Journal of Agricultural Engineering and Food Technology
p-ISSN: 2350-0085; e-ISSN: 2350-0263; Volume 2, Issue 3; July-September, 2015 pp. 155-161

http://www.krishisanskriti.org/Publication.html

Optimization of Recipe for Preparation of Papaya Ready-To-Serve Drink using Response Surface Methodology (RSM)

Akanksha Jain[1], Rakesh Gehlot[2] and Saleem Sidiqqui[3]

[1]Ph.D. Student, Centre of Food Science and Technology (CFST), CCSHAU, Hisar, Haryana-125004
[2,3](CFST), CCSHAU, Hisar, Haryana-125004.
E-mail: [1]akankshajain972@gmail.com, [2]raka66@hau.ernet.in, [3]saleemcfst@gmail.com

Abstract—*In the present study, recipe for preparation of papaya ready-to-serve (RTS) drink was optimized using response surface methodology (RSM). The influence of pulp, TSS (total soluble solids) and acidity percentage on five responses viz., pH, ascorbic acid content, total phenols, carotenoids and overall acceptability of RTS drink was studied by employing a Box-Behnken Design (BBD) with seventeen experimental runs which analyzed the design by second order polynomial quadratic model. Results of ANOVA analysis performed on the experimental runs showed that the product responses of beverages were significantly affected by changes in the percentage of pulp (15 to 20%), TSS (10 to 15%) and acidity (0.18 to 0.30%). The coefficient of determination i.e., R^2 for all responses was higher than 0.96 (approaching to unity). The lack of fit which measured the fitness of the models was not significant in all response parameters, indicating that these models were sufficiently accurate for predicting the responses. Result exhibited that higher percentage of pulp in RTS drink resulted in increased ascorbic acid content, total phenols and total carotenoids, and higher overall acceptability scores of beverages. Furthermore, overall acceptability scores of the beverages were also influenced by positive interaction between TSS and acidity. Through numerical optimization, standardized recipe selected by RSM for papaya RTS drink were 20% pulp, 15% TSS and 0.28% acidity with highest desirability of 0.962. The pH, ascorbic acid content, total phenols, carotenoids and overall acceptability for the optimum recipe predicted by the design were 3.42, 6.35 mg/100 ml, 10.8 mg/100 ml 491 µg/100 ml and 7.9, respectively.*

Keywords*: RTS drink, RSM, Box- Behnken design, optimization*

1. INTRODUCTION

Fruits and vegetables embrace an important status among the health foods because these supply significant quantities of nutrients, especially vitamins, minerals, fiber and sugars. However, due to highly perishable nature and short shelf life of fruits and vegetables, immediate processing into preserved products becomes essential to avoid post-harvest losses [3]. Fruit beverages are convenient to use and their intake helps us to meet the daily requirement of fruits and vegetables in the diet. Present scenario of increasing health awareness, change in dietary habits, taste preferences and lifestyle of present-day consumers has led to increased demand for healthy and nutritive fruit beverages. Today, most of the consumers prefer fruit based functional beverages because these have higher nutritional, medicinal and calorific values over synthetic beverages. Further value enhancement can be done in fruit beverages with the addition of variety of nutraceuticals. Functional beverage sector has therefore been reported to be the fastest growing segment [8]. For the present study, papaya was selected for preparation of fruit drink due to its reasonable price and high nutritive value. It is known as common man's fruit and belongs to *Caricaceae* family. Apart from its attractive colour and luscious taste, papaya is a rich source of minerals like potassium and magnesium and nutrients such as carotenoids, vitamins C, E & flavonoids which acts as antioxidants; vitamins B, folate, pantothenic acid; and fiber [6]. Besides its availability in traditional food products like salads, candy slices and juices, it could be suitably utilized in the development of fast moving consumer goods like RTS drink. As papaya is perishable and under-utilized fruit, it can be preserved by making it as a pulp to be used further for the preparation of RTS drink.

