EFFECT OF THERMOSONICATION ON QUALITY OF SWEET ORANGE JUICE

By

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B.Tech (Agril. Engg.)

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DECLARATION

I, **Ms. GORLE RADHIKA,** hereby declare that the thesis entitled **"EFFECT OF THERMOSONICATION ON QUALITY OF SWEET ORANGE JUICE"** submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Technology in Agricultural Engineering** in the major field of **Processing and Food Engineering** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

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Date: **I.D. No. BEM/20-02**

CERTIFICATE

Ms. **GORLE RADHIKA** has satisfactorily prosecuted the course of research and that the thesis entitled "**EFFECT OF THERMOSONICATION ON QUALITY OF SWEET ORANGE JUICE"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any university.

Date: **(Dr. Ch. Someswara Rao)**

Place: **CHAIRPERSON**

CERTIFICATE

This is to certify that the thesis entitled "**EFFECT OF THERMOSONICATION ON QUALITY OF SWEET ORANGE JUICE"** submitted in partial fulfillment of the requirements for the degree of **"Master of Technology in Agricultural Engineering"** in the major field of **"Processing and Food Engineering"** of the Acharya N. G. Ranga Agricultural University, Lam, Guntur is a record of the bonafide original research work carried out by **Ms. GORLE RADHIKA** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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Place:

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LIST OF SYMBOLS AND ABBREVIATION

TSS	Total soluble solids
et al.	And others
Fig.	Figure
ppm	Parts per million
%	Percentage
mL	Milli litre
mm	Milli meter
nm	Nano meter
°	Degree
°C	Degree Celsius
kHz	Kilo Hertz
W	Watt
CFU	Colony forming units
cm^2	Square centimeter
min	Minutes
g	Grams
RSM	Response Surface Methodology
cm	Centimeter

ABSTRACT

Name of the Author	:	**G. RADHIKA**
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Sweet orange or sweet lime (*citrus sinensis (L.) Osbeck*) is commonly known as "Mosambi" in Indian sub-continent. It is native to Asia and widely cultivated in India. Sweet orange is enjoyed, most either as a juice or whole fruit which is a rich source of vitamin-C and minerals like calcium, phosphorous, potassium and iron.

About 95% of the fruit is essentially sold as fresh due to lack of processing technologies. Thermal processing along with chemical preservatives is widely used for preservation of fruit juices. Thermal processing of juices cause unwanted changes in taste and odour with damage to bio-active components and functional properties. Thermosonication is a good alternative technique to replace the conventional heat treatment process. It is a combined method of applying ultrasound at low temperatures which is an effective non-thermal processing method in inactivating microorganisms and enzymes at less processing time with minimum cost.

The thermosonication of sweet orange juice was carried out at temperature (40,50 and 60°C), amplitude (40,70 and 100%) and sonication time (5, 12.5 and 20 min). Response surface methodology following Box- Behnken design was used to optimize the response parameters i.e., pH, TSS, ascorbic acid and titrable acidity. No significant changes were observed in pH and TSS during thermosonication treatments. Ascorbic acid and titrable acidity showed significant change in thermosonication treatments. The optimized conditions for thermosonicated sweet orange juice were observed as temperature of 40°C, 40% amplitude and sonication time of 9.298 min.

During the storage period, pH, TSS and total sugars of thermosonicated sweet orange juice processed at optimized conditions, slightly increased from 3.8 to 4.1, 11.0 to 13°Brix and from 10.096 to 11.66% at refrigerated storage. The ascorbic acid and titrable acidity of the treated juice decreased from 49.13 to 38.59 mg/100mL and from 1.12 to 0.84% at refrigerant storage. No change in residual activity of PPO and POD of juice during refrigerated storage was observed. The maximum microbial count of 19 x 10^4 CFU/mL and the maximum fungal count of 13.5 x 10^3 CFU/mL were observed in thermosonicated juice sample on 28th day of storage at refrigerated conditions. The shelf life of the thermosonicated sweet orange juice was observed as 24 days at refrigerated storage.

Keywords: Microbial inactivation, polyphenol oxidase, peroxidase, shelf life, sweet orange, thermosonication.

CHAPTER I

INTRODUCTION

Fruits are one of the nature's wonderful medicines packed with minerals, vitamins, anti- oxidants and phyto-nutrients. They also contain many anti-oxidants like polyphenolic flavonoids, vitamin-C and anthocyanins. These compounds, protect human body from oxidant stress, diseases, cancers and help the body to boost the immunity level. Many fruits, when compared to vegetables, have high anti-oxidant value.

Citrus fruits belongs to the family *Rutaceae*. It is an evergreen crop which is sensitive to low temperatures. Juice is present in the juice sacs in citrus fruits. Unlike other fruits, they doesn't have a firm pulp and the juice is easily extracted and made into beverages. Citrus fruits include oranges, which account for about 70% of worldwide production and small citrus fruits, such as mandarins, lemons, limes, clementines, tangerines, satsumas and grapefruit. In India, main citrus fruits of this group are lime/lemon, sweet orange (Mosambi) and orange (Kumar *et al*., 2012).

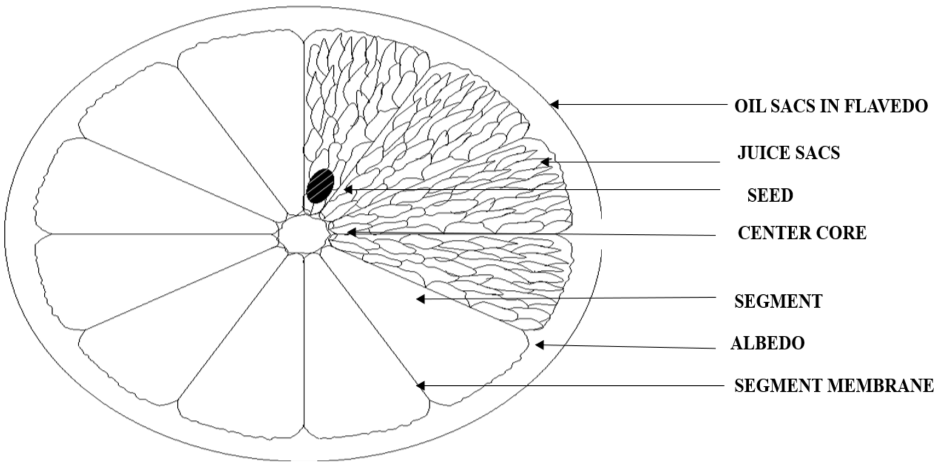


Fig. 1.1 Cross sectional view of a Sweet Orange.

Citrus fruits make up 90% terpenes, 5% oxygenated chemicals, and less than 1% non-volatile components, pigments and waxes. The most prevalent terpene, D-limonene, exhibits antimicrobial effects, most notably antibacterial action against gram-positive bacteria as well as increases the efficiency of sodium benzoate as a preservative (Ahmed *et al*., 2016).

Citrus fruits are covered with a peel to protect the pulp, or edible portion, of the fruit (**Fig 1.1**). The outer layer of the peel is made up of a cuticle covering an epidermal layer (flavedo) containing number of oil glands, filled with an aromatic essential oil having commercial value. In addition to these oil sacs, the flavedo also contains colouring substance, which is not uniformly distributed throughout this layer but is concentrated in small bodies called chromatophores, which is green color in young fruit and gradually changed to yellow or orange color in a matured fruit. A white spongy portion known as the albedo, made of parenchymatous cells lies beneath the flavedo. The cells of albedo layer are loosely arranged with more intercellular spaces and are irregular inshape. This layer contains approximately 20% of pectinous substances, which can be recovered in the form of citrus pectin (Anonymous, 1956). The inner flesh of the fruit consists of segments separated by a thin membrane of epidermal tissue containing juice sacs and seeds. The juice sacs in each segment are attached to the segment wall on one side and in contact with the peel on other side by means of fine threads of varying length. Oil droplets are embedded within the cellular tissue in the central part of each juice vesicle. Yellow chromatophores of crystalline origin may be observed when these juice sacs are crushed and examined microscopically. The central axis (core) of the fruit is composed of a white spongy tissue similar to that found in albedo.

Sweet orange or sweet lime (*citrus sinensis L. (Osbeck*)) is commonly known as "Mosambi" in Indian sub-continent. It is native to Asia and widely cultivated in India, southern Japan, Malaysia, Indonesia, Vietnam and Thailand. Sweet orange is a high value fruit. It is enjoyed mostly either as a juice or whole fruit which is rich source of vitamin-C and minerals like calcium, phosphorous, potassium and iron. It also consists of vitamin-A, B_1 and B_2. The proportions of various components of sweet orange fruit were reported as peel (23.66%), juice (37.95%), pomace (32.09%) and seed (6.3%) (Syed *et al.*, 2012). A freshly squeezed glass of sweet orange is an excellent thirst quencher, energizer and a good replacement for carbonated drinks. It is a multipurpose fruit that tastes sweet and tart and looks like a lemon. Despite belonging to the citrus family, sweet orange juice is quite popular in India, because it is not acidic. The main sweet orange growing states in India are Telangana, Andhra Pradesh, Maharashtra, Madhya Pradesh, Karnataka, Punjab, Haryana and Rajasthan. India is the world's sixth largest producer of citrus fruit (De *et al.,* 2019).

In India, main varieties of sweet orange being cultivated on commercial scale are Mosambi, Blood Red and Sathgudi. Blood red is an important variety in Haryana, Punjab, Rajasthan and Maharashtra whereas Sathgudi is extensively grown in Telangana, Andhra Pradesh and Tamil Nadu.

Sweet orange juice contains carbohydrates (10.5%), proteins (0.6%), fat (0.05%), fibre (0.12%), ash (0.3%), moisture content (88.4%), total soluble solids (TSS) (10.0%), pH (3.7) and ascorbic acid (43.0 mg/100 mL) (Syed *et al*., 2012). The sweetness of citrus juice is due to presence of glucose and fructose. The most important factor governing sugar content is the maturity of the fruit. Reducing and non-reducing sugars are present in equal proportions (Kale and Adsule, 1995). The proteins in citrus are relatively insoluble and are found to be associated with solid portions of the fruit such as seeds, flavedo, albedo and pulp. Amino acids are found in the juice of edible portion of the fruit and in aqueous alcohol extractable fraction of the peel.

Compared to freshly squeezed juice, the flavour and shelf life of aged juice is very less. The presence of enzymes and microbes causes juice to deteriorate. It must be drunk promptly after extraction since enzymatic and oxidative changes degrade natural quality of juice when it is exposed to air. Peroxidase (POD) and polyphenol oxidase (PPO) are oxidative enzymes that change the characteristics of food, reducing nutritional value and colour. The bitter chemical limonine is responsible for the increased bitterness and change in taste of aged juice. Limonine is essentially non-existent in freshly squeezed juice, however precursor compounds to limonine are present. When fresh juice comes into contact with oxygen, enzymes transform the precursors into the bitter limonine. This is referred to as enzymatic bittering, and it is the cause of rancid flavour development.

Generally for the preservation of fruit juices thermal and non-thermal treatments were used. Thermal treatments include low temperature long time (LTLT), high temperature short time (HTST) methods. Non thermal technologies include pulsed electric field (PEF), high hydrostatic pressure (HPP), high pressure homogenization (HPH) and ultrasound treatments.

Thermal processing is commonly used for processing of fruit juices which causes thermal damage to juice and affects the quality parameters. Conventional thermal technologies like pasteurization and sterilization are mostly used for processing of liquid foods. It causes unwanted changes in taste and odour with damage to bio-active components and functional properties during juice processing. Preservation of the orange juice with thermal treatment integrated with the use of chemical preservatives is already in practice. Thermal processing of orange juice adversely affects the nutritional and organoleptic characteristics. Thermal treatments destroy heat-sensitive nutrients, limiting its use in the processing of juices. It assures good shelf- life and stability of fruit juices; however, this process may affect the quality of the juices in terms of nutritional and physicochemical parameters such as: vitamins (C and E), carotenoids, polyphenols, organic acids, pH, and colour. Furthermore, due to increase in knowledge and scientific evidence, customers are more concerned about their health and nutrition and the demand for foods with fresh-like properties have boosted interest in mild processing methods.

Novel food technologies are described as complete or partial alternatives to thermal processing, among which ultrasound applications stand out, which have a proven potential for use in the food industry, especially in the beverage industry. Thermosonication is a good alternative technique to replace the conventional heat treatment process. Thermosonication (TS) is a heat and ultrasound treatment that exposes the product to a moderate amount of heat. The combination of heat and sonication (thermosonication) was found to be more effective for inactivation of microbes than sonication alone (Piyasena *et al*., 2003). TS is the best alternative to conventional thermal treatment and has been shown to have little impact on the quality of most of the juices, such as blackberry and orange juice (Tiwari *et al*., 2009 and Mohideen *et al*., 2015). Many authors have reported improvements in the quality characteristics of different food products treated with thermosonication (Abid *et al*., 2014; Riener *et al*., 2009; Rawson *et al.*, 2011 and Wu *et al.*, 2008). Thermosonication which is a combined method of ultrasound and heat proved to be an effective non-thermal processing method in inactivating microorganisms and enzymes at low temperature at less processing time with minimum cost. Sweet orange juice is rich in vitamin-C and natural antioxidants such as flavonoids and phenylpropanoids. Hence, keeping the above points in mind, the present research was undertaken to study the effect of thermosonication on the quality of sweet orange juice with the following specific objectives:

1. To study the effect of thermosonication on nutritional and sensory quality of sweet orange juice.

2. To optimize the thermosonication parameters for sweet orange juice processing.

3. To study the effect of thermosonication on shelf life of sweet orange juice.

CHAPTER II

REVIEW OF LITERATURE

The review of literature is an important and essential part of scientific investigation. Its main purpose is to determine previous research work and to asset delineation in the development of objectives, hypotheses and research procedures followed. This chapter deals with a detailed study on literature available on thermosonication of juices, process parameters, optimization process, quality parameters and shelf-life studies.

2.1 MATURITY CHARACTERISTICS AND JUICE QUALITY OF SWEET ORANGE

The maturity of fruit is assessed from the colour, juice content, total soluble solids (TSS) and acidity of the juice. The juice content and composition of juice vary widely for a variety grown at different places. The different varieties of oranges have been reported to produce 26.3-59.0% of juice yield, TSS of 8.8-14.8°Brix and 0.64-1.77% of acid content (Sandhu and Minhas, 2006). Before harvest, fruit for processing must meet certain minimum maturity requirements established by the regulatory agencies.

These requirements may vary from one citrus-producing area to another. These requirements are usually based on: colour break, minimum juice content, minimum acid content, minimum percentage of TSS and °Brix: acid ratio.

2.2 CITRUS BITTERNESS

Fresh sweet orange juice is a refreshing, thirst quenching and energy drink providing nutritional requirement of 45 kcal of energy per 100 mL, moderately rich in vitamin C, potassium, bioflavonoids and folic acid (Siddiqui *et al*., 2013).

Low consumption of processed sweet orange juices may be due to high cost of processing and the most important cause is the development of bitterness in sweet orange juice after extraction. The compound limonine is responsible for bitterness in sweet orange juice (Maier and Dreyer, 1965). The non-bitter precursor in citrus fruits was identified as limonin monolactone, which after acid-catalysed conversion formed limonin (Maier and Beverly,1968). Limonin monolactone was stable in the tissues of whole fruit because it was not in direct contact with the juice. It slowly converts into limonin and then, the juice becomes bitter. The majority of the limonin was formed from its non-bitter precursor limonite A-ring lactone.

Limonin content of sweet orange tissues was reported by Siddiqui *et al*. (2013). According to the report, the order of limonin content was seeds (480mg/100 g)>flavedo (38.80 mg/100 g)> albedo (13.80 mg/100 g) > segment wall (0.95 mg/100g)> juice (0.015 mg/100 g). It was reported that the freshly extracted juice contained 0.15 mg/L limonin which was below threshold level and therefore the fresh juice was non-bitter during consumption. After 6 h of storage, the limonin level was increased to 10.2 mg/L. The limonin content of sweet orange juice was increased up to 15.2 mg/L during pasteurization. The adsorbent strongly resins were reported to be effective in reducing limonin content to 1 mg/L.

2.3 NUTRITIONAL AND SENSORY QUALITY OF THERMO-SONICATED JUICES

Shokri *et al.* (2022) conducted a study on thermosonication of broccoli florets prior to fermentation to increase bioactive components in fermented broccoli puree. For thermal pretreatments the broccoli water mixture was kept in water bath at 60°C for 7 min, and TS was performed using an 18 kHz probe at power density of 0.41±0.02 W/g for 7 min at 60°C. TS treated samples have highest sulforaphane yield (7268 µmol/kg dry weight) compared to thermally treated (6227 µmol/kg dry weight) and control fermented sample (3180 µmol/kg dry weight). The measurable residual glucoraphanin content for TS was 1642 µmol/kg dry weight compared to thermally fermented sample (1187 µmol/kg dry weight) and control fermented sample (1047 µmol/kg dry weight). The fermented TS broccoli puree resulted in the highest improvement in total phenol content compared to thermal and control samples.

Strieder *et al.* (2022) studied impact of thermosonication processing on the phytochemicals, fatty acid composition and volatile organic compounds of almond based beverage. 50 mL of almond based beverage was thermosonicated at 50°C using three levels of power (4.6, 8.5 and 14.5 W) and holding times of (5, 10, and 15 min). The acoustic energy increased the working temperature up to 25°C beyond that provided by the external heat source. Higher powers and longer holding times degraded stearic and palmitic fatty acids. Treatment at 14.5 W for 15 min degraded ethyl esters of the samples modifying the natural flavor of the beverage. By maintaining color attributes all TS treatments intensified the blue color of the almond-based beverage.

A study on effect of thermosonication on quality attributes of hog plum juice was conducted by Adebola *et al*. (2021). Freshly prepared hog plum juice (control) and samples of juices were subjected to pasteurization (90 °C for 60 s) and thermosonication (40 kHz, 400 W at (40, 50 and 60°C) each for (5, 10, 20 and 30 min)) and analyzed for physio-chemical, bioactive, microbial and sensory properties. After treatment, no significant changes in pH, total soluble solids and titratable acidity were observed. Thermosonication at 40 and 50°C significantly ($p<0.05$) improved color parameters, cloudiness and browning index. Furthermore, thermosonication increased ascorbic acid (11.40-18.55%), total phenolic content (17.98-18.35%), carotenoids (2.194.30%), flavonoids (10-16%) and antioxidant activity (32.52-48.5) relative to the control. Both treatments significantly reduced the microbial count to non-detectable level after processing, while sensory attributes slightly improved. Thermosonication treatmentat 60 °C decreased most of the quality parameters. Overall thermosonication treatment improved quality, safety and economic potential of hog plum juice and it is a feasible alternative to pasteurization.

Manzoor *et al.* (2021) conducted a study on impact of high intensity thermosonication treatment on spinach juice bioactive compounds, rheological, microbial, and enzymatic activities. 100 mL of spinach juice was subjected to thermosonication at a temperature of 60±1°C for 20 min with 30 kHz frequency at 50% amplitude with different ultrasonic power of 200 W, 400 W and 600 W. Pasteurized (60±1 °C for 30 min) juice and untreated juice were used for control treatments. Results showed that thermosonication improved the bioactive compounds (total flavanols, total flavonoids, total phenolic, carotenoids, chlorophyll and anthocyanins), anti-oxidant activities (DPPH and FRAP assay) in spinach juice. Compared to the untreated and pasteurized samples thermosonication significantly reduced the particle size, improved the suspension stability and rheological properties (shear stress, shear rate and apparent viscosity) of spinach juice. TS treatments inactivated the polyphenol oxidase and peroxidase from 0.97 and 0.034 Abs/min (untreated) to 0.31 and 0.018 Abs/min. Treatment with intensities exhibited complete inactivation of microbial loads, the highest reduction in enzymatic activities, better suspension stability and highest bioactive compounds.

Schuina *et al.* (2021) conducted a study on the effect of thermosonication on pectin methylesterase activity and quality characteristics of orange juice. Orange juice samples were distributed in tubes and incubated in ultrasonic bath at treatment temperature (25, 30, 40, 50 and 60°C) for 10 min at 40 kHz. The pasteurization of the juice sample was carried out at 90°C for 30 s. The results were compared to the untreated control sample. Soluble solids content, pH and color of all treatment samples did not present significant differences when compared to control. Vitamin-C content was profoundly affected by temperature. The lower the temperature used together with sonication, the better was the retention of ascorbic acid. The use of 40 kHz and 60°C thermosonication presented the most significant reduction in pectin methylesterase (PEM) activity. Thermosonication is an alternative preservation method that allows a substantial reduction in PEM activity in orange juice and better vitamin-C retention compared to only thermal treatments.

Wu *et al*. (2021) conducted a study on effect of thermosonication treatment on blue berry juice quality: total phenols, flavonoids, anthocyanin, and antioxidant activity. Thermosonication was performed at 60°C with a frequency (20 kHz), amplitude (50%) for 15 min at three different powers 200, 400 and 600 W. Juice was pasteurized at 60 °C for 30 min. Juices treated with thermosonication had higher color density, L* and lower values of chroma (C), hue angle (h), yellow index (YI). The content of total phenolics, flavonoids and anthocyanins increased by 139.32%, 251.72%, and 94.12%. Compared to raw juice the 1,1 diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical scavenging capacity increased to 65.22% and 51.13% significantly higher than pasteurized sample. The content of delphinidin3-O- glucoside (Dp-G) and cyaniding-3- O-glucoside (Cy-G) were 54.40 mg/L, 28.75 mg/L in juice.

Lafarga *et al.* (2019) studied the effect of thermosonication on the Bioaccessibility of antioxidant compounds and the microbiological, physiochemical and nutritional quality of an anthocyanin- enriched tomato juice. 100 mL of juice sample was heated to 20, 40 and 60°C temperatures, processing duration (0, 5 and 10 min) and ultrasonic frequencies (0, 35 and 130 kHz). Thermosonication for 5 min at 60°C at either 35 or 130 kHz resulted in higher microbial inactivation when compared to thermal pasteurization at 80°C for 1 min. Thermosonication increased the polyphenol, lycopene, anthocyanin and antioxidant capacity retention when compared to the thermal treatment. The anthocyanin content decreased from 1.08 ± 0.04 mg/100 mL before processing to 0.92 ± 0.01 mg/100 mL after thermal pasteurization but the difference was not significant when compared with the thermosonicated juice (1.06 ± 0.03 mg/100 mL). Bioaccessibility of phenolic compounds after a simulated gastrointestinal digestion was lower in processed juices. Compared to the thermally treated ones thermosonicated samples showed higher bioaccessibility.

