

**ELIMINATION OF CERTAIN FOODBORNE PATHOGENS IN
VARIOUS FRUIT JUICES BY COMBINATION OF
ULTRASONICATION AND *EAU DE ROSE***

by

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ABSTRACT

ELIMINATION OF CERTAIN FOODBORNE PATHOGENS IN VARIOUS FRUIT JUICES BY COMBINATION OF ULTRASONICATION AND *EAU DE ROSE*

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Fruit juices can harbor a variety of microorganisms and can be important sources of foodborne pathogenesis. Mostly known as; the pathogenic strains *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp., induce food poisoning owing to the consumption of spoiled 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 *eau de rose*, sole ultrasonication and a combination of *eau de rose* and ultrasonication was used to inactivate these pathogenic species in peach and apricot juices. The combination of 90 dB amplitude at 24 kHz frequency of ultrasound and 20 µl/L *eau de rose* resulted most effective inhibition to total mesophilic aerobic bacteria and the pathogens mentioned above, as per to the control, without disrupting the main chemical and physical properties of the fruit juices.

Keywords: *Eau de rose*, *E.coli* O157: H7, Fruit juice, Food poisoning, *Listeria monocytogenes*, *Salmonella* spp., Ultrasonication.

ÖZET

ULTRASONİKASYON VE GÜL SUYU BİLEŞİMİYLE ÇEŞİTLİ MEYVE SULARINDA BELİRLİ BESİN PATOJENLERİNİN ELİMİNASYONU

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Meyve suları, çeşitli mikroorganizmalar barındırabilirler ve bunlar önemli gıda kaynaklı patojen olabilirler. Bozulmuş meyve sularının ve diğer gıda materyallerinin tüketimine dayalı gıda zehirlenmesinden sorumlu olan *Escherichia coli* O157:H7, *Listeria monocytogenes* ve *Salmonella* spp. en çok bilinen patojenlerdir. Şimdilerde bu enfeksiyonlarla etkin mücadele edebilmek için karışık teknolojiler gıda endüstrisinde büyük önem kazanmıştır. Isıl olmayan yöntemlerin doğal maddelerle kombinasyonu bu teknolojiler için alternatif bir yoldur. Bu çalışmada, yalnızca gül suyu, yalnızca ultrasonikasyon ve gül suyu ve ultrasonikasyon bileşimi, kayısı ve şeftali sularındaki patojenik türleri inaktive etmek için kullanılmıştır. Ultrasonikasyonun 24 kHz frekanstaki 90 dB'lik genliği ve gül suyunun 20 µL/L dozunun bileşiminin, kontrole göre fiziksel ve kimyasal özelliklerini bozmadan toplam mezofilik aerobik bakteriler ve yukarıda sözü geçen patojenler üzerinde en etkili inhibisyona yol açtığı bulunmuştur.

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

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LIST OF ABBREVIATIONS

AC	: Activated Cell
a_w	: Water Activity
<i>B. nigra</i>	: <i>Brassica nigra</i>
CFU	: Colony Forming Unit
CG	: Control Group
CoNS	: Coagulase Negative <i>Staphylococcus</i>
dB	: Decibel
DAEC	: Diffusely Adherent <i>E. coli</i>
DEC	: Diarrhoeagenic <i>E. coli</i>
DNS	: Dinitrosalicylic Acid
E.C.	: Enzyme Classification
EAggEC	: Enteraggregative <i>E. coli</i>
EC	: European Commission
EHEC	: Enterohemorrhagic <i>E.coli</i>
EIEC	: Enteroinvasive <i>E. coli</i>
EMB agar	: Eosin Methylene Blue Agar
EO	: Essential Oils

EPEC	: Enteropathogenic <i>E. coli</i>
ER	: <i>Eau de rose</i>
<i>E. coli</i>	: <i>Escherichia coli</i>
ETEC	: Enterotoxigenic <i>E. coli</i>
FD	: Factor of Dilution
FDA	: Food and Drug Administration
g	: Gram
GLS	: Glucosinolates
GOX	: Glucose oxidase
HC	: Hemorrhagic Colitis
HHP	: High Hydrostatic Pressure
HUS	: Hemolytic Uremic Syndrome
Hz	: Hertz
IR	: Ionizing Radiation
kHz	: Kilohertz
kPa	: Kilo Pascal
kV	: Kilovolts
<i>L. monocytogenes</i>	: <i>Listeria monocytogenes</i>
L	: Liter
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
MS	: Manosonication
Ms	: microseconds
N	: Normality of NaOH
NaCl	: Sodium Chloride
NaOH	: Sodium Hydroxide
Nm	: Nanometer
O.D.	: Optical Density
°C	: Centigrade Degree
PCA	: Plate Count Agar
PEF	: Pulsed Electric Field
<i>S. rouxii</i>	: <i>Saccharomyces rouxii</i>
<i>S. aureus</i>	: <i>Staphylococcus aureus</i>

<i>S. bayanus</i>	: <i>Saccharomyces bayanus</i>
<i>S. biphorus</i>	: <i>Saccharomyces biphorus</i>
<i>S. cerevisiae</i>	: <i>Saccharomyces cerevisiae</i>
SD	: Standard deviation
sp.	: Species
spp.	: Species
Std	: Standard
STEC	: Shiga toxin producing <i>E. coli</i>
TA	: Titratable Acidity
TMAB	: Total Mesophilic Aerobic Bacteria
TSA	: Tryptic Soy Agar
Uc	:Uncountable
US	: Ultrasonication
USDA	: United States Department of Agriculture
V	: Titer Volume of NaOH
VTEC	: Vero cytotoxin producing <i>E. coli</i>
W	: Watts
XLD agar	: Xylose Lysine Deoxycholate Agar
YEA	: Yeast Extract Agar
YM	: Yeasts and Molds

CHAPTER 1

INTRODUCTION

1.1. Food Preservation Methods

The microbial stability and safety of most traditional and novel foods is based on a combination of several factors, called the hurdles. Fundamental importance of hurdles' effect is the preservation of foods. Foods preserved by combined methods remain stable and safe even without refrigeration, and are high in sensory and nutritive properties due to the gentle processes applied. The concept is gaining ground in industrialized as well as in developing countries. In addition, several chemical sanitizers cannot be used in organic food processing and furthermore, consumers' increasingly demand that food industries reduce their use of chemical additives. Therefore, researchers are seeking new ways to reduce the pathogens significantly and chemicals simultaneously to ensure the safety of organic produce (Sagong et al., 2011).

1.2. The juice processing and novel methods

Over the past several decades there has been a growing trend toward adding value to raw agricultural products. Within the globalization of the food industry, the demand for quality juice and juice type beverages has markedly expanded. Consumers have less time to dedicate to food preparation, also seeking natural products that have been minimally processed in order to avoid destruction or partial

removal of the health promoting substances present in fruits. Alternative technologies for inactivating microorganisms without relying on heat are not new concepts (Ross et al., 2003). These novel non-thermal technologies have the ability to inactivate microorganisms at ambient or near-ambient temperatures, thereby avoiding the deleterious effects that heat has on the flavour, colour and nutrient value of foods.

In particular, high hydrostatic pressure (HHP) can alter the structure of proteins and polysaccharides, causing changes in the texture, physical appearance and functionality of foods (Ross et al., 2003). High-intensity ultrasound also can denature proteins and produce free radicals that can adversely affect the flavour of fruit based or high-fat foods.

The irradiation process, ultrasonication, and pulsed electric field (PEF) are the other examples of alternative technology.

1.2.1 Ultrasonication

Sonication is the act of applying sound (usually ultrasound) energy to agitate particles in a sample, for various purposes. In the laboratory, it is usually applied using an *ultrasonic bath* or an *ultrasonic probe*, colloquially known as a *sonicator*. Ultrasound utilizes interaction between high frequency sound waves and matter to obtain information about the composition, structure and dimensions of materials through which it propagates.

Treatments with power-ultrasound (20–100 kHz) are an emerging technology for the preservation of food alone and in combination with other novel processing technologies (Christen et al., 2012). Power-ultrasound creates cavitation, that is, the formation, growth and implosive collapse of bubbles in liquids. During the collapse

of these bubbles, localised hot spots occurs with temperatures of roughly 5000 °C, pressures of about 50 MPa and a lifetime of a few microseconds. The pressure changes resulting from these implosions create shock waves that disrupt the cellular membranes of bacteria resulting in cell lysis (Hartmann et al., 2012).

Destruction of micro-organisms by ultrasound has been studied with a view toward in fruit juice as an alternative supplement to traditional sterilization methods (Villamiel et al., 2000). Thermal treatment can cause undesirable alterations of sensory attributes, i.e. texture, flavor, color, smell.

1.3. Eau de rose

Rose (*Rosa damascena* Mill.) is produced by distilling volatile oils from the flowers. The most common aroma concentrates of rose are rose oil and rose water, derived from steam distillation. *Rosa damascena* Mill (which is known as Gole Mohammadi in Persian) belongs to the family of Rosaceae, many species and variety of which are cultivated throughout the world as an ornamental plant (Loghmani-Khouzani et al., 2007). Turkey is amongst the leading producers of rose oil. The rose oil is produced mainly in Isparta, Burdur and Denizli provinces (Tosun et al., 2002).

In recent years, antioxidant, antibacterial and antimicrobial activities have been reported for *Rosa damascena* essential oil (Ardogan et al., 2002; Achuthan et al., 2003; Ozkan et al., 2004). Also roses are always appreciated because of their inimitable aroma, many uses and of course their beauty. In addition to the different damask rose (*Rosa damascena* Mill.) products (oil, water, concrete, absolute, gulkand etc.), its dried petals are also used for various health purposes (Verma et al., 2011).

Roses have solid (Stearoptene) and liquid (Oleoptene) component. They are rich in corilagin and tellimagrandin (Shiota et al., 2004) and this compounds has significantly antimicrobial activity. Corilagin has strong antioxidative, hepatoprotective (Kinoshita et al., 2007), thrombolytic (Shen et al., 2003), antiatherogenic (Duan et al., 2005), antihypertensive (Cheng et al., 1995) effects that has been reported.

1.4. Fruit Juices

1.4.1. Apricot (*Prunus armeniaca*)

Apricot (*Prunus armeniaca* L.) is classified under the *Prunus* genus of Prunoidae sub-family of the Rosaceae family of the Rosales group. Apricot has an important place in human nutrition and can be used as fresh, dried or processed fruit (Ozsahin et al., 2010). Its scientific name *Prunus armeniaca* (Armenian plum) derives from that assumption. Despite the great number of varieties of apricots that are grown in Armenia today, according to Vavilov, its center of origin would be the Chinese region, where the domestication of apricot would have taken place. Other sources say that the apricot was first cultivated in India in about 3000 BC.

Turkey is the leading apricot producer of the world. According to the FAO statistical database in 2005, 390000 tons of apricot were produced in Turkey contributing to ca. 13 % of the total apricot production in the world (Ozsahin et al., 2010). Malatya region of eastern Turkey is particularly important for cultivation, production and processing of apricots. Moreover, the apricots grown in this region has a reputation for their characteristics and quality. The climate, structure and content of the soil and the other environmental conditions in Malatya region enable

the production of quality apricots with dry matter and sugar content (Ozsahin *et al.*, 2010).

Although the apricot is native to a continental climate region with cold winters, it can grow in Mediterranean climates if enough cool winter weather allows a proper dormancy. The dry climate of these areas is good for fruit maturation. The tree is slightly more cold-hardy than the peach, tolerating winter temperatures as cold as -30°C or lower if healthy. A limiting factor in apricot culture is springfrosts: They tend to flower very early, meaning spring frost can kill the flowers. Furthermore, the trees are sensitive to temperature changes during the winter season.

Cultivators have created what is known as a "black apricot", but this is not a genuine apricot, as it is a hybrid between an apricot and a plum. This fruit is variously called plumcots, apriplums, pluots, or apriums.

An old adage says an apricot tree will not grow far from the mother tree; the implication is that apricots are particular about the soil conditions in which they are grown. They prefer well-drained soils with a pH of 6.0 to 7.0. Some apricot cultivars are self-compatible and do not require pollinizer trees.

1.4.2. Peach (*Prunus persica*)

The peach, *Prunus persica*, is a deciduous tree, native to China and South Asia, that it was first cultivated. It bears an edible juicy fruit also called a peach. The species name *persica* refers to its widespread cultivation in Persia, it was transplanted from Europe. It belongs to the genus *Prunus* which includes the cherry and plum, in the family Rosaceae. The peach is classified with the almond in the subgenus *Amygdalus*, distinguished from the other subgenera by the corrugated seed shell.

Two types are known in Turkey, one with white and the other with yellow flesh. The region of Bursa is famous for its large and juicy peaches that can be split into exposing its seed.

Several researchers have implied that peach is an excellent source for potassium, iron, fiber, vitamin A, vitamin C and other vitamins and also contains high concentrations of phytochemicals such as carotenoids, flavonols and anthocyanins (Hancock and Scorza, 2008; Scorza and Okie, 1991; Crisosto and Costa, 2008). Ascorbic acid (vitamin C), carotenoids and phenolic compounds represent the major sources of antioxidants in peaches (Hajilou and Fakhimrezaei, 2011). The total measured anti-oxidant strength (ORAC value) of 100 g peach fruit is 1814 TE (Trolox equivalents). Fresh peaches are a moderate source of antioxidant, *vitamin C*. It is required for connective tissue synthesis in the body. Consumption of foods rich in vitamin C helps the body develop resistance against infectious agents. Peaches contain health promoting flavonoid polyphenolic antioxidants such as *lutein*, *zea-xanthin* and *β -cryptoxanthin*. These compounds help act as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that play a role in aging and various disease processes.

