Advances in Food & Biosciences
2025, VOL. 1, NO. 1, 2025010105
https://rcpublishers.net

Research Article

Impact of Sonication and Thermosonication on the Microbial and Enzymatic Activities of Citrus Juices

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ABSTRACT

Due to rising consumer awareness towards the quality and safety of food, the demand for safe and nutritious food products has been increased. Similarly, fruit juices are known for their nutritional characteristics, however, it is necessary to preserve nutritional components present in fruit juices. Traditionally, thermal treatment is used to treat the juice and to enhance their shelf life. Although, such treatments are helpful in achieving required microbial and enzymatic safety levels, however, exposure of bioactive components (i.e. carotenoids, vitamin, and anthocyanin) to high temperature would lead to their degradation. Hence, thermal treatment lead to the loss of beneficial nutrients in citrus juices. Alternatively, non-thermal technologies like sonication and thermosonication have been proposed. Therefore, in this study, the effect of these two treatments on the microbial and enzymatic activity of kinnow and sweet lemon juice was studied separately. The sonication was used at 20 kHz for 5 and 10 minutes. While thermosonication was used at 40 °C and 20 kHz frequency for 5 and 10 minutes. As a result, a similar effect of treatments on kinnow and sweet lemon juice was observed. No effect of any treatment was found on the TA of any juice sample. However, it was found that when ultrasound was applied it leads to an increase in CV, NEB, EC, TC, and total anthocyanin content. While thermosonication treatment decreased the EC and total anthocyanin content in both juice samples. The microbial (TPC) activity of kinnow and sweet lemon juice was 4.13 and 4.07 log which decreased to 3.78 and 3.74 log (CFU mL-1) when sonication was applied. And further decreased to 2.92 and 2.86 log when thermosonication was applied. Similarly, minimum enzymatic (PME, PPO, and POD) residual activity achieved after thermosonication was 58.47, 78.82, 72.85% (for kinnow juice), and 63.32, 75.53, 68.72% (for sweet lemon juice). Therefore, in the context of parameters analyzed in this study, thermosonication seems to be the better option since it leads to a significant reduction in both microbial and enzymatic activities. Overall, both thermosonication and sonication have the potential to retain the nutritional properties of kinnow and sweet lemon juices.

Keywords: kinnow; sweet lemon; thermosonication; enzymatic activities; bioactive compounds

INTRODUCTION

Pakistan has a very thriving climate, and under these conditions, various vegetables can grow as well as fruits. Due to the correct environment and suitable climate different types of fruit grown in this area. In northern and northwestern conditions, fruits such as mango, citrus, apple, tomato, grapes, are grown as these regions provide the best cultivation

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https://doi.org/XXX/XX.2025.XXX
Received 20 September 2025; Received in revised form 02 October 2025; Accepted 12 October 2025

conditions for these fruits (Rojas-Grau et al., 2008). Such fruits contribute to the agricultural export of Pakistan whereas citrus fruits are especially at the top of both fruit exports and fruit production. Punjab produces most of the percentage of citrus crops because of favorable growing conditions and availability of adequate water (Ahmad et al., 2018).

Citrus belongs to the genus Citrus L. It belongs to the Rutaceae family and is considered as one of the world's most important fruit crops, mainly grown in tropical and subtropical regions. The global production of these fruits is estimated to be 102 million tons (Zhou et al., 2016). As one of the country's major crops, its total production in Pakistan was about 2.35 million tons in 2017-18, with Punjab producing 97% of the total citrus fruit (GOP, 2017-18a). During 2000-2018, the region under citrus cultivation remained approximately 189 thousand hectares, while the production was estimated to be about 2224 thousand tons (GOP, 2017-18b). Pakistan is currently ranked 12th among the citrus-producing countries, while Brazil is at the top of the list (Nazeer et al., 2019).

Among citrus fruit, Citrus limetta (sweet lemon) variety is grown mainly in southern and southeastern Asia. This fruit has a flattened apex with a thin, light yellow rind. It is rich in nutrients such as vitamin C and minerals, the concentration of which may differ depending on sweet lemon cultivars, maturity, cultural practices, post-harvest practices, and storage conditions (Hashemi et al., 2017).

Another important type of citrus, Kinnow (Citrus reticulate) is Pakistan's significant fruit and the country ranks as the largest exporter of Kinnow. This variety of mandarin was created by an effective cross between a variety of citrus fruit called "willow leaf" and "king". While this successful experiment was performed at the Citrus Research Center, University of California, USA in 1951. Nevertheless, in Pakistan, climatic and soil conditions have given Kinnow a special taste and flavor that makes it distinct from other mandarin varieties (Nazeer et al., 2019).

Citrus fruits are most popular worldwide because of their good taste, attractive color, and aroma (Zou et al., 2016). They are a rich source of nutrients especially beneficial phytochemicals, such as vitamins C, E, and A. Besides, they contain many mineral elements and certain antioxidants, such as flavonoids and phenolic compounds. Among these, vitamin C is known as a natural free radical scavenger that can scavenge many reactive oxygen species (Poprac et al., 2017). Moreover, they also contain pectins, coumarins, carotenoids, limonoids, and other compounds (Nishad et al., 2019). Citrus is known to contain a significant amount of citric acid, vitamin C, trace elements as well as dietary. Citrus fruits, being a rich source of antioxidant compounds, all edible citrus varieties are known for their free radical scavenging activity, which is quite beneficial to human health (Xu et al., 2008). In recent years, more and more attention has been paid to the antioxidant function of citrus fruits and their role in the prevention and treatment of various human chronic and degenerative diseases (Zou et al., 2016).

Owing to anti-oxidant properties, citrus also has the potential for numerous biological functions, i.e. anti-inflammation, anti-aging, and anti-cancer (Ke et al., 2015). More than 170 antioxidants have been identified in citrus fruit, including vitamins, phenolic compounds, minerals, pectin, and terpenoids. Such compounds are documented to be helpful in the prevention of various chronic diseases such as cardiovascular disease, diabetes, and as well as cancer. Usually, these phytochemicals are obtained via fresh fruits or by their derived products (often juices) and are beneficial to human health (Zhang et al., 2015). Besides, citrus also exhibits anti-bacterial, anti-viral, and anti-fungal properties (Ahmed and Azmat, 2019).

On the other hand, when picked, citrus fruits are susceptible to microbiological and biochemical changes resulting in reduced shelf life. Normally, citrus is typically eaten either as fresh or as juice. The market demand for fruit juices is growing instead of consuming them as a whole (Yılmaz et al., 2014). Juices, particularly fruit juices, are used for higher concentrations of bioactive compounds because they are an efficient approach to obtain them. However, due to their perishable nature citrus fruits can be spoiled and wasted thus to enhance their stability and quality characteristics, the application of certain treatments becomes necessary. Fruits are often refined into juices to ensure their continuous supply throughout the year (Anaya-Esparza et al., 2017; Rostagno et al., 2007).

In citrus juices, water, vitamins, minerals, polyphenols, and sugars are present in naturally maintained balance and combination. It is estimated that 8 ounces of orange juice contain 122 calories and 29 g of total carbohydrate. Also, orange juices (like kinnow) contain folate, niacin, potassium, thiamin, magnesium, riboflavin, iron, calcium, vitamin B6, and other minerals. Therefore, the consumption of citrus juice contributes to the necessary dietary intake amount, which is needed for the normal functioning of the human body (Rampersaud and Valim, 2017). On the contrary, the major flavonoids found in sweet lemon are diosmin, hesperidin, and eriocitrin. Though, fresh Kinnow juice is non-bitter, but, it may develop bitterness issue within 3-4 hours, just as in other citrus juices such as grapefruit. This phenomenon is primarily attributed to flavonoid (naringin) and triterpene related derivatives such as limonin. However, appropriate methods such as centrifugation can be used to eliminate this problem in the post-extraction process (Ilame and Singh, 2018).

Nevertheless, as perishable food, fruit juices, including citrus juices, are qualitatively degraded and can be spoiled in a shorter duration of time. The shelf life and quality of juices can be measured either based on microbial growth and enzymatic activities or nutritional contents like total phenolic content, ascorbic acid, etc. Primarily yeast, bacteria, and molds are responsible for the spoilage of juices, which are therefore crucial in the determination of shelf life. Among these, yeasts are predominant microbes because they can thrive even under lower pH, anaerobic conditions, and even under high sugar concentration conditions (Millan-Sango and Valdramidis, 2018).

Moreover, fruit pressing for juice extraction is still performed at the household level. At this point, the consistency and shelf life of the juice is of little concern. But, around the world, fruit juices represent a major multi-billion dollar market that is dominated by orange juice. Thus, when it comes to the production of packaged juices, shelf life enhancement along with the quality product is of significant importance. This is because, the most significant aspect, is the availability of an appropriate quality product to the customer. A substantial loss of goods with considerable commercial value can also be prevented by paying attention to the shelf life of the product (Ashurst, 2016).

For this reason, various processing techniques have been used to maintain natural flavor and nutrition, along with rising shelf life. Thermal treatment (conventionally), i.e. pasteurization, has been used for the processing of fruit juices. This procedure has been used at 90-100 °C and juices have been exposed to this temperature range for 15-60 sec (Rivas et al., 2006). The thermal treatment has been used to effectively kill vegetative cells of spoilage causing microbes and enzymes which consequently result in microbial and cloud stability of juices (Millan-Sango and Valdramidis, 2018). Furthermore, pasteurization treatment at 90 °C for 1 min was found to be effective in the processing of orange juice (Nienaber and Shellhammer, 2001).

To achieve the desired product temperature during traditional processing (application of high temperature), steam is used as a heating medium, while the heating rate is either regulated by two mechanisms, the first being conduction, while the second is known as convection. In the conduction process, the rate of heat transfer is very slow and creates a significant number of time gaps between the point at which the surface of the goods is sterilized and the central part of the food gets sterilized. As a result, the quality defects of the product could result, as the product may be overheated in an attempt to achieve sterilization of its central core component (Awuah et al., 2007).

Typical juice processing involves the washing of the desired quality fruit, followed by pressing the juice for maximum yield. After that, the pulp and debris are removed (can be done by filtration) from the juice, and clear juice is pasteurized to deactivate the enzymatic systems and destroy the problematic microbes to ensure microbial safety. Pasteurized juice can be concentrated, if necessary. The prepared product is then placed in a suitable container (Ashurst, 2016).

In the food industry, traditional thermal treatments, i.e. pasteurization, have been used to kill or reduce microorganisms in food products. Factors such as standardization of instrument design, operating system, control, and maintenance of equipment play a significant role in the destruction of microbes or the reduction of microbial load. Considering the safety status of products, manufacturing lines in the juice or liquid food industries apply pasteurization treatment as a major step, where heat is applied for a predetermined time and temperature ratio (Huang and Wang 2009).

Heat treatment has the potential to kill or reduce both microbial loads and enzymatic activities in food products, and most companies use this approach in batch and continuous processing. While being successful in food safety problems, there is growing concern about the use of conventional heat treatment, as the process causes nutritional quality losses and results in the degradation of many heat-labile nutrients. As heat treatment requires the use of convection and conduction methods, therefore, it is difficult to achieve uniformity with this technique. Furthermore, when the substance is directly heated, the possibility of overheating also increases (Sarkis et al., 2013).

Besides, the ascorbic acid structure is also influenced by thermal treatment during juice processing and can result in non-enzymatic browning due to Maillard reactions. They are also contributing factors for condensation between reducing sugars and amino acids, caramelization, and pigment degradation (Damasceno, 2008). Moreover, as a result of the Maillard reaction, hydroxymethylfurfural (5-HMF) is formed and the presence of this compound is used to indicate the extent of the processing of fruit juices and this compound is considered to be a parameter of quality degradation (Millan-Sango and Valdramidis, 2018).

As a result of increasing consumer awareness towards the consumption of high nutritional food products, the demand for high nutritional profile and extended shelf life products has also increased. Consumer demand is not only to conserve the shelf life, by lowering microbial and enzymatic activities, but also the maintenance of natural features with improved quality characteristics (Aadil et al., 2013). Due to lowering the impact of treatments on food products and overcoming the harmful aspects of food quality and aesthetics posed by thermal treatment, alternative non-thermal technologies are becoming increasingly popular. The emphasis is not only on improving quality (by reducing enzymatic and microbial activities) but also on reducing the nutritional losses of products (Khandpur and Gogate, 2015).

The use of emerging technology has increased over the last decade. Cold plasma, pulsed electric field, ultraviolet, ultrasound, pulsed light, and high-hydrostatic pressure are some of the non-thermal technologies and popular for their use as minimal processing technologies (Barba et al., 2015). They are not only capable of achieving food safety, but can also contribute in terms of food quality. Besides, most of the new technologies are energy-efficient. Therefore, these novel technologies have gained considerable attention as an evolving substitute for conventional thermal processing technologies (Santhirasegaram et al., 2016).

The US is one of the non-thermal techniques that has been the subject for being used in industries and can be used for food processing as well as food analysis. This is a promising food processing technology with the potential to replace thermal-based treatment (Valdramidis et al., 2010). The US is a sound wave with a frequency of 20 kHz or more (José et al., 2014). Interest in this technique has been increased due to its advantages, such as decreased energy usage, improved performance, and decreased processing time (Tiwari et al., 2009). The frequency ranges most widely used for ultrasonic processing of liquid food tends to be 20-25 kHz. US waves primarily cause chemical, biochemical, and physical effects through the formation and collapse of bubbles, which is known as cavitation (Paniwnyk, 2017).

