Optimization of recipe for development of Aonla squash- A Response surface methodology approach

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Abstract: Central Composite Design (CCD) was employed for optimizing the recipe for development of Aonla squash with juice content (25 to 40%), acidity (1.00 to 1.10%) as independent variables and adjusting 50% total soluble solids. Thirteen different experimental combinations given by RSM design were used to investigate the effect of independent process variables on four product responses developed squash. RSM evaluated responses of the 13 squash recipes by second order quadratic equations and found that pH ranged from 2.69-3.00, ascorbic acid from 102-212 mg/100ml, browning from 0.428- 0.579 and overall acceptability scores from 6.25-7.75. ANOVA analysis showed that the product responses of beverages were significantly affected (at 5%) by changes in juice and acidity of the squash recipes. Overall acceptability scores of the beverage increased with the increase in proportion of juice content from 25 to 40%. The coefficient of determination i.e., R^2 for all responses was higher than 0.95 and non-significant lack of fit was observed for all models; which was desirable. Through numerical optimization, standard-ized recipe selected by RSM for aonla squash was with 40% juice and 1% acidity getting highest desirability of 0.991. The pH, ascorbic acid, browning and overall acceptability for the optimum recipe predicted by the design were 2.96, 197.8 mg/100 ml, 0.557 and 7.7, respectively. Thus, present study was first in its kind to optimize aonla squash recipe by using RSM as a tool which gave advantage of not only selecting best recipe but also provided im-pression of the influence of ingredients used in squash's preparation on its physico-chemical profile.

Keywords: Aonla squash, Central composite design, Optimization, Overall acceptability, RSM

INTRODUCTION

Fruits are amongst the first food items known to be consumed prehistorically by human beings and form a part of the staple diet because these are rich in nutri-ents and provide some of the essential minerals, vita-mins. Apart from this, fruits also help in curing a num-ber of diseases. Aonla or Indian gooseberry (*Phyllanthus emblica* L.) is one of the traditional fruits indigenous to India and is considered as "Wonder fruit for health" (Ganachari *et al.,* 2010). Aonla, a member of family Euphorbiaceae and sub family Phyllanthio-dae, is native to India, Ceylon, Malaya and China (Mishra *et al.,* 2010). India ranks first in the World in area and production of aonla crop (Priya and Khatkar, 2013). Its cultivation is concentrated mainly in Uttar Pradesh, Maharashtra, Gujarat, Rajasthan, Andhra Pradesh, Karnataka and Tamil Nadu (Sahu *et al.,* 2010). Aonla fruit is highly nutritious and known as Kaya Kalp according to Hindu mythology as it restores health and vitality. Aonla is a rare example of an edi-ble material that is rich in tannins as well as ascorbic acid (Pathak *et al.,* 2002). The vitamin C content in aonla varies from 200 to 950 mg/100 g depending

upon the variety and size of fruit, which is said to be second highest among all fruits and is next to Barbados Cherry (Ganachari *et al.*, 2010). The main constituents of aonla are tannins, polyphenolic compound 1, 3,6-trigalloylglucose, terehebin, corilagin, phyllantnic, linoleic acid, ellagic acid and lupeol. Tannins contain gallic acid and elagic acid, and glucose retards the oxi-dation of vitamin C rendering its value as antiscorbutic in the fresh fruit as well as in dried products. Aonla is also a source of carbohydrates, carotene, thiamine, riboflavin, and minerals like iron, calcium and phos-phorus. It is valued for its antiscorbutic, diuretic, laxa-tive, antibiotic, acidic, hypoglycaemic, hypolipidemic and cooling properties (Mishra *et al.,* 2010). Aonla, though an underutilized fruit, has enormous potential in the World market. It is almost entirely unknown in the international market and needs to be popularized. Aonla fruits are generally not consumed fresh, as it is highly acidic and astringent (Kumar and Nath, 1993); therefore, it is not popular as a table fruit, but has a great potential in processed forms. Thus, aonla was selected for the present study so that it can be pre-served by making juice for its further use in prepara-tion of squash owing to the increasing trend of popu-

larity among the consumers for fruit based beverages over aerated drinks.

