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Thermosonication an Alternative to Thermal Pasteurization for 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.

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ABSTRACT

Nagpur mandarin is well known globally for its excellent nutritional benefits and flavour. These all characteristics get affected due to a lack of knowledge & processing techniques. Thermal pasteurization is a processing technique mostly used for juice processing before storage. Thermal pasteurization treatment leads to a loss in various physicochemical qualities of juice viz. ascorbic acid content, and carotenoid content. Thermal pasteurization treatment also leads to an increase in limonin content and the browning value of the juice. Therefore, an experiment was carried out to check the effect fascorbic acid and carotenoid content using the ultrasound combined with higher temperatures known as ensthermosensation on Nagpur mandarin (*Citrus reticulata* Blanco) juice as compared. Treatments were also used to observe its effect on limonin, browning, total soluble solids, pH & acidity as compared to thermal pasteurization. Juice samples were thermosonicated at temperatures of 50 ℃, 55 ℃, 60 ℃, and 63 ℃ for 25, 20, 15 and 10 min respectively at a constant

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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. Thermosonication treatments showed better ascorbic acid content and carotenoid content in comparison to thermally pasteurized juice samples. Limonin value and browning value were also less in thermosonicated juice samples. Both treatments do not have any significant effect on Physicochemical characteristics like TSS, pH & acidity. fromThe result indicated that ensthermosensation treatments performed better than thermal pasteurization treatments and can be used as an alternative technique for Nagpur mandarin juice processing.

Keywords: Thermosonication; thermal pasteurization; Nagpur mandarin; juice quality.

1. INTRODUCTION

Citrus is a group of perennials, evergreen flowering trees and shrubs which belong to the family Rutaceae. 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 of 6368 ‘000 MT in the country [1]. 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. 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 MMandarin is 0.025 thousand hectares and production of 0.765 thousand MT.

Nagpur mandarin is well known globally for its excellent nutritional benefits and flavour [2]. These all characteristics get affected during juice processing due to a lack of knowledge & processing techniques. Juices also face low ascorbic acid content & low carotene content after thermal processing. Therefore, it is necessary to develop and standardize a technology to increase and stabilize the ascorbic acid & Carotenoid content with minimum effect on the other quality parameter of juice like the colour taste, etc.

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. 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 [5]. 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 is to check the effect of ensthermosensation over thermal pasteurization on various physicochemical parameters viz. total soluble solid, pH, acidity, carotenoid, limonin, ascorbic acid & browning 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 of 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 Ajandouz et al. [6]. Juice samples were tested for various chemical parameters after the application of different treatments to juice.

2.1 Acidity

acidity of the Nagpur mandarin juice was calculated by taking 5ml of juice & diluting it with 20 ml of distilled water, after further taking 5 ml of aliquot (from diluted juice sample) & titrating this aliquot against 0.1 N NaOH using phenolphthalein as indicator. The end point is denoted by the appearance of light pink colour [7]. The acidity is expressed in per cent (%) and was calculated using the following formulae: -

$$\text{Acidity} = \frac{\begin{array}{c}\text{Titre x Normality of alkali x volume made up x}\\ \text{Equivalent Weight of anthocynin x Equivalent}\\ \text{Weight of anhydrous citri acid x 100}\end{array}}{\begin{array}{c}\text{Volumeof sample taken for estimation x weight or volume of sample}\\ \text{taken x 1000}\end{array}}$$

2.2 pH

The pH of Nagpur mandarin juice was calculated using a digital pH meter. The temperature was kept constant while taking all the observations.

2.3 Total Soluble Solid (TSS)

The total soluble solid of Nagpur mandarin orange juice was directly measured using a digital hand refractometer. Reading was taken at room temperature.

2.4 Browning Index

The browning index was assessed after removing juice pulp by centrifuging the juice sample at 2000 rpm for 20 min. Extracted supernatant after centrifugation was mixed with an equal volume of 95% ethanol. The solution was then filtered through Whatman No. 42 filter paper to obtain a fully clarified extract. The clear extract was then observed for absorbance at 420 nm with a spectrophotometer (Meydav et al, 1977). Browning was measured in optical density (O.D.).

2.5 Ascorbic Acid

L-Ascorbic acid was determined using HPLC. The methodology has two divisions for extraction and analysis of l- Ascorbic acid.

