Inactivation effect of microwave heating on pectin methylesterase in orange juice

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Abstract

Introduction. Enzyme inactivation is a major objective in orange juice production. Conventional heating in elevated temperature causes adverse effects on the final products such as color alterations, flavor damages, ascorbic acid losses. For this, microwave heating (MW) was used in this study as thermal treatment on orange juice production for pectin methylesterase (PME) inactivation, and response surface methodology (RSM) was used for optimization of MW conditions.

Materials and methods. Oranges (*Citrus sinensis* Osb.) of Navel variety were used as raw material in this study. The effects of flow rate and power on PME activity were investigated. After optimization orange juice was produced with using optimized conditions and compared with untreated control juices and conventional thermally heated juices on the aspect of PME inactivation and some quality characteristics.

Results and discussion. The linear effects of flow rate (x_1) and power (x_2), as well as the quadratic effect of flow rate (x_1^2), power (x_2^2) and the interaction effect of flow rate-power (x_1x_2) were significant for PME inactivation by MW. Lack of fit of experimental data was not significant ($P>0.05$) for model. The coefficient of variation (C.V.) was 6.27%. The model showed an adequate precision of 6.788×10^{-3}. The determination coefficient (R^2) was 0.9793, while the adjusted determination coefficient (adjusted R^2) value was 0.9645. R^2 and adjusted R values for the models did not differ dramatically indicating non-significant terms have not been included in the model. Reduction of PME activities was found approximately 93–95% in MW groups. The PME inactivation rate was described satisfactorily as a function of microwave heating conditions. The PME can be inactivating in moderate temperatures by MW (40 mL/min-900W-83 °C) and MW (50 mL/min-900W-75 °C). D values were calculated for two optimum conditions of MW and CH treatments and found as 39.24 sec for MW (40 mL/min-900W), 38.76 sec for MW (50 mL/min-900W) and 70 sec for CH (95 ºC–60 sec). Total pectin content was increased 17.2% after MW application. And the loss of ascorbic acid content for MW sample was found lower than other applications.

Conclusions. A synergistic effect of microwave energy and temperature on orange juice PME inactivation was found under microwave heating conditions. It was determined that MW (50 mL/min-900W) can be applied as a thermal treatment on orange juice production in moderate temperatures (75 °C) for PME inactivation and may improve functional properties of orange juice. And this result is extremely important in terms of cloud stability of orange juices.

Introduction

Orange juice is a widely consumed product owing to its high nutritional value and desirable sensory characteristics [1] and a common problem associated with orange juice (fresh squeezed, concentrated and preserved) is the loss of cloudiness [2], and it is a critical orange juice quality parameter imparting characteristic flavor, color and mouthfeel. The juice cloud, which is composed of finely divided particulates of pectin, cellulose, hemicellulose, proteins and lipids in suspension, is considered a desirable characteristic of orange juice [1, 3, 4]. Cloud stability is affected by the impact of pectic enzymes, particularly pectin methylesterase (PME) [5]. PME, a ubiquitous enzyme in plants, de-esterifies the methoxylated pectin in the plant cell wall. PME (EC 3.1.1.11) is also referred as pectin demethoxylase, pectin methoxylase, pectase, pectinoesterase and pectinesterase, released into juice during extraction [6, 7, 8]. The design for thermal pasteurization of orange juice is based on the thermal destruction characteristics of PME which is thermally stable than many vegetative microorganisms [9, 10]. Conventional thermal processing is the most common method to inactivate PME and to prevent juice cloud precipitation; additionally to control microbial growth in orange juice production. Pasteurization at 90-95 ºC for 60-90 sec is used to inactivate the most tolerant PME isoenzyme. However it can be reduce freshness, affecting sensory and nutritional characteristics of orange juice. This conventional treatment in elevated temperature causes adverse effects on the final products such as color alterations, flavor damages, ascorbic acid losses [11-14].