The present study was planned with the objectives to use RSM as a tool for optimizing recipe of papaya RTS drink and to determine the effect of process factors on response parameters. The major advantage of RSM was that it reduced the number of experimental runs required to provide appropriate information for statistically acceptable results [1]. Here, Box-Behnken (BBD) design was applied in which each process (independent) factor had only three levels, coded as -1, 0 and +1. It was chosen due to its good design properties, appropriate to apply on quadratic model with little collinearity, rotatable design, lesser experimental runs than central composite design (CCD), and insensitive to outliers

2. MATERIALS AND METHODS

2.1. Collection of raw material

Ripe papaya fruits and sugar were purchased from local market of Hisar, whereas citric acid was purchased from SRL, laboratories, Mumbai, India.

2.2. Collection of pulp

The fruits were washed thoroughly with clean running water, peeled off and cut in two halves. The seeds and inner whitish flavonoid layers were discarded and fruit pieces were blended in a mixer to obtain homogeneous pulp (see Fig. 1).

Ripe papaya fruits
↓
Washing
↓
Peeling
↓
Halving
↓
Removing seeds and inner whitish layer
↓
Cutting into pieces
↓
Blending in a mixer
↓
Homogeneous pulp
↓
Addition of sodium benzoate
(1g/kg pulp)
↓
Filling in polypropylene jars
↓
Storage in deep freezer

Fig. 1: Flow sheet for collection of papaya pulp.

2.3. Optimization of recipe for preparing RTS drink using RSM

2.3.1. Experimental design layout. Response surface methodology was applied to design the experiment, a statistical package of design-expert version 9 (Stat-Ease Inc., Minneapolis, MN, USA). In RSM, Box-Behnken design (BBD) was used without blocking with three independent variables (with three levels for each variable) and five dependent variables (see Table 1). The minimum level of each independent variable was selected according to fruit product order (FPO) specifications and then range was selected after carrying out preliminary trials. BBD generated 17 experimental runs (12 were factorial and five were at center point). It gave response surface plots, statistically analyzed experimental data, helped to understand the effect of pulp, TSS and acidity on pH**,** ascorbic acid, total phenols, carotenoids and overall acceptability of prepared RTS drinks and finally optimized the best formulation/recipe for beverage development.

Table 1: Three variable levels for process factors

Process variables (Independent Factors)	Code	Variable level codes		
		-1	0	+1
Pulp (%)	A	15	10	20
TSS (%)	B	10	12.5	15
Acidity (%)	C	0.18	0.24	0.30

2.3.2. Preparation of RTS drink. The percentages of pulp, TSS and acidity were maintained in 17 experimental recipes according to the runs given by BBD design (see Table 2).

For preparing RTS drink, total soluble solids and total acids were first analyzed in papaya pulp. Total soluble solids were estimated at ambient room temperature by Erma hand refractometer (0-32%) and total acids were extracted in water and were estimated by titration against 0.1N sodium hydroxide [7]. On the basis of this analysis, requisite quantities of sugar and citric acid were dissolved in water by heating and then added to the weighed pulp for adjustment of TSS and acidity in RTS drink (w/w basis). The beverages were homogenized in colloidal mill, strained, filled in pre-sterilized glass bottles (200 ml capacity) leaving 2.5 cm headspace and sealed with crown corks. The sealed bottles were processed in boiling water for 25 minutes. The bottles were then cooled in air, labelled and stored at room temperature for analysis of responses (see Fig. 2).

Papaya pulp
↓
Mixing syrup (water, sugar, and citric acid)
↓
Homogenization
↓
Straining
↓
Filling into pre-sterilized glass bottles
(200 ml capacity)
↓
Sealing with crown corks
↓
Processing
(Boiling water for 25 minutes)
↓
Cooling in air and Labelling
↓
Storage at room temperature

Fig. 2: Flow sheet for preparation of papaya RTS drink.

