STUDIES ON THERMOSONICATION OF FRESH CUT APPLE SLICES

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By
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CERTIFICATE

This is to certify that the dissertation entitled "**Studies on Thermosonication of Fresh Cut Apple Slices**" is a bonafide record of the research work carried out by **Malvika Bodh** (Roll No. 11AG10020) under my supervision and tutelage, for the partial fulfillment of the requirements for the award of the degree of Bachelor of Technology in Agricultural and Food Engineering, during the academic session 2014-2015 in the Department of Agricultural and Food Engineering, Indian Institute of Technology, Kharagpur.

Place: Kharagpur (**Dr. P. Srinivasa Rao**)

Date: 06-05-2015 *Associate Professor*

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Place: Kharagpur (Malvika Bodh)

Date:

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LIST OF ABBREVIATIONS AND SYMBOLS

SYMBOLS/ABBREVIATION	DESCRIPTION
w/w	Weight by weight
K	Kelvin
MHz	Megahertz
FRAP	Ferric reducing ability of plasma
°C	Degree Celsius
HTST	High-temperature short-time
PEF	Pulse electric field
kV	Kilo volt
µm	Micrometer
µs	Microsecond
DNA	Deoxyribonucleic acid
HPP	High pressure processing
PME	Pectin methylesterase
PPO	Polyphenol Oxidase
POD	Peroxidase
TS	Thermosonication

ABSTRACT

Enzymatic browning is the second largest cause of quality loss in fruits and vegetables. Enzymatic browning and microbial growth lead to quality losses in apple products. In the present study, freshly cut apple slices were thermosonicated (at 28 KHz and different levels of treatment time and temperature) for the inactivation of enzymes (polyphenolase, peroxidase and pectin methyl esterase). The effect of thermosonication on apple cut slices was studied in terms of the changes in their physicochemical and bioactive changes. The total phenolic content and antioxidant activity of the samples increased with temperature and treatment time. With different temperature and exposure time combinations, the percentage inactivation of apple polyphenolase, peroxidase and pectin methyl esterase was determined. The inactivation obtained by thermosonic treatment for 30 min at 60 °C was 76.9, 90.9 and 80% for PME, PPO and POD respectively. The application of thermosonication was studied to enable less severe thermal treatments and, therefore, improve the quality of the blanched product.

Keywords: Apple slices, Thermosonication, Total phenols, PME, PPO, POD

Chapter I
INTRODUCTION

Apple is the sweet, pomaceous fruit of the apple tree, *Malusdomestica* of the rose family, *Rosaceae* and is the most widely known member of the genus *Malus*. Apples, which are one of the most widely cultivated tree fruits used by humans, grow on deciduous trees which are large (if grown from seeds) or in other cases, small (if grafted onto roots). There are more than 7,500 known cultivars of apples, resulting in a range of desired characteristic, with different cultivars bred for various tastes and uses, including cooking, eating raw and cider production.

In 2010, nearly 69 million tons of apple was grown worldwide. China was the leading producer, growing almost half of this total, with the United States in second and Turkey third, followed by Italy, India and Poland. Freshly cut apples have emerged as a popular snack in food service establishments, which is further boosted by the numerous beneficial health effects thought to result from eating apples. According to United States Department of Agriculture, a typical serving weighs 242 g and contains 126 calories with significant dietary fiber and modest Vitamin C content, with a generally low content of other essential Vitamins.

Trees and fruits are prone to a number of fungal, bacterial problems that are controlled by organic and non-organic means. When the cells of the apple are sliced through or physically damaged (when an apple is dropped), the polyphenol oxidase starts oxidizing the phenols in apples and results in browning of the damaged apple portion (Lu et al., 2007). It reduces the visual quality and also results in undesirable changes in flavour and loss of nutrients (Luo and Barbosa, 1997). Enzymatic browning is quantified using browning indicators through a biochemical index, for e.g. using polyphenol oxidase activity or physical indicators such as color surface.

Ultrasound is a non-thermal method applied to solid, liquid and gas systems for different purposes. The principle aim of this technology is to reduce the processing time, save energy and improve the shelf life and quality of food products (Chemat, 2011). Its instrumentation can be fully automated and it makes rapid and precise measurements (Dolatowski, 2007). The advantages of Ultrasound over heat treatment

include minimization of flavour loss, greater homogeneity and significant energy savings (Earnshaw, 1995).

Ultrasound waves are sound waves which are generated by mechanical vibrations of frequencies higher than 18 kHz, which is beyond the frequency range which can be perceived by the human ear. The upper limit of ultrasound frequency is not sharply defined but is generally taken to be 5 MHz in gases and 500 MHz in liquids and solids. When these waves propagate into liquid media, resulting in the agitation of particles in the sample, alternating compression and expansion cycles are produced. This process is known as Ultasonication (US). During the expansion cycle, high intensity ultrasonic waves make small bubbles which grow in the liquid. When they attain a volume at which they can no longer absorb enough energy, they implode violently. This phenomenon is known as Cavitation (fig.1) (Suneel et al., 2009). During implosion, very high temperatures (approximately 5000 K) and pressures (estimated at 50 MPa) are reached inside these bubbles (Sala et al., 1995).A product with heat sensible components can be treated by ultrasonication as it is operated at low temperature. However, the treatment time is actually long during the inactivation of enzymes and/or micro-organisms, which may cause high-energy requirement. Normally, this treatment will need to be combined with other techniques to optimize the process.

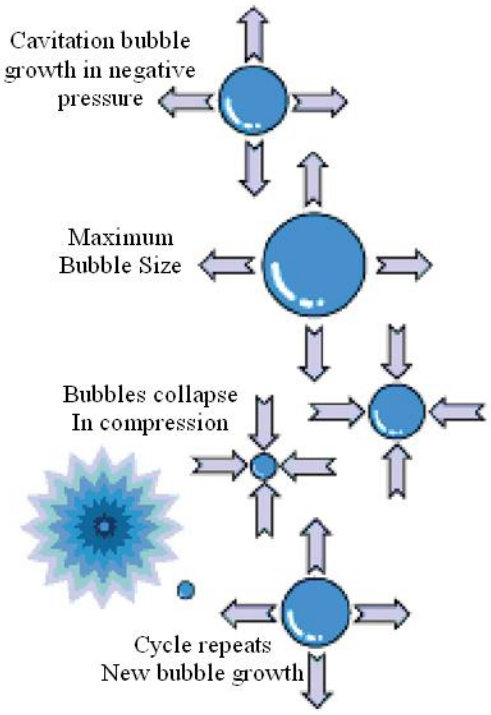

Figure 1: Bubble formation, growth and collapse

Thermosonication (TS) is a combined method of ultrasound and heat where the product is subjected to ultrasound and moderate heat simultaneously. This method produces a greater effect on inactivation of microorganism than heat alone. For example, when thermosonication is used for pasteurization or sterilization purpose, lower process temperatures and processing times are required to achieve the same lethality values as opposed to conventional processes. Microbial inactivation mechanisms of ultrasound is simply

explained by cavitation phenomena that is caused by the changes in pressure (Mason, 1996 and Villamiel, 1999). Earnshaw explained that the extremely rapid creation and collapse of bubbles formed by ultrasonic waves in a medium creates the antimicrobial effect of ultrasound. During the cavitation process, localized changes in pressure and temperature cause breakdown of cell walls, disruption and thinning of cell membranes, and DNA damage via free radical production.

Ultrasound creates continuous vibrations and produces stable cavitation bubbles which collapse due to extreme local increase in pressure (50 MPa) and temperature (5000 K). Also, because of shock waves, strong shear and micro-streaming in the adjacent liquid is observed (Suslick, 1994). All of these factors can cause the modification of the secondary and tertiary structure of protein, due to the breakdown of hydrogen bonding or van der Walls' interaction in the polypeptide chains, which can lead to activity loss of many enzymes. The extreme pressure and temperature also lead to homolytic water molecule cleavage generating high energy intermediates such as hydroxyl and hydrogen free radicals. The free radical formed may react with some amino acid residues that participate in enzyme stability, substrate binding or in the catalytic function with a consequent change in biological activity (Feng, 2011). Such free radicals could also recombine with amino acid residues of the enzymes which are associated with structure stability, substrate binding and catalytic functions. Disruption of tissue due to the ultrasonic application is another important criterion. As the amount of disruption tissue increases, the surface area that is in contact with the enzymes and free radicals increases. For example, oxidases are usually inactivated by sonication while catalyses are affected at low concentrations.

Due to limited information available on application of Thermosonication on *Malusdomestica* family in general and freshly cut apple slices in particular; the present study was undertaken with the following objectives.

Objectives

1. To evaluate the changes in quality attributes of freshly cut apple slices during Thermosonication.
2. To study the inactivation of polyphenol oxidase, peroxidase and pectin methyl esterase enzymes in freshly cut apple slices during Thermosonication.

Chapter II
REVIEW OF LITERATURE

This chapter reviews the research work carried out by various researchers in related areas viz. the effects of HPP, the prevention of browning, thermosonication as an alternative to thermal blanching and its effect on quality improvement and food processing.

2.1 Effects of HPP and Prevention of Browning

Prevention of browning of apple slices has been difficult to achieve because of the rapidity of the enzymatic oxidation of phenolic substrate even under reduced atmospheric pressure storage. To prolong the shelf life of fresh-cut fruits and vegetables different technologies have been used so far as cited by Rojas-Graü. (2009). Among these numerous chemical and physical preservation strategies were explored to reduce enzymatic browning. However, Van de Velde and Kiekens. (2002) reported that almost all chemical treatments (sulfite, citric acid, ascorbic acids, derivatives and benzoate) confer off flavours, and many of the most effective substances added are recognized as unsafe.

