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Development and storage stability of aseptically processed ashgourd-mint leaves juice

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Abstract: Ashgourd-Mint leaves juice was formulated using Response Surface Methodology (RSM) and the storage stability of the juice was investigated. The juice was aseptically processed, sterilized at 95°C for 30 second and packed in 6 layer laminated packet under sterilized environment. The physicochemical, microbiological and sensory characteristics of the juice was evaluated during 8 months storage at room temperature ($28^{o}C \pm 2^{o}C$).The changes in pH, total soluble solids, total acidity (as citric acid) and sensory score of the juice were not remarkable during storage. Loss of vitamin C and β-carotene in the juice samples were 70.2% and 59.1% respectively after 8 months of storage. The result indicated that the juice was acceptable upto 8 months of storage under ambient temperature ($28^{0}C \pm 2^{o}C$).

Keywords: Ashgourd juice (*Benincasa hispida*), mint leaves juice (*Mentha spicata*), storage stability, aseptic processing, RSM

Introduction

Aseptic technology, a HTST thermal process amalgamated with aseptic packing leading to the manufacture of safe, fresh and flavorful food which can be preserved for longer time— without refrigeration or any preservatives. It allows food to retain more color, texture, taste and nutrition. In addition to improved product quality, aseptic packaging with 6 layer laminated packet (4 layer polyethylene, one layer aluminium foil and one layer paper) offers a variety of package shapes, consumer convenience and economy. Aseptic process is based on the principle of ensuring that both food and packaging materials are free of harmful bacteria at the moment the food is packaged. To accomplish this everything in the production chain must be commercially sterile which includes the food and packaging materials, all the machinery involved as well as the environment in which the processing takes place.

Ashgourd (*Benincasa hispida*) has several functional properties. It has been used in traditional Chinese medicine to treat hypertension and inflammation (Huang *et al*., 2004). In Ayurved, *Benincasa hispida* is recommended as an anti-ulcer agent. Researchers from All India Institute of Medical Sciences found the anti-ulcerogenic effect of the fresh juice of *Benincasa hispid*a was dose-dependent in stress-induced model of ulcer and not in other types of models. They explained fresh juice of Benincasa hispida may have a CNS component in prevention of stress induced ulceration. However, antihistaminic, anti-cholinergic effects and prevention of disturbance in gastric micro-circulation as possible modes of action cannot be ruled out (Grover *et al*., 2001). In Korea, ashgourd is used to treat diabetic complications (Lee *et al*., 2005). Ashgourd is anti-mercurial, antidote for alcoholic poisoning, laxative, diuretic and it can cure internal haemorrhages and constipation (CSIR, 1962). Ashgourd contain Calcium (30 mg / 100g), Iron (0.8 mg / 100 g), and vitamin C (1 mg / 100 g) (Bose, 1985). Mint leaves are well known herb and considered stimulant, carminative and antispasmodic. This herb is used as a remedy for vomiting in pregnancy, hysteria, fever, bronchitis and indigestion. It is antibacterial (CSIR, 1962.). Mint Leaves are good source of β-carotene (1620 µg / 100 g), calcium (200 mg / 100 g), Iron (15.6 mg / 100 g), and vitamin C (27mg /100 g). The Mint leaves also contain Riboflavin (80 µg / 100 g), thiamin (50 µg / 100 g) (Bose 1985). Aseptic processing is mainly confined to fruit juices (Friedrich and Lorenz, 1991; Ooghe and Dresselaerts, 1995; Hashimoto *et al.*, 1995; Vercelino-Alves *et al.*, 2001). But the processing of strengthful vegetable, ashgourd juices or traditional vegetable juices by tetrapack processing has not been attempted.