2.5.1 Extraction method

The sample juice was treated with a dilute ortho-phosphoric acid solution in the ratio of 1:1 and then the sample was centrifuged at 5℃. The centrifuged sample was then filtered by 0.45µm nylon filters before being analyzed on HPLC.

2.5.2 Instrument method

L-Ascorbic Acid (Vitamin C) was determined by a normal phase HPLC method. The system consists of an Agilent Technologies (1260 Infinity model) system with four hydraulic pumps, an injection system, a C18 column (4.6 × 250 mm, 5µm), a C18guard column, a DAD detector, and a computerized recorder/integrator. For the determination of L-Ascorbic Acid (Vitamin C), the mobile phase consisted of 20 mM Na_2HPO_4 buffer and acetonitrile (95:5) with a flow rate of

1ml/min. The injection volume of the sample was 5 µl. The detector's wavelength was 220 nm.

2.6 Limonin

Limonin content was determined by taking 25 ml of juice samples, which were centrifuged thrice at 3000 rpm for 10 min. Five milliliters of supernatant was collected after centrifugation. Ten millilitre of chloroform was added to the supernatant on the separating funnel. Four millilitres of lower layer decant were taken from the remaining liquid from the separating funnel. Six millilitres of freshly prepared Burcham reagent were added to 4 ml of separated lower layer and kept at room temperature for 30 min. After 30 min, the upper layer of solution was taken up & then record observation at 503 nm. Limonin was reported in ppm (Wilson, and Crutichfield, 1968).

2.7 Carotenoid

Carotenoid content was estimated by taking 10 ml of juice sample & adding 20 ml of 80% methanol with 0.5 g of elite. Filter it with the Buchner funnel under vacuum pressure. Add 20 ml of acetone to the filtrate & again filter it with a Buchner funnel under vacuum pressure. Take the filtrate and add 5 ml of n-hexane in a separating funnel. Discard the lower acetone layer after a few seconds. Collect the upper n-hexane layer & make up its volume to 10 ml with n-hexane. The observation (absorbance) was recorded at 450 nm. Carotenoid was reported in mg/100ml [8].

3. RESULTS AND DISCUSSION

3.1 TSS, Acidity & pH

Results showed that the thermosensation and thermal pasteurization treatment did not influence the TSS, pH & acidity of Nagpur mandarin juice. The result revealed that acidity was statistically non-significant. These may be attributed to the energy level applied to the samples through thermosensation or thermal pasteurization, which did not change the molecular structure of high molecular weight associated with acidity. These results were in collaboration with Abid et al. [9] on Kasturi lime juice, Aadil et al. [3] on grapefruit juice and Bhat et al. [10] on orange juice. Data observed for the total soluble solid after treatment with thermosensation & thermal pasteurization did not show any significant effect on total soluble solids. Different treatments of thermosensation and thermal pasteurization did not change the molecular structure of high molecular weight associated with total soluble solids (TSS) [11]. These results agree with the findings of Bhat et al. [10] in orange juice. However, heat pasteurization was found to be superior over thermosensation.

Table 1. Effect of thermosonication and Heat Pasteurization on Titrable acidity, TSS, pH of Nagpur mandarin juice

Notation	Treatments	Titrable acidity	TSS (%)	pH
T_0	Control	0.543	8.67	4.06
T_1	Thermosonication at 50 ℃ for 25 min at 40 kHz frequency	0.577	8.57	4.19
T_2	Thermosonication at 55 ℃ for 20 min at 40 kHz frequency	0.593	8.55	4.19
T_3	Thermosonication at 60 ℃ for 15 min at 40 kHz frequency	0.521	8.83	4.11
T_4	Thermosonication at 63 ℃ for 10 min at 40 kHz frequency	0.561	8.6	4.11
T_5	Heat Pasteurization at 65 ℃ for 80 sec	0.628	8.73	4.14
T_6	Heat Pasteurization at 75 ℃ for 60 sec	0.566	8.77	4.13
T_7	Heat Pasteurization at 85 ℃ for 40 sec	0.614	8.83	4.10
T_8	Heat Pasteurization at 95 ℃ for 20 sec	0.552	8.93	4.06
	C.D.	N/A	N/A	N/A

