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Effect of whey protein on sun dried protein enriched kesar mango leather

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Abstract
The study was carried out to 824standardized formulations and optimized process parameters by sun drying techniques. Sun dried mango leather was prepared by varying process variables viz. whey protein (3-7%), sugar (10-20%), citric acid (0.3-0.5%) and thickness of mango pulp (3-6 mm) and its responses like protein content, overall acceptability, ascorbic acid, cutting force and stretchability were analyzed by using Response Surface Methodology. The optimum value of process parameters for sun dried protein enriched mango leather was found as whey protein (4.92%), sugar (12.50%), citric acid (0.45%) and thickness of pulp (6mm) by using RSM and responses of process parameters were found as overall acceptability (7.60) and protein content (9.90%) having desirability of 0.60.

Keywords: Mango, fruit leather, protein enrichment, RSM, sensory attributes

Introduction
Mango (*Mangifera Indica* L.) belonging to the *Anacardiaceae* family, the most important tropical and subtropical fruits of the world, is called as the king of fruits on account of its nutritive value, taste, attractive fragrance and health promoting qualities (Housalmal, 2018) [6]. Mango is an excellent source of vitamin A and C, also having important antioxidant nutrients. The fruit is rich with important minerals like potassium, magnesium, sodium, phosphorus, and sulphur (Sarojini *et al.*, 2009) [10]. The drying of fruit is a low cost processing technology for enhancing the income of farmers by encouraging full utilization of locally available mango and reduces post-harvest losses at farm level. As per the Food Safety and Standards Regulations, 2011, fruit leathers are dried sheets of fruit pulp which have a soft, rubbery texture and a sweet taste. They can be made from most fruits, although mango, apricot, banana and tamarind leathers are amongst the most popular. They may be eaten as snack foods as a healthy alternative to boiled sweets and also used as an ingredients in the manufacture of cookies, cakes and ice cream. Whey protein is a functional ingredient that has been known for its positive health benefits such as immunity enhancement, cholesterol reduction, reducing blood pressure, etc. It is a good food supplement for making fruit leather & energy bars. The use of whey protein in sports and snack products delivers the nutrients that positively affect body compositions (Burrington, 2012) [3]. Recently whey products have become quite popular and are heavily promoted as an ideal protein source (Bhutani, 2010) [2]. Mango leather offer tremendous advantages for protein enrichment owing to simplicity, lower production cost besides better consumer appeal and very popular among children. Food enrichment and fortification are the most cost effective and sustainable strategy to address the problem of malnutrition.

Materials and Methods
Experimental Site
The experiment was conducted in the Department of Processing and Food Engineering, College of Agricultural Engineering and Technology, AAU, Godhra.

Raw Materials
Pasteurized mango pulp of kesar variety without added preservatives was procured from processing plant of PFE Department, CAET, JAU, Junagadh. Whey protein (WPC-80, Arla Make) was procured locally from Anand Market. White sugar (sulphur free, pure and hygienic, Madhur Brand), citric acid and packaging materials (PP and LAF) were procured from local market of Godhra.

Preparation of mango leather
Mango pulp was weighed (100g) by an electronic weighing balance.

The total soluble solids content of the mango pulp was fixed and it ranged between 17-20 ºBrix. Sugar was added to the mango pulp with 10-15g to adjust total soluble solids to 30 ºBrix. Citric acid (@ 0.5%) was added to mango pulp and blended by hand blender. Heat treatment was given to mixture at 80 ºC for 5 minutes. Stainless steel plates were smeared with very thin layer of glycerine to prevent sticking of mango leather after drying. The mango pulp was then spread on smeared plates. Mango pulp was dried in sun up to 17±2% (w.b.) moisture content. After drying, mango leather was cooled at room temperature and packed in bags.

Experimental Plan

Independent process variables			Dependent variable
Whey protein (%)	:	3 – 7	Bio-chemical parameters (protein ascorbic acid), sensory evaluation (taste & flavour, color, texture and overall acceptability), texture analysis (cutting force and stretchability)
Sugar (%)	:	10 – 20	
Citric Acid (%)	:	0.3 – 0.5	
Thickness (mm)	:	3 – 7	

In this experiment, four process variables with five levels each were considered by using central composite rotatable design (CCRD). Thirty experiments were conducted and their interactions were also studied by using RSM as experimental statistical design to see the effect of different independent variables on various bio-chemical, sensory and texture quality of sun dried product (Table 1).

