ELIMINATION OF CERTAIN FOODBORNE PATHOGENS IN VARIOUS FRUIT JUICES BY COMBINATION OF ULTRASONICATION AND MUSTARD SEED

by

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ABSTRACT

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Foodborne infections are reported to cause important problems in human beings worldwide. *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp., mostly known as pathogenic strains are responsible to cause foodborne infections due to the consumption of viable pathogen containing fruit juices and other food materials. Nowadays, in order to combat these infections effectively, hurdle technologies have gained great importance in food industry. Non-thermal methods, in combination with natural substances is an alternative way for these technologies. In this study, sole mustard seed, sole ultrasonication and a combination of mustard seed and ultrasonication were used to inactivate these pathogenic species in apple, alpine strawberry and blackberry juices. It was found that in alpine strawberry and blackberry juices, the combination of ultrasound at a frequency of 24 kHz at the amplitude of 90% and 0.6 g/L mustard seed resulted an

effective inhibition to these important foodborne pathogens mentioned, with respect to the control, without disrupting chemical and physical properties of the fruit juices.

Keywords: Fruit juice, Foodborne infections, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., Ultrasonication, Mustard seed.

ÖZET

ULTRASONİKASYON VE HARDAL TOHUMU KOMBİNASYONUYLA ÇEŞİTLİ MEYVE SULARINDA BAZI GIDA KAYNAKLI PATOJENLERİN YOK EDİLMESİ

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Gıda kaynaklı infeksiyonların insanlarda önemli problemlere sebep olduğu dünya çapında raporlanmıştır. Canlı patojen içeren meyve sularının ve diğer gıda materyallerinin tüketimine dayalı gıda kaynaklı enfeksiyonlardan sorumlu olan *Escherichia coli* O157:H7, *Listeria monocytogenes* ve *Salmonella* spp. en çok bilinen patojenlerdir. Şimdilerde bu enfeksiyonlarla etkin mücadele edebilmek için engeller teknolojisi gıda endüstrisinde büyük önem kazanmıştır. Isıl olmayan yöntemlerin doğal maddelerle kombinasyonu bu teknolojiler için alternatif bir yoldur. Bu çalışmada elma, dağ çileği ve böğürtlen sularındaki patojenik türleri inaktive etmek için yalnızca hardal tohumu, yalnızca ultrasonikasyon ve hardal tohumu ve ultrasonikasyon bileşimi kullanılmıştır. 24 kHz frekanstaki ultrasonikasyonun 90% genliği ve 0.6 g/L hardal tohumunun bileşiminin, dağ çileği ve böğürtlen suyunda kontrole göre, sözü edilen bu önemli gıda patojenlerine karşı,

meyve sularının kimyasal ve fiziksel özelliklerini bozmadan etkili inhibisyonla sonuçlandığı bulunmuştur.

Anahtar Kelimeler: Meyve suyu, Gıda kaynaklı enfeksiyonlar, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., Ultrasonikasyon, Hardal tohumu.

To My Family…

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LIST OF ABBREVIATIONS

AC: Activated Cell

$a_{w:}$ Water Activity

B. nigra: *Brassica nigra*

C. sake: Candida sake

CFU: Colony Forming Unit

CG: Control Group

CoNS: Coagulase Negative *Staphylococcus*

D. naardenensis: Dekkera naardenensis

DAEC: Diffusely Adherent *E. coli*

DEC: Diarrhoeagenic *E. coli*

DNS: Dinitrosalicylic Acid

E.C. : Enzyme Classification

EAggEC: Enteroaggregative *E. coli*

EC: European Commission

EHEC: Enterohemorrhagic *E.coli*

EIEC: Enteroinvasive *E. coli*

EO: Essential Oils

EPEC: Enteropathogenic *E. coli*

FD: Factor of Dilution

FDA: Food and Drug Administration

g: Gram

GLS: Glucosinolates

GOX: Glucose oxidase

HC: Hemorrhagic Colitis

HHP: High Hydrostatic Pressure

HLT: Heat Labile Enterotoxin

HST: Heat Stable Enterotoxin

HUS: Hemolytic Uremic Syndrome

Hz: Hertz

IR: Ionizing Radiation

I1: Primary Injured Cell

I2: Secondary Injured Cell

kGy: Kilogray

kHz: Kilohertz

kPa: Kilo Pascal

kV: Kilovolts

L: Liter

LiCI: Lithium Chloride

LLO: Listeriolysin O

log: Logarithm

m: Volume of Sample

MDa: Mega Dalton

meq. wt.: Milliequivalent Weight of The Standard

min: Minute

mL: Milliliter

mPa.s: Mini Pascal Second

MPa: Mega Pascal

MuS: Mustard Seed

MS: Manosonication

μm: Micrometer

μs: Microseconds

MTS: Manothermosonication

N: Normality of NaOH

NaCl: Sodium Chloride

NaOH: Sodium Hydroxide

ND: Not Detected

nm: Nanometer

O.D.: Optical Density

^{o}C: Centigrade Degree

PCA: Plate Count Agar

PEF: Pulsed Electric Field

PME: Pectin methyl esterase

PI-PLC: Phosphatidylinositol-Specific Phospholipase C

sec: Seconds

sp.: Species

spp.: Species

Std: Standard Deviation

STEC: Shiga Toxin-Producing *E. coli*

TA: Titratable Acidity

TMAB: Total Mesophilic Aerobic Bacteria

TSA: Tryptic Soy Agar

TTP: Thrombotic Thrombocytopenic Purpura

US: Ultrasonication

USDA: United States Department of Agriculture

V: Titer Volume of NaOH

VTEC: Vero Cytotoxin-Producing *E. coli*

W: Watts

WMEO: White Mustard Essential Oil

XLD agar: Xylose Lysine Deoxycholate Agar

YEA: Yeast Extract Agar

YM: Yeasts and Molds

CHAPTER 1

INTRODUCTION

1.1 Food Preservation Methods

Food preservation procedures have gained great interest due to food spoilage that is caused by microorganisms. Although the industry uses food preservation methods to inhibit and inactivate microorganisms, these methods are not enough to supply food safety completely. In the food industry, the most widely used procedure for microbial inactivation is the thermal treatment that causes side effects in the functional, sensory and nutritional properties such as development of undesirable flavour, loss of nutrients in the processed food (Lado and Yousef, 2002; Piyasena et al., 2003; Chemat et al., 2011). Due to increase of fresh fruit consumption and these problems listed above, alternative methods were developed to inactivate microorganisms such as high hydrostatic pressure (HHP), ultrasound under pressure, ionizing irradiation and pulsed electric field (PEF) (Mañas P and Pagán R, 2005; Mason et al., 2005).

Sublethal stress is caused among the surviving cells and spores by control methods such as low pH, low heat, low a_w, low temperature, hydrostatic pressure. When one parameter injures cells and spores, their sensitivity to other parameters increase, and they are killed in the presence. Sublethal injury of microbial cells and spores by one preservation method depending on this increased susceptibility of injured cell and spores to other preservation method that is present in the

combination treatment can play important roles in controling microorganisms. To determine the antimicrobial efficiency of the combinations of different parameters at different doses against target microorganisms in food systems, studying their beneficial effect is important. The hurdle concept suggests that the combinations are more effective when two or more methods are used together, even at a much lower treatment dose (Ray, 2004).

1.2 The Juice Processing and Novel Methods Applicable To Juices

Consumption of ready to eat foods or fruit juices are preferred by consumers in modern life rather than fruit preparation. Minimally processed natural products are preferred by consumers to avoid side effects of processing methods such as destruction or partial removal of the health promoting substances present in fruits. Heat sensitive molecules such as vitamins and aromatic compounds can be destroyed by sterilization and pasteurization (Lado and Yousef, 2002; Piyasena et al., 2003; Chemat et al., 2011). In recent years, freshly squeezed or pasteurized juices have been participating in supermarkets. Orange juice, the most popular fruit juice in the U.S.A., Europe and many other countries (Putnam and Alehouse, 1997) is often sold as “fresh,” being minimally processed and preserved by refrigeration. The incidence of foodborne outbreaks caused by contaminated fresh fruit, vegetables and minimally processed fruit juices started to increase a few years ago (CDC, 2000; Mukherjee et al., 2006).

The use of processing methods such as high pressure (Lee et al., 2002; Alpas et al., 2003; Buzrul et al., 2005; Lee et al., 2006), radiation (Nakauma et al., 2004) or

PEF (Hamilton, 1967) is the most preferred option to avoid the effectiveness of thermal treatment.

The irradiation is the process which gives electromagnetic waves or electrons to foods. Gamma rays of cobalt-60, X-rays or electron beams are the sources of radiation the amount of which can be measured in kGy from irradiation absorbed food. Commercial application of ionizing radiation (IR) treatment on foods was started at the beginning of 1980s, but its success has been prevented by consumer concerns (Mañas and Pagán, 2005).

Pulsed electric field (PEF) is the process which releases short duration (1–100 μs) high electric field pulses (10–50 kV $cm)^{1}$) to a food placed between two electrodes (Mañas and Pagán, 2005).

The HHP which provides pressure from 100 to 1000 MPa not only inactivates microorganisms and denaturates several enzymes, but also affects sensory and quality characteristics of foods (Polydera et al., 2003, Mañas and Pagán, 2005). In spite of the studies of HHP were started at the end of the 19^{th} century, commercial applications of HHP had started (Mañas and Pagán, 2005).

1.2.1 Ultrasonication

Ultrasound is a process of sound waves the frequency of which is above the threshold for human hearing (>16 kHz). In order to use ultrasound in food preservation it is mostly preferred to combine with application of an external hydrostatic pressure of up to 600 kPa [manosonication (MS)] to increase lethality effect of ultrasound. In addition, a combination of MS with temperature [manothermosonication (MTS)] is another choice (Raso et al., 1998a).

Ultrasound equipments use frequencies from 20 KHz to 10 MHz (Piyasena et al., 2003). Ultrasound is classified into low intensity ultrasound with a frequency range of 5-10 MHz and high intensity ultrasound with a range of 20-100 kHz (McClements, 1995; Mason, 1998; Lee et al., 2003). Higher power ultrasound at lower frequencies is referred as power ultrasound (Piyasena et al., 2003). 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 (Bauman et al., 2005; Adekunte et al., 2010).

Power ultrasound is recognised as an alternative method to conventional thermal processing in the food industry (Piyasena et al., 2003, Mason et al., 2005). Ultrasound is a potential technology for the requirements of FDA, concerning a 5-log reduction in important certain microorganisms present in fruit juices (Salleh-Mack and Roberts, 2007, Patil et al., 2009). Ultrasound has been reported to be effective against foodborne pathogens found in guava juice (Cheng et al., 2007), orange juice (Valero et al., 2007), milk and apple cider (D'amico et al., 2006). When sufficient energy is employed, the lag time of yeasts is increased and the growth of yeasts is retarded by ultrasound (Jomdecha and Prateepasen, 2011). Reduced processing time, higher throughput, lower energy consumption, minimal flavour loss and greater homogeneity are the advantages of ultrasonication over thermal treatment (Zenker et al., 2003; Chemat et al., 2011).

Ultrasonic vibration increases heat transfer which triggers microbial inactivation in foods (Sastry et al., 1989; Lima and Sastry, 1990; Sala et al., 1995; Piyasena et al., 2003). However, Valero et al. (2007) reported that the pulp of orange juice increased the resistance of microorganisms to ultrasound. They found that

ultrasound treatment (500 kHz/ 240 W for 15 min) was unable to prevent subsequent growth of microorganisms in orange juice and did not cause ultrasound-related side effects on the quality of juice.

During processing, high power ultrasonication higher than 20 kHz frequency shows biochemical, chemical and mechanical effects that are used to modify the physicochemical properties, enhance shelf life, stability and the quality of foods (Mason, Chemat and Vinatoru, 2011). The destruction of foams, extraction of flavors, enhancement of crystallization, and degassing are the mechanical effects of ultrasonication (Higaki, Ueno, Koyano, and Sato, 2001). Preventing contamination of equipments and removal of bacterial biofilms from surfaces of equipments are the microbiological effects of ultrasonication (Baumann, Martin, and Hao, 2009).

Tomato pectic enzyme (Lopez, Vercet, Sanchez, and Burgos, 1998; Vercet, Sánchez, Burgos, Montañés, and Lopez Buesa, 2002), soybean lipoxygenase (Lopez and Burgos, 1995a), horseradish peroxidase (Lopez and Burgos, 1995b) and orange PME (Vercet, Lopez, and Burgos, 1999) inactivation were increased by the combination of ultrasound with heat and low pressure.

Some researchers have studied the combined effects of ultrasound with other agents against pathogens to inhibit on foods such as lettuce, apples, strawberry and other fresh produce. Seymour et al. (2002) confirmed that *Salmonella typhimurium* (*S. typhimurium*) was reduced as 2.7 log CFU/g by combined treatment of ultrasound and 100 ppm of chlorinated water on strawberry. This was an additional 1.0 log reduction compared with ultrasound or chlorinated water treatment alone for 10 min without affecting quality (Seymour et al., 2002). Scouten and Beuchat (2002) concluded that *Salmonella* and *Escherichia coli* O157:H7 (*E. coli* O157:H7) were

reduced effectively by combined treatments of ultrasound, heat and chemical on alfalfa seeds. The reduction of *Salmonella* and *E. coli* O157:H7 were 2.27 and 1.94 log CFU/g seen after the combined treatment of ultrasound and 1% Ca $(OH)_2$ at 23 °C for 5 min (Scouten and Beuchat, 2002).

Some researchers concluded that pathogens were reduced significantly by ultrasound in a period ranging between 5–60 min. for pathogen inoculated organic fresh lettuce (Mahvi et al., 2005; Seymour et al., 2002; Sagong et al., 2011).

Sagong et al. (2011) considered that the reduction levels of *E. coli* O157:H7, *S. typhimurium*, and *Listeria monocytogenes* (*L. monocytogenes*) of organic fresh lettuce by ultrasonication were increased with pH values that was declined from 2.54 to 2.11 due to the concentration of organic acid from 0.3 to 2.0%. Bacterial counts of these three pathogens at 0.0, 0.3, 0.5, 0.7, 1.0, and 2.0% organic acids for 5 min. of 40 kHz ultrasonication treatment were 0.32, 0.59, 0.80, 1.16, 1.40, and 1.60 log CFU/g, respectively. Whereas *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* were reduced significantly by 20 min. ultrasonication treatment, pathogens were not significantly reduced by 5 and 10 min. treatment. Moreover, color and texture of lettuce samples were significantly degraded after 30 min. treatment of ultrasonication. The combined treatments of ultrasound and organic acids for a period of five minutes, caused an additional 0.8 to 1.0 log reduction of the three pathogens compared to organic acid treatment alone. They also concluded that the combined treatment of ultrasound and 2.0% organic acid (2.67 log CFU/g) was the most effective treatment. They concluded that the combined treatment of ultrasound with organic acids (malic acid, lactic acid, and citric acid) was effective

on reducing these pathogens, compared to treatment with organic acids alone without significantly affecting the quality (Sagong et al., 2011).

1.2.2 The Mechanisms of Ultrasonication

The bubbles generated by ultrasonication cause very high pressures and temperatures, so free radicals and strong mechanical forces are produced against microorganisms and cause inactivation of microorganisms (Suslick, 1990). Bacterial cells could be inactivated by free radicals that is produced by ultrasonication as a similar manner of IR. However, results of experiments that have constructed with free radicals were negligible in comparison with cavitation that generated strong mechanical effects (Allison et al., 1996; Raso et al., 1998a). Haar (1988) concluded that effects of ultrasound were caused by nonthermal interaction mechanisms rather than heating. Cavitation, which was defined as "the formation of tiny gas bubbles in the cells as the result of ultrasound vibration" (Low and Reed, 1994), implied the behavior of bubbles within an acoustic field.

Raso et al. (1998a) studied the effect of equivalent heat, MS and MTS treatments (99% of inactivation) on the degree of cell disruption and they observed that heat-treated cells maintained its full cellular integrity, MS treated cells were completely broken. MTS treated cells demonstrated disruption at a medium degree. These results demonstrated that the envelope of microorganisms are broken down by ultrasonication, by the way microorganisms are inactivated in all type of ultrasonication treatments. A synergistic effect of MS and heat (MTS) has been occurred against some bacterial species such as *Enterococcus faecium* (Pagán et al., 1999c), heat-shocked cells of *L. monocytogenes* (Pagán et al., 1999b) and cells

suspended in a low water activity media (Álvarez et al., 2003). Moderately increased temperatures such as 55–60 ^{o}C weaken cell envelopes and facilitate the effect of ultrasonic waves such as mechanical disruption of the cell (Álvarez et al., 2003). In addition, a synergistic effect of MS and heat has been determined against bacterial spores (Raso et al., 1998b). Dipicolinic acid and some low molecular weight polypeptides of spores are separated from spores by ultrasonication (Palacios et al., 1991). It has also been found that MS sensitize spores of *Bacillus subtilis* to lysozyme (Raso et al., 1998b). Cerny et al. (1984) and Splittstoesser et al. (1998) reported that *D* values at 90 °C were 15 and 16 to 23 min, respectively. Splittstoesser et al. (1998) also reported *D* values of 2.4 to 2.8 min at 95 °C. These data suggest that spores of microorganisms can survive at pasteurization process of juice, the temperature of which is maintained at 86 to 96 °C for 2 min. The addition of nisin (Komitopoulou et al., 1999; Yamazaki et al., 2000), disinfectants (Orr and Beuchat, 2000; Lee et al,. 2004), grape polyphenols (Oita and Kohyama, 2002), enterocin AS-48 (Grande et al., 2005), and natural compounds such as ascorbic acid (Bahçeci and Acar, 2007), eugenol and cinnamaldehyde (Bevilacqua et al., 2008) have also been effective rather than thermal processing (Silva et al., 1999).

1.3 Mustard Seed (*Brassica nigra*)

Even though chemical preservatives are considered by most consumers as unhealthy (Montville et al., 2008; Tajkarimi et al., 2010) the majority of the antimicrobials are considered as chemical preservatives, used in the food industry for food preservation to control and prevent foodborne pathogens (Montville et al., 2008). For this reason, in the food industry natural antimicrobials of the herbs,

spices, fruits, vegetables and some plants have become increasingly important (Tajkarimi et al., 2010).

The family Brassicaceae (Cruciferae) is widely distributed and comprises more than 3000 species in approximately 350 genera. The family's major centers of diversity are southwestern and central Asia and the Mediterranean region. Secondary centers of diversity are in the arctic, western North America, and the mountains of South America (Price et al., 1994), distinguishing with their ability to accumulate the heavy metals in an extremely high degree (Broadley et al., 2001).

Brassica nigra Linn (Koch.) (Cruciferae) is a natural food source for antioxidants. Its radical-scavenging activity may be due to several natural bioactive compounds, such as gallic acid, quercetin, ferulic acid, caffeic acid and rutin, present in the extract. The seeds have a significant amount of fatty oil. In some parts of the world, shoots and leaves are cooked and consumed. *Brassica nigra* is a member of the Brassicaceae family, and used as a food flavouring agent, in addition, has medicinal uses such as, in the treatment of rheumatism and joint pains, in the treatment of indurations of the liver and spleen, and as a laxative in the treatment of throat tumours. There is limited scientific information about *B. nigra* (Rajamurugan et al., 2011).

Some essential oils (EO) have been researched for their antibacterial, antifungal, antiviral, and antioxidant properties (Mourey and Canillac, 2002; Kordali et al., 2005; Sylvestre et al., 2006; Gilles et al., 2010; Gao et al., 2011; Prakash et al., 2011).

Burt (2004) reported that many plant extracts and their essential oils have been inhibited the growth and reduced the number of several foodborne pathogens

such as *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7. Lara-Lledó et al. (2012) concluded that *L. monocytogenes* was reduced from Bologna sausages at 52 day storage (4 °C) to below the detection limit by oriental mustard containing antimicrobial PPG film with vacuum-packaging. Mattson et al. (2011) confirmed that EO volatiles significantly reduced the microbial load of *Salmonella* species on tomato samples to a limit of about 6.0 log CFU/mL (Mattson et al., 2011). Nilson and Holley (2012) found that *E. coli* O157:H7 was reduced by deodorized yellow mustard powder at the concentration of 4% or 6% on dry cured Westphalian hams (Nilson and Holley, 2012). Burt (2004) found that *L. monocytogenes* was effected less or not effected by mustard. The potential use of white mustard essential oil (WMEO) as an antimicrobial in food systems was reported by Ekanayake et al. (2006).

Glucosinolates (GLSs), known as mustard oil glucosides, are a class of nitrogen and sulfur-containing natural products. *Brassicaceae* genera contain glucosinolates (Fahey et al., 2001). Representatives of the *Brassicaceae* family are of particular importance as vegetables, seasonings and relishes, and sources of vegetable oil. GLSs are recognized as the active constituents responsible for many of the physiological activities proposed for the Brassica vegetables (Holst and Williamson, 2004; Verkerk et al., 2009). Upon plant damage, thioglucoside glucohydrolase (E.C.3.2.3.1), or also called myrosinase, hydrolyzes the relatively non-reactive GLS to give an array of biologically active compounds, i.e. isothiocyanates, thiocyanates, nitriles, etc. GLS demonstrate a wide range of antibacterial and antifungal properties with direct or synergistic effect in combination with other compounds (Naidu, 2000a; Tolonen et al., 2004; Almajano et al., 2008;

Graumann and Holley, 2008; Gutierrez et al., 2008). This antimicrobial activity has been proposed to be a part of the crucifers' defense against pathogen attack (Radulovic´ et al., 2011). Allyl isothiocyanate that is one of phenolic compounds in mustard was effective against fungi, and *E. coli* O157:H7 (Suhr and Nielsen, 2003; Turgis et al., 2009). In the past few years isothiocyanates have also been identified as potent cancer prevention agents (Zhang and Talalay, 1994; Holland et al., 1995; Fahey et al., 2001; Halkier and Gershenzon, 2006).

Recently it was shown that the glucosinolates; sinalbin and sinigrin were converted into isothiocyanates, the antimicrobial phenolids by myrosinase-like activity of *S. typhimurium* (02:8421), and *L. monocytogenes* GLM-4 (Herzallah et al., 2011), and *E. coli* O157:H7 (Luciano and Holley, 2011; Luciano et al., 2011). Studies have shown that even with myrosinase inactivated, deodorized or deheated; yellow mustard powder was lethal to *E. coli* O157:H7 (Graumann and Holley, 2008). Graumann and Holley (2008) also showed that in situ produced WMEO (white mustard essential oil) obtained by including ground yellow mustard powder as an ingredient in dry fermented sausage inhibited growth of *E. coli* O157:H7.

Turgis et al. (2009) concluded that pH level inside the bacterial cells that were *E. coli* and *Salmonella* reduced by EOs of mustard.

1.4 Fruit Juices

Natural fruit juices get attention as healthy products rather than other beverages in the worldwide. Fruit juices have health benefits (Suaad and Eman, 2008). Fresh fruit juices have high carbohydrate content, ranging from 5 to 15%, very low protein ($\leq$ 1.0%), and have a low pH, ranging from 2.5 to 4.0 (Ray, 2004).

1.4.1 Alpine Strawberry (*Fragaria vesca* L.)

Alpine strawberry grows in a wide range of areas such as woods and grasslands in Europe, western Asia, North America, and temperate areas in Chile (Alpine strawberry, 2008, Caruso et al.,2011). Each year hundreds of tons of wild fruit are harvested in Turkey and most of these are exported (Turhan and Paydas-Kargi, 2007). Alpine strawberry, among one of these wild fruits has higher production costs, prolonged harvesting time and lower production rather than garden strawberry (Fragaria x ananassa Duch.). Alpine strawberry bearing intense flavour and olfactory property, is used in food, cosmetic and confectionery industry as an ingredient of commercial jam, sauce, liqueur and cosmetic production. It also provides the methyl bromide prohibition to disinfect soil (Caruso et al., 2011; Turhan and Paydas-Kargi, 2007).

The root of alpine strawberry was used in the folk medicine to reveal diarrhea and the stalks for wounds. Fruit has antioxidant properties which prevent cancer. The leaves of alpine strawberry can be utilized as tea for diarrhea, digestive upsets, and to stimulate the appetite (Alpine strawberry, 2008).

Fragaria vesca which is known as wild strawberry belongs to the Rosaceae family, contains flavonoids, tannins, methyl salicylate, volatile oils, and borneol (Agarwal and Paridhavi, 2007). Alpine strawberry which contains salicylic acid is useful in the cure of kidney and liver diseases, as in rheumatism and gout (Phillips and Foy, 1990).

F. vesca contains the ellagitannin agrimoniin which is an isomer of sanguiin H-6 (Vrhovsek et al., 2012). Sanguiin H-6 is a molecule responsible for the antioxidant capacity of raspberries. Sanguiin H-6 which is a dimer of casuarictin

linked by a bond between the gallic acid residue and one of the hexahydroxydiphenic acid units, has sanguisorbic acid ester groups as linking units between glucopyranose moieties (Borges et al., 2010). Strawberries also contain vitamin C (Tulipani et al., 2008).

1.4.2 Blackberry (*Rubus caesius* L.)

Blackberries belong to the Rosaceae family, genus *Rubus*, subgenus *Eubatus* Focke. Blackberries, such as the species *R. occidentalis* L. are grown in the northern hemisphere, especially Europe and North America (Thompson, 1997).

