INVESTIGATION OF MICROWAVE PROCESSING FOR PASTEURIZATION OF VEGAN MILK PRODUCTS

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ABSTRACT

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In this study microwave processing was applied for the pasteurization of three different vegan milk products (almond, soy, and oat milk). For microwave process, 360 Watt power applied for 38 seconds. Also, results were compared with conventional thermal treatment which was carried out in a water bath at 70 °C during come-up times and 30 seconds holding time. For three vegan milk products, dielectric properties were investigated at different temperature values. After the treatments, important quality properties such as color, pH, particle size distribution and zeta potential values were evaluated. After all treatments, more than 5- log reduction of *Salmonella* Enteritidis was observed in three vegan milk products. Dielectric properties generally were significantly changed with temperature and frequency ($p < 0.05$). Color differences of vegan milk samples were not perceptible or perceptible through close observation. The pH values generally were not affected by the heat treatments for soy and oat milk ($p > 0.05$). Only there is a significant increase in the almond milk pH value with the heat treatments ($p < 0.05$). Only soy milk's zeta potential values were significantly affected by the different processes ($p < 0.05$). However, microwave pasteurized samples gave the closest value to untreated samples. Also holding process affected the stability of the oat milk. Particle size diameters were changed after the different processes. According to the results, the holding process is not required for the conventional pasteurization at 70 °C. Microwave heating could be a promising new pasteurization method for vegan milk.

ÖZET

VEGAN SÜT ÜRÜNLERİNİN MİKRODALGA PROSESİ İLE PASTÖRİZASYONUNUN ARAŞTIRILMASI

Bu çalışmada üç farklı vegan süt ürününün (badem, soya ve yulaf sütü) pastörizasyonu için mikrodalga prosesi uygulanmıştır. Mikrodalga işlemi için 360 Watt güç 38 saniye boyunca uygulanmıştır. Ayrıca sonuçlar geleneksel ısıl işlem uygulamasıyla karşılaştırılmıştır. Geleneksel pastörizasyon için 70 °C'ye ulaştıktan sonra ayrıca 30 saniye tutma süresi boyunca ısıl işlem uygulanmıştır. Üç vegan süt ürünü için farklı sıcaklık değerlerinde dielektrik özellikleri incelenmiştir. İşlemlerden sonra renk, pH, partikül boyutu dağılımı ve zeta potansiyel gibi önemli kalite özellikleri değerlendirildi. Tüm uygulamalardan sonra, üç vegan süt ürününde *Salmonella* Enteritidis'te 5 logdan fazla azalma gözlemlenmiştir. Dielektrik özellikler genellikle sıcaklık ve frekansla önemli ölçüde değişim göstermiştir ($p < 0.05$). Vegan süt örneklerinin renk farklılıkları, yakından gözlem yoluyla algılanabilir veya algılanamaz olarak çıkmıştır. pH değerleri, genellikle soya ve yulaf sütüne uygulanan ısıl işlemlerden etkilenmemiştir ($p > 0.05$). Sadece ısıl işlemlerle badem sütü pH değerinde önemli bir artış olmuştur. Zeta potansiyel değerleri genellikle kararlılık için limit'e yakındır. Sadece soya sütünün zeta potansiyel değerleri farklı proseslerden önemli ölçüde etkilenmiştir ($p < 0.05$). Ancak mikrodalga ile pastörize edilen numune, işlem görmemiş numuneye en yakın değeri verirmiştir. Ayrıca, geleneksel yöntemde, tutma süresi yulaf sütünün stabilitesini de etkilenmiştir. Farklı işlemden sonra partikül boyutu dağılımları önemli ölçüde etkilenmiştir ($p < 0.05$). Sonuçlara göre, 70 °C'de geleneksel pastörizasyon için tutma süresi gerekli değildir. Mikrodalga ısıtma, vegan sütler için umut verici yeni bir pastörizasyon yöntemi olabilir.

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CHAPTER 1

INTRODUCTION

Nowadays, most people prefer plant-based diets in developed countries due to environmental concerns, sustainability, and animal welfare and health issues. Thereby, substitute products are developed to catch usual tastes like vegan milk, vegan cheeses, veggie burgers, etc. Soybean, tofu, cereals, dried nuts are popular raw materials used in the vegan food industry. Vegan milk has a great place in the vegan-vegetarian products' sector as a cow's milk substitute, and soy, almond, coconut, hazelnut, and oat milk are the most produced vegan milk products. Recently, the studies have focused on the process of vegan products with the increase in their market share.

In the food industry, thermal processing techniques assure microbiological safety and inhibit some enzyme activities to extend shelf life (Arnoldi, 2001). Generally, conventional methods are applied to milk products, such as pasteurization and sterilization. Pasteurization is one of the oldest methods which destroy pathogen bacteria under 100 °C (Tamime, 2009; Li et al., 2021). However, these old methods cause detrimental effects on food quality and consume more energy than new processing methods.

Microwave heating is one of the novel technology applied to foods for pasteurization. In the electromagnetic spectrum, the microwave region is located between 300 MHz and 300 GHz (Chandrasekaran et al., 2013; Guo et al., 2017). According to ITU (International Telecommunication Union), some specific frequencies can be used for industrial, scientific, and medical (ISM) applications. While 2450 GHz is generally used in house-type microwaves, two different frequencies are preferred in the industrial applications, 896 MHz (UK) and 915 MHz (the USA and China) (Atuonwu and Tassou, 2018). When compared to conventional methods, microwave heating takes a shorter process time since it provides volumetric heating.

The main influential factors in the microwave processing of foods are frequency, power, temperature, mass, and size of food, the water content of food, density, physical geometry, thermal properties, electrical conductivity, and dielectric properties (Konak et al., 2009). The dielectric properties of food indicate the ability to store electromagnetic energy. The dielectric constant is a value that indicates a measure of the ability of foods to absorb electromagnetic waves. The dielectric loss factor is accepted as a measure of a material's ability to convert electromagnetic energy into heat energy (Mudgett, 1986; Wang et al., 2009).

In many food processes, the major point is the inhibition of the pathogen bacteria. For example, *Salmonella* is a foodborne pathogen bacterium that causes gastroenteritis and typhoid fever in humans. In developed countries, *Salmonella* Enteritidis incidence has increased in the last years (Buhinia, 2008). Although contaminated animal origin foods are the primary source of salmonellosis, some outbreaks are associated with fruits and vegetables. Some *Salmonella* outbreaks were reported due to consumption of raw almonds in the USA and Canada between 2000 and 2001, in Sweden between 2005 and 2006 (Chan et al., 2002; Isaacs et al., 2005; Muller et al., 2007; Brandl et al., 2008). Also, there are *Salmonella* outbreaks are linked to low moisture foods, cereals, nuts, etc., caused by handling (Verma et al., 2018). For this reason, it is vital to control raw materials or apply a proper process to eliminate the incidence of any pathogen bacteria in the final products. Besides these, it is crucial to maintain food quality when applied to a new processing method for food products.

In this study, the main aim is to observe 5-log reduction *Salmonella* Enteritidis in inoculated most consumed vegan milk products (almond, soy, oat) with microwave heating and investigate their quality changes. For this purpose, microwave processed vegan milk products were compared with conventional pasteurized products. Also, the important quality factors such as color, pH, particle size and zeta potential values were evaluated. Furthermore, the dielectric properties of three different vegan milk products were measured at 915 MHz and 2450 MHz for different temperatures. To the best of our knowledge, this is the first study that investigates the inhibition of a pathogen bacteria in vegan milk products with microwave heating. There is only one study that focuses on microwave and conventional thermal processing of soymilk to inactivation kinetics of lipoxygenase and trypsin inhibitors activity (Kubo et al., 2021).

CHAPTER 2

LITERATURE REVİEW

2.1. Vegan Milk

Vegan milk means that any plant-based product similar to milk with sensory, functional, and nutritional properties. It should not contain milk fat and other essential dairy nutrients (Elsabie et al., 2016). Many people start to consume vegan milk products due to environmental issues like sustainability and animal welfare or health issues like lactose intolerance. In the production of cow's milk, much more freshwater is needed compared to plant-based milk production. For instance, for a glass of cow's milk, 628 liters of fresh water is required, while 28 liters of milk is necessary for soy milk. Also, carbon footprints are much different in plant-based milk and cow milk. The carbon footprint of cow's milk is three times higher than soy and almond milk (Poore and Nemecek, 2018; Khanal and Lopez, 2021). However, some people believe that they can spend their life healthier with plant-based diets.

