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Development of Pomegranate Juice Blended Tender Coconut Water Beverage

O P Chauhan*, Asha Singh, PS Raju and AS Bawa

Abstract

Pomegranate juice blended tender coconut water ready-to-serve beverage was developed. Response surface methodology was employed to optimize the levels of dependent variables (levels of tender coconut water, pomegranate juice, TSS and citric acid concentration). The responses studied were sensory (colour, flavour, consistency, mouthfeel and overall acceptability), pH and objective CIE colour values (L*, a*, b*). A Central Composite Rotatable Design (CCRD) was used with six experiments at the central point. The data obtained were analysed employing multiple regression technique to generate suitable mathematical models. Quadratic polynomial models were found to fit well in describing the effect of variables on the responses with high correlation coefficients (R^2 87.62 – 99.76), showing the adequacy of the models. An optimum level of the dependent variables (tender coconut water 6.54%, pomegranate juice 6.97%, TSS 12.8^0 Brix and citric acid 0.12%) was achieved through response surface models for getting optimum values of the responses. The experimental and predicted values at optimum conditions showed high correlation coefficients. The beverage prepared at optimum conditions of the variables showed a shelf life of 6 months at 5, 25 and 35^0C under packed condition.

Keywords: Tender coconut water; Pomegranate; Beverage; Response surface methodology; Storage.

*Defence Food Research Laboratory, Siddarthnagar, Mysore, India 570011.
Email: opchauhan@gmail.com

Introduction

Tender coconut water is a very popular drink in the world market, especially for its healing qualities such as oral or intravenous rehydration. It is the natural isotonic beverage with almost the same level of electrolytes as we have in our blood (Suresh *et al.* 1968) and does not destroy red blood cells and is readily accepted by the body (Suresh and Hegde, 1971). It is very helpful in relieving cholera diseases combating intestinal worms and relieving stomach troubles (Jasper, 1979). It is the most nutritious wholesome beverage that the nature has provided. The water of fresh tender coconut has been usually drunk by people with no ill effects. Tender coconut water is not only a thirst quenching but also a mineral drink that cures most of the diseases. Tender coconut water is considered as "unctuous, sweet, increases semen production and digestion, clears the urinary path and strengthen the muscle of cardiovascular system". Sports drinks based on tender coconut water have ample market potential (Anon, 2000). Health drinks based on coconut water are also very popular in different parts of the world (Magda, 1992). It is good for feeding infants suffering from gastro-intestinal diseases (Nurasid *et al*. 1979, Munir and Mustadjab 1980). It also checks urinary infections, has diuretic property and effective in the treatment of kidney, urethral stones and also cures malnourishment (Macalalag and Macalalag, 1987). It is also reported to contain substances capable of inducing rapid proliferation of plant tissue and it is also used as a culture medium for microorganisms.

Coconut water is the liquid endosperm that fills the central cavity enclosed by a solid endosperm. The coconut water, technically the endosperm, is formed in small quantities in the third month of the development of the nut and reaches maximum in the 8th month and declines thereafter as the nut ripens. Its major chemical constituents are sugars and minerals while fat and nitrogenous substances form a minor fraction (Shivashankar, 1991). A drastic change in chemical composition of tender coconut water has been reported by Jayalekshmi *et al.* (1986). As the coconut reaches towards its maturity, fat and protein content increases markedly and water, total solids, sugars, ash and mineral constituents decreases on per unit basis.

Plain tender coconut is available in the market in preserved and packaged form, but, it is not very much acceptable by the consumers because of its bland taste and flavour. Therefore, an attempt was made to develop a refreshing and more acceptable ready-to-serve beverage based on tender coconut water blended with pomegranate juice as pomegranate juice, as such, is an important commercial products and is used for its colour and flavour in a wide range of juices, beverages and other food products (Alper and Acar, 2004). It contains considerable amount of sugars, acids, vitamins, polyphenols and important minerals (Vardin and Fenercioglu, 2003). It is also reported to contain estrogen like activity (Maru *et al.* 2001). It is also useful in reducing oxidative stress, atherogenic modification to LDL and platelet aggregation (Aviram *et al.* 2000).

Therefore, the present study was undertaken to develop a pomegranate juice blended tender coconut water beverage having good consumer acceptability. The levels of the tender coconut water and pomegranate juice were optimized using response surface methodology. The total soluble solids and acidity of the beverage were also optimized as they play major role in the acceptability of the RTS beverages.

Materials and methods

Fruits

Fully mature Lemon and tender (green) coconuts were procured from the local Mysore (India) market for the study. The fruits were sorted and graded and ones with visible infections and mechanical injuries were rejected. The selected fruits of lemons were subjected to surface sanitation wash in chlorinated water (50ppm). The juice was extracted manually from the lemons by cutting into halves and squeezing. Tender coconut water was taken out from the nuts by

penetrating a needle in the nut and sucking the water using a vacuum machine.

