

Pectin Methylesterase Inactivation in *Mosambi* Juice

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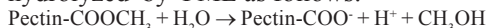
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ABSTRACT Recently, thermosonication has emerged as an alternative technique for thermal treatments in the food industry. Extent of inactivation of pectin methylesterase (PME) was studied in *mosambi* juice during various thermosonication treatments. Extraction of juice was done using a laboratory scale juicer. Thermosonication treatments were carried out at three temperatures: 60, 70 and 80 °C in water bath of a thermosonicator for 5, 10, 15 and 20 min at each temperature. Temperature was measured by thermometer. Treated samples were stored in a deep freezer at -18 °C for PME assay. PME activity of untreated sample was also assayed and residual PME activity and % loss in PME activity was calculated at each time-temperature combination of thermosonication treatments. The extent of inactivation of PME increased with increase in treatment temperature and duration. Thermosonication treatment at 80 °C for 20 min was found best among all thermosonication treatments for PME inactivation.

INTRODUCTION

The enzyme, pectin methyl esterase (PME) becomes activated during extraction of juice from *mosambi* (*Citrus limetta*). PME hydrolyses ester bonds of pectin in citrus juices resulting in decreased cloud stability (Rouse and Atkins 1952). A common problem associated with citrus juices (fresh squeezed, concentrated and preserved) is the loss of cloudiness (Basak and Ramaswamy 1996). In *mosambi* juice cloud stability is necessary to maintain its characteristic flavour, colour and mouthfeel. Cloud is attributed to the suspension of particles composed of a complex mixture of protein, pectin, lipids, hemicellulose, cellulose and other minor components (Klavons et al. 1991; Baker and Cameron 1999).

PME effects on quality aspects such as cloud stability are well known (Cameron et al. 1998; Do Amaral et al. 2005). It is a cell wall bound enzyme and belongs to a family of parallel beta helix proteins. It exists in multiple isozymes which differ in their thermal and pressure stability. Pectin, which is composed of α -1, 4-linked galacturonic acid and galacturonic acid methyl esters, are demethylated by a group of pectinases (Markovic et al. 2002; Nguyen et al. 2003). The galacturonic acid methyl esters are hydrolyzed by PME as follows:



Acid pectin produced during this reaction interacts with calcium ions and forms insoluble pectates that co-precipitate with the pulp particles in the juices and induce cloud loss (Krop and Pilnik 1974; Sio et al. 1995). Juices have

been pasteurized commercially by thermal treatments to inactivate enzymes responsible for loss of cloudiness for a long time but these treatments adversely affect other sensory and nutritional properties of juice. These days consumers prefer minimally processed foods and the use of non-thermal technologies is gaining popularity. In thermosonication method, localised high temperatures up to 5000 K, pressures up to 50,000 kPa, and high shearing effects are generated due to cavitation effect of sound waves which brings about a localised pasteurisation effect (Suslick 1988).

Objective

To study the effect of thermosonication on PME inactivation in *mosambi* juice.

METHODOLOGY

Material

Fresh *mosambis* (*Citrus limetta*) were procured from the local market. Salt, sodium hydroxide, phenolphthalein, hydrochloric acid, pectin used were of analytical grade.

Extraction of Juice

Mosambis were washed with tap water, peeled and cut into halves with knife. Seeds were removed and juice was extracted using a laboratory scale juicer (*Kalsi*). Extracted juice was strained through muslin cloth and was stored at -18 °C in a deep freezer for pectin methylesterase assay.

Treatment of *Mosambi* Juice

Thermosonication treatments were applied at three temperatures 60, 70 and 80 °C in the water bath of a thermosonicator, that is, Cyberlab Powersonic 410 (50 kHz, 400 W, available in Bio and Nano Technology Department, GJU S and T, Hisar) for 5, 10, 15, 20 min at each temperature. The frequency of sound waves was kept constant during each treatment. For each time-temperature combination 50 ml of juice sample was taken in a 250 ml glass beaker. Exposure time was calculated when the sample reached the designated temperature. Temperature was measured by a thermometer. After removal from the water bath, samples were cooled in ice water and then were transferred to the 250 ml plastic beakers and were stored in a deep freezer at -18 °C for PME assay.

