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RESEARCH ARTICLE

Optimization of pulsed electric field processing conditions for passion fruit juice (*Passiflora edulis)* using Response Surface Methodology

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Abstract

A response surface methodology was used to determine the combined effects of pulsed electric fields (PEF) variables such as frequency and pulse width on maximum inactivation of native micro flora and high retention of total carotenoids and color value of passion fruit juices. Native micro flora and total carotenoids were determined in passion fruit juices processed at pulse frequencies from 85 to 100 Hz and pulse widths ranging from 19 to 24 µs. Electric field strength of 38.1 kV/cm with bipolar pulses were maintained constant in all the treatments. Both first-order linear and second-order quadratic expressions used to fit the experimental results. Maximum inactivation of native micro floras were obtained by selecting pulse widths 24 µs at 100 Hz. whereas the lowest inactivation was achieved after applying pulse widths 21.50 µs at 81.89 Hz. Moreover, passion fruit juice had a better color and total carotene was preserved in the optimized PEF processed parameters of pulse widths 24 µs at 100 Hz. Hence, the PEF processing optimization through frequency and pulse width could contribute to assure micro flora inactivation while keeping valuable attributes of quality parameters of juices.

Introduction

In recent scenario, there is a worldwide increasing tendency for the consumption of tropical fruit juices and fruit drinks, due to the consumer interest in healthy products which are practical and ready to be consumed. Yellow passion fruit (*Passiflora edulis*) juice is globally marketed, mainly for its pleasant unique aroma and flavor. Brazil, Ecuador and Colombia are the biggest passion fruit producers in the world (Ricardo Cardoso, *et al.*, 2012). Fruits and vegetables juices are important components of human food, occupying the second place in the food pyramid (Antunes, *et al.*, (2008). Today, consumers demand products to market that are identical to the fresh, to preserve their own physicochemical properties and durability, without reducing the 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 (Gardner, *et al.*, 2000; Sanchez-Moreno, *et al.*, 2003; Tibble, 1998; Williamson, 1999).

Thermal pasteurization is extensively used for inactivation of micro-organisms in foods; there is 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 to foods of electric or magnetic fields, microwave radiation, ionizing radiation, pulsed electric field and high-hydrostatic pressure (Barbosa-Canovas & Gould, 2000; Mertens & Knorr, 1992). As it is a product with a high added value, processing it by a non-thermal technology such as PEF is totally justified.

Pulsed electric field (PEF) is being developed as a non-thermal emerging technology for the preservation of foods. Studies have suggested that PEF treatment is efficient enough to destroy microorganisms in fruit juices at levels equivalent to those achieved by heat pasteurization without greatly affecting their nutritional and sensory properties (Min & Zhang, 2003; Yeom, *et al.*, 2000). On the other hand, some studies have suggested that PEF processing may enhance the antioxidant properties of juices comparing to those untreated (Odriozola-Serrano, *et al.*, 2007; Torregrosa, *et al.*, 2005). There are many published works that study the effect of PEF on juice microorganisms and shelf-life. Inactivation of the juice microbial flora (mesophilic bacteria, molds, and yeast) and the shelf-life when stored under different conditions have been tested (Ayhan, *et al.*, 2001; Hodgins, *et al.*, 2002; Jia, *et al.*, 1999; Min, *et al.*, 2003; Min & Zhang, 2002; Qin, *et al.*, 1998; Yeom, *et al.*, 2000a, b; Zhang, *et al.*, 1996). However, the information regarding the optimization of Pulsed electric field parameters and its effects on the native micro flora, total carotene content, color value and over all acceptability of this product is scarce.

Statistical design tools such as response surface methodology (RSM) are quite effective in optimizing the processing parameters. RSM uses a central composite rotatable design to fit a polynomial model by least-square technique (Hunter, 1959). Basically RSM helps to create a product using regression equations that describe inter-relations between input parameters and product properties. The major constraint on optimizing procedures is that the desired degree of inactivation must be achieved. However, more processing time associated with losses in terms of flavor, color, sensory and nutritional qualities occur. 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 also to study the effects of processing parameters on total carotene, color value, total plate count, coliform, yeast and moulds and over all acceptability using a response surface methodology. We aimed to select the most appropriate PEF treatment to obtain juice with maximum inactivation of native micro flora as well as good of sensory and nutritional quality.

