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Optimization of pulsed electric field parameters for mango nectar processing using response surface methodology

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Abstract

Ready to Drink (RTD) mango nectar was subjected to pulsed electric fields (PEF) processing. The effect of process parameters including pulse frequency (70-120 Hz) and pulse width (15-24 µs) on carotenes, total plate count, coliforms, yeast and moulds and over all acceptability was studied using a response surface methodology. Mango nectar exhibited high retention of carotene content at higher frequencies and pulse widths as compared to lower levels. However, severe PEF treatments reduced carotene content. Maximum inactivation of native micro flora (4.1 log CFU/ml), retention of carotene content (2100 µg/100 ml) and over all acceptability (8.3) scores were obtained when PEF treatments were set up to 38.0 kV/cm for 24 µs using bipolar pulses at 120 Hz.

Introduction

The production of fruit nectars are of great importance in fruit juice industry. They are prepared from fruits which are not suitable for direct processing into juice i.e. fruits in which the colour pigment and flavours constituents of which are bound mainly to pulp particles and their pulpy or cloudy preparations are too thick to be drinkable. The production of mango ranks third among the tropical fruits, the seventh largest among all the fruits, but in terms of consumption, mango ranks first worldwide derived from fresh consumption (Barron *et al*., 2002). Mango is an excellent source of vitamin A and C, as the consumption of 100 g of ripe mango will cover more than 50% of the recommended daily amount of each of these vitamins (Proserco, 2004). It provides a certain amount of other vitamins and minerals such as riboflavin, niacin, calcium, phosphorus and iron (Jimenez *et al*., (2004) as well. Fresh fruits and vegetables are an important component of human food, occupying the second place in the food pyramid (Antunes *et al*., 2008). Today, consumers demand food products that are identical to the fresh so the process should, preserve their own physicochemical properties and provide durability, without reducing the specific properties of food. 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 (Tibble, 1998; Williamson, 1999; Gardner *et al*., 2000; Sanchez-Moreno *et al*., 2003).

Thermal processing is believed to be responsible for depletion of naturally occurring nutrients in food. The overall nutritive and sensory qualities of foods may be affected by processing in several ways, including losses of naturally occurring compounds, improvement of their antioxidant properties, as well as formation of novel compounds by Maillard or other reactions that affect nutritional quality (Nicoli *et al*., 1999). Thermal pasteurization is extensively used for inactivation of micro-organisms in foods; recently a growing interest in the development of alternative approaches in response to the desires of consumers for products which are less organoleptically and nutritionally damaged during processing and less reliant on additives than previously (Gould, 2001). The new approaches mostly involve non-thermal food preservation technologies that offer full or partial (reducing treatment time and/or temperatures) alternatives to heat. They include among other physical procedures the application of electric or magnetic fields, microwave radiation, ionizing radiation, pulsed electric field and high-hydrostatic pressure (Mertens and Knorr 1992; Barbosa-Canovas and Gould 2000). When product is heat pasteurized, it loses a great part of the characteristics that make it different and unattractive to the consumer. As it is a product with a high added value, processing it by a non-thermal technology such as PEF is totally justified.

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Pulsed electric field (PEF) is being developed as a non-thermal emerging technology for the preservation of foods. Studies have been suggested that PEF treatment was efficient enough to destroy microorganisms in fruit juices at levels equivalent to those achieved by heat pasteurisation without greatly affecting their nutritional and sensory properties (Yeom *et al*., 2000; Min and Zhang 2003). In addition, the enzymes commonly present in fruit juices are partially or totally inactivated (Marselles-Fontanet and Martin-Belloso 2007). In this regard, Aguilo-Aguayo *et al*., (2008a) reported complete POD inactivation in tomato juice after applying 5.5-µs bipolar pulses of 35 kV/cm for 2000 µs at 200 Hz. On the other hand, some studies have suggested that HIPEF processing may enhance the antioxidant properties of juices compared to the untreated ones (Torregrosa *et al*., 2005; Odriozola-Serrano *et al*., 2007). Therefore, the objective of this research was to optimize the Pulsed electric field parameters including pulse frequency, pulse width with constant electric field strength and bipolar pulses for preservation of mango nectar and also study the effect of processing parameters on carotene, total plate count, coliforms, yeast and moulds and over all acceptability scores using a response surface methodology. We aimed to select the most appropriate PEF treatment to obtain nectar with maximum inactivation of native micro flora and high retention of carotene content.

