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PROCESS DEVELOPMENT OF KOKUM RTS BEVERAGE USING RESPONSE SURFACE METHODOLOGY (RSM)

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ABSTRACT

A Response Surface Methodology (RSM) was used for the determination of optimum ingredients level to prepare kokum RTS (Ready-To-Serve) beverage. Kokum pulp was extracted using water extraction method at temperature (24.97ºC) and time (30.42 min). The effects of ingredients levels on sensory parameters like colour, flavour, taste, HCA (Hydroxycitric acid) content and anthocyanin content of the prepared beverage were studied by employing a Box- Behnken Design (BBD). The coefficient of determination R^2 for colour, flavour, taste, HCA (Hydroxycitric acid) content and anthocyanin content were 0.9219, 0.8313, 0.7290, 0.9584 and 0.9313 respectively. Analysis of variable (ANOVA) performed on the experimental values showed that pulp quantity, sugar and cumin powder and were the most important factors that affected characteristics of the kokum RTS beverage as it exerted a highly significant influence ($p < 0.05$) on all the dependent variables. Based on surface and contour plots, optimum ingredients level for formation of kokum RTS beverage were pulp, sugar, cumin powder; 57.30g, 35.52g and 13.5g respectively.

Key Words: Kokum, Response Surface Methodology, Box- Behnken Design, Hydroxy Citric Acid, Anthocyanin, Optimization.

INTRODUCTION

Garcinia indica Choisy belonging to the family Guttiferae (in the mangosteen) is an indigenous tree of India. It was originally found only in the western peninsular coastal regions and in the Western Ghats in the states of Maharashtra, Goa, Karnataka and Kerala, India as well as parts of Eastern India in the states of West Bengal, Assam and North Eastern Hill regions.

Kokum is an important culinary agent and is used as an acidulant for curries by people living in Maharashtra, costal Karnataka and Goa, India. In summer the ripe rinds are ground in a blender with sugar and cardamom and consumed as a cooling drink. Addition of kokum is supposed to enhance the taste of coconut-based curries and to remove the unpleasant smell of mackerel and sardines. They are also used in some vegetable dishes and to prepare chutneys and pickles. The Goans regularly prepare kokum kadi or birinda solkadhi. These curries are used with rice or like an after meal digestive drink. Both birindi saar and kokum kadi are supposed to be digestive and to relieve gastric problems. Studies have shown that the rind contains moisture (80.0 g/100 g), protein (1%), tannin (1.7%), pectin (0.9%), Total sugars (4.1%) and fat (1.4%). The seed is very rich in stearic, oleic and stearic triglycerides Phytochemical studies have shown that when compared with any other natural sources, kokum rind contains the highest concentration of anthocyanins (2.4 g/100 g of kokum fruit) (Nayak, Rastogi, et al., 2010; Nayak, Srinivas, et al., 2010). The anthocyanins cyanidin-3-glucoside and cyanidin-3-sambubioside are the major pigment present in kokum and is reported to occur in the ratio of 4:1. Studies have shown that (-)-Hydroxycitric acid (HCA) is the major organic acid in kokum leaves and rinds.

In the Ayurvedic system of medicine, kokum is used to treat illness related to obesity and multiple studies have shown that hydroxycitric acid (also known as garcinia acid) a component of kokum is reported to possess anti-obesity effects (Arseculeratne, S. N., Gunatilaka, A. A. L., & Panabokke, R. G ,1981). Studies have shown that consumption of hydroxycitric acid reduces appetite, inhibits fat synthesis, lipogenesis, decreases food intake and reduces body weight (Preussa et al., 2004; Jena, Jayaprakasha, Singh, & Sakariah, 2002). It also inhibits synthesis of fatty acid and lipogenesis from various precursors (Jena et al., 2002). Concomitantly, it also increases the synthesis of hepatic glycogen thereby activating the glucoreceptors and causing a sensation of reduced appetite and fullness (Lowenstein, 1971; Preussa et al., 2004). Hydroxycitric acid is non toxic as experimental studies have shown that by oral route it did not cause death or systemic or behavioral toxicity even at high dose of 5 g/kg b. wt. When extrapolated to human dose, 5 g/kg b. wt. amounts to about 350 g, which is nearly 233 times more than the recommended dose of 1.5 g/ day (Jena et al., 2002). Cold Water extraction was used to extract kokum pulp from its dried rinds. This method often used to maximize, HCA (Hydroxycitric acid) content.