1.4.3. Foodborne Pathogens in Fruit Juices

Escherichia coli O157:H7, *Salmonella* spp, and *Listeria monocytogenes* are the main pathogens implicated in several foodborne outbreaks related to fresh produce (Sagong et al., 2011). Also yeast species cause foodborne outbreaks.

1.4.3.1. *E.coli* O157:H7

Theodor Escherich first described *E. coli* in 1885, as *Bacterium coli commune*, isolated from the feces of newborns. It was later renamed *Escherichia coli*, and for many years the bacterium was simply considered to be a commensal organism of the large intestine. *E. coli* is the head of the large bacterial family, *Enterobacteriaceae*, the enteric bacteria which is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped, and are about 2.0 microns (μm) long and 0.5 μm in diameter, with a cell volume of 0.6–0.7 (μm). It can live on a wide variety of substrates.

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

Enterohaemorrhagic *E. coli* (EHEC) is a bacterium that can cause severe foodborne disease. EHEC produces toxins, known as verotoxins or Shiga-like toxins because of their similarity to the toxins produced by *Shigella dysenteriae*. EHEC can grow in temperatures ranging from 7°C to 50°C, with an optimum temperature of 37°C. *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). Some EHEC can grow in acidic foods, down to a pH of 4.4, and in foods with a minimum water activity (A_w) of 0.95. Low a_w prevents microbial spoilage in dry sausage. It is destroyed by thorough cooking of foods until all parts reach a

temperature of 70°C or higher. Primary sources of EHEC outbreaks are raw or undercooked ground meat products, raw milk and faecal contamination of vegetables, fruits, juices, drinking water and sprouts was reported to cause outbreaks (Powell et al., 2011). In most cases, the illness is self-limiting, but it may lead to a life-threatening disease including haemolytic uraemic syndrome (HUS), especially in young children and the elderly.

1.4.3.2. *Salmonella* spp.

Salmonella is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and flagella that grade in all directions. They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes (Leyer and Johnson, 1992).

Salmonella is closely related to the *Escherichia* genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever, and foodborne illness. *Salmonella* are a group of food poisoning organisms which are of considerable significance to the food processing industry (Joseph et al., 2000).

Salmonella spp. are known to induce adaptive responses to various stresses, including acids, salts, and temperature, and these adaptive responses may enhance survival in deleterious environments. Foster and Hall demonstrated that *Salmonella typhimurium* expresses an acid tolerance response when exposed to external pHs of 5.5 to 6.0 and that the response protects the cells from more severe acid stress (Leyer and Johnson, 1992).

During harvesting of fruits that are dropped from trees to the ground, 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. *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).

According to the Public Health Agency of Canada, worldwide, more extensively in North America and Europe; higher incidence rate for infants and young children were reported; small outbreaks in general population; large outbreaks in hospitals, institutions, nursing homes, restaurants; but in addition to this; 2-3 million infections annually in USA, most go unreported; *S. enteritidis* and *S. typhimurium* are the most common in North America (Oliver *et al.*, 2005).

1.4.3.3. *Listeria monocytogenes*

Listeria monocytogenes is one of the most important food-borne pathogens causing illness in humans and animals. Because of *Listeria monocytogenes*'s ability to grow and multiply at refrigerated temperatures, it gets an important matter in ready-to-eat foods. Listeriosis cases are often occurred by resulting from the foods contaminated with *Listeria monocytogenes* (Yavuz and Mihriban, 2010).

Listeria monocytogenes is a Gram-positive, rod-shaped, non-spore-forming, and facultative anaerobe bacterium (Vazquez-Boland et al., 2001; Sukhadeo et al., 2009). The pathogen is 1 to 2 μ m long and may exist as single or double cells but display long chains depending on the growth (Arun, 2008). It has the ability to grow in a wide range of temperature (1 to 45°C), although optimal growth occurs at 30 to 37°C (Swaminathan et al., 1995). *L. monocytogenes* is also tolerant and survives in extreme conditions like a wide pH range (4.1 to 9.6), high salt (10%) concentrations and in the presence of antimicrobial agents (Liu et al., 2005; Arun, 2008). *L. monocytogenes* has been described as opportunistic pathogen affecting mainly children, pregnant women, the aged and immune-challenged individuals (Schlech, 2000; Liu, 2006).

There are six species under the genus *Listeria* notably; *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri* and *L. grayi*. Out of these only *L. monocytogenes* have been authentically reported by many researchers as human and animal pathogen (Vazquez-Boland et al., 2001).

In research, various food and environmental samples have been implicated in the spread of *L. monocytogenes*. Thus the pathogen is repeatedly found in meat and meat products, raw milk, soft cheese and pasteurised dairy products, vegetables, and fish and fish products. For example, *L. monocytogenes* has been isolated from sheep, goat and cow milk (Rahimi et al., 2010), chopping boards, cleaning cloths, mincing machine, poultry meat and meat products (Mahmood et al., 2003), cooked meats, cured meats, smoked salmon, soft cheese and vegetables (Vitas et al., 2004), ready-to eat foods (Aurora et al., 2008) and raw and pasteurized egg samples (Rivoal et al., 2010). In the USA, a zero tolerance of *L. monocytogenes* in 25 grams of food have

been recommended (Shank et al., 1996) while in Canada, the tolerance level of below 100/g for some foods and tolerance is zero for others is being adhered to (Farber et al., 1996).

1.4.4. Injured Cell

Cellular injury occurs as a result of physical (ionizing radiation, temperature, mechanic trauma), chemical (drugs, poisons), or biological insults (enzymes, cytokines, viral infections, cell-mediated) or as a result of vital substrate deficiency (Cobb et al., 1996). Injured cells can form colonies on enriched media but not on stressing media (Clark and Ordal, 1969). Injury may be measured by the difference in counts when stressed cells are simultaneously enumerated on selective and nonselective media. Only uninjured cells are recovered on selective medium, whereas the nonselective medium is assumed to recover both injured and uninjured cells (McDonald et al., 1982).

Bacterial injury may be defined simply as the effect of one or more sublethal treatment on a microorganism. By extension, sublethal injury is a consequence of exposure to a chemical or physical process that damages but does not kill a microorganism (Wesche et al., 2008). The term stress has not only been used to describe the effect of sublethal treatments but also in reference to the agents or treatments causing injury. Although there is a tendency to perceive food matrices as metabolically supportive environments, food is frequently bacteriostatic or bactericidal due to intrinsic factors such as water activity (a_w), pH, oxidation-reduction potential, competitive exclusion by protective cultures, and other environmental and processing stresses. The metabolic processes during injury repair vary with the nature of stress and involve the synthesis of ATP, RNA, DNA and

mycopeptides. The structural and functional components known to be damaged by sublethal stresses are the cell wall, cytoplasmic membrane, ribosomal RNA and DNA, as well as some enzymes.

CHAPTER 2

THE AIM AND THE SCOPE OF THE STUDY

The aim of the study is to examine the effects of *eau de rose*, ultrasonication and the combination of them on several foodborne pathogens in apricot and peach juices, to extend their shelf-life. To avoid destruction or partial removal of the health promoting substances present in juices such as antioxidants etc., the behaviour of *eau de rose* as a natural product and the inhibitory effect of ultrasound will be investigated on foodborne pathogens in these fruit juices.

Also, we target to overcome the unwanted side effects of other sterilization techniques that cause functional loss by using either combined or sole ultrasonication method and *eau de rose* as a natural product on foodborne pathogens in fruit juices.

CHAPTER 3

MATERIALS AND THE METHOD

In this study, *eau de rose*, as a natural product, and ultrasonication was used in apricot and peach juices.

3.1. Materials

3.1.1. Eau de rose

Eau de rose was purchased from Gülbirlik A.Ş., Isparta, TURKEY.

3.1.2. Fruits

The peaches and apricots, purchased from a local vendor in Bolu, TURKEY were immediately transported to the laboratory. The defective ones were eliminated and the fruits were washed with tap water and squeezed to obtain their fresh fruit juices by a blender(Moulinex, FRANCE).

3.1.3. The Fruit Juices

The peaches and apricots were processed to clarify their juice in food industry through a subsequent filtration by a sterilized filter paper to separate the pulp before subjecting to *eau de rose* and ultrasonication treatments.

3.1.4. Bacteria

The bacteria (*E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes*) which were inoculated to the fruit juices in order to test the elimination potential of the

combined or alone treatments of ultrasonication and eau de rose, 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 for a period of 1, 5 and 10 minutes at the amplitudes of 30, 60, 90 dB with a frequency of 24 kiloHertz (Hz) (Hielscher Ultrasound Technology, UP 400S Ultrasonic processor, 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 detected temperatures were rising from 15 to 24 °C through ultrasonication process.

3.2.2. The treatment with *eau de rose*

Eau de rose(Gülbirlik A.Ş.) was added to fruit juices at an amount(vol/vol) of 0.1, 0.5, 1.0 and 2.0 ml/L (1µl, 5µl, 10µl and 20µl / 10 mL).

3.2.3. The Effects of The Combined Treatments on The Inhibitory Activity of Foodborne Pathogens

The best effective doses, obtained in Section 3.2.1. of the combined or alone treatments of *eau de rose* and ultrasonication were tested and evaluated on foodborne pathogens in fruit juices.

3.2.4. The treatment with the isolates

The O.D. value of the bacterial suspensions of *Salmonella* spp., *E.coli* O157:H7 and *Listeria monocytogenes* 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 sterilized fruit juice to test the effectiveness of methods.

3.2.5. Determination of the population of viable and injured cells

The injured bacteria was detected in the experiment of the best effective dose selection of *eau de rose*. For this purpose, *E. coli* O157:H7 cells were inoculated with non-selective tryptic soy agar (TSA) and selective agar (TSA supplemented with 1 % NaCl) in order to determine the number of total viable and uninjured cells after the *eau de rose* treatment. 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 taken to determine the number of injured cells (Ray et al., 1998). *Listeria monocytogenes* and *Salmonella* spp. were inoculated to TSA by using a non-selective and TSA supplemented with 4 % NaCl as a selective agar.

Eau de rose-treated cells were serially diluted in distilled water. At first, three dilution tubes, containing 0.1 mL samples were incubated in triplicates on TSA for injured and TSA with NaCl for noninjured cell counts. The plates were then incubated at 37° C for 48 h. Initial inhibition of *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. was calculated by taking 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:

$$[1 - (\text{CFU on TSA with 1\% or 4\% NaCl} / \text{CFU on TSA}) \times 100].$$

3.2.6. The Microbial Identification of Microflora and Microbial Counts

The microflora of fruit juices were identified by the appropriate selective medium of bacteria. The total mesophilic aerobic counts were detected by plate count agar (PCA) (Merck). *Salmonella* identification was carried out by XLD agar (Merck), *E.coli* and gram negative bacteria identification was done by EMB agar (Merck). Mold and yeast identification was carried out by yeast extract agar (Merck). *Listeria monocytogenes* identification was carried out by blood agar (Merck).

The treated and untreated (control) juice samples were diluted with sterile distilled water to enumerate viable cells of the natural micropopulation. Colony counts of total aerobic mesophilic bacteria was determined by standard plate count on plate count agar in triplicates (PCA, Merck). The plates were incubated at 37° C for 24-48 hours in the incubator. Yeast and filamentous fungi counts were carried out by surface plating on yeast extract agar in triplicates (YEA, Merck). The plates were incubated at 25° C for 5 days.

The microbial count values were converted to logarithmic values and in addition, the microbial inhibitions were determined by calculating the growth rate according to the following formula:

Growth Rate = 100 x (mean growth with treatment / mean growth without treatment).

3.2.7. The shelf life of fruit juices

Fruit juices were kept in the refrigerator at + 4°C. In order to detect the microbial quality parameters of shelf life, microbial counts of fruit juices were done at 20th, 40th and 60th days.

3.2.8. The determination of certain physical and chemical properties

3.2.8.1. Total soluble solids (°Brix)

A portable refractometer (Miiler, RHB-10/ATC, Germany) was used for the total soluble solid content of the juices. The measurements were performed at 25 ± 0.5° C and the results were expressed as °Brix.

3.2.8.2. Titratable acidity

In order to detect the titratable acidity (TA), peach and apricot juice samples separately, of 25 mL, and distilled water of 75 mL were placed into a beaker. This solution was subsequently filtrated by filter paper. 25 mL of this filtrated solution was taken to another beaker. 1 g of phenolphthalein was dissolved with 100 mL of 95 % ethyl alcohol at an Erlenmayer flask. Three drops of this phenolphthalein solution were added to the filtrated solution in the beaker. Then this solution was titrated against standardised 0.1 N NaOH to the phenolphthalein end point (pH 8.2 ± 0.1) until the solution got pink. The volume of NaOH was converted to citric acid or malic acid per 100 mL of juice and the total acidity was calculated according to the given formula:

$$TA\% = (N \times V / m) \times 100 \times FD \times \text{meq. wt.}$$

where V is titer volume of NaOH, m is mass of juice (g), meq. wt. is weight of the Standard, N is the normality of NaOH and FD is factor of dilution.