Experimental design

Response surface methodology was used for designing the experiment (Khuri and Cornell 1987) to develop pomegranate juice blended tender coconut water RTS beverage. A Central Composite Rotatable Design (CCRD) was used taking 4 variables at 5 levels each with 6 points at centre. The data pertaining to the coded and uncoded parameter values of the design have been presented in Tables 1 and 2 under which 6 replications at the central point and 2 at the other points were carried out. The effect of four independent variables (pomegranate juice, tender coconut water, TSS and citric acid concentration) were investigated on dependent responses (sensory colour, flavour, consistency, mouthfeel and overall acceptability as well as pH, and CIE L*, a* and b* values.

Optimization

The data obtained for pH, CIE L*, a*, b* values and sensory attributes (colour, flavour, consistency, mouthfeel and overall acceptability) under different experimental conditions were analyzed using multiple regression analyses. Response surface model was developed to represent the data thus collected and to determine the optimal conditions for various parameters. A quadratic polynomial regression model was assumed for predicting the response variable (Y) and the data were fitted to the following second order polynomial equation:

$$Y = \beta_o + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j$$

Where β_0 is the value for the fixed response at the central point of the experiment, β_i, β_j, β_{ii} and β_{ij} are the linear, quadratic and cross product coefficients, respectively.

CIE color coordinates

The CIE colour values (L*, a*, b*) were measured using D-65 illuminant and 10^0 observer using a colour meter (MiniScan XE Plus, Model No. 45/0-S, Hunter Associates Laboratory, Inc., Reston, VA, USA). The equipment was calibrated using white and black standard ceramic tiles and the readings were recorded with inbuilt software (Easy Match QC, Hunter Associates Laboratory, Inc., Reston, VA, USA).

Physico-chemical analysis

Ascorbic acid, titratable acidity, total and reducing sugars were analyzed as per the method described by Ranganna (1999). TSS of the beverage was measured using a hand refractometer (ERMA, Japan) whereas pH was determined using a microprocessor based pH meter (Century, Model CP931, Bangalore, India). Total phenolics and flavonoids were estimated using the method described by Singletton et al (1999) and Zhishen et al (1999), respectively.

Sensory evaluation

The sensory evaluation of the beverage was carried out in terms of colour, flavour, consistency, mouthfeel and overall acceptability by a trained panel consisting of 10 members from the scientific staff of the laboratory with knowledge of consumer preferences using a nine point hedonic scale having a score of 9 for extreme liking and 1 for extreme disliking (Larmond, 1977). The samples were served to the panelists at 11a.m. during each time after duly coding them using a sensory cubical illuminated with white light and maintained at 20^0C.

Storage study

The ingredients were mixed as per the design for the development of the beverage.

Table 1. Process variable and their coded and uncoded levels

Factors	Process variable	-2.00	-1.00	0.00	+1.00	+2.00
A	Tender coconut	2.5	5	7.5	10	12.5

	water (%)					
B	Pomegranate juice (%)	2.5	5	7.5	10	12.5
C	TSS (^{0}Brix)	5	10	15	20	25
D	Citric acid (%)	0.025	0.05	0.125	0.2	0.275

Table 2. Response surface design showing number of experiments at different levels of variables

A	B	C	D	Runs
0	0	0	0	6
±2.0	0	0	0	2
±1	±1	±1	±1	16
0	±2.0	0	0	2
0	0	±2.0	0	2
0	0	0	±2.0	2

Sodium benzoate (150 ppm) was added and the beverage was packed (200 ml pack size) in laminated standi pouches (12µ polyester, 9µ aluminium foil, 15µ nylon and 70µ CPP) and pasteurized in boiling water for 15 min. The samples were kept at 5, 25 and 35ºC for 6 months and were periodically analyzed for physico-chemical, microbiological and sensory attributes.

Microbiological evaluation

The microbiological analysis of the beverage was carried out during storage for standard plate count, yeast and mold count and total coliform count according to APHA (1992) procedures.

Statistical analysis

Contour plotting was carried out to visualize the effect of variables on responses using response surface equation. A series of contour plots were developed to interpret the effect of variables on the responses investigated. A second order polynomial was fitted to the mean data values to obtain regression equations and statistical significance was examined using statistical software (Design Expert Software, version 7.1, Statease, USA). The experimental and computed values were analyzed for coefficient of determination (R^2), standard error and scattered plot. The levels of independent variables were optimized using the same software considering the sensory responses at maximum values. Physico-chemical data and sensory scores of the beverage during storage were analyzed statistically for analysis of variance (ANOVA) at $P < 0.05$ using Statistica 7 software (Stat Soft, Tulsa, OK, USA).