RSM is an attractive tool for carrying out optimization process because it reduced the number of experimental runs required to provide appropriate information for statistically acceptable results (Altan, 2008). Here, central composite design (CCD) was applied. It is composed of a central factorial that forms a cube with sides and has been coded with two units in length (from -1 to +1). It was chosen because it was well suited for fitting a quadratic surface, which usually works well for process optimization. Therefore, the present study was planned with the objective to use RSM as a tool for optimizing recipe for aonla squash and to determine the effect of process factors on response parameters.

MATERIALS AND METHODS

Collection of raw material: Mature aonla fruits cv. Chakaiya was procured from local orchard adjoining Hisar in December, 2014. Sugar was purchased from local market of Hisar, while citric acid was purchased from SRL, laboratories, Mumbai, India.

Collection of aonla juice: Mature aonla fruits were selected for the preparation of squash. The fruits were washed in running water for removal of adhering dirt particles**.** Juice was processed in large scale multipurpose food processing machine without any heat application for maximum retention of ascorbic acid and other nutrients. Flow sheet for collection of juice from aonla fruits has been presented in Fig. 1. Similar mode of processing has been done by Choudhary *et al.* (2013) for juice development.

Experimental design layout: Response surface methodology was applied to design the experiment using statistical package of design-expert version 9.01.03 (Stat-Ease Inc., Minneapolis, MN, USA) (Jain, A. and Grewal R.B., 2015). In RSM, central composite design (CCD) was used without blocking with two independent variables and four dependent variables (see Table 1). CCD design was also used for preparation of probiotic beverage using whey and watermelon juice by Khan and Dalakoti (2013).The minimum level of each independent variable was selected according to Food safety and standards authority (FSSA) regulations, 2011 and then a range was determined after carrying out preliminary trials. CCD generated 13 experimental runs (4 were factorial, 4 were axial and five were at center point). The design gave response surface plots and statistically analyzed experimental data, which helped to understand the effect of juice and acidity per cent on pH**,** ascorbic acid, browning and overall acceptability of squash and finally optimized the best formulation/recipe for squash development.

Preparation of squash: After analyzing the suitable design for optimization and feeding the different levels of independent factors which were also the ingredients

used in squash preparation, the juice and acidity percentages were maintained in 13 experimental recipes according to the runs given by CCD design (Table 2). For preparing squash, total soluble solids and total acids were first analyzed in aonla juice. Total soluble solids were estimated at ambient room temperature by Erma hand refractometer (0-32%) (FSSAI, 2012). Total acids were extracted in water and were estimated by titration against 0.1N sodium hydroxide (Ranganna, 2008). 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 juice for adjustment of TSS and acidity in the squash (w/w basis). The beverages were strained, mixed with sodium benzoate as per permissible limits given by FSSA, filled in pre-sterilized glass bottles (200 ml capacity) leaving 2.5 cm headspace and sealed with crown corks. The beverages were stored at ambient room temperature for analysis of responses (Fig. 2).

Determination of product response/physico-chemical parameters: All developed beverages were subjected to analysis for four physico-chemical parameters by different methods. The pH of squash was determined using Oakton digital pH meter. Ascorbic acid and browning in squash were analyzed by the standard method of AOAC as described in Ranganna, 2008. Sensory evaluation of squash beverages was performed using 9 point hedonic scale described by Ranganna (2008). The overall acceptability of the squash was based on the mean scores obtained from all the sensory characters (colour and appearance, flavour, taste, mouthfeel). Sensory evaluation was conducted by five semi-trained judges. The attributes with mean scores of 6 and above out of 9 were considered acceptable. The treatments were replicated thrice.