Within the liquid, ultrasonic sound waves travel through a series of rarefaction and compression cycles that induced in the molecules of that medium. Upon application of frequencies with adequate wave strength, voids also known as cavities are formed within the liquid. These cavities can expand through a process known as rectified diffusion. In this process, volatile gases, which are already present in the bulk medium enter the created cavities but are not fully expelled during the successive compression phase (Mason et al., 2015). These cavities will expand continuously and ultimately become unstable, resulting in collapse. As a result of this collapse, high pressure, and temperature created at a microscopic level result in shearing forces that cause homogenization, mass transfer, and high efficiency in mixing. Besides, high temperature "hotspots" are also created within the liquid being processed. The energy released as a result of cavitational collapse is responsible for various effects created during food processing operations, such as increased homogeneity, reduced-fat globular size, and enhanced extraction (Tiwari and Mason, 2012). Reduction of enzymatic activities and microbial load, extended shelf life, and reduced nutrient losses are some of the advantages of juice processing by US technology (Paniwnyk, 2017). US treatment affects microbes by puncturing their cell walls and resulting in free radical generation and extrusion of the intracellular matrix that ultimately destroys microbes. Also, cavitation occurs on the cell wall of the microorganism, thus having an anti-bacterial effect (Roobab et al., 2018).

Moreover, US treatment increases the CV (cloud value) of the juice as US processing splits the bigger molecules into smaller ones owing to the high-pressure gradient of the cavitation (Aadil et al., 2013). Peroxidase and Polyphenol oxidase are enzymes generally associated with browning reactions in fruit juices. PPO and POD induced oxidation of phenolic compounds leads to a lack of taste and unwanted brown color in different juice products, which consequently result in the loss of nutritional profile and sensory properties of juices, thus, during the storage periods and processing of the juice products, certain enzymes must be inactivated to ensure the quality of the final product (Saeeduddin et al., 2015).

On the contrary, the US may have a low lethal effect on microorganisms. As the ultimate goal of the modern food industry is to increase quality and improve safety, therefore, a combination of non-thermal techniques has been evaluated. These combinations might be the best way to handle fruit juices, particularly at an industrial level (Li et al., 2017). As a result, researchers found that the US is often more efficient, particularly when combined with mild heat treatment, also known as thermosonication (TS). In TS, more efficient microbial and enzymatic inactivation can be achieved by combining cavitation and heat, as this treatment results in effect on the bacterial membrane as well as macromolecule depolymerization. This combined treatment can be used without losing the quality of the juice being treated (Anaya-Esparza et al., 2017).

In the number of juices such as pineapple, grape, peach, mango, apple, carrot, and cranberry, TS is successful in achieving hygienic conditions. Not only is it efficient for pasteurization, but it can result in a higher quality of juice, as this treatment can preserve bioactive compounds and show improved sensory properties compared to traditional heat treatment (Aguilar et al., 2017). Since ascorbic acid is an essential nutrient of juice and is responsive to various processes, thus, it is used as an indicator for estimating the degradation of quality during processing (Lima et al., 2010). However, variables such as frequency and intensity of sound waves, viscosity, suspended solid material, pH in the medium along with treatment time and temperature need to be considered to get optimal benefits from TS (Anaya-Esparza et al., 2017). TS can be used as a continuous or discontinuous operation. Usually, discontinuous TS is applied through ultrasonic equipment which usually consists of an ultrasonic probe, immersion in an ultrasonic bath, or an external circular water bath. On the other hand, continuous treatment can be applied using equipment consisting of a flow cell with a sonicator probe and temperature control (Chemat et al., 2011). US and TS both have the potential to preserve juice qualities by reducing the activities of certain enzymes and microbes. Sonication can mitigate the degradation effect on titratable acidity, cloud value, and the content of bioactive compounds (anthocyanin and carotenoids). In fruit juices TS can also be useful for a shorter time, lower temperature treatment, resulting in lower enzymatic activity (PME, PPO, and POD) and microbial population as well. However, process parameters need to be optimized (Abid et al., 2014a).

REVIEW OF LITERATURE

Role and importance of citrus fruits: Citrus fruits are widely ranked as the second most valuable fruit category in the world, including mandarins, grapefruit, oranges, etc. Numerous varieties and hybrids have been created for each citrus fruit group. Among the citrus, Kinnow is the most known hybrid fruit of the mandarin group. Kinnow was developed in 1935 in California, USA by crossing two mandarin varieties named Willow Leaf (C. deliciosa) and King (Citrus nobilis). Kinnow, however, has not gained popularity in the United States but has been revolutionized the citrus growing countries such as Pakistan, Bangladesh, and India due to the appropriate environmental conditions found in different parts of these countries. As a result, it has led by occupying vast areas and has resulted in a substantial development in these countries. Kinnow is also known for its attractive orange colors, distant flavor, improved fruit quality, and high juice content in comparison to other citrus fruits (Sharma et al., 2016).

On the contrary, Citrus limetta (generally known as Mosambi) is one of the important citrus plants. It is known throughout the world for its excellent medicinal characteristics and nutritional value (Younis et al., 2015). The chemical composition of sweet lemon depends largely on the environment where it grows and is ideal for both juicing and eating purposes. Like other citrus fruits, it is also considered to be high in vitamin C content. Mosambi is also considered to have soothing powers, i.e. common cold treatment (Jamil et al., 2015).

As one of the most eaten fruit, citrus is the most excellent source of dietary fiber. They are known to contain essential bioactive compounds such as phenolic acids, carotenoids terpenes, ascorbic acid, and flavonoids. They contain flavanone as naringin and hesperidin, β-carotene as major carotenoids, though they also contain limonene (a bioactive terpene). They are also popular for limonoids such as nomilin and limonin. These bioactive compounds have a significant positive impact on human health, especially due to their antioxidant activity (Zhang et al., 2015). In addition, the majority of phenolic acids (i.e. coumaric, caffeic, sinapic, and ferulic) in citrus fruits are found in bound form (Xu et al., 2008).

Citrus is also an excellent source of vitamins, including vitamins D, A, C, B1, and B2. They are a good source of sugars, carbohydrates, and minerals i.e. potassium, sodium, magnesium, iron, and calcium. They are included in those fruits which contain a high amount of citric acid and are therefore known as acidic fruits. Because of its weak organic acidity, citric acid is used as a flavoring agent and also to improve fruit stability. Thus, to reap the benefits of these fruits, people often consumed them in the form of juice (Jamil et al., 2015). Orange juice is estimated to contain 110% of the RDA (recommended dietary allowance) for ascorbic acid. Moreover, it also contains vitamins B1, B6 as well as vitamin A, folic acid, copper, magnesium, potassium, and calcium. The average orange fruit also provides 60 calories (Ahmad et al., 2012). All these are the key ingredients for a healthy diet. Besides, the potential benefits of citrus fruits and juices are well recognized. Citrus fruits are used as diabetic medicines in conventional herbal medicines. They are also renowned for their ethno medicinal use. Such characteristics are due to the presence of limonoids and flavonoids capable of producing anti-tumor and anti-inflammatory effects (Khan et al., 2018). Additionally, their peels have sugar lowering and cholesterol-lowering characteristics and have been found to be abundant in pectin. Hydroxycinnamic acid compounds present in citrus can inhibit low-density lipoproteins and possess anti-cancer and antimicrobial properties. Carotenoid (found in citrus juice) also reduces the risk of cancer, cardiovascular disease, and cataract (Kundusen et al., 2011).

C. Limetta has also been used for medicinal purposes. The fruit can reduce both fever and cholesterol, can treat digestive and inflammatory disorders, and can be used as a blood pressure modulator. The sweet lemon is an important fruit for managing hypertension, thereby reducing the risk of cardiovascular disease (Perez et al., 2010). Similarly, flavonoids in citrus are known to have antioxidant properties and also have anti-microbial and anti-fungal effects. Flavonoids (i.e. diosmin, narirutin, hesperidin, and rutin) can improve wound healing in humans (Damián-Reyn et al., 2017).

Also, multiple studies have shown that citrus fruit juices can be beneficial in the treatment of cancer (colon) (Jaganathan et al., 2014). Moreover, flavonoids and polyphenols present in citrus juices may be protective against excess fat tissue and thus may be useful for the prevention of obesity (Nakajima et al., 2014). There are many other plant-based nutrients found in citrus juices that are helpful in curing a variety of diseases, particularly those related to nutrient deficiency. As secondary plant metabolites, phenolic compounds also lead to the formation of characteristics flavor and color of citrus juices. Flavonols are effective in a similar way. Such characteristics revealed the benefits of citrus juices particularly related to human health (Guerrouj et al., 2016).

Conventional processing of juices: Citrus fruits (sweet lemon and kinnow) are an important component of our diet and can be eaten easily in raw form. For ease of use and longer shelf life, they may be consumed as juices or may be processed into jams or deserts (Guimarães et al., 2010). In order to fulfill the nutritional needs of babies, children and infants fruit may be used in the form of juice, thereby providing essential micro-nutrients. Citrus juices are the most popular fruit juices, throughout the world, accounting for more than 50% of juices on the international market. Though freshly squeezed juice can be consumed, however, serious concerns are posed in this case owing to the contamination by fungi, bacteria, and other pathogenic microorganisms and certain native enzyme systems. As a result, functional ingredients such as bioactive compounds, taste, color, and odor have degraded, affecting the sensory properties and nutritional profile of juices. Therefore, to improve shelf-life and stability, it is important to process juice through some treatments (Guerrouj et al., 2016).

To control the enzymatic and microbial activities in citrus juices, thermal treatment such as pasteurization is used as a traditional method (standard) of juice processing (Aghajanzadeh et al., 2016). Juices are normally subjected to a high temperature of 90-98 °C for approximately 10-60 sec. These pasteurized processed juices are usually stable at room temperature and this treatment improves their shelf life due to the inactivation of enzymes and degradation of microbes. Thermal treatments are also used to preserve the organoleptic properties of juices (Wibowo et al., 2015).

The pasteurization of juices depends mainly on a reduction of 5 log for the most resistant microorganisms. In this process, heat is produced outside and then transferred to the food via the convection or conduction cycle (Chen et al., 2013). Owing to exposure of high temperature (high stress), a continuous increase in membrane permeability induced time-dependent changes (Altunay et al., 2016). As a result, changes in the protein conformation and lipid phase transitions have led to the degradation or death of cells. Moreover, changes in the structure of the membrane primarily depend on the type of thermal treatment being used (Petruzzi et al., 2017). The research was conducted using the high-temperature (thermal treatment) long-duration process for the treatment of acar (vegetable) juice. In this process, the juice was processed at 80 °C for 2 min and resulted in a significant reduction in the microbiota (Pokhrel et al., 2017).

It has been reported that the exposure of juice (grapefruit) to high temperatures may influence its nutritional properties (Uckoo et al., 2013). These researchers have exposed grapefruit juice to an elevated temperature of 85 °C for processing by thermal treatment. The exposure duration was 45 seconds and complete inactivation of microbial growth was observed as a result. Even at refrigeration temperature and storage duration of 21 days, no microbial growth has been observed.

Similarly, Sinchaipanit et al. (2015) have found adverse effects of traditional treatment. Guava juice was used in their research and the juice was subjected to a temperature of 85 °C for 1 min. Likewise other researches, this method also has the potential to reduce microbial growth to an acceptable level. Nonetheless, this analysis showed a decrease of up to 26% in vitamin C content of the juice. Overall, the reduction in vitamin C was in the range of 20-26%. In another research, juice (Jamun) processed via high-temperature treatment and juice was exposed to 80 °C for about 5 minutes. As a result, high rates of anthocyanin degradation have been observed (Shaheer et al., 2014).

The processing of cucumber juice has been done with traditional thermal treatment (Zhao et al., 2013). The juice was exposed to a high temperature of 85 °C while the exposure time was 15 sec. According to this study, the complete inactivation of yeast and mold was observed and even the amount of yeast was below the limit for 50 days of storage duration. Likewise, in another research, juice blends (grape and orange) were also processed by thermal treatment at 95 °C. The blend was enriched with hydrolyzed collagen and the researchers exposed the juice for 18 minutes. Exposure to this high temperature and for a long time results in the full inactivation of the naturally occurring microbiota found in juices (Bilek and Bayram, 2015). However, some drawbacks also arise from the processing of juices by traditional thermal treatment (Biata et al., 2017). The exposure of juices to high temperatures has been shown to have a detrimental impact on nutritional properties, i.e. a reduction in phenolic, flavonoid, and antioxidant content (anthocyanin and carotenoids) and vitamin (Petruzzi et al., 2017).

A beverage blend (litchi, lemon, and coconut) was prepared and then it was treated at 95 °C for 10 min (Jayachandran et al., 2015). Such conditions are successful against endogenous pathogens that cause the degradation of juices. These researchers have found that the quality parameters of the juice have decreased due to these conditions, in particular, a reduction in the ascorbic acid content was reported. Likewise, Santhirasegaram et al. (2015) also processed juice (mango) through thermal treatment. The temperature used for this reason was 90 °C while the juice had been exposed for 1 min. Researchers observed complete inactivation of the spoilage causing microbes, whereas the overall quality decreased as a decline in nutritional content was observed. Zheng and Lu (2011) used heat (90 °C for 1.5 min) treatment for the treatment of juice (pineapple). As a result, the overall ascorbic acid, antioxidant content, and phenolic contents have been substantially reduced. These studies have shown that thermal treatment is effective against spoilage causing microbes and enzymatic systems, and is an effective way to increase the shelf life of juices. However, the negative effects of high-temperature treatment resulted in a deterioration of the quality parameters of the juices. Significant changes in nutritional contents (i.e. phenolic content, ascorbic acid, and anthocyanin), as well as adverse effects on color parameters, have been observed in conventional treatments. As customers become more conscious of nutritional content and diet, there is a need to find a way to preserve the quality of juices. Therefore, non-thermal treatment has been suggested as an alternative to conventional treatment of juices (Roobab et al., 2018).

