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 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. Thermosonication 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 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 ℃, 55 ℃, 60 ℃, and 63 ℃ for 25, 20, 15 and 10 min respectively at a constant frequency of 40 kHz. Juice samples were also thermally pasteurized at 65 ℃, 75 ℃, 85 ℃, and 95 ℃ for 80, 60, 40, and 20 sec. Samples were analyzed after treatment at 0 days and 7 days of refrigerated storage at 4 ℃. Result indicated that thermosensation at 63 ℃ for 10 min at 40 kHz frequency showed much reduced PME activity with inactivation of 72.56% over control whereas thermal pasteurization at 95 ℃ 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.

Key word: 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 (Carbonell *et al*., 2006). 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 (Vercet *et al*., 1999). PME enzyme also degrades the pectin present in the fruits which consists of a polygalacturanic 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 (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 microbial inactivation which is more effective than 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 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 (1985) [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 (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 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 (Versteeg *et al.*, 1980, 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 the table, recorded the highest value for cloud (0.812) under the treatment T_3 (Thermosonication at 60℃ for 15 min at 40 kHz frequency) which was statistically similar to T_2 (Thermosonication at 55 ℃ for 20 min at 40 kHz frequency), T_7 (Heat Pasteurization at 85 ℃ for 40 sec), T_8 (Heat Pasteurization at 95 ℃ for 20 sec) and T_3 (Thermosonication at 63 ℃ 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_0 (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 (2013), has also recorded an increase in the cloud value with the increase in the temperature from 50 ℃ to 60 ℃ 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 (Ohlsson & Bengtsson, 2002; Kuldiloke, 20002; Baslar & Ertugay, 2013). Kuldiloke (2002) 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 (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 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 [Seshadri *et al.*, 2003]. The above phenomenon leads to two consequences; one is the structural modification in pectin molecule which causes a reduction in the accessibility of PME (Tiwari *et al*. 2009) and the other causes the juice to be clearly homogenized and consistent (Abid *et al*. 2013). The study by knorr *et al* (2004) 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.*, 2011, 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 thermosonicatoin 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 T_0 (Control). T_0 (Control) was followed by T_5 (Thermal Pasteurization at 65℃ for 80 sec), T_2 (Thermosonication at 55 ℃ for 20 min at 40 kHz frequency) and T_1 (Thermosonication at 50℃ 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 T_3 (Thermosonication at 60℃ 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 T_3 (Thermosonication at 60 for 15 min at 40 kHz frequency) followed by T_8 (Heat Pasteurization at 65 for 80 sec), T_4 (Thermosonication at 63 for 10 min at 40 kHz frequency) and T_7 (Heat Pasteurization at 65 for 80 sec) having pectin methylesterase enzyme inactivation of 69.31 %, 59.26 % and 55.94 %. Whereas, T_5 (Heat Pasteurization at 65 ℃ for 80 sec) and T_1 (Thermosonication at 50 ℃ 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 (Vora *et al.*, 1999) 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 (Barteri *et al*. 2004, sala *et al.* 1995). Free radicles 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 (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 has been enhanced by the addition of high temperature (Ohlosson & Bengtsson, 2002; Kuldiloke, 2002; Baslar & Ertugay, 2013). PME inactivation kinetics in orange juice has shown an increase from 5% to 62% after increasing ultrasound intensity was increased at the same time (Tiwari *et al*., 2009). Ultrasound treatment of fruit juice shows minimal effect on the 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_1 (Thermosonication at 50 ℃ for 25 min at 40 kHz frequency) with only 33.20 % over control. Sonication at 50 ℃ is not effective against various food enzymes (Butz and Tauscher 2002, Tiwari et al. 2008, 2009). It has also been observed that thermosonicated samples showed maximum PME inactivation under treatment T_3 (Thermosonication at 60℃ for 15 min at 40 kHz frequency) (72.56%) which showed more PME inactivation even if compared to treatment T_8 (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 ℃ (72.56 %) to 63 ℃ (59.26 %). T_3 (Thermosonication at 60 ℃ for 15 min at 40 kHz frequency) shows a decrease in the PME inactivation as compared to T_4 (Thermosonication at 63 ℃ 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 ℃ to 72℃ (Aadil *et al.*, 2015). 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 (Mason,1990). Crelier *et al.* (2001) 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 (Koshani *et al*., 2015). 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 (Sala *et al.*, 1995). Decrease in the efficiency of ultrasound at high temperatures 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 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.*, 1997, Vercet *et al.*, 1999 and Ly-Nguyen *et al.*, 2002. Kuldilok (2000) also showed that a temperature below 60 ℃ has no significant effect on PME inactivation on lemon juice. Whereas, inactivation starts after 70 ℃ and increases rapidly at 90 ℃. 8% of PME inactivation has been observed in orange juice by heat treatment applied at 94.6 ℃ for 30 secs (Yeom *et al*. 2000). whereas 100% PME inactivation has been also observed in orange juice after heat pasteurization at 90 ℃ for 1 min (Elez-Martinez *et al.* 2005). inactivation of 98% has also been observed at 98 ℃ for 21 s (Rivas *et al*. 2006). Barteri *et al*. (2004), 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 thermosonicatoin as compared to the thermal treatment of guava juice (Ali *et al*., 2011). A similar result has also been observed in mosambi juice (Siwach *et al.*, 2012). Thermosonication treatment (US-HP85T for 120s) has shown a 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 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