PME Activity Assay

PME extracts were prepared following the method proposed by Hagerman and Austin (1986). 50 g of juice sample was thawed and homogenized in 100 ml of 8.8 % sodium chloride (NaCl) at a high speed of 13,000 min⁻¹ at 4 °C for 15 s by using Tissue Homogenizer (Yorco). The homogenate was stirred with a magnetic stirrer for 15 min and then centrifuged at 20,000 g for 25 min at 2 °C using Remi C24 Cooling Centrifuge. The supernatant was kept in 150 ml plastic containers and stored at -18 °C pending PME activity assay. PME activity was assayed by an acid base titration of free carboxylic acid groups produced by PME during hydrolysis of a pectin solution at pH 7.5 and at 30 °C following the procedure described by Lee and MacMillan (1968). A 30 ml aliquot of solution containing 0.15 M NaCl and 0.5 % (w/v) pectin was equilibrated to 30 °C and pH was adjusted to 7.5. Following the addition of 600 µl of sample, the pH was readjusted to 7.5 by 1 N sodium hydroxide (NaOH) and was maintained for 10 min by addition of 0.01 N NaOH during enzymic hydrolysis by use of an Cyberscan pH meter. The volume of base added (V_{NaOH}) was monitored as a function of time. All samples were measured in triplicate. According to Crelier et al. (2001) at pH 7.5 the pKa of the carboxylic groups does not have to be taken into account and there is a direct relationship between the amounts of sodium hydroxide injected into the medium and the

µmol of carboxylic groups released. The slope $S = dV_{\text{NaOH}}/dt$ was determined in the linear part of the titration curve by taking two points on the line with coordinates (X1,Y1) and (X2,Y2) and solving for $S = (Y2-Y1)/(X2-X1)$ (Toledo 2000). The slope is directly proportional to the activity of PME per ml of the sample (Asp) which was obtained as follows (Basak and Ramaswamy 1996):

$$\begin{aligned} \text{Asp } (\mu\text{molH}^+\text{min}^{-1}\text{ml}^{-1}) &= S \text{ (ml min}^{-1}) \times N_{\text{NaOH}} (\mu\text{mol ml}^{-1}) / \\ &V_{\text{sp}} \text{ (ml)} \\ &= dV_{\text{NaOH}}/dt \times N_{\text{NaOH}} (\mu\text{mol ml}^{-1}) / \\ &V_{\text{sp}} \text{ (ml)} \end{aligned}$$

Where, S is slope of titration curve, N_{NaOH} is normality of standard NaOH used during titration, dV_{NaOH} is volume of standardized NaOH solution used for titration, V_{sp} is volume of PME solution added into the reaction mixture, dt is reaction time in min.

PME Inactivation Parameters

PME residual activity (RA) and % loss in PME activity were taken as PME inactivation parameters. The residual activity of PME obtained after each thermosonication treatment was calculated as:

$$\text{RA} = A_i/A_0 \times 100$$

Where, A_i is PME activity after thermosonication treatment and A_0 is initial PME activity prior to thermosonication treatment.

After each thermosonication treatment, the % loss in pectin methylesterase activity was calculated as:

$$\text{Loss in pectin methylesterase activity} = 100 - \text{RA}$$

RESULTS

PME Activity of Untreated Sample

PME activity in fresh *mosambi* juice was estimated. No activity of enzyme was found after 1 min during titration of PME extract from untreated sample, as 0.01 N NaOH was not consumed after 1 min to maintain 7.5 pH. The slope of the linear part of the titration curve of PME extract against 0.01 N NaOH was 0.3 (Fig. 1). The activity was found 5 µmolH⁺min⁻¹ml⁻¹ in untreated sample.

