



Impact of Thermo-sonication on Pectin Methylesterase (PME) Inactivation & Cloud Stability of Nagpur Mandarin Juice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i183501

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/104348>

Original Research Article

Received: 25/05/2023

Accepted: 28/07/2023

Published: 29/07/2023

ABSTRACT

Nagpur mandarin is well known globally for its excellent nutritional benefits and flavour. These all characteristics get affected during storage due to a lack of knowledge & processing techniques. One of the most important parameters which decide the quality of fruit juice during storage is cloud stability, which gets affected during storage due to the action of an enzyme named pectin methylesterase. Pectin methylesterase enzyme (PME) is a heat-resistant enzyme that impacts the juice quality by decreasing the cloud stability. Thermo-sonication is a technique to reduce the PME activity in juice by utilizing the principle of heat and ultrasound. Therefore, an experiment was carried out to inactivate the PME utilizing the ultrasound combined with higher temperature known

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as thermosensation on Nagpur mandarin (*Citrus reticulata* Blanco) juice. This study was initiated to calculate the effect of thermosensation treatment on the pectin methylesterase enzyme (PME) activity along with the effect of thermosensation on cloud stability in comparison with various thermal pasteurization treatments. Juice samples were thermosonicated at temperatures of 50 °C, 55 °C, 60 °C, and 63 °C for 25, 20, 15 and 10 min respectively at a constant frequency of 40 kHz. Juice samples were also thermally pasteurized at 65 °C, 75 °C, 85 °C, and 95 °C for 80, 60, 40, and 20 sec. Samples were analyzed after treatment at 0 days and 7 days of refrigerated storage at 4 °C. Result indicated that thermosensation at 63 °C for 10 min at 40 kHz frequency showed much reduced PME activity with inactivation of 72.56% over control whereas thermal pasteurization at 95 °C showed inactivation of 69.31%. The same treatment also showed maximum cloud activity as compared to control and thermally pasteurized juice samples. Hence thermosensation can be used as an alternative processing technique for the inactivation of PME and cloud stability.

Keywords: Productivity; mandarin; flavor; enzyme; pectin.

1. INTRODUCTION

Citrus is a group of perennials, evergreen flowering trees and shrubs which belong to the family Rutaceae. Genus citrus includes a group of fruit crops which includes orange, lemon, grapefruit, pummelo, lime, and many natural hybrids. Citrus fruit is a hesperidium, a specialized berry, globose to elongate with leathery rind surrounding segments filled with pulp vesicles. Citrus fruits contain fragrance mainly due to flavonoids and limonoids contained in the rind. Citrus fruits are rich in ascorbic acid (vitamin C). Fruit juice generally contains a high quantity of citric acid providing them their characteristic sharp flavour. Citrus fruits are eaten fresh, juice pressed, or preserved as a value-added product.

Citrus is grown in more than 100 countries of the world. Among 100 countries, citrus is grown commercially in 53 countries. Nagpur mandarin occupies an area of about 480, '000 Ha of the total area of 1054, '000 Ha under citrus and total production 6368, of '000 MT in the country (NHB, 2019-20). The area under Nagpur mandarin cultivation in Maharashtra alone is 115.003 thousand hectares, with a production of 899.58 thousand MT and productivity of 7.82 t/ha (HAPIS, 2019-20). The total area under citrus fruit in Chhattisgarh is 13.90 thousand hectares with a total production of 103.44 thousand MT with a productivity of 7.44 t/ha. The total area (in Chhattisgarh) under Nagpur Mandarin is 0.025 thousand hectares and production of 0.765 thousand MT (HAPIS, 2019-20).

Nagpur mandarin is well known globally for its excellent nutritional benefits and flavour [1]. These all characteristics get affected during storage due to a lack of knowledge & processing

techniques. One of the most important parameters which decide the quality of fruit juice during storage is cloud stability, which gets affected during storage due to the action of an enzyme named pectin methylesterase. Therefore, it is necessary to develop and standardize a technology to increase and stabilize the cloud activity & antioxidant activity with minimum effect on the other quality parameter of juice like the colour taste, etc.