Plant-based milk is crucial as a raw material of other vegan products such as cheese, yogurt, butter, etc. (Aydar et al., 2020). Thereby, many companies have started to produce dairy milk substitutes to support plant-based diets in developed countries. Soy, oat, rice, and almond-based milk are the most popular dairy substitutes (Hoek et al., 2011; Fuentes and Fuentes, 2017). These products contain healthy fatty acids, carbohydrates, vitamin B, E and antioxidants, and dietary fiber. So, these substitutes are also preferred by consumers with rich nutritional values instead of cow's milk (Alozie Yetunde and Udofia, 2015; Elsabie et al., 2016). Their production is generally based on the obtained liquid from the soaked raw material and stabilization.

Vegan milk is generally defined as an oil-in-water emulsion. These systems compose of oil droplets and solid particles formed with proteins and starch granules. These droplets are covered with the water column (Chen and Tao, 2005). So, providing stability is more brutal in these food products (Briviba et al., 2016). In addition, physicochemical changes cause some quality loss such as creaming, flocculation, coalescence, phase separation (Hasan, 2012). Consequently, besides the appearance and other quality factors, also stability control is crucial in this type of product.

2.1.1. Almond Milk

Almond (*Prunus amygladus*), one of the popular tree nut, is suitable raw material in vegan milk with its nutritional composition (Sang et al., 2002; Maghsoudlou et al., 2016). Its origin is inner Anatolia, the Marmara and the Mediterranean regions of Turkey. Consequently, almond beverages are consumed over the years (Aydin, 2003; Dhakal et al., 2014). Almond milk has high nutritional value with protein and fat content, also contains dietary fiber, α-tocopherol, vitamins and minerals such as magnesium, copper and phosphor (Kundu et al., 2018). In addition, it reduces the plasma LDL cholesterol level, so it is effective in preventing heart diseases (Dhakal et al., 2014).

The production of almond milk depends on the condition of almonds. Both dried and fresh almonds can be used as raw materials. The process steps are altered based on the dried or fresh almond usage.. Dried almonds go to the dry grinding process directly. Fresh almonds are subjected to a roasting process with the heat treatment in the first step. This process leads to decrease in pyrazine and benzaldehyde amounts. The conditions for this process are 95-100 °C, 30 minutes. Then roasted almonds are soaked in water to obtain a soft and swell structure. Following the draining process, the wet milling process is applied. Here, the almonds are ground with added water. The following steps are the same for both fresh and dried almonds; filtration, addition of other additives and sterilization, homogenization, aseptic packaging and cold storage (Callewaert et al., 2012; Aydar et al., 2020).

Generally, vegan milk products are sterilized with the UHT technique. Besides, some studies show preservation or pasteurization of almond milk with alternative methods such as high-pressure processing, Thermo sonication and pulsed electric field (Dhakal et al., 2014 Manzoor et al., 2020; 2021). Dhakal et al. (2014) reported that high-pressure processing and thermal processing affect immunoreactivity differently. High-pressure processing reduced major and allergenic protein in almond, amandine, by half. Also, both treatments caused a reduction in almond milk protein solubility, aggregation and disulfide bond disruption since they affected the protein structure of the product. They state that future searches are required to understand the effect of process conditions. Manzoor et al. (2020) stated that pulsed electric field treatment, a novel processing method, positively affected emulsion stability and phase separation and reduced particle size. In another study reported by Manzoor et al. (2021), the thermosonication process did not change quality parameters significantly without some exceptions. Also, phenolic content, DPPH activity and total antioxidant activity were improved by the thermosonication process. This process provided microbial inactivation and residual enzyme inactivation in almond milk without significant quality changes. So, these studies show that alternative techniques could be applied for the almond milk pasteurization and preservation process without damaging the quality parameters of almond milk.

2.1.2. Soy Milk

Soy products are preferred with a high value of protein, dietary fiber, isoflavones, and omega-3 fatty acid as an alternative to standard products (dairy products). They contain high amounts of minerals such as copper, magnesium, calcium, potassium, zinc, iron, and some essential amino acids (Kundu et al., 2018). Studies show that soy proteins reduce the blood cholesterol level (Gardner et al., 2007).

Soybean (Glycine max) is consumed all over the world as an alternative protein source. It is a legume crop plant and its origin is East Asia (La Menza, 2017). In soy milk production, tofu (okara) is extracted from the fraction of water-extractable part of soybeans and is used as raw material (O'toole, 1999).

Soymilk can be produced in both traditional and industrial methods. Industrial production consists of the following steps; sorting high-grade soybeans then cleaning from dirt, stones, dust, etc. After cleaning, dehulling is applied to remove off-flavor substances like polysaccharides. Also, this process provides easy processing of the beans like decantation. Then, soaking (generally overnight) and blanching in sodium bicarbonate solution is applied. Blanching is a method inactivate the enzymes. After that, a colloid solution (called a slurry) is obtained by the grinding process. The following steps are heat treatment, homogenization, and flavoring in the production line. (Oyeniyi et al., 2014). Homogenization is a crucial step to avoid phase separation in this type of product. Finally, aseptic packaging and storage are the last lines for the product.

As well as in other vegan-vegetable milk products, the UHT process is generally applied in the soy milk industry. Uemura et al. (2010) reported that Radio frequency-flash heating with a teflon film could be applied to reduce four logarithmic units of *Bacillus subtilis* spores in soybean milk. They compared the breaking strength of tofu produced from two different processed (RF-flash heating and conventional process) soybean milk.

Radio-frequency- flash heating treated sample had higher breaking strength than the other sample. In another study reported by Poliseli-Scopel et.al. (2012), soy milk was processed by ultra-high pressure and stored at refrigeration conditions. They indicated that UHPH processed samples had higher microbial inactivation and their quality characteristics were better than conventional pasteurized samples in terms of color, colloidal stability and sensorial properties. Thereby, novel technologies could be applied for the pasteurization of soymilk without causing detrimental effects on the quality.

2.1.3. Oat Milk

Nowadays, oats (Avena sativa L.) are preferred as a result of changing diet habits since they have high nutritional value and gluten-free characteristics. Oat is a high protein source cereal also rich in dietary fiber and β-glucan. β-glucan prevents cardiovascular diseases by controlling blood glucose (Braaten et al., 1994; Butt et al., 2008). Like soybean and almond, it has lowered low-density lipoprotein (LDL) cholesterol levels by means of β-glucan content (Berg et al., 2003; Othman et al., 2011). It is also rich in fat, B complex vitamins, and minerals (Butt et al., 2008). Oat is a promising a healthy beverage with these properties instead of cow's milk.

Oat milk can be produced with enzymatic reactions or extraction methods (Bernat et al., 2015; Demir et al., 2021). In the enzymatic way, rolled oats and water go through a mixing process; after that, α-amylase is added to the oat slurry for gelatinization and liquefaction at 70-75 °C. Following that, the slurry is filtered (Deswal et al., 2014). In the other method, soaking, draining, wet milling, filtration, sterilization, homogenization are the steps followed for the production of oat milk (Bernat et al., 2015; Aydar et al., 2020).

In the production of oat milk, a heat treatment is needed for providing both stability and longer shelf life (Mäkinen et al., 2016; Demir et al., 2021). For industrial products, generally, the UHT process is used to extend shelf life. However, in recent studies, there is some evidence that new technologies could be used instead of conventional methods to provide microbiological safety of oat milk (Demir et al., 2021). Also, the temperature is effective on the rheological properties of oat milk (Deswal et al., 2014). Thereby, both thermal and nonthermal processing methods should be investigated in detail for promising new processing methods in the vegan-vegetable milk industry.

2.2. Food Preservation Methods

In the food industry, the main aim of applying a process is to prevent foodborne illnesses. For this purpose, generally, conventional methods are applied to inactive target pathogenic bacteria in the food product. Currently, traditional methods like pasteurization and sterilization are used as preservation technology. In the light of developing technology and recent studies, novel technologies have potential applications for the future. For example, ultrasound, ultraviolet light, pulsed electric field, x-rays, cold plasma are non-thermal promising methods. Microwave heating and radiofrequency heating are dielectric heating methods that are started to be used instead of conventional methods to process various food products.

2.2.1. Conventional Methods

Thermal processing can be defined as a heat treatment for inhibition of pathogenic and spoilage bacteria in food. Based on the acid level of the food, pasteurization and thermal sterilization are standard methods for industrial applications. Since the twentieth century, pasteurization has been used as typical heat treatment to destroy lethal bacteria and extend shelf life.The process was named as pasteurization in 1862 by the French scientist Louis Pasteur with the invention of the experiment (Hayes and Laudan, 2008).