Effect of process variables on bio-chemical, sensory attributes and textural properties of sun dried protein enriched mango leather

Bio-chemical represents the gross content of important chemical constituents such as protein, ascorbic acid, acidity, TSS and pH. The standard methods have been used for the bio-chemical analysis of mango leather (AOAC, 2005) [1]. The sensory attributes (taste & flavour, color, texture and overall acceptability) of samples were evaluated using a 9 point hedonic scale. The score-card suggested by Ranganna (2004) was used for judging the mango leather. Textural properties (cutting force and strethcability) of mango leather samples was done on TA-HDI Texture Analyzer (Stable micro systems, UK) fitted with 5 kg load cell.

Table 1: Process Variables and responses for optimization of formulation for protein enriched mango leather

S. N.	Process Variables				Responses			
	WPC (%)	Sugar (%)	Citric Acid (%)	Thickness (mm)	Overall Acceptability	Protein Content (%)	Color value	Stretchability (N)
1	4 (-1)	12.50 (-1)	0.35 (-1)	4 (-1)	6.93	6.78	14.10	4.74
2	6 (+1)	12.50 (-1)	0.35 (-1)	4 (-1)	7.11	12.91	16.69	0.41
3	4 (-1)	17.50 (+1)	0.35 (-1)	4 (-1)	7.14	6.89	14.23	0.66
4	6 (+1)	17.50 (+1)	0.35 (-1)	4 (-1)	7.14	8.57	14.49	0.84
5	4 (-1)	12.50 (-1)	0.45 (+1)	4 (-1)	7.61	7.73	14.08	1.27
6	6 (+1)	12.50 (-1)	0.45 (+1)	4 (-1)	7.14	8.96	14.27	1.03
7	4 (-1)	17.50 (+1)	0.45 (-1)	4 (+1)	6.86	6.61	14.52	0.92
8	6 (+1)	17.50 (+1)	0.45 (+1)	4 (-1)	7.25	8.37	14.90	1.11
9	4 (-1)	12.50 (-1)	0.35 (-1)	6 (+1)	7.39	7.74	17.07	1.14
10	6 (+1)	12.50 (-1)	0.35 (-1)	6 (+1)	7.07	7.26	15.75	1.53
11	4 (-1)	17.50 (+1)	0.35 (-1)	6 (+1)	7.11	8.54	16.97	2.98
12	6 (+1)	17.50 (+1)	0.35 (+1)	6 (-1)	7.46	10.04	15.54	2.52
13	4 (-1)	12.50 (-1)	0.45 (+1)	6 (+1)	7.43	8.97	15.26	3.17
14	6 (+1)	12.50 (-1)	0.45 (+1)	6 (+1)	7.54	11.98	15.07	3.30
15	4 (-1)	17.50 (+1)	0.45 (+1)	6 (+1)	7.89	6.67	15.33	2.54
16	6 (+1)	17.50 (+1)	0.45 (+1)	6 (+1)	6.86	9.53	18.64	2.55
17	3 (-2)	15.00 (0)	0.40 (0)	5 (0)	7.39	6.15	14.48	3.80
18	7 (+2)	15.00 (0)	0.40 (0)	5 (0)	6.96	10.83	15.14	3.10
19	5 (0)	10.00 (-2)	0.40 (0)	5 (0)	8.43	10.10	16.51	3.79
20	5 (0)	20.00 (+2)	0.40 (0)	5 (0)	7.71	7.99	14.70	3.99
21	5 (0)	15.00 (0)	0.30 (-2)	5 (0)	7.71	6.45	13.99	4.80
22	5 (0)	15.00 (0)	0.50 (+2)	5 (0)	7.21	8.56	24.46	4.95
23	5 (0)	15.00 (0)	0.40 (0)	3 (-2)	7.54	9.65	15.23	4.71
24	5 (0)	15.00 (0)	0.40 (0)	7 (+2)	7.75	8.94	17.84	4.58
25	5 (0)	15.00 (0)	0.40 (0)	5 (0)	7.61	8.95	15.30	4.51
26	5 (0)	15.00 (0)	0.40 (0)	5 (0)	6.96	8.92	15.26	4.53
27	5 (0)	15.00 (0)	0.40 (0)	5 (0)	6.98	8.97	15.33	4.49
28	5 (0)	15.00 (0)	0.40 (0)	5 (0)	7.01	8.98	15.37	4.54
29	5 (0)	15.00 (0)	0.40 (0)	5 (0)	6.95	9.01	15.25	4.58
30	5 (0)	15.00 (0)	0.40 (0)	5 (0)	6.30	9.30	15.29	4.47

