Pulsed electric field processing of functional drink based on tender coconut water (*Cococus nucifera* L.) - nannari *(Hemidesmus indicus)* blended beverage

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Summary

Tender coconut water (*Cocos nucifera* L.) Nannari extract (*Hemidesmus indicus* L.) ready-to serve (RTS) blended beverage were optimised. Response Surface Methodology (RSM) was employed to optimize the levels of independent variables (levels of tender coconut water, nannari extract and sugar). The responses of pH, ºBrix, CIE colour (L*, a* and b*) value and OAA were studied. The data obtained were analysed by multiple regression technique to generate suitable mathematical models. The developed blended beverage was processed using pulsed electric field (PEF) with electric field 31.2 kV/cm, 20 pulse widths at 100 Hz frequency to minimise nutritional and sensory attributes losses and compared with conventional thermal pasteurization (96 ºC for 360 s) with *p-value* of 8.03. Thermal pasteurization showed a significant ($p<0.05$) decrease in colour value, radical scavenging activity and overall acceptability after treatment and also during storage, when compared to PEF treated tender coconut water-nannari blended beverage. PEF treatment also achieved a 3.01 ± 0.69 log inactivation, similar to thermal pasteurization of native micro flora. PEF treated tender coconut water-nannari blended beverage was stable up to 120 days under ambient storage condition (27-30 °C).

Keywords: blended beverage, tender coconut water, nannari, PEF processing, RSM

Introduction

The coconut (*Cocos nucifera* L.) is an important fruit tree in the tropical regions and the fruit can be made into a variety of foods and beverages. The edible part of the coconut fruit (coconut meat and coconut water) is the endosperm tissue. At first, the cellular endosperm is translucent and jellylike, but it later hardens at maturity to become white flesh (coconut meat). Unlike the endosperms of other plants (e.g., wheat and corn), the cellularization process in a coconut fruit does not fill up the entire embryo sac cavity, but instead leaves the cavity solution-filled. This solution is commonly known as coconut water and it is of cytoplasmic origin (Janick and Paull, 2008). Nutrients from coconut water are obtained from the seed apoplasm (surrounding cell wall) and are transported symplasmically (through plasmodemata, which is the connection between cytoplasms of adjacent cells) into the endosperm (Patrick and Offler, 2001). Coconut water is one of the most nutritious, wholesome beverages. The water of green coconut is delicious, cooling, nutritional and therapeutic beyond exotic (Sulaxana Kumari et al., 2008).

Nannari/Sarsaparilla is an important drug used in Ayurveda, endowed with many medicinal properties. *Hemidesmus indicus* (L.) is referred to as Indian Sarsaparilla and is known as Sariva in Ayurveda. *Hemidesmus indicus* was found in South India and is often substituted for *H. indicus* (Yoganarasimhan, 2000). The root of *H. indicus* possesses antipyretic, antidiarrheal, astringent, diaphoretic, diuretic, refrigerant, tonic properties, etc. It is used in the treatment of biliousness, blood disorders, dysentery, respiratory disorders, skin diseases, syphilis, fever, leprosy, leucoderma, leucorrhoea, itching, bronchitis, asthma, eye diseases, kidney stones and for epileptic fits in children (Longman, 1996). The phytoconstituents and β- sitosterol have been reported in *H. indicus* (Austin and Jagadeeshan, 2002). Earlier studies reported by Ravishankara et al. (2002), Rao et al. (2005), Gopi and Setty (2010), Saravanan and Nalini (2007) proves that *H. indicus* having the high free radical scavenging activity.

Consumers tend to choose any particular juice or beverage due to its unique combination of fresh like nutritive, functional properties and sensory attributes, such as colour, aroma and flavour. Now a day there is a huge consumer demand for juice blends or cocktails, particularly functionalized blended beverages, 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

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flavours, functional properties, vitamins and mineral contents depending on the kind and quality of fruits and vegetables used (De Carvalho et al., 2007). Apart from the nutritional quality improvement, blended juice can be improved. Moreover, 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. Thermal processing, particularly mild heat treatment is commonly used for the juices/beverage to maintain food safety as well as quality by inactivation of microorganisms and enzymes. However, it was well known that mild heat treatment can adversely affect the sensory quality of juices (Lund, 1977). Thermal preservation is believed to be responsible for a depletion of naturally occurring antioxidants in food (Nicoli et al., 1999).

Consequently, the food industry is looking for technologies that are less aggressive to the factors that identify the food as fresh, nutritive, and healthy. One of these technologies that are acquiring importance as an alternative to heat pasteurization is Pulsed Electric Fields Processing (PEF). Pulsed Electric Field (PEF) is largely a non-thermal process that involves the application of high voltage pulses to a fluid medium flowing between a pair of electrodes. Electric current itself is an important destroying agent; the high voltage pulses typically provide electric field intensity in the range of 10 to 80 kV/cm (Korenev and Mundelein, 2004). Consumer demand for functional foods has led to the processing of Tender coconut water-Nannari blend. These products supply antioxidants, vitamins, amino acids, minerals, and other nutritive and functional compounds. There is no systematic study has been undertaken to develop Nannari based functional drink by using RSM. In this study an attempt has been made to develop tender coconut water-nannari blend by using RSM and processed using pulsed electric field (PEF) to minimise nutritional and sensory attributes losses and compared with thermal treatments.