Statistical analysis for optimization: The statistical analysis were performed by central composite design. The following second-order polynomial (quadratic) model was fitted to dependent variables with the experimental data.

$$y = \beta_0 + {}^3\sum_{i=1} \beta_i X_i + {}^3\sum_{i=1} \beta_{ii} X_{ii}{}^2 + {}^3\sum_{i=1} {}^2\sum_{j=i+1} \beta_{ij} X_i X_j$$
(Eq. 1)

The co-efficients of the polynomial in Eq. 1 were represented by β_0 (constant term), X_i (linear effects), X_{ii}^2 (quadratic effects) and $X_i X_j$ (interaction effects). Software generates analysis of variance (ANOVA) tables through which regression coefficients of individual linear, quadratic and interaction terms were determined (Jain *et al.*, 2015). 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 carrying out process of optimization (Saniah and Samsiah, 2012). The 3-D response surfaces and bar

graphs were produced with the help of statistical package, Design-Expert version-9 from which graphically effect on response variables was determined (Deshpande *et al.*, 2008)

After analyzing all the parameters by second order quadratic models in CCD using RSM Dx-9 software, regression equations were created, studied further for optimization and used for understanding the effect of independent factors on four responses. Results have been discussed one by one taking each response/parameter in consideration which provides a clear outlook how each independent variable/ingredient (juice or acidity %) influences the quality and acceptance of squash.

RESULTS AND DISCUSSION

Response parameters of prepared squash: Physico-chemical profile of thirteen aonla squash experiments have been summarized in Table 3. Each parameter is considered as individual model for analysis while describing the effects of independent variables on product parameters which were represented as physico-chemical parameters of the developed product.

Effect of independent variables on product pH: pH is a measure of negative logarithm of hydrogen ion concentration $[-\log(H^+)]$. It is an important attribute, which influences the length of storage time of finished/prepared products. Mean values of pH ranged from 2.69 to 3.00 of the all the prepared squashes. For understanding the effect of independent variables (juice and acidity %) on product pH, regression model fitted to experimental result of pH (see table 3) showed the model F-value of 31.46, which implies that the model is adequate and P-value for lack of fit was 0.86

(P>0.05), which implies that the lack of fit was non-significant *i.e.*, the model was found to be fit and good. The fit of model was also assessed by coefficient of determination *i.e.*, R^2, which was found to be 0.96. Jain *et al.* (2015) also reported non-significant lack of fit in pH model with P-value of 0.44 (P>0.05) and 0.99 R^2 value in developed papaya RTS drink. ANOVA analysis (Table 4) indicates that the pH was significant at 5% level on linear term of juice (A) and acidity (B) percentage. 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:

$$pH = +9.6 - 0.01*A - 10.53*B \quad (R^2 = 0.96)(Eq. 2)$$

In Eq. 2 (demonstrating pH interpretation), negative coefficients of linear terms depict that pH decreased with the increase in quantity of juice and acids. Not a single interaction and quadratic term had any effect on the pH of the squash beverage. Saniah and Hasimah (2008) developed *Morinda citrifolia* citrus-flavoured (calamansi) drink using RSM and found that percentage of citrus juice significantly affected (at 5% level) the pH value of *Morinda* drink compared to *Morinda* juice. The variations in pH with process variables are graphically presented in the 3-D surface plots (Fig. 3).

Effect of independent variables on Ascorbic acid: Ascorbic acid acts as an antioxidant and prevents the oxidative deterioration of other important nutrients in the product. In thirteen experimental recipes generated by RSM, it ranged from 102 to 212 mg/100 ml (see table 3). For understanding the effect of independent variables (juice and acidity %) on product ascorbic acid content, regression model fitted to experimental result of ascorbic acid (see table 3) showed the model had F-value of 2043.75, which implies that the model
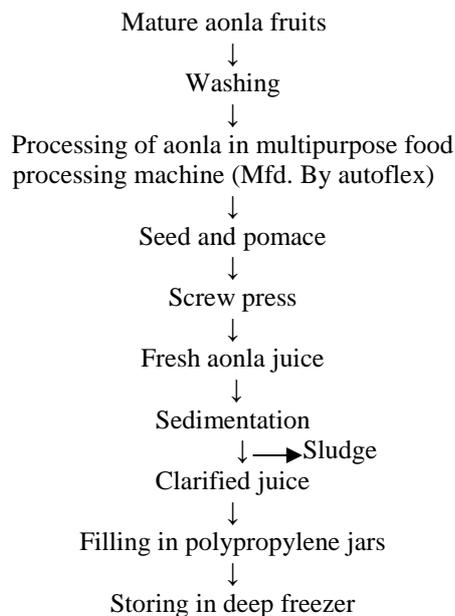
Mature aonla fruits
↓
Washing
↓
Processing of aonla in multipurpose food processing machine (Mfd. By autoflex)
↓
Seed and pomace
↓
Screw press
↓
Fresh aonla juice
↓
Sedimentation
↓→Sludge
Clarified juice
↓
Filling in polypropylene jars
↓
Storing in deep freezer