Berries such as blackberry (*Rubus sp*.) consumed in human diet in the form of yogurts, beverages, jellies, and jams (Seeram, 2006a). They were proved to be rich in polyphenolic compounds like anthocyanins and proanthocyanidins (Fan-Chiang and Wrolstad, 2005; Cuevas-Rodriguez et al., 2010). Berry phenolics include flavonoids (anthocyanins, flavonols, and flavanols), tannins [condensed tannins (proanthocyanidins) and hydrolyzable tannins (ellagitannins and gallotannins)], stilbenoids (e.g., resveratrol), phenolic acids (hydroxybenzoic and hydroxycinnamic acid derivatives), and lignans (Seeram, 2006). Berry phytochemicals play in the prevention and treatment of chronic human diseases (Seeram, 2008). Berries have antioxidant, anti-inflammatory, and anticancer properties (Seeram et al., 2001; Seeram et al.,2002; Seeram et al., 2003; Seeram, 2006b). Blackberries are important fruits for health benefits based on high folic acid, vitamin K, vitamin C, and manganese (Sariburun et al., 2010).

Scalbert et al. (2000) and Manach et al.(2004) demonstrated that anthocyanin has been a major source of apoptosis among the berry phenolics (Scalbert et al.,

2000; Manach et al., 2004). Meyskens et al. (2005), Ramos et al. (2005) and Heo et al. (2005) demonstrated that berry extracts have been shown to have apoptotic effects in human cancer cells (Meyskens et al., 2005; Ramos et al., 2005; Heo et al., 2005). Also, researchers thought that ellagic acid may reduce the harmful effects of estrogen that create breast cancer cells (Papoutsi et al., 2005). Phytochemicals of berry regulate the activities of metabolizing enzymes, modulate nuclear receptors, gene expression, and subcellular signaling pathways; and repair DNA oxidative damage (Seeram, 2006a; Seeram et al., 2006).

1.4.3 Apple (*Malus domestica Borkh*)

The genus Malus belongs to the Rosaceae family, subfamily Pomoideae. There are at least 25 Malus species (Robinson et al., 2001). Apple phytochemicals which have antioxidative, antihypercholesterolemic, and anticarcinogenic properties may reduce the risk of developing coronary heart disease, asthma, and diabetes (Boyer and Liu, 2004). Apples have polyphenolic contents that are flavonols, procyanidins, hydroxycinnamic acids, flavan-3-ols, dihydrochalcones, ascorbic acid, and anthocyanins in the peel of red ones (Guyot et al., 2003; Khanizadeh et al., 2008). These phenolic compounds of apples are effective antioxidants and have anti-microbial, anti-inflammatory, anti-carcinogenic, anti-allergic, anti-platelet, vasodilatory, and neuroprotective properties (Scalbert et al., 2005; Es-Safi et al., 2007; Stevenson and Hurst, 2007). Antioxidant compounds in apples are quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, catechin, epicatechin, procyanidin, cyanidin-3-galactoside, coumaric acid, chlorogenic acid, gallic acid, and phloridzin (Lee et al., 2003). Apple peels contain ursolic acid which increases

skeletal muscle and brown fat, and decreases white fat obesity, glucose intolerance, and fatty liver disease (Kunkel et al., 2012). Apples contain relatively low amounts of vitamin C (Boyer et al., 2004).

1.4.4 The Microflora of Juices

Fruit juices which have certain organic acids, trace elements, water and certain vitamins and sugars act as host for microorganisms to grow. Fruit juices have generally a low pH (pH < 4.5) so, contamination with aerobic acid-tolerant microorganisms, especially yeasts can spoil fruits and their juices. Food spoilage that is undesirable or unacceptable for human consumption due to changed sensory characteristics of foods such as texture, smell, taste, or appearance may be safe to eat, so, spoiled foods may not cause illness because there are no pathogens or toxins present (Sherratt et al., 2006). *Acetobacter, Alicyclobacillus, Bacillus, Clostridium, Gluconobacter, Lactobacillus, Leuconostoc, Saccharobacter, Zymomonas, and Zymobacter* are the main microorganisms to spoil fruit juices. *Pichia membranifaciens*, *Candida maltosa*, *C. sake*, *Saccharomyces bailii*, *S. bisphorus*, *S. cerevisiae*, *S. rouxii*, *S. bayanus*, *Brettanomyces intermedius*, *Schizosaccharomyces pombe*, *Torulopsis holmii*, *Hanseniaspora guilliermondii*, *Schwanniomyces occidentalis*, *Dekkera bruxellensis*, *Torulaspora delbruckii*, *Zygosaccharomyces microellipsodes*, and *D. naardenensis* are also reported to spoil fruit juices (Boekhout et al., 2003; Vasavada, 2003; Keller, 2006).

Spoilage incidents of foods due to spoilage microorganisms are seen generally in summer and spring. Spoilage which can be caused by contaminated fruit and processed fruit products is characterized by off-flavour, odour production, and

sometimes sediment, discolouration or cloudiness occurred on the food. The off-flavour and odour are identified as the chemical compound guaiacol. But the other causes are the halophenols 2,6-dichlorophenol (2,6-DCP) and 2,6-dibromophenol (2,6-DBP) (Parish, 2006, Bhat et al., 2011, Steyn et al., 2011).

1.4.5 The Foodborne Pathogens in Fruit Juices

Consumption of several low pH foods and their unpasteurized juices, such as apple, orange, lemon, carrot, mango juices had been implicated in foodborne diseases and outbreaks caused by *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*. In spite of these pathogens are normally sensitive to low pH that is 4.5 and below, acid-resistant variants of these pathogens are able to survive in low pH foods. Also they are not affected by environmental stress such as low heat, low a_w low temperature, hydrostatic pressure (Ray, 2004). *E. coli* O111, *Cryptosporidium*, *Norovirus*, *Vibrio cholerae*, *Clostridium botulinum* are the other pathogens to cause outbreaks.

1.4.5.1 *Escherichia coli* O157:H7

Escherichia coli (*E. coli*) serotype O157:H7 is a rod-shaped, motile, Gram-negative, facultative anaerobic, non-spore forming, catalase (+) positive, oxidase (-) negative, fermentative and enterohemorrhagic strain that can colonize in the intestine of various host animals (Erkmen and Bozoğlu, 2008; Newell et al., 2010). There are six groups of diarrhoeagenic *E. coli* (DEC) strains, classified as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), diffusely adherent *E. coli* (DAEC) and vero

cytotoxin-producing *E. coli* (VTEC) (enterohemorrhagic *E. coli* (EHEC)) or Shiga toxin-producing *E. coli* (STEC). VTEC and STEC strains are more associated with foodborne outbreaks than the others (Newell et al., 2010).

E. coli O157:H7 causes severe gastrointestinal diseases such as bloody diarrhea, haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS), and traveller's diarrhea (Montville et al., 2008; Robinson et al., 2010).

The enterohemorrhagic *E. coli* (EHEC) has a single strain of serotype O157:H7. EHEC, the life treating pathogen produce shiga toxin like verotoxin (VT1 and VT2) (Erkmen and Bozoğlu, 2008). The symptoms are seen within 2 to 12 days following ingestion of viable EHEC contaminated food. EHEC adheres and colonizes in the large intestine and produce cytotoxic verotoxin which is an enterotoxin. Abdominal pains, bloody or nonbloody and watery diarrhea, vomiting are the symptomes of EHEC (Erkmen and Bozoğlu, 2008). EHEC which has a low infection dose of $\leq$ 10 cells cause hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpurea (TTP) (Cerny et al., 1984; Vojdani et al., 1995; Keller et al., 2006; Parish, 2006; Kiranmayi et al., 2010; Steyn et al., 2011).

Enteroinvasive *E. coli* (EIEC) cause dysentery like shigellosis. Enterotoxin is not produced by EIEC. The symptoms are seen within 8 to 24 hours following ingestion of viable EIEC contaminated food. EIEC invades in the epithelial cells of colon by invasive factors produced by EIEC. EIEC penetrates the cell and multiply to cause epithelial cell death of colon. Ulceration of the mucosa is caused by inflammatory reactions. The symptoms of EIEC are fever, chills, headache, diarrhea with blood, mucus or nonbloody, abdominal and pain (Erkmen and Bozoğlu, 2008).

Enteroaggregative *E. coli* (EAggEC) adheres with the aggregation on HEP-2 cells of intestine. EAggEC produce cytotoxin that damages mucosa. Hemorrhagic necrosis of villi, mononuclear infiltration of submucosa and edema are the lesions that are produced by EAggEC. EAggEC causes infantile diarrhea that lasts more than 14 day. Watery and mucoid diarrhea, low grade fever and in some cases bloody stools are the symptomes of EAggEC (Erkmen and Bozoğlu, 2008).

Diffusely adherent *E. coli* (DAEC) that is seen in children causes mild nonbloody diarrhea. DAEC attaches to HEP or HeLa cells via diffuse-adherent pattern. Enterotoxin is not produced by DAEC. Epithelial cells are not invaded by DAEC (Erkmen and Bozoğlu, 2008).

Enterotoxigenic *E. coli* (ETEC) causes travelers' diarrhea. The symptoms are seen within 12 to 36 hours following ingestion of viable ETEC contaminated food. Ingested vegetative cells pass through the small intestine. ETEC adheres, colonizes and produces enterotoxin in the small intestine. Heat stable enterotoxin (HST) and heat labile enterotoxin (HLT) are released by lysed ETEC. Adenylate cyclase enzyme is activated according to binding of HLT to epithelial cells. cAMP level which is increased by adenylate cyclase in epithelial cells causes dehydration that leads to watery non-bloody and non-mucoid diarrhea (Erkmen and Bozoğlu, 2008). The symptomes are more like in cholera (Ray, 2004). Other symptoms are low grade fever, abdominal pain, nausea and malaise. ETEC toxicoinfection lasts 2 to 3 days. HST is resistant to heating at 100 ^{o}C for 15 min, but HLT can be inactivated at 60 ^{o}C for 30 min (Erkmen and Bozoğlu, 2008).

Enteropathogenic *E. coli* (EPEC) has high mortality rate and does not produce enterotoxin. EPEC are important in infant diarrhea worldwide. The

symptoms are seen following ingestion of EPEC contaminated food. High numbers of cell ingestion is needed to cause symptoms. Lesions are caused by the attachment of EPEC to intestinal epithelial cells (Erkmen and Bozoğlu, 2008). Gastroenteritis, diarrhea, abdominal pain, nausea, headache and chills are the symptoms of EPEC (Ray, 2004; Erkmen and Bozoğlu, 2008). Disease can last for 14 days (Erkmen and Bozoğlu, 2008).

Animal feces that can contaminate processed raw animal product, may also contaminate fruit and vegetable crops with *E. coli* O157:H7. These contaminated products such as vegetables, fruits, juices, drinking water, and sprouts was reported to cause outbreaks (Powell et al., 2011). Ground beef, hamburger that was not properly cooked, and baby spinach were the sources of transmission of *E. coli* O157:H7 to cause an outbreak, resulting in three deaths in Canada and in U.S. (Powell et al., 2011).

In spite of the minimum pH for growth of *E. coli* is 5.0 (Erkmen and Bozoğlu, 2008), *E. coli* O157:H7 has been shown to grow at low pH (4.0–4.5) (Buchanan and Bagi, 1994), survive in very acidic environments (pH 1.5–3.0) (Arnold and Kaspar, 1995; Ray, 2004) and have salt tolerance, surviving in sausage having 4.4–4.8% NaCl (Hinkens et al., 1996; Riordan et al., 1998). Low a_w prevents microbial spoilage in dry sausage, and *E. coli* can grow in a minimum a_w of 0.95 (Sperber, 1983). The combination of low pH and low a_w that were present in dry fermented sausage was found to significantly reduce *E. coli* O157:H7 (1–2 log CFU/mL), but at the high amounts of *E. coli* O157:H7 this was insufficient to eliminate the pathogen (Chacon et al., 2006; Graumann and Holley, 2008).

1.4.5.2 *Salmonella* spp.

Salmonella, a Gram (-) negative, rod-shaped, motile and non-spore-forming, catalase (+) positive, oxidase (-) negative, facultative anaerobic pathogen is in the *Enterobacteriaceae* family (Ray, 2004, Erkmen and Bozoğlu, 2008). The pH range of growth is 5.5 to 9.5, but optimum pH range is 6.5 to 7.5 (Erkmen and Bozoğlu, 2008). *Salmonella* is sensitive to pasteurization temperature. *Salmonella* has been the cause of the outbreaks of salmonellosis (Ray, 2004).

During harvesting of the fruits dropped from trees to the ground during harvesting, are contaminated by *Salmonella* spp., one of the main isolated pathogen from decayed fruits (Wells and Butterfield 1997). Pao et al. (1999) had showed that such surface contamination can be easily transferred to the juice during squeezing of contaminated fruit. Other than animal derived *Salmonella* infections, such as egg, dairy and seafood derived, *Salmonella* also cause fruit juice associated infections. Gastroenteritis that is caused by many strains of *Salmonella* is seen within 12 to 48 hours following ingestion of viable cells contaminated food. *Salmonella* adheres to microvilli of intestinal epithelial cells with its fimbriae, produces thermostable cytotoxin that damages epithelial cells, and enter through the damaged epithelial cells. They proliferate in the epithelial cells, produce and release enterotoxins. Adenylate cyclase enzyme is activated by this enterotoxin. cAMP level which is increased by adenylate cyclase in epithelial cells causes dehydration that leads to watery, bloody or non-bloody diarrhea. Mild fever, abdominal pain, nausea, prostration, muscular weakness, chill, headache and thirst are other symptoms of gastroenteritis. Mortality rate is low (0.1 - 0.2%). Infective dose of *Salmonella* is as low as 10 – 100 cells (Erkmen and Bozoğlu, 2008).

Typhoid and paratyphoid fever which are caused by *Salmonella typhi* (*S. typhi*) and *Salmonella paratyphi* (*S. paratyphi*), respectively, are the systematic diseases of *Salmonella* (Erkmen and Bozoğlu, 2008). *Salmonella typhi* cause typhoid fever which is associated with poor hygiene of handlers. Typhoid fever outbreaks have decreased in developed countries. *Salmonella typhimurium* infection, associated with unpasteurized orange juice were reported (Cerny et al., 1984; Vojdani et al., 1995; Keller et al., 2006; Parish, 2006; Steyn et al., 2011). After penetration of invasive *Salmonella* to the intestinal epithelial cells, the failure of host defense induces invasive *Salmonella* to multiply in the macrophages. When these infected macrophages undergo lysis, *Salmonella* cells that are dispersed into the blood stream and to the whole body cause septicemia. While *Salmonella* is engulfed by macrophages in the infected blood, engulfed cells continue to multiply within macrophages again. This case leads to chronic clinical conditions. Headache, fever, constipation, abdominal pain and rose red spots on the body are the symptoms of septicemia (Erkmen and Bozoğlu, 2008).

1.4.5.3 *Listeria monocytogenes*

Listeria monocytogenes (*L. monocytogenes*) is a Gram (+) positive, rod-shaped, non-spore forming, facultatively anaerobic, catalase (+) positive, oxidase (-) negative and intracellular foodborne pathogenic bacterium that may be found on raw meats and vegetables (Erkmen and Bozoğlu, 2008; Suo et al., 2010). *L. monocytogenes*, the main foodborne pathogen in foods can be undergone lethality treatments, such as ready-to-eat foods stored at refrigeration temperature are generally consumed without heating (Sofos, 2008). So, many contaminated

refrigerated vacuum-packed meat products (pH 6.3) with long shelf-life are able to support substantial growth of *L. monocytogenes* due to its anaerobic conditions (Lara-Lledó et al., 2012).

L. monocytogenes can be present in rotting vegetables, untreated or treated sewage water, sludge, agricultural produce and in certain types of foods (Kerouanton et al., 2010) such as fresh soft cheese, cooked and frozen poultry products, pork products, meat products, hot dogs, smoked fish, vegetables, seafood, and salads (Almeida and Almeida, 2000; Alessandria et al., 2010). *L. monocytogenes* can also able to attach to the surfaces and form biofilms, which increases continuous contamination of the food processing equipments and environment (Alessandria et al., 2010).

L. monocytogenes which is a psychrotroph with optimum growth at 35 to 37 ^{o}C is able to grow at temperatures ranging from 1 to 44 ^{o}C. *L. monocytogenes* is resistant to pH 5.0 and above, high salt, freezing and drying. They are sensitive to pasteurization temperature (71.7 ^{o}C for 15 sec. or 62.8 ^{o}C for 30 min.) (Ray, 2004). *L. monocytogenes* is the main pathogenic bacteria due to its ability to grow at the refrigeration temperatures and acidic conditions of pH 4.4 to 4.6. The infective dose is considered to be 100 to 1000 cells (Ray, 2004). *L. monocytogenes* cause life threatening disease known as listeriosis and invasive form of which complications such as meningitis, meningoencephalitis, septicemia and spontaneous abortion in immunocompromised individuals and pregnant woman, and less frequently endocarditis can be seen (Boekhout et al., 2003; Doganay, 2003; Vasavada, 2003; Keller, 2006). Listeriosis was responsible for 30% of foodborne deaths from 1996 to 2005 and had a high case fatality rate of 16.9% (Barton et al., 2011). In response to

this risk, the United States Department of Agriculture (USDA) adopted a "zero-tolerance" policy for this pathogen in such products (FDA, 2003), although the European Commission (EC, 2005) and Canada (HC, 2011) established a limit of 2 log CFU/g of *L. monocytogenes* in types of these products that do not support *L. monocytogenes* growth (Lara-Lledó et al., 2012). The listeriosis is seen within 1 to 7 days following ingestion of viable *L. monocytogenes* contaminated food. *L. monocytogenes* can pass through stomach acidity. After this passage of *L. monocytogenes* through stomach, *L. monocytogenes* attaches and passes through epithelial cells of intestine. Then, *L. monocytogenes* multiplies and releases listeriolysin O which is a hemolysin and causes intestinal epithelial cell death as a virulence factor (Erkmen and Bozoğlu, 2008). This non-invasive form results in milder food poisoning-like symptoms such as fever, headache and diarrhoea, often referred to as febrile gastroenteritis (Berrada et al., 2006). Then, if the pathogen can spread through bloodstream invades different tissues such as central nervous system, fetus, heart and causes invasive symptoms such as meningitis, meningoencephalitis, septicemia, endocarditis and spontaneous abortion.

The entire *L. monocytogenes* infection is directed by multiple proteins such as internalin A and internalin B (encoded by inlA and inlB), hemolysin (encoded by hly, its product in *L. monocytogenes* being known as listeriolysin O or LLO), phosphatidylinositol-specific phospholipase C (PI-PLC, encoded by plcA), phosphatidylcholine-specific phospholipase C (PC-PLC, encoded by plcB) and actin polymerization protein (actA encoded by actA) (Jaradat et al., 2002).

1.4.5.4 The Pathogenic Yeast and Molds

Varying mycotoxins are produced by several species of molds in fruit juices. Mycotoxins, especially patulin is dangerous for human health in foods. In the apple juices patulin is produced by *P. claviforme, P. roqueforti* var. *carneum, Penicillium expansum, P. griseofulvum, P. funiculosum, P. granulatum,* and *Byssochlamys* spp. Byssotoxin A and byssochlamic acid are produced by *Byssochlamys*. Verruculogen, fumitremorgins, fischerin, and terrein are produced by *Neosartorya.* Ochratoxin A, penicillic acid, and citrinin are the other mycotoxins produced by molds (Vasavada, 2003).

1.4.6 Injured Cell

The microbial cells that are exposed to the stress conditions such as high temperature and pressure, low a_w and pH, not only stop growing, but may be injured or lose viability (Ray, 2004).

Injured cell is defined as the cell which loses some of its vital structures by stress (Busta, 1976, Hartsell, 1951). Each cell of a population which are subjected to the same strength of stress does not undergo the same amounts of injury and each types of stress causes different injuries at different parts of the cell (Jay et al., 2005). When sublethal injury is occurred in the microorganism, many structural and functional damage such as cytoplasmic or inner membrane, cell wall, DNA, RNA, ribosomes, tricarboxylic- acid-cycle or many other enzymes' damage are seen in effected microorganism (Ray, 1979, 1993). The most common affected structure of injured microorganism is the cell membrane of which permeability are damaged and

become susceptible to many antimicrobials and selective agents (Hurst, 1977; Jay et al., 2005; Wu, 2008). Sometimes cell function is altered by changing macromolecules through injury so metabolic activities of these structures were ceased (Ray, 1979; Jay et al., 2005).

Injured microorganisms which constitute a serious threat in food safety due to toxin production and their ability of repair themselves in food can not be ignored. Injured cells which is present in food may not be detected due to lack of its ability to grow in selective media as normal surviving cells (Wu, 2008). This can lead to an overestimation of the lethality of the treatment (Mackey, 2000). Injured cells just able to grow in a non-selective media after their repair. If any cell which doesn't grow in selective media may mislead us whether injured cell is present or not. Because they can repair themselves in processed media or in foods they can be a very dangerous risk at one's health (Damar, 2002).

Injured microorganisms can be detected according to the following principles: They become sensitive to many selective compounds to which uninjured cells are resistant such as NaCI, antibiotics, crystal violet and brillant green dyes, low pH, hydrolytic enzymes such as lysozyme and RNase, surface active compounds such as deoxycholate, bile salts and SDS, some chemicals such as selenite, tetrathionate, bismuthsulfite, LiCI (Ray, 2004) in the media due to the damage of the membrane. Injury is reversible, so, can be repaired in rich nutrients' containing nonselective medium. Repaired cells not only regain their ability to multiply but also regain resistance to selective compounds. In selective medium which has selective compounds, injured cells can not repaired. Injured cells just could be grown in the

selective medium after repaired in the suitable condition (Busta, 1976; Ray, 1979; Foegeding and Ray, 1992).

There are three states of cells subjected to stress. Cells grown as visible colonies on both selective and non-selective medium are defined as active cells (AC). Cells grown as visible colonies on non-selective agar but not on selective agar are defined as primary injured cell (I1), also during prolonged storage visible colonies can be formed on selective media. Cells grown as visible colonies on either non-selective or selective agar are defined as secondary injured cell (I2), also during prolonged storage visible colonies are formed first on non-selective media, then on selective media (Bozoglu, 2004).

CHAPTER 2

THE AIM AND THE SCOPE OF THE STUDY

The aim of the study is to investigate the effects of pH, mustard seed and ultrasonication and the combination of them on several foodborne pathogens in certain fruit juices such as apple, blackberry and alpine strawberry. These fruits were selected due to their economic potential in Bolu province of Turkey in which the study was conducted. To avoid destruction or partial loss of health promoting substances present in juices such as antioxidants etc., the behaviour of mustard seed as a natural product and the inhibitory effect of ultrasound was investigated on foodborne pathogens in these fruit juices.

Due to the unwanted side effects of other sterilization techniques rather than ultrasonication in the nutritional and functional properties of food, such as functional loss of vitamins can occur (Lado and Yousef, 2002; Piyasena et al., 2003; Chemat et al., 2011). We aim to overcome these problems by using either combined or sole ultrasonication method and mustard seed as a natural product on foodborne pathogens in fruit juices.

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

3.1.1 Mustard seed

Mustard seed was purchased from a local vendor in Bolu, TURKEY.

3.1.2 Fruits

The apples, purchased from a local vendor in Isparta, TURKEY were squeezed to obtain the fresh fruit juice and immediately transported to the laboratory. The defective ones were eliminated and the fruits were washed with tap water. The blackberry and alpine strawberry was purchased from a local vendor in Bolu, TURKEY.

3.1.3 The Fruit Juices

Apple, blackberry and alpine strawberry fruits were pressed by blender to squeeze their fresh juices. Due to apples and berries were processed to clear juice in food industry, fresh juices were subsequently filtered by a sterilized filter paper to separate the pulp before subjecting to mustard seed and sonication treatments.

3.1.4 Bacteria

The bacteria (*E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes*) which were inoculated to the fruit juices in the study were obtained from Provincial Directorate of Agriculture, Bolu, TURKEY and all strains were previously isolated from food.

3.2 Method

3.2.1 The Treatment with Ultrasonication

10 mL of each of the fruit juices were ultrasonicated at a constant frequency of 24 kHz, at the amplitude of 30%, 60%, 90% (40μm, 72 μm, and 108 μm, respectively) of the ultrasound wave for a period of 1, 5 and 10 minutes (Hielscher Ultrasound Technology, UP 400S Ultrasonic processor (400 watts, 24 kHz), Germany). Temperature control during ultrasonication treatment was achieved by decreasing excess heat evolved during ultrasonication, using ice bath placed to the periphery of the sample tubes. In each treatment the application temperature was detected. The temperatures were rising from 15 to 24 ^{o}C through ultrasonication process.

3.2.2 The Treatment with The Mustard Seed

Mustard seed was added to fruit juices at concentrations of 0.2, 0.4 and 0.6 g/L.

3.2.3 The Effects of The Combination Treatments on The Inhibitory Activity of Foodborne Pathogens

The best effective doses, obtained in Section 3.2.1. and 3.2.2 of the combination or independent treatments of mustard seed and ultrasonication were tested and evaluated on foodborne pathogens in fruit juices.