Since the recent trend of consumer demand is nutritious, health food, the Ashgourd-Mint leaves blended juice will be a good option for the consumer. The use of RSM in optimization of product ingredients and process variables have been reported by many

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researchers (Ismail *et al.*, 1991; Raghavan *et al*., 1996; Rathi *et al*., 2002; Gill *et al*., 2004; Wadikar *et al,* 2008) and it is the most promising technique for optimization of ingredient compositions. In the present study, development of ashgourd-mint leaves blended juice has been achieved by response surface methodology. The physicochemical, microbiological and sensory characteristics of the juices were evaluated after every 30 days to establish the shelf life of the product.

Materials and Methods

Raw materials

Tender and fresh Mint leaves (*Mentha spicata*), Ashgourd (*Benincasa hispida*) and matured lemons (*Citrus limon*) were purchased from the local market (Mysore, India).

Processing of the Juice

Ashgourd was cleaned with water, deskinned, cut into pieces, blanched in boiling water for 2 min and extracted the juice by juice extractor. Mint 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. The mint leaves were treated with magnesium oxide to retain the green colour of the leaves and potassium metabisulphite was used to prevent browning of the leaves. 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 6.0, Statease Inc. Minneapolis, USA). RSM helps to create a product using regression equations that describes interrelations between input parameters and product properties (Colona *et al.*, 1984). A set of combinations were obtained on the basis of the number of independent factors (variables) decided for ashgourd and mint leaves juice. The parameters that influenced the product quality, acceptability and the shelf life were taken as responses. Independents variables were Mint leaves juice and Ashgourd juice whereas overall sensory scores and β-carotene were taken as the responses (Table 2). The final product was aseptically filled in sterilized 6 layer laminated packet (4 layer polyethylene, one layer aluminium foil and one layer paper) of 200 ml capacity.

Aseptic processing

The optimized quantities of ashgourd juice, mint leaves juice were transferred to a tank for high speed mixing of products. Then added the other ingredients i.e. lemon juice, sugar and salt and mixed well. The product was transferred to buffer tank having cooling facility, passed through the SS-tubes to Tetra-therm with de-aerator, homogeniser and surface heat exchanger at 95ºC for 30 seconds, cooled instantaneously and collected in sterile tank for aseptic storage. Then passed through Tetra-brick aseptic filling machine where the product was auto filled and labeled. Auto filling machine has roll fed packaging material, sterilised with hydrogen peroxide and shaped into a tube. The tube is filled with the product and the packages are shaped and sealed below the surface of the liquid. This means that there is no air space in the package. In the aseptic packaging process the product passes from the UHT (Ultra High Temperature) treatment in a closed system to the packaging machine. There it is packed under aseptic conditions in a packaging material, which is sterile and keeps out light and air. Sterilisation of the packaging material is made in a bath of hydrogen peroxide. Any residue is removed with heat. This leaves the packaging material completely dry and creates a sterile atmosphere in the filling section.

Analytical evaluation

Total acidity was determined by titration with Sodium Hydroxide standard solution and expressed as citric acid (Ranganna, 1986).Total soluble solids were determined by refractometer ATAGO (0-32 (oBrix). 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, 1986). Vitamin C was determined by colorimetric method and β-carotene was determined by column chromatography method (Ranganna, 1986). Microbial analyses were carried out according to 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

Results were analyzed for mean and standard deviation (SD), and the data were statistically examined by analysis of variance at 95% and 99% confidence level. The statistical software was SAS version 6.0 (SAS Institute Inc., Statistical Analysis System, Cary, NC).

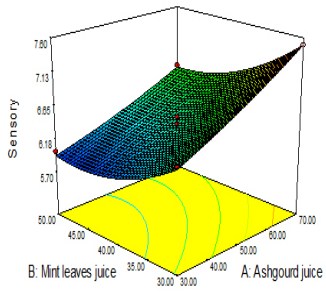

Figure 1. 3D plot depicting effect of mint leaves juice and ashgourd juice on Sensory score

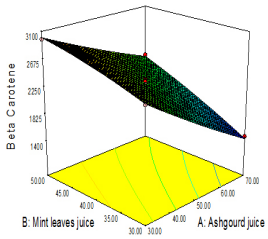

Figure 2. 3D plot depicting effect of mint leaves juice and ashgourd juice on β-Carotene.