Fig. 1. *Flow sheet for collection of juice from aonla fruits.*

Aonla juice
↓
Mixing syrup
(Water, sugar, and citric acid)
↓
Straining
↓
Mixing sodium benzoate @ 600 ppm
↓
Filling into pre-sterilized glass bottles
(200 ml capacity)
↓
Crown corking
↓
Labelling
↓
Storage at room temperature
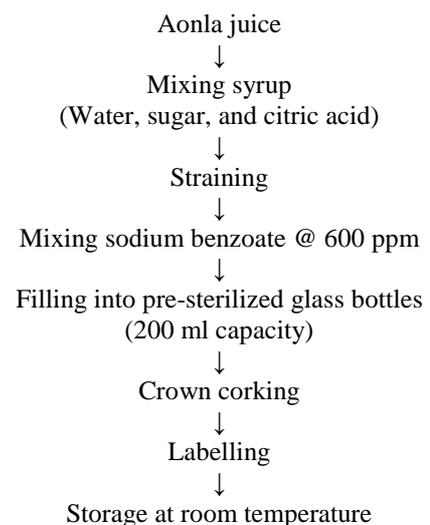
Fig. 2. *Flow sheet for preparation of aonla squash.*
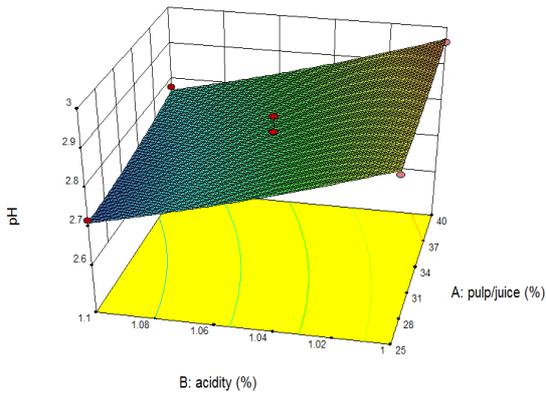
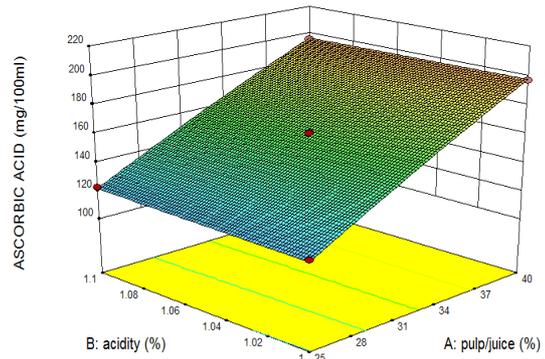**Fig. 3.** *Effect of process variables on pH.*

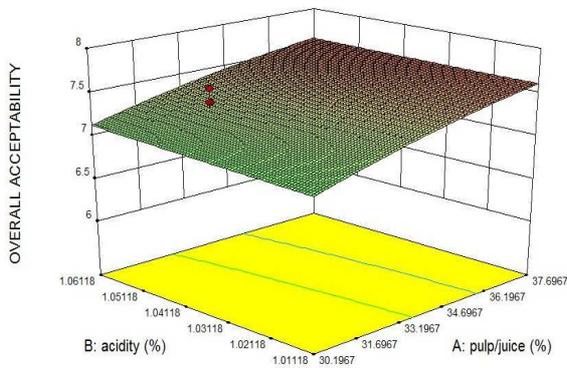**Fig. 4.** *Effect of process variables on ascorbic acid.*