Chitgar *et al.* (2018) studied the stability of barberry juice copigmented with ferulic acid and licorice extract during thermosonication. About 15 mL of barberry juice was pasteurized at 90±1°C for 1 min. 60 mL of juice thermosonicated at 45 °C for 5 min at two amplitude levels of 70% and 100% and a pulse rate of 15 s ON and 5 s OFF at a constant frequency of 20 kHz. Thermal treatment negatively affected the phytochemical compounds; at low amplitude levels no significant changes were observed in thermosonication. During storage a similar trend in the degradation of anthocyanins and color instability of samples was observed. Thermosonication maintained the effectiveness of copigmentation reaction.

Nayak *et al.* (2018) conducted a study on effect of thermosonication on quality attributes of star fruit juice. 50 mL of juice was subjected to thermosonication at different temperatures (25-45°C) for four different time periods 15, 30, 45 and 60 min with 44 kHz constant frequency. Thermal treatment of star fruit juice was carried at 90°C for 60 s. Results showed that the treatments did not show any significant ($p<0.05$) variations in pH, TSS and titrable acidity. A significant ($p<0.05$) increase in total phenolic contents, total flavanoid content, antioxidant activity, ascorbic acid content, cloud index and browning index of thermosonicated juices was observed. There is a significant effect in the reduction of microbial population in both pasteurization and thermosonication treatments.

Aguilar *et al.* (2017) conducted a study on stability of ascorbic acid in fruit juices during thermosonication. Ascorbic acid solutions were prepared at pH values (3, 4, 5 and 6) using critic acid/phosphate buffer solutions. Mandarin (pH=3.72±0.06, 10.6±0.1 °Brix) and orange (pH=3.58±0.10, 10.8±0.1 °Brix) juices obtained by the UHT process were packed in multilayer packages. The beakers containing samples were placed at ultrasonic bath at 700 W power with frequency of 25 kHz and processed for 60 min. Distilled water is used as transmission medium and stainless-steel heat exchanger was used to control sample temperature. Four treatments were conducted: ultrasound processing at 25±44°C (sonication), ultrasound processing at 55±4°C (thermosonication), processing without ultrasound at 55±4°C (thermal treatment) and processing without ultrasound at 25±4°C (control treatment). For the second experiment juice samples were sonicated at 55°C. Results showed that in all the cases ascorbic acid was stable under the treatment.

Sonication did not affect the ascorbic acid content when the solutions had been previously deaereated or degasified.

Guerrouj *et al.* (2016) studied the effect of sonication at mild temperatures on bioactive compounds and microbiological quality of orange juice. Freshly squeezed orange juice was sonicated (for 1, 10, 20 and 30 min at 24 kHz frequency) and thermostatic bath temperature was maintained at 46°C to evaluate its impact on selected physio-chemical and antioxidant properties such as total phenolics, flavonoids, DPPH radical scavenging activity, total carotenoids, ascorbic acid, pH, °Brix and color attributes. The effect of sonication treatments on the microbial load (anerobic mesophilic, yeast and mold) were also evaluated. Sonication of juice samples for 10, 20 and 30 min at temperatures of 43-45°C showed enhancement in most of the bioactive compounds compared to samples treated for 1 min and control samples (untreated). Results indicated that sonication coupled with mild temperatures is a suitable technique for orange juice to improve its safety and nutritional quality.

Abid *et al*. (2014) used thermosonication as a quality improvement technique of apple juice. Fresh juice was thermosonicated in a bath at 25 kHz frequency, 30 min duration with a power density of 0.06 W/cm^3 and ultrasound with sonicator probe at 20 kHz frequency, 10 min duration with 0.30 W/cm^3 power density at various temperatures (20, 40 and 60°C). The highest enzyme and microbial inactivation was obtained at 60°C for 10 min. Compared to the application of ultrasound in-bath at 60°C the total phenolics, ascorbic acid, flavonoids were significantly higher in application of ultrasound with-probe. Use of thermosonication at low temperatures for processing of apple juice inactivated enzymes and microorganisms.

Wu *et al.* (2008) conducted a study on effect of thermosonication on quality improvement of tomato juice. To minimize the quality losses in tomato juice inactivation of pectinmethylesterace (PME) and polygalacturonase (PG) was required. A 200 mL of raw tomato juice was subjected to thermosonication at temperatures of 60, 65 and 70°C in water bath and sonicated at amplitudes of (25%, 50% and 75%) with constant frequency of 24 kHz. At 60, 65 and 70°C for 41.8, 11.7 and 4.3 min exposure the PME activity reduced by 90%. TS treatments with 25-75 µm amplitude had no significant effect on inactivation efficiency between 60 and 70°C. After TS viscosity increased 2-4 fold, the average particle size decreased (<30 µm) compared to the heat treated or untreated juice. The results suggested that TS at 60 and 65°C could be useful to obtain tomato juice with a low residual PME activity and high viscosity.

2.4 OPTIMIZATION OF THERMOSONICATED PARAMETERS OF FRUIT JUICES

Ramfrez *et al.* (2022) conducted a study on optimization of antioxidant activity properties of a thermosonicated beetroot juice and further compared invitro bio accessibility with thermal treatments. Beetroot juice was treated at 50±2°C at 20 kHz compared with two thermal treatments at 70 °C for 20 min (TT1) and 80°C for 10 min (TT2). The optimal conditions were 80% amplitude and 10.5 min. In comparison with the thermal treatments the microbial and enzymatic inactivation was more in TS treated juice. After in vitro digestion processing, juice treated by TS has higher betalains content than TT1, similar total phenol contents and high chelating activity in comparison with juice by thermal treatments.

Wahia *et al.* (2021) conducted a study on quality attributes for optimization of process parameters of orange juice subjected to multi-frequency thermosonication. For 100 mL of orange juice 1 mL *A. acidoterrestris* with an initial load of 10^8 CFU/mL was inoculated. The optimized results of multi-frequency ultrasound (using Box Behnken design-surface response methodology) and further comparison with different mode of ultrasound (mono- and multi- frequency) revealed that 20/40 kHz, 24 min and 64 °C were the best. The *Alicyclobacillus acidoterrestris* (AAT) spores and vegetative cells were inactivated by 2 and 4 logs, without deteriorating orange juice contents. AAT inactivation indicated an inversely proportional relation with reactive oxygen species (ROS) production. The findings suggested that dual-frequency ultrasound technology for fruit beverages promotes beneficial changes in physical properties without any significant effects on the quality of ascorbic acid.

Dundar *et al.* (2019) studied optimization of thermosonication conditions for cloudy strawberry nectar using critical quality parameters. 150 g of nectar was thermosonicated at temperature of 25-75°C for 0.1 to 15 min with a power of 150 W. The PPO inactivation was successfully achieved by thermosonication treatment. Increasing of temperature resulted decrease of browning index and increase of hydroxymethyl furfural. The combination of 59°C and 455 J/g was optimum thermosonication conditions to minimize quality parameters which cause changes like color degradation etc.

Hashemi *et al.* (2019) conducted a study on modeling inactivation of *Listeria* *monocytogenes, Shigella sonnie, Byssochlamys fulve* and *Saccharomyces cerevisiae,* and ascorbic acid and ß-carotene degradation kinetics in tangerine juice by pulsed thermosonication. Thermal and pulsed-ultrasonic treatments were conducted at three temperatures (60, 70 and 80°C) and three ultrasound amplitudes (25, 50 and 75%) up to 25 min with constant frequency 30 kHz. The microbial enumeration and chemical tests were measured at interval of 0, 5, 10, 15, 20 and 25 min. Tangerine juice subjected to pulsed thermosonication were modeled using polynomial, log-logistic, Weibull, biphasic linear and modified gompertz models. The models were assessed using the adjusted determination coefficient and the root mean square error (RMSE). To classify the fitted models Ward's clustering method was applied. Weibull model showed correlation coefficients above 0.98 and RMSE of 0.218. Results showed that the effect of temperature was higher than pulsed- ultrasound amplitude for *Listeria monocytogenes, Shigella sonnie* and *Saccharomyces cerevisiae* and for *Byssochlamys fulve* these two parameters were similar. Pulsed- sonication amplitude was major parameter in decreasing ß- carotene and ascorbic acid in juice. In optimal conditions the treatment had high effecton *Listeria monocytogenes.*

Hussain *et al.* (2019) conducted a study on effects of thermosonication on watermelon rind-honey beverage. The prepared watermelon rind juice was thermosonicated at temperatures of 25, 45 and 65°C for 10, 35 and 60 min at 40 kHz frequency. TS treated samples were optimized using response surface methodology on the physicochemical, vitamin-C and microbial load under different temperatures for a week. TS at 65°C for 60 min significantly affected the quality of the juice stored at 4°C. TS was found to have no significant effect on pH and TSS of the beverage.

Sasikumar *et al.* (2019) conducted a study on thermosonication assisted extraction of blood fruit juice and optimized the process through response surface methodology. 200 mL of juice sample was placed in water bath at a temperature of 42, 54 and 66°C and sonicated at constant frequency of 20 kHz at amplitude of 80% for time intervals of 1, 4 and 7 min. The optimum process conditions were achieved at a temperature of 59.9°C, 118 W/cm^2 power and 7 min time with maximum juice yield of 73.15%, ascorbic acid 38.92 mg/100 mL, total anthocyanin content 371.32 mg/100 mL, lethality of *E. coli* 3.95 log CFU/mL, minimum cloud value of 0.128 and POD activity of 6.86%.

Wahia *et al.* (2019) studied simultaneous optimization of *alicyclobacillus* *acidoterrestris reduction*, pectin methylesterase inactivation and bioactive compounds enhancement affected by thermosonication of orange juice. Samples were thermosonicated at different temperature (45-75 °C), time (20-40 min) and frequency (20-60 kHz). In Box – Behnken design the optimal conditions achieved for TS were 47 °C, 30 min and 20 kHz. At optimal conditions high total phenolic contents (49.085 mg GAE/100 mL), pectin methylesterase enzyme inactivation (94.77%), as well as low *alicyclobacillus acidoterrestris* vegetative cells load (1.00 log reduction) were observed. Compared to the control total flavonoids contents (38.50 mg/100 mL ER), ascorbic acid (441.70 mg/mL), polyphenol oxidase enzyme (56%) of treated orange juice showed significant ($p<0.05$) difference.

Adiamo *et al.* (2018) conducted a study on thermosonication process of carrot juice incorporating orange peel and pulp extracts for optimal functional characteristics. Aqueous extracts of orange peel and pulp with high total phenolic contents (TPC) of 25.94 and 11.38 mg GAE/g were employed in the formulation of functional carrot juice and functional juices were sonicated in ultrasound bath (40-60 °C, 110 W, 40 KHz). Overlaid contour plots prediction showed that the optimal conditions for carrot juice with peel (CJPL) were 125 mL juice volume, 6.50 min ultrasound process time and 52.78 °C ultrasound process temperature for maximum TPC (30.25 mg GAE/100 mL) and DPPH scavenging activity (61.22%). Sample carrot juice with pulp (CJPP) has maximum TPC (30.25 mg GAE/100mL) and DPPH activity (55.87%) under optimal ultrasound process condition of 125 mL juice volume, 5.04 min and 59.99 °C ultrasound process time and temperature, respectively. Optimization of thermosonication showed significant improvements in the quality of functional carrot juice.

2.5 SHELF-LIFE STUDIES OF THERMOSONICATED JUICES

Alcantara *et al.* (2021) studied thermosonication as an alternative method for processing, extending the shelf life, and conserving the quality of pulque: A non-dairy Mexican fermented beverage. Raw pulque was thermosonicated at temperature of 50±2 °C with the different amplitudes and times: 65% amplitude for 5, 7, 10, 13 and 17 min; at 75% amplitude for 3, 6, 9, 12 and 15 min; at 85% amplitude for 2, 4, 6, 8 and 10 min; 95% amplitude for 1, 3, 5, 7 and 9 min. The optimum conditions were selected at 75% amplitude (for 6 and 9 min), at 85% amplitude (for 4 and 6 min), and at 95% amplitude (for 3 and 5 min). Conventional pasteurization (63 °C, 30 min) and raw pulque were used as controls. Results showed that the shelf life of pulque was extended up to 24 days at 4

°C storage. After 24 days, due to the increase of microbial load the quality of beverage decreased. At 24 days of storage TS at 75% and 85% amplitude showed higher content of lactic acid bacteria (6.58-6.77 log CFU/mL) and yeasts (7.08-7.27 log CFU/mL) than conventional pasteurization. Thermosonicated pulque exhibited greater lightness, sensory acceptance, maximum acidity of 0.83 g/lactic acid and an alcohol content of 4.48-4.95% v/v. The thermosonication process preserved sensory and physiochemical properties better than conventional pasteurization.

Medeiros *et al.* (2021) conducted a study on thermosonication for peroxidase inactivation in sugarcane juice. Thermosonication and conventional treatments were compared and both were performed at temperatures of 50-80 °C for 25 min and sonicated at frequency of 20 kHz with an amplitude of 140 µm, 20/10 s on/off pulse durations. The quality of treated juice was monitored for 32 days under refrigerated storage. Results showed that ultrasound lead to faster POD inactivation at 70 and 75 °C when compared to conventional treatment. Ultrasound at 20/10 s on/off for 25 min and 75% power intensity were best conditions for enzyme inactivation (77.3%).

Zhu *et al.* (2021) studied the effect of thermosonication on quality of red pitaya juice. Freshly prepared red pitaya juices were thermosonicated at 475 W and 56 °C for 20 min. Upon TS processing, native microbiota including bacteria, yeasts and molds reduced to less than 10 CFU/mL. Their growth during storage were slow and equal to thermal processed (83 °C, 1.5 min) samples. During storage at 4 °C for 28 days, soluble solid content, pH, activities of polyphenol oxidase and peroxidase, and browning degree remained unchanged. A visible color decay was observed in TS-processed samples at day 10, mainly resulting from decomposition of betacyanins and the growth of residual native microbiota. Compared to thermal-treated juices, better color retention was obtained by TS treatment. Therefore, TS is a promising alternative technology of thermal methods of juice processing, with equal shelf life and better-quality retention effects.

Alvarado *et al. (*2019) conducted a study on application of thermosonication for aloe vera juice processing: impact on the functional properties and main bioactive polysaccharides. 150 mL of aloe vera juice was thermosonicated at temperatures of 25 and 50 °C for total processing time of 6, 12 and 18 min at a constant frequency of 20 kHz with an amplitude of 50% and pulse duration of 5 s ON and 5 s OFF. Acemannan was the predominant polysaccharide in aloevera juice followed by pectins. The degree of methylesterification of pectins was slightly reduced and thermosonication promoted a minor degradation of the acetylated mannose from acemannan than thermal processing. The highest values for swelling (>150 mL/g AIR) and for fat adsorption capacity (120 g oil/g AIR) and high inactivation of *L.plantarum* (75%) were observed when TS was performed at 50 °C for 6 min.

Anaya *et al*. (2017) conducted a study on effect of thermosonication on pathogenic bacteria, quality attributes and stability of soursop nectar during cold storage. 200 mL of nectars were subjected to thermosonication at temperatures of 34, 44 and 54 °C with constant frequency of 24±1 kHz and time 2, 6 and 10 min to obtain total of nine treatments. A reduction of 4.5-5 log CFU/mL was achieved with the TS treatment (24 kHz, average temperature of 50-51 °C, energy density of 1.3-1.4 W/mL for 10 min) in both *Escherichia coli* and *staphylococcus aureas* pathogens. Nectar treated with TS retained 85% of ascorbic acid, polyphenol oxidase inactivation, microbiological and physiochemical stability during 30 days of storage at 4 °C. Sensorially, the thermosonicated nectar was predominantly preferred over a commercial one.

Kiang *et al.* (2013) studied the effect of thermosonication on *Escheria coli* and *Salmonella enteritidis* in mango juice. Inoculated juice samples were sonicated at 50 °C for 10 min and 60 °C for 7 min at 25 kHz. Thermosonication at 60 °C resulted in highest inactivation. Compared to the *Escheria coli* the *Salmonella enteritidis* was found to be more sensitive to thermosonication. Except the samples those subjected to 60 °C for more than 5 min *Salmonella enteritidis* was recovered in all treated samples. Compared to the thermal treatment alone, introducing high intensity ultrasound enhanced the inactivation of pathogens.

Munoz *et al.* (2012) studied the effect on *Escheria coli* inactivation and quality attributes of apple juice treated by combinations of pulsed light and thermosonication. Thermosonication (TS), pulsed light (PL), PL+TS and TS +PL were applied to study the effect on *Escheria coli* inactivation in apple juice. In pulsed light treatment the juice was exposed to two energy dosages i.e., 4.03 J/cm^2 (low dosage) and 5.1 J/cm^2 (High dosage) corresponding to 51.5 and 65.4 J/mL energy inputs. The juice was thermosonicated (24 kHz, 100 µm) at 40 °C for 2.9 min or 50 °C for 5 min corresponding to 1456 and 2531 J/mL energy inputs. The effect of the energy levels and sequence (PL + TS and TS + PL) was studied. When individual technologies (PL and TS) were applied the maximum *Escheria coli* reduction was achieved 2.7 and 4.9 CFU/mL (for TS and PL at high dosage) while the combined treatments (PL + TS and TS + PL) achieved reductions of 6 CFU/mL.

All the treatments significantly changed the color of apple juice and the sequence in which the technologies were applied affected the color significantly ($p<0.05$). The energy level applied did not affect any of the measured quality attributes.

Ribeiro *et al.* (2009) studied shelf life and sensory quality of orange juice after thermosonication and pulsed electric field treatments. Batches of 800 mL of orange juice was sonicated at 55 °C for 10 min followed by continuous PEF for 150 µm at 40 kV/cm field strength. At 94 °C for 26 s HTST pasteurization was used as a control treatment. For sensory evaluation 20 mL of orange juice was served to 37 untrained panelists (16 female, 21 male) and evaluated by hedonic scale (1-9 points). All sensory attributes were rated equivalent for TS/PEF and HTST treated juice ($p>0.05$). Shelf-life study was conducted at 25 °C storage up to 168 days. No significant changes in the physical properties (pH, °Brix and conductivity) were detected after TS/PEF or HTST treatment during 168 days of shelf life ($p>0.05$). The counts for both treatments were consistently within safe levels (<1000 CFU/mL) during the 168 days of storage.

Ribeiro *et al.* (2009) conducted a study on the impact of thermosonication and PEF on *staphylococcus auerus* inactivation and selected quality parameters in orange juice. Batches of 800 mL of orange juice was sonicated at 55 °C for 10 min followed by continuous PEF for 150 µm at 40 kV/cm field strength. At 94 °C for 26 s HTST pasteurization was used as a control treatment. TS/PEF resulted in a comparable inactivation of *S. aureus* to that of conventional HTST. TS/PEF did not affect pH, TSS conductivity and had a minor impact on the juice color than conventional treatment. Non-enzymatic browning was not affected by TS and PEF but significantly affected by conventional treatment. Retention of ascorbic acid was almost complete after TS and PEF (96.0%), but it was lower for HTST (80.5%). Activity of pectin methyl esterase (PME) decreased as PEF field and time duration increased. TS and PEF combination had greater PME activity than HTST (12.9 and 5.0%, respectively).

Tiwari *et al.* (2009) studied the effect of sonication on orange juice quality during storage. Freshly prepared orange juice was sonicated at a frequency of 20 KHz at different amplitude levels (40%, 70% and 100%) and treatment times (2, 6 and 10 min). Samples were stored at 10 °C up to 30 days. The combined effect of treatment time, amplitude level and storage period on pH, °Brix, color values (L*, a*, b*), non-enzymatic browning (NEB), cloud value, titratable acidity and ascorbic acid content were investigated. Storage of sonicated orange juice up to 30 days did not result in significant differences of TSS and titratable acidity. During storage significant changes were observed in juice color, NEB, cloud value, pH and ascorbic acid. Orange juice sonication resulted in improved ascorbic acid and cloud retention during storage.

CHAPTER III

MATERIALS AND METHODS

This chapter provides the detailed information regarding materials used and the methodologies adopted for the thesis work entitled "Effect of thermosonication on quality of sweet orange juice". The equipment and experimental procedures followed during the study have been detailed. This chapter deals with the sample preparation, experimental procedure for determination of some nutritional properties, microbial and sensory evaluation of thermosonicated juice samples, experimental design for thermosonication of sweet orange juice, evaluation of shelf-life of optimized sample as well as control. The experiment work was carried out at Dr. NTR College of Agricultural Engineering, Bapatla.

The research methodology involved in execution of various operations is shown in block diagram (**Fig. 3.1**).

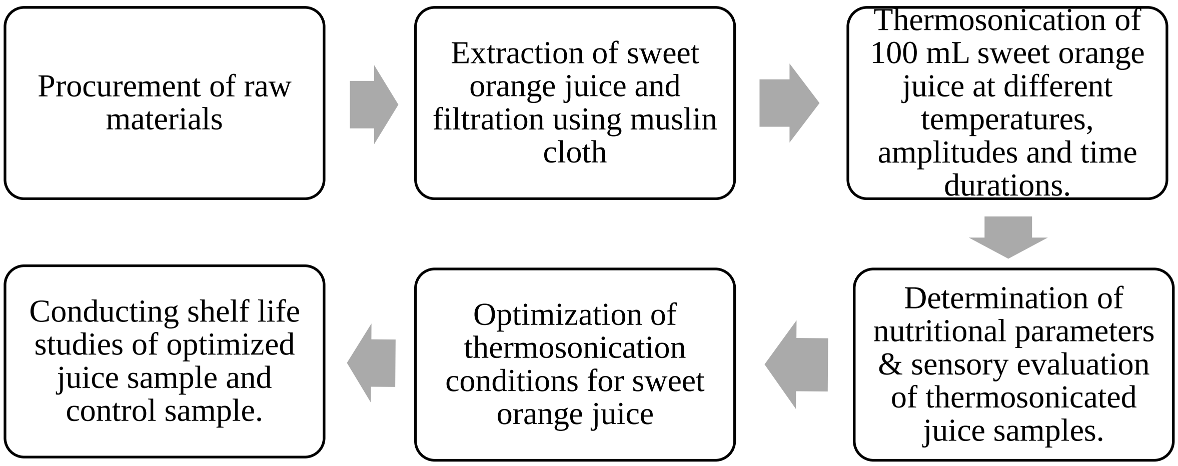


Fig. 3.1 Over-view of research methodology

3.1 PROCUREMENT OF RAW MATERIALS

Freshly harvested sweet orange (**Plate 3.1**) fruits (Variety: Satgudi) used for the study were procured from fruit market, Chirala, Bapatla District. Matured sweet oranges with good floral and melony smell, good appearance and no mechanical damage were selected for the study. The fruits were washed with the tap water to remove all dirt adhering to it and then shade dried at room temperature to remove adhered moisture.

