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Research Article
Effect of Ohmic Heating Parameters on Inactivation of Enzymes and Quality of Not-from-concentrate Mango Juice

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Abstract

Background and Objective: Ohmic heating (OH) is one of the electrothermal technologies that used recently for food processing to inactivate microorganisms and enzymes. In present study, effect of OH parameters (voltage gradients and temperature) on inactivation of polyphenoloxidase (PPO) and pectinmethylesterase (PME) of not-from-concentrate (NFC) mango juice was investigated. Multiple response surface methodology (RSM) was used to optimize the OH parameters, where the effect of voltage gradient and temperature on PPO and PME in the NFC mango juice was evaluated. After optimization, the NFC mango juice was produced with optimized OH conditions. **Methodology:** The PPO and PME activity, total phenolic, total carotenoids, ascorbic acid, cloud value, color as well as physical properties were determined and compared with mango juice reported by conventional heating (CH). **Results:** The PPO activity was completely inactivated for mango juice produced by OH (at 40 V cm^{-1}, 80°C and holding time for 60 sec) and CH (at 90°C holding time for 60 sec), while the inhibition of PME activity were 96 and 90%, respectively. The reduction in the ascorbic acid for OH (11.3%) was significantly lower than for CH (20%) treated samples. The total phenolic content was increased by 8 and 5% for the OH and CH treated samples, respectively. The reduction in total carotenoids level was significantly lower in the OH (10.9%) than in the CH (19.4%) treated sample. **Conclusion:** Overall, OH is a potential mild thermal treatment in the production of mango juice with improved functional properties instead of conventional methods.

INTRODUCTION

Mango (*Mangifera indica* L.) is a common fruit worldwide (known as the fruit queen) due to its phenolic base compounds, in addition to having a good source of ascorbic acid, carotenoids and fibers[1]. Not-from-concentrate (NFC) is one of techniques that is used for producing fruit juices. The NFC is defined as a juice that has not undergone concentration or dilution during processing with the only removal of the insoluble pulp, skin and seeds before pasteurization for the reduction of both microbial load and enzyme activity. After that, the juice is de-aerated by N_2 gas and refrigerated at 0-2°C under strictly controlled conditions to be stored more than 1 year[2,3].

Fruit juices (i.e., mango) are generally heat treated for inactivation of food poisoning, spoilage microorganisms as well as enzymes causing problem (PPO and PME) thus extending the shelf life[4]. The heat treatment of juice adversely affects the final juice quality, especially if the treated temperature is higher than 80°C for a long time[1,2]. Previous studies have reported juice quality changes due to thermal, sonication and ultraviolet treatment on mango juice including off-odor production followed by changes in taste, color (e.g., non-enzymatic browning) and clarity as well as loss of nutritional value (vitamins) and effects on microbial activity [5,6].

After the extraction of juice, browning occurs due to the oxidation of polyphenols. The main reason for this oxidation is polyphenoloxidase (PPO) activity by atmospheric oxygen, catalyzing the aerobic regioselective oxidation of monophenols to o-diphenols followed by dehydrogenation to brown o-quinones[7]. Cloud loss also occurs due to pectic enzymes. Pectinmethylesterase (PME) de-esterifies the methoxylated pectin in the fruits cell wall, which will be released in the juice during extraction[8]. The PME de-esterification of the methyl groups on the galacturonic acid, backbone of pectin, create charged regions complexing with Ca^{2+}, forming gels which precipitate and clarify the juice[9].

A control of enzymatic activity during juice processing is therefore, very important for the product quality. Consumers demand food that retains fresh-like quality with high nutritional value and longer shelf life without using food additives or preservatives. Therefore, the alternative thermal and non-thermal processing methods have resulted in growing interest[10].

Ohmic heating (OH) is considered as one of the upcoming technologies that allows volumetrically heating of food in a short time. The food (which act as an electrical resistance placed between two electrodes) is heated by passing an alternating electric current through it resulted in the conversion of the electrical energy into heat energy[11]. Ohmic heating is similar to high-temperature short time (HTST) treatment with additional benefits. The rapid, mild and uniform heating, which is volumetric in nature has the potential to reduce over-processing by the value of its inside-outside heat transfer pattern[12].

However, there is no reported study available on the NFC mango juice treated by ohmic heating. Therefore, the objective of this study was to investigate the effect of ohmic heating on the inactivation of PPO and PME in NFC mango juice. An optimization of the temperature and electrical field conditions with response surface methodology (RSM) was performed for comparison of mango juice quality parameters with the conventional heating method.

MATERIALS AND METHODS

Chemicals: Citrus pectin, catechol, polyvinyl poly pyrrolidone, NaOH, 2,6-Dichlorophenol-indophenol (DCPIP), sodium bicarbonate, L-ascorbic acid, oxalic acid, folin-ciocalteau reagent, gallic acid, butylated hydroxytoluene (BHT), sodium chloride, phenolphthalein, metaphosporic acid, methanol, hexane, acetone, buffer pH 4 and 7 and sodium carbonate from Sigma Aldrich Chemical Co., Denmark were used.

