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Pigments and antioxidant activity of optimized Ready-to-Drink (RTD) Beetroot (*Beta vulgaris* L.) - passion fruit *(Passiflora edulis var. flavicarpa)* juice blend

T. Kathiravan[*], S. Nadanasabapathi, R. Kumar

Food Engineering and Packaging Division, Defence Food Research Laboratory, DRDO, Mysore-570 011, Karnataka, India

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Summary

A central composite rotatable design (CCRD) was employed to optimize the beetroot-passion fruit blended juice. The independent variables were beetroot juice (30-70 ml), passion fruit juice (10-30 ml) and sucrose (9.00-10.50g). The combined effect of these independent variables on pH, °Brix and overall acceptability were investigated. Results showed that the generated regression models adequately explained the data variation and significantly represented the actual relationship between the independent variables and the responses. The optimized blended juice was prepared in bulk, thermally pasteurized and studied the effects on pigments, antioxidant activity, CIE Color (L*, a*, b*, ΔE*), Browning Index*, native microflora and other physicochemical quality parameters were also evaluated during the storage at 27-30 ± 2 °C. The processing and storage had a significant effect on ($p < 0.05$) degradation in the pigments, antioxidant activity during storage. The decrease in antioxidant activity was correlated ($r = 0.9895$) with a decrease in betalain pigment. The product was safe from microflora after thermal pasteurization and during 180 days of storage with good sensory acceptability.

Keywords: central composite rotatable design, antioxidant activity, CIE Color, pigments

Introduction

Consumer demands for huge juice blends or cocktails, particularly functionalized blended vegetable beverages in present days, due to its nutritional or nutraceutical properties. Juice blending is one of the best methods to improve the nutritional quality of the juice or beverages. It can improve the flavors, functional properties, vitamins and mineral content, which is depending on the kind and quality of fruits and vegetables used (De Carvalho et al., 2007). Apart from improved nutritional quality, blending of juices can be used for promoting the vegetable juice with good taste as well as preservation with mild heat treatment. Furthermore, one could think of a new product development through blending in the form of a natural health drink, which may also be served as an appetizer.

Pigments in fruits and vegetables has been considered to be of nutritional importance in the prevention of chronic diseases, such as cardiovascular disease, various types of cancers, diabetes and neurological diseases (Kalt et al., 1999; Willet, 1994). Beet root is a potential source of valuable water-soluble nitrogenous pigments, called betalains, which comprise two main groups, the red betacyanins and the yellow betaxanthins. They are free radical scavengers and prevent active oxygen-induced and free radical-mediated oxidation of biological molecules (Pedreno and Escribano, 2001). Yellow passion fruit (*Passiflora edulis var. flavicarpa*) juice is globally marketed, mainly for its pleasant unique aroma and flavor. Epidemiological studies have demonstrated that high consumption of fruits and vegetables can provide health benefits due to their antioxidant constituents, including carotenoids, flavonoids, phenolic compounds and vitamins.

Thermal pasteurization is one of the safe method for extending the shelf life of fruit juices by inactivating microorganisms and enzymes, which relies on a mathematical calculation to ensure the safety of the products. Theoretically, this is a combination of the time-temperature profile and the microbial destruction/inactivation. At a pH below 4.5, the risk of growth and toxin production by *Clostridium botulinum* is extremely low and for products with pH values between 4.0 and 4.5, processes are aimed at controlling the survival and growth of spore forming organisms such as *Bacillus coagulans, Bacillus polymyxa, and Bacillus macerans*. A heat process of 9-15 min at 96.0 °C is regarded as adequate for this purpose, when the pH is between 4.0 and 4.3 (Kumar et al., 2013). The antioxidant activity of thermall treated juice had a significant decrese (Kathiravan et al., 2014; Elez-Martinez et al., 2006).

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[*] Corresponding author: kathirtamil56@gmail.com

General practice of optimizing the optimal blend or processing conditions is by means of varying one parameter while keeping the others at a constant level. The major disadvantage of this single-variable optimization method is that it does not include interactive effects among the other independent variables, and therefore it does not represent the net effects of various parameters on their responses (Rai et al., 2004). When many factors are affecting the response, the response surface methodology (RSM) is an effective tool for optimizing the independent variables. Response surface methodologies (RSM) are quite effective in optimizing the blends as well as processing parameters. RSM uses a central composite rotatable design (CCRD) to fit a polynomial model by least-square technique. RSM helps to create a product using regression equations that describe interactions between input parameters and product properties (Kathiravan et al., 2013). The major constraint on optimizing procedures is desired that the overall acceptability. There are some published works on fruit blends and fruit or vegetable blends, pineapple juice blend with carrot and orange juice (Jan and Masih, 2012); fruit juice blending ratios on kinnow juice (Bhardwaj and Mukherjee, 2011); mixed fruit juice spiced beverage (Deka, 2000) and mixed fruit juice spiced RTS beverages (Deka and Sethi, 2001). Most of the researcher optimized the blend by varying one parameter while keeping the others at a constant level. Therefore, blending of two or more fruit and vegetable juices for the preparation of Ready-To-Drink blended beverages is thought to be a convenient and economic alternative for utilization. So far, no work has been carried out on Beetroot (*Beta vulgaris* L.) - passion fruit *(Passiflora edulis var. flavicarpa)* juice blend. Therefore the main objective of this work was to investigate the optimal level of beetroot, passion fruit juice and sucrose using response surface methodology (RSM) and its effect of thermal pasteurization on the stability of pigments, antioxidant activity, native microflora and other physico-chemical properties of Ready-to-Drink (RTD) beetroot-passion fruit blended juice.

Materials and methods

Raw materials

Fresh Beetroots (*Beta vulgaris* L.) were purchased from local market and used immediately for the experiment. Yellow passion fruits (*Passiflora edulis*) were purchased from local horticultural farm at Yelwal, Mysore, India the day before pulping and stored at 4 °C until processing. The beetroot and passion fruits were washed with tap water followed by sterile water.