Plate 3.1 Fresh sweet oranges

3.1.1 Extraction of Juice Content

The sweet orange fruits were cut into two equal halves with a sharp knife. The seeds were scooped with the help of spoon and juice was extracted using hand operated juice press (**Make: Basant; Plate 3.2**) and pre-filtered using muslin cloth.

Plate 3.2 Juice Extractor

3.2 EXPERIMENTAL DESIGN

In current study, the following independent and dependent variables were considered as shown in **Table 3.1.**

Table 3.1 Independent and dependent variables for experimental design

Independent variables	Dependent variables
➢ Temperature (40, 50 and 60 °C)	➢ pH
➢ Amplitude (40, 70 and 100%)	➢ Total soluble solids (˚Brix)
➢ Time duration (5, 12.5 and 20 min)	➢ Total Sugar
	➢ Ascorbic acid
	➢ Titrable acidity
	➢ Poly phenol oxidase (PPO)
	➢ Peroxidase (POD)

The experiments were designed by selecting three independent variables viz., temperature, sonication amplitude levels, sonication period and sonication frequency. The range of independent variables were selected by conducting the experimental trails.

3.2.1 Experimental Design Conditions for Thermosonication of Sweet Orange Juice

Experiments were designed with the help of Design Expert version 13.0.5.0 (Stat Ease Inc., Minneapolis, MN, USA). Thermosonication of sweet orange juice was carried out according to experimental conditions obtained from Design Expert software. For this, four independent process parameters namely temperature, sonication amplitude levels, sonication period and sonication frequency were selected. The range of process factors were i.e., temperature, sonication amplitude levels, sonication period and sonication frequency selected were 40-60°C, 40-100%, 5-20 min and 20 KHz, respectively. Constant sonication frequency was maintained for all the experiments. The response parameters selected were pH, TSS (˚Brix), ascorbic acid and titrable acidity. Further, response surface model namely Box-Behnken design (Box and Behnken, 1960) was selected with the aim of optimizing process parameters. The levels of each variable were established based on a series of preliminary experiments and coded as -1, 0, and 1 (**Tables 3.2** and **3.3**) resulting in a total of 17 experimental runs each experiment was conducted in triplicate and a total of 51 experiments were conducted. Quadratic polynomial models were fitted to the data to obtain the regression equations. The statistical significance of the terms in the regression equations was examined by analysis of variance (ANOVA) for each response. The thermosonicated sample variables were optimised by using the numerical method of response surface methodology (RSM) based on desirability concept.

Table 3.2 Coded values of the independent variables of thermosonicated sweet orange juice

Variables	Codes		
	-1	**0**	**+1**
X1 (°C)	40	50	60
X2 (%)	40	70	100
X3 (min)	5.0	12.5	20

Table 3.3 Experimental treatment combinations obtained by Design Expert

Run	Temperature (°C)	Amplitude (%)	Sonication Time (min)
1	60	100	12.5
2	60	40	12.5
3	40	100	12.5
4	50	70	12.5
5	50	100	5.0
6	50	70	12.5
7	60	70	20.0
8	50	40	20.0
9	50	100	20.0
10	40	40	12.5
11	40	70	5.0
12	50	70	12.5
13	40	70	20.0
14	50	40	5.0
15	50	70	12.5
16	60	70	5.0
17	50	70	12.5

Thermosonication of sweet orange juice was carried out at 17 experimental conditions in three replications and responses of pH, TSS, vitamin-C and titrable acidity were calculated

3.3 SONICATION EQUIPMENT

The laboratory scale ultrasound equipment available at Food Engineering Laboratory of Dr. NTR College of Agricultural Engineering, Bapatla was used for the study. The main components of ultrasound processor are ultrasonic generator, probe, ultrasonic horn and standard accessories. **Plate 3.3** represents the laboratory scale ultrasonic probe sonicator.

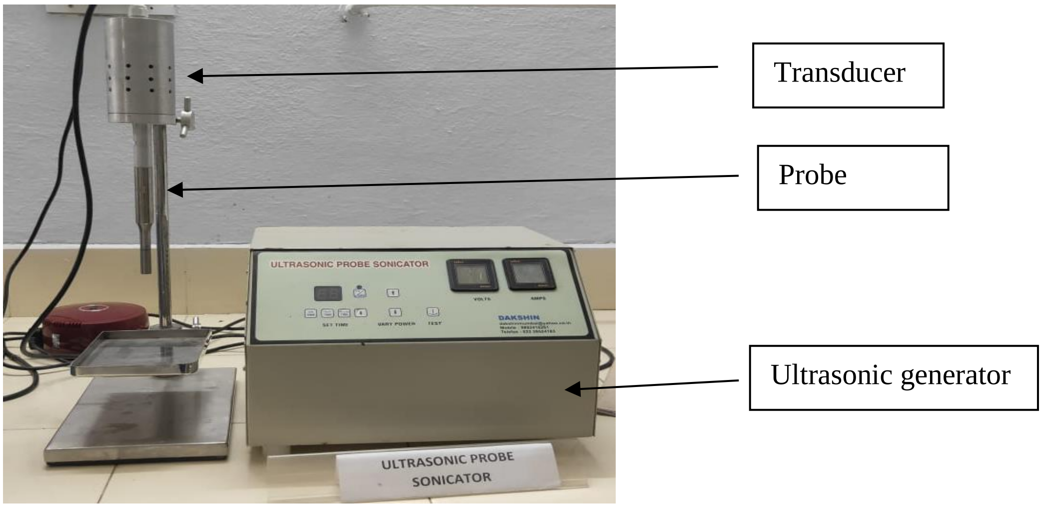


Plate 3.3 Ultrasonic probe sonicator equipment

3.3.1 Description of Ultrasound Probe Sonicator

Ultrasound probe sonicator (Model: DP 120, Make: Dakshin, Mumbai) consists of ultrasonic generator to produce high frequency electrical energy operated at a frequency of 20 kHz. Stainless steel velocity probe of 12 mm diameter fitted with lead zirconate titanate crystals (transducer elements). This velocity probe assembly converted the high frequency electrical energy fed from the ultrasonic generator to mechanical vibrations at the rate of applied electrical frequency. The amplitude of these mechanical vibrations was magnified by this velocity probe. Micro-processor based programmable timer with two-digit display was provided for selecting ON time, OFF time and total time of operation in cyclic mode.

3.3.2 Working Principle

Ultrasound is sound wave transmitted with frequency higher than audible frequency of 20 kHz. During sonication, cycles of pressures form thousands of microscopic vacuum bubbles in the solution. The bubble formed, growed and collapsed into the solution in a process known as cavitation. When ultrasound passes through a liquid medium, they produce gas bubbles prior to acoustic cavitation phenomenon, which is the interaction of ultrasonic waves, liquid and dissolved gas. Oscillations are produced by pressure changes around the dissolved gas nuclei. The quantity of gas and vapor that enters the bubbles during expansion period is more than the quantity that diffuses out of the bubbles during the compression stage. Then the bubbles grow in successive cycles to an unstable size, burst and release very high heat and pressure around the collapsing bubbles localized sterilization effects created due to particle dispersion and cell disruption.

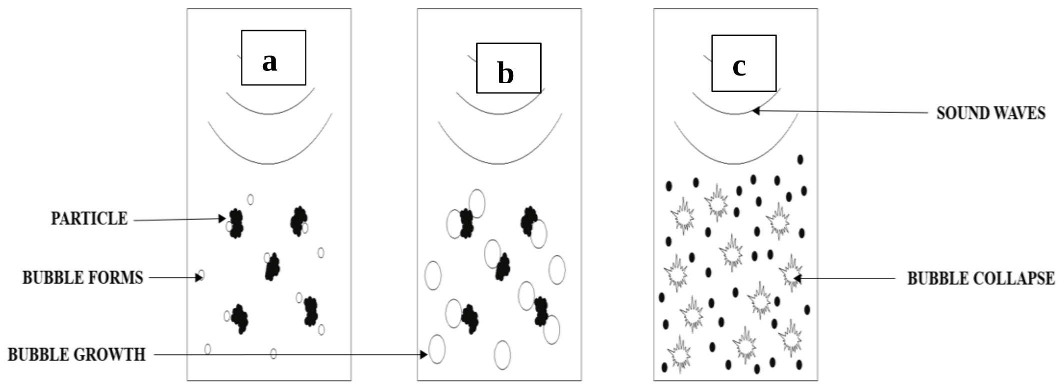


Fig. 3.2 Cavitation phenomenon

(a) Formation of bubbles in juice by sound waves

(b) Growth of bubbles to the maximum size and

(c) Collapse of bubbles, particle dispersion and cell disruption.

3.3.3 Thermosonication of Sweet Orange Juice

Thermosonication is a combined method of heat and ultrasound. Sonication uses sound waves to agitate particles in juices. Ultrasonic transducers produce vibrational energy which convert electrical energy into the physical vibration (sound energy) to break molecules. In this study initially bulk amount of juice was prepared to maintain the properties same throughout the thermosonication process.

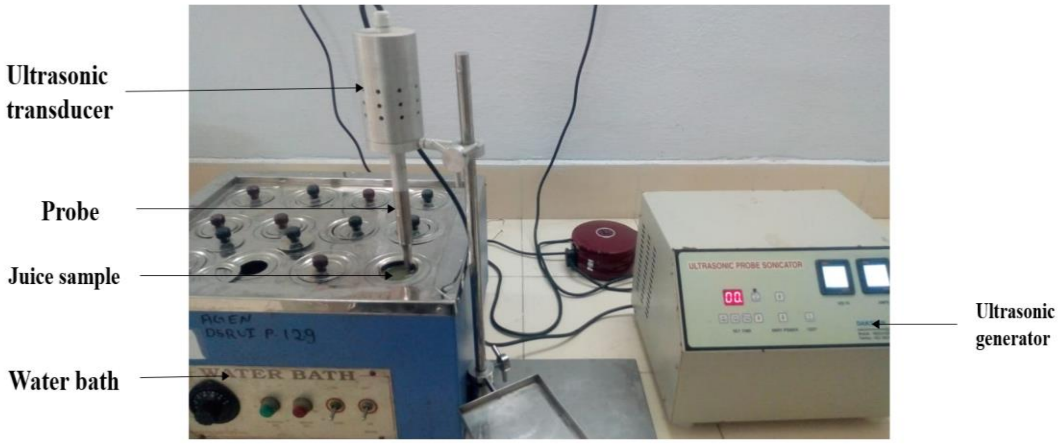


Plate 3.4 Thermosonication equipment

About 100 mL of juice was taken measured in a 100mL measuring cylinder. The beaker filled with juice was placed in a water bath (M.B. Instruments, Delhi) and the temperature of the juice was measured using thermometer. Specified amplitude and sonication time was set in sonicator (**plate 3.4**). After attaining the required temperature of the juice the probe was immersed in to the juice upto 3cm depth. The thermosonication process were carried out at constant frequency 20kHz with pulse durations of 5 s ON and 5 s OFF for all the experiments. After the treatments the treated samples were allowed to cool for further analysis (**plate 3.5**).

Plate 3.5 Control and Treated Samples

3.4 NUTRITIONAL ANALYSIS

The procedure for the estimation of some nutritional properties viz., pH, total soluble solids (TSS), titratable acidity, ascorbic acid, total sugar, polyphenol oxidase (PPO), peroxidase (POD) and microbial count are described in the following paragraphs.

3.4.1 Total Soluble Solids (TSS)

TSS is the amount of total soluble solids present in unit volume of solution. TSS is expressed in terms of ºBrix. Total soluble solids of the extracted juice were determined using Refractometer (Make: Atago, Model: PAL-1, Range: 0-53% ºBrix, Accuracy: ±0.2 °Brix). TSS of fresh sweet orange juice and thermosonicated (at 17 experimental conditions with 3 replications) sweet orange juice was measured. Aliquot of sample (~3 drops i.e., 0.3 mL) was applied on the refractometer prism, avoiding bubbles and large pulp particles. Upon pressing start button, instrument reads the TSS and value is displayed on digital display of instrument **(Plate 3.6**).

Plate 3.6 Refractometer

3.4.2 pH of Juice

pH is a numerical scale that is used to determine whether the material is acidic or basic. pH is defined as negative logarithm of H^+ ion (hydronium ion). The pH of sweet orange juice was measured using digital pH meter. Standardization of pH meter was done by using buffer solution of pH 7. To standardize pH meter, electrode was dipped in buffer solution and if pH meter reading was not exactly 7, knob was adjusted until it reads pH 7. After standardization, the electrode was removed from buffer solution and was dipped in beaker containing sweet orange juice. Reading shown by pH meter (**plate 3.7**) was recorded for 3 replications of 17 experimental conditions.

Plate 3.7 digital pH meter

3.4.3 Titrable Acidity

Titratable acidity of fresh and thermosonicated sweet orange juice was determined by AOAC 2005 method. 10 mL of juice sample was taken into 200 mL conical flask and then distilled water was added to make volume of 50 mL. This mixture was filtered by using whatman filter paper of grade 4. Then, 10 mL of aliquot was taken in 100 mL conical flask and 2 drops of phenolphthalein indicator was added. This aliquot was titrated against 0.1 N NaOH solution until it turned pink colour which persisted for 30 seconds. Difference in volume of NaOH solution before and after titration was taken as titrate value. Titrable acidity of control and thermosonicated sweet orange juice was calculated by using Eq (3.1) given below.

$$\text{Titrable acidity } (\%) = \frac{a \times b \times c \times d \times 100}{e \times w \times 100} \qquad \ldots(3.1)$$

where

a = Titrate value, mL

b = Normality of alkali

c = Volume made up, mL (50 mL)

d = Equivalent weight of citric acid

e = Aliquot, mL

w = Volume of the sample taken, mL

3.4.4 Ascorbic Acid

Ascorbic acid (vitamin-C) is an important indicator of degradation due to thermal processing. The amount of ascorbic acid present in sweet orange was determined by AOAC, 2000 (Method Number: 976.21). Solutions used in this method were 4% oxalic acid (4 g of oxalic acid was taken in a conical flask and the volume was made up to 100 mL), dye solution (52 mg of 2,6-diclorophenol-indophenol and 42 mg of sodium bicarbonate was dissolved in 200 mL of distilled water), stock standard solution (100 mL of 4% oxalic acid and 100 mg of ascorbic acid were dissolved) and working standard solution (added 10 mL of stock solution to 90 mL of 4% oxalic acid to make 100 mL of working standard solution). 10 mL of 4% oxalic acid was mixed with 5 mL of working standard in a conical flask and titrated against dye solution till it turned to pale pink and the volume of dye solution required to turn pink is noted as V_1. Then 2 mL of sample was added to 100 mL of 4% oxalic acid and filtered it using filter paper (Whatman grade 4). 5 mL of filtered solution was taken in to a conical flask and added 10 mL of 4% oxalic acid was added into conical flask. Stir the mixture and titrate it against dye until it turned to pale pink color that persisted for at least 30 s, and the reduced volume of dye is noted as V_2. The ascorbic acid content was calculated using Eq (3.2) given below.

$$\text{Ascorbic acid content } \left({}^{\text{mg}}/_{100}\,\text{mL}\right) = \frac{0.5\text{ mL} \times V_2 \times 100\text{ mL} \times 100}{V_1\text{ mL} \times 5\text{ mL} \times \text{Wt of the sample}} \quad \ldots(3.2)$$

where,

V_1 = Volume of dye required to turn working standard solution to pale pink color, mL

V_2 = Volume of dye required to turn sample solution to pale pink color, mL

3.4.5 Polyphenol Oxidase – (PPO)

PPO is a copper-containing enzyme and primarily causes browning in fruits and vegetables resulting in nutritional loss and less attractive appearance. It catalyses the oxidation of monophenolic compounds to O-diphenols and O-dihydroxy compounds to O-quinones which are the polymerized to dark-colored pigments. The activity of polyphenol oxidase was determined as described by the Zhang and Shao, 2015. Juice sample was centrifuged **(plate 3.8)** at 10,000 g at 4 °C for 10 min (**plate 3.8**). The standard reaction mixture containing 1.5 mL of 40 mmol/catechol and 2.3 mL of 0.1 mol/ phosphate buffer and 0.5 mL of juice sample was placed in a 10 mL test tube and mixed thoroughly. The absorbance of thermosonicated sample was measured at 420 nm with UV-spectrophotometer (**plate 3.9**) for every 1 min interval upto 10 min and the mean absorbance (A_t) of thermosonicated juice sample was calculated. Similar procedure was repeated for control juice sample and the mean absorbance was recorded (A_o). PPO residual activity was calculated using Eq (3.3).

$$\text{Residual activity (\%)} = \frac{A_t \text{ x } 100}{A_0} \quad \ldots(3.3)$$

where,

A_t = Enzyme activity of treated sample

A_0 = Enzyme activity of untreated sample

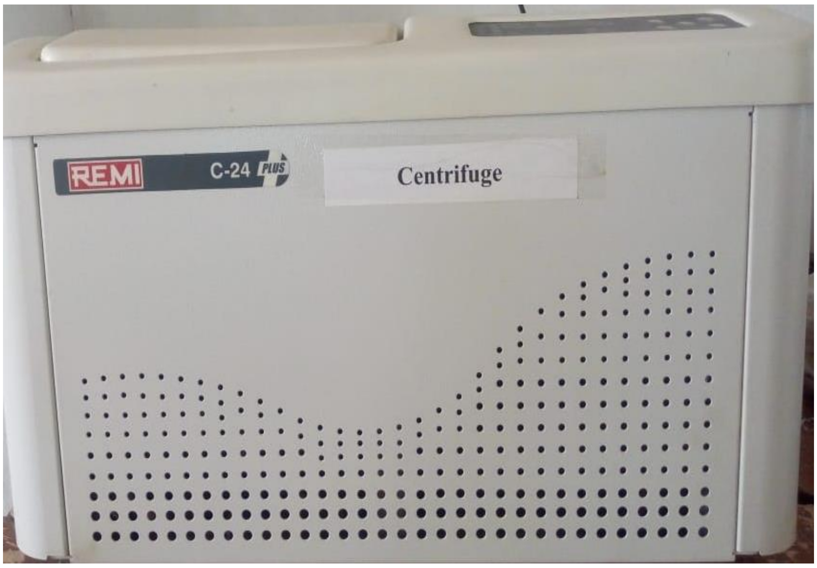


Plate 3.8 Centrifuge

3.4.6 Peroxidase - (POD)

The activity of peroxidase was determined as described by the Zhang and Shao, 2015. Peroxidase is another enzyme involved in enzymatic browning, as it may reduce diphenols and is also involved in the production of lignin. POD activity is limited by the absence of electron compounds such as superoxide radicals, hydrogen peroxide and lipid peroxide. For POD activity determination 19 µl guaiacol, 28 µl 30% H_2O_2**,** 0.5 mL of juice sample and 0.1 mol/phosphate buffer were added in a 10 mL test tube and mixed thoroughly. The absorbance of thermosonicated sample was measured at 470 nm with UV-spectrophotometer (**plate 3.9**) for every 30 s interval upto 2 min and the absorbance (A_t) of thermosonicated juice sample was calculated. Similar procedure was repeated for control juice sample and the mean absorbance was recorded as (A_o). PPO residual activity was calculated using Eq (3.3).

Plate 3.9 UV- visible spectrophotometer

3.4.7 Total Sugars

Total sugar was determined using Lane and Eynon method by titration (Ranganna, 1986). The principle involved is copper in the Fehling solution is reduced to red insoluble cuprous oxide in the presence of invert sugars like glucose and fructose. The quantity of sugar solution required for complete neutralization of a known quantity of Fehling mixture is determined by titration.

3.4.7.1 Reagents used:

- Fehling's mixture: Fehling No. A and B (5 mL each) should be mixed immediately before use of each sample.
- Neutral lead acetate (45%) solution: Dissolve 225 g neutral lead acetate in distilled water and make volume to 500 mL
- Potassium oxalate (22%) solution: Dissolve 110 g potassium oxalate in distilled water and make volume to 500 mL.
- 50% HCL
- Phenolphthalein indicator
- NaoH (40%) solution.

