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Inactivation Kinetics of Pectin Methyl Esterase under Thermosonication and Thermal Pasteurisation Process Conditions in Orange Juice

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ABSTRACT

Pectin methylesterase has a higher thermal resistance than the microorganisms existing in orange juice, and its inactivation is used as a parameter for the pasteurisation process. In this study, the thermal and thermal-ultrasound (thermosonication) inactivation kinetics were determined. Freshly squeezed orange juice (Valencia) was heated at three temperatures (65, 75 and 85°C) or with ultrasound (35 kHz/300 W); four holding times (15, 30, 60 and 120 s) were used for each condition, using laboratory type continuous heat exchanger. The inactivation of pectin methylesterase was influenced by ultrasound and processing temperature. A slower reaction rate constant was found for juices with only heat treatment at the studied temperatures. The highest inactivation level was obtained in juices with combined heat and ultrasound treatment at 85°C. Results indicated that the activation energy for inactivation of pectin methylesterase can be decreased 62% by the combined heat and ultrasound treatments.

Keywords: Pectin methylesterase inactivation, Thermosonication, Kinetics, Orange juice

Portakal Suyundaki Pektin Metil Esterazın Termosonikasyon ve Isıl Pastörizasyon İşlem Koşullarında İnaktivasyon Kinetiği

ÖZ

Pektin metil esteraz (PME) portakal suyunda var olan mikroorganizmalardan daha yüksek ısıl dirence sahiptir ve bundan dolayı portakal suyunun pastörizasyonunda, PME inaktivasyonu ısıl işlem normu olarak kullanılır. Bu çalışmada ısıl işlem veya ısıl işlem+ultrason (termosonikasyon) uygulamaları ile pektin metil esterazın inaktivasyon kinetikleri değerlendirilmiştir. Çalışmada taze sıkılmış portakal suyu (Valencia) 3 farklı sıcaklıkta (65, 75, 85°C) her bir sıcaklık için 4 farklı sürede (15, 30, 60, 120 s) ısıl pastörizasyon veya aynı sıcaklık ve sürelerde ultrason (35 kHz/300 W) uygulaması gerçekleştirilmiştir. Pektin metil esterazın inaktivasyonunda ultrason ve işlem sıcaklığının etkili olduğu tespit edilmiştir. Sadece ısıl işlem uygulanan örneklerde daha düşük inaktivasyon hız sabiti belirlenmiştir. En yüksek inaktivasyon seviyeleri 85 °C' de sıcaklık ve ultrason (termosonikasyon) destekli uygulamadan elde edilmiştir. Sonuçlar, pektin metil esteraz inaktivasyonu için gerekli olan aktivasyon enerjisi değerinin termosonikasyon işlemiyle % 62 oranında azaltılabileceğini göstermiştir.

Anahtar Kelimeler: Pektin metil esteraz inaktivasyonu, Termosonikasyon, Kinetik, Portakal suyu

INTRODUCTION

Global orange production for 2011 was reported to be 54.1 million metric tons (MMT). Orange juice is the most popular juice compared to other non-alcoholic drinks and juices and its global production was forecast 2.46 MMT in 2011 [1]. Moreover, orange juice is an excellent

source of ascorbic acid and is a product desired by many people who are interested in a healthy diet.

Pectin methyl esterase (PME) is an important enzyme that effects quality aspects especially cloud stability in orange juice products. However, the cloud has an important role in turbidity, flavour, aroma and the colour of orange juice [2-6]. It has been reported that orange PME is more heat resistant than the common spoilage microorganisms of orange juice, and inactivation of PME is generally used to determine the intensity of thermal processing during commercial pasteurisation [7-9]. Many studies have been conducted with the objective of inactivating PME by using conventional thermal treatment [10, 11], and industry normally adopts these conditions to pasteurize orange juice (88–95 °C, 15–30 s) [12]. However, conventional thermal treatments cause adverse effects in sensory and nutritional characteristics of orange juice such as colour and flavour changes, loss of bioactive compounds and nutritional value reduction [10,13-19]. Thus, inactivation of PME by non-thermal treatments is becoming a popular method to prevent quality degradation of orange juice by thermal processing. Several studies have demonstrated the inactivation of PME using non-thermal treatments such as a high hydrostatic pressure (HHP) process [16, 20, 21], a pulsed electric field (PEF) process [22-25], or an ultrasound process [26, 27]. Compared to conventional thermal processes, non-thermal treatments have been found to be less detrimental to vitamins, pigments, flavouring agents and other compounds associated with sensory, nutritional and health-related qualities of the product.