(Mamata Mukhopadhyay 2008)

As kokum is an under-utilized fruit, it can be preserved by making it as a pulp to use it for the preparation of RTS (Ready- To- Serve) Beverage. Hence the present study aims to use RSM as a tool for optimizing the ingredients level for kokum RTS beverage formulation for obtaining maximum colour, flavour, taste, HCA content and Anthocyanin content.

MATERIALS AND METHODS

COLLECTION OF FRUITS

Salt rubbed kokum rinds (completely blackish red in colour) sugar and cumin powder were purchased from a local shop in Parrys Market, Chennai.

METHODOLOGY

RTS beverage was prepared by using the clear juice obtained from the kokum pulp with addition of sufficient quantity of sugar followed by pasteurization and cooling. Process of making kokum RTS is given in fig 1.

Formation of kokum pulp
↓
Addition of water to pulp
↓
Straining of the pulp
↓
Addition of sugar, cumin powder to the clear juice
↓
Heating the mixture (Pasteurization)
↓
Straining the juice
↓
Filling into clean dry sterilized bottles
↓
Sealing the filled bottles
↓
Store into refrigerator @ 4°C

Fig 1- Process of making Kokum RTS

EQUIPMENT USED

The laboratory scale mixer consists of two parts- Mixing jar and motor (Preethi Himaachal & Co. Solan, India). A grinding blade made up of stainless steel (1.6 mm thick) with three cutting edges was used to grind the material in the jar.

EXTRACTION OF KOKUM PULP

Purchased kokum rinds were washed in cold water three times for the removal of salt.100 g of the samples was used for each treatment. Equal amount (100ml) of water was added to the washed rinds. The washed rinds were soaked in water at 24.97°C temperature for 30.42 min of time. The kokum pulp was extracted by grinding into the mixer operated at 5000 rpm for 5 min at controlled room temperature (28-32 °C). After extracted, The pulp was strained through 20 mesh stainless steel sieve to get the smooth pulp.

EXPERIMENTAL DESIGN LAYOUT

After several preliminary trials, the levels of ingredients were selected. The independent variables considered were: pulp quantity (40-60g), Sugar quantity (25-45g) and cumin powder quantity (8.5-18.5g). A three-variable (three levels of each variable) box–behnken design (BBD) and a response surface methodology (RSM) were used to understand the interactions of pulp, sugar and cumin powder quantity on the colour, flavour, taste, HCA content and Anthocyanin content of the prepared beverage in 17 runs (Table3.1), of which 12 were IB factorial, and five were at centre point.

Table 2.1 - Processing ingredients and their levels

Ingredients	Levels
Pulp Quantity (g)	40, 50, 60
Sugar (g)	25, 35, 45
Cumin powder (g)	8.5, 13.5, 18.5

RTS BEVERAGE DEVELOPMENT TRIALS

The RTS beverage development trials were carried out and the responses observed also shown in the table 2.2

DETERMINATION OF SENSORY PARAMETERS

The sensory parameters of the prepared beverage samples like colour, flavour , taste were evaluated on the basis of 9 point hedonic scale using B2 Monadic Test of sensory. The samples were given to 20 semi trained panel members.

HCA DETERMINATION

HCA (Hydroxy Citric Acid) content was determined by titration method with help of 0.1N NaOH solution. The end point is formation of black coloured compound named as hydroxy citrate. (G.K. Jayaprakasha, K.K. Sakariah; 2002)

ANTHOCYANIN DETERMINATION

Anthocyanin was determined in terms of cyanidin-3-glucoside by spectrophotometer with pH difference method. (J. AOAC Int. (2005) 88, 1269).

STATISTICAL ANALYSIS FOR OPTIMIZATION OF INGREDIENTS QUANTITY

The response functions y (dependent variables) were colour score, flavour score, taste score, HCA content and Anthocyanin content. The following second-order polynomial model was fitted to the dependent variables with the experimental data (Eq. 2.1).

$$\mathbf{y = ß_0 + \sum_{i=1}^{3} ß_i X_i + \sum_{i=1}^{3} ß_{ii} X_{ii}^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{2} ß_{ij} X_i X_j} \quad \text{--- } \mathbf{(2.1)}$$

The co-efficients of the polynomial were represented by $ß_0$ (constant term), X_i (linear effects), X_{ii}^2 (quadratic effects), and X_i X_j (interaction effects). The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significance of all the terms in the polynomial was judged by computing the P value (Prob.> F) at 0.1%, 1% or 5% significance level. Response surfaces and contour plots were generated with the help of commercial statistical package, Design-Expert (2010) — version 8.1.6 STAT-EASE, Inc., MN, USA.