3.4.7.2 Method

A sample of 20 mL sweet orange juice was taken and the estimation of total sugars was carried out according to the Fig.3.2 and total sugars was calculated using Eq (3.4).

$$\text{Total sugars \%} = \frac{\text{Factor x dilution x 100}}{\text{Titre x Volume of the sample}} \quad \ldots(3.4)$$

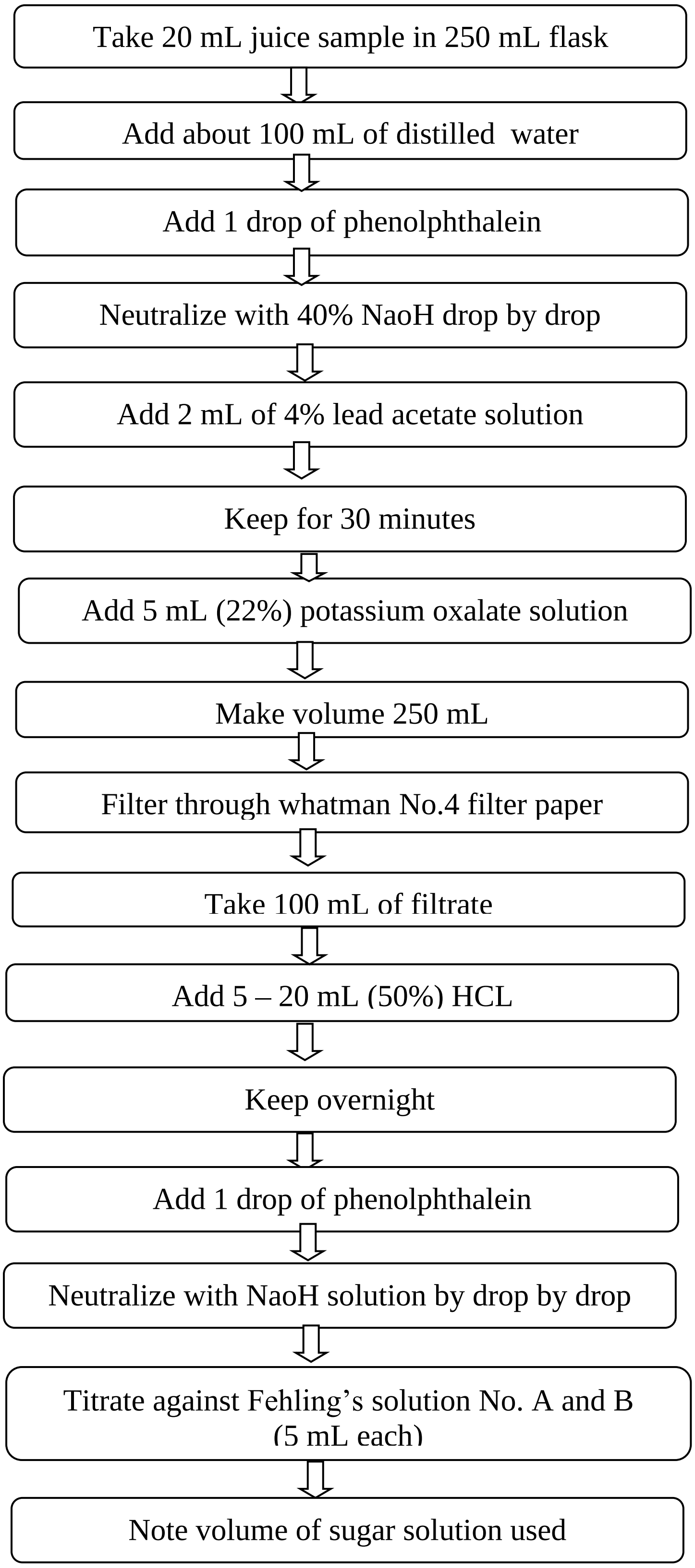


Fig. 3.3 Process flow chart for estimation of total sugars

3.4.8 Microbiological Analysis

Microbiological properties of fresh juice and treated juice samples were determined by total plate count and fungus count according to method described by AOAC (2005).

3.4.8.1 Material required:

Sterilized petri plates, Sterile water blank, Cotton swab, Nutrient agar, Martins Rose Bengal agar.

3.4.8.2 Procedure

Initially the petri plates, cotton swabs and water banks were cleaned with ethanol and sterilized using an autoclave **(plate 3.10)**. Media for both bacterial culture (Nutrient Agar Medium) and fungal culture (MRBA) were prepared according to standard procedure. After autoclaving 20 mL hot media was poured in each plate under sterile conditions and allowed to solidify. 1 mL of juice was diluted up to 10^{-4} serially by using 9 mL sterile water blanks. 1 mL of suspension of suitable dilution was inoculated into each petri plate after solidification and spreaded uniformly over the medium with a sterilized glass spreader and kept for incubation at 35 °C for 24 to 48 hours **(Plate 3.12)**. After 48 hours, plates were observed for growth and numbers of colonies (X) were counted by digital colony counter (**plate 3.13**). The number of colonies was calculated using Eq (3.5).

$$\text{Number of colonies} = X \text{ CFU} \times \text{dilution factor} \qquad \dots (3.5)$$

where X is number of colonies

Plate 3.10 Autoclave

Plate 3.11 Incubator

Plate 3.12 Digital colony counter

3.5 SENSORY EVALUATION OF THERMOSONICATED SWEET ORANGE JUICE

Sensory analysis plays a crucial role for accepting/rejecting of the product by consumers. The sensory analysis of the product was evaluated using quality attributes :texture, color, flavor, taste and overall acceptability. The prepared products at optimized conditions were subjected to sensory evaluation as specified by fuzzy comprehensive model (Das, 2005). Semi trained panelists were selected from the teachers and students of Dr. NTR College of Agricultural Engineering, Bapatla, Andra Pradesh, India in the age group from 18 to 55 years.They were educated to evaluate the colour & appearane, odour, sweetness, sourness and overall acceptability of the thermosonicated sweet orange juices. The scale factors viz., Not satisfactory (NS), Fair (FR), Medium (MD), Good (GD) and Excellent (EX) were assigned to the quality factors for five thermosonicated sweet orange juice samples **(Plate 3.14)**. These five samples were selected among thermosonicated juice samples at different conditions (temperatures, amplitude and time durations) based on their quality parameters (pH, TSS, ascorbic acid and titrable acidity).

3.5.1 Training of Judges and Evaluation Procedure

Twenty five panelists who are non-smokers and non-betel leaf chewers were chosen based on their health, ability to concentrate and learn (Ranganna, 1986). Before the actual sensory evaluation, panelists were educated with quality attributes of thermosonicated sweet orange juice. They were instructed to examine samples before tasting them and to rank them first on colour & appearane and odour. They were informed to wash their mouth with water between testing of each samples. After evaluating the sample, they were instructed to fill appropriate fuzzy scale factor for each of the qualitative attributes of the thermosonicated sweet orange juice. The fuzzy scale variables were awarded as Not satisfactory, Fair, Medium, Good and Excellent. The panelists were asked to rate the juice samples on a fuzzy scale based on their own criteria and preferences. The judge's weightages for the quality parameters of juice sample in general were also included on the score card. The sensory score card used for evaluation of thermosonicated sweet orange juice (Das, 2005) and juice qualities in general are given in **Appendix B**.

3.5.2 Fuzzy Comprehensive Model for Sensory Score

The Fuzzy model was developed by Chen *et al*. (1983) for sensory analysis. The scale factor for each of the quality attributes, such as Not satisfactory, Fair, Medium, Good and Excellent, is included in the evaluation set (Vf). The numerical values given to the sensory scale factors were used in the Fuzzy transformation (Tf) of the factor set (Uf) into the evaluation set (Vf): "Not satisfactory" = 0.1, "Fair" = 0.4, "Medium" = 0.7, "Good" = 0.9 and "Excellent" = 1. The sensory analysis consists of the following estimations.

1. Fuzzy membership function
2. Normalised Fuzzy membership function
3. Normalised Fuzzy membership function matrix
4. Judgement membership function matrix
5. Judgement subset
6. Quality-ranking subset
7. Ranking of the sample

3.5.3 Fuzzy Membership Function (FMF)

Fuzzy membership function (FMF) was estimated by adding each of the individual scale factor assigned to the quality attribute of the sample and dividing it by total number of panelists. An example, in experiment the total number of judges were ten and the total number of scale factor under "Excellent" given by the panelists for particular quality attribute, e.g., "color & appearance" of the first sample was 7, color & appearance will get the membership function as 7/10 = 0.7. Likewise, other quality attributes membership function was calculated as mentioned above.

3.5.4 Normalised Fuzzy Membership Function (NFMF)

Normalised fuzzy membership function was computed by multiplying each of above membership function (FMF) with allotted numerical value of the corresponding "scale factors". As an example, the NFMF formed for membership function under" Excellent" scale factor of the 'colour & appearance' quality attribute (the numerical value allotted to scale factor "Excellent" = 1) will be 1×0.7 = 0.7. Similarly, the NFMF for the other quality attributes were calculated.

3.5.5 Normalised Fuzzy Membership Function Matrix (NFMFM)

Elements of a normalised fuzzy membership function matrix were created by adding the NFMF of each individual scale factor of each product submitted for sensory evaluation. For example, if the normalised fuzzy membership functions (NFMF) for the "colour & appearance" of the first sample were 0.7, 0.6, 0.5, and 0 for the "Excellent," "Good," "Medium," "Fair," and "Not satisfactory," the NFMFM for the sample colour & appearance would be 0.7 + 0.45 + 0 + 0.03 + 0 = 2.4. Similarly, all of the normalised matrix's elements were generated and arranged in a matrix generally called the normalised fuzzy membership function matrix (NFMFM).

3.5.6 Judgement Membership Function Matrix (JMFM)

After forming the normalized fuzzy membership function matrix, the sum of the column values were divided by the greatest value of the column after producing a normalised fuzzy membership function matrix. The retrieved values were used to create the judgement membership function matrix's elements. This matrix was taken into account for determining the judgement rank of samples.

3.5.7 Judgment Subset (JS)

Judgment subset was obtained by average of numerical weightage given by the judges for each individual quality attributes.

3.5.8 Quality-Ranking Subset (QRS)

Finally, comparision was made between individual elements of the JMFM with the corresponding elements of the judgment subset (JS) and minimum of them was taken to form the quality – ranking subset.

3.5.9 Ranking of the Sample

After obtaining quality-ranking subset (QRS), highest was assigned to the sample which had the maximum value in the quality ranking subset. Then the quality attribute having highest value was ranked.

Plate 3.14 Sensory evaluation of thermosonicated sweet orange juice

3.6 SHELF LIFE STUDIES OF THERMOSONICATED SWEET ORANGE JUICE

Shelf life is an established time under a defined set of conditions during which a product is acceptable for use. The three largest variables affecting shelf life are: product characteristics, packaging material and storage conditions. Shelf life studies were carried out for the control and thermosonicated juice processed at optimised conditions sweet orange juice and quality parameters were evaluated during storage. Control and treated juice samples were filled in glass bottels and were sealed tightly placing the aluminium foil and capped with the help of corking machine. All the packed samples in glass bottles were kept for storage studies at ambient (28±2 °C) and refrigerent conditions (4 °C). The nutritional quality characteristics and microbial properties were analyzed for every 4 days interval upto 28 days.

3.7 STATISTICAL ANALYSIS:

Statistical analysis of all the experimental data was carried out by following standard procedures. Box-Behnken Design (BBD) was used and the effect of different independent variables on the dependent variables was analysed.

3.7.1 Optimization

Optimization of process parameters was carried out using Design Expert software version 13.0.6.0. The criteria followed for optmization is shown in **Table 3.4.**

Table 3.4 Criteria for optimization of process parameters

Parameter	**Goal**
Temperature	In range
Amplitude	In range
Time duration	In range
pH	Minimise
TSS(°Brix)	Maximise
Vitamin-C (mg/100 mL)	Maximise
Titrable acidity (%)	Maximise

CHAPTER-IV

RESULTS AND DISCUSSION

This chapter presents the results on the effect of thermosonication on various quality aspects of the sweet orange juice. This chapter also covers the sensory evaluation of thermosonicated sweet orange juice for its final acceptance and storage studies of both control and treated samples.

4.1 EFFECT OF THERMOSONICATION ON QUALITY PARAMETERS OF SWEET ORANGE JUICE

Quality parameters of thermosonicated sweet orange juice and fresh juice were measured according to the procedures described in chapter 3. Thermosonication of sweet orange juice was carried out at process temperatures of 40, 50 and 60 ºC with amplitude levels of 40, 70 and 100% for operating times of 5, 12.5 and 20 min. Thermosonication of sweet orange juice was carried out in triplication for 17 experimental conditions as shown in **Table 3.2**. The pH, TSS, ascorbic acid and titrable acidity values of sweet orange juice was measured after thermosonication (**Table 4.1)**.

Initial pH, TSS, vitamin-C and titrable acidity of fresh sweet orange juice used in the experiments were 3.8±0.00, 11.1±0.00 °Brix**,** 45.60±0.92 mg/100 mL and 0.98±0.03 %, respectively. The pH, TSS, vitamin-C and titrable acidity of the sweet orange juice after thermosonication varied from 3.7±0.10 to 4±0.10, 9.2±0.00 to 11±0.00 °Brix, 22.59±0.66 to 52.32±0.82 mg/100 mL and 0.98±0.01 to 1.44±0.05% depending on the temperature, amplitude and sonication times. There are no significant changes of pH, TSS found in the juice after thermosonication at different experimental conditions. Trend in the pH, TSS values of sweet orange juice are similar to the trend reported for grape fruit juice during thermosonication conducted by Aadil *et al.* (2015). It may also be concluded that there was no significant changes of pH and TSS of sweet orange juice after thermosonication.

Table 4.1 Average experimental values of pH, TSS, ascorbic acid and titrable acidity of control and thermosonicated sweet orange juice.

Run	Temperature (°C)	Amplitude (%)	Time duration (min)	pH	TSS (°Brix)	Vitamin-C mg/100 mL)	Titrable acidity (%)
Control	-	-	-	3.8±0.00	11.1±0.00	45.60±0.92	0.98±0.03
1	60	100	12.5	3.8±0.00	10.0±0.00	31.31±0.95	1.44±0.05
2	60	40	12.5	3.8±0.00	9.5±0.10	33.58±0.67	1.42±0.07
3	40	100	12.5	3.9±0.00	9.7±0.10	40.22±0.80	1.06±0.06
4	50	70	12.5	3.9±0.00	10.2±0.00	50.32±0.72	0.99±0.03
5	50	100	5.0	3.8±0.10	9.9±0.10	40.34±0.80	1.08±0.08
6	50	70	12.5	3.9±0.00	10.2±0.00	50.62±0.65	0.99±0.03
7	60	70	20.0	3.8±0.00	10.0±0.00	35.28±0.76	1.32±0.08
8	50	40	20.0	3.8±0.00	9.6±0.10	39.68±0.16	1.12±0.19
9	50	100	20.0	3.9±0.00	9.5±0.00	22.59±0.66	1.14±0.16
10	40	40	12.5	3.7±0.10	11.0±0.00	45.58±0.86	1.16±0.18
11	40	70	5.0	3.8±0.10	9.2±0.00	47.44±0.64	1.16±0.19
12	50	70	12.5	4.0±0.10	10.2±0.00	51.25±0.56	0.99±0.03
13	40	70	20.0	3.8±0.00	9.6±0.10	36.51±0.75	1.4±0.16
14	50	40	5.0	3.9±0.00	9.9±0.10	52.32±0.82	1.31±0.08
15	50	70	12.5	4.0±0.10	10.1±0.00	50.30±0.44	0.99±0.03
16	60	70	5.0	3.9±0.00	9.3±0.10	36.71±0.77	1.15±0.25
17	50	70	12.5	3.9±0.00	10.2±0.00	50.66±0.56	0.98±0.01

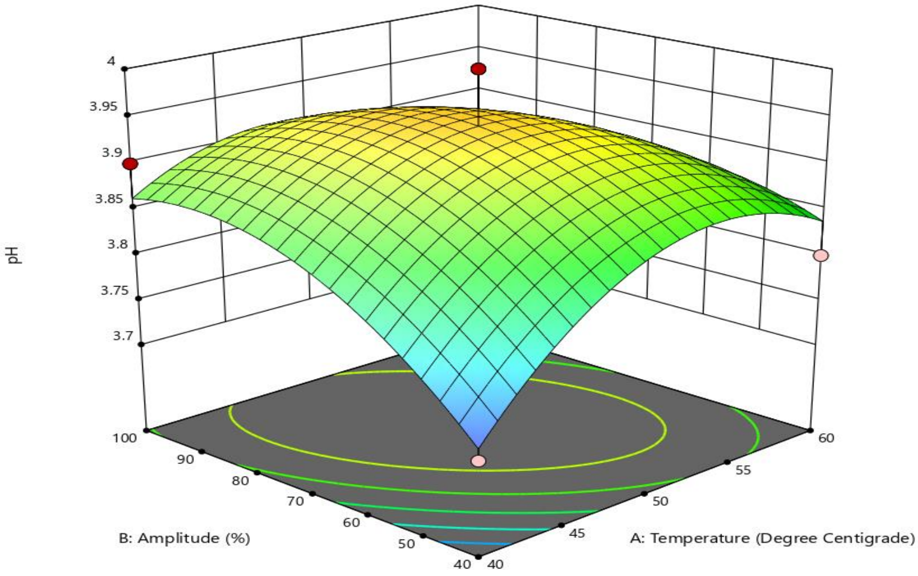
4.1.1 Effect of Thermosonication on pH of Sweet Orange Juice

pH values of thermosonicated sweet orange juice ranged from 3.7±0.10 to 4±0.10 for juice having temperature of 40-60 °C, sonication amplitude 40 – 100% and sonication time of 5–20 min. Average experimental values of pH during thermosonication of sweet orange juice determined at 17 experimental conditions were presented in **Table 4.1**. Higher pH value of 4±0.10 was obtained for juice treated temperature of 50 °C, amplitude 70% and sonication time of 12.5 min. Lower pH value of 3.7±0.10 was obtained for juice treated at temperature of 40 °C, amplitude 40% and sonication time of 12.5 min.

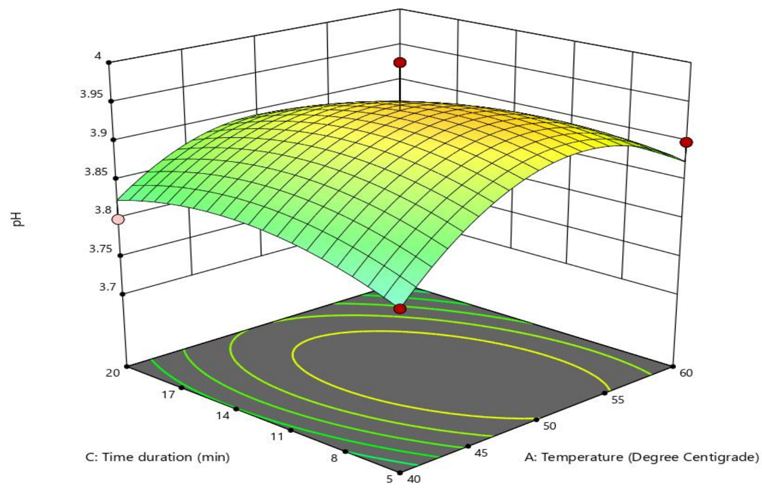
Analysis of variance (ANOVA) was used to assess the variation of dependent process parameter (pH) with the response variables (temperature, amplitude and time duration). ANOVA for pH is presented in **Appendix A (Table 1).** The result of quadratic model indicated that the model was not significant. The model F-value obtained for pH parameter was 3.26 implies the model was not significant.

In present study A^2 is significant model term ($p<0.05$) for pH of sweet orange juice. P-values greater than 0.1000 indicate the model terms are not significant. The lack of fit value for pH parameter has F-value of 0.83 and P-value was >0.0001, this implies that lack of fit is not significant relative to the pure error for pH parameter of sweet orange juice. The regression coefficients for pH of sweet orange juice were presented in **Appendix A (Table 1).**

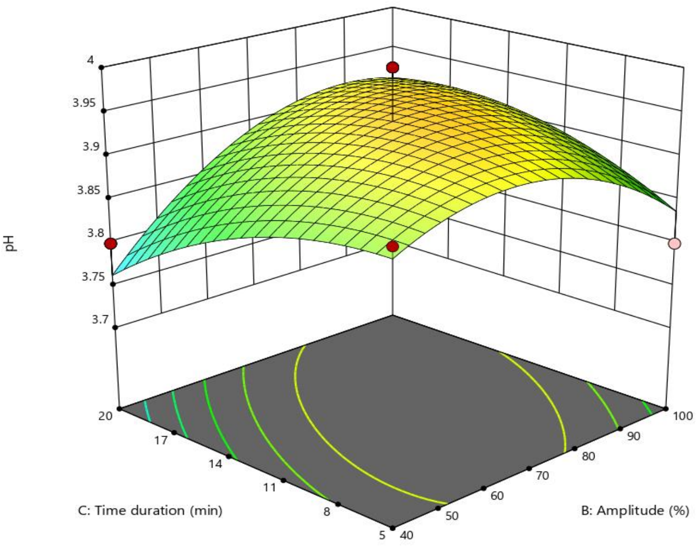
Response surface plots of pH of sweet orange juice in relation to the temperature, amplitude and time duration were presented in **Fig. 4.1**. Based on the data presented in **Table 4.1,** it is evident that application of thermosonication does not have any significant effect on pH of sweet orange juice. The data revealed that juice did not showed any significant change even after sonication up to 60 °C. This may be due to the energy level (ultrasound intensity, time duration and temperature) applied to the samples through thermosonication does not change molecular structures of high molecular weight associated with the pH (Ordonez-santos *et al.,* 2017). These results are in collaboration with Saeeduddin *et al*. (2015) in pear juice, Raju and Deka (2018) in blood fruit juice, Cao *et al.* (2019) in barberry juice and Tiwari *et al.* (2009) in orange juice.


pH
4
3.95
3.9
3.85
3.8
3.75
3.7
100
90
80
70
60
50
40
B: Amplitude (%)
40
45
50
55
60
A: Temperature (Degree Centigrade)

(a)


pH
4
3.95
3.9
3.85
3.8
3.75
3.7
20
17
14
11
8
5
C: Time duration (min)
40
45
50
55
60
A: Temperature (Degree Centigrade)

(b)



(c)

Fig. 4.1 Effect of process parameters on pH of sweet orange juice during thermosonication

(a) pH of sweet orange juice as a function of amplitude and temperature

(b) pH of sweet orange juice as a function of time duration and temperature

(c) pH of sweet orange juice as a function of time duration and amplitude

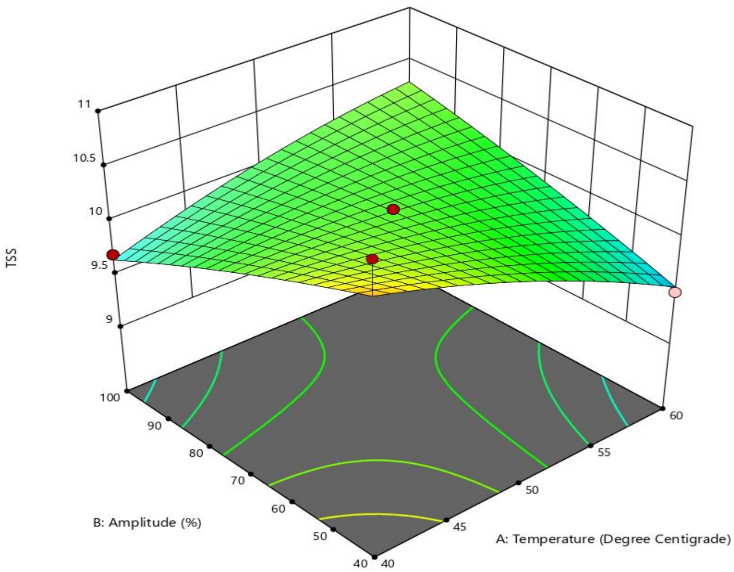
4.1.2 Effect of Thermosonication on TSS of Sweet Orange Juice

TSS values of thermosonicated sweet orange juice ranged from 9.2±0.00 to 11±0.00 for juice thermosonicated temperature of 40-60 °C, sonication amplitude of 40–100% and sonication time of 5–20 min. Average experimental values of TSS during thermosonication of sweet orange juice determined at 17 experimental conditions were presented in **Table 4.1.** Higher TSS value of 11±0.00 was obtained for juice treated at temperature of 40 °C, amplitude 40% and sonication time of 12.5 min. Lower TSS value of 9.2±0.00 was obtained for juice treated at temperature of 40 °C, amplitude 70% and sonication time of 5 min.