3.2.8.3. pH

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

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. Determination of total sugar content

The total sugar content (in fructose) of the fruit juice samples was determined by using dinitrosalicylic acid (DNS) method (Miller, 1959). First of all, DNS solution and 40% sodium potassium tartarate solution were prepared. In order to plot the standard calibration curve, solutions containing 1, 2, 3, 4 % and 5 % D-fructose were prepared. 1 mL of each of these fructose solutions were put into test tubes and 3 mL DNS was added to them. Then these samples were incubated in boiling water bath for 15 - 20 minutes, and subsequently cooled quickly under cool water. Consequently 1 mL from each cooled samples were completed to 40 mL with distilled water. The absorbance was determined by using a spectrophotometer at O.D.540 nm (Shimadzu, PharmaSpec UV-1700, UV-Visible Spectrophotometer, Japan).

3.2.8.5.1. The 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 of distilled water.

3.2.8.6. The Determination of Color

In order to detect the color of juice samples, L^* , a^* , b^* values of 20 mL of each sample was determined by colorimeter. The color values were expressed as L^* (whiteness or brightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness). All measurements were performed in triplicates.

3.2.9. Taste Panel

Thirty panelists rated the fruit juices from 1 (worst) to 10 (best) according to sensory parameters such as color, sweetness, sourness, odor, appearance, flavor, and bitterness. Before the taste panel, each panelist was informed about the parameters and water were served for the palate.

3.3. The Statistical Evaluation

The data were shown as means and analyzed by the SPSS 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.

Bonferonni 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 AND DISCUSSION

4.1. Apricot Juice

4.1.1. The Microbial Analysis of Apricot Juice

The microflora of apricot juices was made up of *Staphylococcus aureus* (*S.aureus*), gram negative bacilli, and certain yeast and mold species in blood agar, in eosine methylene blue (EMB) agar and yeast extract agar (YEA) respectively. No *Salmonella* in XLD agar was observed. All the other 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 *Listeria 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 decrease of microbial load on PCA when compared with the zeroth day (Appendix A.3.). On the other hand there was an increase of the yeast counts. This might be possibly explained by the case that during the period of disappearance of bacteria, yeast is the dominating species.

The results presented in Table 4.1.1.1. and Appendix A.4. stated that when the control and the counts of ultrasound application to the apricot juice was

compared, there was a significant reduction in the logarithmic counts of the total mesophilic aerobic bacteria on PCA agar. When the logarithmic yeast counts were considered it was more than the control in a ten-minute treatment of 90 dB amplitude of 24kHz frequency of ultrasonication, therefore this method was not found to be effective for yeast and molds($p < 0.05$). There was an inverse relationship between bacterial and yeast and mold counts of apricot juice. Yeast and mold counts were increased by ultrasonication, this was probably due to reduced bacterial flora of apricot juice by ultrasonication.

Table 4.1.1.1 The counts of TMAB and YM in the ultrasonicated apricot juice according to doses of ultrasonication

Groups	TMAB			YM		
	Mean	±	Std.	Mean	±	Std.
Control	1.80 ^a	±	.778	.96 ^d	±	.025
30 dB amplitude at 24 kHz	2.39 ^b	±	.840	1.26 ^e	±	.770
60 dB amplitude at 24 kHz	2.06 ^b	±	.639	2.00 ^f	±	.344
90 dB amplitude at 24 kHz	1.33 ^c	±	.641	1.94 ^f	±	.856

Data are means ± SD 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 20µL/ 10mL dose of the *eau de rose* applications to the apricot juice was compared, there was approximately 0.5 log cycle reduction in the logarithmic counts of the total mesophilic aerobic bacteria on PCA agar ($p < 0.05$). This may be due to the effect of possible antimicrobial substances from *eau de rose* into the juice.

According to the results in Table 4.1.1.1., Appendix A.4. and A.5., independently, 20 μ L *eau de rose* and the ten-minute treatment of 90 dB amplitude at 24 kHz frequency of ultrasonication was selected as the best treatment group in the study. The combination treatment was carried out according to these results.

When the combination of 20 μ L *eau de rose* and the ten-minute treatment of 90 dB amplitude at 24 kHz frequency of ultrasonication was considered, it was observed that the total mesophilic aerobic counts of the combination study was found to be 'zero' when compared to the control ($p < 0.05$). The twentieth, fortieth 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 'zero' and significantly different. The results of the combination treatments of the twentieth, fortieth and the sixtieth day results of *eau de rose* and the ultrasonication, as 20 μ L / 10 mL amount and an amplitude of 90 dB at 24 kHz frequency, was found to be very effective on providing the microbial quality of the apricot juice respectively (Figure 4.1).

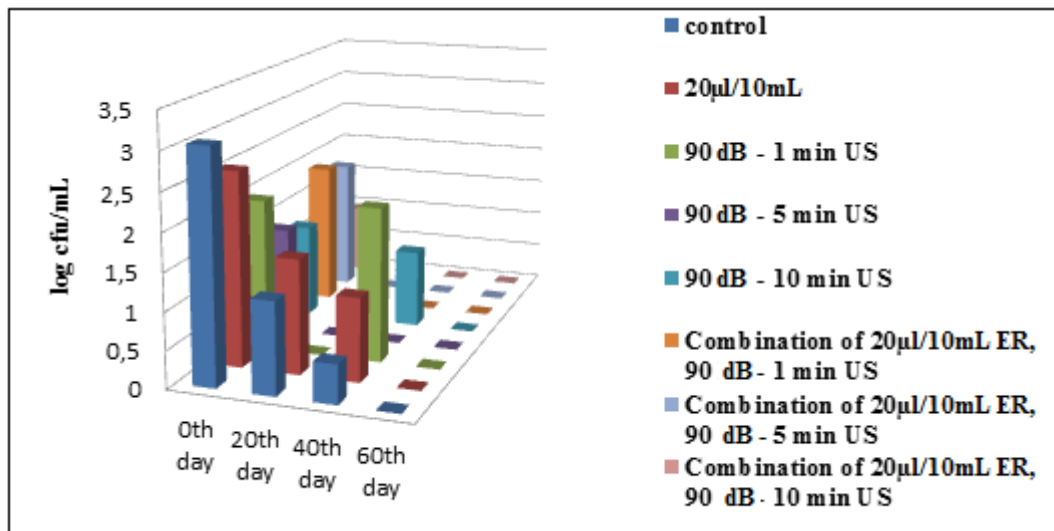


Figure 1 The total mesophilic aerobic bacteria counts of the ultrasonicated (90 dB), *eau de rose* (20µl/10mL) added, and combined *eau de rose* and ultrasonication (90 dB - 20µl/10mL) treated apricot juice.

When the yeast counts of the combination of 20µL *eau de rose* and the ten-minute treatment of 90 dB amplitude at 24 kHz frequency of ultrasonication was considered, there was a little reduction from 0th day to 60th day (Figure 4.2 and Appendix A.6.) ($p < 0.05$). Almost the results were not resembling to the total mesophilic aerobic bacteria counts, in case of the combination; *eau de rose* and the ultrasonication, as 20µL/10 mL amount and 90 dB amplitude at 24 kHz frequency. Due to these results the combination treatment was found to be ineffective on the yeast counts as well.

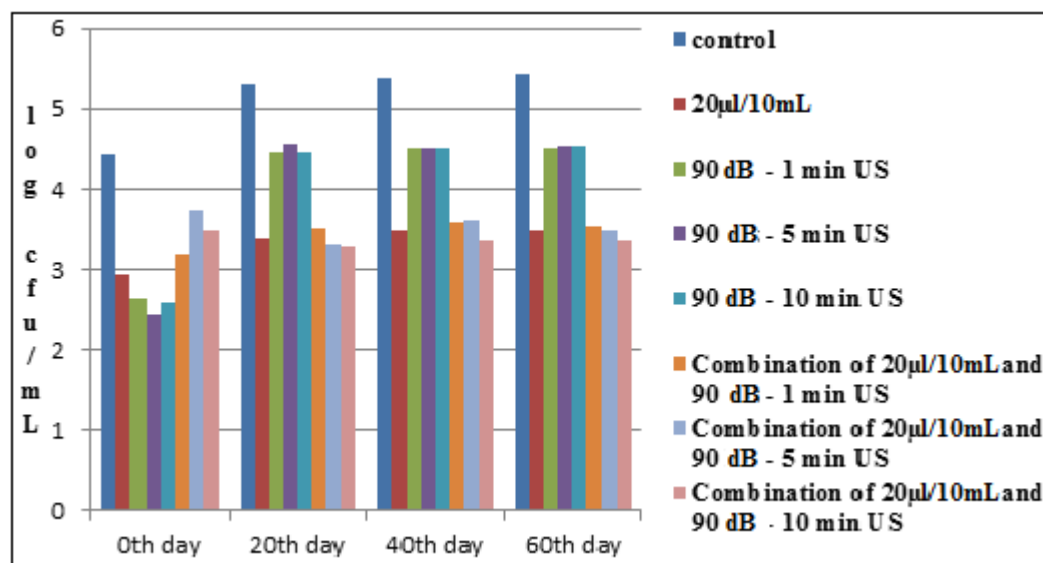


Figure 2 The yeast and mold counts of the ultrasonicated (90 dB), *eau de rose* (20 µl/10mL) added, and combined *eau de rose* and ultrasonication (90 dB – 20 µl/10mL) treated apricot juice.

4.1.2. *E.coli* O157:H7

The *E.coli* O157:H7 added apricot juice was subjected to treatment of ultrasonication with the following doses of 30 dB, 60 dB and 90 dB amplitude at 24 kHz, for a period of one, five and ten minutes (Table 4.1.2.1. and Appendix A.7.). When the logarithmic counts of the study was compared with respect to the control, in each frequency applied, a gradual decrease was observed within the doses of 30 dB, 60 dB and 90 dB amplitude at 24 kHz ultrasonication ($p < 0.05$) (Table 4.1.2.1). A gradual insignificant decrease of *E.coli* O157:H7 was observed within groups while the period of processing time was increased (Appendix A.7) ($p > 0.05$). The 90 dB amplitude at 24 kHz frequency of ultrasonication application for a period of 10 minutes, was found to be significantly different than control and the most effective group as the ‘lowest’ value was obtained (Appendix A.7) ($p < 0.05$).

Table 4.1.2.1 The average counts of *E.coli* O157:H7 inoculated and ultrasonicated apricot juice

Groups	Mean ± Std.
Control	3.25 ^a ± .092
30 dB amplitude at 24 kHz	2.34 ^b ± .789
60 dB amplitude at 24 kHz	1.85 ^{bc} ± .459
90 dB amplitude at 24 kHz	1.24 ^c ± .418

The results in Appendix A.8. indicated that when *eau de rose* was added into the *E.coli* O157:H7 containing apricot juice, an approximate reduction of one and a half (1.5) log cycle was observed when the counts of *E.coli* O157:H7 was considered. No significant difference was seen with respect to the control ($p > 0.05$).

When the combination treatment of 20 μ L *eau de rose* and the ten-minute treatment of 90 dB amplitude at 24 kHz frequency of ultrasonication was considered in *E. coli* O157:H7 inoculated apricot juice, it was observed that the *E.coli* counts were found to be very low when compared to the counts of the control and the *eau de rose* added group (Appendix A.9., Figure 4.3).

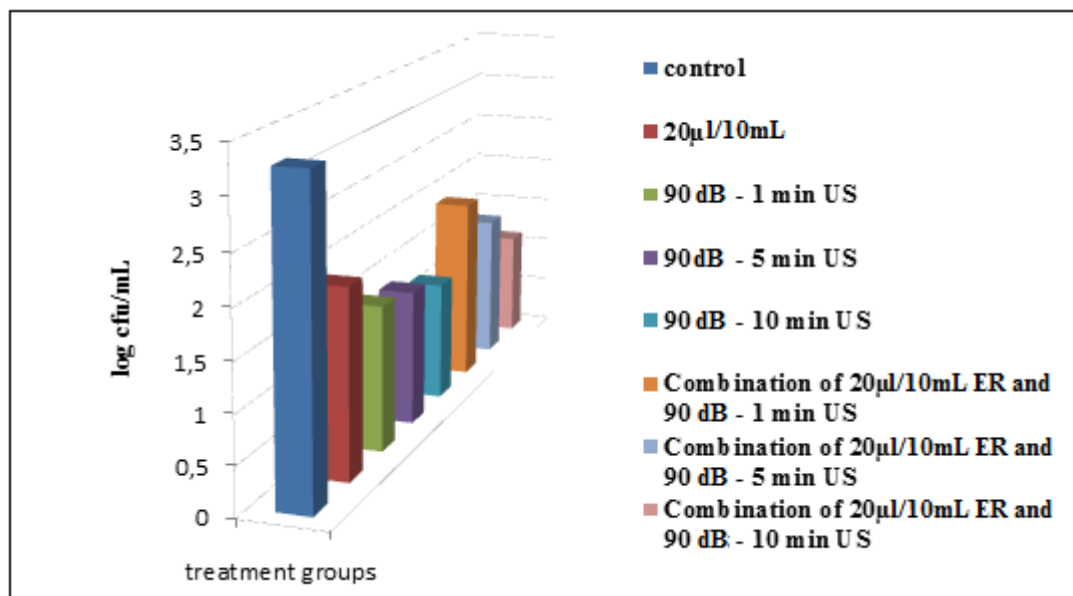


Figure 3 The microbial counts of the combined *eau de rose* (20µl/10mL) added and ultrasonicated (90 dB, 1, 5 and 10 min.) *E.coli* 0157:H7 inoculated apricot juice.