Pasteurization applications vary according to the temperature-time combination. According to processing temperature and time, pasteurization methods can be classified as VAT (batch), high-temperature short time (HTST), higher heat shorter time (HHST), ultra-high temperature, and ultra-pasteurization (UP). VAT pasteurization takes long time in low temperatures. Therefore, it is preferable for the small plants to control process conditions all the time in the batch system. HTST pasteurization is applied in higher temperatures in shorter times. It requires developed control systems such as valves, pumps and heat exchangers. HHST has similar features to HTST, but the product is heated at higher temperatures in shorter times with dissimilar equipment. Finally, in ultra pasteurization the temperature is applied not less than 280 °F. These methods require refrigeration conditions during the product’s shelf life (Amit et al., 2017; IDFA, 2021).

UHT is a more effective method when compared to other techniques. It extends longer shelf life, so UHT pasteurized products can be defined as shelf-stable. It is also needed aseptic packaging and commercially sterile equipment for keeping these conditions. The pasteurized food products with this method don't require refrigeration conditions during their shelf lives. According to temperature-time requirements, pasteurization affects the nutritional values of the food products. Especially vitamin, mineral losses are observed in the product. Sometimes it has a detrimental effect on the beneficial bacteria. These effectuate the applications' limitations (Amit et al., 2017; Shajil et al., 2018).

Pasteurization time-temperature combination varies according to different food products and target bacteria. In addition, the needs in the shelf life and quality parameters determine the process conditions.

2.2.2. Microwave Heating

Microwave heating is a favored thermal processing method with short come up and process time compared to conventional methods (Ahmed and Ramaswamy, 2004). Microwaves are located between 300 MHz and 300 GHz in the electromagnetic spectrum (Tang et al., 2002). It is allowed to microwave heating in specific frequencies for the medical, industrial and scientific applications by the ITU (International Telecommunication Union). These frequencies are 896 MHz (UK) and 915 MHz (the USA and China), 2450 MHz, 5800 MHz and 24125 MHz (Atuonwu and Tassou, 2018; Tucker, 2016). Generally 2450 MHz is used in the domestic type microwave oven while beside the 2450 MHz, 896 MHz (UK) and 915 MHz is applied in the industry (Orsat et al., 2017; Atuonwu and Tassou, 2018; Tang et al., 2018; Hong et.al, 2021).

When microwaves penetrate the food, electromagnetic energy is converted to heat energy in molecular level. Eventually, food is started to heat volumetrically. The heating caused by the molecular friction as a result of dipolar rotations and the conductive migration of dissolved ions. (Alton 1998; Oliveira and Franca, 2002). So, the main heating mechanism is based on dipolar rotation and ionic polarization (Ahmed and Ramaswamy, 2004).

Maxwell's equations explain the electromagnetic field distribution inside the cavity in the microwave system (Geedipalli et al., 2007; Vadivambal and Jayas, 2010).

$$\nabla\, x\, E = jw\mu H \tag{2.1}$$

$$\nabla\, x\, H = jw\varepsilon_0\varepsilon\, E \tag{2.2}$$

$$\nabla . E = 0 \tag{2.3}$$

$$\nabla . H = 0 \tag{2.4}$$

Where E is the electric field strength (V/m), ω is the angular frequency (rad/s), μ is the relative permeability (H/m), H is the magnetic field intensity (A/m), ε_o is the permittivity of free space (F/m), and ε is the complex relative permittivity.

Complex relative permittivity can be explained as $\varepsilon_r = \varepsilon' - i\varepsilon''$

Where ε' is the dielectric constant and ε'' is the dielectric loss factor (Campañone et al, 2014; Topcam et al., 2020). The real parts of the equation, dielectric constant, defines the ability of the storage of electromagnetic field. Imaginary part of the equation defines the ability of the conversion electromagnetic energy to heat energy (İçier and Baysal, 2004; Nelson and Trabelsi, 2012). These properties are affected by the moisture content, temperature, frequency, and product composition (Nelson and Datta, 2001; Renshaw et al., 2021).

Conversion of electromagnetic energy to heat energy can be defined as:

$$P = 2\pi f\, \varepsilon_0\varepsilon''\, E^2 \tag{2.5}$$

Where P = volumetric heating rate (W/m^3).

Penetration depth is a critical property that when food material absorbs the microwaves, it decreases the intensity of them. It is expressed by the following equation:

$$d_p = \frac{\lambda \sqrt{\varepsilon'}}{2\pi \, \varepsilon''} \tag{2.6}$$

Where λ, is the free space wavelength. The penetrations depth is also explained as the distance which is decreased by 1/e (approximately 0.368) from the original value (Ryynänen, 1995, Campañone et al, 2014).

Frequency, power, temperature, mass, and size of food, the moisture content, density, thermal properties, physical geometry, electrical conductivity, and dielectric properties are the effective properties on the microwave heating (Konak et al., 2009). In microwave heating with the system design and geometry can be provided more uniform heating compared to conventional methods (Datta and Hu, 1992; Tucker 2016). Alongside, there are various advantages of microwave heating. It reduces the process time and saves energy. Correspondingly, it preserves the quality characteristics of food by means of nutritional, sensory, color etc.

Several studies showed that microwave heating was applicable for the pasteurization of some liquid food products instead of conventional methods. In a study, microwave pasteurized cloudy apple juice showed better volatile profile compared to the conventional pasteurized one (Siguemoto et al., 2019). In another study, conventional pasteurization and novel processing technologies were compared to each other for the Mandarin juice pasteurization (Cheng et al., 2020). The results have shown that microwave pasteurized juice had better quality in terms of aroma, nutritional quality and aroma. All treatments have also similar effect on the microbiological inhibition. González-Monroy et.al. (2018) reported that the needed time was only 12 s for Pectin methylesterase enzyme inactivation in tamarind beverage with microwave-assisted batch system while 4.67 min in conventional pasteurization. Also MW-assisted pasteurization had not effect on the sensorial and physicochemical properties of the product. It was demonstrated that microwave pasteurization has provided 5-log reduction in *L. monocytogenes* and *E. coli* in apple juice (Siguemoto et al., 2018). Another study in the coconut water, microwave processing was a promising method for inhibition of *B. coagulans* spores (Pinto et et al., 2021). These results emphasize that microwave processing have potential replaceable process instead of conventional methods. It is both effective on the inactivation of the target bacteria and keeping the quality characteristics of the food products.

2.3. *Salmonella*

Salmonella is a foodborne pathogen bacterium named after Daniel E. Salmon and it plays an important role in foodborne illnesses in humans more than a hundred year (FDA, 2020). According to the CDC (Centers for Disease Control and Prevention), *Salmonella* is the most common germ that causes of foodborne illnesses after Norovirus in the United States (CDC, 2021). *Salmonella* belongs to Enterobacteriaceae family. It is a non-spor forming gram-negative bacterium and motile except some species. Its optimum temperature is 35-37 °C but it could grow between 5 and 45 °C (Buhinia, 2008).

According to the recent classification, the genus *Salmonella* are classified into two different species. According to their 16S rRNA sequence relatedness, these are *Salmonella enterica* and *Salmonella bongori*. *S. bongori* do not infect humans. *Salmonella enterica* is further classified into six subspecies mainly based on their genomic sequence and biochemical properties. Differences in the biochemical properties of the flagella, carbohydrate and lipopolysaccharide (LPS) of *S. enterica* and S. *bongori classified* further into 2463 serotypes. *Salmonella* Enteritidis is a non-typhoidal enterica serovar mainly associated with gastroenteritisis in humans (Hurley et al., 2014; Ryan et al., 2017). *S.* Enteritidis causes the 60% of salmonellosis case and have primary role in human salmonellosis (Thorns, 2000; Afshari et al., 2018).