Results and discussion

Effect of dependent variables on sensory attributes

The sensory score for colour, flavour, consistency, mouthfeel and overall acceptability ranged from 5.80 to 7.58, 6.12 to 8.12, 6.03 to 7.68, 5.84 to 7.89 and 6.23 to 8.24; respectively, at different experimental levels of the variables. The sensory colour was significantly ($p \leq 0.0001$) affected by all the four variables (Table 3 and 4). Tender coconut water was found to significantly ($p \leq 0.0001$) reduce the sensory colour scores whereas pomegranate juice, TSS and citric acid were

Table 3. Coefficient of second order polynomial regression model for pomegranate juice blended tender coconut water beverage

Coefficient	Color	Flavor	Consistency	Mouth feel	OAA	pH	L*	a*	b*
Intercept (β_0)	7.54	7.6	7.64	7.85	7.97	3.31	3.89	8.47	0.96
A-TCW (β_1)	-0.10	0.07	-0.03	0.024	0.04	0.085	0.24	-0.01	-0.007
B-Pomegranate juice (β_2)	0.05	0.36	0.34	0.28	0.4	0.03	-0.50	1.39	0.3
C-TSS (β_3)	0.078	0.18	0.05	0.11	0.08	0.02	-0.13	0.65	0.025
D-Citric acid (β_4)	0.15	0.095	0.18	0.14	0.21	-0.026	-0.09	0.64	0..005
AB (β_{12})	0.2	-0.05	-0.12	0.22	-0.03	-0.029	0.015	0.4	-0.06
AC (β_{13})	0.27	0.043	0.22	0.30	0.23	0.009	-0.3	-0.25	2.44
AD (β_{14})	0.02	0.12	0.11	0.17	0.09	0.003	0.14	0.39	-0.16
BC(β_{23})	0.003	0.048	-0.13	0.008	-0.1	-0.008	-0.23	0.089	0.17
BD(β_{24})	0.039	-0.04	0.098	0.27	0.1	0.038	0.18	0.009	-0.18
CD(β_{34})	0.1	0.125	0.17	0.028	0.14	0.034	-0.09	0.37	0.16
A^2(β_{11})	-0.32	-0.18	-0.36	-0.30	-0.31	-0.006	0.2	-0.1	-0.2
B^2(β_{22})	-0.39	-0.12	-0.18	-0.26	-0.20	-0.01	0.59	-1.0	-0.35
C^2(β_{33})	-0.10	-0.32	-0.16	-0.35	-0.30	0.008	-.0.17	-0.29	-0.08
D^2(β_{44})	-0.04	-0.30	-0.24	-0.26	-0.27	-0.003	0.29	0.014	0.29

Table 4. Analysis of variance data for response variables

Source	Sum of squares								
	Colour	Flavour	Consistency	Mouth-feel	OAA	pH	L* value	a* value	b* value
Model	9.31****	9.76***	9.99****	13.18****	12.55****	0.29****	24.53****	95.48****	10.46***
A-TCW	0.07****	0.11	0.017**	0.014*	0.039	0.18****	1.37*	0.0048	0.001
B-Pomegranate juice	0.24****	3.06****	2.77****	1.83****	3.80****	0.022****	5.87****	46.54****	2.18***
C-TSS	0.15****	0.79*	0.063****	0.30****	0.16	0.0057****	0.43	0.10	0.016
D-Citric Acid	0.55****	0.21	0.8****	0.45****	1.097***	0.017****	0.20	9.91****	0.0007
AB	0.64****	0.045	0.21****	0.80****	0.018	0.014****	0.0039	2.56****	0.059
AC	1.19****	0.03	0.78****	1.41****	0.82***	0.0014**	1.46*	1.03***	0.96*
AD	0.008	0.24	0.19****	0.48****	0.13	0.00015	0.32	2.418****	0.43*
BC	0.0002	0.037	0.27****	0.001	0.17	0.0011*	0.87	0.126	0.47*
BD	0.024**	0.03	0.15****	1.18****	0.17	0.023****	0.50	0.0012	0.53*
CD	0.16****	0.25	0.47****	0.013*	0.32*	0.019****	0.13	2.16****	0.43
A^2	2.87****	0.96*	2.58****	2.52****	2.73****	0.00097*	1.11*	0.257	1.12*
B^2	4.22****	0.42	0.95****	1.79****	1.16***	0.0033****	9.62****	29.06****	3.35***
C^2	0.29****	2.82****	0.75****	3.35****	2.39****	0.0017***	0.75	2.34****	0.16
D^2	0.064****	2.42***	1.58****	1.81****	1.93****	0.00032	2.30**	0.006	2.30**
Combined effect of variables									
Linear	1.01	4.17*	3.64*	2.59	5.09*	0.22****	7.87	56.55***	2.20
Interactive	2.02	0.63	2.08	3.88	1.62	0.059****	3.28	8.30	2.87
Quadratic	6.28****	4.96****	4.26****	6.70****	5.83****	0.0068****	13.38****	30.64****	5.39****
R^2 (%)	99.69	87.62	99.76	99.76	95.04	99.49	88.73	99.09	88.15
****Significant (P≤0.0001), ***Significant (P≤0.001), **Significant (P≤0.005), *Significant (P≤0.05)									

