# MANOTHERMOSONICATION (MTS) TREATMENT OF APPLE-CARROT JUICE BLEND: EFFECT ON INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND QUALITY ATTRIBUTES DURING STORAGE

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Food Science and Human Nutrition
with a concentration in Food Science
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2014

Urbana, Illinois

Adviser:

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## ABSTRACT

With advantages such as requiring less time, saving energy and reducing the costs manothermosonication has become increasingly popular in the food processing industry in recent years. My thesis research was conducted to investigate the effect of manothermosonication (MTS) on microbial inactivation and the quality of apple-carrot blended juice during storage.

To examine the effect of MTS on quality of apple-carrot blended juice samples treated with MTS and high temperature - short time (HTST) was examined during a 3-week period under refrigeration conditions. Quality attributes such as brix, total phenolic content, titratable acidity, viscosity, antioxidant capacity, turbidity, pH, and color of the samples were compared.

The MTS treatment helped to achieve 5 log reduction in apple-carrot blended juice in relatively short time, especially at elevated temperatures. At 40$^{o}$C, a maximum of 3.03 log CFU/ml after 75 s treatment at 300 kPa. At 50$^{o}$C, 5 log reduction in the population of *Escherichia coli* O157:H7 was achieved in 60 s under all three pressures (100, 200, and 300 kPa). When temperature was increased to 60$^{o}$C, only 30 s were needed to reduce the survival count of *E. coli* O157:H7 by 5 log cycles at 100, 200, and 300 kPa.

MTS treatment of apple-carrot blended juice significantly affected chemical parameters such as total phenolic content, antioxidant and pH-value. The MTS treatment affected the antioxidant activities of apple-carrot blended juice during three weeks storage and a significant increase in antioxidant activity was recorded for samples treated with MTS during 3 weeks of storage. While the first day antioxidant activities of the samples were low, they were increased when measured on day 7, 14, and 21. Total phenolic content of apple-carrot blended juice treated by HTST was significantly lower compared to the samples treated with MTS. Turbidity of MTS-treated samples decreased significantly during 3 weeks of storage. Overall, apple-carrot blended

juice treated with MTS showed good promise as an alternative to HTST as evidenced by effective microbial inactivation and ability to maintain juice quality.

## ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Hao Feng for his continuous support of my MS study and research, for his patience, motivation, and knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my MS study.

Besides my advisor, I would like to thank the rest of my thesis committee: Dr. Nicki Engeseth and Dr. Michael Miller, for their willingness to serve on my committee and their support and contribution to my thesis preparation. I would also thank to Dr. Graciela Wild Padua for checking my thesis.

My many thanks must also go to Prof. Ibrahim Hayoglu, Dr. Hyoungill Lee, Dr. Hee Kyung Park, Gulcin Yildiz, Brian Jecobson, Todd Kirk, Nick Reitz, and Erman Gungor who help me during my experiments and generously providing guidance on the technical aspect of this thesis. In addition, thanks to my friends and lab mates for their assistances in many aspects. I am so grateful to meet those friends who inspirit my effort in order to overcome the difficulties.

Last but not least important, I owe more than thanks to my family for their love, encouragement throughout my life and financial support. Without their support, it is impossible for me to finish my graduate study.

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# CHAPTER 1

# INTRODUCTION

There is increasing interest in the consumption of juice blends such as carrot juice blending primarily with apple and banana juices. There are several reasons for the increased demand and consumption of vegetable and fruit juice blends (Gao and Rupasinghe, 2012). Blending of juices is reported to enhance the aroma, taste and nutritional value of the juices (Bhardwaj and Pandey, 2011). Juice blending can produce new juice flavors not previously available and help to reduce the production costs. Drinking juice blends is also an efficient way to consume more fruits and vegetables.

In the processing of blended juice, a thermal pasteurization method, normally a high-temperature short-time (HTST) treatment is often used to secure microbial safety of the products. Although HTST effectively inactivates human pathogens in the juice, it generally results in unwanted quality degradation such as nutrient loss, color changes, and sensory property changes in the juices. To secure food safety with minimal quality degradation, non-thermal technologies such as ultrasound treatment have been proposed as alternatives to thermal pasteurization so that the changes of flavor and nutritional value can be minimized during processing. The lethal effect of ultrasound when applied to a liquid medium is attributed to the physical and chemical events in the medium produced by acoustic cavitation, which is the generation, growth, and implosion of tiny bubbles generated when sound waves pass through a liquid. Ultrasound has been tested to inactivate human pathogens in juice products, such as apple cider (Lee et al., 2013). Sonication of apple cider at 57$^{o}$C and 20$^{o}$C achieved 5 log reduction of *E. coli* O157:H7 in about 4.5 and 6 min, respectively. Noticeably, most, if not all, previous work using ultrasound in combination

with heat to inactivate food-borne pathogens required a relatively long time, up to 6 minutes, to achieve 5 log reduction in the population of a target pathogen which may have less practical application in juice processing, as it takes only 15 seconds to achieve a 5-log reduction in the population of a target pathogen when HTST is applied to treat juices. In this work, a high-intensity and short-time (HIST) ultrasound treatment concept was explored to inactivate *Escherichia coli* O157:H7 in carrot and apple juice blend and to maintain quality of the treated product. This was achieved by performing the ultrasound treatment at elevated temperature under a low static pressure, a process termed mano-thermo-sonication (MTS), in the juice samples.

It was hypothesized that MTS will significantly shorten the time needed to achieve a 5-log reduction in the number of inoculated *E. coli* 157:H7 in carrot-apple juice blend compared to previous ultrasound treatment studies, and the quality of MTS treated juice will be as good as or better than the counterpart treated by HTST. The overall objective of this research was to investigate the effects of MTS on microbial inactivation and the quality of apple-carrot blended juice. The first objective was to examine the responses of *E. coli*O157:H**7** to MTS treatment, and to evaluate selected inactivation kinetic models (Weibull, and biphasic linear) for fitting of *E. coli*O157:H**7** inactivation data. The second objective was to evaluate the quality changes in apple-carrot blended juice treated with manothermosonication (MTS) and high temperature-short time (HTST).

The MTS treatment helped to achieve 5 log reduction in apple-carrot blended juice in relatively short time, especially at elevated temperatures. At 40$^{o}$C, a maximum of 3.03 log CFU/ml after 75 s treatment at 300 kPa. At 50$^{o}$C, 5 log reduction in the population of *Escherichia coli* O157:H7 was achieved in 60 s under all three pressures (100, 200, and 300

kPa). When temperature was increased to 60$^{o}$C, only 30 s were needed to reduce the survival count of *E. coli* O157:H7 by 5 log cycles at 100, 200, and 300 kPa.

The quality changes in apple-carrot blended juice treated with MTS and traditional HTST method were examined during a 3-week period under refrigeration conditions. Quality attributes of samples treated with MTS and HTST evaluated included brix, total phenolic content, titratable acidity, viscosity, antioxidant capacity, turbidity, pH, and color. The MTS treatment affected the antioxidant activities of apples-carrot blended juice during three weeks and a significant increase in the antioxidant activity was recorded for samples treated with MTS during 3 weeks of storage. While the first day antioxidant activities of the samples treated with HTST, MTS, and raw were low, they were increased when measured on the seventh, fourteenth and twenty-first days. The total phenolic content of apple-carrot blended juice treated by HTST were significantly lower in juice blends compared to the samples treated with MTS. Turbidity of the MTS-treated samples decreased significantly during 3 weeks of storage. Overall, apple-carrot blended juice treated with MTS showed good promise as an alternative to HTST.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Fruit and vegetable consumption in the U.S

Fruits and vegetables are rich inessential vitamins, minerals, fiber, and other bioactive compounds. It was reported that the diet high in these foods is associated with lower risk for numerous chronic diseases, including certain cancers and cardiovascular diseases (Van Duyn et al., 2000).

Adults in the United States consume fruit about 1.1 times per day and vegetables about 1.6 times per day (State indicator Report on Fruits and Vegetables, 2013). In 1990, the *Dietary Guidelines for Americans* recommended eating at least two servings of fruits and three of vegetables daily (Dietary guidelines, 1990). The MyPlate food guidance system (USDA, 2008) emphasizes the need to "focus on fruits" and "vary your veggies" as building blocks for a healthy diet (www. choosemyplate.gov). Recently, several states attempts to increase fruits and vegetables consumption with the help of improving access and establishing policies. For example, 28 states now have a farm to school/ preschool policy. Twenty-seven states have created state-level food policy councils--coalitions of private and public partners working together to improve access to healthy food.

## 2.2 Quality attributes of apple juice and carrot juice

### 2.2.1 Quality and nutritional value of apple juice

Apple (*Malusdomestica* Borkh.) is an important fruit and it is consumed around 60 million tons globally which places it the third in worldwide fruit production (FDA, 2010). Following onion and tea, apples are one of the major sources for flavonoid intake (Harmanescu et al., 2006). The major flavonoid classes in apple fruit are flavonols such as quercetin 3-glycosides, monomeric and oligomeric flavan-3-ols such as catechin, and epicatechin. In addition, there are significant amounts of hydroxycinnamic acid derivatives which are represented by chlorogenic acid in apple fruit (Nicolas et al., 1994). These flavonoids and chlorogenic acids are the main contributor for the quality of apples. The red color of apples is mainly because of the flavonoid cyanidin-3-galactoside which is located in the skin cells of apple and the reason for browning occurred in processed apple such as juices and ciders is primarily because of the fact that oxidation of chlorogenic acid by oxidative enzymes (Nicolas et al., 1994). Thermal treatments such as pasteurization are used to inactivate human pathogens, spoilage causing microorganisms, and enzymes, resulting an extended shelf life of fruit and vegetable juices. However, heat processing generally causes unwanted changes such as nutrient loss, color changes, and sensory property changes. Non-thermal methods such as ultrasound treatment have been proposed as alternatives for thermal pasteurization so that the changes of flavor and nutritional value can be minimized during processing (Nicolas et al., 1994).

### 2.2.2 Quality and nutritional value of carrot juice

Carrot juice has a high nutritional value. It has several important dietary sources of such as alpha and beta-carotene, zeacarotene, lutein and lycopene (Sharma et al., 2009). Beta carotene is one of the most active carotenoids biologically that act as provitamin A (Sharma et al., 2009). Preservation of carrot juice is difficult since it has low acidity, approximately pH 6, and this range provides suitable environment for the growth of many spoilage and spore forming bacteria (Demir et al., 2004). There are several methods to acidify carrot juice. For example, acidification of carrot juice might be achieved by either fermentation or adding citric acid (Demir et al., 2004). In addition, blanching of carrots in acid may improve the color of carrot juice (Kim et al., 1983). Alternatively and most importantly, blending carrot juice with acidic fruit juices such as apple juice might produce a blend with a lower pH, which can act as a natural barrier against most microorganisms. Thermal treatments such as HTST cause unwanted changes such as nutrient loss, color changes, and sensory property changes.

### 2.2.3 Juice blending

There is an increasing interest in the consumption of blended juice such as carrot juice blending primarily with apples, and bananas. There are several reasons to prefer and consume the blended vegetable and fruit juices (Gao and Rupasinghe, 2012). In order to pursue healthy life, in addition to fruits and vegetables, juices have an important role on the nutritional value of the human body that contains minerals, carbohydrates, and vitamins. For example, blending of juices can enhance the aroma, taste and nutritional value (Bhardwaj and Pandey, 2011). In order to monitor the quality of blended juices, the evaluation of the mineral content of the juices is getting more important (Rupasinghe, 2012).

## 2.3 Microbial food safety of juice products

Food preservation procedures are targeted towards microorganisms. Nowadays, food preservation methods which are used in the industry depend either on the inhibition of microbial growth or on microbial inactivation. Table 2.3 shows the outbreaks of human food-borne disease from various microorganisms associated with apple and carrot juices during the period of 1922–2010. It is the fact that methods which prevent or slow down microbial growth cannot completely ensure food safety since their efficiency depends on the environmental conditions for example the maintenance of the chill chain. Thermal treatment is the most widely used method to inactivate microorganisms in foods. However, the heat that used during thermal treatment leads to unwanted side effects in the sensory, nutritional and functional properties of food. This limitation together with increasing consumer demand for fresh-like foods has lead to the development of alternative methods for microbial inactivation such as ionizing irradiation, ultrasound under pressure, high hydrostatic pressure (HHP), and pulsed electric field (PEF) (Mañas&Pagán, 2005).

