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Optimization of Ingredients for Development of Squash from Seabuckthorn Fruit

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Seabuckthorn fruit was optimized for development of squash by employing response surface methodology as a statistical tool. The ingredients viz., sugar and citric acid were chosen as independent variables, while sensory attribute i.e. taste as dependent variable. Effect of various independent variables on chosen response shows that the ingredient citric acid had more prominent effect on taste score than sugar syrup. The optimum condition to yield maximum score of taste of squash was sugar syrup total soluble solids of 60° brix and citric acid of 0.5 g per 100 g of recipe. Squash contains more natural antioxidants and exhibited more antioxidant activity when compared to commercial products.

Keywords: Squash, Optimization, Response Surface Methodology, Vitamin E, *Hippophae rhamnoides*, Citric Acid

Introduction

Seabuckthorn (*Hippophae rhamnoides*), is a deciduous spiny shrub or small tree between two to four meter high, widely distributed throughout the temperate zone of Asia and Europe[1]. The plant is reported to have considerable medical value[2], being useful for the treatment of skin disorders resulting from bed confinement, stomach and duodenal ulcers, cardiovascular diseases and perhaps growth of some tumors. The plant is primarily valued for its golden-orange fruits which provide anthocyanins, carotenoids and other healthful components[3]. They are also rich in nutrients such as carbohydrates, organic acids, amino acids, vitamin C, E and the vitamin content of seabuckthorn fruit is much higher than any other fruit or vegetable[4-6]. Fresh seabuckthorn fruits are inherently more liable to deterioration under tropical conditions; therefore the present study was undertaken to develop delicious tasty squash from wonderful fruit of seabuckthorn and to give a product of improved nutritional quality with high amount of carotenoids, vitamin E and several other natural antioxidants.

Materials and methods

Raw materials

Seabuckthorn berries (*H. rhamnoides*) were brought from Field Research Laboratory (Leh, Himachal Pradesh, India) by airlifting and kept frozen at (-20°C) until further studies. Food grade commercial sucrose, citric acid and sodium benzoate were purchased from local market.

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Experimental design

A central composite rotatable second order design was adopted to optimize the development of seabuckthorn squash[7]. This includes a total of 14 experimental runs with 2 factorial points, 2 axial points and 1 centre point. The axial points represented the extremes, high and low values of the independent variables and were added to the factorial design to provide for estimation of curvature of the model[7]. Based on the preliminary trial formulations, the two independent variables affecting the quality of seabuckthorn squash were found to be: total soluble solids levels of sugar syrup (X_1) and citric acid (X_2). These coded variables X_1 and X_2 each at five different levels were: -1.414 (lowest level); -1 and 0 (middle levels); +1 and +1.414 (highest level). The levels used for each variable for seabuckthorn squash is given in Table 1 A. The data pertaining to each experiment were statistically analyzed using the PROCRSREG function of the Statistical Analysis System and this software package was used to fit the second order polynomial models[8]. The model proposed for chosen response was given below:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad \text{... (1)}$$

where β_0 is the value of the fitted response at the center point of the design; β_1 and β_2 are the linear regression terms; β_{11} and β_{22} are the quadratic

Table 1 — Variables with their levels

(A) and the central composite design arrangement

Variables	Unit	Symbol	Coded and uncoded variable levels				
			-1.414	-1	0	+1	+1.414
Sugar syrup total soluble solids	°brix	X_1	46.59	50	55	60	63.41
Citric acid	g	X_2	0.43	0.50	0.65	0.80	0.86

(B) for seabuckthorn squash

Experiment number	Variable levels			
	Coded variables		Uncoded variables	
	X1	X_2	Sugar syrup** (°brix)	Citric acid(g)
1	-1	-1	50	0.50
2	+1	-1	60	0.50
3	-1	+1	50	0.80
4	+1	+1	60	0.80
5	-1.414	0	46.59	0.65
6	+1.414	0	63.41	0.65
7	0	-1.414	55	0.43
8	0	+1.414	55	0.86
9	0	0	55	0.65
10	0	0	55	0.65
11	0	0	55	0.65
12	0	0	55	0.65
13	0	0	55	0.65
14	0	0	55	0.65

*For 100 g of squash by keeping pulp as constant variable i.e. 30 g for all experimental runs.

**For preparation of 46.59° brix sugar syrup total soluble solids: 27.70 % sugar was added; for 50° brix sugar syrup: 31.71% sugar was added; for 55° brix sugar syrup: 39.08% % sugar was added; for 60° brix sugar syrup: 47.58% % sugar was added; for 63.41° brix sugar syrup: 55.43% % sugar was added. Water was constantly added @ 31.37 ml during syrup preparation.

regression terms; β_{12} is the cross – product regression terms; X_1 and X_2 are the proportional level of two coded variables. Response surfaces showing the effects of two chosen parameters on selected response of seabuckthorn squash was made using a surfer program of Surfer Access System[9].