found to improve the sensory scores for colour significantly ($p \leq 0.0001$). All the variables affected the sensory colour values at quadratic level with R^2 value of 99.69. Sensory scores for flavour, consistency, mouthfeel and overall acceptability were found to be affected to a greater extent by the level of pomegranate juice (Fig. 1-5). The high R^2 values (87.62, 99.76, 99.76 and 95.04) showed adequacy of the polynomial model for the sensory flavour, consistency, mouthfeel and overall acceptability, respectively. The flavour was significantly ($p \leq 0.0001$) affected more by the level of pomegranate juice followed by TSS, citric acid concentration, and the level of tender coconut water. Pomegranate juice also improved the consistency and mouthfeel of the beverage leading to higher sensory score for overall acceptability of the beverage. The effect of tender coconut water on flavour and overall acceptability of the beverage was found to be non significant. TSS also showed non significant effect on the overall acceptability of the beverage. The beverage prepared using 7.5% of pomegranate juice showed highest sensory scores for all the sensory responses and addition of tender coconut water beyond 7.5% decreased the sensory scores. The dependent variables affected the sensory response for flavour, consistency and overall acceptability significantly ($p \leq 0.05$) at linear level, whereas, the effect was not significant for colour and mouthfeel. The effect was also found to be not significant at interactive levels of the variables for all the sensory responses.

Effect of variables on pH

The beverage samples showed a pH range of 3.05 to 3.45 at different levels of experimental variables. Quadratic model was found to fit well with R^2 value of 99.49 in describing the effect of all the variables on pH. Citric acid and concentration significantly ($p \leq 0.0001$) decreased the pH of the beverages, whereas, other variables had positive effect on ph value (Table 3 and 4). Tender coconut water had more pronounced effect on pH as compared to pineapple juice (Fig. 6). The sample prepared using 0.15% citric acid showed minimum pH value, whereas, those prepared using 0.075% citric acid showed maximum pH value. All the variables affected the pH of the beverages at linear, quadratic and interactive levels. A polynomial model was found to fit well in describing the effect of variables on pH of the beverage developed.

Effect of variables on CIE L*, a*, b* values

Objective colour of the beverage was determined using a colour meter on CIE scale. The L* value which shows the lightness was found to decrease with increase in the level of pomegranate juice, TSS and citric acid. Tender coconut water showed a positive effect, indicating that with the increase in the concentration of tender coconut water L* value of the beverage increased (Fig. 7). The variables affected the L* value significantly ($p \leq 0.0001$) at quadratic level only. The a* value showing redness/greenness of the sample was also highly dependent on the level of pomegranate juice, which increase the a* value (Fig. 8). The TSS and citric acid also showed positive effect on a* value, whereas, tender coconut water showed negative effect decreasing a* value with increase in its concentration. The variable affected a* value significantly ($p \leq 0.0001$) at linear and quadratic levels, whereas, the effect was not significant at interactive level. The yellowness/blueness is represented by b* value and it was found that with the increase in the concentration of pomegranate juice, the b* value increased significantly ($p \leq 0.0001$). Tender coconut water had negative effect on b* value, whereas, TSS and citric acid showed positive effect on b* value, but to a lesser extent as compared to pomegranate juice (Fig. 9). The variables affected the b* value significantly ($p \leq 0.0001$) at quadratic level only. Polynomial model was found to fit well with the experimental CIE colour values with high correlation coefficients (88.73, 99.09 and 88.15 for L*, a* and b*; respectively).