**Table 2.1** Outbreaks of human food-borne disease from various microorganisms associated with apple and carrot juices during the period of 1922–2010 (Danyluk, 2004).

| Type | Product | Pathogen[a] | Year | Location | Venue | Cases (deaths)[b] | Reference |
|---|---|---|---|---|---|---|---|
| Apple | Unpasteurized | *S. Typhi* | 1922 | France | NR | 23 (0) | Paquet, 1923 |
| | Unpasteurized | *S.* Typhimurium | 1974 | USA (NJ) | Farm, small retail outlets | 296 (0) | CDC, 1975 |
| | Unpasteurized | *E. coli* O157:H7 (suspected) | 1980 | Canada (ON) | Local market | 14 (1) | Steele et al., 1982 |
| | Unpasteurized | *E. coli* O157:H7 | 1991 | USA (MA) | Small cider mill | 23 (0) | Besser et al., 1993 |
| | Unpasteurized | *Cryptosporidium* | 1993 | USA (ME) | School | 213 (0) | Millard et al., 1994 |
| | Unpasteurized | *C. parvum* | 1996 | USA (NY) | Small cider mill | 31 (0) | CDC, 1997 |
| | Unpasteurized | *E. coli* O157:H7 | 1996 | USA (CT) | Small cider mill | 14 (0) | CDC, 1997 |
| | Unpasteurized | *E. coli* O157:H7 | 1996 | USA (WA) | Small cider mill | 6 (0) | FDA, 2001 |
| | Unpasteurized | *E. coli* O157:H7 | 1996 | Canada (BC), USA (CA, CO, WA) | Retail | 70 (1) | CDC, 1996; Cody et al., 1999 |
| | Unpasteurized | *E. coli* O157:H7 | 1997 | USA (IN) | Farm | 6 | INS DOH, 1997 |
| | Unpasteurized | *E. coli* O157:H7 | 1998 | Canada (ON) | Farm/Home | 14 (0) | Tamblyn et al., 1999 |
| | Unpasteurized | *E. coli* O157:H7 | 1999 | USA (OK) | NR | 25 | CDC, 2011 |
| | Unpasteurized | *C. parvum* | 2003 | USA (OH) | Farm/Retail | 144 | Vojdani et al., 2008 |
| | Unpasteurized | *E. coli* O111 and *C. parvum* | 2004 | USA (NY) | Farm/Home | 212 | Vojdani et al., 2008 |
| | Unpasteurized | *E. coli* O157:H7 | 2005 | Canada (ON) | NR | 4 | LSDEPC, 2005 |
| | Unpasteurized | *E. coli* O157:H7 | 2007 | USA (MA) | NR | 9 | CDC, 2011 |
| | Unpasteurized | *E. coli* O157:H7 | 2008 | USA (IA) | Fair | 7 | CDC, 2011 |
| | Unpasteurized | *E. coli* O157:H7 | 2010 | USA (MD) | Retail | 7 | FDA, 2010 |
| Carrot | Homemade | *C. botulinum* | 1993 | USA (WA) | Home | 1 (0) | Buzby and Crutchfield, 1999 |
| | Pasteurized | *C. botulinum* | 2006 | USA | Retail | 4 | CDC, 2006 |

a Pathogens abbreviated and associated with outbreaks include S. – *Salmonella*; E. – *Escherichia*; C. parvum – *Cryptosporidium parvum*; C. botulinum – *Clostridium botulinum*.
b The number in parenthesis represents the number of deaths if reported.
e NR – Not Reported

## 2.4 High temperature- short time (HTST) treatment

Heat application named thermal pasteurization is used very frequently to provide safe and secure food materials and lead to reduction in microbial population of foods. Even though thermal treatment is effective in microbial reduction, this process has several negative impacts on nutritional and sensory properties of foods due to the high temperature used during the process. Therefore, some alternative methods to heat treatments are searched for (Lee et al., 2009).

With growing consumer interest in healthier and nutritionally rich food, the juice market has experience major growth recently. Although thermal processes cause significant microbial inactivation, many undesired changes have been reported in the literature (Adekunte et al., 2010). Enzyme deactivation, color change, alterations in taste and also loss of essential vitamins are among the undesired/unwanted changes (Adekunte et al., 2010).

## 2.5 Sonication treatments

Ultrasound is one of the emerging techniques that have shown promise as an alternative to heat treatment. Ultrasound is a kind of sound wave which cannot be detected by human ear because it frequency is above human hearing. Ultrasound consists of high-frequency vibration, which provides mix of fluid and shear forces on a micro-scale (Kentish and Feng, 2014). Ultrasound inhibits and destroys microorganisms due to the phenomenon of cavitation (O'Donnell et al., 2010), as shown in Figure 2.1. Microbial inactivation by ultrasound is caused by the micro-scale physical and chemical events produced by cavitation and therefore it is referred to as a non-thermal process. Ultrasound has found applications in variety areas in the food industry, such as freezing, cutting, tempering, extraction, drying, crystallization, filtration, de-foaming, and homogenization (Adekunte et al., 2010).

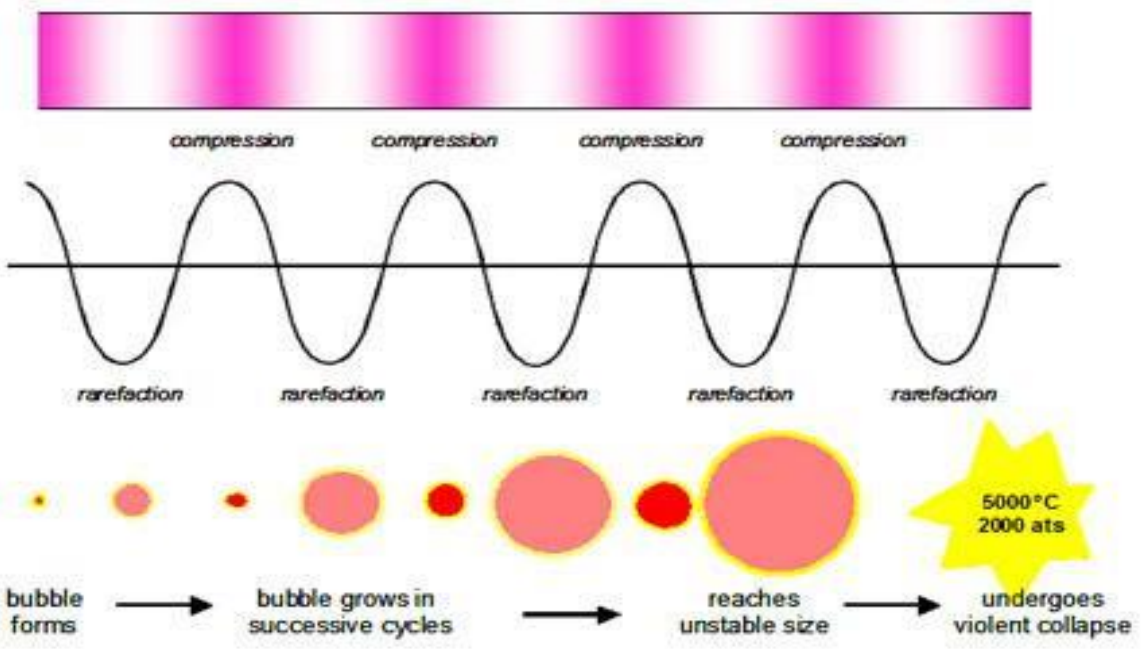


**Figure 2.1 Acoustic cavitation (Cobley et al., 2010).**

Sonication is the application of ultrasound in a liquid medium under sub-lethal temperatures. There are 3 types of sonication treatments namely manosonication (MS), thermosonication (TS), and manothermosonication (MTS). Treatment by ultrasound alone (Sonication) is less effective in microbial inactivation unless the treatment was at high acoustic power density. In order to increase the microbial inactivation rate, a combination of sonication and heat, named thermosonication (TS) was developed to enhance the inactivation efficacy. However, there is an upper temperature limit for TS inactivation of bacteria. Because of the cushioning effect of vapor-filled bubbles at elevated temperatures, TS treatment above a specific temperature did not result in any additional killing compared to a treatment using only heat. The application of static pressure to a TS treatment, called manothermosonication (MTS), was proposed for enhancing the cavitation intensity of the vapor-filled bubbles. MTS treatment was compared to a thermal only treatment at the same temperature, and the tests were conducted with Gram-positive bacteria such as *Listeria monocytogenes* and *Streptococcus faecium* and Gram-

negative organisms such as *Salmonella* cells. It was resulted in an increase in inactivation rate by 3.4–4.0-fold for TS treatment and 5.0–6.3-fold for MTS (Pagan et al., 1999a).In addition, a five-log reduction was achieved in 2.0 min for *L. monocytogenes* treated with MTS in citrate-phosphate buffer at 62 $^{0}$C and 200 kPa (Pagan et al., 1999b) and in 0.9 min in citrate-phosphate buffer for *S. enterica* treated at 60 $^{0}$C and 175 kPa(Pagan et al., 1999a).

# CHAPTER 3

# MANOTHERMOSONICATION (MTS) TREATMENT OF APPLE-CARROT JUICE BLEND FOR INACTIVATION OF *ESCHERICHIA COLI* O157:H7

## 3.1 Introduction

In recent years, there has been increasing interest in the consumption of blended juices such as carrot juice blending primarily with apple and banana juices. There are several reasons for increased consumption of the blended vegetable and fruit juices (Gao and Rupasinghe, 2012). In the effort to pursue a healthy life, juices of fruits and vegetables play an important role providing nutrients to human body, including minerals, carbohydrates, and vitamins (Harmanescu et al., 2006). The blending of juices has an ability to enhance the aroma, taste and nutritional value (Bhardwaj and Pandey, 2011).

Apples (Malusdomestica) are the most important sources of polyphenolic antioxidants, flavanols and their oligomers and polymers (Przybylska et al., 2007). The apple (*Malusdomestica* Borkh.) is an important fruit and it is consumed around 60 million tons globally placing it at the third place in worldwide fruit production (FAO, 2012). Following onion and tea, apples are one of the major sources for flavonoid intake (Singleton et al., 1999).

Because of their pleasant flavor and health benefits such as being a good source for eye disorders, skin care, and indigestion, carrots (Daucuscarota) become a major vegetable in diets. Carrot juice has a high nutritional value, being an important dietary source of carotenoids such as alpha and beta-carotene, zea carotene, lutein and lycopene (Singleton et al., 1999). Beta carotene, one of the most biologically active carotenoids, acts as provitamin A (Singleton et al., 1999).

Preservation of carrot juice is difficult because of its low acidity, which provides ideal environment for the growth of many human pathogens such as *E.coli* 0157:H7, *Salmonella spp*., and *Listeria monocytogenes*) (Demir et al., 2004). There are several methods to acidify carrot juices. For example, acidification of carrot juice could be achieved by either fermentation or adding citric acid (Demir et al., 2004). Alternatively, blending carrot juice with acidic fruit juices such as apple juice could produce a blend with a lower pH that can act as a natural barrier against most microorganisms.

Acoustic energy density (AED) is used for measurement of sound energy input into the treated juice (Kimura et al., 1996). Increasing temperature while using ultrasound causes several phenomena such as microstreaming, transiet cavitation, and microjetting. In other words, all of these applications of ultrasound will result in temperature rising. Even though changes (increasing) in temperature are not very high, it is important not to ignore this effect in system design (Kimura et al., 1996). It was showed that increasing AED cause increase in inactivation efficacy of the *E.coli* in black mulberry juice, which was in agreement with the previous studies (Guerrero *et al*., 2001; Salleh-Mack and Roberts, 2007; Wang *et al.,* 2010). Positive correlation in AED and inactivation rate of *E. coli* might be explained by mechanical disruption of cell wall via increasing in cavitation, localized instant overheating and production of free radicals (Guerrero *et al.*, 2001; Condon *et al*., 2005; Salleh-Mack and Roberts, 2007).