Beetroot and passion fruit juice preparation

Beetroot was peeled out and sliced, the slices were ground in a wet grinder and then pulped by using hydraulic press (D.K. Barry & Co (P) Ltd, New Delhi, India) and the extracted juice was again filtered using a four layer cheese cloth to remove remaining pomace. Passion fruits were cut into two half pieces, the pulp was removed, pulped using a mixer grinder without breaking the seed, filtered through four layer cheese cloth and transferred into sterile stainless steel vessels prior to processing. The beetroot and passion fruit juice of (1:1) dilution was blended according to the experimental design.

Experimental design

Beetroot and passion fruit juice level was optimized by using Response Surface Methodology (RSM) (Kathiravan et al., 2013). A face-centered central composite response surface analysis was used to optimize the independent variables (levels of beetroot juice, passion fruit juice and sugar). The selected responses were pH, ºBrix and overall acceptability (OAA). The beetroot juice, passion fruit juice and sugar were blended according to the experiment run. The order of assays within a block was randomized and performed in triplicate. The variables and their levels of central composite rotatable design (CCRD) were represented in the Table 1. The experimental design was performed in one block of experiments. The results for the central composite rotatable design (CCRD) were used to fit second-order polynomial equation. However, the regression analysis of the responses was conducted by fitting suitable models represented by (1) and (2):

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i \tag{1}$$

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i \neq j=1}^{n} \beta_{ii} x_i x_{ij} \tag{2}$$

where:

β_0 was the value of the fitted response at the center point of the design, i.e., point (0,0,0) in case beetroot juice, passion fruit juice and sucrose; 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. All the individual desirability functions obtained for each response were combined into an overall expression, which is defined as the geometric mean of the individual functions.

Table 1. Variables and their levels for central composite rotatable design for beetroot-passion juice blend

Variables		Range of Levels			
		Low Actual	Low Coded	High Actual	High Coded
A	Beetroot Juice (ml)	30.00	-1.000	70.00	1.000
B	Passion fruit Juice (ml)	10.00	-1.000	30.00	1.000
C	Sugar (Sucrose) (g)	9.00	-1.000	10.50	1.000

The higher the desirability value, the more adequate is the system. In the present study, desirability functions were developed in order to obtain target pH, °Brix with higher overall acceptability (OAA). Design Expert 7.0.0 software (Stat Ease Inc., Minneapolis, MN) was used to generate the quadratic models that fit the experimental data, draw the response surface plots and optimize beetroot juice, passion fruit juice and sugar. Analysis of Variance (ANOVA) was performed to obtain the coefficients of the final equation for better accuracy. Three-dimensional surface plots were drawn to illustrate the interactive effects of two factors on the dependent variables, while keeping constant the other variables. All variables of the polynomial regression at a significance level of $p < 0.05$ were included in the model, and the coefficient of determination (R^2) was generated in order to assess the adequacy of the model. The response surfaces were generated from the equation of the second order polynomial, using the values of each independent variable to the maximum quadratic response.

Thermal pasteurization

Beetroot-passion fruit juice blend of 200 ml was filled into sterile pre-fabricated multilayer laminate pouches consisting of 12 μm Polyethylene terephthalate / 15μm Nylon / 9μm Aluminium foil / 80μm Cast. polypropylenes (Total thickness 116μm) of 200 ml capacity with a dimension of 15 X 20 cm and sealed using impulse sealing machine (Model: HP Impulse Sealer, M/S Sunray Industries Mysore, India). Filled pouches were subjected to in-pack thermal pasteurization at 96 °C (product temperature) for 720s using a steam jacketed kettle. For heat penetration studies, the pouches were fixed with thermocouple glands through which copper-constantan thermocouples were placed at the geometrical center of the steam jacketed kettle. A reference thermocouple was also placed and monitored in the steam jacketed kettle. Thermocouple outputs was connected to a data logger (Model: CTF 9004, M/s. Ellab, Denmark). The temperature of the samples and steam jacketed kettle was measured from the thermo-electro-motive-force at regular intervals of 60 seconds. Once the treatment over the whole baskets were removed and placed in the tap water for 2-5 min. *P-value* was determined with a 6D process of inactivation for *Bacillus coagulans* has been used for this study to complete the inactivation of native micro flora (Kumar et al., 2013). The *Z Value*, *D_{96} Value* and the reference temperature were feed into Ellab Val suit Pro software prior to the processing, during processing the time-temperature profile monitored and recorded and *P-value* was calculated with the help of Ellab Val suit Pro software for thermal pasteurized juices. The thermal processed pouches were tested for sterility; the samples were used for further analysis. All the experiments were performed in triplicate.

Methods of analysis

Total soluble solids (°Brix)

The soluble solids (°Brix) were measured using a hand Refractometer (RF.5580 Euromex Brix hand Refractometer). Measurements were performed at 25.0 ± 2 °C. The refractometer prism was cleaned with distilled water after each analysis.

pH

The pH was determined by a pH 700 Digital meter at 25.0 ± 2 °C (Model: Eutech Instruments, Singapore). The pH meter was standardized using pH buffer of 4.0, 7.0 and 10.2.

Acidity

The titratable acidity (TA) was determined by a method described by Kathiravan et al. (2014). Titrating 1 ml of each sample (diluted to 20 ml final volume with deionized water) using 0.1 mol L^{-1} NaOH. Results were expressed as percentage of malic acid 100 ml sample.

CIE Color (L, a*, b*, ΔE* and Browning Index*)*

The CIE Color *(L*, a* and b*)* values were measured using a Hunter Lab Scan Spectrophotometric colorimeter controlled by a computer that calculates color ordinates from the reflectance spectrum. (Hunter Lab Color Flex EZ 45/0° color spectrophotometer, USA). The results were expressed in accordance with the CIELAB system with reference to illuminate D_{65} and with a visual angle of 10°. The samples were placed in an optical glass tray, using the white plate of the colorimeter as the background (Standard white plate no. CFEZ0503 X=79.05, Y=84.00, Z=87.76). This background was used to standardize the measurements. The measurements were made through a diaphragm 30 mm. The color values were expressed as *L** (whiteness or brightness/darkness), *a** (redness/greenness) *b** (yellowness/ blueness). Total color difference (ΔE*) and Browning Index (BI*) which indicates the magnitude of color change after treatment was calculated using Eq. (3 and 4) (Tiwari et al., 2009; Palou et al., 1999). Color values were recorded as the mean of triplicate readings.