OPTIMIZATION

Numerical and graphical optimization was carried out for the independent variables to obtain the beverage with Maximum colour, flavour, taste, and HCA, anthocyanin content using Design-Expert software. Conventional graphical method was applied to obtain Maximum pulp of all responses. Predictive models were used to graphically represent systems. Contour plots of the response variables were utilized to select optimum ingredients level of pulp, sugar, cumin powder quantity for the production of kokum RTS beverage.

RESULTS AND DISCUSSIONS

STATISTICAL ANALYSIS

Table 3.1, 3.2 summarizes the results of each dependent variable with their coefficients of determination (R^2). The statistical analysis indicates that the proposed model was adequate, possessing no significant lack of fit and with very satisfactory values of the R^2 for all the responses. The R^2 values for color, flavour, taste, HCA content, Anthocyanin content were 0.9219, 0.8313, 0.7290, 0.9584 and 0.9313 respectively. The closer the value of R^2 to unity, the better is the empirical models fit the actual data. On the other hand, the smaller the value of R^2 the less relevance the dependent variables in the model have in explaining the behaviour of variations (Little & Hills, 1978; Mendenhall, 1975).

EFFECT OF INGREDIENTS ON RESPONSE VARIABLES

COLOUR

ANOVA (Table 3.1) indicated that the colour was highly significant at 5% level on linear term of pulp quantity. Quadratic terms of process variables were having significant effect at 0.1%, 1% level. By neglecting the non significant terms in Eq.2.1 and with the coded values of independent variables, the following equation (Eq. 3.1) describes the effect of significant process variables on colour of the produced beverage.

Colour = $8.2 - 0.175 * X_1 - 0.41 * X_1^2 - 0.58 * X_2^2 - 0.34 * X_3^2$ ($R^2 = 0.95$) ------------------------------ (3.1)

Where, X_1 is the pulp quantity in grams; X_2 is the sugar quantity; X_3 is the cumin powder quantity. The first-order term of pulp quantity, (Eq.3.1) indicated that colour increased with increase of this variable. The quadratic terms suggested that increase of these variables resulted in decrease of colour. The variation of colour with pulp, sugar level were graphically presented in the 3-D surface plot and contour plots (Fig. 3.1a, 3.1b).

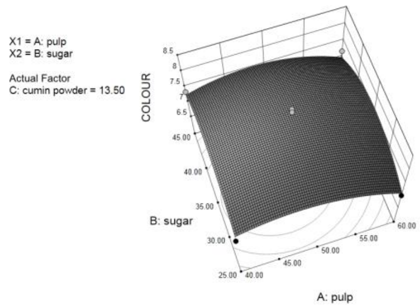

Fig.3.1a 3-D Response surface plots

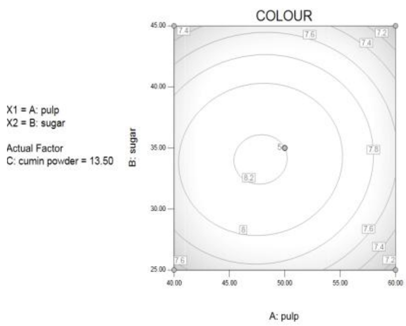

Fig.3.1 b Contour plots

FLAVOUR

ANOVA (Table 3.1) indicated that flavour content was significant at 5% level on quadratic terms of pulp, sugar, cumin powder quantity. Interaction term is not having any effect on the flavour of the beverage. By neglecting the non significant terms in Eq. 2.1 and with the coded values of independent variables, the following equation (Eq. 3.2) describes the effect of significant process variables on flavour of the produced beverage.

Flavour = $8.13 - 1.07 * X_1^2 - 1.18 * X_2^2 - 1.03 * X_3^2$ ($R^2 = 0.83$) -- (3.2)

The negative coefficients of the quadratic terms of process variables (Eq.3.2) indicated that flavour content increased with decrease of these variables. The variation of flavour with sugar, pulp and cumin powder were graphically presented in the 3-D surface plot and contour plots (Fig. 3.2a, 3.2b).

