RESEARCH ARTICLE OPEN ACCESS

Kokum Fruit Bar Development via Response Surface Methodology (RSM).

Pritam Bafna*, Mrs. N. Manimehalai
Fruit and Vegetable Laboratory, Department of Food and Process Engineering, SRM University, Kattankulathur, India

Abstract
A response surface methodology (RSM) was used for the determination of optimum ingredients level to prepare kokum fruit bar. 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 texture, overall acceptability and calcium content of the prepared fruit bar were studied by employing a Box- Behnken Design (BBD). During the experimental trials the pulp quantity is kept fixed. The coefficient of determination R^2 for texture, overall acceptability and calcium content were 0.8298, 0.9239 and 0.9842 respectively. Analysis of variable (ANOVA) performed on the experimental values showed that sugar and milk powder were the most important factors that affected characteristics of the kokum fruit bar 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 fruit bar were pulp, sugar, milk powder; 50g, 40g and 9.39g respectively.
Key words: Kokum, Fruit Bar, Response Surface Methodology, Box- Behnken Design, Calcium, Optimization

I. 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 the adjoining 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. 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, Rastogiet 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 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. This curry is used with rice or like an after meal digestive drinks. Both birindi saar and kokum kadi are supposed to be digestive and to relieve gastric problems. As kokum is a highly under-utilized fruit, it can be preserved by making it as a processed food to use it during off-season. (Krishnamurthy, Ravindranath, B. 1982)

1.2 Objective of Research*:*

Perishable nature of kokum and unavailability of the preserved product in the market gave rise to this research. This study was planned keeping in view the nutritional importance of kokum and to utilize it by preserving as Fruit bar. This kokum fruit bar provides both nutritional and phytochemical benefits to human population. Also its industrial application will help to catch worldwide consumers of kokum.

II. Materials and Methods

2.1 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.

2.2 Methodology

Fruit bar was prepared by drying the boiled kokum pulp with addition of sufficient quality of sugar and other ingredients. The process involves in fruit bar preparation is shown in Fig 1.

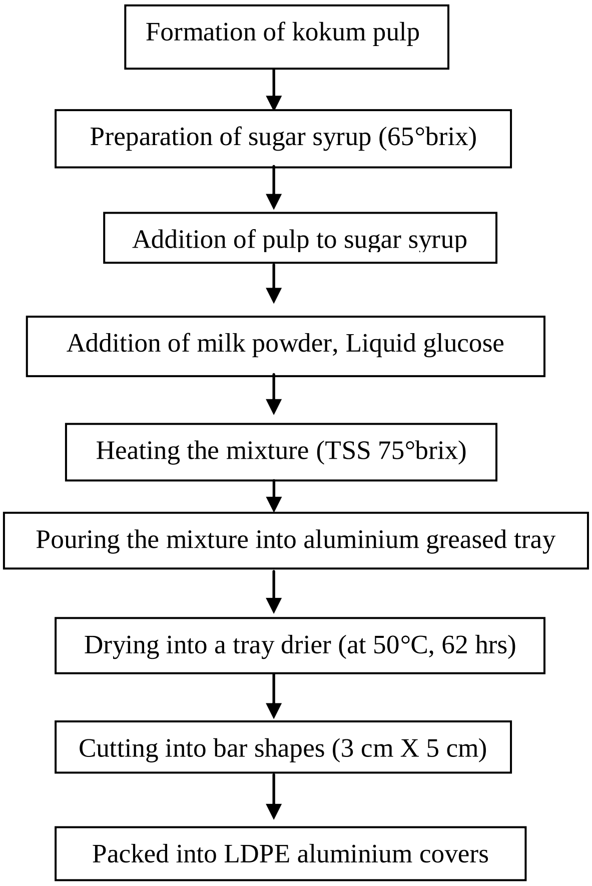


Fig 1.Process of making Kokum Fruit Bar.

2.2.1 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.

2.2.2 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.

2.3 Experimental Design Layout

The levels of ingredients were selected based on basic trials. By keeping the pulp quantity constant as 50gmthe independent variables considered were: Sugar quantity (40-60g) and milk powder quantity (5.0-15.0g). A two -variable (three levels of each variable) 3-level factorial design and a response surface methodology (RSM) were used to understand the interactions of sugar and milk powder quantity on the texture overall acceptability and calcium content of the prepared fruit bar in 13 runs (Table2.2), of which 4 were centre edge, four were factorial, and five were at centre point.