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (3)$$

$$\text{Browning Index} = [100\ (X-0.31)]\ 10.72 \qquad (4)$$

where: - $X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$

Betalain (Betacyanin and Betaxanthin) pigment analysis

The betalain content (Betacyanin and Betaxanthin) was quantified by a method described by Kathiravan et al. (2014). The blended juice was diluted (30 times) with deionized water and the absorbance of the diluted juice read at 538 nm and 480 nm is using a spectrophotometer (Model: UV-Spectrophotometer, Spectronic® Genesys™ 2 Instruments, USA). The betalain content was calculated using an equation proposed by Cai and Corke (1999). The betacyanin and betaxanthin evaluated as equivalents to betanin and indicaxanthin respectively. Betacyanin content was calculated using Eq. (5):

$$\text{Betacyanin [mg/l]} = A \times DF \times Mw \times 100/ \varepsilon \times L \qquad (5)$$

where in *A* is the absorption value of betanin λ max (538 nm) corrected by the absorption at 700 nm, *DF* is a dilution factor, *Mw* is the betanin molecular weight (550 g mol^{-1}), *ε* is the betanin molar extinction coefficient (60,000 L mol^{-1} cm^{-1}) and *L* is the path length (1.0 cm) of the cuvette. Betaxanthin content was calculated using Eq. (6):

$$\text{Betaxanthin [mg/l]} = A \times DF \times Mw \times 100/ \varepsilon \times L \qquad (6)$$

where:
A is the absorption value of indicaxanthin λ max (480 nm) corrected by the absorption at 700 nm, *Mw* is the indicaxanthin molecular weight (308 g mol^{-1}), *ε* is the indicaxanthin molar extinction coefficient (48,000 L mol^{-1} cm^{-1}).

Antioxidant (% of DPPH radical scavenging) activity

The percentage of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the blended juice was determined by a method described by Kathiravan et al., (2014). The hydrogen atom or electron donation abilities of the juice were measured from the bleaching of a purple-colored methanol solution of stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). Briefly, 0.1 ml of samples or 0.1 ml of methanol (control) were mixed with 2.9 ml of 0.004% DPPH solution (10 mg in 250 ml of methanol prepared freshly) and methanol used as a blank. The mixture was vortexed thoroughly for 1 min and left at 37 °C temperature for 30 min in darkness and then the spectrophotometer absorbance was read against blank at 517nm (Model: UV Spectrophotometer, Spectronic® Genesys™ 2 Instruments, USA).
The capability to scavenge the DPPH radicals, DPPH scavenging activity (SA_{DPPH}), was calculated using Eq. (7):

$$\textbf{Scavenging Activity}_{\textbf{DPPH}} = \frac{(A_{Control} - A_{Sample})}{(A_{Control})} \text{ X } 100 \qquad (7)$$

where:
$A_{control}$: absorbance of the control reaction (containing all reagents except the juice)
A_{sample}: absorbance in the presence of the juice

Total carotenoids

Total carotenoids were determined spectrophotometrically (UV-Spectrophotometer, Spectronic® Genesys™ 2 Instruments, Made in USA) by a method described by Kumar et al. (2013). A 5g blended juice sample was mixed with 20ml of acetone and kept in dark for 10-15min, then the contents were filtered through a sintered funnel under suction and 20 ml of acetone was added twice to extract the pigments followed by the addition of 20ml of hexane to extract the pigment completely. The combined extract was transferred to a separating funnel. After 5 min the aqueous layer was completely discarded and transferred the hexane layer to 250ml volumetric flask and the volume was made up to the mark with hexane. A pinch of anhydrous sodium sulphate was added and absorbance was read at 450nm against hexane as blank. The total carotene was expressed as µg /100 ml. The total carotenoid content of each sample was estimated calculated using Eq. (8):

$$\text{Total Carotene}_{\mu g/100ml} = \frac{A_{450}\,X\,250\,X\,1000\,X\,100}{250\,X\,\text{Wt of sample}} \qquad (8)$$

Micro flora and sensory analysis analysis

Microbial sensory analysis was carried out. For the microbial counts, samples were serially diluted, plated in total count agar (PCA) for total plate (aerobic) counts, and in acidified Potato dextrose agar (PDA) for mould and yeast counts. Plates were incubated at 30 °C for 48h and 5 days for Total Plate Counts and Moulds and Yeast respectively. Violet Red Bile Agar was used for Coliforms. Sensory quality was determined using 9 points Hedonic scale rating. For sensory taste and odour evaluation and overall acceptability, 20 semi-trained volunteers were selected. The 100 ml samples (treated blended juice) were presented to the judges. The judges rated the preferred samples in comparison with control (untreated) (Kathiravan et al., 2013).

Statistical analysis

Data were analyzed by the least-squares method and response surfaces were generated using the Design Expert® 7.0.0 software (Stat Ease Inc., Minneapolis, MN). Analysis of variance (ANOVA) was used to test the significance of each variable (α = 0.05) and to verify the adequacy of the model. Interaction effects were determined using LS means (α = 0.05). All assays were carried out in triplicate. All the experiments were conducted in triplicate. The data were analyzed by Analysis of Variance (ANOVA) (SAS version 9.1 software (SAS Institute, Cary, NC, USA) during storage period. Significant differences ($p < 0.05$) between treatment means were determined by the Student-Newman-Keuls's test.

Results and discussion

Table 2 shows a Central Composite Rotatable Design (CCRD) with three independent variables (beetroot juice, passion fruit juice and sucrose) including five replicates of the center point which were used to study the response (pH, °Brix and OAA) model and to find out the optimum combination of the those variables.