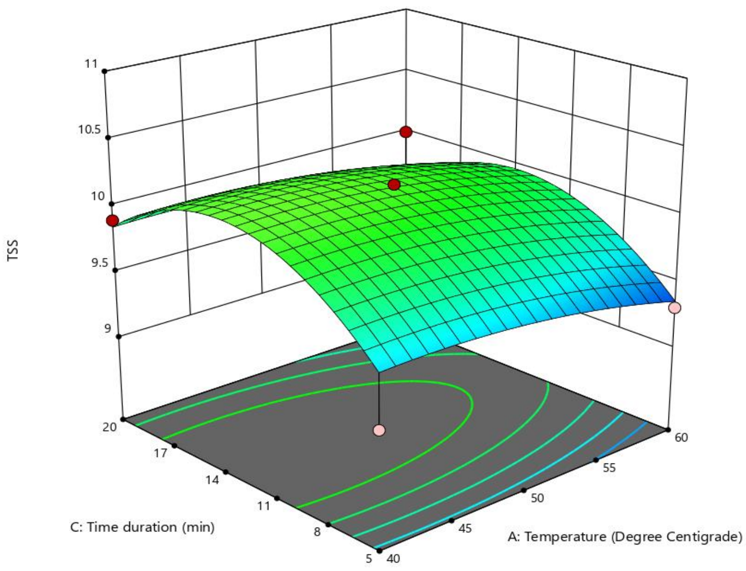
Analysis of variance (ANOVA) was used to assess the variation of dependent process parameter (TSS) with the response variables (temperature, amplitude and time duration). ANOVA for TSS is presented in **Appendix A (Table 2).** The result of quadratic model indicated that the model was not significant. The model F-value obtained for TSS parameter was 2.22 implies the model was not significant.

In present study AB and C^2 are significant model term ($p<0.05$) for TSS of sweet orange juice. P-values greater than 0.1000 indicate that the model terms are not significant. The lack of fit value for TSS parameter has F-value of 129.17and P-value >0.0001, this implies that lack of fit is not significant relative to the pure error for TSS parameter of sweet orange juice. The regression coefficients for TSS of sweet orange juice were presented in **Appendix A (Table 2)**.

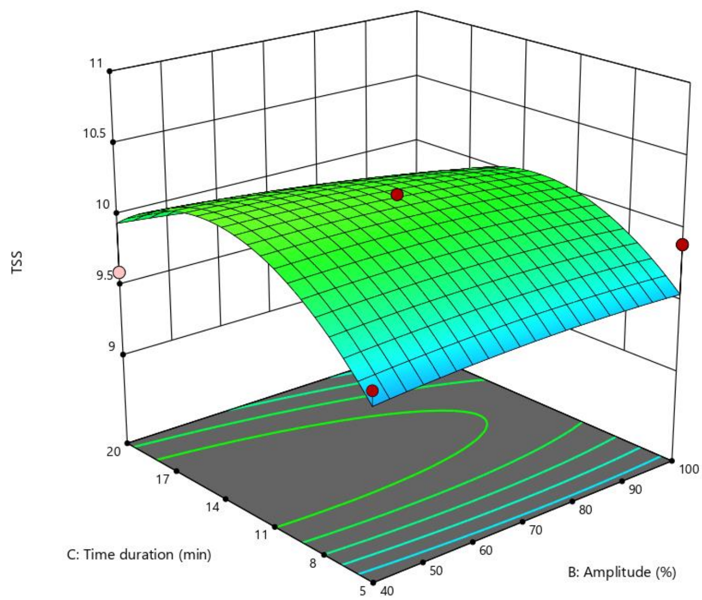
Response surface plots of TSS of sweet orange juice in relation to the temperature, amplitude and time duration were presented in **Fig. 4.2**. Based on the data presented in **Table 4.1** it is evident that application of thermosonication does not have any significant effect on TSS of sweet orange juice. The data revealed that juice did not showed any significant change even after sonication up to 60 °C. This may be due to the energy level applied to the samples through thermosonication did not alter molecular structure of high molecular weight associated with TSS (Ordonez-santos *et al.*, 2017). These results are in collaboration with Saeeduddin *et al*. (2015) in pear juice, Raju and Deka (2018) in blood fruit juice, Cao *et al.* (2019) in barberry juice and Tiwari *et al.* (2009) in orange juice.



(a)



(b)



(c)

Fig. 4.2 Effect of process parameters on TSS of sweet orange juice during thermosonication

(a) TSS of sweet orange juice as a function of amplitude and temperature

(b) TSS of sweet orange juice as a function of time duration and temperature

(c) TSS of sweet orange juice as a function of time duration and amplitude

4.1.3 Effect of Thermosonication on Ascorbic Acid of Sweet Orange Juice

Ascorbic acid values of thermosonicated sweet orange juice ranged from 22.59±0.66 to 52.32±0.82 mg/100 mL for juice thermosonicated at having temperature of 40-60 °C, sonication amplitude of 40 – 100% and sonication time of 5–20 min. Average experimental values of vitamin-C during thermosonication of sweet orange juice determined at 17 experimental conditions were presented in **Table 4.1**. Higher vitamin-C value of 52.429±0.82 mg/100 mL was obtained for juice treated at temperature of 50 °C, amplitude 70% and sonication time of 12.5 min. Lower vitamin-C value of 22.807±0.66 mg/100 mL was obtained for juice treated at temperature of 50 °C, amplitude 100% and sonication time of 20 min.

Analysis of variance (ANOVA) was used to assess the variation of dependent process parameter (vitamin-C) with the response variables (temperature, amplitude and time duration). ANOVA for vitamin-C is presented in **Appendix A (Table 3).** The result of quadratic model indicated that the model was significant. The model F-value obtained for vitamin-C parameter was 7.76 implies the model is significant. This value indicate that model was significant for Vitamin-C.

In present study A, B, C, A^2, B^2 and C^2 are significant model term ($p<0.05$) for vitamin-C of sweet orange juice. P-values greater than 0.1000 indicate the model terms are not significant. The lack of fit value for vitamin-C parameter has F-value of 78.08, this implies that lack of fit is significant relative for vitamin-C parameter of sweet orange juice. The regression coefficients for vitamin-C of sweet orange juice were presented **Appendix A (Table 3).** Coefficient of regression (**R^2**) for ascorbic acid was 0.9088. A negative predicted **R^2** (-0.4363) implies that the overall mean may be a better predictor of the response than the current model. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 7.593 indicates an adequate signal.

Regression equation representing the variation of vitamin-C with different independent parameters is shown in Eq (4.1).

$$\text{Ascorbic acid (mg/100 mL)} = -100.55155+5.54860A+0.834236B+552927C+0.002408AB+0.029237AC-005507BC-0.064943A^2-0.007413B^2-0.093446C^2. \quad \ldots (4.1)$$

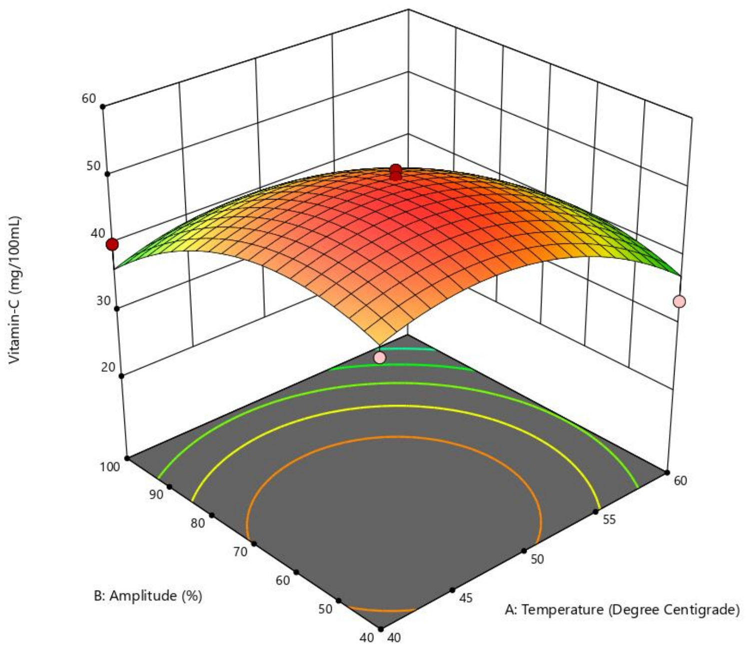
Where,

A = Temperature, °C

B = Amplitude, %

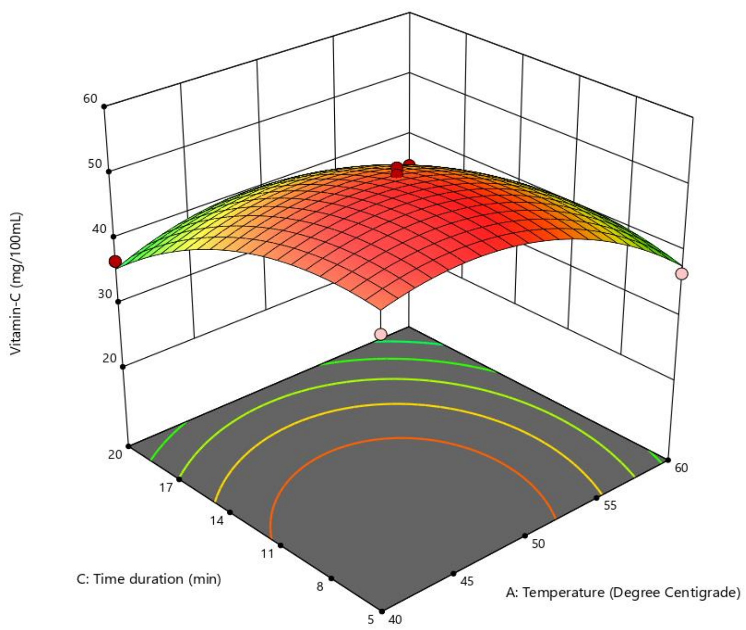
C = Sonication time, min.

Response surface plots of vitamin-C of sweet orange juice in relation to the temperature, amplitude and time duration were presented in **Fig. 4.3.** Results showed that a significant increase in ascorbic acid was observed during thermosonication but at high temperature, amplitude and time duration a rapid decrease of ascorbic acid was recorded. The ascorbic content of thermosonicated sweet orange juice increased with increase in temperature (40-50 °C) at lower amplitudes and sonication time duration. The increase in ascorbic acid content may be due to elimination of dissolved oxygen during cavitation which is essential for ascorbic acid degradation. Similar observations were made by Cheng *et al.,* 2007 during sonication of guava juice. The increase in ascorbic acid in ultrasound processed juice could be due to the mechanical rupture of the cell wall, which results in more ascorbic acid release into the juice (Wang *et al*., 2019). At higher treatment temperatures (60 °C), the ascorbic acid content decreased irrespective of amplitude and sonication time durations. This may be due to chemical decomposition of ascorbic acid at higher temperatures. Abid *et al*. (2014) reported that at lower ultrasound treatment ascorbic acid content of apple juice increased with increase of temperature and reduction in ascorbic content was observed at high temperatures. Similar trend was observed by Riberio *et al*. (2009) in orange juice. Ascorbic acid degradation is influenced more by the oxygen concentration than the temperature (Aguilar *et al.*, 2017). Martinez *et al.*, 2015 reported that at mild temperatures sonication helps to preserve some important nutritional components in the product. The increase in the ascorbic acid is due to the removal of dissolved oxygen from the juice (Aadil *et al*., 2013, Cheng *et al*., 2007). Saeeduddin *et al.*, (2015) concluded that ultrasonication degasses any liquid and the treatment favours retention of vitamin-C in juice due to the removal of dissolved oxygen responsible for vitamin-C degradation. Adekunte *et al.,* (2010) reported that degradation can also be related to oxidation reaction promoted by the interaction of hydroxyl radicals produced by cavitation during sonication.



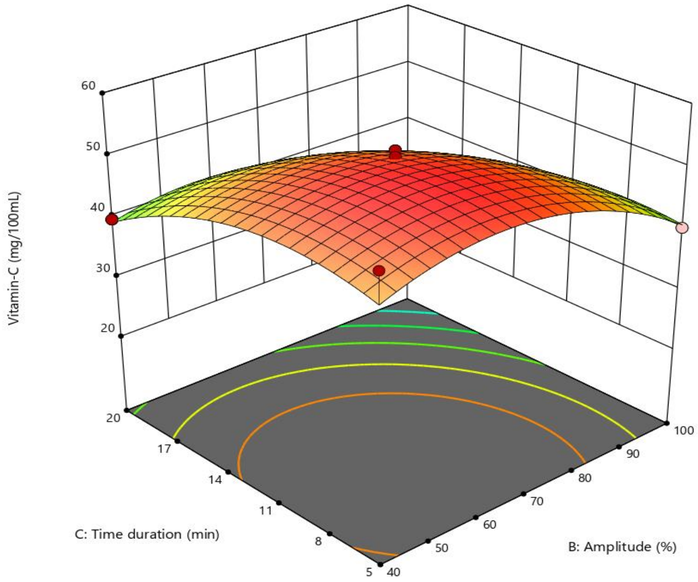
Vitamin-C (mg/100mL)
60
50
40
30
20
100
90
80
70
60
50
40
40
45
50
55
60
B: Amplitude (%)
A: Temperature (Degree Centigrade)

``(a)



Vitamin-C (mg/100mL)
60
50
40
30
20
20
17
14
11
8
5
40
45
50
55
60
C: Time duration (min)
A: Temperature (Degree Centigrade)

(b)



(c)

Fig. 4.3 Effect of process parameters on ascorbic acid of sweet orange juice during thermosonication

(a) Ascorbic acid of sweet orange juice as a function of amplitude and temperature

(b) Ascorbic acid of sweet orange juice as a function of time duration and temperature

(c) Ascorbic acid of sweet orange juice as a function of time duration and amplitude

4.1.4 Effect of Thermosonication on Titrable Acidity of Sweet Orange Juice

Titrable acidity values of thermosonicated sweet orange juice ranged from 0.98±0.01 to 1.435±0.05% for juice thermosonicated at temperature of 40-60 °C, sonication amplitude of 40–100% and sonication time of 5–20 min. Average experimental values of titrable acidity during thermosonication of sweet orange juice determined at 17 experimental conditions are presented in **Table 4.1**. Higher titrable acidity value of 1.435±0.05% was obtained for juice treated at temperature of 60 °C, amplitude 100% and sonication time of 12.5 min. Lower titrable acidity value of 0.98±0.01% was obtained for juice treated at temperature of 50 °C, amplitude 70% and sonication time of 12.5 min.

Analysis of variance (ANOVA) was used to assess the variation of dependent process parameter (titrable acidity) with the response variables (temperature, amplitude and time duration). ANOVA for titrable acidity is presented in **Appendix A (Table 4).** The result of quadratic model indicated that the model was significant. The model F-value obtained for titrable acidity parameter was 5.70 implies the model is significant for titrable acidity.

The model terms A, A^2 and B^2 are found significant model term ($p<0.05$) for titrable acidity of sweet orange juice. P-values greater than 0.1000 indicate the model terms are not significant. The lack of fit value for titrable acidity parameter has F-value of 668.68 this implies that lack of fit is significant relative for titrable acidity parameter of sweet orange juice. The regression coefficients for titrable acidity of sweet orange juice were presented in **Appendix A (Table 4).** Coefficient of regression ($\mathbf{R^2}$) for titrable acidity was 0.8799. A negative predicted $\mathbf{R^2}$ (-0.9186) implies that the overall mean may be a better predictor of the response than the current model. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 6.653 indicates an adequate signal.

Regression equation representing the variation of titrable acidity with different independent parameters is shown in Eq (4.2).

$$\text{Titrable acidity (\%)} = 5.97144-0.152696A-0.027923B-0.063600C +0.000081AB+0.000560AC+000249BC +0.001502A^2+0.000140B^2+0.000736C^2. \quad \dots (4.2)$$

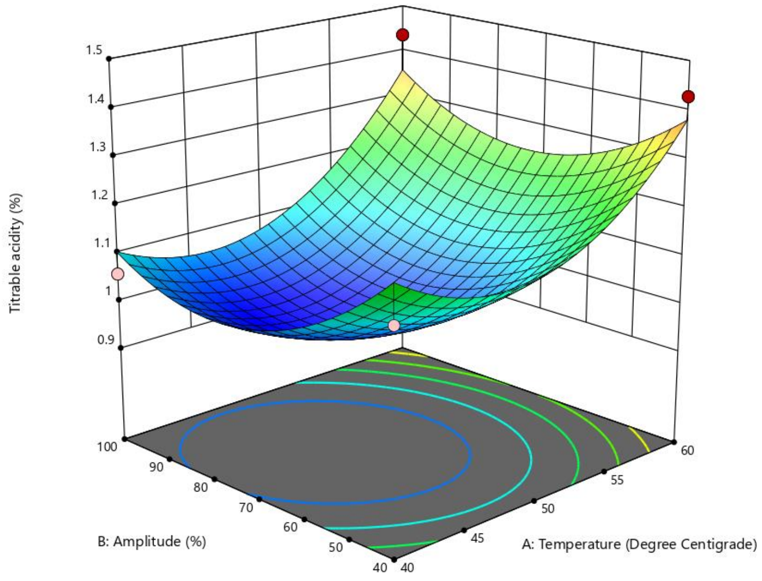
Where,

A = Temperature, °C

B = Amplitude, %

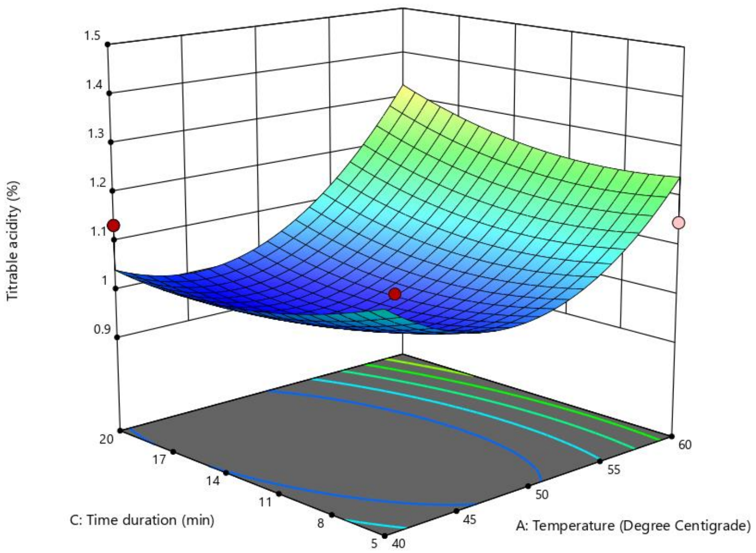
C = Sonication time, min.

Response surface plots for titrable acidity were presented in **Fig. 4.4**. Titrable acidity of sweet orange juice in relation to the temperature, amplitude and time duration were presented in **Fig. 4.4.** Results showed that a significant increase of titrable acidity was observed during thermosonication. The increase of titrable acidity of the treated samples may be due to the possible release of galacturonic acid from the pectin structure due to cavitation phenomenon. Similar results were also reported earlier in the research studies of Bora *et al*., 2017 and Alves *et al.,* 2020 for tangerine juice.



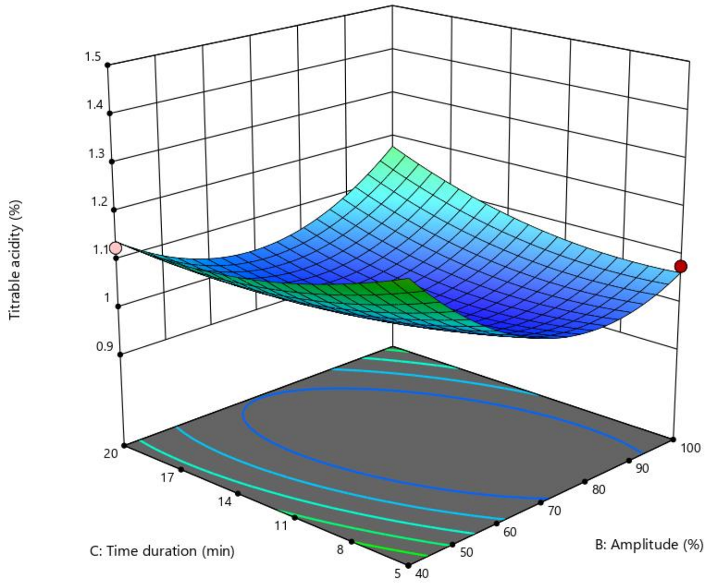
Titrable acidity (%)
B: Amplitude (%)
A: Temperature (Degree Centigrade)

(a)



Titrable acidity (%)
C: Time duration (min)
A: Temperature (Degree Centigrade)

(b)



(c)

Fig. 4.4 Response surface plots showing effect of process parameters on titrable acidity of sweet orange juice during thermosonication

(a) Titrable acidity of sweet orange juice as a function of amplitude and temperature

(b) Titrable acidity of sweet orange juice as a function of time duration and temperature

(c) Titrable acidity of sweet orange juice as a function of time duration and amplitude

4.2 SENSORY EVALUATION OF CONTROL AND THERMO-SONICATED SWEET ORANGE JUICE USING FUZZY LOGIC CONCEPT

The sensory scores of thermosonicated sweet orange juice were analysed as described in **Section 3.5**. The sensory and quality attribute terms namely, colour & appearance, odour, sweetness, sourness and overall acceptability for 5 samples are presented in **Table 4.2**. In sensory evaluation, T_1(TS:40 °C, 40% amplitude and 12.5 min duration), T_2(TS:40 °C, 70% amplitude and 5 min duration), T_3(TS:50 °C, 40% amplitude and 5 min duration), T_4 (TS:50 °C, 70% amplitude and 12.5 min duration) were thermosonicated juice samples and T_5 was control sample. These samples were selected based on their quality parameters. The sample T_1 secured highest score for colour & appearance, and T_5 sample scored highest for odour, sweetness, sourness and overall acceptability as shown in **Table.4.4.** fuzzy membership function (FMF), normalised fuzzy membership function (NFMF) and judgement membership function (JMF) were calculated as explained in **Section 3.5**. The obtained results of NFMF and JMF values were shown in **Table 4.3 and 4.4.**

The results of the judgement membership function were compared with panellist's average weightage for each of the quality attribute. The weightage average values for each of the quality parameters were determined and are shown in **Table 4.5**.