Effect of Thermosonication on PME Inactivation

PME Inactivation at 60 °C

Volume of 0.01 N NaOH utilised during titration against PME extracts obtained from the

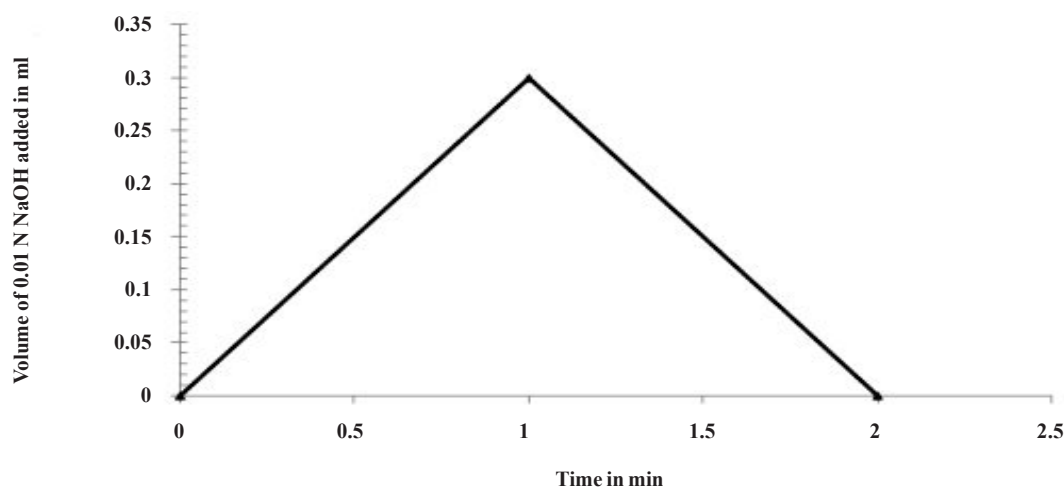


Fig. 1. Titration curve of PME extract of untreated sample

mosambi juice samples, which were thermosonicated for 5, 10, 15 and 20 min was 0.11, 0.10, 0.09, repeatedly 0.09 ml, respectively. Slope of the curve also declined along with falloff in the ingested volume of 0.01 N NaOH during titration, indicating a subsequent reduction in PME activity.

The PME activities were 1.83, 1.66, 1.50, $1.50 \mu\text{molH}^+\text{min}^{-1}\text{ml}^{-1}$ and PME residual activities were 36.6, 33.2, 30.0 and repeatedly 30.0 % at 5, 10, 15 and 20 min thermosonication treatment times, respectively (Table 1).

Table 1: Effect of thermosonication treatments carried out at different time-temperature combinations on PME inactivation in mosambi juice

Time-temperature combination		PME activity in $\mu\text{molH}^+\text{min}^{-1}\text{ml}^{-1}$	% loss in PME activity	Residual activity of PME in %
Temperature ($^{\circ}\text{C}$)	Time (min)			
60	5	1.83	63.4	36.6
60	10	1.66	66.8	33.2
60	15	1.50	70.0	30.0
60	20	1.50	70.0	30.0
70	5	1.00	80.0	20.0
70	10	0.83	83.4	16.6
70	15	0.66	86.8	13.2
70	20	0.66	86.8	13.2
80	5	0.66	86.8	13.2
80	10	0.50	90.0	10.0
80	15	0.33	93.4	6.6
80	20	0.16	96.8	3.2

PME Inactivation at 70 $^{\circ}\text{C}$

The volume of 0.01 N NaOH used during titration and the slope of the titration curves at

four mentioned time-temperature combinations at 70 $^{\circ}\text{C}$ decreased along with the increase of severity of the thermosonication treatment due to prolonged treatment times. Results presented in Table 1 showed a drastic PME inactivation during 70 $^{\circ}\text{C}$. The residual PME activity values measured at 5, 10, 15 and 20 min were 20.0, 16.6, 13.2 and repeatedly 13.2 % respectively. These were comparatively lower than those obtained during 60 $^{\circ}\text{C}$ treatment. Thus, it can be said that the rate of PME activity loss increased remarkably with increase in temperature during thermosonication treatment.