The regression analysis was performed for the result of the responses of central composite rotatable design (CCRD) to fit suitable models. The coefficient of determination (R^2) was generated in order to assess the adequacy of the model. The pH response of the beetroot-passion juice blend was fitted with quadratic model, whereas linear model was fitted with responses °Brix and overall acceptability (OAA) of the beetroot-passion juice blend.

The *p* value indicates the *p* > F-values which should be less than 0.05 for the model to be significant. The *p* values of the resulted responses were found to be less than 0.05. The models of the each response were significant. The effects of selected independent variables on the responses were reported (Table 3). Visualization of the response surfaces for pH, °Brix and overall acceptability (OAA) were represented in Fig. 1-3.

Response surface model fitting

Table 3 shows the analysis of the variance (ANOVA) of the responses in the models and their corresponding R^2. Response surface models developed in this study for predicting the pH was adequate, whereas the response surface models developed for °Brix and OAA found to be slightly inadequate compared to model of pH. For the best fit of response surface models, R^2 should be at ≥ 0.80. The R^2 values for these responses were more than 0.80, representing that the regression models fitted excellently. The response of the pH R^2 value was found to be 0.9683, whereas the R^2 values of °Brix and OAA (0.7845 and 0.4461 respectively) were low, viewing that a high proportion of variability were not explained by the models (Table 3). Sin et al. (2006) also suggested that the $R^2 > 0.80$ for a good fit of response model. Our results accordance with the author who studied the optimization of pulsed electric field processing conditions for passion fruit juice, also found that all response models good fit with an R^2 of more than 0.80 (Kathiravan et al., 2013). The probability (*p*) values of all regression models were less than 0.0001 except in response of OAA (0.0210).

Table 3 clearly indicating that pH was significantly affected by the quadratically ($p > 0.0001$), whereas °Brix and overall acceptability (OAA) was significantly affected linearly ($p > 0.0001$, $p > 0.0210$). The interaction between the independent variables (beetroot juice, passion juice and sucrose) showed no significant effect on the responses ($p > 0.05$). The linear model of °Brix and overall acceptability (OAA) R^2 found to be 0.7845 and 0.4461 respectively. It is verifying that the variables were not much affected the °Brix and overall acceptability (OAA) of the blended juice, whereas the pH describes the quadratic model with accuracy (R^2 0.9683) (Table 3). The pH of the beetroot-passion fruit juice blend was significantly ($p > 0.05$) affected by the independent variable (passion fruit juice), when the volume of the passion fruit juice increased, the pH of the blended juice decreased considerably (Table 2). Calle et al. (2002) also reported that the yellow passion fruit has intense and special aroma and flavor, but only limited amounts of juice can be added to any food product or juice blend, because of its high acidity in nature.

Table 2. Experiment for central composite rotatable design of beetroot-passion juice blend

Run Order	Factors			Responses		
	Beetroot Juice (mL)	Passion fruit Juice (mL)	Sucrose (g)	pH	°Brix	Sensory (OAA)
1	70.00	10.00	9.00	4.31	11	7.8
2	50.00	20.00	9.75	3.39	16	6.1
3	16.36	20.00	9.75	3.2	19	6.2
4	30.00	30.00	10.50	3.1	17	6
5	50.00	20.00	9.75	3.39	16	6.1
6	50.00	20.00	11.01	3.42	18	7.9
7	70.00	30.00	10.50	3.37	12	7.9
8	50.00	36.82	9.75	3.12	15	5.5
9	50.00	3.18	9.75	4.8	17	5.5
10	50.00	20.00	8.49	3.41	15	6.2
11	50.00	20.00	9.75	3.39	16	6
12	30.00	10.00	9.00	3.62	14	5.5
13	50.00	20.00	9.75	3.39	16	5.5
14	70.00	30.00	9.00	3.25	10	6.9
15	50.00	20.00	9.75	3.39	16	6.8
16	30.00	30.00	9.00	3.18	16	5.5
17	30.00	10.00	10.50	3.6	18	6.2
18	70.00	10.00	10.50	4.31	13	8.6
19	50.00	20.00	9.75	3.39	16	7.8
20	83.64	20.00	9.75	3.5	10	6.5

Table 3. Regression coefficients, R^2, adjusted R^2 and probability (p) values for the beetroot-passion juice blend

Term Model	Response		
	pH	°Brix	OAA
F Value	33.97	19.42	4.30
P > *F*	< 0.0001*	< 0.0001*	0.0210*
Mean	3.53	15.05	6.53
Standard deviation	0.11	1.32	0.79
C V %	3.06	8.76	12.11
R squared	0.9683	0.7845	0.4461
Adjusted *R* Squared	0.9398	0.7441	0.3423
Predicted *R* Squared	0.7576	0.6318	0.1212
Adequate Precision	20.922	14.266	6.698

*Significant at 0.05 level

Fig. 1 representing the contour plots for the effect of beetroot juice and passion juice on pH. It was observed that the pH of the blended juice decreased as increased in passion juice. Hence passion fruit juice content is increased, resulting in a decrease in pH.

Fig. 2a indicates that °Brix increased gradually with increasing volume of the juice. However, the effect of beetroot and passion fruit juice on °Brix was not much more effective. The effect of sucrose and beetroot juice on °Brix was presented in the Fig. 2b. °Brix content was increased linearly by increasing in sucrose content; the sucrose was significantly ($p < 0.05$) affecting the °Brix of the blended Beetroot-passion fruit juice. Fig. 2c showed the effect of sucrose and passion juice on °Brix content; it also followed a same trend as Fig. 2b.