Table 2.1 - Processing ingredients and their levels

Ingredients	Levels
Sugar (g)	40, 50, 60
Milk powder (g)	5, 10, 15

2.4 Fruit Bar Development Trials

The fruit bar development trials were carried out and the responses observed also shown in the table 2.2

2.4Determination of Sensory Parameters

The sensory parameters of the prepared fruit bar samples like texture and overall acceptability 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.

Table 2.2 - Effect of process variables on texture, overall acceptability and calcium content of prepared kokum fruit bar.

Run	Factor 1 Sugar (g) X_1 (x_1)	Factor 2 Milk powder (g) X_2 (x_2)	Response 1 Texture	Response 2 Overall Acceptability (OA)	Response 3 Ca content (mg/100g)
1	40 (-1)	10 (0)	8.52	8.69	94
2	50 (0)	10 (0)	8.34	8.5	92
3	60 (+1)	10 (0)	8.17	8.27	93
4	50 (0)	10 (0)	8.32	8.43	92
5	50 (0)	10 (0)	8.3	8.5	93
6	40 (-1)	5 (-1)	8.1	8.32	84
7	60 (+1)	5 (-1)	7.27	7.86	85
8	60 (+1)	15 (+1)	8.36	8.38	105
9	50 (0)	10 (0)	8.37	8.41	92

10	50 (0)	10 (0)	8.28	8.37	93
11	40 (-1)	15 (+1)	7.85	8.46	104
12	50 (0)	15 (+1)	8.47	8.23	105
13	50 (0)	5 (-1)	8.3	8.12	84

Liquid glucose = 2g

x represents the coded level of variables; X represents the actual level of variables.

2.5 Calcium Content Determination

Calcium is determined as calcium pectate. Pectin extracted from plant material is saponified with alkali and precipitated as calcium pectate from an acid solution by addition of calcium chloride. The calcium pectate precipitate is washed until free from chloride, dried and weighed.

2.6 Statistical Analysis for Optimization of Ingredients Quantity

The response functions y (dependent variables) were sugar quantity and milk powder quantity. The following second-order polynomial model was fitted to the dependent variables with the experimental data (Eq.1.1)

$$\mathbf{y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_{12} + b_{12} X_1^2 + b_{22} X_2^2} \quad \textbf{(1.1)}$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1 and b_2 (linear effects), b_{11} and b_{22}(quadratic effects), and b_{12} (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 0.001, 0.01 or0.05 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, USA.

2.7Optimization

Numerical and graphical optimization was carried out for the independent variables to obtain the fruit bar with Maximum texture, Overall acceptability scores and calcium 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 kokum pulp sugar, milk powder quantity for the production of kokum fruit bar.

III. Results and Discussions

3.1 Statistical Analysis

Table 3.1, 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 texture, overall acceptability and calcium content were 0.8298, 0.9239 and 0.9842 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).

Table 3.1 Regression coefficients and ANOVA of the second-order polynomial model for the response variables texture, OA and calcium content (in coded units).

Run	**df**	**Estimated Variables**			**F- values**			**P value Prob. > F**		
		Texture	OA	Ca cont	Texture	OA	Ca cont	Texture	OA	Ca cont
Model	5	8.39	8.44	92.55	6.83	17.01	238.88	0.0128	0.0009	0.0001
X_1	1	-0.11	-0.16	0.17	2.39	28.35	0.32	0.1661	0.0011**	0.5920
X_2	1	0.17	0.13	10.17	5.43	18.24	1172.92	0.0526	0.0037**	0.0001***
$X_1 X_2$	1	0.34	0.095	0.001	14.33	6.66	0.001	0.0068 *	0.0364*	0.9875
X_1^2	1	-0.22	0.058	0.57	4.45	1.73	1.69	0.0728	0.2297	0.23465
X_2^2	1	-0.18	-0.25	1.57	3.01	31.03	12.86	0.01256	0.0008***	0.0089**
Lack-of-fit								0.0786	0.1954	0.1743
R^2		0.8298	0.9239	0.9842						
Pred. R^2		0.7883	0.8696	0.9616						

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

3.2 Effect of Ingredients on Response Variables

3.2.1 Texture

ANOVA (Table 3.1) indicated that the texture was highly significant at 1% level on interaction term of Sugar and milk powder quantity. Quadratic terms of process variables were not having any significant effect. By neglecting the non significant terms in Eq. 1.1and with the coded values of independent variables, the following equation (Eq. 1.2) describes the effect of significant process variables on texture of the produced fruit bar.

