ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEVELOPMENT OF A NOVEL BABY FOOD FROM PERSIMMON PUREE AND HAZELNUT FLOUR BY ULTRASONICATION AND EAU DE ROSE ADDITION AND THE INVESTIGATION OF ITS CERTAIN CHEMICAL, PHYSICAL AND MICROBIOLOGICAL PROPERTIES

DOCTOR OF PHILOSOPHY

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APPROVAL OF THE THESIS

Development of a Novel Baby Food From Persimmon Puree and Hazelnut Flour By Ultrasonication and Eau De Rose Addition and The Investigation of Its Certain Chemical, Physical and Microbiological Properties submitted by **H. Dilek ULUSAN** in partial fulfillment of the requirements for the degree of Doctor of Philosophy in **Department of Biology, Abant Izzet Baysal University** by,

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November 04, 2016

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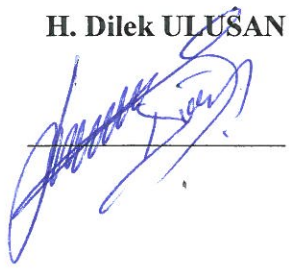
Director, **Graduate School of Natural and Applied Sciences**

To my family

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

H. Dilek ULUSAN



ABSTRACT

DEVELOPMENT OF A NOVEL BABY FOOD FROM PERSIMMON PUREE AND HAZELNUT FLOUR BY ULTRASONICATION AND EAU DE ROSE ADDITION AND THE INVESTIGATION OF ITS CERTAIN CHEMICAL, PHYSICAL AND MICROBIOLOGICAL PROPERTIES
PHD THESIS
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ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
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(SUPERVISOR:PROF. DR. SEYHUN YURDUGÜL)

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Complementary feeding practices are fundamental to infant's nutrition, health, and survival during the first two years of life. Persimmon fruit is an important source of phenolic compounds, vitamin C, antioxidants, glucose, dietary fibre, and carotenoids and has been shown to have positive impact on cardiovascular and immune systems, decrease certain type of cancers and help maintain a healthy vision. On the other hand, hazelnuts play a major role in human nutrition and health due to their special nutritional value. Hazelnut flour is a good source of protein and fat. Aim of the present study is to prepare a novel healthy and nutrition complementary baby food. Persimmon puree and hazelnut flour are treated with ultrasonication technique and eau de rose addition to prepare such a product. Ultrasonication is a non – thermal treatment method to eliminate microorganisims without damaging the nutritionally important compounds in the food. On the other hand, eau de rose has well known anti-bacterial properties. In wiew of these properties, it is expected that baby food prepared from persimmon puree and hazelnut flour and treated with ultrasonication and eau de rose will be a healthy, nutritious complementary baby food. Main finding of our study can be summarized as follows: Ultrasonication and eau de rose treatment improve microbial safety and positively effects biochemical composition of the prepared baby food. Protein, fat, and sugar content and pH of the food were found to be unaffected by the combination treatment. Moreover, no allergen and aflatoxin were detected. Vitamin C levels were decreased in treatment groups with respect to control. The taste panel results were evaluated and it was found that there were no generally extreme differences between the treatment groups of baby food.

KEYWORDS: Baby Food, Persimmon, Ultrasonication, Hazelnut Flour.

ÖZET

ULTRASONİKASYON VE GÜL SUYU UYGULAMALARIYLA TRABZON HURMASI VE FINDIK UNU İÇEREN BEBEK MAMASININ GELİŞTİRİLMESİ VE MİKROBİYOLOJİK, KİMYASAL, FİZİKSEL ÖZELLİKLERİNİN İNCELENMESİ
DOKTORA TEZİ
H. DİLEK ULUSAN
ABANT İZZET BAYSAL ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ
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Tamamlayıcı beslenme uygulamaları yaşamın ilk iki yılı boyunca bebeklerin beslenme, sağlık ve yaşam için temeldir. Trabzon hurması işlevsel bir meyve olup birçok yemekte kullanılabilir. Sağlık açısından çok sayıda yararı olup kalp ve bağışıklık sistemi, bazı kanserlerin riskinde azalma ve sağlıklı görmenin sağlanması gibi yararlar sayılabilir. Trabzon hurması fenolik bileşikler, C vitamini, antioksidan, glikoz, diyet lif ve karotenoid bakımından önemli bir kaynaktır. Öte yandan, fındık unu çok özel besin değeri olması nedeni ile insan beslenmesinde ve sağlığında önemli rol oynamaktadır. Fındık unu protein ve yağ için iyi bir kaynaktır. Bu çalışmada, ultrasonikasyon ve gül suyu ile muamele edilerek oluşturulan bebek mamasının bebeğin yaşam ve beslenmesine pozitif katkıda bulunulması amaçlanmıştır. Belirli mikroorganizmaları yok etmek için kullanılan ve ısıl olmayan bir yöntem olan ultrasonikasyon ek gıdalarda besin kaybını önleyebilir. Buna ek olarak gül suyu antibakteriyel özelliklere sahiptir. Bu özelliklerin ışığında, ultrasonikasyon ve gül suyu ile muamele edilen bu tamamlayıcı gıda bebek sağlığı için uygun olabilir. Bu çalışmaya göre; ultrasonikasyon ve gül suyunun beraberinde kullanılması (2mL ER/10g+90dB+10 min.) başlangıçta mikrobiyal güvenliği sağlamakta olup biyokimyasal açıdan bebek mamasını pozitif yönde etkileyebilir. Kontrol grubu ile kıyaslandığında uygulama gruplarında pH, yağ, şeker ve protein içeriği açısından anlamlı farklılıklar bulunamamıştır. Yapılan testler sonucu mamada alerjen ve aflatoksin olmadığı bulunmuştur. Vitamin C açısından uygulama gruplarında kontrol grubuna göre azalma gözlenmiştir. Tat paneli sonuçlarında uygulama grupları arasında aşırı farklı sonuçlar gözlenmemiştir.

ANAHTAR KELİMELER: Bebek Maması, Trabzon hurması, Ultrasonikasyon, Fındık unu.

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LIST OF ABBREVIATIONS AND SYMBOLS

CF	**:** Complementary Foods
cfu	**:** Colony Forming Unit
CPS	**:** Consumer Product Safety - Health Canada
dB	**:** Decibel
DF	**:** Dietary Fibre
DFCD	**:** Danish Food Composition Databank of the Technical University of Denmark
DNS	**:** Dinitrosalicylic Acid
ER	**:** Eau de Rose
EMB agar	**:** Eosin Methylene Blue Agar
E. coli	**:** *Escherichia coli*
FDA	**:** Food and Drug Administration
FW	**:** Fresh Weight
g	**:** Gram
HHP	**:** High Hydrostatic Pressure
HMWPT	**:** High Molecular Weight Persimmon Condensed Tannin
HPLC	**:** High Performance Liquid Chromatography
Hz	**:** Hertz
ISO	: International Organization for Standardization
ILP	**:** Intense Light Pulse
IR	**:** Infrared Radiation
kcal	**:** Kilocalorie

kGy	**:** Kilogray
kHz	**:** Kilohertz
kPa	**:** Kilo Pascal
L	**:** Liter
L. monocytogenes	**:** *Listeria monocytogenes*
LC-PUFA	**:** Long Chain Polyunsaturated Fatty Acids
Mg	**:** Milligram
MHz	**:** Megahertz
Min	**:** Minute
MKTTn	**:** Muller-Kauffmann Tetrathionate-novobiocin broth
mL	**:** Milliliter
MPa	**:** Megapascal
MS	**:** Manosonication
MTS	**:** Manothermosonication
Log	**:** Logarithm
MUFAs	**:** Monounsaturated Fatty Acids
N.D.	**:** Not Determined
NNDSR	**:** National Nutrient Database for Standard Reference of the United States Department of Agriculture
nm	**:** Wavelength
^{o}C	**:** Centigrade Degree
O.D.	**:** Optical Density
Pas	**:** Proanthocyanidins

PCA	**:** Plate Count Agar
PEF	**:** Pulsed Electric Fields
PL	**:** Pulsed Light Treatment
PUFAs	**:** Polyunsaturated Fatty Acids
ppb	**:** parts per billion
ppm	**:** parts-per million
RDA	**:** Recommended Daily Allowance
SS agar	**:** Salmonella - Shigella Agar
S. cerevisiae	**:** *Saccharomyces cerevisiae*
spp.	**:** Species
TMAB	**:** Total Mesophilic Aerobic Bacteria
US	: Ultrasonication
UV	**:** Ultraviolet
mg	**:** Microgram
µs	**:** Microsecond
VRB	**:** Violet Red Bile Agar
VRBG	**:** Violet Red Bile Glucose Agar
W	**:** Watts
WHO	**:** World Health Organization
XLD agar	**:** Xylose Lysine Deoxycholate Agar
YEA	**:** Yeast Extract Agar
YM	**:** Yeasts and Molds

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1. INTRODUCTION

Adequate nutrition during early stages of life is of key importance for growth and development and for preventing adult diseases. Full breast-feeding for up to 6 months along with timely introduction of complementary food and continued breast-feeding thereafter is recommended as the optimal form of infant feeding (Rebhan et al., 2009).

Since 2001, the World Health Organization recommendation is to introduce complementary foods from the seventh month of life, rather than in the fifth or sixth month as previously recommended. The European Society of Paediatric Gastroenterology, Hepatology and Nutrition supports exclusive or full breast-feeding for about 6 months as a desirable goal and recommends the introduction of complementary feeding not before 17 weeks and no later than 26 weeks. The American Academy of Pediatrics recommends that solid foods should not be introduced before 4 to 6 months of age (Schiess et al., 2010). The timing of introduction of complementary food to an infant's diet is variable throughout the world. The American and European Committees on Nutrition recommend that complementary foods should be introduced at about 6 months of age (Kattelman et al., 2001).

Complementary foods (CF), commonly known as weaning foods, are semi-solid or solid foods that are used to transfer infants from breast milk to an adult and/or elder diet. Their nutritional content is important to the growth and development of children, particularly in developing countries (Bonda et al., 2005).

Depending on the source of proteins, these formulas may be derived from bovine caseine, bovine whey protein, a mixture of caseine and whey proteins, or a mixture of soy proteins and collagen. There is no consensus on the appropriateness of soy formulas for the treatment and prevention of nutritional allergies and current opinion seems to favour hydrolyzed protein formulas (Maldonadoa et al., 1998).

Diets that are rich in fruit and vegetables have been shown to reduce the risk of chronic disease and various cancers. Because consumption of fruit and vegetables

helps to protect against obesity, it is of imminent importance to develop better strategies to increase children's willingness to consume these healthful foods (Osborne et al., 2012). There is much evidence that dietary fibre (DF) may contribute to present and future health benefits in young children. For example, DF has a major influence on the bacterial colonization of the gastrointestinal tract and its maturation, in promoting laxation, and in establishing healthy eating patterns. Foods high in dietary fibre have also been associated with higher satiety, lower incidences of obesity and improved micronutrient intake in children (Brooksa et al., 2006). Also, diet contains high levels of vitamin C to enhance absorption of iron, zinc and copper (Foote and Marriott, 2003).

Persimmon fruit is a good source of carbohydrates, organic acids, vitamins (mainly A and C), minerals, phenolic compounds, dietary fiber, and carotenoids (Vázquez-Gutiérrez et al., 2011; Baltacıoğlu and Artık, 2013). Phenolic compounds have been reported to exhibit antioxidant, anticarcinogenic, antimutagenic and cardioprotective effects. Together with ascorbic acid they protect body tissues against oxidative stress. Fructose and glucose were the main sugars in persimmon fruits, comprising more than 90% of the total sugars. Sucrose was detected at minor levels (Baltacıoğlu and Artık, 2013).

Condensed tannins, also known as proanthocyanidins (PAs) in persimmon are important natural products for human beings because of their strong antioxidant activities with beneficial effects on human health (Ikegami et al., 2007; Navarro et al., 2014). For the fruits with high antioxidant activities, Petridis et al. (2010) showed that in descending antioxidant activity, the order was: cornelian cherry > jujube > cherry > red grape > blackberry > pear > persimmon > plum > white grape > peach > pomegranate > apple > nectarine > kiwifruit > quince > fig > apricot (Petridis et al., 2010). High molecular weight persimmon condensed tannin (HMWPT) is the main antioxidant in persimmon pulp (Tian et al., 2012).

The persimmon is also a good source of biologically active compounds such as ascorbic acid and condensed tannins and these are related to various physiological functions including a protective role against oxidative stress-related diseases, and antimutagenic and anticarcinogenic capacities. The persimmon is a nutritionally important fruit since it has an elevated sugar content; this is higher than in other

extensively consumed fruits such as apples, peaches, pears and oranges. The most abundant sugars found in persimmons are sucrose, glucose and fructose. Such variability is most likely due to the genetic differences among the investigated cultivars (Del Bubba et al., 2009; Giordani et al., 2011).

Persimmons resulted rich in sugars (about 12.5 g/100 g FW (Fresh Weight)), being fructose, glucose and sucrose as the major components, and in total vitamin C, for which 100–150 g of fresh persimmon supplies the recommended daily amount. The main carotenoid components are β-cryptoxantin (193 μg/100 g FW), β, β-carotene (113 μg/100 g FW) and β, ε-carotene (30 μg/100 g FW). Persimmons are also a good source of polyphenolic compounds such as p-coumaric acid, catechin, epicatechin, epigallo catechin, and condensed proanthocyanidins (Giordani et al., 2011; Dembitsky et al., 2011). Nutritional properties include 0.60 g protein, 5.40 pH, 27 mg phosphorus, 203 mg potassium, 16 mg calcium, 11 mg magnesium, 10 mg sodium and 3.15 mg/100 g of tannins (Celik, 2007). Jang et al. (2010) indicated that persimmon fruit is known to have many bioactive compounds such as polyphenols, carotenoids, dietary fiber and minerals. For example, persimmon contains 70 mg of vitamin C, while apple contains only 4 mg in 100 g of its flesh part (Jang et al., 2010).

Total, soluble, and insoluble dietary fibers, total phenols, epicatechin, gallic and p-coumaric acids, and concentrations of Na, K, Mg, Ca, Fe, and Mn in whole persimmons, their pulps, and peels are significantly higher than in whole apples, pulps, and peels. The relatively high contents of dietary fibers, total and major phenolics, main minerals, and trace elements make persimmon preferable for an antiatherosclerotic diet. Total polyphenols in fresh persimmons were higher than in dried fruits (1.3 vs. 0.9 and 0.8 mg/100 g FW, respectively) (Dembitsky et al., 2011). The bioactivity of persimmon is attributed to its water soluble dietary fibers, minerals, trace elements and phenolics, which determine total antioxidant activity of the fruits (Parka et al., 2006; Zhou et al., 2011).

Non-thermal methods, in combination with natural substances can be an alternative way in complementary food sterilization. In a previous study conducted in our laboratories, 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 without disrupting the main chemical and physical properties of the fruit juices (Aydın, 2013).

Finally, sterilization of the complementary food plays an important role on the health of the related consumer. Babies are vulnerable to contamination and sensitive to allergy. That's why the composition of the baby food is important. Soy and whey proteins were generally used in the development of the baby food as a source of protein. In this study, hazelnut flour will be used as a protein source. This mixture will contain high concentration of vitamin C, sugar, and dietary fiber. Vitamin C enhances the absorption of iron and zinc. Also, dietary fiber is important for gastrointestinal tract. In view of these properties, this complementary food treated with ultrasonication and eau de rose addition could be suitable for all possible consumers.

1.1 Food Preservation Method

Thermal pasteurization and sterilization are predominantly used in the food industry for their efficacy and product safety record (O'Donnell et al., 2010; Bermúdez-Aguirre and Corradini, 2012). Excessive heat treatment may cause undesirable protein denaturation, non-enzymatic browning and loss of vitamins and volatile flavor compounds (Chouliara et al., 2010). Effect of pasteurization has been reported for mulberry fruit extract, pineapple juice and cashew apple juice leading to a decrease in the levels of bioactive components such as total anthocyanin, ascorbic acid and carotenoid (Rawson et al., 2011). Advances in technology allowed optimization of thermal processing for maximum efficacy against microbial contaminants and minimum deterioration of food quality. High-temperature short-time pasteurization and ultra-high temperature sterilization, for example, minimize vitamin losses in milk in comparison with batch pasteurization and conventional commercial sterilization, respectively. Products processed by modern thermal technologies still lack the fresh flavor and texture. Non-thermal alternative technologies have been intensively investigated in the past 30 years (Lado and Yousef, 2002). In the last decade, non-thermal technologies for inactivating microorganisms have also been developed in response to the worldwide interest for more fresh and natural food

products. Novel non-thermal technologies such as ultrasounds (US), high pressure processing (HPP), pulsed electric fields (PEF) and pulsed light treatment (PL) have the ability to inactivate microorganisms at near-ambient temperatures, avoiding thermal degradation of the food components, and consequently preserving the sensory and nutritional quality of the fresh-like food products (Pereira and Vicente, 2010; Alexandre et al., 2013).

1.2 Non Thermal Technology

Alternative technologies for inactivating microorganisms without relying on heat are not new concepts, but their development for use as food preservation treatments has received considerable attention only recently (Muñoz et al., 2012), in response to consumer demands for more 'fresh' and 'natural' food products. 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 (Ross et al., 2003; Bermúdez-Aguirre and Corradini, 2012; Ottley, 2000). Non-thermal processes with cell membrane permeabilizing features such as high pressure treatment, high electric field pulse application, the use of supercritical carbon dioxide, or the subjection to ultrasound in combination with moderate heat and pressure hold promise not only for gentle preservation but also to modify plant foods. Such modifications include the improvement of mass transfer and subsequent increases in juice yield, increased recovery as well as retention of desirable metabolites from plant materials, or enhanced water removal during dehydration operations (Knorr, 2003). Some of these non-thermal technologies tested in food processing are high hydrostatic pressure, pulsed electric fields, ultrasound, ultraviolet light, cold plasma, irradiation, pulsed light, dense phase carbon dioxide and shock waves (Bermúdez-Aguirre and Corradini, 2012).

1.2.1 Ultrasonication

The “Father of Ultrasonics”, Paul Langevin, was the first to transmit sound waves in water ostensibly as a method of detecting icebergs to avoid another Titanic disaster (Kwiatkowska et al., 2011). Ultrasound is defined as sound waves having frequency that exceeds the hearing limit of the human ear (~20 kHz). Some animals utilize ultrasound for navigation (dolphins) or hunting (bats) using the information carried by back-scattering sound waves. Ultrasound is one of the emerging technologies that were developed to minimize processing, maximize quality and ensure the safety of food products (Gao et al., 2014b; Ramos et al., 2013; Pakbin et al., 2014). Ultrasound is applied to impart positive effects in food processing such as improvement in mass transfer, food preservation, assistance of thermal treatments and manipulation of texture and food analysis (Awad et al., 2012; Chemat et al., 2011) and microbial inactivation (Bermúdez-Aguirre and Corradini, 2012; Cameron et al., 2008; Bermúdez-Aguirre et al., 2009). Ultrasonication promotes cell membrane permeability and disrupt the bacterial cell wall (Peternel, 2013), alters surface potential resulting in activation of calcium channels; weakens the cell wall/membrane promoting permeability and selectivity and accelerating nutrition or molecule transport and/or increases the dissolution rate of moderately soluble compounds and enhances mass transfer inside and outside of the cell (Kwiatkowska et al., 2011). When a liquid medium is subjected to high power ultrasound, cavitation occurs (Geciova et al., 2002). Cavitation is the production of microbubbles in liquid subjected to a large negative pressure. Due to rectified diffusion, dissolved gas in the liquid becomes free gas in the form of microbubbles (Pingret et al., 2013; Tiwari et al., 2009c; Russel, 2002; Cheng et al., 2007; Cameron et al., 2008; Kasaai, 2013; Zheng and Sun, 2006; São José et al., 2014). Typical treatments are for 1–30 s using 20–40 kHz ultrasound (Russel, 2002). These microbubbles grow during the ultrasound rarefaction cycle, reaching an unstable size, and eventually collapse violently. This could be due to several factors such as collision of the microbubbles, presence of foreign bodies and gradients in the pressure waves (Montalbo-Lomboy et al., 2010; Muñoz et al., 2012; Forghani and Oh, 2013; O’Donnell et al., 2010; Ercan and Soysal, 2011; Kasaai, 2013; Vercet et al., 2001; Gao et al., 2014b). Depending on the ultrasound intensity, food viscosity can either increase or decrease, the effect being temporary or permanent (Soria and Villamiel, 2010).

Ultrasound is a process of sound waves, the frequency of which is above the threshold for human hearing (>16 kHz). 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). As an example, 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). 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 infrared radiation (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, manosonication (MS) and manothermosonication (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 *Listeria monocytogenes* (Pagán et al., 1999b) and cells suspended in a low water activity media (Álvarez et al., 2003b). Moderately increased temperatures such as 55–60ºC weaken cell envelopes and facilitate the effect of ultrasonic waves such as mechanical disruption of the cell (Álvarez et al., 2003b). 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).

Ultrasound was also reported to increase the effectiveness of antibiotics against biofilm cells. Lower-frequency in sonication is remarkably more efficient than higher frequency for reducing biofilm cells viability (Srey et al., 2013). Ultrasound processing enhances enzymatic reactions at low power levels e.g. a-amylase, invertase and amyloglucosidase for starch, sucrose and glycogen hydrolysis respectively (O'Donnell et al., 2010). Ultrasound can not only increase the freezing rate, but also improve the quality of the frozen products (Zheng and Sun, 2006).

Ultrasound has been used successfully to induce transfer of genetic material into live animal and plant cells. Intense ultrasound is known to damage macromolecules such as enzymes, probably from unfolding and scrambling the native protein and breaking the chain into radicals or small peptides (Chisti, 2003). The functional properties of Bovine Serum Albumin were altered by ultrasonication and surface activity of Bovine Serum Albumin was increased (Gülseren et al., 2007).