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 99% injury rate is obtained (Table 4.1.2.2).

Table 4.1.2.2 Percent injured cell counts of *E.coli* 0157:H7 after *eau de rose* treatment

	20µl / 10mL
Growth on TSA	3784
Growth on TSA with 1% NaCl	92
Injured cell(%)	99

4.1.3. *Salmonella* spp.

The *Salmonella* spp. added apricot juice was subjected to treatment of ultrasonication with the following doses of 30 dB, 60 dB and 90 dB amplitudes at 24kHz frequency for a period of one, five and ten minutes (Appendix A.10, Table 4.1.3.1). When the logarithmic counts of the study was compared with respect to the counts of *E.coli* 0157:H7, an approximate reduction of 2.0 log cycle in the counts of *Salmonella* spp. among amplitudes of ultrasonication ranging from 30 dB to 90 dB at 24kHz frequency (Table 4.1.3.1) ($p < 0.05$). 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 4.1.2.1 of *E.coli* 0157:H7; the 90 dB amplitude at 24 kHz frequency of ultrasonication application for 10 minutes, was found to be the most effective group. An independent ten minutes' of an amplitude of 90 dB at 24 kHz frequency treatment was also found to be effective too, due to its 'zero' value 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 amplitude of 90 dB at 24 kHz ultrasonication treatment for 10 minutes revealed a 2.76 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..

Table 4.1.3.1 The average counts of *Salmonella* spp. inoculated and ultrasonicated apricot juice

Groups	Mean	±	Std.
Control	2.76 ^a	±	.152
30 dB amplitude at 24kHz	1.48 ^b	±	.592
60 dB amplitude at 24kHz	1.14 ^{bc}	±	.567
90 dB amplitude at 24kHz	.67 ^c	±	.429

When 20µl / 10 mL *eau de rose* was added into the *Salmonella* spp. containing apricot juice, it was found that an approximate reduction of 2.5 log cycle was obtained when the counts of *Salmonella* spp. was considered (Appendix A.11). The most effective dose of *eau de rose* was 20µl / 10mL ($p < 0.05$). When compared with *E.coli* 0157:H7 inoculated- *eau de rose* added and the *Salmonella* inoculated apricot juice followed by ultrasonication treatment, it was found to be too much effective.

When the combination of 20µl / 10 mL *eau de rose* and the ten-minute treatment of 90 dB amplitude at 24kHz frequency of ultrasonication was considered in *Salmonella* spp. inoculated apricot juice, it was observed that (Figure 4.4.) the *Salmonella* spp. counts were found to be 'zero' when compared to the counts of the control and the sole *eau de rose* added and ultrasonicated group (Appendix A.12). A significant difference was observed between the control, 20µl / 10 mL *eau de rose* and the combination of 20µl / 10 mL *eau de rose* and the ten-minute treatment of 90 dB amplitude at 24kHz frequency of ultrasonication ($p < 0.05$).

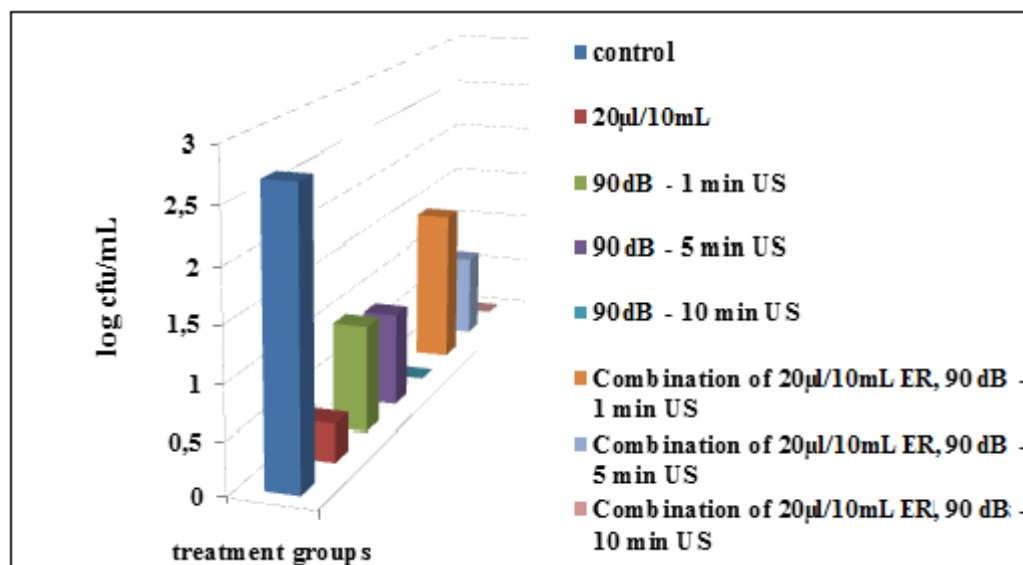


Figure 4 The microbial counts of the combined *eau de rose* (20µl / 10mL) added and ultrasonicated (90 dB amplitude at 24 kHz., 1, 5 and 10 min.) *Salmonella* spp. inoculated apricot juice.

The results of the injured cell determination in the effected population of *Salmonella* spp. revealed that almost all the cells in the medium were found to be injured and a 98% injury rate is obtained, as similarly in the case of *E.coli* 0157:H7 added apricot juices (Table 4.1.3.2).

Table 4.1.3.2 Percent injured cell counts of *Salmonella* spp. after *eau de rose* treatment

	20µl / 10mL
Growth on TSA	203
Growth on TSA with 1% NaCl	4.33
Injured cell(%)	98

4.1.4. *Listeria monocytogenes*

The *Listeria monocytogenes* added apricot juice was subjected to treatment of ultrasonication with the following doses of the amplitudes of 30 dB, 60 dB and 90 dB at 24kHz, for a period of one, five and ten minutes (Appendix A.13). The effect of the amplitude of 30 dB at 24kHz and 60 dB amplitude at 24kHz ultrasonication treatment was almost the same with the control but in fact, less than an amplitude of 90 dB at 24kHz treatment of ultrasonication (Table 4.1.4.1.) ($p < 0.05$). When the logarithmic counts of the study was compared with respect to the control, in each frequency applied, a gradual insignificant decrease was observed within groups ($p > 0.05$) while the period of processing time was increased except an amplitude of 90 dB at 24kHz ultrasonication treatment for 10 min., this was approximately 3.00 log cycles (Appendix A.13) ($p < 0.05$). According to Scherba, there is no difference in the inactivation of Gram-negative bacteria (*P. aeruginosa* and *E. coli*) compared with the inactivation of Gram-positive bacteria (*S. aureus* and *B. subtilis*). These researchers proposed that ultrasound damaged cells by an unknown mechanism, in which damage originated in the cytoplasm rather than the cell wall, thereby affecting Gram-negative and Gram-positive bacteria in the same manner (Scherba et al 1991). Conversely, in my study *Listeria monocytogenes* was found to be more resistant to ultrasonication processing than *Salmonella* and *E.coli* 0157:H7.

Table 4.1.4.1 The average counts of *Listeria monocytogenes* inoculated and ultrasonicated apricot juice

Groups	Mean	±	Std.
Control	3.22 ^a	±	.076
An amplitude of 30 dB at 24kHz	2.97 ^a	±	.337
An amplitude of 60 dB at 24kHz	2.92 ^a	±	.195
An amplitude of 90 dB at 24kHz	1.15 ^b	±	.864

When 20µl / 10mL *eau de rose* was added into the *Listeria monocytogenes* containing apricot juice, it was found that an approximate reduction of more than 2.0 log cycle was obtained (Appendix A.14). No significant difference was observed with respect to the control ($p > 0.05$). When compared with *E.coli* 0157:H7 and *Salmonella* spp. inoculated- *eau de rose* added apricot juice it was found to be almost similar but statistically insignificant ($p > 0.05$).

When the combination of 20µl / 10mL *eau de rose* and the ten-minute treatment of an amplitude of 90 dB at 24kHz frequency of ultrasonication was considered in *Listeria monocytogenes* inoculated apricot juice, it was observed that (Figure 4.5) the *Listeria monocytogenes* counts were found to be decreased approximately 3.50 log cycles when compared to the counts of the control ($p < 0.05$) and 2.50 log cycles with the *eau de rose* added group ($p > 0.05$) respectively (Appendix A.15).

The gram positive bacteria contains high peptidoglycan content in the cell wall and therefore this is possibly decreasing the effect of combination treatment on *Listeria monocytogenes*, a severe pathogenic strain.

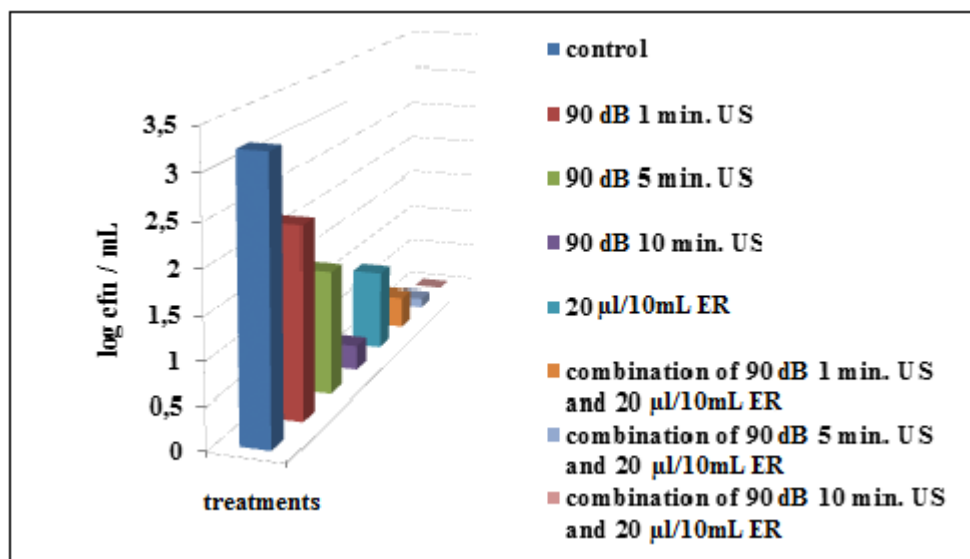


Figure 5 The microbial counts of the combined *eau de rose* (20µl / 10mL) added and ultrasonicated (an amplitude of 90 dB at 24kHz., 1, 5 and 10 min.) *Listeria monocytogenes* inoculated apricot juice.

As seen in Table 4.1.4.2 the results of the injured cell determination for the effected population of *Listeria 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 4.1.4.2 Percent injured cell counts of *Listeria monocytogenes* after *eau de rose* treatment

	20µl / 10mL
Growth on TSA	1825
Growth on TSA with 1% NaCl	50
Injured cell(%)	75

4.1.5. The Results of Physical and Chemical Analysis of Apricot Juice

The pH of the apricot juice was found to be 3.60, 3.61, 3.71, 3.70, 3.87, 3.89, 3.73, 3.75, for the control, an amplitude of 90 dB at 24kHz – 1 min., an amplitude of 90 dB at 24kHz – 5 min., and an amplitude of 90 dB at 24kHz – 10 min., 20µL of sole ultrasonication and *eau de rose* treatments, and the combination treatments of 20µL *eau de rose* with an amplitude of 90 dB at 24kHz – 1 min., an amplitude of 90 dB at 24kHz – 5 min., and an amplitude of 90 dB at 24 kHz – 10 min. of ultrasonication respectively (Table 4.1.5.1) ($p > 0.05$). It was found to be low when compared to the apricot juice, therefore the low pH assists the inhibitory potential of not only the total mesophilic aerobic bacteria, yeast and mold species, but also, particularly *Listeria monocytogenes*, *E.coli* 0157:H7 and *Salmonella* spp.

Table 4.1.5.1 Physical and chemical analysis of nontreated and treated apricot juices

	Control	An amplitude of 90 dB at 24kHz – 10 min.	20µl / 10mL ER	Combination
Ph	3.42 ± .021	3.70 ± .014	3.86 ± .014	3.74 ± .014
Total Soluble Solids (Brix%)	6.00 ± .000	6.00 ± .000	6.00 ± .000	6.00 ± .000
Titrateable Acidity	.58 ± .014	.54 ± .014	.51 ± .014	.47 ± .014
Total Sugar Content (g/mL)	.59 ± .050	.54 ± .057	.61 ± .021	.59 ± .071
Viscosity (mPa.s)	97.05 ± 4.172	86.95 ± .636	97.00 ± .424	79.20 ± 1.697
L*	36.48 ± .007	37.22 ± .014	36.58 ± .014	38.16 ± .014
a*	7.04 ± .000	7.09 ± .042	7.26 ± .007	5.84 ± .028
b*	19.55 ± .000	19.50 ± .042	19.22 ± .028	20.03 ± .014

Data are means ± SD from two replicates.