Porta et al. (2013) suggests Modified Atmospheric packaging (MAP) as a dynamic system with 2 gas fluxes which allows the respiration of fresh products and gas exchange through a packaging film. MAP gas composition, which is generally low in O_2 and high in CO_2, primarily depends on package total surface, product weight, respiration rate, film gas transmission capacity and storage temperature. However, food packaging by MAP at low temperature is usually not sufficient to significantly extend the shelf life of the majority of the fresh cut fruits and vegetables.

Olivas and Barbosa-Cánovas (2005) studied the application of edible coatings, which is increasingly being demonstrated as a relatively new and simple technique, effective in preventing the appearance and textural deterioration of several products. The use of different type of films, based on a variety of single biopolymers or on their combinations, is observed to be extremely advantageous even though all data obtained so far indicates that coatings need to be tailored and optimized for each kind of food.

Wu et al. (2012) studied the effect of HPP (600MPa) on apple cubes in pineapple juice which resulted in a visible color change during four weeks of storage at a temperature

of 4 °C. The treatment significantly reduced the PPO activity, while the PME activity was not affected. Pineapple juice in combination with pressure can be used as a preservation method for minimally processed apples. The study revealed that dipping the sample into 0.5% w/w Ascorbic acid, 0.5% w/w Citric acid and 0.5% w/w Calcium Chloride for 5 min, reduced the changes in color and firmness of apple wedges during HPP treatments and retained good sensory attributes.

2.2 Thermosonication as an alternative to thermal blanching and its effect on quality improvement and inactivation

However, the approach used in the present study will be focused on the thermosonication technique. Cruz et al. (2010) studied the impact of thermal blanching and thermosonication treatments on Watercress (*Nasturtium officinale*) quality, with a focus on TS process optimisation. The TS treatment was found to be better than heat blanching, since it inactivated watercress peroxidase at less severe thermal conditions and consequently retained vitamin C content at higher levels while enhancing the green colour. The treatment of 92 °C for 2 sec proved to be an optimal condition, but was considered poor in terms of industrial process feasibility. Hence, 86 °C and 30 sec was determined to be a better choice in terms of product quality, with about 75% vitamin C content; ~8% a-value (improvement toward green) and higher feasibility.

Alexandre et al. (2011) studied the application of Thermosonication and Ultraviolet Radiation processes as an alternative to blanching for some fruits and vegetables. It was observed that thermosonication allowed better quality retention, when compared to heat blanching at the same temperatures. Additionally, the impact of thermosonication on microbial load reductions was statistically significant and thermosonicated samples retained quality attributes better than heat blanched ones at the same temperatures ($p < 0.05$). Hence, it can be concluded that thermosonication is a promising process and may be a favorable alternative to the conventional thermal treatment.

Aadil et al. (2015) studied thermosonication as a potential technique which influences the quality of grape juice. The micro-organism activity was completely inactivated in the treatment (60 °C for 60 min).The TS treatment (60 °C) for 60 min exposure reduced the PME, PPO and POD activity by 91, 90 and 89%, respectively.

Additionally, a significant increase in the electrical conductivity (EC), non enzymatic browning (NEB), cloud value, viscosity, total carotenoids, colour attributes and bioactive compounds was also observed (as compared to the control).

Wu et al. (2008) studied the effect of thermosonication on quality improvement of tomato juice. The tomato juice was subjected to TS treatment (24 kHz), at amplitudes of 25, 50 and 75 μm at 60, 65 and 70 °C for 41.8, 11.7 and 4.3 min exposures respectively, which reduced the PME activity by 90%. For the same decrease in PME activity, at the same temperatures, the heat only treatment required 90.1, 23.5 and 3.5 min respectively. Additionally, the average particle size decreased noticeably (30 μm) and viscosity increased 2-4 fold, as compared to the heat treated or untreated juice (180 μm). The inactivation of PME and PG is required to minimize quality loss in tomato products. Hence, these results suggest that thermosonication demonstrates beneficial effects at 60 and 65 °C and can be considered as potential alternative process to conventional "cold break" and "hot break" treatments. However, it is not beneficial at 70 °C and this might be explained by the impairment of cavitation at higher temperatures.

Walkling-Ribeiro et al. (2009) studied the impact of thermosonication and pulsed electric fields on *Staphylococcus aureus* inactivation and selected quality parameters in orange juice. TS treatment (55 °C for 10 min) was applied in combination with PEF (40 kV/cm for 150 μs) and resulted in a comparable inactivation of *S. aureus* to that achieved by conventional heat treatment (HTST). The TS/PEF did not affect the pH or °Brix and had a milder impact on the juice color than thermal treatment. Additionally, the non-enzymatic browning index was significantly affected by HTST ($p < 0.05$) but not by TS and PEF. The residual activity of PME decreased as PEF field strength and treatment time increased; however, applying TS and PEF in combination left a greater residual PME activity than HTST (12.9% vs. 5.0% respectively).

Cruz et al. (2004) studied the effect of heat and thermosonication on the inactivation kinetics of peroxidase in Watercress for the temperature range of 40-92.5°C. In the heat blanching processes, a first-order biphasic inactivation model was observed while the enzyme kinetics in TS treatment showed a first-order model. The application of thermosonication (temperatures > 85 °C) and same blanching times, led to higher enzyme inactivation as compared with the heat blanching processes. These results

allow the application of shorter blanching times at this range of temperatures, leading to a product with a higher quality, or minimized processing.

Arroyo et al. (2011) studied the combined effect of synergistic combination of heat and ultrasonic waves under pressure (manothermosonication or MTS) on the survival of a strain of *Cronobacter sakazakii* inactivation in apple juice. The inactivation by ultrasound under pressure was found to be independent of temperature, below 45 °C. However, above 64 °C, the lethal effect of ultrasound under pressure was negligible as compared to the lethality of heat treatment at the same temperature. The findings revealed that between 45 and 64 °C, the lethality of the combined process (MTS) was higher than expected if heat and ultrasound under pressure processes acted simultaneously but independently, that is, a synergistic effect was observed, with the maximum synergistic effect (38.2%) found at 54 °C.

2.3 Effects of Sonication in Food Processing

Knorr et al. (2004) studied the application and potential of ultrasonics in food processing. The activity of endogenous enzymes of fresh lemon juice was effectively decreased when UST was applied, with more than 90% inactivation shown at 80 °C. Thus, UST is a method which offers the promise of destabilising undesirable enzymes at short time and moderate temperatures. Moreover, the reduction of pectinmethylesterase (PME) activity in lemon juice resulted in highly improved cloud stability when stored after UST treatment (4 °C for 18 days).Colour measurements during the storage of orange juice after heat or UST treatment indicated similar overall changes of *a* and *b* values, but significantly lighter products (*L*-values) resulting from the UST treatment. The stabilisation in lightness was attributed to a lower enzyme activity and a lower partial precipitation of suspended, insoluble particles in the juice.

Annegowda et al. (2011) studied the effects of sonication treatments (time intervals of 0, 15, 30, 45 and 60 min.) and extraction solvents on phenolics and other antioxidant compounds in star fruits extracted in methanol and water. With regard to the aqueous extracts, the control samples, viz. extract obtained without sonication or ‘0 min’, showed significantly higher phenolics and antioxidant activities as compared to extracts obtained after sonication treatments. At prolonged sonication intervals (15, 30, 45 and 60 min), a significant ($p < 0.05$) time dependent decrease in the percentage

inhibition of DPPH radicals, FRAP value, antioxidant capacity, total phenolics, total tannins, and total flavonoids was recorded. However, for methanolic extracts, the aforementioned evaluated parameters showed significant increase ($p < 0.05$) at the treatment time of 30 min, which later decreased slightly (at the 45 and 60 min intervals). However, the values were still significantly higher than the control samples ('0 min') ($p < 0.05$). Annegowda et al. (2011) contention is that sonication as a physical mode of food processing, holds high potential to be explored for industrial applications as an effective environment-friendly method for enhancing the extractability of natural antioxidants. Thus, they could effectively play a significant role in preventing several physiological and degenerative diseases in consumers.

Norazlin and Nyuk. (2014) studied the application of thermosonication treatment in processing and production of high quality and safe-to-drink fruit juices. The TS treatment was used to inactivate various endogenous enzymes such as polyphenoloxidase, peroxidase, pectin methylesterase and polygalacturonase, which are released during processing and must be inactivated as quickly as possible to avoid food spoilage. Thermosonication was also considered useful in acting against the thermo-resistant enzymes which are difficult to denature by thermal treatment alone as the use of extreme heat could lead to adverse changes in juice quality. Here, since enzymes are more thermo-resistant than microorganisms in citrus juices, the inactivation of enzymes promises the achievement of required number of microbial destruction for spoilage prevention.

Wu et al. (2008) also considered thermosonication as a potential alternative for cold break and hot break treatments of tomato juice. The enzymes in tomato are traditionally inactivated by thermal treatment (hot break and cold break). The hot break process involves rapid heating of the pulp to temperature range of 95 to 102 °C immediately after or during crushing, while the cold break process involves heating of the pulp to temperature range of 60 to 71 °C. Although the hot break process causes complete enzymes inactivation and high juice consistency, the severe heat treatment leads to loss of flavour, nutritional value and juice colour (darkening). Thermosonication was considered appropriate to replace hot break treatment in tomato juice production for minimal colour change, development of unpleasant flavour and nutrient losses. Thermosonication could enhance the inactivation rates of both PME

and PG in fruit juices leading to improvement of the juice rheological properties, with PME inactivation of 98.5 and 98.9% in tomato juice achieved by TS at 60 and 65 °C respectively. PME was almost inactivated and PG was 90% inactivated after 18 mins of TS treatment of tomato juice at frequency of 20 kHz, amplitude of 64 μm and temperature of 75 °C.