Results and Discussions

Effect of process variables on protein content of sun dried mango leather

Protein content of mango leather ranged from 6.15 to 12.91%. The maximum protein content (12.91%) of mango leather at coded point (1, -1, -1, 1) was about 2.10 times more than the minimum protein content (6.15%) of mango leather at coded point of (-2, 0, 0, 0).

The model F value of 2.46 implies that the model is significant (P<0.05). R^2 and adjusted R^2 values of the model are 0.70 and 0.41 respectively. The adequate precision value of 5.93 indicates that the model can be used to predict the response within the design space as it is greater than 4.0 (Table 2). It may be seen from Fig. 1 that the increase in whey

protein proportion in mango leather formulation resulted in increase in protein content and there is no effect of sugar on protein content of mango leather. Mir and Nath (2000) [7], Gayathri and Uthira (2008) [5] and Chauhan N. (2013) [4] have also observed similar behavior with increase in other source of protein.

Table 2: ANOVA for effect of process variables on protein content of protein enriched mango leather

Source	Coefficient of model terms	Sum of Squares	df	Mean Square	F Value	Prob > F
Model	-21.89					
A-WPC	4.43^{***}	30.49	1	30.49	21.28	0.0003
B-SUGAR	0.60^{*}	5.35	1	5.35	3.73	0.0725
C-CITRIC ACID	123.55^{ns}	0.77	1	0.77	0.54	0.4737
D-THICKNESS	-4.69^{ns}	0.26	1	0.26	0.18	0.6772
AB	-0.05^{ns}	0.27	1	0.27	0.19	0.6687
AC	0.03^{ns}	0.00	1	0.00	0.00	0.9951
AD	-0.24^{ns}	0.96	1	0.96	0.67	0.4269
BC	-2.90^{ns}	2.11	1	2.11	1.47	0.2437
BD	0.11^{ns}	1.42	1	1.42	0.99	0.3350
CD	8.81^{ns}	3.11	1	3.11	2.17	0.1616
A^2	-0.13^{ns}	0.47	1	0.47	0.33	0.5758
B^2	0.00128^{ns}	0.00	1	0.00	0.00	0.9725
C^2	-150.79^{ns}	3.90	1	3.90	2.72	0.1199
D^2	0.07^{ns}	0.14	1	0.14	0.10	0.7619
Complete Model						
Regression		49.40	14	3.53	2.46	0.0472
Lack of Fit		21.40	10	2.14	109.75	< 0.0001
Pure Error		0.10	5	0.02		
Residual		21.49	15	1.43		
Total		70.90	29			
R^2		0.70		Adeq. Precision		5.93
Adjusted R^2		0.41				

Level of Significance: * P<0.1, $^{**}P$<0.05, *** P<0.01, ^{ns} not significant; df: degrees of freedom.