3.2.4 The Bacterial Culture and Inoculum Preparation

The bacterial suspensions of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* were prepared in sterile distilled water and the final concentrations of the bacterial suspensions were adjusted to a value of approximately 0.5 Mc Farland by using a densitometer (Biosan, DEN-1 BS101040, Latvia). 0.1 mL of bacterial suspensions were added into 9.9 mL of each of the pasteurized fruit juice to test the effectiveness of both of these methods. Fruit juices were pasteurized by heating at 145 °F (63 °C) for 30 minutes (Rich, 2003) prior to the inoculation of bacteria. After ultrasonication, mustard seed and combination treatments were done, 1 mL of samples were serially diluted in 9 mL of sterile peptone water. 0.1 mL samples of first three dilution tubes were inoculated in triplicates on PCA using standard spread plate technique. The plates were then incubated at 37 °C for 48 h (Anonymous, 2005). The microbial counts were converted to logarithmic values (Cemeroğlu, 2007).

3.2.5 The Determination of The Population of Viable and Injured Cells

For this purpose, *E. coli* O157:H7 cells were inoculated to non-selective tryptic soy agar (TSA) and selective agar (TSA supplemented with 1% NaCl) using standard spread plate technique in order to determine the number of total viable and uninjured cells after the mustard seed treatment, respectively (Damar et al., 2002). The difference between the number of total viable (c.f.u. on non-selective agar) and uninjured (c.f.u. on selective agar) cells was calculated to determine the number of injured cells (Ray, 1996). *L. monocytogenes* and *Salmonella* spp. were inoculated to non-selective TSA and TSA supplemented with 4% NaCl as a selective agar using standard spread plate technique (Beuchat et al., 1986; Novak and Juneja, 2001).

Mustard seed-treated cells were serially diluted in sterile peptone water. 0.1 mL samples of first three dilution tubes were inoculated in triplicates on TSA for non-injured and TSA with NaCl for injured cell counts using standard spread plate technique. The plates were then incubated at 37 °C for 48 h. Initial inhibition of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. was calculated by the difference between the number of cells on non-selective agar and selective agar and recorded as final inhibition. The percentage injury was calculated according to the given formula (Novak and Juneja, 2001) :

[1 – (CFU on TSA with 1% or 4% NaCI/CFU on TSA) ×100].

3.2.6 The Microfloral Analysis of Fruit Juices

3.2.6.1 The Determination of Microflora of Fruit Juices

The total mesophilic aerobic counts were detected by plate count agar (PCA) (Merck). *Salmonella* and *Shigella* determination was carried out by XLD agar (Merck), *E. coli* and Gram (-) negative bacteria determination was done by EMB agar (Merck), *S. aureus* and *L. monocytogenes* by blood agar (Merck) and the mold and yeast by yeast extract agar (Merck). *Alicyclobacillus acidoterrestris* was identified by Ali agar (Merck). For *Alicyclobacillus acidoterrestris* identification, juice sample was added into two Ali and two PCA medium. One of each Ali and PCA medium which contained the juice sample, were incubated at 25 ^{o}C for 24-48 hours. The other Ali and PCA medium which contained the juice sample were incubated at 50 ^{o}C for 24-48 hours. After these incubations, the bacteria observed in Ali medium, rather than PCA was identified as *Alicyclobacillus acidoterrestris*.

3.2.6.2 The Microbial Counts

The treated and untreated (control) juices, kept in closed jars were aseptically opened and 1 mL of samples, obtained from these juices were serially diluted in 9 mL of sterile peptone water to enumerate viable cells of the natural micropopulation. 0.1 mL samples of first three dilution tubes were inoculated in triplicates on plate count agar (PCA, Merck) and yeast extract agar (YEA, Merck) using standard spread plate technique. The plate count agar plates were incubated at 37^{o}C for 24-48 hours in the incubator. The yeast extract agar plates were incubated at 25°C for 5 days. The microbial counts were converted to logarithmic values (Cemeroğlu, 2007).

3.2.7 The Shelf Life of Fruit Juices

Fruit juices were kept in the refrigerator at + 4^{o}C. Microbial counts of fruit juices were done after 20, 40 and 60 days in order to detect the shelf life.

3.2.8 The Physical and Chemical Analysis

3.2.8.1 Total Soluble Solids (^{o}Brix)

The total soluble solid content of the juices were determined by using a hand refractometer (Portable Refractometer, FG108/118, Beijing, China (Mainland)). Refractive indexes were recorded and converted to ^{o}Brix. The measurements were performed at 25 ± 0.5 °C in triplicates (Cemeroğlu, 2007).

3.2.8.2 Titratable Acidity

In order to determine the titratable acidity (TA), samples of 25 mL from each of the apple and alpine strawberry juice and 75 mL distilled water were mixed in a beaker. For the blackberry juice, a sample of 10 mL was completed to 150 mL by distilled water. Then this solution was filtered by filter paper and 1 g of phenolphthalein was dissolved by 100 mL of 95 % ethyl alcohol and three drops of this phenolphthalein solution were mixed with 25 mL of this filtered solution. The mixture was titrated with 0.1 N NaOH to the end point of phenolphthalein (pH 8.2 ± 0.1), till pink color was observed. The volume of NaOH was expressed as either citric acid or malic acid per 100 mL of juice based on the method of Cemeroğlu (2007) and the total acidity was calculated according to the given formula:

TA%: = (N × V / m) × 100 × FD × meq. wt.

According to this equation V is the titrated volume of NaOH, m is mass of juice (g), meq. wt. is the weight of the standard, N is the normality of NaOH and FD symbolizes the factor of dilution.

The values of 0.067, 0.064 and 0.064 were used as milliequivalent weight of the standard (meq. wt.) for malic acid of apple juice, citric acid of alpine strawberry juice and blackberry juice in the formula, respectively (Cemeroğlu, 2007).

3.2.8.3 The pH Analysis

The pH of fruit juice samples was measured using a digital pH meter (WTW Inolab pH 720, Germany) (Cemeroğlu, 2007).

3.2.8.4 Viscosity

The viscosity of the samples was measured by a viscometer (AND Vibro Viscometer, SV-10, Japan).

3.2.8.5 The Determination of Fructose Content

The fructose content of the fruit juice samples was determined by using dinitrosalicylic acid (DNS) method (Miller, 1959). The standard solutions which contained 1, 2, 3, 4 % and 5 % of D-fructose were prepared (Appendix 42). 1 mL from each of these fructose solutions were treated with 3 mL DNS. Subsequently 1 mL of juice samples were added to this mixture in test tubes and then these samples were incubated in boiling water bath for 30 minutes. Following the cooling of the test

tubes, 1 ml aliquot from the incubated sample was completed to 40 mL with distilled water and the absorbance was determined by using a spectrophotometer at O.D.540 nm (Shimadzu, PharmaSpec UV-1700, UV-Visible Spectrophotometer, Japan). A linear standard curve for fructose was constructed using the absolute amounts of fructose plotted against spectrophotometric values to determine the amount released from each sample (Appendix A.1.).

3.2.8.6 The Color Determination

The color of the juice samples were determined by a colorimeter according to CIE $L^*A^*B^*$ system (Konica Minolta, CR-400, Japan). The color values were expressed as L^* (whiteness or brightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness) (Lopez-Nicolas and Garcia-Carmona, 2007). All measurements were performed in triplicates.

3.2.9 The Taste Panel

Thirty panelists rated the fruit juices in fresh form according to the criteria of odor, colour, appearance, flavor, bitterness, sourness, sweetness and overall evaluation with a 10 point scale per replication. A score of 10 represented most liked, and 1 corresponded to least liked. Each panelist was informed about the criteria before evaluation. Bread and water were served for the palate.

3.3 The Statistical Evaluation

The data were shown as means and analyzed by the IBM SPSS Statistics 21 program. Anova Scheffe test was used to detect the significance between treatment groups and different concentrations and process times of each treatment group. Kruskal-Wallis test was used in the chemical analysis of fruit juices to detect the significance between treatment groups. Pearson correlations was used to correlate related chemical parameters.

General Linear Model Repeated Measures test was used to detect the significance between storage periods during shelf-life determination.

The significant level was set to $p < 0.05$ at the beginning of the study.

CHAPTER 4

RESULTS

4.1 The Application of Ultrasonication and Mustard Seed in Alpine Strawberry Juice

4.1.1 The Microbiological Analysis of Alpine Strawberry Juice

The native microflora of alpine strawberry juices was found to be composed of *Staphylococcus aureus* (*S. aureus*), Gram (-) negative bacilli, and certain yeast and mold species due to their presence in blood agar, EMB and yeast extract agar (YEA) respectively. No growth of *Salmonella* in XLD and *Alicyclobacillus* spp. in BAT (Ali) agar was recorded. The microbial counts were tabulated in Appendix A.3.

In the fruit juices, salmonellosis and *E. coli* related diarrhea were claimed to cause serious infections (Parish, 1997), therefore *L. monocytogenes*, an acid resistant strain was reported to be the most common microorganism present (Jordan et al., 2001). Due to these findings, these three microorganisms were targeted throughout the study. It was observed that there was a loss of bacterial counts on PCA when compared with the zeroth day (Appendix A.3). On the other hand there was an increase in the yeast counts.

The results presented in Table 4.1 and Appendix A.4. stated that when the control and the counts of ultrasound application to the alpine strawberry juice was compared, there was a significant reduction in the logarithmic counts of the total mesophilic aerobic bacteria on PCA agar. The reduction was approximately more

than one and a half log cycles when the treatment of ultrasonication at amplitudes of 60 and 90% for 10 minutes was considered ($p < 0.05$) (Table 4.1). When the logarithmic yeast counts were considered it was greater than the control in the treatment of ultrasonication at an amplitude of 90% for ten-minute, therefore this method was not found to be effective for the reduction of yeast and molds count ($p < 0.05$). Interestingly, there was an unknown inverse relationship between bacterial and yeast and mold counts of alpine strawberry juice.

Table 4.1. The counts of total mesophilic aerobic bacteria (TMAB) and yeast/molds (YM) in the ultrasonicated alpine strawberry juice according to doses of ultrasonication

	TMAB			YM		
Groups	**Mean**	**±**	**Std.**	**Mean**	**±**	**Std.**
Control	2.18^{a}	±	.505	2.75^{a}	±	.075
30%	2.09^{a}	±	.875	3.70^{b}	±	.215
60%	1.05^{b}	±	.047	3.73^{b}	±	.212
90%	$.48^{b}$	±	.660	3.52^{c}	±	.190

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

The results tabulated in Appendix A.5. showed that when the control and each dose of the mustard seed applications to the alpine strawberry juice was compared, there was a significant reduction in the logarithmic counts of the total mesophilic aerobic bacteria on PCA agar ($p < 0.05$). No bacteria was observed during the enumeration, when 0.6 g/L black mustard seed was added to 10 mL of alpine strawberry juice with respect to control ($p < 0.05$). In addition, there was an infinitesimal decrease in the yeast counts with the same amount of black mustard seed treatment, but this decrease was limited to an amount of 0.1 log cycles ($p > 0.05$).

According to the results in Table 4.1., Appendix A.4. and A.5., independently, 0.6 g/L black mustard seed and treatment of ultrasonication at the amplitude of 90% for ten-minute was selected as the best treatment group in the study. The combination treatment was carried out according to these results.

When the combination of 0.6 g/L black mustard seed and ultrasonication at the amplitude of 90% for 10 minutes treatment was considered, it was observed that the total mesophilic aerobic counts of the combination study was found to be undetectable level when compared to the control ($p < 0.05$). The twentieth, fourtieth and the sixtieth day results of the total mesophilic aerobic bacteria were peerly evaluated (Figure 4.1 and Appendix A.6.), and therefore, when compared with the control, it was found to be undetectable level and significantly different ($p < 0.05$). The results of the combination treatments of the twentieth, fourtieth and the sixtieth day results of the black mustard seed and the ultrasonication, as 0.6 g/L amount and 90% amplitude, was found to be very effective on providing the microbial quality of the alpine strawberry juice respectively (Figure 4.1).

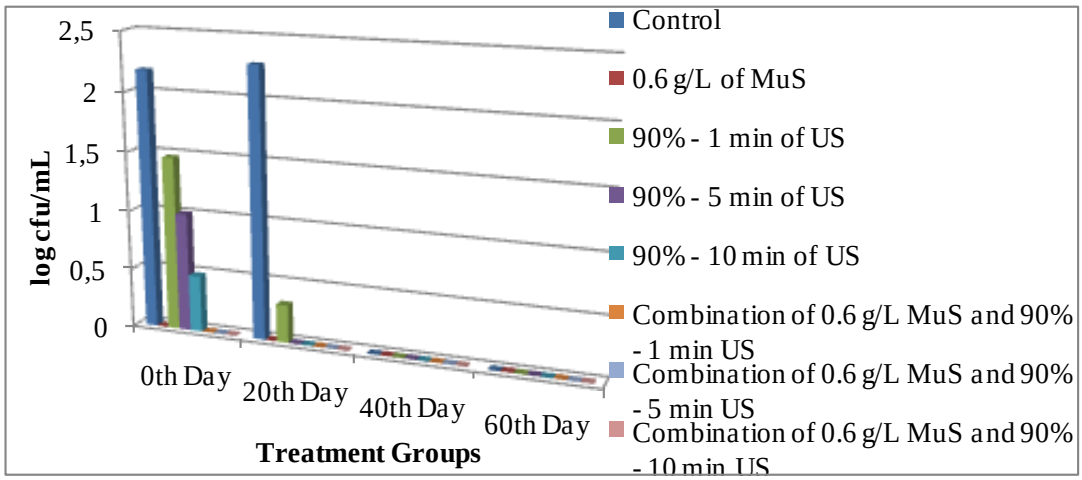

Figure 4.1. The total mesophilic aerobic bacteria counts of the ultrasonicated (90%), black mustard seed (0.6 g/L) added, and the combination of black mustard seed and ultrasonication (90% - 0.6 g/L) treated alpine strawberry juice.

When the yeast counts of the combination of 0.6 g/L black mustard seed and ultrasonication treatment at an amplitude of 90% for ten-minute was considered, there was a significant reduction up to undetectable level in 20^{th}, 40^{th} and 60^{th} days, not only descending from the initial value of approximately 2.81 log colony forming units, obtained in 0^{th} day of the study, but also the difference between the control and the treatment was approximately 4.11 log cycles, indicating a powerful process (Figure 4.2 and Appendix A.6.) ($p < 0.05$). Almost the results were resembling to the total mesophilic aerobic bacteria counts, in case of the combination; in the twentieth, fourtieth and the sixtieth day results of the black mustard seed and the ultrasonication, as 0.6 g/L amount and 90% amplitude. Due to these results the combination treatment was synchronously found to be very effective on the yeast counts as well, other than its success on total mesophilic aerobic bacteria.

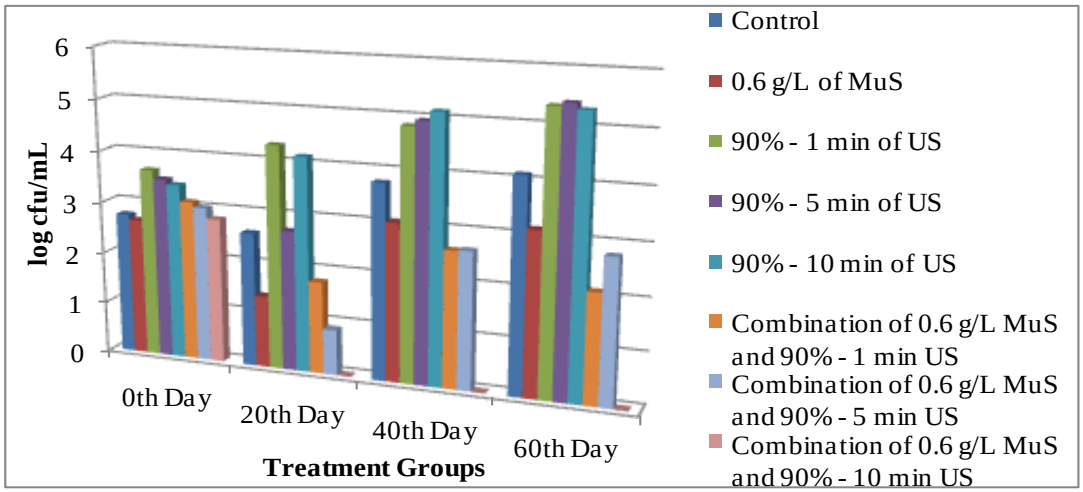

Figure 4.2. The yeast and mold counts of the ultrasonicated (90%), black mustard seed (0.6 g/L) added, and the combination of black mustard seed and ultrasonication (90% - 0.6 g/L) treated alpine strawberry juice.

4.1.2 The Application of Ultrasonication and Mustard Seed against *E. coli* O157:H7 in Alpine Strawberry Juice

The *E. coli* 0157:H7 added alpine strawberry juice was subjected to treatment of ultrasonication with the following amplitudes of 30, 60 and 90%, for a period of one, five and ten minutes (Table 4.2. and Appendix A.7.). When the logarithmic counts of the study was compared with respect to the control, in each amplitude applied, a gradual decrease was observed within the amplitudes of 30, 60 and 90% ultrasonication ($p < 0.05$) (Table 4.2). A gradual insignificant decrease of *E. coli* 0157:H7 was observed within groups while the period of processing time was increased (Appendix A.7) ($p > 0.05$). The treatment of ultrasonication at amplitude of 90% for 10 minutes, was found to be significantly different than control and the most effective group as undetectable level was obtained (Appendix A.7) ($p < 0.05$).

Table 4.2. The average counts of *E. coli* O157:H7 inoculated and ultrasonicated alpine strawberry juice.

Groups	Mean ± Std.
Control	2.95^{a} ± .042
30%	2.40^{a} ± .927
60%	1.23^{b} ± .935
90%	$.52^{b}$ ± .797

Data are means ± SD from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

The results in Appendix A.8. indicated that when black mustard seed (*Brassica nigra*) was added into the *E. coli* 0157:H7 containing alpine strawberry juice, an approximate reduction of one and a half (1.5) log cycle was observed when

the counts of *E. coli* 0157:H7 was considered. No significant difference was seen with respect to the control ($p > 0.05$).

When the combination treatment of 0.6 g/L black mustard seed and treatment of ultrasonication at amplitude of 90% for 10 minutes was considered in *E. coli* 0157:H7 inoculated alpine strawberry juice, it was observed that the *E. coli* 0157:H7 counts were found to be undetectable when compared to the counts of the control and the black mustard seed added group (Appendix A.9, Figure 4.3). From the table presented in Appendix A.9 and Figure 4.3., it was also seen that in the combination case, when the treatment was compared with the sole ultrasonication application, the one minute processing time was also enough to eradicate the *E. coli* 0157:H7 population from the alpine strawberry juice. In five and ten minutes' time no *E. coli* 0157:H7 in the juice was found due to the powerful effect of combination on this severe pathogenic strain and this treatment was found to be significantly different from the control ($p < 0.05$).

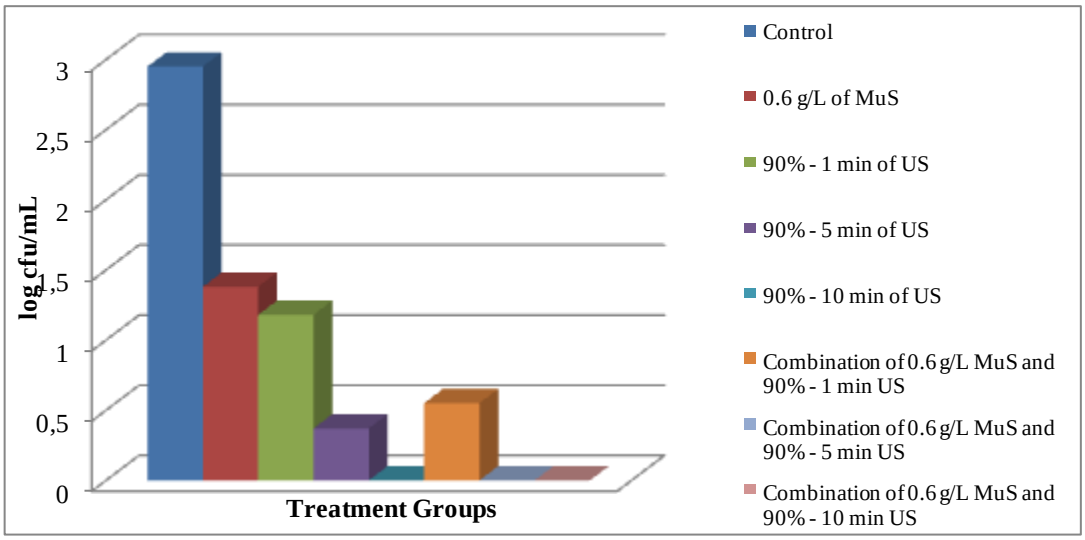

Figure 4.3. The microbial counts of the combination of black mustard (*Brassica nigra*) seed (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min.) *E. coli* 0157:H7 inoculated alpine strawberry juice.

The results of the injured cell determination in the effected population revealed that all the cells in the medium were found to be injured and a 100% injury rate is obtained (Table 4.3.).

Table 4.3. The percent injured cell counts of *E.coli* 0157:H7 after mustard seed treatment

	0.6g/L MuS treatment
Growth on TSA	58
Growth on TSA with 1% NaCI	0
Injured cell	100

4.1.3 The Application of Ultrasonication and Mustard Seed against *Salmonella* spp. in Alpine Strawberry Juice

The *Salmonella* spp. added alpine strawberry juice was subjected to treatment of ultrasonication with the following amplitudes of 30, 60 and 90% for a period of one, five and ten minutes (Appendix A.10, Table 4.4.). When the logarithmic counts of the study was compared with respect to the control, a gradual decrease was observed among the amplitudes of ultrasonication ranging from 30 and 90% (Table 4.4) ($p < 0.05$). Moreover, in each amplitude applied, a gradual insignificant decrease was also observed within groups while the period of processing time was increased with respect to the control (Appendix A.10) ($p > 0.05$). Likewise the results in Figures 4.3 and Table 4.2. of *E. coli* 0157:H7; the 90% amplitude of ultrasonication application for 10 minutes, was found to be the most effective group as undetectable level was obtained. An independent treatment of ultrasonication at amplitude of 90% for 5 minutes was also found to be effective too, due to its undetectable level and in

addition, short time processing was found to be much more feasible when the industrial scale of this process was considered (Figure 4.4, Appendix A.10). As a result, the 90% ultrasonication treatment for 10 minutes revealed a 3.13 log cycle decrease when compared with the control, and was found to be significantly different ($p < 0.05$), indicating that ultrasonication can be proposed to industrial applications as it was found to be very effective on the destruction of *Salmonella* spp., another important foodborne pathogen like *E. coli* 0157:H7 (Figure 4.3.).

Table 4.4. The average counts of *Salmonella* spp. inoculated and ultrasonicated alpine strawberry juice.

Groups	Mean ± Std.
Control	3.13^{a} ± .118
30%	2.73^{a} ± .297
60%	$.86^{b}$ ± .861
90%	$.31^{b}$ ± .611

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

When 0.6 g/L black mustard seed (*Brassica nigra*) was added into the *Salmonella* spp. containing alpine strawberry juice, it was found that an approximate reduction of 0.5 log cycle was obtained when the counts of *Salmonella* spp. was considered (Appendix A.11). The most effective dose of mustard seed was 0.6 g/L ($p < 0.05$). The effects of 0.2 and 0.4 g/L mustard seed were the same ($p > 0.05$) and less than 0.6 g/L mustard seed ($p < 0.05$) (Appendix A.11). When compared with *E. coli* 0157:H7 inoculated-mustard seed added and the *Salmonella* inoculated alpine strawberry juice followed by ultrasonication treatment, it was not found to be too much effective.

When the combination of 0.6 g/L black mustard seed and ultrasonication at an amplitude of 90% for 10 minutes was considered in *Salmonella* spp. inoculated alpine strawberry juice, it was observed that (Figure 4.4.) the *Salmonella* spp. counts were found to be undetectable, compared to the counts of the control and the sole black mustard seed added and ultrasonicated group (Appendix A.12). A significant difference was observed between the control, 0.6 g/L of mustard seed and the combination of 0.6 g/L black mustard seed and the ten-minute treatment of 90% amplitude of ultrasonication ($p < 0.05$). The results were almost in line with the *E. coli* 0157:H7 treatment of the alpine strawberry juice, as ten minutes' ultrasonic treatment was effectively able to kill the population of *Salmonella* spp. either solely or in combination with the black mustard seed (*Brassica nigra*).

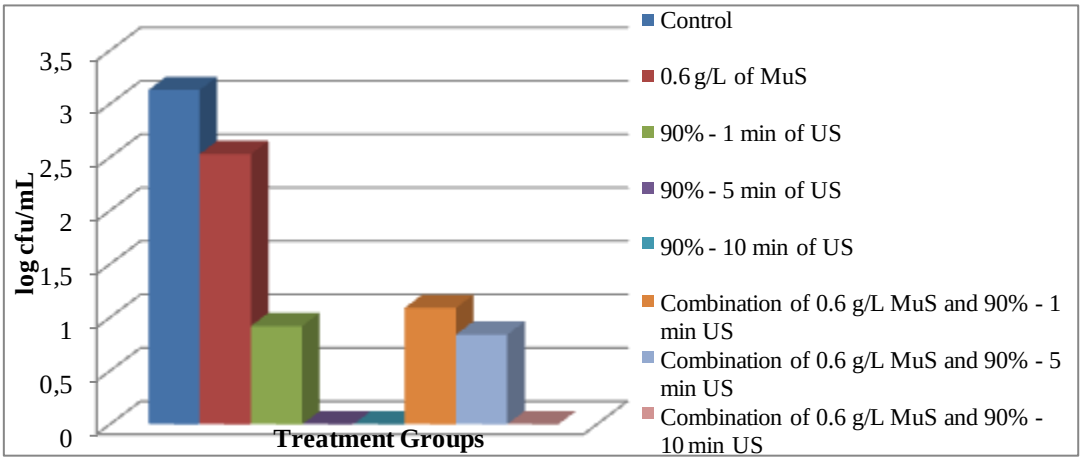

Figure 4.4. The microbial counts of the combination of black mustard (*Brassica nigra*) seed (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min.) *Salmonella* spp. inoculated alpine strawberry juice.