4. Conclusion

Based on the result obtained in the present study, it is inferred that the application of treatment T_3 (Thermosonication at 60 ℃ for 15 min at 40 kHz frequency) showed maximum pectin methylesterase enzyme (PME) inactivation. On the other hand, the treatment T_3 (Thermosonication at

60 ℃ 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_3 (Thermosonication at 60 ℃ 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.

REFERENCES

Aadil, R. M., Zeng, X., Abbasi, M. S., Khan, M. S., Khalid, S., Jabbar, S., and Abid, M. 2015. Influence of power ultrasound on the quality parameters of grapefruit juice during storage. Science letters, 3: 6-12.

Aadil, R. M., Zeng, X., Zhang, Z., Wang, M., Han, Z., Jing, H., and Jabbar, S. 2015. Thermosoniation: a potential technique that influence the quality of grapefruit juice. International Journal of Food Science and Technology, 50: 1275-1282.

Abdullah, N., and Chin, N. L. 2014. Application of Thermosonication Treatment in Processing and Production of High Quality and Safe-to-Drink Fruit Juices. Agriculture and Agricultural Science Procedia, 2:320–327.

Agcam, E., Polat, S., Akyildiz, A., and Fenercioglu, H. 2016. Inactivation kinetics of pectin methyl esterase under thermosonication and thermal Pasteurization process condition in orange juice, 14(2):85-91.

Ali, G., Russly, A.R., Jamilah, B., Azizah, O. and Mandana, B. 2011. Effect of heat and thermosonication on kinetics of peroxidase inactivation and vitamin C degradation in seedless guava (*Psidium guajava L.*). International Food Research Journal, 18(4).

Anaya-Esparza, L. M., Velázquez-Estrada, R. M., Roig, A. X., García-Galindo, H. S., Sayago-Ayerdi, S. G., & Montalvo-González, E. 2017. Thermosonication: An alternative processing for fruit and vegetable juices. Trends in Food Science & Technology, 61:26–37.

Anonymous. 2020. http://aps.dac.gov.in/

Barteri, M., Diociaiuti, M., Pala, A. and Rotella, S. 2004. Low frequency ultrasound induces aggregation of porcine fumarase by free radicals production. Biophysical chemistry, 111(1): 35-42.

Başlar, M. &Ertugay, M.F. 2013. The effect of ultrasound and photosonication treatment on PPO activity, total phenolic component, and color of apple juice. International Journal of Food Science & Technology, 48(4): 886-892.

Butz, P. and Tauscher, B., 2002. Emerging technologies: chemical aspects. Food research international, 35(2-3): 279-284.

Carbonell, J. V., Contreras, P., Carbonell, L., & Navarro, J. L. 2006. Pectin methylesterase activity in juices from mandarins, oranges and hybrids. European Food Research and Technology, 222(1–2): 83–87.

Chemat, F., Zill-e-Huma & Khan, M.K. 2011. Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrasonic Sonochemistry, 8(4): 813-35.

Crelier, S., Robert, M.C., Claude, J. and Juillerat, M.A., 2001. Tomato (*Lycopersicon esculentum*) pectin methylesterase and polygalacturonase behaviors regarding heat-and pressure-induced inactivation. Journal of Agricultural and Food Chemistry, 49(11): 5566-5575.

Dabir, M.P. and Ananthanarayan, L., 2017. Effect of thermosonication on peroxidase, pectin methylesterase activities and on bioactive compounds in custard apple juice. Journal of Food Measurement and Characterization, 11(4):1623-1629.

Elez-Martinez, P., Soliva-Fortuny, R. C., & Martin-Belloso, O. 2005. Comparative study on shelf life of orange juice processed by high intensity pulsed electric fields or heat treatment. European Food Research & Technology, 222:321-329.

Ertugay, M. F., and Baslar, M. 2014. The effect of ultrasonic treatment on cloudy quality related quality parameters in apple juice. Innovative Food Science and Emerging Technologies, 26: 226-231.

Gomez, K. A. and Gomez A. A. 1984. Statistical procedure for agricultuual Research. Edn 2, John Wiley & sons Newyork : 241-271.

Kimbal, D. A. (1999). Citrus processing, a complete guide (2nd ed.). Gaithersburg, Maryland: An Aspen Publication, 264–273.

Knorr, D., Zenker, M., Heinz, V. and Lee, D.U. 2004. Applications and potential of ultrasonics in food processing. Trends in Food Science & Technology, 15(5): 261-266.