Materials and methods

Raw materials

Fresh ripe yellow passion fruit (*Passiflora edulis*) purchased from local farm at Yelwal, Mysore India the day before pulping and stored at 4^0C until processing. The fruits were washed with tap water followed by sterile water.

Passion fruit juice preparation

Fruits were cut into two half pieces, pulp was removed, pulped using mixer grinder without breaking the seed, filtered through mesh filters and poured into sterile stainless steel vessel prior to processing. The fresh pulp was diluted (1:1) with sterile water and then total soluble solids (^{0}Brix) were adjusted (15 ^{0}Brix) with sucrose.

Pulsed electric fields 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. 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 (-5^0 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 unit. Recorded temperatures did not exceed 40^0 C. The passion fruit juice 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 40.5 ltr/hr. It was treated at frequencies between 85 and 100 Hz, applying 19-24 µs pulse width in constant electric field strength 38.1 kV/cm and bipolar mode for all the treatments. Volume of (200ml) processed samples of each batches were filled in sterile (thermally) pre-fabricated multilayer laminated pouches consisting of 12 µm Polyethylene terephthalate / 9µm Aluminium foil / 15µm Nylon / 80µm Cast. Polypropylene (Total thickness 116µm) of 200 ml capacity 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 response surface methodology (RSM) analysis was used to evaluate the effect of the different PEF processing variables on native micro flora, total carotene content and CIE color value as well as on over all acceptability (OAA) of passion fruit juice. A face-centered central composite response surface analysis was used to determine the effect of frequency and pulse width on the native micro flora, total carotene content, color value and OAA of passion fruit juice. The selected responses were total plate count, coliform, yeast and moulds, total carotene, CIE color value (L*, a*, b*) and sensory over all acceptability. The independent variables were pulse frequency (from 85 to 100 Hz) and pulse width (from 19 to 24 µs). The levels for each independent parameter were chosen considering sample load resistant and equipment limitations Table 1. The experimental design along with each experimental condition is shown in Table 2. The experimental design was performed in one block of experiments. The order of assays within block was randomised and performed in triplicate. The results for 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) & (2)

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i \quad (1)$$

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

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 frequency-pulse width; βi, βii and βij 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 PEF treatment. 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 PEF parameters was done following the method of Derringer and Suich (1980) with few modifications. All the individual desirability functions obtained for each response were combined into an overall expression, which is 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 native micro flora of Passion fruit juice with the high levels total carotene content and CIE color value. 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 (Montgomery 2001; Sin, *et al.,* 2006).

Physico-Chemical analysis

pH

The pH was determined with a 700 Digital pH meter at 23^0C (Eutech Instruments, Made in Singapore). The pH meter was standardized using pH buffer of 4.0, 7.0 and 10.2.

Soluble solids (^{0}Brix)

The soluble solids (^{0}Brix) were measured using a hand Refractometer (RF.5580 Euromex Brix hand Refractometer). Measurements were performed at 25.0 ± 2^0 C. The refractometer prism was cleaned with distilled water after each analysis.

CIE colour lab (L* a* b*)

The CIE color value *(L* a* b*)* was 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 $45/0^0$ color spectrophotometer, Made in USA). The results were expressed in accordance with the CIE LAB system with reference to illuminant D_{65} and with a visual angle of 10^0. 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.

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 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 addition of 20ml of hexane to extract the pigment completely. The combined extract was transfer to a separating funnel. After 5 min the aqueous layer was completely discarded and transferred the hexane layer to 250ml volumetric flask and 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 carotenoids content of each sample was estimated according to the following equation: Absorbance x 250 x1000 x 100 / 250 x Wt of sample. The total carotene was expressed as µg /100 ml.

Micro flora analysis

Microbial analysis was carried out following the method of 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 30^0C 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 rating according to method of Ranganna (1999). For sensory taste and odor evaluation and overall acceptability, 20 untrained volunteers were selected. The 100 ml samples (Treated juices) were presented to the judges. The judges rated the preferred samples in comparison with control (untreated).