For both food processing industry and consumers, food-borne pathogen contamination is a main concern. *Escherichia coli* O157:H7 is a major virulent food-borne pathogen (Sharma et al., 2009). *Enterohaemorrhagic Escherichiacoli* (EHEC) that consist of the well-known pathogenic strain O157:H7 is a primarily source of food-borne outbreaks (Przybylska et al., 2007). It is estimated that around 9000 human illnesses and 70 deaths per year in the United

States occur because of this food-borne pathogen (Przybylska et al., 2007). Most important symptomatic effects of EHEC include bloody diarrhea, hemolytic uremic syndrome (HUS), and thrombotopenicpurpura (TTP). There are many food products that have been associated with this pathogen such as and most importantly milk, ground beef, lettuce, spinach, tomatoes, carrot juice and apple cider (Rosnah, 2012). Since the acid tolerance of *E coli* is between 3.7-4.7, it can survive in apple-carrot juice blend (pH: 4-4.5) (Samelis et al., 2003).

Treatments at high temperatures to inactivate human pathogens, a process termed pasteurization are often used to achieve a 5-log reduction in the population of a target pathogen, as required by FDA's. However, thermal processing generally causes unwanted changes such as nutrient loss, color changes, and sensory property changes (Harmanescu, et al., 2006). Non-thermal methods such as ultrasound treatment have been proposed as alternatives to thermal pasteurization so that the changes of flavor and nutritional value can be minimized during processing. Compared with diagnostic ultrasound, power ultrasound with a lower frequency range of 20 to 100 kHz and a higher sound intensity of 10 to 1000 $W/cm^2$ is used for microbial control in food applications (Baumann, 2005). Ultrasound has been identified as a potential technology to meet the FDA requirement of a 5 log colony forming units (CFU) reduction in pertinent microorganisms found in fruit juice and sufficient to inactivate food borne spoilage microorganisms, such as *Saccharomyces cerevisiae*, pertinent to fruit juice. In order to increase the microbial inactivation rate, a combination of sonication and heat, named thermosonication (TS), was developed to enhance treatment effectiveness. However, there is an upper temperature limit for TS inactivation of bacteria. Because of the cushioning effect of vapor-filled bubbles at elevated temperatures, TS inactivation above this temperature did not result in any additional killing compared to a treatment using only heat (Salleh-Mack et al., 2007). The application of

static pressure to a TS treatment, which is called manothermosonication (MTS), was proposed for enhancing the cavitation intensity of the vapor-filled bubbles. MTS treatment was compared to a thermal only treatment at the same temperature, and MTS inactivation tests were conducted with Gram-positive cells such as *Listeria monocytogenes* and *Streptococcus faecium* and Gram-negative cells such as *Salmonella*. MTS resulted in an increase in inactivation rate by 3.4–4.0-fold and 5.0–6.3-fold, respectively (Pagan et al., 1999a, b). In addition, a five-log reduction was achieved in 2.0 min for *L. monocytogenes* treated with MTS at 62 $^{0}$C and 200 kPa (Pagan et al., 1999b) and in 0.9 min for *S. enterica* treated at 60 $^{0}$C and 175 kPa in liquid whole egg using citrate-phosphate buffer (Pagan et al., 1999a). Generally, most previous ultrasound inactivation tests were performed in phosphate water.

Although a number of investigations using ultrasound to inactivate microorganisms under selected treatment conditions have been conducted, a detailed study into the interaction between bacteria and ultrasound under ultrasound–heat–pressure combinations (MTS) in a juice blend has not been reported in the literature. No report has documented kinetic modeling study to examine the effect of temperature, pressure, and ultrasound on inactivation of vegetative bacterial cells in a juice blend. This work was therefore undertaken to examine the responses of *E. coli* O157:H**7** to MTS treatment, and to evaluate the selected inactivation kinetic models (Weibull, and biphasic linear) for fitting of *E. coli* O157:H**7** inactivation data.

## 3.2 Materials and methods

### 3.2.1 MTS Treatment

Manothermosonication treatment was conducted using a continuous flow laboratory scale MTS system (Figure 3.1) developed at University of Illinois (Lee et al., 2013). Temperature readings were obtained with Ultrasound (20 kHz and 100% power) generated by a VC-750 ultrasound generator (Sonics & Materials, Inc., Newtown, CT, U.S.A) was transferred through a 13 mm-diameter standard probe with replaceable tip into the sono-reactor. A thermal couple (TMQSS-062G-6, Omega Engineering Inc., Stamford, CT, U.S.A) was used to monitor the inside temperature (±1 $^{0}$C) of a custom-made double-jacketed sono-reactor via circulating cooling water through the jacket. The temperature of the circulating water was determined by mixing tap water with water from a water bath with preset temperature. Nitrogen gas was used as the pressure source. The sample was sonicated at three temperatures (40, 50, and 60 $^{0}$C) and three pressures (100,200, and 300 kPa). Three replications were used for each condition. In continuous mode, 79 mL of juice sample in a bottle placed in a ice bath was pumped by a peristaltic pump (Model 7523-20, Masterflex, Vermon Hill, IL, U.S.A) into the reactor. The flow rate was determined for each experiment based on the length of the time between sample entering the system and sample flowing out from the system. The flow rates used in the experiments were in the range of 9.3 ml/s to 50.4 ml/s. A section of precision pump tubing was connected to polyethylene tubing with a valved in-line coupling insert, which provided pressure seal and fast assembly capacity. After that, the juice sample was introduced through the polyethylene tubing into the reactor. The treated juice samples was collected from two outlets of the reactor, and mixed in a cross-type tube fitting. The distance between replaceable tip and chamber bottom was 13 mm.

### 3.2.2 Sample preparation

Juices of Red Delicious apples and carrots were freshly pressed with using a juice extractor (Bullet Express Multifunction Food Processor, Model: BE 110, Pacoima, CA, USA). The carrots were first peeled with a sterile stainless steel knife. Apples then were peeled and put into the juice extractor. The apple-carrot juice blend was made of ninety-percent (90%) apple juice and ten-percent (10%) carrot juice, having a pH of 4–4.5. The juice blend samples were filtered with a 0.2 mm filter (Tri Clover Compatible Filter, CA, U.S.A) to remove the pulp. The MTS was conducted by pumping juice samples with a peristaltic pump (Model 7523-20, Masterflex, Vermon Hill, IL, U.S.A) through the custom-designed sono-reactor. The juice samples was exposed to sonication at three different temperatures (60, 50 and 40$^{o}$C) under three different static pressures (100, 200, and 300 kPa) for five residence times of 15, 30, 45, 60, and 75 seconds. The flow speeds were determined for each experiments based on the length of the time between sample inlet to the system and sample outlet from the system. For sanitizing whole system, 1 L hot water (about 100 $^{0}$C) was circulated through the whole MTS system. Plate counting method was performed on *Escherichia coli* O157:H7 before and after each treatment. For the experiment, 0.9 ml peptone water was added to 0.1 ml sample. Acoustic energy density (AED) is a measure of sound energy per unit volume of treated juice and it is useful for scaling up (Tiwari and Mason, 2011). In this experiment, the AED was in the range of 1.73 to 6.64 W/ml.

### 3.2.3 Preparation of inoculum

A frozen stock culture of non-pathogenic *Escherichia coli* O157:H7 collected at FSHN at the University of Illinois at Urbana-Champaign. The bacterial strain was previously prepared by repeated sub-culturing on plate containing 50 mg/L of nalidixic acid (Sigma Aldrich, St. Louis,

MO). Nalidixic acid blocks DNA replication in susceptible gram-negative bacteria. The cell culture was inoculated to tryptic soy agar (TSA) (Sigma Aldrich, St. Louis, MO) plates which were supplemented with 50 mg/L of nalidixic acid and incubated at 37 $^0$C for 24 h. The culture on the tryptic soy agar (TSA) plate was stored in a refrigerator until the sample preparation. A loop of cell culture was transferred to 250 ml of tryptic soy broth (TSB) (Sigma Aldrich, St. Louis, MO) and incubated at 37 $^0$C for 24 h in order to provide a cell density of $10^8$ CFU (Colony-forming unit) per ml (CFU/ml). The cell cultures were harvested by centrifugation (Sorvall Instruments Model RC5C centrifuge, SM10 rotor) at 10,000 g for 10 min at 4 $^0$C in a high speed centrifuge and washed with sterile 0.1% Peptone water (PW). The washing procedure system was repeated three times (Lee et al., 2009).

Plate counting method was performed on *Escherichia coli* O157:H7 before MTS treatment and after treatment as well, and log reduction of *Escherichia coli* O157:H7 was determined.

### 3.2.4 *Escherichia coli* O157:H7 inactivation models

The following kinetic models were used for fitting inactivation curves of *Escherichia coli* O157:H7. In addition, the following criteria were used to evaluate the models:

$R^2$: The closer to 1 the $R^2$ value, the better fit of the model.

$R^2$= 1- RSS/ TSS

where:

RSS is the residual sum of squares and TSS is the total sum of squares.

#### 3.2.4.1 First-order model

The first-order model is a one-parameter model, which assumes that all cells in a population have identical resistance to a lethal treatment.

The model is given by the following equation:

$$log\frac{N}{NO} = -\frac{t}{D}$$

where:

N0: is the initial population (CFU/ml),

N: is the residual population at time (CFU/ml), and

D: is decimal reduction time (min), which is the time, required for 1log10 reduction of the microbial population.

**3.2.4.2 Weibull model**

Model is basically depending on the assumption of different resistances of the cells in a population and the resistance to stress follows a Weibull distribution (Mafart, 2002). This model is given by:

$$log\frac{N}{NO} = (\frac{t}{\delta})^{\beta}$$

where;

d: is the characteristic time (min),

β: is a fitting parameter defining the curve shape (with β < 1 and β> 1 corresponding to concave-upward and concave-downward survival curves, respectively. If β= 1, the Weibull model reduces to a log-linear model).

**3.2.4.3 Gompertz model**

A three parameter model is determined by the following equation:

$$log\frac{N}{NO} = A.\exp(-\exp(b + c.t) - a.\exp(-\exp(b))$$

where:

a, b, and c are fitting parameters.

#### 3.2.4.4 Biphasic linear model

This model predicts that the population is divided into two populations. One of them is treatment-resistant and the other one is treatment-sensitive (Cerf, 1977).

$$log\frac{N}{NO} = log_{10}[(1-f).10^{-\frac{t}{Dsens}} + f.10^{-\frac{t}{Dres}}]$$

where:

(1-f) and f: are the fractions of treatment-sensitive and treatment-resistant population, respectively.

Dsens and Dres: are the decimal reduction times (min) of the two populations, respectively (min).

#### 3.2.4.5 Log-logistic model

The log-logistic model is given by the following three-parameter equation:

$$log\frac{N}{NO} = \frac{A}{1+e^{4a(t-logt)/A}} - \frac{A}{1+e^{4a(t-logt_0)/A}}$$

where:

A: is the upper asymptote-lower asymptote ($log_{10}$CFU/ml),

r: is the maximum inactivation rate (log10(CFU/ml)/$log_{10}$-min), and

s: is the log time to the maximum inactivation rate ($log_{10}$min).

### 3.2.5 Statistical analysis

Three replications were used for each treatment for all measurements, unless otherwise stated. The results were analyzed by analysis of variance using the General Linear Models (PROC GLM) procedure in SAS (version 9.1, SAS Institue, Inc., Cary, North Carolina, USA).

Differences among the mean values were obtained by Fisher's least significant difference (LSD) test at alpha=0.05.

## 3.3 Results and discussion

### 3.3.1 Microbial inactivation

The microbial reduction of apple-carrot blended juice samples treated with MTS at 40, 50, and 60 $^{0}$C are shown in Tables 3.1, 3.2, and 3.3, respectively.

The treatment at 50$^{o}$C and 300 kPa resulted in a 4.81 log CFU/ml reduction in the *E. coli* O157:H7 count in 45 s. Increasing the temperature to 60$^{o}$C at 200 kPa and 300 kPa significantly enhanced the microbial reduction, reaching 5 log CFU/ml in 30s. The static pressure in the sono-reactor helped to inactivate *E. coli* cells at both temperatures. The enhancement in *E. coli* inactivation at 60$^{o}$C + 200 kPa and/or 300 kPa MTS treatment was more than additive effect of 50$^{o}$C MTS treatment. When 60 s and 75 s were applied, 5 log CFU/ml reduction was achieved in treatments at 50$^{o}$C and 60$^{o}$C. By combining several factors at the same time (applying temperature, pressure, and time), a synergetic effect was assured.

