

# **Impact of Thermo-sonication on Pectin methylesterase (PME) inactivation & cloud stability of Nagpur mandarin Juice**

## **ABSTRACT**

Nagpur mandarin is well known globally for its excellent nutritional benefits and flavor. These all characteristics get affected during storage due to 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 impact 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 as thermo-sonication on Nagpur mandarin (*Citrus reticulata* Blanco) juice. This study was initiated with the objective of calculating the effect of thermo-sonication treatment on the pectin methylesterase enzyme (PME) activity along the effect of thermo-sonication on cloud stability in comparison with various thermal pasteurization treatment. Juice samples were thermo-sonicated at temperature 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 day and 7 days of refrigerated storage at 4 °C. Result indicated that thermo-sonication at 63 °C for 10 min at 40 kHz frequency showed much reduced PME activity with an 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 thermo-sonication can be used as an alternative processing technique for the inactivation of PME and cloud stability.

## **1. INTRODUCTION**

Citrus is group of perennials, evergreen flowering trees and shrubs which belongs to 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 flavor. 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, '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 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 flavor (Carbonell *et al.*, 2006). These all characteristics get affected during storage due to 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 color taste, etc.

Pectin methylesterase (PME) is an important enzyme which is more heat resistant than common food spoilage microorganism & 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 important role in turbidity, flavor, aroma & color 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 (Vercet *et al.*, 1999). PME enzyme also degrades the pectin present in the fruits which consist of 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 increased number of carboxyl group 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 (color, flavor, nutrients). Sonication is considered as a prominent technology and a magnificent alternative to the thermal processing for the food processing industry and the most valuable source in less energy and reduced processing time (Aadil *et al.*, 2015). Sonication, when combined with heat is called thermo-sonication (Anaya-Esparza *et al.*, 2017). Thermosonication is a treatment that is the combination of ultrasound and heat. The effect of this combination is for the microbial inactivation which is more effective than the sonication without heat treatment (Aadil *et al.*, 2015). It is more effective as lower temperatures are used in comparison to thermal treatment (Anaya-Esparza *et al.*, 2017). Thermosonication with ultrasound is an alternative processing technique to enhance the inactivation of enzymes by the formation, growth and explosion of bubbles in liquid (Abdullah & Chin, 2014). 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 (Anaya-Esparza *et al.*, 2017). 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. MATERIAL & METHODS**

The current experiment was conducted for examining the effect of thermosonication 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 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. Juice was extracted from fruits using the orange juice extractor. After extraction of juice, it was filtered using sieve & muslin cloth to separate the pulp present in the juice. Juice samples were then thermosonicated & thermally pasteurized at different temperature & frequency for different time interval. Treated juice & control were studied to evaluate the effect of treatments on various physicochemical quality parameters just after treatment. The experiment comprised of 9 treatments and three replications designed under CRD at 5 per cent level of significance, includes various thermosonication treatments (50 °C/ 25 minute, 55 °C/ 20 minute, 60 °C/ 15 minute and 63 °C/ 10 minute) at 40kHz frequency and thermal treatments (65 °C/ 80 second, 75 °C/ 60 second, 85 °C/ 40 second and 95 °C/ 20 second) along with control. Data were then subjected to various statistical analysis of variance as given by Gomez & Gomez (1985) <sup>[7]</sup>. Juice samples were tested for various chemical parameters after the application of different treatment to juice.

### 2.1. Pectin methylesterase (PME) activity:

Pectin methylesterase enzyme activity was determined by the procedure as given by Kimball (1991).

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 dissolve.
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 exact the 0.1 ml of 0.05 N NaOH solution and note the time to regain the same pH of 7.8.

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

### 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 (Versteeg *et al.*, 1980, and Tiwari *et al.*, 2008).

## 3. RESULTS AND DISCUSSION

### 3.1. Cloud value

Cloud value is one of the most important quality parameters for the juice. Data presented in table, recorded highest value for cloud (0.812) under the treatment T<sub>3</sub> (Thermosonication at 60°C for 15 min at 40 kHz frequency) which was statistically similar with T<sub>2</sub> (Thermosonication at 55 °C for 20 min at 40 kHz frequency), T<sub>7</sub> (Heat Pasteurization at 85 °C for 40 sec), T<sub>8</sub> (Heat Pasteurization at 95 °C for 20 sec) and T<sub>3</sub> (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 T<sub>0</sub> (Control).