Table 2: Response surface experimental design in terms of coded levels and actual levels

Sr. No.	Coded values			Actual values		
	A	B	C	Pulp (%)	TSS (%)	Acidity (%)
1	0	-1	1	15	10	0.3

2	1	0	1	20	12.5	0.3
3	-1	1	0	10	15	0.24
4	1	0	-1	20	12.5	0.18
5	0	0	0	15	12.5	0.24
6	1	-1	0	20	10	0.24
7	0	-1	-1	15	10	0.18
8	-1	0	1	10	12.5	0.3
9	1	1	1	20	15	0.3
10	-1	-1	0	10	10	0.24
11	0	1	1	15	15	0.3
12	0	0	0	15	12.5	0.24
13	0	0	0	15	12.5	0.24
14	0	0	0	15	12.5	0.24
15	0	1	-1	15	15	0.18
16	-1	0	-1	10	12.5	0.18
17	0	0	0	15	12.5	0.24

2.3.3. Determination of product response parameters. The beverages were subjected to analysis for five parameters by different methods. The pH of the RTS beverage was determined using a digital pH meter by oaklon. Ascorbic acid was analyzed by the method of [7], total carotenoids by method of [9] and total phenols by the method of [2]. Sensory evaluation of beverages was performed using 9 point hedonic scale as described by [7]. The overall acceptability of the beverages was based on the mean scores obtained from all the sensory characters (colour and appearance, flavour, taste, mouthfeel). The attributes with mean scores of 6 and above out of 9 were considered acceptable. The treatments were replicated thrice.

Table 3. 17: Experimental recipes with response parameters of prepared papaya RTS beverages

Run No.	Pulp (%)	TSS (%)	Acidity (%)	pH	Ascorbic mg/100ml	Total phenols mg/100ml	carotenoids µg/100ml	O.A scores
1	15	10	0.3	3.45	5.83	8.69	370.5	7.2
2	20	12.5	0.3	3.43	6.57	11.1	495.6	8.0
3	10	15	0.24	3.58	3.49	5.79	248.3	7.0
4	20	12.5	0.18	3.73	6.14	11.49	488.9	7.8
5	15	12.5	0.24	3.54	5.22	8.25	367.8	7.6
6	20	10	0.24	3.5	6.74	10.86	508.5	7.8
7	15	10	0.18	3.78	4.86	8.54	375.2	7.2
8	10	12.5	0.3	3.49	3.58	5.73	245.7	6.9
9	20	15	0.3	3.40	6.62	10.78	489.5	8.2
10	10	10	0.24	3.59	2.79	5.67	252.3	6.4
11	15	15	0.3	3.46	5.61	8.42	372.5	7.9
12	15	12.5	0.24	3.56	5.42	8.05	360.9	7.5
13	15	12.5	0.24	3.53	4.96	8.41	365.2	7.3
14	15	12.5	0.24	3.53	5.24	8.32	358.5	7.4
15	15	15	0.18	3.76	5.66	8.6	369.1	7.2
16	10	12.5	0.18	3.79	3.48	5.61	245.9	6.9
17	15	12.5	0.24	3.56	5.32	8.3	369.2	7.4

2.3.4. Statistical analysis for optimization. The following second-order polynomial (quadratic) model was fitted to the dependent variables with the experimental data

$$y = ß_0 + {}^{3}\sum_{i=1} ß_i X_i + {}^{3}\sum_{i=1} ß_{ii} X_{ii}^2 + {}^{3}\sum_{i=1} {}^{2}\sum_{j=i+1} ß_{ij} X_i X_j \quad \textbf{(Eq.1)}$$

The co-efficient of the polynomial in Eq.1 were represented by $ß_0$ (constant term), X_i (linear effects), X^2_{ii} (quadratic effects) and X_i X_j (interaction effects). The analysis of variance (ANOVA) tables were generated and regression coefficients of individual linear, quadratic and interaction terms were determined. The adequacy of the model was determined using p-value, lack of fit test and coefficient of determination *i.e.*, R^2 after model evaluation. The model was generally considered adequate when the calculated F-value was more than significant p-value (Prob.> F) at 5% significance level. Model should show high R^2 values and non-significant lack of fit for further optimization. 3-D response surfaces and contour plots were produced with the help of statistical package, Design-Expert 9 from which graphically effect on response variables was determined.

3. RESULTS AND DISCUSSION

3.1. Response parameters of prepared RTS drinks

The beverages were evaluated for different parameters and data have been summarized in Table 3. After analyzing all the parameters by second order quadratic models in BBD using RSM Dx-9 software, regression equations were created, studied further for optimization and used for understanding the effect of independent factors on five responses.