There are many published works that study the effect of PEF on microorganisms and shelf-life in fruit juices. Inactivation of the microbial flora (mesophilic bacteria, moulds, and yeast) and the shelf-life when stored under different conditions have been tested (Ayhan et al., 2001; Min and Zhang 2002; Rodrigo et al., 2001; Rodrigo et al., 2003a; Rodrigo et al., 2003b; Yeom et al., 2000). The main objective of this study was the development of a functional drink based on tender coconut water-Nannari blend using RSM and also study the effect of pulsed electric field (PEF) processing compared with conventional thermal pasteurization.

Materials and methods

Raw materials

Tender coconut (six month maturity) procured from the Local Market, Mysore, India. The nannari root was collected from Coimbatore, India. The tender coconut and nannari root were washed with tap water followed by sterile water.

Collection of tender coconut water and extraction of nannari extract

Tender coconut surface was cleaned with sterile water; the nut was cut open and water collected through muslin cloth in a sterile stainless steel vessel prior to processing. The nannari root was surface cleaned and 1 kg root was soaked overnight in 2 litre of sterile water under 45 °C, next day the extract was filtered through double layer muslin cloth and stored in a sterile stainless steel vessel prior to processing.

Experimental design

A Response Surface Methodology (RSM) was employed (Kathiravan et al., 2013) to optimize the levels of independent variables (levels of tender coconut water, nannari extract and sugar). A face-centred central composite response surface analysis was used to optimize the independent variables (levels of tender coconut water, nannari extract and sugar). The selected responses were pH, ºBrix, CIE colour (L*, a* and b*) value and overall acceptability (OAA). The levels for each independent parameter were represented in the Table 1. The experimental design along with each experimental condition is shown in Table 2.

Table 1. Experimental ranges and levels of independent variables used in RSM in terms of actual and coded factors for Tender coconut water-Nannari blended beverage

Variables		Range of Levels			
		Low Actual	Low Coded	High Actual	High Coded
A	Tender Coconut Water (ml)	80.00	-1.000	93.00	1.000
B	Nannari Extract (ml)	2.00	-1.000	7.00	1.000
C	Sugar (g)	2.00	-1.000	5.00	1.000

Table 2. Design of experiment of Tender coconut water-Nannari blended beverage

Run Order	Factors			Responses					
	[a]TCW	[b]NE	Sugar	pH	°Brix	CIE colour value			[c]OAA
						L*	a*	b*	
1	86.50	4.50	3.50	4.90	6.00	52.32	0.21	9.40	7.40
2	86.50	4.50	6.02	5.00	9.00	52.30	0.20	9.42	7.40
3	75.57	4.50	3.50	4.80	6.00	50.10	0.32	10.02	7.00
4	86.50	4.50	3.50	4.90	6.00	52.32	0.21	9.40	7.40
5	80.00	7.00	5.00	4.80	7.00	49.53	0.39	12.82	7.00
6	86.50	8.70	3.50	5.10	6.00	50.32	0.42	13.28	7.20
7	86.50	4.50	3.50	4.90	6.00	52.32	0.21	9.40	7.40
8	93.00	2.00	2.00	4.80	6.00	52.01	0.23	8.13	8.10
9	93.00	7.00	5.00	4.90	8.00	51.12	0.29	11.52	8.20
10	86.50	4.50	3.50	4.90	6.00	52.32	0.21	9.40	7.40
11	86.50	4.50	3.50	4.90	6.00	52.32	0.21	9.40	7.40
12	80.00	7.00	2.00	5.00	5.00	49.02	0.40	12.01	7.60
13	86.50	4.50	0.98	4.80	5.00	52.21	0.23	9.32	7.40
14	80.00	2.00	5.00	4.70	7.00	51.32	0.23	8.98	8.00
15	86.50	0.30	3.50	4.70	6.00	58.12	0.01	2.32	9.80
16	86.50	4.50	3.50	4.90	6.00	52.32	0.21	9.40	7.40
17	93.00	7.00	2.00	4.90	5.00	51.34	0.30	11.42	8.00
18	93.00	2.00	5.00	4.70	9.00	51.52	0.22	8.12	7.90
19	80.00	2.00	2.00	4.70	5.00	51.32	0.24	8.13	7.80
20	97.43	4.50	3.50	4.70	7.00	52.00	0.20	9.80	6.90

[a]Tender coconut water
[b]Nannari extract
[c]9 point hedonic scale over all acceptability

The experimental design was performed in one block of experiments. The tender coconut water, nannari extract and sugar were blended according to the experiment run. The order of assays within a block was randomised and performed in triplicate. The results of the central composite designs 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 centre point of the design, i.e., point (0,0,0) in case tender coconut water, nannari extract and sugar; 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.

Analysis of Variance (ANOVA) was performed to obtain the coefficients of the final equation for better accuracy. Design Expert 7.0.0 software (Stat Ease Inc., Minneapolis, MN) was used to generate quadratic models that fit the experimental data, draw the response surface plots and optimise Tender coconut and nannari blend. Three-dimensional surface plots were drawn to illustrate the interactive effects of two factors on the dependent variable, while keeping constant the other variables. The optimisation of independent parameters was done following the method of Derringer and Suich 1980. 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. The higher the desirability value, the more adequate is the system. In the present study, desirability functions were developed in order to obtain maximum OAA with good colour value.

Thermal Pasteurization

Tender coconut water-nannari blend (200ml) 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). Pouches were subjected to in-pack thermal pasteurization at 96 °C for 360 s using a steam jacketed kettle. For heat penetration studies pouches were fixed with thermocouple glands through which copper-constantan thermocouples were placed at the geometrical centre of the steam jacketed kettle. A reference thermocouple was also placed and monitored in the steam jacketed kettle. Thermocouple outputs were 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. The thermal processed pouches were tested for sterility; the samples were used for further analysis. All the experiments were performed in triplicate.