Juice material: Fresh mango fruits (*Mangifera indica* L. cv. Kent) were purchased from a local supermarket in Copenhagen, Denmark. The unblemished fruits were washed, dried by tissue paper, each mango was peeled and the stone was discarded. Mango pulp was macerated using a domestic juice extractor (Braun, Multiquick 3, Hungary) and then centrifuged (Sigma 3MK, Labrzentrifugen, GmbH Germany) at 12,000 rpm for 10 min at 4°C. The supernatant was filtered using a steel sieve with an approximate pore diameter of 2 mm to obtain the juice and remove any remaining fibers. The filtered mango juice was divided into 3 parts i.e., fresh mango juice, conventional heating (CH) and ohmic heating (OH). All samples were cooled quickly to 4°C using an ice bath and stored at -18°C to stop all reactions until further analysis.

Processing methods

Conventional heating: Mango juice (80 mL) was heated at 90°C for 60 sec in a clean 250 mL glass bottle using a shaker water bath (Julabo, SW22, Germany). The temperature was measured during the experiments by a thermocouple (Pico, TC-08, UK) within the center vertical axis of the mango juice bottles without agitation.
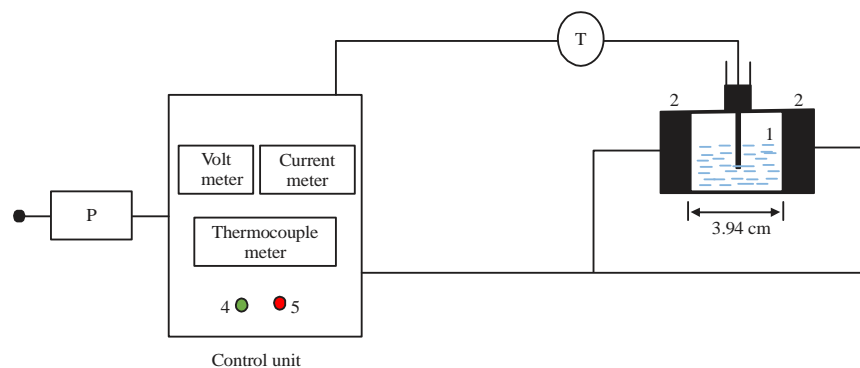
Fig. 1: Diagram of the experimental lab scale ohmic heating set (P: Power supply (0-230 V, 60 Hz), 1: Ohmic heating chamber, T: Titanium electrodes, 3-Thermocouple probe (K-type), 4: Start for power, 5: Stop for power)

Table 1: Three level factorial with experimental values of responses variable (PPO and PME activity of mango juice)

Run orders	Voltage gradient (χ_1) (V cm^{-1})	Temperature (χ_2) (°C)	PPO activity (U mL^{-1} min^{-1})	PME activity (U mL^{-1} min^{-1})
1	35 (0)	70 (0)	10.11±0.3	3.11±0.2
2	30 (-1)	80 (+1)	0.00	1.55±0.2
3	30 (-1)	60 (-1)	23.81±0.4	4.29±0.3
4	30 (-1)	70 (0)	9.82±0.3	3.32±0.2
5	35 (0)	70 (0)	10.05±0.2	2.97±0.1
6	35 (0)	70 (0)	9.95±0.3	2.98±0.2
7	40 (+1)	70 (0)	9.25±0.2	2.25±0.1
8	35 (0)	80 (+1)	0.00	1.35±0.2
9	35 (0)	70 (0)	9.92±0.3	3.10±0.2
10	40 (+1)	60 (-1)	21.85±0.2	2.97±0.3
11	40 (+1)	80 (+1)	0.00	0.85±0.2
12	35 (0)	60 (-1)	23.24±0.3	3.52±0.3

In the 2nd and 3rd column: The coded values of the test parameters are in parenthesis and the real (un-coded) values are outside the parenthesis, Data are expressed as means±standard deviation (n = 3)

Ohmic heating: An ohmic heater (BCH ltd., Lancashire, UK) with an ohmic unit consisting of a holding cell made of W500 grade polyethylene-polypropylene with variable size adjustment and mountings for temperature loggers (K-type) was used. Experimental ohmic heating set is shown in Fig. 1. A maximal supply at 230 v using alternating current (60 Hz, sinusoidal) was installed with the ohmic heater, a titanium electrode with high corrosion resistance in chloride environments[13]. A distance between the electrodes at 3.945 cm and a width of the chamber at 9.5 cm was set. After the system was closed, 80 mL of mango juices were added to the ohmic heater and heated at the set voltage gradient (e.g., 30, 35 and 40 V cm^{-1}) until it reached the desired final temperature (e.g., 60, 70 and 80°C). The experiments were performed in triplicate. After the treatment, the samples were cooled quickly to 4°C in an ice bath.