Koshani, R., Ziaee, E., Niakousari, M. and Golmakani, M.T., 2015. Optimization of Thermal and Thermosonication Treatments on Pectin Methyl Esterase Inactivation of Sour Orange Juice (*Citrus aurantium*). Journal of food processing and preservation, 39(6): 567-573.

Kuldiloke, J. 2002. Effect of ultrasound, temperature and pressure treatments on enzyme activity and quality indicators of fruit and vegetable juices.

Lopez, P., Sanchez, A.C., Vercet, A. and Burgos, J. 1997. Thermal resistance of tomato polygalacturonase and pectinmethylesterase at physiological pH. ZeitschriftfürLebensmitteluntersuchung und-Forschung A, 204(2): 146-150.

Ly-Nguyen, B., Van Loey, A.M., Fachin, D., Verlent, I., Indrawati, and andHendrickx, M.E. 2002. Partial purification, characterization, and thermal and high-pressure inactivation of pectin methylesterase from carrots (*Daucus carrota L*.). Journal of agricultural and food chemistry, 50(19): 5437-5444.

Mason, T. J. 1990. Introduction. In T. J. Mason (Ed.), Chemistry with ultrasound (pp. 1–26). New York: Elsevier Applied Science.

Ohlsson, T. and Bengtsson, N. 2002. Minimal processing of foods with non-thermal methods. Minimal processing technologies in the food industry, 34-60.

Rao, M.A. (1999). Rheology of fluid and semisolid foods: Principles and applications. New York: Aspen Publishers.

Rivas, A., Rodrigo, D., Martinez, A., Barbosa-Cánovas, G.V. and Rodrigo, M. 2006. Effect of PEF and heat pasteurization on the physical–chemical characteristics of blended orange and carrot juice. LWT-Food Science and Technology, 39(10): 1163-1170.

Sala, F.J., Burgos, J., Condon, S., Lopez, P. and Raso, J. 1995. Effect of heat and ultrasound on microorganisms and enzymes. In New methods of food preservation (pp. 176-204). Springer, Boston, MA.

Seshadri, R., Weiss, J., Hulbert, G. J., and Mount, J. 2003. Ultrasonic processing influences rheological and optical properties of high-methoxyl pectin dispersions. Food Hydrocolloids, 17(2): 191–197.

Siwach, R. 2012. Pectin Methylesterase Inactivation in Mosambi Juice. Journal of Life Science, 4(2):81-85.

Tiwari, B. K., Muthukumarappan, K., Donnell, C. P. O., & Cullen, P. J. 2008. Effect of sonication on the kinetics of orange juice quality parameters. Journal of agricultural and food chemistry, 56: 2423-2428.

Tiwari, B. K., Muthukumarappan, K., Donnell, C. P. O., and Cullen, P. J. 2009. Inactivation kinetics of pectin methylesterase and cloud retention in sonicated orange juice. Innovative Food Science and Emerging Technologies, 10: 166-171.

Valero, M., Recrosio, N., Saura, D., Munoz, N., Marti, N., and Lizama, V. 2007. Effect of ultrasonic treatment in orange juice processing. Journal of Food Engineering, 80: 509-516.

Vercet, A., Burgos, J. and López-Buesa, P. 2001. Manothermosonication of foods and food-resembling systems: effect on nutrient content and nonenzymatic browning. Journal of Agricultural and Food Chemistry, 49(1): 483-489.

Vercet, A., Lopez, P., & Burgos, J. (1999). Inactivation of Heat-Resistant Pectinmethylesterase from Orange by Manothermosonication. Journal of Agricultural and Food Chemistry, 47(2):432–437.

Versteeg, C., Rombouts, F.M., Spaansen, C. H. &Pilnik, W. (1980). Thermostability and orangejuice cloud destabilizing properties of multiple pestinesterases from orange. Journal of Food Science, 45(4): 969-971.

Villamiel, M. and de Jong, P. 2000. Influence of high-intensity ultrasound and heat treatment in continuous flow on fat, proteins, and native enzymes of milk. Journal of Agricultural and Food Chemistry, 48(2): 472-478.

Vora, H.M., Kyle, W.S.A. and Small, D.M. 1999. Activity, localization and thermal inactivation of deteriorative enzymes in Australian carrot (*Daucus carota L*) varieties. Journal of the Science of Food and Agriculture, 79(8): 1129-1135.

Wu, J., Gamage, T. V., Vilkhu, K. S., Simons, L. K., & Mawson, R. 2008. Effect of thermosonication on quality improvement of tomato juice. Innovative Food Science & Emerging Technologies, 9:186–195.

Yeom, H.W., Streaker, C.B., Zhang, Q.H. and Min, D.B. 2000. Effects of pulsed electric fields on the activities of microorganisms and pectin methyl esterase in orange juice. Journal of Food Science, 65(8): 1359-1363.