However, using only non-thermal treatments has not been effective for commercial production. So, use of non-thermal technologies with conventional thermal heat treatments was another approach used to avoid quality degradation of orange juice by thermal processing [28]. The use of ultrasound in pasteurisation continues to be of great interest to the food industry. Investigations on ultrasound effectiveness have also shown the inactivation of enzymes such as pectinmethylesterase, polyphenoloxidases and peroxidases responsible for deterioration of fruit and vegetable juices. But, pectin methylesterase have not been inactivated even after 1 hour of exposure to the cavitating 20 kHz ultrasound at room temperature at pH 7. However, the inactivation effect of the ultrasonic treatment with temperature above 50°C has been found much greater than the inactivation of heat treatment at the same temperature. Therefore, it has been suggested to combine the ultrasonic treatment with temperature to increase the inactivation rate [29]. In combination with heat, ultrasound can accelerate the rate of sterilisation of foods, and a combination of heat and ultrasound treatment has been called thermosonication in the literature [30].

In this study, we attempted to determine the inactivation kinetics of PME in orange juice as well as different temperatures and different treatment times under only heat treatment or combined conditions of ultrasound and heat (thermosonication). Aiming at a better quantitative comparison of different treatments and an optimal process design that describes the PME inactivation rate as a function of ultrasound and temperature conditions, we explored the dependence of activation energy on the applied conventional heat and thermosonication treatments.

MATERIALS and METHODS

Orange Juice Preparation

Oranges (Valencia) were purchased at a local market and stored at 4°C before juice extraction. Fresh juice was squeezed using a table top citrus juice machine (Cancan, Adana, Turkey). Orange juice was passed through 1-mm stainless steel sieves to remove seeds and coarse pulp. The juice was immediately processed by pasteurisation units. The general properties of orange juice were given in Table 1.

Table 1. General properties of orange juice

Properties	Orange juice
Brixº	12.29±0.36
pH	3.36±0.08
Total acidity (%)	2.06±0.09

Inactivation Process

The continuous process method used a special design pasteuriser (Figure 1), which consists of: a magnetic stirrer (1); a peristaltic pump (2); a heater and ultrasound unit (3), which includes an ultrasonic water bath (Bandelin Sonorex, RK 1028 H, Germany) and glass holding tubes; a cooler unit (4), which includes a water bath (PolyScience, USA) and holding tubes.

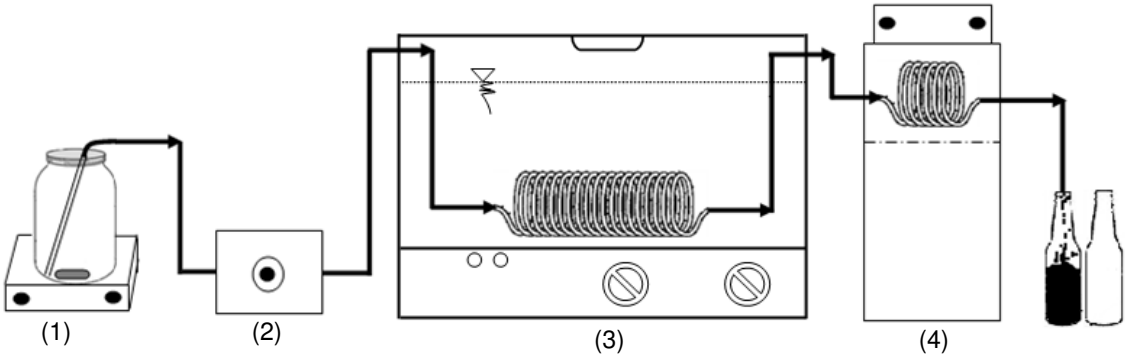

Figure 1. Special design continue pasteuriser for thermosonication or thermal treatment

The fresh orange juice is forced through the heater section where hot water inside of the water bath heats the juice to the intended temperature (65, 75 and 85°C). The juice, at the intended temperature and under pressure, flows through the holding tube where it is held for the intended time (15, 30, 60 and 120 s). After, the warm juice was passed through the cooling section where it was cooled to approximately 15-20°C.