Terefe et al. (2009) studied the effects on apparent (juice) viscosity of thermosonic treated products, which had a greater viscosity than conventional heat treated products. A lower activity of PME improves cloud stability in juice. The inactivation of PME activity at a medium temperature range and higher viscosity are attributed to mechanical effects of thermosonication. Both PME and PG are synergistic in pectin breakdown leading to browning of fruits and vegetables and damaged tissues and loss of quality by the oxidation of phenolic compounds. The degree of polymerisation of pectic substances affects juice viscosity. Pectin reduction leads to a large decrease in tomato juice viscosity which causes less fouling of heat exchanger and thus, the pumping process gets easier. The cavitation effects of ultrasound functions reduce juice yield, lead to colour loss and give improvement in terms of the enzymes inactivation and microbial destruction. For a shorter processing time, it can be categorised as minimal processing for freshness and health purposes.

Abid et al. (2014) suggests that polyphenoloxidase (PPO) and peroxidase (POD) are two major enzymes causing browning that need to be inactivated for colour preservation. The inactivation of PPO and POD in apple juice was significantly higher in thermosonication treatment with probe sonicator (60 °C for 10 min) with values of 93.9 and 91% respectively. In cantaloupe melon juice, ultrasonic treatment alone could give significant decrease in PPO and POD activities. It was also determined that the time required to decrease initial PPO activity by 90% is shorter for thermosonication inactivation as compared to the time duration for conventional thermal inactivation.

Chapter III
MATERIALS AND METHODS

3.1 RAW MATERIAL

Apple samples of average weight were procured from the local market, Kharagpur. The samples were then washed thoroughly and dried clean. An apple slicer was used to cut wedges of uniform size. The cut slices were vacuum packed in LDPE films and then stored under refrigerated conditions till further processing.

3.2 METHODOLOGY

3.2.1 Effect of thermosonication on physicochemical and bioactive components

A full factorial design was used with the total number of 20 thermosonication treatments. Three replications of the experiment were performed and mean values reported. The frequency of thermosonicator was fixed at 28 kHz and the process parameters were temperature (30, 40, 50 and 60 °C) and time (0, 5, 10, 20 and 30 min).

Responses:

a) pH, TSS, TA, Texture, BI

b) Colour

c) Total phenols

d) Antioxidant activity

Outcome: Physicochemical & biochemical characterization.

3.2.2 Effect of thermosonication on enzymes

A full factorial design was used with the total number of 15 thermosonication treatments**.** Two replications of the experiment were performed and mean values were reported. The frequency of thermosonicator was fixed at 28 kHz and the process parameters were temperature (40, 50 and 60 °C) and time (0, 5, 10, 20 and 30 min).

Responses:

Relative activities (%) of

a) PME
b) POD
c) PPO

Outcome: Thermosonic inactivation residual activity percentage of PME, POD and PPO.

3.3 THERMOSONICATION

The samples were treated in a professional, digital ultrasonic cleaner with heating (Model: UD 80 SH -3L; Make: EUMAX), having a tank size: 176 mm length, 164 mm depth and 65 mm height. It has a digital timer of 0-30 min and a heating range of 0-70 degree. The outer case, tank and lid are all made of stainless steel. One single 28 kHz transducer is installed.

The samples are treated at 30, 40, 50 and 60 ^{0}C, for a holding time of 5, 10, 20 and 30 min at a frequency of 28 KHz and one set of sample was kept untreated as control .Then the samples were vacuum packed in LDPE films and stored under refrigeration.

OBJECTIVE 1: To evaluate the changes in the quality attributes of freshly cut apple slices during Thermosonication.

3.4 Determination of Physicochemical and Microbiological characteristics.

3.4.1 Color measurement

The colour of Apple slices was measured using the Portable Colorimeter (Model: Spectro-guide 45/0 gloss; Make: BYK Gardener, Germany) in a reflection mode. Colour is expressed in L* (brightness), a* (+a, red; -a, green), and b* (+b, yellow: -b, blue) coordinates, standard illuminant D65, and observer angle 10°. The measuring orifice is 8 mm wide. The results were expressed as the mean of three measurements from three different samples of cut apples (three measurements are taken on each sample at different locations).

Fig 2: (a) Colorimeter; (b) pH meter

3.4.2 pH

A sample of 20 g was crushed using a mortar and pestle and the pH reading was taken (Model: CL 46+; Make: Toshcon Industries Pvt. Ltd.) by inserting a pH probe into the sample.

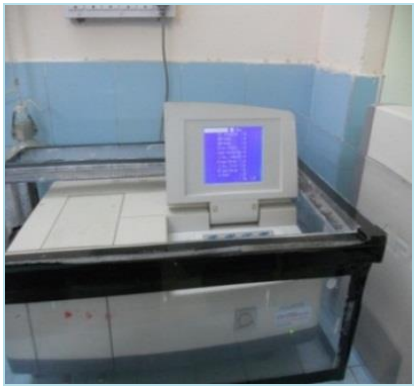

Fig 3: (a) Digital Refractometer; (b) UV-visible spectrophotometer

3.4.3 Titratable acidity

For the measurement of titratable acidity, 4 g of apple puree was taken and diluted to 40 mL with distilled water. The 40 mL of diluted apple puree was then taken for titration, mixed with 2-4 drops of 1% phenolphthalein as indicator and titrated with 0.1 N NaOH solution. Titrate values were noted and titrable acidity in mL/100 mL of sample was calculated.

3.4.4 Total soluble solids

Total soluble solid (TSS) of the sample was determined using a digital hand held refractometer (Model: PAL-1; Make: Atago, Japan) having a range of 0-53%. The sample was extracted and a drop of filtrate was placed on the sample slot. The total soluble solids was recorded and expressed in °Brix.

3.4.5 Browning Index

The browning index was calculated by using the L*, a* and b* values as described in Rattanathanalerk et al., 2005. The formula for calculating Browning Index is provided below.

$$BI = \frac{[100(x * 0.31)]}{0.172} \quad \text{…..(3.1)}$$

where,

$$x = (a* + 1.75L*)/(5.645L* + a* - 3.012b*) \quad \text{.....(3.2)}$$

3.5 Determination of Bioactive components

3.5.1 Total phenolic content

The ethanol extract of apple wedges was used for analysis of total phenolics and DPPH free-radical scavenging activity. It was prepared by shaking 5 g of apple puree with 15 mL of 80% ethanol (1:3 w/w ratio), for 3 hours at ambient temperature (27±1 °C) maintained in a water bath and the extract was stored at -20 °C for subsequent analysis.

Total phenolic content (TP) was determined by following the procedure proposed by Singleton and Rossi. (1965). Briefly, blue colour developed using the Folin-Ciocalteu reagent (FCR) in an alkaline medium (20% sodium carbonate) and its absorbance was measured at 750 nm in a UV-visible spectrophotometer (Model: UV1700; Make: Shimadzu, Japan). Gallic acid was taken as standard for phenols and TP content was expressed as mg Gallic acid equivalent (GAE)/100 g sample.

3.5.2 DPPH free-radical scavenging activity

DPPH free-radical scavenging activity is determined by following the method given by Brand-Williams et al. (1995) with slight modifications. Briefly, there are changes in colour of DPPH solution (36 µg/ mL in methanol) from purple to yellow which result from the addition of different concentrations of ethanolic extract of sample (11-167 mg/ mL extract) and keeping the solution in dark for 30 min (measured at 517 nm). This colour change was used to calculate the total antioxidant capacity. All the above procedures were carried out for the control sample and thermosonically treated samples and the changes were subsequently studied.

OBJECTIVE 2: To study the percentage inactivation of polyphenol oxidase, peroxidase and pectin methyl esterase in freshly cut apple slices

3.6 Inactivation of PME

50ml of pectin solution was prepared and taken in a beaker. The pH of the solution was made 7.5 using 0.2 N NaOH and 4 g of treated apple sample puree was added to the solution. Subsequently, the pH of this solution was again made 7.5 using 0.2 N NaOH. As the pH reached close to 7.5, stopwatch was turned on.0.1 mL of 0.02 N NaOH was added when the pH reduced to 7.45.The number of times that 1 mL of 0.02 N NaOH is added was diligently noted and this procedure was continued for 15 min.

3.6.1 PME Activity

The residual activity (RA) of PME obtained after each thermosonic treatment was defined as:

$$RA = \frac{A}{A0} * 100 \qquad .....(3.3)$$

$$\text{PME unit:} \frac{\{(\text{ml of NaOH})*(\text{N of NaOH})*1000\}}{\{(\text{g of sample})*(\text{time in min})\}} \qquad .....(3.4)$$

3.7 Inactivation of PPO and POD

3.7.1 Preparation of crude PPO and POD extracts

The enzyme extracts were made by homogenization of 1 g of apple slices puree with 1 mL of 0.2 M Sorenson's phosphate buffer (pH = 6.5), containing 1 M NaCl, 4% PVPP and 1% Triton x-100. The homogenate was centrifuged at 14,000 rpm for 25 min at 4 °C and the supernatant was used to determine enzymes activity

3.7.2 Assay of polyphenol oxidase (PPO)

For the PPO assay, the reaction mixture consisted of 200 µL of enzyme extract and 1.25 mL of 0.1 M Catechol (Himedia) in 0.2 M Sorenson's buffer (pH 6.5) solution. The blank was prepared in the same way except that 0.2 M Sorenson's buffer (pH 6.5) was used instead of crude enzyme extract. The absorbance of the assay mixture was measured at every 10 sec interval for 10 min at 420 nm and 25 ^{o}C, using a UV-

visible spectrophotometer. The activity of the enzyme was expressed as the slope of the linear portion of the curve that is the change of absorbance.sec^{-1}.g^{-1} fresh weight of sample.