Pectin methylesterase (PME) is an important enzyme which is more heat resistant than common food spoilage microorganisms & enzymes. Pectin methylesterase affects the quality aspects especially cloud stability in orange juice & its products. Cloud stability is one of the most important parameters to be affected by PME. Cloud has an important role in the turbidity, flavour, aroma & colour of orange juice. PME catalyzes the de-esterification of pectin molecules which then interact through the calcium bridges leading to the loss of the cloudiness and phase separation in orange juices [2]. PME enzyme also degrades the pectin present in the fruits which consists of a polygalacturonic acid backbone with a variable number of the methyl groups esterified to the galacturonic acid residues. PME catalyzes the removal of methyl groups leaving an increased number of carboxyl groups that can bind cations & cross-link the pectin chain. In juices, these cross-linked pectin aggregates & settles down leading to cloud loss. This degradation by PME leads to loss of viscosity.

Thermal pasteurization is a common method used to destroy the micro-organism and inactivate the enzymes in fruit juice. This heat treatment may cause undesirable changes in the properties of juice such as physical, biological,

chemical, and organoleptic (colour, flavour, nutrients). Sonication is considered a prominent technology and a magnificent alternative to thermal processing for the food processing industry and the most valuable source of less energy and reduced processing time [3]. Sonication, when combined with heat is called thermo-sonication [4]. Thermosonication is a treatment that is the combination of ultrasound and heat. The effect of this combination is for microbial inactivation which is more effective than sonication without heat treatment [5]. It is more effective as lower temperatures are used in comparison to thermal treatment [4]. Thermosonication with ultrasound is an alternative processing technique to enhance the inactivation of enzymes by the formation, growth and explosion of bubbles in liquid [6]. In ultrasound, a phenomenon called cavitation occurs due to the propagation of sound waves. Cavitation leads to the formation and collapse of bubbles, which in synchronization with heat, promote cell disruption along with bacterial and enzymatic inactivation [4]. The current objective of the experiment is to check the effect of thermal pasteurization and thermosonication treatment on pectin methylesterase (PME) activity and cloud stability of Nagpur mandarin juice.

2. MATERIALS AND METHODS

The current experiment was conducted for examining the effect of thermosensation over thermal pasteurization on various physicochemical quality parameters of Nagpur mandarin juice at post-harvest management & processing lab under ICAR-Central Citrus Research Institute, Nagpur, Maharashtra, during the year 2019-20.

Fully matured, uniform fruits of Nagpur mandarins were harvested from the experimental field under the ICAR-CCRI, Nagpur and used for the experiment. Those fruits which were free from mechanical injury were selected for the experiment and the immature, damaged and -off-type fruits were discarded. For extraction of juice, mandarin fruits were peeled manually. The juice was extracted from fruits using the orange juice extractor. After extraction of juice, it was filtered using a sieve & muslin cloth to separate the pulp present in the juice. Juice samples were then thermosonicated & thermally pasteurized at different temperatures & frequencies for different time intervals. Treated juice & control were studied to evaluate the effect of treatments on various physicochemical quality parameters just

after treatment. The experiment comprised 9 treatments and three replications designed under CRD at a 5 per cent level of significance, including various thermosensation treatments (50°C/ 25 minutes, 55°C/ 20 minutes, 60°C/ 15 minutes and 63°C/ 10 minute) at 40kHz frequency and thermal treatments (65°C/ 80 seconds, 75°C/ 60 second, 85°C/ 40 second and 95°C/ 20 second) along with control. Data were then subjected to various statistical analyses of variance as given by Gomez & Gomez [7]. Juice samples were tested for various chemical parameters after the application of different treatments to juice.

2.1 Pectin methylesterase (PME) activity

Pectin methylesterase enzyme activity was determined by the procedure given by Kimball [8].

Reagents:

1. 1 % Pectin Solution: Mix 10 gm of Pectin & 15.3 gm NaCl in 1 Litre of distilled water. Heat the solution for complete dissolution.
2. 0.05 N NaOH: Take 0.5 gm NaOH in 250 ml of distilled water.
3. 2 N NaOH: Take 4 gm NaOH in 50 ml of distilled water.

PME activity was determined by mixing 10 ml of juice sample and 40 ml of pectin solution. Add 2.0 N NaOH solution to maintain the pH up to 7.0. Then add 0.05 N NaOH solution to maintain pH up to 7.8. and then add exactly the 0.1 ml of 0.05 N NaOH solution and note the time to regain the same pH of 7.8.

$$\text{PME activity} = \frac{(0.05 \text{ N NaOH}) \times 0.10 \text{ ml NaOH}}{\text{The volume of the sample (10 ml)} \times \text{Time (Minutes)}}$$

2.3 Cloud Activity

The Nagpur mandarin juice samples were centrifuged at 3000 rpm for 10 min and the absorbance of the supernatant was measured at 660 nm using a spectrophotometer to determine the cloud values [9,10].