As consumers are highly demanding minimally processed and fresh-like food products, the use of emerging technologies is gaining popularity. The food industry is interested in emerging technologies which inactivates enzymes and microorganisms without significant adverse effects on flavor and nutrients [15]. Microwave heating is an emerging technology in food processing, which bases its preservation properties in thermal reduction of the microbial counts in foods. Heating takes place due to the interaction of electromagnetic radiation at certain frequencies with dielectric materials; this is also called volumetric heating due to, in contrast with convection or conduction mechanisms, microwave radiation directly penetrates the material causing volumetric heat generation in the material, resulting in high-energy efficiency and lower heating times [16, 17]. This technology has found many applications in the food industry, as it has important advantages over conventional heating, such as reduced processing time, high-energy efficiency, and improved food quality [17]. Microwave heating as an alternative method for fruit juice pasteurization has now gained better acceptance as it offers several advantages over the conventional method. The advantages for the use of microwave energy in fruit juice processing are (i) heating the juice directly, (ii) no heat-transfer films, (iii) improved temperature control, (iv) rapid startup and cooldown, and (v) less heat lost to the environment.[18] Microwave pasteurization of fruits and fruit juices, e.g. citrus juices, involving enzyme inactivation has not been commonly studied. There have been few reports on PME inactivation using microwave energy [7, 14, 16–18, 20–22]. However, no optimization data for microwave inactivation of PME has been reported.

Therefore the objective of this study was to investigate the effect of microwave heating on the inactivation of PME on orange juice and to optimize the moderate temperature conditions for electrical field application with response surface methodology (RSM) and to compare it with the conventional thermal treatment. Then effects of MW treatment at optimized conditions on the PME activity and some quality characteristics were determined by physical and chemical analyses.

Materials and methods

Material. Oranges (*Citrus sinensis* Osb.) of Navel variety were used as raw material in this study. The oranges were purchased from Zumdieck Frozen and Canned Food Company (Salihli-Manisa, TURKEY). They were stored in a refrigerator at 7 ºC and 80–90% humidity for maximum of 48 hours before processing.

Processing methods.

Orange juice production: After washing and peeling applications oranges were processed to orange juice by using a juice extractor (Moulinex, JU5000-800W) and all groups (control, MW and CH) were sieved (mesh 3.5 mm). Then orange juices were divided into three groups; (i) control group (without any treatment) and (ii) MW application group, process conditions (flow rate and power) were optimized by using RSM; (iii) conventional thermal treatment group (CH) to determine PME inactivation and quality effects;

MW application: MW heating was used for the pasteurization of the orange juices. A modified MW oven (Model Arçelik MW 595, Istanbul, Turkey) with 2450 MHz operated at 540 to 900 W was used. The heating region of the MW oven contained a 3-m-long silicon hose (diameter of hose 8 mm-inside; 11 mm-outside) and a peristaltic pump for controlling flow (Watson Marlow [505U] Ltd., Falmouth, Cornwall, U.K.). Entrance and exit temperatures were measured by thermocouples. The maximum difference among the measured temperatures was approximately 1 °C. The experiments were replicated three times. The average temperature of the replicated heating experiments was accepted or used as the measured temperature values. Flow rates between 40 and 80 mL/min at 540, 720, and 900 W were studied. Flow rates and power were optimized for pasteurization process by RSM and the PME activity was taken as a response. By the ANOVA, flow rate and power were found to be significantly important on PME activity at 95% confidence interval. Model was tested for lack of fit.

CH application: Conventional thermal treatment was realized in a water bath (DKZ Series). Shelf stable orange juices, processing times for thermal pasteurization are equivalent to 90-95 °C for 60-90 sec [23, 24]. As given in literature; 200 mL bottled orange juice were heated until 95 °C and kept at the same temperature for 60 sec. The temperature was measured in the juice within the center vertical axis of the bottles without agitation. And the water bath temperature was 100 °C; all of the bottled orange juices were come up to 95 °C in 10 minutes and kept at the same temperature for 60 sec. Then orange juice was processed at optimized conditions to compare quality characteristics. Processed orange juices by MW were filled into 200 mL sterile bottles in aseptic conditions and closed leaving the minimum amount of headspace volume. After production of orange juices, all samples were cooled +4^{o}C in an ice bath and analyses were conducted after production. After heating treatments decimal reduction times (D values) of PME were calculated for MW optimum points and conventional heating treatments.