3.7.2 Assay of peroxidase (POD)

For the POD assay, the reaction mixture consisted of 100 μL of enzyme extract, 160 μL of 1% (v/v) hydrogen peroxide solution and 320 μL of 5% pyrocatechol (Himedia) in 0.2 M Sorenson's buffer (pH 6.5) solution. The blank was prepared in the same way except that 0.2 M Sorenson's buffer (pH 6.5) was used instead of crude enzyme extract. The absorbance of the assay mixture was measured at every 10 sec interval for 10 min at 420 nm and 25 ^{o}C using a UV-visible spectrophotometer. The activity of the enzyme was expressed as the slope of the linear portion of the curve that is the change of absorbance.sec^{-1}.g^{-1} fresh weight of sample.

Chapter IV
RESULTS AND DISCUSSION

In this chapter, the results of different experiments conducted are presented under various sections.These sections include the effect of independent parameters on physicochemical and biochemical properties of apple slices.

4.1 Effect of Thermosonication on Quality of Apple Slices

4.1.1 Color

Color plays a major role in the overall acceptability of food products and is derived from the natural pigments in fruits and vegetables, many of which change with maturation and ripening. The brown color imparted to freshly cut apple slices is due to enzymatic and non-enzymatic browning. Color measurement of fruits is generally correlated with quality attributes like flavor and contents of pigments because of the simplicity in its measurement. Thermosonicated samples showed changes in color when compared with untreated samples. The L* value, which is an index of visual whiteness, showed significant decrease with increase in treatment time which indicates that the color of samples change from orange to brown.

Table 1: Color changes in apples slices during thermosonication

Treatment		**Time (min)**				
		0	**5**	**10**	**20**	**30**
30 °C	L*	77.550	75.008	73.248	70.808	63.968
	a*	00.543	01.340	01.223	01.528	01.633
	b*	26.905	29.713	33.035	32.838	32.868
40 °C	L*	77.235	74.353	70.378	70.565	65.073
	a*	01.340	02.770	05.038	03.210	06.858
	b*	26.380	32.665	35.688	33.298	40.955
50 °C	L*	78.860	72.328	74.408	72.398	71.090
	a*	00.803	02.595	02.383	02.783	03.298
	b*	25.575	37.093	38.345	39.295	40.970
60 °C	L*	75.973	72.060	65.903	61.948	61.043
	a*	02.040	03.053	06.530	07.863	08.533
	b*	24.603	31.383	33.040	34.128	36.615

On the other hand, a* values, normally used as an index of visual redness, increased as treatment time increased. Similarly, the b* values also increased with increase in treatment time. Similar results were obtained by Lunadei (2011) for apple samples stored at 7.5 °C for 9 days. However, it should also be noted that a certain amount of browning was unavoidable in sample preparation as well.

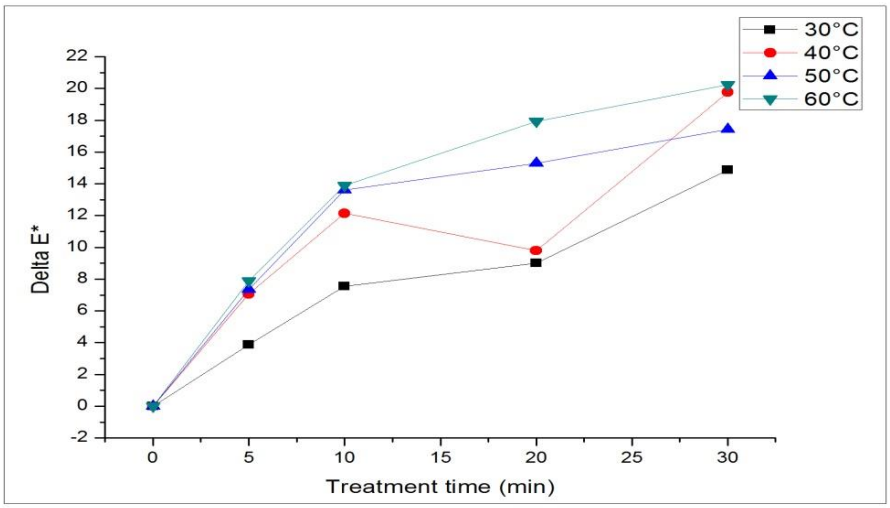

Fig. 4: Effect of thermosonication on Δ*E** of apple slices

4.1.2 Browning Index

The formula for calculating Browning Index is

$$BI = [100(x * 0.31)]/0.172 \quad \text{.....(4.1)}$$

where,

$$x = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*) \quad \text{.....(4.2)}$$

The browning index increased (fig. 5) with increasing treatment time and the rise in temperature from 30 to 60 °C**.** It increased up to 12.9% at 30 °C and 25.6% at 60 °C thermosonication treatment. Thus, it can be concluded that thermosonication treatment increased the Browning Index in freshly cut apple slices.

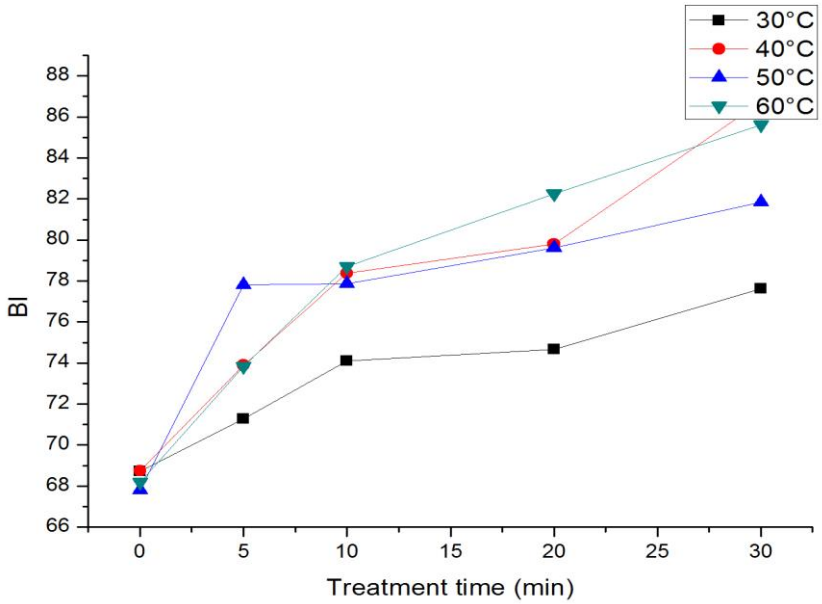

Fig. 5: Effect of thermosonication on *BI* of apple slices

4.1.3 Total soluble solids

The results regarding the effects of thermosonication on °Brix are presented in fig. 6. No significant changes were observed in °Brix of samples of treated and untreated apple slices (Walkling-Ribeiro, 2009). It has been reported that sonication treatment causes a significant decrease in °Brix of kasturi lime juice (Mason et al., 2005). However, there was no correlation to the findings of Mason et al. (2005) as we found non-significant effect of thermosonication on the °Brix of apple juice, which is also in agreement with the observation of ultrasound-treated apple–carrot juice blends (Gao and Rupasinghe, 2012)

