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Statistical Optimization of Processing Variables using Response Surface Methodology (RSM) for Sensory Evaluation of Aloe Vera Chocolate Preparation

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ABSTRACT

The aim of this present research work was to evaluate the potential use of Aloe Vera for the production of chocolate. Response Surface Methodology (RSM) was used to optimize the process variables like sugar (800-1200 g), skim milk powder (900 – 1100 g), Cocoa powder (200 – 300 g)and Aloe Vera juice (50 – 150 ml). The design contains a total of 31 experimental runs involving replications of the central points and organized in a randomized factorial design. Data obtained from RSM on Aloe Vera chocolate production were subjected to the analysis of variance (ANOVA) and were analyzed using a second order polynomial equation. Maximum sensory analysis of color, Taste, Aroma and Texture in the Aloe Vera chocolate production was obtained from ingredients at the optimized process conditions. The optimum condition for taste is Sugar – 1000 g, Skim milk powder – 1019 g, Cocoa powder - 252.5 g and Aloe Vera juice - 82.5 ml. Chocolate produced under the optimum conditions for taste was again subjected to evaluation of sensory values and the results were compared with the RSM predictions. **Keywords:** Aloe Vera, food processing, CCD, Chocolate, Skim milk powder, cocoa powder



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INTRODUCTION

Chocolate is a widely consumed dairy product. When dealing with dairy foods, sensory quality is always involved on some level. The best raw ingredients make the best finished products so sensory quality is critical aspect of dried ingredients and fluid milk [1]. Sensory analysis is the examination of a product through the evaluation of the attributes perceptible by the four organoleptic attributes such as color, taste, aroma and texture. Sensory analysis allows establishing the organoleptic profile of diverse products [2]. The sensory characters lie in the continuous phase lipid composition, which influences melting and mouth feel properties. Chocolate triglycerides are dominated by mono unsaturated oleic acid and fatty acids, saturated stearic (34%) and palmitic (27%) acids. Chocolates are solid at ambient temperature and have a melting point of 37 $^{\circ}$ C (oral temperature). During consumption they give a smooth suspension of particulate solids of milk fat and cocoa butter [3-4]. Rheological properties of chocolate are important in production process for obtaining high quality products with fine texture and sensory characters [5]. Chocolates with high palatability and high viscosity have a pasty mouth feel [6]. Viscosity relates to composition, processing strategy and particle size distribution. Also the apparent viscosity in aqueous solutions influences flavor by mouth and taste intensity during consumption [7]. Recently greater importance has been given to chocolates due to its nutritional benefits arising out of it. Cocoa solid which has poly phenols in excess is seen as a common item in the European and American diet [8]. Poly phenols are reported as a beneficial compound in cardio and vascular protection through their anti oxidative activity [9-10].

The aloe Vera juice is an odorless and colorless hydrocolloid with several natural substances that are beneficial. It is a diverse mixture of coagulating agent, astringent, antibiotic, cell growth stimulator and pain inhibitor. Aloe Vera contains more than 70 essential ingredients including protein, minerals, vitamins and enzyme. Aloe Vera, when taken internally helps in digestion and curing stomach ulcer, heart burn, colitis, and other viral infections. Also it fights cold, flu and other intestinal problems, reduces allergies and tumors, aids in detoxification and also helps during arthritis and chronic fatigue conditions. It has sugar and cholesterol lowering activity [11]. The objective of the present work is to produce chocolate using aloe vera. This is the first study which focuses on the production of chocolate using aloe vera. The quality of the chocolate depends on the proportion of the mixture which can be tested by sensory analysis. The proportions can be standardized using a mathematical tool called Response Surface Methodology (RSM). The RSM is an innovative approach to model a system with the collection of statistical techniques wherein interactions between multiple processes variables can be identified with fewer experimental trials. The RSM experimental design is an efficient approach to deal with a large number of variables and there are several reports on application of RSM for the evaluation of sensory analysis [12-15].