Figure 1. Colour as a function of dependent variables

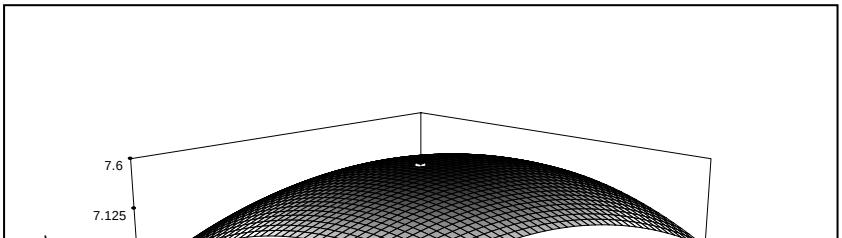


Figure 2. Flavour as a function of dependent variables

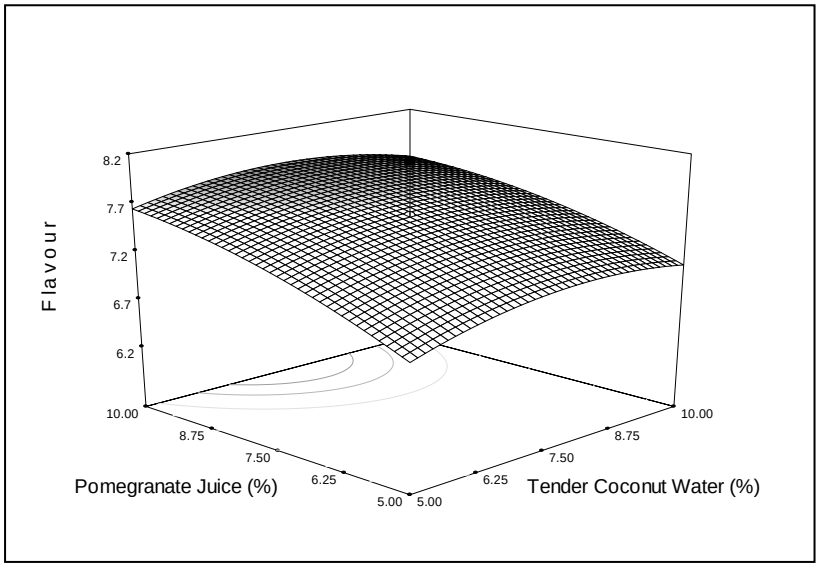


Figure 3. Consistency as a function of dependent variables

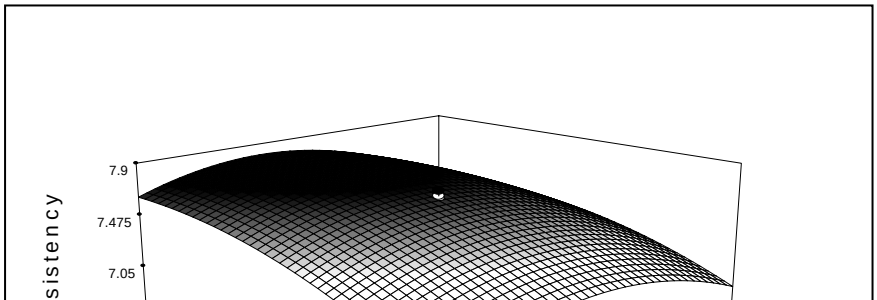


Figure 4. Mouthfeel as a function of dependent variables Mouthfeel

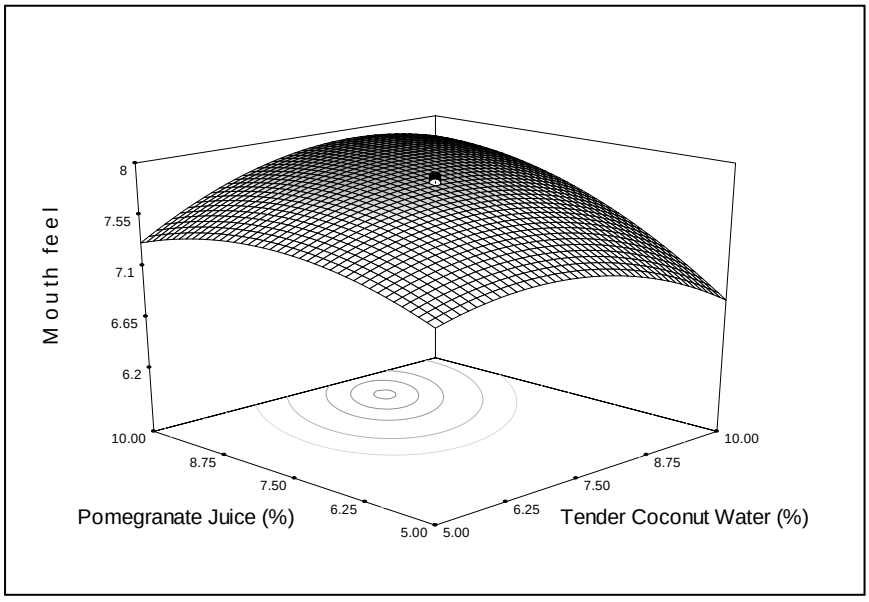


Figure 5. Overall acceptability as a function of dependent variables

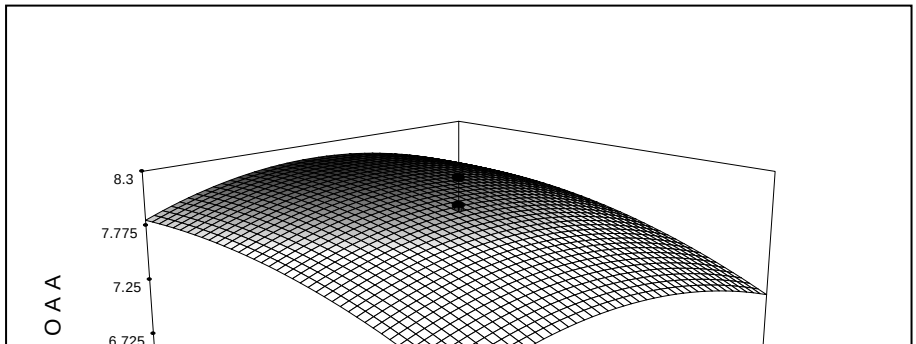