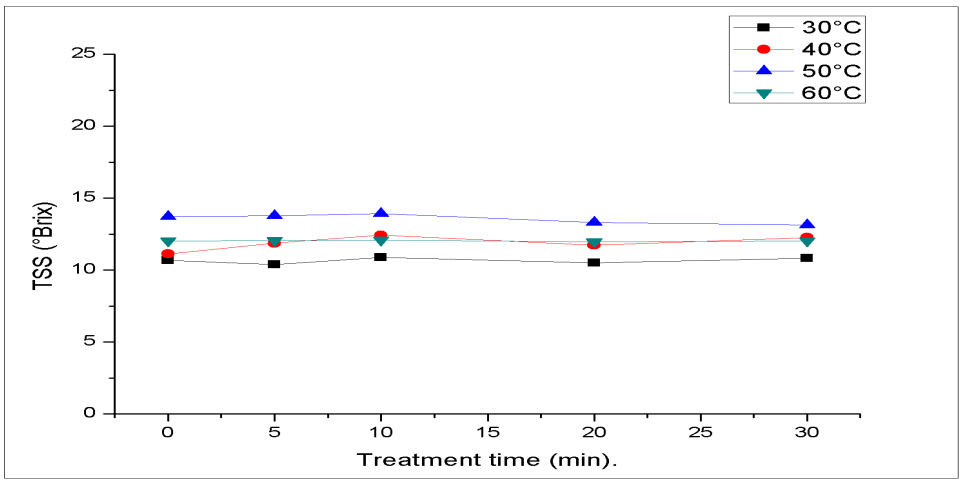

Fig. 6: Effect of thermosonication on TSS of apple slices

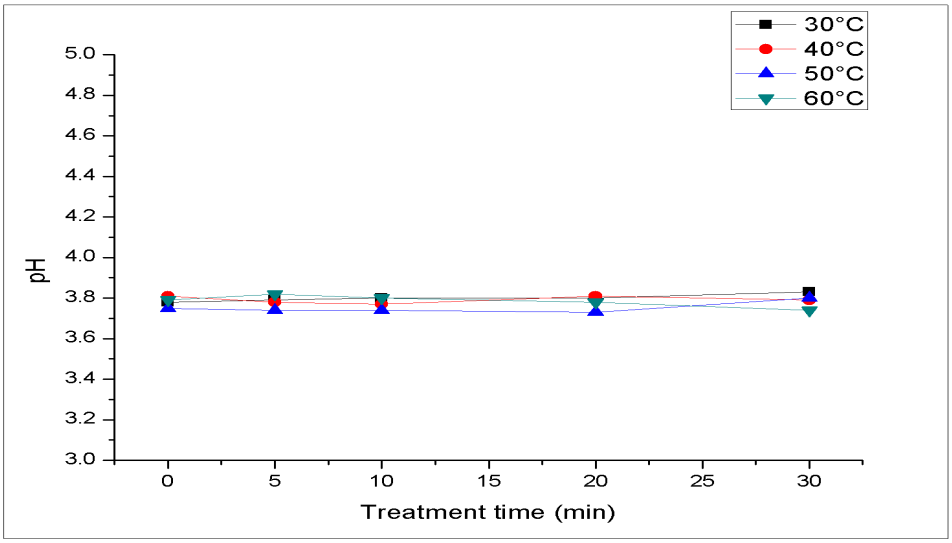

Fig. 7: Effect of thermosonication on pH of apple slices

4.1.4 pH

pH is defined as the measure of the strength of acid in a solution. The determination of pH is one of the most frequently used physical quality control methods. In treated apple slices, no significant change in pH was observed (Tiwari et al., 2008). The change in pH was of the order of 2 decimal points, with the pH observed (30 °C) as 3.78 varying to 3.73, in untreated and treated samples respectively. The possible reason for this behaviour could be the fact that ultrasonication in combination with thermal treatment is not sufficient to initiate the chemical breakdown process. pH plays an important role in phenomenon such as gelation, enzyme activity, protein denaturation and microbial inactivation kinetics and most microorganisms show increased susceptibility and inability to recover from sub-lethal injuries at low pH values (Hoover et al., 1989).

4.1.5 Titratable Acidity

Titratable Acidity (TA) is a measure of the amount of acid present in a solution. It is expressed as grams/liter (g/L) and is obtained by multiplying to percent TA by 10. So, a TA of 0.60% is expressed as 6g/L. In the conducted research, no significant changes in the TA were observed in the thermosonicated apple slices. Additionally, our results regarding pH and acidity of thermosonicated apple juice are in accordance with the observations of sonicated kasturi lime juice (Mason et al., 2005).

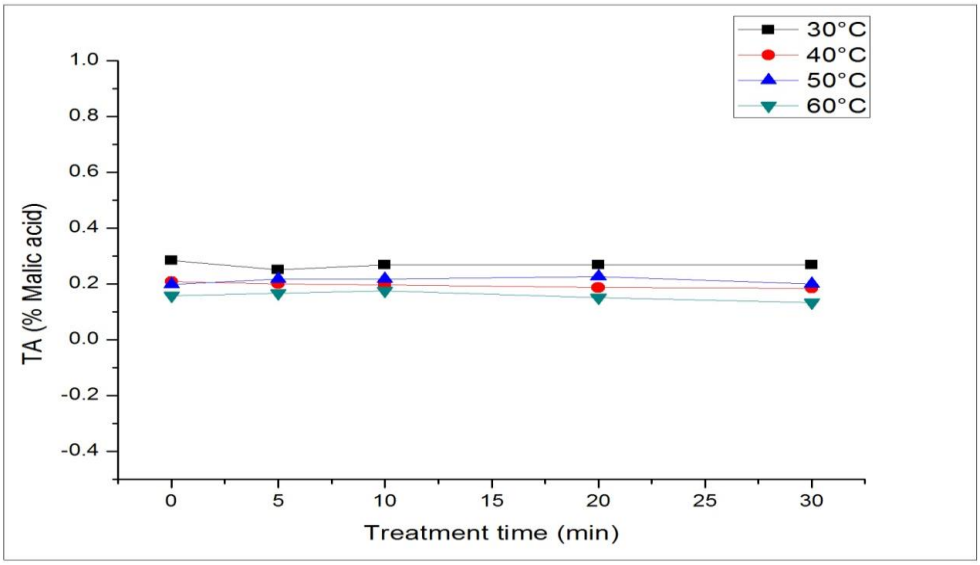

Fig. 8: Effect of thermosonication on TA of apple slices

4.1.6 Total Phenolic Content

Fruits are an important source of phenolic compounds in our diet, and these phenolic compounds play a significant role in flavour and colour development and also have a major role in human health by controlling the threat of many diseases (Annegowda et al., 2012). Fresh samples, taken in this experiment, had a total phenolic content in the range of 3.45-9.85909 mg GAE/100 mL of sample. The graphs (fig. 9) below show the variation in total phenolic contentment with respect to temperature and time. The graph at 30 °C shows an increase in the total phenolic content of apple slices with increase in dwell time, with the TPC increasing by 10.5-30.3% on thermosonication. The total phenolic content of thermosonically treated apple slices showed an increasing trend from 30 to 50 °C. However, at 60 °C the total phenolic content started decreasing. Our results regarding an increase in total phenolic content with an increase in temperature in freshly cut apple slices are not in conformity with the observation of Rawson et al. (2011), who reported that the total phenolic content decreased with increase in processing temperature from 25 to 45 °C in thermosonically treated watermelon juice.

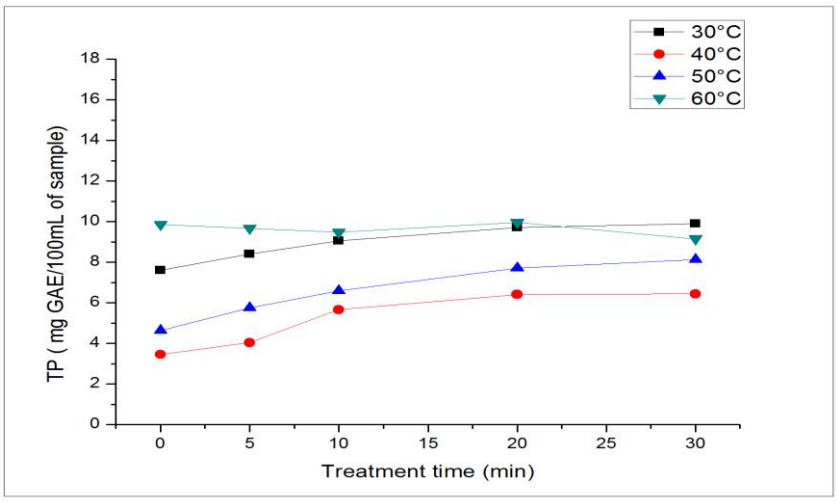

Fig. 9: Effect of Thermosonication in Total Phenolic content of apple slices

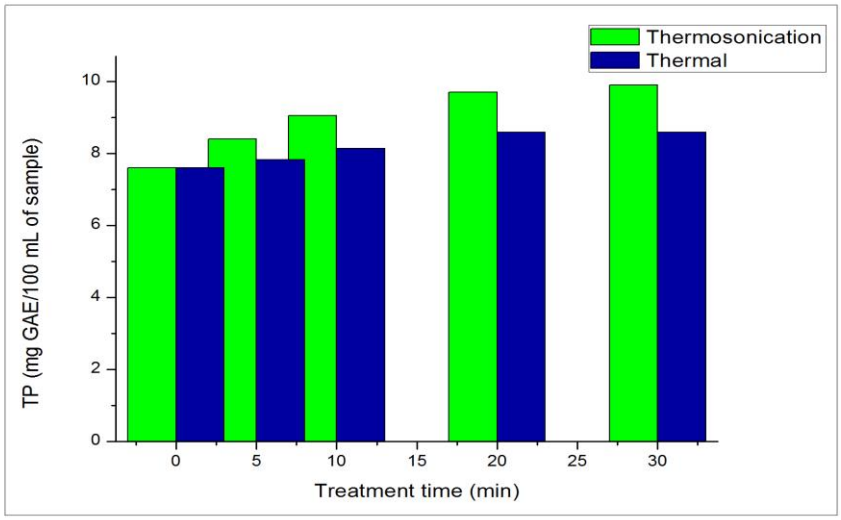

Fig. 10: (a) Effect of Thermosonication and Thermal treatment on Total Phenolic content of apple slices at 30 °C

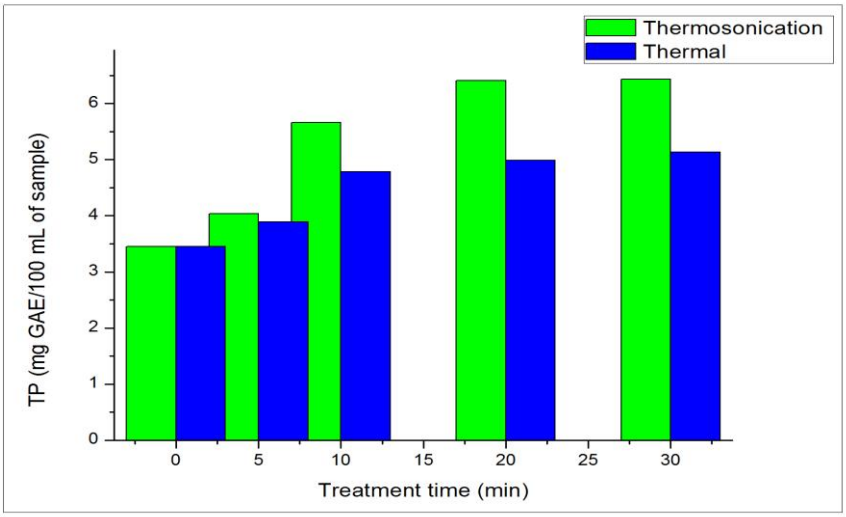

Fig. 10: (b) Effect of Thermosonication and Thermal treatment on Total Phenolic content of apple slices at 40 °C