MATERIALS AND METHOD

Chocolate Masses

The chocolate ingredients like Sugar, Skim milk powder, Cocoa powder, Butter, Flavor and Aloe Vera juice were obtained from essence market.

Sample Preparation

The coco powder was mixed with skim milk powder. Sugar was dissolved in water till complete saturation in a boiling pan and the mixture was heated with occasional stirring. Aloe Vera juice was then added to the mixture. Once the mixture attains 110°C, the cocoa powder and skimmed milk powder were added. Then, Butter and flavor were added. After 20 minutes the whole mass is oared into a frame on an oiled slab. Then it was cut into appropriate size and wrapped in waxed paper.

Independent vari	Coded levels							
	Code	-2	-1	0	1	2		
Sugar ,g	А	800	900	1000	1100	1200		
Skim milk powder ,g	В	900	950	1000	1050	1100		
g, Cocoa powder	С	200	225	250	275	300		
Aloe Vera juice ,ml	D	50	75	100	125	150		

Optimization of Aloe Vera Chocolate Production for Sensory Analysis

The Response surface methodology (RSM) consists of a group of empirical techniques used for evaluation of relationship between clusters of controlled experimental factors and measured response. The RSM was employed to optimize the components like sugar, Skim Milk powder, Cocoa powder and Aloe Vera juice which enhance the Aloe Vera chocolate preparation. The ranges of these four variables are given in Table-1.

Experiments were performed according to the central composite design (CCD) in the RSM. The design of experiment was given in Table-2. The statistical software package "Minitab 15" was used to analyze the experimental data. All variables were taken at a central coded value of zero. The minimum and maximum ranges of variables investigated are listed in (Table-1). Upon the completion of experiments, the average maximum sensory evaluation of aloe vera chocolate for color, taste, aroma and texture was taken as the response (Y). A multiple regression analysis of the data was carried out for obtaining an empirical model that relates the response measured to the independent variables.

A second-order polynomial equation is

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1, i < j}^{k=1} \sum_{j=2}^k \beta_{ij} X_i X_j,$$
 (1)

Table 2: Experimental design matrix for screening of important variables

Run no	Α	В	С	D
1	-1	-1	1	1
2	0	0	0	0
3	-1	1	1	1
4	0	0	0	0
5	0	0	0	0
6	1	-1	-1	-1
7	0	0	-2	0
8	0	0	0	0
9	1	1	-1	-1
10	-1	-1	-1	1
11	0	0	0	-2
12	0	-2	0	0
13	0	0	2	0
14	2	0	0	0
15	-1	1	-1	-1
16	0	0	0	0
17	-1	1	1	-1
18	-2	0	0	0
19	0	2	0	0
20	1	1	1	-1
21	0	0	0	0
22	1	-1	1	-1
23	-1	-1	-1	-1
24	-1	-1	1	-1
25	1	-1	-1	1
26	1	1	1	1
27	0	0	0	0
28	0	0	0	2
29	-1	1	-1	1
30	1	-1	1	1
31	1	1	-1	1

Where Y is the measured response, $\beta 0$ is the intercept term, βi are linear coefficients, βii are quadratic coefficient, $\beta i j$ is interaction coefficient, and Xi and Xjare coded independent variables. The optimal concentrations of the critical variables were obtained by analyzing response surface methodology. The statistical analysis of the model was represented in the form of analysis of variance (ANOVA).

Sensory Evaluation

Sensory evaluation was carried out by a 50 member untrained panel (35 males and 15 females) consisting of faculty and graduate students of the laboratory of Food process and technology at

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the Department of Technology, Annamalai university. Panelists were chosen adopting the following criteria: age: 20-35 years, non smokers, non allergic to any food, frequent chocolate consumption. The Aloe Vera Chocolate was kept in small plastic containers coded with 3-digit random numbers and tightly capped. The samples were evaluated based on color, taste, aroma and texture using a 9 point hedonic scale where: 9=Excellent, 8 = very good, 7 = good, 6 = Just a little good, 5=May be good or may be bad, 4=Just a little bad, 3=Bad, 2=very bad and 1=worst [16].