Experimental design

Two different treatments (traditional heat treatment and thermosonication) were applied for PME inactivation. Both inactivation treatments were performed at 65, 75 and 85°C and for each temperature the following holding times were chosen 15, 30, 60 and 120 s. The setting of these holding times was allowed by the previous calibration of the flow rate of product and pump rotations. The thermosonication treatments were set up by applying ultrasound 35 kHz with the intended temperatures and times.

PME Activity Measurement

PME activity of the sample was carried out by modifying the method of Kimball et al. [5]. For the measurement of PME activity, 10 mL of orange juice was mixed with 20 mL of 1% pectin-salt substrate (0.1M NaCl) and incubated at 30 °C. The solution was adjusted to pH 7.0 with 2.0 N NaOH, and then the pH of solution was re-adjusted to pH 7.7 with 0.05 N NaOH. After the pH reached 7.7, 0.10 mL of 0.05 N NaOH was added. Time was measured (t) until pH of the solution regained pH of 7.7. PME activity (%) was calculated by the following formulas where (t) is time in min; A_0 is the PME activity of the untreated sample, which was determined immediately after processing to avoid the effects of storage time, and A_t is the PME activity after the treatments.
PME activity (A) was calculated by the following formulas:

$$A = \frac{(0.05N - NaOH).(0.10ml - NaOH)}{(t^{'})(10 - ml\ sample)}$$

$$Residual\ PME\ Activity\ (\%) = \left({A_t}/{A_0}\right)100$$

Kinetic Formula

In this study, PME inactivation kinetics is described according to the first order differential equation. A_0 is the initial PME activation, A is the residual PME activation, t is time (s) and finally k is the reaction rate constant (s^{-1}).

$${dA}/{dt} = -kA \quad (1)$$

$$ln\left({A}/{A_0}\right) = -kt \quad (2)$$

The Arrhenius equation, which expresses relationships between temperature and reaction rate, was expressed as follows:

$$k = k_0.e^{\frac{-Ea}{R.T}} \quad (3)$$

$$\ln(k) = \ln(k_0) - E_a/(R.T) \quad (4)$$

where k is the reaction rate constant at a certain temperature (s^{-1}), T is temperature (K), E_a is activation energy ($Jmole^{-1}$), R is the universal gas constant ($Jmole^{-1}K^{-1}$), and k_0 is the Arrhenius constant (s^{-1}). D is defined as the time required in certain heat or thermosonication conditions for 90% reductions in PME activity. The z value is defined as the necessary increase of temperature to ensure tenfold reduction in the value of D. The Q_{10} value is defined as the rate of change of chemical reaction as a consequence of increasing the temperature by 10°C.

$$D = 2.303/k \quad (5)$$

$$z = T_2 - T_1/(logD_1 - logD_2) \quad (6)$$

$$Q_{10} = 10^{\left(\frac{10}{z}\right)} \quad (7)$$

RESULT and DISCUSSION

Thermal and Thermosonication Inactivation of PME in Orange Juice

The effects of the thermal treatment (HP) and the thermosonication treatment (US-HP) for inactivation of PME in the orange juice are presented in Figure 2. The data for isothermal inactivation percentage of PME obtained from applied thermal treatments at 65°C (HP65T) for 15, 30, 60, 120 s were determined as 5.46, 9.56, 17.22 and 30.62% respectively; at 75°C (HP75T) for the same times were determined as 18.53, 32.42, 53.50 and 63.65%; and at 8°C (HP85T) for same times were determined as 27.08, 42.37, 63.99 and 81.63% (Table 2).