* $p < 0.05$

According to Table 4.1.5.1 no any difference was found to be present in the selected treatment groups of total soluble solid content of the apricot juices with respect to the control ($p > 0.05$). This might be due to the release of possible antimicrobial substances to the apricot, by the help of ultrasonication, and the *eau de rose* 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 decreased due to 20 μ l ER treatment ($p > 0.05$). 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 insignificantly decreased due to 20 μ l ER treatment ($p > 0.05$) but the other selected groups showed insignificant increase when compared with the control ($p > 0.05$) and b^* (blue/yellow) value was found to be little increased in all treatment groups with respect to the control, but these results were insignificant ($p > 0.05$) (Table 4.1.5.1). So, there are no significant changes present in the main physical and chemical properties of non-treated and treated apricot juices statistically ($p > 0.05$).

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

4.1.6. Taste Panel of Apricot Juice

Thirty panellists rated the apricot 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 most liked, and 1 corresponded to least liked (Table 4.1.6.1).

67% of panelists were greater than twenty years old. The age of 30% panelists were twenty and 3% panelists lower than twenty. 80% of panelists were female and 20% were male. 27% of panelists were smokers and 73% were nonsmokers. 93% of panelists were student and 7% were research assistant. According to Table 4.1.6.1, the scores of all of the treatment groups of apricot juice were almost the same. There were no extreme results between the treatment groups of apricot juice.

Table 4.1.6.1 Sensory parameters of nontreated and treated apricot juice

	Control	An amplitude of 90 dB at 24 kHz for 10min.	20µl / 10mL ER	Combination	Total
Color	7.33 ± .809	7.70 ± .805	7.23 ± .662	7.27 ± .835	7.38 ± .998
Sweetness	7.33 ± .305	7.57 ± .762	7.70 ± .817	7.90 ± .375	7.63 ± .955
Sourness	2.43 ± .028	2.07 ± .305	1.83 ± .424	1.87 ± .277	2.05 ± .810
Odor	7.73 ± .784	7.57 ± .769	7.43 ± .636	7.37 ± .092	7.53 ± .186
Appearance	7.97 ± .661	8.30 ± .402	8.10 ± .071	7.87 ± .450	8.06 ± .707
Flavor	7.43 ± .718	7.97 ± .540	7.87 ± .028	8.30 ± .300	7.89 ± .830
Bitterness	1.00 ± .000	1.13 ± .346	1.03 ± .183	1.00 ± .000	1.04 ± .201
Overall	7.40 ± .472	7.83 ± .508	7.73 ± .403	7.90 ± .332	7.72 ± .669

Data are means ± SD

4.2. The Peach Juices

4.2.1. The Microbial Analysis of Peach Juice

The microflora of peach juices was found to be composed of *Staphylococcus aureus* (*S.aureus*), *gram negative bacilli*, and certain yeast and mold species in blood agar, in eosine methylene blue (EMB) agar and yeast extract agar (YEA) respectively. No growth record of *Salmonella* in XLD agar were obtained (Appendix A.16).

It was observed that (Appendix A.16.) there were insignificant growth of total mesophilic aerobic bacteria on PCA at zeroth and twentieth day, whereas at fortieth and sixtieth day no growth was observed. On the other hand there was an increase in the yeast and mold counts and it was gradual up to the fortieth day. This might be possibly explained by the case that during the period of disappearance of bacteria, yeast is found to be the dominating species (das Chagas Oliveira Freire and Offord, 2002). At the sixtieth day the yeasts were found to be uncountable.

When the control and the ultrasound application to the peach 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 of ten minutes (Table 4.2.1.1, Appendix A.17). A slight increment was observed at the one and five minute treatment of the sonication when compared with the control but ten minutes' treatment increased the total mesophilic aerobic counts of the ultrasonicated peach juices. There was not so much difference for the yeast counts between the treatments and the control (Appendix A.17). No statistical difference was observed for all of the treatments ($p > 0.05$).

Table 4.2.1.1 The counts of TMAB and YM in the ultrasonicated peach juice according to doses of ultrasonication

	TMAB			YM		
Groups	Mean	±	Std.	Mean	±	Std.
Control	.17 ^a	±	.300	3.83 ^d	±	.083
An amplitude of 30 dB at 24kHz	.44 ^a	±	.699	3.30 ^e	±	.116
An amplitude of 60 dB at 24kHz	.30 ^a	±	.609	3.59 ^f	±	.078
An amplitude of 90 dB at 24kHz	.14 ^a	±	.294	3.58 ^f	±	.060

When the control and the *eau de rose* application to the peach juice was compared, between control and other treatments, the same results were obtained with 20µl ER and a slight increase of total mesophilic aerobic bacteria on PCA agar with 1, 5 and 10µl / 10mL ER were observed. No significant difference was observed between treatments (Appendix A.18). In addition, there was a statistically supported insignificant decrease in the yeast counts with the same amount of ER treatment, an initial value of 3.83 log cycles, reaching up to 3.20 at the end ($p < 0.05$) (Appendix A.18). The gradual increase of the amount of the *eau de rose* showed a gradual decrease in the log cycles of yeast and molds ($p < 0.05$) (Appendix A.18).

When the combination of 20µl *eau de rose* and the ten-minute treatment of an amplitude of 90 dB at 24 kHz frequency of ultrasonication was considered, it was observed that the total mesophilic aerobic count values of the combination study was found to be 'zero' in 20th, 40th and 60th days when compared to the counts of the control in the zeroth day which was also zero (Figure 4.6., Appendix A.19). These results indicated that certain antimicrobial compounds present in the peach juice or the pH itself, or both; could be able to inhibit the total mesophilic aerobic bacteria in addition to ultrasonication and *eau de rose*, which would not possibly permit an extra contamination or growth. So, they were not found to be significantly different.

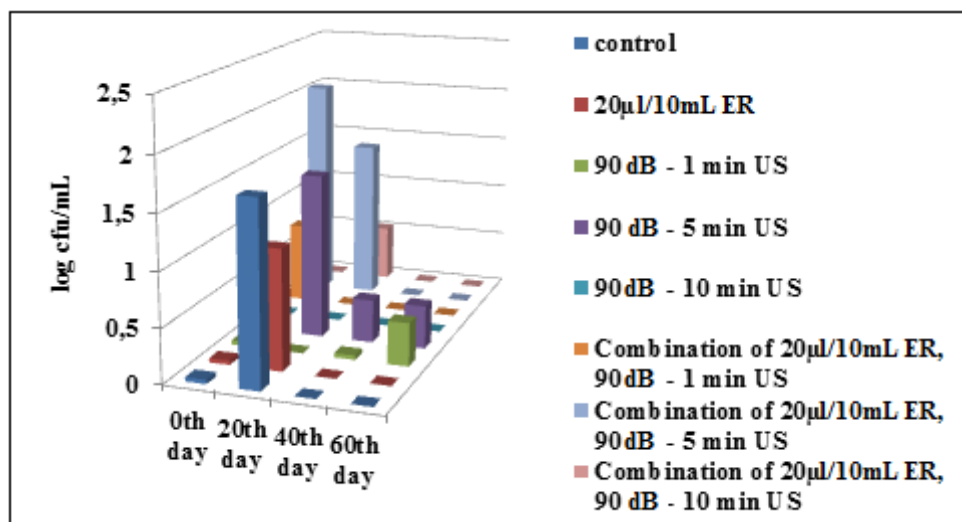


Figure 6 The total mesophilic aerobic bacteria counts of the ultrasonicated (an amplitude of 90 dB at 24 kHz.), *eau de rose* (20µl / 10mL) added, and combined *eau de rose* and ultrasonication (an amplitude of 90 dB at 24kHz. - 20µl / 10mL) treated peach juice.

When the yeast counts was considered, there was a gradual increment in 0th, 20th, 40th and 60th days for control and each treatment. These results were found to be significantly different ($p < 0.05$). (Figure 4.7.) (Appendix A.19).

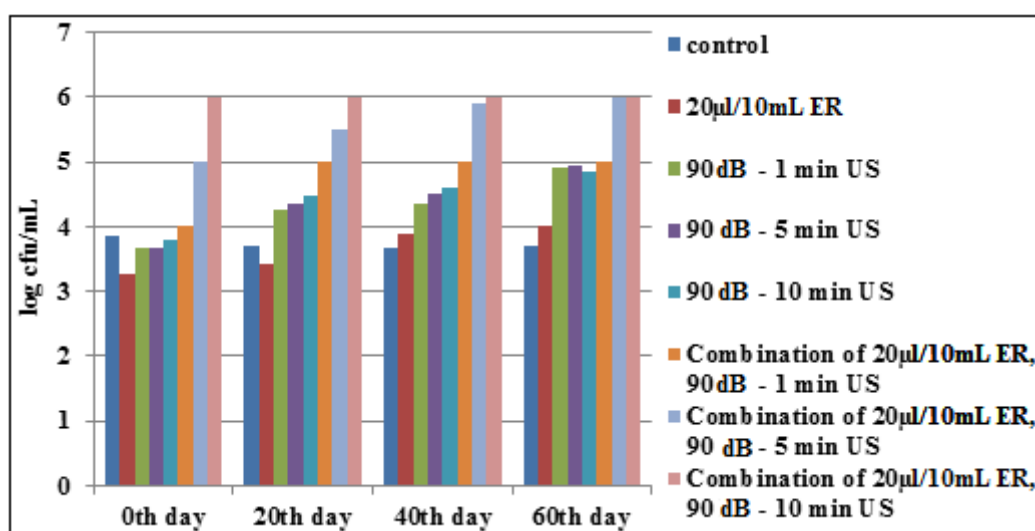


Figure 7 The yeast and mold counts of the ultrasonicated (an amplitude of 90 dB at 24kHz.), *eau de rose* (20µl / 10mL) added, and combined *eau de rose* and ultrasonication (an amplitude of 90 dB at 24kHz. - 20µl / 10mL) treated peach juice.

4.2.2. *E.coli* O157:H7

The *E.coli* O157:H7 added peach juice was exposed to ultrasonication with the following doses of the amplitudes of 30, 60 and 90 dB at 24kHz, for a period of one, five and ten minutes, respectively (Appendix A.20). When the logarithmic counts of the study was compared with respect to the control, a gradual decrease was observed when ultrasonic frequency was increased (Table 4.2.2.1) ($p < 0.05$). 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 amplitude of 90 dB at 24kHz frequency of ultrasonication application for 10 minutes, was found to be the most effective group as lowest values of microbial counts were obtained (Appendix A.20). The significant reduction was found to be approximately four log cycles ($p < 0.05$). When compared with the *E.coli* O157:H7 added apricot juice (Table 4.1.2.1, Appendix A.7.) a reduction of 2 log cycles was observed ($p < 0.05$), so it is two folds' more effective than the apricot.

Table 4.2.2.1 The average counts of *E.coli* O157:H7 inoculated and ultrasonicated peach juice

Groups	Mean	±	Std.
Control	3.71 ^a	±	.120
An amplitude of 30 dB at 24kHz	2.99 ^a	±	.628
An amplitude of 60 dB at 24 kHz	1.14 ^b	±	.672
An amplitude of 90 dB at 24kHz	.44 ^b	±	.492

When *eau de rose* was added into the *E.coli* 0157: H7 containing peach juice, it was found that an approximate maximum reduction of 3.55 log cycle was obtained when the control counts of *E.coli* 0157: H7 was considered ($p < 0.05$) and there was a significant difference between control and the treatment groups (Appendix A.21.) ($p < 0.05$). The antibacterial effects of 10 μ l and 20 μ l / 10mL *eau de rose* were almost significantly the same ($p < 0.05$) but 1 μ L and 5 μ l / 10mL *eau de rose* were different compared with the control (Appendix A.21.).

When the combination of 20 μ l *eau de rose* and the ten-minute treatment of an amplitude of 90 dB at 24kHz frequency of ultrasonication was evaluated in *E .coli* 0157:H7 inoculated peach juice, it was observed that, except the combination of 10 minutes' case, a gradual decrease were obtained. Only the combination of 10 minutes' was found to be zero (Appendix A.22.). Statistically these results were found to be significantly the same with control ($p < 0.05$), but not with the 10 minutes' treatment ($p > 0.05$).

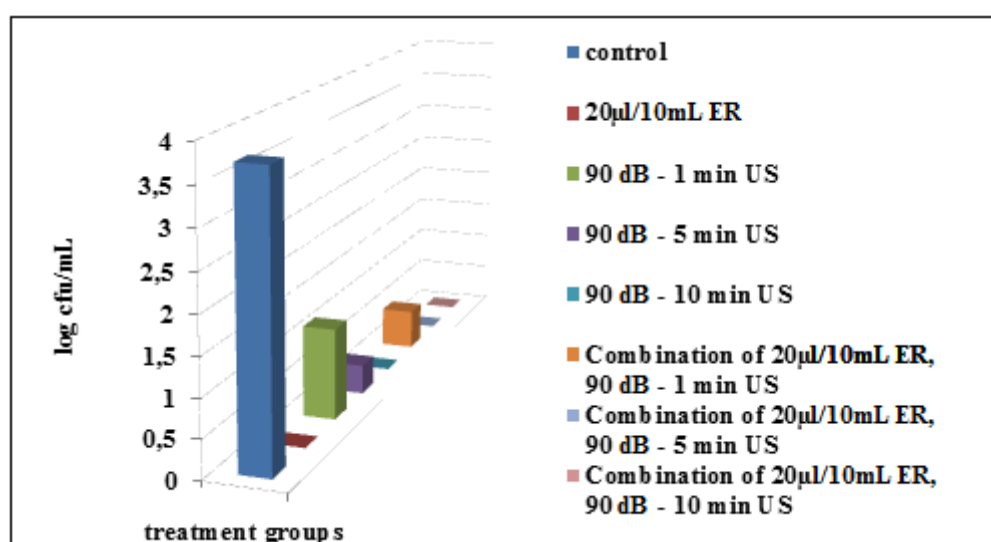


Figure 8 The microbial counts of the combined *eau de rose* (20 μ l / 10mL) added and ultrasonicated (90 dB at 24kHz., 1, 5 and 10 min.) *E.coli* 0157:H7 inoculated peach juice.