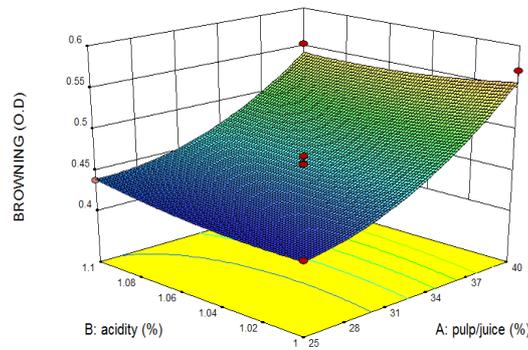**Fig. 5.** *Effect of process variables on overall acceptability scores.*

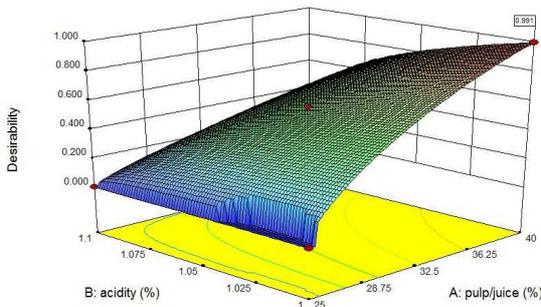**Fig. 6.** *Effect of process variables on browning.*

Fig. 7a. *3-D graph showing desirability (0.991) of optimum recipe.*

Fig. 7b. *Bar graph showing desirability of each response parameters for optimization.*

is significant and P-value for lack of fit was 0.17 (P>0.05), which implies that the lack of fit was non-significant. Coefficient of determination *i.e.,* R^2 was also very high (0.99) but when Devaki and Premavalli (2012), developed fermented ashgourd beverage, they reported lower R^2 value of 0.93 and lower Vitamin-C content from 0.02 to 0.21 mg/100g as compared to aonla squash which was due to lower content of ascorbic acid present in ashgourd as compared to aonla fruit whereas Jaiswal *et al.* (2008) prepared aonla squash using 45% pulp, 50% TSS and 1% acidity and reported
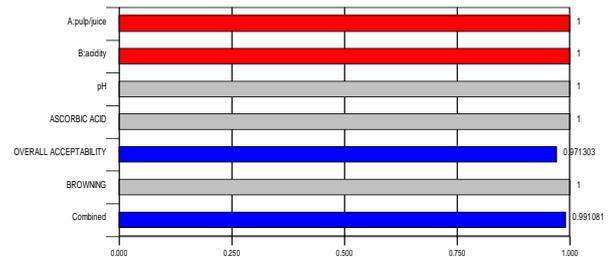
Table 1. Factors for response surface study and their levels**.**

Process variable	Code	Variables level codes				
		-1.414	-1	0	1	1.414
Juice (%)	A	21.9	25	32.5	50	43.1
Acidity (%)	B	0.97	1	1.05	1.1	1.12

78.30 mg/ 100ml ascorbic acid content. ANOVA analysis (see Table 4) indicates that the ascorbic acid was highly significant at 5% level on linear term of juice (A) and quadratic term of (A^2). By neglecting the non-significant terms in Eq. 1 and with the coded values of independent factors, the following equation (Eq.

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

Run	Space type	Coded values		Actual values	
		X_1	X_2		
		A	B	Juice (%)	Acidity (%)
1.	Factorial	1.000	-1.000	40.0	1.00
2.	Axial	0.000	1.414	32.5	1.12
3.	Axial	1.414	0.000	43.1	1.05
4.	Center	0.000	0.000	32.5	1.05
5.	Factorial	-1.000	-1.000	25.0	1.00
6.	Center	0.000	0.000	32.5	1.05
7.	Factorial	-1.000	1.000	25.0	1.10
8.	Axial	-1.414	0.000	21.9	1.05
9.	Center	0.000	0.000	32.5	1.05
10.	Axial	0.000	-1.414	32.5	0.97
11.	Center	0.000	0.000	32.5	1.05
12.	Center	0.000	0.000	32.5	1.05
13.	Factorial	1.000	1.000	40.0	1.10

Table 3. 13 Experimental recipes with response parameters of aonla squash.