Bun	Colo	r	Tast	e	Arom	Aroma		re
no	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	6.6	6.537	6.45	6.437	6.45	6.437	6.6	6.283
2	8.5	8.586	8.95	8.929	8.95	8.929	9	9
3	6	6.369	5.65	6.01	5.65	6.01	5.5	5.731
4	8.65	8.586	9	8.929	9	8.929	9	9
5	8.75	8.586	9	8.929	9	8.929	9	9
6	6.75	6.394	6.5	6.201	6.5	6.201	6.4	6.323
7	5.95	6.435	6.4	6.445	6.4	6.445	6.45	6.644
8	8.35	8.586	8.75	8.929	8.75	8.929	9	9
9	8	7.538	7.4	7.277	7.4	7.277	7.45	7.383
10	6.45	6.285	6.4	6.418	6.4	6.418	6.45	6.281
11	7.2	7.61	7	7.364	7	7.364	7.2	7.394
12	6.45	6.994	6.95	7.439	6.95	7.439	7.05	7.31
13	7.1	7.127	6.95	6.98	6.95	6.98	7.1	7.135
14	5	5.71	5	5.245	5	5.245	5.95	5.952
15	7.2	7.227	7.25	7.368	7.25	7.368	7.2	7.09
16	8.45	8.586	9	8.929	9	8.929	9	9
17	8	7.879	7.35	7.362	7.35	7.362	7.4	7.092
18	7	6.802	6.5	6.33	6.5	6.33	6.2	6.427
19	8	7.969	8.5	8.087	8.5	8.087	7.85	7.819
20	7.8	7.977	7.75	7.793	7.75	7.793	7.55	7.873
21	8.55	8.586	8.85	8.929	8.85	8.929	9	9
22	6.15	5.671	6.05	5.82	6.05	5.82	6.45	6.075
23	8.55	8.446	8.6	8.495	8.6	8.495	8.95	8.817
24	7.95	7.935	7.81	7.591	7.81	7.591	7.95	8.081
25	5.5	5.096	5.05	4.902	5.05	4.902	5.1	5.025
26	7.75	7.329	7.25	7.218	7.25	7.218	8	7.75
27	8.85	8.586	8.95	8.929	8.95	8.929	9	9
28	4.7	4.802	5	4.712	5	4.712	4.7	4.735
29	5	4.954	5	5.093	5	5.093	5	4.992
30	5.15	5.135	5.5	5.443	5.5	5.443	5.25	5.515
31	6.1	6.127	5.5	5.78	5.5	5.78	6.5	6.523

Table 3: Central composite design (CCD) of factors in coded levels with sensory analysis of foods in the production of Aloe Vera chocolate using RSM

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RESULTS AND DISCUSSIONS

Optimization of process variables in chocolate production using aloe vera by rsm.

The results obtained were given in Table-3. A polynomial model is proposed for sensory analysis of foods in the production of aloe vera chocolate. The four outputs, namely color, taste, aroma and texture of the product are given as polynomials in equations (2), (3), (4) and (5) respectively

$$Y = 8.58571 - 0.27292A + 0.24375B + 0.17292C - 0.70208D - 0.58237A^{2} - 0.27612B^{2} - 0.45112C^{2} - 0.59487D^{2} + 0.59062AB - 0.05313AC + 0.21563AD + 0.29063BC - 0.02812BD + 0.19063CD (2)$$

 $Y = 8.7643 - 0.1521A + 0.2562B + 0.1521C - 0.7438D - 0.5520A^{2-} 0.4333B^{2-} 0.5583C^{2} - 0.5583D^{2} + 0.6219AB + 0.1031AC + 0.1219AD + 0.1219BC - 0.1094BD + 0.1094CD$ (3)