(1.2) **Texture = 8.39 + 0.34 * X_1X_2(R^2 = 0.82)**

Where, $X_1$is the sugar quantity in grams; $X_2$is the milk powder quantity. The interaction term of pulp quantity, (Eq. 1.2) indicated that colour increased with increase of this variable. The quadratic terms suggested that increase of these variables resulted in increase 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, 3.2b).

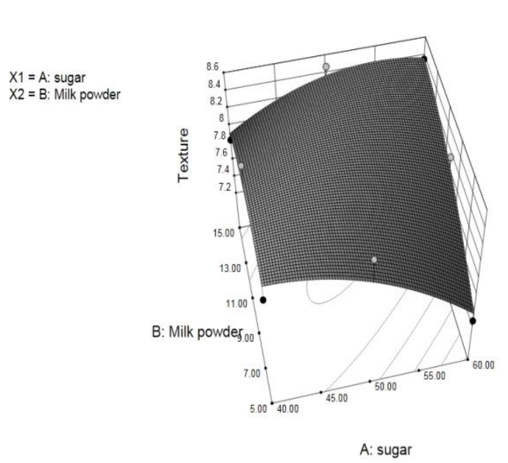


Fig. 3.1a 3-D Response surface plots

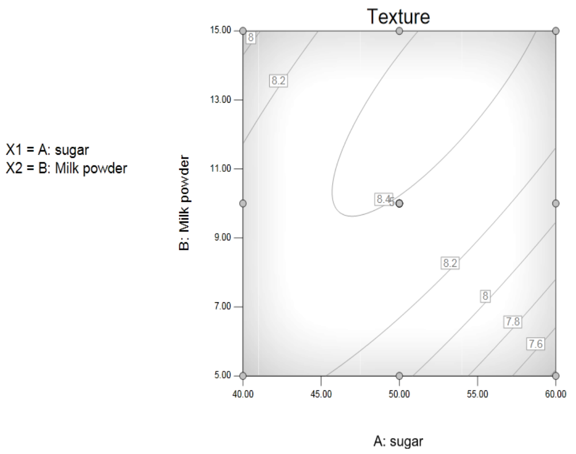


Fig. 3.2b Contour plots

Showing the effect of sugar, milk powder quantity on texture of fruit bar

3.2.2Overall Acceptability

ANOVA (Table 3.1) indicated that the Anthocyanin content was significant at 1% level on linear terms of sugar quantity and milk powder quantity. Quadratic terms of process variable; milk powder quantity was having significant effect at 0.1% level on overall acceptability of the fruit bar. Interaction term also had the significant effect of 5% level on overall acceptability. By neglecting the non significant terms in Eq. 1.1and with the coded values of independent variables, the following equation (Eq. 1.3) describes the effect of significant process variables on overall acceptability of the produced fruit bar.

(1.3) **O.A. = 8.44 - 0.16 * X_1+ 0.13 * X_2 + 0.095 * X_1X_2 – 0.25 * X_2^2 (R^2 = 0.92)**

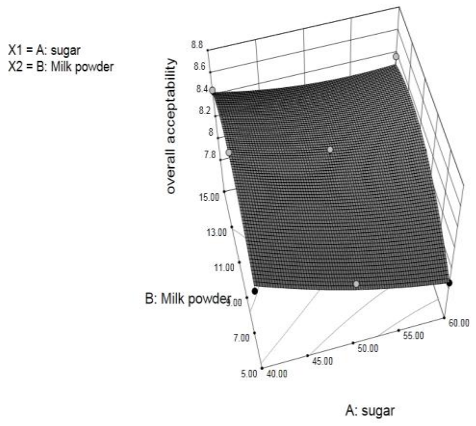
The positive coefficients of the linear term of milk powder quantity (Eq. 1.3) indicated that overall acceptability content increased with increase of milk powder. The quadratic terms suggested that increase of this variable resulted in decrease of overall acceptability. The interaction term showed that increase in the term causes increase of overall acceptability. The variation of Overall acceptability with sugar and milk powder were graphically presented in the 3-D surface plot and contour plots (Fig. 3.3a, 3.4b).

3.2.3 Calcium Content

ANOVA (Table 3.1) indicated that the calcium content was significant at 0.1% level on linear term of milk powder quantity. Quadratic terms of process variable; milk powder quantity was having significant effect at 5% level on calcium content. No Interaction term also had any significant effect. By neglecting the non significant terms in Eq. 1.1and with the coded values of independent variables, the following equation (Eq. 1.4) describes the effect of significant process variables on calcium content of the produced fruit bar.