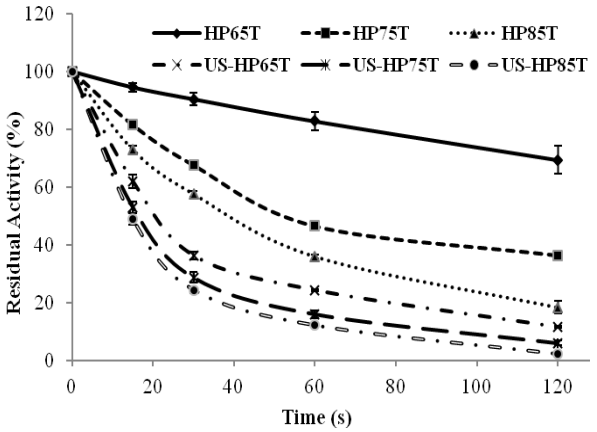

Figure 2. Isotherms inactivation curves of PME in the orange juice for different process conditions (HP65T, HP75T, HP85T: Heat treatment at 65, 75, 85°C, respectively. US-HP65T, US-HP75T, US-HP85T, Thermosonication treatments (35kHz-65, 75, 85°C, respectively)

Table 2. Effect of different processing conditions inactivated of PME (%) in orange juice

Treatment Time (s)	HP65T	HP75T	HP85T	US-HP65T	US-HP75T	US-HP85T
15	5.46±1.53	18.53±1.23	27.08±1.31	38.09±2.26	47.03±1.96	51.15±1.75
30	9.56±2.13	32.42±1.27	42.37±1.03	63.77±1.39	71.30±1.82	75.70±1.36
60	17.22±3.18	53.50±1.22	63.99±0.64	75.78±0.34	83.98±1.35	87.95±0.85
120	30.62±4.72	63.65±0.58	81.63±2.04	88.57±0.30	94.02±0.77	97.90±0.08

Kuldiloke [29] showed that the temperature below 60°C has no significant inactivation effect on the lemon PME. However, the inactivation started above 70°C and dramatically increased at 90°C. Yeom et al. [23] achieved 89% inactivation of PME in orange juice by the heat treatment applied at 94.6°C for 30 s. Elez-Martínez et al. [31] inactivated 100% orange juice PME through traditional heat pasteurisation (90°C, 1 min). In a similar study, 98% of PME inactivation was reported at 98°C for 21 s [15]. Thermal treatment is the most common and widely employed pasteurisation and sterilisation technique for the inactivation of microorganisms and enzymes in the food industry. However, high process temperature and time reduce nutrient value of food and increase the cost of the process.

Thermosonication inactivation percentages of PME in the orange juice were determined as 38.09, 63.77, 75.78 and 88.57 at 65°C (US-HP65T) for 15, 30, 60 and 120 s respectively; 47.03, 71.30, 83.98 and 94.02% at 75°C (US-HP75T) for 15, 30, 60 and 120 s respectively; 51.15, 75.70, 87.95 and 97.90% at 85°C (US-HP85T) for 15, 30, 60 and 120 s respectively.

Ultrasound alone or in combination with heat or pressure or both heat and pressure is reported to be effective against various food enzymes [30, 32, 33]. Tiwari et al. [26] studied the effect of ultrasound treatments on PME inactivation kinetics in orange juice and reported that PME inactivation was increased by 5 to 62% when the ultrasound intensities was increased at same times. It is also reported that ultrasonic processing of fruit juices has minimal effect on the quality of orange juice [34] and enhances cloud value and stability of orange juice during storage [26].

Cameron, Baker and Grohmann [35] isolated four isoenzymes of PME in orange juice and studied their effects on juice cloud stability, concluding that the most heat-resistant form, representing only 7.9% of the total enzyme, had the greatest influence on juice cloud stability. If only heat treatment is used for inactivation of this isoenzyme form, it will cause loss of food quality. However, in this study, the combined method significantly increased inactivation percentage of PME in the orange juice. In particular, the thermosonication treatment (US-HP85T for 120 s) showed 16.62% more PME-inactivation percentage than thermal treatment alone (HP85T for 120 s).