3.2 Ascorbic Acid (Vitamin C)

Ascorbic acid content is a very important quality parameter of the juice. Data in Table 2 revealed that various treatments exerted significant influence over the ascorbic acid content of juice after the treatment. Data presented in Table 2 recorded maximum ascorbic acid content (19.371 mg/100ml) under treatment T_2 (Thermosonication at 65℃ for 20 min at 40 kHz frequency) which was statistically at par with T_1 (Thermosonication at 50 ℃ for 25 min at 40 kHz frequency) having the ascorbic acid content of 19.34 mg/100ml. The minimum ascorbic acid content (16.082 mg/100ml) of Vitamin C was recorded under treatment T_8 (Thermal Pasteurization at 95℃ for 20 sec). In addition to these, maximum ascorbic acid content (18.014 mg/100ml) among thermally pasteurized treatment was observed under treatment T_5 (Heat Pasteurization at 65 ℃ for 80 sec).

Ascorbic acid content is primarily related to the nutritional quality of orange juice [12]. A significant reduction in the ascorbic acid content has been recorded among the thermally pasteurized juice with a maximum reduction of Ascorbic acid under treatment T_8 (Thermal Pasteurization at 95℃ for 20 sec) and minimum ascorbic acid reduction was recorded under treatment T_5 (Heat Pasteurization at 65℃ for 80 sec. This shows that the loss of the ascorbic acid content increased with the increase in temperature from 65℃ and 95℃. A similar result was also observed by Saeeduddin et al. [13] in pear juice. According to Saeeduddin et al*.,* 2015 this trend in loss of ascorbic acid with an increase in temperature for thermal pasteurization indicates the thermo-sensitivity of ascorbic acid. Ascorbic acid is thermolabile, photosensitive and prone to degradation due to various processing conditions [14]. Verbeyst et al., (2012), have also studied the effect of matrix on the degradation of vitamin C during pasteurization. Thermal pasteurization can also cause ascorbic acid degradation by oxidation reaction in most fruit derivatives (Leskova et al*.,* 2006).

On the other hand, a significant increase in ascorbic acid content was also recorded by thermosonication treatments but these treatments also showed a decrease in the ascorbic acid content with the increase of temperature from 50℃ to 63℃. Such increase in ascorbic acid content at lower ultrasound treatment and reduction with increase of thermosensation temperature has already been observed by Abid et al. [4] on apple juice, Tiwari et al*.* [15], Tiwari et al. (2009b), Walking-Ribeiro et al*.* [16] in orange juice, Gardner et al. [17], Rawson et al. [18], and Spanos and worsted [19]. Thus, sonication at a mild temperature helps to preserve some important nutritional components in the product [20]. The increase in the ascorbic acid is supposed to be due to the removal of dissolved oxygen from the juice [21] (Knorr, Zenker, Heeinz & Lee, 2004, Aadil et al., 2013). Oxygen is a critical factor affecting the stability of ascorbic acid (Solomon, Svanberg, & Sahlstrom, 1995). Higher temperatures may induce the chemical decomposition of ascorbic acid at higher rates [13]. Ultrasonication degasses any liquid and the treatment favours the retention of vitamin C in juice due to the removal of dissolved oxygen responsible for vitamin C degradation [13].

The data recorded under the present investigation are following Saeeduddin et al. [13] in pear juice, Bhat et al. [10] in kasturi lime & Cheng et al*,* [21] in guava juice. Dias et al. [22] on sour soap, Aadil et al*.* (2013) in grapefruit juice, Abid et al*,* [9] in apple juice, Santhiradegaram et al*.* [23] on choking mango juice, Nayak et al. [24] in star fruit juice & Guerrouj et al*.* (2014) in orange juice.

In contrast, Cao et al. [25], in Barbery juice, observed a decrease in the ascorbic acid content of the ultrasound treated samples as compared to the control. similar results were also observed by Adekunte et al. [2] in tomato juice. The loss was attributed to sonochemical reactions and extreme physical conditions occurring during sonication. It has been seen that the hydrogen ions (H^+), free radicals (O^-, OH^-), and hydrogen peroxide (H_2O_2) formed during the sonolysis of water molecules present in juice [2,26] (Petrier, Combet, and Mason, 2007). The degradation can also be related to oxidation reactions promoted by the interaction of hydroxyl radicals produced by cavitation during ultrasonic processing [2].