(1.4) **Calcium content = 92.55 +10.17 * X_2 + 1.57 * X_2^2(R^2 = 0.98)**

The positive coefficients of the linear term of process variables (Eq. 1.4) indicated that calcium content increased with increase of this variable. The quadratic terms suggested that increase of milk powder resulted in increase of calcium content. The variation of calcium content with sugar and milk powder were graphically presented in the 3-D surface plot and contour plots (Fig. 3.5a, 3.6b)



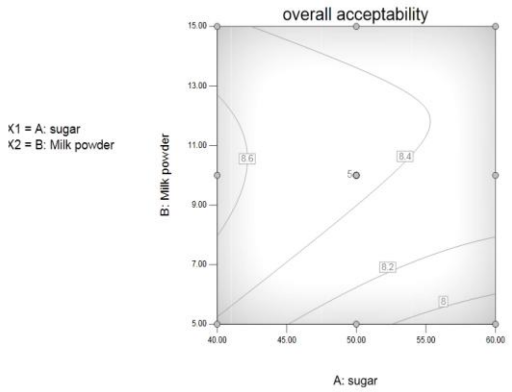
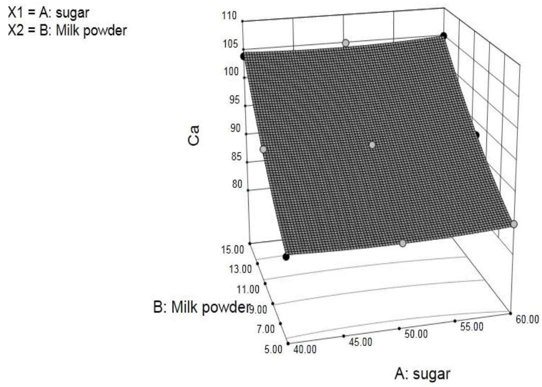


Fig. 3.3a 3-D Response surface plots Fig. 3.4b Contour plots
Showing the effect of sugar, milk powder quantity on overall acceptability of fruit bar



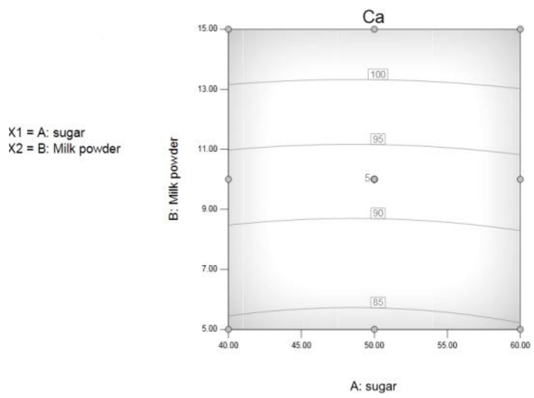


Fig. 3.5a 3-D Response surface plots Fig. 3.6b Contour plots
Showing the effect of sugar, milk powder quantity on calcium content of fruit bar

3.3 Numerical Optimization

Figs. 3.7 – 3.9show the optimum levels of the process parameters to obtain the fruit bar with maximum texture, overall acceptability score and calcium content, respectively by numerical optimization. The contours indicate that when the fruit bar was prepared with pulp, sugar, milk powder; 50g, 40g and 9.39g respectively gives the predicted value of maximum texture score 8.29, overall acceptability score 8.68 and calcium content 91.73mg/100g.

.