Based on frequency range, the applications of ultrasound in food processing, analysis and quality control can be divided into low and high energy (Ercan and Soysal, 2011; Soria and Villamiel, 2010; Kasaai, 2013; Zheng and Sun, 2006; Bermúdez-Aguirre and Barbosa-Cánovas, 2012). Low energy (low power, low intensity) ultrasound has frequencies higher than 100 kHz at intensities below 1 W/cm^2, which can be utilized for non-invasive analysis and monitoring of various food materials during processing and storage to ensure high quality and safety (Soria and Villamiel, 2010; Kasaai, 2013; Gao et al., 2014b). Low power ultrasound has been used to nondestructively support genetic improvement programs for livestock and for evaluating the composition of raw and fermented meat products, fish and poultry. It is

also used for the quality control of fresh vegetables and fruits in both pre- and postharvest, cheese during processing, commercial cooking oils, bread and cereal products, bulk and emulsified fat based food products, food gels, aerated and frozen foods. Other applications include the detection of honey adulteration and assessment of the aggregation state, size and type of protein (Awad et al., 2012).

Low energy ultrasound applications include stimulation of activity of living cells, surface cleaning of foods, effects on enzymes, ultrasonically assisted extraction, crystallization, emulsification, filtration, drying and freezing processes as well as tenderization of meat (McClements, 1995; Knorr et al., 2004; Bilek and Turantaş, 2013).

High energy (high power, high-intensity) ultrasound uses intensities higher than $1W/cm^2$ at frequencies between 20 and 500 kHz, which are disruptive and induce effects on the physical, mechanical or chemical/biochemical properties of foods (Awad et al., 2012; Kasaai, 2013; McClements, 1995; Knorr et al., 2004; Bilek and Turantaş, 2013). These effects are promising in food processing, preservation and safety. This emerging technology has been used as alternative to conventional food processing operations for controlling microstructure and modifying textural characteristics of fat products (sonocrystallization), emulsification, defoaming, modifying the functional properties of different food proteins, inactivation or acceleration of enzymatic activity to enhance shelf life and quality of food products, microbial inactivation, freezing, thawing, freeze drying and concentration, drying and facilitating the extraction of various food and bioactive components (Awad et al., 2012). Higher-power ultrasound at lower frequencies (20 to 100 kHz), is referred to as "power ultrasound" and has the ability to cause cavitation, which has uses in food processing to inactivate microorganisms (Bilek and Turantaş, 2013).

High energy ultrasound has been applied for degassing of liquid foods, the induction of oxidation/reduction reactions, extraction of enzymes and proteins, enzyme inactivation and the induction of nucleation for crystallization (Knorr et al., 2004).

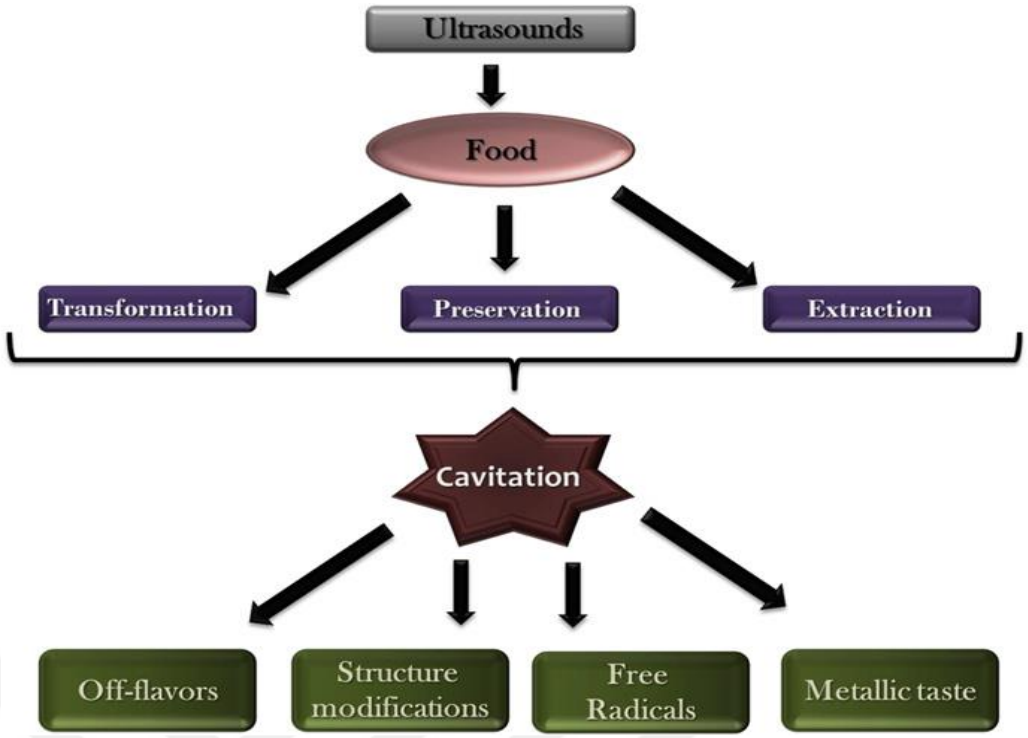

Figure 1. Chemical effects generated by cavitation phenomena (Pingret et al., 2013).

Ultrasound can increase the survival rate of fish, increase the productivity for large scale farm crops and stable emulsions generated using ultrasound are used in the cosmetic, pharmaceutical, textile and food industries. Ultrasound can be used as an alternative in extraction. The mechanical effect of ultrasound provides a greater penetration of solvent into the cellular materials and result in the disruption of biological cell walls to facilitate the release of contents. The advantages of sonochemistry application in food processing increase both the extraction yield and rate, resulting in reduced extraction time and a higher throughput. Ultrasound enhances the extraction yield of lycopene from tomato by 26% on the average of 6 replications at the coded level (Eh and Teoh, 2012).

1.2.2 The Mechanisims of Ultrasonication

The use of ultrasound in bacterial inactivation was first reported in the 1920s (Harvey and Loomis, 1929), and the fundamental mechanism of bacterial inactivation was explored during the 1950s and 1960s (Davies, 1959; Earnshaw et al., 1995; Gao et al., 2014a).

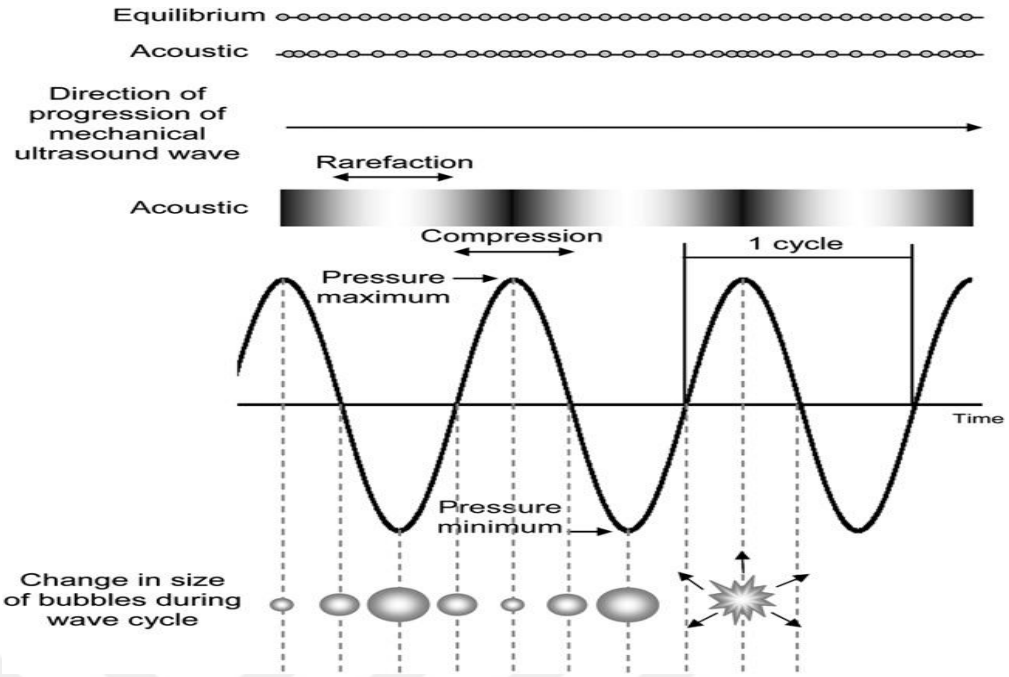

Figure 2. Ultrasonic cavitation (Soria and Villamiel, 2010).

Bactericidal effect was first described by Scherba et al. who, in 1991, wrote a study describing bactericidal ultrasound effect on *Escherichia coli, Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* in planktonic form. The exposure durations examined for the bacterial experiments were 1, 2, 4, 8, 16, and 32 minutes. Results of this work show how all bacteria species analysed were killed by ultrasound application, with a rate directly proportional to the exposure time and intensity level. The principal ultrasonic effect, at low frequency, responsible for bactericidal power seemed to be the cavitational effect with no differences between Gram - or Gram+ bacteria (Scherba et al., 1991).

Amplitude, pressure, exposure time, volume processed, temperature, food composition, viscosity, and concentration of solids are the parameters describing the ultrasonic liquid processing as an energy function and acoustic waves intensity. Ultrasonication depends on main parameters of the process. First, the frequency is inversely proportional to the bubble size. Therefore, low frequency ultrasound produces large cavitation bubbles culminating to locally higher temperatures and pressures. Second, increasing the external pressure, it reduces the number of cavitation bubbles as a result of elevating the cavitation (Mohammadi et al., 2014).

Ultrasonic waves with large amplitudes facilitate the displacement of molecules and collapse pressure. The viscosity of the medium can affect cavitation. In highly viscous foods, ultrasound diffusion is difficult, thus reducing the frequency at which cavitation occurs. Lower frequencies are more effective in this case, as the ultrasound waves can pass through the viscous product. The pH of the medium or food is another condition that can affect ultrasound efficiency. At a lower pH, inactivation rate of microorganisms is increased (São José et al., 2014).

Other proposed mechanisms of ultrasound on microbial inactivation occur by free radical formation. These compounds can help facilitate different applications of ultrasound. During bubble collapse, energy is released, which can contribute to the sonolysis of water. In this reaction, OH^-, H^+ and hydrogen peroxide are generated, which have important bactericidal effects. DNA is the primary target of these free radicals in the bacterial cell, which produce breakages and fragmentation along the extension of the DNA (São José et al., 2014).

1.3 Baby Food – Complementary Food

The first 2 years of life are critical to the growth and development of children. Feeding practices and food consumption contribute to infants' and young children's nutrient adequacy (Briefel et al., 2010). Breast-feeding and complementary feeding practices are fundamental to children's survival and development. In many developing countries, nutritional problems in infants and young children are closely linked to these practices. Among other things, feeding practices have an impact on physical growth, which is regarded as one of the best indicators of children's well-being. However, the relation between the quality of feeding practices during early age and nutritional status are difficult to establish, and, depending on the context and overall living conditions, the influence of feeding factors on children's nutritional status can vary considerably. In addition, feeding practices are often complex, change with a child's age, and are seldom all positive or all negative. It is therefore not easy to assess the global quality of feeding practices at the scale of the individual child (Sawadogo et al., 2006).

One to three years of age is a critical period for the acquisition of food preferences. During these 'toddler years' children experience developmental gains in

body function, language, and motor and social skill, and establish a large proportion of their food preferences. Given that early introduction or exposure to fruits and vegetables is positively associated with increased intake and variety of these foods consumed later in childhood (Howard et al., 2012).

During the first year of life, infants' show transition from a diet of breast milk and/or infant formula to one that includes solid foods and other beverages (Fein et al., 2008). The breast milk and milk are major contributors of energy and most nutrients in the diets of infants and toddlers. Among toddlers, juices and fruit flavored drinks are the second and third most important sources of energy. Fortified foods make substantial contributions to intakes of many essential nutrients, and these contributions increase as children age. For example, among toddlers, fortified grain-based foods make substantial contributions to intakes of vitamin A, iron and folate, relative to foods that are naturally rich in these nutrients. Supplements also make substantial contributions to intakes of vitamins and selected minerals, particularly among toddlers (Fox et al., 2006).

Most infant formulas are derived from animals or plants and therefore are mostly milk-based or soy-based formulations. Because of the nutritional properties of soybean, dairy-like products, or infant formulas based on soybean, are proposed as one of the most interesting alternatives for adults or children who are allergic to animal proteins. Consumption of soybeans is reported to provide immense health benefits but soybean is known to have high levels of neurotoxins, as well as aluminium and silicon (Ikema et al., 2002).

Table 1. Reference values for the average daily energy and nutrient intakes in populations of healthy European children (Koletzko, 2008).

Age	**1<4 Years**
Vitamin C (mg/day)	55
Iron (mg), male/female	8
Vitamin A (mg retinol equivalent)	0.6
Vitamin D (mg)	5
Vitamin K (mg), male/female	15
Energy (kcal/kg), male/female	100
Protein (g/kg), male/female	1.2
Fat (% of energy)	30-35
Essential fatty acids (% of energy)	3.5
Calcium (mg)	600
Magnesium (mg), male/female	80

Protein source, macronutrient composition and content of long chain polyunsaturated fatty acids (LC-PUFA) of infant formulae may influence infant growth (Fleddermann et al., 2014). The modification of protein source, macronutrient composition and content of LC-PUFA of infant formulae may influence infant growth and development, modified formula must be tested to ensure supporting adequate growth (Fleddermann et al., 2014).

Microorganisms and contamination are important for baby food since microorganisms can be isolated from a wide spectrum of food and food ingredients. The ubiquitous microorganism *Enterobacter sakazakii* is a rare contaminant of infant formula and may cause severe systemic infectionsin baby. *Enterobacter sakazakii* was isolated from plant food and food ingredients like cereal, fruit and vegetables, legume products, herbs and spices as well as from animal food sources like milk, meat and fish and products made from these foods (Friedemann, 2007; Iversen and Forsythe, 2004; Tesfai et al., 2014).

The CPS (Health Canada) and the WHO (World Health Organization) recommend the introduction of complementary foods at about 6 months of age for the healthy born infant. The energy contribution from complementary foods at 6-8 months of age to total dietary intake is ~20% and this increases to 50% at 1 year of age. Complementary food should be both energy dense and micronutrient rich. Infants are ready to begin complementary food, when they have good head and neck control, are able to be safely placed in a seated position and are showing signs of both hunger and satiety such as turning their head away when full (O'Connor and Sharon Unger, 2013).

Complementary feeding (i.e, solid foods and liquids other than breast milk) should not be introduced before 17 weeks and no later than 26 weeks. There is no convincing scientific evidence that avoidance or delayed introduction of potentially allergenic foods, such as fish and eggs, reduces allergies, either in infants considered at increased risk for the development of allergy or in those not considered to be at increased risk (Agostoni et al., 2008).

1.3.1 Fruit Baby Food

Commercial fruit baby food is a preserved fruit product usually made with fruit purees, sugar, water and variable additives. Since infants between 6 month and 3 years of age are rather limited in their food choices, the commercial fruit baby foods serve as the important source of energy, basic nutrients, fiber, vitamins and minerals and establish their taste and eating patterns. Fruit baby foods are perceived as the fiber, ascorbic acid, polyphenols and other antioxidants sources based on the fruit (vegetable) content and composition. The other important factors affecting the nutritive value are conditions of processing and all parameters which could cause the reduction of nutrients in products, such as oxidation, non enzymatic browning, the effect of contaminants (metals, migrants from packaging, etc.). These reactions are usually affected by heating, therefore the heat damage during the blanching, boiling, pasteurisation or sterilisation, storage conditions during the retail, etc. are important for the nutritive value of baby food as well (Čížková et al., 2009).

Fruit-based infant foods, together with cereals, are the first foodstuffs introduced in the diet of the unweaned infant, contributing to cover the vitamin

requirements, especially those of vitamin C. Ascorbic acid (AA) has multiple biochemical roles, though it primarily acts as a water-soluble antioxidant in the human body. The recommended dietary allowances for vitamin C are 40 or 50 mg/day in unweaned infants from 0 to 6 or 7 to 12 months of age, respectively. On the other hand, the maximum amount of added vitamin C allowed in fruit based infant foods is 125 mg/100 kcal (Bosch et al., 2013).

Dietary fibre (DF) may contribute to present and future health benefits in young children. For example, DF has a major influence on the bacterial colonization of the gastrointestinal tract and its maturation, in promoting laxation, and in establishing healthy eating patterns. Foods high in dietary fibre have also been associated with higher satiety and therefore lower incidences of obesity and improved micronutrient intake in children (Brooks et al., 2006).

1.3.1.1 Food Allergies and Food Sensitivities

Food allergy or hypersensitivity is a form of food intolerance characterized by reproducible symptoms with each exposure to the offending food and evidence of an abnormal immunologic reaction to the food. The most common foods associated with allergic reactions include eggs, cow's milk, wheat, soy, peanuts, tree nuts, fish, and shellfish. Infants with a strong family history of food allergy (ie, those whose parents or siblings have or had significant allergies) should be breastfed for as long as possible and should not receive complementary foods until after 6 months of age. Introduction of the major food allergens should be delayed until well after the first year of age (Butte et al., 2004).

Attempts to reduce the risk for the development of allergy using dietary modification have generally focused on the delayed introduction or elimination of foods identified as potentially most allergenic, although there is also increasing interest in the active prevention of atopy using specific dietary components. There is good evidence that certain foods are more allergenic than others. They include eggs, fish, nuts, and seafood. There is also observational evidence that the early (<4 months) introduction of more than 4 foods is associated with an increased risk of atopic dermatitis, both in the short term and, more important, at 10-year follow-up. The

evidence that delaying or avoiding the introduction of allergenic foods prevents or delays the development of allergy (Agostoni et al., 2008).

1.4 Persimmon (Diospyros spp.)

Diospyros spp. (Persimmon) is a fruit belonging to the Ebenaceae family (Nugraheni et al., 2013; Giordani et al., 2011; Zhang et al., 2013; Rashed et al., 2014; Chung et al., 2015). Persimmon is widely grown in many regions of the world. China and Japan are known to be land of origin and big producers and consumers of persimmon (Navarro et al., 2014; Del Bubba et al., 2009; Karhan et al., 2003; Khan et al., 2007). Fuyu and hachiya varieties are very popular in Turkey as well as in the world. Although, it is not known when persimmon have cultured first in Turkey, it has a total production of 10.000 tons per year in Mediterranean and Black Sea region (Karhan et al., 2003; Evrenosoğlu et al., 2011). According to the available records, persimmon was introduced to Turkey from Russia via the route of Black Sea. Persimmon has four species (*Diospyros kaki* L., *Diospyros lotus* L., *Diospyros virginiana* L., *Diospyros oleifera* Cheng). (Bibi et al., 2007). *D. kaki* is widely grown in many parts of Turkey (Yıldız et al., 2007). There are generally two types of *Diospyros kaki* fruit: astringent and non-astringent (Chung et al., 2015; Oh et al., 2013). Astringent species cannot be eaten when firm because of high levels of soluble tannins. Nonastringent *Diospyros kaki* are not actually free of tannins (Nugraheni et al., 2013). Persimmon tannin were divided into hydrolysable tannin and condensed tannin (Zhang et al., 2011; Wu and Hwang, 2002). It contains many bioactive compounds, such as sugars, vitamins, flavonoids, tannins, terpenoids, steroids, naphthoquinones, dietary fiber, carotenoids, minerals, amino acids, and lipids (Oh et al., 2013). Astringency is caused by procyanidine which are also known as condensed tannins (Bibi et al., 2007).

Persimmon contains large amount of condensed tannins. Persimmon tannins comprising a large variety of structures, give many health function and is the key factors that affect product quality in processing (Zhang et al., 2011). Persimmon has a positive influence on the level of plasma lipids and antioxidant activity in an animal model (rats and mice) fed cholesterol (Chol)-containing diets (Gorinstein et al., 2011).

Persimmon has been traditionally used to treat conditions such as coughs, hypertension (Gua et al., 2008), dyspnea, paralysis, frostbite, burns (Veberic et al., 2010) and bleeding, disease of the bowels, chronic dysentery (Rashed et al., 2014). Foods containing condensed tannin, such as persimmon fruit, have been associated with antioxidant activity, anti-inflammatory activity and atherosclerosis prevention. It has hypocholesterolemic, antitumor, hypolipidemic (Zou et al., 2012) antioxidant antidiabetic and protects DNA against oxidative damage (Tian et al., 2012; Veberic et al., 2010; Nugraheni et al., 2013; Lee et al., 2012; Yaqub et al., 2013). Glucose, fructose, sucrose and total sugar content levels in *Diospyros kaki L*. cultivar were analyzed in a study. Generally, glucose was found in the highest concentrations, followed by fructose and sucrose. In persimmon fruit, three organic acids were determined, the predominant one being malic acid, followed by citric acid and fumaric acid. Amongst the phenolic compounds in fully ripe persimmon fruit, catechin and gallic acid were predominant (Veberic et al., 2010).

The standard value of total sugar concentration in persimmon fruits is about 12.5g/100 g FW, according to the National Nutrient Database for Standard Reference of the United States Department of Agriculture (NNDSR) and the Danish Food Composition Databank of the Technical University of Denmark (DFCD), being higher than those of other extensively consumed fruits such as apples, peaches, pears and oranges. The main sugars in the flesh of mature fruit are fructose, glucose and sucrose; while galactose and arabinose are minor components (Giordani et al., 2011).

Consumption of one piece of kaki fruit (200–400 g) would give a mineral intake providing 1–10% of the recommended daily allowance (RDA) for calcium, 1–30% for copper and potassium, 1–15% from iron and magnesium, up to 1% of sodium, and up to 4% of zinc (Mir-Marqués et al., 2015). Allergy to persimmon (*Diospyros kaki*) is very rare (Anliker et al., 2001).

1.5 Hazelnut

Hazelnut (*Corylus avellana L.*) belonging to the Betulaceae family is a very popular tree nut worldwide. Hazelnut is Turkey's most important agricultural crop since Turkey is one of the main producers of hazelnuts in the world. Turkey has the largest hazelnut production area with 550–650 thousand hectares that includes the 83% of total world area (Aydemir et al., 2014; Delgado et al., 2010). Although hazelnut is being raised mainly for its fruit and oil, hazelnut bagasse and hazelnut shells have a great importance as a source of energy (Demiral and Şensöz, 2008). Hazelnuts have a high fat content of monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs), the major compounds in hazelnut fatty acids (Delgado et al., 2010).

