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Effect of Storage on Physico-Chemical, Microbiological and Sensory Quality of Bottlegourd-Basil Leaves Juice

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ABSTRACT

Bottlegourd (*Lagenaria siceraria*) and Basil (*Ocimum sanctum*) leaves blended juice was formulated with the help of Response surface methodology. The central composite design with 15 experimental combinations was used to optimize the product. The surface plots are used to represent the effects of variations in the ingredient levels. The physico-chemical, microbiological safety and sensory characteristics of the bottlegourd-basil blended juice in glass bottle were evaluated during 6 months at room temperature (28+2°C). There were no remarkable changes in pH, total soluble solids, total acidity (as citric acid) and sensory scores of the juice during storage. Loss of vitamin C and β-carotene were 74 and 57%, respectively after 6 months of storage. The result revealed that the blended juice was acceptable for 6 months and was microbiologically safe.

Key words: Pasteurization, multiple regression equations, vitamin C product optimization, nutrient loss, overall acceptability

INTRODUCTION

The bottle gourd (*Lagenaria siceraria*) is greenish colour bottleshaped or round shaped vegetable, belongs to Cucurbitaceae family. Bottlegourd is diuretic, cooling, purgative, brain tonic and useful in cough, fever, inflammations, dropsy and scalding of urine. Basil (*Ocimum sanctum*) is an erect much branched softly pubescent undershrub, 30 to 60 cm high plant belongs to Lamiaceae family which is digestive, diuretic, expectorant, stomachic and useful in asthma, bronchitis, catarrhal fever, hiccough, vomiting, ringworm and skin diseases (Prajapati *et al*., 2003). Essential oil of basil have antimicrobial, antifungal and antioxidant activities (Lopez *et al*., 2005; Oxenham *et al*., 2005; Bozin *et al*., 2006). Basil leaves are good source of β-carotene (2500 μg/100 g) and ascorbic acid (67 mg/100 g) (Yamawaki *et al.,* 1993; Chen *et al*., 1993). Bottlegourd-basil juice is a good source of minerals and vitamins and provides health benefit to consumer. This ready-to-drink, thirst quenching blended juice will satisfy the consumer demand for nutritious, health food. Bottlegourd-basil juice is not available commercially and research were also not carried out on preservation of the juice. Ingredients composition of bottlegourd-basil juice was optimized by Response Surface Methodology (RSM) to achieve good sensory acceptability of the product. The blended juice in glass bottle was pasteurized in hot water at 95°C for 15 min to achieve long shelf life of the product. The production of vegetable juices i.e., lettuce, celery, persely, celery, tomato, carrot, cabbage, chilies) have been reported by Shen *et al*. (1994) however, limited research carried out on long term preservation of vegetable and herb juice. Research on preservation and storage studies were mostly confined to carrot, pumpkin, tomato, spinach and beetroot juice (Ayranci and Tuetuencueler, 1993; Hsin and Swi-Bea, 1996; Chen *et al.*, 1996; Bong and Bock, 1998; Su-Yeun *et al.*, 2000; Dhaliwal and Hira, 2001).

Response Surface Methodology (RSM) and Central composite design of experiments are used in statistical optimization of products or processes. Several researchers have reported the use of RSM in product ingredient optimization (Ismail *et al.*, 1991; Raghavan *et al.*, 1996; Rathi *et al.*, 2002; Gill *et al.*, 2004; Wadikar *et al.*, 2008).

In the present study, bottlegourd-basil leaves blended juice was developed by RSM and the physico-chemical, microbiological and sensory characteristics of the juice were evaluated after every 30 days during 6 months.

MATERIALS AND METHODS

Raw materials: Tender and fresh Basil leaves, Bottlegourd and matured lemons (*Citrus limon*) were purchased at the local market (2005, Mysore, India). Four killogram (8.83 pound) basil leaves was processed for development of the bottlegourd-basil blended juice and 2 kg (4.41 pound) basil leaves was processed for production of final optimized juice which was required for storage studies of the juice.