Materials and Methods

Raw materials

Fresh ripe Mallika variety (hybridization of the Indian mango varieties Neelum and Dasheri) mango *(Mangifera indica)* were purchased from the local market at Mysore India the day before pulping and stored at 4°C until processing. The fruits were washed with tap water followed by sterile water.

Mango Nectar preparation

Mangoes were deskinned manually pulped using pulper, diluted, filtered through muslin cloth and poured into sterile stainless steel vessel prior to processing. The fresh pulp was diluted (1:1.5) with sterile water and adjusted total soluble solids (20°Brix) with sucrose followed by acidification (pH 4.36) with citric acid.

Pulsed electric fields processing

PEF treatments were performed 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. The 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 an AKG - cooling system (-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 (Model: Digital touch screen oscilloscope Siemens, Made in 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 RTD mango nectar was pumped through the system using a peristaltic pump (Type SK 20F-80 L 14 TF T 10/ 1-S. Getriebebow Nord Bargteheid, Germany) at a flow rate of 41 ltr/hr. It was treated at frequencies between 70 and 120 Hz, applying 15-24 µs pulse width in constant bipolar mode for all the treatments. 200ml of processed samples from each batch were filled in sterile (thermally) pre-fabricated multilayer laminated pouches consisting of 12 µm Polyethylene terephthalate / 9µm Aluminium foil / 15 µm Nylon / 80 µm Cast. Polypropylene (Total thickness 116 µm) pouches with a dimension of 15 X 20 cm under sterile conditions and hermetically sealed using impulse sealing machine (Model: HP Impulse Sealer, M/s Sunray Industries Mysore, India). The experiments were preformed in triplicate.

Experimental design

A face-centered central composite response surface analysis was used to determine the effect of frequency and pulse width on the native micro flora of RTD mango nectar. The selected responses were total plate count, coliforms, yeast and moulds, carotene and over all acceptability. The independent variables were pulse frequency (from 70 to 120 Hz) and pulse width (from 15 to 24 µs). The experimental design along with each experimental condition has been given shown in Table 1. The experiment was performed in one block of experiments. The order of assays within block was randomised and performed in triplicate. Experimental data were fitted to a polynomial response surface function. The second-order response function was predicted by the following equation (1):

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i \neq j=1}^{n} \beta_{ii} x_i x_{ij} \quad \text{.....(1)}$$

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 frequency-pulse width; 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 7.1 software (Stat Ease Inc., Minneapolis, MN) was used to generate quadratic models that fitted the experimental data, drew the response surface plots and optimized PEF treatment. Three-dimensional surface plots were drawn to illustrate the interactive effect of the two factors on the dependent variables, while keeping the other variables constant. The optimisation of PEF parameters was done following the method of Kathiravan *et al*., (2013). All the individual desirability functions obtained for each response were combined into an overall expression, which was defined as the geometrical 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 inactivation of native micro flora in RTD mango nectar with maximum retention of carotene content. All the variables of 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 equations of the second order polynomial, using the values of each independent variable giving to the maximum quadratic response (Montgomery 2001; Sin *et al*., 2006).

PhysicChemical analysis

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 with a 700 Digital pH meter at 23°C (Eutech Instruments, Made in Singapore). The pH meter was standardized using pH buffer of 4.0, 7.0 and 10.2.

CIE colour lab ($L^* a^* b^*$)

The CIE *($L^* a^* b^*$)* values were measured using a Hunter Lab Scan Spectrophotometric colorimeter controlled by a computer that calculated color ordinates from the reflectance spectrum. (Hunter Lab Color Flex EZ 45/0° color spectrophotometer, Made in USA). The results were expressed in accordance with the CIELAB system with reference to illuminant D65 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.

Viscosity

Viscosity was measured using 100 ml of Mango nectar with rotatory viscometer (Model Rheotech GmbH, Viscosimeter RC 01/02, Made in Germany) having a precision cylindrical spindle (R^2) rotating (UL) adapter. Mango nectar was placed in the UL adapter and viscosity determined at 100 rpm. The viscosity was expressed in centipoises (cP).