Hazelnuts play a major role in human nutrition and health due to their very special nutritional value. One hundred grams of hazelnut provides 600–650 kcal. Hazelnut kernel includes carbohydrate at 10–22%. It also includes organic acids but in small quantities and the most abundant organic acid in hazelnut kernel is malic acid. The protein content of hazelnut kernel changes between 10% and 24%. The average niacin, vitamin B1, vitamin B2, vitamin B6, ascorbic acid, folic acid, retinol and total tocopherol contents of hazelnut kernels were 1.45 mg/100 g, 0.28 mg/100 g, 0.05 mg/100 g, 0.5 mg/100 g, 2.45 mg/100 g, 0.043 mg/100 g, 3.25 mg/100 g and 26.9 mg/100 g, respectively. The amount of the essential amino acids, mostly as arginine (2003 mg/100 g) and leucine (1150 mg/100 g), and the non-essential amino acids, mostly as glutamic acid (2714 mg/100 g) and aspartic acid (1493 mg/100 g) were also determined in the hazelnut varieties. Mineral compositions of the hazelnut varieties, e.g., K, Mn, Mg, Ca, Fe, Zn, Na and Cu were (averagely) measured as 863 mg/100 g, 186 mg/100 g, 173 mg/100 g, 5.6 mg/100 g, 4.2 mg/100 g, 2.9 mg/100 g, 2.6 mg/100 g and 2.3 mg/100 g, respectively (Köksal et al., 2006).

Hazelnuts are also used as a complementary protein source in combination with legume foods and to increase protein values of cereal products by incorporating nuts as flour, protein isolates and meal (Kalemoğlu et al., 2004). Turkish hazelnut meal is a good source for protein extraction (Aydemir et al., 2014). In all hazelnut varieties, however, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine are determined as the essential amino acids, and arginine and leucine are found as the most abundant (Köksal et al., 2006). Hazelnuts are used in a

large variety of confectionery foods like chocolates, nougat, cookies, cereal mixtures, muesli bars and chocolate spreads (Fæste et al., 2006).

Hazelnut kernels are a good source of fat (50–73%) and contain unsaturated fatty acids (linoleic, linolenic, oleic acids, palmitic and stearic), essential for human health. Hazelnut oil decreases the cholesterol level in blood and also controls adverse effects of hypertension. The main fatty acids in hazelnut varieties were oleic (79.4%), linoleic (13.0%) and palmitic acid (5.4%) (Köksal et al., 2006).

The lack of dietary fiber in diets has been associated with constipation, diverticulosis, cardiovascular diseases and cancer. Hazelnut testa is named as antioxidant dietary fiber (Anil, 2007). Hazelnuts contain dietary fibre as well as beneficial nutrients, such as plant proteins, essential minerals (potassium, calcium, magnesium, selenium), vitamin E, vitamins of B complex, as well as unsaturated fatty acids, plant sterols, phytochemicals and micronutrients. Nuts have been shown to contain substances such as tocopherols and polyphenols that significantly reduce the risk of coronary heart disease, some types of cancer, and several other diseases and physiological conditions and syndromes. Total phenol concentrations ranged from 70 to 478 mg gallic acid equivalents per kg hazelnut kernels (Jakopic et al., 2011).

1.6 Eau de Rose

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).

Rose water is well known for its natural healing properties as a good anti-septic, anti bacterial, anti-inflammatory product. It has anti-viral and anti-bacterial properties, rose water can be used as a disinfectant to keep our home or workplace free from bacteria and germ (Raaz and Rani, 2013). Rose water is a liquid preparation obtained by hydrodistillation of fresh rose flowers. Under the Indian System of Medicine, various rose preparations are being used as an astringent, tonic, mild laxative, antibacterial and in treatment of sore throat, enlarged tonsils, cardiac troubles, eye

diseases, gall stones, cooling effect and as vehicle for other medicines (Agarwal et al., 2005).

Roses have solid (Stearoptene) and liquid (Oleoptene) components. They are rich in corilagin and tellimagrandin (Shiota et al., 2004) and these compounds have a significant antimicrobial activity. Corilagin has strong antioxidative, hepatoprotective (Kinoshita et al., 2007), thrombolytic (Shen et al., 2003) and antiatherogenic (Duan et al., 2005) activities.

2. AIM AND SCOPE OF THE STUDY

In this study, baby food or complementary food that contain hazelnut flour and persimmon fruit will be subjected to non – thermal processing, ultrasonication, and/or eau de rose addition to produce appropriate feed for the babies of 1 year old and up to 4 years. The expectation is that it would make a positive contribution to the nutrition and health of baby.

The basic hypothesis is that, this complementary food, treated with ultrasonication and addition of eau de rose is going to effect the development of the baby positively. The nutritional content of baby food is important with relevance to the growth and development of children, particularly in developing countries. Adequate nutrition during early life is of key importance for growth and development. On development of novel baby food concerning the treatment with ultrasonication, up to now, there has not been a study performed yet. Also, up to date, no combined use of both persimmon puree and hazelnut flour in baby food have been reported.

In addition, no study or similar one has been reported on ultrasonication and the mixture of the persimmon puree and hazelnut flour to obtain a baby food.

The Eastern Black Sea region of Turkey is one of the main hazelnut producers in Turkey and in the world. Hazelnuts play a major role in human nutrition and health due to their very special nutritional value. Hazelnuts are a good source of protein and fat. In addition, persimmon fruit is an important source of phenolic compounds, vitamin C, antioxidants, glucose, dietary fibre and carotenoids. This kind of a baby food containing dietary fibre, phenolic compounds, vitamin C, antioxidants, glucose and protein can serve a good nutrition choice especially suffering from constipation. Constipation is a significant distress for baby. Dietary fibre may be a major influence on the bowel movement in gastrointestinal tract.

3. MATERIALS AND METHODS

3.1 Materials

The hazelnut flour and *eau de rose* (Rosense^{TM}, 100% natural) were purchased from GÜRSOY, Ordu, TURKEY and Gülbirlik A.Ş., Isparta, TURKEY, respectively. The persimmons, 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 mashed to obtain baby food by a blender (Moulinex, FRANCE). 100 g from persimmon fruit and 20 g from hazelnut flour were mixed in a sterile container. The final weight of the mixture was 120 g.

3.2 The Treatment (Method)

The treatment groups of the baby food samples were as follows:

Table 2. The treatment groups of the baby food samples.

Treatment Groups	Amount / Freguency
Ultrasonication	90 dB, 60 dB and 30 dB
Eau de rose (ER)	0.5, 1 and 2 mL
Control (no treatment) (Mixing ratio)	120 g baby food

Table 3. Treatment(T) groups.

Codes	Treatment Groups
(T1)	10 g baby food (Control)
(T2)	0.5 mL ER/10 g baby food
(T3)	1 mL ER/10 g baby food
(T4)	2 mL ER/10 g baby food
(T5)	10 g baby food + 30 dB(decibel) + 5 minute
(T6)	10 g baby food + 30 dB(decibel) + 10 minute
(T7)	10 g baby food + 60 dB(decibel) + 5 minute
(T8)	10 g baby food + 60 dB(decibel) + 10 minute
(T9)	10 g baby food + 90 dB(decibel) + 5 minute
(T10)	10 g baby food + 90 dB(decibel) + 10 minute
(T11)	Combination of 2 mL ER/10 g baby food +90 dB (decibel)+ 5 minute
(T12)	Combination of 2 mL ER/10 g baby food +90 dB (decibel)+10 minute

3.2.1 Eau de rose application

Eau de rose (Gülbirlik A.Ş.) was added to baby food sample at an amount of 0.5, 1 and 2 mL/10 g.

3.2.2 The treatment with ultrasonication

10 g of each of the baby food was ultrasonicated for a period of 5 and 10 minutes at the amplitudes of 30, 60, 90 dB (decibel) with a frequency of 24 kiloHertz (kHz) (Hielscher Ultrasound Technology, UP 400S Ultrasonic processor, Germany). The combination of the best effective dose of sole ultrasonication and sole eau de rose application was determined. Temperature control during ultrasonication treatment was achieved by decreasing excess heat evolved, 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 15ºC to 24ºC through ultrasonication process.

3.3 Microbiological Analysis

According to Turkish Food Codex Regulation on Microbiological Criteria of the Ministry of Food, Agriculture and Livestock, *Salmonella* spp., *Listeria monocytogenes*, *Enterobacteriaceae* spp., *Bacillus cereus* should be analyzed for additional food or complementary food for infants and children.

The treated and untreated (control) baby food samples were diluted with sterile distilled water to enumerate viable cells of the natural micropopulation. The microbial counts were converted to logarithmic values. 0.1 mL samples of the first three dilution tubes were inoculated in triplicates on the agar plates and the cell counts were expressed as cfu/g.

3.3.1 The Total Mesophilic Aerobic Bacteria

Plate count agar (PCA) (Merck, Germany) was used to enumerate the total mesophilic aerobic bacteria (ISO 4833). The plates were incubated at 37ºC ± 1ºC for 24-48 hours in triplicates.

3.3.2 The Yeast/Mold

Yeast and filamentous fungi counts were carried out by surface plating on yeast extract agar in triplicates (YEA) (Merck, Germany). The plates were incubated at 25ºC ± 1ºC for 5 days.

3.3.3 *E.coli*

Eosin Methylene-Blue agar (EMB) (Merck, Germany) was used to enumerate *Escherichia coli*. The plates were incubated at 37ºC ± 1ºC for 24 hours.

3.3.4 The Total Coliforms

Violet Red Bile agar (VRB) (Liofilchem, Italy) was used for detecting total coliform bacteria (ISO 4832). The plates were incubated at 37ºC ± 1ºC for 24 hours.

3.3.5 *Enterobactericeae* spp.

Violet Red Bile Glucose agar (VRBG) (Liofilchem, Italy) was used for detecting *Enterobactericeae* spp. The plates were incubated at 37ºC ± 1ºC for 24 hours.

3.3.6 *Bacillus cereus*

Bacillus cereus Chromogenic agar (Liofilchem, Italy) was used to enumerate *Bacillus cereus* from food samples. The plates were incubated at 37ºC ± 1ºC for 24 hours.

3.3.7 *Listeria monocytogenes* (TS EN ISO 11290-1:2004)

The TS EN ISO 11290-1:2004 conventional method was followed for the detection of *Listeria monocytogenes*. Baby food sample was enriched with Half Fraser Broth (1:10 ratio) (Liofilchem, Italy) and was incubated at 30ºC ± 1ºC for 24 hours. Then 1 mL of incubated Half Fraser Broth was transferred to 10 mL Fraser Broth (Liofilchem, Italy) and incubated at 30ºC ± 1ºC for 24 hours. After incubation, a loopful amount of colonies was subsequently streaked into Palgam (Liofilchem, Italy) and *Listeria monocytogenes* Chromogenic Agars (Liofilchem, Italy) and incubated at 37ºC ± 1ºC for 24 hours. The suspected colonies on Palgam agar and *Listeria monocytogenes* Chromogenic Agar were incubated on blood agar at 37ºC ± 1ºC for 24 hours. The colonies which hemolyzed on blood agar were transferred to Microgen Listeria Identification Kit (Microgen, England) for the identification of *Listeria monocytogenes*.

3.3.8 For Determination of *Salmonella* spp. (TS EN ISO 6579:2002)

The TS EN ISO 6579:2002 conventional method was used for the isolation of *Salmonella* spp. Baby food sample was enriched with peptone broth (Liofilchem, Italy) (1:10 ratio) and incubated at 37ºC ± 1ºC for 24 hours. Then, separately, 0.1 mL of incubated peptone broth was transferred to 10 mL Rappaport Vassiliadis Soy Broth (RVS) (Liofilchem, Italy) and incubated at 41.5ºC ± 1ºC for 24 hours and 1 mL of incubated peptone broth was transferred to 10 mL Muller-Kauffmann Tetrathionate-novobiocin broth (MKTTn) (Liofilchem, Italy) and incubated at 37ºC ± 1ºC for 24 hours. 0.1 mililiters of the two broths mentioned above was transferred to Xylose Lysine Deoxycholate (XLD) (Merck, Germany) agar and Salmonella – Shigella Agar (SS) (Liofilchem, Italy). The colonies which were red with black centers on the agars were identified with GNA Enterobacter Identification Kit (Microgen, England).

3.4 The shelf life

Baby food samples were kept in the refrigerator at +4ºC. In order to detect the microbial quality parameters of shelf life, microbial counts of baby food samples were done at 30^{th}, 60^{th} and 90^{th} days.

3.5 The Physical and Chemical Analysis

3.5.1 The pH

The pH of the baby food samples was measured by using a digital pH meter (WTW Inolab pH 720, Germany).

3.5.2 Total Soluble Solids (ºBrix)

A portable refractometer (Miiler, RHB-10/ATC, Germany) was used for the detection of total soluble solid content of the baby food. The measurements were performed at 25ºC ± 0.5ºC and the results were expressed as ºBrix.

3.5.3 Viscosity

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

3.5.4 Color Determination

The color of the baby food was 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). All measurements were performed in triplicates.

3.5.5 Determination of Total Sugar Content

The total sugar content of the baby food sample was determined by using dinitrosalicylic acid (DNS) (Sigma, USA) method (Miller, 1959). The standard solutions which contained 1, 2, 3 and 4% of D-fructose (Merck, Germany) were prepared. 1 mL from each of these fructose solutions were treated with 3 mL DNS

(Sigma, USA). Subsequently 1 g of baby food was 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.

3.5.5.1 Preparation of DNS Solution

10.6 g of DNS (Sigma, USA) (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.

3.5.6 Fat Determination by Soxhlet Method

The fat content of the baby food was determined by Soxhlet method (Soxhlet, 1879). The samples were placed in a porous cellulose thimble. Petroleum ether was used as an organic solvent in this experiment. The thimble was placed in an extraction chamber, which is suspended above in a flask, previously dried at 105ºC, weighed and coded as W1, containing the solvent and below a condenser. After heating the flask, the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts six hours, the flask containing the solvent and lipid was removed. The solvent in the flask was then evaporated and the mass of the remaining lipid was measured. The flasks were placed in an oven at 105ºC and dried until a constant weight was reached (1-2 hours). Then the flasks were cooled in a desiccator and the flask and its content were weighed. It was evaluated as W2. The percentage of lipid in the initial sample was calculated according to the equation (Eq.1):

Eq.1: % Crude fat = $\frac{W2-W1}{m}$ x100

Weight of empty flask (g) = W1

Weight of flask and extracted fat (g) = W2

Weight of sample = m

3.5.7 Determination of Total Nitrogen (Kjeldahl method)

The Kjeldahl method was used to determine nitrogen content (Magomya et al., 2014). The baby food samples; weighed in a Kjeldahl digestion flask by boiling with 25 mL of concentrated H_2SO_4 and a Kjeldahl digestion tablet (catalyst) until the mixture was clear. After digesting the mixture by observing light green color, for a period of 30 minutes, 25 mL of 4% boric acid solution was put into a 250 mL volumetric flask. The digest was then filtered into a 250 mL volumetric flask and the solution was made up to mark with distilled water and connected for distillation. Ammonia was steam distilled from the digest to which it had been added to 50 mL of 40% sodium hydroxide solution and released from the acid digest by the addition of sodium hydroxide. 150 mL of the distillate was collected in a conical flask. Distillate was titrated with 0.1 N HCl solution. The amount of hydrochloric acid used was proportional to the amount of nitrogen originally present in the test portion, calculated according to Eq.2.

% Nitrogen was calculated as follows:

$$\text{Eq. 2: \% N} = \frac{(V_2 - V_1)\text{mL x Normality x 0.014 g Nitrogen x 100}}{\text{Weight of sample (g)}}$$

V_1 = mL of 0.1 N HCl consumption for the blind
V_2 = mL of 0.1 N HCl consumption for the sample
Normality = normality of HCl solution
% Protein= % N x 6.25

3.5.8 Vitamin C Analysis

5 g baby food was put in a test tube and 5 mL 6% Meta-phosphoric acid solution (Merck, Germany) was added. The mixture was centrifuged at 4000 rpm for 15 minutes. 1 mL of clear supernatant was taken from centrifuge tube and supplemented with 6% Meta-phosphoric acid solution to obtain a 10 mL mixture. This mixture was filtered through a filter (Millipore Millex – HV Hydrophilic PVDF 0.45 µm) into HPLC (PerkinElmer, Flexar, USA). HPLC analysis of vitamin C were performed in a C_{18} column (COL – AQ C_{18} 5 µm 250 x 4.6 mm, USA). For the mobile phase, 0.05 M KH_2PO_4 (Potassium dihydrogen phosphate) was used at 1 mL/minute flow rate. The mobile phase, 0.05 M KH_2PO_4 was filtered by a filter (Millipore, Type GNWP 0.20 µm) with vacuum pump (Millipore XF54 230 50). After filtration of the mobile phase, it was placed in a water bath (Bandelin, Sonorex) to avoid air bubbles. Quantitations were performed at DAD detector (Photodiode Array Detector) in a wavelength of 254 nm. For the identification of the peaks and the amount of vitamin C, different concentrations (15-30-45-60-75 ppm) of L-ascorbic acid (Sigma, China) were used as standards.

3.5.9 The Allergen Tests

AgraStrip® Hazelnut Allergen Tests were used for the detection of trace amount of allergens in a low ppm range. The range of detection of AgraStrip® Hazelnut Allergen Tests is 5 – 10000 ppm. 0.2 g of baby food sample was treated with an extraction buffer and shaked vigorously by hand for 1 minute. 400 µL of this mixture was transferred to an incubation vial and shaked vigorously by hand for 15 second then left to stand at room temperature for 5 minute. AgraStrip® Hazelnut Allergen Kit (Romer, USA) was inserted to vial. After 5 minutes, the result was read immediately.

3.5.10 The Analysis of Aflatoxins

10 g of baby food sample was treated with 20 mL of 50% ethanol solution. Ethanol and sample should be in a ratio of 1:2 (w:v) and shaked vigorously for 1 minute. Microwell sealer was placed to an appropriate number of microwells in a microwell holder. A 50 µL of sample was transferred to microwell sealer. AgraStrip® Total Aflatoxin Assay Kit (Romer, USA) was put into one well. After 5 minutes, the result was obtained.

AgraStrip® Total Aflatoxin Test is an immunochromatographic assay that determines a qualitative level for the presence of total aflatoxin (B_1, B_2, G_1 and G_2). The cut off level of the assay is 4 ppb aflatoxin based on an immunoassay format. Antibody-particle complex is dissolved and mixed with sample extract.

3.5.11 Rheological Assessment

Rheological tests were carried out by using a Malvern Kinexus Pro rheometer (Malvern Instruments Ltd, UK). This rheometer has 40 mm diameter parallel plate. Samples were placed into the bottom plate using a 1.5 mm gap. The gap between upper and lower plates was kept at 1.5 mm. All frequency sweeps were obtained in controlled strain mode between 0.1 and 10 Hz. The parameters in this study were start frequency (10 Hz), end frequency (0.1 Hz), shear strain (0.1000%), sample per decade (10). Elastic modulus (G′), viscous modulus (G″) and phase angle (δ) were measured. All the experiments were carried out at 25ºC.

Physical and chemistry analyzes were repeated twice.

3.5.12 Taste Panel

Fifty panelists rated the baby food from 1 (worst) to 9 (best) (9=extremely like, 7=like somewhat, 5=neither like nor dislike, 3=dislike somewhat, 1=extremely dislike) 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 was served for the palate.

3.5.13 The Statistical Evaluation

The data were shown as means and analyzed by the SPSS program (IBM SPSS Statistics 22.0 - August 2013). 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 baby food to detect the significance between treatment groups. The significant level was set to $p<0.05$ at the beginning of the study.

4. RESULTS AND DISCUSSION

4.1 The microflora of baby food

When the microflora of the baby food was analyzed, no *Salmonella* spp., *total coliform, Enterobactericeae* spp., *Bacillus cereus, Listeria monocytogenes* and *E.coli* were recorded in the appropriate media in zeroth day. The microbial results were tabulated in Table 4. According to these findings our baby food is suitable to Turkish Food Codex Regulation on Microbiological Criteria of the Ministry of Food, Agriculture and Livestock.

Table 4. The mean and standard deviation results of total mesophilic aerobic bacteria and yeast/mold in 0^{th} day and microflora of baby food.

TreatmentGroups	**Total Bacteria** Mean ± Standard Deviation	**Yeast Mold** Mean ± Standard Deviation	***Total coliform***	***Enterobactericeae* spp.**	***Bacillus cereus***	***Listeria monocytogenes***	***E.coli***	***Salmonella* spp.**
Control (T1)	3.50 ± 0.082 ^{a}	3.67 ± 0.163 ^{a}	N.D	N.D	N.D	N.D	N.D	N.D
0.5 mL ER/10 g (T2)	3.07 ± 0.023 ^{ab}	3.10 ± 0.173 ^{bc}	N.D	N.D	N.D	N.D	N.D	N.D
1 mL ER/10 g (T3)	3.15 ± 0.058 ^{ab}	3.20 ± 0.173 ^{ab}	N.D	N.D	N.D	N.D	N.D	N.D
2 mL ER/10 g (T4)	3.00 ± 0.000 ^{bc}	3.15 ± 0.212 ^{bc}	N.D	N.D	N.D	N.D	N.D	N.D
30 dB+5 min. (T5)	3.10 ± 0.141 ^{ab}	3.15 ± 0.212 ^{bc}	N.D	N.D	N.D	N.D	N.D	N.D
30 dB+10 min. (T6)	3.00 ± 0.000 ^{bc}	3.39 ± 0.219 ^{ab}	N.D	N.D	N.D	N.D	N.D	N.D
60 dB+5 min. (T7)	3.00 ± 0.000 ^{bc}	3.30 ± 0.000 ^{ab}	N.D	N.D	N.D	N.D	N.D	N.D
60 dB+10 min. (T8)	3.29 ± 0.078 ^{ab}	3.26 ± 0.035 ^{ab}	N.D	N.D	N.D	N.D	N.D	N.D
90 dB+5 min. (T9)	3.19 ± 0.110 ^{ab}	3.50 ± 0.035 ^{ab}	N.D	N.D	N.D	N.D	N.D	N.D
90 dB+10 min. (T10)	2.95 ± 0.495 ^{bc}	3.41 ± 0.184 ^{ab}	N.D	N.D	N.D	N.D	N.D	N.D
Comb. 5 min. (T11)	2.60 ± 0.000 ^{dc}	2.70 ± 0.000 ^{de}	N.D	N.D	N.D	N.D	N.D	N.D
Comb.10 min. (T12)	2.48 ± 0.000 ^{d}	2.30 ± 0.000 ^{d}	N.D	N.D	N.D	N.D	N.D	N.D

N.D.: Not Determined

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

When the control and the combinations groups were compared, a few numbers of total mesophilic aerobic bacteria on PCA agar in combination groups were observed. There was a significant reduction up to one log cycle in the logarithmic counts of the total mesophilic aerobic bacteria on PCA agar. The yeast colony count of control group was found to be higher than the combination groups in yeast extract agar plate, therefore (T12) reached approximately up to 1.3 log cycle reduction with respect to the control (Table 4, Figure 3).