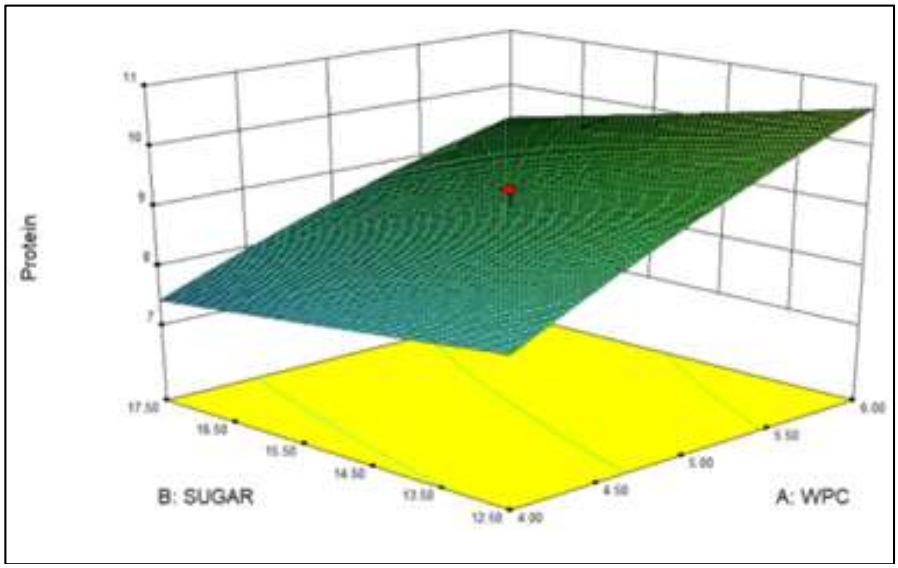

Fig 1: Variation of protein content with respect to whey protein and sugar in protein enriched mango leather

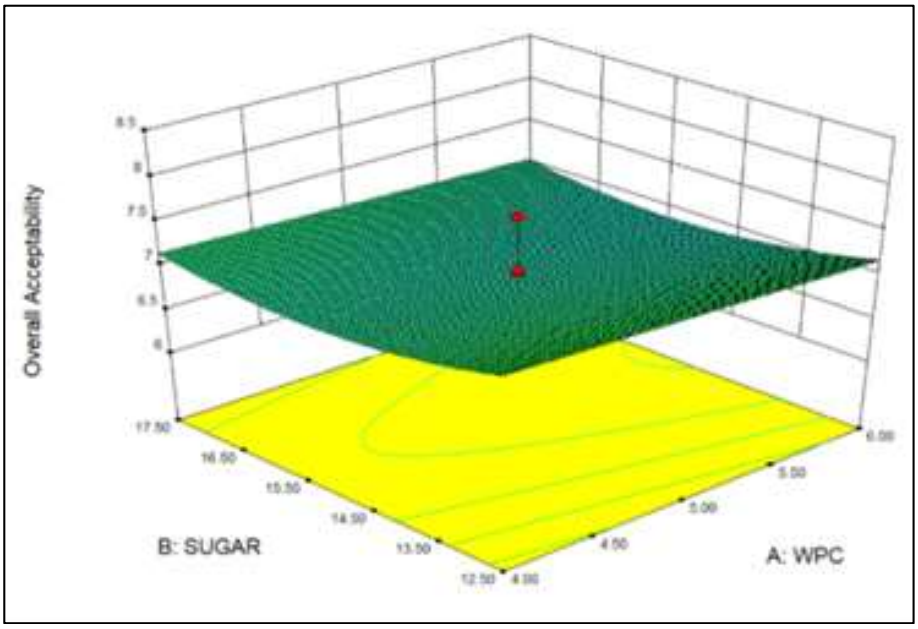

Fig 2: Variation of overall acceptability with respect to whey protein and sugar in protein enriched mango leather

Effect of process variables on overall acceptability of sun dried mango leather

Overall acceptability of protein enriched mango leather ranged from 6.86 to 8.43. The maximum score at coded point (0, -2, 0, 0) was about 1.23 times more than the minimum score at the coded point (1, 1, 1, 1).

From ANOVA table that F-value of 0.96 indicates that the model is non-significant (Table 3). F-values of squares term of sugar content (B^2) at p values of 0.013 (P<0.05) showing that the term are significant. It may be seen from Fig. 2 that the increase or decrease in sugar content in mango leather may affect the overall acceptability of the product and there is no effect of whey protein on overall acceptability of mango leather.