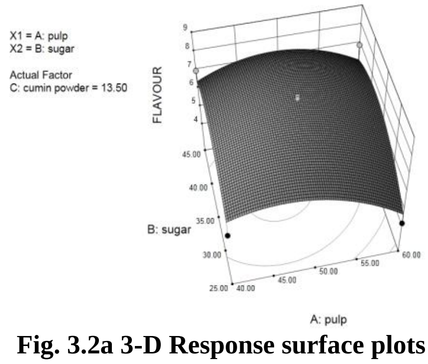

Fig. 3.2a 3-D Response surface plots

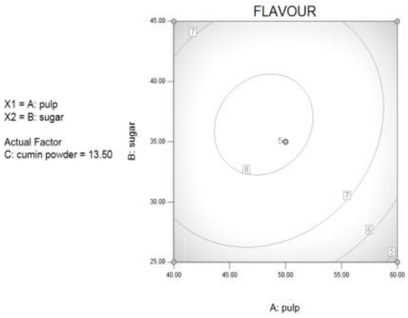

Fig. 3.2b Contour plots

TASTE

ANOVA (Table 3.1) indicated that the taste was highly significant at 5% level on quadratic term of pulp and sugar quantity. Not a single interaction term had any effect on the taste of the beverage. By neglecting the non significant terms in Eq. 2.1 and with the coded values of independent variables, the following equation (Eq.3.3) describes the effect of significant process variables on taste of the produced beverage.

Taste = $8.42 - 1.32 * X_1^2 - 1.41 * X_2^2$ **(**$R^2 = 0.72$**) – (3.3)**

The quadratic term of pulp quantity, sugar quantity (Eq. 4.7) indicated that taste increased with decrease of these variables. The variation of colour with pulp, sugar level were graphically presented in the 3-D surface plot and contour plots (Fig. 3.3a, 3.3b).

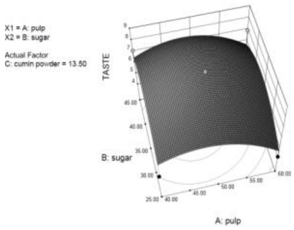

Fig.3.3a 3-D Response surface plots

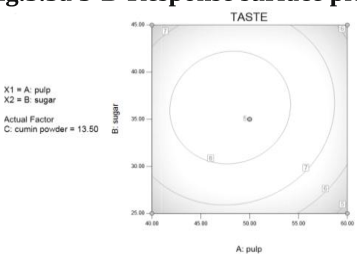

Fig.3.3b Contour plots

HCA CONTENT

ANOVA (Table 3.2) indicated that the HCA content was significant at 0.1% level on linear term of pulp quantity. Quadratic terms of process variables; pulp quantity, cumin powder quantities were having significant effect at 0.1%, 5% level on HCA content of the beverage. Interaction term also had the significant effect of 5% level on HCA content. By neglecting the non significant terms in Eq. 2.1 and with the coded values of independent variables, the following equation (Eq. 3.4) describes the effect of significant process variables on HCA content of the produced beverage. The positive coefficients of the linear term of pulp quantity and quadratic terms of pulp quantity and cumin powder (Eq. 3.4) indicated that HCA content increased with increase of this variable. While the interaction between sugar and cumin powder resulted in decrease of HCA content. The variation of HCA content with sugar, pulp and cumin powder were graphically presented in the 3-D surface plot and contour plots (Fig.3.4a, 3.4b).

HCA content = $0.84 + 0.15 * X_1 - 0.067 * X_1X_3 + 0.13 * X_1^2 + 0.066 * X_3^2$ **(**$R^2 = 0.95$**) -------------------------- (3.4)**

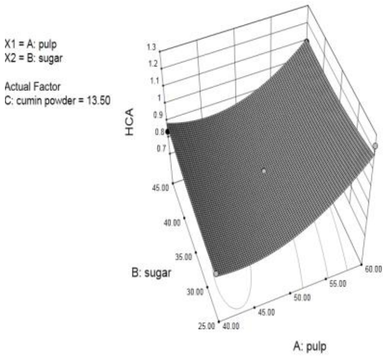

Fig. 3.4a 3-D Response surface plots

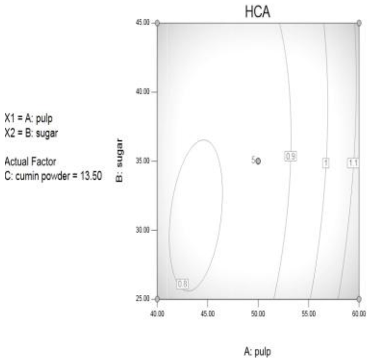

Fig.3.4b Contour plots

ANTHOCYANIN CONTENT

ANOVA (Table 3.2) indicated that the Anthocyanin content was significant at 0.1% level on linear term of pulp quantity while at 5% level on cumin powder. Quadratic terms of process variable; cumin powder quantity was having significant effect at 5% level on anthocyanin content of the beverage. No Interaction term also had any significant effect. By neglecting the non significant terms in Eq. 2.1 and with the coded values of independent variables, the following equation (Eq. 3.5) describes the effect of significant process variables on anthocyanin content of the produced beverage.