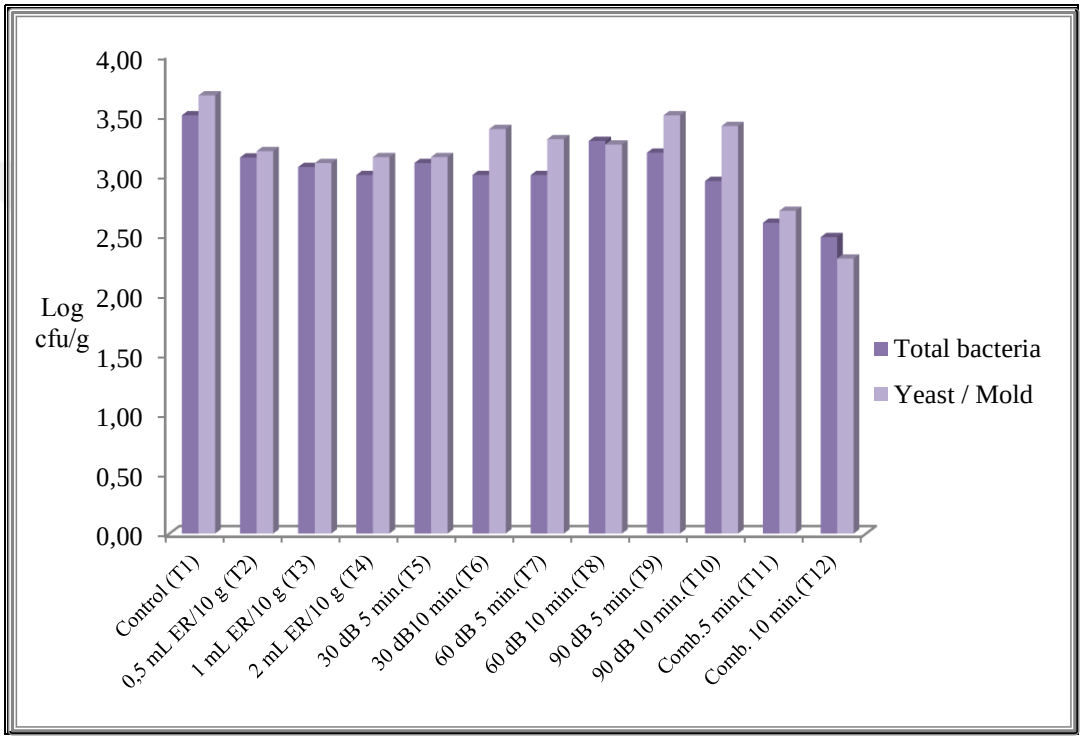

Figure 3. The microbial counts of 0^{th} day.

The results presented in Table 4 and Figure 3 stated that there was a significant reduction of total mesophilic aerobic bacteria counts and yeast/mold counts at (T11) and (T12) according to the control ($p<0.05$). Considering the microbial counts, non-linear inactivation curves were observed between treatment groups. This may be due to the sensitivity of microorganisms to the ultrasonication, reported by Sagong et al. (2011a), and the antimicrobial activity of eau de rose.

Sagong et al. (2011a) declared that the pathogens were reduced significantly by ultrasound, of which the treatment doses were 5–60 min. in pathogen inoculated organic fresh lettuce. Certain studies revealed that the combination of ultrasound and high pressure treatment increased inactivation of *E. coli* due to additional effect of ultrasound. At the elevated temperature over 50ºC, sensitivity of microorganisms was increased by ultrasonication (Earnshaw et al., 1995; Zenker et al., 1999; Villamiel & de Jong, 2000).

The microbial reduction effect of ultrasound occurred primarily during the first 5 min. and did not significantly increase even after a 10 min. treatment in different samples such as parsley, lettuce, cabbage, carrot, cucumber, strawberry, onion, and pepper. The study showed that there was a synergistic effect enhanced by approximately 0.7–1.7 logarithmic unit in the reduction of total viable count, yeast and mold count, *E. coli O157:H7*, and *Salmonella* spp. when ultrasound was combined with some antimicrobial chemical agents, depending on the concentrations used, the ultrasound experimental conditions, the strains of microorganisms, and the type of vegetable. In literature, there are limited studies which determined the antimicrobial effect of ultrasound alone, approximately reduces 0.6–1.5 $\log_{10}$ cfu/g mL in general experimental conditions (Bilek and Turantaş, 2013).

Figure 3 showed that there were differences between the treatment groups in 0^{th} day. The total mesophilic aerobic bacteria count in the (T12) was found to be significantly different from the other groups. The (T10) was significantly lowered the total mesophilic aerobic bacteria count than the (T9) ($p<0.05$). The period of processing time and frequency of ultrasonication may play an important role on the bacterial counts. When total mesophilic aerobic bacteria counts of the ten-minute and five minute treatment of 30 dB and 60 dB frequency of ultrasonication were compared, there was a non-linear inactivation curve. According to Figure 3, the total mesophilic aerobic bacteria count in the (T4) decreased significantly with respect to (T2), (T3) and the control (T1).

The bactericidal efficiency of ultrasound on Gram-positive versus Gram-negative bacteria is controversial. Some studies report Gram-positive bacteria to be more resistant to cavitation than Gram-negative bacteria, while others found no significant differences in their resistance to inactivation by ultrasound. Sonication of

orange juice (50W, 20 kHz, 25ºC), combined with its subsequent concentration and storage (48 h) at high osmotic pressure (10.9 MPa) reduced *Salmonella* spp. by 5 log. Moreover, sonication did not affect the key physicochemical and functional attributes of the juice (Chandrapala et al., 2012). In another study, *Listeria monocytogenes* was found to be almost resistant in different fruit juices (Kirmusaoglu, 2013).

Power ultrasound has been reported to be sufficient to meet the FDA's mandatory 5 log reduction of foodborne pathogens in fruit juices. Ultrasound alone or in combination with mild temperature is reported to be effective against *Escherichia coli* in model fluids (Salleh-Mack & Roberts, 2007). For fruit juice processing, the parameters such as fruit juice type, presence of pulp and viscosity will be important factors in determining the inactivation rate and treatment time to achieve the desired log reduction (Patil et al., 2009). Ultrasound have been successfully used for microbial inactivation in high lipid containing food samples as a replacement technology for those conventional methods with the advantages of using milder temperatures that prevent degradation of thermo sensible compounds in a reduced treatment time (Pingret et al., 2013).

Sagong et al. (2011b) studied the impact of ultrasonication at 40 kHz on safety and quality features of lettuce. The authors obtained microbial reductions between 0.9–1.6, 0.8–1.7 and 0.9–1.9 log-cycles for *E. coli*, *Salmonella typhimurium* and *L. monocytogenes*, respectively. No impact on colour and texture was detected.

These results were consistent with the studies conducted by Khanal et al. (2014) and Gao et al. (2014a) in terms of reduction of microorganisms. Khanal et al. (2014) studied inactivation of thermoduric aerobic spore formers in milk by ultrasonication. In this study, ultrasonication at 80% amplitude for 10 minute however, inactivated the vegetative cells of *Bacillus coagulans* and *Anoxybacillus flavithermus* in skim milk by 4.53, and 4.26 logs, respectively (Khanal et al., 2014).

Gao et al. (2014a) studied the inactivation of bacteria and yeast using high frequency ultrasound treatment. Three species of bacteria, *Enterobacter aerogenes*, *Bacillus subtilis* and *Staphylococcus epidermidis* as well as a yeast *(Aureobasidium pullulans)* were compared. The study showed that high-frequency ultrasound is highly efficient in inactivating the bacteria in both their exponential and stationary growth

phases, and inactivation rates of more than 99% were achieved. The rod-shaped bacterium *Bacillus subtilis* was also found to be sensitive to the mechanical effects of acoustic cavitation. Most bacterial inactivation is achieved during the first 10 minute of ultrasound treatment (Gao et al., 2014a; Gao et al., 2014b). Log of the inactivation ratio decreased linearly with sonication time, and the rate of inactivation increased with the increase in sonication power (Gao et al., 2014c).

When the logarithmic counts of the combination groups were compared with respect to the control (Table 4), there was a significant decrease of yeast and mold counts. Referring to Table 4, there were no linear inactivation curves for the total mesophilic aerobic bacteria counts. In combination, the reduction might be possibly due to the increasing amplitude of ultrasonication and processing time, similar to the findings of Adekunte et al. (2010a) and Tsukamoto et al. (2004a).

Ultrasound inactivation of yeast in a study showed a>5 log reduction with increasing amplitude (μm) level in 10 min or less. Extrinsic control parameters of amplitude level (μm) and processing time (min) had a significant effect ($p<0.05$) on the inactivation of yeast in tomato juice. A 5 log reduction was achieved in 7.5 min at an amplitude of 61 μm. Sonication alone, at a moderate temperature, can achieve the desired 5 log reduction in yeast cells (Adekunte et al., 2010a). Another study, conducted by Adekunte et al. (2010b), showed that for yeast inactivation, tomato juice samples were sonicated at amplitude levels ranging from 24.4 to 61.0 μm at a constant frequency of 20 kHz for different treatment times (2 to 10 min) and pulse durations of 5 second on and 5 second off. Significant reductions in yeast ($p<0.05$) were observed at higher amplitudes and processing times (Adekunte et al., 2010b).

The yeast inactivation could be due to a combination of physical and chemical mechanisms which occur during cavitation. Formation of free radicals and H_2O_2, due to sonication, aids in microbial inactivation (Oyane et al., 2009). Bactericidal effects on yeast cells were reported during the initial stages of sonication, with the bactericidal and bacteriostatic effects gradually increasing with time and intensity of sonication (Tsukamoto et al., 2004a).

Guerrero et al. (2001) reported that, at moderate temperatures, D values of *S.cerevisiae* in Saboraud broth were reduced by the simultaneous effect of ultrasound.

According to the same study the yeast and mold counts showed unstable or unproportional to amplitude of ultrasonication. These results may be related to yeast structure, studied by Earnshaw (1998). The effect of bacterial inactivation depends on action time, intensity, frequency sensitivity of cells, kind of sonication environment, and presence of additional substances (Dolatowski et al., 2007). Earnshaw (1998) concluded that large cells, such as yeast (5-20 mm) are more susceptible to the effects of cavitation, due to having a larger surface area than smaller sized. In general, spores of bacteria (e.g. *Bacillus and Clostridium* spp.) are more resistant to cavitational effects than vegetative cells, which are in growth phase (Earnshaw, 1998). Because of their larger size and different cell wall structure, disruption of yeasts is generally easier than bacteria (Geciova et al., 2002).

Another similar study, Mohideen et al. (2015) reported that sonicated and unsonicated blueberry juices were analyzed for coliforms, total aerobes, yeasts and molds. Continuous flow sonication successfully reduced microbial counts. Sonication at higher intensities significantly ($p<0.05$) reduced microbial counts. The highest log reduction in total aerobes (1.36 log cycles) was achieved at high flow rate (93.5 min/mL) with high intensity (100 amplitude) sonication condition compared to untreated and control blueberry juice samples. Increased ultrasound intensity (amplitude) resulted in greater reduction in all aerobic bacteria, total coliforms, and yeast ($p<0.05$) (Mohideen et al., 2015).

In this study, both ultrasonication and eau de rose were found to be not only effective on the total mesophilic aerobic bacteria but also the yeast and the mold species, especially in the beginning of the experiments. These findings are more or less in line with the studies mentioned above.

4.2 The Shelf Life Results

Microbial counts of baby food samples were examined in 0^{th}, 30^{th}, 60^{th} and 90^{th} days.

Table 5. The mean and standard deviation results of total mesophilic aerobic bacteria and yeast/mold in 0^{th}, 30^{th}, 60^{th} and 90^{th} days.

	TreatmentGroups	**Total Bacteria** Mean ± Standard Deviation	**Yeast Mold** Mean ± Standard Deviation
0^{th} Day	Control (T1)	3.50 ± 0.082 ^{a}	3.67 ± 0.163 ^{a}
	2 mL ER/10 g (T4)	3.00 ± 0.000 ^{bc}	3.15 ± 0.212 ^{bc}
	90 dB+10 min.(T10)	2.95 ± 0.495 ^{bc}	3.41 ± 0.184 ^{ab}
	Comb. 5 min. (T11)	2.60 ± 0.000 ^{dc}	2.70 ± 0.000 ^{de}
	Comb. 10 min. (T12)	2.48 ± 0.000 ^{d}	2.30 ± 0.000 ^{d}
30^{th} Day	Control (T1)	6.34 ± 0.057 ^{c}	8.26 ± 0.000 ^{a}
	2 mL ER/10 g (T4)	6.68 ± 0.057 ^{b}	7.48 ± 0.000 ^{a}
	90 dB+10 min.(T10)	6.82 ± 0.085 ^{b}	7.52 ± 0.014 ^{a}
	Comb. 5 min. (T11)	6.67 ± 0.021 ^{b}	7.78 ± 0.000 ^{a}
	Comb. 10 min. (T12)	7.18 ± 0.000 ^{a}	7.14 ± 0.658 ^{a}
60^{th} Day	Control (T1)	6.00 ± 0.000 ^{c}	7.12 ± 0.007 ^{c}
	2 mL ER/10 g (T4)	6.85 ± 0.000 ^{b}	7.83 ± 0.007 ^{a}
	90 dB+10 min.(T10)	7.00 ± 0.000 ^{ab}	7.12 ± 0.007 ^{c}
	Comb. 5 min. (T11)	7.55 ± 0.078 ^{a}	7.31 ± 0.007 ^{b}
	Comb. 10 min. (T12)	7.25 ± 0.346 ^{ab}	7.01 ± 0.007^{d}
90^{th} Day	Control (T1)	5.48 ± 0.000 ^{e}	6.30 ± 0.000 ^{d}
	2 mL ER/10 g (T4)	6.95 ± 0.007 ^{c}	7.56 ± 0.035 ^{b}
	90 dB+10 min.(T10)	6.85 ± 0.000 ^{d}	7.75 ± 0.064 ^{a}
	Comb. 5 min. (T11)	7.08 ± 0.000 ^{b}	7.17 ± 0.021 ^{c}
	Comb. 10 min. (T12)	7.17 ± 0.021 ^{a}	7.16 ± 0.064 ^{c}

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

Table 5 indicated that there were significant changes in treatment groups and shelf life period, 0^{th}, 30^{th}, 60^{th} and 90^{th} days.

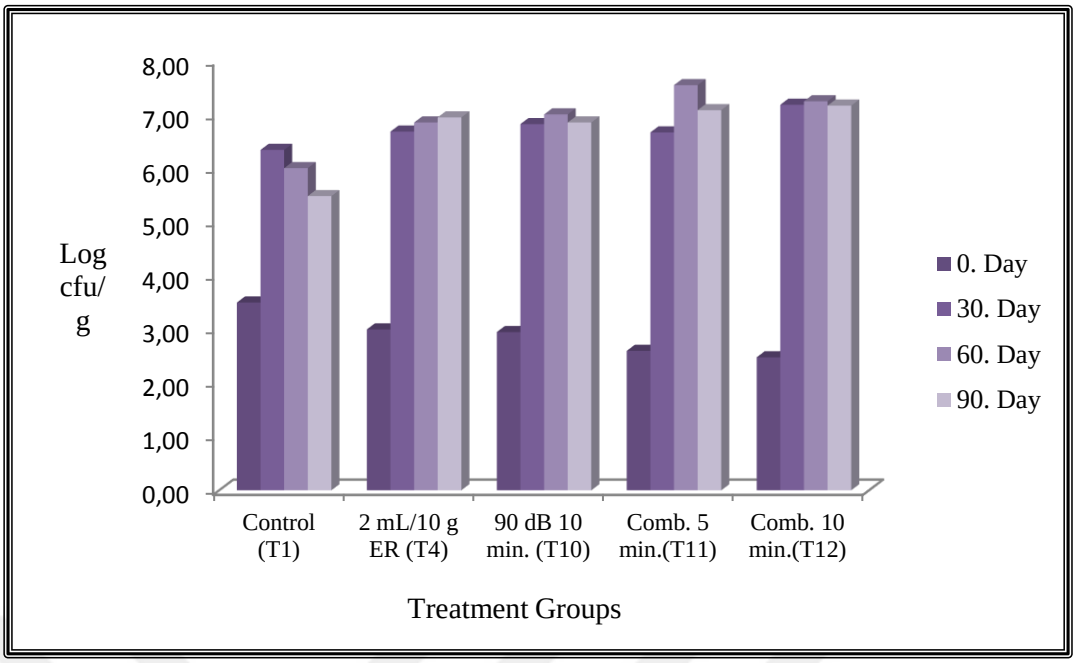

Figure 4. The total mesophilic aerobic bacteria counts in the shelf life.

According to Table 5, the total mesophilic aerobic bacteria count in the treatment groups was higher than the control group (Figure 4). This may be due to the amount of water in treatment groups and the competition between microbial populations. Water serves as an appropriate medium for confluent growth of total mesophilic aerobic bacteria and probably this may be related with the increase in water activity.

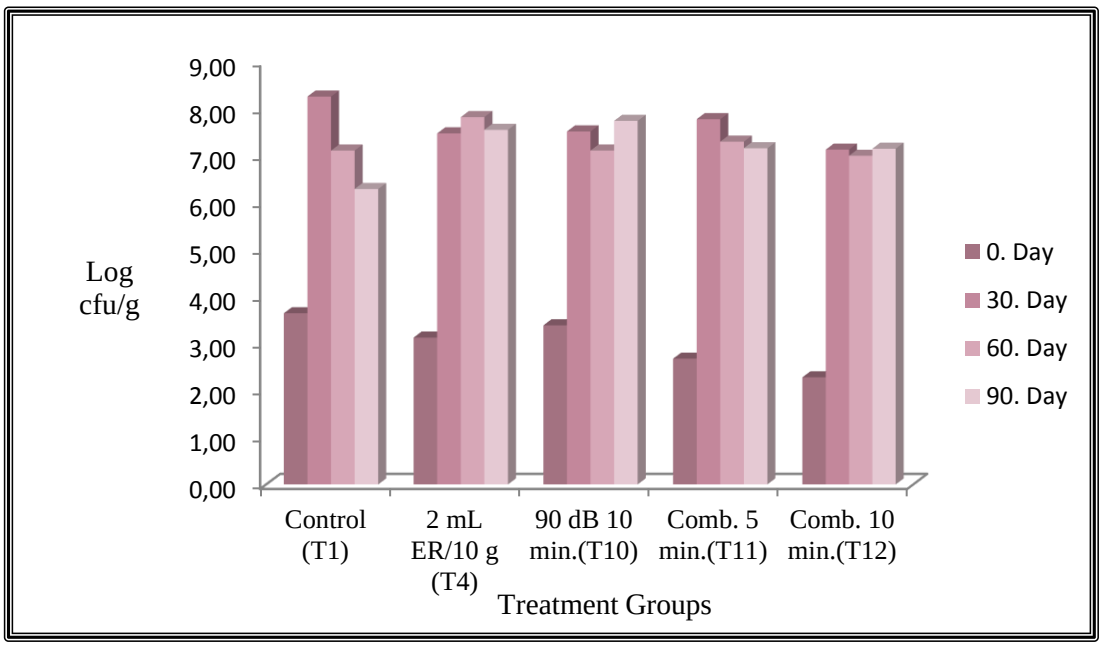

Figure 5. The yeast/mold counts in the shelf life.

On the other hand, the yeast/mold colony count in the control group was higher than the treatment groups of 30 and 60 days with respect to the total mesophilic aerobic bacteria (Figure 5). The competition in microbial populations may be effective on the results.

Figure 4 and 5 showed the differences between total mesophilic aerobic bacteria and the yeast/mold counts in treatment groups in 30^{th}, 60^{th} and 90^{th} days. The results of the control group differed significantly from treatment groups. At the same time, differences in treatment groups for yeast/mold counts in 30^{th} day were not found to be significant. The differences in combination groups for total mesophilic aerobic bacteria counts were found to be significant with respect to control group.

When the logarithmic counts of the study were compared with respect to the control, a significant decrease was observed in (T12) (Table 5) ($p<0.05$). The differences in treatment groups for total mesophilic aerobic bacteria count and yeast/mold count were found to be significant ($p<0.05$).

When compared within 0^{th}, 30^{th}, 60^{th} and 90^{th} days, the count of total mesophilic aerobic bacteria in 0^{th} day differed significantly from the counts in 30^{th}, 60^{th} and 90^{th} days ($p<0.05$). Within 30^{th}, 60^{th} and 90^{th} days, a difference was observed but this difference was found to be statistically insignificant ($p>0.05$) (Appendix A.4).

For yeast/mold counts, when compared within 0^{th}, 30^{th}, 60^{th} and 90^{th} days, the counts of yeast/mold in 0^{th} day differ statistically from 30^{th}, 60^{th} and 90^{th} ($p<0.05$). There were differences within 30^{th}, 60^{th} and 90^{th} days, but these differences were found to be statistically insignificant ($p>0.05$) (Appendix A.6).

Upon analyzing the results of shelf life as a whole, the results of total mesophilic aerobic bacteria within shelf life groups were similar to the results of yeast/mold.

4.3 The Results of Physical and Chemical Analysis

The color, total soluble solid content, viscosity, sugar, fat, protein and pH were analyzed (L*, a*, b*). When the results of the physical and chemical analysis were compared, there were significant differences in color, total soluble solid content and viscosity ($p<0.05$). A slight insignificant difference was present in sugar, fat, pH and protein within treatment groups with respect to the control ($p<0.05$).

4.3.1 The Color Test Results

Table 6 indicated the mean values of color (L*, a*, b*) in treatment groups. Referring to Table 6, significant changes were observed in the results of color. The lightness/darkness (L*) value in control group was significantly higher than (T9), (T10) and (T12) (Table 11). The lightness/darkness (L*) value was found to be the same in (T6), (T7) and (T4). The lightness/darkness (L*) may be affected by ultrasonic amplitude level and processing time, reported by Adekunte et al. (2010a).