Methods of analysis.

Response measurement techniques: RSM was used for optimization of MW conditions (Myers and Montgomery, 1995) [25]. The effects of flow rate and power (independent variables) were investigated on PME activity (response) of orange juice. A central composite rotatable design was used in designing the MW treatment of two variables at five levels (Design Expert 7.0.0 STAT-EASE, 2005). PME activities were determined after MW applications.

The model adequacies were checked by R^2, adjusted-R^2, predicted-R^2 and prediction error sum of squares (PRESS) [25]. A good model will have a large predicted R^2, and a low PRESS. After model fitting was performed, residual analysis was conducted to validate the assumptions used in the ANOVA. This analysis included calculating case statistics to identify outliers and examining diagnostic plots such as normal probability plots and residual plots. Maximization and minimization of the polynomials thus fitted was performed by desirability function method and mapping of the fitted responses was achieved using Design Expert Version 7.0.0 software.

Chemical and physical analysis: PME activity and quality characteristics of orange juices were investigated and samples were analyzed to determine the following:

PME activity was measured by continuous recording of the titration of carboxyl groups released from a pectin solution using a pH meter (WTW InoLab, Weilheim, Germany) and 0.01 M NaOH. Routine assays were performed with a 0.5% pectin (Sigma-Aldrich Corp., St. Louis, Mo., U.S.A.) solution (25 mL) containing 0.117 M NaCl (pH 7.0) at 30 °C. An activity unit (U) of PME is defined as the amount of enzyme required to release 1 μmol of carboxyl groups per minute [26, 27].

Pectin content was investigated according to AOAC (1968) [28]. The method is based on the extraction of pectin with ethanol after centrifugation (4000 rpm, 15 min, 20 ºC) (CFC free Universal Hettich Zentrifugen, Tuttlingen, Germany); the precipitated part was treated with 5 mL NaOH and completed to 100 mL with deionized water. After filtration, samples were prepared with 0.5 mL carbazol (Merck, Darmstadt, Germany) and 0.5 mL ethanol. Sulfuric acid (Merck) (6 mL) was added to both samples, then they were placed in a water bath at 85 ºC for 5 min. Absorbance values were taken at 525 nm with a Varian Cary 50 Scan (Sydney, Australia) spectrophotometer and the pectin content was calculated with the calibration curve that was made by using gallacturonic acid anhydrate standards (Sigma-Aldrich Corp.).

Ascorbic acid was determined according to Hışıl (2004) [29]. 10 mL of orange juice were homogenized in a Waring Blender (Waring Products Inc., Connecticut, USA) with 90 mL of 0.4% aqueous oxalic acid solution, filtrated through Whatman No 1 paper. 1 mL of filtrated sample was mixed with 9 mL 2,6 dichlorophenol-indophenol (Merck KGaA, Germany), and immediately the absorbance was measured with a spectrophotometer (Varian Cary 50 Scan, Australia) at 518 nm against the solution of sample mixed with distilled water (1:10). Standard curve was constructed by using L(+) ascorbic acid (Carlo Erba Reagenti SpA) solutions with known concentrations, and ascorbic acid content of the samples calculated against the standard curve.

Total soluble matter (ºBrix) of juices was measured with a refractometer at 20 ºC (RFM 330; Bellingham+Stanley Limited, Tunbridge Wells, Kent, U.K.) [30]. The pH values of orange juices were measured with pH meter-WTW InoLab at 20 ºC [30]. The color (L*, a*, b*) values of orange juice were measured with a HunterLab Colorflex colorimeter (Hunter Associates Laboratory, Reston, Va., U.S.A.) and total color differences (ΔE) and chroma (ΔC) values were calculated according to control group. Orange juices were placed on the light port using a 5 cm diameter glass dish with cover. Color parameters were recorded as L* (lightness), a^* (redness) and b^* (yellowness) and total color differences (ΔE) values were calculated according to equations 1.

$$\Delta E = [(L-L_{ref})^2+(a-a_{ref})^2 + (b-b_{ref})^2]^{0.5} \quad (1)$$

Statistical methods: Results were statistically analyzed by analysis of variance (ANOVA) using the software SPSS 13 (SPSS Inc., Chicago, IL, USA) with the Duncan test

to evaluate differences between treatments at levels of significance $P \leq 0.05$. Each experiment was repeated at least three times; means and standard deviations of results were calculated.