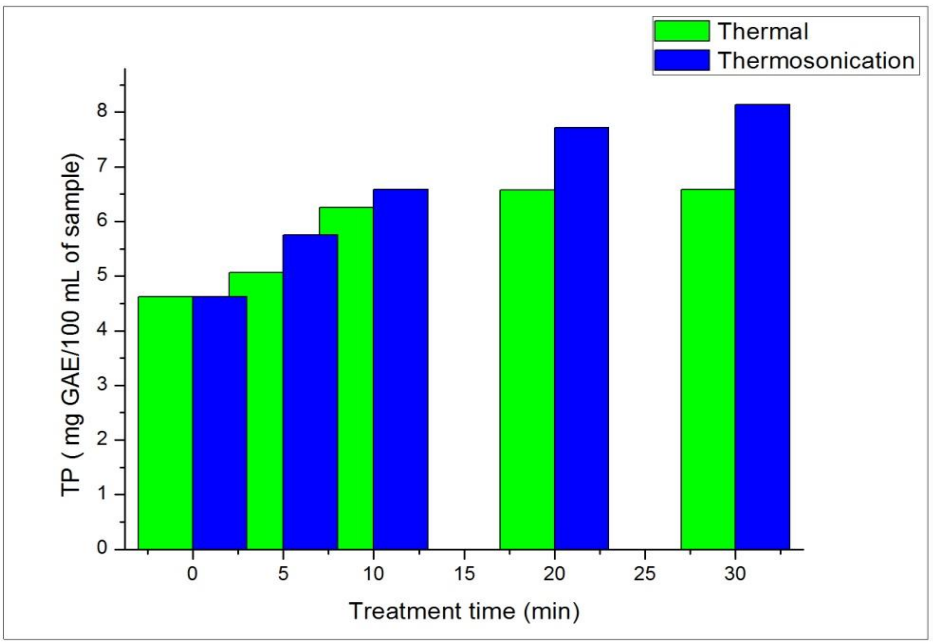

Fig. 10: (c) Effect of Thermosonication and Thermal treatment on Total Phenolic content of apple slices at 50 °C

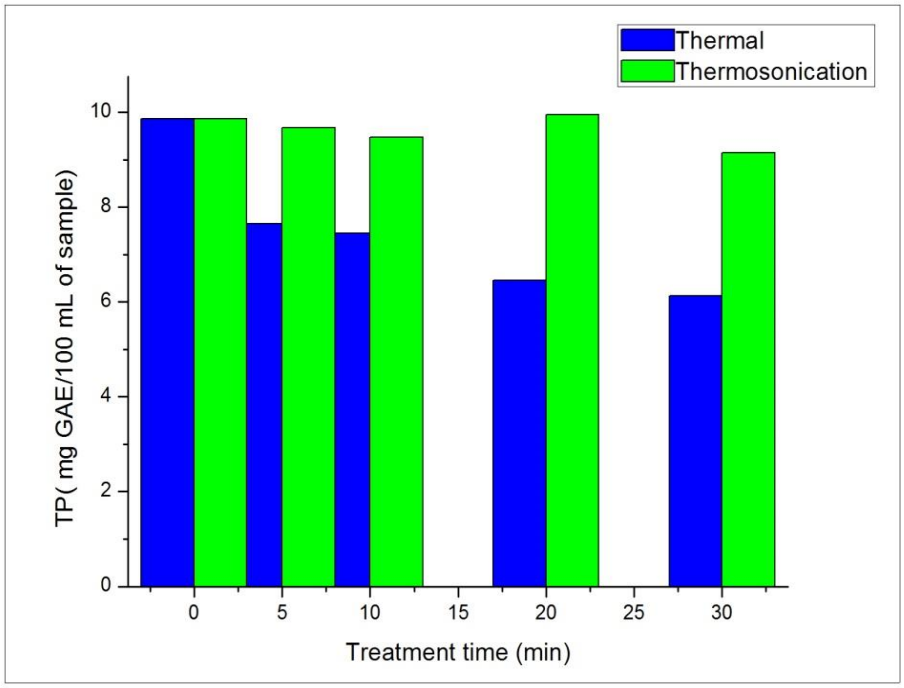

Fig. 10: (d) Effect of Thermosonication and Thermal treatment on Total Phenolic content of apple slices at 60 °C

A comparative study of thermal treatment and thermosonication was also undertaken and the results inferred (fig. 10) were that the total phenolic content increment was more in case of thermosonication treatment than thermal treatment alone. The increase at 30 °C thermosonication was 13.2% more, and at 60 °C the retention was 33.1%

more than that of thermal treatment alone. Hence, it was concluded that thermosonication treatment was useful and more effective in releasing the total phenols at moderate temperatures below 60 °C. This suggests that both thermal and thermosonication treatments facilitate in the release of phenols from the food matrix. These released phenols are then solubilised, due to the cavitations produced during sonication, which increases the extraction and availability of these compounds. The agitation and heat treatment also causes the increase in solubility but at higher temperatures, these phenolic compounds are degraded.

4.1.7 Total Antioxidant Capacity

The antioxidant capacity of a substance is defined as its ability to scavenge reactive oxygen species and electrophiles, which are formed during normal processes in the organism, but their accumulation leads to disease such as cancer.The trend followed by total antioxidant capacity is as depicted in fig. 11. The antioxidant activity increased with time for 30, 40 and 50 °C. However, for 60 °C, the antioxidant activity showed a decreasing trend. The increase in antioxidant capacity might be attributed to the increase in ascorbic acid and polyphenolic compounds in the apple slices, due to cavitations produced during sonication which increases the extraction and availability of these compounds. More the polyphenolic compound more the antioxidant capacity will be. But, at higher temperatures like 60 °C, the decrease is due to the degradation of antioxidants at higher temperature.

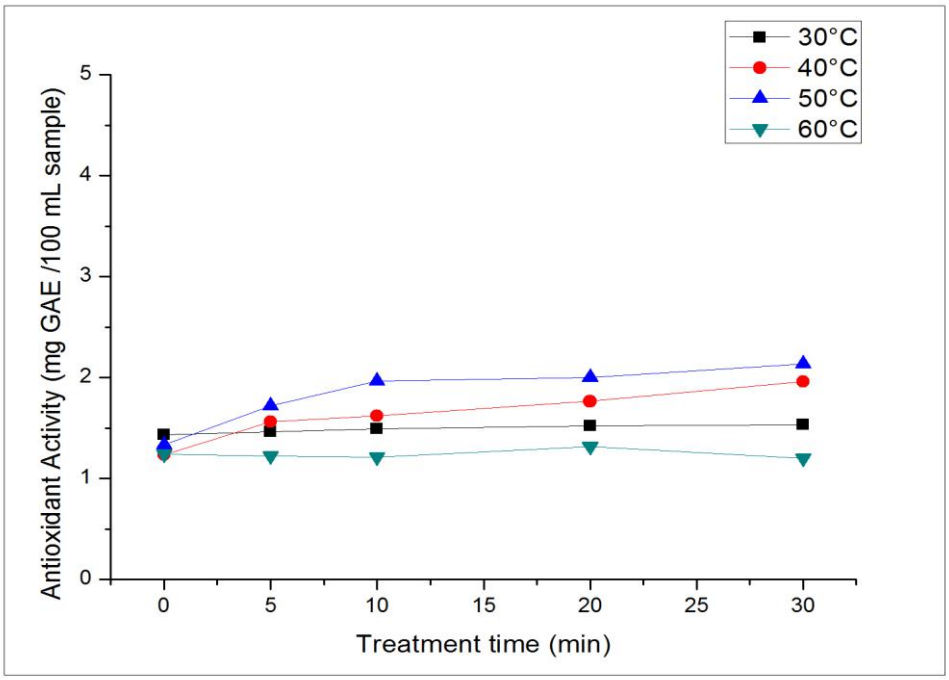

Fig. 11: Effect of Thermosonication on Antioxidant Activity of apple slices

A study for comparing the antioxidant activity with thermosonication and thermal treatment was undertaken, with the conclusion that both thermal treatment and thermosonication treatments favoured antioxidant activity. However, the increase in thermosonication was 7.2% more than in thermal treatment alone at 30 °C.

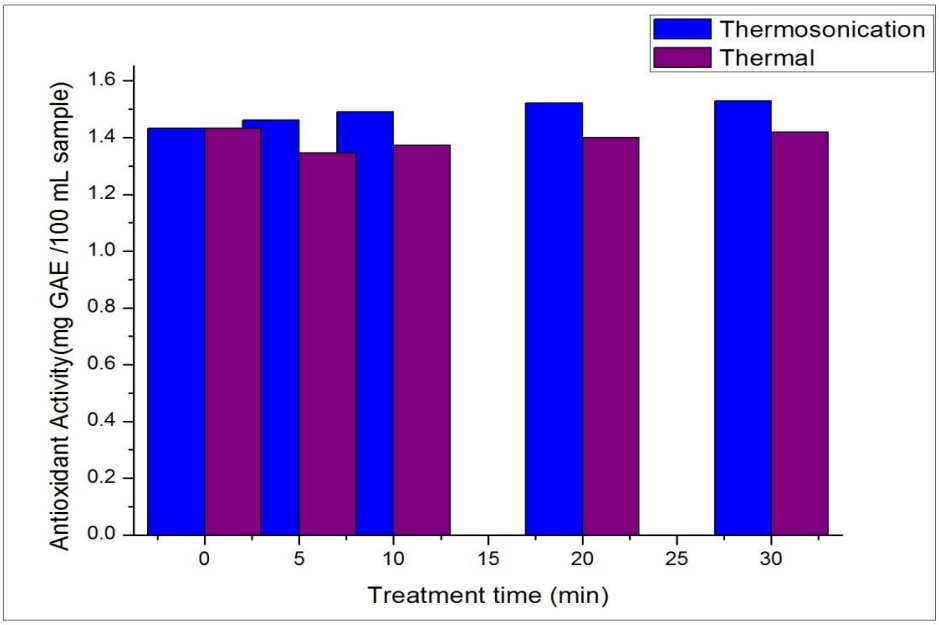

Fig. 12: (a) Effect of Thermosonication and Thermal treatment on Antioxidant Activity of apple slices at 30 °C