Table 6. The color analysis of baby food.

TreatmentGroups	Mean ± Standard Deviation		
	L*	a*	b*
Control (T1)	51.14 ± 0.007 ^{a}	8.57 ± 0.057 ^{a}	31.79 ± 0.014 ^{a}
0.5 mL ER/10 g (T2)	51.58 ± 0.000 ^{b}	7.71 ± 0.014 ^{bcde}	31.50 ± 0.014 ^{ab}
1 mL ER/10 g (T3)	51.61 ± 0.113 ^{b}	7.86 ± 0.049 ^{cde}	31.55 ± 0.092 ^{ab}
2 mL ER/10 g (T4)	52.32 ± 0.028 ^{c}	7.68 ± 0.035 ^{bcde}	31.63 ± 0.375 ^{ab}
30 dB+5 min. (T5)	50.94 ± 0.099 ^{a}	7.87 ± 0.318 ^{cde}	30.88 ± 0.127 ^{c}
30 dB+10 min. (T6)	52.10 ± 0.113 ^{c}	7.88 ± 0.226 ^{cde}	31.51 ± 0.170 ^{ab}
60 dB+5 min. (T7)	52.25 ± 0.049 ^{c}	7.99 ± 0.000 ^{ed}	31.19 ± 0.177 ^{bc}
60 dB+10 min. (T8)	51.86 ± 0.000 ^{d}	8.11 ± 0.049 ^{ae}	31.56 ± 0.000 ^{ab}
90 dB+5 min.(T9)	50.24 ± 0.007 ^{e}	7.33 ± 0.049 ^{bf}	28.98 ± 0.035 ^{de}
90 dB+10 min.(T10)	49.75 ± 0.028 ^{f}	7.51 ± 0.021 ^{bcf}	28.45 ± 0.007 ^{e}
Comb. 5 min. (T11)	54.67 ± 0.021 ^{g}	7.08 ± 0.014 ^{f}	31.49 ± 0.014 ^{ab}
Comb. 10 min.(T12)	50.42 ± 0.021 ^{e}	7.54 ± 0.035 ^{bcdf}	29.52 ± 0.035 ^{d}

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

According to other studies, in contrast to these results, the lightness/darkness (L*) values in color assay results in ultrasonication groups of alpine strawberry juices were found to be almost the same in the treatment groups with respect to the untreated

(control) juice (Kirmusaoglu, 2013). Similarly, ultrasound application has almost no effect on the evolution of colour scoring in orange juices (Valero et al., 2007). These different results may be related to the chemical properties of fruits or samples.

A decrease in a* values of blueberry juices is related to anthocyanin degradation and formation of Maillard reaction products (Mohideen et al., 2015). Ultrasonication treatment may lead to anthocyanin degradation in persimmon fruits.

Valero et al. (2007) studied the effects of ultrasonic treatments in orange juice processing. Storage in refrigerated temperature conditions of orange juices processed by ultrasound and mild pasteurization, resulted in microbial populations at the first examination step (14 days) ranging from 12 to 610 cfu mL^{-1}, with the highest values in the high frequency ultrasound-treated samples. Survey of the ultrasound-treated orange juices stored at 5ºC and 12ºC showed changes in the initial total counts of yeast and other fungi. Likewise, ultrasound treatment has almost no effect on the evolution of colour scoring in the analyzed orange juices.

Table 6 pointed out that the a* (red/green) value was found to be low in (T9), (T10), (T11) and (T12) with respect to the control (T1), whereas it was almost the same in (T2), (T3), (T4), (T5), (T6), (T7) and (T8) groups. The a* (red/green) value in (T1) was found to be statistically different from (T9), (T10), (T11) and (T12). In the same way, ultrasonication frequency and processing time might be effective on a* (red/green) value.

These results are similar with Bhat et al. (2011) and Dias et al. (2015). The juice samples sonicated for 60 minute (at 20ºC, 25 kHz frequency), showed the lowest value for lightness (L*) and red–green component (a*) (Bhat et al., 2011). Another study showed that the sonicated soursop juice samples showed lower a* and b* values by Dias et al. (2015).

The b* (blue/yellow) value in control (T1) was found to be statistically higher than (T9), (T10) and (T12) ($p<0.05$). When compared with the control (T1), b* (blue/yellow) value was found to be slightly decreased in all groups with respect to the control (T1) (Table 6).

Colour values (L*, a*, b*), total colour difference, chroma, colour index were significantly ($p<0.05$) influenced by both ultrasonic amplitude level and treatment time. The colour values (L*, a*, b*) in (T9) and (T10) groups were lower than the control (T1). Sonication led to a decrease in L*, a*, b*. The sonication of tomato juice revealed also color modifications which related to a decrease in carotenoid pigments by isomerization (Adekunte et al., 2010a). Likewise, color degradation in sonicated orange juice was reported by Tiwari et al (2008c). Ultrasonication treatment (80% amplitude for 10 min.) caused significant reduction ($p<0.05$) in brightness and greenness of milk (Khanal et al., 2014). It was in line with our findings since the greater power of sonication and its long duration slightly decreased the L*, a*, b* values.

4.3.2 Total Soluble Solids (Brix%) Results

Table 7 showed the differences, mean and standard deviation of total soluble solid content of the treatment groups. When the total soluble solids results (Brix%) were considered, the results of the treatments of (T5), (T6), (T7), (T8), (T9) and (T10) were almost found to be statistically the same with the control (T1) ($p<0.05$). But, the results of (T2), (T3), (T4), (T11) and (T12) were slightly and significantly decreased with respect to control due to addition of eau de rose.

Table 7. The differences, mean and standard deviation of total soluble solids (Brix%).

TreatmentGroups	**Brix (%)** Mean ± Standard Deviation
Control (T1)	20.00 ± 0.000 [a]
0.5 mL ER/10 g (T2)	19.75 ± 0.354 [ab]
1 mL ER/10 g (T3)	19.00 ± 0.001 [b]
2 mL ER/10 g (T4)	15.50 ± 0.000 [c]
30 dB+5 min. (T5)	20.00 ± 0.000 [a]
30 dB+10 min. (T6)	20.25 ± 0.354 [a]
60 dB+5 min. (T7)	20.00 ± 0.001 [a]
60 dB+10 min. (T8)	20.00 ± 0.001 [a]
90 dB+5 min.(T9)	20.00 ± 0.001 [a]
90 dB+10 min.(T10)	20.25 ± 0.354 [a]
Comb. 5 min. (T11)	15.75 ± 0.354 [c]
Comb. 10 min.(T12)	15.75 ± 0.354 [c]

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

Based on these results, ultrasonication frequency and processing time did not affect but addition of eau de rose decreased the total soluble solids' content (Table 7).

The effect of sonication on anthocyanins, total phenolic content, and antioxidant capacity of pomegranate juices was studied by Alighourchi et al. (2013). The pomegranate juices were continuously sonicated at different amplitudes (50, 75 and 100%) and times (0, 3, 6 and 9 min.) at 25ºC ± 1ºC. The results showed that different intensities and treatment period had no significant effect on pH, acidity and °Brix. The degradation percentage of total anthocyanin content was ranging between 0.38-9.75%, while it was increased between 0.44-7.32% at various amplitude levels and treatment period. Furthermore, the antioxidant activity of all juices compared to the control showed no significant difference ($p<0.01$).

Santhirasegaram et al. (2013) investigated the effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica*) juice. In this study, freshly squeezed Chokanan mango juice was thermally treated (at 90ºC for 30 and 60 s) and sonicated (for 15, 30 and 60 min. at 25ºC, 40 kHz frequency, 130 W) to compare the effect on microbial inactivation, physicochemical properties, antioxidant activities and other quality parameters. After sonication and thermal treatment, no significant changes occurred in pH, total soluble solids and titratable acidity.

Effects of ultrasound treatments on quality of grapefruit juice were investigated by Aadil et al. (2013). Sonication did not induce any change in the pH, titratable acidity and ºBrix of grapefruit juice even after a treatment for 60 and 90 min (Aadil et al., 2013). No significant changes in pH, acidity and ºBrix were also observed in sonicated apple juice (Zhang et al., 2012) and blueberry juice (Mohideen et al., 2015).

Considering only the results of ultrasonication treatments, they are similar to the findings of Aadil et al. (2013); Zhang et al. (2012); Santhirasegaram et al. (2013) and Alighourchi et al. (2013). The infinitesimal amount of decrease was possibly due to the dilution of the samples with eau de rose.

4.3.3 The Results of Viscosity

According to Table 8, the viscosities of (T6), (T8), (T10) were significantly greater than (T1), (T5), (T7) and (T9). The treatment time of ultrasonication may affect the viscosity of the baby food. The viscosities of (T4) and (T12) showed a significant increase with respect to the control (T1) and other treatment groups. The increased concentration of rose water and elongated ultrasonication treatment time may have led to a hydrocolloidal formation but the appearance was not changed with respect to the control and other treatment groups.

Table 8. The differences, mean and standard deviations of viscosity.

TreatmentGroups	**Viscosity** (mPa.s) Mean ± Standard Deviation
Control (T1)	1.97 ± 0.042 ^{ef}
0.5 mL ER/10 g (T2)	1.26 ± 0.014 ^{h}
1 mL ER/10 g (T3)	1.23 ± 0.001 ^{h}
2 mL ER/10 g (T4)	2.81 ± 0.021 ^{b}
30 dB+5 min. (T5)	2.10 ± 0.057 ^{e}
30 dB+10 min. (T6)	2.66 ± 0.014 ^{c}
60 dB+5 min. (T7)	1.98 ± 0.021 ^{ef}
60 dB+10 min. (T8)	3.72 ± 0.042 ^{a}
90 dB+5 min. (T9)	1.88 ± 0.007 ^{f}
90 dB+10 min. (T10)	2.48 ± 0.064 ^{d}
Comb.5 min. (T11)	1.47 ± 0.035 ^{g}
Comb.10 min. (T12)	3.61 ± 0.035 ^{a}

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

Abid et al. (2014) reported that a significant ($p<0.05$) increase was observed in viscosity of apple juice sonicated for 30 and 60 min. as compared to non-sonicated control juice sample. This increase in viscosity is directly attributed to the sonication treatments that cause extraction of bound form of macromolecules and increase their concentration in the colloidal system and make the juice more viscous (Abid et al., 2014). Our results show similarity to the study of Abid et al. (2014).

4.3.4 Total Sugar Results

For the total sugar analysis, the most effective groups in microbiological analysis were selected between 12 treatment groups. When the total sugar content assays were considered (Table 9, Appendix B1), a slight decrease was observed in all of the treatment groups with respect to control, but this difference was found to be insignificant.

Table 9. The mean and standard deviation results of sugar analysis (%).

		Sugar (%) Mean ± Standard Deviation
Treatment Groups	Control (T1)	6.42 ± 0.276 [a]
	2 mL ER/10 g (T4)	4.24 ± 0.877 [a]
	90 dB+5 min. (T9)	6.01 ± 1.273 [a]
	90 dB+10 min. (T10)	5.70 ± 0.304 [a]
	Comb. 5 min. (T11)	5.22 ± 0.431 [a]
	Comb. 10 min. (T12)	5.23 ± 0.219 [a]

The sugar contents in the (T11), (T12) and (T4) were slightly and insignificantly decreased due to addition of rose water. Similarly, when the control and the ultrasound treatment were compared, there was an insignificant decrease in ultrasonic treatment groups ($p>0.05$). Ultrasonication power and treatment time may lead to insignificant reduction in sugar concentration. It was also in line with the °Brix measurements.

According to a study, sugars are one of the important quality parameter of apple juice which affects the sensory characteristics. Persimmon approximately contains 8% fructose. The fructose concentration is almost equal to glucose concentration (Aksu et al., 1994).

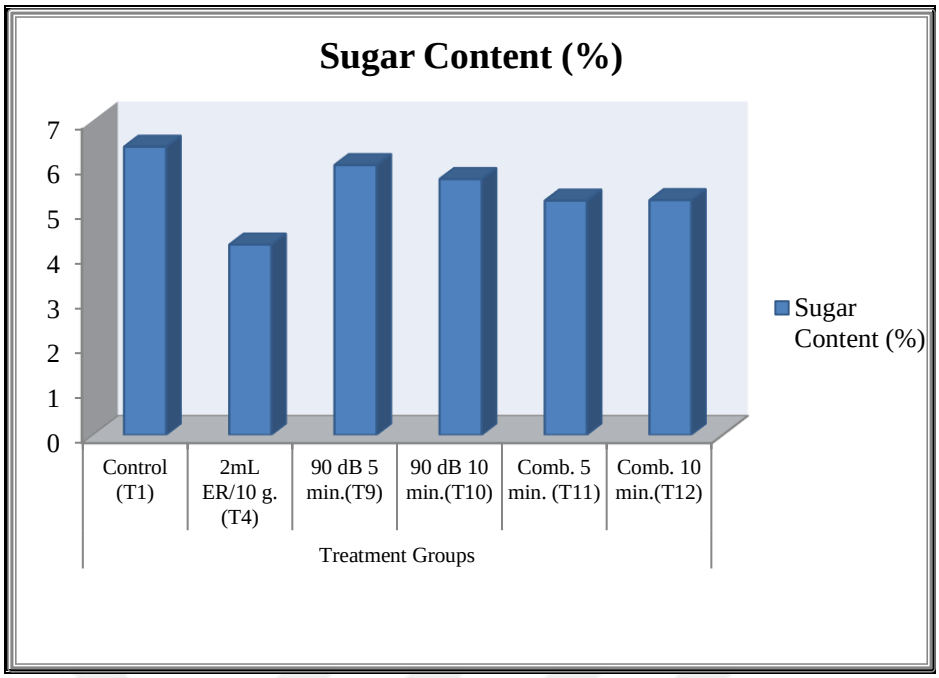

Figure 6. The sugar content results.

4.3.5 Fat Determination

For the fat determination assays, the most effective groups in microbiological analysis were selected between 12 treatment groups. When the control and the ultrasound treatment were compared, no difference was observed between ultrasonication treatments of (T9) and (T10) (Table 10).

Table 10. The mean and standard deviation of fat determination.

		Fat (%) Mean ± Standard Deviation
Treatment Groups	Control (T1)	2.27 ± 0.190 [a]
	2 mL ER/10 g (T4)	2.01 ± 0.000 [a]
	90 dB+5 min. (T9)	2.51 ± 0.925 [a]
	90 dB+10 min. (T10)	2.54 ± 0.460 [a]
	Comb. 5 min. (T11)	1.82 ± 0.003 [a]
	Comb. 10 min. (T12)	1.42 ± 0.278 [a]

The fat contents in the (T11), (T12) and (T4) groups were slightly and insignificantly decreased due to addition of rose water and possible degradation of fat globules by ultrasonication (Table 10).

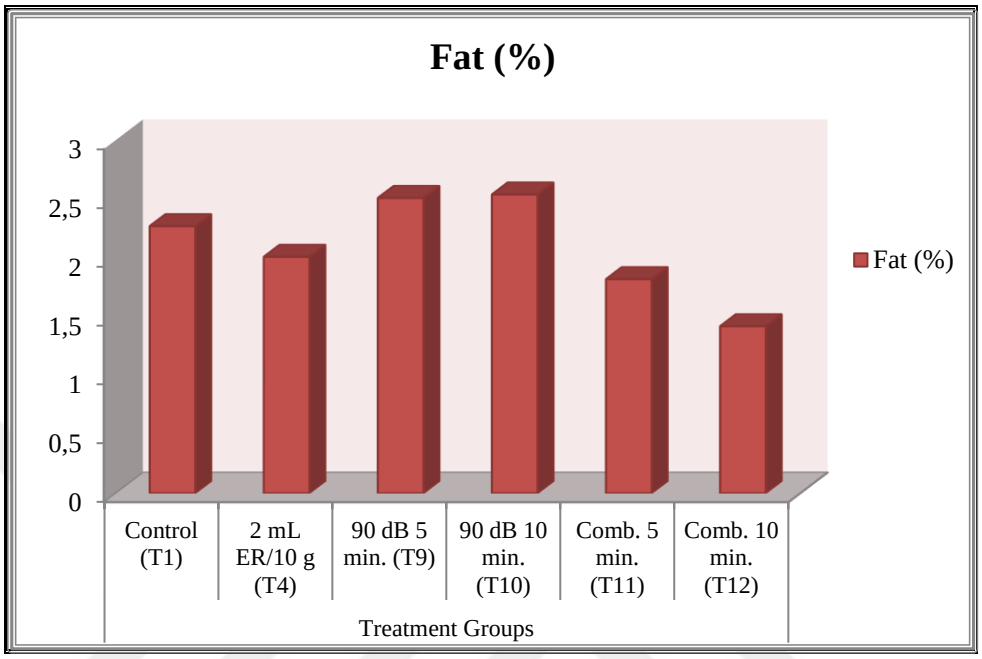

Figure 7. The fat content results.

Increase of the ultrasonic power leads to decrease in size of fat globules in coconut milk's samples (Iswarin and Permadi, 2012). Leong et al. (2014) reported that the application of ultrasound results in significantly improved fat separation ($p<0.05$) compared with the controls at the preheated temperatures of 25ºC and 40ºC, for both 600 kHz and 1 MHz frequency, after 5 minute sonication. However, in our studies, an insignificant slight increase was found when the ultrasonic power and its duration of application were increased.

4.3.6 Protein Determination

For the protein analysis, the most effective groups in microbiological analysis were selected between 12 treatment groups. Referring to Table 11, no significant differences ($p<0.05$) in protein content were observed in treatment groups.

Table 11. The mean and standard deviation of protein determination.

		Protein (%) Mean ± Standard Deviation
Treatment Groups	Control (T1)	0.74 ± 0.168[a]
	2 mL ER/10 g (T4)	0.51 ± 0.064 [a]
	90 dB+5 min. (T9)	0.79 ± 0.001 [a]
	Comb. 5 min. (T11)	0.62 ± 0.049 [a]
	Comb. 10 min. (T12)	0.93 ± 0.001 [a]

The protein content of (T4) had the lowest value due to addition of eau de rose or water concentration, when compared with other groups (Figure 8). It was found to be slightly increased in (T12) with respect to the other groups, but these results were insignificant ($p<0.05$). This increase in (T12) may be related to an increase in viscosity (Table 8). Ultrasonic treatment and processing time may lead to aggregation of proteins.

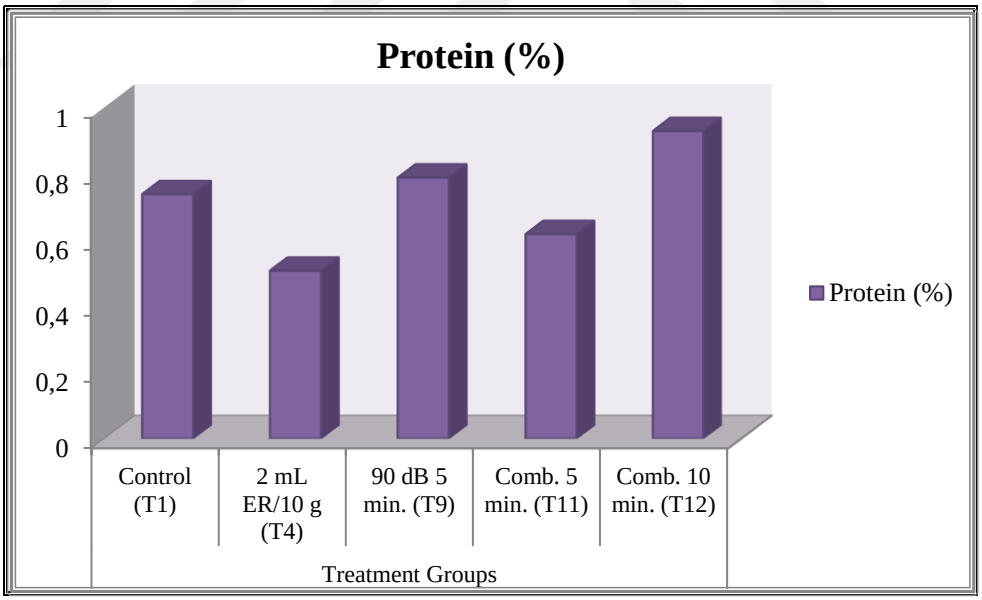

Figure 8. The protein analysis results.

Ultrasound treatment was reported to be effective for the destruction of *E. coli, Pseudomonas fluorescens* and *Listeria monocytogenes* with no detrimental effect on the total protein or casein content of pasteurized milk (Chemat et al., 2011).

Both classical and ultrasound-assisted lysis increased the concentration of carbohydrates in the model media, but only ultrasound markedly increased the release of proteins in wine. Ultrasound could not only inactivate the yeast cells, but could also provoke the rupture of the cells, thus increasing the release of proteins (and other cytoplasmic compounds not quantified) to the model wine medium (García Martín et al., 2013).

Chandrapala et al. (2011) reported that the sonication process had little effect on the structure of proteins in whey protein concentrate solutions. It is found that the effects of ultrasound are minimal on the secondary and tertiary structures of proteins. Sonication does result in some structural changes of individual proteins. Prolonged sonication resulted in protein re-aggregation. Only minor changes to the protein secondary structural and surface hydrophobicity were observed (Chandrapala et al., 2011). Cavitation also triggers some physico-chemical changes to milk, such as aggregation (McCarthy et al., 2014).

4.3.7 The pH Results

Table 12 showed the mean and standard deviation of pH in treatment groups.

Table 12. The mean and standard deviation of pH.