 $Y = 8.92857 - 0.27125A + 0.16208B + 0.13375C - 0.66292D - 0.78516A^{2} - 0.29141B^{2} - 0.55391C^{2} - 0.72266D^{2} + 0.55062AB + 0.13062AC + 0.19437AD + 0.22438BC - 0.04938BD + 0.23062CD (4)$

$$Y = 9.0000 - 0.1188A + 0.1271B + 0.1229C - 0.6646D - 0.7026A2 - 0.3589B2 - 0.5276C2 - 0.7339D2 + 0.6969AB + 0.1219AC + 0.3094AD + 0.1844BC + 0.1094BD + 0.1844CD (5)$$

ANOVA for the response surface methodology was shown in Table-4 to 7 for color, taste, aroma and texture respectively. The F value of 20.32 for color, 43.64 for taste, 50.89 for aroma and 68.14 for texture implies the model is significant. Generally values of "prob> F" and less than 0.05 indicate that model term is significant. Values greater than 0.1 indicate that the model terms are not significant.

In the present work, all the linear, interactive effects of A*B, A*D, B*C and square effects of A, B, C and D were significant for sensory values of color. For sensory values of taste, all the linear, interactive effects of A*B and square effects of A, B, C and D were significant. For sensory values of aroma, all the linear, interactive effects of A*B, A*D, B*C, C*D and square effects of A, B, C and D were significant. For sensory values of texture, all the linear, interactive effects of A, B, C and D were significant. For sensory values of texture, all the linear, interactive effects of A *B, A*D, B*C, C*D and square effects of A, B, C and D were significant.

The coefficients of determination (R^2) for sensory values were found to be 0.9468 for color, 0.9745 for taste, 0.9780 for aroma and 0.9835 for texture. The predicted R^2 value, 0.7100 for color, 0.8777 for taste, 0.8777 for aroma and 0.9050 for texture, was in reasonable agreement with the adjusted R^2 value of 0.9002 for color, 0.9522 for taste, 0.9588 for aroma and 0.9691 for texture.





Figure 1: Predicted response versus actual value (color, taste, aroma, texture)

		Color	Taste				
Source	Regression Coefficient	t-statistic	P-value	Regression Coefficient	t-statistic	P-value	
Intercept	8.58571	56.591	<0.000	8.7643	83.181	<0.000	
А	-0.27292	-3.331	<0.004	-0.1521	-2.673	<0.017	
В	0.24375	2.975	<0.009	0.2562	4.503	<0.000	
С	0.17292	2.11	0.051	0.1521	2.673	<0.017	
D	-0.70208	-8.569	<0.000	-0.7438	-13.071	<0.000	
A*A	-0.58237	-7.758	<0.000	-0.552	-10.589	<0.000	
B*B	-0.27612	-3.678	<0.002	-0.4333	-8.311	<0.000	
C*C	-0.45112	-6.01	<0.000	-0.5583	-10.709	<0.000	
D*D	-0.59487	-7.925	<0.000	-0.5583	-10.709	<0.000	
A*B	0.59062	5.886	<0.000	0.6219	8.923	<0.000	
A*C	-0.05313	-0.529	0.604	0.1031	1.48	0.158	
A*D	0.21563	2.149	0.047	0.1219	1.749	0.099	
B*C	0.29063	2.896	<0.011	0.1219	1.749	0.099	
B*D	-0.02812	-0.28	0.783	-0.1094	-1.569	0.136	
C*D	0.19063	1.9	0.076	0.1094	1.569	0.136	

Table 4: Regression analysis and corresponding t and p-value of second order polynomial model for the optimization of sensory analysis of foods in the production of aloe vera chocolate for color and taste

S = 0.401400, Press = 14.0415, R-Sq = 94.68%, R-Sq (pred) = 71.00%, R-Sq (adj) = 90.02% for color.