3. RESULTS AND DISCUSSION

3.1 Cloud Value

Cloud value is one of the most important quality parameters for the juice. Data presented in the Table 1, recorded the highest value for cloud (0.812) under the treatment T₃

Table 1. PME activity & cloud activity as influenced by different thermosensation and thermal pasteurization treatment in Nagpur mandarin juice

Notation	Treatment	PME Activity	Cloud Value
T ₀	Control	1.01 × 10 ⁻⁴	0.399
T ₁	Thermosonication at 50 °C for 25 min at 40 kHz frequency	5.76 × 10 ⁻⁵	0.496
T ₂	Thermosonication at 55 °C for 20 min at 40 kHz frequency	6.72 × 10 ⁻⁵	0.704
T ₃	Thermosonication at 60 °C for 15 min at 40 kHz frequency	2.77 × 10 ⁻⁵	0.812
T ₄	Thermosonication at 63 °C for 10 min at 40 kHz frequency	4.11 × 10 ⁻⁵	0.611
T ₅	Heat Pasteurization at 65 °C for 80 sec	7.74 × 10 ⁻⁵	0.578
T ₆	Heat Pasteurization at 75 °C for 60 sec	4.61 × 10 ⁻⁵	0.597
T ₇	Heat Pasteurization at 85 °C for 40 sec	4.44 × 10 ⁻⁵	0.628
T ₈	Heat Pasteurization at 95 °C for 20 sec	3.09 × 10 ⁻⁵	0.613
	C.D.	1.21 × 10 ⁻⁵	0.203
	SE(m)	4.09 × 10 ⁻⁶	0.068

(Thermosonication at 60°C for 15 min at 40 kHz frequency) which was statistically similar to T₂ (Thermosonication at 55 °C for 20 min at 40 kHz frequency), T₇ (Heat Pasteurization at 85 °C for 40 sec), T₈ (Heat Pasteurization at 95 °C for 20 sec) and T₃ (Thermosonication at 63 °C for 10 min at 40 kHz frequency) having the cloud value of 0.704, 0.628, 0.613 and 0.611. The minimum cloud value (0.399) was recorded with T₀ (Control).

In the present investigation, data presented in Table 1 showed a significant increase in the cloud value of juice in response to various thermosonicated and thermally pasteurized Nagpur mandarin juice after the treatment as compared to untreated Nagpur mandarin juice (control). Cloud is related to the suspension of particles composed of a complex mixture of protein, pectin, lipid, hemicellulose, cellulose, and another component (Sheshdari et al. 2003). Ertugay and Baslar [11], has also recorded an increase in the cloud value with the increase in the temperature from 50°C to 60°C combined with sonication treatment (thermosensation) in apple juice due to greater fragmentation of large particles at higher treatment temperature. Many studies have revealed that the effect of sonication has been enhanced by the addition of high temperature [12-14]. Kuldiloke [13] reported that the quality of clouds in citrus juice improved with an increase in temperature because, at higher temperatures, large molecules are fragmented due to the formation of more transient bubbles. According to Rao 1999, the ultrasound treatment breaks the larger molecule into smaller ones, due to the cavitation process which creates high-pressure gradient which causes proper homogenization of juice and the number of suspended particles also increases due to the breakage of the bigger molecule which lowers the distance among particle by the enlargement of surface area and thus improves the clouds in the juice. Similar findings were also observed by Ertugay & Balsar [14] in apple juice, Aadil et al. [3] in grapefruit juice, Dabir et al. [15] in custard apple juice and Tiwari et al. [16] in orange juice.