Treatment Groups	**pH** Mean ± Standard Deviation
Control (T1)	6.22 ± 0.184[a]
0.5 mL ER/10 g (T2)	6.16 ± 0.134 [a]
1 mL ER/10 g (T3)	6.17 ± 0.099 [a]
2 mL ER/10 g (T4)	6.15 ± 0.141 [a]
30 dB+5 min. (T5)	6.20 ± 0.205 [a]
30 dB+10 min. (T6)	6.20 ± 0.171 [a]
60 dB+5 min. (T7)	6.19 ± 0.219 [a]
60 dB+10 min. (T8)	6.17 ± 0.163 [a]
90 dB+5 min. (T9)	6.20 ± 0.177 [a]
90 dB+10 min. (T10)	6.15 ± 0.156 [a]
Comb. 5 min. (T11)	6.15 ± 0.177 [a]
Comb. 10 min. (T12)	6.15 ± 0.156 [a]

The pH of the baby food was found to be **6.22 ± 0.184**, 6.16 ± 0.134, **6.17 ± 0.099**, 6.15 ± 0.141, **6.19 ± 0.205**, 6.20 ± 0.171, **6.19 ± 0.219**, 6.17 ± 0.163, **6.20 ± 0.177**, 6.15 ± 0.156, **6.16 ± 0.177**, 6.15 ± 0.156 for the (T1), (T2), (T3), (T4), (T5), (T6), (T7), (T8), (T9), (T10), (T11) and (T12) respectively. There was no significant difference between the control and the treatment groups (Table 12) ($p<0.05$). The results are in line with a study conducted by Adekunte et al. (2010) in which the effects of ultrasound amplitude level and treatment time on tomato juice were investigated and no significant differences ($p<0.05$) in pH, Brix(%) or titratable acidity were observed in sonicated samples.

Alexandre et al. (2012) studied that efficacy of non-thermal technologies and sanitizer solutions on microbial load reduction and quality retention of strawberries. The application of ozone and ultrasound treatments, before storage of strawberries under refrigerated conditions, was efficient in controlling the growth of microbial contamination and attained a product with satisfactory quality retention. After treatments, no significant changes in pH values were detected.

The pH of kasturi lime fruit juice showed non-significant changes after sonication treatments (at 20ºC, 25 kHz frequency), even after 60 minute of treatment time ($p>0.05$). Additionally, sonication did not induce any significant changes in the titratable acidity, even after 60 minute of treatment time (Bhat et al., 2011).The pH ($p>0.05$) of treated skim milk (80% amplitude for 10 min.) was not affected (Khanal et al., 2014).

The pH results are similar to the results of Alexandre et al. (2012), Alighourchi et al. (2013) and Bhat et al. (2011).

4.3.8 Vitamin C Determination

For Vitamin C analysis, the most effective groups in microbiological analysis were selected between 12 treatment groups. According to Table 13, Appendix C1, C2, C3, C4, C5, C6, C7 and Figure 9, the amount of vitamin C in the control was higher than the treatment groups.

In this study, ascorbic acid concentration may be influenced by both ultrasonic amplitude level, treatment time and eau de rose concentration. When compared to treatment groups, ascorbic acid concentration in (T12) was found to be significantly different from other groups ($p>0.05$). The results were significantly different in all groups. Ascorbic acid is thermolabile and highly sensitive to various processing conditions.

Vitamin C is the least stable of all vitamins and easily destroyed during processing and storage (Ercan and Soysal, 2011).

Table 13. The amount of Vitamin C.

		Vitamin C (mg/100g) Mean ± Standard Deviation
Treatment Groups	Control (T1)	64.50 ± 0.141^{a}
	2 mL ER/10 g (T4)	51.79 ± 0.127^{b}
	90 dB+10min. (T10)	61.80 ± 0.148^{c}
	90 dB+5 min. (T9)	57.03 ± 0.120^{d}
	Comb. 5 min. (T11)	50.11 ± 0.134^{e}
	Comb. 10 min. (T12)	47.29 ± 0.113^{f}

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

Previous studies also showed degradation of vitamin C in sonicated fruit juice. Tiwari et al. (2009a) reported a maximum degradation of 5% in the ascorbic acid content of orange juice when sonicated at the highest acoustic energy density (0.81 W/mL) and treatment time (10 min).

Sonication also affected color and decreased ascorbic acid content. Sonication degraded ascorbic acid in a time-dependent manner. Ascorbic acid degradation during sonication has been related to advanced oxidative processes (Go´mez-Lo´ pez et al., 2010).

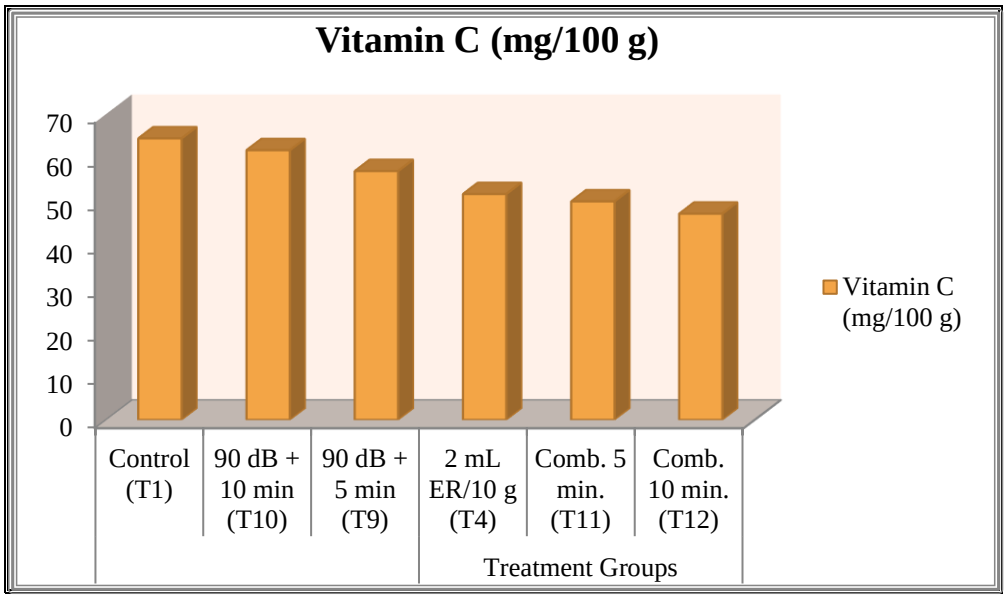

Figure 9. Vitamin C levels of treatment groups compared to control.

Adekunte et al. (2010a) reported that ascorbic acid was significantly ($p<0.05$) effected by both ultrasonic amplitude level and treatment time. Ascorbic acid content was found to be influenced by amplitude level ($p<0.0001$) and treatment time ($p<0.0001$). Ascorbic acid degradation is mainly due to sonochemical reactions and the extreme physical conditions which occur during sonication. It is known that hydrogen ions (H^+), free radicals (O, OH, HO_2) and hydrogen peroxide (H_2O_2) are formed during the sonolysis of water molecules present in juice samples. The ascorbic acid degradation during ultrasonic processing could be related to oxidation reactions, promoted by the interaction with free radicals formed during sonication. Hydroxyl radicals produced by cavitation may be involved in the degradation of ascorbic acid (Adekunte et al., 2010a).

Valdramidis et al. (2010) indicated that the largest ascorbic acid reduction was observed at the highest amplitude (61.0 μm) and processing temperature (30ºC). However this reduction was less than 15% loss of the initial ascorbic acid content of the unprocessed juice.

Ascorbic acid degradation and a decrease in total phenolics content were observed in the same samples and the suggested mechanisms for those reactions were pyrolysis and oxidation by OH radicals formed by cavitation (Pingret et al., 2013).

The results of this study are similar with Tiwari et al. (2009a), Go´mez-Lo´ pez et al. (2010), Adekunte et al. (2010a), Valdramidis et al. (2010).

In general, no extreme detrimental effect of the treatments on the physical and chemical quality of the baby food was observed.

4.3.9 The Allergen and Aflatoxins Results

AgraStrip® Hazelnut Allergen Tests were used in the study and negative results were obtained since no allergenic substances were determined.

Ultrasonication could result in changes of native protein structure, thus it may lead to allergenicity or vice versa, allergenicity may be reduced by ultrasonication.

Hazelnuts have been shown to contain different allergenic proteins. These are Cor a 1 and Cor a 8 and the 2S albumin Cor a 14, Cor a 9 (D'Andrea et al., 2011).

Pulsed ultraviolet light, high intensity ultrasound, nonthermal atmospheric plasma, gamma irradiation, genetic modification, physical, chemical and enzymatic processing are some of the nonthermal treatments shown to have the ability to alter the allergenicity of different food allergens. Very little information is available on the effect of high intensity ultrasound treatment on food allergenicity. A study reported 24% decrease in allergenicity of soy proteins through 10 minute high intensity ultrasound treatment by a 37kHz ultrasonic processor (Tammineedi and Choudhary, 2014).

Li et al. (2011) studied the effect of power ultrasound on the allergenicity and texture properties of shrimp (*Penaeus vannamei*). Raw and boiled shrimps were treated with power ultrasound (30 kHz, 800 W) at 0ºC and 50ºC for 0, 2, 8, 10, and 30 minutes. The results showed that the ultrasound treatment had a greater effect on the allergenicity of the boiled shrimps than of the raw ones. The allergenicity of the boiled shrimps treated at 0ºC and 50ºC decreased by nearly 50% and 40%, respectively, with 10 min. of the treatment duration. Allergenicity can be reduced by power ultrasound (Li et al., 2011).

In addition; AgraStrip® Total Aflatoxin Tests gave negative results. Aflatoxins were not found in this study.

Aflatoxins are a group of mycotoxins with highly toxic, mutagenic, carcinogenic and immuno-suppressive properties. Mycotoxins are a large and varied group of mold-secondary metabolites. Aflatoxins are produced by common fungi *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nominus* ubiquitous in a wide variety of agricultural commodities, such as cereals, nuts, dried fruits and in feed stuffs of plant. Eighteen aflatoxins have been identified to date, but only four of them have been found in food and feed: B_1, B_2, G_1 and G_2. Aflatoxin B_1 is the most potent hepatocarcinogen known for mammals (Manoochehri et al., 2014; Mortazav et al., 2015).

Mortazav et al. (2015) reported that aflatoxin solutions with concentrations of about 17.7 ppb aflatoxin were treated by ultrasound irradiation at constant frequency of 20 kHz with intensities 20, 60 and 100% for 10, 20 and 30 minutes. Results showed that the amount of aflatoxins decreased about 41% at constant frequency of 20 kHz with an intensity of 60% for 10 minutes.

4.3.10 The Taste Panel of Baby Food

Schwartz et al. (2011) reported the role of taste in food acceptance at the beginning of complementary feeding. In this study, the role of taste in new food acceptance was highlighted at the beginning of complementary feeding. Apart from taste, flavor and texture are other sensory determinants of food preferences in children (Schwartz et al., 2011).

Emerging research indicates that the sensory properties of different foods given early in life can shape later taste preferences and food choices (Foterek et al., 2015).

Table 14. The sensory parameters of treatment groups.

	Treatment Groups	Mean ± Standard Deviation
Color	Control (T1)	7.56 ± 1.618[a]
	90 dB+10 min. (T10)	7.84 ± 1.621 [a]
	2 mL ER/10 g (T4)	7.04 ± 1.916 [a]
	Comb. 10 min. (T12)	7.40 ± 1.666 [a]
Sweetness	Control (T1)	6.92 ± 1,275 [ab]
	90 dB+10 min. (T10)	7.56 ± 1.567 [b]
	2 mL ER/10 g (T4)	6.44 ± 1.668 [b]
	Comb. 10 min. (T12)	6.76 ± 2.046 [ab]
Sourness	Control (T1)	1.88 ± 1.573 [a]
	90 dB+10 min. (T10)	1.72 ± 1.443 [a]
	2 mL ER/10 g (T4)	1.84 ± 1.462 [a]
	Comb. 10 min. (T12)	1.96 ± 1.818 [a]
Odor	Control (T1)	5.68 ± 2.931 [a]
	90 dB+10 min. (T10)	6.00 ± 2.893 [a]
	2 mL ER/10 g (T4)	5.88 ± 2.430 [a]
	Comb. 10 min. (T12)	6.24 ± 2.646 [a]
Appearance	Control (T1)	6.68 ± 2.152 [a]
	90 dB+10 min. (T10)	7.04 ± 2.040 [a]
	2 mL ER/10 g (T4)	6.64 ± 2.008 [a]
	Comb. 10 min. (T12)	6.64 ± 2.275 [a]
Flavor	Control (T1)	7.36 ± 1.793 [ab]
	90 dB+10 min. (T10)	7.52 ± 1.843 [a]
	2 mL ER/10 g (T4)	6.32 ± 2.084 [b]
	Comb. 10 min. (T12)	6,84 ± 2.280 [ab]
Bitterness	Control (T1)	1.44 ± 1.163 [a]
	90 dB+10 min. (T10)	1.48 ± 1.182 [a]
	2 mL ER/10 g (T4)	1.92 ± 1.676 [a]
	Comb. 10 min. (T12)	1.56 ± 1.146 [a]
Overall	Control (T1)	7.20 ± 1.355 [ab]
	90 dB+10 min. (T10)	7.68 ± 1.789 [a]
	2 mL ER/10 g (T4)	6.32 ± 1.789 [b]
	Comb. 10 min. (T12)	6.88 ± 2.076 [ab]

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

(9=extremely like, 7=like somewhat, 5=neither like nor dislike, 3=dislike somewhat, 1=extremely dislike)

Fifty panelists rated the baby food in the taste panel according to the criteria of color, sweetness, sourness, odor, appearance, flavor, bitterness and overall evaluation with a 5 point scale per replication (Table 14). A score of 9 represented most liked, and 1 corresponded to least liked, 3 to disliked somewhat, 5 to neither liked nor dislike and 7 to liked somewhat. Other than control, the highest doses of ultrasonication and eau de rose and their combination were analyzed.

The average age of panelists in this study was 25.48 ± 6.59. The 96% of panelists were greater than twenty years old. The 4% of panelists were lower than twenty. The age of the panelists was generally between the range of 21 and 25 years of age. The 68% of panelists were female and 32% of panelists were male. The 20% of panelists were smokers and the 80% of panelists were nonsmokers.

According to Table 14, there were significant differences in sweetness, flavor and overall between sensory parameters. On the other hand, no significant differences in color, sourness, odor, appearance and bitterness. Ultrasonication treatment and addition of eau de rose did not affect on color in appearance. These results indicated parallel results in terms of color with study by Go´mez-Lo´ pez et al. (2010).

In generally, the 70% of panelists for overall parameters of combination group gave 7 or 9 points. The 82% of panelists for overall parameters of control gave 7 or 9 points.

Microbiological and sensory quality of sonicated calcium-added orange juice were analyzed by Go´mez-Lo´pez et al. (2010). Sonication of this juice did not cause statistically significant differences ($p>0.05$) on panel-tested color, aroma and flavor even at extreme treatment conditions (89.25 mm 75%, 10 min.) (Go´mez-Lo´ pez et al., 2010).

If the significant difference in sensory parameters, flavor, sweetness and overall were considered, it was found to be significantly affected ($p<0.05$). The (T4) was found to be statistically lower than (T10) according to the results in terms of flavor, sweetness and overall. Addition of eau de rose might have affected the flavor, sweetness and overall.

Table 14 designated the significant differences between flavor, sweetness and overall results in treatment groups. According to Table 14, a slight insignificant increase was found to be present in (T10) with respect to the control ($p<0.05$). Ultrasonication treatment may have helped to release of aromatic compounds in persimmon fruit or baby food.

Ultrasound processing is also reported to enhance extraction yield of bioactive compounds by about 6 and 35% depending on the processing conditions (Rawson et al., 2011). This may be effective on the preference of the treatments by panelists.

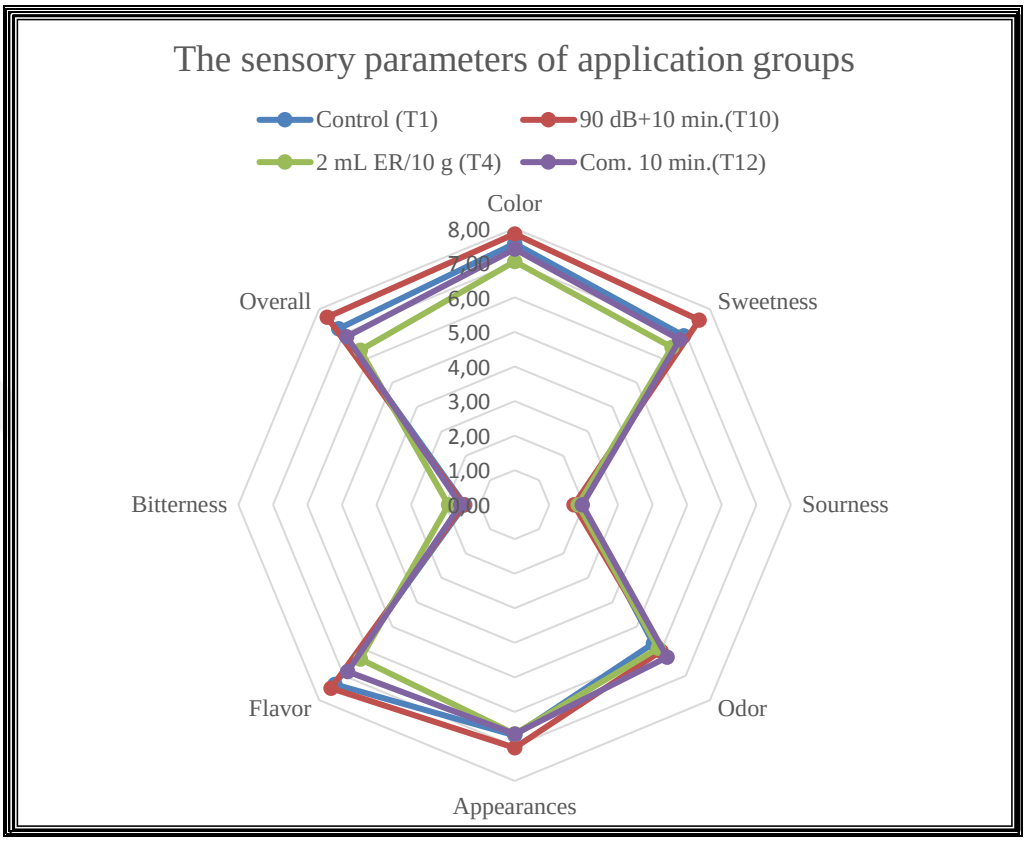

Figure 10. The sensory parameters of treatment groups.

The taste panel results can be visualised in the form of a spider-web graphic. Referring to Figure 10, there were no extreme differences between taste profile of groups.

4.3.11 Rheological Assessment

Rheology is defined as the science of deformation and flow. Rheology studies the response of materials when subjected to force. The effect of ultrasound on food rheology is mainly due to the cavitation phenomenon. Mechanical stress, generated by shock waves derived from bubble implosion or from microstreaming derived from bubble size oscillation, may also break large macromolecules or particles. Possible effects of ultrasound treatment (shear forces generated due to the collapse of cavitation bubbles) may affect on the concentration, structure, molecular weight, hydrogen bonding, other intra-and intermolecular forces of the protein and the viscosity of samples (Ahmed et al., 2010).

Elastic component of a material is described by storage modulus (G′), while the viscous component is described by loss modulus (G″) and G* is defined as the complex shear modulus. They are related to phase angle as follows: $\tan \delta = G''/G'$ (Elezović et al., 2015).

G′ value is a measure of the deformation energy stored in the sample during the shear process, representing the elastic behavior of a sample. In contrary, G″ value is a measure of the deformation energy used up in the sample during the shear and lost to the sample afterwards, representing the viscous behavior of a sample. If G′ is much greater than G″, the material will behave more like a solid; that is, the deformations will be essentially elastic and recoverable. However, if G″ is much greater than G′, the energy used to deform the material is dissipated viscously and the material is behavior is liquid-like. The δ value could be used to explain whether the material was solid or liquid and if δ is 90^o, the material behaves like a true liquid and if 0^o a true solid. If the δ is between 0^o and 90^o ($0 < \delta < 90$), the material is called viscoelastic (Wan et al., 2011; Tabilo-Munizaga and Barbosa-Ca´novas, 2005).

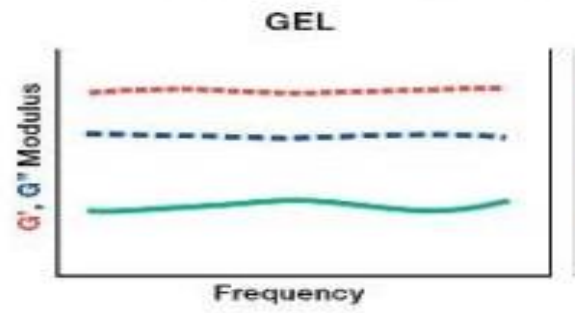

Figure 11. Behaviour of gel (Malvern Instrument, 2013)

In our study, the control (T1), (T9), (T10), (T4), (T11) and (T12) were analyzed by rheometer. The most effective groups in microbiological analysis were selected between 12 treatment groups. The rheology graphics of these groups were similar to gel graphic (Figure 11, Appendix D1, D2, D3, D4, D5 and D6). Considering the rheological graphics of groups in general, ultrasound treatment did not result in significant changes on the elastic (G′) and viscous component (G″) of rheological properties of samples. Fluid property was generally stable and there was no significant difference between groups. G′ value or line generally remained the same. These results are in line with the findings of Jambrak et al. (2011) and Çevik et al. (2014).

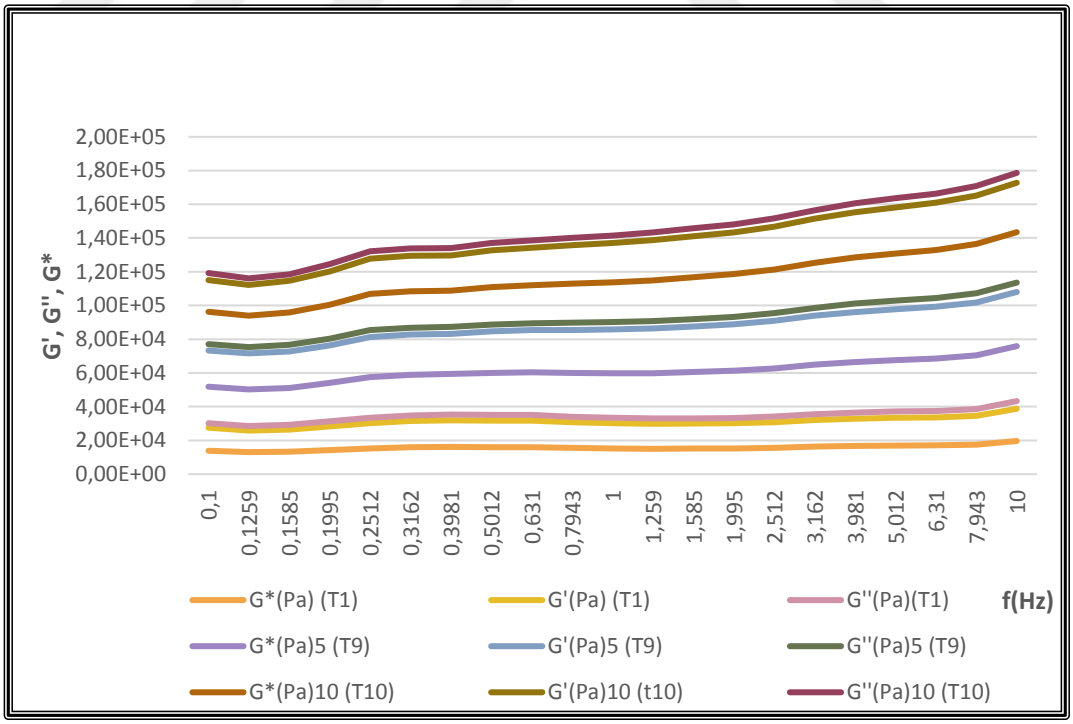

Figure 12. The differences of the (T1), (T9) and (T10) groups.