Thermal and Thermosonication Inactivation Kinetics of PME in Orange Juice

The effects of treatment types and treatment times on the residual activity (RA, %) of PME under the experimental conditions investigated are shown in Figure 2. The results show that when the combined method was used, PME activity decreased as a function of heat temperature (°C) and holding time much faster than heat treatment alone. The inactivation kinetics as a function of treatment time at each method were best fitted to a first-order inactivation model (Table 3, Fig. 2). According to first-order reaction kinetic, the reaction rate constant (k) value was calculated to be 0.181 min^{-1} for HP65T, 0.502 min^{-1} for HP75T and 0.833 min^{-1} for HP85T. D value is defined as the time required in certain temperature conditions for a 90% reduction in PME activity and was calculated to be 12.98, 4.59 and 2.77 min for HP65T, HP75T and HP85T, respectively. The k values were calculated as being 1.029 min^{-1} for US-HP65T, 1.342 min^{-1} for US-HP75T and 1.859 min^{-1} for US-HP85T. Comparing these data with thermal treatments obtained in the orange juice at the same conditions, it can be observed that the k values are much higher. Results indicate that the PME in the orange juice is more thermally resistant and the k values less sensitive to heat. D values were calculated as being 2.24, 1.72 and 1.24 min for US-HP65T, US-HP75T and US-HP85T, respectively. z value is defined as the increase required for temperature to ensure a tenfold reduction in the D value and was calculated to be in the range 65 to 85°C. It was 77.89°C for thermosonication treatment and 30.12°C for traditional heat treatment. Raviyan et al. [27] reported that the D value of tomato PME was reduced from 299.0 min for thermal treatment (61°C) to 1.5 min for thermosonication treatment (61°C, 20 kHz) at the same temperature. Also, researchers demonstrated that the D value significantly decreased when the treatment's temperature and cavitation energy was increased.

Activation energy (Ea) is generally defined as the energy required per mole of the reactant for the realisation of a reaction. Ea values at different treatments were calculated from k values. k values which determined at different temperature was plotted against the temperature and the slope of the line was used for calculation Ea values (Figure 3). The Ea values were calculated to be 77.43 kJ mol^{-1} and 29.74 kJ mol^{-1} for heat treatment and the thermosonication, respectively. Activation energy decreased with applying thermosonication (ranging from 77.43 kJ mol^{-1} to 29.74 kJ mol^{-1} at 65-85°C). The difference in these results was due to the synergist effect of ultrasound and heat on the PME inactivation. Q_{10} value is defined as the rate of change of chemical reaction as a consequence of increasing the temperature by 10°C. In this study,Q_{10} values were calculated to be 2.16 and 1.34 for heat and the thermosonication treatments, respectively.

Table 3. The inactivation kinetics as a function of treatment time at each method and kinetic data

Samples	Reaction Order		Kinetic Data*				
	0.	1.	K (min^{-1})	D (min)	z (°C)	Ea (kJmol^{-1})	Q_{10}
HP65T	0.993	0.999	0.181±0.033	12.98±2.30			
HP75T	0.859	0.930	0.502±0.009	4.59±0.09	30.12±2.23	77.43±5.89	2.16±0.12
HP85T	0.873	0.985	0.833±0.058	2.77±0.31			
US-HP65T	0.727	0.941	1.029±0.017	2.24±0.04			
US-HP75T	0.674	0.952	1.342±0.067	1.72±0.09	77.89±1.66	29.74±0.64	1.34±0.01
US-HP85T	0.652	0.985	1.859±0.029	1.24±0.02			