Salmonellosis is caused generally by meat and meat products, egg and egg products, dairy products and also sometimes by the vegetables, fruits and nuts (Silva and Gibbs, 2012; CDC, 2021). There have been some outbreaks related to raw almond consumption in the United States and Canada between 2000- 2004 and Sweden from 2005 to 2006. (Chan et al., 2002; Isaacs et al., 2005; Muller et al., 2007; Brandl et al., 2008). Due to these outbreaks, minimum 5-log reduction of *Salmonella* is a requirement for packaged almonds described as pasteurized (Andrews and Hammack 2007; Du et al., 2010). For this reason, recent studies are focused on the reduction of Salmonella in almonds with different methods (Almond Board California, 2007; Du et al., 2010). However, there have been some *Salmonella* cases associated to some low moisture foods, cereals, nuts, etc., caused by handling (Verma et al., 2018). Cross contamination with animal origin products cause the spread of the pathogen bacteria and results with salmonellosis**.**

2.4. Quality Attributes

2.4.1. Color

Color is one of the crucial quality factor that affects the consumer preference for the food product. It is a perceptual response to reflected and emitted light from the object in the visible region of the electromagnetic spectrum (Wu and Sun, 2013). By the *Commission Internationale de I'Eclairage* (CIE), color's three elements are defined as the light source, the visual sensitivity of observer, the reflectivity of the sample. Color is recognized by the human with the three primary colors, red, green, and blue. (CIE, 1986; Mendoza et.al, 2006; Castro et.al, 2019). Currently, different color spaces and numbers are used to define and evaluate these color values in two and three dimensional space (Leon et, al, 2006). Generally, following color models are used to analyze color values; CIE $L^*a^*b^*$, XYZ, Hunter Lab, and RGB (Red, Green, Blue) (Joshi, 2002; Kılıç, et al, 2007). Among these color spaces CIE $L^*a^*b^*$ color space is defined as standard model by the CIE in 1976. L^* (lightness), ranges from 0 to 100, $a*$(from green to red) and $b*$(from blue to yellow) are other chromatic components and their range is from −120 to 120 (Papadakis et al., 2000; Pedreschi et al, 2016).

Color can be analyzed qualitatively but a standard measurement is required for the many research. For this reason, several techniques are developed to evaluate the food color. Colorimeters and spectrophotometers are general instruments to measure color in the food industry. Colorimeters are widely preferred with the ease of use and calibration, rapidness, compact and mobile (Nguyen et.al, 2022) Colorimeters give results in XYZ, RGB and $L^*a^*b^*$ color space for the color measurement readings (Trussell et al., 2005; Mendoza et al., 2006).

2.4.2. pH

The pH is a term used to define the concentration of hydrogen ions in aqueous media and it is named by Sørensen in 1909 (Buck et al., 2002). The pH is explained by the following equation (IUPAC, 1997).

$$pH = -\log[H^{+}] \tag{2.7}$$

The pH value is crucial to evaluate microbial flora and heat resistance of the food. In a solution, the pH value can be analyzed with a strip of litmus paper, an indicator or pH meters. The pH meters offer sensitive measurements and the measurements are based on the voltage difference between reference electrode and glass electrode that measures the H_3O^+ concentration (Jennings et al., 2010). The pH value of the food products can be affected by the food processing especially from pasteurization. As a result of the pasteurization a decrease seems in the pH value (Paredes-Sabja et al., 2007; Dhakal et al., 2014).

2.4.3. Zeta Potential

Emulsions, suspensions, liposomes, and nanoparticles are dispersed systems that crucial groups for food processing. They consist of electrically charged particles and play role in the interaction through the medium (Cano-Sarmienito et al., 2018). Soft drinks, cream, mayonnaise, salad dressings, milk, and butter are common examples for the food emulsions (Li et al, 2020). Emulsions are unstable systems, due to this feature, many applications are limited in the food industry (Shao et al., 2020).

Zeta potential is a significant parameter that measures of the electric interactions in food systems (Cano-Sarmienito et al., 2018). It is a potential difference between the medium and stationary phase in the dispersed particles. The value is affected by the ionic strength, the concentration of any additives, temperature and most significantly pH (Lu and Gao, 2010). Emulsion stability can be analyzed by the zeta potential (Tekin et al., 2020).

There are several studies that use zeta potential as emulsion stability in vegan milk products which are oil-in-water emulsions (Bernat et al., 2015; An, 2019; Weston et al., 2020; Zhao et al., 2020).

2.4.4. Particle Size Distribution

In emulsions which are particulate systems, it is very important to analyze size distribution to evaluate food safety, quality and performance of final product (Świrniak, and Mroczka, 2021). Particle size distribution is crucial to determine emulsion stability, flocculation and creaming (Huang et al., 2001). Naturally, particles have different size and generally their shape is not spherical. Also they have different specifications in terms of density, conductivity and refractive index (Merkus, 2009).

Particle size distribution can be measured with dynamic light scattering. It is based on the interaction of light with the electric field of a particle. In light scattering measurements, particles are evaluated as sphere having the diameter. The technique is analyzed with the scattering light from the particles in the Brownian motion. (Mailer et al., 2015).

In dynamic light scattering instruments, the diameter is determined by the diffusion coefficient of the particles in the liquid medium. The relation between diffusion coefficient and particle diameter lay on the Stokes-Einstein equation (Palmer and von Wandruszka, 2001):

$$\frac{kT}{3\pi\eta D}\ x\ 10^{12} \qquad (2.8)$$

Where x is hydrodynamic diameter of an equivalent spherical particle (nm), k is Boltzmann constant (1.38×10^{-23} J.K^{-1})' T is absolute temperature (K)' η is viscosity of the medium (mPa.s) and D is diffusion coefficient (m^2.s^{-1}).

In particle size distribution, surface area mean diameter ($D_{3,2}$) is sensitive the presence of finest particles whereas the volume mean diameter ($D_{4,3}$)is sensitive to the presence of large particles and aggregates. These diameters are defined by the equation 2.9 and 2.10.

For volume weighted particle size distributions, results are generally given as maximum particle size for a specific percentage volume of the sample. The most used percentiles are the D_{v10}, D_{v50} and D_{v90} (Poliseli-Scopel et al., 2012; Malvern Instruments, 2015).

$$D[3,2] = \frac{\sum_1^n D^3{}_i v_i}{\sum_1^n D^2{}_i v_i} \tag{2.9}$$

$$D[4,3] = \frac{\sum_1^n D^4{}_i v_i}{\sum_1^n D^3{}_i v_i} \tag{2.10}$$

CHAPTER 3

MATERIAL AND METHODS

3.1. Materials

Sugar-free vegan milk samples (almond milk, soy milk and oat milk) were purchased from a local market to provide a standard product profile in the experiments.

3.2. Methods

3.2.1. Preparation of *Salmonella* Inoculum and Inoculation of Vegan Milks

The vegan milk samples (soy, almond, and oat) were kept at room temperature (20 ± 2 °C) before the microbial inoculum. *S.* Enteritidis ATCC 13076 was obtained from the culture collection of the Ankara University Food Microbiology Laboratory (Department of Food Engineering, Ankara, Turkey). After inoculation, *S*. Enteritidis was cultured in Tryptic Soy Broth (BD, the USA) and incubated at 37°C for 24 hours. At the end of the incubation time, strains were transferred into sterile 50 mL centrifuge tubes and centrifuged at 2600 g for 30 min. Then, the supernatant was removed from the pellet and it was washed twice in 9 mL 0.1% of peptone water. Following that, the pellet was diluted with 3 mL % 0.1 peptone water, and 1 mL aliquots from the Salmonella culture were inoculated in 9 mL vegan milk samples. The samples were diluted again with 90 mL vegan milk samples again to reach approximately 10^6 CFU/mL *Salmonella* Enteritidis strain in the samples (Possas et al., 2018; Coskun et al., 2021).

3.2.2. Pasteurization of Vegan Milk Samples

10 mL vegan milk samples were filled to sterile 15 mL centrifuge tubes for the pasteurization process.

3.2.2.1. Microwave Pasteurization

The experiments were performed in a house-type inverter microwave oven (Siemens HF25g5L2, Germany) to provide standard experimental conditions. This inverter oven supplies power continuously during the experiment in 360, 600 and 900 Watts. In higher power levels, it is hard to control experiments and samples since some points reach the higher temperature in very short time. This is the disadvantage for ensuring microbiological safety in desired level. So, after various trials, microwave pasteurization conditions were determined as 38 seconds in 360 Watt as optimum. A stand was designed with a 3D printer to hold samples in centrifuge tubes in the mid of the microwave oven. Before the experiments, in vegan milk samples the temperature near the cold points was measured with fiber optics (FISO Technologies INC. Québec, Canada) (Figure 1).