	Independent factors (Major ingredients of squash)		Dependent/ response factors (Physico-chemical profile of prepared squash)			
Run No.	Juice (%)	Acidity (%)	pH	Ascorbic acid (mg/100 ml)	Overall acceptability scores (out of 9)	Browning (O.D @ 440 nm)
1.	40.0	1.00	2.96	197.4	7.75	0.571
2.	32.5	1.12	2.69	161.1	7.60	0.467
3.	43.1	1.05	2.88	212.0	7.70	0.579
4.	32.5	1.05	2.82	161.5	7.50	0.442
5.	25.0	1.00	2.91	120.5	6.25	0.422
6.	32.5	1.05	2.83	160.2	7.35	0.455
7.	25.0	1.10	2.72	122.8	6.50	0.438
8.	21.9	1.05	2.81	101.8	6.25	0.428
9.	32.5	1.05	2.8	161.0	7.30	0.458
10.	32.5	0.97	3.0	159.8	7.25	0.461
11.	32.5	1.05	2.87	159.4	7.30	0.459
12.	32.5	1.05	2.8	160.7	7.10	0.468
13.	40.0	1.10	2.78	195.5	7.70	0.553

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

	Estimated p and F value of different models							
Parameters	pH		Ascorbic acid		Overall acceptability		Browning	
Coefficient	p-value	F value	p-value	F value	p-value	F value	p-value	F value
Intercept (β_0)	0.0001	31.46	< 0.0001	2043.75	0.0003	23.59	< 0.0001	48.04
A-Juice (β_1)	*0.0169**	9.72	*< 0.0001**	*10195.65*	*< 0.0001**	*103.61*	*< 0.0001**	*202.53*
B-Acidity (β_2)	*< 0.0001**	145.41	*0.4562*	*0.62*	*0.1801*	*2.22*	*0.8522*	*0.037*
AB (β_{12})	*0.8389*	0.045	*0.0930*	*3.78*	*0.3935*	*0.83*	*0.1950*	*2.05*
A^2 (β_{11})	*0.3082*	1.21	*0.0037**	*18.26*	*0.0135**	*10.74*	*0.0006**	*35.00*
B^2 (β_{22})	*0.3082*	1.21	*0.9259*	*9.301E-003*	*0.7585*	*0.10*	*0.1709*	*2.33*
Lack of Fit**	0.86	0.25	0.17	2.87	0.29	1.77	0.21	2.39
R^2	0.96		0.99		0.94		0.97	
Predicted- R^2	0.89		0.996		0.74		0.95	
Adjusted- R^2	0.92		0.998		0.90		0.85	

3) describes the effect of significant process variables on ascorbic acid of the squash beverages:
Ascorbic acid = +160.59+38.20*A-1.73*A^2 (R^2=0.99)

(Eq. 3)

In Eq. 3 (demonstrating ascorbic acid interpretation), positive and negative coefficients of linear and quad-

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

Factors	Goal	Lower Limit	Upper Limit	Importance
A:Juice	maximize	25	40	5
B:Acidity	minimize	1	1.1	4
pH	in range	2.69	3	3
Ascorbic acid	in range	101.8	212.07	4
Overall acceptability	maximize	6.25	7.75	4
Browning	in range	0.422	0.579	4

Table 6: Five solutions generated by Response surface statistical package.

Run No.	Juice (%)	Acidity (%)	pH	Ascorbic acid (mg/100 ml)	O.A. scores*	Browning	Desirability
1	40	1.0	2.96	197.8	7.70	0.557	0.991
2	39.5	1.0	2.96	195.4	7.68	0.549	0.974
3	40	1.01	2.94	197.6	7.70	0.553	0.954
4	40	1.02	2.93	197.5	7.70	0.551	0.936
5	40	1.02	2.92	197.5	7.70	0.551	0.934

*O.A. = Overall acceptability sensory scores (out of 9), Run No. 1- selected for optimization
Run. No.1 is selected as optimized recipe

ratic terms depict that ascorbic acid content increased with the increase in quantity of juice (A) and decreased with the quadratic variable (A^2). Similar results were recorded by Devaki and Premavalli (2012), who developed bittergourd fermented beverage using RSM and reported that fermentation time (A) and curd concentration (B), which are the process variables in the experiment had significant positive effect (at 5% level) on vitamin C content at linear level. The variations in ascorbic acid with process variables were graphically presented in the 3-D surface plots (Fig. 4).