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Figure 2: Contour plot showing the interactive effects of Cocoa powder and Skim milk powder on sensory analysis of aloe vera chocolate for color



Figure 3: Contour plot showing the interactive effects of Sugar and Aloe Vera juice on sensory analysis of Aloe Vera chocolate for color



Figure 4: Contour plot showing the interactive effects of Sugar and Skim milk powder on sensory analysis of Aloe Vera chocolate for taste









Figure 6: Contour plot showing the interactive effects of Aloe Vera juice and Sugar on sensory analysis of aloe vera chocolate for texture



Figure 7: Contour plot showing the interactive effects of Cocoa powder and Skim milk powder on sensory analysis of aloe vera chocolate for texture



The above model can be used to predict the sensory analysis of aloe vera chocolate production within the limits of the experimental factors. Figure 1 shows that the actual response values agree well with the predicted response values. The interactive effects of variables on sensory analysis of aloe vera chocolate production in the form of contour plots were shown in Figures 2 - 7. The experimental conditions for different sensorial score obtained and their response for the sensorial color, taste, aroma and texture were given in Table-4 to 7.

Course	Color					Taste				
Source	SOS	DF	MS	F	Р	SOS	DF	MS	F	Р
Regression	45.8456	14	3.27469	20.32	<0.000	45.4815	14	3.39153	43.64	<0.000
Linear	15.7613	4	3.94031	24.46	<0.000	15.9621	4	3.99052	51.35	<0.000
Square	21.7684	4	5.4421	33.78	<0.000	24.3035	4	6.07586	78.19	<0.000
Interaction	8.3159	6	1.38599	8.6	<0.000	7.2159	6	1.20266	15.48	<0.000
Residual Error	2.5779	16	0.16112	-	-	1.2434	16	0.07771	-	-
Lack-of-Fit	2.3944	10	0.23944	7.83	<0.010	0.9698	10	0.09698	2.13	0.184
Pure Error	0.1836	6	0.0306	-	-	0.2736	6	0.0456	-	-
Total	48.4235	30	-	-	-	48.7248	30	-	-	-

 Table 5: Analysis of variance (ANOVA) for the quadratic polynomial model for the optimization of sensory analysis of foods in the production of Aloe Vera chocolate for color, Taste

S = 0.278765, Press = 5.95836, R-Sq = 97.45%, R-Sq (pred) = 87.77%, R-Sq (adj) = 95.22% for taste.

Table 6: Regression analysis and corresponding *t* and p- value of second order polynomial model for the optimization of sensory analysis of foods in the production of Aloe Vera chocolate for Aroma and Texture

		Aroma	Texture				
Source	Regression Coefficient	t-statistic	P-value	Regression Coefficient	t-statistic	P-value	
Intercept	8.92857	84.654	<0.000	9	99.494	<0.000	
А	-0.27125	-4.762	<0.000	-0.1188	-2.431	<0.027	
В	0.16208	2.846	<0.012	0.1271	2.601	<0.019	
С	0.13375	2.348	<0.032	0.1229	2.516	<0.023	
D	-0.66292	-11.638	<0.000	-0.6646	-13.604	<0.000	
A*A	-0.78516	-15.046	<0.000	-0.7026	-15.699	<0.000	
B*B	-0.29141	-5.584	<0.000	-0.3589	-8.018	<0.000	
C*C	-0.55391	-10.615	<0.000	-0.5276	-11.789	<0.000	
D*D	-0.72266	-13.849	<0.000	-0.7339	-16.397	<0.000	
A*B	0.55062	7.893	<0.000	0.6969	11.647	<0.000	
A*C	0.13062	1.872	0.08	0.1219	2.037	0.059	
A*D	0.19437	2.786	<0.013	0.3094	5.171	<0.000	
B*C	0.22438	3.216	<0.005	0.1844	3.082	<0.007	
B*D	-0.04938	-0.708	0.489	0.1094	1.828	0.086	
C*D	0.23062	3.306	< 0.004	0.1844	3.082	<0.007	

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S = 0.279051, Press = 6.93765, R-Sq = 97.80%, R-Sq (pred) = 87.77%, R-Sq (adj) = 95.88% for aroma.