Processing and formulation of the juice: Bottlegourd was cleaned with water, de-skinned, cut into pieces, blanched in boiling water for 2 min and extracted the juice by mechanical juice extractor. Basil leaves were separated from stalk, cleaned, cut into small pieces, blanched in boiling water containing magnesium oxide (0.1%) and potassium metabisulphite (0.1%) for 2 min and extracted the juice by mechanical juice extractor. Ingredients composition of the juices were optimized by RSM, a statistical design tools which uses a central composite rotatable design to fit a polynomial model by least square technique (Design expert® 8.0, Statease Inc., Minneapolis, USA, www.statease.com). For the experimental design the number points were obtained on the basis of the number of independent factors (variables) decided for a product. The parameters that influenced the product characteristics were taken as responses. In the present context, bottlegourd juice, Basil leaves Juice and lemon juices were selected as independent variables while the overall sensory scores and acidity of the product were the responses. Food grade sugar and salt were added to the juice to improve the taste and flavour. The final product was filled in sterilized 200 mL opaque white glass bottle. After filling, bottles were sealed with air tight cap and were pasteurized in hot water at 95°C for 15 min, cooled.

The product was stored at room temperature (28±2°C) for 6 months for establishing its shelf life with periodic evaluations after every 30 days.

Analytical evaluation: Total acidity was determined by titration with Sodium Hydroxide standard solution and expressed as citric acid (Ranganna, 2000). Total soluble solids were determined by refractometer ATAGO (0-32 (°Brix). The pH values were measured by pH meter (WTW 340i, Fisher Bioblock Scientific, France) (AOAC, 1985). Total, non-reducing and reducing sugars were determined according to Lane and Eynon method (Ranganna, 2000). Vitamin C was determined by colorimetric method and β-carotene was determined by column chromatography method (Ranganna, 2000). Microbial analyses were carried out according to Compendium of

Methods for the Microbiological Examination of Foods, American Public Health Association (APHA, 2001). All estimations were carried out in triplicates at 30 days interval and the mean values were reported.

Sensory analysis: Initially and periodically, samples were evaluated by a panel of 30 semi-trained members for colour, flavour, taste and overall acceptability of the juices. The tests were performed using 9-point hedonic scale, where 9 was like extremely and 1 was dislike extremely.

Statistical analysis: The data obtained was analysed statistically for mean and standard deviation. The statistical software was SAS® version 6.0 (SAS, 1990). The statistical software package Design-Expert® version 6.0 (Stat-Ease, 2006) was used to construct the experimental design as well as to analyse the data.

RESULTS AND DISCUSSION

Ingredient compositions of bottlegourd-basil juice, was optimized from 20 different formulations based on sensory scores and acidity. Lemon juice, Basil leaves juice and bottlegourd juice, were independent variables and the overall sensory scores and acidity of the product were selected as the response. With in-house trials, a juice blend was made with moderate acceptability rating and then the scope of ingredient levels was studied by using central composite design for optimization. The experimental ranges of the levels of the major ingredients (independent variables) used in RSM with actual and coded factors for bottlegourd-basil juice are given in Table 1. Generally, the coding is done for the uniformity within the design and to avoid the bias. The coding is from -1 to +1 through 0 for minimum, maximum and the centre point, respectively. The actual levels ranged from 50 to 75, 25 to 50 and 5 to 10 mL for bottle-gourd, basil leaves and lemon juices, respectively.

The experimental design with actual levels of different independent variables having 20 combinations and the observed responses for juice are given in Table 2. The acidity response ranged between 0.2 and 0.42%, whereas the sensory score response ranged between 6.0 and 7.9.

The effects of independent variables on the responses as represented in perturbation graphs (Fig. 1), reveals that bottlegourd and basil leaves juice showed the lowering effect with reference to acidity while lemon juice levels proportionately elevated acidity of the juice blend. However, the sensory score was affected considerably by changes in the level of all the three variables (Fig. 2). The increase in the level of basil leaves had a reduced sensory response, whereas it showed a corresponding increase with increase in the level of bottlegourd juice. The elevated as well as reduced levels of lemon juice resulted into lowered sensory response.

The analysis of variance calculated for each selected model as well as response, to assess how well the model represented the data has been tabulated for the products (Table 3). The high model

Table 1: Experimental ranges and levels of independent variables used in RSM in terms of actual and coded factors for bottlegourd-basil leaves juice

	Range of levels					
Variables (mL)	Actual	Coded	Actual	Coded	Actual	Coded
Bottlegourd juice	50.0	-1	62.5	0	75.0	+1
Basil leaves juice	25.0	-1	37.5	0	50.0	+1
Lemon juice	5.0	-1	7.5	0	10.0	+1

Table 2: Design of experiments with actual levels of variables in bottlegourd-basil juice