Pulsed electric field processing

PEF treatments were carried out using a pilot scale continuous PEF system (Model: ELCRACK® HVP 5, DIL, German Institute of Food Technologies, Quackenbruck, Germany) with bipolar square-wave pulses through an electrode gap of 7 mm (Kathiravan et al., 2013). The system maximum voltage was 80 kV, the maximum frequency was 1 kHz and the pulse width was adjustable between 4 and 32 µs. The system consisted of co-linear treatment chambers followed by a refrigerated cooling module (-5 °C). The characteristics of the electric pulses delivered such as shape, polarity, width, difference of potential as well as the electric current generated across the electrodes and the pulse frequency were monitored using a digital oscilloscope (Digital touch screen oscilloscope Siemens-Denmark). Temperatures were monitored by two thermocouples (Testo AG-Lenzkirch-Germany) with a pipe wrap type probe attached to the surface of the stainless-steel tubes at the inlet and outlet points of the unit. Recorded temperatures did not exceed 35 °C. The blended beverage was pumped through the system using a peristaltic pump (Type SK 20F-80L14TF T10/1-S Getriebebau NORD GmbH & Co KG-Germany) at a flow rate of 41.5 ltr/hr. The blended beverage was treated with electric field 31.2 kV/cm, 20 pulse widths at 100 Hz frequency. Processed samples were filled with sterile (thermally) pre-fabricated multilayer laminated pouches hermetically sealed. The experiments were performed in triplicate.

Method of analysis

pH

The pH was determined with a 700 Digital pH meter at 23 °C (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 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 $mL^{-}1$ samples (Araujo et al., 2011).

Total Soluble solids (°Brix)

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

CIE colour (L a* b*)*

The CIE colour value (Luminosity (L*), Darkness (-L*), Redness (a*), Greenness (-a*), Yellowness (b*) and Blueness (-b*) was measured using a Hunter Lab Scan Spectrophotometric colorimeter (Kathiravan et al., 2013) controlled by a computer that calculates colour ordinates from the reflectance spectrum. (Hunter Lab Color Flex EZ-USA). The results were expressed in accordance with the CIE LAB system with reference to illuminant 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 30 mm diaphragm.

Antioxidant (% of DPPH radical scavenging) activity

The percentage of 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity of the beetroot juice was determined by a method described by Kathiravan et al. (2014). The hydrogen atom or electron donation abilities of the sample were measured from the bleaching of a purple-coloured 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 vortexes thoroughly for 1 min and left at 37 °C temperature for 30 min in darkness, and then the spectrophotometer (Spectronic® Genesys™ 2 Instruments-USA) absorbance was read against blank at 517nm. The capability to scavenge the DPPH radicals, DPPH scavenging activity (SA_{DPPH}), was calculated using the following equation:

$$SA_{DPPH} (\%) = (A_{control} - A_{sample})/A_{control} \times 100$$

where:

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

Micro flora analysis

Microbial analysis was carried out following the method of Kathiravan et al. (2013). For the microbial counts, samples were serially diluted, plated in total count agar (PCA) for total plate (aerobic) counts. Plates were incubated at 30 °C for 48h for Total Plate Counts.

Sensory quality

Sensory quality was determined using 9 point Hedonic scale rating (Kathiravan et al., 2013). For sensory taste and odour evaluation and overall acceptability, 20 untrained panellists were selected. The 100 ml samples were presented to the panellists. The panellists rated the preferred samples in comparison with control (untreated).

Statistical analysis

Data were analysed 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.

Results and discussion

The °Brix and overall acceptability responses of the tender coconut water-nannari beverage were fitted with quadratic model and other responses were fitted only with linear models. The p-value given in the parameters for each response were for the model significance. The p-value indicates the p > F-values which should be less than 0.05 for the model to be significant. The effect of changes in levels of selected variables on the response parameters has been represented in Fig. 1-6.

Effects of variables on pH and °Brix in tender coconut water-nannari blended beverage

The pH of the tender coconut water-nannari blended beverage was not much affected by the independent variables (Tender coconut water and Nannari extract), when the volume of the nannari extract increased the pH of the blended beverage was also slightly increased (Table 2). The linear model of pH, R^2 found to be 0.5537, it proves that the variables not much affected the pH of the blended beverages. Second-order polynomial models describe with accuracy (R^2 0.9865) changes in °Brix of the tender coconut water-nannari blended beverage) (Table 3), but here the variable of sugar strongly influenced the °Brix content. The result indicated that pH and ºBrix had a significant ($p<0.05$) change occurs with respect to nannari extract and sugar increase respectively (Fig. 1 and 2). Multiple regression equations (in terms of coded factors) as obtained in response of pH and ºBrix have been represented as follows:

pH $Y = + 4.85 - 4.992E - 003 * A + 0.10 * B + 2.662E - 003 * C$

ºBrix $Y = + 6.00 + 0.42 * A - 0.15 * B + 1.22 * C - 0.25 * A * B + 0.25 * A * C + 0.000 * B * C + 0.17 * A^2 - 6.400E\text{-}003 * B^2 + 0.35 * C^2$

Table 3. ANOVA and model statistics for the Tender coconut water-Nannari blended beverage