Experimental design: The effects of voltage gradient and temperature (independent variables) on PPO and PME activity (responses) of the NFC mango juice were investigated using response surface methodology (RSM). A 3-level factorial design (3^2) was used: Voltage gradient (30, 35 and 40 V cm^{-1}) and temperature (60, 70 and 80°C).

The 3^2 factorial design was set up for 2 factors, with 3 coded levels (-1, 0 and +1) as illustrated in Table 1. The significant terms in the models were found by analysis of variance (ANOVA) for the responses and validation of the equation was investigated by model ANOVA statistics. The regression coefficients were used to make statistical calculations to generate response surface plot from the model (trial version of design expert version 10.0.6 software). The generalized second-order polynomial model was used in the response surface analysis, which is described by Eq. 1:

$$Y = a_0 + a_1\chi_1 + a_2\chi_2 + a_{12}\chi_1\chi_2 + a_{11}\chi_1^2 + a_{22}\chi_2^2 \qquad (1)$$

where, Y is the response variable, χ_1 (voltage gradient) and χ_2 (temperature) are the independent variables. Regression coefficients are: a_o is for intercept, a_1 and a_2 are for the linear term, a_{11} and a^2_2 are for the quadratic term and a_{12} is for cross

product (interaction) term. The experimental data were fitted to a second-order polynomial model Eq. 1 to obtain the regression coefficients.

The adequacy of the model was checked using the R^2, adjusted-R^2, predicted-R^2 (should be above 0.90) and prediction error sum of squares (PRESS), where a large predicted R^2 and a low PRESS show a good model fitting[14]. Moreover, the effects of factors were compared at a particular point in the design space using the perturbation plot. Response surface and contour plots were then generated.

A desirability function was used for the optimization of OH parameters (voltage gradient and temperature) for PPO and PME inactivation. For each response (y), a desirability function d (y) ranging from 0-1 and completely dependent on closeness to the lower and upper limits. The desirability value ranges are from 0 (representing a completely undesirable value of y) to 1 (completely desirable or ideal response value). Depending on whether a particular response is to be maximized, minimized or assigned to a target value, numerous desirability functions can be used[15].

In this study, the main objective of optimization is minimizing the PPO and PME activity (response, y), therefore, the desirability function is described by Eq. 2:

$$d(y) = \begin{cases} 1 & y \leq L \\ \dfrac{(y\text{-}L)}{(U\text{-}L)} & L < y < U \\ 0 & y \geq U \end{cases} \qquad (2)$$

where, L and U are the lower and upper limit values of the response y, respectively. Minimization of the polynomials by desirability function method was carried out using a trial version of design expert version 10.0.6 software.

Physical analysis: Total soluble solids (TSS) was ready by using two drops of prepared mango juice samples were placed in a portable refractometer (Model No. p 300003, UK) and the TSS was read directly from the refractometer at the room temperature.

The pH value of mango juice was measured at room temperature using 10 mL of mango juice placed in a 50 mL beaker with a magnetic stirrer. The digital pH-meter was (model 420A, Orion Benchtop pH meter, Allometrics Inc.) calibrated before use with pH 4 and 7 buffer solutions.

The electric conductivity (EC) of mango juice samples were measured at the room temperature using a conductivity meter (WTW82362 Weinheim, LF323 Instrument, Germany).

The color parameters L* (lightness), a* (redness), b* (yellowness) and ΔE (total color differences) of mango juice was measured using a colorimeter (Model CR-200, Konica Minolta, Japan). ΔE was calculated using Eq. 3:

$$\Delta E = \sqrt{(L_o - L^*)^2 + (b_o - b^*)^2 + (a_o - b^*)^2} \qquad (3)$$

where subscript "o" refers to the values of fresh mango juice used as the reference and a larger ΔE indicated a greater color change from the fresh sample.

Cloud value, 5 mL of mango juice were centrifuged (Sigma 1A, AGB Scientific Ltd, Dublin, Ireland) at 3000 rpm for 10 min at room temperature. Cloud value was measured as the supernatant absorbance (250 µL) at 650 nm using a microplate reader (Biotek Synergy 2 Microplate reader, U.S.A) with distilled water serving as a blank[16].

Chemical analysis: For titratable acidity (% TA), 10 mL of mango juice were mixed with 40 mL of distilled water and titrated against standardized 0.1 M NaOH to the phenolphthalein end point (pH 8.2±0.1) using an auto-titrator (Dos Bio-5, 665 Dosimat, metrohm, Swiss). The volume of NaOH was converted to grams of citric acid per 100 mL of juice. Titratable acidity (TA) was calculated using Eq. 4:

$$TA = \frac{V \times 0.1M\ NaOH \times F \times 100}{m} \qquad (4)$$

where, V is the titration volume of NaOH, m is a mass of juice sample (g) and F is the factor of citric acid = 0.064 [17].