Lee et al. (2013) reported that the times needed to achieve a 5-log reduction of *Escherichia coli* K12 in apple cider were 1.4, 2.5, and 3.8 minutes for the MTS, MS and TS treatments, respectively. Under the experimental conditions used in Lee et al. (2013), the ultrasound treatment at 59$^{o}$C (lethal temperature) and elevated static pressure, a process known as MTS, was the most effective in microbial load reduction (Lee et al., 2013). Based on the findings from our work a 5-log reduction was achieved for 1.4 minutes (75 s) at 50 $^{0}$C. At 60 $^{0}$C, a 5-log reduction was achieved in 30s at all 3 pressure values. Using multiple hurdles (temperature, pressure, and time) was more effective than using only one hurdle. A reduction in

the time to reach 5 log reduction will help to save energy and increase throughout. In another study, *Escherichia coli* K12 cells suspended in apple cider were treated by MTS (400 kPa/ 59 $^0$C), thermosonication (TS, 100 kPa/59 $^0$C), and manosonication (MS, 400 kPa/55 $^0$C) for up to 4 min. A 5-log reduction was achieved in 1.4 min by MTS, 3.8 min by TS, and 2.5 min by MS (Lee et al., 2013). Lee at al. (2013) concluded that the inactivation rates reported in their work were higher than those documented in the literature. In Lee et al. (2013), a 5-log reduction of *E. coli* K12 in phosphate buffer (pH 7) by TS at 61 $^0$C was achieved within 0.75 min. Similarly, our work also showed that the 5-log reduction was achieved 0.75 min at 60 $^0$C.

### 3.3.2 Kinetic modeling

Totally, five microbial survival models (First-order, Weibull, ModifiedGompertz, Biphasic linear and Log-logistic) were used to analyze the *E. coli*O157:H7 inactivation data. The inactivation data and model prediction are presented in Figure 3.2 for 40 $^0$C, Figure 3.3 for 50 $^0$C, and Figure 3.4 for 60 $^0$C. Table 3.4 shows the statistical indices of the kinetic models used to fit inactivation data for *E. coli* O157:H7by MTS at three temperatures (40, 50, and 60$^0$C) and three pressures (100, 200, and 300 kPa). Based on the statistical indices and visual observations, the Weilbull and Biphasic models were better models for fitting of the data for MTS treatment as shown by high $R^2$ values. The non-linear kinetic models, including the Modified Gompertz, First-order, and Log-logistic models did not provide satisfactory fit to data from MTS compared the Weibull and Biphasic models.

In order to describe non-linear inactivation kinetics, several models, such as Weibull, Modified Gompertz, Biphasic linear, and Log-logistic models have been proposed and used to fit non-linear inactivation data of several microorganisms for inactivation by heat, high pressure processing or pulsed electric field. Most ultrasonic inactivation studies employed first-order

kinetic parameters (D-values and z-values) in order to describe the reduction on microbial survival count. There are many studies shows that inactivation of microorganisms may not follow first-order kinetics, especially for inactivation with non-thermal processing methods, especially for non-thermal technologies (Mafart, 2002). The finding reported in this work shown that our data also did not follow the first-order kinetics.

### 3.4 Conclusion

The MTS treatment helped to achieve 5 log reduction in apple-carrot blended juice in relatively short time, especially at elevated temperatures. At 40$^{o}$C, a maximum of 3.03 log CFU/ml after 75 s treatment at 300 kPa. At 50$^{o}$C, 5 log reduction in the population of *Escherichia coli* O157:H7 was achieved in 60 s under all three pressures (100, 200, and 300 kPa). When temperature was increased to 60$^{o}$C, only 30 s were needed to reduce the survival count of *E. coli* O157:H7 by 5 log cycles at 100, 200, and 300 kPa.

## 3.5 Figures and Tables

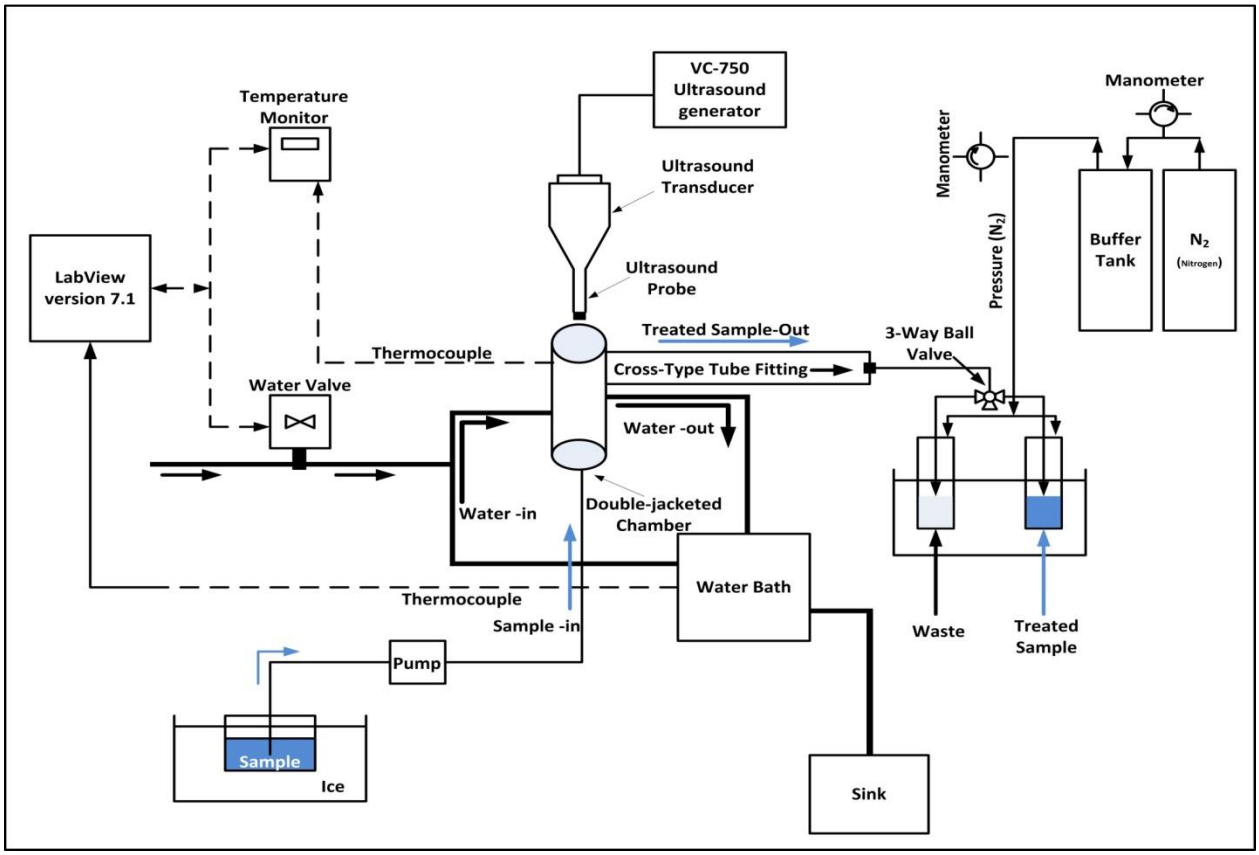


**Figure 3. 1 Laboratory scale manothermosonication (MTS) system** (Lee et al., 2013).

**Figure 3.2** Fitting of the inactivation data of *E. coli* O157:H7treated by MTS at 40ºC for 15, 30, 45, 60, and 75 s with the Weibull, First Order, Modified Gompertz, Biphasic linear and Log-logistic models.

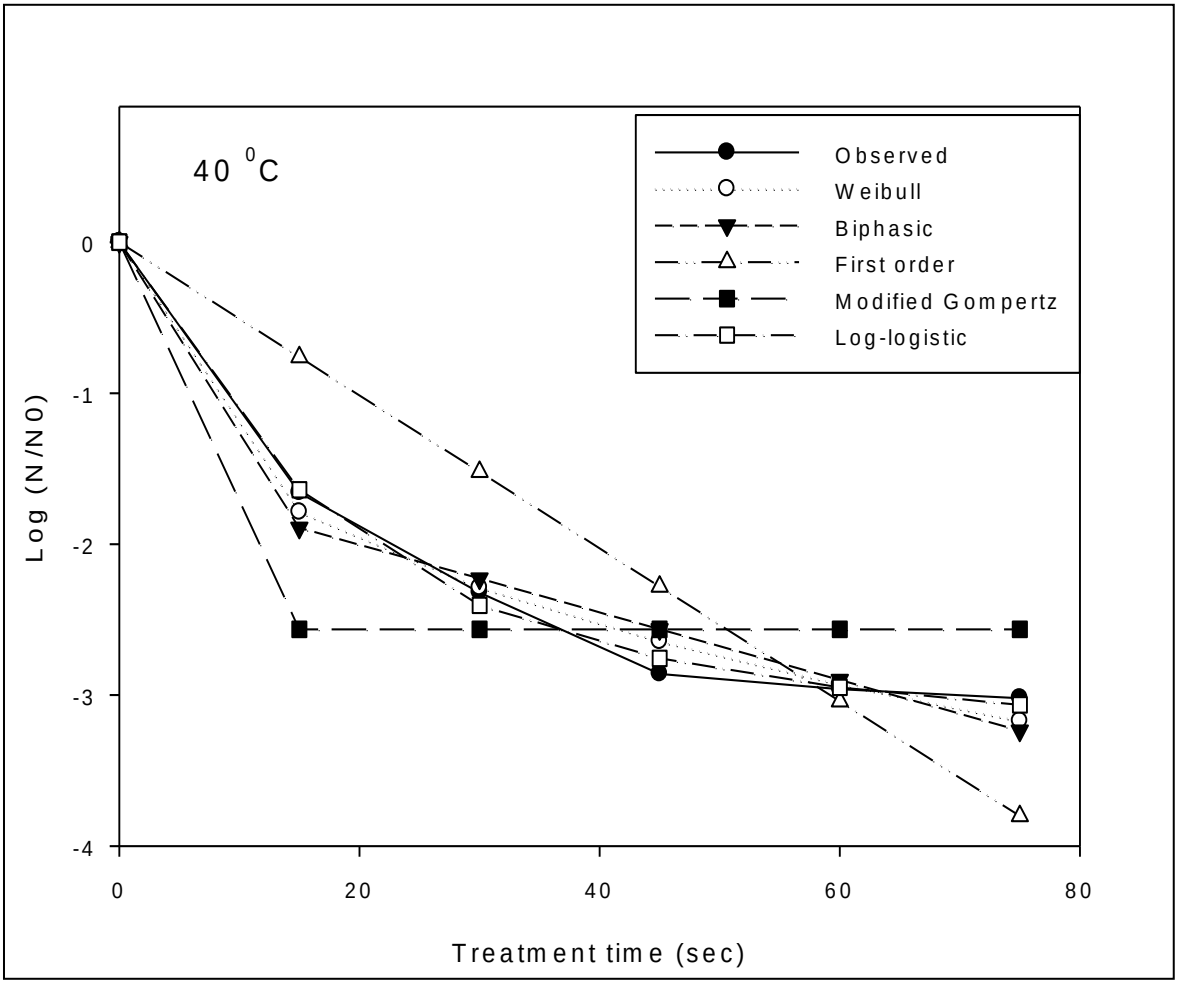

**Figure 3.2. (cont.) (a)** Fitting of the inactivation data of *E. coli* O157:H7 treated by MTS at 200 kPa and 40ºC with the Weibull and biphasic linear models