Ascorbic acid

Ascorbic acid content in mango nectar was estimated following the method of Ranganna (1999). A 10 ml sample of Mango nectar was mixed with 20% Meta phosphoric acid in a pestle and mortar, and transferred to a 100 ml volumetric flask by decantation. Extraction was repeated thrice with a few ml of Meta phosphoric acid each time and made up to the volume with distilled water. 10 ml of vitamin C extract was titrated against the standard 2, 6 dichlorophenol indophenol dyes. The Ascorbic acid content of each sample was estimated according to the following equation: Ascorbic acid mg/100ml = T.V x dye factor x volume made x 100 / volume taken for titration x sample weight. The Ascorbic acid content was expressed as mg /100 ml.

Total carotenoids

Total Carotenoids were determined spectrophotometrically (UV- Spectrophotometer, Spectronic® Genesys™ 2 Instruments, Made in USA) following the method of Ranganna (1999). A 5g sample was mixed with 20 ml of acetone and kept in dark for 10-15 min, 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 addition of 20 ml 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 250 ml volumetric flask and volume was made up to the mark with

hexane. A pinch of anhydrous sodium sulphate was added and absorbance read at 450 nm against hexane as blank. The carotenoid content of each sample was estimated according to the following equation: Absorbance x 250 x1000 x 100 / 250 x Wt of sample. The carotene was expressed as µg /100 ml.

Micro flora analysis

Microbial analysis was carried out as per Rivas *et al.*, (2006). 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 mold and yeast counts. Plates were incubated at 30 °C for 48h and 5 days for Total Plate Counts and Molds and Yeast respectively. Violet Red Bile Agar was used for Coliforms.

Sensory quality

Sensory quality was determined using 9 point Hedonic scale according to Ranganna, (1999). For sensory taste and odor evaluation, 20 semi trained panelists were selected. The 100 ml samples (Treated nectar fresh and processed) were presented. The panelists rated the preferred samples in comparison with control (untreated) for over all acceptability (OAA).

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 ($\alpha = 0.05$) and to verify the adequacy of the model. Interaction effects were determined using LS means ($\alpha = 0.05$). All assays were carried out in triplicate.

Results and Discussion

Effects of PEF treatment conditions on carotene content in RTD Mango nectar

All the responses of the RTD mango nectar were fitted into quadratic model. The p-value given in the parameters for each response are for the model significance. The p-value indicated the p > F- values which should be less than 0.05 for model to be significant. The effect of changes in levels of selected variables on the response parameters has been represented. Carotene content of fresh RTD mango nectar was 2230 µg/ 100 ml. Nectars samples treated with optimized parameters of 38.0 kV/cm, pulse width 24 µs at 120Hz with bipolar pulses exhibited the carotene retention of 94.2% (Table 1, assay 4). On the other hand, carotene loss was higher than 8% when PEF treatment was setup at 38.0 kV/cm for 19.50 µs at 130.36 Hz applying with bipolar pulses (Table 1, assay 11). Second-order polynomial models described with accuracy the changes in carotene content of RTD mango nectar (R^2) of 0.9512 Table 2. Multiple regression equations (in terms of coded factors) as obtained for response of carotene have been represented as follows:

$$\text{Carotene } Y = +2168.00 - 41.24^{*}A - 14.56^{*}B + 2.50^{*}A^{*}B - 21.62^{*}A^{2} - 6.12^{*}B^{2}$$

As a result of the synergy between frequency (Hz) and pulse width (µs) ($p < 0.05$), carotene depletion was achieved by increasing both the variables (Figure 1). The results indicated that the carotene degradation occurred with respect to frequency and pulse width increase. Our results were in accordance with Odriozola-Serrano *et al*., (2009b) who studied the PEF-treated (35 kV/cm for 1500 µs with 4 µs bipolar pulses at 100 Hz) tomato juice samples and reported a significant decrease in carotene content.