3.1.1. Effect on pH. ANOVA (see Table 4) indicated that the pH was highly significant at 5% level on linear term of pulp (A) and acidity (C) percentage and quadratic term of acidity (C^2). By neglecting the non-significant terms in Eq. 1 and with the coded values of independent factors, the following equation (Eq. 2) describes the effect of significant process variables on pH of the prepared beverages

$$pH = +3.55-0.038*A-0.16*C-0.067*C^2 \; (R^2 = 0.99) \quad \textbf{(Eq. 2)}$$

In Eq. 2, negative coefficients of linear and quadratic terms depict that pH decreased with the increase in quantity of pulp and acidity. Not a single interaction term had any effect on the pH of the beverage. Saniah and Hasimah (2008) [10] developed *Morinda citrifolia* citrus-flavoured (calamansi) drink using RSM and found that percentage of citrus juice significantly affected the pH value of *Morinda* drink compared to *Morinda* juice. They reported that pH was very low (2.85) for the mixture of 5% calamansi juice and 10% *Morinda* juice. The variations in pH with process variables were graphically presented in the 3-D surface plots (see Fig. 3).

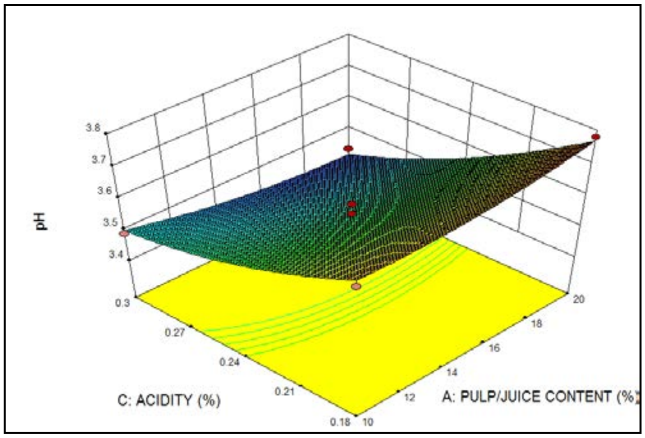

Fig. 3: Effect of process variable on pH.

3.1.2. Effect on ascorbic acid. ANOVA (see Table 4) indicated that the ascorbic acid was highly significant at 5% level on linear term of pulp (A) and acidity (C) percentage and quadratic term of pulp (A^2). By neglecting the non-significant terms in Eq. 1 and with the coded values of independent factors, the following equation (Eq. 3) describes the effect of significant process variables on ascorbic acid of papaya RTS drink

Ascorbic acid= +5.22+1.55*A+0.20*C-0.47*A^2 (R^2=0.98) (Eq.3)

The positive coefficients of the linear term indicated that ascorbic acid content increased with increase in quantity of pulp and acidity but quadratic terms of pulp quantity suggested that ascorbic acid content increased with the decrease of this variable, whereas no interaction term had any significant effect. Devaki and Premavalli (2012) [4] developed ash gourd fermented beverage using RSM and reported that yeast concentration, one of the process variable in their experiment had a significant (p<0.001) positive effect on vitamin C content at linear level. The variation of ascorbic acid with process variables were graphically presented in 3-D surface and contour plot (see Figure. 4).

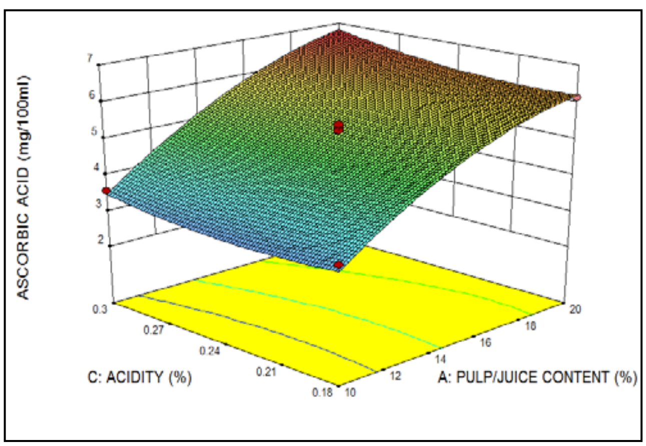

Fig. 4: Effect of process variable on ascorbic acid.

