradation of molecular components. It measures the amount of soluble polysaccharide released from the starch component after extrusion [29]. The water solubility index of extrudates varied in the range of (4.85% -10.45%, 4.51-9.74% and 4.55-9.87) with an average value of 6.81%, 6.54% and 6.64% for yellow, white and red respectively. The maximum water absorption index at coded point (1,1,1) was about 2.1546, 2.15965 and 2.1692 times more than the minimum water solubility index at the coded point of (-1,-1,-1) for yellow white and red honey based extrudate respectively. The Model F-value are 78.88, 94.12, and 101.60, which implies that the model were significant ($P < 0.05$). And in this case X_1, X_2, X_3, X_1X_3, X_2^2, X_3^2 for yellow, X_1, X_2, X_3, X_1X_2, X_2X_3, X_1^2, X_2^2, X_3^2 for white and X_1, X_2, X_3, X_1X_3, X_2X_3, X_2^2, X_3^2 for red are significant model terms.

Response model selected for representing the variation of water solubility index for further analysis are;

$$WSI = +6.50+0.38 * X_1+0.43 * X_2+1.74 * X_3+0.15 * X_1 * X_2+0.28 * X_1 * X_3-0.093 * X_2 * X_3-0.10 * X_1^2-0.92 * X_2^2+1.78 * X_3^2 \quad (26)$$

$$WSI = +6.27+0.23 * X_1+0.39 * X_2+1.68 * X_3+0.15 * X_1 * X_2+0.27 * X_1 * X_3-0.21 * X_2 * X_3-0.33 * X_1^2-0.92 * X_2^2+1.92 * X_3^2 \quad (27)$$

$$WSI = +6.40+0.19 * X_1+0.37 * X_2+1.73 * X_3+0.10 * X_1 * X_2+0.27 * X_1 * X_3-0.22 * X_2 * X_3-0.10 * X_1^2-1.00 * X_2^2+1.74 * X_3^2 \quad (28)$$

Where X_1, X_2 and X_3 are the coded values temperature, screw speed and feed proportion (rice flour + wheat flour): honey, respectively. And equation 26, 27 and 28 shows water solubility index of yellow, white and red honey based extrudate respectively.

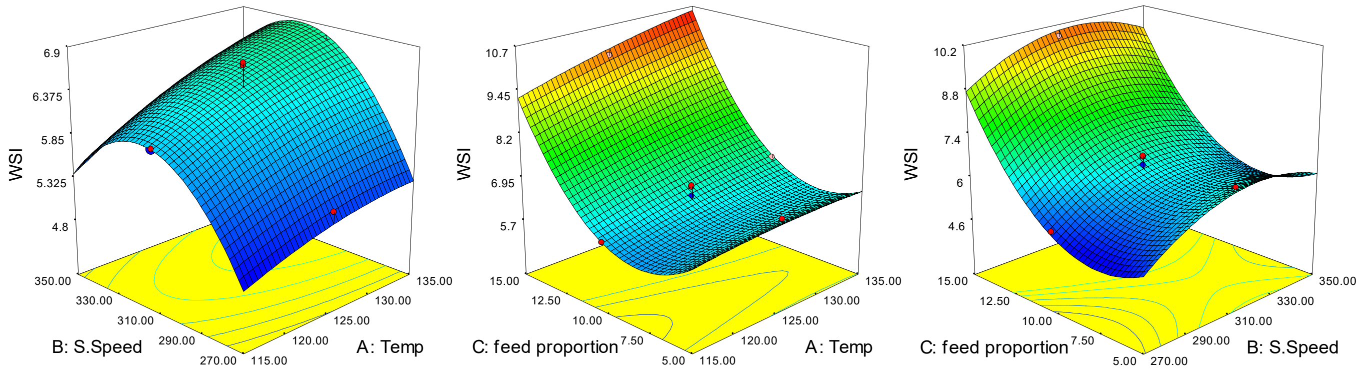

Figure 7. *Response surface plots for the WSI* as a function of (a) temperature and speed (b) temperature and feed proportion (c) speed and feed proportion.*