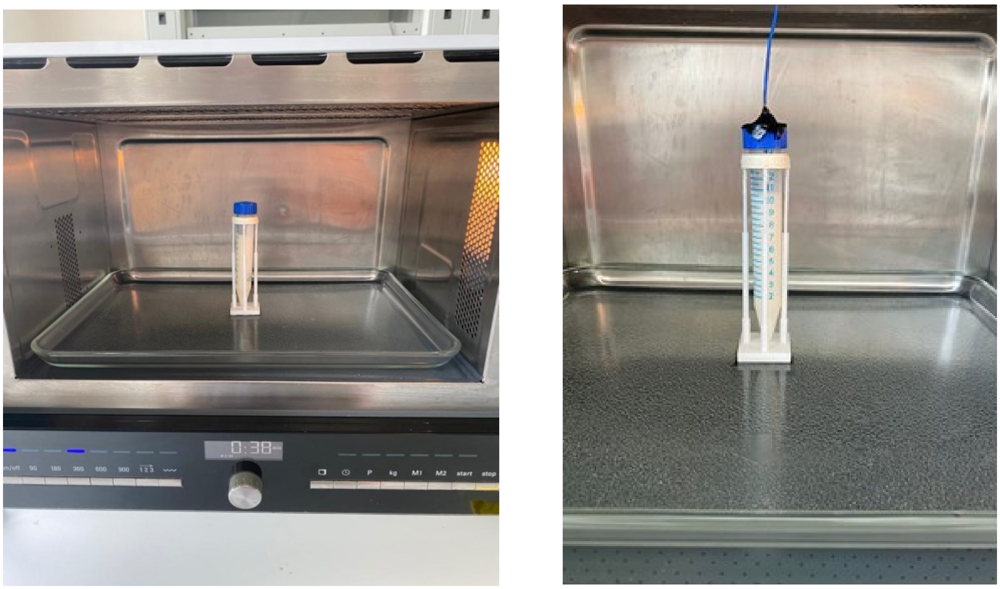


Figure 1. Microwave pasteurization experimental setup

3.2.2.2. Conventional Pasteurization

Conventional pasteurization was applied at 70 °C in a water bath (Nuve ST 30, Turkey) to catch a similar temperature in the final product at (360 W- 38 seconds) microwave pasteurization. Also, 71 °C is a critical temperature for one of the essential proteins, β-Conglycinin, denaturation (Pathomrungsiyounggul et al., 2010). For this reason, process was planned not to exceed 70°C.

Come-up times were calculated after experiments. After different come–up times, vegan milk samples were held at 70°C for 30 seconds. Thus, come up times are approximately 480, 465, and 480 seconds for soy, almond, and oat milk, respectively (From 20 °C to 70 °C).

During the conventional pasteurization, the temperature near the cold point of the samples and water temperature were measured with Type-T 36 gauge thermocouples (Figure 2).

Figure 2. Conventional pasteurization experimental setup

3.2.3. Microbial Analysis

Inoculated milk samples were filled aseptically as 10 mL into 15 mL centrifuge tubes before the pasteurization processes. For the control and processed samples, serial dilutions were prepared using 0.1% peptone water. From each dilution, 0.1 mL sample was spread on the Xylose Lysine Tergitol-4 Agar (XLT4) (Sigma, Canada) (two plates per dilution). The plates were incubated at 37 °C for 24 hours and the viable cells of *S.* Enteritidis were determined in untreated (control) and processed samples (Villa-Rojas et al., 2013).

3.2.4. Dielectric Properties

For dielectric properties measurements, around 100 mL of the samples were kept at water bath until to reach the desired temperature. Dielectric properties of vegan milk samples in different temperature values (22, 8, 50, 60, 70, and 80 °C) were measured with

Network analyzer E5061B (Agilent Technologies ES061B ENA Series, Santa Clara, USA) (Topcam et al., 2018). The frequency range of the analyzer is 100 kHz- 3 GHz.

3.2.5. Quality Analysis

In this study, color, the pH, particle size, and zeta potential were evaluated as quality attributes for conventional and microwave pasteurization processes.

3.2.5.1. Color Analysis

Control and processed samples were filled into the sample cup in the same amount (approximately 20 mL). To assess color quality of samples, L*(lightness), a* (red/green value) and b* (blue/yellow value) values were evaluated with a CR-400 Chroma meter (Konica Minolta, UK). Instrument specifics are; the illuminant C and 2° observer angle. Total color difference is calculated from the following formula (Pathare et al., 2013):

$$\Delta E = \sqrt{((L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2)} \qquad (3.1)$$

3.2.5.2. pH Analysis

The pH values of the control and processed samples were evaluated with a pHmeter (Adwa AD8000 pH meter, Hungary).

3.2.5.3. Zeta Potential

Vegan milk samples were diluted as described by Shi and Guo (2016) with deionized water with a ratio of 1/10 (v/v). The zeta potential values of the samples was measured in Zeta-sizer (Particulate Systems – NanoPlus, LabWrench, Canada).

3.2.5.4. Particle Size Distribution

As described in the previous section, same diluted vegan milk samples were injected into capillary cells. Then, particle size distribution were analyzed with the Zeta-Sizer (Particulate Systems – NanoPlus, LabWrench, Canada).

3.2.6. Statistical Analysis

All pasteurization processes were conducted in duplicate. After these pasteurization processes, all analyses were conducted with three replicates. Results were given as mean and their standard deviations. Result of the experiments were compared by means with one-way ANOVA using Minitab 19 software (Minitab Inc., State College, PA, USA). The difference in mean values were compared with Tukey's comparison test and two sample t-test at 95% of confidence level.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Temperature Profiles of Vegan Milk samples

In 70 °C water bath, temperature values were measured with thermocouples during the conventional pasteurization experiments. Figures 3, 4, 5 represent the temperature profiles in come up times and holding times for almond, soy and oat milk respectively, for three parallels. Come up times for the soy milk and oat milk are the same (480 s), but for almond milk the temperature has reached to 70 °C 15 seconds before this time. Also figures showed the water temperature in the water bath in case of the determining experimental error. All three vegan milk sample had approximately the same temperature profile during the experiment.