3.1.3. Effect on total phenols. ANOVA (see Table 4) indicated that the total phenols were significant at 5% level only on linear term of pulp (A). By neglecting the non-significant terms in Eq. 1 and with the coded values of independent factors, the following equation (Eq. 4) describes the effect of significant process variables on total phenols of the papaya RTS drink

Total phenols= +8.28+2.69*A (R^2=0.99) (Eq.4)

The positive coefficients of the linear term indicated that total phenols in RTS drink increased with increase in pulp quantity whereas no interaction and quadratic terms had any significant effect. The variation of total phenols with process variables were graphically presented in the 3-D surface and contour plot (see Fig. 5).

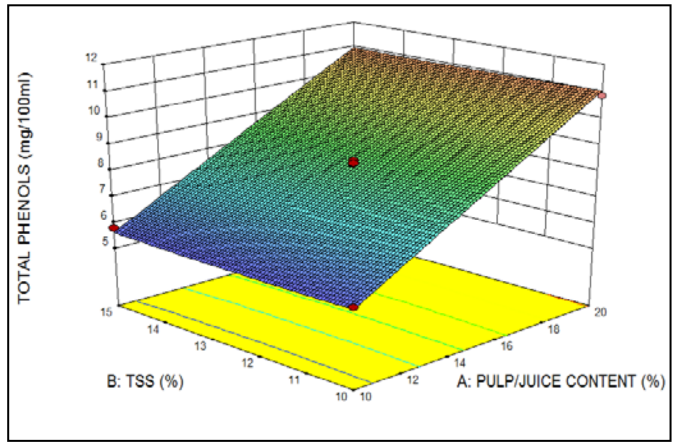

Fig. 5: Effect of process variables on total phenols.

3.1.4. Effect on total carotenoids. ANOVA (see Table 4) indicated that total carotenoids were highly significant at 5% level on linear term of pulp (A) and quadratic term of TSS (B^2). By neglecting the non-significant terms in Eq. 1 and with the coded values of independent factors, the following equation (Eq. 5) describes the effect of significant process variables on total carotenoids of papaya RTS drink

Total carotenoids= +364.63+123.39*A+6.2*B^2 (R^2=0.99) (Eq.5)

The positive coefficients of the linear and quadratic terms indicated that total carotenoids in RTS drink increased with the increase in these variables, whereas no interaction term had any significant effect. Majumdar *et al.* (2012) [5] also reported that β-carotene content was significantly affected with the increased quantity of mint leaves juice in the ash gourd (A)-mint leaves (B) blend juice and β-carotene content was also affected with the interaction of both process variables (AB). The variations in total carotenoids with process variables were graphically presented in the 3-D surface and contour plot (see Fig. 6).

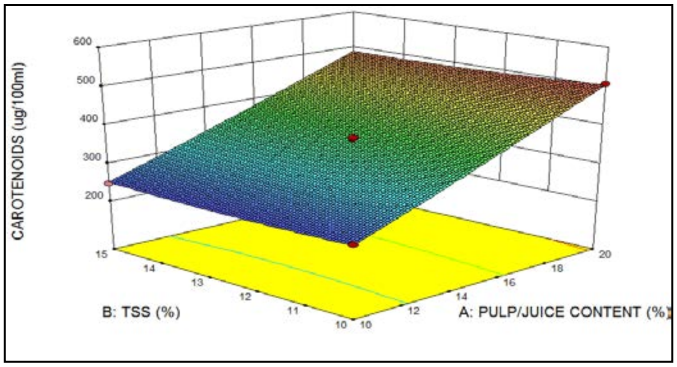

Fig. 6: Effect of process variables on carotenoids.