Impact of sonication on juices: Sonication has been used for juice treatment (strawberry) to access its effect on the microbial and physicochemical properties of the juice. 20 kHz of frequency at constant room temperature has been used for this purpose. The juice has been subjected to US treatment for 30, 15, and 0 (control sample) minutes. Sonication did not affect properties such as titratable juice acidity, pH, and TSS. Similarly, no effect on water activity and the color was observed as compared to untreated (control) juice. That study also revealed an improvement in turbidity and cloudiness (cloud value) due to the use of US, while the viscosity of the juice declined with the rise in the length of sonication treatment. Moreover, improvement of the bioactive compound was observed, particularly when the juice was treated with the US for 30 min. However, in this case, a total reduction in microbial load was not obtained, thereby indicating that the US alone is not sufficient for microbial load and may be useful for improved bioactive (anthocyanin) components of juices (Bhat and Goh, 2017). Bora et al. (2017) used the sonication technique to test its effect on the quality of banana juice. The processing duration of up to 30 min was used for this analysis. The researchers also use the enzymatic pre-treatment method. It has been found that using the US is the most effective approach for obtaining excellent yields of banana juice. The researchers revealed that US treatment increases the clarity of the juice. Thus, the US can be used to increase the yield of juice and to further boost the quality of bananas.

Many researchers have studied the impact of US on fruit juices. Research on guava juice treated with sonication and carbonation to evaluate the effect of the treatment on physicochemical properties (such as acidity, stability, color, pH, TSS, and ascorbic acid content), polyphenol oxidase and microbial stability (Cheng et al., 2007). It was found that the amount of vitamin C (ascorbic acid) increased significantly after US treatment compared with the control sample. In addition, this treatment also enhanced polyphenol oxidase activity. Cloudiness was also higher compared to the control that could be attributed to an increased amount of colloids, smaller particle size, and higher phenolic compounds. However, this coupling carbonation and US treatment were not enough at room temperature to kill or reduce the microbial load. Therefore, microbial safety could be a problem for this application, which must be kept in mind while applying sonication treatment for commercial level production. Generally, the physical and nutritional characteristics of fruit juices can be altered with sonication treatment (Ferrante et al., 2007)

Tiwari et al. (2008) processed orange juice with US treatment at various intensities of 8.61, 9.24, 10.16, 17.17, and 22.79 W/cm2. The ultrasonic equipment (probe type) was used for this purpose at a constant frequency of 20 kHz. Moreover, the treatment time of 2, 4, 6, 8, and 10 min was used for the processing of juices. These researchers have not noticed any significant change in titrable acidity, pH, and Brix of orange juice. However, US treatment results in major changes in cloud value, color parameters, and browning index. Thus, the preservation of basic quality parameters, such as TSS and pH, was observed in this study.

Gómez-López et al. (2010) applied US treatment on orange juice added with calcium. This procedure was used at a frequency of 20 kHz and with processing durations of 2, 4, 6, 8, and 10 min. The content of ascorbic acid, sensory characteristics, hunter color value was evaluated along with microbial stability checks. The wave amplitude was 89.25 μm (8 min) for final treatment. In addition, the storage study was also carried out at 2 different (4 and 10 °C) temperatures. Microbial load reduced as a result of this treatment. Also, degradation of juice sensory qualities was observed after the application of sonication treatment. Moreover, significant deterioration of calcium added orange juice was observed during storage. After 10 days of storage, the sensory panel rejected the ultrasonic sample mainly due to the off-odor problem. However, a controlled sample (without any treatment) was rejected only after 6 days of storage. Thus, US treatment increases shelf life by 4 days. In addition, these authors also observed the degradation of color properties and a decrease in ascorbic acid content. Despite some drawbacks, these authors proposed sonication as a possible technique to improve juice shelf-life and nutritional content (Cao et al., 2010).

Sonication has also been used for the treatment of Citrus microcarpa (Kasturi lime) fruit juice (Bhat et al., 2011). In this study, the juice was processed at 25 kHz for the duration of 0, 30, and 60 min, and the impact of US on the treated sample was assessed. It was observed that even 60 min sonication did not cause major changes in titrable acidity, pH, and TSS of Kasturi lime juice. Moreover, an increase in total flavonoid, phenolic, ascorbic acid, flavonol, and antioxidant potential has been observed. US treatment of 60 min results in an increase in bioactive compounds compared to 30 min of treatment, which means that an improvement in treatment time often leads to an increase in bioactive compounds in juices. In addition, this method also results in a significant reduction of the microbial load (viable count). Thus, according to Bhat et al. (2011), sonication treatment can be used not only to maintain the quality attributes of fruit juices but also to achieve the desired safety and quality standards.

Sonication treatment has been shown to be successful against different enzymes found in juices. Fonteles et al. (2012) observed that a reduction of the major enzymatic activities of cantaloupe melon juice can be attained by using an ultrasonic processor operated at 19 kHz with 500 W power. For this purpose, the juice was exposed to an ultrasonic treatment with 75, 226, and 376 W/cm2 intensities. It was observed that applying US treatment at 376 W/cm2 for 10 min would significantly inactivate different enzymes, such as polyphenol oxidase, peroxidase and could reduce ascorbate peroxidase activity completely. While no change in color was reported in this study, however, authors observed a 30% reduction in phenolic compounds. Instead, sonication treatment also enhanced the homogeneity of melon (cantaloupe) juice. These studies show the capabilities of sonication treatment.

Aadil et al. (2013) applied sonication treatment at 28 kHz using an ultrasonic bath and treated grapefruit juice. The treatment has been applied for 90, 60, and 30 minutes. Improvements were seen in the selected parameters, although no significant effects on acidity, total soluble solids (TSS), and pH were reported. However, a substantial increase in electrical conductivity was observed and this was due to the fact that the use of sonication increases the vitamin and mineral content. Nevertheless, after sonication, an increase in ascorbic acid was noticed due to the removal of trapped oxygen by cavitation. In addition, sonication also increases the CV of the juice, due to the break-up of larger molecules into smaller ones. Overall, an increase in phenolic compounds and vitamins has been observed.

Abid et al. (2013) also treated the apple juice with an ultrasonic bath cleaner at 25 kHz frequency. The temperature was kept constant at 20 °C, exposing juice for 0, 30, 60, and 90 min. US power was set at 70%. The water circulation (1.5L/min) kept the temperature of the sample maintained. The juice was immediately poured into sterilized and airtight bottles covered with aluminum foil after treatment was applied. Subsequently, treated sonicated juice was stored for further study while storage temperature was maintained at 4 °C. The authors observed the effects of US on the microbial quality and physicochemical properties of apple juice. Like other researches, the US treatment was unable to make substantial changes in apple juice pH, titrable acidity, and TSS. The increase was observed for total flavonols, phenolic content, and flavonoid. Moreover, increases in CV, free radical (DPPH) scavenging activity and antioxidant capacity were also observed under similar conditions. The US has also been able to reduce microbial activities suggesting that this method can be effective in processing apple juice whilst retaining product quality attributes.

The juice extracted from pomegranate and arils was used to assess the effect of US on the blended juice. Sonication was performed at 100, 75 and 50% amplitude and constant room temperature conditions were used. The juice was processed for the duration of 0 (control), 3, 6, and 9 minutes, and the effect on the different physiochemical properties of the juice was tested. Decreased total anthocyanin was detected, which ranged from 0.38 to 9.75%. However, an increase in anthocyanin at some time and amplitude was observed, which was between 0.44 and 7.32%. In resemblance to other studies, this treatment did not affect the acidity, pH, and TSS despite different durations and strengths. At the treatment duration of 9 min and 100% amplitude, an increase in total phenolic was observed between 5.40 and 42.52%. Interestingly, the antioxidant activity of each sample has not changed in comparison to control (Alighourchi et al., 2013).

Similarly, Golmohammadi et al. (2013) subjected red raspberry (from squashed berries) puree to US treatment, whereas 20 kHz of frequency was used. Microstreaming at 490 and 986 kHz frequency ranges were investigated in order to determine the total antioxidant activity, phenolic, and anthocyanin content. The purée fruit was processed for 30 minutes of treatment. With a frequency of 49 kHz, the temperature has risen to 56 °C. The frequency levels between 20 and 490 kHz have an important impact on anthocyanin, antioxidant, and total phenolics of puree. Sonication treatment did not cause any major changes in anthocyanin and antioxidant activity when applied (20 kHz) for the duration of 30 and 10 min. However, using the 20 kHz 986 frequency, 9.5 and 10% increase in phenolic content was reported after 30 min of duration. After 10 minutes of treatment, anthocyanin and antioxidant were also increased by 12.6% and 17.3% respectively. US treatment at 20 kHz (frequency) and treatment duration of up to 10 min was also found to be appropriate for the extraction of bioactive components. However, the application of various amplitudes may also affect the fruit of the raspberry.

Santhirasegaram et al. (2013) investigated the impact of sonication treatment on the juice (mango). The US was applied at 40 kHz of frequency for the duration of 60 and 30 and 15 min, while the temperature was held at 25 °C. In this research, various quality and physicochemical properties of the sonicated sample were compared with thermal treatments. Such authors have observed no major changes in titratable acidity, pH, and TSS in both thermal and US treated samples. The sonication application improved the polyphenol and carotenoid content by 30-35% and 4-9%, respectively. Moreover, radical scavenging activities of the sample also increased after the treatment. In addition, a substantial reduction in microbial counts was also observed in both sonicated and thermally treated juices. These researchers concluded that the US has the potential to maintain the quality attributed to mango so that it can be used as an alternative treatment, but further studies are required to ensure safety standards.

Šimunek et al. (2013) applied US processing on apple juice and nectar. The sonicator frequency was kept at 20 kHz during different processing durations (3, 6, 9 min). The sonication amplitude used in this study was 60, 90, and 120 μm. These authors have reported that the sensory properties of raw juice and US treated juice are different from each other. It was also found that treatment via the US also results in the generation of certain aromatic compounds that were previously absent in the sample. Ultrasound-based sterilization was used to replace thermal treatment in fruit and vegetable juices in order to evaluate their microbiological and quality parameters. The most widely used sonication frequency in this regard is 20 kHz. Different samples of vegetable juice such as spinach juice were treated with sonication treatment. The goal was to increase juice shelf life without having a significant impact on the nutritional content of the juice. The juice was put in 5 L container and its impact on microbiological and quality parameters was evaluated. It has been observed that the sonication is very useful for the treatment of such vegetable juices and can be used to enhance the shelf-life of spinach juice. These researchers concluded, that the initial cost of ultrasonic technology is the major obstacle in this implementation at the industrial level, however, further research is needed to make sonication technology as an alternative to thermal treatment.

Ertugay and Başlar (2014) assessed the US impact on apple juice parameters related to cloud quality. Sonication treatment at 25 kHz was performed with an ultrasonic processor with a probe of around 22 cm for the duration of 5 and 10 min. It has been found that US has the potential to reduce coarse apple juice particles and can improve the quality of its cloudiness. The rise in cloudiness level and cloud stability was observed using sonication at high temperature, amplitude, and time, which was 16.9 times, and 9.8 times respectively. The US treated apple juice was stored at 25 °C for 4 months and cloud stability levels of the juice were observed without any further processing. This procedure also contributes to the complete inactivation of both yeast and mold, which shows the efficacy of sonication processing.

In another study, Dias et al. (2015) analyzed the effects of sonication on the quality of soursop juice. These researchers exposed the juice at a constant frequency of 19 kHz, with an exposure duration of 2-10 min. The amplitude level ranged from 20 to 100% of the total input power of 500 W. On the other hand, the applied sonication power was 75, 118, 224, 330, and 373 W/cm2. These authors have observed a decrease in the enzymatic activity of polyphenol oxidase (PPO). This procedure has been able to maintain good phenolic compounds even at high intensity. In addition, sonication also increases vitamin C content. It was found that an increase in intensity and exposure period also results in an increase in the temperature of the juice. At optimal (330 W/m2 power intensity and 9 min) processing conditions the sensory results of the US treated samples showed overall acceptance of the juice.

Similarly, Mohideen et al. (2015) applied continuous US treatment for the processing of juice (blueberry). The purpose of this research was to determine the microbial and physicochemical properties of the juice after sonication treatment. Sonicated samples were compared to non-treated samples (control). The authors have found that the processing of blueberry juice using the US can retain the nutritional quality of the juice, in particular the anthocyanin (antioxidant) content. This technique was also capable of improving the bioactive compound in juices. Besides, the color of the juice was also retained as a result of this treatment. More important than that, in this situation, the Brix, pH, and titratable acidity of the juice remained unchanged. It was revealed that microbes could be destroyed by a higher intensity continuous flow sonication system. The highest log reduction was observed in the aerobic microbial count where the sonication treatment was able to reduce the aerobic count by 1.33 log. The authors, therefore, concluded that US can be used for the processing of juice (blueberry) to retain the nutritional content of the juice.