Course	Aroma					Texture				
Source	SOS	DF	MS	F	Р	SOS	DF	MS	F	Р
Regression	55.4818	14	3.96298	50.89	<0.000	54.641	14	3.90293	68.14	<0.000
Linear	13.3727	4	3.34317	42.93	<0.000	11.6887	4	2.92219	51.02	<0.000
Square	34.685	4	8.67126	111.36	<0.000	32.1338	4	8.03344	140.25	<0.000
Interaction	7.424	6	1.23734	15.89	<0.000	10.8184	6	1.80307	31.48	<0.000
Residual Error	1.2459	16	0.07787	-	-	0.9165	16	0.05728	-	-
Lack-of-Fit	1.1916	10	0.11916	13.17	0.003	0.9165	10	0.09165	-	-
Pure Error	0.0543	6	0.00905	-	-	0	6	0	-	-
Total	56.7277	30	-	-	-	55.5574	30	-	-	-

Table 7: Analysis of variance (ANOVA) for the quadratic polynomial model for the optimization of sensory analysis of foods in the production of Aloe Vera chocolate for Aroma and Texture

S = 0.239330, Press= 5.2788, R-Sq = 98.35%, R-Sq (pred) = 90.50%, R-Sq (adj) = 96.91% for texture.

Table 8: The optimum conditions for maximum sensory	score obtained using RSM
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Sensory output	Sugar (g)	Skim milk powder (g)	Cocoa powder (g)	Aloe vera juice (ml)
Color	986	1021	255.5	85.5
Taste	1000	1019	252.5	82.5
Aroma	982	1007	250.5	88.5
Texture	974	993	249.5	87.5

Figure 2 shows the significant interaction between skim milk powder and cocoa powder for color. The sensory score value of color increases with increase in skim milk powder and cocoa powder to about 1021 g and 255.5 g respectively and thereafter sensory score decreases with further increase in skim milk powder and cocoa powder. The same trend was observed in Figure 3. Which shows an increase in sugar and Aloe Vera juice resulted in increase in sensory score value of color up to 986 g and 85.5 ml respectively. The sensory value of the four outputs, namely, color, taste, aroma and texture decreased when the proportions of the ingredients vary from the above mentioned values. Figure 4 shows the significant interaction between sugar and skim milk powder for taste. The sensory score value of taste increased with increase in sugar and skim milk powder to about 1000 g and 1019 g respectively and decreases with further increase in sugar and skim milk powder.

Figure 5 shows the significant interaction between skim milk powder and cocoa powder for aroma. The sensory score value of aroma increased with increases in skim milk powder and cocoa powder to about 1007 g and 250.5 g respectively and its sensory score decreases with



further increase in skim milk powder and cocoa powder. Figure 6 shows significant interaction between sugar and aloe vera juice for texture. The sensory score value of texture increased with increase in sugar and Aloe Vera juice to about 974 g and 87.5 ml respectively and further increase in skim milk powder and cocoa powder decreased the sensory score of the texture. The same trend was observed in Figure 7 which shows an increase in skim milk powder and cocoa powder resulted in increase in texture up to 993 g and 249.5 g respectively. The optimum conditions for the best sensory score of the four outputs determined by RSM are given in Table-8.

An experimental run was conducted by taking the operating parameters that yielded best sensory value for taste. The chocolate produced was tested with the panelists and the scores were compared with the predicted value. The overall scores were 8.90 for color, 9.00 for taste, aroma and texture.

CONCLUSIONS

The present investigation deals with the production of aloe vera chocolate using sugar, skim milk powder, cocoa powder and aloe vera juice. Response Surface Methodologydesign was used to test the relative importance of sensory outputs of the chocolate in terms of color, taste, aroma and texture. The proportion of the four ingredients for the best sensory value of taste is given higher priority and the experiment run for the optimum conditions obtained from RSM gave a sensory score of 8.90 for color, 9.00 for taste, aroma and texture. The R² value, greater than 0.9, for all the four cases indicates the applicability of RSM approach in determining the optimum conditions for production of aloe vera chocolate.

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