Anthocyanin content = $95.10 + 13.16 * X_1 - 4.19 * X_3 - 5.21 * X_3^2$ **(**$R^2 = 0.93$**) ---------------------------- (3.5)**

The positive coefficients of the linear term of pulp quantity Eq. 3.5) indicated that anthocyanin content increased with increase of this variable. While the negative coefficients of linear and quadratic term of cumin powder indicated that increase of these variables resulted in decrease of anthocyanin content. The variation of Anthocyanin content with sugar, pulp and cumin powder were graphically presented in the 3-D surface plot and contour plots (Fig. 3.5a, 3.5b).

Table 3.1 Regression coefficients and ANOVA of the second-order polynomial model for the response variables colour, flavour and taste (in coded units)

Run	df	Estimated Variables			F-Value			P value Prob.> F		
		Colour	Flavour	Taste	Colour	Flavour	Taste	Colour	Flavour	Taste
Model	9	8.20	8.13	8.42	9.18	3.83	3.46	0.0040	0.0451	0.0437
X_1	1	-0.17	-0.46	-0.54	6.31	2.88	2.38	0.0403*	0.1332	0.1573
X_2	1	-0.099	0.40	0.38	2.01	2.16	1.17	0.1992	0.1851	0.3085
X_3	1	250E003	-0.13	-0.44	8.05E003	0.24	1.54	0.9310	0.6361	0.2464
$X_1 X_2$	1	0.040	0.31	0.14	0.16	0.67	0.0801	0.6968	0.4415	0.7842
$X_1 X_3$	1	0.015	-0.097	0.17	0.023	0.064	0.052	0.8833	0.8079	0.6412
$X_2 X_3$	1	-0.017	-0.052	0.20	0.032	0.018	0.0853	0.8640	0.8975	0.7132
X_1^2	1	-0.40	-1.07	-1.32	18.12	8.04	7.41	0.0038**	0.0252*	0.0235*
X_2^2	1	-0.58	-1.18	-1.41	36.02	9.87	8.46	0.0005***	0.0163*	0.0173*
X_3^2	1	-0.34	-1.03	-0.58	12.36	7.56	1.44	0.0093**	0.0285*	0.2614
Lack - of- fit								0.0780	0.0675	0.0857
R^2		0.9291	0.8313	0.7290						
Pred. R^2		0.8215	0.7458	0.6750						

*** Significant at 0.001 level, ** Significant at 0.01 level; * Significant at 0.05 level

Table 3.2 - Regression coefficients and ANOVA of the second-order polynomial model for the response variables HCA, Anthocyanin (in coded units)

Variables	df	Estimated variables		F values		P value Prob. > F	
		HCA content	Anthocyanin content	HCA content	Anthocyanin content	HCA content	Anthocyanin content
Model	9	0.84	95.10	17.90	10.55	0.0005	0.026
X_1	1	0.15	13.16	91.60	76.85	0.0001***	0.0001***
X_2	1	1.250E-003	2.30	6.361E-003	2.35	0.9387	0.1694
X_3	1	-0.019	-4.19	1.43	7.78	0.2705	0.0269*
$X_1 X_2$	1	-0.038	-1.25	2.86	0.35	0.1345	0.5746
X_1X_3	1	-0.067	-0.025	9.27	1.386E-004	0.0187*	0.9909
X_2X_3	1	0.035	1.65	2.49	0.60	0.1583	0.4626
X_1^2	1	0.13	0.24	38.19	0.013	0.0005***	0.9119
X_2^2	1	0.031	-1.44	2.06	0.48	0.1944	0.5097
X_3^2	1	0.066	-5.21	9.33	6.34	0.0184*	0.0399*
Lack-of-fit						0.0671	0.0875
R^2		09584	0.9313				
Pred. R^2		0.9048	0.8731				