Results and discussion

Optimization ranges of MW application were determined by pretesting. By the aim of choosing the suitable flow rate interval some experiments were made between flow rate ranges of 20-100 mL/min at 900 W. Under 40 mL/min flow rate orange juice outlet temperatures were measured upper than conventional heating temperatures (95 °C). Above 80 mL/min application, PME was activated because of the low temperature (~55 °C). And the effects of power on PME inactivation were determined by three power levels as 540, 720 and 900 W. So independent variables (flow rate and power) interval were chosen as 40–80 mL/min; 540-900 W for RSM. By the ANOVA, flow rate and power were found significantly important on the PME inactivation of orange juice at 95% confidence interval. Model was tested for lack of fit. Table 1 shows the experimental design and responses.

Table 1

Central composite rotatable design with experimental values of response variables

Run #	Flow rate (mL/min) *(x_1)*	Power (W) *(x_2)*	PME activity* (μmol/min/mL)
1	60	720	0.243±0.02
2	60	540	0.281±0.01
3	88	720	0.373±0.03
4	40	540	0.173±0.01
5	60	720	0.276±0.01
6	32	720	0.130±0.03
7	60	900	0.179±0.02
8	60	720	0.276±0.02
9	80	540	0.366±0.04
10	60	720	0.276±0.02
11	80	900	0.260±0.02
12	40	900	0.098±0.01
13	60	720	0.281±0.01

* Results are presented as means ±SD (n = 3).

The linear effects of flow rate (x_1) and power (x_2), as well as the quadratic effect of flow rate (x_1^2), power (x_2^2) and the interaction effect of flow rate-power (x_1x_2) were significant for PME inactivation by MW. Lack of fit of experimental data was not significant ($P>0.05$) for model. The coefficient of variation (C.V.) was 6.27%. Adequate precision compares the model predicted values with its associated error, its signal to noise ratio. Ratios greater than 4 indicate adequate model discrimination. The model showed an adequate precision of 6.788×10^{-3}. The determination coefficient (R^2) was 0.9793, while the adjusted determination coefficient (adjusted R^2) value was 0.9645. R^2 and adjusted R values for the models did not differ dramatically indicating non-significant terms have not been included in the model. There was a high correlation between the experimental and predicted

values. These statistical parameters confirm the consistency of model, indicating that it is reliable to predict PME inactivation. Using the regression coefficients from the adjusted model (Table 2) the following model equation was generated (x_1: flow rate, mL/min; x_2: power, W):

$$\text{PME activity (μmol/min/mL)} = 0.27 + 0.087\,x_1 - 0.041\,x_2 - 7.5x10^{-0.03}\,x_1\,x_2 - 0.014\,x_1^2 - 0.025\,x_2^2 \quad (2)$$

(x_1: flow rate; x_2: power)

Table 2

Anova table showing the variables as quadratic terms on response variable and coefficient for the prediction model

Source	DF**	Coefficient	Sum of squares	p-value*
Model	5	+0.27	0.080	< 0.0001
x_1	1	0.087	0.061	< 0.0001
x_2	1	-0.041	0.013	< 0.0001
$x_1 x_2$	1	$-7.5x10^{-0.03}$	$2.250x10^{-4}$	0.3659
x_1^2	1	-0.014	$1.307x10^{-3}$	0.0526
x_2^2	1	-0.025	$4.186x10^{-3}$	0.0042
Residual	7		$1.686x10^{-3}$	
Lack of fit	3		$7.487x10^{-4}$	0.4572
Pure error	4		$9.369x10^{-4}$	
Total	12		0.081	
R^2		0.9793		
Adj-R^2		0.9645		
Pred-R^2		0.9167		
PRESS		$6.788x10^{-3}$		
CV		6.27		