The results of the injured cell determination in the effected population revealed that almost half of the cells in the medium were found to be injured and a 100% rate of injury was obtained (Table 4.2.2.2.).

Table 4.2.2.2 Percent injured cell counts of *E.coli* 0157:H7 after *eau de rose* treatment

	20µl / 10mL
Growth on TSA	93
Growth on TSA with 1% NaCl	0
Injured cell(%)	100

4.2.3. *Salmonella* spp.

The *Salmonella* spp. added peach juice was subjected to treatment of ultrasonication with the following doses of the amplitudes of 30, 60 and 90 dB at 24 kHz, for a period of one, five and ten minutes (Table 4.2.3.1, Appendix A.23.). When the logarithmic counts of the study was compared with respect to the control, a gradual decrease was observed when ultrasonic amplitudes at 24 kHz frequency were increased (Table 4.2.3.1) ($p < 0.05$). The best effective amplitude at 24 kHz frequency of ultrasonication was found to be 90 ($p < 0.05$) (Table 4.2.3.1). 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 amplitude of 90 dB at 24 kHz frequency of ultrasonication application for

10 minutes, was found to be the most effective group as ‘zero’ value was obtained and significantly different from the control ($p < 0.05$) (Appendix A.23). As a result, ten minutes’ ultrasonication treatment at an amplitude of 90 dB at 24 kHz revealed an approximate value of 3.82 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., (Appendix A.23).

Table 4.2.3.1 The average counts of *Salmonella* spp. inoculated and ultrasonicated peach juice

Groups	Mean	±	Std.
Control	3.82 ^a	±	.140
30 dB amplitude at 24kHz	2.71 ^b	±	.315
60 dB amplitude at 24kHz	1.42 ^c	±	.821
90 dB amplitude at 24kHz	.25 ^d	±	.398

When 20µl / 10mL *eau de rose* was added into the *Salmonella* spp., containing peach juice, it was found that an approximate maximum reduction of 3.82 log cycle was obtained, compared with the control (Appendix A.24) ($p < 0.05$). The effects of 1µl, 5µl and 10µl / 10mL doses of *eau de rose* were insignificant when compared with the control counts of *Salmonella* spp. inoculated peach juice ($p < 0.05$) (Appendix A.24).

When the combination of 20µl *eau de rose* and the ten-minute treatment of an amplitude of 90 dB at 24kHz frequency of ultrasonication was considered in

Salmonella spp. inoculated peach juice, it was observed that (Figure. 4.9., Appendix A.25) except sole amplitude of 90 dB at 24kHz for 1 min., an amplitude of 90 dB at 24kHz for 5 min. and combination of 1 min. with 20 µl *eau de rose* treatment of the *Salmonella* spp. counts were found to be ‘zero’ when compared to the counts of the control (Appendix A.25). The results indicated that ten minutes’ ultrasonic treatment was effectively able to kill the population of *Salmonella* spp. as solely or in combination with the *eau de rose*.

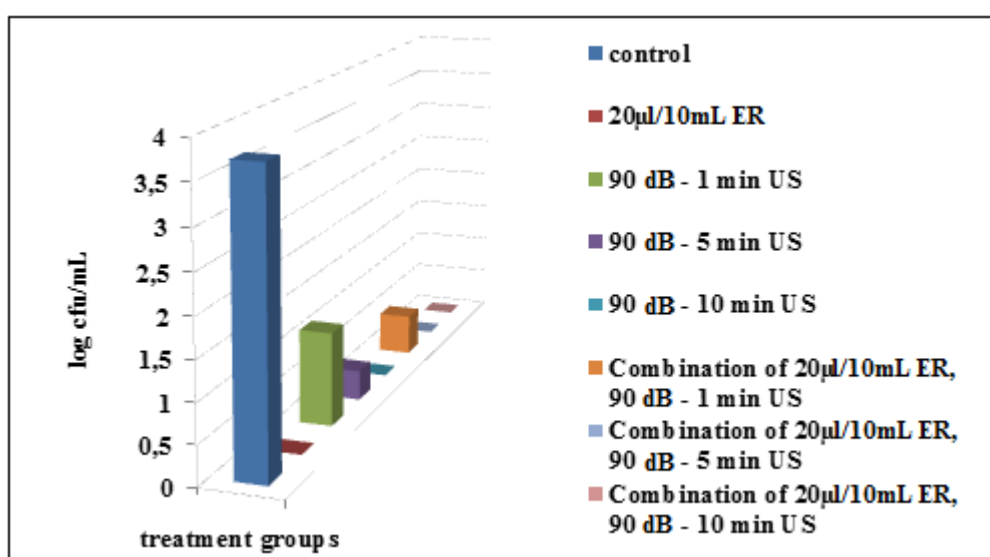


Figure 9 The microbial counts of the combined *eau de rose* (20µL / 10mL) added and ultrasonicated (an amplitude of 90 dB at 24kHz., 1, 5 and 10 min.) *Salmonella* spp. inoculated peach juice.

The results of the injured cell determination in the *Salmonella* spp. added peach juice revealed that all the cells in the medium were found to be injured and a 100% injury rate is obtained (Table 4.2.3.2).

Table 4.2.3.2 Percent injured cell counts of *Salmonella* spp. after *eau de rose* treatment

	20µl / 10mL
Growth on TSA	687
Growth on TSA with 1% NaCl	0
Injured cell(%)	100

4.2.4. *Listeria monocytogenes*

The peach juice inoculated with *Listeria monocytogenes* was subjected to treatment of ultrasonication with the following doses of an amplitude of 30, 60 and 90 dB at 24kHz, for a period of one, five and ten minutes respectively (Appendix A.26) (Table 4.2.4.1). When the logarithmic counts of the study was compared with respect to the control, a gradual decrease was observed while amplitudes of 24kHz frequency 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 3.10 log cycle, whereas a value of 'zero' was obtained in ten minutes of an amplitude of 90 dB at 24kHz ultrasonication treatment of the peach juice. The average counts of *Listeria monocytogenes* inoculated and ultrasonicated peach juice with the amplitudes of 60 and 90 dB at 24kHz frequency, and the processing times was found to be significantly different from control ($p < 0.05$) (Table 4.2.4.1, 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 4.2.4.1 The average counts of *Listeria monocytogenes* inoculated and ultrasonicated peach juice

Groups	Mean	±	Std.
Control	3.83 ^a	±	.142
30 amplitude at 24kHz	3.53 ^a	±	.143
60 amplitude at 24kHz	2.75 ^b	±	.406
90 amplitude at 24kHz	1.43 ^c	±	.917

When 20µl / 10mL eau de rose was added into the *Listeria monocytogenes* containing peach juice, it was found that an approximate reduction of 1.66 log cycle was obtained with respect to control (Appendix A.27) ($p < 0.05$). If the counts of ER added *Listeria monocytogenes* inoculated peach juice was compared with the sole eau de rose added one containing *Salmonella* spp. (Appendix A.24), the effect of sole eau de rose was found to be not much powerful on *Listeria monocytogenes*. It was also in line with the results presented in Appendix A.21 reflecting the *E.coli* 0157:H7 inoculated-eau de rose added group.

When the combination of 20µl eau de rose and the ten-minute treatment of an amplitude of 90 dB at 24kHz. frequency of ultrasonication was considered in *Listeria monocytogenes* inoculated peach juice, it was observed that (Figure. 4.10., Appendix A.28). ‘Zero’ value was only seen in combination of ten minutes’ group and value of 3.83 log cycle when compared with the control (Figure. 4.10., Appendix A.28). All treatments were found to be significantly different from control ($p < 0.05$).

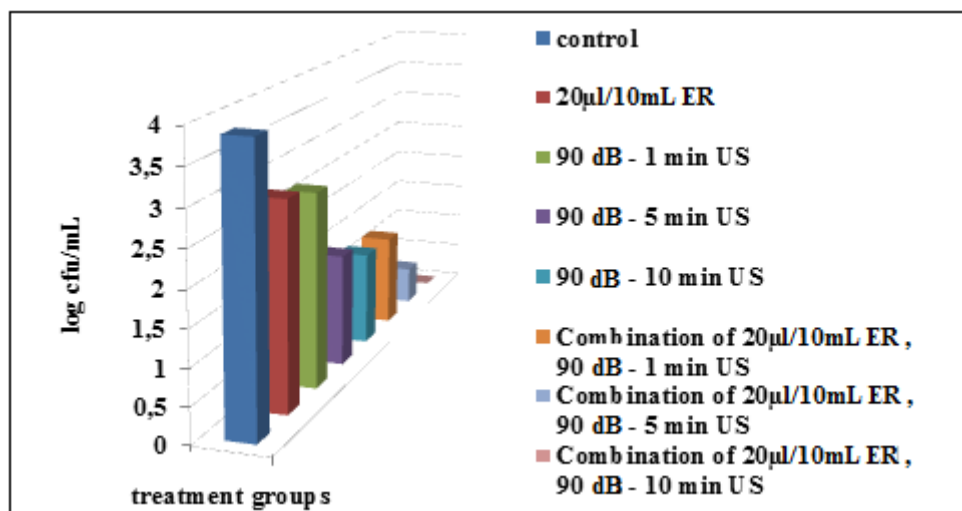


Figure 10 The microbial counts of the combined *eau de rose* (20µl / 10mL) added and ultrasonicated (an amplitude of 90 dB at 24kHz frequency, 1, 5 and 10 min.) *Listeria monocytogenes* inoculated peach juice.

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 *Listeria monocytogenes* (Table 4.2.4.2).

Table 4.2.4.2 Percent injured cell counts of *Listeria monocytogenes* after *eau de rose* treatment

	20µl / 10mL
Growth on TSA	279
Growth on TSA with 1% NaCl	0
Injured cell(%)	100

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

The pH of the peach juice was found to be 4.54, 4.53, 4.52, 4.52, 4.51, 4.37, 4.38, 4.39, 4.40, 4.39 and 4.41 for the control, 1, 5, 10 and 20 μ l / 10mL of eau de rose, an amplitude of 90 dB at 24kHz – 1 min., an amplitude of 90 dB at 24kHz – 5 min., and an amplitude of 90 dB at 24kHz – 10 min. of sole ultrasonication treatments, and the combination treatments of 20 μ l / 10mL of eau de rose with an amplitude of 90 dB at 24kHz – 1 min., an amplitude of 90 dB at 24kHz – 5 min., and an amplitude of 90 dB at 24 kHz – 10 min. of ultrasonication respectively (Table 4.2.5.1) ($p > 0.05$). It was found to be high when compared to the apricot juice, therefore the low pH assists the inhibitory potential of not only the total mesophilic aerobic bacteria, yeast and mold species, but also, particularly *Listeria monocytogenes*, *E.coli* 0157:H7 and *Salmonella* spp.

Table 4.2.5.1 Physical and chemical analysis of nontreated and treated peach juices

	Control	An amplitude of 90 dB at 24kHz – 10 min.	20 μ l/10mL ER	Combination
pH	4.55 $\pm$.014	4.40 $\pm$.007	4.53 $\pm$.014	4.41 $\pm$.007
Total Soluble Solids (Brix)	5.50 $\pm$.000	5.50 $\pm$.000	5.50 $\pm$.000	5.50 $\pm$.000
Titrateable Acidity	.18 $\pm$.007	.14 $\pm$.000	.13 $\pm$.000	.11 $\pm$.014
Total Sugar Content (g/mL)	.62 $\pm$.057	.72 $\pm$.007	.68 $\pm$.078	.74 $\pm$.028
Viscosity (mPa.s)	29.00 $\pm$ 3.678	26.95 $\pm$.919	36.50 $\pm$.849	30.40 $\pm$ 1.979
L	29.67 $\pm$.014	33.15 $\pm$.007	30.03 $\pm$.007	31.62 $\pm$.007
a*	3.00 $\pm$.007	2.33 $\pm$.000	2.87 $\pm$.057	1.38 $\pm$.035
b*	11.05 $\pm$.007	13.20 $\pm$.000	11.66 $\pm$.042	11.71 $\pm$.007

Data are means $\pm$ SD from two replicates.

According to Table 4.2.5.1 no increase or decrease was found to be present in the selected treatment groups of total soluble solid content of the peach juices with respect to the control, likewise the case of peach (Table 4.1.5.1) ($p > 0.05$). 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 increased in the treatment groups than the control ($p > 0.05$). The viscosity was said to be decreased due to the amplitude of 90 dB at 24kHz ultrasonication treatment but the other selected groups showed an increase 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 slightly increased in the treatment groups with respect to the control, whereas a^* (red/green) value was found to be slightly decreased in all treatment groups, with respect to the control of peach juice, and b^* (blue/yellow) value was found to be slightly increased in all treatment groups with respect to the control (Table 4.2.5.1) ($p > 0.05$). In general, no detrimental effect of the treatments on the physical and chemical quality of the peach 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. Taste Panel of Peach Juice

Thirty panellists rated the peach 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 4.2.6.1). A score of 10 represented most liked, and 1 corresponded to least liked.