Ascorbic acid content in the mango juice was determined based on the 2,6-Dichlorophenol-indophenol (DCPIP) visual titration method[18]. About 5 mL of mango juice was immediately added into 10 mL of 3% metaphosphoric acid to halt any degradation of ascorbic acid and then titrated with standardized dye solution (0.25 g L^{-1} DCPIP). An auto-titrator (Dos Bio-5, 665 Dosimat, metrohm, Swiss) was used to deliver dye to the sample to a pink endpoint (color should persist for ≥15 sec). The results obtained were expressed as mg of ascorbic acid per 100 mL.

Total phenolic content was measured according to folin-ciocalteu method with some modifications[19]. About 5 mL of mango juice was mixed with 5 mL of 80% methanol in a 15 mL centrifuge tubes (sarstedt) and then the tubes were centrifuged at 4000 rpm for 20 min at 4°C (Sigma 4-16KS, Germany). For analysis, 100 µL appropriately diluted sample or

standard solution (10-100 μg mL^{-1}) at various concentrations was mixed with 100 μL folin-ciocalteu reagent and 3 mL deionized water and vortexed. After incubation for 10 min at room temperature, 100 μL of 20% sodium carbonate solution was added with immediate mixing and incubated at room temperature for 2 h in the dark. The mixture absorbance of 250 μL was then measured at 765 nm using a microplate reader (Biotek Synergy 2 Microplate reader, U.S.A). Gallic acid was used as standard and total phenolic contents of the samples were expressed as mg of gallic acid per 100 mL.

Total carotenoids content was measured according to Lee and Castle[20] with some modifications. About 5 mL of apple juice and 10 mL of hexane/methanol/acetone, 50/25/25, v/v with 0.1% BHT were mixed and centrifuged for 10 min 4000 rpm at 4°C. The absorbance of the supernatant phase was measured at 450 nm. The total carotenoid content was calculated as μg g^{-1} β-carotene using an extinction coefficient of 2505 in hexane[21].

The PPO activity was determined by the method of Trejo-Gonzalez and Soto-Valdez[22] with the following modification. About 5 mL of mango juice were mixed with 5 mL of 0.2 M sodium phosphate buffer (pH 6.8) containing 2% (w/v) polyvinyl polypyrrolidone (PVPP) and then centrifuged (Sigma 3 MK, Labrzentrifugen, GmbH made in Germany) at 10,000 rpm at 4°C for 30 min. About 0.5 mL of enzyme extract was mixed with 2.5 mL of 0.05 M catechol in 0.05 M sodium phosphate buffer (pH 6.8) and the reaction mixture incubated for 10 min at 25°C. The increase in the absorbance at 420 nm was measured by microplate reader. The PPO activity (1 unit) was defined as the increase in the absorbance by 0.001 min^{-1}.

Pectinmethylesterase (PME) was determined by the method described by Rouse and Atkins[8] and Ting and Rouseff[23]. About 2 mL of mango juice were mixed with 20 mL of a 1% citrus pectin substrate solution in 0.2 M sodium chloride. The mixture was titrated to pH 7.5 with 0.2 M NaOH. An auto-titrator (Dos Bio-5, 665 Dosimat, metrohm, Swiss) was used to deliver 0.05 M NaOH to the sample to maintain the pH at 7.5 for 10 min during hydrolysis at 30°C. The volume of 0.05 M NaOH consumed during this time was recorded. The PME activity expressed as PME units per mL was calculated by the formula Eq. 5:

$$PME(U\ mL^{-1}\ min^{-1}) = \frac{(mL\ of\ NaOH) \times (NaOH\ molarity)}{(mL\ of\ sample) \times (10\ min)} \times 10^4 \quad (5)$$

Microbiological analysis: Total plate count and mold and yeast were determined using plate count agar and potato dextrose agar medium, respectively. Plates were incubated at 35°C for 48±2 h for total plate count and were placed in the dark at 22-25°C for 5 days for mold and yeast[24].

Statistical analysis: Results of physical and chemical characteristics (section 3.2) were statistically analyzed by analysis of variance using the software SPSS 13 (SPSS Inc., Chicago IL, USA). One-way ANOVA was applied with the Duncan's test to evaluate differences between the treatments at levels of significance (p≤0.05). Each experiment was repeated atleast 3 times, means and standard deviations were calculated.

RESULTS AND DISCUSSION

Effect of ohmic heating parameters on the PPO activity: Ohmic heating parameters-voltage range of 30-40 V cm^{-1} and temperature range of 60-80°C were selected (Table 1) for RSM to evaluate the effect of OH parameters on PPO and PME activities and to optimize process parameters. This was done due to the observation that a voltage gradient of 40 V cm^{-1} lead to adverse color changes in the mango juice (preliminary experiment, data not given) and a similar result was reported for orange juice by Demirdoven and Baysal[25]. On the other hand, temperatures > 80°C at 40 V cm^{-1} causes juice bubbles leading to the loss of the juice during heating and further deterioration of the color and other quality characteristics[25,26]. The holding time for all OH treatments was set to 60 sec avoiding long treatment time leading to further quality deterioration[25]. Table 1 presents the 3^2 factorial design (at different voltage gradients and temperature) and the measured responses (the PPO and PME activity).