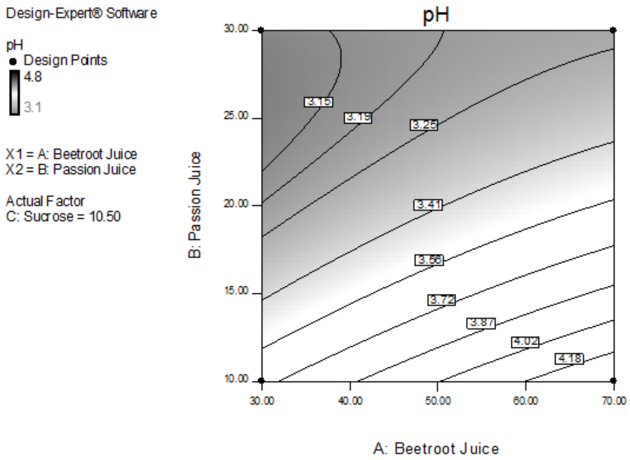

Fig. 1. Contour plots for pH as a function of beetroot and passion juice

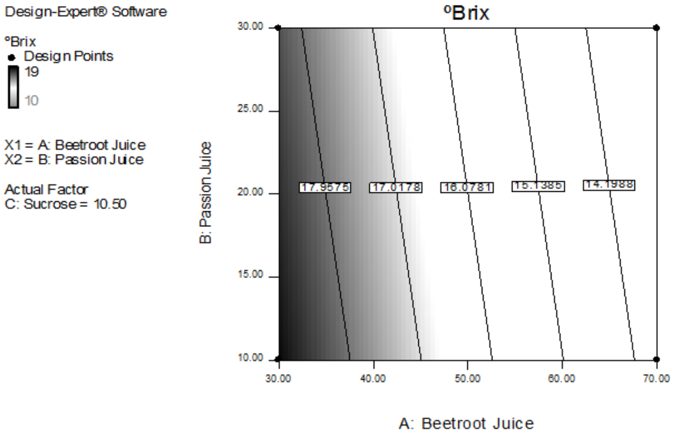

Fig. 2a. Contour plots for °Brix as a function of beetroot and passion juice

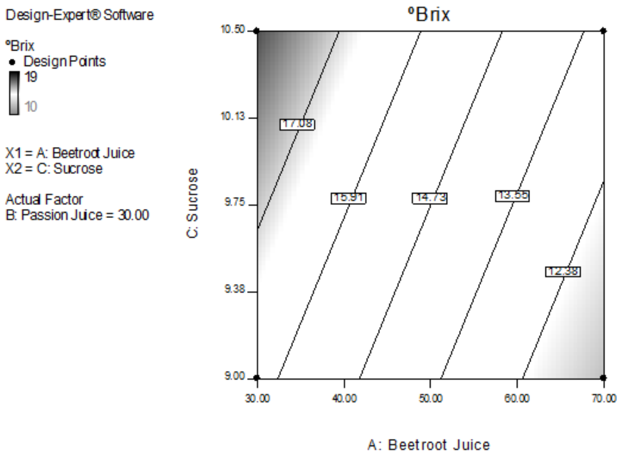

Fig. 2b. Contour plots for °Brix as a function of sucrose and beetroot juice

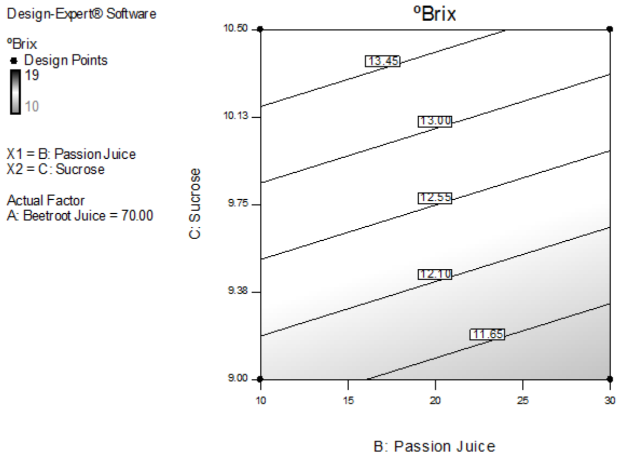

Fig. 2c. Contour plots for °Brix as a function of sucrose and passion fruit juice

Consumer acceptance is one of the most important criteria for any newly developed product. Overall acceptability (OAA) is representing the consumer acceptance; it was also taken as one of the responses for the blended beetroot-passion fruit juice. The regression analysis of the response was conducted and found to be fit with a linear model for overall acceptability response. The analysis of variance was calculated and represented the data (Table 3). Fig. 3 depicts the contour plots for overall acceptability (OAA) as a function of beetroot and passion fruit juice. The combination of the beetroot and passion juice was gradually increasing the overall acceptability (OAA) scores. The escalating trepidation of consumers about their health and new lifestyles that are fascinating them towards increased fresh vegetable juice. Vegetable juices provide antioxidant compounds and a complex mixture of other natural substances that promote antioxidant capacity which leads to health benefits. Beet root ranks among the 10 most powerful vegetables with respect to its antioxidant capacity (Kathiravan et al., 2014). Even though the beetroot has many advantages and health benefits, it also has certain disadvantages. The beetroot juice has nitrate after taste while drinking due to higher content present. Blending of beetroot juice with passion fruit juice leads to an increases in the overall acceptability (OAA) of beetroot-passion fruit juice blend (Table 2). De Carvalho et al. (2007) also reported that juice blending is one of the best methods to improve the nutritional and sensory quality of the juices. It can improve the vitamin and mineral content depending on the kind and quality of fruits and vegetables used for blending.

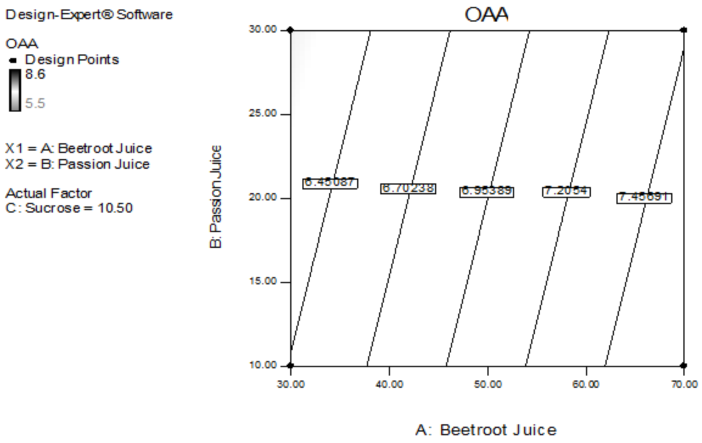

Fig. 3. Contour plots for OAA as a function of beetroot and passion fruit juice

Second-order polynomial, and linear equations can be obtained by the Design Expert® 7.0.0 software. Multiple regression equations (in terms of coded factors) as obtained for responses fitted models represented as follows:

Y_1 = +3.39 +0.16 * A -0.42 * B +2.696E-003 * C - 0.13 * A * B +0.028 * A* C + 7.500E-003 * B * C - 0.013 * A^2 +0.20 * B^2 + 0.010 * C^2

Y_2 = +15.05-2.50 * A -0.32 * B +1.03 * C

Y_3 = +6.53 +0.62 * A -0.13 * B + 0.43 * C

Here, Y_1 is pH, Y_2 is °Brix, Y_3 is OAA, A is Beetroot juice, B is Passion fruit juice and C is Sucrose.

Optimization of beetroot-passion fruit juice blend and thermal pasteurization

The numerical optimization technique is implemented to find out the best optimum combination for the beetroot-passion fruit juice blend. It is one of the best technique radically reduces the amount of time and effort required for the investigation of multifactor and multiple-response systems. Wadikar et al. (2010) studied the development of ginger based ready-to-eat appetizers and optimized the variable levels by desirable maximization of the necessary response by numerical optimization procedure. An acceptable compromise is made based on the following criteria: $Y_1 \geq 3.37$, $Y_2 \geq 13.4$ and $Y_3 \geq 7.5$. The contour plots for each of the responses generated are shown in Fig. 1-3 and can be compared visually. The independent variable levels were optimized as 70.0 ml of beetroot juice, 30 ml of passion fruit juice with 10.50 g of sucrose (sugar). The blended Beetroot-passion fruit juice was prepared using optimized composition were verified for the predicted values and the actual values for the responses. Since these values (Table 4) were almost similar, the fitted models are suitable for predicting the response. The optimized Beetroot-passion fruit juice blend was prepared in bulk and exposed to thermal pasteurization using the equipment and conditions described in thermal pasteurization section. Total process time (*fh*), and *p*-value of thermal pasteurization was achieved as 720 s and 669 s. Further the processed Beetroot-passion fruit blended juice was analyzed for its effect of thermal pasteurization and also changes during ambient storage on physico-chemical, pigments, free radical scavenging activity (Antioxidant activity), sensory and micro flora content using the methods described in the method of analysis section.

Table 4. Predicted responses vs. actual response

Values	Response		
	pH	°Brix	Sensory (OAA)
Predicted	3.37	13.42	7.51
Actual	3.36	12.0	7.8

Effect of thermal pasteurization and storage on physico-chemical and micro flora of beetroot-passion fruit juice blend

The physico-chemical properties like pH, acidity, °Brix, CIE Color (L*, a*, b*, ΔE*), Browning Index* and microflora were evaluated for its thermal pasteurization effect and storage stability. The result indicated that the pH (4.24 ± 0.02) and total soluble solids 13 °Brix of thermally pasteurized blended juice were higher than the control (4.15 ± 0.05 and 12 for pH and °Brix respectively), the acidity of the blended juice reduced (0.1008 ± 0.0008) when compare to control (0.1009 ± 0.0007). The decrease in total titratable acidity related to the increase found in pH. During the ambient storage pH, acidity and total soluble solids remained invariable and also no significant ($p > 0.05$) changes were observed during the storage of 180 days (data not shown). Our results accordance with authors who have studied the thermal pasteurization and storage on physico-chemical parameters of RTD beetroot juice (Kathiravan et al., 2014); apple cider juice (Tandon et al., 2003); blended orange and carrot juice (Rivas et al., 2006); orange juice (Yeom et al., 2000) and Valencia and navel orange juice (Bull et al., 2004) reported that pH, acidity and soluble solids (°Brix) of pasteurized or heated juices were no significant ($p > 0.05$) changes throughout the storage time. pH is one of the main quality characteristics in the all beverages and juice / juice blends (Sanchez-Moreno et al., 2006). Beetroot-passion blended juice found to be no significant ($p > 0.05$) change and it prove that blended juice quality was maintained during entire storage.

The thermal pasteurization of blended beetroot-passion juice microflora was completely inactivated and remained sterile throughout the storage, whereas the control had a microbial load of 6.40, 6.23 and 3.23 log CFU/mL for the total plate count, coliforms, yeast and moulds, respectively. Our results were also in accordance with authors who studied the thermal processed grape juice (Fontanet et al., 2013) and thermally pasteurized Beetroot juice (Kathiravan et al., 2014) they also reported that thermal pasteurization able to inactivate 6.53 log CFU/mL of native microflora.

Table 5 representing the effect of thermal pasteurization on the CIE Color of L*, a*, b*, ΔE* and browning index (BI*). The control (untreated) blended juice sample had a redness (a*), yellowness (b*) / blueness (-a*), luminosity (L*) and browning index (BI*) values were 4.29 ± 0.01, -0.62 ± 0.01, 3.71 ± 0.01 and 94.121 ± 0.684 respectively. Thermal pasteurization significantly ($p < 0.05$) affecting the redness (a*), yellowness (b*) and luminosity (L*) value of blended juice, the total color difference (ΔE*) was found to be 1.233 ± 0.071. The luminosity (L*) values for 15-60th day and 90-150th day for the redness (a*) values 15-30th day, 45-60th day and 135-150th day storage shows non significantly ($p > 0.05$) changes, whereas yellowness (b*), total color difference (ΔE*) and browning index (BI*) shows significant ($p < 0.05$) difference throughout storage. The increase in the total color difference (ΔE*) associated with degradation of betalain pigment (Betaxanthin and Betacyanin) due to thermal pasteurization and the storage effect of blended juice. Chandran et al. (2012) also reported that the betacyanin content change to yellowish-brown from deep violet-red color during heat treatment. Authors who studied the heat pasteurization of fruit juices (Zhang et al., 1997); RTD mango nectar (Kumar et al., 2013) and RTD Beetroot juice (Kathiravan et al., 2014) also found a significant ($p < 0.05$) color degradation in the heat pasteurized juice compared to control (untreated) samples during storage.