67% of panelists were greater than twenty years old. The age of 30% panelists were twenty and 3% lower than twenty. 80% of panelists were female and

20% were male. 27% of panelists were smokers and 73% were nonsmokers. 93% of panelists were student and 7% were assistant. According to Table 4.2.6.1 scores of all of the treatment groups of peach juice were almost the same ($p > 0.05$). There were no extreme results between treatment groups of peach juice.

Table 4.2.6.1 Sensory parameters of nontreated and treated peach juice

	Control	An amplitude of 90 dB at 24kHz. 10 min.	20µl/10mL ER	Combination	Total
Color	6.83 ± .759	6.77 ± .834	7.10 ± .855	6.77 ± .845	6.87 ± .822
Sweetness	5.83 ± .669	6.43 ± .907	7.20 ± .650	7.63 ± .993	6.77 ± .804
Sourness	1.47 ± .183	1.23 ± .030	1.13 ± .140	1.03 ± .272	1.22 ± .156
Odor	5.73 ± .817	6.07 ± .300	5.63 ± .776	6.83 ± .643	6.07 ± .634
Appearance	6.80 ± .254	6.73 ± .412	7.37 ± .890	7.07 ± .558	6.99 ± .529
Flavor	5.90 ± .928	6.27 ± .919	7.10 ± .788	7.70 ± .108	6.74 ± .686
Bitterness	1.03 ± .740	1.07 ± .172	1.03 ± .277	1.07 ± .000	1.05 ± .297
Overall	6.27 ± .697	6.57 ± .932	7.43 ± .230	7.80 ± .220	7.02 ± .519

Data are means ± SD

CHAPTER 5

CONCLUSION

Fruit juices have a tendency to spoilage due to improper processing and storage problems. In this study *Salmonella* spp., *E.coli* 0157:H7 and *Listeria monocytogenes* was studied for their inhibition potential in apricot and peach juices. Although *E.coli* 0157:H7 was reduced up to three log cycle inhibition in apricot juice, which was almost closer to a complete destruction; it was completely destroyed in peach juice. This study have revealed that *Salmonella* and *Listeria monocytogenes* was also found to be completely inhibited when 20µl / 10mL *eau de rose* was combined with an amplitude of 90 dB at 24kHz frequency of ultrasonication in peach and apricot juice. In general no any chemical or physical change was observed in all fruit juices treated with ultrasonication and *eau de rose*. The low pH of the fruit juices therefore, may play a role in these inhibitions. Consequently, these findings indicated that ultrasonication and *eau de rose* can show a recommendable status in industrial processing of fruit juices due to their effectiveness and low cost, therefore it was verified by the taste panel and the statistical analysis.

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URL 1:

<http://en.wikipedia.org/wiki/Sonication>

URL 2:

<http://www.turkishculture.org/pages.php?SearchID=388>

URL 3:

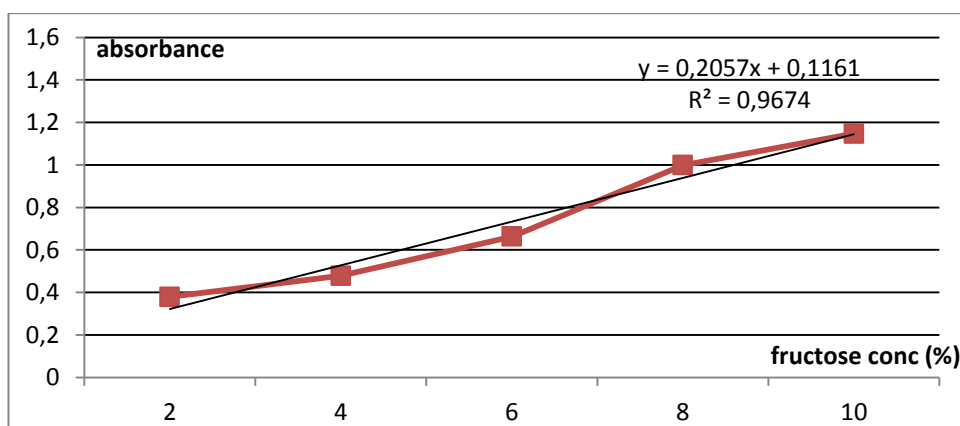
<http://en.wikipedia.org/wiki/Salmonella>

APPENDICES

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

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



The amount of total sugar content (fructose conc.) can calculate that each samples's absorbance value put in the equation on graph.

Graph equation : $y = 0,2057x + 0,1161$

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

		0. Day	20. Day	40. Day	60. Day
TMAB	Mean	1167	17	0.33	1
	log	3.06	1.22	0.52	0
YM	Mean	27180	203666	240550	261423
	log	4.43	5.31	5.38	5.42

A.4 The microbial counts of the ultrasonicated apricot juice

Groups		TMAB		YM	
		Mean $\pm$	Std.	Mean $\pm$	Std.
Control		1.80 ^a $\pm$	.778	.96 ^d $\pm$	.025
30 dB at 24kHz	1 min	2.97 ^b $\pm$	.113	1.39 ^e $\pm$	.386
	5 min	2.19 ^b $\pm$	1.056	.68 ^e $\pm$	1.178
	10 min	2.02 ^b $\pm$	.959	1.69 ^e $\pm$	.142
60 dB at 24 kHz	1 min	2.13 ^b $\pm$	.700	1.85 ^f $\pm$	.185
	5 min	2.08 ^b $\pm$	.840	1.90 ^f $\pm$	.129
	10 min	1.96 ^b $\pm$	.645	2.25 ^f $\pm$	.535
90 dB at 24 kHz	1 min	1.74 ^a $\pm$	.588	1.92 ^f $\pm$	.930
	5 min	1.06 ^c $\pm$	.915	1.89 ^f $\pm$	1.016
	10 min	1.19 ^c $\pm$	.251	2.01 ^f $\pm$	1.010

Data are means $\pm$ SD from three replicates

Values followed by different letters in the same column are significantly different (P<0.05)

A.5 The microbial counts of *eau de rose* added apricot juice

Control	TMAB	YM
	Mean std.	Mean std.
Control	1.80 ^{ab} ± .778	.96 ^t ± .025
1µl / 10mL ER	3.01 ^c ± .268	3.06 ^{de} ± .206
5µl / 10mL ER	2.68 ^{bc} ± .314	3.07 ^{de} ± .156
10µl / 10mL ER	2.69 ^{bc} ± .223	3.22 ^e ± .072
20µl / 10mL ER	1.34 ^a ± .294	2.84 ^d ± .140

**A.6 The shelf-life determination studies of the ultrasonicated (90 dB at 24kHz),
eau de rose (20µl / 10mL) added, and combined eau de rose and ultrasonication
(90 dB at 24kHz. - 20µL / 10mL) treated Apricot Juice: The TMAB and YM
counts**

	Groups	0.day	20. day	40.day	60. day
T M A B	90 dB at 24kHz - 1 min of US	1.74 ^a ± .588	0.16 ^b ± .277	0.84 ^b ± 1.454	0.00 ^b ± .000
	90 dB at 24kHz - 5 min of US	1.06 ^c ± .915	0.16 ^d ± .277	0.00 ^d ± .000	0.16 ^d ± .277
	90 dB at 24kHz - 10 min of US	1.20 ^d ± .251	0.00 ^d ± .000	0.51 ^d ± .878	0.00 ^d ± .000
	20µl / 10mL ER	1.34 ^e ± .295	0.67 ^e ± 1.155	0.78 ^e ± .761	0.00 ^e ± .000
	Combination; 90 dB at 24 kHz - 1 min US + 20µL ER	0.99 ^e ± .932	0.17 ^e ± .300	0.00 ^e ± .000	0.00 ^e ± .000
	Combination; 90 dB at 24kHz - 5 min US + 20µL ER	1.42 ^f ± .855	0.00 ^f ± .000	0.17 ^f ± .300	0.00 ^f ± .000
	Combination; 90 dB at 24 kHz - 10 min US + 20µL ER	0.74 ^g ± .650	0.00 ^g ± .000	0.00 ^g ± .000	0.00 ^g ± .000
	Control	1.80 ^h ± .778	1.12 ^h ± .352	0.43 ^h ± .412	0.16 ^h ± .277
Y M	Control	.96 ⁿ ± .025	3.98 ⁿ ± .025	3.99 ⁿ ± .026	3.99 ⁿ ± .050
	90 dB at 24kHz - 1 min of US	1.93 ^k ± .930	2.69 ^k ± .516	2.94 ^k ± .250	3.07 ^k ± .221
	90 dB at 24kHz - 5 min of US	1.89 ^k ± 1.016	2.59 ^k ± .477	2.95 ^k ± .182	3.44 ^k ± .474
	90 dB at 24kHz - 10 min of US	2.01 ^{kl} ± 1.010	3.01 ^{kl} ± .051	3.19 ^{kl} ± .487	3.13 ^{kl} ± .268
	20µl / 10mL ER	2.84 ^{klm} ± .140	3.02 ^{klm} ± .179	3.10 ^{klm} ± .070	3.16 ^{klm} ± .040
	Combination; 90 dB at 24kHz - 1 min US + 20µL ER	3.15 ^{klm} ± .085	3.00 ^{klm} ± .162	2.98 ^{klm} ± .115	3.32 ^{klm} ± .045
	Combination; 90 dB at 24kHz - 5 min US + 20µL ER	3.24 ^{lm} ± .050	3.12 ^{lm} ± .104	3.09 ^{lm} ± .125	3.34 ^{lm} ± .150
	Combination; 90 dB at 24kHz - 10 min US + 20µL ER	3.31 ^m ± .096	3.14 ^m ± .203	3.33 ^m ± .074	3.58 ^m ± .036

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

Groups		Mean $\pm$ std.
Control		3.25 ^a $\pm$.092
30 dB amplitude at 24 kHz	1 min.	2.92 ^a $\pm$.079
	5 min.	2.09 ^b $\pm$.902
	10 min.	2.02 ^b $\pm$.959
60 dB amplitude at 24 kHz	1 min	1.81 ^b $\pm$.886
	5 min	1.89 ^b $\pm$.133
	10 min	1.85 ^b $\pm$.190
90 dB amplitude at 24 kHz	1 min	1.37 ^c $\pm$.386
	5 min	1.31 ^c $\pm$.468
	10 min	1.03 ^c $\pm$.484

A.8 The counts of the *E.coli* 0157:H7 inoculated and *eau de rose* added apricot juice

Groups	Mean ± std.
Control	3.25 ^a ± .092
1 µl / 10mL ER	2.73 ^{ab} ± .356
5 µl / 10mL ER	2.39 ^{bc} ± .293
10 µl / 10mL ER	2.41 ^{bc} ± .355
20 µl / 10mL ER	1.83 ^c ± .193

A.9 The microbial counts of the combined *eau de rose* (20µl / 10mL) added and ultrasonication (90 dB amplitude at 24 kHz., 1, 5 and 10 min.) treated *E.coli* 0157:H7 inoculated apricot juice

Groups		Mean	±	std.
Control		3.25 ^a	±	.092
20 µl <i>eau de rose</i>		1.37 ^{bc}	±	.386
90 dB amplitude at 24 kHz	1 min	1.31 ^{bc}	±	.468
	5 min	1.03 ^{bc}	±	.484
	10 min	1.83 ^b	±	.193
Combination	90 dB amplitude at 24 kHz - 1 min of US + 20µl <i>eau de rose</i>	1.22 ^{bc}	±	.646
	90 dB amplitude at 24 kHz - 5 min of US + 20µl <i>eau de rose</i>	1.26 ^{bc}	±	.416
	90 dB amplitude at 24 kHz - 10 min of US + 20µl <i>eau de rose</i>	.26 ^c	±	.445

A.10 The counts of the *Salmonella* spp. inoculated and ultrasonicated apricot juice

Groups		Mean ± std.
Control		2.26 ^a ± .153
30 dB amplitude at 24kHz	1 min.	2.02 ^a ± .517
	5 min.	1.06 ^b ± .561
	10 min.	1.36 ^b ± .312
60 dB amplitude at 24kHz	1 min	1.46 ^b ± .372
	5 min	1.19 ^b ± .638
	10 min	.79 ^c ± .635
90 dB amplitude at 24kHz	1 min	.98 ^c ± .146
	5 min	.78 ^c ± .315
	10 min	.26 ^c ± .450

A.11 The counts of the *Salmonella* spp. inoculated and *eau de rose* added apricot juice

Groups	Mean $\pm$ std.
Control	2.76 ^a $\pm$.153
1 μl / 10mL ER	1.65 ^{ab} $\pm$ 1.196
5 μl / 10mL ER	1.93 ^{ab} $\pm$.641
10 μl / 10mL ER	1.08 ^{ab} $\pm$.479
20 μl / 10mL ER	0.33 ^b $\pm$.289

A.12 The microbial counts of the combined *eau de rose* (20µl / 10mL) added and ultrasonication (90 dB amplitude at 24kHz., 1, 5 and 10 min.) treated *Salmonella* spp. containing apricot juice