*p-value < 0.05 is significant at a = 0.05. Lack of fit is not significant at p-value > 0.05.
** DF: Degress of freedom

Optimum condition for MW of orange juices was determined to obtain minimum PME activity. Second order polynomial models obtained in this study were utilized for response in order to determine the specified optimum conditions. These regression models are valid only in the selected experimental domain. In this study, flow rate and power were selected in the range of 32–88 mL/min, 540–900 W respectively. Figure 1 shows the effect of flow rate and power PME inactivation of the orange juice at MW application. By applying desirability function method, two solutions were obtained for the optimum covering the criteria. The first one is 40 mL/min for flow rate and 900 W for power. The second one is 50 mL/min for flow rate and 900 W for power. The results indicate that the high powers on lower flow rates can decreases PME activities and both solutions gave nearly same desirability values (0.9). So, the factor level combinations obtained both solutions were selected as the optimum. Production of orange juices was made at two optimum point for determining PME activities and quality properties. Predicted value by RSM was suitable with the observed value of PME activities as shown in Table 3.

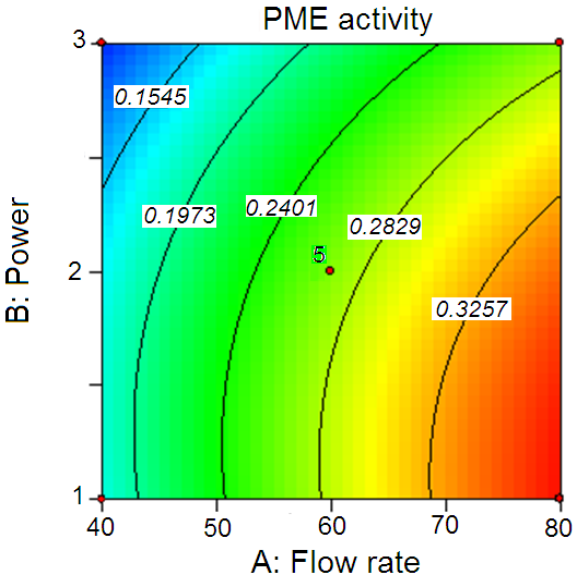

Figure 1. **The effect of voltage gradient and temperature on pectin methylesterase inactivation of the orange juice at microwave heating applications**

Table 3

Predicted and observed responses at optimum level of variables

Sample	Predicted value of response, PME activities (µmol/min/mL)	Observed response, PME activities** (µmol/min/mL)	Reduction of PME activities (%)*
MW (40 mL/min-900W)	0.0734	0.087±0.05[a]	95.36
MW (50 mL/min-900W)	0.1221	0.130±0.04[b]	93.04
CH (95 °C-60 sec)	------	0.260±0.03[c]	86.08
Control	------	1.868±0.06[d]	-------

* Reduction of PME activities (%) were calculated according to control PME activities

**Statistically significant difference shown levels a, b ($P \leq 0.05$); results are presented as means ±SD ($n = 3$).

PME activities of orange juices were found as 0.087 µmol/min/mL MW (40 mL/min-900W) and 0.130 µmol/min/mL MW (50 mL/min-900W) whereas control group has 1.868 µmol/min/mL PME activities. In CH (95 °C-60 sec) group PME activities of orange juices were found as 0.260µmol/min/mL. It was found statistically significant ($P < 0.05$). Reduction of PME activities were found as 95.36%, 93.04%, 86.08% for MW (40 mL/min-900W), MW (50 mL/min-900W), CH (95 °C-60 sec); respectively (Table 3).

Results were in agreement with Nikdel et al., (1993) [18]. They found 98.5-99.5% reduction of PME activity during MW (>75 °C with 10–15 sec) compared to its activity in fresh orange juice. This compared to 99.0% inactivation by traditional pasteurization for 15 sec at 90.5 °C. Villamiel et al., (1998) reported that continuous microwave heating compares favourably with conventional heating at PME inactivation temperatures [14] .