Cloud loss mainly occurs due to the enzymatic precipitation of clouds in the juices due to the initiation of a sequence of events by PME. Cloud loss in orange juice is a result of the degree of esterification of the pectin backbone. The application of ultrasound breaks the linear pectin molecule, reducing its molecular weight and resulting in a weaker network increasing in cloud

value [17]. The above phenomenon leads to two consequences; one is the structural modification in pectin molecule which causes a reduction in the accessibility of PME [16] and the other causes the juice to be clearly homogenized and consistent (Abid et al. 2013). The study by Knorr et al. [18] has also reported that ultrasound treatment significantly decreases the activity of the endogenous enzyme, due to a higher pressure gradient which dissociates larger molecules into smaller ones and other structural changes. According to Chemat et al. ultrasound breaks the particle in the juice by acoustic cavitation. Acoustic cavitation leads to the mechanical division of larger biopolymers under localized extreme physical conditions such as very high temperature, pressure, or the formation of shock waves. In the current study reduction in PME activity has been observed due to sonication and thermal pasteurization treatment, which may be also attributed to an increase in cloud value.

3.2 Pectin Methylesterase Enzyme (PME) Activity

One of the most important parameters which decide the quality of fruit juice during storage is cloud stability, which gets affected during storage due to the action of an enzyme named pectin methylesterase.

The data presented in Table 1 indicated that the pectin methylesterase enzyme (PME) activity was statistically significant and different thermosonication and thermal pasteurization treatments significantly influenced the enzyme activity. Pectin methylesterase enzyme (PME) activity, as depicted in Table 1 ranged between 2.77×10^{-5} to 1.01×10^{-4} . The initial data recorded after different thermosensation and thermal pasteurization treatments of Nagpur mandarin juice showed that the highest value for pectin methylesterase enzyme (PME) activity (1.01×10^{-4}) was recorded under treatment T₀ (Control). T₀ (Control) was followed by T₅ (Thermal Pasteurization at 65°C for 80 sec), T₂ (Thermosonication at 55 °C for 20 min at 40 kHz frequency) and T₁ (Thermosonication at 50°C for 25 min at 40 kHz frequency) having the pectin methylesterase enzyme activity of (7.74×10^{-5}), (6.72×10^{-5}) and (5.76×10^{-5}) respectively. The minimum pectin methylesterase activity (2.77×10^{-5}) was recorded under treatment T₃ (Thermosonication at 60°C for 15 min at 40 kHz frequency).

However, if we investigate the PME inactivation, after the application of different thermosensation and thermal pasteurization treatments, it can be observed from Table 1 that the pectin methylesterase enzyme activity reduced significantly as compared to the control, which represents pectin methylesterase enzyme inactivation. The highest PME inactivation (72.56 %) over control has been observed by T₃ (Thermosonication at 60 for 15 min at 40 kHz frequency) followed by T₈ (Heat Pasteurization at 65 for 80 sec), T₄ (Thermosonication at 63 for 10 min at 40 kHz frequency) and T₇ (Heat Pasteurization at 65 for 80 sec) having pectin methylesterase enzyme inactivation of 69.31 %, 59.26 % and 55.94 %. Whereas, T₅ (Heat Pasteurization at 65 °C for 80 sec) and T₁ (Thermosonication at 50 °C for 25 min at 40 kHz frequency) showed the least PME inactivation at 23.26 % and 33.20 % respectively.

Pectin methylesterase enzyme may have a major role in either the development or loss of textural characteristics [19] and pectic acids are mostly consumed to ease maceration, clarification, improve the cloud stability and most important to increase the yield of fruit juice. An increasing trend for enzyme deactivation was observed with the increase in treatment temperature. A significant increase in PME inactivation has been observed in the thermosonicated and thermally pasteurized juice samples as compared to untreated juice samples (control).

Thermosonicated samples showed an increase in PME inactivation as compared to control. Sonication causes the inactivation of the enzyme through various effects including the localized extreme increase in temperature and pressure accompanying the collapse of cavitation bubbles, strong shear force and shock waves created by stable cavitating bubbles and generation of free radicals that oxidize amino acid residue that participate in the stability and catalytic activity of the enzyme [20,21]. Free radicals generate during the sonication treatment also plays a major role in enzyme inactivation. Ultrasound alone or in combination with heat or pressure or both heat and pressure is reported to be effective against various food enzymes [22,23,2]. However, the result also showed that ultrasound alone could not inactivate enzymes to a desired level [24]. Many studies have revealed that the effect of sonication has been enhanced by the addition of high temperature (Ohlsson & Bengtsson, 2002; [13,14]. PME inactivation kinetics in orange juice has shown an increase

from 5% to 62% after increasing ultrasound intensity was increased at the same time [16]. Ultrasound treatment of fruit juice shows minimal effect on the quality of orange juice [25] and sonication also enhances cloud value and stability of orange juice during storage [16].