Term Model	Response					
	pH	°Brix	L*	a*	b*	[b]OAA
F Value	6.62	81.19	4.84	13.43	24.78	3.28
P > *F*	< 0.0041[a]	< 0.0001[a]	< 0.0139[a]	0.0001[a]	< 0.0001[a]	0.0392[a]
Mean	4.85	6.35	51.81	0.25	9.58	7.64
Standard deviation	0.084	0.19	1.42	0.052	1.04	0.44
C V %	1.72	2.98	2.73	21.19	10.82	5.73
R squared	0.5537	0.9865	0.4759	0.7158	0.8229	0.7467
Adjusted *R* Squared	0.4700	0.9743	0.3776	0.6625	0.7897	0.5187
Predicted *R* Squared	0.2227	0.8974	0.0562	0.5131	0.6767	-0.9609
Adequate Precision	9.053	30.774	7.107	12.095	17.439	7.794

[a]Significant at $p < 0.05$
[b]9 point hedonic scale over all acceptability

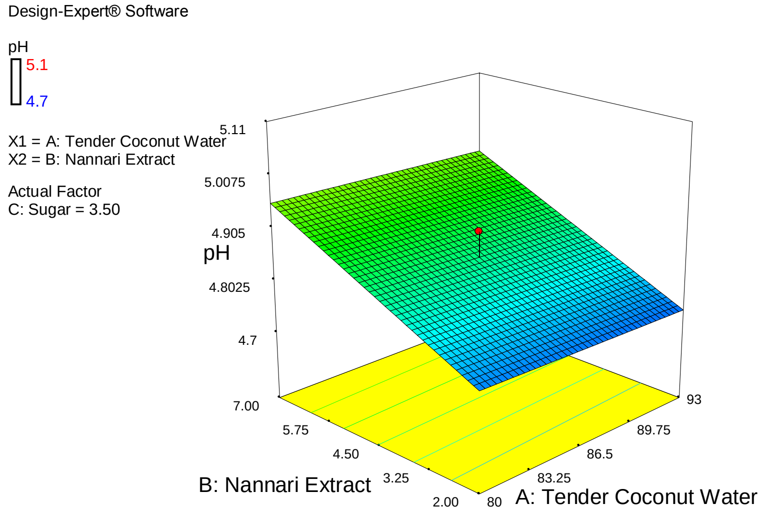

Fig. 1. 3D plot depicting effect of Tender coconut water and Nannari extract on pH of Tender coconut water-Nannari blended beverage

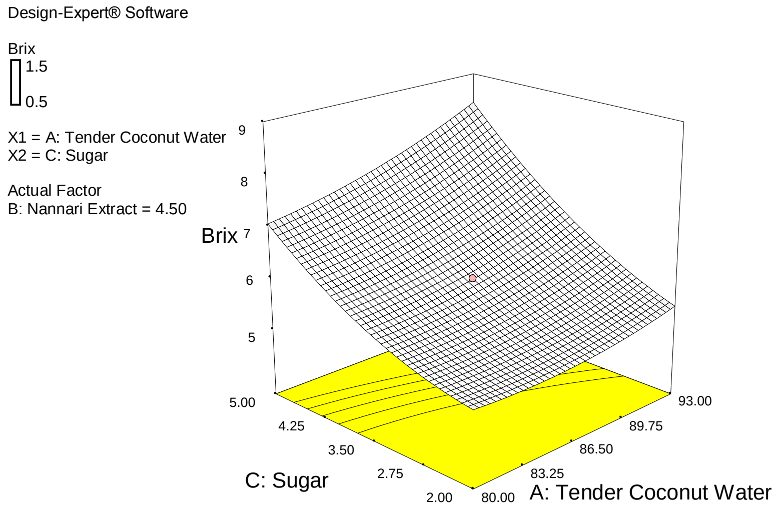

Fig. 2. 3D plot depicting effect of Tender coconut water and sugar on °Brix of Tender coconut water-Nannari blended beverage

Effects of variables on CIE colour values (L, a* and b*) in tender coconut water-nannari blended beverage*

The tender coconut water had a*, b* and L* values were -0.59, 1.36 and 59.43 respectively. When the nannari extract was added into the tender coconut water, it had a significant ($p<0.05$) changes in the a*, b* and L* values of blended beverage (Table 2 and 3). Multiple regression equations were obtained for CIE colour value (L*, a* and b*) represented as follows:

$$L^* \; Y = +\,51.81 + 0.59 * A - 1.34 * B - 3.561E\text{-}003 * C$$

$$a^* \; Y = +\,0.25 - 0.031 * A + 0.084 * B - 6.623E\text{-}003 * C$$

$$b^* \; Y = +\,9.58 - 0.23 * A + 2.40 * B + 0.14 * C$$

The regression analysis indicates that the proposed linear model was fit to L* a* and b* values, L* value describes the significant ($p<0.05$) changes with accuracy of R^2, 0.4759. Values of a* and b* describes the significant ($p <0.05$) changes with accuracy of R^2, 0.7158 and R^2 0.8229 (Table 3). The nannari extract ($p<0.05$) significantly changes the L*, a* and b* values of the blended beverage (Fig. 3, 4 and 5).