Table 3: ANOVA for effect of process variables on overall acceptability of protein enriched mango leather

Source	Coefficient of model terms	Sum of Squares	df	Mean Square	F Value	Prob > F
Model	15.17					
A-WPC	0.82^{ns}	0.11	1	0.11	0.67	0.4244
B-SUGAR	-0.90^{ns}	0.16	1	0.16	0.94	0.3472
C-CITRIC ACID	-5.21^{ns}	0.00	1	0.00	0.01	0.9104
D-THICKNESS	-0.91^{ns}	0.17	1	0.17	0.98	0.3377
AB	0.01^{ns}	0.00	1	0.00	0.02	0.8999
AC	-1.51^{ns}	0.09	1	0.09	0.54	0.4722
AD	-0.06^{ns}	0.06	1	0.06	0.36	0.5552
BC	-0.60^{ns}	0.09	1	0.09	0.54	0.4722
BD	0.01^{ns}	0.01	1	0.01	0.03	0.8621
CD	0.19^{ns}	0.00	1	0.00	0.01	0.9284
A^2	-0.01^{ns}	0.00	1	0.00	0.00	0.9468
B^2	0.03^{**}	1.31	1	1.31	7.78	**0.0138**
C^2	26.38^{ns}	0.12	1	0.12	0.71	0.4131
D^2	0.11^{ns}	0.35	1	0.35	2.05	0.1725
Complete Model						
Regression		2.27	14	0.16	0.96	0.5242
Lack of Fit		1.66	10	0.17	0.97	0.5520
Pure Error		0.86	5	0.17		
Residual		2.52	15	0.17		
Total		4.80	29			
R^2		0.47		Adeq. Precision		4.12
Adjusted R^2		-0.02				

Level of Significance: * P<0.1, $^{**}P$<0.05, *** P<0.01, ^{ns} not significant; df: degrees of freedom.

Effect of process variables on color value L* of sun dried mango leather

Color value (L*) of protein enriched mango leather ranged from 13.99 to 24.66. The maximum color value (L*) of mango leather at coded point of (0, 0, +2, 0) was about 1.76 times more than the minimum protein content of mango leather at coded point of (0, 0, -2, 0) (Table 4). The Model F-value of 1.04 indicates that the model is non-significant. F-values of term citric acid (C), thickness (D) and square term of citric acid (C^2) at p values of 0.0804, 0.0898 and 0.0523 (P<0.1) showing that all the terms are significant.

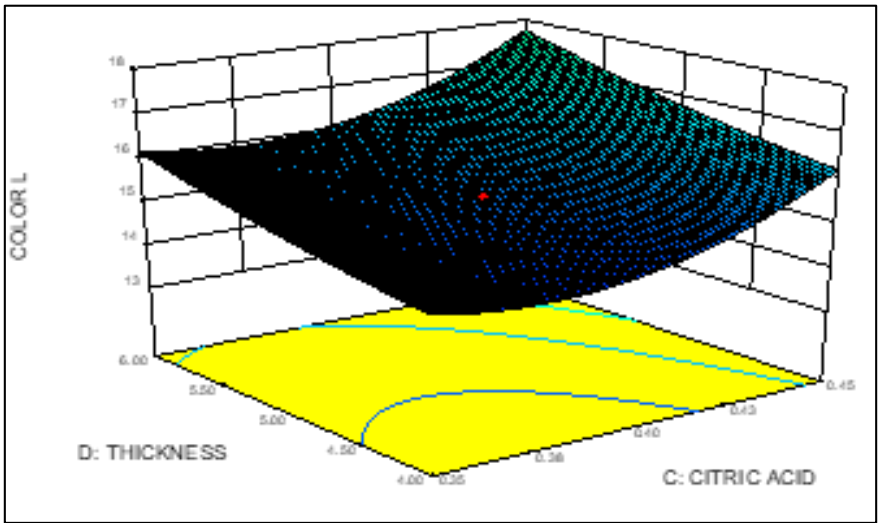

Fig 3: Variation of color value L* with respect to citric acid and thickness in protein enriched mango leather