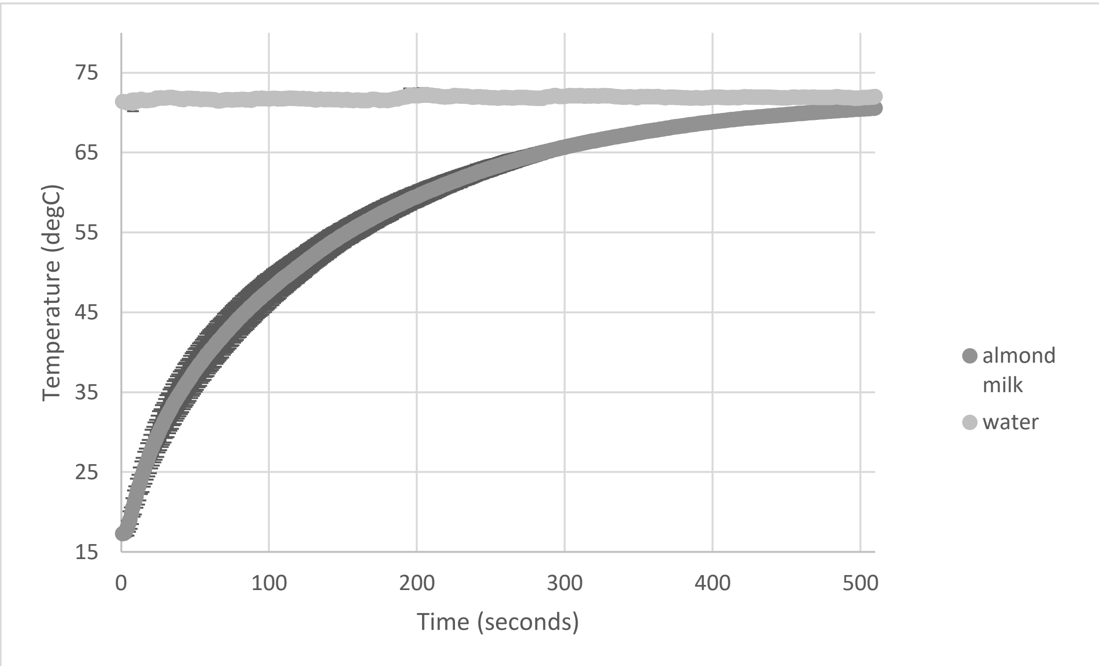


Figure 3. Temperature profile of almond milk during conventional pasteurization

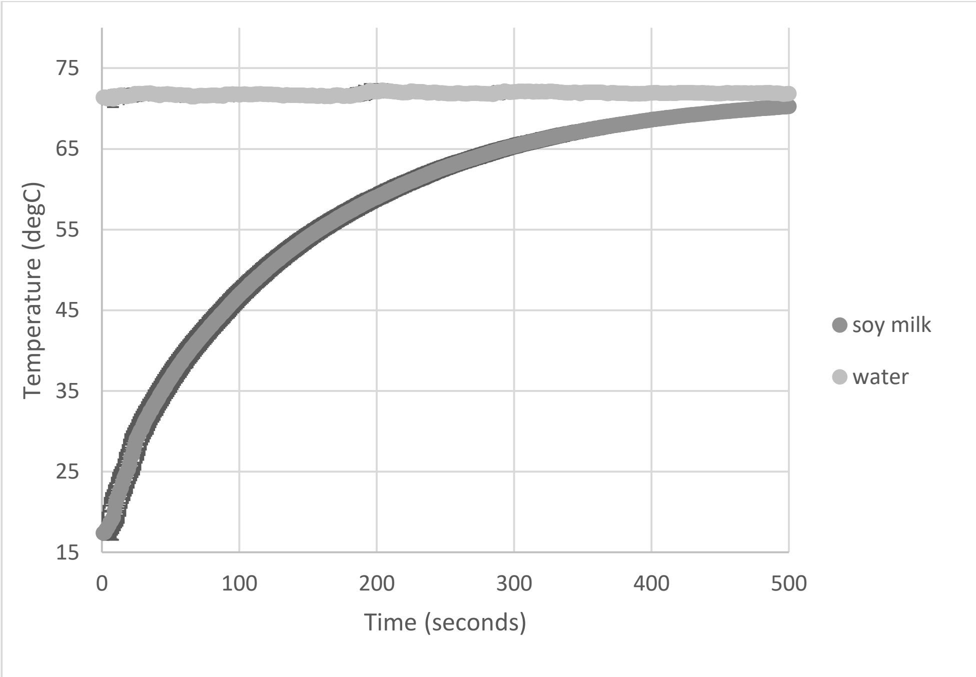


Figure 4. Temperature profile of soy milk during conventional pasteurization

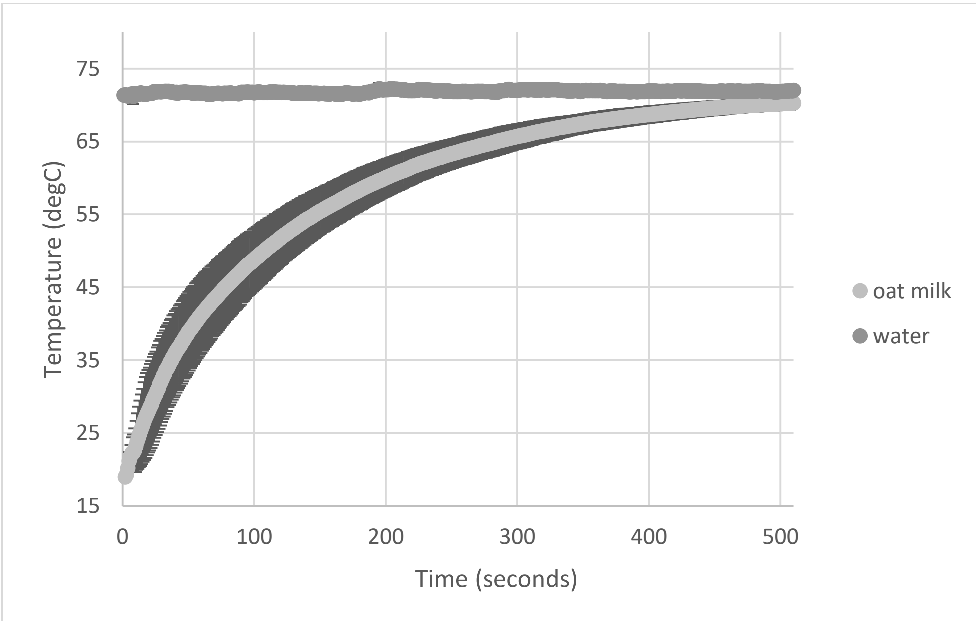
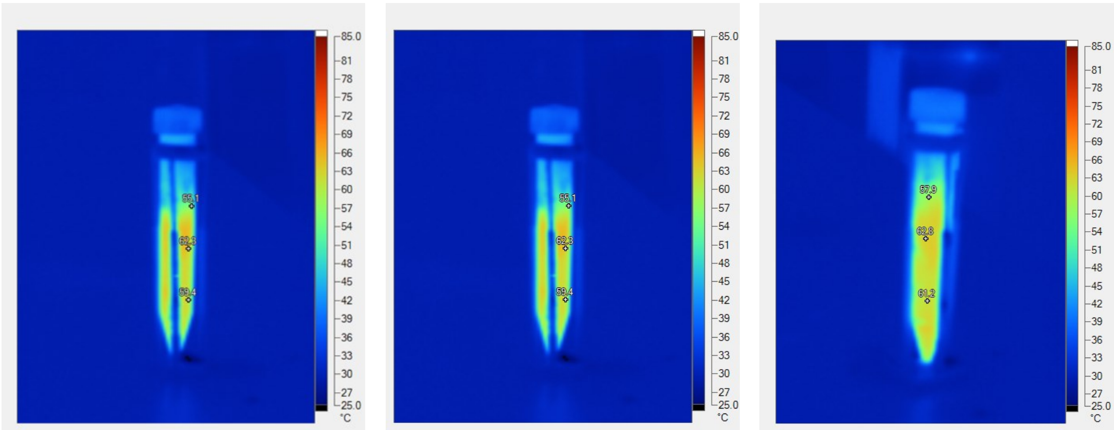


Figure 5. Temperature profile of oat milk during conventional pasteurization

According to infrared camera images (Figure 6), the final temperature profiles through the tubes seem uniform. But there is a problem in these images. When the images were captured, the tubes were removed from the water bath and this could cause an immediate temperature decrease since the temperature of water in water bath and room temperature are not the same.



(a) (b) (c)

Figure 6. Infrared camera images of the vegan milk products, soy milk (a), almond milk (b), oat milk (c) after conventional pasteurization

Figures 7, 8, and 9 indicate the temperature profiles of soy, almond and oat milks respectively in the microwave pasteurization. According to the results, all vegan milk samples had similar trend during pasteurization. Final temperature values of all vegan milk samples were around 70- 75°C. Striking parallel about the graphs, after reaching 45 °C, temperature values were started to increase sharply during the treatment. Also oat milk's final temperature value was higher than the other vegan milk samples.