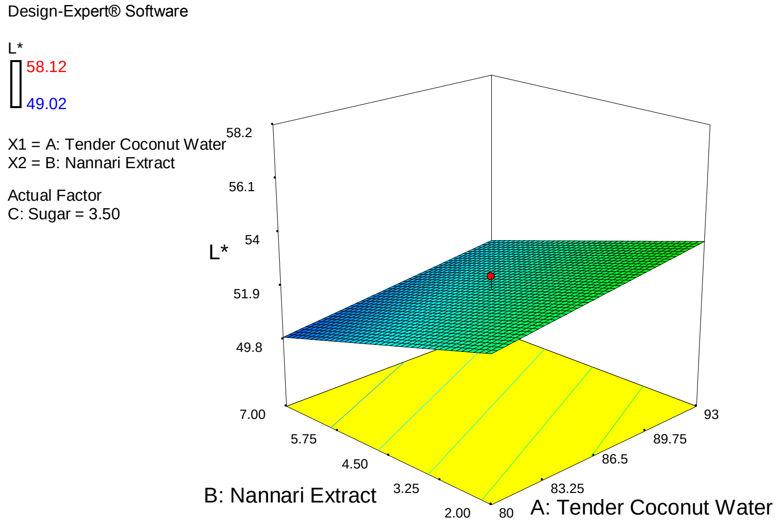

Fig. 3. 3D plot depicting effect of Tender coconut water and Nannari extract on L* value of Tender coconut water-Nannari blended beverage

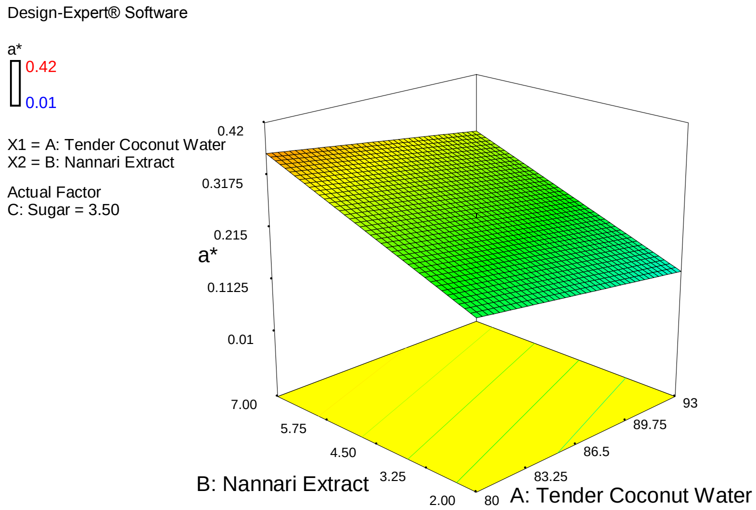

Fig. 4. 3D plot depicting effect of Tender coconut water and Nannari extract on a* value of Tender coconut water-Nannari blended beverage

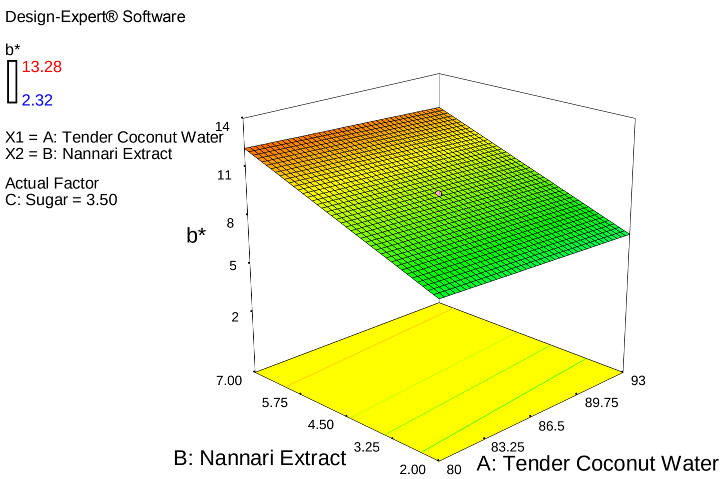

Fig. 5. 3D plot depicting effect of Tender coconut water and Nannari extract on b* value of Tender coconut water -Nannari blended beverage

Effects of variables on OAA in tender coconut water-nannari blended beverage

Sensory score (OAA) is the most important criteria for overall acceptability of any beverages/juices; it was also taken as a response for tender coconut water-nannari blended beverage. The regression analysis of the response was conducted by fitting quadratic models as suitable for sensory response. The analysis of variance was calculated and represented the data (Table 3). Multiple regression equations as obtained for sensory score (OAA) represented as follows:

OAA (Sensory) $Y = +7.39 + 0.12 * A - 0.39 * B - 0.029 * C + 0.18 * A * B + 0.050 * A * C - 0.050 * B * C - 0.12 * A^2 + 0.43 * B^2 + 0.041 * C^2$

The effect of independent variables (tender coconut water and sugar) had been depicted in response surface plots (Fig. 6). Sensory score (OAA) was mainly affected by level of sugar content and the score increased with a decrease in their levels within the experiments range. The independent variables significantly $p<0.05$ affected the overall acceptability of the tender coconut water-nannari blended beverage (Table 3).