Table 2 presents the results of the ANOVA analysis and regression models built on the results of the factorial experiment (Table 1 and Eq. 1). It shows the effect of voltage gradient and temperature on the PPO and PME activity in the mango juice at 95% confidence interval. The models show a good fit according to the measured PPO and PME activity: A highly significant and having less variation around the mean (R^2 is 0.999 for PPO and 0.979 for PME and adj-R^2 is 0. 999 for PPO and 0.971 for PME). This means that 99.9% (for PPO) and 97.9% (for PME) of the response variability could be explained by the fitted model and the adj-R^2 and R^2 did not differ dramatically-implying a high degree of correlation between the experimental and predicted values. The lack-of-fit was not significant (p > 0.05). Therefore, based on the obtained results, the models (Eq. 6 and 7), equations are satisfactory in predicting the effect of the voltage gradient and temperature on the PPO and PME activities in the tested experimental ranges.

Table 2: Analysis of variance (ANOVA) and significance coefficient for PPO and PME activity of mango juice

Sources	Coefficient estimate		Sum of squares		df		p-value	
	PPO	PME	PPO	PME	PPO	PME	PPO	PME
Model	9.96	2.96	801.54	10.68	5	3	<0.0001	<0.0001
χ_1	-0.42	-0.52	1.07	1.59	1	1	0.0010	<0.0001
χ_2	-11.48	-1.17	791.20	8.24	1	1	<0.0001	<0.0001
$\chi_1 \chi_2$	0.49		0.96		1		0.0014	
χ_{12}	-0.34		0.31		1		0.0194	
χ_2^2	1.75	-0.53	8.13	0.85	1	1	<0.0001	0.0006
Residual			0.18	0.22	6	8		
Lack of Fit			0.16	0.21	3	5	0.0740	0.0662
Df = 11								
R^2	0.999	0.979						
Adj- R^2	0.999	0.971						
Pred-R^2	0.998	0.940						
CV	1.64	6.23						
PRESS	1.52	0.65						

p-value is significant at p<0.05, χ_1 is coded voltage gradient and χ_2 is coded temperature, df: Degress of freedom, CV: Coefficient of variation, PRESS: Predicted residual sum of squares, PME: Pectinmethylesterase, PPO: Polyphenoloxidase

Using surface response plots of the polynomial model, the negative linear effect of voltage gradient (χ^1) and temperature (χ^2) were found to be significant for the response variable (PPO and PME activity) and the quadratic effect of temperature (χ_2^2) on PPO and PME activity was also found to be significant (p<0.05). The effect of interaction ($\chi_1 \chi_2$) and the quadratic of voltage gradient (χ_{12}) was significant for PPO activity (p>0.05), however, these terms were insignificant for PME activity.

The non-significant variables were removed and the fitted second order polynomial equations are presented as Eq. 6 and 7 for PPO and PME, respectively:

$$PPO(U\,mL^{-1}\,min^{-1}) = Y_1 = \begin{bmatrix} 9.96 - 0.42\chi_1 - 11.48\chi_2 + \\ 0.49\chi_1\chi_2 - 0.34\chi_1^2 + 1.75\chi_2^2 \end{bmatrix} \quad (6)$$

$$PME(U\,mL^{-1}\,min^{-1}) = Y_2 = 2.96 - 0.52\chi_1 - 1.17\chi_2 - 0.53\chi_2^2 \quad (7)$$

Where:

χ_1 = Voltage gradient (V cm^{-1})

χ_2 = Temperature ($^{\circ}$C), are the coded values

As illustrated in Fig. 2a and 3a, the PPO and PME activity decreases with increasing voltage gradient and temperature, respectively. The model equations, perturbation and 3D response surface plots show the significant influence of temperature (factor B) on PPO activity with increasing the temperature (Fig. 2b), while on PME was significantly increased in high temperature more than at low temperature (Fig. 3b). On the other hand, the effect of voltage gradient (A in figure) is lower than temperature (B in figure) on PPO and PME activity (Fig. 2b and 3b), respectively. The effect of temperature is larger than the effect of voltage gradient on

the inactivation of both enzymes (PPO and PME, see perturbation plots (Fig. 2b and 3b). However, for PME inactivation, the effect of temperature is depending on the level of the voltage gradient, that means the effect of temperature (rate of inactivation of PME) is enhanced with increasing the voltage gradient. The obtained model (second order polynomial models, Eq. 6 and 7) were used to optimize the response (PPO and PME activities) to determine the optimum conditions. The optimum condition for OH of NFC mango juice was obtained at the minimum PPO and PME activity by applying desirability function. Ohmic heating (OH) at 40 V cm^{-1} and 80°C was selected as an optimum condition for inactivation of PPO and PME in the mango juice, which gave the best result for the PPO (zero U mL^{-1} min^{-1}) and PME (0.74 U mL^{-1} min^{-1}) inactivation (Table 3). The obtained optimum OH condition was used to produce the juice and then compared to the CH in section 3.2.