*Data calculated according to the first order kinetics

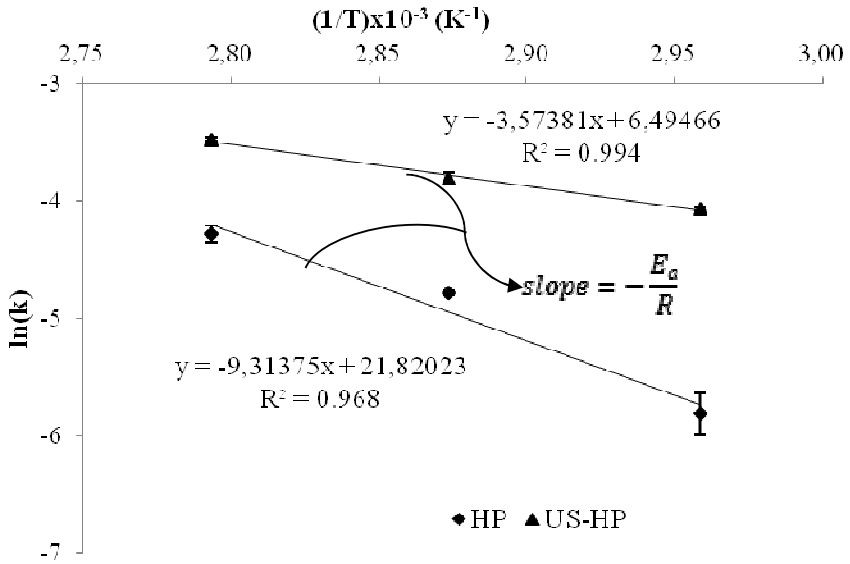

Figure 3. The activation energy (*Ea*) values were calculated from slope of the line (HP: Heat treatment; US-HP: Thermosonication treatment)

Tajchakavit and Ramaswamy [36] reported that the enzyme inactivation data to be characterised by the conventional thermal treatment: D_{60} = 154 s (z = 17.6°C for heat sensitive and 31.1°C for heat-resistant fraction between 60 and 90°C). Terefe et al. [37] reported *D* values of 14.6-2.8 min and 89.00-4.04 min between 60-75°C for thermosonication and thermal treatments, respectively. E_a and z values (between 50 and 75°C) were calculated as being 130 kJ/mol and 16.6°C for thermosonicated and 193 kJ/mol and 11.4°C for thermally pasteurised PME extracted from the tomato. Wu et al. [38] determined the D values in tomato juice after thermosonication treatment at 60, 65 and 70°C for 41.8, 11.7 and 4.3 min, respectively. In addition, the only heat treatments at 60, 65 and 70°C for 90.1, 23.5 and 3.5 min, respectively inactivated PME by 90%. Researchers noted that thermosonication at 60 and 65°C could be useful to obtain tomato juice with a low residual PME activity and high viscosity. Lopez et al. [39] reported that the manothermosonication treatment at the same temperature inactivated the tomato PME at higher rates than when it was heated at 62.5°C. The *D* values of manothermosonication treatments were calculated as being 4.30 and 0.85 min at 37 and 62.5°C, respectively and the *D* value of thermal treatment was calculated to be 45.0 min at 62.5°C. It was reported that manothermosonication inactivation is synergistic phenomenon since the rate of inactivation due to this treatment is much higher than the sum of the rate inactivation by ultrasonic waves at 37°C and the inactivation rate by heat at 62.5°C.

Different researchers report different *D*, z and *Ea* values for the inactivation of PME. The main reason for this PME's relationship with the media is extracted and treated. In general, after the enzyme has been extracted from a particular material, the enzyme is inactivated in a buffer. The inactivation kinetics values obtained with the buffer media, cannot fully reflect the real values of inactivation of the real media. Thus, the method to be applied to food preservation will not be able to realise an adequate and effective level.

In this study thermosonication treatments of z values were higher than thermal treatments. This situation may be explained, an important part of PME-resistant fractions can be inactivated by thermosonication process. Remaining resistant fractions of PME after the application of this process were highly resistant to inactivation, therefore a higher z value was calculated.

CONCLUSION

In conclusion, combining the ultrasonic treatment with heat treatment increased the inactivation rate of orange juice PME. Also, the activation energy for inactivation of PME in orange juice can be decreased by the combined heat and ultrasound treatments. Inactivation degrees of PME by the heat (85°C, 120s) and thermosonication (35kHz-120s, 85°C) treatments were determined 81.60 and 97.93%, respectively. Activation energy was decreased with applying thermosonication treatment approximately 61.6% than conventional heat treatment.

In this study, the effect of thermosonication process on post-processing quality loss of orange juice was not investigated. However during storage period, orange juice undergoes a number of deterioration reactions resulting in quality degradation of the product. The results reported in this work showed that combined treatment can be used as an alternative to orange juice pasteurisation. The quality parameters and microbiologic inactivation of the orange juice, which is processed with thermosonication, have yet to be investigated.

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