The average weightage values for colour & appearance, odour, sweetness and sourness were observed as 0.204, 0.234, 0.270 and 0.139, respectively (**Table 4.6**). Highest JMF values of 0.270 was observed for sweetness and lowest JMF value of 0.139 was observed for sourness. Therefore, sweet orange juice with more sweetness and sourness is most likely preferred by the panelists. The preference order of quality attributes for juice samples by the panelists according to JMF values is as follows sweetness >odour> colour & appearance> sourness. The quality ranking subset value was determined by comparing the average value of quality aspects and the judgement membership function formed. All the quality ranking subset (QRS) values were allotted to all the samples for all quality attributes and presented in **Table 4.7**.

Ranking of the samples was carried out based on the quality ranking subset value (QRS) of the samples. For sample T_1, the highest value (0.203) of the quality ranking subset values (0.203, 0.184, 0.163, 0.179, and 0.186) was observed for colour & appearence. Similarly, quality-ranking values for colour & appearance T_2, T_3, T_4, and T_5 were 0.199, 0.200, 0.195, and 0.204 respectively.

It was observed that fresh juice (T_5) got better quality rankings followed by T_2. The quality ranking order for juice samples is as follows T_5 (control)> T_2 (TS:40 °C, 70% amp and 5 min duration)> T_1 (TS:40 °C, 40% amp and 12.5 min duration)> T_3 (TS:50 °C, 40% amp and 5 min duration)> T_4 (TS:50 °C, 70% amp and 12.5 min duration).

Table 4.2 Scale factor, fuzzy membership function (FMF) and normalised membership function (NFMF) for quality attributes of thermosonicated sweet orange juice

Row No.	Quality Attributes	Scale Factor	T_1	T_1 FMF	T_1 NFMF	T_2	T_2 FMF	T_2 NFMF	T_3	T_3 FMF	T_3 NFMF	T_4	T_4 FMF	T_4 NFMF	T_5	T_5 FMF	T_5 NFMF
1	Colour & Appearance	EX	7	0.28	0.28	9	0.36	0.36	12	0.48	0.48	5	0.2	0.2	9	0.36	0.36
2		GD	15	0.6	0.54	10	0.4	0.36	9	0.36	0.324	15	0.6	0.54	11	0.44	0.396
3		MD	2	0.08	0.056	5	0.2	0.14	2	0.08	0.056	4	0.16	0.112	5	0.2	0.14
4		FR	1	0.04	0.016	1	0.04	0.016	1	0.04	0.016	0	0	0	0	0	0
5		NS	0	0	0	0	0	0	1	0.04	0.004	1	0.04	0.004	0	0	0
6		**TOTAL**	**25**		**0.892**	**25**		**0.876**	**25**		**0.88**	**25**		**0.856**	**25**		**0.896**
8	Odour	EX	3	0.12	0.12	8	0.32	0.32	4	0.16	0.16	0	0	0	8	0.32	0.32
9		GD	15	0.6	0.54	11	0.44	0.396	11	0.44	0.396	13	0.52	0.468	12	0.48	0.432
10		MD	4	0.16	0.112	5	0.2	0.14	5	0.2	0.14	8	0.32	0.224	5	0.2	0.14
11		FR	2	0.08	0.032	1	0.04	0.016	4	0.16	0.064	0	0	0	0	0	0
12		NS	1	0.04	0.004	0	0	0	1	0.04	0.004	4	0.16	0.016	0	0	0
13		**TOTAL**	**25**		**0.808**	**25**		**0.872**	**25**		**0.764**	**25**		**0.708**	**25**		**0.892**
15	Sweetness	EX	5	0.2	0.2	8	0.32	0.32	3	0.12	0.12	1	0.04	0.04	9	0.36	0.36
16		GD	5	0.2	0.18	9	0.36	0.324	6	0.24	0.216	8	0.32	0.288	9	0.36	0.324
17		MD	9	0.36	0.252	4	0.16	0.112	10	0.4	0.28	9	0.36	0.252	6	0.24	0.168
18		FR	5	0.2	0.08	4	0.16	0.064	6	0.24	0.096	4	0.16	0.0640	1	0.04	0.016
19		NS	1	0.04	0.004	0	0	0	0	0	0	3	0.12	0.012	0	0	0
20		**TOTAL**	**25**		**0.716**	**25**		**0.82**	**25**		**0.712**	**25**		**0.656**	**25**		**0.868**
22	Sourness	EX	4	0.16	0.16	3	0.12	0.12	1	0.04	0.04	0	0	0	4	0.16	0.16
23		GD	11	0.44	0.396	10	0.4	0.36	7	0.28	0.252	9	0.36	0.324	16	0.64	0.576
24		MD	7	0.28	0.196	7	0.28	0.196	14	0.56	0.392	7	0.28	0.196	4	0.16	0112
25		FR	2	0.08	0.032	4	0.16	0.064	3	0.12	0.048	6	0.24	0.096	1	0.04	0.016
26		NS	1	0.04	0.004	1	0.04	0.004	0	0	0	3	0.12	0.012	0	0	0
27		**TOTAL**	**25**		**0.788**	**25**		**0.744**	**25**		**0.732**	**25**		**0.628**	**25**		**0.864**
31	Overall Acceptance	EX	6	0.24	0.24	10	0.4	0.4	4	0.16	0.16	1	0.04	0.04	4	0.16	0.16
32		GD	13	0.52	0.468	9	0.36	0.324	10	0.4	0.36	10	0.4	0.36	16	0.64	0.576

33	MD	2	0.08	0.056	5	0.2	0.14	7	0.28	0.196	6	0.24	0.168	4	0.16	0.112
34	FR	3	0.12	0.048	1	0.04	0.016	3	0.12	0.048	4	0.16	0.064	1	0.04	0.016
35	NS	1	0.04	0.004	0	0	0	1	0.04	0.004	4	0.16	0.016	0	0	0
36	**TOTAL**	**25**		**0.816**	**25**		**0.88**	**25**		**0.768**	**25**		**0.648**	**25**		**0.864**

Table 4.3 Normalised membership function (NFMF) for quality attributes of thermosonicated sweet orange juice

Quality Attribute	T_1	T_2	T_3	T_4	T_5
Colour &Appearance	0.892	0.876	0.88	0.856	0.896
Odour	0.808	0.872	0.764	0.708	0.892
Sweetness	0.716	0.82	0.712	0.656	0.868
Sourness	0.788	0.744	0.732	0.628	0.864
Overall acceptance	0.816	0.88	0.768	0.648	0.864
Total NFMF	**4.02**	**4.192**	**3.856**	**3.496**	**4.384**

Table 4.4 Judgment membership functions (JMF) for quality attributes of thermosonicated sweet orange juice

Quality Attribute	T_1	T_2	T_3	T_4	T_5
Colour &Appearance	0.203	0.199	0.200	0.195	0.204
Odour	0.184	0.198	0.174	0.161	0.203
Sweetness	0.163	0.187	0.162	0.149	0.197
Sourness	0.179	0.169	0.166	0.143	0.197
Overall acceptance	0.186	0.200	0.175	0.147	0.197

Table 4.5 Scale factor, fuzzy membership function (FMF) and normalised membership function (NFMF) for quality attributes of thermosonicated sweet orange juice

Row No.	Quality Attribute	Scale factor	S_1	FMF	NFMF
1		EX IMP	3	0.12	0.012
2		H IMP	3	0.12	0.024
3		IMP	12	0.48	0.336
4		S IMP	3	0.12	0.108
5	Colour & Appearance	N IMP	4	0.16	0.16
6		TOTAL	25		**0.64**
7		EX IMP	0	0	0
8		H IMP	4	0.16	0.032
9		IMP	8	0.32	0.224
10		S IMP	10	0.4	0.36
11	Odour	N IMP	3	0.12	0.12
12		TOTAL	25		**0.736**
13		EX IMP	0	0	0
14		H IMP	0	0	0
15		IMP	10	0.4	0.28
16		S IMP	8	0.32	0.288
17	Sweetness	N IMP	7	0.28	0.28
18		TOTAL	25		**0.848**
19		EX IMP	4	0.16	0.016
20		H IMP	10	0.4	0.08
21		IMP	7	0.28	0.196
22		S IMP	4	0.16	0.144
23	Sourness	N IMP	0	0	0
24		TOTAL	25		**0.436**

Note: EX IMP: Extremely important, H IMP: Highly important, IMP: Important, S IMP: Slightly important, and N IMP: Not important

Table 4.6 Judgment membership functions (JMF) of thermosonicated sweet orange Juice

Quality Attribute	S_1	JMF
Colour & Appearance	0.64	0.204
Odour	0.736	0.234
Sweetness	0.848	0.270
Sourness	0.436	0.139

Table 4.7 Quality ranking subset values of thermosonicated sweet orange juice

Quality Attribute	Average weightage (JMF)	T_1	T_2	T_3	T_4	T_5
Colour & Appearance	0.204	0.203	0.199	0.200	0.195	0.204
Odour	0.234	0.184	0.198	0.174	0.161	0.203
Sweetness	0.270	0.163	0.187	0.162	0.149	0.197
Sourness	0.139	0.179	0.169	0.166	0.143	0.197
Overall acceptance	0.150	0.186	0.200	0.175	0.147	0.197
Quality Ranking		**III**	**II**	**IV**	**V**	**I**

4.3 OPTIMIZATION OF PROCESS PARAMETERS OF THERMOSONICATED SWEET ORANGE JUICE

The sweet orange juice was thermosonicated and the effect of different process parameters on the quality characteristics of the thermosonicated sweet orange juice were studied. Optimization of the thermosonication process parameters was done using the three independent variables *viz.*, temperature (40, 50 and 60 °C), amplitude (40, 70 and 100%), and sonication time (5, 12.5 and 20 min). The optimization was done using the Box-Behnken experimental design (Response surface methodology) in Design Expert software. Desirability ranges were fixed from zero to one for any given response. Zero represents that more than one response fall outside desirable limits and a value of one indicates the ideal case (Maran *et al.*, 2013).

From the desirability analysis, the optimal level of various parameters were found. The optimal processing conditions for the thermosonicated sweet orange juice were found as temperature of 40 °C, amplitude of 40% and sonication time of 9.29 min. The desirability of the optimization of thermosonicated sweet orange juice was found to be 0.785.

4.4 QUALITY PARAMETRS OF OPTIMALLY THERMOSONICATED SWEET ORANGE JUICE

Optimum processing conditions based on the Box Behnken design of Response Surface Methodology (RSM), for the thermosonication of sweet orange juice were obtained at temperature of 40 °C, amplitude of 40% and thermosonication time of 9.29 min. Sweet orange juice was thermosonicated at optimized conditions and the quality parameters of optimally thermosonicated sweet orange juice are tabulated in **Table 4.8.** The pH and TSS were found to be 3.8 and 11°Brix. The vitamin-C and titrable acidity values of the juice were 49.122 mg/100 g and 1.12%, respectively. The pH, TSS, ascorbic acid and titrable acidity of sweet orange juice thermosonicated at optimum conditions were close to that of responses obtained by optimization.

Table 4.8 Quality parameters of optimally thermosonicated sweet orange juice

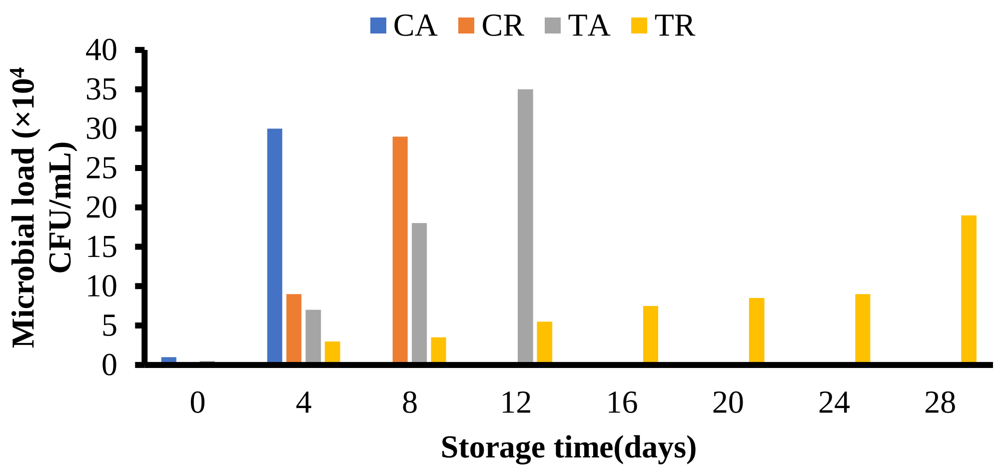
Quality parameter	Optimized value	Measured value
pH	3.723	3.8
TSS(°Brix)	10.514	11
Ascorbic acid (mg/100 mL)	48.735	49.122
Titrable acidity (%)	1.276	1.12

4.5 SHELF-LIFE STUDIES OF THERMOSONICATED SWEET ORANGE JUICE

The thermosonicated sweet orange juice at optimum conditions as well as fresh sweet orange juice was stored at room temperature of 28±2 °C and at refrigerated temperature of 4 °C**.** Variation of various properties of the juice with storage time was discussed below. The shelf-life studies were started at 18th June, 2022 and the quality of juice samples was determined were conducted for every 4 days interval.

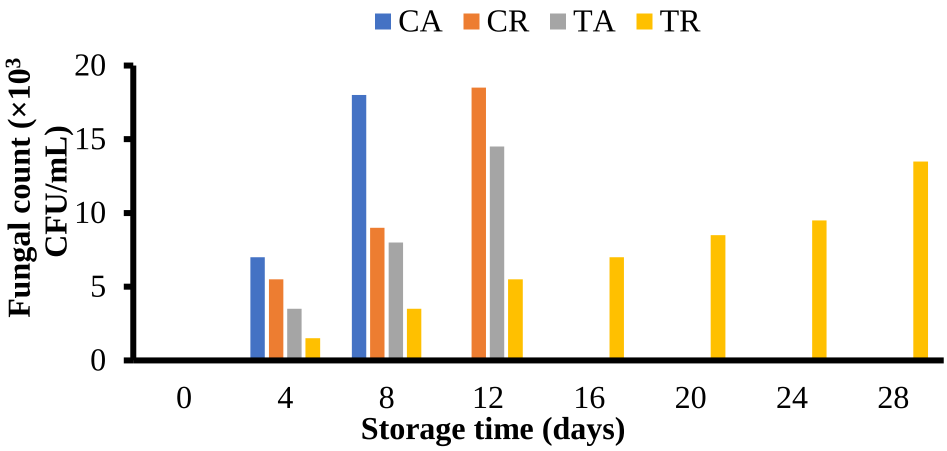
4.5.1 Microbial and Fungal Count

The microbial and fungal count of thermosonicated and fresh juice during storage is shown in **Figures 4.5 and 4.6**. It was observed that in all treatments, microbial and fungal growth increased with increase in the storage period. The fungal count was not observed in all juice samples on the initial day of storage. According to Food Safety and Standards Authority of India the acceptable limit of total plate count is < 10^5 CFU/g and the fugal count is < 10^4 CFU/g. The control juice sample stored at ambient temperature reached the maximum microbial count of 30 x 10^4 CFU/mL on 4th day of storage and maximum fungal count of 18 x 10^3 CFU/mL on 8th day of storage whereas at refrigerated conditions the maximum microbial count of 29 x 10^4 CFU/mL on 8th day of storage and maximum fungal count of 18.5 x 10^3 CFU/mL on 12th day of storage. Therefore, the storage life of control juice stored at ambient temperature is less than 4 days and storage life of control juice stored at refrigerated conditions is less than 8 days. The thermosonicated juice sample stored at ambient temperature reached the maximum microbial count of 18 x 10^4 CFU/mL on 8th day of storage and maximum fungal count of 14.5 x 10^3 CFU/mL on 12th day of storage whereas at refrigerated conditions the maximum microbial count of 19 x 10^4 CFU/mL on 28th day of storage and maximum fungal count of 13.5 x 10^3 CFU/mL on 28th day of storage. The obtained results indicated that the shelf life of the thermosonicated sweet orange juice is up to 24 days of storage under refrigerated conditions. Ultrasonic treatment enhanced transfer of heat and facilitate breakdown of microorganism's clumps making them susceptible to heat. Cavitation occurred during sonication leads to collapse of induced microbubbles leading to localized decontamination effect (Mason *et al.*, 1991). Similar results were reported by Alcantara *et al*. (2021) for pulque beverage.



NOTE: CA: Control Ambient; CR: Control Refrigerate; TA: Treatment Ambient; TR: Treatment Refrigerate

Fig. 4.5 Effect of storage period on microbial count of control and thermosonicated juice

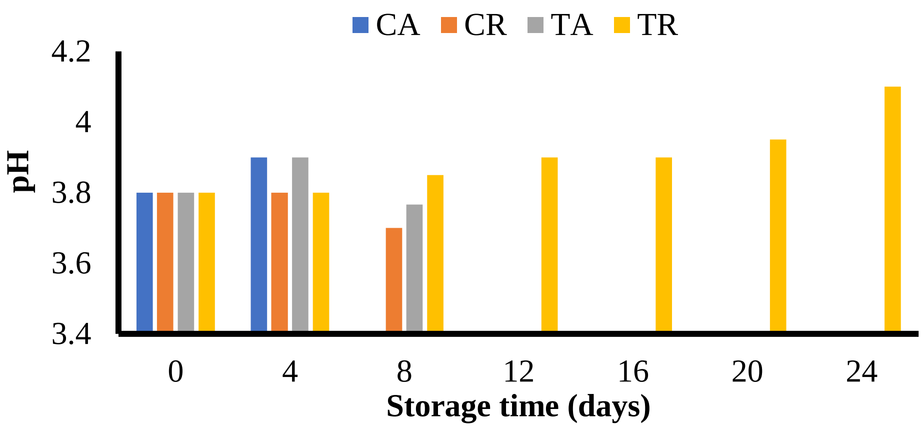


NOTE: CA: Control Ambient; CR: Control Refrigerate; TA: Treatment Ambient; TR: Treatment Refrigerate

Fig. 4.6 Effect of storage period on fungal count of control and thermosonicated juice

4.5.2 pH

The effect of pH of thermosonicated and fresh sweet orange juice during storage is shown in **Fig. 4.7**. pH almost remained stable during the storage period of 28 days in refrigerated and ambient conditions. The initial pH of juice was 3.8 and it was noticed that pH was slightly increased at refrigerated temperature to 4.1 in thermosonicated juice sample during storage. The increase in pH may be due to acid hydrolysis of some polysaccharides into disaccharides like starch into sucrose, fructose, and glucose etc., these reactions increase the sweetness and decrease the sourness as a result pH increases. Similar results were reported by Rai *et al*. (2008) for mosambi juice and Bruijn *et al*. (2003) for apple juice.

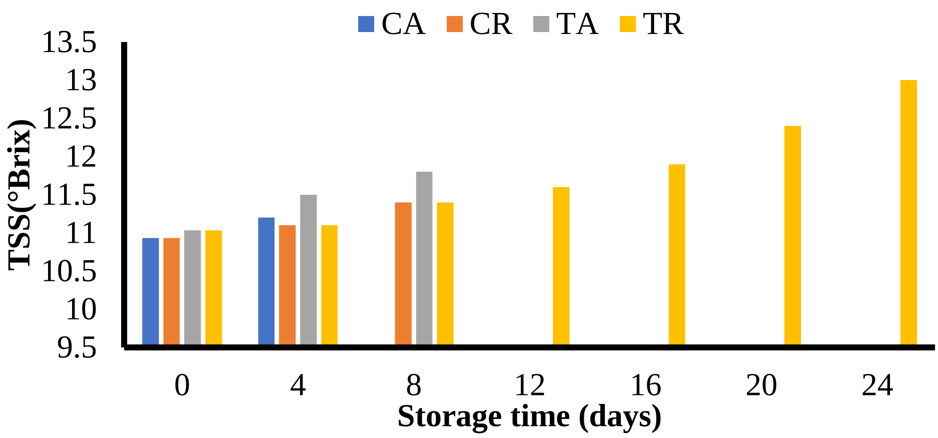


NOTE: CA: Control Ambient; CR: Control Refrigerate; TA: Treatment Ambient; TR: Treatment Refrigerate

Fig. 4.7 Effect of storage period on pH of control and thermosonicated juice

4.5.3 Total Soluble Solids (TSS)

The effect of TSS of thermosonicated and fresh sweet orange juice during storage is shown in **Fig. 4.8**. During the storage period, the TSS gradually increased from initial day to end of the storage period. During the storage period, TSS of thermosonicated sweet orange juice varied from 11.0 to 13 °Brix at refrigerated temperature. The increase of TSS during storage period, might be due to slow hydrolysis of polysaccharides, acids and pectin substances to simple substances like sugars. Similar results were reported by Ranote *et al*. (1993) in kinnow orange.