3.1.5. Effect on overall acceptability. ANOVA (see Table 4) indicated that the overall acceptability was significant at 5% level on linear term of pulp (A), TSS (B), acidity (C) and interaction terms of AB and BC. By neglecting the non-significant terms in Eq. 1 and with the coded values of independent factors, the following equation (Eq. 6) describes the effect of significant process variables on overall acceptability of the papaya RTS drink

Overall acceptability= +7.45+0.50*A+0.14*B+0.11*C-0.19*AB+0.17*BC(R^2=0.96) (Eq.6)

The positive coefficients of the linear terms indicated that overall acceptability of papaya RTS drink increased with the increase in pulp, TSS and acidity. Interaction between TSS and acidity resulted in an increase in overall acceptability scores, while interaction between pulp and TSS exhibited a slight decrease in overall acceptability scores of RTS drink. Similar results were reported by Majumdar *et al.* (2012), [5] who developed aseptically processed ash gourd-mint leaves juice using RSM and found that sensory scores increased with higher percentage of ash gourd juice in the blend. The variation in overall acceptability of RTS drink with process variables has been graphically presented in the 3-D surface and contour plot (see Fig. 7).

In all above predictive models, predicted R^2 were in reasonable agreement with the adjusted R^2 (Adj-R^2) with the difference of less than 0.2. All R^2 values were high, close to 1, which was desirable (see Table 4).

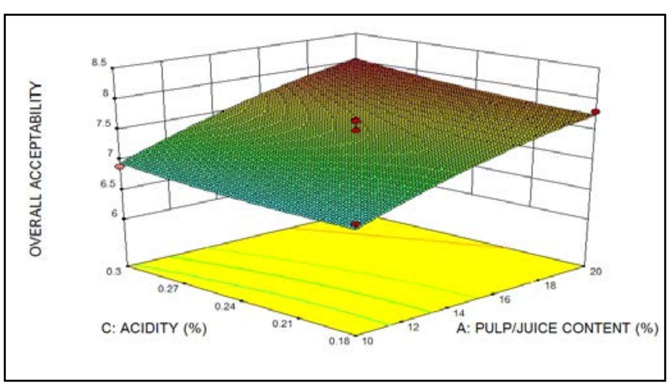

Fig. 7: Effect of process variables on overall acceptability scores.

3.2. Optimum conditions for standardization of recipe for papaya RTS drink using RSM

The ANOVA results of the second order polynomial response models in Table 4 and regression equations (Eq. 2 to 6) indicated that the Dx-9 RSM software could adequately be used to standardize the process under the desired operating conditions.

Table 4: Regression coefficients and ANOVA of the second-order polynomial quadratic models.

	Estimated p and F value									
Parameter	**pH**		**Ascorbic acid**		**Total phenols**		**Carotenoids**		**Overall acceptability**	
Coefficient	**p-value**	**F value**	**p-value**	**F value**	**p-value**	**F value**	**p value**	**F value**	**p – value**	**F value**
Intercept β_0	<0.0001	113.2	< 0.0001	53.62	< 0.0001	195.89	< 0.0001	601.66	0.0003	21.23
A-PULP β_1	0.0003*	43.5	< 0.0001*	380	< 0.0001*	1588.6	< 0.0001*	4830.96	< 0.0001*	115.2
B-TSS β_2	0.4858	0.54	0.2169	1.84	0.8668	0.030	0.0706	4.54	0.0195*	9.10
C-ACIDITY β_3	<0.0001*	831.8	0.0336*	6.95	0.4876	0.54	0.8662	0.031	0.0428*	6.10
AB β_{12}	0.9123	0.013	0.0506	5.56	0.7659	0.096	0.1272	2.99	0.0283*	7.59
AC β_{13}	0.7213	0.14	0.3132	1.18	0.1425	2.73	0.6913	0.17	0.5168	0.47
BC β_{23}	0.5480	0.44	0.0664	4.72	0.2604	1.50	0.5927	0.31	0.0293*	7.46
A^2 β_{11}	0.6582	0.21	0.0032*	19.23	0.8248	0.053	0.1994	2.01	0.1331	2.89
B^2 β_{22}	0.8918	0.02	0.5124	0.48	0.5907	0.32	0.0365*	6.65	0.0747	4.38
C^2 β_{33}	<0.0001*	64.97	0.1104	3.34	0.0566	5.20	0.8074	0.064	0.4816	0.55
Lack of Fit**	**0.44**	**1.09**	**0.21**	**2.31**	**0.16**	**2.95**	**0.40**	**1.23**	**0.56**	**0.79**
R^2	**0.99**		**0.987**		**0.99**		**0.99**		**0.96**	
Predicted- R^2	**0.94**		**0.84**		**0.93**		**0.98**		**0.74**	
Adjusted- R^2	**0.98**		**0.96**		**0.99**		**0.99**		**0.91**	