The results of the injured cell determination in the effected population of *Salmonella* spp. revealed that all of the cells in the medium were found to be injured and a 100% injury rate is obtained, as similarly in the case of *E. coli* 0157:H7 added alpine strawberry juices (Table 4.5.).

Table 4.5. The percent injured cell counts of *Salmonella* spp. after mustard seed treatment

	0.6g/L MuS treatment
Growth on TSA	409
Growth on TSA with 4% NaCI	0
Injured cell	100

4.1.4 The Application of Ultrasonication and Mustard Seed against *Listeria monocytogenes* in Alpine Strawberry Juice

The *L. monocytogenes* added alpine strawberry juice was subjected to treatment of ultrasonication with the following amplitudes of 30, 60 and 90%, for a period of one, five and ten minutes (Appendix A.13). The effect of 30% and 60% amplitudes of ultrasonication treatment was almost the same with the control but in fact, less than treatment of ultrasonication at amplitude of 90% (Table 4.6) ($p < 0.05$). When the logarithmic counts of the study was compared with respect to the control, in each amplitude applied, a gradual insignificant decrease was observed within groups ($p > 0.05$) while the period of processing time was increased except the treatment of ultrasonication at amplitude of 90% for 10 min. This was up to a maximum amount of approximately 3.5 log cycles (Appendix A.13) ($p < 0.05$). *L. monocytogenes* was found to be more resistant to ultrasonication processing than *Salmonella* and *E. coli* 0157:H7. The treatment of ultrasonication at the amplitude of 90% for 10 minutes, was found to be the most effective group as similarly with the other experiments in this study, and it was supported by the statistical difference when compared with the control ($p < 0.05$).

Table 4.6. The average counts of *Listeria monocytogenes* inoculated and ultrasonicated alpine strawberry juice.

Groups	Mean ± Std.
Control	3.34^{a} ± .118
30%	3.29^{a} ± .188
60%	2.75^{a} ± .178
90%	1.93^{b} ± 1.127

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

When 0.6 g/L black mustard seed (*Brassica nigra*) was added into the *L. monocytogenes* containing alpine strawberry juice, it was found that an approximate significant reduction of more than one and a half (1.5) log cycle was obtained (Appendix A.14) ($p < 0.05$). The effects of 0.4 g/L and 0.6 g/L of mustard seed against *L. monocytogenes* were the same. No significant difference was observed in the 0.2 g/L of mustard seed with respect to the control ($p > 0.05$).

If the counts of *Salmonella* spp. was considered, the effect of mustard seed was found to be more effective against *Listeria monocytogenes* ($p < 0.05$). When compared with *E. coli* 0157:H7 inoculated-mustard seed added alpine strawberry juice it was found to be almost similar but statistically insignificant ($p > 0.05$).

When the combination of 0.6 g/L black mustard seed and ultrasonication at an amplitude of 90% for ten-minute was considered in *L. monocytogenes* inoculated alpine strawberry juice, it was observed that the *L. monocytogenes* counts were found to be decreased approximately three log cycles when compared to the counts of the control ($p < 0.05$) and two log cycles with the black mustard seed added group ($p > 0.05$) respectively (Appendix A.15). The combination of 0.6 g/L black mustard seed and ultrasonication at an amplitude of 90% for 10 minute was the best one among all

of the treatments to eradicate this important pathogen from alpine strawberry juice ($p > 0.05$) (Appendix A.15).

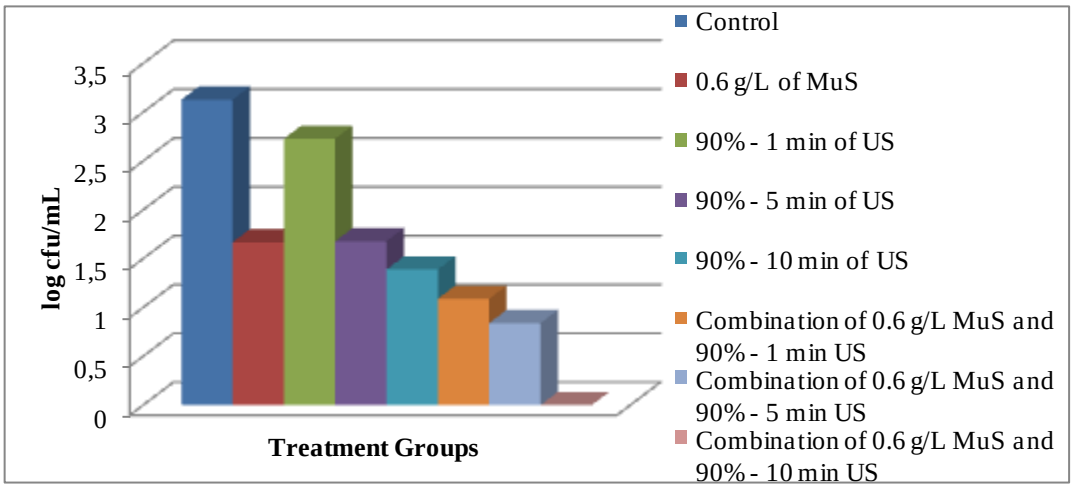

Figure 4.5. The microbial counts of the combination of black mustard (*Brassica nigra*) seed (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min.) *Listeria monocytogenes* inoculated alpine strawberry juice.

As seen in Table 4.7. the results of the injured cell determination for the effected population of *L. monocytogenes* stated that a rate of 75% of the cells in the medium were found to be injured, different than the case of *E. coli* 0157:H7 and *Salmonella* spp.

Table 4.7. The percent injured cell counts of *Listeria monocytogenes* after mustard seed treatment

	0.6g/L MuS treatment
Growth on TSA	201
Growth on TSA with 4% NaCI	50
Injured cell (%)	75

4.1.5 The Results of Physical and Chemical Analysis of Alpine Strawberry Juice

The pH of the alpine strawberry juice was found to be 3.63, 3.63, 3.63, 3.62, 3.66, 3.66, 3.66, 3.66, 3.66, and 3.66, for the control, 0.2, 0.4 and 0.6 g/L of black mustard seed, 90% – 1 min., 90% – 5 min., and 90% – 10 min. of sole ultrasonication treatments, and the combination treatments of 0.6 g/L black mustard seed with 90% – 1 min., 90% – 5 min., and 90% – 10 min. of ultrasonication respectively (Table 4.8).

Table 4.8. The physical and chemical properties of nontreated and treated alpine strawberry juices

	Control	**0.6 g/L of MuS**	**90% - 10 min**	**Combination**	**P**
pH	3.63 ± .000	3.62 ± .000	3.66 ± .000	3.66 ± .000	.072
Total Soluble Solids (Brix)	6.00 ± .000	6.50 ± .000	6.50 ± .000	6.50 ± .000	.072
Titratable Acidity	.640 ± .007	.614 ± .000	.620 ± .008	.630 ± .007	.118
Total Sugar Content (g/mL)	2.51 ± .000	2.36 ± .000	2.41 ± .000	2.36 ± .000	.072
Viscosity (mPa.s)	88.15 ± 2.616	101.8 ± 10.182	78.00 ± .000	66.55 ± 6.576	.080
L*	32.20 ± .028	32.18 ± .000	32.92 ± .014	33.48 ± .000	.087
a*	21.92 ± .057	22.27 ± .028	20.98 ± .000	21.19 ± .042	.080
b*	8.98 ± .028	8.89 ± .014	9.80 ± .028	10.11 ± .014	.083

Data are means ± Std. from two replicates

*$p < 0.05$

According to Table 4.8. a slight insignificant increase was found to be present in the selected treatment groups of total soluble solid content of the alpine strawberry juices with respect to the control ($p > 0.05$). This might be due to the release of possible antimicrobial substances to the alpine strawberry pulp, by the help of ultrasonication, and the mustard seed treatments, either in combination or sole case. When the titratable acidity and the total sugar content assays were considered, a

slight insignificant decrease was observed in all of the treatment groups with respect to control ($p > 0.05$). The viscosity was said to be insignificantly increased due to 0.6 g/L black mustard seed treatment ($p > 0.05$) but the other selected groups showed insignificant decrease when compared with the control ($p > 0.05$). The lightness/darkness (L*) value in color assay results in all treatment groups were found to be almost the same in the treatment groups with respect to the control, whereas a* (red/green) value was found to be the same in all treatment groups, and b* (blue/yellow) value was found to be slightly and gradually increased in all treatment groups with respect to the control, but these results were insignificant ($p > 0.05$). From the statistical point of view, there were no significant changes present in the main physical and chemical properties of nontreated and treated alpine strawberry juices ($p > 0.05$).

The treatment groups in the physical and chemical assays were selected due to their effectiveness in microbial assays.

4.1.6 The Sensorial Properties of Alpine Strawberry Juice

Thirty panellists rated the alpine strawbery juice in the taste panel according to the criteria of color, sweetness, sourness, odor, appearance, flavor, bitterness and overall evaluation with a 10 point scale per replication. A score of 10 represented the most liked, and 1 corresponded to least liked (Table 4.9). The ages of 60% of panelists were found to be greater than twenty and 40% of panelists were twenty and lower than twenty. 80% of panelists were female and 20% were male. 13.3% of panelists were smokers and 86% were nonsmokers. 96.7% of panelists were student and 3% were research assistant (Table 4.9). According to Table 4.9, the scores of all

of the treatment groups of alpine strawberry juice were almost the same. There were no extreme results between the treatment groups of alpine strawberry juice.

Table 4.9. The sensory parameters of nontreated and treated alpine strawberry juices

	Control	0.6 g/L of MuS	90% - 10 min	Combination	Total
Color	8.40 ± .814	8.23 ± .956	7.90 ± .900	7.97 ± .999	8.13 ± .931
Sweetness	8.63 ± .809	8.23 ± .762	8.07 ± .998	8.10 ± .662	8.26 ± .835
Sourness	1.10 ± .305	1.65 ± .661	1.41 ± .825	1.57 ± .817	1.43 ± .707
Odor	9.07 ± .785	8.16 ± .820	8.07 ± 1.033	8.20 ± .714	8.38 ± .926
Appearance	8.63 ± .718	8.52 ± .769	7.90 ± .900	8.27 ± .828	8.33 ± .843
Flavor	8.70 ± .952	8.10 ± 1.012	7.93 ± 1.033	8.27 ± .640	8.25 ± .955
Bitterness	1.00 ± .000	1.19 ± .402	1.03 ± .186	1.10 ± .403	1.08 ± .306
Overall	8.63 ± .718	8.19 ± .910	8.07 ± .842	8.20 ± .761	8.28 ± .830

Data are means ± Std.

4.2 The Application of Ultrasonication and Mustard Seed in The Blackberry Juices

4.2.1 The Microflora of Blackberry Juices

When the microbial counts were considered, yeast and mold were determined in the native microflora of blackberry juices in yeast extract agar. No growth was recorded in PCA, blood, EMB, XLD and BAT (Ali) agar (Appendix A.16).

It was observed that (Appendix A.16.) there were no total mesophilic aerobic bacteria on PCA when compared between the zeroth and the sixtieth day interval. On the other hand there was an increase in the yeast and mold counts and it was gradual up to the fourtieth day. Between fourtieth and the sixtieth days the increase in the yeast counts was almost stabilized.

When the control and the ultrasound application to the blackberry juice was compared, there was no difference for the total mesophilic aerobic counts on PCA

agar among the control and the treatment, as since no bacterium was recorded to be grown in both groups (Table 4.10, Appendix A.17). A slight reduction was observed at the one minute treatment of the sonication when compared with the control but interestingly five and ten minutes' treatment increased the yeast content of the ultrasonicated blackberry juices. There was not so much difference for the yeast counts between the ten-minute ultrasonication group and the control (Table 4.10, Appendix A.17). No statistical difference was observed for all of the treatments ($p > 0.05$).

Table 4.10 The average counts of TMAB and YM in the ultrasonicated blackberry juice.

	TMAB	YM
Groups	**Mean ± Std.**	**Mean ± Std.**
Control	ND	2.66^{a} ± .299
30%	ND	1.70^{a} ± 1.046
60%	ND	1.51^{a} ± 1.170
90%	ND	1.39^{a} ± 1.140

Data are means ± Std. from three replicates
ND refers not detected
Values followed by different letters in the same column are significantly different ($P < 0.05$).

When the control and the mustard seed application to the blackberry juices were compared, there were no total mesophilic aerobic bacteria on PCA agar in each case. Probably this was due to the antimicrobial compounds present in the blackberry juice, leading to a preliminary inhibition of the bacteria. No significant difference was observed between treatments (Appendix A.18). In addition, there was a statistically supported significant decrease in the yeast counts with the same amount of black mustard seed treatment, an initial value of 2.66 log cycles, reaching up to

undetectable level at the end ($p < 0.05$) (Appendix A.18). The gradual increase of the amount of the black mustard seed showed a gradual decrease in the counts of yeast and molds ($p < 0.05$) (Appendix A.18).

When the combination of 0.6 g/L black mustard seed and ultrasonication at an amplitude of 90% for 10 minutes was considered, it was observed that the total mesophilic aerobic count values of the combination study was found to be undetectable in 20^{th}, 40^{th} and 60^{th} days when compared to the counts of the control in the zeroth day which was also undetectable (Figure 4.6., Appendix A.19). These results indicated that certain antimicrobial compounds present in the blackberry juice could be able to inhibit the total mesophilic aerobic bacteria in addition to ultrasonication and the black mustard seed, which would not possibly permit an extra contamination or growth. So, they were not found to be significantly different. When the yeast counts was considered, there was a significant reduction up to undetectable level from a value of 5.54 log cycle for 0.6 g/L mustard seed treatment and the combined treatment of 0.6 g/L mustard seed and 90%-one minute ultrasonication on 60^{th} day (Figure 4.7.) (Appendix A.19). These results were found to be significantly different ($p < 0.05$). Combinations of each processing time of ultrasonication with 0.6 g/L of mustard seed was more effective than the sole treatments of ultrasonication ($p < 0.05$) (Appendix A.19).

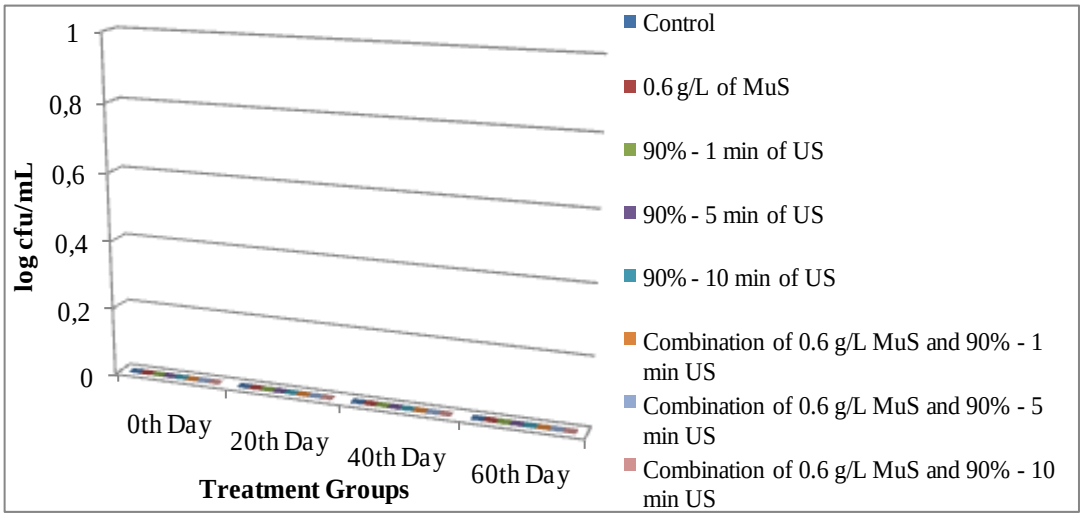

Figure 4.6. The total mesophilic aerobic bacteria counts of the ultrasonicated (90%), black mustard (*Brassica nigra*) seed (0.6 g/L) added, and the combination of black mustard (*Brassica nigra*) seed and ultrasonication (90% - 0.6 g/L) treated blackberry juice.

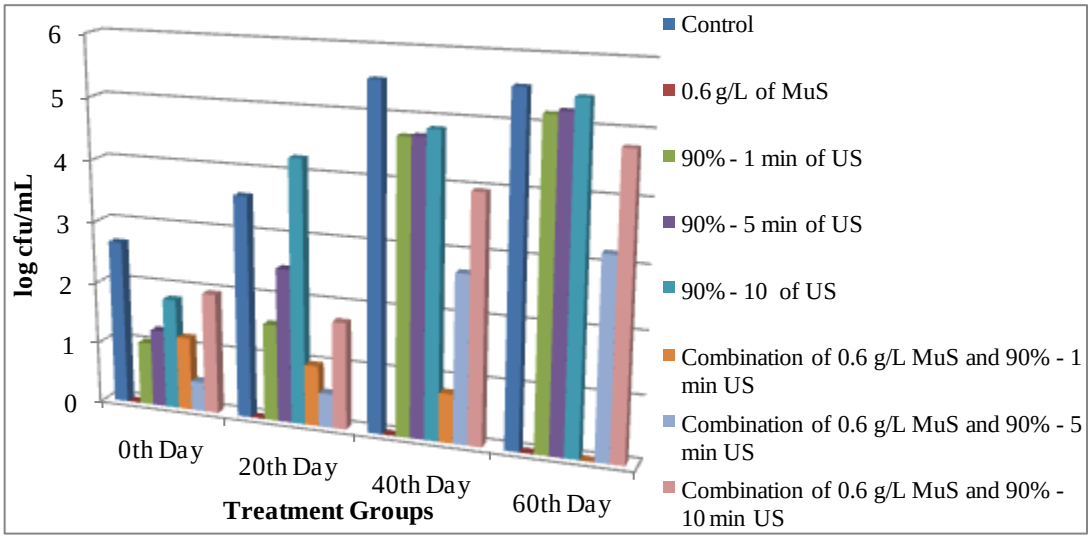

Figure 4.7. The yeast and mold counts of the ultrasonicated (90%), black mustard seed (0.6 g/L) added, and the combination of black mustard (*Brassica nigra*) seed and ultrasonication (90% - 0.6 g/L) treated blackberry juice.

4.2.2 The Application of Ultrasonication and Mustard Seed against *E. coli* O157:H7 in Blackberry Juice

The *E. coli* 0157:H7 added blackberry juice was exposed to ultrasonication with the following amplitudes of 30, 60 and 90%, for a period of one, five and ten minutes (Appendix A.20). When the logarithmic counts of the study was compared with respect to the control, a gradual decrease was observed when amplitude of ultrasonication was increased (Table 4.11) ($p < 0.05$). Most commonly, a gradual insignificant decrease was observed within groups ($p > 0.05$) while the period of processing time was increased, especially in 10 minutes' time (Appendix A.20). Likewise the other groups, the 90% amplitude of ultrasonication application for 10 minutes, was found to be the most effective group as undetectable levels of microbial counts were obtained (Appendix A.20). The significant reduction was found to be approximately three log cycles ($p < 0.05$). Almost the same result was obtained in the *E. coli* 0157:H7 added alpine strawberry juice (Table 4.2, Appendix A.7.) with a reduction of 2.95 log cycles ($p < 0.05$).

Table 4.11. The average counts of *E.coli* O157:H7 inoculated and ultrasonicated blackberry juice, according to doses of ultrasonication.

Groups	Mean ± Std.
Control	2.94^{a} ± .060
30%	1.54^{b} ± .992
60%	$.42^{c}$ ± .722
90%	$.05^{c}$ ± .160

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

When black mustard seed (*Brassica nigra*) was added into the *E. coli* 0157:H7 containing blackberry juice, it was found that a maximum approximate reduction of 2.94 log cycle was obtained when the control counts of *E. coli* 0157:H7 was considered ($p < 0.05$). No *E. coli* 0157:H7 was present in 0.006 g/mL black mustard seed treated blackberry juice and there was a significant difference between control and the treatment groups (Appendix A.21.) ($p < 0.05$). The antibacterial effects of 0.2, 0.4, and 0.6 g/L mustard seed were significantly found to be almost the same ($p < 0.05$).

When the combination of 0.6 g/L black mustard seed and ultrasonication at an amplitude of 90% for 10 minutes was considered in *E. coli* 0157:H7 inoculated blackberry juice, it was observed that, except the sole 90% - one minute ultrasonication processing, almost all of the *E. coli* counts were found to be undetectable (Appendix A.22, Figure 4.8.) when compared to the counts of the control ($p < 0.05$). It was also clear that in the combination case, when the treatment was compared with the sole ultrasonication application, the five minutes' processing time was also found to be enough to eradicate the *E. coli* population from the blackberry juice (Appendix A.22). In one, five and ten minutes' processing time there was no *E. coli* 0157:H7 in the juice due to the powerful effect of combination on this extremely pathogenic strain. Statistically these results were found to be significantly different than control ($p < 0.05$), but not than the sole treatments of mustard seed and ultrasonication at amplitude of 90% ($p > 0.05$).

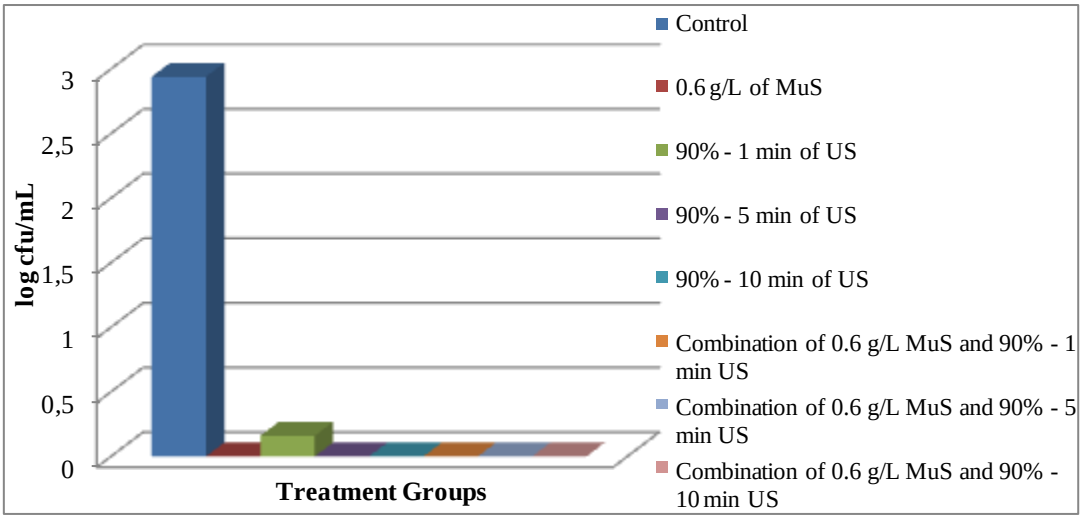

Figure 4.8. The microbial counts of the combination of black mustard (*Brassica nigra*) seed added (0.6 g/L) and ultrasonicated (90%, 1, 5, and 10 min.) *E. coli* 0157:H7 inoculated blackberry juice.

The results of the injured cell determination in the effected population revealed that all of the cells in the medium were found to be injured and a 100% rate of injury was obtained (Table 4.12).

Table 4.12. The percent injured cell counts of *E.coli* 0157:H7 after mustard seed treatment

	0.6g/L MuS treatment
Growth on TSA	233
Growth on TSA with 1% NaCI	0
Injured cell (%)	100

4.2.3 The Application of Ultrasonication and Mustard Seed against *Salmonella* spp. in Blackberry Juice

The *Salmonella* spp. added blackberry juice was subjected to treatment of ultrasonication with the following amplitudes of 30, 60 and 90%, for a period of one,

five and ten minutes (Table 4.13, Appendix A.23.). When the logarithmic counts of the study was compared with respect to the control, a gradual decrease was observed when amplitudes of ultrasonication were increased (Table 4.13) ($p < 0.05$). The most effective amplitude of ultrasonication was found to be 90% ($p < 0.05$) (Table 4.13). A gradual insignificant decrease was observed within groups ($p > 0.05$) while the period of processing time was increased (Appendix A.23). Likewise the other groups; the application of ultrasonication at amplitude of 90% for 10 minutes, was found to be the most effective group as undetectable level was obtained and significantly different from the control ($p < 0.05$) (Appendix A.23). At the same amplitude, a five minutes' treatment was also found to be effective and much more feasible when industrial scale of this process was considered and statistically, significantly different from the control too. As a result, the treatments of ultrasonication at an amplitude of 90% for five and ten minutes' revealed an approximate value of three log cycle decrease when compared with the control ($p < 0.05$) and this shows that ultrasonication can be proposed to industrial applications as it was found to be very effective on the destruction of *Salmonella* spp., another important foodborne pathogen similar to the *E. coli* 0157:H7 case (Appendix A.23).