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

The responses of the total carotenoids content, coliforms, yeast and moulds, CIE color values of a* and b* were fit with quadratic model, where as TPC, colour value of L* and over all acceptability was fit with only linear model. The *p-value* given in the parameters for each response are the model significance. The *p-value* indicates 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 in Fig 1-6.

Effects of PEF treatment conditions on Native micro flora

PEF treated passion fruit juice residual micro floras (logs CFU/ml) were 3.0-4.5, 0.0-3.2 and 0.0-2.7 for TPC, coliforms and yeast and moulds respectively. The highest inactivation (TPC-4.079 coliforms-4.1 and yeast and moulds-3.95) was observed after treating passion fruit juice 24 µs pulse width, at 100 Hz frequency (Table 2) where as the fresh passion fruit juice had a native micro flora of total plate count (TPC), coliforms and yeast and moulds were 7.079, 4.1 and 3.954 logs CFU/ml respectively. The passion fruit juice treated at 38.1 kV/cm, 24 µs pulse width at 100 Hz shown a maximum log inactivation of native micro flora with maximum retention (86.56 %) of total carotene content, due to that, this parameter was optimized to process the passion fruit juice. The 4.0 log inactivation in PEF treated passion fruit juice was accordance with other authors (Mosqueda-Melgar, *et al.*, 2007) who reported that the populations of *Salmonella enteritidis*, *E. coli* and *L. monocytogenes* were reduced up to 3.71, 3.70 and 3.56 log units, respectively, when watermelon juice was submitted to a PEF treatment set up at 35 kV/cm for 1727 µs using 4 µs bipolar pulses at 188 Hz. Aguilo-Aguayo, *et al.,* (2008) also reported PEF treatments were effective in reducing the population of pathogenic microorganisms and inactivating spoilage micro flora. Multiple regression equations as obtained for response of native micro flora (TPC, coliforms and yeast and moulds) have been represented as follows;

TPC $Y = +3.57 - 0.26 * A - 0.31 * B$

Coliforms $Y = +1.80 - 0.56 * A - 0.93 * B + 0.050 * A * B + 0.094 * A^2 - 0.33 * B^2$

Yeast & Moulds $Y = +1.70 - 0.43 * A - 0.88 * B + 0.075 * A * B - 6.250E\text{-}003 * A^2 - 0.36 * B^2$

The statistical analysis indicates that the proposed quadratic model was fit to be only TPC, coliforms and yeast and moulds, whereas for TPC the linear model found to be fit. It was adequate ($p < 0.0003$, $p < 0.0001$ and $p < 0.0001$) with satisfactory determination coefficient ($R^2 = 0.8050$, $R^2 = 0.9891$ and $R^2 = 0.9691$) respectively Table 3. The frequency and pulse width shown a significant ($p < 0.05$) inactivation of native micro flora of the juice (Figure 2, 3 & 4). The variables of frequency and pulse width were optimized based on maximum log inactivation with high retention of total carotene content, CIE color value and over all acceptability.

Effects of PEF treatment conditions on carotene content

Passion fruit juice with optimized parameters of 38.1 kV/cm, pulse width 24 µs at 100 Hz exhibited the total carotene content retention of 86.56% (Table 2, assay 5). Whereas total carotene content of fresh passion fruit juice was 1962.39µg/ 100 ml. The maximum retention (94.74%) was achieved when PEF treatment was set up at 38.1 kV/cm pulse width 24 µs at 100 Hz; however it had a residual micro flora of 4.5 log of total plate count. (Table 2, assay 9). Second-order polynomial models describes with accuracy the changes in total carotene content of Passion fruit juice ($R^2 = 0.9596$) Table 3. Multiple regression equations (in terms of coded factors) was obtained for response of total carotene content has been represented as follows;

Total carotene content $Y = +1782.12 - 26.99 * A - 38.89 * B + 2.86 * A * B + 9.53 * A^2 - 13.91 * B^2$

The synergy between frequency (Hz) and pulse width (µs) was significantly ($p < 0.05$) affected the total carotene content by increasing both the variables (Figure 1). The result indicates that total carotene content degradation occurs with respect to frequency and pulse width increase. Authors of Odriozola-Serrano, *et al.,* (2009) also studied significant decrease in carotene content in the PEF-treated (35 kV/cm for 1500 µs with 4µs bipolar pulses at 100 Hz) tomato juices. It also had a significant decrease in carotene content.