Effect of thermal pasteurization and storage on pigments, free radical scavenging activity (Antioxidant activity) and sensory quality of beetroot-passion fruit juice blend

Plant pigments are secondary metabolites of plants. They are chemical compounds that absorb light in the wavelength of the visible region. Color is produced due to a molecule specific structure in the pigment, called chromophore. The healthbenefit of natural pigments has been concentrating by many researchers, especially those of carotenoids and anthocyanins, whose antioxidant properties have been extensively studied. Betalains, because of their relative scarceness in nature, have not been much explored as bioactive compounds, but some studies have indicated their potential as antioxidant pigments (Azeredo, 2009). Thermal pasteurization and storage effect on total betalain pigment (Betaxanthin and Betacyanin), total carotenoids, antioxidant activity and sensory quality (OAA) were analyzed and presented in the Table 6.

Table 5. The effect of pasteurization and storage on CIE L *, a*, b*, ΔE* and BI* of RTD beetroot-passion blended juice

Storage days	L *	a*	b*	ΔE*	BI*
Control	3.71 ± 0.01^{f}	4.29 ± 0.01^{k}	-0.62 ± 0.01^{m}		94.121 ± 0.684^{l}
0	4.15 ± 0.04^{e}	5.28 ± 0.07^{j}	-0.04 ± 0.01^{l}	1.233 ± 0.071^{l}	134.004 ± 0.401^{k}
15	4.25 ± 0.02$^{d, e}$	5.74 ± 0.17^{i}	1.56 ± 0.02^{k}	2.680 ± 0.092^{k}	232.226 ± 3.425^{j}
30	4.34 ± 0.01$^{d, e}$	5.79 ± 0.03^{i}	2.07 ± 0.02^{j}	3.146 ± 0.027^{j}	263.918 ± 0.822^{i}
45	4.38 ± 0.01$^{d, e}$	5.97 ± 0.01^{h}	2.21 ± 0.01^{i}	3.366 ± 0.004^{i}	276.053 ± 0.844^{g}
60	4.48 ± 0.01^{d}	6.06 ± 0.06^{h}	2.32 ± 0.01^{h}	3.523 ± 0.034^{h}	279.579 ± 1.102^{g}
75	4.68 ± 0.01^{e}	6.16 ± 0.02^{g}	2.35 ± 0.01^{h}	3.558 ± 0.063^{h}	270.697 ± 0.680^{h}
90	4.76 ± 0.02^{c}	6.26 ± 0.02^{f}	2.75 ± 0.01^{g}	4.044 ± 0.016^{g}	297.376 ± 1.668^{f}
105	4.83 ± 0.02^{c}	6.38 ± 0.02^{e}	2.93 ± 0.04^{f}	4.274 ± 0.030^{f}	308.302 ± 4.785^{e}
120	4.86 ± 0.01^{c}	6.59 ± 0.08^{d}	3.08 ± 0.02^{e}	4.508 ± 0.053^{e}	321.339 ± 1.570^{d}
135	4.88 ± 0.01^{c}	6.82 ± 0.06^{c}	3.26 ± 0.02^{d}	4.784 ± 0.055^{d}	338.445 ± 2.430^{b}
150	4.94 ± 0.02^{c}	6.88 ± 0.01^{c}	3.67 ± 0.02^{c}	5.165 ± 0.025^{c}	370.154 ± 3.750^{a}
165	5.60 ± 0.07^{b}	7.06 ± 0.02^{b}	3.76 ± 0.02^{b}	5.524 ± 0.033^{b}	328.945 ± 5.506^{c}
180	6.22 ± 0.01^{a}	7.37 ± 0.02^{a}	3.91 ± 0.06^{a}	6.079 ± 0.082^{a}	305.224 ± 4.441^{e}

All values are means ± standard deviation of data from three independent experiments. Different lowercase letters (a-m) in the same column indicate significant difference ($p<0.05$).

Table 6. Thermal pasteurization and storage effects on pigments, antioxidant activity and OAA of RTD Beetroot-passion blended juice

Storage days	Betaxanthin (mg/L)	Betacyanin (mg/L)	Total Carotenoids µg/100mL	Antioxidant activity (%)	OAA
Control	126.330 ± 0.490^{a}	135.270 ± 0.066^{a}	200.250 ± 0.26^{a}	67.427 ± 0.153^{a}	8.10 ± 0.10^{a}
0	80.353 ± 0.143^{b}	94.437 ± 1.812^{b}	150.203 ± 0.072^{b}	51.573 ± 0.353^{b}	8.07 ± 0.10^{b}
15	79.180 ± 0.572^{c}	88.210 ± 0.286^{b}	148.160 ± 0.173^{c}	48.440 ± 0.182^{c}	8.07 ± 0.12^{b}
30	74.860 ± 0.364^{d}	$75.050 \pm 18.014^{c, d}$	145.493 ± 0.248^{d}	45.330 ± 0.336^{d}	8.03 ± 0.06^{b}
45	65.100 ± 0.806^{e}	77.917 ± 0.577^{c}	140.247 ± 0.147^{e}	43.217 ± 0.058^{e}	$8.00 \pm 0.10^{b, c}$
60	60.117 ± 0.135^{f}	$72.460 \pm 0.466^{c, d, e}$	138.647 ± 0.195^{f}	41.363 ± 0.373^{f}	$7.93 \pm 0.06^{b, c}$
75	58.443 ± 0.396^{g}	$69.050 \pm 0.808^{d,e, f}$	132.827 ± 0.057^{g}	38.447 ± 0.482^{g}	$7.87 \pm 0.06^{c, d}$
90	47.187 ± 0.324^{h}	$65.527 \pm 0.396^{e, f, g}$	130.483 ± 0.204^{h}	36.127 ± 0.304^{h}	$7.73 \pm 0.15^{d, e}$
105	35.760 ± 0.193^{i}	$61.217 \pm 0.174^{f, g}$	128.350 ± 0.200^{i}	33.383 ± 0.115^{i}	$7.67 \pm 0.12^{e, f}$
120	31.253 ± 0.305^{j}	59.713 ± 0.058^{g}	125.553 ± 0.110^{j}	32.627 ± 0.133^{j}	$7.63 \pm 0.06^{e, f, g}$
135	28.507 ± 0.418^{k}	58.483 ± 0.153^{g}	115.740 ± 0.100^{k}	30.317 ± 0.115^{k}	$7.63 \pm 0.06^{e, f, g}$
150	27.530 ± 0.085^{l}	$57.297 \pm 0.332^{g, h}$	110.647 ± 0.115^{l}	27.657 ± 0.203^{l}	$7.57 \pm 0.06^{f, g}$
165	26.307 ± 0.169^{m}	$49.507 \pm 0.150^{h, i}$	88.183 ± 0.115^{m}	25.683 ± 0.300^{m}	$7.50 \pm 0.00^{g, h}$
180	25.627 ± 0.142^{n}	42.420 ± 0.121^{i}	70.503 ± 0.225^{n}	21.317 ± 0.058^{n}	7.40 ± 0.10^{h}