Carbonell-Capella et al. (2016) assessed the US impact on juice blend. Blend juice was prepared for nutritional enhancement using the papaya and mango while stevia was also added in this juice blend. These researchers also compare sonication treat with the treatment of the pulsed electric field. The researchers found that PEF is ideal for treatment, however, sonication treatment was able to maintain more flavonol, flavonoid and phenolic content and other biologically active compounds found in juices. Additionally, because of the release of intracellular juice content, sonication was able to increase the bioactive compounds.

Guerrouj et al. (2016) used US treatment for the processing of juice (orange). The sonication was applied at 25 kHz for the duration of 1, 10, 20, and 30 min, and the nutritional and physicochemical properties of the juice were evaluated. During this procedure, the juice temperature rose to 43-45 °C. The viable microbial count of the sample was found to have decreased significantly. In addition, this treatment increased the total anthocyanins, carotenoids, phenolic, flavonoids, and ascorbic acid content, thereby increasing the overall bioactive content of the samples. It also indicates an increase in the anti-oxidant level of the juice (orange) sample. But, the microbial load reduction was not adequate for the preservation of fruit juice. However, the authors proposed that the sonication treatment could be used as an alternative treatment to maintain the nutritional content of juices (Khandpur and Gogate, 2016).

In another research, Rojas et al. (2016) processed juice (peach) using US treatment. The sonication equipment used had a maximum power of 1000 W and the frequency was set at 20 kHz. The juice has been exposed to US for the duration of 0 (control), 3, 6, 10, and 15 min. Provided that, the temperature was held at 22°C. These researchers observed important changes in the physicochemical properties of juice (pear). US treatment disrupts the cellular structure resulting in the release of the intracellular structure. It has been observed that sonicated samples have reduced polysaccharide size, reduced particle size, disruption of whole cells accompanied by dispersion of contents. These effects are treatment time-dependent. Such modifications determine the turbidity, rheological properties, and pulp sedimentation in the treated juice sample. Researchers concluded that the US could be used to enhance the physical properties of peach juice.

Saeeduddin et al. (2016) used pear juice and treated it with the sonication technique. The sonicator frequency used was 25 kHz with 70 percent amplitude, whereas the overall sonication power was 500 W. The juice was treated with US for the duration of 0 (control), 15, 30, 45, and 60 min while the temperature was kept at room temperature. Their research verified that the US treatment did not affect titrable acidity, Brix, and pH. Although, an improvement in total phenolic, flavonoid, ascorbic acid (vitamin C), and antioxidant (such as anthocyanin and carotenoids) values was observed. However, sugar content and minerals such as Na, K, Fe, and Mg have also been improved. However, there has been no increase in calcium. Similarly, microbial activities and the amount of copper and phosphorus have been shown to decrease. According to these researchers, 60-minutes of ultrasonic treatment showed optimum results in terms of microbial inactivation and physiochemical quality.

Similarly, Chitgar et al. (2017) also used US treatment for the processing of barberry juice. They used a batch sonication method with a 20 kHz frequency and a 13 mm probe. The temperature was maintained at 25 °C with the help of circulating (flow rate 0.5 L/min) water. The amplitude of the sonication system was 70 and 100% while the processing time was 10-15 min. Moreover, the pulse duration was set at 5 sec On and 5 sec Off. After treatment, the juice was put in a sterilized bottle and stored at 4 °C. As a result, there was a significant decrease in the microbial load of barberry juice. Sonication also improved the overall antioxidant and phenolic content of the juice. Less effect on anthocyanin and juice color was observed when treated at a 70% amplitude relative to thermal treatment. It was found during storage that, in thermally treated samples the anthocyanin content decreased more rapidly than in the US treated samples. These authors concluded that the non-destructive impact of the US could lead to its use as an alternative to thermal treatment. However, temperature, time, and other processing conditions would have to be optimized to fully utilize the ultrasonic treatment capabilities and further studies are required for this purpose.

Ordóñez-Santos et al. (2017) used ultrasonic treatment (40, 20, and 10 min) for the processing of gooseberry juice. For this purpose, an ultrasonic cleaner with a frequency of 42 kHz and a maximum power of 240 W was used. These researchers have found that US treatment induces a substantial increase in the total content of carotenoid, phenolic, and retinol in the juice sample. Interestingly, a large decline in ascorbic acid has been observed in this situation. Besides, the color of the US treated juice was changed from yellow to yellow-red. Such studies have suggested the use of US as a possible alternative to thermal treatment.

Tremarin et al. (2017) introduced apple juice to the US to determine its impact on microbial quality. These authors evaluated the inactivation of licyclobacillus acidoterrestris. On the other hand, these researchers also used ultraviolet (UV) treatment to make a distinction between these two treatments. The thermal treatment was used in this process as a control sample. The result shows that the US can reduce the activity of the microbe, but it was lower than the result obtained by ultraviolet treatment. For both cases, the inactivation rate was not adequate. However, when these treatments were combined, a better result was achieved which could lead to the conclusion that sonication treatment can help reduce the microbes in apple juice.

Tomadoni et al. (2017) used the US as an innovative treatment for the processing of strawberry juice. For this purpose, these authors applied 40 kHz of sonication for the duration of 10 and 30 min and an ultrasonic bath with a capacity of 6.5 L was used for this treatment. The temperature was kept at 20 °C and the results were compared with conventionally (thermal) treated (90 °C for 60 sec) juice. Compared to untreated (control) and thermally processed samples, ultrasound affected the color and off-odor scores. However, the flavor attributes (acidity and sweetness) were greatly influenced by the treatment used. On the other hand, no major change in physicochemical parameters was observed, as the US did not affect pH, total acidity, and TSS. In this study, higher levels of anti-oxidant activity and polyphenols were found in sonication treated samples indicating higher nutritional properties of the juice. Furthermore, sonication also reduces significant microbial activity compared to untreated (control) samples, resulting in the increased shelf life of the juice. The shelf life (in storage) of the US treated sample was more than 10 days. As a whole, thermal treatment was effective in decreasing further microbial loads while sonication was successful in preserving the nutritional quality of the juice (strawberry).

Recently, Campoli et al. (2018) used the sonication approach to process guava juice. The frequency of sonication equipment was held constant at 20 kHz with a maximum output of 1000 W. Processing time for this treatment ranged between 3 and 9 minutes. It has been found that sonication treatment of guava cells results in the release of cell contents, thus, altering juice properties. As a result, an improvement in the bio-accessibility of lycopene content was observed despite the fact that this treatment reduced the lycopene content. Sonication treatment also reduced the particle size of juice components which results in improved physical stability of the guava juice. As a result, no major color changes were observed even during storage.

Non-thermal technologies are gaining popularity among researchers to address the drawbacks caused by heat treatment (Aadil et al., 2013). Among them, the US is a novel non-thermal treatment involving the application of sound waves having a frequency of more than 20 kHz. The US has shown potential for its application in the food processing industry and can achieve the required degree of microbial protection while maintaining the nutritional quality of fruit juices. The effects produced by the US are linked to the cavitation cycle (Yamini et al., 2012). As the US moves through a liquid, the pressure fluctuation is generated as a result of alternating rarefaction and compression cycles, which depend primarily on the application of adequate sound wave strength. Upon achieving adequate intensity, the US results in the generation of bubbles (cavities) in liquid. Such bubbles undergo various processes, resulting in enlargement, followed by the collapse of these bubbles, creating hotspots of high pressures (up to 500 atm) and high temperatures (about 5000 K). Most significantly, the controlled application of the US is sufficient for the desired level of inactivation and a minimal reduction in nutritional quality (Khandpur and Gogate, 2015).

Impact of thermosonication on juices: Although sonication treatment was found to be successful in preserving the quality of fruit juices, it was not effective in reducing the adequate microbial load (Guerrouj et al., 2016). Some researchers, therefore, recommended that sonication should be used with other non-thermal (i.e. pulsed electrical field) treatments or with mild heat (thermoultrasound, TS) treatment in order to decrease microbial loads up to safety level and for reducing the impact on the nutritional characteristics of juice (Chitgar et al., 2017).

Wu et al. (2008) treated tomato juice with the help of TS at amplitudes of 25, 50, and 75 µm. The frequency of ultrasonic equipment was 24 kHz and temperatures were 60, 65, and 70 °C. Thermosonication at 70 °C for 4.3 min reduced the activity of PME by 90%. The same results were achieved when treatment conditions of 60 and 65 °C were applied for 41.8, 11.7 min respectively. It was found that different intensities application does not have any effect on the inactivation of PME. Moreover, TS decreased the particle size of juice and also result in increased viscosity of the juice.

Rawson et al. (2011) utilized TS for the treatment of watermelon juice. They took freshly squeezed juice and exposed it to an ultrasonic frequency of 20 kHz with a processing time of 2-10 min. The temperature was maintained in the range of 25-45 °C. The amplitude level of ultrasonic equipment was 24.1-60 µm and the pulse cycle was 5 s on/off. These authors revealed, at low temperature higher retention of ascorbic acid and lycopene can be observed. The ascorbic acid, phenolics, and lycopene content were in decreasing trend as the intensity of the US and exposure time were increasing. Thus, a significant effect of sonication with thermal treatment was observed on quality parameters of watermelon juice.

Pineapple, grape and cranberry juices were subjected to TS performed at 24 kHz with a power of 400 W (Bermúdez-Aguirre et al., 2012). The temperature used for this research was 40 °C, 50 °C and 60 °C while the time duration was 10 min. It was found that processing at 60 °C is effective for the inactivation of Saccharomyces cerevisiae. However, significant changes in color and pH were observed which indicates that US may promote the chemical reaction and can extract some components. It was observed that pineapple juice required a higher amount of energy for this treatment while the lowest energy is required by the grape juice. Thus, ultrasonic treatment can be viable for pasteurization purposes (Bermúdez-Aguirre et al., 2012).

TS has been employed in musambi juice in order to inactivate PME (Siwach and Kumar, 2012). These authors sonicated the juice and applied temperature of 60, 70, and 80 °C for 5, 10, 15, and 20 min. It was found that the processing time of 20 min and the temperature range of 80 °C is suitable for a sufficient reduction of PME. Saad et al. (2013) processed two fruit juices (guava and apple), using the TS method. This research was carried out at 20 kHz and the exposure time for juices was 10, 5, and 2 min. Moreover, the variable temperature used for this study was 50, 35, and 20 °C. The amplitude level was maintained at 40, 30, and 21% and the pulse duration was 5 s On and 5 s Off. It was found that TS did not affect juice acidity, pH, and TSS. Maximum retention of quality parameters was achieved at 40% amplitude with 10 min of treatment duration. In addition, stability in the color of juices was also achieved in this treatment. On the other hand, this approach has been effective in both reducing browning and turbidity. Thus, US treatment with mild temperatures can also be used for the preservation of guava and apple juices.

Abid et al. (2014a) applied TS to apple juice and evaluated its quality parameters. Thermosonication was conducted in ultrasonic baths at the frequency of 25 kHz for 30 min. They also applied sonication (20 kHz sonicator) for the duration of 6 and 10 min. The temperatures used for this treatment were 60, 40, and 20 °C. Significant decreases in polyphenolase (PPO), peroxidase (POD), and pectin methylesterase (PME) have been observed. In addition, this treatment was also effective against the microbial load. In this study, the sonication treatment with a probe was found to be effective against enzymes at 60 °C. Complete inactivation of microbial activities was also achieved at this temperature. TS with both forms of sonicators has been found to be effective in the retention of bioactive compounds (phenolic compounds, ascorbic acid, flavonoids, and flavonols). Moreover, treatment with ultrasonic probe equipment was more effective in the preservation of bioactive compounds compared to ultrasonic baths. These researchers concluded that low-temperature TS is an efficient way of preserving the nutritional quality and to inactivate certain enzymes and microbes of apple juice.

Cruz-Cansino et al. (2015) used TS for the processing of pear (purple cactus) juice. The juice was subjected to a sonication probe of 13 mm with a frequency of 20 kHz (80% amplitude). In addition, the processing duration was 15 to 25 min. The juice was compared with the untreated (control) sample and thermally processed juice sample, which was treated at 70 °C for 30 min. The aim of this study was to assess the impact of TS on microbiological activities, shelf life, antioxidant, and color properties of juices. In all cases, a significant decline in microbial counts has been observed. However, a lower reduction in the activities of PME (enzyme) was found relative to pasteurized juice samples. Moreover, TS treated pear juice retained higher levels of vitamin C and showed a higher level of antioxidant value relative to other juices. A minimal increase in PME activities was observed during storage. However, sonicated samples showed similar activities of total plate count as thermally (pasteurized) treated juice. After 14 days of storage duration, a rise in phenolic content was also observed, although higher antioxidant activities were achieved at the end of storage.

Herceg et al. (2015) utilized the method of TS and applied it for the treatment of strawberry juice. The juices were treated using the ultrasonic probe and frequency maintained at 20 kHz. Variable temperature (25, 40, and 55 °C) and processing time of 3, 6, and 9 minutes were used. On the other hand, pasteurization purpose was achieved by using temperature 85

°C for 2 minutes. As a result of thermal treatment, inactivation of all microbes, and reduction of anthocyanin content (7.1-7.4% reduction) were observed. TS reduced microorganisms in the range of 58 to 94% while lower reduction of anthocyanin was observed in this case. Thus, thermosonication was effective in the retention of valuable components. It was revealed that increasing temperature and processing time inactive microorganisms more effectively but also decrease more anthocyanin present in strawberry juice.

