Optimization of Process Parameters for Extraction of Kokum (*Garcinia Indica)* Fruit Pulp using Response Surface Methodology (RSM)

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Abstract – A response surface methodology (RSM) was used for the determination of optimum water temperature and soaking time to produce an acceptable smooth kokum pulp. Kokum pulp was extracted using water extraction method at different temperature (13-33°C) and time (10-50 min.) The effects of the extraction conditions on pulp recovery and HCA (Hydroxy citric acid) content were studying by employing a Central Composite Rotatable Design (CCRD). The coefficient of determination R for pulp recovery and HCA content were 0.94 and 0.86 respectively. Analysis of variable (ANOVA) performed on the experimental values showed that temperature and soaking time were the most important factors that affected characteristics of the kokum pulp as it exerted highly significant influence ($p < 0.05$) on all the dependant variables. An increase in soaking time and water temperature used for extraction of pulp resulted increase in pulp recovery and HCA content of the kokum pulp but to a certain level. Based on surface and contour plots optimum conditions for formation of smooth kokum pulp were 24.97°C temperature of water and soaking time of 30.42 min.

Keywords – Kokum, Response Surface Methodology, Central Composite Rotatable Design, Pulp recovery, Hydroxy citric acid, Optimization

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1. 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)

For production of clarified sapodilla juice, hot water extraction is a preferred method as the method maximizes juice yield, colour and flavour extracted (McLellan1996). Luh and Wood roof (1975) reported that the hot water extraction method could also inactivate enzymes in the juice. RSM has been used extensively for optimizing processes in the tropical fruit juice production (Lee et al., 2006; Sin et al., 2006; Wong, Yusof, Mohd Ghazali, & Che Man, 2003; Yusof, Talib, Mohamed, & Bakar, 1988). As kokum is an under-utilized fruit, it can be preserved by making it as a pulp to use it during off-season. Hence the present study aims to use RSM as a tool for optimizing the processing conditions (extraction temperature and time) for obtaining maximum pulp recovery and HCA content from dried kokum rind. These conditions will serves as a preliminary basis for conducting further studies on development of preserved products from kokum fruit.

2. Materials and methods

2.1 Collection of Fruits

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

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 different temperature for various periods of time (Table 1). 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. The smooth pulp was then pasteurized at 60°C for 10 min before packing into the aluminium covers.

Table 1. Process Parameters and their levels

Parameters	Levels
Water Temperature (°C)	13, 18, 23, 28, 33
Soaking time (Min)	10, 20, 30, 40, 50

2.3 Grinding device

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.

2.4 Extraction Trials

To determine the effect of water temperature used for soaking and soaking time on Pulp Recovery (PR), Hydroxy Citric Acid content (HCA), Central Composite Rotatable Design (CCRD) was used. The extraction trials were carried out as shown in the table2.

2.5 Determination of pulp Recovery

The percentage of pulp recovery (%w/w) is calculated (Mamiro peter, Fweja Leonard; 2005) as the weight of smooth pulp divided by weight of whole rinds.

$$\text{Pulp Recovery} = \frac{\text{Weight of smooth pulp after straining}}{\text{Weight of whole rinds taken}} \times 100$$

2.6 HCA determination

HCA (Hydroxy Citric Acid) content was determined by titeration 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).

Table- 2. Effect of process variables on PR. and HCA of extracted kokum pulp.

Run	Factor1 Water Temp (°C) $X_1(x_1)$	Factor 2 Soaking time (Min) X_2 (x_2)	Response 1 Pulp Recovery (%)	Response 2 HCA (g/100g)
1	15.93 (-2)	30 (0)	75.43	10.13
2	23 (0)	30 (0)	87.71	12.6
3	23 (0)	15.86 (-2)	70.17	9.81
4	23 (0)	30 (0)	91.22	13.2
5	23 (0)	44.14 (+2)	73.68	10.21
6	23 (0)	30 (0)	94.73	15.2
7	28 (+1)	40 (+1)	80.7	11.69
8	23 (0)	30 (0)	94.73	15.18
9	18 (-1)	20 (-1)	66.66	8.91
10	28 (+1)	20 (-1)	80.7	11.76
11	30.07 (+2)	30 (0)	91.22	13.22
12	18 (-1)	40 (+1)	82.45	12.12
13	23 (0)	30 (0)	92.98	14.56

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

2.7. Experimental design and statistical analysis

The ranges of experimental parameters were selected based on preliminary trials. The independent variables considered were: Water Temperature (13-33°C), Soaking time (10-50 min). A two-variable (five levels of each variable) CCRD and a response surface methodology (RSM) were used to understand the interactions of Water temperature, soaking time on the quality of pulp recovery and HCA in 13 runs, of which 4 were factorial, four were axial, and five were at centre point (Montgomery 2001). The response functions y (dependent variables) were pulp recovery (PR) and Hydoxy Citric Acid (HCA) content. The following second-order polynomial model

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2$$

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 statistically by computing the P value (Prob.> F) at 0.1%, 1% and 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.