In the present investigation, data presented in table 1 showed 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 particle composed of a complex mixture of protein, pectin, lipid, hemicellulose, cellulose, and another component (Sheshdari *et al.* 2003). Ertugay and baslar (2013), have also recorded increase in the cloud value with the increase in the temperature from 50 °C to 60 °C combined with sonication treatment (thermosonication) in apple juice due to greater fragmentation of large particle in apple juice at higher treatment temperature. Many studies have revealed that the effect of sonication have been enhanced by addition of high temperature (ohlsson & Bengtsson, 2002; Kuldiloke, 20002; Baslar & Ertugay, 2013). Kuldiloke (2002) reported that quality of cloud in citrus juice improved with an increase in the temperature because at higher temperature, large molecules are fragmented due to formation of more transient bubble. According to Rao 1999, the ultrasound treatment breaks the larger molecule in to smaller ones, due to cavitation process which creates high pressure gradient which causes proper homogenization of juice and the number of suspended particles also increases due to breakage of bigger molecule which lower 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 (2014) in apple juice, Aadil *et al.* (2014) in grapefruit juice, Dabir *et al.* (2017) in custard apple juice and Tiwari *et al.* (2008) in orange juice.

Cloud loss mainly occurs due to the enzymatic precipitation of cloud in the juices due to the initiation of sequence of events by PME. Cloud loss in orange juice is result of degree of esterification of pectin backbone. Application of ultrasound breaks the linear pectin molecule, reducing its molecular weight and resulting in weaker network resulting in increase in cloud value [Seshadri *et al.*, 2003]. These above phenomenon leads to the two consequences; one is structural modification in pectin molecule which causes reduction in accessibility of PME (Tiwari *et al.* 2009) and the other causing the juice to be clearly homogenized and consistent (abid *et al.* 2013). Study by knorr *et al* (2004) has also been reported that ultrasound treatment significantly decreases the activity of endogenous enzyme, due to higher pressure gradient which dissociates larger molecule into smaller once and other structural changes. According to Chemat *et al.*, 2011, ultrasound breaks the particle in the juice by acoustic cavitation. Acoustic cavitation leads to mechanical division of larger biopolymer under localized extreme physical condition 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 increase in cloud value.

### 3.2. Pectin Methyltransferase 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 methyltransferase.

The data present in table 1 indicated that the pectin methyltransferase enzyme (PME) activity was statistically significant and different thermosonication and thermal pasteurization treatment significantly influenced the enzyme activity. Pectin methyltransferase enzyme (PME) activity, as depicted in table 1 ranged between  $2.77 \times 10^{-5}$  to  $1.01 \times 10^{-4}$ . The initial data recorded after different thermosonication and thermal pasteurization treatments of Nagpur mandarin juice showed that the highest value for pectin methyltransferase enzyme (PME) activity ( $1.01 \times 10^{-4}$ ) was recorded under treatment T<sub>0</sub> (Control). T<sub>0</sub> (Control) was followed by T<sub>5</sub> (Thermal Pasteurization at 65°C for 80 sec), T<sub>2</sub> (Thermosonication at 55 °C for 20 min at 40 kHz frequency) and T<sub>1</sub> (Thermosonication at 50°C for 25 min at 40 kHz frequency) having the pectin methyltransferase enzyme activity of ( $7.74 \times 10^{-5}$ ), ( $6.72 \times 10^{-5}$ ) and ( $5.76 \times 10^{-5}$ ) respectively. The minimum pectin methyltransferase activity ( $2.77 \times 10^{-5}$ ) was recorded under treatment T<sub>3</sub> (Thermosonication at 60°C for 15 min at 40 kHz frequency).

However, if we investigate the PME inactivation, after the application of different thermosonication and thermal pasteurization treatments, it can be clearly observed from table 1 that the pectin methyltransferase enzyme activity reduced significantly as compared to control, which represents pectin methyltransferase enzyme inactivation. The highest PME inactivation (72.56 %) over control has been observed by T<sub>3</sub> (Thermosonication at 60 for 15 min at 40 kHz frequency) followed by T<sub>8</sub> (Heat Pasteurization at 65 for 80 sec), T<sub>4</sub> (Thermosonication at 63 for 10 min at 40 kHz frequency) and T<sub>7</sub> (Heat Pasteurization at 65 for 80 sec) having pectin methyltransferase enzyme inactivation of 69.31 %, 59.26 % and 55.94 %. Whereas, T<sub>5</sub> (Heat Pasteurization at 65 °C for 80 sec) and T<sub>1</sub> (Thermosonication at 50 °C for 25 min at 40 kHz frequency) showed least PME inactivation 23.26 % and 33.20 % respectively.