Tajchakavit and Ramaswamy (1995) found that the comparison of continuos-flow MW and thermal inactivation kinetics of PME at 60 °C indicated the MW inactivation rate to be 7.5 and 3.5 times faster than convectional thermal inactivation rate under batch type and continuous flow heating conditions indicating the possibility for some additive non-thermal effects [31]. Tajchakavit and Ramaswamy (1997) reported that the PME decimal reduction times for microwave heating were 38, 12, 4.0 and 1.3 sec at 55, 60, 65 and 70 °C, respectively [22]. And as compared with about 150 and 37 sec at 60 and 70 °C respectively, during conventional heating. In the literature, there are few reports on MW heating of orange juice for PME inactivation but there are some other studies on PME inactivation for orange juice by using high pressure, PEF etc. Yeom et al. (2002) found a 90% orange PME activity reduction with 125 pulses of 2-μs-pulse-width at 25 kV/cm [32] and Elez-Martinez et al. (2003) reached an 80% activity reduction of orange PME after 375 pulses of 4-μs-pulse-width at 35 kV/cm [33]. Moreover, Rodrigo et al. (2003) achieved an 81.3% PME reduction in orange-carrot juice after a 350-μs HIPEF treatment of 35 kV/cm [34]. Giner et al. (2005) also observed an 86.8% PME inactivation in a commercial pectolytic enzyme preparation [35]. Different results achieved by employing techniques can be related to different temperature/time history of orange juice samples. In addition, this difference is the result of thermostable PME ratio of total-PME. The relative ratio of the thermostable PME to total-PME can vary between 0 and 33% depending on the citrus cultivars. In the case of oranges, the percentage of PME fractions depends on the variety of the oranges [36-38], geographic location, growth practice, post-harvest handling, seasonal differences [37], fruit tissues [8], and experimental changes in protocol [39]. A 5% thermostable PME fraction was observed for Valencia oranges [38]. In Navel oranges, Van den Broeck et al. (2000) [40] and Versteeg et al. (1980) [7] also found a 5% heat-stable PME fraction. In addition, according to Rombouts et al. (1982) [36], the thermostable PME represented 6% of the total activity in Navel oranges, 11% in Salustiana oranges and 7% in Shamouti oranges. And our results shows additional PME inactivation rates for Navel oranges by MW heating.

Thermal heating histories and D values of microwave heating (optimum points) and conventional thermally heated orange juices were shown in Table 4. D values were calculated for two optimum conditions of MW and CH treatments and found as 39.24 sec for MW (40 mL/min-900W), 38.76 sec for MW (50 mL/min-900W) and 70 sec for CH (95 ºC – 60 sec). D value defined as the heating time required to result in 90% inactivation of initial activity. As seen from the results, to inactivate 90% of PME at MW conditions need shorter time than CH. And it shows the additional effects of electromagnetic energy. D values of PME inactivation in orange juice obtained following microwave heating were remarkably smaller than those obtained from conventional thermal inactivation. This indicates that microwave heating is more effective than conventional heating in inactivating PME in orange juice with some contributory nonthermal effects. Wicker and Temelli (1988) reported the D value of 0.225 sec at 90 °C for orange juice [41]. The calculated ranges of D values are 10-390 sec at 55 °C and 6-36 sec at 70 °C. In comparison with these values, the D values obtained under microwave heating conditions are smaller by an order of magnitude. This indicates that microwaves cause inactivation of PME in some way which cannot be solely explained by thermal effects. In other words, these results confirm the contributory nonthermal effects of microwaves resulting in enhanced inactivation of PME in orange juice as also observed in our study. Versteeg et al. (1980) showed three forms of PME in Navel oranges, one of which, the high molecular weight PME, was reported to be the most heat resistant fraction D value of 24 sec at 90 °C; of the other two forms of PME isozymes, one was more rapidly inactivated (D60 of 47 sec) than the other (D70 of 27 sec) [7]. These studies indicate that PME inactivation kinetics depend on several

factors: variety and composition (acidity, total solids, pulp content) of juice as well as heating method.