Results and Discussion

Development of juice by RSM

The optimization of ingredients was carried out by using design expert software package. The experimental designs with different independent variables i.e. ashgourd juice and mint leaves juice and the sensory score and β-carotene content as responses are given in Tables 1 and 2 . The variation in the level of mint leaves juice and ashgourd juice equally influenced the sensory score. The b-carotene content decreased with increase in the level of ashgourd juice and directly proportional to the level of mint leaves juice in the final juice blend. The results for the central composite designs were used to fit second order polynomial equation. The response surface plots of these models have been plotted as a function of two variables where the effect of all the variations in levels of independent factors in the designs, on different responses can be visualized for each response (Figures 1 and 2).

The regression analysis of the responses was conducted and polynomial quadratic model was found suitable represented by generic equation 1.

$$Y = \beta o + \sum_{i=1}^{n} \beta i\, Xi + \sum_{i=1}^{n} \beta ii\, Xi2 + \sum_{i\neq j=1}^{n} \beta ij\, XiXij \quad \text{……..(1)}$$

where,

βo was the value of the fitted response at the centre point of the design, i.e., point (0,0), βI, βii, and βij were the linear, quadratic and cross product (interaction effect) regression terms respectively and n denoted the number of independent variables. Equations (in terms of coded factors) generated for both the responses in this product have been represented as follows.

Sensory score =+6.38+0.44A -0.34 B -0.075AB+0.048 A2+0.22 B2

Beta Carotene =+2332.00-472.01A+312.60 B+94.50 A B+26.88 A2-39.62 B2

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 (Table 3) which shows a clear fit to the quadratic model with a square of correlation to be

Table 1. Experimental ranges and levels of independent variables used in RSM in terms of actual and coded factors for Ashgourd –Mint leaves Juice

	Variables (g)	Range of Levels					
		Actual	Coded	Actual	Coded	Actual	Coded
Ashgourd–Mint leaves Juice							
A	Ashgourd Juice	30.0	-1	50.0	0	70.0	+1
B	Mint leaves juice	30.0	-1	40.0	0	50.0	+1

Table 2. Design of experiments of ashgourd-mint leaves Juice

Run	Ingredients		Response	
	Ashgourd juice (ml)	Mint leaves juice (ml)	Sensory score	β-carotene µg / ml
1	50.00	54.14	6.3	2700
2	78.28	40.00	7.1	1678
3	30.00	50.00	6	2980
4	30.00	30.00	6.5	2555
5	50.00	40.00	6.5	2300
6	50.00	25.86	7.3	1800
7	50.00	40.00	6.2	2370
8	50.00	40.00	6.4	2340
9	70.00	30.00	7.5	1475
10	21.72	40.00	5.8	3088
11	70.00	50.00	6.7	2278
12	50.00	40.00	6.5	2300
13	50.00	40.00	6.3	2350

Table 3. ANOVA and model statistics for the ashgourd-mint leaves juice

Term	Response	
	Sensory Score	β -Carotene
Model	Quadratic	Quadratic
F. Value	51.90	373.56
P>F	0.0001	0.0001
Mean	6.55	2324.15
S.D.	0.10	37.44
C.V.	1.60	1.61
R squared	0.9737	0.9963
Adjusted R squared	0.9550	0.9936
Predicted R Squared	0.9419	0.9816
Adequate Precision	23.253	63.423

Table 4. Effect of storage on physico-chemical changes of aseptically processed Ashgourd-mint leaves juice.