3.3 Carotenoid

Carotenoid is a very important quality parameter in Nagpur Mandarin Juice. Results indicated that the carotenoid content of Nagpur mandarin juice was statistically significant. Data depicted in the table showed that the maximum carotenoid content (2.926 mg/100ml) was registered under treatment T_0, (Control), which was followed by Treatment T_3 (Thermosonication at 60℃ for 15 min at 40 kHz frequency), treatment T_2 (Thermosonication at 55℃ for 20 min at 20kHz frequency) and treatment T_6 (Heat Pasteurization at 75 ℃ for 60 sec), having the carotenoid content 2.835, 2.721, and 2.671 mg/100 ml respectively. Whereas, the minimum carotenoid content (2.468 mg/100 ml) was registered under the treatment T_8 (Heat Pasteurization at 95 ℃ for 20 sec). In addition to these, among thermosonication samples, treatment T_3 recorded the highest carotenoid content (2.72 mg/100 ml), whereas, among thermally pasteurized samples, treatment T_6 recorded the highest carotenoid content (2.671 mg/100 ml). It was observed that thermosonication samples registered higher carotenoid content after control as compared to thermal pasteurization.

In the present study, a significant decrease in the carotenoid content has been observed in the thermosonicated and thermally pasteurized samples of Nagpur mandarin juice as compared to the control. However, it has also been observed that after control, thermosonicated samples showed higher carotene content as compared to thermal pasteurization. These might be attributed to the sonication treatment well known for extracting some components due to its disrupting effect on cell walls [20]. This may also be accounted for the mechanical disruption of the cell wall, enhancing free carotenoids in the juice [4]. Similar results were also observed by Abid et al. [9] in apple juice, Aadil et al. [3] in grapefruit juice, Martinez-flores et al. [20] in carrot juice, & Guerrouj et al. [27] in orange juice where they observed higher carotene content in thermosonicated samples as compared to thermally pasteurized samples. In contrast, some other studies have also shown an increase in total carotenoid content, as in orange juice-milk beverages treated with high pressure [11]. Similarly, an increase in carotenoid content has also been seen in orange juice treated with high pressure as compared to pasteurized juice [28].

3.4 Limonin

The data in Table 2 presents limonin content as influenced by different thermosonication and thermal treatments of Nagpur mandarin juice, recorded after the application of treatment. Nagpur mandarin juice treated with various treatments showed a significant response to the limonin. The data presented for limonin in Table 2 ranged between 7.121 ppm to 9.512 ppm. Data showed that the maximum value for limonin 9.512 ppm was recorded under treatment T_8 (Thermal Pasteurization at 95℃ for 20 sec) which was found statistically at par with treatment T_7 (Heat Pasteurization at 85℃ for 40 sec), T_6 (Heat Pasteurization at 75 ℃ for 60 sec), T_5 (Thermal Pasteurization at 65℃ for 80 sec) and T_4 (Thermosonication at 63 ℃ for 10 min at 40 kHz frequency) having the limonin 9.062 ppm, 9.046 ppm, 8.639 ppm and 8.465 ppm respectively. The minimum limonin content (7.121) was recorded under the treatment T_1 (Thermosonication at 50℃ for 25 min at 40 kHz frequency).

A significant increase in limonin content in the thermally pasteurized juice samples has been observed as compared to untreated juice (Control). The increase in limonin content was more pronounced in treatment with higher processing temperature which may be attributed to its low solubility in water. Heating increases its concentration [29] and accelerates the conversion of the non-bitter precursor, α-limonin monolactone to limonin [30]. Whereas low limonin content at low processing temperature may be attributed to the inhibition of oxidation of D-ring lactone into limonin It has also been studied that heat treatment and acidification of juice speed up the formation of limonin to the maximum level but does not change the maximum constant value (Zhang et al., 2018).

3.5 Browning

The data obtained in Table 2 represented the significant influence of different treatments on the browning of Nagpur mandarin juice. The data depicted for the browning of juice on Table 2 ranged between 0.277 to 0.539. The data recorded a maximum browning value (0.539) under treatment T_8 (Thermal Pasteurization at 95℃ for 20 sec). Treatment T_8 was statistically at par with T_4 (Thermosonication at 63 ℃ for 10 min at 40 kHz frequency) and T_3 (Thermosonication at 60 ℃ for 15 min at 40 kHz frequency) having the browning value of 0.527 and 0.494 respectively. The minimum browning value of 0.277 was recorded under treatment T_0 (Control).