Figure 6. pH as a function of dependent variables

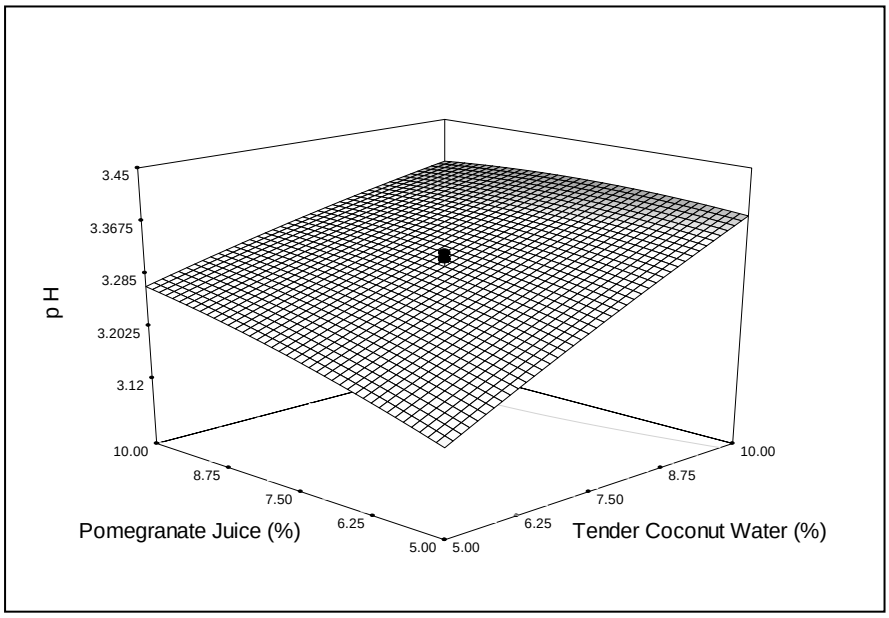


Table 5. Predicted and actual response values at optimized conditions

Responses	Predicted Value	Actual value
Colour	7.52	7.53
Flavour	7.49	7.48
Consistency	7.53	7.52
Mouth feel	7.72	7.70
OAA	7.84	7.82

pH	3.26	3.26
L* value	3.95	3.98
a* value	8.29	8.26
b* value	-0.018	-0.017

Table 6. Changes in physico-chemical, sensory and microbiological parameters of pomegranate juice blended tender coconut water beverage during storage