Effects of PEF treatment conditions on native micro flora in RTD Mango nectar

Fresh RTD mango nectar had a native micro flora total plate count (TPC), coliforms and yeast and moulds was 6.89, 4.4 and 4.01 logs CFU/ml respectively. The survival of log CFU/ml was 3.4-4.1, 0.0-4.0 and 0.0-3.8 for TPC, coliforms and yeast and moulds respectively. The optimized parameters of 38.0 kV/cm, 24 µs pulse width at 120 Hz with bipolar pulse showed a maximum log inactivation of native micro flora in RTD mango nectar (Table 1 assay 4). Our results are in accordance with the authors Mosqueda-Melgar *et al.,* (2007) and Aguilo-Aguayo *et al*., (2008b) who reported that the PEF treatments were effective in reducing the population of pathogenic microorganisms and inactivating spoilage enzymes. Mosqueda-Melgar *et al*., (2007) reported that the populations of *Salmonella enteritidis*, *E. coli* and *L. monocytogenes* were reduced to 3.71, 3.70 and 3.56 log units, respectively, when watermelon juice was submitted to a HIPEF treatment set up at 35 kV/cm for 1727 µs using 4-µs bipolar pulses at 188 Hz. Multiple regression equations as obtained for response of native micro flora have been represented as follows;

$$\text{TPC } Y = +3.71 - 0.41^{*}A - 0.14^{*}B - 0.11^{*}A^{*}B - 0.19^{*}A^{2} - 0.048^{*}B^{2}$$

$$\text{Coliforms } Y = +3.50 - 1.54^{*}A - 0.26^{*}B + 0.17^{*}A^{*}B - 0.97^{*}A^{2} - 0.42^{*}B^{2}$$

Table 1. Central composite response surface designs followed to evaluate carotene content, native micro flora and OAA of PEF-treated RTD Mango nectar

Assay number[a]	Factors		Responses				
	f[b]	*p*[c]	Carotene[d]	TPC[e]	Coliforms[e]	Yeast & Moulds[e]	OAA[f]
1	70.00	15.00	2186	3.8	3.7	3.6	8.6
2	120.00	15.00	2120	3.4	0.0	1.0	8.4
3	59.64	19.50	2198	4.1	4.0	3.8	8.6
4	120.00	24.00	2100	2.85	0.0	0.0	8.3
5	95.00	19.50	2168	3.71	3.5	3.2	8.5
6	95.00	19.50	2168	3.71	3.5	3.2	8.5
7	95.00	25.86	2132	3.51	2.6	2.2	8.4
8	70.00	24.00	2156	3.68	3.0	2.8	8.5
9	95.00	19.50	2168	3.71	3.5	3.2	8.5
10	95.00	19.50	2168	3.71	3.5	3.2	8.5
11	130.36	19.50	2051	2.65	0.0	0.5	8.0
12	95.00	19.50	2168	3.71	3.5	3.2	8.5
13	95.00	13.14	2179	3.8	3.6	3.51	8.6

[a] Run order
[b] Frequency (Hz)
[c] Pulse width (µs)
[d] µg/100mL
[e] log CFU/mL
[f] 9 Point Hedonic scale

Table 2. ANOVA and model statistics for the PEF Process optimization of RTD Mango nectar

Term Model	Response				
	Carotene[d]	TPC[c]	Coliforms[c]	Yeast & Moulds[c]	OAA[b]
F Value	27.30	23.56	21.76	27.52	12.70
P > *F*	0.0002[a]	0.0003[a]	0.0004[a]	0.0002[a]	0.0021[a]
Mean	2150.92	3.56	2.65	2.57	8.45
Standard deviation	11.69	0.12	0.50	0.36	0.067
C V %	0.54	3.46	18.79	14.10	0.79
R squared	0.9512	0.9439	0.9395	0.9516	0.9007
Adjusted *R* Squared	0.9164	0.9038	0.8964	0.9170	0.8298
Predicted *R* Squared	0.6532	0.6011	0.5701	0.6558	0.2940
Adequate Precision	16.636	13.859	13.966	15.425	11.880

[a] Significant at $p < 0.05$
[b] 9 Point Hedonic scale
[c] log CFU/mL
[d] µg/100mL

Yeast & Moulds $Y = +3.20 -1.26 {}^{*}A -0.46{}^{*}B -0.050{}^{*} A{}^{*}B -0.69{}^{*}A^2 -0.34{}^{*}B^2$

The statistical analysis indicated that the proposed quadratic model for TPC, coliforms and yeast and moulds was adequate ($p < 0.0003$, $p < 0.0004$ and $p < 0.0002$) with satisfactory determination coefficient (R^2) of 0.9439, 0.9395 and 0.9516 respectively (Table 2). The frequency and pulse width significantly ($p < 0.05$) affected the inactivation of native micro flora in the nectar (Figure 2). The variables were optimized based on maximum inactivation as well as carotene content and sensory scores for OAA.