Physical and chemical characteristics of mango juice: Table 3 shows the experimental effects of CH and OH on enzyme (PPO and PME) activities in mango juice. A significant decrease in the PPO and PME activity in both CH and OH compared to control (fresh juice) was obtained. The PME enzyme is more resistant to heating than PPO in the mango juice: The PPO activity was completely inactivated in both treatments (CH at 90°C and OH at 40 V cm^{-1}, 80°C), while the inhibition of PME activity was 89.9 and 95.7% for CH and OH, respectively. The complete inhibition of PPO activity in the mango juice may be due to the effect of heat on protein (isozyme with a lower thermostability) of PPO[27]. Generally, inactivation of PPO has been detected at lower temperatures (inactivation start in the range 60-65°C (for mango juice than
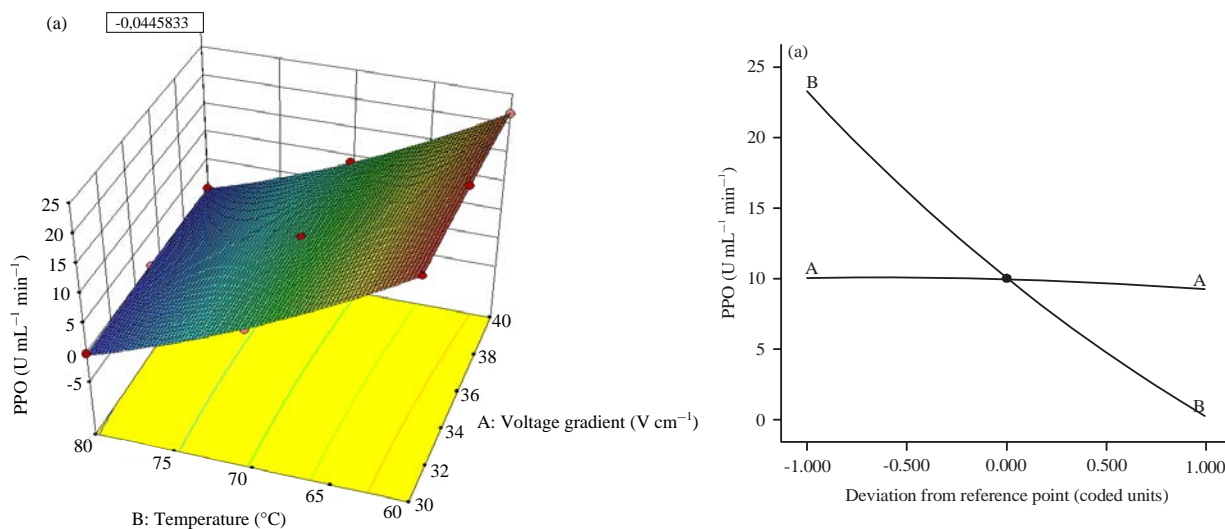
Fig. 2(a-b): (a) Effect of ohmic heating (OH) parameters (voltage gradient and temperature) on the PPO activity-response surface and contour plots. Blue indicates lower PPO activity and red indicates higher PPO activity and (b) Perturbation plot showing the relative significance of factors on the PPO activity
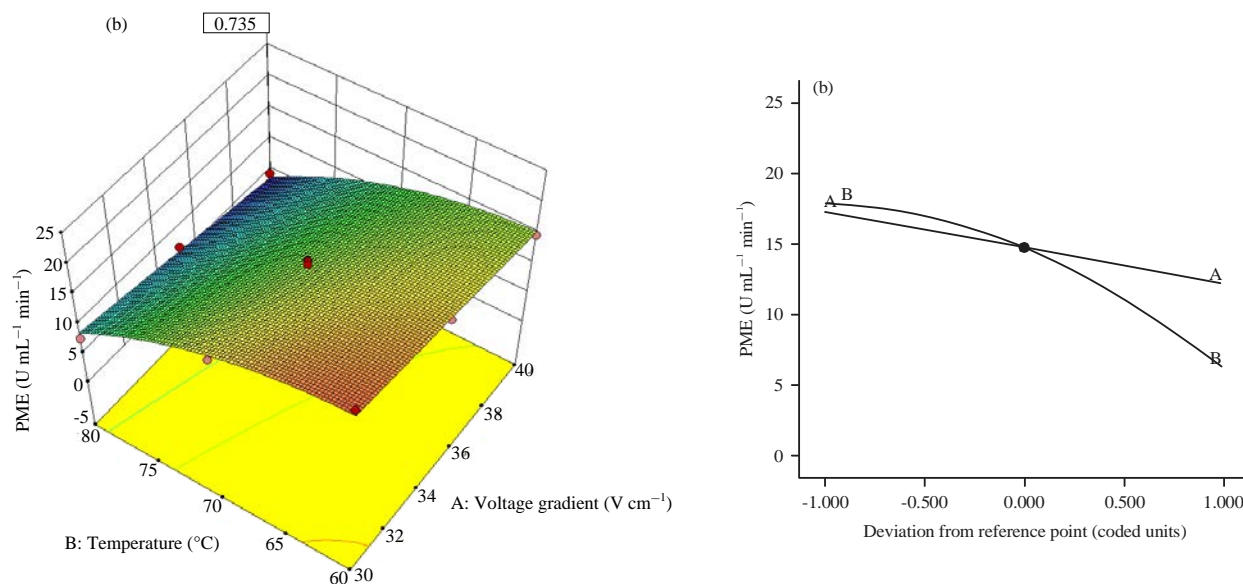