For optimization process, desired criterion for each factor and response parameter was set from the numerical optimization menu. The goal of experiment was to obtain papaya RTS drink with maximum pulp, TSS, ascorbic acid, carotenoids and overall acceptability, while acidity was targeted to 0.28%, pH and total phenols values were kept in the range (see Table 5).

Table 5: Criteria established for different factors in numerical optimization process.

Factors		Lower Limit	Upper Limit
Name	**Goal/ criteria**		
A:Pulp/juice content	maximize	10	20
B:TSS	maximize	10	15
C:Acidity	is target = 0.28	0.18	0.3
pH	is in range	3.4	3.78
Ascorbic acid	maximize	2.79	6.74
Total phenols	is in range	5.61	11.49
Carotenoids	maximize	245.76	508.51
Overall acceptability	maximize	6.4	8

All the above given criteria were combined and program generated solutions with good set of conditions having maximum desirability (see Table 6). Papaya RTS drink recipe was optimized with 20% pulp, 15% TSS and 0.28% acidity, and selected for standardization with highest desirability of 0.962 (see Fig. 8a and 8b). The optimum solution indicate that when the beverage was prepared with 20% pulp, 15% TSS and 0.28% acidity, it gave predicted value of 3.42 pH, 6.35 mg/100 ml ascorbic acid content, 10.8 mg/100 ml total phenols, 491 µg/100 ml total carotenoids and 7.9 overall acceptability score.

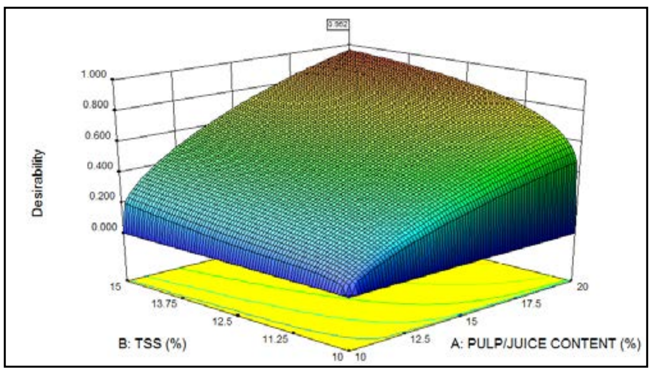

Fig. 8a: 3-D graph showing desirability (0.962) of optimum recipe.

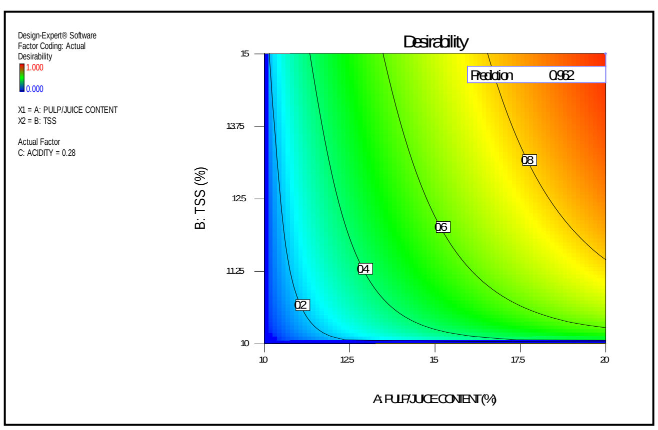

Fig. 8b: Contour plot showing operating conditions of optimum recipe.

Table 6: Solutions generated by RSM.