Effect of PEF treatment conditions on OAA (Sensory score) of RTD Mango nectar

Sensory score (OAA) is the most important criteria for acceptability of any product; it was also taken as a response for the RTD mango nectar. The regression analysis of the response was conducted by fitting quadratic models as suitable for the sensory response. The analysis of variance was calculated and has been presented in Table 2. Multiple regression equations as obtained for sensory score (OAA) are as follows;

Sensory (OAA) $Y = +8.50 -0.16{}^{*}A -0.060{}^{*}B +0.000{}^{*} A{}^{*}B -0.088{}^{*}A^2 +0.012{}^{*}B^2$

The effect of two independent variables has been depicted in response surface plots. Sensory score (OAA) was mainly affected by the level of frequency and pulse width. The score increased with decrease in their levels within the experimental range. Studies

Table 3. Effect of PEF processing on physico-chemical characteristics and micro flora of RTD Mango nectar

Parameters		Values	
		Control[a] (un treated)	PEF treated[a]
pH		4.36 ± 0.01	4.39 ± 0.01
Acidity (as per citric acid %)		0.213 ± 0.005	0.22 ± 0.005
°Brix		20 ± 0.0	20 ± 0.0
Total (%)		17.8 ± 0.1	17.7 ± 0.1
Reducing (%)		6.9 ± 0.07	6.6 ± 0.05
Ascorbic acid (mg/100ml)		9.2 ± 0.03	8.241 ± 0.007
Carotene (µg/100ml)		2230 ± 3.05	2100 ± 1.15
Viscosity	Shear Force (cp)	102 ± 0.00	103 ± 0.00
	Shear rate (%)	26.5 ± 0.00	25.0 ± 0.00
	Temp (°C)	26.4 ± 0.00	26.5 ± 0.00
CIE Color Lab	L*	28.63 ± 0.01	28.53 ± 0.005
	a*	0.02 ± 0.00	0.14 ± 0.00
	b*	15.57 ± 0.01	14.62 ± 0.005
Sensory Score[b]	Colour	8.5 ± 0.05	8.2 ± 0.05
	Appearance	8.6 ± 0.05	8.2 ± 0.15
	Texture	8.3 ± 0.05	8.4 ± 0.00
	Flavour	8.7 ± 0.05	8.6 ± 0.05
	OAA	8.5 ± 0.05	8.4 ± 0.02
Survival micro flora log CFU/ml	Total plate count	6.89 ± 0.01	2.85 ± 0.01
	Coliforms	4.4 ± 0.05	0.00 ± 0.00
	Yeast and moulds	4.01± 0.00	0.00 ± 0.00

[a] Mean ± SD
[b] 9 Point Hedonic scale

reported by Min and Zhang (2003) also showed similar trends, PEF treated RTD mango nectar had a slightly affected sensory scores.

Optimal PEF treatment conditions for RTD mango nectar and model validation

Carotene and native micro flora seemed to be more affected by PEF treatments. The combination of PEF critical parameters leads to RTD mango nectar with the maximum inactivation as well as high retention of carotene content (94.2%) and sensory score (OAA) of 8.3 with minimum survival log CFU/ml of 2.85, while the values for TPC, coliforms and yeast and moulds were nil when applying 38.0 kV/cm, 24 µs pulse width using bipolar pulse at 120 Hz with high desirability (0.885) was applied. The RTD mango nectar was processed using the optimized processing parameters and verified the predicted values and the actual values for the responses. Predicted value of carotene, TPC, coliforms, yeast & moulds, OAA and desirability found to be 2086.96 µg/ 100 ml, 2.82 log CFU/ml, 0.47 log CFU/ml, 0.41 log CFU/ml, 8.20 and 0.885 respectively. Whereas the actual value also found to be similar as predicted 2098.01 µg/ 100 ml, 2.84 log CFU/ml, 0.00 log CFU/ml, 0.00 log CFU/ml, 8.30 and 0.890 for carotene, TPC, coliforms, yeast & moulds, OAA and desirability respectively.