Parameters	Pomegranate -TCW						
	Initial	3 Months			6 Months		
		RT	5º C	37º C	RT	5º C	37º C
TSS (^{0}Brix)	12.8^a	12.8^a	12.8^a	12.7^b	12.7^b	12.8^a	12.6^c
pH	3.26 ^{a}	3.26^a	3.26^a	3.24^c	3.24^c	3.25^b	3.23^d
Acidity (% citric acid)	0.12 ^{a}	0.12^a	0.12^a	0.13^c	0.12^a	0.12^a	0.126^d
Total phenolics (mg/100 ml)	7.13 ^{a}	7.12^a	7.12^a	7.11^b	6.99^c	7.11^b	6.56^d
Total flavonoids (mg/100 ml)	1.20^a	1.19^a	1.19^a	1.16^b	1.13^d	1.15^c	1.12^e
Total Sugar (%)	12.52^a	12.52^a	12.52^a	12.50^b	12.5 ^{a}	12.51^a	12.50^b
Reducing Sugar (%)	2.35^a	2.41^c	2.37^b	2.47^e	2.40^c	2.36^b	2.45^d
Ascorbic acid (mg/100 ml)	2.02^a	1.94^c	1.98^b	1.86^e	1.91^d	1.95^c	1.82^f
CIE colour values							
L*	3.98^a	3.93^c	3.98^a	3.89^d	3.88^d	3.96^b	3.84^e
a*	8.26^a	8.27^b	8.26^a	8.28^b	8.29^c	8.28^b	8.30^d
b*	-0.017^a	-0.016^b	-0.017^a	-0.015^a	-0.015^c	-0.016^b	-0.014^d
Sensory attributes							
Colour	7.53^a	7.52^b	7.53^a	7.51^c	7.49^d	7.50^c	7.46^e
Flavour	7.48^a	7.46^b	7.47^a	7.44^c	7.42^d	7.45^b	7.42^d
Consistency	7.52^a	7.51^b	7.52^a	7.48^d	7.50^c	7.51^b	7.47^e
Mouth feel	7.70^a	7.68^b	7.70^a	7.64^c	7.64^c	7.68^b	7.62^d
OAA	7.82^a	7.80^b	7.81^a	7.78^d	7.77^e	7.79^c	7.76^f
Microbiological quality							
TPC (cfu/ml)	Nil	Nil	Nil	Nil	Nil	Nil	Nil
CC (cfu/ml)	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Y& M (cfu/ml)	Nil	Nil	Nil	Nil	Nil	Nil	Nil

*Values with different superscript in the same row differ significantly ($p<0.05$)