A significant increase in browning has been observed in the thermosonicated as well as thermally pasteurized juice samples as compared to untreated juice sample (Control). It might be due to the increase in browning reaction with the increase in temperature because browning depends on ascorbic acid, reducing sugar and enzyme activity. similar findings were reported by [6,31]. Increase in NEB is due to sonication which may be attributed to the breakdown of the carotenoid pigment. similar changes were also reported by Ugarte-Romero et al. [32]. Marcy et al. [33] has observed that ascorbic acid degradation is also responsible for the browning of citrus juices. This degradation of ascorbic acid has been observed due to the application of thermal pasteurization in the present experiment. This has already been depicted in Table 2. Destruction of ascorbic acid provides reactive carbonyl groups, which can be precursor to non-enzymatic browning [34]. Furfural formation resulting from the decomposition of ascorbic acid causes non-enzymatic browning. Non-enzymatic browning in orange juice has already been reported due to particulate fraction (Zarate-Rodriguez et al., 2000).

The enzymatic discolouration is also responsible for the browning of juice which is caused by the action of enzymatic browning, caused by the action of polyphenol oxidase (PP0) which catalyzes the oxidation of phenolic compounds [34] and pectin methylesterase (PME). As thermal pasteurization inactivates the PME activity, browning in the thermally pasteurized sample is not due to an enzyme, but due to non-enzymatic biochemical reactions like Millard condensation, pigment destruction and caramelization of sugar [35, (Ibarz et al. 2000, & Landl et al. 2010). A similar process may also be related to the sonication treatment under the present investigation which also inactivates the PME activity.

Various browning-related studies based on A_{420nm} measurement in different juice like citrus juice [36], apple juice [37], pear juice (Ibarz, Pagan and Garza, 1999), and pear juice concentrate [7] have indicated that the non-enzymatic browning predominates over pigment destruction. The result recorded under the present experiment was in agreement with Tiwari et al. [15] in orange juice, Aadil et al. [3] in grapefruit juice, Lagnika et al. (2017) in pineapple juice, Cruz-casino et al. [38] in purple cactus pear juice and Tiwari et al. [39] in orange juice [40-47].

Table 2. Effect of thermosonication on ascorbic acid, carotene, limonin & browning of Nagpur mandarin juice

Notation	Treatments	Ascorbic acid (mg/100ml)	Carotene (mg/100ml)	Limonin (ppm)	Browning (OD)
T_0	Control	18.010	2.926	8.020	0.277
T_1	Thermosonication at 50 ℃ for 25 min at 40 kHz frequency	19.349	2.651	7.121	0.413
T_2	Thermosonication at 55 ℃ for 20 min at 40 kHz frequency	19.371	2.721	7.419	0.461
T_3	Thermosonication at 60 ℃ for 15 min at 40 kHz frequency	18.610	2.835	7.641	0.494
T_4	Thermosonication at 63 ℃ for 10 min at 40 kHz frequency	18.325	2.505	8.465	0.527
T_5	Heat Pasteurization at 65 ℃ for 80 sec	18.014	2.590	8.639	0.395
T_6	Heat Pasteurization at 75 ℃ for 60 sec	17.222	2.671	9.046	0.424
T_7	Heat Pasteurization at 85 ℃ for 40 sec	16.741	2.546	9.062	0.464
T_8	Heat Pasteurization at 95 ℃ for 20 sec	16.082	2.468	9.512	0.539
	C.D.	0.676	0.139	1.218	0.049
	SE(m)	0.226	0.046	0.407	0.016

4. CONCLUSION

Based on the results obtained in the present study, it is inferred that the application of thermosonication for the processing of Nagpur mandarin showed better physicochemical & biochemical quality characteristics as compared to thermal pasteurization. It can be concluded from the observation that thermosonication technique can be used as an alternative technique to thermal pasteurization for the processing of Nagpur mandarin juice to obtain a better physicochemical quality parameter. However, the behaviour of different physicochemical parameters must be compared with thermally pasteurized juice during storage. 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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