Effect of PEF processing and on physical properties of RTD Mango nectar

The effect of optimized PEF processing on the Physico-chemical properties of RTD Mango nectar was evaluated and the data presented in Table 3. The total soluble solids, pH and acidity of RTD mango nectar did not showed much change after PEF processing. A similar study has been reported by Rivas *et al*., (2006) indicating negligible change in pH and °Brix of PEF processed blended orange and carrot juice. Yeom *et al.*, (2000) also did not observe any significant changes in PEF treated orange juice during storage. The color is one of the important parameters for quality of nectars. Color degradation of PEF treated RTD mango nectar was investigated using Hunter color instrument with CIE color lab values. The carotene degradation is one of the important factors to cause color change in PEF treated mango nectar. The PEF treated samples did not reveal any marked degradation of carotene content; color values were also not much affected when compared to control samples. Similar study was performed by Zhang *et al*., (1997) and he reported that PEF treatment did not much affect the color values of the juices. The reliability of RTD mango nectar expressed in viscosity is essential part for the quality of mango nectar. The viscosity of control RTD mango nectar sample was 102 ± 0.00 and 126.5 ± 0.00 for Shear force (cp) and shear rate (%) respectively. After the PEF treatment it was changed to 103 ± 0.00 cp, 125.0 ± 0.00% respectively.

Ascorbic acid content of fresh RTD mango nectar was found to be 9.2 ± 0.03 mg/100ml. while PEF treated sample had 8.241 ± 0.007. Ascorbic acid is a highly labile vitamin which degrades very easily on exposure to heat, air and light. The PEF processing

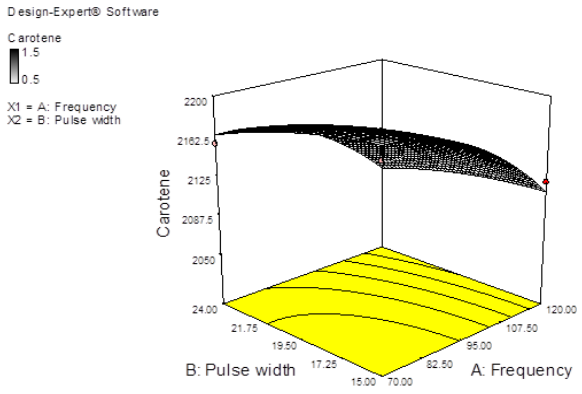

Figure 1. 3D plot depicting effect of frequency and pulse width on carotene of Mango nectar

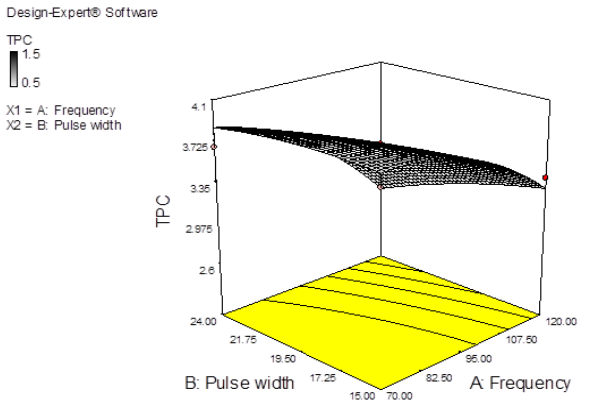

Figure 2. 3D plot depicting effect of frequency and pulse width on total plate count (TPC) in Mango Nectar

had a significant ($p < 0.05$) degradation effect in the ascorbic acid content. Our results are in accordance with authors Elez-Martinez and Martin-Belloso (2007) and Odriozola-Serrano *et al.*, (2009a) who evaluated the Vitamin C losses in pulsed electric field treated orange and strawberry juices respectively. There is a need to study the effect of PEF on physico-chemical changes and microbiological quality characteristics of RTD mango nectar under different storage conditions.

Conclusion

PEF processing had a statistically significant effect on carotene, inactivation of native micro flora and sensory score (OAA) of RTD mango nectar. Second-order equations were developed to properly fit the experimental data. Native micro flora was strongly inactivated within the range of assayed conditions. Minimum survival logs CFU/ml (2.85) were obtained when applying bipolar treatments at 120 Hz and pulse width of 24 µs. The higher survival log of CFU/ml were achieved (4.1 TPC) in the product treated under the bipolar treatments at 59.64 Hz and pulse width 19.50 µs. Hence, frequency and pulse width can contribute to achieve optimal processing conditions for PEF to obtain mango nectar with high commercial acceptability.

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