Pectin methyltransferase enzyme may have a major role in either development or loss of textural characteristics (Vora *et al.*, 1999) and pectic acids are mostly consumed to ease maceration, clarification, improve the cloud stability and most important is to increase the yield of fruit juice. An increasing trend for enzyme deactivation was observed with the increase of treatment temperature. Significant increase in the 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 inactivation of enzyme through various effect 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 radical that oxidize amino acid residue that participate in the stability and catalytic activity of enzyme (Barteri *et al.* 2004, sala *et al.* 1995). 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 enzyme (Chemat *et al.*, 2011, Vercet *et al.*, 1999 and vercet *et al.*, 2001). However, the result also showed that ultrasound alone could

not inactivate enzymes to a desired level (Villamiel & De Jong, 2000). Many studies have revealed that the effect of sonication have been enhanced by addition of high temperature (ohlsson & Bengtsson, 2002; Kuldiloke, 2002; Baslar & Ertugay, 2013). PME inactivation kinetics in orange juice has shown increase from 5% to 62% after increasing ultrasound intensity was increased at same time (Tiwari *et al.*, 2009). Ultrasound treatment of fruit juice shows minimal effect on quality of orange juice (Valero *et al.* 2007) and sonication also enhances cloud value and stability of orange juice during storage (Tiwari *et al.* 2009).

However lower PME inactivation has been observed in T<sub>1</sub> (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 enzyme (Butz and Tauscher 2002, Tiwari *et al.* 2008, 2009). It has also been observed that thermosonicated samples showed maximum PME inactivation under treatment T<sub>3</sub> (Thermosonication at 60°C for 15 min at 40 kHz frequency) (72.56%) which showed more PME inactivation even if compared to treatment T<sub>8</sub> (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 thermosonication temperature from 60 °C (72.56 %) to 63 °C (59.26 %). T<sub>3</sub> (Thermosonication at 60 °C for 15 min at 40 kHz frequency) shows a decrease in the PME inactivation as compared to T<sub>4</sub> (Thermosonication at 63 °C for 10 min at 40 kHz frequency). Increase in inactivation is more pronounced at lower temperature. 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 (Aadil *et al.*, 2015). These can be attributed that at higher temperature, vapor pressure increases inside the bubbles which introduces a cushioning effect hence produces less effective collapse (Mason,1990). Crelier *et al.* (2001) also noticed that PME is more heat sensitive at low temperature. At higher temperature the effect of sonication on enzyme inactivation was no longer effective (Koshani *et al.*, 2015). This is attributed to the increased vapor pressure of water at higher temperature, resulting in the collapse of bubbles and hence less violent cavitation. These leads to lower the viscosity which leads to decreased violence of implosion (Sala *et al.*, 1995). Decrease in the efficiency of ultrasound at high temperature has also been reported by Wu *et al.* (2008).

Thermal treatment has also been found to increase the PME inactivation of juice as compared to control (untreated juice sample). High temperature causes changes in the enzyme structure, such as hydrogen bond breaking, opening the secondary and tertiary structures of enzyme and thermal deamination. Amino acid such as asparagine and glycine lead to 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 temperature 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.*, 1997, Vercet *et al.*, 1999 and Ly-Nguyen *et al.*, 2002. Kuldilok (2000) also showed that the 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 (Yeom *et al.* 2000). whereas 100% PME inactivation has been also observed in orange juice after heat pasteurization at 90 °C for 1 min (Elez-Martinez *et al.* 2005). inactivation of 98% has also been observed at 98 °C for 21 s (Rivas *et al.* 2006). Barteri *et al.* (2004), observed progressive oxidation of cystein residues and aggregation of the enzyme from which they concluded that the inactivation of 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. 1.5-3 times increase in PME inactivation has been observed by thermosonicatoin as compared to thermal treatment of guava juice (Ali *et al.*, 2011). Similar result has also been observed in mosambi juice (Siwach *et al.*, 2012). Thermosonication treatment (US-HP85T for 120s) has shown 16.62% more PME-inactivation percentage than thermal treatment alone (HP85T for 120s) (Agcam *et al.* 2016). 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.

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

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

#### 4. Conclusion

Based on result obtained in the present study, it is inferred that the application of treatment T<sub>3</sub> (Thermosonication at 60 °C for 15 min at 40 kHz frequency) showed maximum pectin methylesterase enzyme (PME) inactivation. On the other hand, the treatment T<sub>3</sub> (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 T<sub>3</sub> (Thermosonication at 60 °C for 15 min at 40 kHz frequency) responded well in terms pectin methylesterase enzyme inactivation and cloud value of juice. However, these parameters need to be explored during the storage for production of a high-end value-added Nagpur mandarin juice.

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