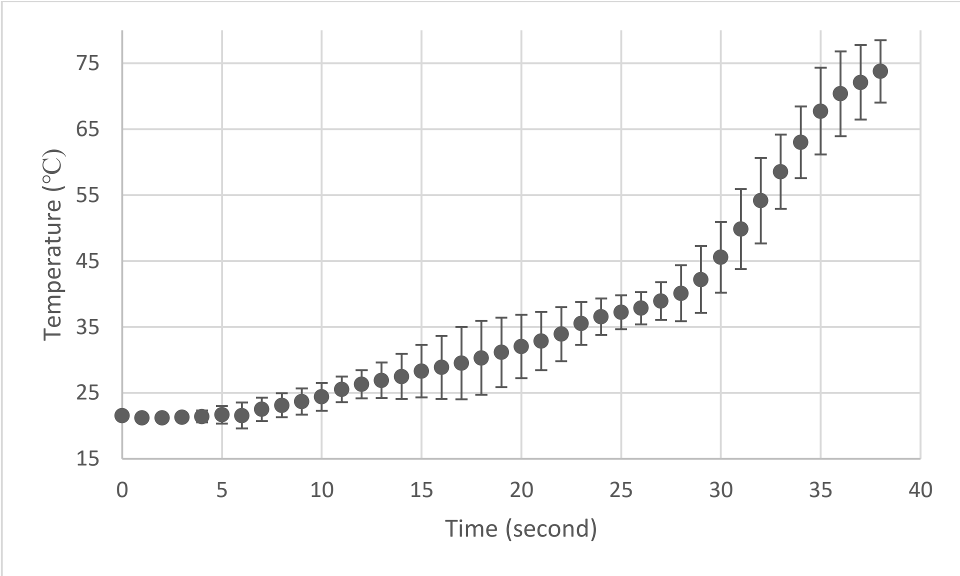


Figure 7. Temperature profile of soy milk during microwave pasteurization

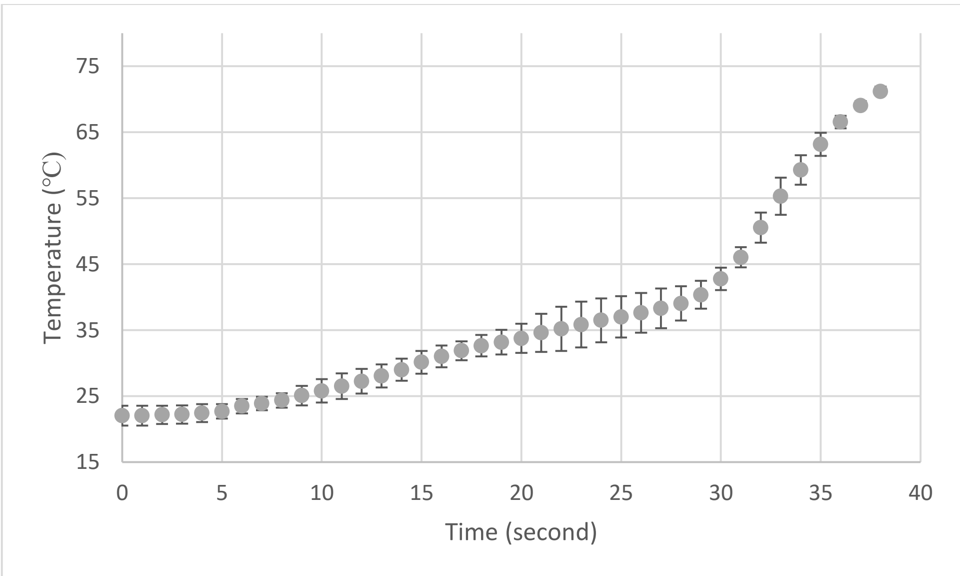


Figure 8. Temperature profile of almond milk during microwave pasteurization

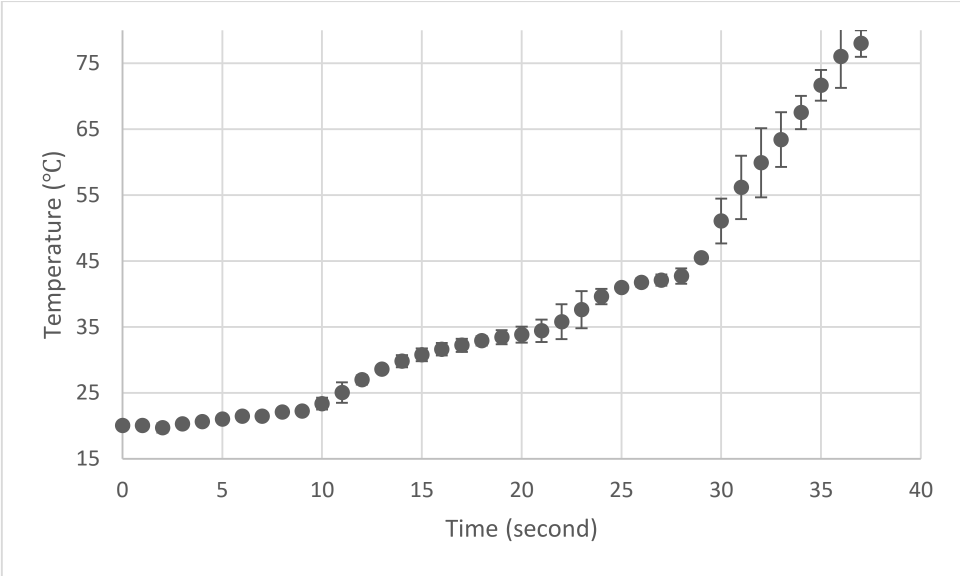
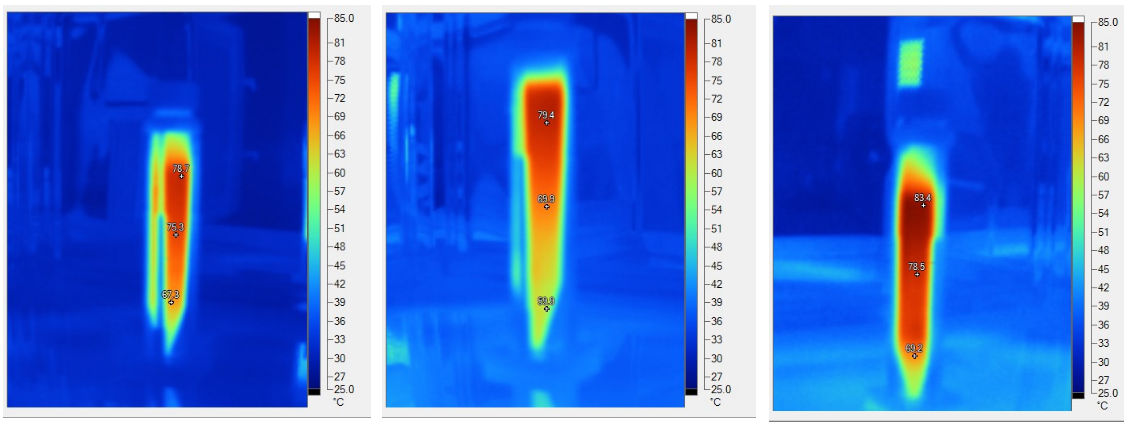


Figure 9. Temperature profile of oat milk during microwave pasteurization

Figure 10 indicates the final temperature profile through the tubes. In microwave pasteurization, the upper side of the product was warmer than the bottom side of the product. To solve this problem, after the treatment, products were shaken manually before the microbiological analysis.



(a) (b) (c)

Figure 10. Infrared camera images of the vegan milk products, soy milk (a), almond milk (b), oat milk (c) after microwave pasteurization

4.2. Dielectric Properties

Dielectric properties explain the ability of the storage of electromagnetic energy and conversion of these energy to heat energy. Tables 1, 2 and 3 gives the dielectric properties of soy, almond and oat milk respectively. In the food processes generally 915 MHz and 2450 MHz are applied for microwave applications. So, the results were given in these two main frequency values. The results generally show that the dielectric properties of vegan milk samples were affected from both frequency and temperature values ($p < 0.05$). In the region of polar dispersion, the dielectric constant increase with the increase in the temperature value. However, dielectric loss factor either increase or decrease in this region (İçier and Baysal, 2004).

In vegan milk samples there is a noticeable decrease at 80 °C in the dielectric constant values at both frequencies. Also there was noticeable decrease in the dielectric constant values of almond and soy milk from 38 °C to 50 °C. This movement was observed between 50 and 60 °C for oat milk. In microwave heating profiles there was significant changes in the slopes of the graphs at around 40 °C for soy and almond milk. For the oat milk, this change was observed at 50 °C. This is most probably related to dielectric properties were changed at this temperature interval. The magnitudes of the results were in agreement with the results reported by Kataria et al., 2018 and Kubo et al., 2021. However, in their studies dielectric constant linearly decrease with the temperature. Dielectric properties are generally effective on the heating profile of the food products. However, in this process, conventional heat transfer is more effective than other factors through the tubes during microwave heating. So, dielectric properties could not explain the heating profile of samples by themselves.

Table 1. Dielectric properties of soy milk

Temperature (°C)	Permittivity	Frequency (MHz) 915	2450
22 °C	ε'	$69.32 \pm 0.42^{a,A}$	$69.15 \pm 0.45^{a,A}$
	ε''	$13.37 \pm 0.25^{a,A}$	$11.98 \pm 0.50^{a,B}$
38 °C	ε'	$69.04 \pm 0.98^{a,A}$	$68.16 \pm 0.97^{a,A}$
	ε''	$8.75 \pm 0.14^{b,A}$	$8.64 \pm 0.07^{b,A}$
50 °C	ε'	$61.43 \pm 2.77^{b,A}$	$64.13 \pm 0.6^{b,A}$
	ε''	$12.99 \pm 1.81^{a,A}$	$8.28 \pm 0.50^{b,B}$
60 °C	ε'	$68.33 \pm 1.85^{a,A}$	$65.57 \pm 0.33^{b,B}$
	ε''	$14.86 \pm 1.75^{a,A}$	$8.18 \pm 0.39^{b,B}$
70 °C	ε'	$67.40 \pm 3.44^{a,A}$	$65.12 \pm 1.10^{b,A}$
	ε''	$13.54 \pm 1.82^{a,A}$	$8.97 \pm 1.07^{b,B}$
80 °C	ε'	$61.02 \pm 0.02^{b,A}$	$60.95 \pm 0.02^{c,B}$
	ε''	$12.62 \pm 0.25^{a,A}$	$11.11 \pm 0.69^{a,B}$

Results were given as “means ± standard error”. The least significant difference was determined by Tukey’s comparison and two sample t- test. Means that have different superscripts indicate significantly different results ($p < 0.05$). Lowercase letters specifies the differences between different temperature values, uppercase letters specifies the differences between different frequency values.