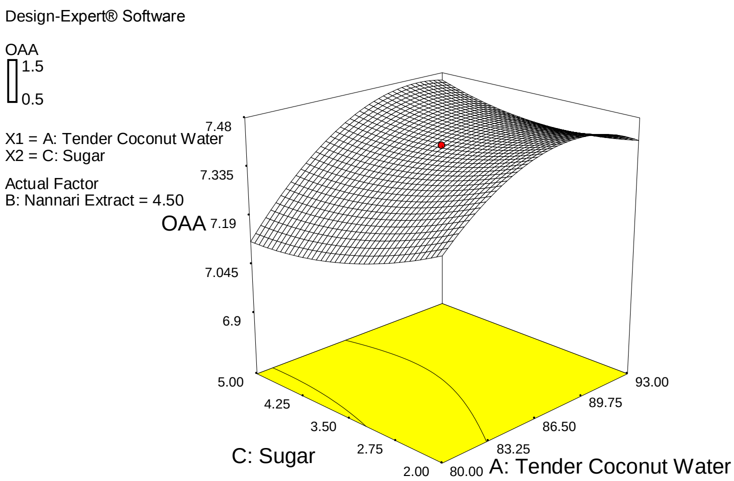

Fig. 6. 3D plot depicting effect of Tender coconut water and sugar on OAA of Tender coconut water-Nannari blended beverage

Optimized tender coconut water-nannari blended beverage and processing

The optimized independent variable levels were 93.0 ml of tender coconut water, 7 ml of nannari extract with 5 g of sugar. The blended beverage was prepared using the optimized beverage composition. The predicted response values and the actual obtained response values for the optimized products (Table 4) were almost similar; hence the fitted models were suitable for predicting the responses. The optimized product tender coconut water-nannari blended beverage was exposed to thermal pasteurization and pulsed electric field (PEF) processing using the equipment and conditions described in "Materials and methods" section. Total process time (*fh*), and *p*-value of thermal pasteurization was achieved as 10 and 8.03 min. The processed products were analysed for their physico-chemical, free radical scavenging activity (Antioxidant activity) and micro flora content using the methods described in the method of analysis section during storage period.

Table 4. Predicted responses vs. actual response for Tender coconut water-Nannari blended beverage

Values	Response					
	pH	°Brix	L*	a*	b*	[a]OAA
Predicted	4.94	8.00	51.05	0.29	11.90	7.61
Actual	4.91	8.00	51.12	0.30	11.75	7.80

[a]9 point hedonic scale over all acceptability

Effect of thermal pasteurization, pulsed electric field processing and storage on quality parameters of tender coconut water-nannari blended beverage under storage

The Optimized blended beverage of tender coconut water-nannari was processed using conventional thermal pasteurization and pulsed electric field processing and analysed the quality parameters for its storage stability and acceptance. The thermal pasteurization and pulsed electric field processing effect on quality parameters during storage was evaluated and presented in Table 5 and 6 respectively.

Table 5. Effect of thermal pasteurization on physico-chemical, antioxidant properties and micro flora of Tender coconut water-nannari blended beverage during storage

Storage days	pH	[b]Acidity	°Brix	CIE Colour values			[c]RSA	[d]OAA	[e]TPC
				L*	a*	b*			
Control	[a]4.91 ± 0.01	0.072 ± 0.001	8.0 ± 0.0	51.25 ± 0.11	0.31 ± 0.01	11.75 ± 0.01	34.87 ± 0.32	7.83 ± 0.06	3.01 ± 0.69
0	5.01 ± 0.01	0.048 ± 0.001	8.1 ± 0.0	49.79 ± 0.30	0.38 ± 0.01	11.22 ± 0.10	20.91 ± 0.01	7.17 ± 0.06	Nil
15	5.02 ± 0.01	0.048 ± 0.012	8.1 ± 0.0	49.05 ± 0.70	0.42 ± 0.01	11.02 ± 0.01	17.84 ± 0.02	7.13 ± 0.06	Nil
30	5.07 ± 0.06	0.049 ± 0.012	8.1 ± 0.0	49.04 ± 0.02	0.46 ± 0.01	10.95 ± 0.05	16.31 ± 0.01	7.10 ± 0.10	Nil
45	5.07 ± 0.06	0.047 ± 0.011	8.1 ± 0.0	48.92 ± 0.01	0.47 ± 0.03	10.57 ± 0.01	15.51 ± 0.01	7.03 ± 0.06	Nil
60	5.06 ± 0.06	0.047 ± 0.011	8.1 ± 0.0	48.81 ± 0.01	0.56 ± 0.02	10.44 ± 0.02	16.82 ± 1.75	6.93 ± 0.06	Nil
75	5.07 ± 0.00	0.046 ± 0.006	8.1 ± 0.0	48.70 ± 0.03	0.58 ± 0.01	10.25 ± 0.01	14.85 ± 0.01	6.77 ± 0.06	Nil
90	5.08 ± 0.06	0.049 ± 0.007	8.1 ± 0.0	48.64 ± 0.03	0.63 ± 0.01	10.02 ± 0.01	14.36 ± 0.01	6.73 ± 0.06	Nil
105	5.06 ± 0.06	0.048 ± 0.006	8.1 ± 0.0	48.46 ± 0.01	0.62 ± 0.01	09.98 ± 0.01	13.81 ± 0.02	6.67 ± 0.06	Nil
120	5.07 ± 0.06	0.047 ± 0.007	8.1 ± 0.0	48.32 ± 0.01	0.63 ± 0.02	09.54 ± 0.03	13.15 ± 0.04	6.57 ± 0.06	Nil

[a]Mean ± SD (n=3)
[b]% of acetic acid
[c]% of Radical Scavenging Activity (RSA)
[d]9 point Hedonic scale over all acceptability
[e]Total Plate Count (TPC) in log^{10}

Table 6. Effect of PEF processing on physico-chemical, antioxidant properties and micro flora of Tender coconut water-nannari blended beverage during storage