PME Inactivation at 80 $^{\circ}\text{C}$

The volume of 0.01 N NaOH added and the slope of the titration curves was reduced remarkably after giving 5, 10, 15 and 20 min thermosonication treatments at 80 $^{\circ}\text{C}$. Only 0.4, 0.3, 0.2 and 0.1 ml of 0.01 N NaOH was consumed at respective thermosonication treatment times respectively.

Satisfactory PME inactivation was achieved during 80 $^{\circ}\text{C}$. A progressive loss of PME activity was reported at 80 $^{\circ}\text{C}$ when the duration of treatment time was increased from 5 to 20 min. The lowest PME activity ($0.16 \mu\text{molH}^+\text{min}^{-1}\text{ml}^{-1}$) among all thermosonication treatments carried out, was noticed at 80 $^{\circ}\text{C}/20$ min. Only 3.2 % residual PME activity remained after thermosonication treatment given at 80 $^{\circ}\text{C}$ for 20 min (Table 1). The results further revealed that severe conditions were necessary for desirable and satisfactory PME inactivation.

Thermosonication treatments given at 60 °C resulted into reduction in PME activity upto 70 %. As the severity of treatment was increased, a greater loss in PME activity was obtained. Treatment applied at 70 °C for 20 min caused 86.8 % loss in PME activity. 96.8 % loss was achieved during treatment carried out at 80 °C for 20 min, thus leaving only a minor 3.2 % residual activity of PME (Table 1).

DISCUSSION

The pectin methylesterase activity in fresh mosambi juice was found 5 $\mu\text{molH}^+\text{min}^{-1}\text{ml}^{-1}$. TS treatments applied at different time-temperature combinations inactivated PME to different extents and the results obtained are in line with the observations made by others.

Ali et al. (2011) applied thermosonication treatment to guava juice and observed 1.5-3 times increase in inactivation rate of peroxidase in comparison to thermal treatments. In present study also thermosonication treatment was found better in comparison to thermal treatments. Siwach (2012) observed that thermal treatment given at 80 °C for 20 min resulted into minimum residual activity of PME (26.6 %) while similar duration thermosonication treatment in present study decreased PME residual activity to 3.2 %.

Wu et al. (2008) observed that at 70°C the thermally treated tomato juice samples and thermosonicated tomato juice samples showed similar reductions in enzyme activities and there was no additional advantage of using thermosonication at 70°C for the inactivation of pectin methylesterase under their experimental conditions. Thermosonication provided additional advantage only at lower temperatures at 60 and 65°C. In contrast in the present study conducted, a progressive loss of pectin methylesterase activity was observed with increase in thermosonication treatment temperature. The difference may be due to presence of different constituents in both the juices thus affecting its heat stability accordingly. Another reason may be the presence of different fractions of pectin methylesterase in *mosambi* and guava juices, differing in their heat stabilities.

Thus, findings of this study would help to design the processing conditions in order to reduce the severity of conventional thermal treatments prevalent in juice industry and, therefore,

improving the quality of the thermally treated product. Further, there is a need to assess the effect of thermosonication on quality parameters of mosambi juice in addition to PME activity. Unfortunately, no literature was found regarding thermosonication of mosambi juice, so the results presented herein cannot be fully explained with current knowledge or understanding of effect of thermosonication on pectin methylesterase inactivation especially in mosambi juice.

CONCLUSION

Thermosonication can act as a substitute for thermal treatments in the food industry for the processing of juices. This technique inactivates the enzyme, pectin methylesterase at much lower temperature in comparison to traditionally used thermal treatments in the industry. Thermosonication treatment at 60 °C for 5 min resulted into minimum inactivation. As the treatment duration and temperature was increased, an increase in inactivation was observed. Treatment given at 80 °C for 20 min was found to be the best among the entire treatments (96.8% loss).

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