Fig. 3(a-b): (a) Effect of ohmic heating (OH) parameters (voltage gradient and temperature) on the PME activity-response surface and contour plots. Blue indicates lower PME activity and red indicates higher PME activity and (b) Perturbation plot showing the relative significance of factors on the PME activity

for other fruits like apple, plum, avocado and pear[28]. Also, polyphenoloxidase of mango pulp was rapidly deactivated after 1 min of pasteurization at 93°C[29].

In general, the reduction of PPO and PME activity was due to the effect of the heat during OH and CH treatment. In addition to OH, the effect of a voltage gradient could influence biochemical reactions by changing molecular spacing and increasing interchain reactions[30]. In the literature, no study

was found on OH heating of mango juice. Two studies on orange juice reported results similar to this study, namely, that the reduction (compared to fresh orange juice) of PME activity was larger in OH than CH treated samples[25,31]. Cloud value was significantly increased by 63.5% (from 1.332 A to 2.179 A) for OH and 46.6% (from 1.332-1.954 A) for CH treated mango juice. The increase in the cloud value means a better cloud stability which indicates the increased inactivation of pectic

Table 3: PPO and PME activities of ohmic and conventionally heated mango juice

Heating treatments	Observed PPO activity (U mL^{-1} min^{-1})	Inhibition of PPO (%)	Predicted PPO activity (U mL^{-1} min^{-1})	Observed PME activity (U mL^{-1} min^{-1})	Inhibition of PPO (%)	Predicted PME activity (U mL^{-1} min^{-1})	Cloud value (A)
Fresh juice	44.65±2.66	0		19.77±0.6[a]	0.0		1.332±0.24[c]
CH	0	100		1.99±0.3[b]	89.9		1.954±0.11[b]
OH	0	100	0	0.85±0.2[c]	95.7	0.74	2.179±0.14[a]

Different letters [a,b,c]mean statistical significant difference (p<0.05), the results represent the mean±standard deviation, A: Absorbance, CH: Conventional heating (at 90°C for 60 sec), OH: Ohmic heating (40 V cm^{-1} at 80°C for 60 sec)

Table 4: Effect of ohmic and conventional heating on ascorbic acid, total phenolic compounds (TPC), total carotenoids content (TCC), cloud value and color values (L*, a*, b*and ΔE) of mango juice

Heating treatments	Ascorbic acid (mg/100 mL)	TPC (mg/100 mL)	TCC (mg/100 g)	L*	a*	b*	ΔE
Fresh juice	23.39±0.74[a]	32.36±0.13[a]	9.88±0.10[a]	46.80±0.03[a]	-6.20±0.07[b]	13.83±0.04[a]	-
CH	18.93±0.15[c]	33.48±0.05[a]	7.96±0.06[c]	46.44±0.05[b]	-6.43±0.05[c]	13.62±0.04[b]	0.48±0.05
OH	20.37±0.23[b]	33.63±0.17[a]	8.80±0.04[b]	46.92±0.09[a]	-5.98±0.05[a]	13.96±0.09[a]	0.30±0.14

Different letters [a,b,c]mean statistical significant difference (p<0.05), the results represent the mean±standard deviation, CH: Conventional heating (at 90°C for 60 sec), OH: Ohmic heating (40 V cm^{-1} at 80°C for 60 sec)

Table 5: Titrable acidity (TA), pH, TSS, electric conductivity (EC), total plate count (log CFU mL^{-1}) and mold and yeast (log CFU mL^{-1}) of mango juice

Heating treatments	pH	TA (as a citric acid %)	TSS	EC (S m^{-1})	Total plate count (log CFU mL^{-1})	Mold and yeast (log CFU mL^{-1})
Fresh juice	3.22±0.03[a]	0.64±0.02[a]	12.5	0.279±0.002[a]	3.14±0.2	2.97±0.2
CH	3.20±0.03[a]	0.63±0.01[a]	12.4	0.283±0.002[a]	nd	nd
OH	3.21±0.02[a]	0.63±0.02[a]	12.5	0.286±0.003[a]	nd	nd

Different letters [a,b,c]mean statistical significant difference (p<0.05), the results represent the mean±standard deviation, nd: Not detected, CH: Conventional heating (at 90°C for 60 sec), OH: Ohmic heating (40 V cm^{-1} at 80°C for 60 sec)

enzymes (especially PME). This is clear in Table 3, with the increased PME inactivation in the OH (95.7%) compared to CH (89.9%) leading to increased cloud value in the OH compared to CH.