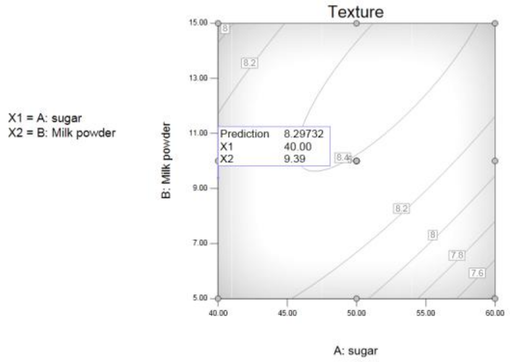


Fig. 3.7 Contour plot for Texture

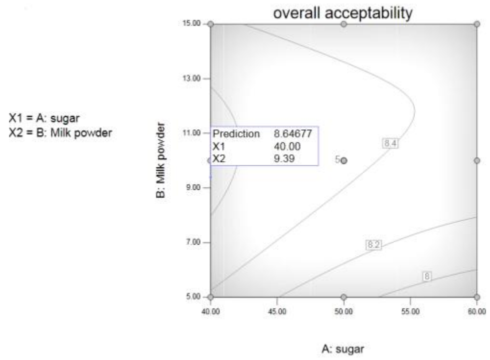


Fig.3.8 Contour plot for overall acceptability

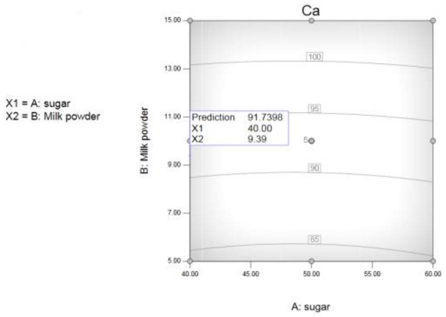


Fig. 3.9 Contour plot for calcium content

3.4 Optimized Ingredients Level for Kokum Fruit Bar Production

In graphical optimization overlay plot (Fig. 3.10) was obtained by applying superimposing surface methodology to contour plots of the response variables to select optimum levels of ingredients. The optimum was obtained at pulp, sugar, milk powder; 50g, 40g and 9.39g respectively from the overlay plot.

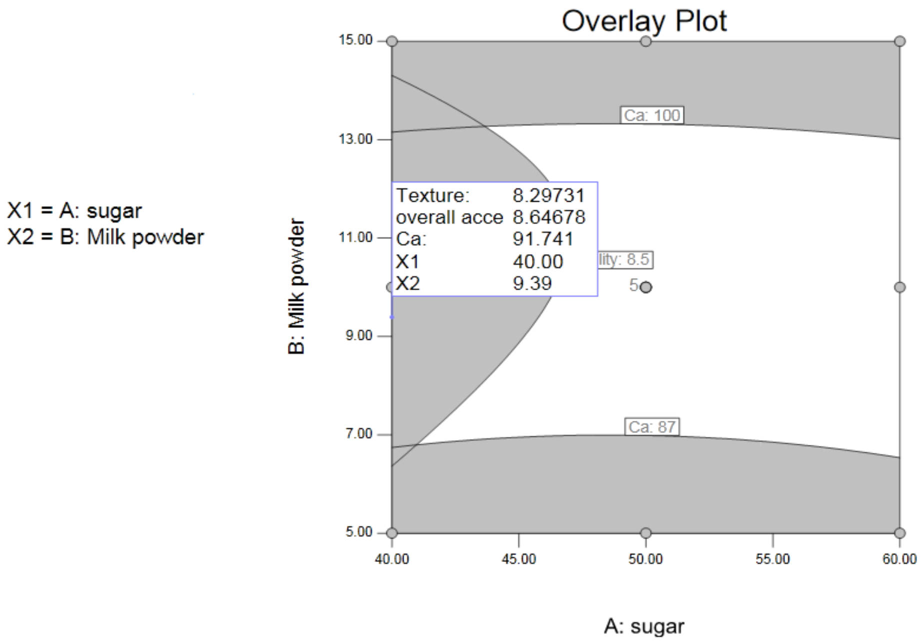


Fig. 3.10 Optimium ingredients level for kokum fruit bar poroduction

IV. Conclusions

The different levels of ingredients for preparation of kokum fruit bar showed that all these variables markedly affect texture, overall acceptability score and calcium content of the prepared fruit bar. 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 fruit bar. The optimized ingredients level achieved after the numerical and Graphical optimization for maximum texture, overall acceptability score and calcium content were, pulp, sugar, milk powder; 50g, 40g and 9.39g respectively. The desirability of 0.87 was achieved at this optimum point. The predicted values of texture, overall acceptability score and calcium content were 8.29, 8.68, 91.73mg/100g respectively, at optimum ingredients level.

References

[1] Agarwal, G. and S. Mangaraj, (2005). Studies on physico-chemical changes in selected fruits during storage, Beverage and Food World, 32(11): 72-75.

[2] Anon, D., Sunil Sachdeva and S. Singh, (1995). Methods of determination of sensory quality of foods. J. Food Science and Technology, 32(7): 67-68.

[3] Anonymous, (1996). Adapted from Herald of health, Fruits - Natures Tonic, Nutrition, pp: 56-57.

[4] Aruna, K., R. Dhanalakshmi and V. Vimala, (1998). Development and storage stability of Cereal-based papaya powder. J. Food Science and Technology, 35(3): 250-254.