TS was also used for the treatment (using sonication cleaner) of grapefruit juice (Aadil et al., 2015). The sonication frequency was set at 28 kHz and the experiment was performed at variable (20, 30, 40, 50, and 60 °C) temperatures. The power was 70% with a maximum output (420 W) and the treatment duration was up to 60 min. These authors reported that microbial activity (total plate count, yeast, and mold) was completely inactivated at 60 °C and 60 min. On the other hand, under similar conditions (60 °C and 60 min), the enzymatic activity of peroxidase (POD), pectin methylesterase (PME) and polyphenolase (PPO) decreased by 90%, 91%, and 89% respectively. In addition, TS was also efficient for the retention of bioactive compounds (phenols, flavonoids, flavonoids) and the enhancement of anti-oxidation activities. These authors concluded that US with heat treatment at lower temperatures could be useful for the processing of juices (grapefruit) and that better microbial protection was observed compared to sonication treatment alone (Aadil et al., 2015).

Jabbar et al. (2015) checked the effect of TS on the carrot juice processing and compared it with thermally treated carrot juice. The sonication was applied at a frequency of 20 kHz with a 70% amplitude and intensity of 48 W/cm2. To complete TS, the temperature applied was 20, 40, and 60 °C while exposure time was 5 and 10 min. Additionally, the pulse cycle was set at 5s on and 5s off. On the other hand, thermal treatment (using a water bath) for 1 min was applied at 80 °C. The result revealed that thermal treatment along with sonication is effective for reducing enzymes (PME, PPO, POD) as well as against microbial load (total plate count, yeast, and mold). The highest inactivation of both microbes and enzymes was achieved at 60 °C for both TS and thermal treatment. However, at a similar temperature, TS samples were effective in the retention of various bioactive compounds. Moreover, improvement in coloring pigment was also observed at 60 °C. Other quality parameters like pH, TSS, and titratable acidity were remaining unchanged irrespective of treatment applied. These authors suggested that TS (60 °C) could be a thermal treatment alternative for carrot juice production, to reduce its microbial and enzymatic activities and for retention of bioactive compounds.

TS was applied with essential oil (cinnamon leaf), treated at mild temperature (50 °C), for the processing of orange and pomegranate juice (Sánchez-Rubio et al., 2016). The frequency and intensity of the sonication treatment were 24 kHz and 33.31 W m/L and the processing duration was 30 minutes. The temperature was maintained at 50 °C. The purpose of this study was to examine the effect of TS on Saccharomyces cerevisiae during storage and post-processing. Juice samples were kept at refrigeration temperature (5 °C) for 28 days. This research has been successful in achieving a significant decrease in the yeast count. The most effective result was that combined (50 °C for 30 min) treatment and it achieved the most reduction in the yeast population. These researchers achieved a reduction in S. cerevisiae during the storage time of 28 days. On the contrary, during the storage, no reduction in the yeast population was achieved compared with the control sample (Sánchez-Rubio et al., 2016). US alone can also be effective in carrot juice processing. (Zou and Jiang, 2016).

Sulaiman et al. (2017) evaluated the effect of US, thermal treatment (thermoultrasound), and pressure treatment on the strawberry puree. The thermal treatment was applied at 65 °C for 15 min. While the US frequency was 24 kHz and the temperature was maintained at 33°C. Moreover, the pressure treatment of 600 MPa at 60 °C was also applied. This treatment was able to inactivate the PPO enzyme. The processed product was also placed in storage at refrigeration temperature. No further reactivation of the enzyme was found in this case. Moreover, no change in pH and TSS were observed which indicates the microbial safety of the product. It was observed that pressure and US were able to inactivate enzyme more effectively as compared to thermal treatment. Moreover, it was observed that US treatment alone treatment can enhance antioxidant activities and have the ability to retain the color attributes of the product.

Türken and Erge (2017) utilized TS and applied at a moderate temperature for the treatment of sour cherry juice. The aim was to evaluate the effect on US on some chemical and microbiological properties of sour cherry juice. Ultrasonication was applied with varying amplitude levels with 50%, 75%, and 100%. The treatment was applied for 2, 6, and 10 min while keeping the frequency at a constant level of 20 kHz. At the same time, the effect of different temperatures (20, 30, 40 °C) was also evaluated. It was found that TSS, titratable acidity, and pH of the juice were not affected by any time, temperature, and ultrasonic amplitude. Upon increasing temperature and amplitude, the increase in anthocyanin content was also reported. As the temperature rise, the significant increase in phenolic content also occurred. It was found that the ultrasonic amplitude also impacted the antioxidant capacity of sour cherry juice. Moreover, by increasing time, temperature, and amplitude level, an increasing trend in color parameters was also found. These results showed the ability of ultrasonic treatment especially upon combining mild heat treatment. In addition, decreased activity of Escherichia Coli was also observed. Rise of every parameter used in this study which suggests the significant effect of this treatment on juice microbes.

Researchers have also assessed the impact of TS on different parameters of star fruit juice (Nayak et al., 2018). For this study, the juice was treated using an ultrasonic bath at various (25–45 °C) temperatures and a maximum ultrasonic power of 500 W. The star fruit juice was treated at a fixed frequency of 44 kHz and a duration of 15, 30, 45 and 60 min. Dark conditions were maintained in order to avoid any light interaction. After processing, the sonicated samples were poured into sterilized bottles and kept at a refrigeration temperature of 4 °C. On the other side, traditional thermal treatment was used and the juice sample was pasteurized at 90 °C for 60 sec. The pasteurization temperature was in compliance with industrial requirements for the reduction of 5 logs of microbes. The authors found that there was no major improvement in titrable acidity, TSS, and pH relative to raw juice. But, an increase in bioactive compounds have been observed. In addition, the findings showed that a large increase in cloud value and the browning index was also observed as a result of this treatment. It was concluded that TS and pasteurization have been effective in reducing microbial loads (Nayak et al., 2018).

Liao et al. (2018) used TS to improve the shelf life of fresh apple juice. In order to improve the preservation impact, the researchers also used nisin. Variable temperatures (37, 42, 47, and 52 °C) were used for the duration of 5 and 40 min. The role of temperature in the deactivation of mold, yeast, and aerobic bacteria was found to be significant. After TS at 52 °C and 30 min, the original quality of fresh juice as ascorbic acid (89% retention) content was reported. The change in color was not noticeable though pH, acidity, and Brix were not affected by any treatment applied. The authors have been successful in extending the shelf life of the juice for up to 15 days (8 °C storage temperature). In addition, the application of nisin results in the inactivation of aerobic bacteria, with no noticeable impact on mold and yeast. This study indicates the possible use of TS to increase the shelf life of apple juice.

Impact of sonication with other non-thermal techniques: Various other treatments have been employed aiming to process juice like orange juice. These may include treatment of US with other non-thermal treatments, thus, explained the potential of other technologies. A combination of sonication and high-pressure processing has been applied for the treatment of prebiotic cranberry juice (Gomes et al., 2017). The juice used in this study was fortified with fructo-oligosaccharides in order to enhance its nutritional value. Ultrasonic treatment was applied with an intensity of 600 and 1200 W/L for 5 minutes. Subsequently, high-pressure processing was applied for 5 min at 450 MPa. The physicochemical analysis was carried to evaluate pH, TSS, acidity, anthocyanin content, color, organic acid, antioxidant capacity, and fructo-oligosaccharides. It was found that both treatments were able to maintain the fructo-oligosaccharides content which represents retention of fortified materials. Moreover, more than 90% of organic acids were retained while improvement in anthocyanin content was observed especially when combined treatment was applied. Other than these, no major change in pH, TSS, and instrumental color occurred. This study showed that high pressure and US can be applied for the treatment of prebiotic cranberry juice (Gomes et al., 2017).

Leizerson and Shimoni (2005) explained the scope of ohmic heating and its effect on orange fruit juice quality. The quality of ohmic heating treated juice was compared to heat pasteurization at 90 °C. In an ohmic heating system temperature employed was 90, 120, and 150 °C, and the processing time was 1.13, 0.85, and 0.68 sec. The authors found that ohmic results in complete inactivation of microbes like bacteria, yeast, and mold. Similar results were found in the case of thermal treatment only. Also, the pectinesterase activity was reduced by 98% as the result of ohmic treatment. It was revealed that ohmic and thermal treatment decrease the microbial count by at least 2-3 orders of magnitude as compared to untreated (fresh) orange juice sample which was 102.5 colony-forming units (CFU)/mL. However, more studies are required to achieve more accurate results. The reduction of 15% in vitamin C content was observed in thermal treatment. On the contrary, ohmic heating successfully retained a higher amount of flavor compound. Moreover, sensory evaluation of treated and untreated samples showed no significant difference. Thus, it was concluded that orange juice can be pasteurized using ohmic heating with minimal sensory deterioration (Vikram et al., 2005).

Similarly, Yildiz et al. (2009) studied ohmic heating as an alternative method for pomegranate juice. This study aimed to develop a non-thermal technique to get benefits from more nutritious juice. The results were compared to conventional thermal treated juice samples. The application of ohmic heating was conducted with a voltage gradient of 10-40 V/cm and 50 Hz. The juice samples were heated in the temperature range of 20 and 90 °C while the processing time was 0, 3, 6, 9, and 12 min). Initially, change in rheological properties, color, and total phenolic contents were observed, however, during the holding period, no significant change occurred. These authors suggested ohmic heating as alternative fast-heating method for juices, especially fruit juices. Similarly, ohmic treatment was employed in grape juice processing. The processing conditions were voltage of 10-15 V/cm while temperature was varied from 25-80 °C. These authors observed no difference in basic physiochemical parameters of juice, whoever, an increase in electrical conductivity of juice was observed. Moreover, temperature of the sample was also increased as the result of this treatment (Assawarachan, 2010). These studies showed that other non-thermal treatment can be proven effective for processing of fruit (orange) juices (Darvishi et al., 2013).

Abid et al. (2014b) used high hydrostatic pressure with ultrasonication treatment for apple juice treatment. This study was carried out for assessing the impact of combined treatment on the physicochemical characteristics of juice (apple). The US treatment was applied at 25 kHz, 70% amplitude at room temperature. The ultrasonic treatment was applied for 60 min. On the other hand, high hydrostatic pressure was applied with 250, 350, and 450 MPa pressure while this treatment was applied for 10 min. When exposed to 450 MPa, the highest inactivation of juice enzymes was achieved. As a result of this treatment, improvement in antioxidant activity, antioxidant capacity, color as well as ascorbic acid content were achieved. It was concluded that combine pressure and US treatment ineffective in reducing microbes and enzymes while keeping the nutritional value of apple juice.

US with high pressure is another novel method that can treat juices. Attempts have been made to inactivate the mold using combine US and high-pressure treatment (Kim et al., 2016). In this study, a specific acidic food spoiling mold was targeted to inactivate. The apple juice was used as a sample in this case. The authors used ultrasonic treatment of 24 kHz at an intensity of 0.33 W/L. On the other, high-pressure processing was used at 600 MPa. The thermal treatment was also combined in this method which was applied at 75 °C. The authors found that when the temperature is used along with pressure it results in higher inactivation of the mold. However, thermosonication did not cause any significant destruction of the mold. These results showed that pressure could be an alternative way to protect high acidic foods like juice as it is capable of higher spore inactivation.

Bot et al. (2017) performed an experiment to check the feasibility of high-pressure homogenization and US on tomato juice. The pressure of 150 MPa while US treatment was applied for 30 minutes. Different concentration of juice was used in this study. This treatment was the reason for the consistency of tomato juice. The sample with 10 TSS was more effected by high-pressure treatment as compared to sonication processing. This study showed the ability of combined treatment for juice processing.

Thus, sonication can be combined with other treatments to find a novel way for the treatment of fruit juices. These treatments can be used to inactivate enzyme (i.e. PPO), destruct microbes (total microbial count, yeast, and mold). Most importantly, they can retain the quality attributes of fresh juice. Also, they can be used to enhance bioactive compounds (carotenoid, phenol, polyphenols, flavonols, flavonoids and ascorbic acid and organic acid, etc.) availability. However, the optimization of treatment is still needed in order to make it effective at the commercial level. Overall, these treatments are energy savings, cost-effective, and can scale up to industrial level but further studies are required in order to make the initial equipment cost more feasible for industrial level and to fully understand its effect on specific food bioactive components (Anaya-Esparza et al., 2017).

Aadil et al. (2018) used the combined ultrasound and pulsed electric filed aiming to treat grapefruit juice. At first, the pulsed electric field (80 mL/min flow rate) was employed, and a pulse frequency of 1 kHz, 20 kV/cm EF strength. The temperature was maintained at 40 °C. Then the juice was subjected to an ultrasonic bath (28 kHz frequency) with 600 W power for 30 min. As a result, the microbial load was reduced significantly. Moreover, this combined treatment increased carotenoids, lycopene, anthocyanin contents. Ascorbic acid was also retained as the result of these treatments suggesting that such combined treatment can be effective against spoilage causing microorganisms and can also retain bioactive components present naturally in fruit juice.

MATERIALS AND METHODS

Chemicals and reagents: This study was carried out in the laboratories of the National Institute of Food Science and Technology (NIFSAT), Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad.

Fresh and fully mature citrus fruits including Kinnow and sweet lemon were bought from the local market of Faisalabad. The good quality fruits were brought to the laboratory where they were further processed.