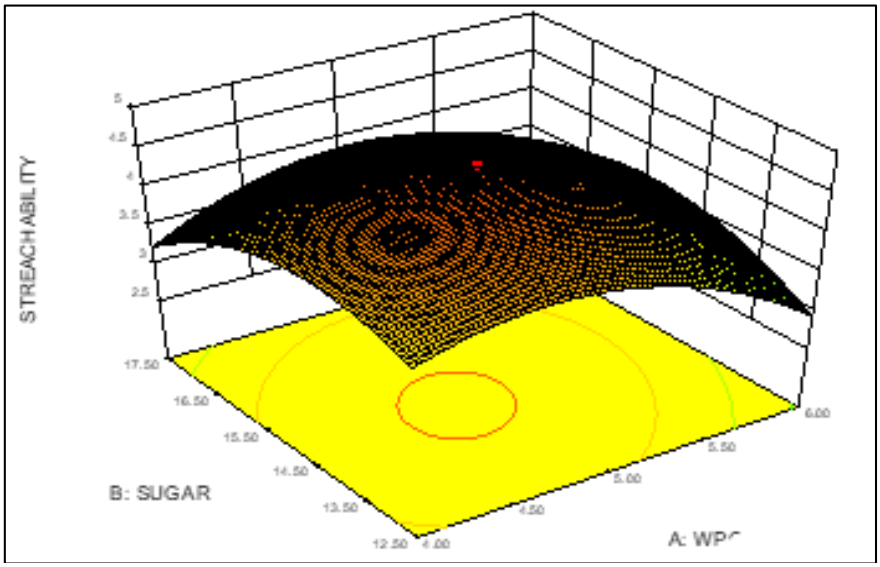

Fig 4: Variation of stretchability with respect to whey protein and sugar in protein enriched mango leather

Table 4: ANOVA for effect of process variables on color value L*of protein enriched mango leather

Source	Coefficient of model terms	Sum of Squares	df	Mean Square	F Value	Prob > F
Model	84.96					
A-WPC	+1.98 ^{ns}	1.09	1	1.09	0.28	0.6056
B-SUGAR	-1.61 ^{ns}	0.07	1	0.07	0.02	0.8959
C-CITRIC ACID	-319.61*	13.76	1	13.76	3.52	**0.0804**
D-THICKNESS	-1.53*	12.86	1	12.86	3.29	**0.0898**
AB	+0.03 ^{ns}	0.10	1	0.10	0.02	0.8766
AC	+4.49 ^{ns}	0.81	1	0.81	0.21	0.6565
AD	-0.19 ^{ns}	0.58	1	0.58	0.15	0.7053
BC	+3.54 ^{ns}	3.14	1	3.14	0.80	0.3843
BD	+0.11 ^{ns}	1.17	1	1.17	0.30	0.5922
CD	+0.89 ^{ns}	0.03	1	0.03	0.01	0.9297
A^2	-0.31 ^{ns}	2.60	1	2.60	0.66	0.4278
B^2	-0.017 ^{ns}	0.33	1	0.33	0.08	0.7767
C^2	+318.37*	17.38	1	17.38	4.44	**0.0523**
D^2	+0.12 ^{ns}	0.42	1	0.42	0.11	0.7483
Complete Model						
Regression		56.97	14	4.07	1.04	0.4681
Lack of Fit		58.67	10	5.87	2933.27	< 0.0001
Pure Error		0.01	5	0.00		
Residual		58.68	15	3.91		
Total		115.65	29			
R^2		0.49		Adeq. Precision		4.77
Adjusted R^2		0.02				

Level of Significance: * P<0.1, $^{**}P$<0.05, *** P<0.01, ^{ns} not significant; df: degrees of freedom.

It was found that citric acid has significant effect ($p< 0.05$) on color value (L*). This might be due to the better sugar-acid blend of the product. Prasad *et. al.,* (2009) [8] reported that the addition of citric acid to a level of 0.45% in banana pulp improved the colour. Thickness had also significant effect ($p< 0.05$) on color value (L*) because of higher thickness require more time of drying and resulted in increase in color value L* (Fig. 4.3).