Parameter	Storage Period in Months								
	0	1	2	3	4	5	6	7	8
pH	4.4±0.01	4.39±0.01	4.37±0.01	4.34±0.01	4.32±0.01	4.31±0.01	4.29±0.01	4.27±0.01	4.26±0.01
Total Acidity %	0.15±0.01	0.16±0.01	0.16±0.01	0.17±0.01	0.18±0.01	0.18±0.01	0.2±0.01	0.2±0.01	0.21±0.01
TSS ,°Brix	9.17±0.02	9.2±0.04	9.23±0.02	9.3±0.01	9.3±0.02	9.3±0.02	9.3±0.03	9.4±0.01	9.43±0.02
Total Sugars %	8.36±0.01	8.32±0.01	8.28±0.00	8.26±0.01	8.23±0.01	8.22±0.01	8.20±0.02	8.18±0.02	8.17±0.01
Reducing Sugars %	1.73±0.01	1.82±0.02	1.86±0.02	1.91±0.03	2.00±0.01	2.10±0.04	2.22±0.02	2.34±0.03	2.42±0.01
Non Reducing Sugars %	6.63±0.02	6.50±0.02	6.42±0.02	6.35±0.02	6.23±0.02	6.12±0.02	5.98±0.02	5.84±0.02	5.75±0.02
Vitamin C mg / 100 ml	37.0±0.1	34.0±0.12*	29.6±0.11*	27.4±0.1*	25.2±0.14**	21.5±0.12**	17.8±0.15**	14.0±0.1**	11.0±0.15**
β-carotene µg/100 ml	1270±2.2	1110±3.4*	921±2*	851±2.5**	763±3.2**	708±3**	645±3.5**	585±2.5**	520±2.2**

*values significantly different from initial value at 95% confidence level
**values significantly different from initial value at 99% confidence level

Table 5. Sensory scores of aseptically processed ashgourd-mint leaves Juice

Months	0	1	2	3	4	5	6	7	8
Colour	7.5±0.23	7.4±0.23	7.2±0.23	7.1±0.23	7.1±0.23	7.0±0.23	7.0±0.23	7.0±0.20	6.8±0.20*
Aroma	7.4±0.16	7.4±0.16	7.3±0.16	7.3±0.16	7.2±0.16	7.2±0.16	7.1±0.16	7.1±0.12	7.1±0.12
Taste	7.5±0.20	7.5±0.20	7.3±0.20	7.3±0.20	7.3± .20	7.2± .20	7.2±0.20	7.2±0.25	7.2±0.21
Overall acceptability score	7.5±0.21	7.5±0.21	7.4±0.21	7.3±0.21	7.3±0.21	7.2±0.21	7.2 ±.21	7.2± 0.3	7.2± .25

*values significantly different from initial value at 95% confidence level

nears. The response optimization was achieved by maximizing the both responses considering the final product characteristics. The optimized juice contains ashgourd and mint leaves juice in the ratio of 7:3. Food grade sugar (10 gm / 100 ml), salt (0.5 gm/100 ml) and lemon juice (6 ml / 100 ml) were added to the juice to improve the taste and flavour. Acidity of the final optimized juice was 0.15%.

Storage stability of ashgourd-mint leaves blended juice changes in pH and acidity in ashgourd-mint leaves blended juice during storage is represented in table-4. Acidity and pH values did not show significant difference (P>0.05) at 95% confidence level. It was observed that mean pH values ranged from 4.27 to 4.4 and the mean values of total acidity ranged from 0.15 to 0.21 g / 100 ml in ashgourd-Mint leaves juice during storage. Practically this slight variation did not affect the product quality or acceptance as reflected in the sensory tests. Changes of total soluble solids (increased from 9.17oBrix to 9.43 oBrix) content were also negligible during storage (Table 4).

It was observed that the reducing sugars increased (from 1.73 % to 2.42%) while the non-reducing sugars decreased (from 6.63% to 5.75%) during storage (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 (decreased from 8.36 to 8.17). Dhaliwal and Hira (2004) reported that pasteurization and storage of carrot-spinach and carrot-pineapple blended juice for 6 months showed minor variations of pH values, total acidity, total solids and total sugars.