When the rheology graphics of the (T1), (T9) and (T10) groups were evaluated together, G*, G′ and G″ values in (T10) group were slightly higher than (T1) and (T9) groups.

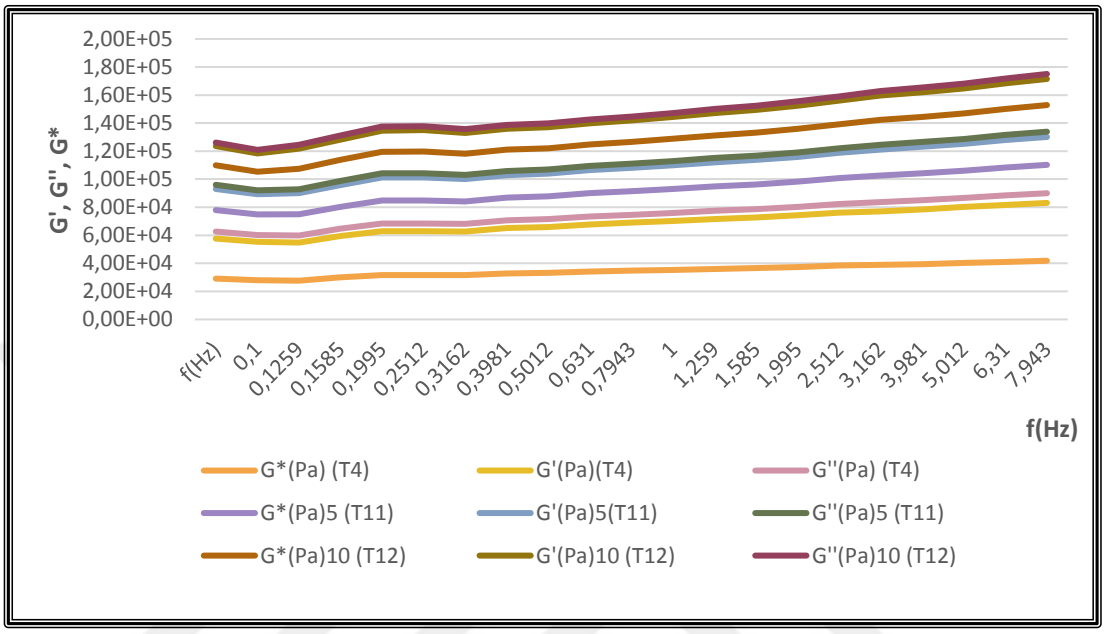

Figure 13. The differences of the (T4), (T11) and (T12) groups.

Similarly, when the rheology graphics of the (T4), (T11) and (T12) groups were evaluated together, G*, G′ and G″ values in (T12) group were slightly higher than (T4) and (T11) groups. As a results; ultrasonication treatment time may affect on the G*, G′ and G″ values. It was observed that ultrasonication visually led to aggregation in the sample. This result may be related to increased G*, G′ and G″ values. The variability of the rheological findings may be associated with molecular structure, composition, nature and content of the samples.

Šimunek et al. (2014) investigated the rheological properties of ultrasound treated apple, cranberry and blueberry juice and nectar. Ultrasonication (20 kHz) was carried out with 60, 90 and 120 µm amplitudes. Juice and nectar samples were treated by ultrasound for 3, 6 and 9 minute at 20ºC, 40ºC and 60ºC. Rheological properties are expressed with consistency coefficient (k) and flow behavior indices (n), the consistency coefficient (k) in treated samples of apple, cranberry and blueberry juices and nectars, decreases and increases depending on the applied ultrasound treatment.

Statistically, the interaction of treatment time and temperature of sample of apple juice was found to be significantly effective on flow behavior index (n) for ultrasound treatment (Šimunek et al., 2014).

In another study, Çevik et al. (2014) reported the rheological properties of red beet root juice squeezed from ultrasonicated red beet root slices. In this study, ultrasound at a constant power (1500 W) and fixed frequency (20 kHz) were applied to the red beet root slices (25ºC) for different application periods (0, 5, 10, 15 and 20 minute). The k (consistency coefficient) and n (flow behavior index) values of red beetroot juices were not affected from the duration of ultrasonication applied to the slices. Ultrasound treatment did not result in any changes on the rheological properties of red beetroot juice (Çevik et al., 2014).

Jambark et al. (2010) indicated that the consistency coefficient decreases stepwise jointly with the increasing ultrasound power (Jambark et al., 2010). Another study showed that ultrasound treatment causes changes in the flow behaviour of soymilk. This effect has been shown as statistically significant ($p<0.05$) increases in flow behaviour indices (n). The changes in flow behaviour after ultrasound treatment are a consequence of changes in the binding capacity for water: the hydrophilic parts of amino acids are opened toward water surroundings, leading to higher binding of water molecules (Fahmi et al., 2011).

Jambrak et al. (2011) explained the treatment of whey protein model systems (with or without milk powder or sucrose) with 30 kHz ultrasound, the changes in consistency coefficients (k) were observed, but there were no significant changes in flow behaviour indices (n).

Rojas et al. (2016) studied peach juice processed by the ultrasound technology. As the ultrasound processing time increased from 0 to 15 minute, the consistency index (k) was reduced to 70% of the original value. On the other hand, the flow behaviour index (n) was increased.

5. CONCLUSION AND RECOMMENDATIONS

Ultrasonication is generally associated with cell disruption of bacteria, yeast, fungi, algae and protozoa. With regard to the main yeast in the wine making process, *Saccharomyces cerevisiae*, Tsukamoto et al. (2004b) showed that 100 yeast cells per mL were almost perfectly inactivated within 180 s of ultrasonic irradiation; the inactivation rate was increased with the increase of amplitude of the ultrasonic wave and with the decrease of the initial cell numbers. Similarly, it is found that the yeast viability of *Saccharomyces cerevisiae*, after 20 minute of high power ultrasound exposure, was reduced to 25% (García Martin et al., 2013).

Wordon et al. (2012) researched that comparative real-time analysis of *Saccharomyces cerevisiae* cell viability, injury and death induced by ultrasound (20 kHz) and heat for the application of hurdle technology. When *Saccharomyces cerevisiae* was treated with ultrasound (20 kHz, 124 μm), only viable and dead cell populations were detected. Scanning electron microscopy showed extensive boundary damage to cells after 1 minute sonication. After 5 minute of treatment, ~92% of the cell population was dead.

Bermúdez-Aguirre et al. (2009) reported the modeling the inactivation of *Listeria innocua* in raw whole milk treated under thermo-sonication. Ultrasound was used to study the inactivation of *Listeria innocua* and mesophilic bacteria in raw whole milk. Tested amplitudes of ultrasonic waves were 0, 40, 72, 108 and 120 μm, with a constant temperature of 63ºC and treatment time of 30 minute. After using ultrasound at 60, 90 or 100% in combination with temperature, a 5 log-reduction was obtained after 10 minute inactivation of mesophilic bacteria was similar to those for *Listeria* spp. (Bermúdez-Aguirre et al., 2009).

Various research groups have demonstrated the inactivation of pathogenic and spoilage microorganisms (*Escherichia coli*, *Listeria* spp.), enzymes (pectin methyl esterase, polyphenol oxidase) with reduced effects on quality or nutritional parameters including ascorbic acid in orange juice (Tiwari et al., 2008a), ascorbic acid and anthocyanin content in strawberry (Tiwari et al., 2008b) and blackberry juice (Tiwari et al., 2009a; Tiwari et al., 2009b).

Consequently, the baby food made in this study did not contain pathogenic microorganisms in its normal microflora. The use of the combination of ultrasound and eau de rose can improve microbial safety. At the same time, this baby food containing dietary fibre, phenolic compounds, vitamin C, antioxidants, glucose, protein can serve a good nutrition choice especially suffering from constipation. According to our study, treatment with ultrasonication and eau de rose can affect the development of baby positively in terms of microbial quality and biochemical composition. When compared to control, there were no significant differences in treatment groups in terms of pH, fat, sugar and protein content. No allergen and aflatoxin were detected. Ultrasonication may affect the vitamin C stability. The taste panel results were evaluated and it was found that there were no extreme differences between treatment groups of baby food. Further studies can be performed on shelf life extension by using different novel processing technologies and/or other possible natural substances.

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APPENDICES

7. APPENDICES

Appendix A.1. The statistical analysis of the total mesophilic aerobic bacteria and yeast and mold by ANOVA.

	ANOVA					
		Sum of Squares	df	Mean Square	F	Sig.
Total Bacteria/0thDay	Between Groups	2.217	11	.202	11.464	.000
	Within Groups	.316	18	.018		
	Total	2.533	29			
Yeast Mold/0thDay	Between Groups	4.070	11	.370	19.116	.000
	Within Groups	.348	18	.019		
	Total	4.418	29			
Total Bacteria/30thDay	Between Groups	.738	4	.185	65.690	.000
	Within Groups	.014	5	.003		
	Total	.752	9			
Yeast Mold/30thDay	Between Groups	1.398	4	.349	4.038	.079
	Within Groups	.433	5	.087		
	Total	1.830	9			
Total Bacteria/60thDay	Between Groups	2.707	4	.677	26.836	.001
	Within Groups	.126	5	.025		
	Total	2.833	9			
Yeast Mold/60thDay	Between Groups	.855	4	.214	4274.800	.000
	Within Groups	.000	5	.000		
	Total	.855	9			
Total Bacteria/90thDay	Between Groups	3.863	4	.966	9657.350	.000
	Within Groups	.000	5	.000		
	Total	3.863	9			
Yeast Mold/90thDay	Between Groups	2.470	4	.618	315.056	.000
	Within Groups	.010	5	.002		
	Total	2.480	9			

Appendix A.2. The differences of total mesophilic aerobic bacteria counts with in shelf life time (0^{th}, 30^{th}, 60^{th} and 90^{th} days).

(Tests of Within-Subjects Effects) Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Toplam Bacteria	Sphericity Assumed	112.890	3	37.630	153.835	.000
	Greenhouse-Geisser	112.890	1.245	90.682	153.835	.000
	Huynh-Feldt	112.890	1.347	83.786	153.835	.000
	Lower-bound	112.890	1.000	112.890	153.835	.000

Appendix A.3. The within-subjects factors in shelf life period (0^{th}, 30^{th}, 60^{th} and 90^{th} days).

(Within-Subjects Factors) Toplam.bacteria	Dependent Variable
1	Total.Bacteria.0^{th} day
2	Total.Bacteria.30^{th} day
3	Total.Bacteria.60^{th} day
4	Total.Bacteria.90^{th} day

Appendix A.4. The comparison of different days for total mesophilic aerobic bacteria in shelf life time (0^{th}, 30^{th}, 60^{th} and 90^{th} days).

Pairwise Comparisons		Mean Difference (I-J)	Std. Error	Sig.a	95% Confidence Interval for Difference	
(I) Total bacteria	(J) Total bacteria				Lower Bound	Upper Bound
1	2	-3.822	.215	.000	-4.546	-3.098
	3	-4.013	.301	.000	-5.025	-3.001
	4	-3.789	.329	.000	-4.897	-2.681
2	1	3.822	.215	.000	3.098	4.546
	3	-.191	.137	1.000	-.652	.270
	4	.033	.148	1.000	-.465	.531
3	1	4.013	.301	.000	3.001	5.025
	2	.191	.137	1.000	-.270	.652
	4	.224	.087	.184	-.070	.518
4	1	3.789	.329	.000	2.681	4.897
	2	-.033	.148	1.000	-.531	.465
	3	-.224	.087	.184	-.518	.070

Appendix A.5. The differences of yeast/mold counts within shelf life period (0^{th}, 30^{th}, 60^{th} and 90^{th} days).

Tests of Within-Subjects Effects		Type III Sum of Squares	df	Mean Square	F	Sig.
Source						
Yeast/Mold	Sphericity Assumed	141.953	3	47.318	230.317	.000
	Greenhouse-Geisser	141.953	1.718	82.642	230.317	.000
	Huynh-Feldt	141.953	2.084	68.114	230.317	.000
	Lower-bound	141.953	1.000	141.953	230.317	.000

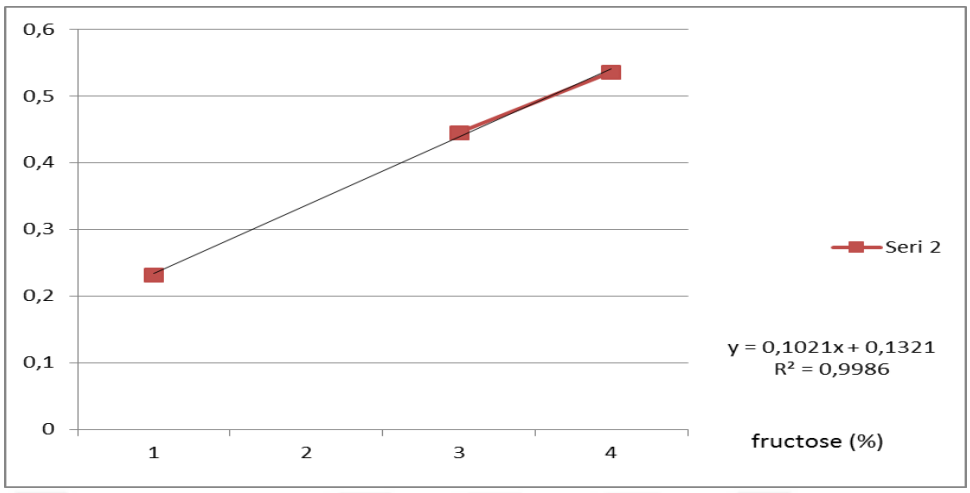
Appendix A.6. The comparison of different days for yeast/mold in shelf life period (0^{th}, 30^{th}, 60^{th} and 90^{th} days).

Pairwise Comparisons		Mean Difference (I-J)	Std. Error	Sig.a	95% Confidence Interval for Difference	
(I) Yeast/Mold	(J) Yeast/Mold				Lower Bound	Upper Bound
1	2	-4.604	.139	.000	-5.071	-4.137
	3	-4.242	.176	.000	-4.834	-3.650
	4	-4.153	.252	.000	-5.001	-3.305
2	1	4.604	.139	.000	4.137	5.071
	3	.362	.176	.419	-.230	.954
	4	.451	.276	.824	-.479	1.381
3	1	4.242	.176	.000	3.650	4.834
	2	-.362	.176	.419	-.954	.230
	4	.089	.159	1.000	-.447	.625
4	1	4.153	.252	.000	3.305	5.001
	2	-.451	.276	.824	-1.381	.479
	3	-.089	.159	1.000	-.625	.447

Appendix A.7. The Anova results of sensory parameters.

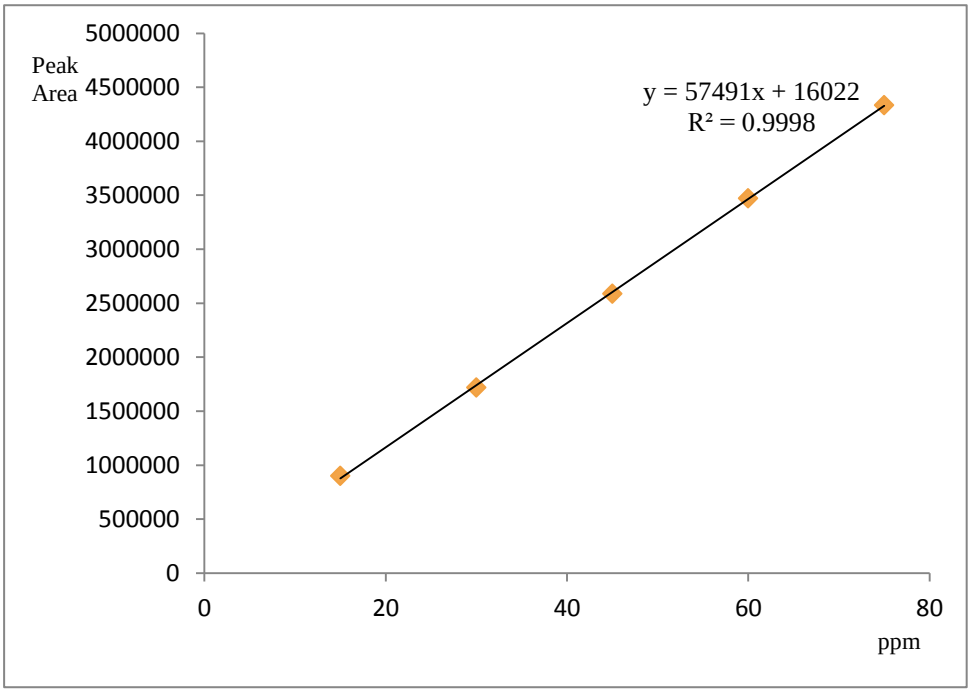
ANOVA						
		Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Color	Between Groups	16.720	3	5.573	1.907	.130
	Within Groups	572.960	196	2.923		
	Total	589.680	199			
Sweetness	Between Groups	33.280	3	11.093	4.016	.008
	Within Groups	541.440	196	2.762		
	Total	574.720	199			
Sourness	Between Groups	1.500	3	.500	.200	.896
	Within Groups	490.000	196	2.500		
	Total	491.500	199			
Odor	Between Groups	8.220	3	2.740	.367	.777
	Within Groups	1463.280	196	7.466		
	Total	1471.500	199			
Appearance	Between Groups	5.660	3	1.887	.419	.739
	Within Groups	881.840	196	4.499		
	Total	887.500	199			
Flavor	Between Groups	44.380	3	14.793	3.663	.013
	Within Groups	791.600	196	4.039		
	Total	835.980	199			
Bitterness	Between Groups	7,200	3	2.400	1.397	.245
	Within Groups	336.800	196	1.718		
	Total	344.000	199			
Overall	Between Groups	48.880	3	16.293	5.192	.002
	Within Groups	615.040	196	3.138		
	Total	663.920	199			

Appendix B.1. Standard curve of fructose (g/mL).

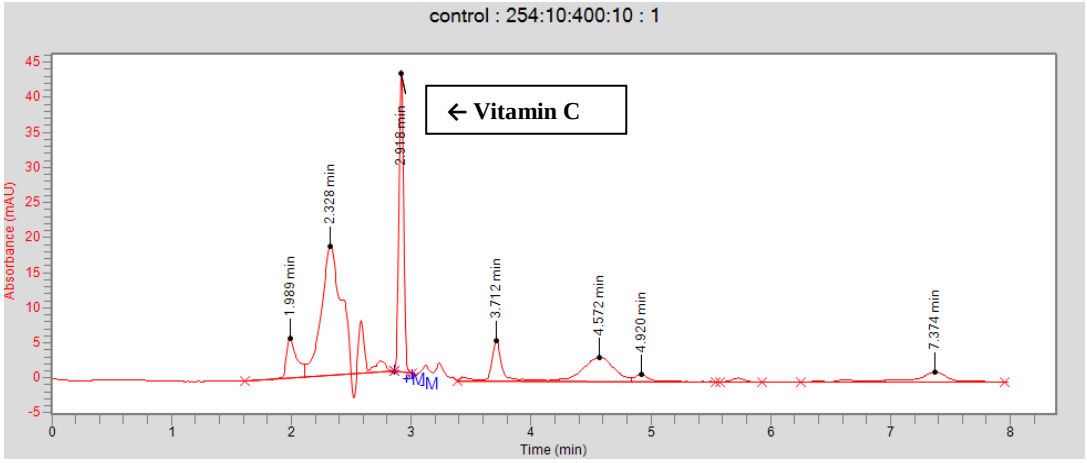


Graph equation: y = 0.1021x + 0.1321

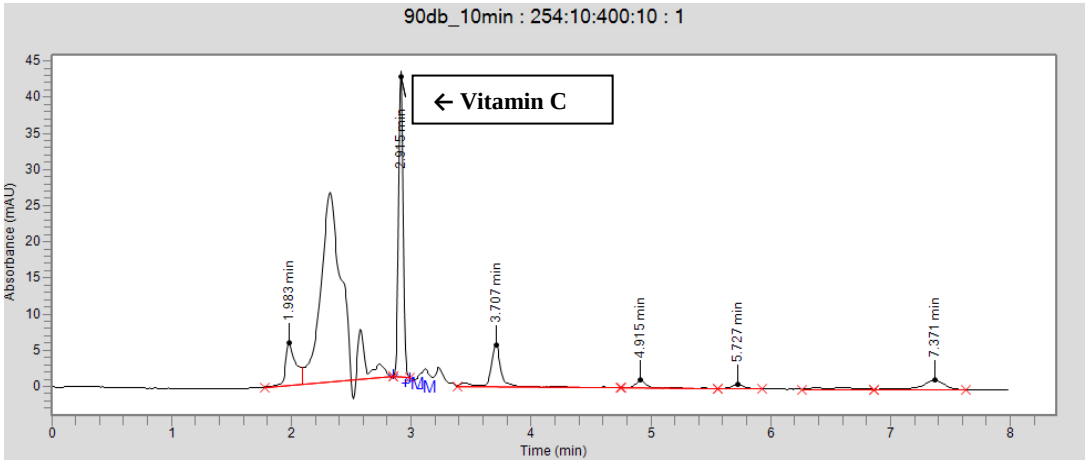
Appendix C.1. Standard curve of Vitamin C (ppm (mg/L)).