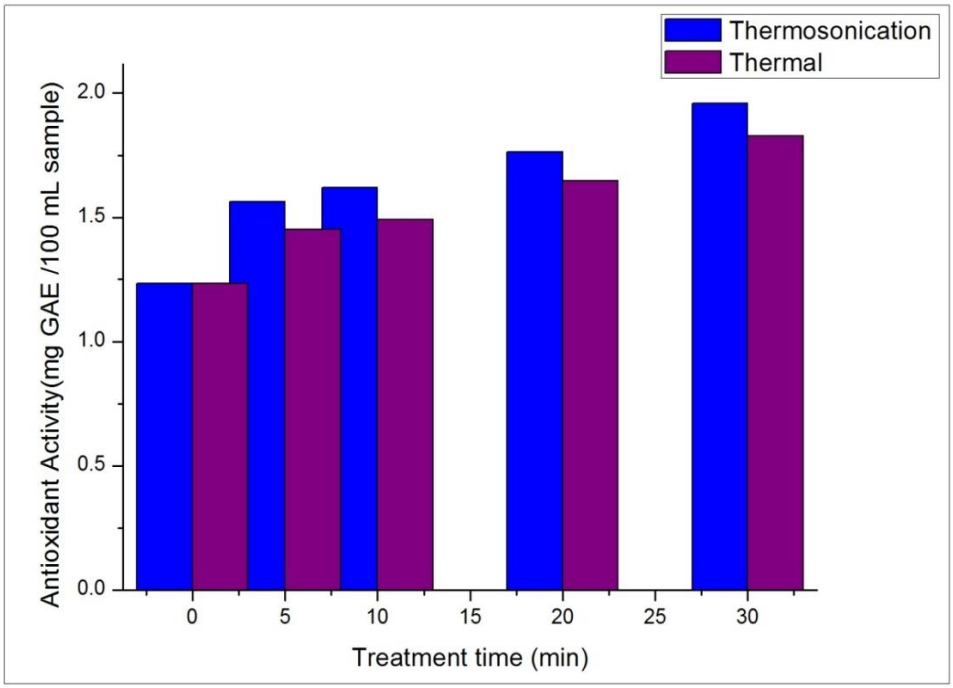

Fig. 12: (b) Effect of Thermosonication and Thermal treatment on Antioxidant Activity of apple slices at 40 °C

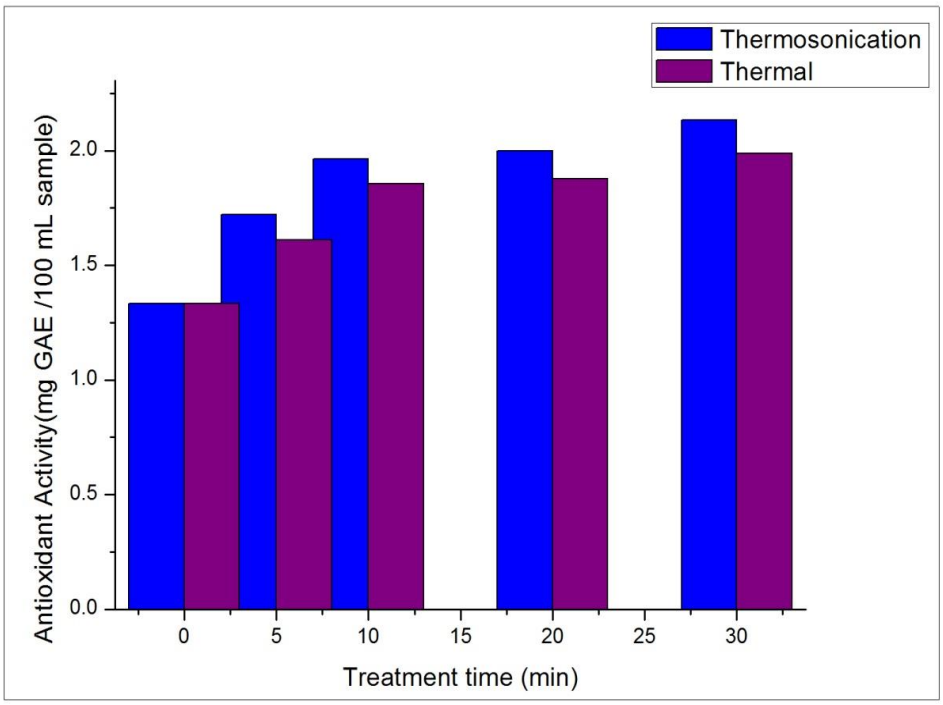

Fig. 12: (c) Effect of Thermosonication and Thermal treatment on Antioxidant Activity of apple slices at 50 °C

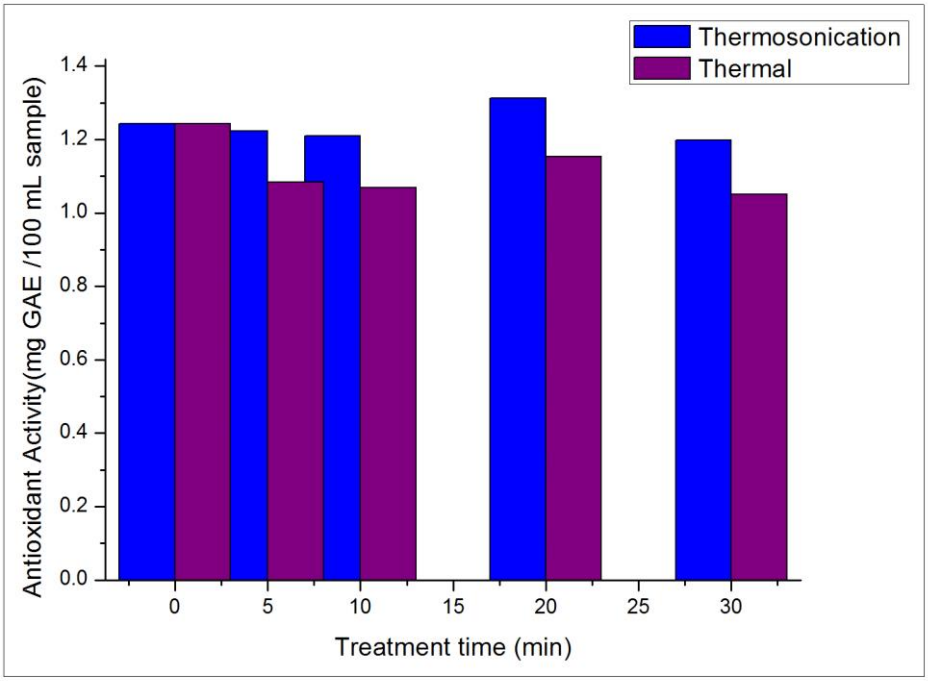

Fig. 12: (d) Effect of Thermosonication and Thermal treatment on Antioxidant Activity of apple slices at 60 °C

4.2.1 PME inactivation

Pectin methylesterase may have a major role in either the development or the loss of textural characteristics (Vora et al., 1999). PME is also responsible for the softening of plant tissues occurring in post-harvest storage of fruits and vegetables. The duration of the action of PME during fruit and vegetable processing must be controlled in order to prevent the detrimental, post-processing effects of the enzyme. The application of sonication is being studied for the inactivation of enzymes and micro-organisms in the recent years. Sonication alone can cause microbial inactivation but sonication with heat (thermosonication) provides a greater effect for the inactivation of micro-organism in which an additive or even synergistic effect can be observed (Lopez-Malo et al., 2005) and enable shorter processing time, perhaps resulting in enhanced juice quality (Feng et al., 2009).

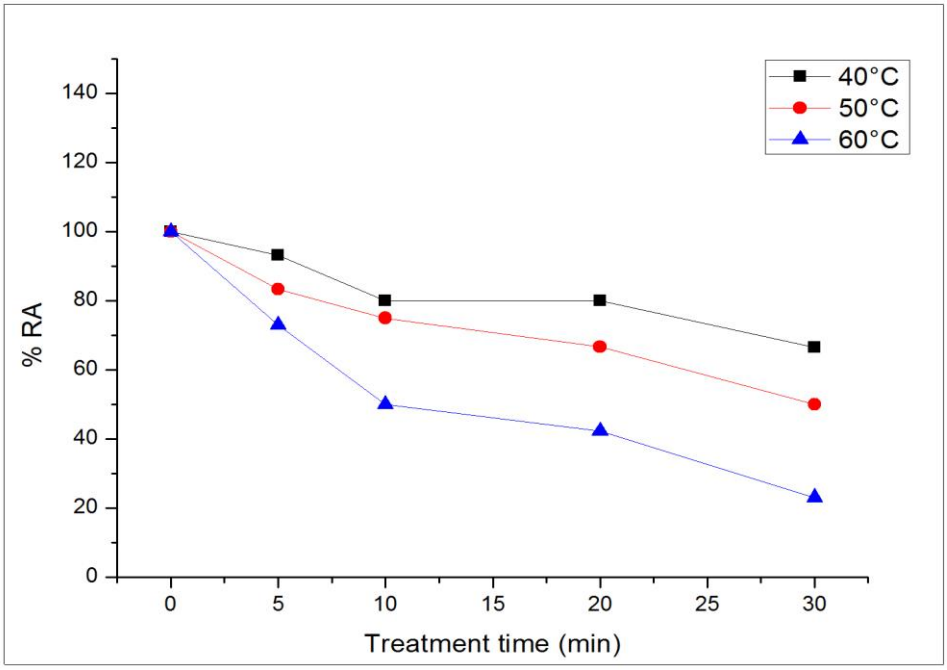

Fig. 13: Effect of thermosonication treatment on PME of apple slices

The residual activity of PME at 40 °C thermosonication treatment, after 30 min of treatment time is 66.5%. At 50 °C thermosonication treatment, after 30 min of treatment the residual activity obtained is 50% and at 60 °C it is 23.1%.The inactivation caused was observed to be the highest at 60 °C (76.9%). With increase in temperature and treatment time, PME inactivation shows an increasing trend. The inactivation of PME in thermosonicated samples may contribute to the maintenance of the degree of esterification in pectin at its original state and/or increase in the temperature should increase the inactivation rate. The enzyme effects of sonication treatment are frequently recognised to numerous sonochemical, biochemical, physical and mechanical processes induced by cavitation (Sehgal et al., 1980).