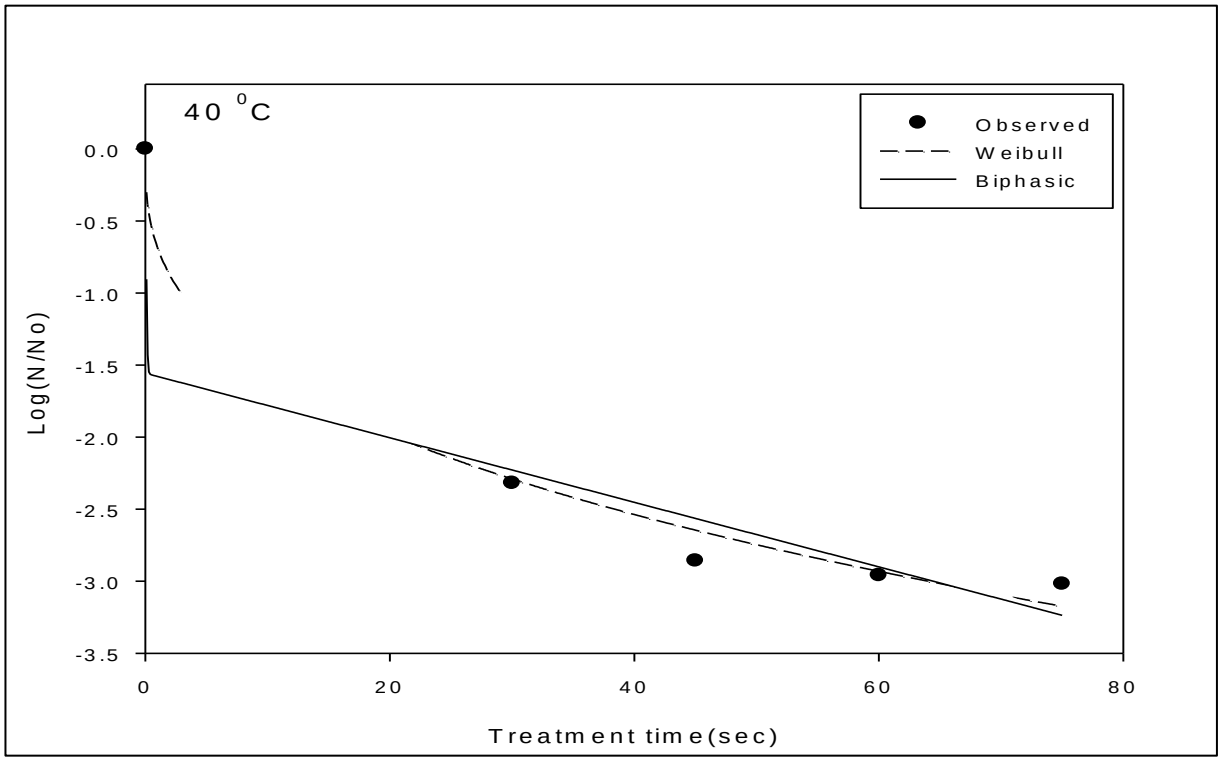

**Figure 3.3** Fitting of the inactivation data of *E. coli* O157:H7 treated by MTS at 50ºC with the Weibull, First Order, Modified Gompertz, Biphasic linear and Log-logistic models.

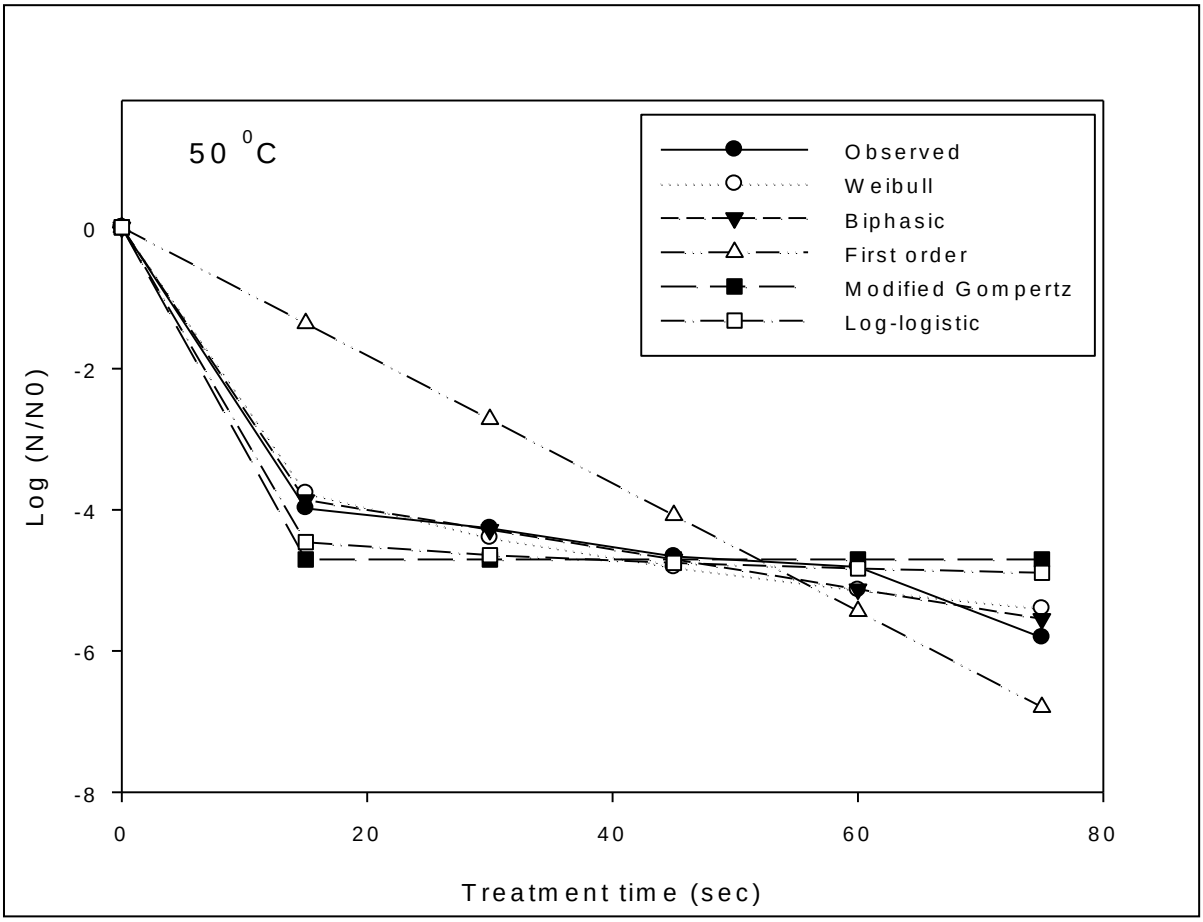

**Figure 3.3. (cont/) (a)** Fitting of the inactivation data of *E. coli* O157:H7 treated by MTS at 200 kPa and 50ºC with the Weibull and Biphasic linear models

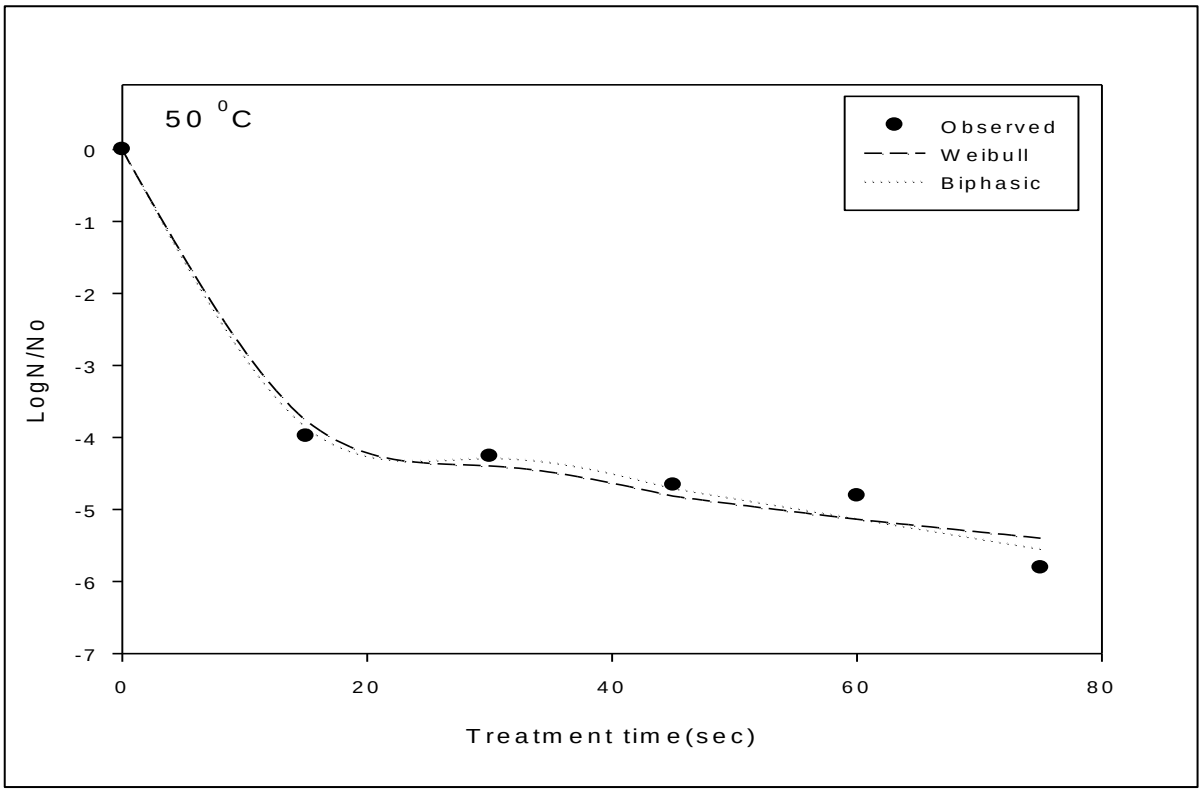

**Figure 3.4** Fitting of the inactivation data of *E. coli* O157:H7 treated by MTS at 60ºC with the Weibull, First order, Modified Gompertz, Biphasic linear and Log-logistic models.

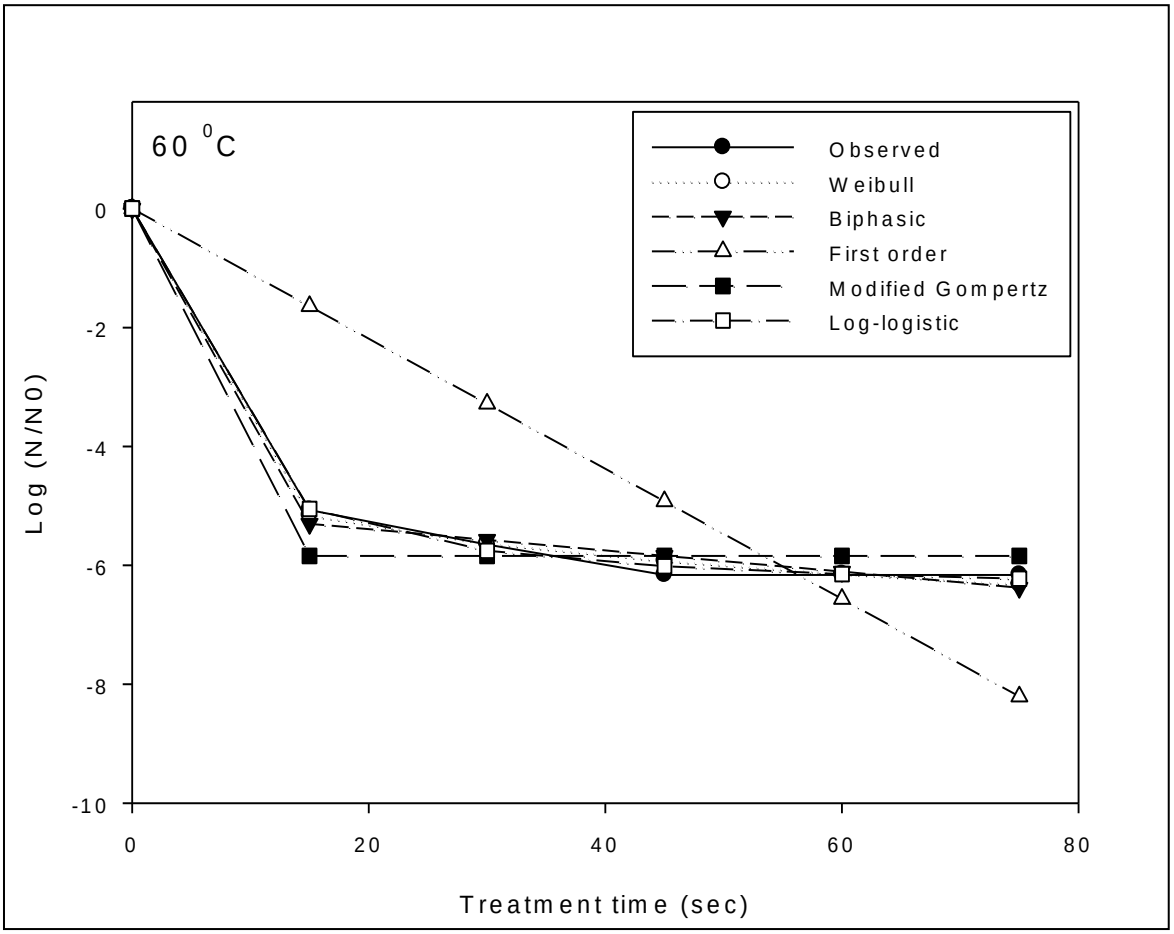

**Figure 3.4. (cont.) (a)** Fitting of the inactivation data of *E. coli* O157:H7 treated by MTS at 200 kPa and 60ºC with the Weibull and biphasic linear