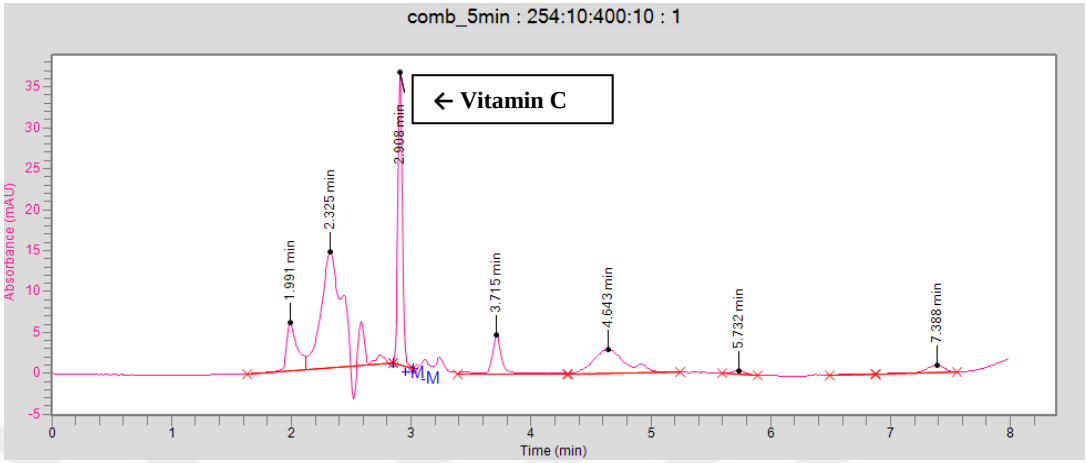
Appendix C.2. The Vitamin C analysis of the control group in HPLC.



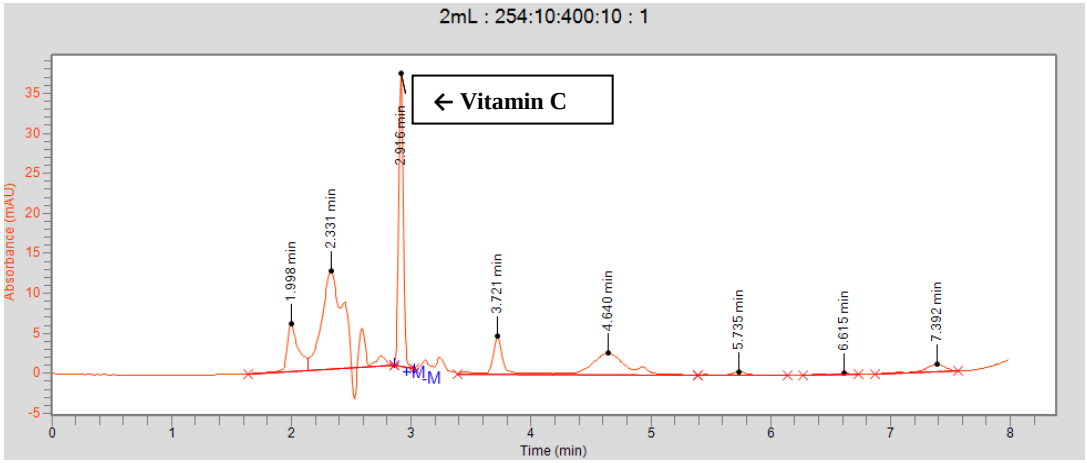
Appendix C.3. The Vitamin C analysis of the 90 dB+10 min group in HPLC.



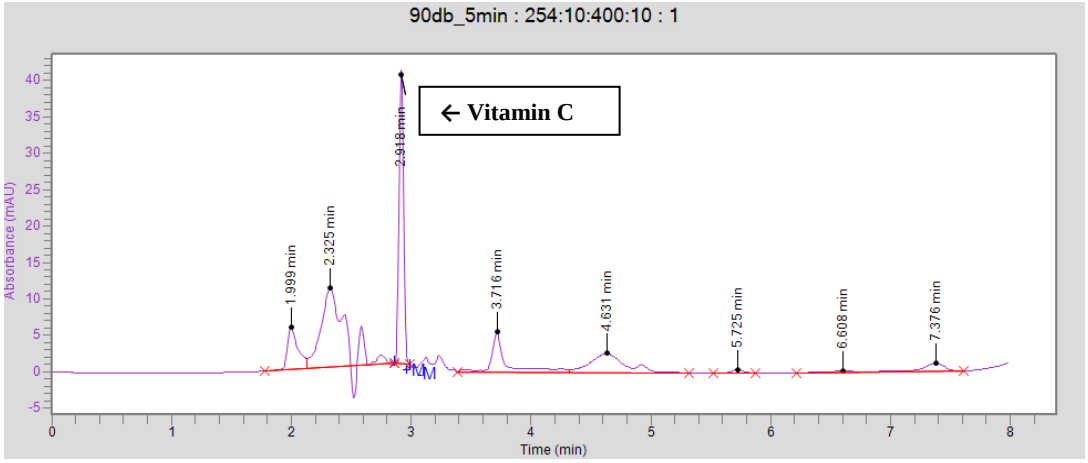
Appendix C.4. The Vitamin C analysis of the combination 5 min. group in HPLC.



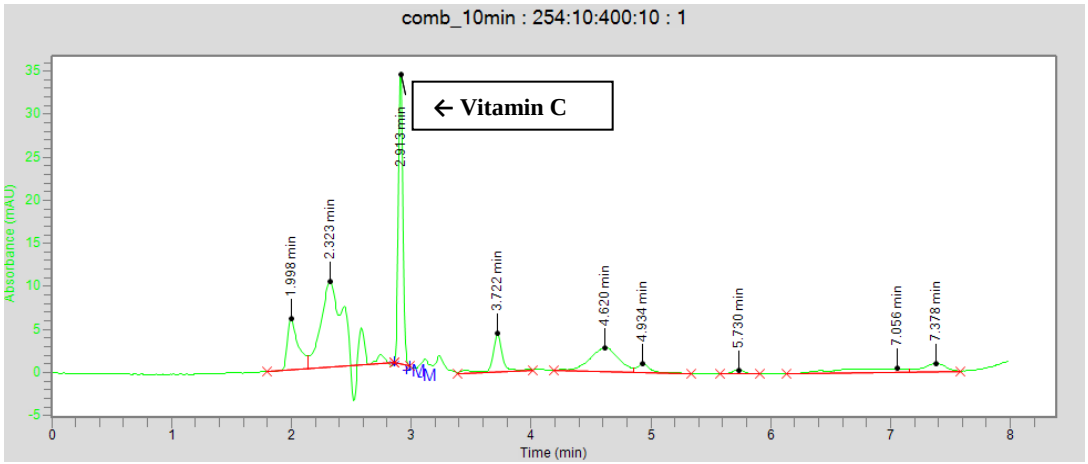
Appendix C.5. The Vitamin C analysis of the 2mL ER/10g group in HPLC.



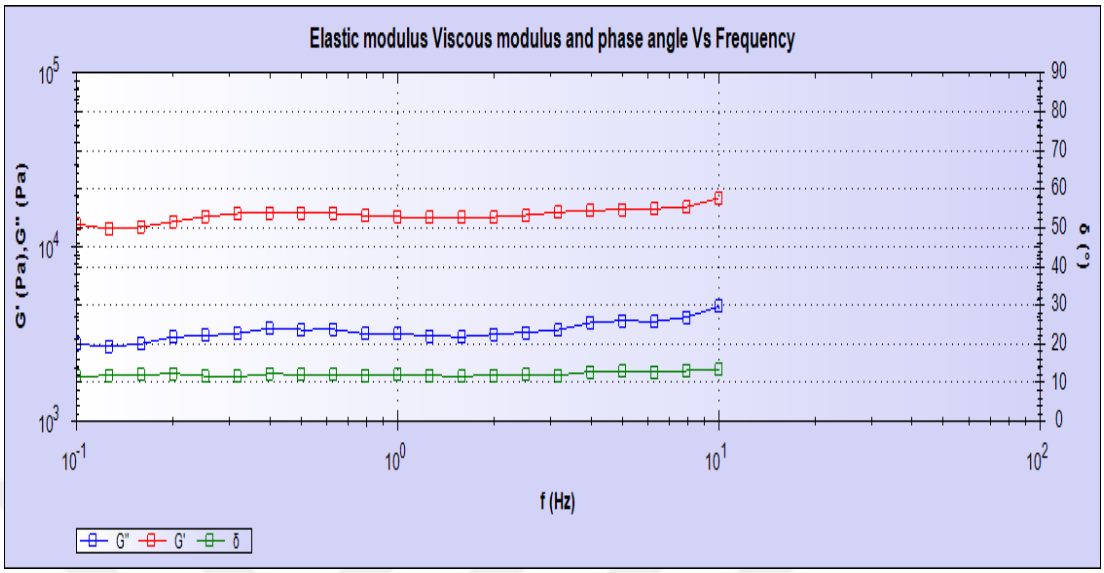
Appendix C.6. The Vitamin C analysis of the 90 dB+5 min. group in HPLC.



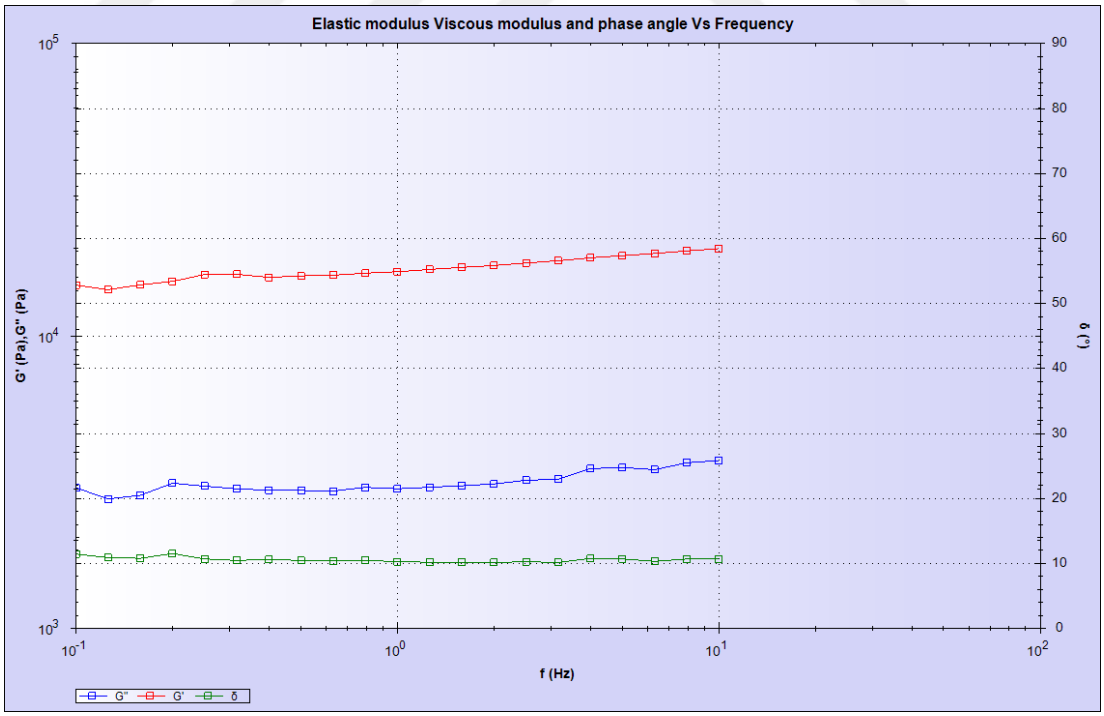
Appendix C.7. The Vitamin C analysis of the combination 10 min. group in HPLC.



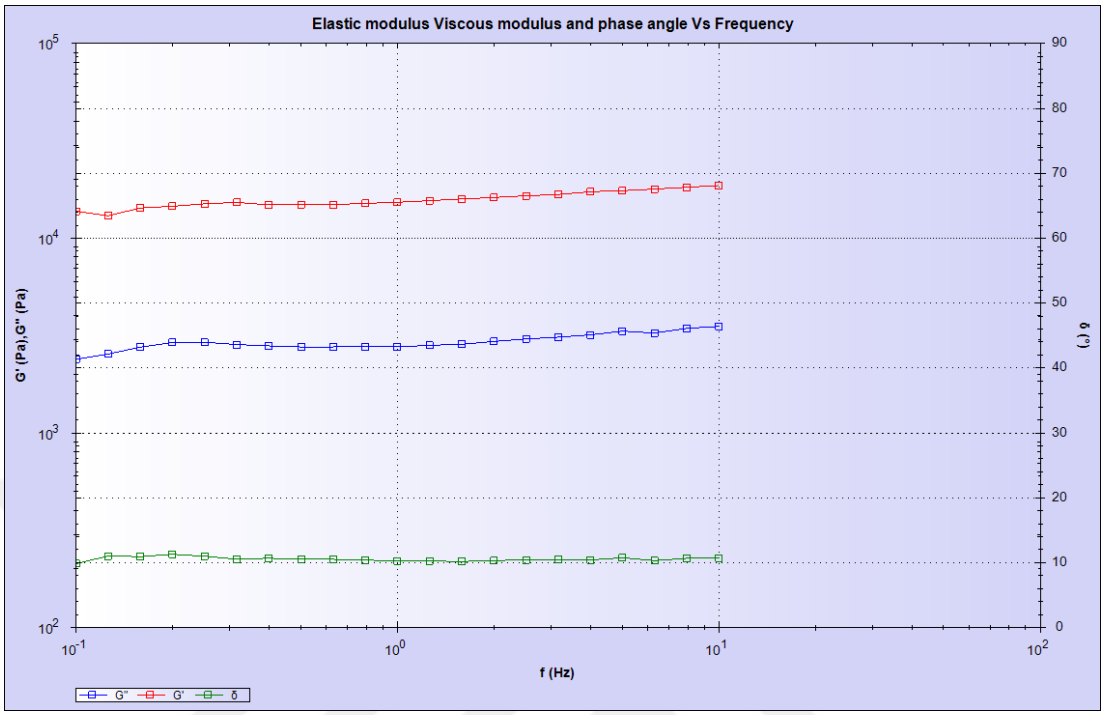
Appendix D.1. The rheological properties (Elastic modulus and Viscous Modulus) of control group.



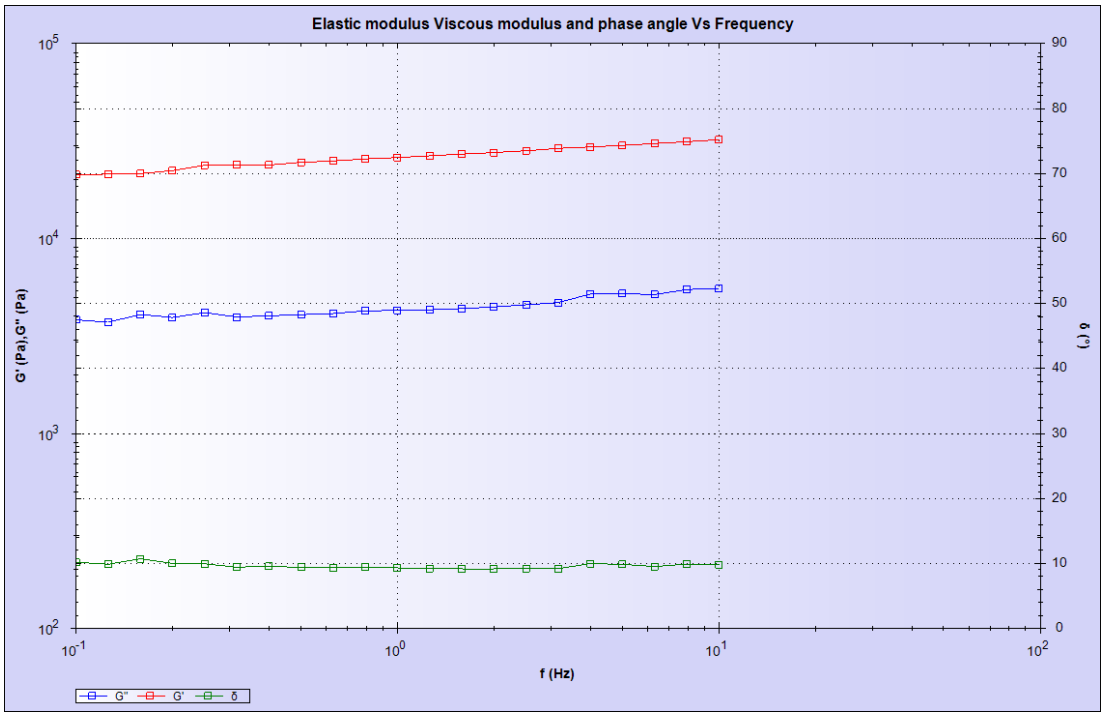
Appendix D.2. The rheological properties (Elastic modulus and Viscous Modulus) of combination 5 min.



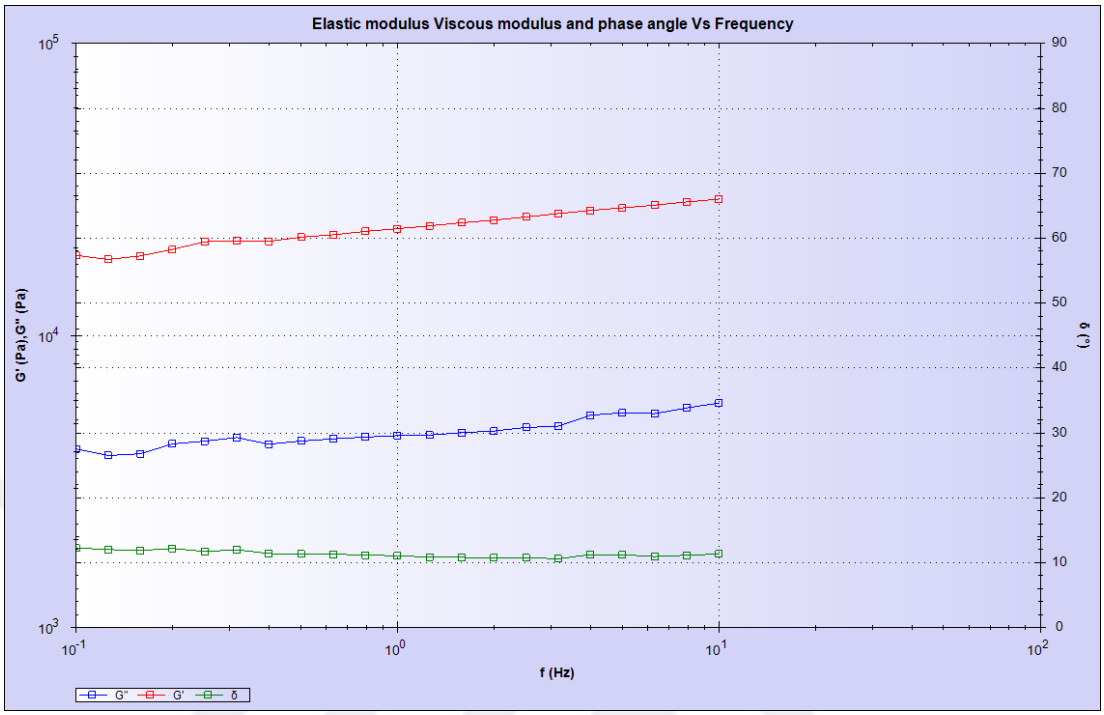
Appendix D.3. The rheological properties (Elastic modulus and Viscous Modulus) of combination 10 min.



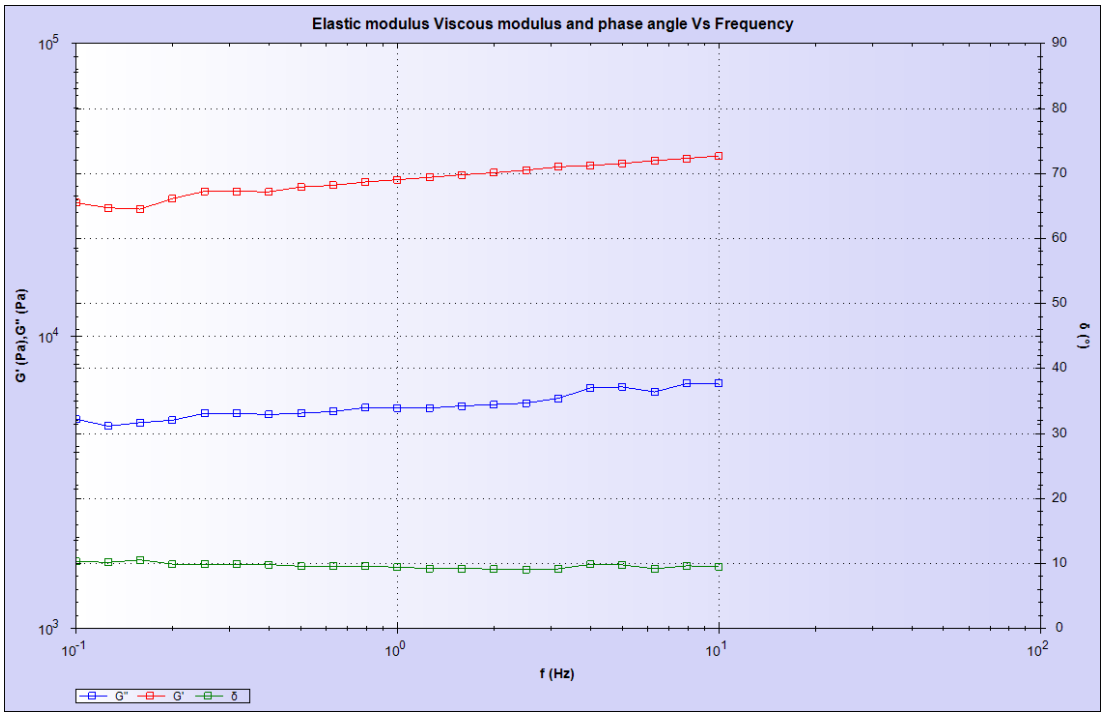
Appendix D.4. The rheological properties (Elastic modulus and Viscous Modulus) of 90 dB 5 min. group.



Appendix D.5. The rheological properties (Elastic modulus and Viscous Modulus) of 90 dB 10 min. group.



Appendix D.6. The rheological properties (Elastic modulus and Viscous Modulus) of 2mL ER/10 g group.



8. CURRICULUM VITAE

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