***Significant at 0.001 level; **Significant at 0.01 level; *Significant at 0.05 level

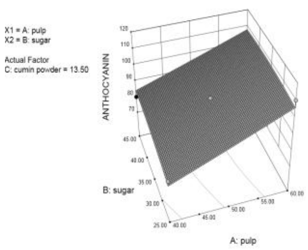

Fig. 3.5a 3-D Response surface plots

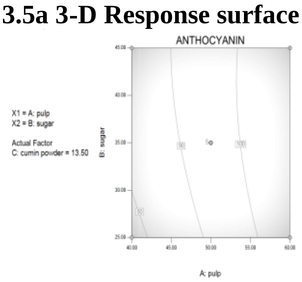

Fig. 3.5b Contour plots

NUMERICAL OPTIMIZATION

Figs. 3.6- 3.10 show the optimum levels of the process parameters to obtain the beverage with maximum colour, flavour, taste score and HCA, Anthocyanin content, respectively by numerical optimization. The contours indicate that when the beverage was prepared with pulp, sugar, cumin powder; 57.30g, 35.52g and 13.5g respectively gives the predicted value of maximum colour score 7.84, flavour score7.25, taste score7.34, HCA content 1.01 g/100ml and Anthocyanin content 104.89 mg/100ml.

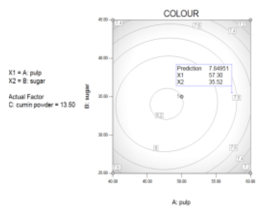

Fig. 3.6 Contour plot for colour

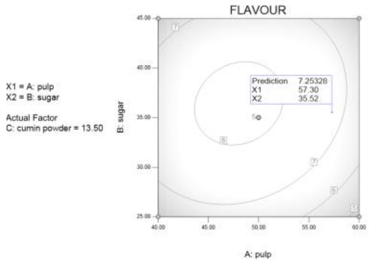

Fig. 3.7 Contour plot for flavour

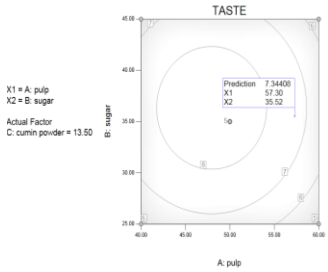

Fig. 3.8 Contour plot for Taste

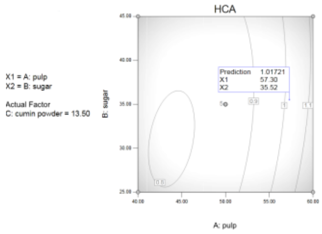

Fig.3.9 Contour plot for HCA

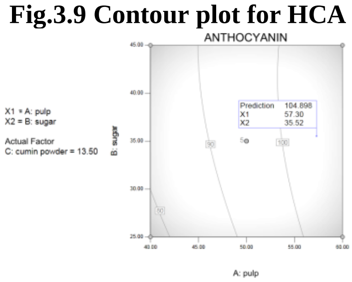

Fig.3.10 Contour plot for Anthocyanin

OPTIMIZED INGREDIENTS LEVEL FOR KOKUM RTS BEVERAGE PRODUCTION

In graphical optimization overlay plot (Fig. 3.11) was obtained by applying superimposing surface methodology to contour plots of the response variables to select optimum level of ingredients. The optimum level of ingredients for pulp, sugar, cumin powder; 57.30g, 35.52g and 13.5g respectively were obtained from the overlay plot.

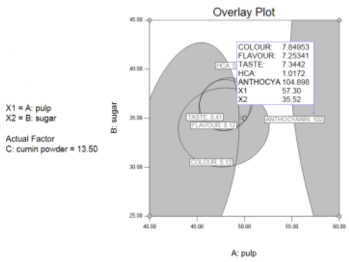

Fig. 3.11 Optimum ingredients level for kokum RTS beverage formulation

CONCLUSIONS

The different levels of ingredients preparation of kokum RTS beverage showed that all these variables markedly affect colour, flavour, taste, HCA content and nthocyanin content of the prepared beverage. These can be related to the ingredients levels by using second order polynomials. Using the contour plots, the optimum set of the ingredients are obtained graphically for the production of kokum RTS beverage. The optimized ingredients level achieved after the numerical and Graphical optimization for maximum colour, flavour , taste score, HCA content and anthocyanin content were, pulp, sugar, cumin powder; 57.30g, 35.52g and 13.5g respectively. The desirability of 0.859 was achieved at this optimum point. The predicted values of colour, flavour, taste, HCA content and anthocyanin content were 7.84, 7.25, 7.34, 1.01 g/100ml, 14.89mg/100ml respectively, at optimum ingredients level.

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