Similarly, we can establish a correlation to TS treatment in tomato juice where 90% reduction in PME activity at above 60 °C was observed (Wu et al., 2008). In another study, tomato juice was subjected to TS treatment and the PME activity was determined to be reduced by 90% at 60, 65 and 70 °C for 41.8, 11.7 and 4.3 min exposure, respectively (Wu et al., 2008). In addition, TS treatment was proved to be more efficient in the inactivation of the PME than heat treatment (Leighton, 1998).Thus, on the basis of these observation, it can be concluded that thermosonication is an effective treatment for inactivation of PME.

4.2.2 PPO and POD inactivation

PPO and POD are the main enzymes involved in the phenolic oxidation of numerous fruits and vegetables (Jiang et al., 2004). Polyphenolase is a copper-containing enzyme which is certainly present in all fruits and vegetables products. Aadil (2013) stated that for grapefruit juice, there was a non-significant change in the PPO inactivation at 20 °C for 30 and 60 min, but with the increase in temperature, a significant change of PPO activity was observed while 90% PPO inactivation was observed at 60 °C for 60 min. It was reported that the combined effect of ultrasound and heat was found to synergistically enhance the inactivation kinetics of PPO. TS treatment at 60 °C for 60 min exposure reduced PME, PPO and POD activity by 91, 90 and 89% respectively (Cheng et al., 2013).

In our study, we observed that at lower temperatures POD residual activity exhibited an increasing trend suggesting that thermosonication at lower temperatures (30 °C) favoured the activation of POD enzyme. However, at higher temperatures POD activity decreased to 60 and 20% at 50 and 60 °C thermosonic treatment respectively.

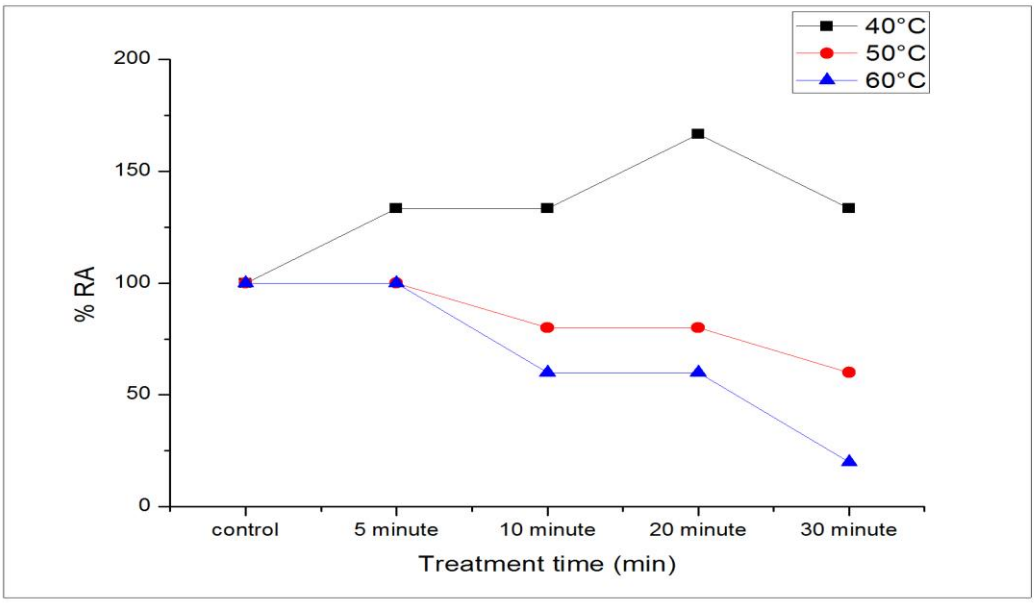

Fig. 14: Effect of Thermosonication on POD

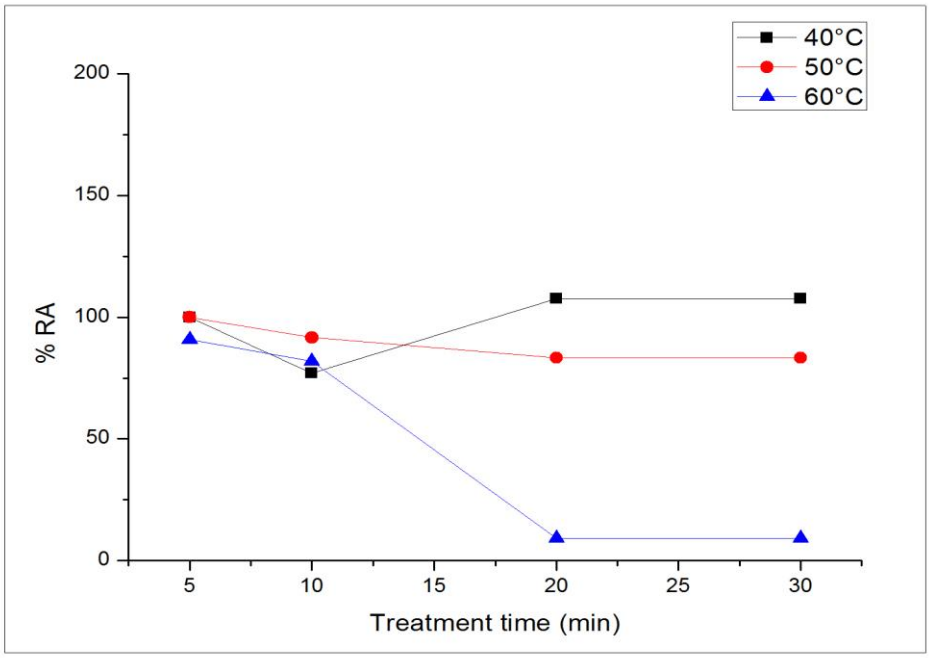

Fig. 15: Effect of thermosonication on PPO

The increase in POD activity may be related to the formation of hydrogen peroxide in the liquid media by ultrasound application because this enzyme is activated by an increase in hydrogen peroxide levels, which is the main substrate for PODs. The reduction of activity is related to the conformation changes in the tertiary structure, as in the active site three-dimensional structure affecting the enzyme-substrate interaction.

The activity of PPO also followed a similar trend with inactivation of 16.7% and 90.9% at 50 and 60 °C thermosonic treatment respectively. However, for 40 °C thermosonic treatment, the enzyme was activated. Thus, thermosonication at lower temperature favoured the activation of PPO while the inactivation of PPO and POD was favoured with increase in temperature and treatment time.

Chapter V

SUMMARY AND CONCLUSIONS

Packaged, fresh cut apple slices market has experienced rapid growth in recent years, as advances in processing technology and changes in the marketplace have coincided. Industry surveys show convenience as the major driver for growth of this category. Thermal treatment, ascorbic acid treatment, Modified Atmospheric packaging (MAP) and edible coatings are used for this purpose but all the mentioned treatments have their demerits in terms of protecting the commodity from deterioration. Hence, thermosonication treatment was undertaken to examine if it was advantageous in the case of fresh cut apple slices.

Fresh cut apple slices were packed in LDPE films and were treated for 5-30 min at 30, 40, 50 and 60 °C. All the samples were analyzed for titratable acidity, Browning index, physical (pH, TSS, color, texture) and biochemical (Total phenolic content, antioxidant activity) characteristics. The results revealed that there were no significant changes in pH, TSS and titratable acidity. The browning index decreased with increase in thermosonication temperature and treatment time. Similarly, the hardness of the samples also decreased with the increase in treatment time and temperature. The total phenolic content as well as the antioxidant capacity increased at 30, 40 and 50 °C, but decreased at higher temperature (60 °C). Thermosonicated samples also showed higher release of total phenols and antioxidant capacity.

PPO, PME and POD activity assay was conducted as the second objective of this project. The enzyme assays were performed for 5-30 min at 40, 50 and 60 °C. The inactivation observed in PME was sufficient at 60 °C. However, the activities of PPO and POD increased at lower temperatures and the inactivation of PPO and POD occurred at higher temperatures. Inactivation in both the enzymes was sufficient at 60 °C for 30 min.

Based on the results of the present investigation, following major conclusions could be drawn:

1. Thermosonication treatment resulted in slightly more browning of treated samples as compared to the untreated samples.
2. After Thermosonication as well as thermal treatment, total phenolic content and antioxidant capacity increased in comparison to control, at temperatures <60 °C. However, the increase observed in thermosonication treatment was more than that of thermal treatment.
3. Thermosonication was effective in the inactivation of PME, PPO and POD at 60 °C and it led to an increase in activity of PPO and POD at 40 °C.
4. Thermosonication has proved to be a better technology for processing of fresh cut apple slices as compared to thermal treatment alone.

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