Effects of PEF treatment conditions on CIE color lab (L*, a* & b*)

The color degradation of PEF treated passion fruit juice was investigated using CIE color values (L*, a* & b*). The major causes of color change in passion fruit juice due to degradation of carotene content and non-enzymatic browning. The control juice sample had a redness (a*), yellowness (b*) and luminosity (L*) values were 13.95, 47.35 and 34.86 respectively. The redness (a*), yellowness (b*) and luminosity (L*) value of passion fruit juice was significantly increased ($p < 0.05$) after PEF treatment (Table 2 & 3). The decrease in the luminosity (L*) values was evidently indicate that the slight browning occurred in passion fruit juice. Increased in redness (a*) and yellowness (b*) values clearly indicated that decrease in total carotene content. Our results accordance with Zhang, *et al.,* (1997) who found significant color degradation in the PEF treated juice samples. Rivas, *et al.,* (2006) also found changes in color value in PEF treated blended orange and carrot juice. Multiple regression equations was obtained for CIE color value (L*, a* & b*) represented as follows;

L* Value $Y = +33.52 - 0.24 * A - 0.26 * B$

a* Value $Y = +13.43 - 0.25 * A - 0.42 * B - 0.14 * A * B + 5.625E\text{-}003 * A^2 - 0.19 * B^2$

b* Value $Y = +44.92 - 0.35 * A - 0.31 * B + 0.28 * A * B + 0.22 * A^2 + 0.019 * B^2$

The regression analysis indicates that the proposed quadratic model was fit to a* & b* values, whereas for L*value the linear model found to be fit. L* value describes the significant ($p < 0.05$) changes with accuracy of R^2

0.9309. Values of a* and b* describes the significant ($p < 0.05$) changes with accuracy of R^2 0.9766 and R^2 0.9361. The frequency and pulse width significantly ($p < 0.05$) affected the L*, a* & b* values of the juice (Figure 5, 6).

Effects of PEF treatment conditions on Over all acceptability (Sensory score)

Over all acceptability (OAA) is the most important criteria for over all acceptability of any product, it was also taken as a response for the passion fruit juice. The regression analysis of the response was conducted by fitting linear model as suitable for sensory response. The analysis of variance was calculated and represented the data (Table 3). Multiple regression equations as obtained for over all acceptability represented as follows;

Sensory (OAA) Y = +8.29 -0.091* A -0.14 * B

The effect of two independent variables has been depicted in response surface plots (figure not shown). Over all acceptability was significantly ($p > 0.05$) affected by level of frequency and pulse width and the score increased with decrease in their levels within the experiments range (Table 3). Studies reported by Min, & Zhang, (2003) also showed similar trends.

Optimal critical PEF treatment conditions for Passion fruit juice and model validation

The combination of PEF critical parameters leads to passion fruit juice with the maximum log inactivation (4.1) was achieved. The total carotene content was also found to be retention of 86.56% (Table 2, assay 5) with good color and sensory qualities. The electric field strength of 38.1 kV/cm, 24 µs pulse width at 100 Hz good enough to inactivate the 4.1 log reduction of native micro flora in passion fruit juice. The passion fruit juice had a load resistance of 260 Ω, due to the higher load resistance the frequency of 100 Hz is enough for maximum inactivation of native micro flora whereas other juice like beetroot juice having low load resistance it need more frequency. The passion fruit juice was processed using the optimized frequency and pulse width and verified for the predicted values and the actual values for the responses. Since these values (Table 4) were almost similar, the fitted models were suitable for predicting the response. There is the good scope to study the physic-chemical changes and micro flora activity of PEF treated Passion fruit juice in different packaging systems under different storage conditions.