Effect of independent variables on overall acceptability: Sensory evaluation is important because it indicates the overall acceptability of a product. Overall acceptability scores of the experimental recipes ranged from 6.25 to 7.75. For understanding the effect of independent variables (juice and acidity %) on product overall acceptability scores, regression model fitted to experimental result of overall acceptability scores (see table 3) showed the model had F-value of 23.59, which implies that the model is adequate and P-value for lack of fit was 0.29 (P>0.05), which implies that the lack of fit was non-significant. Coefficient of determination i.e., R^2 was also high (0.94). ANOVA analysis (Table 4) indicates that the overall acceptability of aonla squash was highly significant at 5% level on linear term of juice (A) and quadratic term of (A^2). Previous investigation by Melo *et al.* (2013) optimized peach nectar (low calorie) *via.* RSM and found that F-test value and R^2 for overall liking was 101.2 and 0.99, respectively while Majumdar *et al.* (2012) reported 51.90 F-value and 0.97 R^2 value for sensory scores in developed ashgourd-mint leaves juice using RSM. By neglecting the non-significant terms in Eq. 1 and with the coded values of independent factors, the following equation (Eq. 4) describes the significant effect of process variables on

overall acceptability of the squash beverages:

Overall acceptability = $+7.31+0.59*A-0.21*A^2$ ($R^2 = 0.94$) (Eq. 4)

In Eq. 4 (demonstrating overall acceptability interpretation), positive and negative coefficients of linear and quadratic terms depict that overall acceptability scores of aonla squash increased with the increase in quantity of juice and decreased with the quadratic variable (A^2). Similarly, Kumar and Singh (2012) developed mango based whey beverage in which effect of quantity of mango pulp, stabilizer and sucrose were studied on overall acceptability of beverage using response surface methodology (RSM). They reported that increase in mango pulp (A) and sucrose (C) caused significant effect (at 5% level) on overall acceptability scores of the beverage and more increase was found with mango pulp than sucrose.

The variations in overall acceptability scores with process variables are graphically presented in the 3-D surface plots (Fig. 5).

Effect of independent variables on browning: Non-enzymatic browning (NEB) reactions generally lead to colour change either due to ascorbic acid decomposition or oxidation in ascorbic acid rich beverages. OD ranged from 0.422 to 0.579. NEB of aonla juice was found to be 0.035 OD at the time of juice preparation to very high OD of 0.822 after 8 months room temperature storage as reported by Bhattachaterjee *et al.* (2012). For understanding the effect of independent variables (juice and acidity %) on product overall acceptability scores, regression model fitted to experimental result of browning (see table 3) showed the model had F-value of 48.04, which implies that the model is significant and P-value for lack of fit was 0.21 (P>0.05), which implies that the lack of fit was non-significant (see table 4). Coefficient of determination i.e., R^2 was also high (0.97).

ANOVA analysis (Table 4) indicates that the browning of aonla squash was significant at 5% level on linear term of juice (A) and quadratic term of (A^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 significant effect (at 5% level) of process variables *i.e* are juice and acidity on browning of the prepared beverages:

Browning = $+0.46+0.06*A+0.027*A^2$ (R^2 = 0.97) (Eq. 5)

In Eq. 5 (demonstrating browning interpretation), positive coefficients of linear and quadratic terms depicts that browning of aonla squash increased with increase in quantity of juice variable (A) and quadratic variable of juice (A^2). Acidity percentages have been found no effect on browning of prepared aonla squash. The variations in browning with process variables are graphically presented in the 3-D surface plots (Fig. 6).

Optimum conditions for standardization of recipe for aonla squash using RSM: Dx-9 RSM software could adequately be used to standardize the process under the desired operating conditions as observed from the ANOVA results of the second order polynomial response models in Table 4 and regression equations (Eq. 2 to 5).