Figure 7. CIE *L value as a function of dependent variables**

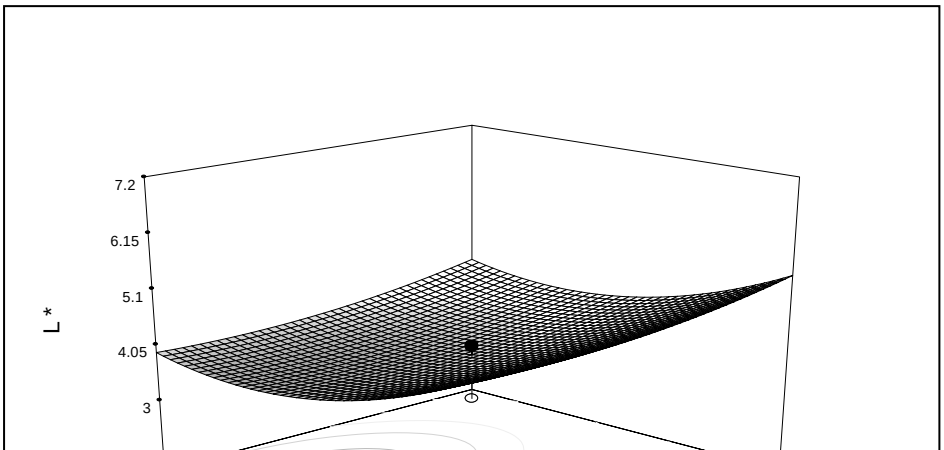


Figure 8. CIE *a value as a function of dependent variables**

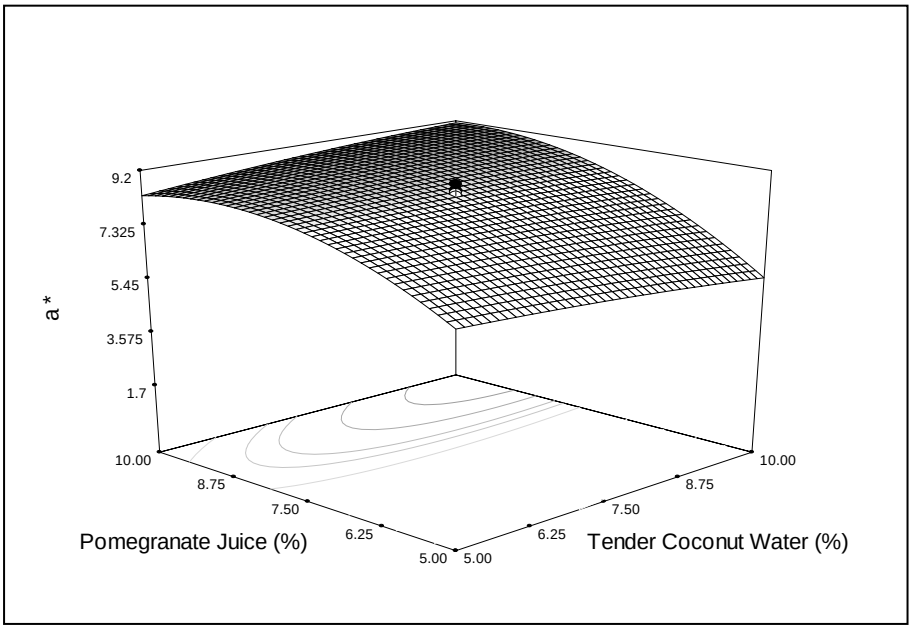
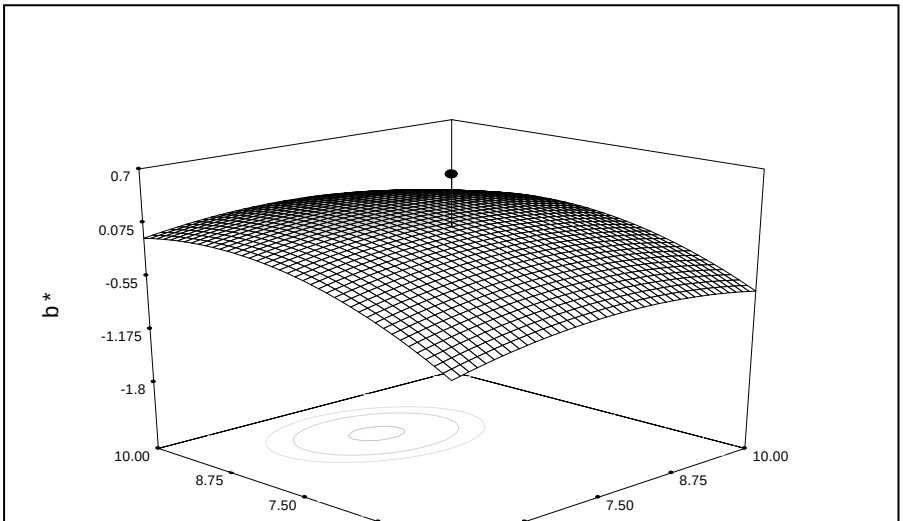


Figure 9. CIE *b value as a function of dependent variables**



Optimization of process variables

The variables were optimized for getting optimal values of responses using Design Expert Software (Version 7.1, Statease, USA). The compromised optimized levels of variables obtained for tender coconut water, pomegranate juice, TSS and citric acid were 6.54%, 6.97%, 12.8^0 Brix and 0.12%, respectively. A high correlation could be established between the predicted and the actual response values under these optimized conditions (Table 5). Riaz and Elahi (1992) studied the effects of different levels of pomegranate juice (5, 10 and 20%) on physico-chemical and consumer acceptance of carbonated pomegranate beverage and found that beverage containing 10-20% pomegranate juice had maximum acceptability. In the present study some percentage of the pomegranate juice had been supplemented by tender coconut water for getting good consumer acceptability.

Storage study

Pomegranate juice blended tender coconut water RTS beverage was prepared at the optimum levels of the independent variables and stored at 5, 25 and 35^0C. Various physico-chemical data obtained during storage study revealed that the prepared beverage was stable for a period of 6 months at all the three temperatures. TSS, pH, total phenolics, total flavonoids, total sugar and ascorbic acid decreased significantly ($p<0.05$) whereas, acidity and reducing sugars increased significantly ($p<0.05$) during storage for 6 months (Table 6). The CIE L* and b* values decreased with increase in the storage duration. The a* value increased during storage showing increase in redness of the beverage. The sensory scores for colour, flavour, consistency, mouthfeel and overall acceptability decreased with increase in the storage duration at all the three temperatures, but, the sensory scores remained within acceptable limit. The changes in various physico-chemical parameters and sensory attributes were found to be more at higher temperatures. The samples stored at 5^0C showed minimum changes in physico-chemical parameters. Marti et al. (2002) also observed changes in ascorbic acid and anthocyanin content during storage of pomegranate juice at 5 and 25^0C for 5 months and reported less change at 5^0C. The beverage samples were found free of microbial counts during entire storage period showing microbial stability for a period of 6 months at all the three temperatures.

Conclusion

All the dependent variables (tender coconut water, pomegranate juice, TSS and citric acid concentration) affected the sensory, pH and objective colour responses at quadratic level. Polynomial model fits well in describing the effect of variables on all the responses studied. Pomegranate juice affected the sensory attributes and CIE colour coordinates (L*,a*,b*) to a greater extent as compared to tender coconut water, TSS and citric acid concentration. The optimized beverage was found shelf stable at 5, 25 and 35^0C for a period of 6 months on the basis of physico-chemical and microbiological attributes. The developed beverage showed good consumer acceptability even after 6 months of storage and the optimum levels of the variables can be made use of in commercial production of the pomegranate juice blended tender coconut water beverage.

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