No.	Pulp	TSS	Acidity	pH	Ascorbic acid	Total phenols	Carotenoids	O A scores*	Desirability
1	**20**	**15**	**0.28**	**3.4**	**6.35**	**10.85**	**491.2**	**7.9**	**0.962**
2	20	14.9	0.28	3.4	6.35	10.85	491.2	7.9	0.961
3	20	14.9	0.28	3.4	6.35	10.85	491.1	7.9	0.961
4	19.9	15	0.28	3.4	6.35	10.82	489.6	7.8	0.959
5	20	14.8	0.28	3.4	6.36	10.85	491	7.9	0.959
6	19.9	15	0.27	3.4	6.34	10.85	491.1	7.8	0.959
7	20	15	0.28	3.4	6.35	10.85	491.2	7.9	0.957
8	20	14.9	0.27	3.4	6.34	10.85	491.1	7.8	0.956
9	19.8	15	0.28	3.4	6.34	10.77	487.3	7.8	0.956
10	20	14.7	0.28	3.4	6.37	10.85	490.8	7.9	0.956

O.A.*= Overall acceptability sensory scores

4. CONCLUSION

It can be concluded that RSM can be effectively applied for optimizing recipe for development of papaya RTS drink. With the application of Box-behnken design, the effect of pulp, TSS and acidity on pH, ascorbic acid, total carotenoids, total phenols and overall acceptability of RTS drink can be determined using second order quadratic polynomial models with good results in the residual analysis. Present study showed that all independent factors markedly affected one or the other product response parameters. The optimized levels of independent factors achieved after numerical optimization were 20, 15 and 0.28% for pulp, TSS and acidity, respectively. The desirability of 0.962 was achieved at this optimum point (run no. 1 was chosen from table 6)

5. ACKNOWLEDGEMENT

The present work was investigated under the guidance of elite members of advisory committee; Dr. Rakesh Gehlot; Dr. Saleem Siddiqui, Dr. S.K Sehrawat and Dr. Manju Tonk, Professor at CCS, Haryana Agricultural University, Hisar, Haryana-125004 for their inexhaustible encouragement and sincere co-operation.

REFERENCES

[1] Altan, A., McCarthy, K.L. and Maskan, M., "Evaluation of snack foods from barley-tomato pomace blends by extrusion processing", *Journal of Food Engineering*, 84, 2008, pp. 231-242.

[2] Amorium, H.V., Dougall, D.K. and Sharp, W.R., "The effect of carbohydrate and nitrogen concentrations of phenol synthesis in plant scarlet rose cells grown in tissue culture", *Physiological Plantarum*, 39**,** 1997, pp**.** 91-95.

[3] Bhardwaj, R.L. and Pandey, S., “Juice blends- a way of utilization of under-utilized fruits, vegetables, and spices: A Review”, *Critical Reviews in Food Science and Nutrition,* 51, 2011, pp. 563-570.

[4] Devaki, C.S. and Premavalli, K.S., ‘Development of Fermented Beverage Using RSM and Nutrients Evaluation’, *Journal of Food Res*earch, 1, 3, 2012, pp.138-147.

[5] Majumdar, T.K., Wadikar, D.D. and Bawa, A.S., “Development and storage stability of aseptically processed ash gourd-mint leaves juice”, *International Food Research Journal,* 19, 3, 2012, pp.823-828.

[6] Ramachandran, P. and Nagarajan, S., “Quality characteristics, nutraceutical profile and storage stability of aloe gel-papaya functional beverage blend”, *International Journal of Food Sci*ence, 2014, pp. 1-7.

[7] Ranganna, S., *Handbook of Analysis and Quality Control for Fruit and Vegetable Products,* New Delhi, Tata McGraw Hills Publishing Co. Ltd., 2009.

[8] Roberts. W., ‘Benefitting beverages’, *Prepared Foods*, 56, 2009, pp. 32–35.

[9] Rodriguez-Amaya, D.B., *A Guide to Carotenoids Analysis in Foods*. Washington, ILSI Press, 1999, p. 63.

[10] Saniah, K. and Hasimah, H.A., “Development of *Morinda citrifolia* citrus-flavoured drink using Response Surface Methodology (RSM)”, *Journal of Tropical Agriculture,* 36, 1, 2008, pp. 87–89.