2.8 Optimization

Numerical and graphical optimization was carried out for the independent variables to obtain the pulp with Maximum recovery and maximum HCA content using Design-Expert software. Conventional graphical method was applied to obtain Maximum recovery and maximum HCA. Predictive models were used to graphically represent the systems. Contour plots of the response variables were utilized to select optimum combinations of water temperature and soaking time for the production of kokum fruit pulp.

was fitted to the dependent variables with the experimental data (Eq.1).

3. Results and discussion

3.1 Statistical analysis

Table 3 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 Pulp recovery and HCA content were 0.94 and 0.86, respectively. The closer the value of R^2 to unity, the better the empirical models fit the actual data. (W.C. Lee; S. Yusof 2005) 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).

$$\text{Pulp Recovery} = +92.27 + 4.33 * X_1 + 2.59 * X_2 - 3.95 * X_1X_2 - 4.47 * X_1^2 - 10.17 * X_2^2 \ (R^2 = 0.94) \quad (2)$$

Table- 3. Regression coefficients and ANOVA of the second-order polynomial model for the response variables

Variables	DF	Estimated variables		F values		P value Prob. > F	
		Pulp Recovery	HCA	Pulp Recovery	HCA	Pulp Recovery	HCA content
Model	14	92.27	14.15	23.94	8.64	0.0003***	0.0066**
X_1	1	4.33	0.85	16.98	5.69	0.0045**	0.0486*
X_2	1	2.59	0.46	6.10	1.69	0.0428*	0.2343
$X_1 X_2$	1	-3.95	-0.82	7.07	2.65	0.0326*	0.1473
X_1^2	1	-4.47	-1.17	15.78	9.35	0.0054**	0.0184*
X_2^2	1	-10.17	-2.0	81.62	27.45	< 0.0001***	0.0012**
Lack-of-fit						0.4612	0.7959
R^2		0.944	0.860				
Pred. R^2		0.778	0.62				

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

3.2 Effect of grinding on Process Variables

3.2.1 Pulp Recovery

PR is commonly used to calculate the yield. Pulp recovery varied from 66.66 to 94.73% (Table 2). ANOVA (Table 3) indicated that the PR was significant at 1% and 5% level on linear terms of water temperature, soaking time respectively. Interaction term and quadratic terms of process variables are having significant effect at 5%, 1% level respectively. By neglecting the non significant terms in Eq. 1 and with the coded values of independent variables, the following equation (Eq. 2) describes the effect of significant process variables on pulp recovery of the produced pulp.

Where, X_1 is the water temperature in °C; X_2 is the soaking time in min. The positive coefficients of the first-order terms of water temperature, soaking time (Eq. 2) indicated that pulp recovery increased with increase of these variables. The quadratic terms suggested that excessive increase of these variables resulted in decrease of PR.

The variation of PR with water temperature and soaking time are graphically presented in the 3-D surface plot and contour plots (Fig. 1a, 1b). As the Temperature of water and soaking time were increased, the pulp recovery increased significantly more the pulp recovery indicates that more amount of smooth pulp was produced. (Fig. 1b)

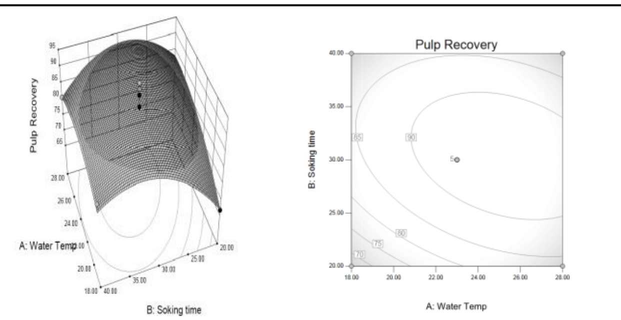

Fig.1a 3-D Response surface plots Fig.1b Contour plots

Showing the effect of soaking time and water temperature on pulp recovery

3.2.2 HCA content

HCA content is one of the important parameter of kokum fruit which is commonly used to indicate the acidity of the pulp. HCA content varied from 8.91 to 15.20 g/100g (Table 2). ANOVA (Table 3) indicated that HCA content was significant at 5% level on linear terms of water temperature. Interaction term is not having any effect and quadratic terms of process variables are having significant effect at 1% level. By neglecting the non significant terms in Eq. 1 and with the coded values of independent variables, the following equation (Eq. 3) describes the effect of significant process variables on HCA content of the produced pulp.

$$HCA = +14.15 + 0.85 * X_1 - 1.17 * X_1^2 - 2.00 * X_2^2 \quad (R^2 = 0.86)$$

Where, X_1 is the water temperature in °C; X_2 is the soaking time in min. The positive coefficients of the first-order terms of water temperature, soaking time (Eq. 3) indicated that HCA content increased with increase of these variables. But this increase in HCA content is to a certain level (23°C; 30 min) after which further increment in process variables shows the declination of HCA content of the pulp (Table 2). The quadratic terms suggested that excessive increase of these variables resulted in decrease of HCA.