[5] Ashwah, E.I., F.A. Abd, N.M. Baki, S.K. Samahy, E.I. Abd and M.H. Fadeel, (1982). Effect of storage on the characteristics of the concentrated orange and lime juice. Agricultural Research Review, 38(3): 275-288.

[6] Bhattacharyya, B.K. and D. Bhattacharjee, (2007). Bactriocin: A biological food preservative, J. Food Science and Technology, 44(5): 459-464.

[7] Chandrajit Singh, G.S. Poonia and M.S. Toor, (1994).Distribution pattern of fruit processing industry in Punjab. Indian Food Packers, Jan-Feb., XL VIII(1): 47.

[8] Das, A.K., (1991). Policy issues on processed food industry. Indian Food Industry, 10(1): 14-25.

[9] Food Science and Technology, (1999). Use of ozone to improve the safety of fresh fruits and vegetables, 53(10): 58-61.

[10] Graumlich, T.R., (1981). Survival and recovery of thermally stressed Yeast in Orange juice. J. Food Science, 46(5): 1410-1411.

[11] Jain, M.S. and G. Agawal, (2005). Studies on Physico-chemical changes in selected fruits during storage, Beverage and Food World, 32(11): 72-75.

[12] Jayaprakasha, G. K., &Sakariah, K. K. (2002). Determination of organic acids in leaves and rinds of Garcinia indica (Desr.) by LC. Journal of Pharmaceutical and Biomedical Analysis, 28, 379-384.

[13] Johnson, M. and M. Hessel, (1982). Stability of Ascorbic acid in ready to drink juices.Varfoda, 34(5): 267-279.

[14] Kalra, S.K. and D.K. Tandon, (1985). Physico-chemical changes in mango pulp during ambient storage in glass containers, J. Food Science and Technology, 22: 343-345.

[15] Krishnamurthy, N., Lewis, Y. S., &Ravindranath, B. (1982). Chemical constituents of Kokum fruit rind. Journal of Food Science and Technology, 19(3), 97-100.

[16] Maheshwari, B., & Reddy, S. Y. (2005). Application of kokum (Garcinia indica) fat as cocoa butter improver in chocolate. Journal of the Science of Food and Agriculture, 85, 135-140.

[17] Nayak, C. A., Rastogi, N. K., &Raghavarao, K. S. M. S. (2010). Bioactive constituents present in Garcinia indica Choisy and its potential food applications: A review. International Journal of Food Properties, 13, 441-453.

[18] Otto, K., (1984) Minimum shelf life of fruit juices. Flurssigesobst, FRG, 51(11): 574-580.

[19] Pritam G. Bafna, (2012). Optimization of Process Parameters for Extraction of Kokum (Garcinia Indica) Fruit Pulp using Response Surface Methodology (RSM) , International Journal of Scientific & Engineering Research, 3 (8), 1-7

[20] Ranganna, S., (1986). Sensory evaluation General Instruction for microbiological examination. Hand book of Analysis and the quality control for fruits and vegetable products. II edition, Tata McGraw Hill Publishing Company Ltd., New Delhi, pp: 670-686.

[21] Rao, M.A. Mir and N. Nirankar(1993). Storage changes in Fortified Mango Bars. J. Food Science and Technology, 30(4): 279-282.

[22] Reimer, T. and M. Karel, (1978). Shelf life studies of Vitamin C during food storage. Chemistry Abstracts, 89(13): 1061-66.

[23] Sandhu, K.S., K.S. Bhatia and F.G. Shukla, (1998). Physico chemical changes during storage of kinnowmandarian, orange and pineapple juice concentrates. Food Science and Technology, 22: 343-345.

[24] Saxena, J.S. and J.K. Afanam, (1980). Studies on the determination of optimum conditions of preservation of fresh vegetables in solidified sulphited brine, Indian Food Packer, 34(6):9.

[25] Sindhu, S., K. Bhumbla and C. Joshi, (1984). Preservation of Tomato juice under acid condition. J. Food science and Agriculture, 35: 345-352.

[26] Singh Jashkaran, A.K. Singh and H.K. Singh, (2005). Studies on physico-chemical characteristics of pomegranate (punicagranatum L.) fruit, Beverage and Food World, 32(5): 42-43.

[27] Sudheer, K.P. and S.K. Dash, (1999). Osmo-solar dehydration of Fruits and Vegetables- An Overview, Indian Food Packers, 53(3): 28-35.

[28] 28. Tripathi, A.R., L.S. Diwate, Kute and J.K. Chavan, (2004) Beverage and FoodWorld, Preparation of Toffees from Papaya pulp. 31(9): 65-66.