Squash preparation

Processing of seabuckthorn berries

The frozen seabuckthorn berries were thawed to room temperature, sorted and washed to remove any adhering stalks and leaves. The cleaned berries were partially crushed in mixer grinder at lower speed, seeds were separated and removed manually from it and once again crushed well at higher speed to get coarse pulp. The fine pulp was obtained by filtering the seabuckthorn coarse pulp through the stainless steel sieve having screen size of 30-mesh. The fine pulp was pasteurized at 85°C for 30 min in order to destroy the yeasts and moulds which could be present in berries, cooled and utilized for further product development.

Development of seabuckthorn squash using response surface methodology

The seabuckthorn squash was developed as per the standard procedure of Girdharilal *et al*.[10] using response surface methodology (RSM). They were developed by combination of ingredients viz., sugar syrup and citric acid, which are being used to obtain taste to individual treatment with a view to decrease the level of citric acid. The optimum level of single treatment was used as center value for the RSM experiments. The total soluble solids level of sugar syrup ranged between 46.59 to 63.41 °brix and citric acid level was in the range of 0.43 to 0.86 g/100 g of squash recipe. Desired amount of seabuckthorn fruit pulp was mixed with the various above experimental combinations. It was then added with sodium benzoate as a preservative, filled in pre-sterilized glass bottles, sealed with a metal foil crown cap and utilized for nutritional analysis. The different variables and their levels, central composite design arrangement for preparation of seabuckthorn squash was given in Table 1 (A and B).

Physico-chemical analysis

Total soluble solids were determined using hand refractrometer (Erma, Tokyo, Japan). The titrable acidity was estimated as per the method described by Ranganna[11]. The method of Lane and Eynon was followed for the determination of total and reducing sugar[11]. The 2, 6-dichlorophenol-indophenol titration method described by Ranganna[11] was used for the estimation of ascorbic acid content. The total carotenoids were determined using an ultra violet – visible recording spectrophotometer at 450 nm (UV 1601, Shimadzu Corp, Columbia, USA) as per the method described by Ranganna[11]. The total anthocyanins were estimated as per the procedure of Ranganna[11], spectrophotometrically at 535 nm. The total phenol content was measured with the Folin-Ciocalteu's reagent, spectrophotometrically according to the procedure described by Ranganna[11]. Vitamin E was estimated using Ferric Chloride-Dipyridyl, spectrophotometrically according to Ranganna[11]. Antioxidant activity was estimated by DPPH method, spectrophotometrically as described by Pisoschi and Negulescu[12].

Sensory evaluation

A total panel of 15-judges from different age groups comprising of male and female, ranging from 20-50's were selected from the laboratory in order to evaluate the squash in terms of colour, aroma, taste, body and overall acceptability using a 9-point hedonic scale, 9 being "extremely like" and 5 being "neither like nor dislike".

Results and Discussion

Response results of seabuckthorn squash

The results for response i.e. taste score of seabuckthorn squash varied from 6.12 to 8.40. The maximum taste score of squash was of the sample prepared, which had the combination of ingredients viz., sugar syrup total soluble solids (60˚brix) and citric acid 0.50 g/ 100 g of squash ; and minimum taste score was from combination sugar syrup of total soluble solids 55° brix and citric acid of 0.86 g/ 100 g of squash, respectively. The recorded low taste score in sample could be due to use of higher amount of citric acid during squash preparation. This might have added sour taste to the final developed product and simultaneously reduced the taste score to a greater extent[10,13].

Assessment on model of taste score for ingredients sugar syrup and citric acid

Second order polynomial equation was fitted to the experimental data. The main results of this multiple regression was developed for dependent variable, with the corresponding coefficients of determination (Taste, $R^2 = 0.78$). The linear terms of sugar syrup total soluble solids (β_1) and citric acid (β_2) significantly ($p < 0.05$) influenced the taste score of seabuckthorn squash. The equation obtained was:

$$Y = 7.18 + 0.25 X_1 - 0.71 X_2$$

The equation for Y i.e. taste score reveals that the ingredients i.e. sugar syrup total soluble solids and citric acid were the factors which influence the taste score. Among these two ingredients, citric acid exhibited more remarkable effect[9,13].

Effect of sugar syrup and citric acid on taste score of squash

The taste score of seabuckthorn squash increased with the decrease in levels of both sugar syrup total soluble solids and citric acid. Citric acid had more prominent effect on the taste score than sugar syrup. The citric acid level reduction changes lead to a sharp increase in the taste score of the final product[13]. A similar result of increase in organoleptic score was already reported as a result of decrease in ingredient i.e. citric acid during preparation of functional beverages from orange juice and soya milk [14].