Table: 1 Experimental ranges and levels of independent variables used in RSM in terms of actual and coded factors for PEF Process optimization of Passion fruit juice

Variables		Range of Levels			
		Low Actual	Low Coded	High Actual	High Coded
A	Frequency (Hz)	85	-1.000	100	1.000
B	Pulse width (µs)	19	-1.000	24	1.000

Table: 2 Central composite response surface designs followed to evaluate total carotene, native micro flora, color and OAA of PEF-treated passion fruit juice

Assay[a] number	Factors		Responses							
	f[b]	*p*[c]	Total[d] Carotene	TPC[e]	Coliforms[e]	Yeast & Moulds[e]	[f]L*	[f]a*	[f]b*	[g]OAA
1	81.89	21.50	1829.78	4.0	2.8	2.5	33.89	13.76	45.89	8.5
2	92.50	25.04	1710.0.	3.3	0.0	0.0	33.22	12.56	44.71	8.1
3	92.50	21.50	1782.12	3.5	1.8	1.7	33.51	13.43	44.92	8.3
4	92.50	21.50	1782.12	3.5	1.8	1.7	33.51	13.43	44.92	8.3
5	100.00	24.00	1698.80	3	0.0	0.0	33.00	12.29	44.67	8.0
6	92.50	17.96	1801.78	3.8	2.3	2.1	33.72	13.62	45.20	8.4
7	85.00	24.00	1762.85	3.3	1.0	0.5	33.39	13.18	44.75	8.2
8	92.50	21.50	1782.12	3.5	1.8	1.7	33.51	13.43	44.92	8.3
9	85.00	19.00	1859.21	4.5	3.2	2.7	34.12	13.82	46.21	8.5
10	92.50	21.50	1782.12	3.5	1.8	1.7	33.51	13.43	44.92	8.3
11	103.11	21.50	1775.76	3.4	1.2	1.0	33.14	13.21	44.82	8.2
12	100.00	19.00	1783.74	3.6	2.0	1.9	33.67	13.51	45.02	8.4
13	92.50	21.50	1782.12	3.5	1.8	1.7	33.51	13.43	44.92	8.3

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

Table: 3 ANOVA and model statistics for the PEF Process optimization of Passion fruit juice

Term Model	Response							
	Total[d] carotene	TPC[c]	Coliforms[c]	Yeast & Moulds[c]	[e]L*	[e]b*	[e]c*	OAA[b]
F Value	33.29	20.64	127.06	43.89	67.31	58.37	20.51	43.56
P > *F*	< 0.0001[a]	0.0003[a]	< 0.0001[a]	< 0.0001[a]	< 0.0001[a]	< 0.0001[a]	0.0005[a]	<0.0001[a]
Mean	1779.43	3.57	1.65	1.48	33.52	13.32	45.07	8.29
Standard deviation	11.02	0.18	0.13	0.20	0.086	0.088	0.15	0.051
C V %	0.62	4.99	7.71	13.43	0.26	0.66	0.34	0.61
R squared	0.9596	0.8050	0.9891	0.9691	0.9309	0.9766	0.9361	0.8970
Adjusted *R* Squared	0.9308	0.7660	0.9813	0.9470	0.9170	0.9598	0.8905	0.8764
Predicted R^2	0.7130	0.5847	0.9225	0.7802	0.8475	0.8334	0.5456	0.7739
Adequate Precision	19.656	13.307	37.868	22.167	24.120	22.806	15.226	18.989

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

Table: 4 Predicted responses vs. actual response for the PEF Process optimization of Passion fruit juice

Values	Response							
	Total Carotene	TPC	Coliforms	Yeast & Moulds	L*	a*	b*	OAA
Predicted	1714.72	2.99	0.12	0.10	33.01	12.43	44.77	8.06
Actual	1701.04	3.01	0.00	0.00	33.02	12.30	44.65	8.00

Figure 1 3D plot depicting effect of Frequency and Pulse width on total carotene content of passion fruit juice