RSM gives the best recipe from different recipes (13 in number) after establishing certain criteria or conditions for each factor for preparation of good quality and acceptable beverage. Deshpande *et al.* (2008) also successfully optimized chocolate flavoured peanut-soy beverage using RSM as applied to only consumer acceptability data using 9-point hedonic scale from 28 beverage formulations whereas our study employed four product responses to judge the beverage quality and optimization process. For optimization process, preferred criterion for each independent factor and response parameters was set from the numerical optimization menu displayed at the left hand side of the CCD design. Criteria can be set manually, else program will consider automatically. But, preferably conditions should be set manually with certain objectives keeping in mind maximum nutrition, acceptability of consumer and different taste preferences of the major population. In the present study, goal of experiment for selection of best recipe was to obtain aonla squash with maximum juice content and overall acceptability scores and minimum acidity, while pH, ascorbic acid and browning values were kept in the range (Table 5).

All the above given criteria were combined and program generated five solutions with the help of 'solutions' option on the left hand side of the CCD design but amongst them run no.1 from table 6 was selected with 40% juice and 1.0% acidity showing highest desirability and appeal. Thus, aonla squash recipe prepared with 40% juice, 1.0% acidity and adjusting 50% TSS was selected for standardization with highest desirability of 0.991 (Fig. 7a and 7b). The optimum solution no. 1 with maximum desirability of

0.991 (should approach to unity) from table no. 6 indicated that the selected aonla squash beverage recipe cv. chakaiya when prepared with 40% juice, 50% TSS and 1% acidity, it gave predicted value of 2.96 pH, 197.8 mg/100 ml ascorbic acid content, 0.557 browning and 7.7 overall acceptability score.

Rebouças *et al.* (2014) also optimized prebiotic beverage from broken cashew nut kernels hydrosoluble extract and passion fruit juice with the addition of 3% sugar using Response surface methodology (CCD) in order to optimize acceptance of its sensory attributes. Formulation with 14% oligofructose (A) and 33% passion fruit juice (B) were optimized using RSM from 9 experimental recipes. Likewise; present study employs two independent factors such as juice % (A) and acidity % (B) for preparation of aonla squash with the application of CCD design which gives 13 formulation/recipes contrary to 9 formulations prepared by Rebouças *et al.* (2014). This difference could be due to number of varied response or dependent parameters taken for study. Our study optimized not only the overall acceptability of the aonla squash (indicator of sensory acceptance) but also the chemical profile too which is essential to predict quality of the aonla squash. Formulation with 40 % juice, 1% acidity, with the addition of 50% sugar were found best amongst 13 formulations.

Conclusion

It can be concluded from the present investigation that central composite design and response surface methodology can be successfully applied for optimizing aonla squash recipe. The different levels of ingredients such as percentages of juice (25 to 40%) and acidity (1.0 to 1.10%) employed for the preparation of aonla squash showed that these two variables markedly affected pH, ascorbic acid content, browning and overall acceptability scores of the prepared beverage. These can be related to the ingredients levels by using second order polynomials which showed that model p-values for all response/ physico-chemical variables were less than 0.05 which make it significant at 5% level. Results showed that higher percentage of aonla juice (upto 40%) in squash resulted in increased ascorbic acid content, browning and higher overall acceptability scores of prepared beverages. Study showed that sensory panelists generally responded with a high level of acceptance for squash formulations containing 40% juice and 1.0% acidity with highest scores of 7.75 (out of 9). For selection of best recipe/formulation amongst thirteen recipes, recipe with 40% juice and 1.0% acidity with highest appeal or desirability of 0.991 was chosen by attractive statistical package *i.e* RSM with the help of numerical optimization option. Thus, present study was former to use response surface methodology as a tool for optimizing aonla squash recipe of variety chakaiya, which gave advantage of not only

selecting best formulation from different recipes but also gave imprint of the effect of ingredients used in preparation (juice and acidity contents) on squash's physico-chemical profile with the help of regression equations and 3D-surface plots. This information would further help in developing newer and qualitative beverages.

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