However lower PME inactivation has been observed in T₁ (Thermosonication at 50°C for 25 min at 40 kHz frequency) with only 33.20% over control. Sonication at 50°C is not effective against various food enzymes [26,10,16]. It has also been observed that thermosonicated samples showed maximum PME inactivation under treatment T₃ (Thermosonication at 60°C for 15 min at 40 kHz frequency) (72.56%) which showed more PME inactivation even if compared to treatment T₈ (Heat Pasteurization at 65 for 80 sec) (69.31%). Under current investigation, it has also been reported that the PME inactivation decreases with the increase in thermosensation temperature from 60°C (72.56 %) to 63°C (59.26%). T₃ (Thermosonication at 60°C for 15 min at 40 kHz frequency) shows a decrease in the PME inactivation as compared to T₄ (Thermosonication at 63°C for 10 min at 40 kHz frequency). The increase in inactivation is more pronounced at lower temperatures. A similar reduction in PME inactivation was also observed in tomato juice, where a reduction in PME inactivation was seen from 61°C to 72°C [3]. These can be attributed that at the higher temperature, vapour pressure increases inside the bubbles which introduces a cushioning effect and hence produce less effective collapse [27]. Crelier et al. [28] also noticed that PME is more heat sensitive at low temperatures. At higher temperatures, the effect of sonication on enzyme inactivation was no longer effective [29]. This is attributed to the increased vapor pressure of water at higher temperatures, resulting in the collapse of bubbles and hence less violent cavitation. This leads to lower viscosity which leads to decreased violence of implosion [21]. Decrease in the efficiency of ultrasound at high temperatures has also been reported by Wu et al. [30].

Thermal treatment has also been found to increase the PME inactivation of juice as compared to the control (untreated juice sample). High temperature causes changes in the enzyme structure, such as hydrogen bond breaking, opening the secondary and tertiary structures of the enzyme and thermal deamination. Amino acids such as asparagine and glycine lead to the reduction of RA (%). These changes may result

in loss of enzyme shape by becoming a random coil resulting in substrate not fitting into the active sites appropriately. Thermal treatments at low temperatures did not reduce PME activity, considerably. However, on rising the treatment temperature and treatment time the PME activity reduced hence increasing the PME inactivation. These results agree with Lopez et al., [31], Vercet et al., [32] and Ly-Nguyen et al., [33]. Kuldilok [13] also showed that a temperature below 60 °C has no significant effect on PME inactivation on lemon juice. Whereas, inactivation starts after 70 °C and increases rapidly at 90°C. 8% of PME inactivation has been observed in orange juice by heat treatment applied at 94.6°C for 30 secs [33]. Whereas 100% PME inactivation has been also observed in orange juice after heat pasteurization at 90°C for 1 min [34]. inactivation of 98% has also been observed at 98°C for 21 s [35]. Barteri et al. [20], observed progressive oxidation of cysteine residues and aggregation of the enzyme from which they concluded that the inactivation of the enzyme was due to the formation of disulfide-linked aggregates, formed as a result of oxidation of the cysteine residue by the free radicle has also been reported in ultrasonic-induced inactivation of trypsin. A 1.5-3 times increase in PME inactivation has been observed by thermosonication as compared to the thermal treatment of guava juice [36]. A similar result has also been observed in mosambi juice [37]. Thermosonication treatment (US-HP85T for 120s) has shown a 16.62% more PME-inactivation percentage than thermal treatment alone (HP85T for 120s) [38-40]. Thermosonication shows better PME inactivation as compared to thermal treatment may be attributed to the synergistic effect of ultrasound and heat on the PME inactivation.

4. CONCLUSION

Based on the result obtained in the present study, it is inferred that the application of treatment T₃ (Thermosonication at 60°C for 15 min at 40 kHz frequency) showed maximum pectin methylesterase enzyme (PME) inactivation. On the other hand, the treatment T₃ (Thermosonication at 60 °C for 15 min at 40 kHz frequency) also increased the cloud activity. Hence, from the above experimental results it can be inferred that among all the treatments, treatment T₃ (Thermosonication at 60 °C for 15 min at 40 kHz frequency) responded well in terms of pectin methylesterase enzyme inactivation and cloud value of the juice.

However, these parameters need to be explored during the storage for the production of a high-end value-added Nagpur mandarin juice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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