Table 4 presents the effects of OH and CH on the ascorbic acid, total phenolic compounds (TPC), total carotenoids content (TCC), cloud value and color values (L*, a*, b*and ΔE) of mango juice.

Ascorbic acid content in the OH treated sample (20.4 mg/100 mL) is significantly larger than the CH treated sample (18.9 mg/100 mL). The OH treatment is a better processing method to retain the heat-sensitive ascorbic acid than conventional heating, because OH is a fast heating (33 sec to reach 80°C, at 40 V cm^{-1}) process compared to the conventional heating (570 sec to reach 90°C) process. Moreover, the increase in the voltage gradient during OH treatment shortens the heating time (i.e., increase heating rate) to the desired temperature.

The total phenolic content (TPC) in the OH (33.6 mg/100 mL) and CH (33.5 mg/100 mL) treated samples increase slightly (but not significantly) compared to the fresh juice (32.4 mg/100 mL). The increase in the total phenolic content could be attributed to the increased extractability of total phenolic components due to the changes in the tissue matrix induced by heating according to (McInerney *et al.*)[32] or a disruption of complexes between polyphenols and proteins[33]. During OH, the alternating current

has a synergistic effect on releasing total phenolic contents resulting in a slight increase in phenolic content[34].

Total carotenoids of mango juice were significantly decreased in the OH (8.80 mg/100 g) and the CH (7.96 mg/100 g) compared to the fresh mango juice (9.88 mg/100 g). Similarly, total carotenoids of fresh mango (Kent) to be 9.6 mg /100 g was reported[35]. The degradation in the total carotenoids for OH and CH was caused by heat treatment. A significant reduction of total carotenoids after conventional thermal treatment, however for orange juice was reported[36,37].

The color values (L*, a*, b* and ΔE) in the OH treated samples increase slightly (but not significantly) however, the color values of the CH treated samples decrease significantly compared to the fresh juice. The ΔE value was 0.30 for OH and 0.48 for CH. The larger ΔE in the CH indicates a higher decrease in the color compared to the reference sample (fresh), while ΔE in the OH has an increase in the color values compared to a fresh sample. The changes in the color values (L*, a*, b* and ΔE) during thermal treatments might be due to certain chemical changes (i.e., condensation or degradation of phenolic compounds or more extract for some pigments that effect on the color values and this depends on the time of heat treatment)[31].

From Table 5, no significant differences between OH and CH treated samples in the pH, TA, TSS and EC (as citric acids %) were observed. Also, no significant differences between OH

and CH treated orange samples in the TSS content were observed[25,38]. No microbial growth was detected for both OH and CH. The decrease in the microbial load was due to the effect of thermal heating for CH and effect of thermal heating with electric field during OH treatment[39].

This study demonstrated a significant improvement in quality characteristics of NFC mango juice treated by OH compared to CH. Also, the results indicate that OH can be applied as a thermal treatment on NFC mango juice production in mild temperatures for PPO and PME inactivation with improving functional properties. The study showed a need for further studies about OH effects during storage conditions and combinations with other technologies.

CONCLUSION

In this study, optimal ohmic heating conditions (i.e., temperature, 60-80°C and voltage gradient, 30-40 V cm^{-1}) for PPO and PME inactivation in the mango juice were a voltage gradient of 40 V cm^{-1} and a temperature of 80°C. An improvement in the mango juice quality was achieved by the optimized OH process condition compared to CH treatment. PPO activity was completely inactivated for both OH holding for the 60 sec) and CH (at 90°C holding for 60 sec), while the inhibition of PME activity was 96 and 90%, respectively. The loss of ascorbic acid and carotenoids contents in the OH treated samples were significantly lower than the CH treated samples. The color values (L*, a*, b* and ΔE) was improved using OH compared to CH treatment. Overall, the results indicate that OH can be used as a potential mild thermal treatment for pasteurization-inactivating enzymes (PPO and PME) and showing the antimicrobial effect to extend the shelf life and improving the quality characteristics of NFC mango juice.

SIGNIFICANT STATEMENT

This study discovers that ohmic heating is a potential mild thermal treatment that can be beneficial for the production of not-from-concentrate (NFC) mango juice with improved functional properties instead of conventional methods. This study will help the researchers to uncover the critical areas of NFC juices production by electrothermal technologies that many researchers were not able to explore. Thus a new theory on these electrothermal technologies and possibly combinations with other technologies may be arrived at.

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