Groups		Mean	±	Std.
Control		2.76 ^a	±	.153
20µl eau de rose		.33 ^{bc}	±	.289
90 dB amplitude at 24kHz	1 min	.98 ^b	±	.146
	5 min	.78 ^{bc}	±	.315
	10 min	.26 ^{bc}	±	.450
Combination	90 dB amplitude at 24kHz - 1 min of US + 20µl eau de rose	.85 ^b	±	.415
	90 dB amplitude at 24kHz - 5 min of US + 20µl eau de rose	.59 ^{bc}	±	.162
	90 dB amplitude at 24 kHz - 10 min of US +20µl eau de rose	.00 ^c	±	.000

A.13 The counts of the *Listeria monocytogenes* inoculated and ultrasonicated apricot juice

Groups		Mean ± std.
Control		3.22 ^a ± .076
30 dB amplitude at 24kHz	1 min.	3.18 ^a ± .050
	5 min.	3.06 ^a ± .085
	10 min.	2.67 ^a ± .483
60 dB amplitude at 24kHz	1 min	3.07 ^a ± .117
	5 min	2.90 ^a ± .205
	10 min	2.81 ^a ± .204
90 dB amplitude at 24kHz	1 min	2.02 ^b ± .523
	5 min	1.17 ^b ± .429
	10 min	.25 ^b ± .445

A.14 The counts of the *Listeria monocytogenes* inoculated and eau de rose added apricot juice

Groups	Mean ± std.
Control	3.22 ^a ± .076
1 µl / 10mL ER	3.12 ^a ± .131
5 µl / 10mL ER	2.87 ^{ab} ± .182
10 µl / 10mL ER	2.42 ^b ± .390
20 µl / 10mL ER	0.94 ^c ± .104

A.15 The microbial counts of the combined *eau de rose* (20µl / 10mL) added and ultrasonication (90 dB amplitude at 24kHz, 1, 5 and 10 min.) treated *Listeria monocytogenes* containing apricot juice

Groups		Mean	±	std.
Control		3.22 ^a	±	.076
20µl eau de rose		.94 ^{cd}	±	.104
90 dB amplitude at 24kHz	1 min	2.02 ^b	±	.523
	5 min	1.17 ^{bc}	±	.429
	10 min	.25 ^{cd}	±	.445
Combination	90 dB amplitude at 24 kHz - 1 min of US + 20µl eau de rose	1.06 ^{bc}	±	.263
	90 dB amplitude at 24 kHz - 5 min of US + 20µl eau de rose	.70 ^{cd}	±	.612
	90 dB amplitude at 24 kHz - 10 min of US +20µl eau de rose	.00 ^d	±	.000

A.16 The total mesophilic aerobic bacteria (TMAB), and yeast/mold counts of the Peach Juice (Control)

		0. Day	20. Day	40. Day	60. Day
TMAB	Mean	1.10	47.60	0.00	0.00
	log	0.04	1.68	0.00	0.00
YM	Mean	7230	7298	9235	Uc
	log	3.85	3.86	3.96	Uc

A.17 The microbial counts of the ultrasonicated Peach juice

Groups		TMAB		YM	
		Mean $\pm$	Std.	Mean $\pm$	Std.
Control		.17 ^a $\pm$	.300	3.83 ^d $\pm$	.083
An amplitude of 30 dB at 24kHz	1 min	.53 ^a $\pm$	.923	3.26 ^e $\pm$	.143
	5 min	.79 ^a $\pm$	.780	3.40 ^e $\pm$	.098
	10 min	.00 ^b $\pm$	.000	3.25 ^e $\pm$	.045
An amplitude of 60 dB at 24kHz	1 min	.41 ^a $\pm$	.704	3.53 ^f $\pm$	.107
	5 min	.51 ^a $\pm$	.876	3.60 ^f $\pm$	.021
	10 min	.00 ^b $\pm$	.000	3.63 ^f $\pm$	.067
An amplitude of 90 dB at 24kHz	1 min	.26 ^a $\pm$	.450	3.56 ^f $\pm$	.060
	5 min	.17 ^a $\pm$	.300	3.64 ^f $\pm$	.042
	10 min	.00 ^b $\pm$	.000	3.57 ^f $\pm$	.060

A.18 The microbial counts of the *eau de rose* added Peach juice

Control	TMAB		YM	
	Mean	std.	Mean	std.
Control	.17 ^a	± .300	3.83 ^d	± .083
1µl / 10mL ER	.79 ^a	± .780	3.69 ^d	± .181
5µl / 10mL ER	.59 ^a	± .524	3.75 ^d	± .055
10µl / 10mL ER	.45 ^a	± .415	3.56 ^{de}	± .082
20µl / 10mL ER	.17 ^{ab}	± .300	3.20 ^e	± .326

A.19 The shelf-life determination studies of the ultrasonicated (an amplitude of 90 dB at 24 kHz), *eau de rose* (20µl / 10mL) added, and combined *eau de rose* and ultrasonication (an amplitude of 90 dB at 24 kHz. - 20µl / 10mL) treated Peach Juice: The TMAB and YM counts

	Groups	0.day	20. day	40.day	60. day
T M A B	An amplitude of 90 dB at 24kHz - 1 min of US	.26 ^{ab} ± .450	.00 ^b ± .000	.16 ^{ab} ± .277	.17 ^{ab} ± .300
	An amplitude of 90 dB at 24 kHz - 5 min of US	.17 ^{ab} ± .300	1.43 ^{ab} ± .375	.17 ^{ab} ± .300	.00 ^b ± .000
	An amplitude of 90 dB at 24 kHz - 10 min of US	.00 ^b ± .000	.00 ^b ± .000	.00 ^b ± .000	.00 ^b ± .000
	20µl ER	.17 ^{ab} ± .300	.16 ^{ab} ± .277	.16 ^{ab} ± .277	.00 ^b ± .000
	Combination; an amplitude of 90 dB at 24kHz - 1 min US + 20µl ER	.16 ^{ab} ± .277	.03 ^b ± .058	.00 ^b ± .000	.00 ^b ± .000
	Combination; an amplitude of 90 dB at 24 kHz - 5 min US + 20µl ER	.05 ^b ± .092	.00 ^b ± .000	.00 ^b ± .000	.00 ^b ± .000
	Combination; an amplitude of 90 dB at 24 kHz - 10 min US + 20 µl ER	.00 ^b ± .000	.00 ^b ± .000	.00 ^b ± .000	.00 ^b ± .000
	Control	.17 ^a ± .300	.83 ^a ± .835	.00 ^b ± .000	.00 ^b ± .000
Y M	Control	3.83 ^d ± .083	4.34 ^d ± .082	5.14 ^d ± .113	6.11 ^d ± .163
	90 dB amplitude at 24kHz - 1 min of US	3.56 ^e ± .060	4.15 ^e ± .121	4.44 ^e ± .155	5.16 ^e ± .065
	90 dB Amplitude at 24kHz - 5 min of US	3.64 ^{ef} ± .042	4.09 ^{ef} ± .426	4.53 ^{ef} ± .140	5.39 ^{ef} ± .085
	90 dB amplitude at 24kHz - 10 min of US	3.57 ^f ± .060	4.36 ^f ± .190	4.59 ^f ± .076	5.76 ^f ± .196
	20µl ER	3.20 ^g ± .326	3.37 ^g ± .229	3.61 ^g ± .025	3.81 ^g ± .185
	Combination; 90 dB amplitude at 24kHz - 1 min US + 20µl ER	3.55 ^h ± .154	3.67 ^h ± .062	3.78 ^h ± .021	3.88 ^h ± .068
	Combination; 90 dB amplitude at 24kHz - 5 min US + 20µl ER	3.61 ^h ± .064	3.72 ^h ± .060	3.81 ^h ± .038	3.89 ^h ± .057
	Combination; 90 dB amplitude at 24kHz - 10 min US + 20µl ER	3.68 ⁱ ± .056	3.80 ⁱ ± .081	3.94 ⁱ ± .047	4.48 ⁱ ± .230

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

Groups		Mean ± std.
Control		3.71 ^a ± .120
An amplitude of 30 dB at 24kHz	1 min.	3.57 ^a ± .080
	5 min.	3.19 ^a ± .106
	10 min.	2.23 ^a ± .317
An amplitude of 60 dB at 24kHz	1 min	1.39 ^b ± .518
	5 min	1.27 ^b ± .587
	10 min	.75 ^b ± .921
An amplitude of 90 dB at 24kHz	1 min	.85 ^b ± .647
	5 min	.33 ^b ± .289
	10 min	.16 ^b ± .277

A.21 The counts of the *E.coli* 0157:H7 inoculated and *eau de rose* added Peach juice

Groups	Mean ± std.
Control	3.71 ^a ± .120
1µl / 10mL ER	2.92 ^b ± .165
5µl / 10mL ER	1.71 ^c ± .362
10µl / 10mL ER	.77 ^d ± .240
20µl / 10mL ER	.16 ^d ± .277

A.22 The microbial counts of the combined *eau de rose* (20µl / 10mL) added and ultrasonication (an amplitude of 90 dB at 24kHz., 1, 5 and 10 min.) treated *E.coli* 0157:H7 inoculated Peach juice

Groups		Mean	±	std.
Control		3.71 ^a	±	.120
20µl <i>eau de rose</i>		.16 ^b	±	.277
An amplitude of 90 dB at 24kHz	1 min	.85 ^b	±	.647
	5 min	.33 ^b	±	.289
	10 min	.16 ^b	±	.277
Combination	An amplitude of 90 dB at 24kHz - 1 min of US + 20µl <i>eau de rose</i>	.17 ^b	±	.300
	An amplitude of 90 dB at 24kHz - 5 min of US + 20µl <i>eau de rose</i>	.16 ^b	±	.277
	An amplitude of 90 dB at 24kHz - 10 min of US +20µl <i>eau de rose</i>	.00 ^c	±	.000

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

Groups		Mean ± std.
Control		3.82 ^a ± .140
An amplitude of 30 dB at 24kHz	1 min.	2.87 ^b ± .162
	5 min.	2.83 ^b ± .187
	10 min.	2.43 ^b ± .397
An amplitude of 60 dB at 24kHz	1 min	2.06 ^c ± .446
	5 min	1.45 ^c ± .646
	10 min	.78 ^c ± .899
An amplitude of 90 dB at 24kHz	1 min	.59 ^c ± .525
	5 min	.16 ^c ± .277
	10 min	.00 ^d ± .000

A.24 The counts of the *Salmonella* spp. inoculated and *eau de rose* added Peach juice

Groups	Mean ± std.
Control	3.82 ^a ± .140
1µl / 10mL ER	3.22 ^{ab} ± .275
5µl / 10mL ER	3.12 ^b ± .164
10µl / 10mL ER	2.89 ^{bc} ± .187
20µl / 10mL ER	.00 ^d ± .000

A.25 The microbial counts of the combined *eau de rose* (20µl / 10mL) added and ultrasonication (an amplitude of 90 dB at 24kHz., 1, 5 and 10 min.) treated *Salmonella* spp. containing Peach juice

Groups		Mean	±	std.
Control		3.82 ^a	±	.140
20µl eau de rose		.00 ^b	±	.000
An amplitude of 90 dB at 24kHz	1 min	.59 ^{cd}	±	.525
	5 min	.16 ^d	±	.277
	10 min	.00 ^d	±	.000
Combination	An amplitude of 90 dB at 24kHz - 1 min of US + 20µl eau de rose	1.15 ^c	±	.458
	An amplitude of 90 dB at 24kHz - 5 min of US + 20µl eau de rose	.27 ^{cd}	±	.473
	An amplitude of 90 dB at 24kHz - 10 min of US +20µl eau de rose	.00 ^d	±	.000

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

Peach juice

Groups		Mean ± std.
Control		3.83 ^a ± .142
An amplitude of 30 dB at 24kHz	1 min.	3.69 ^a ± .050
	5 min.	3.46 ^a ± .101
	10 min.	3.44 ^a ± .108
An amplitude of 60 dB at 24kHz	1 min	3.00 ^b ± .101
	5 min	2.87 ^b ± .165
	10 min	2.39 ^b ± .558
An amplitude of 90 dB at 24kHz	1 min	2.25 ^c ± .199
	5 min	1.05 ^c ± .993
	10 min	.73 ^c ± .643

A.27 The counts of the *Listeria monocytogenes* inoculated and eau de rose added

Peach juice

Groups	Mean ± std.
Control	3.83 ^a ± .142
1µl / 10mL ER	3.66 ^a ± .080
5µl / 10mL ER	3.32 ^a ± .272
10µl / 10mL ER	2.70 ^b ± .257
20µl / 10mL ER	2.17 ^c ± .140

A.28 The microbial counts of the combined *eau de rose* (20µl / 10mL) added and ultrasonication (An amplitude of 90 dB at 24kHz., 1, 5 and 10 min.) treated *Listeria monocytogenes* containing Peach juice

Groups		Mean	±	std.
Control		3.83 ^a	±	.142
20µl eau de rose		2.17 ^{bc}	±	.140
An amplitude of 90 dB at 24kHz	1 min	2.25 ^b	±	.199
	5 min	1.05 ^{bcd}	±	.993
	10 min	.73 ^{cd}	±	.643
Combination	An amplitude of 90 dB at 24kHz - 1 min of US + 20µl eau de rose	.84 ^{bcd}	±	.845
	An amplitude of 90 dB at 24kHz - 5 min of US + 20µl eau de rose	.49 ^d	±	.023
	An amplitude of 90 dB at 24kHz - 10 min of US +20µl eau de rose	.00 ^e	±	.000