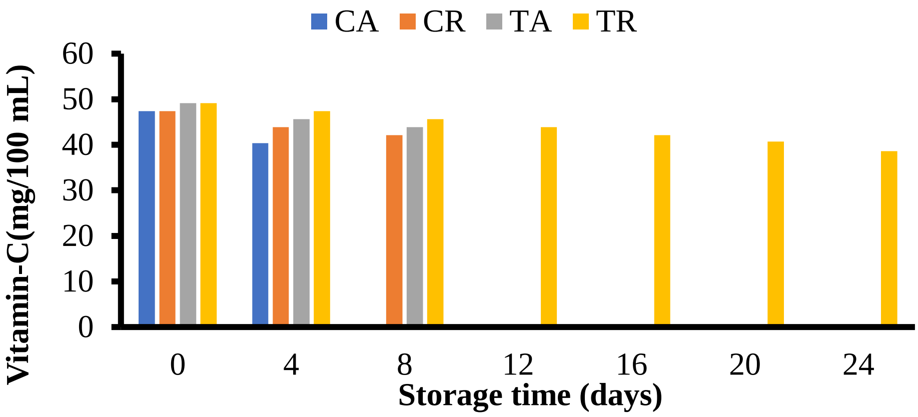


NOTE: CA: Control Ambient; CR: Control Refrigerate; TA: Treatment Ambient; TR: Treatment Refrigerate

Fig. 4.8 Effect of storage period on TSS of control and thermosonicated juice

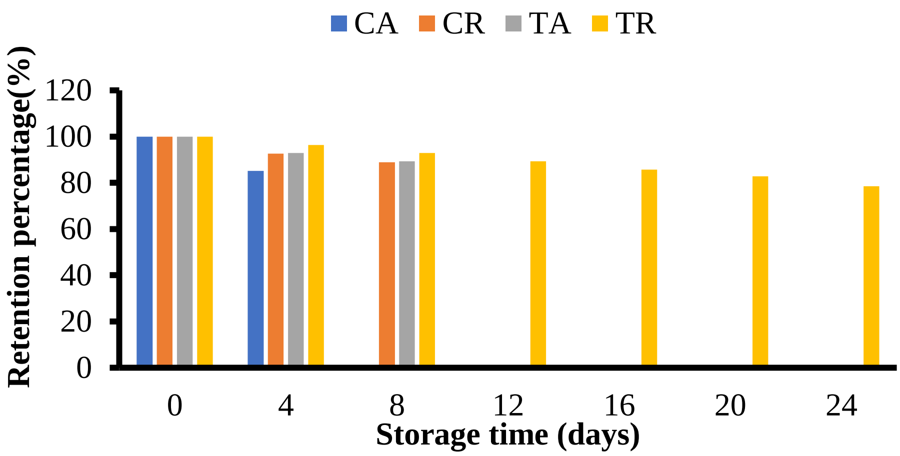
4.5.4 Ascorbic Acid

The variation of vitamin-C content of thermosonicated and fresh sweet orange juice during storage is shown in **Fig. 4.9**. During the storage period, the ascorbic acid gradually decreased from initial day to end of the storage period. Ascorbic acid of thermosonicated sweet orange juice during storage period varied from 49.13 to 38.59 mg/100 mL at refrigerated temperature. The retention percentage of ascorbic acid during storage decreased during storage period as shown in **Fig. 4.10.** The thermosonicated sample stored at ambient temperature retained 89.26% percentage of ascorbic acid at 8 days of storage. The thermosonicated sample stored at refrigerated temperature retained 75% percentage of ascorbic acid after 24 days of storage at refrigerated condition. Retention of ascorbic acid in fruit juices has been employed as an indicator of quality and shelf life of juice the ascorbic acid retention decreases to below 50% as reported by Abid *et al*., 2014. The decrease in ascorbic acid content of sweet orange during the storage might be due to thermal degradation during processing and subsequent oxidation effect of light and storage temperature. Degradation of ascorbic acid into dehydro ascorbic acid furfural and hydroxyl furfural is a common phenomenon observed during storage as reported by (Upale, 2005). Major problem associated with the quality of orange juice was the loss of ascorbic acid during storage (Lee and Coats, 1999). Similar results were reported by Rai *et al*. (2008) and Polydera *et al.* (2003).



NOTE: CA: Control Ambient; CR: Control Refrigerate; TA: Treatment Ambient; TR: Treatment Refrigerate

Fig. 4.9 Effect of storage period on ascorbic acid of control and thermosonicated juice

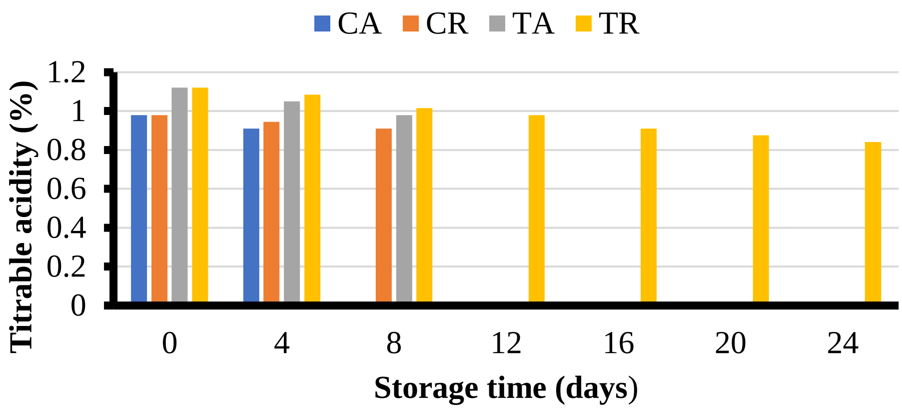


NOTE: CA: Control Ambient; CR: Control Refrigerate; TA: Treatment Ambient; TR: Treatment Refrigerate

Fig. 4.10 Effect of storage period on retention percentage ascorbic acid of control and thermosonicated juice

4.5.5 Titrable Acidity

The effect of titrable acidity of thermosonicated and fresh sweet orange juice during storage is shown in **Fig. 4.11**. During the storage period, the titrable acidity gradually decreased from initial day to end of the storage period. During the storage period, titratable acidity of thermosonicated sweet orange juice varied from 1.12 to 0.84 % at refrigerated temperature. The decrease in titrable acidity during storage period, might be due to conversion of acids into salts and sugars by enzymes. Similar observations were noticed by Jain *et al.*, 1986.

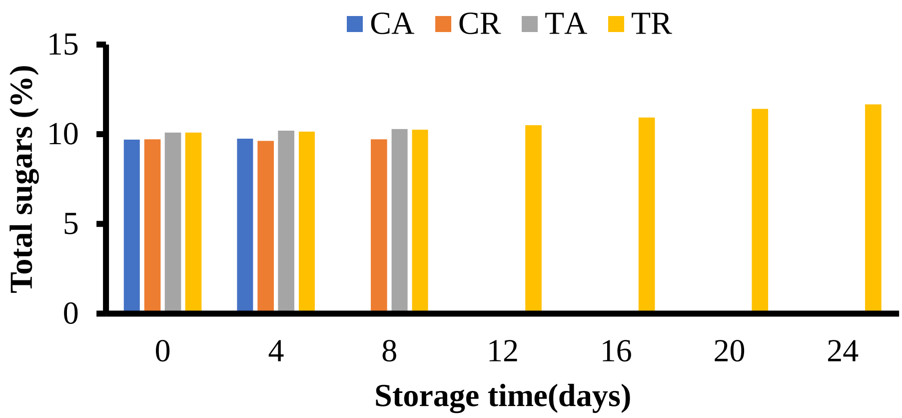


NOTE: CA: Control Ambient; CR: Control Refrigerate; TA: Treatment Ambient; TR: Treatment Refrigerate

Fig. 4.11 Effect of storage period on titrable acidity of control and thermosonicated juice

4.5.6 Total Sugars

The effect of total sugars of thermosonicated and fresh sweet orange juice during storage is shown in **Fig. 4.12**. During the storage period, the total sugars gradually increased from initial day to end of the storage period. During the storage period, total sugars for thermosonicated sweet orange juice varied from 10.096 to 11.66 % at refrigerated temperature. Total sugars were increased during storage, this might be due to the acid hydrolysis of polysaccharides to mono and oligosaccharides. Similar results have been reported by Ilamaran and Amutha (2007) in banana and sapota juice.



NOTE: CA: Control Ambient; CR: Control Refrigerate; TA: Treatment Ambient; TR: Treatment Refrigerate

Fig. 4.12 Effect of storage period on total sugars of control and thermosonicated juice

4.5.7 Polyphenol Oxidase (PPO)

Polyphenol oxidase (PPO) of thermosonicated and fresh sweet orange juice during storage is shown in **Fig. 4.13**. PPO residual activity gradually decreased during storage period. PPO residual activity slightly decreased from 85.31% to 81.25% during storage at refrigerated conditions. No browning was observed for juice during storage at refrigerated temperature. Less activity of polyphenol oxidase at refrigerated temperatures was reported by Ranveer *et al.* (2010) tamarind juice pulp. Ascorbic acid degradation is a key chemical reaction responsible for browning. During processing non enzymatic reactions between amino acids and carbohydrates also cause browning of food matrix which is continued during storage (Friedman 1996). The obtained results are in agreement with Aadil *et al*. (2015) for grape fruit juice.

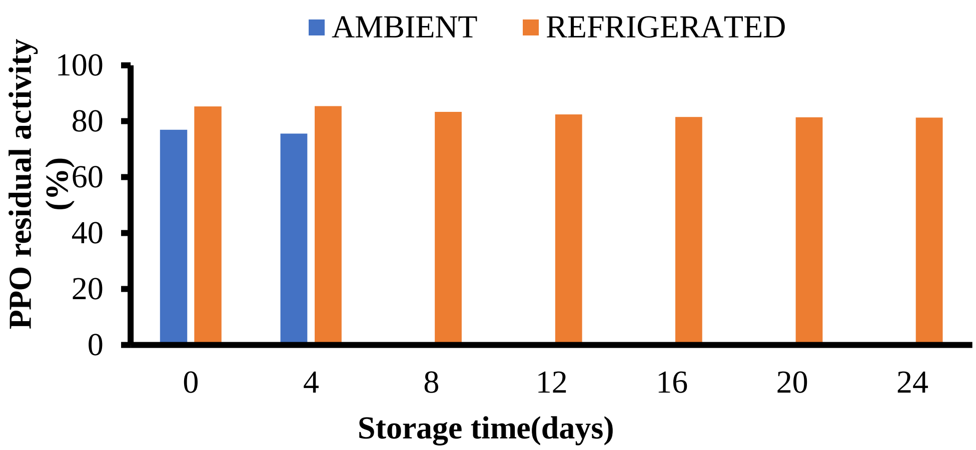


Fig. 4.13 Effect of storage period on polyphenol oxidase at ambient and refrigerated temperature

4.5.8 Peroxidase (POD)

Peroxidase (POD) of thermosonicated and fresh sweet orange juice during storage is shown in Fig. 4.14. POD residual activity gradually decreased during storage period. The POD residual activity slightly decreased from 80.64% to 79.01% during 24 days of storage at refrigerated conditions. No browning was observed for juice stored at refrigerated temperature. Less activity of peroxidase during refrigerated storage was reported by Ranveer *et al.* (2010) for tamarind juice pulp. Ascorbic acid degradation is a key chemical reaction responsible for browning. During processing non enzymatic reactions between amino acids and carbohydrates also cause browning of food matrix which is continued during storage (Friedman 1996). The obtained results are in agreement with Aadil *et al*. (2015) for grape fruit juice.

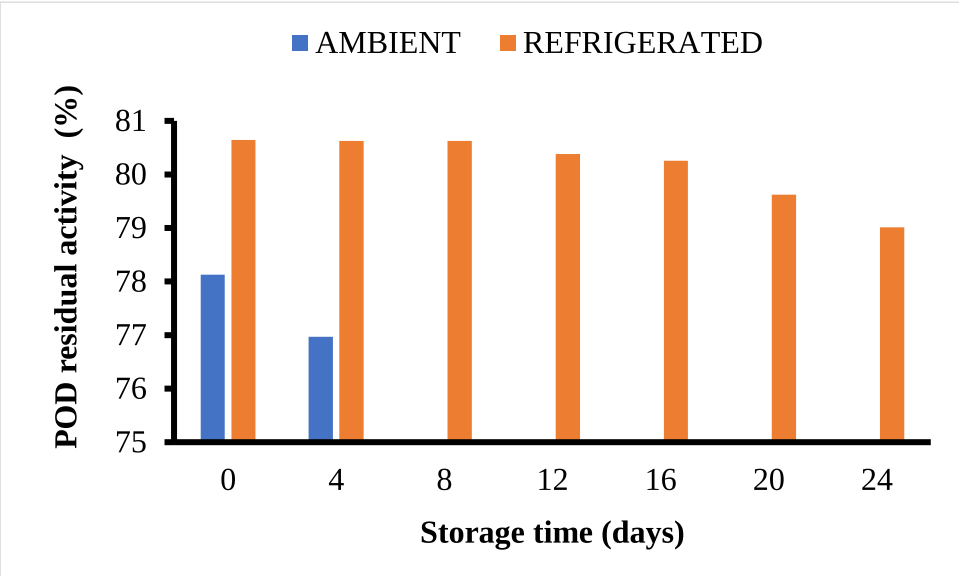


Fig. 4.14 Effect of storage period on peroxidase at ambient and refrigerated temperature

CHAPTER-V

SUMMARY AND CONCLUSIONS

Sweet orange is rich source of vitamin-C and minerals like calcium, phosphorous, potassium and iron. It also consists of vitamin A, B, and B2. Andhra Pradesh and Telangana produce more than 80% of total India's production of the sweet oranges and due to seasonality of the crop, glut situation prevails during the season bringing lower remunerative price to the farmers. About 95% of the fruit is essentially sold as fresh for juice purpose due to lack of processing technologies. Thermal processing causes unwanted changes in taste and odour with damage to bio-active components and functional properties during juice processing. Thermosonication is a good alternative technique to replace the conventional heat treatment process. It is a combined method of ultrasound and heat proved to be an effective non-thermal processing method in inactivating microorganisms and enzymes at low temperature at less processing time with minimum cost. Extensive study of literature revealed that minimal work was carried out on thermosonication of sweet orange juice. keeping the above points in mind, the present research was undertaken to study the effect of thermosonication on the quality of sweet orange juice.

An experiment entitled "EEFECT OF THERMOSONICATION ON QUALITY OF SWEET ORANGE JUICE" was carried out following Box- Behnken design with 17 treatments and 3 replications each. The major results obtained and discussed in the preceding chapter have been summarized below.

The important findings of the study are discussed below.

1. pH of sweet orange juice was not influenced significantly by the application of different thermosonication treatments. Among all the treatments the higher pH value of 4±0.10 was obtained for juice treated temperature of 50°C, amplitude 70% and sonication time of 12.5 min. Lower pH value of 3.7±0.10 was obtained at juice treated at temperature of 40°C, amplitude 40% and sonication time of 12.5 min. This may be due to the energy level applied to the samples through thermosonication did not change molecular structure of high molecular weight associated with physiochemical properties
2. TSS of sweet orange juice was not influenced significantly by the application of different thermosonication treatments. Among all the treatments higher TSS value of 11±0.00

was obtained at juice treated at temperature of 40°C, amplitude 40% and sonication time of 12.5 min. Lower TSS value of 9.2±0.00 was obtained at juice treated at temperature of 40°C, amplitude 70% and sonication time of 5 min. This may be due to the energy level applied to the samples through thermosonication did not change molecular structure of high molecular weight associated with physiochemical properties

3. Ascorbic acid of sweet orange juice is significantly influenced by the application of different thermosonication treatments. Among all the treatments higher Vitamin-C value of 52.429±0.82 mg/100 mL was obtained for juice treated at temperature of 50°C, amplitude 70% and sonication time of 12.5 min. Lower Vitamin-C value of 22.807±0.66 mg/100 mL was obtained for juice treated at temperature of 50°C, amplitude 100% and sonication time of 20 min. Ascorbic acid degradation is influenced more by the oxygen concentration than the temperature.
4. Titrable acidity of sweet orange juice is significantly influenced by the application of different thermosonication treatments. Among all the treatments higher titrable acidity value of 1.435±0.05% was obtained for juice treated at temperature of 60°C, amplitude 100% and sonication time of 12.5 min. Lower titrable acidity value of 0.98±0.01% was obtained for juice treated at temperature of 50°C, amplitude 70% and sonication time of 12.5 min. The increase of titrable acidity of the treated samples may be due to the possible release of galacturonic acid from the pectin structure due to cavitation phenomenon.
5. Sensory evaluation revealed that fresh juice and thermosonicated juice treatment at low temperature and amplitude had good acceptance compared with treatments at high temperature and amplitude.
6. The sensory quality ranking order for juice samples is as follows T5 (control)> T2 (TS:40°C,70% amplitude and 5 min duration)> T1 (TS:40°C,40% amplitude and 12.5 min duration)>T3 (TS:50°C,40% amplitude and 5 min duration)>T4 (TS:50°C,70% amplitude and 12.5 min duration). The average weightage values of T5 were nearer to T1 which was close agreement to the optimized value.
7. The optimal conditions for processing of thermosonicated sweet orange juice were observed at a temperature of 40°C, amplitude of 40% and sonication time of 9.298 min. The pH, TSS, ascorbic acid and titrable acidity of thermosonicated sweet orange juice obtained at optimized conditions are observed as 3.8, 11°Brix, 49.122 mg/100 mL and 1.12 % respectively. Similarly experimental values at optimized conditions were observed as pH (3.723), TSS (10.514 °Brix), ascorbic acid (48.735 mg/100 mL) and

titrable acidity (1.276%). The results obtained at optimum conditions are in close agreement with responses obtained at optimized conditions.

8. The microbial and fungal growth increased with increase in the storage period. Based on the obtained results it was observed that the storage life of control juice stored at ambient temperature is less than 4 days and storage life of control juice stored at refrigerated conditions is less than 8 days. The thermosonicated juice sample stored at ambient temperature reached the maximum microbial count on 8th day of storage. Thermosonicated sample stored at refrigerated conditions the maximum microbial count of 19 x 10^4 CFU/mL on 28th day of storage and maximum fungal count of 13.5 x 10^3 CFU/mL on 28th day of storage were observed.
9. Gradual increase in TSS of juice was observed during storage. TSS of thermosonicated sweet orange juice varied from 11.0 to 13 °Brix at refrigerated temperature. The increase of TSS during storage period, might be due to slow hydrolysis of polysaccharides, acids and pectin substances to simple substances like sugars.
10. During the storage period, the ascorbic acid gradually decreased. Ascorbic acid of thermosonicated sweet orange juice during storage period varied from 49.13 to 38.59 mg/100 mL at refrigerated temperature. The thermosonicated sample stored at refrigerated temperature retained 75% percentage of ascorbic acid after 24 days of storage at refrigerated condition. The decrease in ascorbic acid content of sweet orange during the storage might be due to thermal degradation during processing and subsequent oxidation effect of light and storage temperature.
11. Titrable acidity of thermosonicated sweet orange juice during the storage period varied from 1.12 to 0.84 % at refrigerated temperature. The decrease in titrable acidity content during storage period might be due to conversion of acids into salts and sugars by enzymes.
12. During the storage period, total sugars of thermosonicated sweet orange juice varied from 10.096 to 11.66 % at refrigerated temperature. The increase in total sugars might be due to the acid hydrolysis of polysaccharides to mono and oligosaccharides.
13. PPO and POD residual activity of treated juice gradually decreased during ambient storage. The PPO residual activity decreased from 85.31% to 81.25% during 24 days of storage at refrigerated conditions. The POD residual activity slightly decreased from 80.64% to 79.01% during 24 days of storage at refrigerated conditions. No browning was observed for juice during storage at refrigerated temperature. This may be due to non-enzymatic reactions between amino acids and carbohydrates during processing which is continued during storage.

14. The sensory quality of thermosonicated sweet orange juice processed at temperature of 40°C, 70% amplitude for 5 min duration ranked second after control sample, followed by the juice treated at same temperature of 40°C, 40% amplitude for 12.5 min duration. The optimal conditions for processing of thermosonicated sweet orange juice were observed at a temperature of 40°C, amplitude of 40% and sonication time of 9.298 min.

Based on sensory evaluation results and optimized conditions obtained from experimental data, the sweet orange juice thermosonicated at low temperature (40°C) for minimum duration resulted in best sensory and nutritional quality.

SUGGESTIONS FOR FUTURE WORK

Following suggestions may be taken into consideration for the future work.

1. Effect of different packaging materials on storage of thermosonicated sweet orange juice shall be carried out.

2. Effect of thermosonication on quality parameter of different juices or RTS beverages shall be studied.

3. Sensory evaluation can also be done during storage studies.

4. Effect of chemical preservatives on shelf life of thermosonicated sweet orange juice shall be carried out.

LITERATURE CITED

Aadil, R. M., Zeng, X. A., Zhang, Z. H., Wang, M. S., Han, Z., Jing, H. and Jabbar, S. 2015. Thermosonication: A potential technique that influences the quality of grapefruit juice. *International Journal of Food Science & Technology*. 50(5): 1275-1282.

Aadil, R. M., Zeng, X. A., Han, Z. and Sun, D. W. 2013. Effects of ultrasound treatments on quality of grapefruit juice. *Food Chemistry*. 141(3): 3201-3206.

Abid, M., Jabbar, S., Hu, B., Hashim, M. M., Wu, T., Lei, S., Khan, M. A. and Zeng, X. 2014. Thermosonication as a potential quality enhancement technique of apple juice. *Ultrasonics Sonochemistry*. 21(3): 984-990.

Adebola, O. O., Adeboyejo, F. O., Okekunbi, T. A. and Aderibigbe, O. R. 2021. Effect of thermosonication on quality attributes of hog plum (*Spondias mombin L.*) juice. *Ultrasonics Sonochemistry*. 70: 105-316.

Adekunte, A. O., Tiwari, B. K., Cullen, P. J., Scannell, A. G. M. and O'donnell, C. P. 2010. Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chemistry*. 122(3): 500-507.

Adiamo, O. Q., Ghafoor, K., Al-Juhaimi, F., Mohamed Ahmed, I. A. M. and Babiker, E. E. 2018. Thermosonication process for optimal functional properties in carrot juice containing orange peel and extracts. *Food Chemistry*. 245: 375-381.

Aguilar, K., Garvin, A., Ibarz, A. and Augusto, P. E. 2017. Ascorbic acid stability in fruit juices during thermosonication. *Ultrasonics Sonochemistry*. 37: 375-381.

Ahmed, A. K., Mahmood, T., Siddiqui, H. H. and Juber, A. 2016. Phytochemical and pharmacological properties on citrus limetta (Mosambi). *Journal Of Chemical and Pharmaceutical Research*. 8(3): 555-563.

Alcantara-Zavala, A. E., de Dios Figueroa-Cardenas, J., Perez-Robles, J. F., Arambula-Villa, G. and Miranda-Castilleja, D. E. 2021. Thermosonication as an alternative method for processing, extending the shelf life, and conserving the quality of pulque: A non-dairy Mexican fermented beverage. *Ultrasonics Sonochemistry*. 70: 105-290.