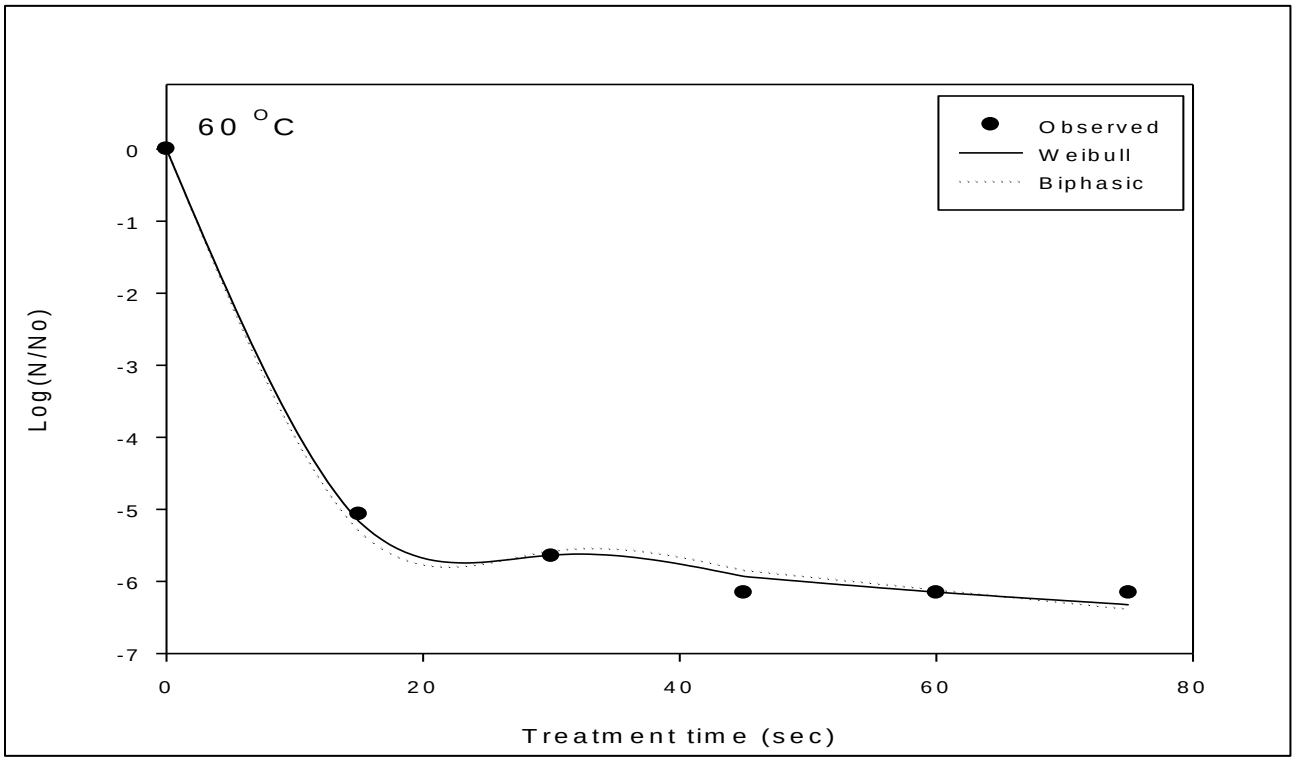

**Table 3.1** Apple–carrot blended juice treated by MTS at 40$^{o}$C (CFU/ml)

| **40 $^{0}$C** | **100 kPa** | **200 kPa** | **300 kPa** |
|---|---|---|---|
| **15s** | $1.56^{b,x}$ | $1.66^{b,x}$ | $1.78^{b,x}$ |
| **30s** | $2.22^{ab,x}$ | $2.33^{ab,x}$ | $2.43^{ab,x}$ |
| **45s** | $2.80^{a,x}$ | $2.87^{a,x}$ | $2.95^{a,x}$ |
| **60s** | $2.93^{a,x}$ | $2.97^{,x}$ | $2.98^{a,x}$ |
| **75s** | $2.97^{a,x}$ | $3.02^{a,x}$ | $3.03^{a,x}$ |

$^{a-b}$Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

$^{x}$Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 3.2** Apple–carrot blended juice treated by MTS at 50$^{o}$C (CFU/ml)

| **50 $^{0}$C** | **100 kPa** | **200 kPa** | **300 kPa** |
|---|---|---|---|
| **15s** | $3.92^{d,x}$ | $3.98^{c,x}$ | $4.11^{c,x}$ |
| **30s** | $4.19^{d,x}$ | $4.26^{bc,x}$ | $4.42^{b,x}$ |
| **45s** | $4.51^{c,x}$ | $4.66^{b,x}$ | $4.81^{a,x}$ |
| **60s** | $5.12^{b,y}$ | $6.08^{a,x}$ | $6.08^{a,x}$ |
| **75s** | $6.08^{a,x}$ | $6.08^{a,x}$ | $6.08^{a,x}$ |

[a-d]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x-y]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 3.3** Apple–carrot blended juice treated by MTS at 60$^{o}$C (CFU/ml)

| **60 $^{0}$C** | **100 kPa** | **200 kPa** | **300 kPa** |
|---|---|---|---|
| **15s** | $4.78^{c,,x}$ | $4.82^{c,x}$ | $4.89^{c,x}$ |
| **30s** | $5.03^{bc,x}$ | $5.18^{b,x}$ | $5.30^{b,x}$ |
| **45s** | $5.36^{b,y}$ | $6.17^{a,x}$ | $6.17^{a,x}$ |
| **60s** | $6.17^{a,x}$ | $6.17^{a,x}$ | $6.17^{a,x}$ |
| **75s** | $6.17^{a,\ x}$ | $6.17^{a,x}$ | $6.17^{a,x}$ |

[a-c]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x-y]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 3.4** Statistical index of the kinetic models used to fit inactivation data for *E.coli* O157:H7 by MTS at three temperatures (40, 50, and $60^0$C) and three pressures (100, 200, and 300 kPa)

| **Treatment Conditions** | | **Statics** | **Kinetic Models** | | | | |
|---|---|---|---|---|---|---|---|
| **Temp (C)** | **Pressure (kPa)** | **Index** | **Weibull** | **First Order** | **Modified Gompertz** | **Biphasic Linear** | **Log-logistic** |
| **40°** | 100 | $R^2$ | 0.986 | 0.688 | 0.780 | 0.968 | 0.982 |
| | 200 | $R^2$ | 0.987 | 0.646 | 0.805 | 0.970 | 0.984 |
| | 300 | $R^2$ | 0.994 | 0.872 | 0.861 | 0.999 | 0.999 |
| **50°** | 100 | $R^2$ | 0.977 | 0.568 | 0.865 | 0.991 | 0.316 |
| | 200 | $R^2$ | 0.975 | 0.609 | 0.839 | 0.984 | 0.294 |
| | 300 | $R^2$ | 0.999 | 0.603 | 0.983 | 0.999 | 0.958 |
| **60°** | 100 | $R^2$ | 0.993 | 0.361 | 0.939 | 0.996 | 0.874 |
| | 200 | $R^2$ | 0.992 | 0.320 | 0.941 | 0.988 | 0.879 |
| | 300 | $R^2$ | 0.996 | 0.675 | 0.964 | 0.999 | 0.899 |

# CHAPTER 4

# TREATMENT OF APPLE-CARROT JUICE BLEND WITH MANOTHERMOSONICATION (MTS) AND ITS EFFECT ONJUICE QUALITY DURING STORAGE

## 4.1 Introduction

Recently, the juice market has experienced a major growth, which was triggered by a growing interest of consumers in healthier and nutritionally rich diet. The first priority in juice processing is to secure the microbial safety and for that purpose, different thermal processing method, such as high temperature short time (HTST) treatment have been developed and applied to juice pasteurization. Although the thermal processes are effective in destruction of harmful microorganisms, many undesired changes have been reported in different studies HTST pasteurization was reported to negatively impact the nutritional and sensory properties of foods because high temperature is applied during the process. Therefore, some alternative methods with minimal heat application have been under investigation in recent years (Lee et al., 2009). Ultrasound is one such technique that was proposed as an alternative to heat treatment (Kentish and Feng, 2014). The mode of action in an ultrasound treatment is cavitation, which refers to the generation, growth, and rapid implosion of tiny gas- or vapor-filled bubbles in a liquid. Microbial inactivation by the physical and chemical events produced by cavitation is caused by non-thermal lethal factors, and ultrasound treatment is thus regarded as a non-thermal treatment. Although a number of publications have documented the use of MTS on decreasing microbial load and improving product quality (Lee et al., 2009; Arroyo et al., 2011b; Condón et al., 2011), no study has been published investigating the effect of MTS on quality of apple-carrot juice

blend. There are several studies to investigate the effect of ultrasound on apple-carrot juice blend or other blended juices such as apple-cranberry, but no study has used MTS to treat the juice blends (Caminiti et al., 2011).

The purpose of this study was to examine the quality changes in apple-carrot juice blend treated with manothermosonication (MTS) and high temperature-short time (HTST) during storage for three weeks under refrigeration conditions. Quality attributes evaluated included brix, total phenolic, titratable acidity, viscosity, antioxidant capacity, turbidity, pH, and color.

## 4.2 Materials and methods

### 4.2.1 HTST treatment

Apple-carrot blended juice samples were pasteurized at 72˚C ± 2˚C for 15 s using a HTST/UHT system (Armfield company, FT74XTS, Hampshire, England) (Figure 4.1). In the heating section of the heat exchanger the product was heated to the target temperature by pressurized hot water. The Plate HeatExchanger (FT74P) included a re-generation stage, where the hot outgoing product issued to pre-heat the incoming product. The Tubular Heat Exchanger (FT74T) used a portion of the pressurized hot water at a lower flow rate to pre-heat the product in a dedicated pre-heat section. The pressurized hot water was generated by an electric heater contained in a heating vessel. A pump circulated the hot water through the heat exchanger and back to the pressurized heating vessel. A pressure relief valve provided protection against excess pressure. Each heat exchanger incorporated a holding tube between the final heating section and the cooling section. The holding tube held the product at the process temperature for a defined time at the nominal process flow rate. The sensor was used to control power to the hot water heater, by providing the control input for a three-term controller. In this way, the desired temperature of product could be maintained. The product was cooled in the cooling section of the

heat exchanger, using a flow of colder chilled water. A combined flow meter and control valve was used to regulate and measure the flow of cooling water. A back pressure valve at the product outlet from the heat exchanger was used in order to create a pressure within the heat exchanger. This prevents the product boiling at the elevated processing temperatures.

### 4.2.2 Sample preparation

Juices of Red Delicious apples and carrots were freshly pressed with using a juice extractor (Bullet Express Multifunction Food Processor, Model: BE 110, Pacoima, CA, USA). The carrots were first peeled with a sterile stainless steel knife. Apples then were peeled and put into the juice extractor. The apple-carrot juice blend was made of ninety-percent (90%) apple juice and ten-percent (10%) carrot juice, having a pH of 4–4.5. The juice blend samples were filtered with a 0.2 mm filter (Tri Clover Compatible Filter, CA, U.S.A) to remove the pulp.

The manothermosonication (MTS) (20 kHz, 100%) will be conducted by pumping juice samples through a custom-designed sono-reactor. The HTST treatment was conducted by HTST/UHT system at 72 $^{0}$C for 15 seconds.