The variation of HCA with Water temperature and soaking time are graphically presented in the 3-D surface plot and contour plots (Fig. 2a, 2b). As the Temperature of water and soaking time were increased, the HCA increased significantly. More the HCA content indicates that more the acidity of the pulp. (Fig. 2b)

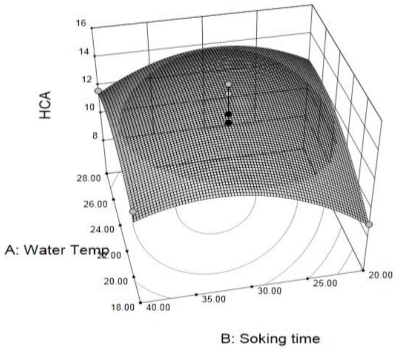

Fig.2a 3-D Response surface plots

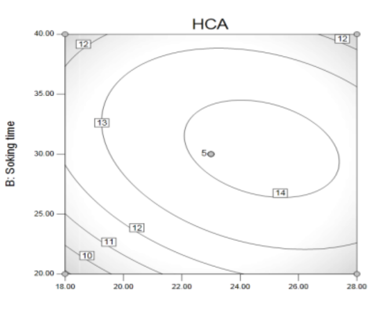

Fig.2b Contour plots

Showing the effect of soaking time water temperature on HCA content

3.3. Optimization

Figs. 3-4 show the optimum conditions of the extraction process to yield maximum pulp recovery, HCA content, respectively by numerical optimization. Fig. 3 shows contour plots for the response pulp recovery. The contours indicate that when the pulp was extracted with water at a temperature 24.97°C and soaking time of 30.42 min gives the predicted value of maximum pulp recovery which is 93.31%. Fig. 4 shows contour plots for the response HCA content. The contours indicate that when the pulp was extracted with water at a temperature 24.97°C and soaking time of 30.42 min gives the predicted value of maximum HCA content which is 14.30g/100g.

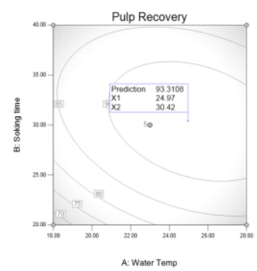

Fig.3 Contour plot for Pulp Recovery

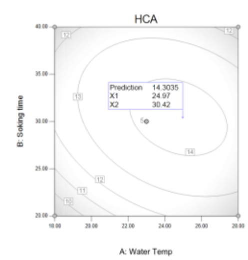

Fig.4 Contour plot for HCA content

Overall Pulp Recovery and HCA content increased with higher temperature and longer extraction time (Figs. 3–4, Table 2) up to a certain level. In graphical optimization overlay plot (Fig. 5) was obtained by applying superimposing surface methodology to contour plots of the response variables to select optimum combination of water temperature and soaking time for the production of smooth kokum pulp. The optimum was obtained at 24.97°C for 30.42 min which gave Pulp Recovery (93.31%), HCA content (14.30g/100g).

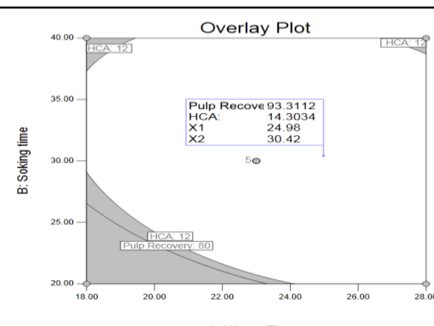

Fig. 5 Optimum conditions for kokum pulp extraction

4. Conclusions

Soaking, which is the main process in extraction of fruit pulp, was optimized with maximum PR and HCA content so that the smooth pulp obtained having more yield along with more HCA content. The different extraction time and temperature for extraction of kokum pulp showed that all these variables markedly affect the PR and the HCA content of the smooth kokum pulp. These can be related to the extraction conditions by using second order polynomials. Using the contour plots, the optimum set of the operating variables are obtained graphically in order to obtain the desired levels of these properties of the smooth kokum pulp which is suitable for the further preserved product development. The optimum conditions achieved after the numerical and Graphical optimization for maximum pulp recovery and HCA content were, water temperature (24.97°C) and soaking time (30.42 min). The desirability of 0.807 was achieved at this optimum point. The predicted values of pulp recovery and HCA content were 93.311% and 14.303 g/100g respectively, at optimum process conditions while the experimental values were 92.15% and 14.50g/100g respectively.

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