Optimization of squash

Numeric and graphic optimizations were carried out for the process parameters of the seabuckthorn fruit squash[8]. The desired goals for each variable and response were chosen and the limit for each variable was narrowed down to obtain an optimal region. Each goal was chosen to be is: to maximize and is at target based on the sensory score of the developed product. The software has generated one optimum conditions of independent variables with the predicted values of responses, in the maximum amount of sugar syrup total soluble solids = 60 °brix and citric acid is at target=0.50 g/ 100 g for achieving the maximum score of taste=8.37. The optimized squash was developed for further studies using above ingredients.

Verification of second order polynomial model

The seabuckthorn fruit squash was prepared using the derived optimum formulation conditions to check the validity of the second order polynomial model[8]. The actual values i.e. experimental values for taste score were determined and compared with the predicted values of the second order polynomial model. The actual values i.e. 8.40, which was found to be in close agreement to the predicted values i.e. 8.37 and they are also within the acceptable limit

Table 2 — Nutritional composition ** of optimized seabuckthorn squash in comparison with other commercial squashes

Composition	Seabuckthorn squash	Pineapple squash*	Grape squash*
Total soluble solids (°brix)	50.00 ± 0.00	55.00 ± 0.00	50.00 ± 0.00
Acidity (%)	1.21 ± 0.02	1.32 ± 0.03	1.23 ± 0.04
Total sugars (%)	49.54 ± 0.41	44.57 ± 0.54	48.32 ± 0.60
Reducing sugars (%)	14.86 ± 0.86	11.30 ± 0.43	15.72 ± 0.71
Vitamin C (mg/100g)	105.06 ± 0.50	4.24 ± 0.45	3.59 ± 0.32
Vitamin E (mg/100g)	45.71 ±1.84	ND	14.40 ±1.12
Total carotenoids (mg/100g)	1.63 ± 0.93	0.07 ± 0.02	0.04 ± 0.01
Total anthocyanins (mg/100g)	0.31 ±0.10	0.03 ±0.01	0.05 ±0.02
Total phenols (mg/100g)	136.05 ± 2.02	12.07 ± 1.65	13.97 ± 1.81
Antioxidant activity (%)	45.73 ± 0.52	3.32 ± 0.39	6.14 ± 0.45

*Commercial products
**Values are mean ± standard deviation of triplicate analysis.
ND: not detectable.

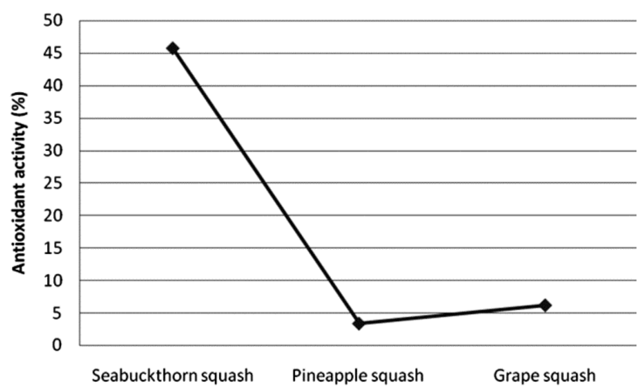

Fig.1 — Antioxidant activity of seabuckthorn fruit squash in comparison with commercial squashes

indicating the suitability of the model in predicting quality attributes of seabuckthorn fruit squash.

Nutritional composition and antioxidant activity of squash

The nutritional composition and antioxidant activity of seabuckthorn squash in comparison with other commercial squashes were presented in Table 2 and Figure 1. Seabuckthorn squash found to contain significant vitamin C and carotenoids content of 30 times higher when compared to commercial squashes ($p<0.05$). The other constituent's viz. total phenols were also found to be 10 times higher, total anthocyanins of 5 times and vitamin E contents of 2 times higher than commercial products. This may be due to presence of higher amount of natural antioxidants like vitamin C, E, phenols, carotenoids and anthocyanins in seabuckthorn fruit, when compared to the squash prepared from pineapple and grapes. The presence of these functional components significantly contributed to the higher antioxidant activity in the developed product[3, 13]. Vitamin E content was below the detectable limits in commercial pineapple squash. Chauhan *et al*.[4] also reported similar results of higher antioxidative nutrients viz., vitamin C and carotenoids in seabuckthorn squash while comparing with the other fruit squashes viz., orange, apple and papaya.

Conclusion

The studies indicated that the tasty delicious and nutrient enhanced squash may be developed naturally from wonderful fruit of seabuckthorn. This product is much superior when compared to commercial squashes as far as the contents of vitamin C, E, carotenoids, anthocyanins, total phenols and antioxidant activity. Therefore this type of product may be recommended as an antioxidant booster for the consumers in health point of view after its clinical evaluation. Since it's a lab study, the test/trials are required before product commercialization for further intended use of human consumption.

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