Storage days	pH	[b]Acidity	°Brix	CIE Colour values			[c]RSA	[d]OAA	[e]TPC
				L*	a*	b*			
Control	[a]4.91 ± 0.01	0.072 ± 0.001	8.0 ± 0.0	51.25 ± 0.11	0.310 ± 0.010	11.75 ± 0.01	34.87 ± 0.32	7.83 ± 0.06	3.01 ± 0.69
0	4.94 ± 0.01	0.068 ± 0.001	8.0 ± 0.0	50.24 ± 0.25	0.330 ± 0.010	11.65 ± 0.01	30.57 ± 0.06	7.80 ± 0.10	Nil
15	4.93 ± 0.01	0.068 ± 0.001	8.0 ± 0.0	49.96 ± 0.05	0.330 ± 0.010	11.64 ± 0.01	30.17 ± 0.06	7.80 ± 0.17	Nil
30	4.94 ± 0.01	0.068 ± 0.001	8.0 ± 0.0	49.97 ± 0.02	0.333 ± 0.012	11.65 ± 0.01	29.83 ± 0.06	7.80 ± 0.10	Nil
45	4.93 ± 0.01	0.067 ± 0.001	8.0 ± 0.0	49.94 ± 0.03	0.333 ± 0.012	11.53 ± 0.02	29.43 ± 0.06	7.80 ± 0.17	Nil
60	4.94 ± 0.01	0.068 ± 0.001	8.0 ± 0.0	49.95 ± 0.02	0.337 ± 0.029	11.55 ± 0.01	29.27 ± 0.06	7.80 ± 0.10	Nil
75	4.95 ± 0.01	0.067 ± 0.002	8.0 ± 0.0	49.94 ± 0.03	0.340 ± 0.017	11.52 ± 0.01	28.50 ± 0.17	7.80 ± 0.10	Nil
90	4.93 ± 0.01	0.068 ± 0.001	8.0 ± 0.0	49.87 ± 0.01	0.333 ± 0.006	11.52 ± 0.01	27.70 ± 0.10	7.77 ± 0.15	Nil
105	4.92 ± 0.01	0.071 ± 0.001	8.0 ± 0.0	49.80 ± 0.01	0.340 ± 0.010	11.51 ± 0.01	27.10 ± 0.10	7.73 ± 0.21	Nil
120	4.90 ± 0.01	0.076 ± 0.001	8.0 ± 0.0	49.82 ± 0.02	0.343 ± 0.015	11.50 ± 0.01	26.33 ± 0.06	7.60 ± 0.44	Nil

[a]Mean ± SD (n=3)
[b]% of acetic acid
[c]% of Radical Scavenging Activity (RSA)
[d]9 point Hedonic scale over all acceptability
[e]Total Plate Count (TPC) in log^{10}

Effect of thermal pasteurization, pulsed electric field processing and storage on Total soluble solids (°Brix), pH and Total titratable Acidity

The thermal pasteurization effect and storage of the total soluble solids (°Brix) of Control (Untreated) and processed tender coconut water-nannari blended beverage was studied. The result indicated that the total soluble solids (°Brix) of thermally pasteurized tender coconut water-nannari blended beverage were higher than the control. The total soluble solids remained almost invariable and no significant ($p>0.05$) changes were observed during the storage. Our results were accordance to Maciel et al. (1992) who studied the effects of different storage conditions on preservation of coconut water. Tandon et al. (2003) who also studied the storage quality of pasteurized apple cider reported that the higher soluble solids of pasteurised juice are due to water evaporation and no significant ($p>0.05$) changes during storage. In juices or liquid products °Brix is used to indicate the percentage of soluble solids and is one of the important factors for grading the quality of the juice (McAllister, 1980). There was no significant ($p<0.05$) change in the TSS (°Brix) on PEF processing and storage period of the tender coconut water-nannari blended beverage. There are no comparable literature sources concerning to the pulsed electric field processing and storage effect on tender coconut water/tender coconut water-nannari blended beverage so the PEF treated other juices was compared. Similarly, PEF-treated orange juice (35 kV/ cm, 59 μs) stored for 112 days at 2 and 22 °C did not showed any variation in the TSS (°Brix) (Ayhan et al., 2001). On the other hand, shelf-life studies on pasteurized orange juice showed no changes up to 6 months storage after that there is a decrease in sucrose content (Martin et al., 1995).

pH is one of the main quality characteristics in the all beverages and juice/juice blends (Sanchez-Moreno et al., 2006). The pH of tender coconut water-nannari blended beverage was higher than that of control (Table 5). The increase in pH might be due to the ascorbic acid degradation by thermal pasteurization. The pH of the tender coconut water-nannari blended beverage found that no significant ($p>0.05$) changes were observed throughout the storage. It leads to maintain good quality. Our results were accordance to Maciel et al. (1992) who studied the effects of different storage conditions on preservation of coconut water. The pH of the tender coconut water found to be non-significant ($p>0.05$) changes during storage. Rivas et al. (2006) also reported no pH variations in thermally treated juice (blended orange and carrot juice) during storage. Another studies reported by Yeom et al. (2000) observed that non-significant ($p>0.05$) changes in the heated orange juice during storage. The total titratable acidity of tender coconut water-nannari blended beverage was decreased slightly after thermal pasteurization and did not found any changes throughout the storage. The decrease in total titratable acidity related to the increase found in pH after thermal treatment (Table 5). Bull et al. (2004) studied the thermally processed Valencia and Navel orange juice found no significant ($p>0.05$) modifications of total titratable acidity throughout the storage time. The total acidity of also did not show any significant changes (Table 6) It showed a slight increase in total acidity, it related to the decrease found in pH. Our results accordance with Rivas et al. (2006) he also did not found much variations in total acidity of PEF treated juice when compared with an untreated sample.