Table 4.13. The average counts of *Salmonella* spp. inoculated and ultrasonicated blackberry juice.

Groups	Mean ± Std.
Control	2.85^{a} ± .289
30%	2.51^{a} ± .363
60%	$.85^{b}$ ± .831
90%	$.26^{c}$ ± .549

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

When 0.6 g/L black mustard seed (*Brassica nigra*) was added into the *Salmonella* spp. containing blackberry juice, it was found that an approximate reduction of 2.85 log cycle was obtained, compared with the control (Appendix A.24) ($p < 0.05$). The effects of 0.2, 0.4, and 0.6 g/L doses of mustard seed were almost the same against *Salmonella* spp. inoculated blackberry juice and undetectable levels were observed in statistical means ($p < 0.05$) (Appendix A.24).

When the combination of black mustard seed and ultrasonication at the amplitude of 90% for 10 minutes was considered in *Salmonella* spp. inoculated blackberry juice, it was observed that (Figure. 4.9., Appendix A.25) almost all of the *Salmonella* spp. counts were found to be undetectable when compared to the counts of the control and the 90% - 1 minute ultrasonication treatment (Appendix A.25). The results were almost in line with the *E. coli* 0157:H7 treatment of the blackberry juice (Figure 4.8), as since ten minutes' ultrasonic treatment was effectively able to kill the population of *Salmonella* spp. as solely or in combination with the black mustard seed (*Brassica nigra*).

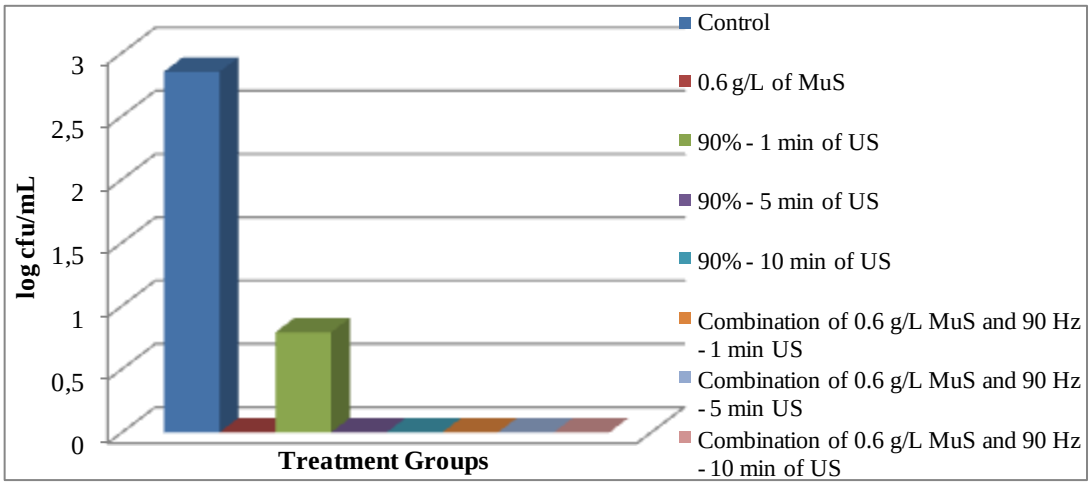

Figure 4.9. The microbial counts of the combination of black mustard (*Brassica nigra*) seed (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min.) *Salmonella* spp. inoculated blackberry juice.

The results of the injured cell determination in the *Salmonella* spp. added blackberry juice revealed that all of the cells in the medium were found to be injured and a 100% injury rate is obtained (Table 4.14).

Table 4.14. The percent injured cell counts of *Salmonella* spp. after mustard seed treatment

	0.6g/L MuS treatment
Growth on TSA	7
Growth on TSA with 4% NaCI	0
Injured cell (%)	100

4.2.4 The Application of Ultrasonication and Mustard Seed against *Listeria monocytogenes* in Blackberry Juice

The blackberry juice inoculated with *L. monocytogenes*, was subjected to treatment of ultrasonication with the following amplitudes of 30, 60 and 90%, for a period of one, five and ten minutes (Appendix A.26) (Table 4.15). When the logarithmic counts of the study was compared with respect to the control, a gradual decrease was observed while the amplitudes of ultrasonication were increased (Table 4.15) ($p < 0.05$). A gradual insignificant decrease was observed within groups ($p > 0.05$) while the period of processing time was increased (Appendix A.26). The control showed 1.43 log cycle, whereas undetectable level was obtained in all 90% ultrasonication treatment of the blackberry juice. Consequently the sole ultrasonication treatment was found to be effective (Appendix A.26). The average counts of *L. monocytogenes* inoculated and ultrasonicated blackberry juice with the amplitude of 60% and 90%, and the processing times was found to be significantly

different from control ($p < 0.05$) (Table 4.15, Appendix A.26). This reduction was found to be important as due to the resistant nature of the gram positive *Listeria* spp. with its thick peptidoglycan structure.

Table 4.15. The average counts of *Listeria monocytogenes* inoculated and ultrasonicated blackberry juice.

Groups	Mean ± Std.
Control	1.43^{a} ± 1.243
30%	1.30^{a} ± .988
60%	$.45^{b}$ ± .692
90%	ND

Data are means ± Std. from three replicates
ND refers not detected
Values followed by different letters in the same column are significantly different ($P < 0.05$).

When 0.6 g/L black mustard seed (*Brassica nigra*) was added into the *L. monocytogenes* containing blackberry juice, it was found that an approximate reduction of 1.43 log cycle was obtained with respect to control (Appendix A.27) ($p < 0.05$). If the counts of mustard seed added *L. monocytogenes* inoculated blackberry juice was compared with the sole mustard seed added one containing *Salmonella* spp. (Appendix A.24), the effect of mustard seed was found to be as much as powerful on *L. monocytogenes*. It was also in line with the results presented in Appendix A.21 reflecting the *E. coli* 0157:H7 inoculated-mustard seed added group.

Both sole black mustard seed addition with an amount of 0.6 g/L, sole ultrasonication at amplitude of 90% and combination treatment of the black mustard seed and ultrasonication was found to be successful when compared with the control value of 1.43 log cycle (Figure 4.10., Appendix A.28). Undetectable level was obtained in all groups and the combination too, so *L. monocytogenes* cannot

withstand to all of these effective treatments, as well as the low pH and antimicrobials in blackberry juices even though it has a thick cell wall due to high peptidoglycan content (Figure 4.10, Appendix A.28). All treatments were found to be significantly different from control ($p < 0.05$).

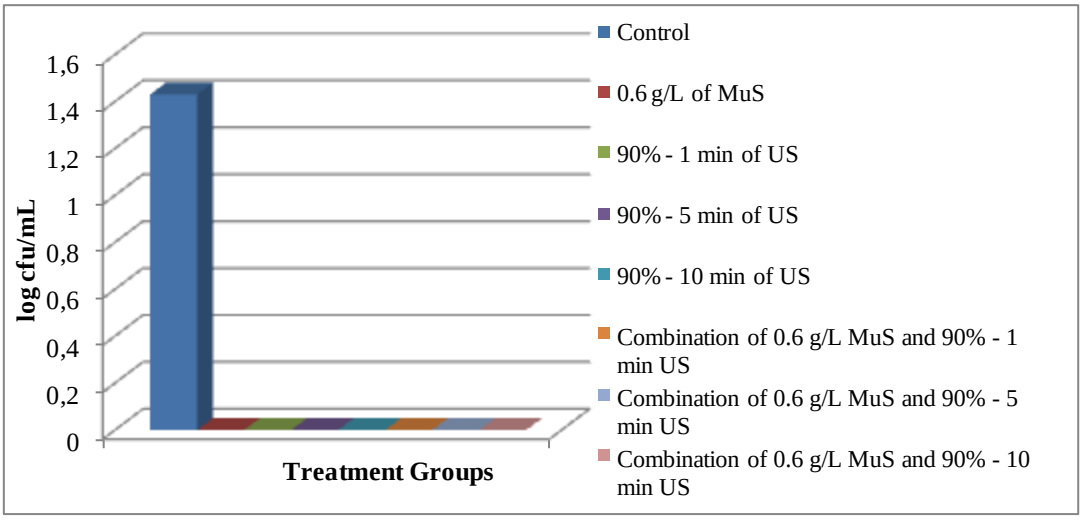

Figure 4.10. The microbial counts of the combination of black mustard (*Brassica nigra*) seed (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min.) *Listeria monocytogenes* inoculated blackberry juice.

The results of the injured cell determination in the effected population revealed that all the cells in the medium were found to be injured and a 100% injury rate was obtained for *L. monocytogenes* (Table 4.16).

Table 4.16. The percent injured cell counts of *Listeria monocytogenes* after mustard seed treatment

	0.6g/L MuS treatment
Growth on TSA	2
Growth on TSA with 4% NaCl	0
Injured cell (%)	100

4.2.5. The Results of Physical and Chemical Analysis of Blackberry Juice

The pH of the blackberry juice was found to be 3.12, 3.11, 3.10, 3.09, 3.09, 3.09, 3,09, 3.10, 3.10 and 3.09 for the control, 0.2, 0.4 and 0.6 g/L of mustard seed, 90% – 1 min., 90% – 5 min., and 90% – 10 min. of sole ultrasonication treatments, and the combination treatments of 0.6 g/L mustard seed with 90% – 1 min., 90% – 5 min., and 90% – 10 min. of ultrasonication respectively (Table 4.17) ($p > 0.05$). It was found to be low when compared to the alpine strawberry juice, therefore the low pH assists the inhibitory potential of not only the total mesophilic aerobic bacteria, yeast and mold species, but also, particularly *L. monocytogenes, E. coli* 0157:H7 *and Salmonella* spp.

Table 4.17. The results of the physical and chemical properties of nontreated and treated blackberry juices

	Control	**0.6 g/L of MuS**	**90% - 10 min**	**Combination**	**p**
pH	3.12 ± .000	3.09 ± .000	3.09 ± .000	3.09 ± .000	.072
Total Soluble Solids (Brix%)	6.00 ± .000	6.50 ± .000	6.50 ± .000	6.50 ± .000	.072
Titratable Acidity	1.095 ± .028	.998 ± .000	1.075 ± .000	1.037 ± .000	.084
Total Sugar Content (g/mL)	1.83 ± .000	1.78 ± .000	1.25 ± .000	1.39 ± .000	.072
Viscosity (mPa.s)	55.05 ± 2.475	62.20 ± 2.546	52.70 ± .283	46.70 ± 1.838	.083
L*	22.54 ± .000	22.47 ± .014	23.00 ± .000	23.23 ± .000	.075
a*	11.48 ± .085	1.09 ± .014	13.47 ± .057	14.00 ± .028	.083
b*	4.25 ± .014	3.93 ± .014	4.57 ± .042	4.94 ± .028	.083

Data are means ± Std. from two replicates.
*$p < 0.05$

A slight insignificant increase was found to be present in the selected treatment groups of total soluble solid content of the blackberry juices (Table 4.17) with respect to the control, likewise the case of alpine strawberry (Table 4.8) ($p >$

0.05). This might be due to the release of possible antimicrobial substances or some other compounds like fibers etc. to the blackberry pulp by the help of ultrasonication, and the mustard seed treatments, either in combination or sole case. When the titratable acidity assay was considered, a slight decrease was observed in the treatment groups with respect to the control ($p > 0.05$). Total sugar content was slightly decreased in the treatment groups except the control ($p > 0.05$). The viscosity was said to be increased due to 0.6 g/L black mustard seed treatment but the other selected groups showed a decrease with respect to the control ($p > 0.05$). The lightness/darkness (L*) value in color assay results in all treatment groups were found to be almost the same in the treatment groups with respect to the control, whereas a* (red/green) value was found to be the same in all treatment groups, except in the case of a decline, observed in 0.6 g/L black mustard seed addition, with respect to the control of blackberry juice, and b* (blue/yellow) value was found to be the same in all treatment groups with respect to the control (Table 4.17) ($p > 0.05$). In general, no detrimental effect of the treatments on the physical and chemical quality of the blackberry juice was observed.

The selection of the treatment groups in the physical and chemical assays were done due to their effectiveness in microbial assays.

4.2.6 The Sensorial Properties of Blackberry Juice

Thirty panellists rated the blackberry juice in the taste panel according to the criteria of color, sweetness, sourness, odor, appearance, flavor, bitterness and overall evaluation with a 10 point scale per replication (Table 4.18). A score of 10 represented most liked, and 1 corresponded to least liked. The ages of 60% of the

panelists were found to be greater than twenty and 40% of panelists were twenty and lower than twenty. 70% of panelists were female and 30% were male. 20% of panelists were smokers and 80% were nonsmokers. 96.7% of panelists were student and 3% were found to be research assistant. According to Table 4.18, the scores of all of the treatment groups of blackberry juice were almost the same ($p > 0.05$). There were no extreme results between the treatment groups of blackberry juice.

Table 4.18. The Sensorial parameters of nontreated and treated blackberry juices

	Control	0.6 g/L of MuS	90% - 10 min of US	Combination	Total
Color	9.10 ± .759	9.13 ± .819	8.97 ± .89	8.97 ± .890	9.04 ± .834
Sweetness	7.13 ± 1.008	6.87 ± .819	7.13 ± 1.008	7.00 ± .788	7.03 ± .907
Sourness	2.17 ± 1.206	2.20 ± 1.126	2.17 ± 1.206	2.17 ± 1.117	2.18 ± 1.150
Odor	6.63 ± 1.245	6.50 ± 1.408	6.63 ± 1.273	6.63 ± 1.299	6.60 ± 1.293
Appearance	9.03 ± .669	8.97 ± .928	8.87 ± .776	8.93 ± .740	8.95 ± .776
Flavor	7.73 ± 1.202	7.67 ± 1.093	7.73 ± 1.202	7.80 ± .997	7.73 ± 1.113
Bitterness	1.03 ± .183	1.07 ± .254	1.03 ± .183	1.07 ± .254	1.05 ± .219
Overall	7.83 ± 1.117	7.67 ± 1.155	7.87 ± 1.137	7.77 ± 1.040	7.78 ± 1.101

Data are means ± Std.

4.3 The Application of Ultrasonication and Mustard Seed to Apple Juice

4.3.1 The Microbial Analysis of Apple Juice

The microflora of apple juice was found to be consisted of *Staphylococcus aureus* (*S. aureus*), gram positive cocci, and certain yeast and mold species in blood, and yeast extract (YEA) agars, respectively. No growth of *Salmonella* in XLD and *Alicyclobacillus* spp. in BAT (Ali) agar was recorded. All the other microbial counts were tabulated below (Table 4.19) and presented in detail in Appendix A.29.

It was observed that there was an increase of total mesophilic aerobic bacterial counts on PCA when compared with the zeroth day (Appendix A.29).

Table 4.19. The average counts of TMAB and YM in the ultrasonicated apple juice.

	TMAB			YM		
Groups	**Mean**	**±**	**Std.**	**Mean**	**±**	**Std.**
Control	2.51[a]	±	.433	3.36[a]	±	.683
30%	2.40[a]	±	.444	3.17[a]	±	.672
60%	1.88[a]	±	1.073	2.67[a]	±	.747
90%	1.55[a]	±	1.173	1.54[b]	±	1.207

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

The results presented in Table 4.19 and Appendix A.30 stated that when the control and the counts of ultrasound application to the apple juice was compared, there was an insignificant reduction in the logarithmic counts of the total mesophilic aerobic bacteria on PCA agar ($p > 0.05$). The reduction was approximately only one log cycles when a ten-minute treatment of 90% amplitude of ultrasonication was considered ($p > 0.05$) (Appendix A.30). When the yeast counts were considered it was lower than the control in a ten-minute treatment of 90% amplitude of ultrasonication, therefore that dose of ultrasonication was found to be effective for yeast ($p < 0.05$) (Appendix A.30). Also undetectable levels were obtained at the end of 60 days'period (Appendix A.29) due to the presence of the enhanced acid-resistant bacteria which might synchronously dominate the yeast and mold. An approximate 2.5 log cycle decrease was observed with respect to the amplitude of 90% ultrasonic treatment for ten minutes, but it was not found to be significantly different (Appendix A.30).

The results tabulated in Appendix A.31 showed that when the control and the mustard seed application to the apple juice was compared, there was an insignificant reduction in the logarithmic counts of the total mesophilic aerobic bacteria on plate count agar ($p > 0.05$). In addition, there was a sharp decrease in the yeast counts with the same amount of black mustard seed treatment, approximately about three log cycles and this decrease was found to be significantly different in statistical means ($p < 0.05$) (Appendix A.31). All doses of mustard seed treatments had the same effect against yeast and molds ($p > 0.05$) (Appendix A.31).

When the combination of 0.6 g/L black mustard seed and ultrasonication at the amplitude of 90% for 10 minutes was considered, it was observed that the yeast and mold counts of the combination study was found to be almost undetectable when compared to the counts of the control in the zeroth and the twentieth days (Figure 4.11 and Appendix A. 32). The results of the combination treatments of the fourtieth and the sixtieth day of the black mustard seed and the ultrasonication, was found to be very effective on destructing the yeast and molds of the apple juice respectively ($p < 0.05$). When the total mesophilic aerobic counts was considered, there was an increase up to a value of 5.54 for control group in 60^{th} day, slightly different from the value of 5.07 log colony forming units, obtained in 60^{th} day of the study for the combination of 90% - 10 minutes ultrasonication and 0,6 g/L mustard seed treatment, but this was not significant ($p > 0.05$). Only the 90% - one minute ultrasonication treatment, unexpectedly, was found to be the most effective treatment in general (Figure 4.12 and Appendix A.32). Due to these results the combination treatment was found to be very effective on the yeast counts as well, other than its success on total mesophilic aerobic bacteria should be questioned.

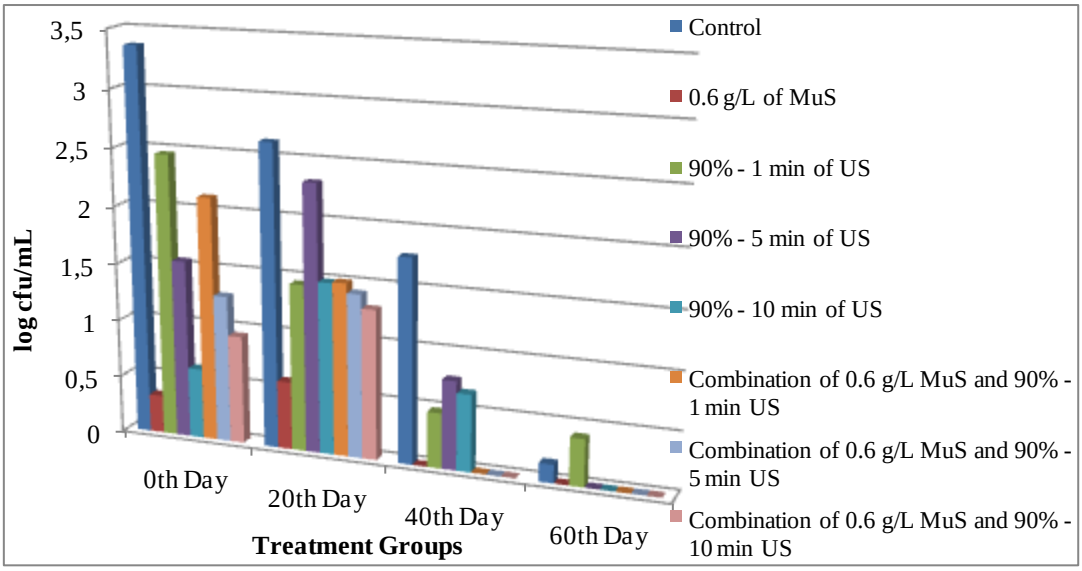

Figure 4.11. The yeast and mold counts of the ultrasonicated (90%), black mustard seed (0.6 g/L) added, and the combination of black mustard seed and ultrasonication (90% - 0.6 g/L) treated apple juice.

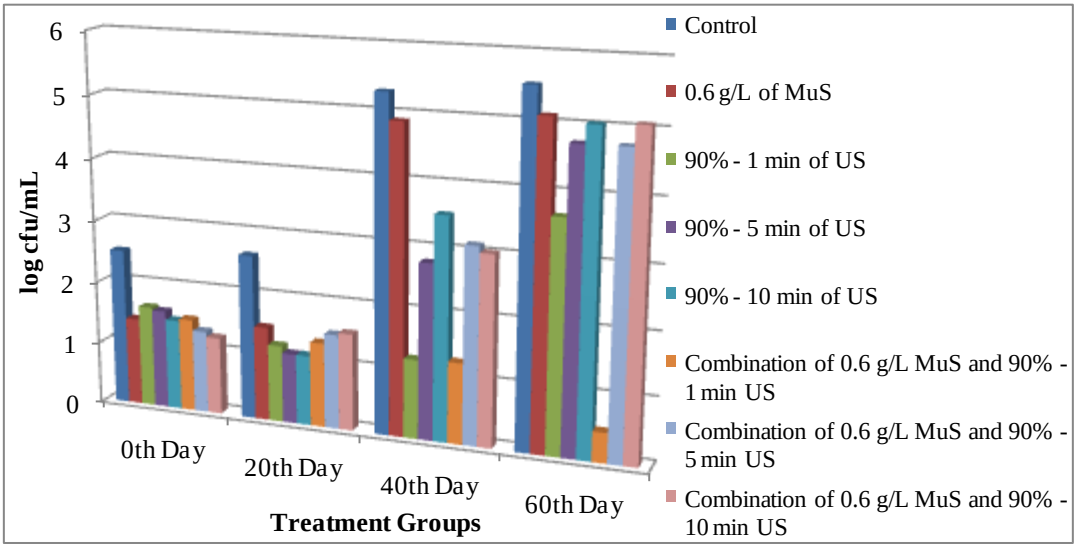

Figure 4.12. The total mesophilic aerobic bacteria counts of the ultrasonicated (90%), black mustard seed (0.6 g/L) added, and the combination of black mustard seed and ultrasonication (90% - 0.6 g/L) treated apple juice.

4.3.2 The Application of Ultrasonication and Mustard Seed against *E. coli* O157:H7 in Apple Juice

The *E. coli* 0157:H7 added apple juice was subjected to treatment of ultrasonication with the following amplitudes of 30, 60 and 90%, for a period of one, five and ten minutes. When the logarithmic counts of the study was compared with respect to the control, in each amplitude, an infinitesimal decrease was observed within groups while the period of processing time was increased. The most effective amplitude of ultrasonication was found to be 90% (Table 4.20) ($p < 0.05$). The effect of ultrasonication treatment at the amplitude of 30% was almost the same with ultrasonication at the amplitude of 60% and control ($p > 0.05$), lesser than ultrasonication treatment at the amplitude of 90% ($p < 0.05$) (Table 4.20). The 90% amplitude of ultrasonication application for a period of 10 minutes, was found to be significantly different than control but the decrease is limited approximately only one log cycle, referring to an inefficient case (Table 4.20, Appendix A.33) ($p < 0.05$).

Table 4.20. The average counts of *E. coli* O157:H7 inoculated and ultrasonicated apple juice.

Groups	Mean ± Std.
Control	4.15^{a} ± .137
30%	4.11^{a} ± .084
60%	4.04^{a} ± .051
90%	3.75^{b} ± .305

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

The results tabulated in Appendix A.34. indicated that when black mustard seed (*Brassica nigra*) was added into the *E. coli* 0157:H7 containing apple juice, it

was found that only an approximate significant reduction of 0.2 log cycle was obtained when the counts of *E. coli* 0157:H7 was considered in the 0.6 g/L mustard seed treatment ($p < 0.05$).

When the combination treatment of 0.6 g/L black mustard seed and ultrasonication at the amplitude of 90% for 10 minutes was considered in *E. coli* 0157:H7 inoculated apple juice, it was observed that the *E. coli* counts were found to be reduced approximately two log cycles when compared to the counts of the control and the black mustard seed added group (Appendix A.35., Figure 4.13.) ($p < 0.05$). From the table presented in Appendix A.35 it was also seen that in the combination case, just the combination of 90% - 10 min ultrasonication and 0.6 g/L mustard seed was found to be effective and significantly different from the control and sole treatment of mustard seed and sole treatment of 90% - 10 min ultrasonication in statistical point of view to reduce the *E. coli* population as approximately two log cycle from the apple juice ($p < 0.05$).

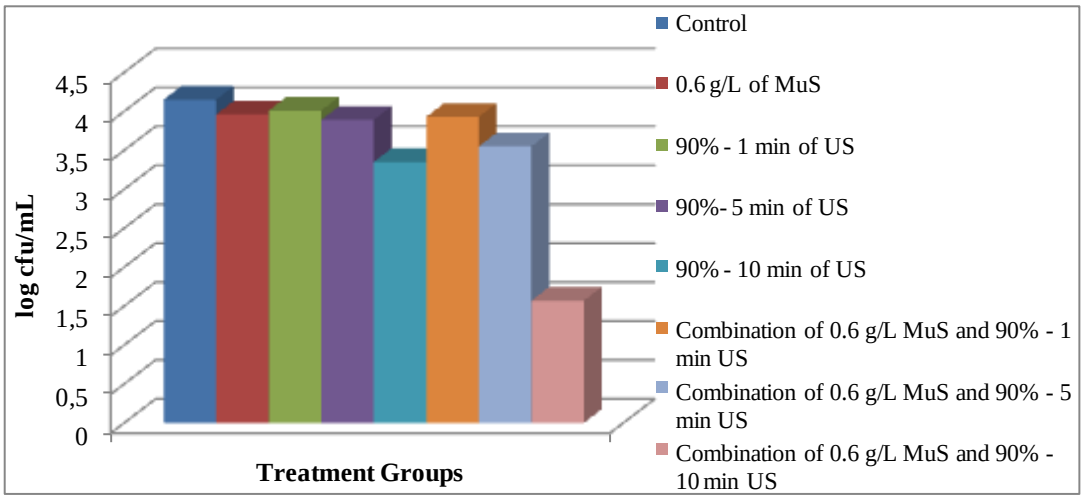

Figure 4.13. The microbial counts of the combination of black mustard (*Brassica nigra*) seed added (0.6 g/L) and ultrasonicated (90%, 1, 5, and 10 min.) *E. coli* 0157:H7 inoculated apple juice.