Sample preparation

Peeling and juice extraction: The peel of both citruses was removed manually and then fresh juice of fruits was obtained by squeezing the fruits with the aid of juice squeezer. Before any application of sonication or thermosonication, juices were filtered through a double-layer muslin cloth and filtered juice was used for further treatments.

Sonication treatment: The sample of both juices was separately treated using an ultrasonic homogenizer. Firstly, juices were poured in the 250 mL beaker and placed in an ultrasonic chamber while the probe was immersed in juice without touching the wall of the beaker. The juice samples were processed under constant ultrasound frequency of 20 kHz (70%) for 5 and 10 minutes. All samples were treated under darkness in order to avoid any intervention caused by light.

Thermosonication treatment: TS was applied at 40 °C for 5 and 10 minutes using the ultrasonic homogenizer with 420 W power, 70% amplitude, and 20 kHz. The sample was taken in 250 mL beaker and treatments were applied in dark conditions. Both sonicated and thermosonicated samples were poured in airtight and sterilized bottles for further analysis.

Titratable acidity (TA): TA of juice samples was analyzed according to the procedure (with some modifications) followed by Abedelmaksoud et al. (2018). Firstly, the acidity of the juice was determined by taking a 20 ml sample in the 250 ml beaker and 80 ml of distilled water was added in it. This solution was then titrated against a standardized 0.1 N NaOH to the phenolphthalein endpoint (pH 8.2 ± 0.1). The volume of NaOH used in the titration process was then converted to g citric acid/100 ml of juice and titratable acidity (TA) was calculated by using the following equation:

$$TA (\%) = (V \times 0.1\ NaOH \times 0.067 \times 100)/m \quad \text{Eq. 1}$$

Where V is the volume of NaOH used in liter and m is the mass of citrus juice (ml).

Electrical conductivity (EC): The electrical conductivity (EC) of sweet lemon and kinnow juice was measured by using a digital conductivity meter (DDS-307A, INSEA Scientific Instrument Co., Ltd., Shanghai, China).

Cloud value: The cloud value of both citrus juices was determined by following the method outlined by Ertugay and Baslar (2014) with some minor changes. About 5 mL of juice sample was centrifuged at 3000 rpm for 10 min and the temperature was maintained at 20 °C during the process. A spectrophotometer with distilled water acting as blank, was used to assess cloud value. The absorption of supernatant was taken at 660 nm.

Non-enzymatic browning (NEB): Non-enzymatic browning of both citrus juices was assessed by the method Velazquez-Estrada et al. (2019) with some changes. The 5 mL juice sample was centrifuged at 12,500 g for 10 min at room temperature. The supernatant of the juice was collected and clarified by using a 0.45 µm filter. The absorbance was determined at 420 nm in the spectrophotometer and the value obtained was considered as the non-enzymatic browning index.

Microbial analysis: The microbiological analysis of sweet lemon and kinnow juice was done by the standard procedure of FDA's Bacteriological Analytical Manual (Guerrouj et al., 2016; FDA, 2001). A pour plate method was applied to determine the total plate count (TPC) while using a nutrient agar media. Whereas total yeast and mold content was enumerated by a pour plate method using potato dextrose ager (PDA) media. The result was expressed as log colony-forming unit per milliliter (CFU mL^{-1}) of the juice.

Total carotenoids: Total carotenoid contents were evaluated by the procedure describe by Aadil et al. (2018). In a separating funnel mixing of 25 mL of the sample juice and 80 mL of n-hexane / acetone (1:1, v / v) was done. The organic phase was separated after shaking well and the aqueous phase was extracted repeatedly until colorless. Anhydrous sodium sulfate was used to dehydrate the organic phase. For production of the standard curve, different concentrations of standard b-carotene solutions were prepared. At ambient temperature the absorbance was measured at 450 nm using a spectrophotometer. The findings were expressed as µg of β-carotene equivalent per mL of sample.

Total anthocyanins: Total anthocyanin content was determined by the pH-differential method as explained by Aadil *et al.* (2018). This method involves using two buffer systems: sodium acetate buffer (0.4 $_{M}$, pH 4.5) and potassium chloride buffer (0.025 $_{M}$, pH 1.0). 9 mL of each buffer solution was mixed with 1 mL of juice sample (sweet lemon and kinnow juice), and the absorbance was measured using a spectrophotometer at 700 and 520 nm, respectively. Total anthocyanins content was calculated using the following equation:

$$Total\ anthocyanins\ (mgL^{-1}) = \frac{Abs \times Mw \times DF \times 1000}{\varepsilon \times 1} \quad \text{Eq. 2}$$

where Abs = $(Abs520\text{-}Abs700)_{pH = 1.0}$ - $(Abs520\text{–} Abs700)_{pH = 4.5}$, MW = molecular weight, DF = dilution factor, 1 = path length (1 cm), pigment contents were calculated as malvidin-3-O-glucoside using an extinction coefficient e of 28 000 L mol^{-1} cm^{-1} and a molecular weight of 493.2 g mol^{-1}, 1000 = conversion from g to mg

Enzymatic activity (PME, PPO and POD): The residual activity of these enzymes was determined according to the methods followed by Aadil et al. (2015).

Pectin methylesterase (PME) activity: Residual activity of PME was calculated by measuring free carboxylic groups formed by the action of the enzyme on pectin. The mixture of the reaction included a 10 mL centrifugal sample (10 000 g at 4 °C for 10 min) and a 40 mL pectin solution of 1 % (w/v) in 0.15 M (w/v) NaCl. By adding 100 µL of 0.05 mol L^{-1} (w/v) NaOH, the mixture retained the pH of 7.7 and the time needed to achieve that pH (7.7) was recorded (50 ° C ± 2).

The PME unit is defined as the amount of enzyme that released 1 mmol of carboxyl groups in 1 min. The following equation was used to calculate the percent residual activity of PME:

$$Residual\ activity\ (\%) = 100 \times A_t/A_0 \quad \text{Eq. 3}$$

where A_t is the enzyme activity of the treated sample, and A_0 is the enzyme activity of the control sample.

Polyphenoloxidase (PPO) activity: The PPO residual activity was calculated by centrifuging the sample at 10 000 g for 10 min at 4 °C. The total volume of the reaction mixture was 5 mL, which contained 1.5 mL of sample, 0.2 M (w/v) potassium phosphate buffer (pH 6.8) and 0.5 mL of 0.05 M (w/v) catechol. The increase in absorbance at 410 nm was noted after every 60 sec for 10 min using the spectrophotometer. The percentage RA of PPO was calculated using Eq. 3.

Peroxidase (POD) activity: Pyrogallol was used as a substrate to measure the POD residual activity. The total volume of reaction mixture, after the centrifugation (10 000 g, 10 min, 4 °C), was 3 mL containing a 2.2 mL sample, a 5% pyrogallol (w / v) 0.32 mL, a 100 mM potassium/ phosphate buffer (pH 6) 0.32 mL and a 0.147 M (v / v) H2O2 0.16 mL. The reaction was initiated by hydrogen peroxide addition and the increase in absorbance was recorded using a spectrophotometer at 420 nm in 3 minutes. Eq. 3 was used to calculate the percentage of POD residual activity.

Statistical analysis: All experiments were performed in triplicate, and data was analyzed using one-way ANOVA. For significant differences, Duncan's multiple comparison test was applied. Statistical analyses were conducted with IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of treatments on TA of kinnow juice and sweet lemon juice**:** Data related to the impact of SC and TS on TA of kinnow juice is given in Table 1(a) and 1(b). The TA of control (T0, untreated) sample of kinnow juice was 0.16%. It is seen that sonication treatment did not have any significant ($p < 0.05$) impact on TA of kinnow juice, even after the processing time of 5 and 10 min at 20 °C. Similarly, the TA of kinnow juice samples treated with TS remains approximately the same as compared to control samples. TS treatment of 5 and 10 min at 40 °C had not caused any significant ($p < 0.05$) effect on TA. Recorded TA for T3 and T4 was 0.17 and 0.16% respectively.

These results were in accordance with the research of Ruiz-De Anda et al. (2019). These authors treated the orange and celery juice blend at 20 kHz while the processing time (0-90 min, 20 ± 5 °C) was much higher than used in our study. They showed that sonication did not affect the TA of juice even after processing time of 90 min. Moreover, the study of Demir and Kılınç (2019) showed that TS treatment for the processing (batch TS) of pumpkin juice did not cause any significant effect on TA. In contrast to our study, these authors treated the pumpkin juice at 37 kHz and the temperature used was up to 60 °C. Thus, no significant change in TA would mean that these non-thermal treatments (sonication and thermosonication) are effective in retaining the natural balance of TA in kinnow juice.

On the other hand, TA of sweet lemon juice showed the same effect as the TA of kinnow juice. Table 2(a) and 2(b) represent the values of TA for sweet lemon juice. In this study, the TA of the control sample was 0.14 % which was not treated with US treatment of 5 and 10 minutes. The TA of T1 and T2 samples were 0.14 and 0.13 % respectively and showed non-significant effects. Similarly, after TS processing of sweet lemon juice, the TA was not changed and observed TS values for T3 and T4 were also 0.14 %. Thus, the values of TA after treatments and increased processing time were not changed significantly ($p < 0.05$). These results represent a similar effect concerning other juice as mentioned by Ruiz-De Anda et al. (2019). Thus, it was concluded that even the changing parameters of treatment and processing time (up to 10 min) did not cause any significant change in TA which represent these non-thermal techniques are effective processing method which did not bring change in TA of citrus juices. Similar results were also reported by Liao et al. (2018). These authors use apple juice for TS treatment and concluded that the TA of juice was not influenced by treatment while the processing time also did not cause any significant change in TA of juice. Similarly, Abid et al. (2013) applied sonication on fresh apple juice to evaluate the quality parameters of fresh apple juice. Researchers found non-significant change in TA of apple juice. However, the treatment conditions were different from our study. They applied sonication at 25 kHz, 20 °C for up to 90 min.

Effect of treatments on electrical conductivity (ms/cm): Table 4.3 (a) and 4.3 (b) represent mean values regarding the impact of US and TS on kinnow juice. In this study, the mean value of EC in untreated juice of kinnow (T_0) was 3.03 (ms/cm). However, after the application of US significant ($p < 0.05$) increase in EC was observed which was increased to 3.21. The increase in the processing time of ultrasonic treatment also increased the EC value. This increase is attributed to the fact that ultrasonic treatment results in the breakage of the cell membrane and the release of intracellular (vitamins and minerals) materials (Rojas et al., 2016). On the other hand, as the temperature increase (40 °C) in the case of TS, a significant ($p < 0.05$) decrease in EC value was observed as compared to control. For 5 min thermosonication, the value of electrical conductivity was 2.96, which goes on decreasing as the treatment time increased to 10 min, thus T_4 of kinnow juice sample observed a minimum value of 2.89. This decrease can be explained by the fact that increasing temperature also degrades nutrient contents such as vitamins and minerals.

In the case of sweet lemon juice, the effects were similar to that of kinnow juice. Table 4.4 (a) and 4.4 (b) represent the values of sweet lemon juice. Initially, the EC of sweet lemon juice was increased from 3.01 (T_0) to a maximum of 3.19 (T_2) as a result of US treatment. After the application of TS, a significant decreasing trend was seen with increasing time or processing. Thus, the mean values of T_3 and T_4 were observed to be 2.94 and 2.84 respectively. These results showed a significant increase ($p < 0.05$) in EC after the application of sonication while a decrease in values was due to the application of combined heat and sonication treatment. Similar to previous juice variety (kinnow juice) results, the decrease could be due to the heat effect which degrades the vitamins and minerals present in sweet lemon.

In both cases (kinnow and sweet lemon juice), the results were in accordance with the study of Zou and Jiang (2016). These researchers also reported a significant ($p < 0.05$) increase in EC value due to the application of ultrasonication, however, they used carrot juice. They applied treated carrot juice at 40 kHz for up to 60 min. On the other side, Aadil *et al.* (2015) also observed a decrease in EC value due to an increase in time under the application of TS. These authors conducted a study on grapefruit juice and in this study, they used 28 kHz for 30 and 60 min at 20-60 °C. Hence, it can be concluded that time and temperature significantly affect electrical conductivity in juices. The temperature effect can be explained, as the researchers found that the application of US at a temperature above 30 °C (TS) can bring down vitamin and mineral content in grapefruit juice mainly due to the time of processing applied (Aadil *et al.*, 2015). Thus, a decrease in EC after the TS treatment was also observed in this study.

Effect of treatments on cloud value: In the case of kinnow juice, the cloud value was 0.45 in the control sample. When the treatment of US was applied for 5 and 10 min, the obtained result was 0.68 and 0.83 respectively. Even the treatments of TS (T$_3$ and T$_4$) shown the cloud value of 0.99 and 1.14 respectively. These results are the representation of a significant ($p < 0.05$) increase in the cloud value of all samples. Table 4.5 (a) and 4.5 (b) are representing data (ANOVA and mean values) related to the impact of US and TS on the cloud value of kinnow juice. These results were similar to those shown by Bhat and Goh (2017). The study of these authors showed that US treatment at 25 kHz for 15 and 30 minutes caused significant ($p < 0.05$) changes in strawberry juice. Our study also showed significant results at lower frequency (20 kHz). The study of Saad et al. (2013) also showed that increased temperature up to 50 °C also results in significant change in cloud value of guava juice. These authors also suggested the TS as a novel treatment for processing of juices.