All values are means ± standard deviation of data from three independent experiments. Different lowercase letters (a-n) in the same column indicate significant difference ($P < 0.05$).

Blended juice had more part of beetroot juice than passion fruit juice, it leads to the more betalain pigment. The Betalain is an important pigment component it possesses antioxidant ability and provides the protection against free radicals. It is also considered an indicator quality of juices, the higher degradation of betalain content leads to consumer dissatisfaction.

Thermal pasteurization leads to a significant ($p < 0.05$) degradation by 36.39 and 30.18% of betaxanthin and betacyanin content (Table 6). Further betaxanthin content was decreased significantly ($p < 0.05$) compared to betacyanin content during storage. Whereas the betacyanin content of the beetroot-passion blended juice initially decreased non significantly ($p > 0.05$) and followed gradually decrease. Degradation by 68.10 and 55.08% for betaxanthin and betacyanin content were observed during 180 day of storage. Betacyanin content was found to be more stable compared to betaxanthin content. Our results were in accordance with authors who studied the stability of betalains and reported that betacyanins have found to be more stable than betaxanthins, room temperature (Sapers and Hornstein, 1979) heating (Singer and von Elbe, 1980; Herbach et al., 2004) and upon thermal pasteurization (Kathiravan et al., 2014). Similarly Fan yung and Khotivari (1975); Herbach et al. (2004) also observed that degradation of betalain pigment during heating. Kavitha Ravichandran et al. (2010), studied the effect of different treatments on red beet and reported that betalain content was decreased during boiling and roasting. Total carotenoids of the beetroot-passion blended juice was decreased up to 24.99% during thermal pasteurization of blended Beetroot-passion fruit juice. Further, it was reduced significantly ($p < 0.05$) up to 53.06% at 180 days of storage. Other author (Kumar et al., 2013) studied the thermal processing of mango nectar and reported up to 63.05% of total carotenoid degradation occurred during the 180 days of storage. Subagio and Morita, (2001) also reported that depending on the severity of the thermal treatments used in the processing of foods, isomerization and oxidative degradation of carotenoids can be induced. Betalain pigments of beetroot juice possess antioxidant activity *in vitro*, which was revealed by their ability to inhibit lipid peroxidation in membranes and in the linoleate system (Kanner et al., 2001). The 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity (Antioxidant activity) of the blended juice was also performed to find out the reduction during thermal pasteurization. The untreated (control) blended beetroot-passion fruit juice antioxidant activity was found to be 67.427 ± 0.153 %. Significant ($p < 0.05$) reduction (23.51%) of antioxidant activity was occurring during thermal pasteurization. Further it was reduced up to 58.66% during storage period. The decrease in antioxidant activity was correlated ($r = 0.9895$) with a decease in betalain pigment. Czapski et al. (2009) also reported that the antioxidant capacity of beet juice is strongly correlated with contents of red pigments. The high antioxidant capacity was due to their higher pigment contents. Kathiravan et al. (2014) also observed the drastic decrease in the antioxidant activity of the thermally pasteurized RTD Beetroot juice. Another author who observed the thermally treated orange juice had a significant ($p < 0.05$) decrease in the antioxidant activity (Elez-Martinez et al., 2006). The sensory score analysis of the blended juice was performed based on the 9 point hedonic scale rating given by the panelist, the overall acceptability (OAA) scores of the blended juice were shown in Table 6. The thermally processed, blended juice was significantly ($p < 0.05$) reduced when compared to control blended juice (8.10 ± 0.10). The sensory score (OAA) was initially reduced non significantly ($p > 0.05$) whereas during 75 days storage the reduction was increased significantly ($p < 0.05$), however the blended Beetroot-passion fruit juice was accepted (7.40 ± 0.10) after 180 days of ambient storage. Our study in accordance with Min and Zhang (2003) who studied the thermally processed juice and found that sensory score was decreased significantly.

Conclusions

The present study demonstrated that vegetable and fruit juice could be optimized by the experiments of central composite rotatable design (CCRD). In pH model the variable with the largest effect is the quadratic of the passion fruit juice, in the °Brix and sensory model it is the linear term of the sucrose and beetroot-passion fruit juice respectively. The numerical optimization is one of the best techniques to find out the best optimum combination. According to the RSM results and the numerical optimization technique the Beetroot juice 70 ml; Passion juice 30 ml and sucrose 10.50g can be recommended as the best optimum combination of the blend. Predicted responses vs. actual response prove that the adequacy of the above models is satisfactory. The thermal pasteurization effect on beetroot-passion fruit blended juice had a decisive impact and significant ($p < 0.05$) degradation in the pigments, antioxidant activity during storage. The product was safe from microflora upto 180 days of ambient storage with good sensory acceptability. Beetroot-passion fruit blended juice is one of the best method to minimize the postharvest loss of fresh produce.

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