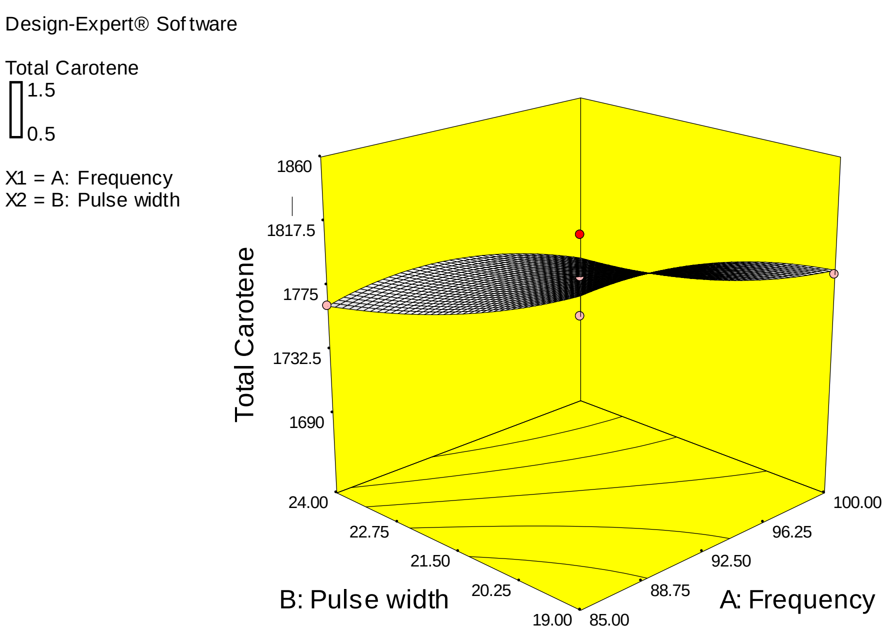


Figure 2 3D plot depicting effect of Frequency and Pulse width on TPC of passion fruit juice

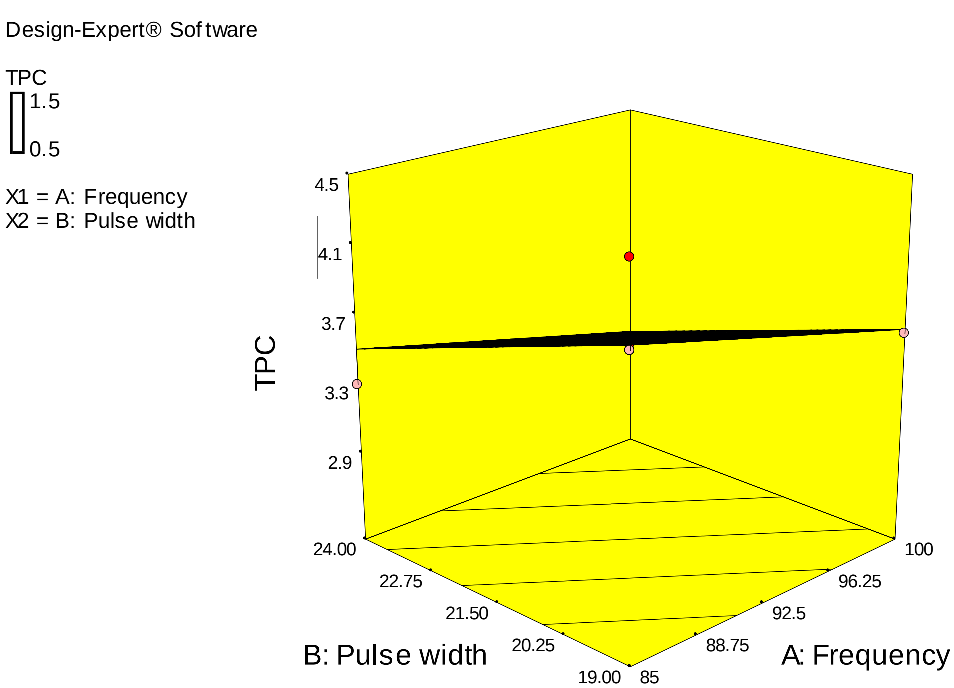


Figure 3 3D plot depicting effect of Frequency and Pulse width on Coliforms of passion fruit juice