The results of the injured cell determination of the *E. coli* 0157:H7 population revealed that less than half of the cells in the medium were found to be injured and a 40% injury rate is obtained (Table 4.21).

Table 4.21. The percent injured cell counts of *E. coli* 0157:H7 after mustard seed treatment in apple juice.

	0.6g/L MuS Treatment
Growth on TSA	184
Growth on TSA with 1% NaCI	111
Injured cell (%)	40

4.3.3 The Application of Ultrasonication and Mustard Seed against *Salmonella* spp. in Apple Juice

The *Salmonella* spp. added apple juice was subjected to treatment of ultrasonication with the following amplitudes of 30, 60 and 90%, for a period of one, five and ten minutes (Appendix A.36, Table 4.22). When the logarithmic counts of the study was compared with respect to the control, a gradual decrease was observed when the amplitudes of ultrasonication were increased (Table 4.22) ($p < 0.05$). A slight gradual insignificant decrease was observed within groups ($p > 0.05$) while the period of processing time was increased, except ultrasonication treatment at amplitude of 90% for 10 minutes in which a significant effect was observed against *Salmonella* spp. (Appendix A.36) ($p < 0.05$). The ultrasonication treatment at amplitude of 90% for 10 minutes, was found to be the most effective group due to an approximate 1.50 log cycle decrease (Appendix A.36) when compared with the

control and found to be significantly different ($p < 0.05$), in addition, it was effective on the destruction of *Salmonella* spp., like *E. coli* 0157:H7, another important foodborne pathogen (Figure. 4.13.).

Table 4.22. The average counts of *Salmonella* spp. inoculated and ultrasonicated apple juice.

Groups	Mean ± Std.
Control	4.96^{a} ± .000
30%	4.75^{a} ± .068
60%	4.67^{a} ± .074
90%	4.20^{b} ± .558

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

When 0.6 g/L black mustard seed (*Brassica nigra*) was added into the *Salmonella* spp. containing apple juice, it was found that an approximate significant reduction of 0.5 log cycle was obtained when the counts of *Salmonella* spp. was considered (Appendix A.37) ($p < 0.05$). If this was compared with *Salmonella* inoculated apple juice followed by an ultrasonication treatment at amplitude of 90% for 10 minutes, it was not found to be too much effective.

The combinations of 0.6 g/L black mustard seed and the one and five-minute treatments of 90% amplitude of ultrasonication that were significantly different from control ($p < 0.05$) had the same effects with sole treatment of 0.6 g/L mustard seed and sole ultrasonication treatments at amplitude of 90% for 1 and 5 minutes ($p > 0.05$) (Appendix A.38). When the combined treatment of 0.6 g/L black mustard seed and ultrasonication at the amplitude of 90% for 10 minutes was considered in *Salmonella* spp. inoculated apple juice, it was observed that (Figure.4.14.) the *Salmonella* spp. counts were found to be reduced significantly only up to one log

cycle when compared to the counts of the control and the sole black mustard seed added and ultrasonicated group (Appendix A.38) ($p < 0.05$). The results were compared with the *E. coli* 0157:H7 treatment of the apple juice (Appendix A.35, Figure 4.13.) and 10 minutes' ultrasonic treatment at an amplitude of 90% was not found to be effective as *E. coli* 0157:H7 treatment to kill the population of *Salmonella* spp.

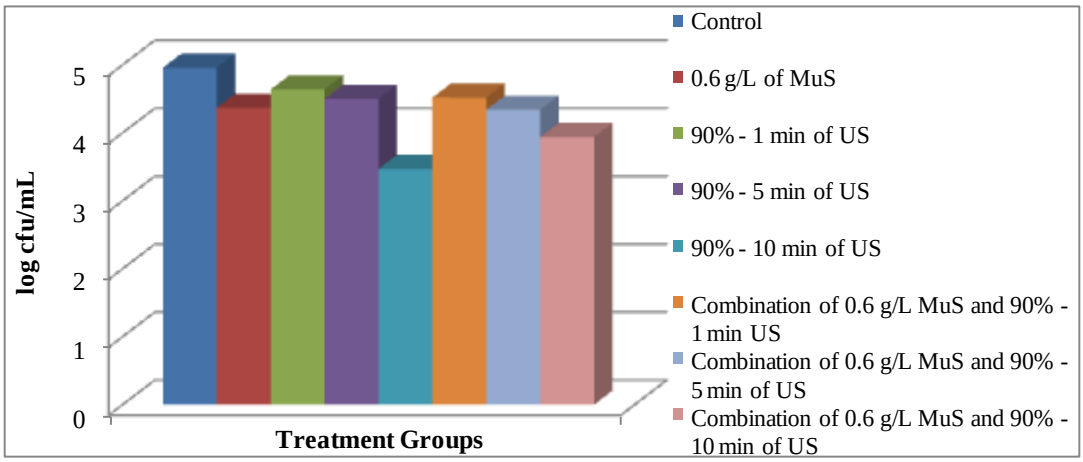

Figure 4.14. The microbial counts of the combination of black mustard (*Brassica nigra*) seed added (0.6 g/L) and ultrasonicated (90%, 1, 5, and 10 min.) *Salmonella* spp. inoculated apple juice.

The results of the injured cell determination in the *Salmonella spp.* added apple juice revealed that 91% of the cells in the medium were found to be injured (Table 4.23).

Table 4.23. The percent injured cell counts of *Salmonella* spp. after mustard seed treatment

	0.6g/L MuS Treatment
Growth on TSA	76000
Growth on TSA with 4% NaCI	7000
Injured cell (%)	91

4.3.4 The Application of Ultrasonication and Mustard Seed against *Listeria monocytogenes* in Apple Juice

The apple juice inoculated with *Listeria monocytogenes* was subjected to treatment of ultrasonication with the following amplitudes of 30, 60 and 90%, for a period of one, five and ten minutes (Appendix A.39) (Table 4.24). When the logarithmic counts of the study was compared with respect to the control, in each amplitude, a gradual decrease was observed (Table 4.24) ($p < 0.05$). A slight gradual insignificant decrease was observed within groups ($p > 0.05$) while the period of processing time was increased, except the treatments of ultrasonication at the amplitude of 90% for one, five and ten minutes, showing significant effects against *L. monocytogenes* (Appendix A.39) ($p < 0.05$). It was limited up to a 0.26 log cycle decrease (Appendix A.39).

Table 4.24. The average counts of *Listeria monocytogenes* inoculated and ultrasonicated apple juice.

Groups	Mean ± Std.
Control	5.41^{a} ± .035
30%	5.39^{a} ± .018
60%	5.35^{a} ± .054
90%	5.24^{b} ± .128

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($P < 0.05$).

When 0.6 g/L black mustard seed (*Brassica nigra*) was added into the *L. monocytogenes* containing apple juice, it was found that an approximate significant reduction of 0.16 log cycle was obtained with respect to control (Appendix A.40) (p

< 0.05). All of the mustard seed doses showed almost the same effect against *L. monocytogenes* ($p > 0.05$). In addition, the effect of mustard seed was not found to be as much as powerful. It was also in line with the results presented in Appendix A.39 and Table 4.24, almost resembling the *L. monocytogenes* inoculated-ultrasonicated group; as since *L. monocytogenes* resist the treatments due to its strong, peptidoglycan fortified Gram (+) positive cell wall structure.

The combinations of 0.6 g/L black mustard seed and the one, five and ten minute treatments of 90% amplitude of ultrasonication were significantly different from control ($p < 0.05$) and showed almost the same effects with sole treatment of 0.6 g/L mustard seed and sole treatments of ultrasonication at amplitude of 90% for one, five and ten minute against *L. monocytogenes* ($p > 0.05$) (Appendix A.41). Both sole 0.6 g/L black mustard seed (*Brassica nigra*) addition, sole ultrasonication at amplitude of 90% and, the combination treatment of the black mustard seed and ultrasonication was found to be not successful when compared with the control, only differing a 0.15 log cycle (Figure 4.15). *L. monocytogenes* withstands to all of these effective treatments, and the low pH in apple juices too, as it has a thick cell wall due to high peptidoglycan content **(**Appendix A.40).

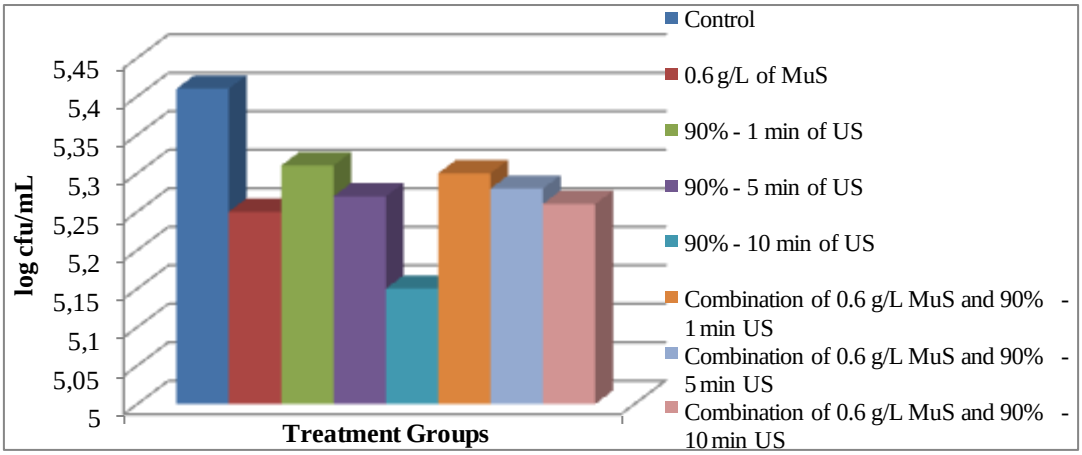

Figure 4.15. The microbial counts of the combination of black mustard (*Brassica nigra*) seed (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min.) *Listeria monocytogenes* inoculated apple juice.

The results of the injured cell determination for *L. monocytogenes* in the effected population revealed that only 1% the cells in the medium were found to be injured (Table 4.25).

Table 4.25. The percent injured cell counts of *Listeria monocytogenes* after mustard seed treatment

	0.6g/L MuS Treatment
Growth on TSA	212000
Growth on TSA with 4% NaCl	209667
Injured cell (%)	1

4.3.5 The Results of Physical and Chemical Analysis of Apple Juice

The pH of the apple juice was found to be 4.32, 4.34, 4.34, 4.33, 4.35, 4.34, 4.35, 4.34, 4.34, and 4.33 for the control, 0.2, 0.4 and 0.6 g/L of mustard seed, 90% – 1 min., 90% – 5 min., and 90% – 10 min. of sole ultrasonication treatments, and the combination treatments of 0.6 g/L mustard seed with 90% – 1 min., 90% – 5 min.,

and 90% – 10 min. of ultrasonication respectively (Table 4.26) ($p > 0.05$). There was no difference between the control and the treatment groups. When compared with the alpine strawberry and blackberry, the pH of apple juice was not so acidic as them, therefore this might have decreased its own inhibitory potential of bacteria in the processes mentioned above.

Table 4.26. The physical and chemical properties of nontreated and treated apple juices

	Control	**0.6 g/L of MuS**	**90% - 10 min**	**Combination**	**p**
pH	4.33 ± .000	4.33 ± .000	4.35 ± .000	4.33 ± .000	.072
Total Soluble Solids (%Brix)	8.00 ± .000	8.50 ± .000	8.50 ± .000	8.50 ± .000	.072
Titratable Acidity	.16 ± .000	.15 ± .000	.15 ± .000	.16 ± .000	.072
Total Sugar Content (g/mL)	2.27 ± .000	1.83 ± .000	2.12 ± .000	1.93 ± .000	.072
Viscosity (mPa.s)	11.10 ± .424	15.65 ± .212	10.20 ± .566	9.16 ± 1.188	.104
L*	33.13 ± .099	32.34 ± .156	37.33 ± .000	37.48 ± .000	.078
a*	6.06 ± .000	5.76 ± .127	3.06 ± .014	3.53 ± .028	.080
b*	17.11 ± .071	16.34 ± .368	16.81 ± .000	17.6 ± .000	.078

Data are means ± Std. from two replicates

*$p < 0.05$

A slight increase was found to be present in the selected treatment groups of total soluble solid content of the apple juices with respect to the control ($p > 0.05$). This might be due to the release of possible antimicrobial substances to the apple juice, by the help of ultrasonication, and the black mustard (*Brassica nigra*) seed treatments, either in combination or sole case. When the titratable acidity and the total sugar content assays were considered, in general, a slight decrease was observed in the treatment groups with respect to the control ($p > 0.05$). The viscosity was said to be increased due to 0.6 g/L black mustard (*Brassica nigra*) seed treatment but the

other selected groups showed a decrease with reference to the control ($p > 0.05$). The lightness/darkness (L*) value in color assay results a slight decrease in the independent treatment group of 0.6 g/L black mustard (*Brassica nigra*) seed addition, but it was elevated in 90% - 10 minutes ultrasonic processing, and the combination case with respect to the control, whereas a* (red/green) value was found to be decreased in 0.6 g/L black mustard seed (*Brassica nigra*) addition and the independent 90% - 10 minutes ultrasonic processing and the combination case, with respect to the control of apple juice, and b* (blue/yellow) value was found to be almost the same in all treatment groups with respect to the control (Table 4.26) ($p > 0.05$).

The selection of the treatment groups in the physical and chemical assays were done due to their effectiveness in microbial assays.

4.3.6 The Sensorial Properties of Apple Juice

Thirty panelists rated the apple juice in the taste panel according to the criteria of color, sweetness, sourness, odor, appearance, flavor, bitterness and overall evaluation with a 10 point scale per replication (Table 4.27, Table 4.28). A score of 10 represented most liked, and 1 corresponded to least liked. The ages of 63.3% of panelists were found to be greater than twenty and 36.7% were twenty and lower than twenty. 76.7% of panelists were female and 23.3% were male. 16,7% of panelists were smoker and 83.3% were non-smoker. 96.7% of panelists were students and 3.3% were research assistants. According to Table 4.27 scores of all the treatment groups of apple juice were almost the same ($p > 0.05$). There were no extreme differences between the treatment groups of apple juice.

Table 4.27. The sensory parameters of nontreated and treated apple juices

	Control	0.6 g/L of MuS	90% - 10 min of US	Combination	Total
Color	8.17 ± .791	7.77 ± .858	8.43 ± 1.135	7.57 ± .971	7.98 ± .996
Sweetness	7.87 ± 1.252	7.20 ± 1.324	8.47 ± .900	7.23 ± 1.135	7.69 ± 1.262
Sourness	1.00 ± .000	1.00 ± .000	1.00 ± .000	1.00 ± .000	1.00 ± .000
Odor	7.40 ± 1.192	7.00 ± 1.313	7.90 ± 1.094	6.93 ± 1.337	7.31 ± 1.282
Appearance	8.30 ± 1.149	7.80 ± 1.270	8.80 ± 1.031	7.67 ± 1.241	8.14 ± 1.245
Flavor	8.07 ± 1.081	7.17 ± .834	8.17 ± .747	7.17 ± .699	7.64 ± .968
Bitterness	1.00 ± .000	1.13 ± .346	1.03 ± .183	1.00 ± .000	1.04 ± .201
Overall	7.93 ± 1.081	7.30 ± .952	8.53 ± .819	7.27 ± .868	7.76 ± 1.061

Data are means ± Std.

Table 4.28. The correlation between acidity and color of all juices

		Titratable Acidity	L*	a*	b*
Titratable Acidity	Pearson Correlation	1	-0.899	.485	-0.989
	Sig. (2-tailed)		*.000	*.016	*.000
	N	24	24	24	24
L*	Pearson Correlation	-0.899	1	-0.162	.877
	Sig. (2-tailed)	*.000		.451	*.000
	N	24	24	24	24
a*	Pearson Correlation	.485	-0.162	1	-0.554
	Sig. (2-tailed)	*.016	.451		*.005
	N	24	24	24	24
b*	Pearson Correlation	-0.989	.877	-0.554	1
	Sig. (2-tailed)	*.000	*.000	*.005	
	N	24	24	24	24

* $p < 0.05$

According to table 4.28 there was a correlation between titratable acidity and L, a, b values of fruit juices ($p < 0.05$). There was a direct relationship between titratable acidity and L, a, b values of fruit juices.

CHAPTER 5

DISCUSSION

5.1 The Microfloral and Shelf Life Results

The primary sources for microbial contamination are water, employees, cross contamination of equipments and poorly cleaned fruits which are contaminated when they drop down from the tree. Determination of the food-borne pathogens which cause food-borne diseases in the juices and prevention of spoilage of them are strictly required. Due to this purpose, this study was designed to identify the possible food-borne microorganisms, especially in the alpine strawberry and the blackberry juices which might be an alternative income for the inhabitants of Bolu region of Turkey. In our study, *S. aureus* grew in the microflora of alpine strawberry and apple juices. These juices might be contaminated during squeezing process of fruits by *S. aureus* that is associated with hand hygiene. Bagde and Tumane (2011) showed that *S. aureus* and *E. coli* were detected in apple, pineapple and mosambi juice. They also detected *S. typhi* in one of the fruit juice too. Gallotannins which are present in blackberry have antibacterial effect against *S. aureus* (Engels et al., 2012). If any *S. aureus* contamination was occured in blackberry juice, *S. aureus* could be inhibited by gallotannins. TMAB grew in the microflora of juices except blackberry juice due to low pH and gallotannin content of blackberry (Lee et al., 2009, Engels et al., 2012). Yeast and molds grew in the microflora of juices.

Raybaudi-Massilia et al. (2009) concluded that essential oils possess a strong antimicrobial activity against spoiling and pathogenic microflora of fruit juices, being the effect greater at low pH. USFDA (2011) concluded that fruit juices that have low pH represent strong antimicrobial activity by means of EOs. Low pH is related with improvement of EO action by increasing hydrophobicity of EO. Gutierrez et al. (2008, 2009) had showed that antibacterial efficacy of thyme and oregano EOs was very high when pH was 4.33–5.32. Tserennadmid et al. (2011) had showed that yeasts were affected slightly in the low pH values that range between 4.0 and 7.0. In our study, the blackberry juice was found to be more acidic than the other juices, and this property might help the inhibition of the bacteria starting from first to 60^{th} day of storage, due to an increase through improvement of possible essential oil action. There are also probable rich content of phenolic antimicrobial substances in blackberry juices (Kaume et al., 2012). Our study demonstrated that in spite of low pH of blackberry juice, yeast and molds grew gradually up to the fourtieth day but bacteria didn't grow due to molds and yeasts are able to grow at lower pH than do bacteria. This might be possibly explained by the cases that yeast and molds have ability to grow at lower pH in which many bacteria can't grow (Ray, 2004), and during the period of disappearance of bacteria, yeast is found to be the dominating species (das Chagas Oliveira Freire and Offord,, 2002). The pH range of growth for molds, yeasts, Gram (+) positive and Gram (-) negative bacteria are 1.5 to 9.0, 2.0 to 8.5, 4.0 to 8.5 and 4.5 to 9.0, respectively (Ray, 2004). The cell wall of bacteria contains polysaccharide, peptidoglycan, whereas yeasts contain a β (1→3) glucan that may resist to environmental stress (Alcamo, 1997; Cabib et al., 2001). The similar result was revealed by Kiang et al. (2012) who demonstrated that any growth

of *Salmonella enteritidis* (*S. enteritidis*) was detected after incubation in mango juice (pH: 3.1), in spite of the growth of *S. enteritidis* was detected following incubation in universal pre-enrichment broth.

Jasson et al. (2007) and Lara-Lledó et al. (2012) confirmed that *L. monocytogenes* was effected by oriental mustard extract film throughout the shelf-life study, and microbial counts were below the detection limit of 1.6 log CFU/g at 52 day storage at 4 °C (75% shelf-life). In most cases, the results in our study is almost similar with the effects reported by these researchers. No bacteria was observed through the shelf life when 0.6 g/L mustard seed treatment of alpine strawberry juice was analyzed with respect to control ($p < 0.05$). There was an infinitesimal increase in the yeast counts with the same amount of mustard seed treatment ($p > 0.05$). In blackberry, there was a significant decrease in the yeast counts with treatment of 0.6 g/L mustard seed, an initial value of 2.66 log cycles, reaching up to undetectable level at the end ($p < 0.05$). When the yeast counts was considered, there was a significant reduction up to undetectable level from a value of 5.54 log cycle for 0.6 g/L mustard seed treatment and the combined treatment of 0.6 g/L mustard seed and 90% - 1 min ultrasonication on 60^{th} day. In apple juice, there was a sharp decrease in the yeast counts with the treatment of 0.6 g/L mustard seed through shelf life, approximately about three log cycles ($p < 0.05$). Mustard seed reduced yeast and mold counts to undetectable level.

Wong et al. (2008) demonstrated that *Salmonella* spp. was reduced by sonication in orange juice after a short storage period, compared with a nonsonicated *Salmonella* spp. containing orange juice. Gao and Rupasinghe (2012) demonstrated that total aerobic counts that were subjected to the ultrasound at a frequency of 20

kHz for 10 min at 25°C ± 1°C were lower in juice with lower pH (apple-carrot ratio of 90:10, v/v) than the juices with higher pH after one month storage at 4 °C. A similar observation was also recorded in our experiment that the counts of TMAB that were subjected to the ultrasound at a frequency of 24 kHz with amplitude of 90% for 10 min and combination of the ultrasound at a frequency of 24 kHz with amplitude of 90% for 10 min and 0.6 g/L mustard seed were reduced to undetectable level in alpine strawberry juice, of which its pH was lower than apple juice after 60th day storage at 4°C, whereas the counts of TMAB were higher in apple juice. In our study, it was observed that there was an increase of total mesophilic aerobic bacterial counts through storage for all treatments of apple juice when compared with the zeroth day. This might be explained by the presence of certain bacterial growth stimulating and promoting substances or enhanced acid resistance of these bacteria, such as in case of *E. coli* 0157:H7 (Patil et al., 2010) in the apple juice. On the other hand there was a decrease in the yeast counts. This might be possibly explained by the case that during the period of appearance of bacteria, yeast disappears. das Chagas Oliveira Freire and Offord (2002) obtained similar results in their study, conducted on roasted and salted Brazilian nut kernels and black pepper. Guerrero et al. (2005) demonstrated a 3 log reduction of *S. cerevisiae* by sonication for 30 min at 45 °C using 95.2 μm in apple juice. Our study demonstrated approximately 3 log reduction of yeast and molds by ultrasonication at the frequency of 24 kHz with amplitude of 90% for 10 min. Interestingly, in another study conducted by Lu et al. in 2010, it was reported that the inactivation of yeasts was worse than that of other bacteria either in the bottle apple juice or in the self-produced apple juice and moreover, the results were in line with many authors' findings, who had reported *S.*

cerevisiae were more resistant to UV than many bacteria such as *E. coli, S. enteritidis*, *L. brevis* and *S. aureus* (Lue et al., 2010). Due to all of these reasons, there is a need for alternative processing treatments that can achieve a 5 log reduction of these pathogens in apple juice (Mazzotta, 2001). In our study, there was a loss of bacterial counts in alpine strawberry juice when compared with the zeroth day. This might be explained by the presence of certain antimicrobial substances such as essential oils and owing to the high acidity (pH 3.2 – 4.1) of alpine strawberry juice. Essential oils possess a strong antimicrobial activity against spoiling and pathogenic microflora of juices, being the effect greater at low pH (Raybaudi-Massilia et al., 2009). The growth of most pathogens were inhibited by fruit juices that have high acidity ranging from pH 3.2 to 4.1 (Lee et al., 2009). On the other hand there was an increase in the yeast counts except combined treatment of 0.6 g/L mustard seed and ultrasonication at amplitude of 90% for 10 min. This might be possibly explained by the case that during the period of disappearance of bacteria, yeast is the dominating species. das Chagas Oliveira Freire and Offord, (2002) obtained similar results in their study, conducted on roasted and salted Brazilian nut kernels and black pepper.

Utkun and Kunduhoglu (2012) concluded that no microbial growth (*E. coli* O157:H7 and *L. monocytogenes*) was seen in orange juice samples treated with ultrasonication at a frequency of 20 kHz for 5 min and EO constituents (1000 μL/L) during 48 days of storage at 4 and 25 °C.