On the contrary, the effect of US and thermoultrasound on the cloud value of sweet lemon juice has been shown in Table 4.6 (a) and 4.6 (b). The result showed that initial values for untreated juice have a mean of 0.42. Upon application of sonication for 5 min, the cloud value recorded was 0.65. When the treatment time increased to 10 min, the cloud value was changed to 0.84. While the application of TS for 5 and 10 min has seen the values of 0.96 and 1.08 respectively. These results showed the same effect as previously discussed in the case of kinnow juice. Overall, the cloud value of sweet lemon juice was affected by both treatment and processing time, thus showing a significant ($p < 0.05$) increase in juice samples. The results were similar in accordance with the experiment performed by Nayak et al. (2018). These authors applied sonication with mild heat treatment on star fruit juice and even at a frequency higher than used in our study (used ultrasonic frequency of 44 kHz) and raised the temperature to 45 °C. They showed that processing time of 45 and 60 min also result in a significant ($p < 0.05$) increase in the cloud value of star fruit juice.

From these results of kinnow and sweet lemon juice, it can be concluded that non-thermal technologies like US and TS can increase the cloud value of citrus juice. The difference between the maximum and minimum values showed a significant ($p < 0.05$) effect of both of these treatments which mean the cloud value of juices is dependent on treatment conditions, processing time as well as temperature. However, the effect of heat could bring changes at the high-temperature condition which must be taken into account while application of combined heat and sonication treatment. The increasing cloud value could be due to thermosonic treatments that could divide large molecules into smaller ones by the high-pressure gradient of cavitations (Nayak et al., 2018). Ultimately, the juice is properly homogenized and larger molecules breaking, increases the number of suspended particles, decreases the distance between the particles by increasing the surface area, thus improving the juice cloud (Abid et al., 2013). Temperature also increased the cloud value of juices as reported in other literature (Cervantes-Elizarrarás et al., 2017).

Effect of US and TS on non-enzymatic browning of juices: Table 4.7(a) and 4.7 (b) shows values obtained for kinnow juice. In the case of kinnow juice, control treatment showed a mean value of 0.24. Upon application of US for 5 minutes (T$_1$), the value was significantly increased ($p < 0.05$) to 0.28, and further application for 10 min have seen a maximum mean value of 0.31. The NEB was also increased when TS was applied for 5 min and further increased significantly ($p < 0.05$) upon processing for 10 min. The maximum value of NEB for thermoultrasonic treated kinnow juice was 0.37. The significant ($p < 0.05$) increase in NEB of kinnow juice was highly correlated with the degradation of ascorbic acid. These outcomes are in compliance with the results of (Fustier et al., 2011).

The initial value of NEB for sweet lemon juice was 0.21,

Table 1. Effects of SC and TS treatments on the physicochemical properties of kinnow (K) and sweet lemon (SL) juice.

Sample	TA	EC (ms/cm)	Cloud value	NEB
K-T$_0$	0.16 ± 0.002^{a}	3.03 ± 0.018^{bc}	0.45 ± 0.003^{e}	0.24 ± 0.014^{d}
K-T$_1$	0.16 ± 0.001^{a}	3.14 ± 0.066^{ab}	0.68 ± 0.015^{d}	0.28 ± 0.010^{c}
K-T$_2$	0.16 ± 0.003^{a}	3.21 ± 0.054^{a}	0.83 ± 0.013^{c}	0.31 ± 0.013^{bc}
K-T$_3$	0.17 ± 0.002^{a}	2.96 ± 0.027^{cd}	0.99 ± 0.019^{b}	0.34 ± 0.017^{ab}
K-T$_4$	0.16 ± 0.003^{a}	2.89 ± 0.049^{d}	1.14 ± 0.030^{a}	0.37 ± 0.008^{a}
Values with different letters in the same column (a-e) are significantly different from each other.				
SL-T$_0$	0.14 ± 0.002^{a}	3.01 ± 0.012^{c}	0.42 ± 0.004^{e}	0.21 ± 0.012^{d}
SL-T$_1$	0.14 ± 0.004^{a}	3.09 ± 0.038^{b}	0.65 ± 0.013^{d}	0.24 ± 0.013^{cd}
SL-T$_2$	0.13 ± 0.004^{a}	3.19 ± 0.012^{a}	0.84 ± 0.021^{c}	0.27 ± 0.017^{bc}
SL-T$_3$	0.14 ± 0.003^{a}	2.94 ± 0.019^{d}	0.96 ± 0.014^{b}	0.31 ± 0.016^{b}
SL-T$_4$	0.14 ± 0.005^{a}	2.84 ± 0.021^{e}	1.08 ± 0.027^{a}	0.36 ± 0.011^{a}

Values are means ± standard deviations. Values within each column with different letters are significantly different ($p < 0.05$).

which was increased after US application for 5 and 10 minutes (T1 and T2) up to the level of 0.24 and 0.27 respectively. Table 4.8(a) and 4.8 (b) show the effect of US and TS on NEB of sweet lemon juice. Then, at T3, an increase in NEB was seen and it increased to a value of 0.31. However, when TS was applied for 5 minutes, a significant increase ($p < 0.05$) in value was seen as compared to the value of the control sample. A further increase in time sees further rise in NEB, thus a maximum value of 0.36 was seen in the case of a T4 sample. These results were significantly ($p < 0.05$) different between control and treated juice. The increase in NEB is due to the degradation of ascorbic acid as a result of sonication treatment.

The rise in NEB was observed when US and TS treatment applied to kinnow and sweet lemon juice. A similar result has been revealed by Santhirasegaram et al. (2013), however, these authors used the mango juice for processing through ultrasonic treatment. Also, the frequency used for mango juice treatment (40 kHz) was higher than used in this study, and the processing time used was up to 30 and 60 minutes. Like our study, they found that the time of processing increased the NEB of mango juice, which is also similar in case of this study. However, the temperature effect in TS treatment showed a slow rise in NEB as compared to US treatment. In another recent study, a significant increase in NEB was also reported as a result of US treatment. However, these authors applied US (20 kHz, up to 10 min) treatment on bayberry juice (Cao et al., 2018). The increase in NEB has been previously reported in case of grapefruit juice in which authors used 28 kHz frequency with variable frequency and temperature of up to 60 °C (Aadil et al., 2015). Thus, the effect of TS on citrus fruit juices reported in this study was also similar. This confirmed that NEB of citrus juice (i.e. kinnow and sweet lemon) will increase as a result of US and TS treatment.

Effect of treatments on microorganisms (log CFU mL^{-1}): The data regarding the impact of treatment (US and TS) on microorganisms of kinnow juice is represented in table 4.9(a) and 4.9(b). It was observed that microorganisms (TPC, Y, and M) of untreated (control), kinnow juice sample was 4.13 and 3.72 log (CFU mL^{-1}). This value was significantly decreased ($p < 0.05$) when ultrasonication treatment was applied for 5 min (T_1) up to 3.97 and 3.56 log. While 10 min ultrasonication treatment further decreases the activity of TPC and Y and M with increased treatment time. Thus, the T_2 sample of kinnow juice showed a further decline in the activity of microbes (mean value 3.78 and 3.39 log). Furthermore, it was observed that the application of TS also significantly decrease ($p < 0.05$) the activity of TPC and Y and M as compared to control value. When thermosonication was applied for 5 min, the log value of microbes was decreased to 3.46 and 2.97 log while increasing the processing time to 10 min also further decrease ($p < 0.05$) the log value to 2.92 and 2.65 which was the maximum achieved value in all kinnow juice samples. These findings regarding the ultrasonic effect on these juices were similar as explained by Li et al. (2017). These authors used mandarin (Citrus unshiu) juice in their study and treated it for sonication treatment at 25 kHz for 40 min at 50°C and found that sonication significantly reduced ($p < 0.05$) the microbial activity in mandarin juice. And this decrease in TPC and Y and M activity can again be attributed to the cavitation process during US treatment.

Data regarding the microbial activity of sweet lemon juice is given in Table 4.10(a) and 4.10(b). In sweet lemon juice, the control value of T_0 was 4.07, and 3.84 log (CFU mL^{-1}). The microbial (TPC and Y and M) activity showed a significant decrease ($p < 0.05$) with increasing time of processing for ultrasonic treatment. Therefore, microbial activity in T_1 and T_2 of sweet lemon juice were 3.92 and 3.65 log and 3.74 and 3.42 log respectively. Similarly, the effect of TS caused a significant decrease in values. Therefore, T_3 and T_4 samples of sweet lemon juice have seen a major decrease in values which were 3.35 and 3.07 log and 2.86 and 2.78 log respectively. These results represent a significant decline ($p < 0.05$) in microbial activity of sweet lemon juice. Also, authors have reported a similar effect in other fruit juice. Abid *et al.* (2013) showed that increasing the ultrasonic processing time (60-90 min) also caused a significant decline in the microbial activity of apple juice. However, this research used more processing time and treated the juice with more frequency (25 kHz) as compared to that used in our study.

Table 2. Effects of SC and TS treatments on microorganisms (log CFU mL^{-1}) of kinnow (K) and sweet lemon (SL) juice

Sample	TPC	Y&M
K-T_0	4.13±0.104^{a}	3.72±0.106^{a}
K-T_1	3.97±0.111^{ab}	3.56±0.093^{ab}
K-T_2	3.78±0.103^{b}	3.39±0.083^{b}
K-T_3	3.46±0.097^{c}	2.97±0.101^{c}
K-T_4	2.92±0.100^{d}	2.65±0.090^{d}

Values are means ± standard deviations. Values within each column with different letters are significantly different ($p < 0.05$).

Sample	TPC	Y&M
SL-T_0	4.07±0.102^{a}	3.84±0.096^{a}
SL-T_1	3.92±0.099^{ab}	3.65±0.100^{ab}
SL-T_2	3.74±0.113^{b}	3.42±0.091^{b}
SL-T_3	3.35±0.109^{c}	3.07±0.103^{c}
SL-T_4	2.86±0.105^{d}	2.78±0.111^{d}

Results are shown as mean values ± SD in triplicate. Values in the same columns showing the letters differ significantly ($p < 0.05$).

Thus, the effect of US and TS treatment seems to be similar to both juices (kinnow and sweet lemon) used in this study. Similar effects were reported by Mohideen *et al.* (2015), the study of which shows a significant decline in microbial activity of blueberry juice after the US (20 kHz, 25 °C) treatment. Similarly, Nayak *et al.*, (2018) shown that temperature increase with TS treatment would significantly affect the microbial (TPC and Y and M) activity. Moreover, increasing the time of processing would also cause more reduction of these compounds. The result of these authors regarding TS (44 kHz, up to 60 min) was similar to our study and showed that when the temperature is more than 45 °C it would completely inactivate the bacterial population. The combination of chemical and physical activities during the cavitation process may lead to a reduction in the activities of TPC and Y and M. The cavitation process has been reported to cause the production of free radicals and increased localized heat, which might lead to the killing of microorganisms. (Aadil *et al*., 2018). On the other hand, the loss in nutrients as the result of thermosonication is still lower as compared to a nutrient loss of thermal treatment which led to the point that thermosonication is also effective in retaining the nutritional profile of juice (Anaya-Esparza *et al*., 2017).

***Effect of treatments on total carotenoids (µg mL^{-1})*:** The data regarding the impact of treatment on TC of kinnow juice is represented in table 4.11 (a) and 4.11 (b). It was observed that the TC of untreated (control), kinnow juice sample was 1.17 (µg mL^{-1}). This value was significantly increased ($p < 0.05$) when ultrasonication treatment was applied for 5 min (T_1) up to 1.34 µg mL^{-1}. While 10 min ultrasonication treatment further confirms the increase in value with increased treatment time. Thus, the T_2 sample of kinnow juice had the maximum TC (mean value 1.40 µg mL^{-1}) under SC treatment. Furthermore, it was observed that the application of TS also significantly increased ($p < 0.05$) the TC content as compared to control value. When thermosonication was applied for 5 min, the value of TC was increased to 1.47 µg mL^{-1} while increasing the processing time to 10 min also further increased ($p < 0.05$) the TC content to 1.57 µg mL^{-1} which was the maximum achieved value in all kinnow juice samples. These findings regarding the ultrasonic effect on both these juices were similar as explained by Ordóñez-Santos et al. (2017). These authors used cape gooseberry juice in their study and treated it with sonication treatment at 42 kHz for 10-40 min and found that sonication enhanced ($p < 0.05$) the TC content present in cape gooseberry juice. And this increase in TC content can again be attributed to the release of intracellular material due to the effect produced by sound waves.

On the other hand, a significant ($p < 0.05$) impact of treatment on TC content of sweet lemon juice was also observed. The data regarding sweet lemon is represented in table 4.12 (a) and 4.12 (b). The control value of sweet lemon juice was quite similar to the control value of kinnow juice however was a bit lowered (1.11 µg mL^{-1}). The quantity of TC was increased significantly ($p < 0.05$) due to the implication of sonication and a further increase was observed in the T_2 sample (1.47). Similarly, an increasing trend was detected in the case of TS treatment as compared to control value. The maximum increase in value was obtained when sweet lemon juice was processed using thermoultrasound treatment for 10 min. Thus, the value of T_4 was 1.62 µg mL^{-1}. Thus, significant effect ($p < 0.05$) on all treatment present in all the samples.