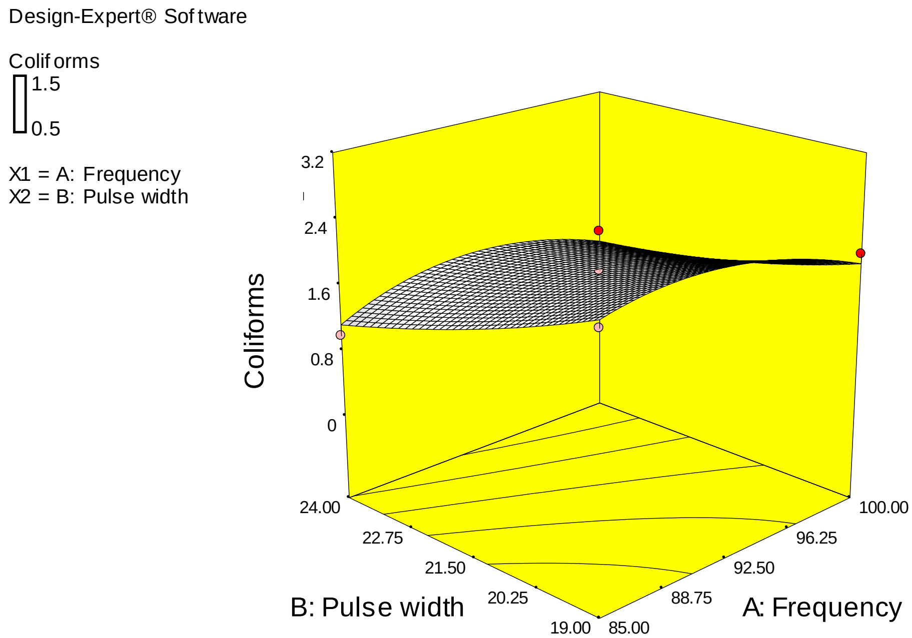


Figure 4 3D plot depicting effect of Frequency and Pulse width on Yeast & Moulds of passion fruit juice

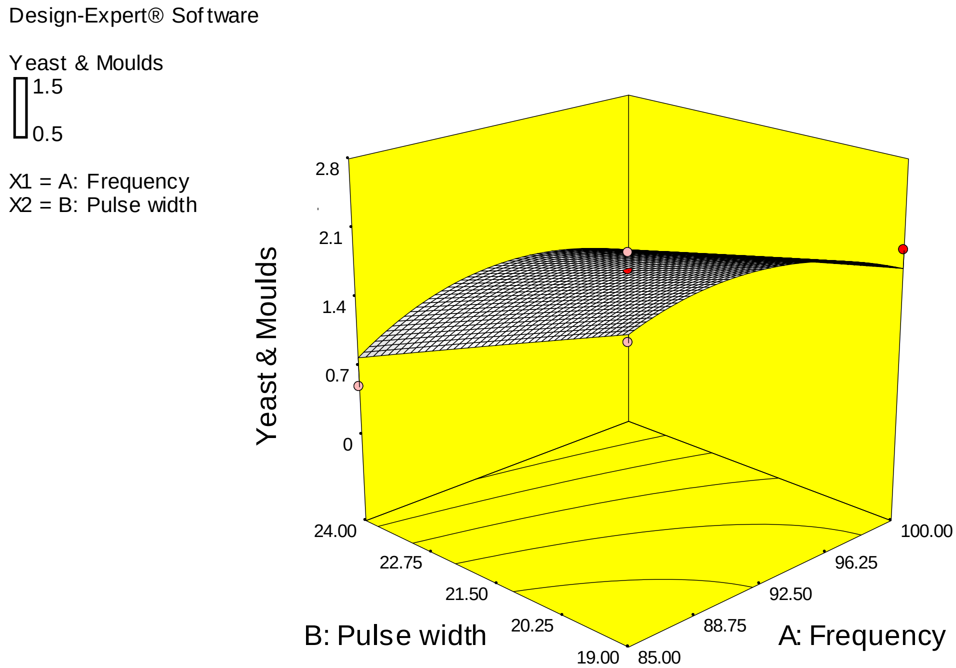


Figure 5 3D plot depicting effect of Frequency and Pulse width on L* value of passion fruit juice