Yang et al. (2011) found that combined treatment of salicylic acid (0.05 mM) and ultrasound at frequency of 40 kHz, 8.8 W/L for 10 min reduced the *Penicillium expansum* in peach without causing physical and chemical quality change after six

days of storage at 20 ^{o}C. We found that combined treatment of the ultrasonication at frequency of 24 kHz with amplitude of 90% for 10 min and 0.6 g/L mustard seed had started to reduce counts of yeast and mold in alpine strawberry and apple juices after 20 day of storage. The counts of yeast and molds in apple juice were inhibited completely through storage by the combination of 0.6 g/L mustard seed and ultrasonication at the amplitude of 90% for 10 minutes when compared to the counts of the control in the zeroth and the twentieth days. The counts of yeast and molds in the alpine strawberry juice were significantly reduced up to undetectable level by the combination of 0.6 g/L mustard seed and ultrasonication at amplitude of 90% for 10 minutes in 20^{th}, 40^{th} and 60^{th} days, not only descending from the initial value of approximately 2.81 log colony forming units obtained in 0^{th} day of the study, but also the difference between the control and the treatment was approximately 4.11 log cycles, indicating a powerful process (Figure 4.2 and Appendix A.6.) ($p < 0.05$). This may be due to the release of possible antimicrobial substances from the mustard seed into the juice, reported by Fahey et al. in 2001 by means of ultrasonication.

5.2 The Treatment of Ultrasonication

Some researchers found that type of juice, viscosity and pulp of juice were necessary factors to determine processing times of ultrasonication required and inactivation rate to meet FDA recommendations (Wong et al., 2008; Patil et al., 2009; Yuan et al., 2009; Bermúdez-Aguirre and Barbosa-Cánovas, 2012). In our study, any significant reduction of bacteria was seen depending on increased processing time of ultrasonication in each fruit juices. Some researchers demonstrated that enhanced amplitude levels of ultrasonication increased inhibition

of foodborne pathogens (Hua and Thompson, 2000; Ugarte-Romero et al., 2007). These studies were in accordance with our study that those revealed pathogens decreased with increasing amplitude levels of ultrasonication. The high amplitude level of ultrasonication such as 90% increased inhibition of pathogens.

Our study showed that the treatment of ultrasonication at the amplitude of 90% for 10 min in blackberry and alpine strawberry juices were more effective than apple juice. Marx et al. (2011) concluded that low pH affected membrane permeability and reduce cell resistance to ultrasound. Tsong (1991) considered that water influx and drastic increase in turgor pressure which leads to cell lysis may be caused by organic acid found in juices. Wong et al. (2008) and Wordon et al. (2011) concluded that sensitivity of bacteria to low pH was increased by nonlethal intracellular injuries caused by ultrasonic cavitation and changes in cell membrane structure caused by ultrasonication. Sagong et al. (2011) considered that the reduction levels of *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* of organic fresh lettuce by ultrasonication were increased with decreased pH values. These researchers explain why the effect of ultrasound against bacteria was higher in blackberry and alpine strawberry juices than apple in our study. The maximum reduction of *Salmonella* spp. caused by ultrasonication at the amplitude of 90% for 10 min was higher than *E. coli* O157:H7 as 1.49 and 0.80 log in apple juice, 3.13 and 2.96 log in alpine strawberry juice, respectively. This result was supported by Kiang et al. (2012) who concluded that *S. enteritidis* was found to be more sensitive to thermosonication than *E. coli* O157:H7.

In alpine strawberry juice, inhibition of ultrasound treatment with the amplitude of 90% for 10 min against *L. monocytogenes* was lower than *E. coli*

O157:H7 and *Salmonella* spp. due to rigid Gram (+) positive cell wall of *L. monocytogenes* that can resist environmental stress more than cell wall of Gram (-) negative bacteria (Patterson and Loaharanu, 2000; Ray, 2004). So, *Listeria monocytogenes* was found to be more resistant to ultrasonication processing than *Salmonella* spp. and *E. coli* 0157:H7. This result was in line with a study conducted on prosthetic infections of the implants, in which the Gram (+) positive bacteria such as *Listeria* spp. was found to be resistant to ultrasound processing (Monsen et al., 2009). So, *L. monocytogenes* can withstand and survive in the acidic environment. According to Ray (2004), Gram (+) positive bacteria is able to survive at the lower pH environment than Gram (-) negative bacteria. Our results were also supported by Feng et al. (2008) who demonstrated that Gram (+) positive and coccal cells were more resistant to ultrasonication at frequency of about 20 kHz treatment than Gram (-) negative and rod shaped bacteria.

The treatment of ultrasonication at the amplitude of 90% for 10 min reduced *E. coli* O157:H7 and *Salmonella* spp. to undetectable level in alpine strawberry juice without causing significant physical and chemical quality change. Acid that is present in juice can pass easily through damaged cell wall caused by ultrasonication. This can be revealed as synergistic effect on the inactivation of these pathogens (Ross et al., 2003). These results were supported by Wong et al. (2008) and Wordon et al. (2011) who concluded that sensitivity of bacteria to low pH was increased by nonlethal intracellular injuries caused by ultrasonic cavitation and changes in cell membrane structure caused by ultrasonication. Conversely to our study, Birmpa et al. (2013) determined that reductions of *E. coli, S. enteritidis* and *S. aureus* caused by ultrasonication with the frequency of 37 kHz were significant after 30-45 min except

for *Listeria* which was after 10 min in strawberry ($p < 0.05$). The maximum reductions of *E. coli, S. aureus, S. enteritidis* and *L. innocua* caused by the treatments of ultrasonication were 3.04, 2.41, 5.52 and 6.12 log CFU/mL in strawberry, respectively. The color of strawberry did not change significantly by the treatment of ultrasonication for time periods (up to 45 minutes) ($p > 0.05$).

In blackberry juice, the treatment of ultrasonication at an amplitude of 90% for 10 min reduced *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* to undetectable level without causing significant physical and chemical quality change. Acid that is present in juice can pass easily through damaged cell wall caused by ultrasonication. This can be revealed as synergistic effect on the inactivation of these pathogens (Ross et al., 2003). These results were supported by many researchers. Lee et al. (2009) concluded that the growth of most pathogens were inhibited by fruit juices that have high acidity ranging from pH 3.2 to 4.1. Marx et al. (2011), concluded that low pH affected membrane permeability and reduce cell resistance to ultrasound. Tsong (1991) considered that water influx and drastic increase in turgor pressure which leads to cell lysis may be caused by organic acid found in juices. Wong et al. (2008) and Wordon et al. (2011) concluded that sensitivity of bacteria to low pH was increased by nonlethal intracellular injuries caused by ultrasonic cavitation and changes in cell membrane structure caused by ultrasonication. Our results can also be explained by the presence of gallotannin in the blackberry (Seeram, 2006) that have antibacterial activity against some pathogens such as *S. enteritidis* (Kiang et al., 2012) and *S. aureus* (Engels et al., 2012) and were also supported by some researchers who concluded that pathogens were reduced significantly by the ultrasonication with the frequency of 40 kHz of which processing

times were 5–60 min. in pathogen inoculated organic fresh lettuce (Mahvi et al., 2005; Seymour et al., 2002; Sagong et al., 2011).

In most cases, the results in our study is almost similar with the effects reported in this chapter, conducted by different researchers.

5.3 The Treatment of Mustard Seed

Burt (2004) and Oussalah et al. (2007) indicated that some essential oils have a strong antimicrobial activity against the *S. aureus, E. coli* O157:H7, *S. typhimurium,* and *L. monocytogenes.* Sofia et al. (2007) concluded that extract and oil extract of mustard among various spices which included garlic, cinnamon, mint, ginger, and clove had inhibitory effects against *E. coli.* Graumann and Holley (2008) have shown that even with myrosinase inactivated, deodorized or deheated yellow mustard powder was lethal to *E. coli* O157:H7. Nilson and Holley (2012) found that *E. coli* O157:H7 was reduced by deodorized yellow mustard powder at the concentration of 4% or 6%. Sofia et al. (2007) demonstrated that mustard at 1% concentration had inhibitory action against *E. coli, S. aureus* by using disc diffusion method. The inhibition zones of up to 25.6 mm at 3% concentration of mustard seed were revealed. This antimicrobial activity of mustard can be due to allyl isothiocyanates that are present in mustard (Sofia et al., 2007). Jasson et al., 2007 confirmed that *L. monocytogenes* was effected by oriental mustard extract film throughout the shelf-life study, and microbial counts were below the detection limit of 1.6 log CFU/g at 52 day storage (75% shelf-life). Similar observations were also recorded in our study.

In blackberry juice, the treatment of 0.6 g/L mustard seed reduced *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* to undetectable level with 100% injury rate according to alpine strawberry and apple juices without causing significant physical and chemical quality change. USFDA (2011) concluded that fruit juices that have low pH represent strong antimicrobial activity by means of EOs. Burt (2004) implied that physical conditions such as low pH increase the action of EOs. Gutierrez (2008, 2009) had showed that antibacterial efficacy of thyme and oregano EOs was very high when pH was 4.33–5.32. Seeram (2006) found that berry phenolics include gallotannin that has antibacterial activity against some pathogens, such as *S. enteritis* (Kiang et al., 2012) and *S. aureus* (Engels et al., 2012). These studies of researchers supported our results that were due to gallotannin content of blackberry and low pH of blackberry juice increased the inhibitory effect of mustard seed.

The maximum reductions of *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* by the treatment of 0.6 g/L mustard seed were 0.19, 0.59, and 0.16 log CFU/mL in apple juice ($p < 0.05$), 1.57 ($p > 0.05$), 0.6 and 1.7 log CFU/mL in alpine strawberry juice ($p < 0.05$), 2.94, 2.85 and 1.43 log CFU/mL in blackberry juice ($p < 0.05$), respectively, without causing significant physical and chemical quality change. With the treatment of 0.6 g/L mustard seed, the percent injuries of *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* were 100, 100 and 75% in alpine strawberry juice, 100, 100 and 100% in blackberry juice, 40, 91 and 1% in apple juice, respectively. The lower reductions and lower injury rates of bacteria were seen in apple juice may be due to higher pH of apple juice than the others that provide optimum pH to the growth of bacteria (Ray, 2004). The lowest rate of

injured bacteria was achieved in *L. monocytogenes* in apple and alpine strawberry juices as 1 and 75%, respectively, due to its strong peptidoglycan Gram (+) cell wall. This was supported by Patterson and Loaharanu (2000). Our results were also supported by Burt (2004) who concluded that *L. monocytogenes* was affected less or not affected by mustard seed. *E. coli* O157:H7 was injured less than *Salmonella* spp. as 40% rather than 91% in apple juice, respectively. Our results were also in line with the findings of Kiang et al. (2012) and Roering et al. (1999) who demonstrated *E. coli* O157:H7 was more resistant than *S. enteritidis* and *S. typhimurium* in the environmental stres. Roering et al. (1999) demonstrated that *E. coli* O157:H7 resisted better in unpasteurized apple cider (pH 3.3–3.5) and simulated gastric fluid (pH 1.5) than *S. typhimurium.* Kiang et al. (2012) demonstrated that *E. coli* O157:H7 survived better than *S. enteritidis* in thermosonicated mango juice (pH 3.1). They considered that this might be due to the ability of *E. coli* O157: H7 to withstand and survive in acidic conditions. The higher injury rates of bacteria in alpine strawberry and blackberry juices compared to apple juice may be due to the low pH of alpine strawberry and blackberry juices increased the inhibitory effect of mustard seed or synergistic effect of mustard seed with low pH of juices. Earlier studies showed that glucosinolates represent antibacterial and antifungal effect directly or synergistically in combination with other compounds (Naidu, 2000a; Tolonen et al., 2004; Almajano et al., 2008; Graumann and Holley, 2008; Gutierrez et al., 2008). Burt (2004) demonstrated that physical conditions such as low pH increase the effect of EOs. USFDA (2011) concluded that fruit juices that have low pH represent strong antimicrobial activity by means of EO.

5.4 The Treatment of Combination

Some researchers also studied combination treatments of ultrasonication or mustard seed with different factors. Solomakos et al. (2008) confirmed that combinations of EOs and nisin increased antimicrobial activities against *L. monocytogenes*. Some researchers concluded that combination of ultrasound and high pressure treatment increased inactivation of *E. coli* due to additional effect of ultrasound. At the increased temperature over 50 ^{o}C, sensitivity of microorganisms were increased by ultrasonication (Earnshaw et al., 1995, Zenker et al., 1999, Villamiel and de Jong, 2000). In our study, any temperature rising by ultrasonication over 50 ^{o}C was observed. Temperature rising was observed from 15 to 24 ^{o}C during ultrasonication process in our study. So, bacterium were affected only by treatments not by temperature.

Sagong et al. (2011) concluded that the maximum reductions of *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* that were caused by combined treatment of ultrasonication with the frequency of 40 kHz for 5 min and 2% organic acid were 2.75, 3.18, and 2.87 log CFU/mL, respectively. The combined treatment of ultrasonication and organic acids resulted in additional 0.8 to 1.0 log reduction of *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* compared to sole treatments, without causing significant quality change (color and texture) on lettuce during seven days of storage (Sagong et al., 2011). Utkun and Kunduhoglu (2012) concluded that no significant reductions of *E. coli* O157:H7 and *L. monocytogenes* were seen when the combined treatments of sonication at a frequency of 20 kHz for 5 min and EOs performed at low temperatures in orange juice (4 and 25 °C) ($p > 0.05$). The reductions of *L. monocytogenes* that were caused by the combined treatment of

ultrasonication and EO constituents (200 μL/L) at 4 and 25 °C ranged between 0.34-0.54 and 0.21-0.39 log CFU/mL, respectively ($p > 0.05$). Significant ($p < 0.05$) inactivations of *E. coli* O157:H7 and *L. monocytogenes* were caused by combined treatments at 45 °C compared with the control. Utkun and Kunduhoglu (2012) concluded that the significant reductions of *E. coli* O157:H7 that were caused by the combined treatment of sonication with eugenol or linalool (1000 μL/L) at 45 °C were 5.92 and 5.85 log cycles, respectively. Kiang et al. (2012) investigated thermal treatment and thermosonication with the frequency of 25 kHz at 50°C for 10 min and at 60 °C for 7 min against *E. coli* O157:H7 and *S. enteritidis* in mango juice. Kiang et al. (2012) demonstrated that *S. enteritidis* that was subjected to more than 5 min. thermosonication with the frequency of 25 kHz and 200 W at 60 °C in mango juice was not recovered after treatment. The reduction of *E. coli* O157:H7 caused by ultrasonication for 7 min at 60 °C was 5.0 log cycles. The reduction of *E. coli* O157:H7 caused by thermal treatment was only 4.4 log cycles. Approximately 9.0 log cycle reduction of *S. enteritidis* caused by ultrasonication for 3 and 7 min at 60 °C and 50 °C was observed (Kiang et al., 2012). Kiang et al. (2012) demonstrated that pathogen inactivations were increased by high-intensity ultrasound compared to thermal treatment alone. Muñoz et al. (2012) demonstrated that the combined treatment of pulsed light and thermosonication at the frequency of 24 kHz for 5 and 2.9 min was more effective against *E. coli* in apple juice than the sole treatments. The maximum reductions of *E. coli* that were caused by the treatment of thermosonication and pulsed light were 2.7 and 4.9 log CFU/mL, respectively, while reduction of combined treatments was 6 log CFU/mL that reveals an additive effect for both treatments when acting in combination. Muñoz et al. (2011) had also

described the additive effect of both technologies when they combined in orange juice. In our study, the maximum reductions of *L. monocytogenes* that were caused by the treatments of 0.6 g/L mustard seed, and ultrasonication at the amplitude of 90% for 10 min were 1.67 and 1.95 log CFU/mL in alpine strawberry juice, respectively, while reduction of combined treatment was 3.34 log CFU/mL that reveals an additive effect for treatments when acting in combination. The counts of *L. monocytogenes* in the treatments of ultrasonication at the amplitude of 90% for 10 min, 0.6 g/L mustard seed and combinations of these were 1.39, 1.67 log CFU/mL, and 0, respectively. The combined treatment of 0.6 g/L black mustard seed and ultrasonication at amplitude of 90% for 10 minutes was the best one among all of the treatments to eradicate this important pathogen from alpine strawberry juice ($p > 0.05$). In combination, the reduction might be possibly due to the help of the release of certain possible antimicrobial substances such as glucosinolates (Fahey et al., 2001), fatty oil and several natural bioactive compounds, such as gallic acid, quercetin, ferulic acid, caffeic acid and rutin (Rajamurugan et al., 2011) from the mustard seed by the help of ultrasonic processing. The maximum reductions of *E. coli* O157:H7 that were caused by the treatments of 0.6 g/L mustard seed, and ultrasonication at the amplitude of 90% for 10 min were 0.19 and 0.8 log CFU/mL in apple juice, respectively, while the reduction of combined treatment was 2.58 log CFU/mL that reveals a significant synergistic effect for treatments when acting in combination. These synergistic effects between ultrasonication and mustard seed might be possibly due to glucosinolates that are present in mustard seed. Earlier studies support our results that glucosinolates represent antibacterial and antifungal effect directly or synergistically in combination with other compounds (Naidu,

2000a; Tolonen et al., 2004; Almajano et al., 2008; Graumann and Holley, 2008; Gutierrez et al., 2008). These studies explain why inactivation of combination treatment was higher than sole treatments of mustard seed and ultrasonication.

The maximum reductions of *Salmonella* spp. that were caused by the treatments of 0.6 g/L mustard seed, and ultrasonication at the amplitude of 90% for 10 min were 0.59 and 1.49 log CFU/mL in apple juice, respectively, while reduction of combined treatment was 1.02 log CFU/mL that reveals an antagonistic effect for treatments when acting in combination. The maximum reductions of *L. monocytogenes* that were caused by the treatments of 0.6 g/L mustard seed, and ultrasonication with the amplitude of 90% for 10 min were 0.16 and 0.26 log CFU/mL in apple juice, respectively, while reduction of combined treatment was only 0.15 log CFU/mL that reveals an antagonistic effect for treatments when acting in combination.

In blackberry juice, any synergistic or additional effects of combination could not be observed against *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* due to sole treatments, already reduced bacteria to undetectable level in combination. But the presence of synergistic effect between ultrasonication and high amount of acids which originated from both blackberry juice and mustard seed can be revealed based on Ross et al. (2003). Ross et al. (2003) revealed that acid present in juice can pass easily through damaged cell wall caused by ultrasonication. This can be revealed as synergistic effect on the inactivation (Ross et al., 2003).

The maximum reductions of *E. coli* O157:H7 that were caused by the treatments of 0.6 g/L mustard seed, and ultrasonication at the amplitude of 90% for 10 min were 1.57 and 2.95 log CFU/mL in alpine strawberry juice, respectively,

while reduction of combined treatment was 2.95 log CFU/mL. The maximum reductions of *Salmonella* spp. that were caused by the treatments of 0.6 g/L mustard seed, and ultrasonication at the amplitude of 90% for 10 min were 0.6 and 3.13 log CFU/mL in alpine strawberry juice, respectively, while reduction of combined treatment was 3.13 log CFU/mL. Seymour et al. (2002) confirmed that reduction of *S. typhimurium* that was caused by the combined treatment of ultrasound for 10 min and 100 ppm of chlorinated water was 2.7 log CFU/g on strawberry. This was an additional 1.0 log reduction compared with sole treatments of ultrasound for 10 min or chlorinated water without affecting quality of strawberry (Seymour et al., 2002). In our study, any synergistic or additional effects of combination could not be observed against *E. coli* O157:H7, and *Salmonella* spp. in alpine strawberry juice due to sole treatment of ultrasonication, already reduced bacteria to undetectable level in combination. The presence of synergistic effect between ultrasonication and high amount of acids which originated from alpine strawberry juice can be revealed based on Ross et al. (2003).

In most cases, the results in our study is almost similar with the effects reported in this chapter, conducted by different researchers.

CHAPTER 6

CONCLUSION

Ultrasonication can modify physical, mechanical, and chemical properties of food, lower energy consumption, reduce reaction time and increase reaction yield. Ultrasonication can be referred as green technology due to its low energy consumption according to other food processing methods. By the use of ultrasonication and its combinations, the destruction of health promoting substances such as vitamins present in fruits and juices can be also prevented.

Consequently the use of the combination of ultrasound and mustard seed can improve microbial safety while maintaining the quality of organic fruit juices. The use of chemical preservatives in food industry have been reduced due to their side effects such as toxicity of chemicals in food products. Use of mustard seed as a natural product in fruit juices can not only be an effective antimicrobial application in food preservation, processing and safety, but also it can fight against certain pesticides which might be used in the fruits; the raw material, before harvesting or during storage due to its free-radical scavenging activity. According to our study, the high acidity found to be also helpful in alpine strawberry and blackberry juices to the ultrasonication process and together with the black mustard seed to eliminate the important foodborne pathogens *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp., it was applicable to industry, due to the undetectable level obtained in most of the studies, as well as the non-thermal point of view of ultrasonication, a low-cost process with relevance to the thermal processing. Due to the higher pH of apple juice

than blackberry and alpine strawberry juices, the ultrasonication and black mustard seed applications were not recommended for use in the industrial level for the elimination of bacteria, but another hurdle technology must be in trial to help the involvement of ultrasonication or mustard seed treatments in the apple juice industry as well.

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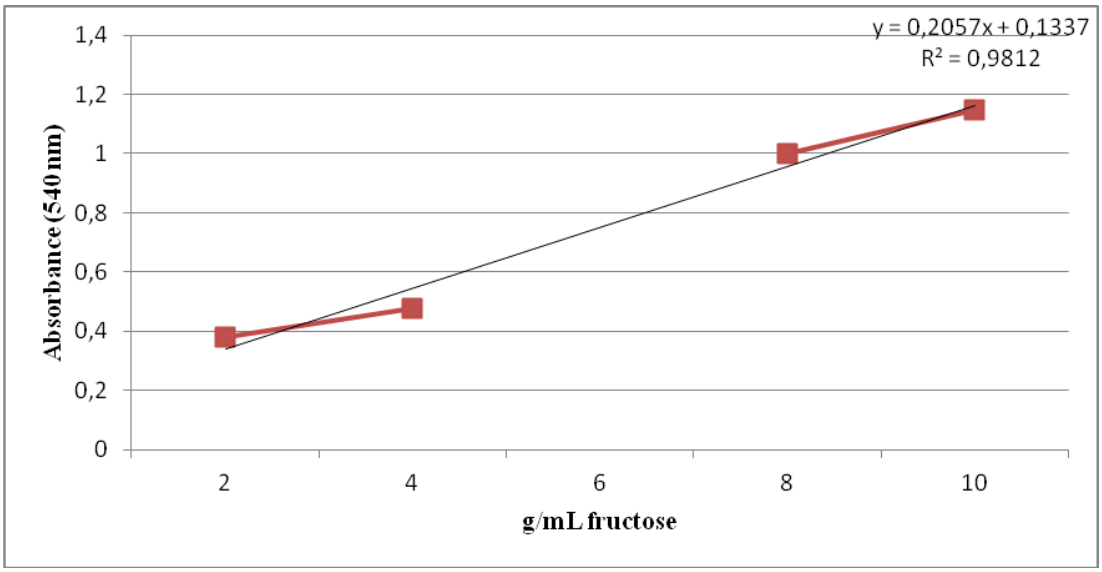
APPENDICES

Appendix A.1 The Standards of Fructose (g)

Std – 2	Std - 4	Std – 6	Std – 8	Std - 10
0.379	0.478	0.663	0.999	1.147

Appendix A.2 Standard curve of fructose (g/mL).