Alvarado-Morales, G., Minjares-Fuentes, R., Contreras-Esquivel, J. C., Montanez, J., Meza-Velazquez, J. A. and Femenia, A. 2019. Application of thermosonication for Aloe vera (*Aloe barbadensis Miller*) juice processing: Impact on the functional properties and the main bioactive polysaccharides. *Ultrasonics Sonochemistry*. 56: 125-133.

Alves, L. D. L., dos Santos, R. L., Bayer, B. L., Devens, A. L. M., Cichoski, A. J. and Mendonca, C. R. B., 2020. Thermosonication of tangerine juice: Effects on quality characteristics, bioactive compounds, and antioxidant activity. *Journal of Food Processing and Preservation*. 44(12): 14914.

Anaya-Esparza, L. M., Mendez-Robles, M. D., Sayago-Ayerdi, S. G., Garcia-Magana, M. D. L., Ramirez-Mares, M. V., Sanchez-Burgos, J. A. and Montalvo-Gonzalez, E. 2017. Effect of thermosonication on pathogenic bacteria, quality attributes and stability of soursop nectar during cold storage. *Journal of Food*. 15(4): 592-600.

Anonymous. 1956. Chemistry and technology of citrus, citrus products and by products. In: *Agriculture Handbook* No. 98. United States Department of Agriculture, Washington D. C.

AOAC. 2000. "*Official Methods of Analysis*" Association of Official and Analytical Chemists Washington DC, USA.

AOAC. 2005. "*Official Methods of Analysis*" Association of Official and Analytical Chemists Washington DC (17^{th} ed), USA.

Bora, S. J., Handique, J. and Sit, N. 2017. Effect of ultrasound and enzymatic pre-treatment on yield and properties of banana juice. *Ultrasonics Sonochemistry*. 37: 445-451.

Box, G.E. and Behnken, D.W. 1960. Some new three level designs for the study of quantitative variables. *Technometrics*. 2(4): 455-475.

Bruijn, J. P. F., Venegas, A., Martınez, J. A. and Borquez, R. 2003. Ultrafiltration performance of Carbosep membranes for the clarification of apple juice. *LWT-Food Science and Technology*. 36(4): 397-406.

Cao, J., Spielmann, M., Qiu, X., Huang, X., Ibrahim, D. M., Hill, A. J., Zhang, F., Mundlos, S., Christiansen, L., Steemers, F. J. and Trapnell, C. 2019. The singlecell transcriptional landscape of mammalian organogenesis. *Nature*. 566(7745): 496-502.

Chen, Y. Y., Liu, Y. F. and Wang, P. Z. 1983. Models of multifactorial evaluation. *Fuzzy Mathematics*. 1: 61-70.

Cheng, L. H., Soh, C. Y., Liew, S. C. and Teh, F. F. 2007. Effects of sonication and carbonation on guava juice quality. *Food chemistry*. *104*(4): 1396-1401.

Chitgar, M. F., Aalami, M., Kadkhodaee, R., Maghsoudlou, Y. and Milani, E. 2018. Effect of thermosonication and thermal treatments on phytochemical stability of barberry juice copigmented with ferulic acid and licorice extract. *Innovative Food Science & Emerging Technologies*. 50: 102-111.

Das, H. 2005. Sensory evaluation using fuzzy logic. *Food Processing Operations Analysis*. Asian Books Private Limited, New Delhi: 383-402.

De, L. C., Sharma, Y. P. and Bujarbaruha, K. M. 2019. Citrus in north eastern region. *National Symposium on Citriculture.*

Dundar, B., Agcam, E. and Akyildiz, A. 2019. Optimization of thermosonication conditions for cloudy strawberry nectar with using of critical quality parameters. *Food Chemistry*. 276: 494-502.

Friedman, M. 1996. Food browning and its prevention: an overview. *Journal of Agricultural and Food chemistry*. 44(3): 631-653.

Guerrouj, K., Sanchez-Rubio, M., Taboada-Rodriguez, A., Cava-Roda, R. M. and Marin-Iniesta, F. 2016. Sonication at mild temperatures enhances bioactive compounds and microbiological quality of orange juice. *Food And Bioproducts Processing*. 99: 20-28.

Hashemi, S. M. B., Roohi, R., Mahmoudi, M. R. and Granato, D. 2019. Modeling inactivation of *Listeria monocytogenes, Shigella sonnei, Byssochlamys fulva* and *Saccharomyces cerevisiae*,and ascorbic acid and β-carotene degradation kinetics in tangerine juice by pulsed-thermosonication. *Lwt*. 111: 612-621.

Hussain, N., Azhar, N. and Rajoo, S. G. S. 2019. Effects of thermosonication on watermelon rind-honey beverage. *Italian Journal of Food Science.* 31(4).

Ilamaran, M. and Amutha, S. 2007. Effect of total soluble solids and CO_2 pressure on physico-chemical and sensory qualities of carbonated banana and sapota beverages. *Journal of Food Science and Technology-Mysore*. 44(2): 178-182.

Jain, S. P., Tripathi, V. K., Ram, H. B. and Singh, S. 1986. Effect of storage conditions on storage life of some important squashes. Ind. Food Pack, 2: 36-41.

Kale, S. and Adsule, P. G. 1995. Citrus. Handbook of fruit science and technology (production, composition, storage and processing), edited by: Salunkhe, D. K. and Kadam, S. S., Marcel Dekker Inc.47.

Kiang, W. S., Bhat, R., Rosma, A. and Cheng, L. H. 2013. Effects of thermosonication on the fate of *E scherichia coli* O157: H7 and *Salmonella Enteritidis* in mango juice. *Letters in Applied Microbiology*. 56(4): 251-257.

Kumar, B., Mistry, N. C., Singh, B. and Gandhi, C. P. 2012. Commodity wise status (Chap 2). *Indian Horticulture Database* 2011, Published by National Horticulture Board, Ministry of Agriculture, Gurgaon. 55.

Lafarga, T., Ruiz-Aguirre, I., Abadias, M., Vinas, I., Bobo, G. and Aguilo-Aguayo, I. 2019. Effect of thermosonication on the bioaccessibility of antioxidant compounds and the microbiological, physicochemical, and nutritional quality of an anthocyanin-enriched tomato juice. *Food and Bioprocess Technology*. 12(1): 147-157.

Lee, H. S. and Coats, G. A., 1999. Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: a storage study. *Food Chemistry*. 65(2): 165-168.

Maier, V. P. and Beverly, G. D. 1968. Limoninmonolactone, the non-bitter precursor responsible for delayed bitterness in certain citrus juices. *Journal of Food Science*. 33: 488-92.

Maier, V. P and Dreyer, D. L. 1965. Citrus bitter principles. Occurrence of limonene in grapefruit. *Journal of Food Science*. 30: 874-875.

Manzoor, M. F., Xu, B., Khan, S., Shukat, R., Ahmad, N., Imran, M., Rehman, A., Karrar, E., Aadil, R. M. and Korma, S. A. 2021. Impact of high-intensity thermosonication treatment on spinach juice: Bioactive compounds, rheological, microbial, and enzymatic activities. *Ultrasonics Sonochemistry*. 78: 105740.

Mason, T. J. and Peters, D. 1991. Practical sonochemistry (Vol.18). New York: Ellis Horwood.

Medeiros, J. K., Sarkis, J. R., Jaeschke, D. P. and Mercali, G. D. 2021. Thermosonication for peroxidase inactivation in sugarcane juice. *LWT*. 140: 110730.

Mohideen, F. W., Kevin, M. S., Juan, L., Zhang, J., Alexander, C., Chotiko, A., Alfedo, P. D. and Subramaniam, S. 2015. Effect of continuous sonication on microbial counts and physio-chemical properties of blueberry juice. *Food Science and Technology*. 60: 563-570.

Munoz, A., Caminiti, I. M., Palgan, I., Pataro, G., Noci, F., Morgan, D. J., Cronin, D. A., Whyte, P., Ferrari, G. and Lyng, J. G. 2012. Effects on *Escherichia coli* inactivation and quality attributes in apple juice treated by combinations of pulsed light and thermosonication. *Food Research International*. 45(1): 299-305.

Maran, J. P., Manikandan, S., Thirugnanasambandham, K., Nivetha, C. V. and Dinesh, R. 2013. Box-Behnken design based statistical modelling for ultrasound-assisted extraction of corn silk polysaccharide. *Carbohydrate Polymers*. 92(1): 604-611.

Martinez-Flores, H. E., Garnica-Romo, M. G., Bermudez-Aguirre, D., Pokhrel, P. R. and Barbosa-Canovas, G. V. 2015. Physico-chemical parameters, bioactive compounds and microbial quality of thermo-sonicated carrot juice during storage. *Food chemistry*. 172: 650-656

Nayak, P. K., Chandrasekar, C. M. and Kesavan, R. K. 2018. Effect of thermosonication on the quality attributes of star fruit juice. *Journal of Food Process Engineering*. *41*(7): 12857.

Ordonez-Santos, L. E., Martinez-Giron, J. and Arias-Jaramillo, M. E., 2017. Effect of ultrasound treatment on visual color, vitamin C, total phenols, and carotenoids content in Cape gooseberry juice. *Food chemistry*. 233: 96-100.

Piyasena, P., Mohareb, E. and McKellar, R. C. 2003. Inactivation of microbes using ultrasound. *International Journal of Food Microbiology*. 87: 207-216.

Polydera, A. C., Stoforos, N. G. and Taoukis, P. S. 2003. Comparative shelf life study and vitamin C loss kinetics in pasteurised and high pressure processed reconstituted orange juice. *Journal of Food Engineering*. 60(1): 21-29.

Puri, M., Kaur, A., Singh, R. S. and Jagat, K. 2008. Immobilized enzyme technology for debittering citrus fruit juices. *Food Enzymes: Application of New Technologies*. Transworld research network, Kerala, India. 91-103.

Rai, P., Rai, C., Majumdar, G. C., Dasgupta, S. and De, S., 2008. Storage study of ultrafiltered mosambi (*Citrus sinensis (L.) Osbeck*) juice. *Journal of food processing and preservation*. 32(6): 923-934.

Ramfrez-Melo, L. M., del Socorro Cruz-Cansino, N., Delgado-Olivares, L., Ramirez-Moreno, E., Zafra-Rojas, Q. Y., Hernandez-Traspena, J. L. and Suarez-Jacobo, A. 2022. Optimization of antioxidant activity properties of a thermosonicated beetroot (*Beta vulgaris L.*) juice and further in vitro bioaccessibility comparison with thermal treatments. *LWT*. 154: 112780.

Raju, S. and Deka, S. C. 2018. Influence of thermosonication treatments on bioactive compounds and sensory quality of fruit (*Haematocarpus validus*) juice. *Journal of Food Processing and Preservation*. 42(8): 13701.

Ranganna, S. 1986. *Handbook of Analysis and Quality Control for Fruit and Vegetables Products*. 2nd Edition, Tata McGraw-Hill Publishing Co. Ltd., New Delhi. 868-924.

Ranveer, R. C., Sahoo, A. K., Ghosh, J. S., Jadhav, D. Y. and Mali, A. M. 2010. Phytochemical detection and in vitro evaluation of tamarind fruit pulp for potential antimicrobial activity. *International Journal of Tropical Medicine*. *5*(3): 68-72.

Ranote, P. S., Saini, S. P. S. and Bawa, A. S. 1993. Shelf-life of processed Kinnow juice. *Research and Industry*. 38(1): 15-18.

Rawson, A., Tiwari, B. K., Patras, A., Brunton, N., Brennam, C., Cullen, P. J. and O'Donnell, C. 2011. Effect of thermosonication on bioactive compounds in watermelon juice. *Food Reaserch International*. 44: 1168-1173.

Ribeiro, W. M., Noci, F., Cronin, D. A., Lyng, J. G. and Morgan, D. J. 2009. Shelf life and sensory evaluation of orange juice after exposure to thermosonication and pulsed electric fields. *Food and Bioproducts Processing*. 87(2):102-107.

Ribeiro, W. M., Noci, F., Riener, J., Cronin, D. A., Lyng, J. G. and Morgan, D. J. 2009. The impact of thermosonication and pulsed electric fields on *Staphylococcus aureus* inactivation and selected quality parameters in orange juice. *Food and Bioprocess Technology*. 2(4): 422-430.

Riener, J., Ribeiro, M. W., Noci, F., Cronin, D. A., Lyng, J. G. and Morgan, D. J. 2009. The impact of thermosonication and pulced electric fields on *staphylococcus aureas* inactivation and selected quality parameters in orange juice. *Food and Bioprocess Technology*. 2(4): 422-430.

Saeeduddin, M., Abid, M., Jabbar, S., Wu, T., Hashim, M. M., Awad, F. N., Hu, B., Lei, S. and Zeng, X. 2015. Quality assessment of pear juice under ultrasound and commercial pasteurization processing conditions. *LWT-Food Science and Technology*. 64(1): 452-458.

Sasikumar, R., Chutia, H. and Deka, S.C. 2019. Thermosonication assisted extraction of blood fruit (*Haematocarpus validus*) juice and process optimization through response surface methodology. *Journal of Microbiology,Biotechnology and Food Sciences*. 9(2): 228-235.

Sandhu, K. S. and Minhas, K. S. 2006. Oranges and Citrus Juices (Chap. 10.). *Handbook of Fruits and Fruit Processing*. Edited by Y. H. Hui. Blackwell Publishing, Oxford, U.K. 316,334.

Schuina, G. L., Moraes, V. P., Silva, P. I. and Carvalho, R. V. 2021. Effect of thermosonication on pectin methylesterase activity and on quality characteristics of orange juice. *Revista Ciencia Agronomica*. *52*.

Shokri, S., Jegasothy, H., Hliang, M. M., Augustin, M. A. and Terefe, N. S. 2022. Thermosonication of Broccoli Florets Prior to Fermentation Increases Bioactive Components in Fermented Broccoli Puree. *Fermentation*. 8(5): 236.

Siddiqui, A. N., Kulkarni, D. N., Kulkarni, K. D. and Mulla, M. Z. 2013. Studies on debittering of sweet orange juice. *World Journal of Dairy and Food Science*. 8(2): 185-189.

Strieder, M. M., Neves, M. I. L., Belinato, J. R., Silva, E. K. and Meireles, M. A. A. 2022. Impact of thermosonication processing on the phytochemicals, fatty acid composition and volatile organic compounds of almond-based beverage. *LWT*. 154: 112579.

Syed, H. M., Ghatge, P. U., Machewad, G. and Pawar, S. 2012. Studies on Preparation of Squash from Sweet Orange. 1: 311.doi:10.4172/scientificreports.311

Tiwari, B. K., O'Donnell, C. P., Muthukumarappan, K. and Cullen, P. J. 2009. Effect of sonication on orange juice quality parameters during storage. *International Journal of Food Science & Technology*. 44(3): 586-595.

Upale, K. B. 2005. Studies on processing of jamun juice and its beverages. *M. Sc. (Horti.) Thesis, Univ, Agri, Sci, Dharwad*.

Wahia, H., Zhou, C., Fakayode, O. A., Amanor-Atiemoh, R., Zhang, L., Mustapha, A. T., Zhang, J., Xu, B., Zhang, R. and Ma, H. 2021. Quality attributes optimization of orange juice subjected to multi-frequency thermosonication: *Alicyclobacillus acidoterrestris* spore inactivation and applied spectroscopy ROS characterization. *Food Chemistry*. 361: 130108.

Wahia, H., Zhou, C., Sarpong, F., Mustapha, A. T., Liu, S., Yu, X. and Li, C. 2019. Simultaneous optimization of *Alicyclobacillus acidoterrestris* reduction, pectin methylesterase inactivation, and bioactive compounds enhancement affected by thermosonication in orange juice. *Journal of Food Processing and Preservation*. 43(11): 14180.

Wang, J., Wang, J., Ye, J., Vanga, S. K. and Raghavan, V. 2019. Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure. *Food Control*. 96:128-136.

Wu, J., Gamage, T.V., Vilkhu, K.S., Simons, L.K. and Mawson, R. 2008. Effect of thermosonication on quality improvement of tomato juice. *Innovative Food Science & Emerging Technologies*. 9(2):186-195.

Wu, Y., Xu, L., Liu, X., Hasan, K. F., Li, H., Zhou, S., Zhang, Q. and Zhou, Y. 2021. Effect of thermosonication treatment on blueberry juice quality: Total phenolics, flavonoids, anthocyanin, and antioxidant activity. *LWT*. 150:112021.

Zhu, W., Ai, Y., Fang, F. and Liao, H. 2021. Application of thermosonication in red pitaya juice processing: impacts on native microbiota and quality properties during storage. *Foods*. 10(5):1041.

Zhang, X. and Shao, X., 2015. Characterisation of polyphenol oxidase and peroxidase and the role in browning of loquat fruit. *Journal of Food Sciences*. 33(2): 109-117.

APPENDICES

Appendix-A

Table 1 ANOVA for pH of thermosonicated sweet orange juice

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0817	9	0.0091	3.26	0.0668	not significant
A-Temperature	0.0013	1	0.0013	0.4487	0.5244	
B-Amplitude	0.0050	1	0.0050	1.79	0.2222	
C-Time duration	0.0013	1	0.0013	0.4487	0.5244	
AB	0.0100	1	0.0100	3.59	0.1000	
AC	0.0025	1	0.0025	0.8974	0.3750	
BC	0.0100	1	0.0100	3.59	0.1000	
A^2	0.0287	1	0.0287	10.29	0.0149	
B^2	0.0139	1	0.0139	5.00	0.0605	
C^2	0.0044	1	0.0044	1.60	0.2469	
Residual	0.0195	7	0.0028			
Lack of Fit	0.0075	3	0.0025	0.8333	0.5413	not significant
Pure Error	0.0120	4	0.0030			
Cor Total	0.1012	16				

Std. Dev.	0.0528	**R^2**	0.8073
Mean	3.86	**Adjusted R^2**	0.5595
C.V. %	1.37	**Predicted R^2**	-0.3714
		Adequate Precision	5.6200

Table 2 ANOVA for TSS of thermosonicated sweet orange juice

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.23	9	0.2483	2.22	0.1529	not significant
A-Temperature	0.1250	1	0.1250	1.12	0.3256	
B-Amplitude	0.0450	1	0.0450	0.4023	0.5461	
C-Time duration	0.1250	1	0.1250	1.12	0.3256	
AB	0.8100	1	0.8100	7.24	0.0310	
AC	8.882E-16	1	8.882E-16	7.940E-15	1.0000	
BC	0.0400	1	0.0400	0.3576	0.5687	
A^2	0.0341	1	0.0341	0.3049	0.5980	
B^2	0.0067	1	0.0067	0.0602	0.8132	
C^2	1.01	1	1.01	9.04	0.0198	
Residual	0.7830	7	0.1119			
Lack of Fit	0.7750	3	0.2583	129.17	0.0002	significant
Pure Error	0.0080	4	0.0020			
Cor Total	3.02	16				

Std. Dev.	0.3345	**R^2**	0.7405
Mean	9.89	**Adjusted R^2**	0.4069
C.V. %	3.38	**Predicted R^2**	-3.1133
		Adequate Precision	5.2629

Table 3 ANOVA for Vitamin-C of thermosonicated sweet orange juice

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1091.51	9	121.28	7.76	0.0066	significant
A-Temperature	135.59	1	135.59	8.67	0.0216	
B-Amplitude	166.48	1	166.48	10.65	0.0138	
C-Time duration	224.84	1	224.84	14.38	0.0068	
AB	2.09	1	2.09	0.1335	0.7256	
AC	19.23	1	19.23	1.23	0.3041	
BC	6.14	1	6.14	0.3926	0.5508	
A^2	177.58	1	177.58	11.36	0.0119	
B^2	187.44	1	187.44	11.99	0.0105	
C^2	116.33	1	116.33	7.44	0.0294	
Residual	109.47	7	15.64			
Lack of Fit	107.63	3	35.88	78.08	0.0005	significant
Pure Error	1.84	4	0.4595			
Cor Total	1200.98	16				

Std. Dev.	3.95	**R^2**	0.9088
Mean	42.12	**Adjusted R^2**	0.7917
C.V. %	9.39	**Predicted R^2**	-0.4363
		Adequate Precision	7.5933

Table 4 ANOVA for Titrable acidity of thermosonicated sweet orange juice

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.2989	9	0.0332	5.70	0.0159	significant
A-Temperature	0.0822	1	0.0822	14.10	0.0071	
B-Amplitude	0.0107	1	0.0107	1.84	0.2170	
C-Time duration	0.0000	1	0.0000	0.0042	0.9501	
AB	0.0024	1	0.0024	0.4035	0.5455	
AC	0.0071	1	0.0071	1.21	0.3076	
BC	0.0125	1	0.0125	2.15	0.1858	
A^2	0.0950	1	0.0950	16.29	0.0050	
B^2	0.0665	1	0.0665	11.41	0.0118	
C^2	0.0072	1	0.0072	1.24	0.3023	
Residual	0.0408	7	0.0058			
Lack of Fit	0.0407	3	0.0136	668.68	< 0.0001	significant
Pure Error	0.0001	4	0.0000			
Cor Total	0.3397	16				

Std. Dev.	0.0763	**R^2**	0.8799
Mean	1.14	**Adjusted R^2**	0.7254
C.V. %	6.69	**Predicted R^2**	-0.9186
		Adequate Precision	6.6534

Appendix-B

SCORE CARD FOR EVALUATION OF THERMOSONICATED SWEET ORANGE JUICE

Product: SWEET ORANGE JUICE Made on:

Tested on:

Please rate the samples for quality attributes according to the five point scale
Excellent -5; Good -4; Medium -3; Fair -2; Not satisfactory -1

Sensory quality attributes of sweet orange juice	Sample description	Sensory scale factors				
		Not satisfactory	Fair	Medium	Good	Excellent
Apeearence						
	T1					
	T2					
	T3					
	T4					
	T5					
Odour						
	T1					
	T2					
	T3					
	T4					
	T5					
Sweetness						
	T1					
	T2					
	T3					
	T4					
	T5					
Sourness						
	T1					
	T2					
	T3					
	T4					
	T5					
Overall acceptability						
	T1					
	T2					
	T3					
	T4					
	T5					

Sensory scale factors					
Sensory quality attributes of sweet orange juice	Not at all important	Somewhat important	important	Highly important	Extremely
Appearance					
Odour					
Sweetness					
Sourness					
Overall acceptability					