Table 4

Termal heating histories of microwave and conventional heated orange juices

Heating treatments	F (mL/min)	P (W)	T_{in} (ºC)	$t_{h:}$ (sec)	T_{out} (ºC)	t_t (sec)	$T_{c\text{-}out}$ (ºC)	D* (sec)
Microwave	40	900	25±2	52	83±1	52	+4±1	39.24±0.2[a]
Microwave	50	900	24±2	45	75±1	45	+4±1	38.76±0.5[a]
Conventional heating	___	___	24±2	60	95±1	660	+4±1	70.00±0.2[b]

F: flow rate; P: power $T_{in:}$ inlet temperature; $T_{out:}$ outlet temperature after heating treatments; $T_{c\text{-}out:}$ outlet temperature after cooling;
$t_{h:}$ total heating time; $t_{t:}$ total treatment time; D: Decimal reduction times of PME;
*Statistically significant difference shown levels a, b ($P \leq 0.05$); results are presented as means ±SD ($n = 3$).

Results of chemical and physical analyses of orange juice which produced at optimum conditions were shown in Table 5. Pectin content is important for cloudiness of orange juice. Total pectin content was increased 2.6% and 17.2% for MW (40 mL/min-900W) and MW (50 mL/min-900W) respectively. The difference between pectin content of samples was found statistically significant ($P \leq 0.05$). Yıldız and Baysal (2006) [42]; investigated the effects of alternative current on pectin content in tomato samples and found 3.23% pectin content at 68 V/cm for 23 sec application (78ºC) and 3.15% pectin content at 48 V/cm for 40 sec at (82ºC). Rayman et al. (2011) investigated the effect of electroplasmolysis on carrot juice and the total pectin content was increased 14.78% after electroplasmolysis applications [43]. Demirdöven and Baysal (2009) found 18.4% increase in pectin content of orange juices after electroplasmolysis application at 27 V/cm [15].

Table 5

Results of chemical analyses of microwave and conventionally heated orange juices

Sample	Total pectin (GA-AH mg/ L)*	Ascorbic acid (mg/100mL)*	°Brix*	pH*
MW (40 mL/min-900W)	418.67±1.5[a]	50.4±0.02[b]	12.4±0.1[a]	3.8±0.1[a]
MW (50 mL/min-900W)	478.2±2.3[b]	51.3±0.10[c]	12.3±0.1[a]	3.8±0.1[a]
CH (95 °C-60 sec)	411.1±1.0[c]	40.5±0.00[a]	12.5±0.1[a]	3.7±0.2[a]
Control	407.9±1.3[d]	55.4±0.04[d]	12.3 ±0.1[a]	3.7±0.2[a]

*Statistically significant difference shown levels a, b compared with same column ($P \leq 0.05$); results are presented as means ±SD ($n = 3$)

Ascorbic acid was found in control sample as 55.4 mg/100 mL where, it was observed 40.5 mL/100 mL in CH group. Ascorbic acid contents of MW samples were found as 50.4 mg/100 mL and 51.3 mL/100 mL for MW (40 mL/min-900W) and MW (50 mL/min-900W) respectively. High ascorbic acid content of MW samples can be explained by moderate temperature applications. And also it can be explained by increasing in cell

permeability and components can be transferred to orange juice easily. The difference between ascorbic acid content of samples was also found statistically significant ($P \leq 0.05$). The content of vitamin C in fresh orange juice has been widely studied and the results obtained in the present work are in the range of those published in the literature, which varied from 25 mg/100 mL to 68 mg/100 mL [44–50]. Vikram et al. (2005) investigated the effects of different electro-heating method on vitamin C degradation [50]. They evaluated that the destruction of vitamin C was influenced by the method of heating and the temperature of processing as found in the present study. They found highest vitamin C degradation during microwave heating due to uncontrolled temperature generated during processing and ohmic heating gave the best result facilitating better vitamin retention at all temperatures. Lima et al. (1999), examined ascorbic acid degradation in pasteurized orange juice during conventional and ohmic heating [51]. They also found that the type of heating had no significant effect on vitamin C degradation. They measured a decrease of 21–23% in ascorbic acid during thermal treatments at 90 °C for 30 min.