Effect of thermal pasteurization, pulsed electric field processing and storage on CIE Colour (L a* and b*) values*

The colour of the pasteurized tender coconut water-nannari blended beverage was investigated using CIE colour parameters (L* a* and b*). The colour was not much change after processing, a little change (Table 5) was happened in the change yellowness (b*) of the beverage, it may be due to the thermal processing effect. Table 5 represents the CIE colour values of the thermally pasteurized tender coconut water-nannari blended beverage during storage and control (Untreated). The control juice sample had a*, b* and L* values were 0.310 ± 0.010, 11.75 ± 0.01 and 51.25 ± 0.11 respectively. The a*, b* and L* value of blended beverage had a significant ($p<0.05$) changes under the storage. Zhang et al. (1997) also found a colour degradation in the heat pasteurized juice samples. No significant changes were found in the a* and b* in L* values when compare to thermally processed blended beverage. PEF treated sample did not found much variation during (Table 6), but thermally processed samples showed a significant ($p<0.05$) changes due to the heat applied to the samples. Zhang et al. (1997) also found a better colour preservation in the PEF treated samples when compared to heat pasteurized samples.

Effect of thermal pasteurization, pulsed electric field processing and storage on antioxidant activity

The nannari extract has an excellent *in vitro* free radical scavenging activity, good flavour and excellent medicinal properties (Saravanan and Nalini, 2007). The nannari extract alone had a radical scavenging activity of 80.71 ± 0.17 %. Zahin et al. (2009) who studied the *in vitro* antioxidant activity and total phenolic content of four Indian medicinal plants found that 80.00 of free radical scavenging activity of nannari root. The antioxidant activity of control (untreated) blended beverage had a 34.87 ± 0.32 %, after thermal treatment, it was reduced to 20.91 ± 0.01 %, during the storage time the antioxidant activity was reduced significantly ($p<0.05$) (Table 5). Though the nannari extract alone had higher activity, but the blended beverage has only 7 ml of extract. The combination of nannari and coconut water, ascorbic acid showed the scavenging activity of 34.87 ± 0.32 %. Our results were in accordance with Elez-Martinez et al. (2006) who observed that thermally treated orange juice had a significant decrease in antioxidant activity. There are no comparable literature sources concerning to the thermal pasteurization and the storage effect of antioxidant activity of tender coconut water-nannari blended beverage/nannari extract. PEF processed tender coconut water-nannari blended beverage had a retention of 88% of radical scavenging activity, where as thermally pasteurized samples showed only 60%. Similar results were reported by Plaza et al. (2006), who observed that the PEF process did not much affect the antioxidant capacity of treated orange juice with respect to the untreated sample. Our results are in accordance with Elez-Martinez et al. (2006) who observed that PEF treated orange juice had a higher antioxidant capacity than that thermally treated one.

Effect of thermal pasteurization, pulsed electric field processing and storage on micro flora and sensory analysis

The native micro flora (Total Plate Count (TPC) of tender coconut water-nannari blended beverage had a 3.01 ± 0.69 log CFU/mL, hence the tender coconut water basically sterile in nature until cross contamination (Nadanasabapathi and Kumar, 1999). The initial load of 3 logs may be due to the nannari root. The thermal pasteurization with *p*-value of 8.03 min good enough to inactivate the initial micro flora load and throughout the storage also not detected any micro floral growth. Our results were in accordance with the author (Chowdhury et al., 2009) who studied processing and preservation of green coconut water; also not found any microbial growth in thermally processed tender coconut water (total process time (*fh*) of 10 min for 90 °C). The micro flora was completely inactivated by PEF treatment, and up to 120 days there was no microbial growth in PEF treated samples. Our result was in accordance with Mosqueda-Melgar et al. (2007) who reported that PEF treated watermelon juice (35 kV/cm, pulse width, 4 µs, with bipolar pulses at 188 Hz) had a 3.71 logs inactivation.

The sensory score was based on the 9 point hedonic scale rating given by the panellist, the overall acceptability (OAA) scores of the control were higher rating (7.83 ± 0.06) (Table 5). During storage time, the sensory score was significantly ($p<0.05$) reduced. It was due to the thermal pasteurization effect. Chowdhury et al. (2009) also found that the thermally processed tender coconut water had changed in the sensory score during the storage. Min and Zhang (2003) and Dunn and Pearlman (1987) also found that thermally processed juice had a significant decreased in the sensory score. PEF treated samples were more acceptable than treated by thermal (Table 6). Min et al. (2003); Dunn and Pearlman (1987) confirmed the high sensory quality of PEF treated juice when compared with thermally processed juice.

Conclusions

The optimisation process was aimed at identifying the suitable blend with the combination of tender coconut water-nannari extract with good colour and overall acceptability as ready to serve beverage (RTS). The pilot scale continuous PEF system was effective in inactivating the native micro flora in the tender coconut water-nannari blended beverage, while maintaining the nutritional quality. PEF treatment significantly extended the shelf-life up to 120 days at ambient temperature. The colour stability and antioxidant activity of the blended beverage did not change significantly during the storage, when compared to the conventional thermal pasteurization. The response surface methodology (RSM) seemed to be a suitable statistical tool for defining optimum concentration for real food commodities and PEF is a promising technology for inactivation of the native micro flora and retention of quality in tender coconut water-nannari extract beverage.

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Conflict of interest

The authors claim no conflict of interest.

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