It is observed that from the equations (26, 27 and 28) and Figure 7 that the coefficients of X_1 and X_2 and X_3 were positive, therefore increase in temperature and screw speed and feed proportion may increase the water solubility index for all varieties of honey based extrudates. But initially increase in feed proportion may decrease the water solubility index of the product to some extent then it increases continuously there as when the screw speed increases, WSI increase and eventually it decreases after some point. The coefficients of x_1^2, and x_2^2 were negative therefore they show negative quadratic effect on water absorption index while X_3^2 shows positive quadratic effect.

3.3. Results of Sensory Analysis

The extrudates were evaluated for various sensory attributes, color, appearance, flavor, crispness and overall acceptability as follows and for yellow white and red, where most of products had a mean value greater than 5, indicating that they were mostly liked by the panelists. The one way ANOVA of hedonic scores for the sensory attributes revealed that significant difference ($p<0.05$) exists between extruded snacks. But there is no significant difference between the sensory values of yellow, white and red honey.

Results showed that barrel temperature, screw speed and feed proportion had significant effect ($p<0.05$) on all the characteristics of sensory attributes of the extrudates and the highest mean value for colour (8.63) appearance (8.59), flavors (8.62), crispiness (8.39) and overall acceptability (8.69) were obtained at 125°C barrel temperature, 310 rpm screw speed and 10% feed proportion.

3.4. Optimum Points Obtained from Extrusion Conditions

The optimum values were determined by design expert 7.0 software using response surface methodology by choosing desired goals for each conditions that all processing parameters were kept within range and lateral expansion, L* and water absorbability index were maximized while bulk density, a*, b* and water solubility index were minimized.

Table 7. *Optimum points obtained from Extrusion Conditions.*

Extrudate type (honey-based)	Optimum Values Obtained on Independent Variables			Desirability	Remark
	Barrel temperature, ºC	Screw speed, rpm	Feed proportion,%		
Yellow	124.68	310.35	9.69	0.899	Selected
White	125.12	309.88	9.89	0.913	Selected
Red	124.19	309.79	9.66	0.881	Selected

As it is shown Table 7 the optimum process variables selected are almost similar with that sensory attribute values (125°C barrel temperature, 310 screw speed and 10% feed proportion) and these conditions are accepted as the optimum points for honey based extruded product development.

Table 8. *Proximate composition of the selected extrudate with optimum condition 125BT, 310SS and 10H%.*

Sample	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	CHO (%)	Energy (kcal/100)
Yellow	10	6.33	0.5	1.97	1	80.2	350.62
White	7.1	6.37	0.42	1.98	0.98	83.15	361.86
Red	7.5	6.28	0.36	1.88	1.21	82.77	359.44

The values are triplicate means.

Comparing Table 8 with Tables 3, it is revealed that due to incorporation of honey in the extruded product, the protein content significantly decreased and carbohydrate content and energy content increased when compared with both the raw flours and the blends. The white honey based extrudate has highest carbohydrate and energy content compared to other extrudates (Table 8).

4. Conclusion

Honey-based extruded product was developed by blending honey with wheat and rice flours at different proportions with varying the extrusion parameters. The optimum condition obtained for the development of honey based extrudate indicates that 10% honey could be incorporated, hence be considered as a source of carbohydrate, aroma building component and source of other health-related values like remedy for stomach ulcers, boost the immune system, cardiovascular health, help to reduce kidney problems, remedy of asthma, antimicrobial, antioxidant effects, ant-mutagenic and antitumor and anti-inflammatory and other activities. Under the process condition studied, extrusion at barrel temperature of 125°C, screw speed of 310 rpm and feed proportion of 10% was found to produce better quality extrudate with maximum lateral expansion, less dense product. Using the RSM, the combined effect of the three process variables, barrel temperature, screw speed and honey proportion on extrudate responses were predicted, which was difficult to achieve with conventional methods. All product quality attributes were influenced by all process variables even though the extent varies.

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