Effect of process variables on stretchability of sun dried mango leather

Stretchability of protein enriched mango leather ranged from 0.41 to 4.95 N. The maximum stretchability value of mango leather at coded point of (0, 0, +2, 0) was about 9.14 times more than the minimum protein content of mango leather at coded point of (+1, -1, -1, -1) (Table 4.5). The F-value of 0.84 indicates that the model is non-significant. F-values of square term of whey protein (A^2) and sugar (B^2) at p values of 0.0520 and 0.1000, respectively show that both the terms are significant ($P<0.1$).It was found from Figure 4 that increase or decrease in whey protein and sugar affect the stretchability of protein enriched mango leather. It was observed that with the increase of sugar, stickiness of mango leather was also increases. This is may be due to hygroscopic nature of sugar, upon cooling which produce sticky surface found in mango leather (Srivastava and Kumar, 2006) [11]. Thickness had also significant effect ($p< 0.05$) on color value (L*) because of higher thickness require more time of drying and resulted in increase in color value L*.

Table 5: ANOVA for effect of process variables on stretchability of protein enriched mango leather

Source	Coefficient of model terms	Sum of Squares	df	Mean Square	F Value	Prob > F
Model	-12.58 ^{ns}					
A-WPC	+1.29 ^{ns}	1.274	1	1.27	0.49	0.4949
B-SUGAR	+1.56 ^{ns}	0.179	1	0.18	0.07	0.7970
C-CITRIC ACID	+38.78 ^{ns}	0.078	1	0.08	0.03	0.8647
D-THICKNESS	-2.34 ^{ns}	3.003	1	3.00	1.15	0.2997
AB	+0.10 ^{ns}	0.985	1	0.99	0.38	0.5477
AC	+5.39 ^{ns}	1.161	1	1.16	0.45	0.5144
AD	+0.27 ^{ns}	1.140	1	1.14	0.44	0.5182
BC	-0.42 ^{ns}	0.043	1	0.04	0.02	0.8994
BD	+0.13 ^{ns}	1.802	1	1.80	0.69	0.4184
CD	+7.14 ^{ns}	2.038	1	2.04	0.78	0.3902
A^2	-0.65*	11.592	1	11.59	4.45	0.0520
B^2	-0.09*	8.001	1	8.00	3.07	0.1000
C^2	-11.7.54 ^{ns}	2.368	1	2.37	0.91	0.3553
D^2	-0.35 ^{ns}	3.386	1	3.39	1.30	0.2719
Complete Model						
Regression		30.496	14	2.18	0.84	0.6280
Lack of Fit		39.037	10	3.90	2568.24	< 0.0001
Pure Error		0.008	5	0.00		
Residual		39.045	15	2.60		
Total		69.541	29			
R^2		0.44		Adeq. Precision		2.67
Adjusted R^2		-0.09				

Level of Significance: * $P<0.1$, $^{**}P<0.05$, *** $P<0.01$, ^{ns} not significant; df: degrees of freedom.

Optimization of process parameter of sun-dried protein enriched mango leather

Optimization of process parameters was done using Design Expert 8.0.7.1 software. Numerical optimization was carried out by putting the values of process parameters within the experimental range and by setting desirable goals for the responses. Optimization of process parameters for preparation of sun dried protein enriched mango leather was performed on the basis of its dominant quality attributes such as overall acceptability and protein content. The optimum range of process parameters for sun dried mango leather was found as: whey protein (4.92%), sugar (12.50%), citric acid (0.45%) and thickness of pulp (6mm). Corresponding to optimum values of process parameters, predicted values of responses were found as sensory score in term of overall acceptability (7.60) and protein content (9.90%) having desirability (0.60).

Conclusions

Sun dried mango leather was prepared by varying process variables *viz*. whey protein (3-7%), sugar (10-20%), citric acid (0.3-0.5%) and thickness of mango pulp (3-6 mm) and its responses like protein content, overall acceptability, ascorbic acid, colour value – L*, a*, b*, cutting force and stretchability of protein enriched mango leather were analyzed by using Response Surface Methodology for model fitting and determination of statistical significance of the model terms and optimum value of process parameters for sun dried protein enriched mango leather was found as: whey protein (4.92%), sugar (12.50%), citric acid (0.45%) and thickness of pulp (6mm) by using RSM and responses of process parameters were found as overall acceptability (7.60) and protein content (9.90 g/100g).

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