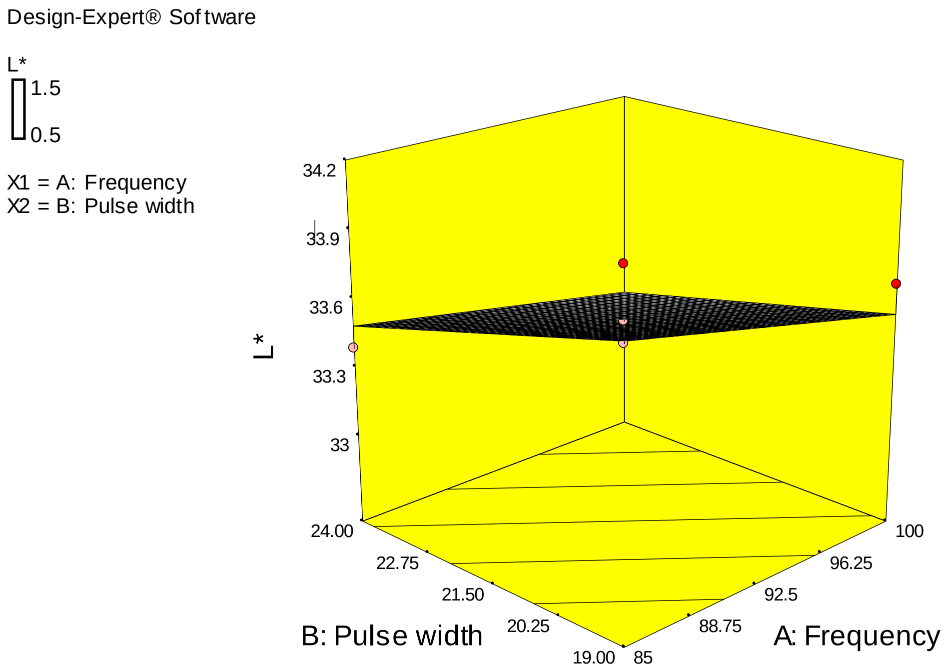
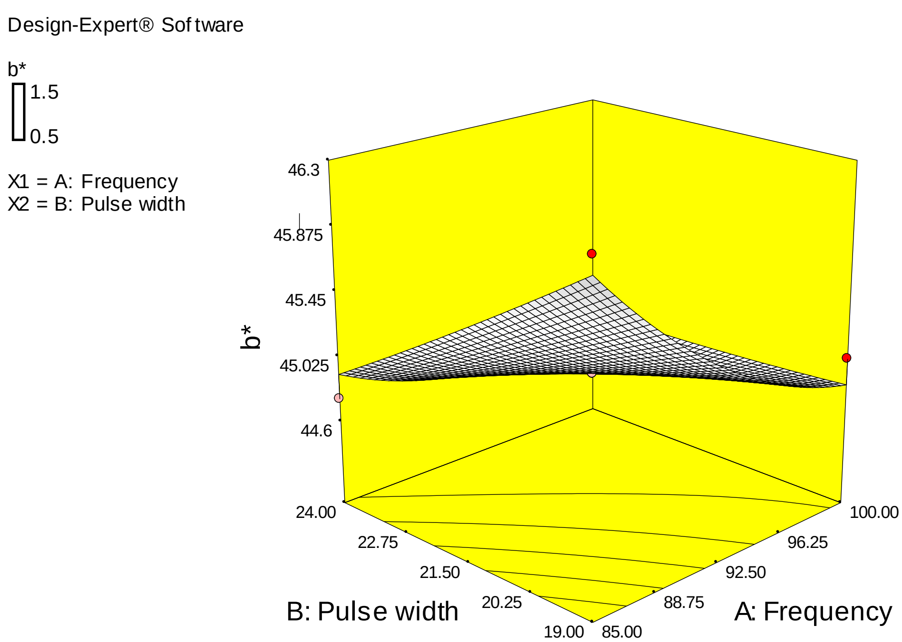


Figure 6 3D plot depicting effect of Frequency and Pulse width on b* value of passion fruit juice



Conclusion

Pulse frequency and pulse width significantly affected the native micro flora activity and total carotene content, CIE color value and over all acceptability of passion fruit juice. The lower the PEF-processing parameters increased the retention of carotene and color but in the same time it has the lower inactivation of native micro flora. Native micro flora was strongly inactivated within the range of assayed conditions. Minimum survival logs CFU/ml (3.0) were obtained when applying electric field strength of 38.1 kV/cm, pulse width 24 s at 100 Hz with bipolar pulses. As the passion fruit juice has been shown to be an excellent source of carotene and antioxidant capacity is highly related to its content, optimised parameters could be used during production of juice with high amount of nutrients and bio active compounds.

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