Run order	Bottlegourd juice	Basil juice	Lemon juice	Acidity (%)	Sensory score
	-------------------------	(mL)	-------------------------		
1	62.50	37.50	7.50	0.26	7.3
2	50.00	50.00	5.00	0.26	6.0
3	62.50	37.50	3.30	0.20	6.8
4	41.48	37.50	7.50	0.35	6.2
5	75.00	25.00	10.00	0.35	7.3
6	75.00	50.00	10.00	0.28	7.5
7	83.52	37.50	7.50	0.24	7.9
8	62.50	37.50	7.50	0.25	7.5
9	50.00	25.00	10.00	0.42	6.5
10	62.50	58.52	7.50	0.25	6.1
11	62.50	37.50	7.50	0.24	7.7
12	75.00	50.00	5.00	0.22	6.6
13	62.50	37.50	7.50	0.26	7.3
14	75.00	25.00	5.00	0.24	7.3
15	62.50	37.50	7.50	0.26	7.6
16	50.00	25.00	5.00	0.28	7.0
17	50.00	50.00	10.00	0.36	6.0
18	62.50	16.48	7.50	0.34	7.4
19	62.50	37.50	7.50	0.27	7.4
20	62.50	37.50	11.70	0.41	6.3

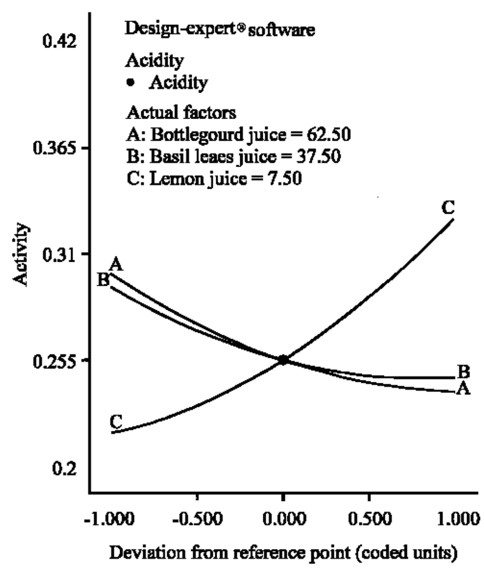

Fig. 1: Perturbation graph showing the effect of independent variables on acidity of the bottlegourd-basil juice blend

F Values of 71.94 and 21.56 reveals that the polynomial model is a significant fit. The values of Prob>F less than 0.0500 indicate model terms are significant. In this case A, B, C, AC, BC, A^2, B^2, C^2 are significant model terms which indicates the significant interaction effect on the responses. All the R squared values are high and are reasonably in agreement with each other. This indicates the model used highly suitable for predicting the response through the equations.

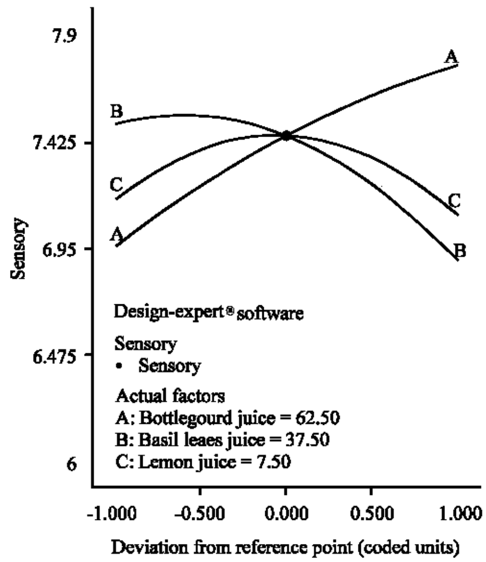

Fig. 2: Perturbation graph showing the effect of independent variables on sensory score of the bottlegourd-basil juice blend

Table 3: ANOVA and model statistics for the bottlegourd-basil juice

	Response	
Term model	Acidity quadratic	Sensory score quadratic
F-value	71.94	21.56
P>F	0.0001	0.0001
Mean	0.29	7.00
SD	0.011	0.18
CV%	3.71	2.60
R^2	0.9848	0.9510
Adjusted R squared	0.9711	0.9069
Predicted R squared	0.9298	0.7283
Adequate precision	29.222	15.259

The regression analysis of the responses was conducted by fitting second order models represented by the generic Eq. 1 as the first order equation was not fit.

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i \neq j=1}^{n} \beta_{ij} X_i X_{ij}$$

where, β_{oo} was the value of the fitted response at the center point of the design, i.e., point (0, 0, 0) in case of bottlegourd-basil leaves juice; β_i, β_{ii} and β_{ij} were the linear, quadratic and cross product (interaction effect) regression terms respectively and n denoted the number of independent variables.