The researchers, Zou and Jiang (2016) have already reported an increase in TC content due to the application of sonication in carrot juice. In case of the increasing trend shown by TS, the results of this study can be co-related to the impact of heat treatment as TS shows a greater impact on disrupting protein-carotenoid complexes and cell membranes than US treatment. This increasing trend of TS has already mentioned by another study in which carrot juice was used for treatment purposes (Jabbar *et al*., 2015). These authors also explained that increased processing time of TS would increase the TC content of juice as happened in this study (sweet lemon and kinnow juice). Moreover, loss of TC in case of thermal treatment in still higher than the losses obtained as the result of thermoultrasound. This happens due to the mild heat treatment used in thermoultrasound. This is why, authors have suggested combination treatment as a potential treatment to replace conventional processing (Jabbar *et al*., 2015).

Effect of treatments on total anthocyanins (mg L^{-1}): One of the important parameters linked to antioxidant value in juices (kinnow and sweet lemon). Anthocyanins are potent antioxidants and exhibit anti-inflammatory, neuroprotective, and analgesic properties. Oxygen, pH, light, temperature, intra-molecular and intermolecular interaction with other compounds and metal ions are significant factors influencing the stability and color of anthocyanins (Korte et al., 2009). In this study, total anthocyanins content is expressed in the form of mg L^{-1}.

Table 4.13(a) and 4.13(b) shows the data regarding the impact of treatment applied to kinnow juice. The initial values of juice showed a total anthocyanin content of approximately 1.44 (mg L^{-1}). US treatment did not cause any significant effect on total anthocyanins content. In this study, the US treatment for 5 and 10 min did not show any significant change in total anthocyanins rather they remained stable when compared with control untreated juice samples, thus, the value of T_1 and T_2 were 1.46 and 1.47 respectively. Similar to other bioactive compounds, the application of TS at 40 °C for 5 and 10 min, caused a significant decrease in total anthocyanins content. Therefore, the values of T_3 and T_4 of kinnow juice were 1.39 and 1.34 respectively. These results are in accordance with Dubrović *et al*. (2011), who treated strawberry juice with TS treatment and found that TS decreases the total anthocyanins content in the juice.

Data regarding the total anthocyanins contents of sweet lemon juice is given in table 4.14(a) and 4.14(b). In sweet lemon juice, the control value of T_0 was 1.36 (mg L^{-1}). This anthocyanin content showed non-significant change ($p < 0.05$) with increasing time of processing for ultrasonic treatment. Therefore, total anthocyanins content in T_1 and T_2 of sweet lemon juice were 1.37 and 1.38 respectively. On the contrary, the effect of TS caused a significant decrease in values. Therefore, T_3 and T_4 samples of sweet lemon juice have seen a decrease in values which was 1.33 and 1.29 respectively. These results represent highly significant changes in total anthocyanins content of sweet lemon juice. Also, authors have reported a similar effect in other fruit juice. Abid *et al*. (2014c) showed that increasing the ultrasonic processing time (30-60 min) also has a non-significant impact on total anthocyanins content of apple juice. However, this research used more processing time and treated the juice with more frequency (25 kHz) as compared to that used in our study.

Table 3. Effects of SC and TS treatments on phytochemicals of kinnow (K) and sweet lemon (SL) juice.

Sample	Carotenoids (µg mL^{-1})	Anthocyanins (mg L^{-1})
K-T$_0$	1.17 ± 0.017^{e}	1.44 ± 0.015^{a}
K-T$_1$	1.34 ± 0.010^{d}	1.46 ± 0.015^{a}
K-T$_2$	1.40 ± 0.019^{c}	1.47 ± 0.012^{a}
K-T$_3$	1.47 ± 0.012^{b}	1.39 ± 0.018^{b}
K-T$_4$	1.57 ± 0.014^{a}	1.34 ± 0.024^{c}

Results are shown as mean values ± SD in triplicate. Values in the same columns showing the letters differ significantly ($p < 0.05$).

Sample	Carotenoids (µg mL^{-1})	Anthocyanins (mg L^{-1})
SL-T$_0$	1.11 ± 0.011^{e}	1.36 ± 0.017^{a}
SL-T$_1$	1.39 ± 0.027^{d}	1.37 ± 0.002^{a}
SL-T$_2$	1.47 ± 0.022^{c}	1.38 ± 0.014^{a}
SL-T$_3$	1.55 ± 0.022^{b}	1.33 ± 0.023^{ab}
SL-T$_4$	1.62 ± 0.037^{a}	1.29 ± 0.025^{b}

Values are means ± standard deviations. Values within each column with different letters are significantly different ($p < 0.05$).

Thus, the effect of US and TS treatment seems to be similar to both juices used in this study. Similar effects were reported by Tiwari *et al*. (2009), the study of which shows a non-significant effect of US on total anthocyanins in red grape juice. Similarly, Chitgar *et al.* (2018) shown that temperature increase with TS treatment would significantly affect total anthocyanins content. Moreover, increasing the time of processing would also cause more reduction of these compounds. The result of these authors regarding TS was similar to our study and showed that when the temperature is more than 30 °C it would start degrading antioxidant compounds like anthocyanins. Thus effective measurement is necessary before the application of combined heat and US treatment (TS). These authors suggest that temperature must be kept low in order to retain the nutritional quality of juices. On the other hand, the loss in nutrients as the result of thermosonication is still lower as compared to the nutrient loss of thermal treatment which led to the point that thermosonication is also effective in retaining the nutritional profile of juice (Anaya-Esparza *et al*., 2017).

Effect of treatments on enzymatic activity: Enzymes such as pectin methylesterase (PME), polyphenoloxidase (PPO) and peroxidase (POD) are responsible for the deterioration of fruits and vegetables. Processing time, temperature, US intensity, food matrix composition, and pH are factors that lead to inactivation of enzymes. In this study, the activity of these enzymes in kinnow and sweet lemon juice was evaluated after the US and TS treatment. In this study, the enzymatic activity is expressed in the form of residual activity (%).

Table 4. Effects of SC and TS treatments on enzymatic activities of kinnow (K) and sweet lemon (SL) juice

Sample	PME	PPO	POD
K-T$_0$	100.00 ± 0.000^{a}	100.00 ± 0.000^{a}	100.00 ± 0.000^{a}
K-T$_1$	99.26 ± 0.282^{a}	98.32 ± 0.602^{a}	98.91 ± 0.099^{a}
K-T$_2$	97.79 ± 1.287^{a}	98.27 ± 0.630^{a}	97.66 ± 0.704^{a}
K-T$_3$	76.43 ± 1.340^{b}	87.25 ± 1.529^{b}	86.16 ± 1.510^{b}
K-T$_4$	58.47 ± 1.249^{c}	78.82 ± 1.716^{c}	72.85 ± 1.286^{c}

Results are shown as mean values ± SD in triplicate. Values in the same columns showing the letters differ significantly ($p < 0.05$).

Sample	PME	PPO	POD
SL-T$_0$	100.00 ± 0.000^{a}	100.00 ± 0.000^{a}	100.00 ± 0.000^{a}
SL-T$_1$	98.67 ± 0.598^{a}	98.91 ± 0.322^{a}	98.94 ± 0.500^{a}
SL-T$_2$	97.46 ± 0.394^{a}	97.97 ± 0.398^{a}	97.49 ± 0.564^{a}
SL-T$_3$	81.64 ± 1.521^{b}	85.34 ± 1.496^{b}	81.54 ± 1.429^{b}
SL-T$_4$	63.32 ± 1.378^{c}	75.53 ± 1.583^{c}	68.72 ± 1.408^{c}

Values are means ± standard deviations. Values within each column with different letters are significantly different ($p < 0.05$).

Table 4.15(a) and 4.15(b) shows values obtained for kinnow juice. In the case of kinnow juice, control residual activity (%) of enzymes (PME, PPO, and POD) was taken as 100%. Upon application of US for 5 min (T$_1$) and 10 min (T$_2$), non-significant change ($p < 0.05$) was observed in the inactivation kinetics of enzymes. The residual activity of enzymes (PME, PPO, and POD) for T$_1$ was 99.26, 98.32, 98.91%, and T$_2$ were 97.79, 98.27, 97.66% respectively. The residual enzymatic activity was decreased when TS was applied for 5 min and further decreased significantly ($p < 0.05$) upon processing for 10 min at 40 °C. With the increase in temperature, residual enzymatic activity decreased significantly ($p < 0.05$). The maximum inactivation of enzymes in kinnow juice was achieved at T$_4$ and residual activity was 58.47, 78.82, 72.85 respectively. The combined effect of the US and heat (TS) was shown to synergistically improve the kinetics of enzyme (PME, PPO, and POD) inactivation.

Similar to kinnow juice, control residual activity (%) of enzymes (PME, PPO, and POD) for sweet lemon juice was taken as 100%, which saw a slight (non-significant) decrease after US application for 5 minutes (T$_1$) up to the level of 98.67, 98.91, 98.94%. Table 4.16(a) and 4.16(b) shows the effect of US and TS on residual enzymatic activity (%) of sweet lemon juice. Then, at T$_3$ (40 °C), a significant decrease in residual enzymatic activity (%) was seen and achieved the values of 81.64, 85.34, 81.54%. However, when TS (40 °C) was applied for 5 minutes, a significant decrease ($p < 0.05$) in values was achieved as compared to the value of control samples. A further increase in time (10 min) sees a further decrease in enzymatic activity, thus a maximum inactivation 63.32, 75.53, 68.72% respectively, was achieved in the case of the T_4 sample. These results were significantly ($p < 0.05$) different between control and treated juice.

A significant decrease in enzymatic activity (PME, PPO, and POD) was observed when TS treatment applied to kinnow and sweet lemon juice. The similar, result has been revealed by Cao *et al*. (2018), however, these authors used bayberry juice for processing through sonication treatment. Also, the frequency used for bayberry juice treatment (20 kHz) was similar to this study, and the processing time used was up to 12 minutes. They found that the time of SC processing and temperature decreased the residual activity of enzymes, which is also similar in the case of our study. In another recent study, a significant decrease in residual activity of enzymes was also reported as a result of TS treatment. However, these authors applied US (20 kHz, up to 10 min, 65 °C) treatment on pear juice (Saeeduddin *et al*., 2015). The decrease in enzymatic residual activity has been previously reported in the case of grapefruit juice in which authors used 28 kHz frequency with variable frequency and temperature of up to 60 °C (Aadil *et al*., 2015). Thus, the effect of TS on citrus fruit juices reported in this study was also similar. This confirmed that residual enzymatic activity of citrus juice (i.e. kinnow and sweet lemon) will decrease as a result of TS treatment. The heat and mechanical forces in the ultrasound have a synergistic effect on enzyme inactivation and thus reduction was achieved at a lower temperature as compared to other thermal techniques.

Conclusion: This study investigated the effects of sonication (SC) and thermosonication (TS) on the microbial, enzymatic, and physicochemical properties of kinnow and sweet lemon juices. The findings highlight the potential of these non-thermal technologies as sustainable alternatives to conventional heat treatments. Titratable acidity remained stable under all treatments, indicating that the natural acid balance of the juices was preserved. Sonication enhanced electrical conductivity (EC), cloud value (CV), non-enzymatic browning (NEB), and bioactive compounds such as carotenoids and anthocyanins, primarily due to cavitational disruption of cellular structures and the subsequent release of intracellular materials. Thermosonication further improved CV, NEB, and carotenoid content, but led to reductions in EC and anthocyanins, reflecting the influence of mild heat on certain sensitive compounds. Both treatments effectively reduced microbial loads, but their impacts on enzymatic activity differed. Sonication alone decreased microbial activity but was less effective against enzymes such as PME, PPO, and POD. In contrast, thermosonication achieved significant inactivation of both microbes and enzymes, with the strongest effects observed under T4 conditions (40 °C, 20 kHz, 10 minutes). The enhanced performance of TS can be attributed to the synergistic action of ultrasound-induced cavitation and mild heating. These results confirm thermosonication as a promising approach for citrus juice preservation, offering microbial safety, enzymatic stability, and improved quality while minimizing nutrient losses compared to conventional thermal methods. However, reductions in anthocyanin content suggest that process parameters require further optimization. Future research should explore large-scale applications, economic feasibility, and potential integration with other emerging non-thermal technologies. Overall, thermosonication presents a viable, forward-looking solution for producing safe, high-quality, and nutritionally valuable citrus juices that align with consumer demand for minimally processed beverages.

CRediT authorship contribution statement: **Muhammad Talha Afraz:** Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing; **Noor Ul Huda:** Conceptualization, Investigation, Writing – review & editing; **Asmara Afraz:** Conceptualization, Writing – review & editing; **Sultan Mehmood Ghani:** Methodology, Writing – review & editing; **Muhammad Faisal Manzoor:** Conceptualization, Writing – review & editing; **Muhammad Mohsin Raza:** Writing – review & editing.

Funding Statement

Declaration of Competing Interest: The authors declare no conflict of interest, financial or otherwise, nor any personal relationships that could have influenced the findings or interpretations presented in this paper.

Acknowledgments: The authors thank the National Institute of Food Science and Technology, University of Agriculture for using its infrastructure.

Data availability: Data will be made available on request.

SDGs addressed: SDG 3 – Good Health and Well-Being; SDG 9 – Industry, Innovation and Infrastructure; SDG 12 – Responsible Consumption and Production;SDG 9 – Industry, Innovation and Infrastructure; SDG 12 – Responsible Consumption and Production.

Policy referred: WHO/FAO Codex Alimentarius; National Food Safety and Security Policies (Pakistan); UN Sustainable Consumption and Production frameworks.

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