Vitamin C and β-Carotene values showed significant difference (P<0.05) from initial values up to 3 months of storage and showed highly significant (p<0.01) thereafter. The initial Vitamin C content decreased from 37 mg /100 ml to 15mg / 100ml (70.2% loss) in ashgourd-mint leaves juice during storage (Table 4).The initial β-Carotene content of the ashgourd-mint leaves juice decreased from 1270 µg /100ml to 520 µg /100ml (59.1% loss) .during 8 months of storage (Table 4). Sterilization of the ashgourd-mint leaves juice at 95ºC for 30 second under aseptic condition resulted in 7% loss of vitamin C and 12% loss of β-carotene. During refrigerated storage of the juice (at 4ºC) loss of β-carotene and vitamin C was 20% and 40% respectively, after 8 months. Losses of vitamin C and β-Carotene during storage of tomato, amla, carrot, carrot-spinach, carrot-pineapple and carrot-beetroot juice have been reported by various authors (Kwasneiwska *et al*., 1987; Aggarwal *et al*., 1995; Dietz and Gould, 1986; Nagra and Khan, .1988; Dhaliwal and Hira, 2004 & 2001). According to their study, the losses of vitamin C and β-carotene were ranging from 80 to 88.7% and 52 to 61.4% respectively which is correlating with our study.

The ashgourd-mint leaves juice was evaluated for microbiological quality. Coliform, Spores, Yeast and Mold count were nil upto 8 months of storage. Total Plate Count (TPC) was nil up to 6 months of storage. However TPC was 12 cfu/ml after 8 months of

storage. The result indicated that the microbiological quality of the product was satisfactory and the product was safe.

Initially and periodically the juice samples were evaluated for sensory quality (Table5). The statistical analysis of sensory scores revealed that no significant difference was found ($p>0.05$) for the taste, flavour and overall acceptability of the product during the storage period. However, sensory score of colour was significantly different from initial value after 8 months of storage ($p<0.05$). Mean values ranged from 6.8 to 7.5, 7.2 to 7.5, 7.1 to 7.4 and 7.2 to 7.5 for colour, taste, aroma and overall acceptability respectively indicating good acceptability of the product .

Storage studies of aseptically processed ashgourd juice (with the addition of lemon juice, sugar and salt) during 8 months were carried out by Majumdar *et al.* (2010). The change of pH values and total acidity was negligible during storage. It was observed that mean pH values ranged from 4.27 to 4.4 and the mean values of total acidity ranged from 0.15 to 0.22 g/100ml in ashgourd juice during storage. Changes of total soluble solids (oBrix) content of the juice were also negligible during storage. Due to addition of lemon juice (6%) the product contains 9 mg/ 100 ml of vitamin C which decreased to 3.06 mg/ 100 ml (66% loss) during storage. There was negligible change of total sugar and the total sugar content decreased from 8.31% to 8.19% during 8 months of storage. The product was microbiologically safe during storage.

The aseptically processed ashgourd-mint leaves juice had longer shelf life (8 months) than normal thermal processed ashgourd-mint leaves juice (six months shelf life). Moreover, sensory quality and nutrient retention during storage was better in aseptically processed ashgourd-mint leaves juice than normal thermal processed ashgourd-mint leaves juice (Majumdar *et al.*, 2010).

Conclusions

Optimisation of the ingredients composition with RSM for the formulation of Ashgourd-Mint leaves juice lead to the development of nutritious and delicious juice blend. The juice had good sensory characteristics and good acceptance during storage. Loss of vitamin C of the juice was 70.2% and loss of β-carotene was 59.1%. The product was microbiologically safe during storage. The aseptically processed Ashgourd-Mint leaves blended juice had shelf life of 8 months with good acceptability.

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