There are no significant differences between the samples for water soluble matters and pH values ($P > 0.05$). Effects of PEF application on brix were investigated by some researchers. Rivas et al. (2006) applied thermal pasteurization and PEF to the blended orange–carrot juice and found brix values: 9.5 and 10.4 for control and pasteurized samples [52]. Torregrosa et al. (2006) who studied PEF determined comparable brix values for the pasteurized juice and juice treated by PEF [53]. Like the mentioned previous study Cserhalmi et al. (2006) reported that in citrus juices PEF treatment (50 pulses at 28 kV/cm) did not change the brix value significantly [54] as we found.

The color values (L*, a* and b*), and the total color difference (ΔE) of samples in CIE Lab system are summarized in Table 6. The difference between L* values of MW (50 mL/min-900W) samples was found as statistically significant ($P \leq 0.05$). The difference between a* and b* values of samples and the color differences (ΔE) of all samples were found as statistically important ($P \leq 0.05$). By MW and CH treatments a* and b* values decreased compared to control group, this can be explained by color of samples became lighter after thermal applications. Same effects were observed after electrical application in apple juices [55]. And the lower ΔE value measured on MW (50 mL/min-900W) heated samples.

Table 6

Color values of microwave and conventionally heated orange juices

Sample	L*	a*	b*	ΔE
MW (40 mL/min-900W)	56.1 ± 0.01^{a}	11.5 ± 0.04^{a}	57.7 ± 0.08^{a}	3.1 ± 0.2^{a}
MW (50 mL/min-900W)	56.4 ± 0.01^{b}	12.2 ± 0.02^{b}	58.5 ± 0.02^{b}	1.9 ± 0.1^{b}
CH (95 °C-60 sec)	56.1 ± 0.01^{a}	11.8 ± 0.01^{c}	58.4 ± 0.05^{c}	2.3 ± 0.1^{c}
Control	56.1 ± 0.01^{a}	13.2 ± 0.06^{d}	60.2 ± 0.01^{d}	------

*Statistically significant difference shown levels a, b compared with same column ($P \leq 0.05$), results are presented as means ±SD ($n = 3$)

Demirdöven and Baysal (2009) found statistically significant decrease in L*, a* and b* values of orange juices after electroplasmolysis application [15]. And Demirdöven and Baysal (2012) found statistically significant difference between a*, b* and ΔE values of ohmic and conventional heated orange juices [56]. In a previous study after treatment of PEF in citrus juices ΔE values were determined as 0.45 for grapefruit; 0.59 for lemon; 0.47 for orange and 2.44 for tangerine juices. L* value of PEF treated (50 pulses at 28 kV/cm)

tangerine juice was found as 20.76 where control has 22.16 L* value [54]. Rivas et al.[52] (2006) in blended orange–carrot juice investigated the effects of HTST (98ºC, 21 sec) and PEF (25 kV/cm, 280 μs) treatments and found L* values for control, pasteurized and PEF treated; 62.80; 62.65; 63.08, respectively.

Conclusion

In this study; the effect of microwave heating on the inactivation of PME in orange juice and to optimize the microwave heating conditions for electrical field application with response surface methodology (RSM) in moderate temperatures (60-85 °C) were investigated. A synergistic effect of microwave energy and temperature on orange juice PME inactivation was found under microwave heating conditions. The PME inactivation rate was described satisfactorily as a function of microwave heating conditions. The PME can be inactivating in moderate temperatures by MW (40 mL/min-900W- 83 °C) and MW (50 mL/min-900W-75 °C). The flow rate and power combinations necessary to inactivate the labile fraction of PME can be estimated allowing selection of optimal process conditions that should also provide sensorial quality. Reduction of PME activities was found approximately 93-95% in MW groups where conventional thermally heated juice has 86% reduction value. Total pectin content was increased 17.2% after MW (50 mL/min-900W) applications. And the lost of ascorbic acid content of MW (50 mL/min-900W) sample was lower than other applications. Due to these results, there was an important improvement in functional properties particularly in pectin content of orange juice; and this result is extremely important in terms of cloud stability of orange juices. And it was determined that MW (50 mL/min-900W) heating can be applied as a thermal treatment on orange juice production in moderate temperatures (75 °C) for PME inactivation and improving functional properties of orange juice.

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