Multiple regression equations (in terms of coded factors) generated for the responses are as follows:

$$\text{Acidity} = +0.25-0.030*A-0.024*B+0.056*C-0.0012*AB-0.0087\,AC-0.011*BC+0.014*A^2+0.014*B^2+0.018*C^2$$

$$\text{Sensory} = +7.45+0.41*A-0.31*B-0.032*C+0.13*AB+0.18*AC+0.18*B*C-0.093*A^2-0.25*B^2-0.32*C^2$$

The optimized levels of independent variables and the predicted response values are: Bottlegourd juice = 75.0 mL, Basil Leaves Juice = 33.5 mL, Lemon Juice = 7.8 mL, Acidity = 0.25%, Sensory score = 7.8 on 9 point hedonic scale. The optimization criterion was to maximize the sensory score. The desirability of the optimized variable levels maximizing the sensory score has been represented in Desirability plot (Fig. 3) with respect to bottlegourd and basil leaves juice. The maximum desirability of the optimized formulation was found with elevated level of bottlegourd juice. The juice blend was prepared as per the optimized composition and the predicted responses were validated with the actual observed responses of sensory score and acidity. These were almost nearer to each other. Hence, the fitted models were highly suitable for predicting the responses. Considering the optimized levels of independent variables and fixed variables, the product was processed and stored for shelf life evaluation.

The changes of pH values and total acidity were negligible during storage and the values did not show significant difference ($p>0.05$) at 95% confidence level. During storage the pH decreased to 3.89 from initial pH 4.0 (Table 4) and the mean values of total acidity increased from 0.25 to 0.36% (Table 4) during storage. The total soluble solids increased from 11.3 to 11.5% showing a negligible change within 6 months of storage (Table 4). During the storage period, the reducing sugars increased while the non-reducing sugars decreased (Table 4). These changes may be attributed to sucrose inversion in the presence of acidic environment. Total sugar values did not show significant difference ($p>0.05$) at 95% confidence level, because the increase of reducing sugar was immediately followed by a decrease in non-reducing sugar. Vitamin C and **β**-carotene values

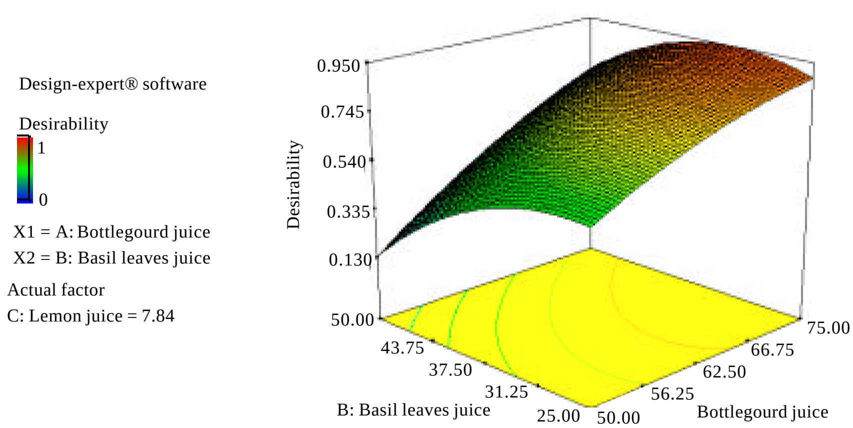

Fig. 3: Desirability of the optimized variable levels in bottlegourd-basil leaves juice

Table 4: Effect of storage on physico-chemical changes of bottlegourd-basil juice

	Storage period (months)						
Parameters	0	1	2	3	4	5	6
pH	4.0±0.01	3.98±0.01	3.97±0.01	3.95±0.01	3.93±0.01	3.91±0.01	3.89±0.01
Total acidity (%)	0.25±0.01	0.25±0.01	0.27±0.01	0.29±0.01	0.31±0.01	0.34±0.01	0.36±0.01
TSS (°Brix)	11.3±0.02	11.31±0.01	11.33±0.02	11.35±0.01	11.4±0.02	11.41±0.02	11.50±0.03
Total sugars (%)	10.4±0.01	10.36±0.01	10.33±0.00	10.3±0.01	10.3±0.01	10.3±0.01	10.29±0.02
Reducing sugars (%)	2.13±0.01	2.22±0.02	2.31±0.02	2.41±0.03	2.51±0.01	2.62±0.04	2.70±0.02
Non reducing sugars (%)	8.27±0.02	8.14±0.02	8.02±0.02	7.89±0.02	7.79±0.02	7.68±0.02	7.59±0.02
Vitamin C (mg/100 mL)	90±0.1	75±0.12	63±0.11	52±0.1	45±0.14	34±0.12	23.40±0.15
β-arotene (µg/100 mL)	2550±0.16	2189±0.14	1783±0.18	1656±0.12	1478±0.15	1275±0.12	1096±0.15