After treatment with MTS and HTST, the apple-carrot blended juice samples were kept under refrigeration conditions (0-4 $^{0}$C) for three weeks, and analyses were done at day 0, 7, 14 and 21.

### 4.2.3 Color measurement

Color measurements of apple-carrot blended juice were conducted with a reflectance colorimeter (LabScan XE, HunterAssociates Laboratories, Inc., Reston, Va, USA) based on the L*, a*, and b* values. 10mL samples were placed to a35-mm plastic dish (Corning tissue culture dish, Corning, NY, USA). For each sample, three color readings (L, a, and b values) were taken at room temperature. The averaged L, a, and b values were reported. For each treatment, colors

of 3 replicated samples were measured. Color readings were taken once a week during the 3-week storage.

#### 4.2.4 pH and titratable acidity

For pH measurement, pH changes of the apple-carrot blended juices treated with MTS and HTST were measured using an Accumet Research AR15 pH meter (Fisher Scientific, USA). Three replications were used. The pH readings were taken once a week during the 3-week storage period.

For titratable acidity measurement, an Accumet Research AR15 pH meter was used to titrate the solution until pH 8.2 using 0.1 N NaOH as the titrant.

#### 4.2.5 Brix and Viscosity

Brix was determined with a hand held refractometer (Model: Refracto 30 GS,Mettler Toledo, Schwerzenbach, Switzerland) at room temperature.

Viscosities of apple-carrot blended juices were determined using a Brookfield Digital Viscometer Model DV-E England, UK.). 50 ml mixture was poured into the beaker and allowed to rotate at room temperature for 1 min to measure the viscosity.

#### 4.2.6 Total phenolic content

Total phenolic content of the MTS- and HTST-treated apple-carrot blended juice, and raw juice were evaluated using a modified colorimetric method (Singleton and Rossi, 1965). The method involved the reduction of Folin - Ciocalteau reagent (Sigma Chemical, St. Louis, Missouri, U.S.A) by phenolic compounds, with a concomitant formation of a blue complex. In this study, 1 ml of the extract was mixed with 75 ml of distilled water and 5 ml of Folin-Ciocalteau reagent. After waiting 3 minutes, 10 ml of saturated sodium carbonate and 0.95 ml of distilled water were added. Then, the mixture was kept for 60 min at room temperature, and the

absorbance was read at 720 nm using spectrophotometer (Lambda 1050 UV/VIS/NIR Spectrometer, PerkinElmer, Waltham, MA, USA). The measurement was compared to a standard curve prepared with a cathedol solution (Sigma Chemical). The total phenolic content was expressed as milligrams of cathedol equivalents per gram of fresh weight (mg CAE/ FW g).

### 4.2.7 Antioxidant capacity

1,1-diphenyl-2picryl hydrazyl radical (DPPH) was used to determine the antioxidant activity of the MTS-treated, HTST-treated and raw apple-carrot blended juice (Ebrahimzadeh et al., 2010). The sample extracts were prepared in different volumes (0.02 %, 0.04 %, 0.06 %, 0.08 %, and 0.1%). DPPH solution was added to sample extracts, and vortexed. It was incubated in the dark around 15 minutes. At the end of the time, the absorbance of the solutions was read of absorbance at 517 nm with spectrophotometer (Lambda 1050 UV/VIS/NIR Spectrometer, PerkinElmer, Waltham, MA, U.S.A). Absorbance of DPPH radical without sample was used as a control. The antioxidant capacity of the MTS- and HTST-treated apple-carrot blended juice, and the raw apple-carrot blended juices were compared.

### 4.2.8 Turbidity

Turbidity of apple-carrot blended juice treated with MTS and HTST was measured by absorbance at 600 nm with a spectrophotometer (Lambda 1050 UV/VIS/NIR Spectrometer, PerkinElmer, Waltham, MA, U.S.A).

### 4.2.9 Statistical analysis

Three replications were used for each treatment in all measurements, unless otherwise stated. The results were analyzed by analysis of variance using the General Linear Models (PROC GLM) procedure in SAS (version 9.1, SAS Institute, Inc., Cary, North Carolina, U.S.A).

Differences among the mean values were obtained by Fisher's least significant difference (LSD) test at alpha=0.05.

## 4.3 Results and discussion

### 4.3.1 Color changes

The changes in *L* (lightness), *a* (redness) and *b* (yellowness) values for the apple-carrot blended juice treated with the MTS, HTST, and no treatments during a three-week period were shown in Tables 4.2, 4.3, and 4.4, respectively. The *L* (lightness) values of all treated (MTS and HTST) apple-carrot blended juice were significantly higher than the raw samples for all storage times. The highest *L* value was observed for the samples treated with HTST during storage. No significant changes were observed between untreated apple-carrot blended juice and the samples treated with the MTS and HTST in their *a* (redness) and *b* (yellowness) values during 3 weeks of storage. The *L* values decreased significantly with the storage time in apple-carrot blended juices for all treatments (Raw, MTS, and HTST). The highest *L* value was observed for the samples on day 0, and the lowest *L* value was observed for the last (third) week samples.

Usually, a decrease in *L* value indicates browning development (Rico et al., 2007). In this study, the *L* (lightness) values of apple-carrot juice blend samples for all treatments (Raw, MTS, and HTST) decreased, while *a* (redness) and *b* (yellowness) increased during the storage, indicating browning activities. The non-linear effect of cavitation exhibited in the form of temperature rising. This increase on temperature leads to increase the rate of browning in the sample right after treatment (Lee, H., 2013).

### 4.3.2 pH and titratable acidity

The pH changes measured at room temperature for the Raw, MTS-, and HTST-treated apple-carrot blended juice samples are tabulated in Table 4.4. The pH values of the raw juices,

HTST-treated and MTS-treated apple-carrot blended juices were in the range of 4.13 to 4.33, 4.10 to 4.29, 4.05 to 4.23 during 3 weeks of storage, respectively. An increase in pH values was recorded for all types of treatment (HTST, MTS and Raw). The pH values increased significantly with the storage time in the samples for all the treatments (Raw, MTS, and HTST). The lowest pH value was observed for the samples on day 0, and the highest pH value was observed in the day 21 samples.

The titratable acidity (TA) values of the Raw, MTS, and HTST-treated apple-carrot blended juice samples are shown in Table 4.5. The TA values of the raw apple-carrot blended juice were in the range of 28.14 to 42.54 during 3 weeks of storage. The TA values of the HTST and MTS-treated apple-carrot blended juices were in the range of 29.48 to 33.5 and 37.52 to 44.20 during 3 weeks of storage, respectively. The TA values decreased significantly during storage in the apple-carrot blended juice samples for all methods (Raw, MTS, and HTST). The highest TA value was observed for the samples on day 0, while the lowest TA value was observed for the last (twenty-first) day samples. The lowest TA values were observed for the apple-carrot blended juice treated with HTST for all storage times. On the other hand, the highest TA values were observed in the raw juice for days 0 and 7 compared to the other treatments. Especially a significant decrease during days 14 and 21 was observed in the raw juices. In total, a significant decrease from 42.54 to 28.14 was observed in the raw juices. In this study, a decrease in the pH value of apple-carrot blended juice treated with MTS was observed compared to pH values of raw juice blends.

### 4.3.3 Brix and viscosity

The brix changes of the Raw, MTS, and HTST-treated apple-carrot juice blends are illustrated in Table 4.6. The brix values of the raw juice, HTST- and MTS-treated ones were in

the range of 9.65 to 9.70, 9.45 to 9.70, and 9.50 to 9.65 during 3 weeks of storage, respectively. A significant decrease in brix values was recorded in the apple-carrot blended juice for MTS and HTST treatments during 3 weeks of storage. The highest brix values were observed in the apple-carrot blended juice treated all treatments (Raw, MTS, and HTST) in day 0, and the lowest brix values were observed for the last (twenty-first) day samples. However, the changes in brix were not significant for the raw apple-carrot blended juice.

The viscosity changes of the raw, MTS, and HTST treated apple-carrot blended juice samples are shown in Table 4.7. The viscosity values of the raw apple-carrot blended juices were in the range of 1.25 to 1.45 during storage. The viscosity values of the HTST- and MTS-treated juices were in the range of 1.15 to 1.40 and 1.35 to 1.55 during storage, respectively. The viscosity values increased significantly during storage time in apple-carrot blended juice samples for all methods (Raw, MTS, and HTST). The lowest viscosity value was observed for the samples on day 0, and the highest viscosity value was observed for the last (twenty-first) day samples. Among the treatments, the HTST-treated juice blends showed the lowest viscosity during the storage.

It was reported that the viscosity of the sample sonicated at 58°C showed a significant increase as compared to the control. In this study, the viscosity values of the apple-carrot blended juices treated with MTS was higher compared to the raw juice blends. This could be attributed to an increase in solubilization of pectin in cell walls (Zhan et al., 2005).

### 4.3.4 Total phenolic

Total phenolic changes of the Raw, MTS, and HTST-treated apple-carrot blended juice samples are demonstrated in Table 4.8. The phenolic content values of the raw apple-carrot blended juices were in the range of 0.1724 to 0.1730 during storage. The phenolic content values

of the HTST and MTS-treated apple-carrot juice blends were in the range of 0.1454 to 0.1712 and 0.1754 to 0.1809 during storage, respectively. The phenolic content values in all juice samples increased during storage. The highest phenolic content values were observed on day 21 for all treatments, while the lowest phenolic content values were observed on day 0.

Phenolic compounds are very important and beneficial to human health as they play a significant role in controlling the risk of many physiological and degenerative diseases in the human body. In this study, there was a significant increase in total phenols in all the sonicated juice samples as compared to control. An increase in total phenolic content study was also observed in sonicated kasturi lime juice (Bhat et al., 2011). This increase might be attributed to the release of bound form of phenolic contents due to breakage of cell wall by the cavitation related activities. It could also be due to the presence of hydroxyl groups produced by sonication, and their interactions with aromatic ring of phenolic compounds.

### 4.3.5 Antioxidant capacity

Antioxidant capacity changes of the Raw, MTS, and HTST-treated samples are presented in Table 4.9.The antioxidant capacity values of the raw apple-carrot blended juices were in the range of 18.1 to 24.2. The antioxidant values of the HTST and MTS-treated apple-carrot blended juices were in the range of 6.6 to 12.0 and 15.9 to 20.0, respectively. The antioxidant capacity increased significantly during storage in all treatments (Raw, MTS, and HTST). The lowest antioxidant value was observed at day 0, and the highest on day 21. Among the treatments, the HTST-treated samples exhibited the lowest antioxidant capacity during the storage.

In fruits and vegetables, especially in citrus fruits, phenolic compounds and vitamin C are the major components responsible for DPPH free radical scavenging activity and antioxidant capacity. It was reported that a significant increase exists in total antioxidant capacity in all

sonicated juice samples (Bhat et al., 2011). In our experiment, the antioxidant capacity increased significantly during storage in all treatments (Raw, MTS, and HTST). This increase might be attributed to an increase in phenolic compounds as a result of acoustic cavitation. In our tests, we did see an increase in total phenolic content during storage in samples of all treatments. Previous studies also shows that there is a positive relationship between total phenolic content and antioxidant activity in many plant species such as lime, orange, and carrots (Duh, Tu, & Yen, 1999; Gulcin, 2004). Significant increase in the antioxidant activity is related to presence of high concentration of total polyphenol content in vegetables and fruits.

### 4.3.6 Turbidity

Turbidity changes in apple-carrot blended juices treated by the Raw, MTS, and HTST are shown in Table 4.10. The turbidity values for the raw apple-carrot blended juice changed from 3.22 to 3.31 during storage. Turbidity values for samples treated with HTST changed from 3.22 to 3.26 and that from the MTS treatment were from 3.04 to 3.14. The turbidity values decreased significantly during storage in samples of all treatments (Raw, MTS, and HTST). The lowest turbidity was observed in the samples on day 21, and the highest turbidity value was observed at day 0. Among the treatments, the MTS treated samples had the lowest turbidity over the entire storage times. On the other hand, the raw apple-carrot blended juice samples showed the highest turbidity during 3 weeks of storage.