Appendix A.3 The total mesophilic aerobic bacteria (TMAB), and yeast/mold counts of the Alpine Strawberry Juice (Control)

	0. Day	**20. Day**	**40. Day**	**60. Day**
TMBA	2.33 log	2.34 log	ND	ND
YM	3.23 log	2.61 log	3.78 log	4.17 log

ND refers not detected

Appendix A.4 The microbial counts of the ultrasonicated alpine strawberry juice

Groups		TMAB			YM		
		Mean	±	Std.	Mean	±	Std.
Control		2.18[a]	±	.505	2.75[a]	±	.075
30%	**1 min**	2.52 [a]	±	.295	3.73[b]	±	.146
	5 min	1.45 [a]	±	1.269	3.53[b]	±	.132
	10 min	2.30[a]	±	.630	3.85[b]	±	.266
60%	**1 min**	1.33 [a]	±	1.266	3.84[b]	±	.236
	5 min	1.31[a]	±	1.161	3.81[b]	±	.190
	10 min	.51[b]	±	.878	3.55[b]	±	.113
90%	**1 min**	.94[b]	±	.911	3.66[b]	±	.123
	5 min	.33[b]	±	.577	3.50[b]	±	.126
	10 min	.16 [b]	±	.277	3.41[ab]	±	.257

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.5 The microbial counts of the black mustard seed (*Brassica nigra* seed) added alpine strawberry juice

	TMAB			YM		
Groups	**Mean**	**±**	**Std.**	**Mean**	**±**	**Std.**
Control	2.18[a]	±	.505	2.75[a]	±	.075
0.2 g/L of MuS	.94[b]	±	.821	3.08[a]	±	.473
0.4 g/L of MuS	.43[b]	±	.751	2.96[a]	±	.329
0.6 g/L of MuS	ND			2.65[a]	±	.165

Data are means ± Std. from three replicates
ND refers not detected
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.6 The shelf-life determination studies of the ultrasonicated (90%), black mustard seed (0.6 g/L) added, and the combination of black mustard seed and ultrasonication (90% - 0.6 g/L) treated alpine strawberry juice: The TMAB and YM counts

	Groups	0. day	20. day	40. day	60. day
TMAB	**90% - 1 min of US**	$1.47^{l} \pm .422$	$.32^{m} \pm .277$	ND	ND
	90% - 5 min of US	$1.00^{n} \pm .000$	ND	ND	ND
	90% - 10 min of US	$.48^{o} \pm .000$	ND	ND	ND
	0.6 g/L of MuS	ND	ND	ND	ND
	Combination (90% - 1 min US, 0.6 g/L MuS)	ND	ND	ND	ND
	Combination (90% - 5 min US, 0.6 g/L MuS)	ND	ND	ND	ND
	Combination (90% - 10 min US, 0.6 g/L MuS)	ND	ND	ND	ND
	Control	$2.18^{u} \pm .505$	$2.28^{u} \pm .295$	ND	ND
YM	**90% - 1 min of US**	$3.66^{a} \pm .123$	$4.32^{ab} \pm .676$	$4.84^{bc} \pm .321$	$5.35^{c} \pm .050$
	90% - 5 min of US	$3.50^{a} \pm .126$	$2.71^{a} \pm .920$	$4.95^{b} \pm .390$	$5.42^{b} \pm .115$
	90% - 10 min of US	$3.41^{a} \pm .257$	$4.14^{ab} \pm .628$	$5.13^{bc} \pm .519$	$5.31^{c} \pm .040$
	0.6 g/L of MuS	$2.65^{ab} \pm .165$	$1.39^{a} \pm .805$	$3.05^{b} \pm .052$	$3.15^{b} \pm .705$
	Combination (90% - 1 min US, 0.6 g/L MuS)	$3.10^{a} \pm .274$	$1.78^{a} \pm .761$	$2.63^{a} \pm .061$	$2.12^{a} \pm .821$
	Combination (90% - 5 min US, 0.6 g/L MuS)	$3.01^{a} \pm .266$	$.88^{b} \pm .786$	$2.65^{a} \pm .350$	$2.79^{a} \pm .700$
	Combination (90% - 10 min US, 0.6 g/L MuS)	$2.81^{a} \pm .386$	ND	ND	ND
	Control	$2.75^{a} \pm .080$	$2.60^{a} \pm .160$	$3.77^{b} \pm .115$	$4.11^{b} \pm .300$

Data are means ± Std. from three replicates
Values followed by different letters in the same line of TMAB and YM except lines of mean are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.7 The counts of the *E. coli* 0157:H7 inoculated and ultrasonicated alpine strawberry juice

Groups		Mean	±	Std.
Control		2.95[a]	±	.042
30%	**1 min**	2.92 [a]	±	.174
	5 min	2.62[a]	±	.096
	10 min	1.64[a]	±	1.434
60%	**1 min**	1.33[b]	±	1.152
	5 min	1.27[b]	±	1.102
	10 min	1.09[b]	±	.954
90%	**1 min**	1.18[b]	±	1.021
	5 min	.37[b]	±	.641
	10 min	ND		

Data are means ± Std. from three replicates
Values followed by different letters in the same column of each dose of ultrasonication are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.8 The counts of the *E. coli* 0157:H7 inoculated and black mustard seed (*Brassica nigra*) added alpine strawberry juice

Groups	Mean ± Std.
Control	2.95[a] ± .042
0.2 g/L of MuS	1.74 [a] ± 1.507
0.4 g/L of MuS	1.59 [a] ± 1.374
0.6 g/L of MuS	1.38 [a] ± 1.193

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.9 The microbial counts of the combination of black mustard seed (*Brassica nigra*) (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min) *E. coli* 0157:H7 inoculated alpine strawberry juice

Groups		**Mean ± Std.**
Control		2.95[a] ± .042
0.6 g/L of MuS		1.38[a] ± 1.193
90%	**1 min**	1.18[b] ± 1.021
	5 min	.37[b] ± .641
	10 min	ND
Combination	**90% - 1 min of US + 0.6 g/L of MuS**	.55[b] ± .958
	90% - 5 min of US + 0.6 g/L of MuS	ND
	90% - 10 min of US + 0.6 g/L of MuS	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.10 The counts of the *Salmonella* spp. inoculated and ultrasonicated alpine strawberry juice

Groups		Mean	±	Std.
Control		3.13[a]	±	.118
30%	**1 min**	2.88[ab]	±	.193
	5 min	2.81 [ab]	±	.012
	10 min	2.50[abc]	±	.436
60%	**1 min**	1.28[abcd]	±	1.114
	5 min	.94[bcd]	±	.821
	10 min	.37[cd]	±	.641
90%	**1 min**	.92[bcd]	±	.807
	5 min	ND		
	10 min	ND		

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.11 The counts of the *Salmonella* spp. inoculated and black mustard seed (*Brassica nigra*) added alpine strawberry juice

Groups	Mean ± Std.
Control	3.13^{a} ± .118
0.2 g/L of MuS	2.96^{b} ± .038
0.4 g/L of MuS	2.82^{b} ± .115
0.6 g/L of MuS	2.53^{c} ± .101

Data are means ± Std from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.12 The microbial counts of the combination of black mustard seed (*Brassica nigra*) (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min) *Salmonella* spp. inoculated alpine strawberry juice

Groups		**Mean ± Std.**
Control		3.13^{a} ± .118
0.6 g/L of MuS		2.53^{b} ± .101
90%	**1 min**	$.92^{bc}$ ± .807
	5 min	ND
	10 min	ND
Combination	**90% - 1 min of US + 0.6 g/L of MuS**	1.09^{bc} ± .954
	90% - 5 min of US + 0.6 g/L of MuS	$.84^{bc}$ ± .773
	90% - 10 min of US + 0.6 g/L of MuS	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.13 The counts of the *Listeria monocytogenes* inoculated and ultrasonicated alpine strawberry juice

Groups		Mean ± Std.
Control		3.34[a] ± .118
30%	**1 min**	3.32[a] ± .204
	5 min	3.25[a] ± .244
	10 min	3.30[a] ± .190
60%	**1 min**	2.88[a] ± .101
	5 min	2.80[a] ± .173
	10 min	2.56[a] ± .067
90%	**1 min**	2.73[a] ± .079
	5 min	1.68[a] ± 1.462
	10 min	1.39[b] ± 1.202

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.14 The counts of the *Listeria monocytogenes* inoculated and black mustard seed (*Brassica nigra*) added alpine strawberry juice

Groups	Mean ± Std.
Control	3.34^{a} ± .118
0.2 g/L of MuS	2.89^{a} ± .200
0.4 g/L of MuS	1.80^{b} ± 1.565
0.6 g/L of MuS	1.67^{b} ± 1.445

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.15 The microbial counts of the combination of black mustard seed (*Brassica nigra*) (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min) *Listeria monocytogenes* inoculated alpine strawberry juice

Groups		**Mean ± Std.**
Control		3.13^{a} ± .118
0.6 g/L of MuS		1.67^{b} ± 1.445
90%	**1 min**	2.73^{a} ± .079
	5 min	1.68^{b} ± 1.462
	10 min	1.39^{b} ± 1.202
Combination	**90% - 1 min of US + 0.6 g/L of MuS**	1.09^{b} ± .954
	90% - 5 min of US + 0.6 g/L of MuS	$.84^{b}$ ± .773
	90% - 10 min of US + 0.6 g/L of MuS	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.16 The total mesophilic aerobic bacteria, and yeast/mold counts of the blackberry juice (Control)

	0. Day	**20. Day**	**40. Day**	**60. Day**
TMAB	ND	ND	ND	ND
YM	2.74 log	3.60 log	5.52 log	5.54 log

ND refers not detected

Appendix A.17 The microbial counts of the ultrasonicated blackberry juice

Groups		TMAB	YM
		Mean ± Std.	**Mean ± Std.**
Control		ND	2.66[a] ± .299
30%	**1 min**	ND	1.31[a] ± 1.263
	5 min	ND	2.12[a] ± .359
	10 min	ND	1.69[a] ± 1.471
60%	**1 min**	ND	1.30[a] ± 1.136
	5 min	ND	1.44[a] ± 1.251
	10 min	ND	1.80[a] ± 1.559
90%	**1 min**	ND	1.03[a] ± .892
	5 min	ND	1.26[a] ± 1.097
	10 min	ND	1.87[a] ± 1.623

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.18 The microbial counts of the black mustard seed (*Brassica nigra*) added blackberry juice

Groups	TMAB	YM
	Mean ± Std.	**Mean ± Std.**
Control	ND	2.66^{a} ± .299
0.2 g/L of MuS	ND	1.40^{a} ± 1.221
0.4 g/L of MuS	ND	1.20^{b} ± 1.087
0.6 g/L of MuS	ND	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.19 The shelf-life determination studies of the ultrasonicated (90%), black mustard seed (0.6 g/L) added, and combination of black mustard seed and ultrasonication (90%. - 0.6 g/L) treated blackberry juice: The total mesophilic aerobic bacteria, and yeast and mold counts

	Groups	0. day	20. day	40. day	60. day
TMAB	**90% - 1 min of US**	ND	ND	ND	ND
	90% - 5 min of US	ND	ND	ND	ND
	90% - 10 min of US	ND	ND	ND	ND
	0.6 g/L of MuS	ND	ND	ND	ND
	Combination (90% - 1 min US, 0.6 g/L MuS)	ND	ND	ND	ND
	Combination (90% - 5 min US, 0.6 g/L MuS)	ND	ND	ND	ND
	Combination (90% - 10 min US, 0.6 g/L MuS)	ND	ND	ND	ND
	Control	ND	ND	ND	ND
YM	**90% - 1 min of US**	$1.03^{g} \pm .892$	$1.57^{g} \pm 1.358$	$4.70^{h} \pm .212$	$5.17^{h} \pm .015$
	90% - 5 min of US	$1.26^{i} \pm 1.097$	$2.49^{ik} \pm 2.169$	$4.72^{jk} \pm .191$	$5.23^{j} \pm .050$
	90% - 10 min of US	$1.8^{l} \pm 1.623$	$4.25^{m} \pm .300$	$4.84^{m} \pm .290$	$5.44^{m} \pm .035$
	0.6 g/L of MuS	ND	ND	ND	ND
	Combination (90% - 1 min US, 0.6 g/L MuS)	$1.19^{n} \pm 1.053$	$.98^{n} \pm .853$	$.79^{n} \pm .762$	ND
	Combination (90% - 5 min US, 0.6 g/L MuS)	$.48^{o} \pm .826$	$.55^{o} \pm .958$	$2.70^{p} \pm .382$	$3.20^{p} \pm .080$
	Combination (90% - 10 min US, 0.6 g/L MuS)	$1.96^{r} \pm .660$	$1.73^{r} \pm 1.499$	$3.96^{s} \pm .509$	$4.76^{s} \pm .075$
	Control	$2.66^{t} \pm .299$	$3.59^{u} \pm .115$	$5.52^{v} \pm .015$	$5.54^{v} \pm .060$

Data are means ± Std. from three replicates
Values followed by different letters in the same line of TMAB and YM except lines of mean are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.20 The counts of the *E. coli* 0157:H7 inoculated and ultrasonicated blackberry juice

Groups		Mean	±	Std.
Control		2.94[a]	±	.060
30%	**1 min**	1.49[ab]	±	1.295
	5 min	2.11[ab]	±	.623
	10 min	1.04[ab]	±	1.002
60%	**1 min**	1.10[ab]	±	.971
	5 min	.16[b]	±	.277
	10 min	ND		
90%	**1 min**	.16[b]	±	.277
	5 min	ND		
	10 min	ND		

Data are means ± SD from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$)
ND refers not detected

Appendix A.21 The counts of the *E. coli* 0157:H7 inoculated and black mustard seed (*Brassica nigra*) added blackberry juice

Groups	Mean ± Std.
Control	2.94^{a} ± .060
0.2 g/L of MuS	$.84^{b}$ ± .773
0.4 g/L of MuS	$.67^{b}$ ± .777
0.6 g/L of MuS	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.22 The microbial counts of the combination of black mustard seed added (*Brassica nigra*) (0.6 g/L) and ultrasonicated (90%, 1, 5, and 10 min) *E. coli* 0157:H7 inoculated blackberry juice

Groups		**Mean ± Std.**
Control		2.94^{a} ± .060
0.6 g/L of MuS		ND
90%	**1 min**	$.16^{b}$ ± .277
	5 min	ND
	10 min	ND
Combination	**90% - 1 min of US + 0.6 g/L of MuS**	ND
	90% - 5 min of US + 0.6 g/L of MuS	ND
	90% - 10 min of US + 0.6 g/L of MuS	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.23 The counts of the *Salmonella* spp. inoculated and ultrasonicated blackberry juice

Groups		Mean ± Std.
Control		2.85[a] ± .289
30%	**1 min**	2.73[a] ± .079
	5 min	2.50[ab] ± .436
	10 min	2.31[ab] ± .446
60%	**1 min**	1.22[c] ± 1.054
	5 min	.92[c] ± .807
	10 min	.41[c] ± .710
90%	**1 min**	.79[c] ± .762
	5 min	ND
	10 min	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.24 The counts of the *Salmonella* spp. inoculated and black mustard seed (*Brassica nigra*) added blackberry juice

Groups	Mean ± Std.
Control	2.85^{a} ± .289
0.2 g/L of MuS	$.77^{b}$ ± .949
0.4 g/L of MuS	$.67^{b}$ ± .777
0.6 g/L of MuS	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.25 The counts of the combination of black mustard seed (*Brassica nigra*) (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min) *Salmonella* spp. inoculated blackberry juice

Groups		**Mean ± Std.**
Control		2.85^{a} ± .289
0.6 g/L of MuS		ND
90%	**1 min**	$.79^{b}$ ± .762
	5 min	ND
	10 min	ND
Combination	**90% - 1 min of US + 0.6 g/L of MuS**	ND
	90% - 5 min of US + 0.6 g/L of MuS	ND
	90% - 10 min of US + 0.6 g/L of MuS	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.26 The counts of the *Listeria monocytogenes* inoculated and ultrasonicated blackberry juice

Groups		Mean ± Std.
Control		1.43[a] ± 1.243
30%	**1 min**	1.38[a] ± 1.200
	5 min	1.37[a] ± 1.186
	10 min	1.15[a] ± 1.004
60%	**1 min**	.52[b] ± .906
	5 min	.47[b] ± .814
	10 min	.37[b] ± .641
90%	**1 min**	ND
	5 min	ND
	10 min	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.27 The counts of the *Listeria monocytogenes* inoculated and black mustard seed (*Brassica nigra*) added blackberry juice

Groups	Mean ± Std.
Control	1.43^{a} ± 1.243
0.2 g/L of MuS	$.49^{b}$ ± .854
0.4 g/L of MuS	ND
0.6 g/L of MuS	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.28 The counts of the combination of black mustard seed (*Brassica nigra*) (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min) *Listeria monocytogenes* inoculated blackberry juice

Groups		**Mean ± Std.**
Control		1.43^{a} ± 1.243
0.6 g/L of MuS		ND
90%	**1 min**	ND
	5 min	ND
	10 min	ND
Combination	**90% - 1 min of US + 0.6 g/L of MuS**	ND
	90% - 5 min of US + 0.6 g/L of MuS	ND
	90% - 10 min of US + 0.6 g/L of MuS	ND

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.29 The total mesophilic aerobic bacteria (TMAB), and yeast/mold counts of the apple juice (Control)

	0. Day	20. Day	40. Day	60. Day
TMAB	2.66 log	2.85 log	5.31 log	5.54 log
YM	3.72 log	2.80 log	2.41 log	ND

ND refers not detected

Appendix A.30 The microbial counts of the ultrasonicated apple juice

Groups		TMAB			YM		
		Mean	**±**	**Std.**	**Mean**	**±**	**Std.**
Control		2.51[a]	±	.433	3.36[a]	±	.683
30%	**1 min**	2.54[a]	±	.408	3.42[a]	±	.773
	5 min	2.46[a]	±	.319	3.24[a]	±	.473
	10 min	2.20[a]	±	.652	2.86[a]	±	.858
60%	**1 min**	2.47[a]	±	.050	3.07[a]	±	.681
	5 min	1.65[a]	±	1.432	2.49[a]	±	.870
	10 min	1.53[a]	±	1.325	2.46[a]	±	.813
90%	**1 min**	1.62[a]	±	1.407	2.46[a]	±	.550
	5 min	1.58[a]	±	1.373	1.55[b]	±	1.359
	10 min	1.45[a]	±	1.271	.61[b]	±	1.057

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.31 The counts of the black mustard seed (*Brassica nigra*) added apple juice

	TMAB			YM		
Groups	**Mean**	**±**	**Std.**	**Mean**	**±**	**Std.**
Control	2.51[a]	±	.433	3.36[a]	±	.683
0.2 g/L of MuS	2.30[a]	±	.203	.94[b]	±	.821
0.4 g/L of MuS	1.54[a]	±	1.342	.84[b]	±	.773
0.6 g/L of MuS	1.40[a]	±	1.217	.33[b]	±	.577

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.32 The shelf-life determination studies of the ultrasonicated (90%), black mustard seed (0.6 g/L) added, and the combination of black mustard seed and ultrasonication (90% - 0.6 g/L) treated apple juice: The TMAB and YM counts

	Groups	0. day	20. day	40. day	60. day
TMAB	**90% - 1 min of US**	$1.62^{a} \pm 1.407$	$1.23^{a} \pm 1.065$	$1.26^{a} \pm 1.166$	$3.66^{a} \pm .010$
	90% - 5 min of US	$1.58^{b} \pm 1.373$	$1.12^{b} \pm .979$	$2.79^{bc} \pm .550$	$4.74^{c} \pm .035$
	90% - 10 min of US	$1.45^{de} \pm 1.271$	$1.12^{d} \pm .979$	$3.54^{ef} \pm .196$	$5.03^{f} \pm .030$
	0.6 g/L of MuS	$1.40^{g} \pm 1.217$	$1.50^{g} \pm 1.323$	$4.89^{h} \pm .040$	$5.11^{h} \pm .030$
	Combination (90% - 1 min US, 0.6 g/L MuS)	$1.49^{i} \pm 1.289$	$1.35^{i} \pm 1.185$	$1.29^{i} \pm 1.261$	$.48^{i} \pm .826$
	Combination (90% - 5 min US, 0.6 g/L MuS)	$1.32^{j} \pm 1.141$	$1.51^{j} \pm 1.312$	$3.12^{jk} \pm 1.030$	$4.75^{k} \pm .045$
	Combination (90% - 10 min US, 0.6 g/L MuS)	$1.24^{l} \pm 1.074$	$1.55^{l} \pm 1.348$	$3.02^{lm} \pm 1.477$	$5.07^{m} \pm .025$
	Control	$2.51^{n} \pm .433$	$2.63^{n} \pm .520$	$5.31^{o} \pm .006$	$5.54^{o} \pm .006$
YM	**90% - 1 min of US**	$2.46^{e} \pm .550$	$1.45^{e} \pm 1.260$	$.48^{e} \pm .826$	$.41^{e} \pm .710$
	90% - 5 min of US	$1.55^{fg} \pm 1.359$	$2.32^{g} \pm .224$	$.77^{f} \pm .949$	ND
	90% - 10 min of US	$.61^{i} \pm 1.057$	$1.49^{i} \pm 1.297$	$.67^{i} \pm .777$	ND
	0.6 g/L of MuS	$.33^{j} \pm .577$	$.59^{j} \pm 1.016$	ND	ND
	Combination (90% - 1 min US, 0.6 g/L MuS)	$2.12^{k} \pm .352$	$1.50^{kn} \pm 1.300$	ND	ND
	Combination (90% - 5 min US, 0.6 g/L MuS)	$1.28^{o} \pm 1.109$	$1.42^{o} \pm 1.230$	ND	ND
	Combination (90% - 10 min US, 0.6 g/L MuS)	$.94^{p} \pm .821$	$1.30^{p} \pm 1.137$	ND	ND
	Control	$3.36^{r} \pm .683$	$2.63^{r} \pm .471$	$1.77^{rs} \pm 1.187$	$.16^{s} \pm .277$

Data are means ± Std. from three replicates
Values followed by different letters in the same line of TMAB and YM except lines of mean are significantly different ($p < 0.05$).
ND refers not detected

Appendix A.33 The counts of the *E. coli* 0157:H7 inoculated and ultrasonicated apple juice

Groups		Mean ± Std.
Control		4.15[a] ± .137
30%	**1 min**	4.12[a] ± .105
	5 min	4.12[a] ± .085
	10 min	4.09[a] ± .095
60%	**1 min**	4.07[a] ± .080
	5 min	4.02[a] ± .025
	10 min	4.02[a] ± .015
90%	**1 min**	4.01[a] ± .015
	5 min	3.89[a] ± .015
	10 min	3.35[b] ± .020

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.34 The counts of the *E. coli* 0157:H7 inoculated and black mustard seed (*Brassica nigra*) added apple juice

Groups	Mean	±	Std.
Control	4.15[a]	±	.137
0.2 g/L of MuS	4.14[a]	±	.050
0.4 g/L of MuS	4.08[a]	±	.015
0.6 g/L of MuS	3.96[b]	±	.085

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.35 The counts of the combination of black mustard seed added (*Brassica nigra*) (0.6 g/L) and ultrasonicated (90%, 1, 5, and 10 min) *E. coli* 0157:H7 inoculated apple juice

Groups		**Mean ± Std.**
Control		4.15^{a} ± .137
0.6 g/L of MuS		3.96^{b} ± .085
90%	**1 min**	4.01^{a} ± .015
	5 min	3.89^{b} ± .015
	10 min	3.35^{b} ± .020
Combination	**90% - 1 min of US + 0.6 g/L of MuS**	3.93^{b} ± .015
	90% - 5 min of US + 0.6 g/L of MuS	3.55^{b} ± .250
	90% - 10 min of US + 0.6 g/L of MuS	1.57^{c} ± 1.404

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.36 The counts of the *Salmonella* spp. inoculated and ultrasonicated apple juice

Groups		Mean ± Std.
Control		4.96[a] ± .000
30%	**1 min**	4.79[ab] ± .010
	5 min	4.74[ab] ± .085
	10 min	4.71[ab] ± .080
60%	**1 min**	4.69[ab] ± .105
	5 min	4.68[ab] ± .075
	10 min	4.66[ab] ± .065
90%	**1 min**	4.64[b] ± .045
	5 min	4.50 [b] ± .150
	10 min	3.47[c] ± .096

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.37 The counts of the *Salmonella* spp. inoculated and black mustard seed (*Brassica nigra*) added apple juice

Groups	Mean	±	Std.
Control	4.96[a]	±	.000
0.2 g/L of MuS	4.66[b]	±	.060
0.4 g/L of MuS	4.58[b]	±	.100
0.6 g/L of MuS	4.37[c]	±	.095

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.38 The counts of the combination of black mustard seed (*Brassica nigra*) (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min) *Salmonella* spp. inoculated apple juice

Groups		**Mean ± Std.**
Control		4.96^{a} ± .000
0.6 g/L of MuS		4.37^{b} ± .095
90%	**1 min**	4.64^{b} ± .045
	5 min	4.50^{b} ± .150
	10 min	3.47^{c} ± .096
Combination	**90% - 1 min of US + 0.6 g/L of MuS**	4.52^{b} ± .090
	90% - 5 min of US + 0.6 g/L of MuS	4.34^{b} ± .080
	90% - 10 min of US + 0.6 g/L of MuS	3.94^{c} ± .060

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.39 The counts of the *Listeria monocytogenes* inoculated and ultrasonicated apple juice

Groups		Mean ± Std.
Control		5.41[a] ± .035
30%	**1 min**	5.39[a] ± .015
	5 min	5.39[a] ± .020
	10 min	5.38[a] ± .025
60%	**1 min**	5.36[a] ± .060
	5 min	5.35[a] ± .050
	10 min	5.35[a] ± .075
90%	**1 min**	5.31[b] ± .105
	5 min	5.27[b] ± .115
	10 min	5.15[b] ± .146

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.40 The counts of the *Listeria monocytogenes* inoculated and black mustard seed (*Brassica nigra*) added apple juice

Groups	Mean ± Std.
Control	5.41^{a} ± .035
0.2 g/L of MuS	5.32^{b} ± .040
0.4 g/L of MuS	5.28^{b} ± .035
0.6 g/L of MuS	5.25^{b} ± .055

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.41 The counts of the combination of black mustard seed (*Brassica nigra*) (0.6 g/L) added and ultrasonicated (90%, 1, 5, and 10 min) *Listeria monocytogenes* inoculated apple juice

Groups		**Mean ± Std.**
Control		5.41^{a} ± .035
0.6 g/L of MuS		5.25^{b} ± .055
90%	**1 min**	5.31^{b} ± .105
	5 min	5.27^{b} ± .115
	10 min	5.15^{b} ± .146
Combination	**90% - 1 min of US + 0.6 g/L of MuS**	5.30^{b} ± .025
	90% - 5 min of US + 0.6 g/L of MuS	5.28^{b} ± .045
	90% - 10 min of US + 0.6 g/L of MuS	5.26^{b} ± .040

Data are means ± Std. from three replicates
Values followed by different letters in the same column are significantly different ($p < 0.05$).

Appendix A.42 Preparation of DNS Solution

10.6 g of DNS (2-hydroxy-3,5 dinitro benzoic acid), 19.8 g of sodium hydroxide, 7.6 g of phenol, 8.3 g of sodium metabisulphide, 306 g of sodium potassium tartarate were dissolved in 1416 g distilled water.

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