showed significant difference ($p<0.05$) during storage. Vitamin C content decreased from 90 mg/100 mL to 23.4 mg/100 mL (74% loss, Table 4) and **β**-carotene content of the juice decreased from 2550 µg/100 mL to 1096.5 µg/100 mL during 6 months storage period (57% loss, Table 4). Dhaliwal and Hira, (2004) reported that there was minor changes in pH values (3.9 to 3.6), total acidity (0.39% to 0.42%), total solids (12.78 to 13.28%) and total sugars (6.58 to 6.55%), however, significant loss ($p<0.05$) of vitamin C (80 to 88.75%) and **β**-carotene (52 to 61.4%) during 6 months storage of carrot-spinach and carrot-pineapple blended juice at ambient temperature. Majumdar *et al.* (2009) found marginal changes in acidity (0.25 to 0.3%), pH(4 to 3.9), total sugars (10.22 to 10.26%) and remarkable loss of vitamin C (74%) during 6 months storage of cucumber-litchi-lemon juice at room temperature (28±2). Krishnaveni *et al.* (2001) also reported marginal changes in pH (5.75 to 5.61), acidity (0.25 to 0.27%), total sugars (15.67 to 14.72%) and remarkable decrease of vitamin C (64.1% loss) and **β**-carotene (50% loss) during 6 months storage of jack fruit (*Artocarpus heterophyllus*) beverage at room temperature. Losses of **β**-carotene was 56% during 6 months storage of guava and papaya beverage (Tiwari, 2000). These studies are supporting the result of present experiment and the similar kind of result found by various authors during storage of mango, amla, carrot-spinach, carrot-pineapple and carrot-beetroot juice (Beerh *et al.*, 1989; Aggarwal *et al.*, 1995; Dhaliwal and Hira, 2001). However, Tiwari, (2000) reported 26.47% loss of vitamin C during 6 months storage of guava and papaya beverage at room temperature which is a contradictory result compared to the present experiment. Losses in vitamin C content of apple juice and pineapple juice stored for 12 months in the warehouse under ambient conditions (33°C) were 45.8 and 49.8%, respectively (Ewaidah, 1992). Cortes *et al.* (2005) reported loss of 4.1% vitamin C during 132 days storage of orange-carrot juice at -40°C. However, lower losses of vitamin C occurred due to low temperature storage of the sample.

The Bottlegourd-Basil leaves juice was evaluated for its microbiological quality. Coliform, Spores, Yeast and Mold count were nil upto 6 months of storage. Total Plate Count (TPC) was nil upto 5 months of storage. However TPC was 5 cfu mL^{-1} after 6 months of storage.The result indicated that the microbiological quality of the product was satisfactory.

The statistical analysis of sensory scores revealed that no significant difference was found ($p>0.05$) for the colour, taste, flavour and overall acceptability of the product during the storage period. Sensory scores of colour, flavour, taste and overall acceptability was good and very good during storage. It was observed that sensory scores of taste and flavour were better than sensory scores of colour. Mean values ranged from 7.0 to 7.9, 7.2 to 8.0, 7.1 to 7.8 and 7.1 to 7.9 for colour, taste, flavour and overall acceptability, respectively indicating good acceptability of the product (Table 5).

Table 5: Sensory scores of bottlegourd-basil leaves juice during storage

	Storage period (months)						
Variables	0	1	2	3	4	5	6
Colour	7.9±0.16	7.9±0.16	7.9±0.16	7.7±0.20	7.5±0.11	7.3±0.16	7.0±0.22
Flavour	7.8±0.20	7.8±0.20	7.6±0.20	7.4±0.12	7.3±0.20	7.3±0.20	7.1±0.16
Taste	8.0±0.25	7.8±0.25	7.6±0.25	7.5±0.22	7.5±0.18	7.3±0.12	7.2±0.12
OAA	7.9±0.20	7.8±0.20	7.6±0.20	7.5±0.13	7.3±0.25	7.2±0.21	7.1±0.26

CONCLUSION

The physico-chemical changes did not have remarkable effect on the sensory characteristics and acceptance of the bottlegourd-basil leaves blended juice during storage. Loss of vitamin C and β-carotene were 74 and 57%, respectively after 6 months of storage. The product was microbiologically safe during 6 months of storage with good acceptability.

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