The turbidity values of the samples treated with ultrasound at 40 °C and 60 °C were significantly lower than the control and thermal treatment at 60 °C. Among the treatments, the MTS-treated samples had the lowest turbidity during 3 weeks of storage. The turbidity values decreased with time in samples of all treatments (Raw, MTS, and HTST). No difference between the control and the heat treatment at 60 °C was found. The turbidity of apple cider is a measure

of cloudiness and is related to suspended particles. For the single frequency (20 kHz) system used in this study, standing waves could be formed in the treatment chamber and particles in the ultrasonic wave field would move to a pressure node and get enriched, which would cause a separation of the particles, resulting in reduction in turbidity (Groschl, 1998). On the other hand, ultrasound treatment may also break down the particles to cause a reduction in particle size and to change the transmittance. Both would contribute to a decrease in the turbidity of juice samples.

### 4.4 Conclusion

MTS treatment of apple-carrot blended juice significantly altered chemical parameters such as total phenolic content, antioxidant capacity and pH-value. A significant increase in the antioxidant activity was recorded for samples treated with MTS during 3 weeks of storage. While the first day antioxidant activities of the samples were low, they were increased when measured on the seventh, fourteenth and twenty-first days. Total phenolic content of apple-carrot blended juice treated by HTST were significantly lower compared to the samples treated with MTS. Turbidity of MTS treated samples decreased significantly during 3 weeks of storage. Overall, apple-carrot blended juice treated with MTS showed to be a promising alternative to HTST as evidenced by its ability to maintain juice quality.

## 4.5 Figures and Tables

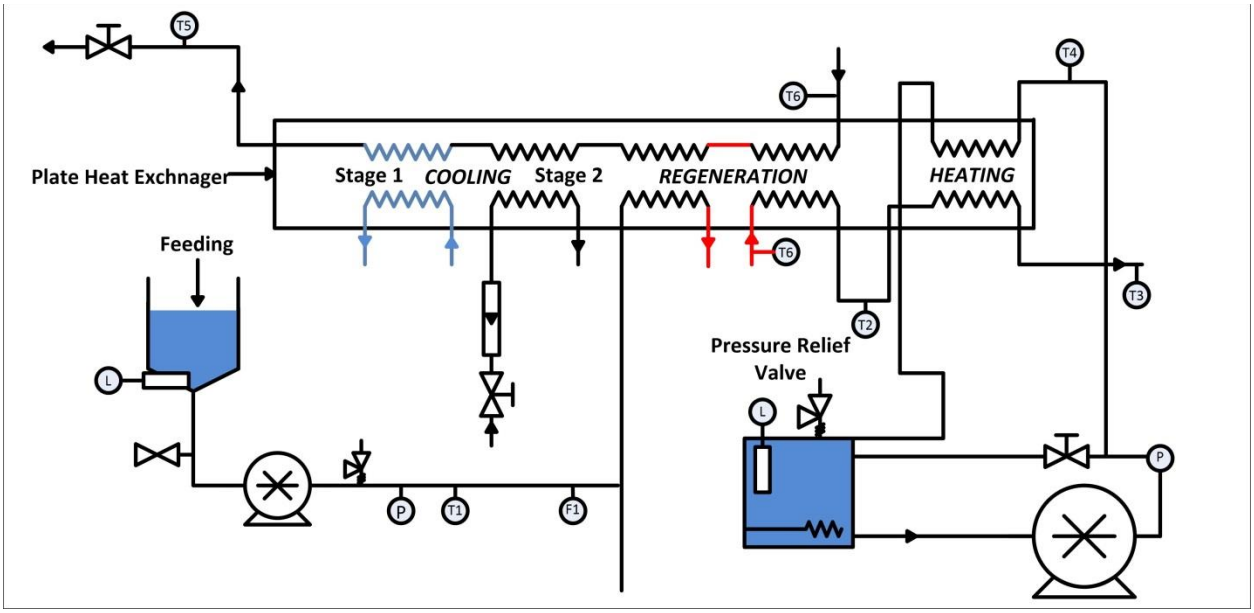


**Figure 4.1** FT74XTS HTST/UHT system

**Table 4.1** Changes in *L* (lightness) values in apple-carrot blended juice treated by MTS, HTST, and no treatment (Raw) during a three-week period.

| **L (Lightness) values** | **Day 0** | **Day 7** | **Day 14** | **Day 21** |
|---|---|---|---|---|
| **RAW** | $25.91^{a,x}$ | $25.18^{b,xy}$ | $24.29^{b,y}$ | $22.38^{b,z}$ |
| **HTST** | $26.78^{a,x}$ | $26.61^{a,x}$ | $25.90^{a,xy}$ | $24.97^{a,y}$ |
| **MTS** | $26.32^{a,x}$ | $26.11^{a,x}$ | $24.60^{b,xy}$ | $22.76^{b,y}$ |

[a-b]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x-z]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 4.2** Changes in *a* (redness) values for apple-carrot blended juice treated with MTS, HTST, and no treatment (Raw) during a three-week period

| **a (redness) values** | **Day 0** | **Day 7** | **Day 14** | **Day 21** |
|---|---|---|---|---|
| **RAW** | $9.43^{a,x}$ | $10.03^{a,x}$ | $10.60^{a,x}$ | $10.52^{a,x}$ |
| **HTST** | $11.05^{a,x}$ | $11.11^{a,x}$ | $11.16^{a,x}$ | $11.97^{a,x}$ |
| **MTS** | $10.09^{a,x}$ | $10.16^{a,x}$ | $10.26^{a,x}$ | $10.81^{a,x}$ |

[a]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 4.3** Changes in *b* (yellowness) values for apple-carrot blended juice treated with MTS, HTST, and no treatment (Raw) during a three-week period

| b (yellowness) values | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **RAW** | $20.28^{a,x}$ | $21.04^{a,x}$ | $21.94^{a,x}$ | $22.63^{a,}$ |
| **HTST** | $21.91^{a,x}$ | $22.87^{a,x}$ | $22.96^{a,x}$ | $23.42^{a,x}$ |
| **MTS** | $20.51^{a,x}$ | $20.63^{a,x}$ | $21.28^{a,x}$ | $22.66^{a,x}$ |

[a]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 4.4** Changes in pH for apple-carrot blended juice treated with MTS, HTST, and no treatment (Raw) during a three-week period

| pH | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **RAW** | $4.13^{a,y}$ | $4.18^{a,y}$ | $4.23^{a,xy}$ | $4.33^{a,x}$ |
| **HTST** | $4.10^{a,y}$ | $4.13^{a,y}$ | $4.15^{a,y}$ | $4.29^{a,x}$ |
| **MTS** | $4.05^{a,y}$ | $4.09^{a,y}$ | $4.14^{a,xy}$ | $4.23^{a,x}$ |

[a]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x-y]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 4.5** Changes in titratable acidity (TA) for apple-carrot blended juice treated with MTS, HTST, and no treatment (Raw) during a three-week period (g/L)

| Titratable Acidity | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **RAW** | $42.54^{a,x}$ | $40.20^{a,y}$ | $33.5^{b,z}$ | $28.14^{b,t}$ |
| **HTST** | $33.5^{b,x}$ | $30.82^{b,y}$ | $29.48^{c,y}$ | $29.48^{b,y}$ |
| **MTS** | $44.20^{a,x}$ | $40.54^{a,xy}$ | $40.20^{a,xy}$ | $37.52^{a,y}$ |

[a-b]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x-t]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 4.6** Changes in brix for apple-carrot blended juice treated with MTS, HTST, and no treatment (Raw) during a three-week period (%)

| Brix | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **RAW** | $9.70^{a,x}$ | $9.70^{a,x}$ | $9.65^{a,x}$ | $9.65^{a,x}$ |
| **HTST** | $9.70^{a,x}$ | $9.55^{b,y}$ | $9.50^{b,y}$ | $9.45^{b,y}$ |
| **MTS** | $9.65^{a,x}$ | $9.60^{ab,x}$ | $9.60^{a,x}$ | $9.50^{b,y}$ |

[a-b]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x-y]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 4.7** Changes in viscosity for apple-carrot blended juice treated with MTS, HTST, and no treatment (raw) during a three-week period (N s/$m^2$)

| Viscosity | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **RAW** | $1.25^{ab,y}$ | $1.25^{b,y}$ | $1.40^{ab,x}$ | $1.45^{ab,x}$ |
| **HTST** | $1.15^{b,y}$ | $1.30^{ab,xy}$ | $1.35^{b,x}$ | $1.40^{b,x}$ |
| **MTS** | $1.35^{a,y}$ | $1.35^{a,y}$ | $1.50^{a,x}$ | $1.55^{a,x}$ |

$^{a-b}$Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

$^{x-y}$Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 4.8** Changes in total phenolic contents for apple-carrot blended juice treated with MTS, HTST, and no treatment (raw) during a three-week period (mg)

| Total Phenolic Contents | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **RAW** | $0.17^{a,x}$ | $0.17^{a,x}$ | $0.17^{ab,x}$ | $0.17^{a,x}$ |
| **HTST** | $0.14^{b,y}$ | $0.15^{b,xy}$ | $0.16^{a,x}$ | $0.16^{a,x}$ |
| **MTS** | $0.17^{a,x}$ | $0.17^{a,x}$ | $0.18^{a,x}$ | $0.18^{a,x}$ |

[a-b]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x-y]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 4.9** Changes in antioxidant capacity for apple-carrot blended juice treated with MTS, HTST, and no treatment (Raw) during a three-week period

| Antioxidant Capacity (%) | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **RAW** | $18.1^{a,z}$ | $20.8^{a,y}$ | $21.0^{a,y}$ | $24.2^{a,x}$ |
| **HTST** | $6.6^{c,z}$ | $7.0^{c,z}$ | $9.3^{b,y}$ | $12.0^{c,x}$ |
| **MTS** | $15.9^{b,y}$ | $17.0^{b,y}$ | $19.3^{ab,x}$ | $20.0^{b,x}$ |

[a-c]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x-z]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

**Table 4.10** Changes in turbidity for apple-carrot blended juice treated with MTS, HTST, and no treatment (Raw) during a three-week period

| Turbidity | Day 0 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|
| **RAW** | $3.31^{a,x}$ | $3.29^{a,xy}$ | $3.30^{a,x}$ | $3.22^{a,y}$ |
| **HTST** | $3.27^{ab,x}$ | $3.23^{b,y}$ | $3.23^{a,y}$ | $3.22^{a,y}$ |
| **MTS** | $3.14^{b,x}$ | $3.11^{c,x}$ | $3.11^{b,x}$ | $3.04^{b,y}$ |

[a-c]Treatment means within treatments (columns) with the same letter in each sample are not significantly different ($p<0.05$).

[x-y]Treatment means within time (rows) with the same letter in each sample are not significantly different ($p<0.05$).

# CHAPTER 5

## OVERALL CONCLUSIONS AND FUTURE WORK

The work reported in this thesis provided insight into the effect of manothermosonication (MTS) on microbial inactivation and the quality of an apple-carrot juice blend. Juice samples inoculated with *E. coli* 0157:H7 were exposed to sonication at 40, 50 and 60$^{o}$C under three different static pressures (100, 200, and 300 kPa) for 15, 30, 45, 60, and 75 seconds. Quality changes in apple-carrot juice blend treated with MTS and traditional HTST (High temperature-short time) method were examined immediately after treatment and during storage for 3weeks under refrigeration conditions. Quality attributes of the apple-carrot juice blend treated with MTS and HTST evaluated included brix, total phenolic contents, antioxidant capacity, turbidity, pH, and color.

This work demonstrated that MTS was effective in reduction of *E. coli* O157:H7 population in apple-carrot juice blend to meet the FDA requirement, achieving a 5 log CFU/g in 30 s at 60 $^{0}$C to 60s at 50 $^{0}$C. The apple-carrot juice blend treated by MTS had quality parameters close to that of raw juice and significantly better than the HTST juices. Overall, MTS treatment was a promising alternative to HTST as evidenced by its effective microbial inactivation and quality retention ability.

For future studies, the surface morphology of the *E. coli* O157:H7 cells treated by MTS will be investigated using different types of microscopes such as SEM (Scanning electron microscope), ESEM (Environmental scanning electron microscope), and TEM (Transmission electron microscope) to elucidate the inactivation mechanisms. A sensory evaluation of MTS-treated juices by a trained panel will also be conducted. Finally, studies using MTS to treat

different juice blends, such as orange and peach, will be conducted to fully understand the benefits of using MTS in juice pasteurization for ensuring the microbial safety while maintaining product quality.

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