Table 2. Dielectric properties of almond milk

Temperature (°C)	Permittivity	Frequency (MHz)	
		915	**2450**
22 °C	ε'	$68.50 \pm 2.27^{b,A}$	$68.04 \pm 2.01^{a,A}$
	ε''	$11.71 \pm 0.60^{bc,A}$	$11.28 \pm 0.88^{a,A}$
38 °C	ε'	$71.97 \pm 1.33^{a,A}$	$66.35 \pm 0.38^{abc,B}$
	ε''	$14.26 \pm 2.33^{ab,A}$	$9.29 \pm 1.52a^{b,B}$
50 °C	ε'	$65.66 \pm 1.25^{b,A}$	$63.75 \pm 0.77^{c,B}$
	ε''	$13.27 \pm 0.93^{abc,A}$	$10.12 \pm 0.37^{ab,B}$
60 °C	ε'	$67.30 \pm 0.70^{b,A}$	$67.57 \pm 0.05^{ab,A}$
	ε''	$12.47 \pm 0.44^{bc,A}$	$8.32 \pm 0.79^{b,B}$
70 °C	ε'	$66.77 \pm 1.12^{a,A}$	$64.83 \pm 2.02^{bc,A}$
	ε''	$15.84 \pm 0.67^{a,A}$	$10.29 \pm 0.36^{ab,B}$
80 °C	ε'	$60.10 \pm 0.04^{c,A}$	$60.94 \pm 0.03^{d,B}$
	ε''	$10.97 \pm 1.077^{c,A}$	$9.65 \pm 0.92^{ab,A}$

Results were given as "means ± standard error". The least significant difference was determined by Tukey comparison and two sample t- test. Means that have different superscripts indicate significantly different results ($p < 0.05$). Lowercase letters specifies the differences between different temperature values, uppercase letters specifies the differences between different frequency values.

Table 3. Dielectric properties of oat milk

Temperature (°C)	Permittivity	Frequency (MHz)	
		915	**2450**
22 °C	ε'	51.7±3.52^{b,A}	55.02±3.35^{ab,A}
	ε''	13.30±0.78^{ab,A}	6.75±0.51^{d,B}
38 °C	ε'	55.89±1.32^{ab,A}	54.17±0.61^{ab,A}
	ε''	6.71±0.56^{d,A}	8.67±0.19^{ab,B}
50 °C	ε'	60.27±3.94^{a,A}	59.12±3.91^{a,A}
	ε''	8.97±0.69^{c,A}	8.20±0.42^{,c,A}
60 °C	ε'	54.7±2.41^{b,A}	53.71±2.08^{b,A}
	ε''	9.74±0.25^{c,A}	8.97±0.14^{a,B}
70 °C	ε'	54.74±0.62^{b,A}	53.28±0.20^{b,B}
	ε''	12.95±0.75^{b,A}	7.86±0.27^{c,B}
80 °C	ε'	51.23±0.432^{b,A}	51.57±0.38^{b,A}
	ε''	14.27±0.08^{a,A}	8.24±0.16^{b,c,B}

Results were given as "means ± standard error". The least significant difference was determined by Tukey's comparison and two sample t- test. Means that have different superscripts indicate significantly different results ($p < 0.05$). Lowercase letters specifies the differences between different temperature values, uppercase letters specifies the differences between different frequency values

4.3. Microbial Analysis Results

Table 4 indicates the initial average inoculum population of *Salmonella* Enteritidis and the populations after the microwave and conventional pasteurization processes in different vegan milk samples. According to the results, all heat treatments gave satisfied inactivation of *Salmonella* Enteritidis in vegan milk samples. For soy milk, approximately 5.39 log reduction was observed in microwave pasteurization, however, approximately 5.77 log and approximately 6.62 log reductions were observed for the almond and oat milk samples, respectively. In the past years, there were several outbreaks due to *Salmonella* Enteritidis in almonds. According to Almond Board California here should be minimum 5-log reduction of *Salmonella* in the packaged almonds. It was the starting point of this study.

Table 4. *Salmonella* Enteritidis ATTC 13076 inoculum population and changed after different heat treatments

Treatment	Soy milk	Almond milk	Oat milk
	logN (mean CFU/ml ± SD)	logN (mean CFU/ml ± SD)	logN (mean CFU/ml ± SD)
Initial population	6.40±0.43	5.77±0.11	6.62±0.27
After MWP	1.09±0.55	ND	1.26±00
After CP	ND	ND	ND
After CP-H	ND	ND	ND

Abbreviations: CFU: Colony Forming Unit, ND: Not detected; U (Untreated), MWP (Microwave pasteuriziation), CP (Conventional pasteurization in come up time), CP-H (Conventional pasteurization with holding time)

Several studies showed that the microwave heating is promising technology to inactivation of *Salmonella* Enteritidis in shell eggs (Lakins et al., 2008), in salmon and whiting (Alakavuk et al., 2021), raw poultry (Pucciarelli and Benassi, 2005), and potato omelette (Valero et al., 2014).

In the industry, many companies apply the same procedures of cow's milk processing to vegan milk. Actually, there are not many researches investigating the microbiological risks in the vegan milk samples. In this study, the risks coming from the raw material was tried to eliminate. To compare, microwave heating and conventional pasteurization process, final temperatures of microwave heated and conventionally pasteurized samples were approximately the same. All treatments performed in this study caused above 5-log reduction of *Salmonella* Enteritidis in three vegan milk product. So, microwave heating can be applied instead of conventional methods. More gentle conditions can be tried for the future researches. But the critic point is that there could be unheated parts in microwave heating since the non-uniformity of temperature profiles.

4.4. Quality Attributes

4.4.1. Color Measurement Results

Impacts of the heat treatments on the color change are represented in Table 5. Pasteurization had significant changes on L*, a* and b* values ($p < 0.05$). Both microwave and conventional pasteurization caused small changes in the values. However, the color values were close to each other. Minaker et al., (2021) reported the standard ΔE values for the human perception. As stated there, when $\Delta E<1$, it is not perceptible by the human eye and when $1<\Delta E<2$ perceptible through close observation. As a result, color changes were acceptible for the all treatments. Among the vegan milk samples, microwave pasteurized oat milk had higher color difference than conventional ones. It is most probably about the final temperature reached higher degrees than the other vegan milk samples in the microwave pasteurization. The result could be related with the Maillard reaction which is responsible for formation of melanoidins which are brown polymers (Žilić et al., 2014).

Table 5. Color measurement results

		L*	a*	b*	ΔE
Soy milk	U	66.51±0.28[a]	-1.79±0.01[b]	3.58±0.25[bc]	
	MWP	65.52±0.18[c]	-1.64±0.02[a]	3.45±0.17[c]	1.01
	CP	65.84±0.09[b]	-1.64±0.02[a]	3.78±0.06[ab]	0.72
	CP-H	65.80±0.08 [bc]	-1.64±0.02[a]	3.84±0.09[a]	0.77
Almond milk	U	78.94±0.18[a]	-1.32±0.02[c]	3.34±0.04[a]	
	MWP	77.97±0.26[b]	-1.34±0.01[c]	3.01±0.08[b]	1.02
	CP	77.69±0.09[b]	-1.26±0.01[b]	3.04±0.03[b]	1.29
	CP-H	78.02±0.09[b]	-1.22±0.01[a]	3,02±0.03[b]	0.98
Oat milk	U	80.36±0.13[a]	-3.25±0.02[b]	10.64±0.03[a]	
	MWP	78.33±0.23[c]	-3.27±0.04[b]	10.15±0.03[c]	2.09
	CP	79.89±0.12[b]	-3.16±0.01[a]	10.49±0.03[b]	0.50
	CP-H	79.55±0.07[b]	-3.12±0.01[a]	9.58±0.04[d]	1.34

Results were given as "means ± standard error". The least significant difference was determined by Tukey's comparison test. Means that have different superscripts indicate significantly different results ($p < 0.05$).

Abbreviations: L* (lightness), a* (red/green value), b* (yellow/blue value), ΔE (total color difference)

4.4.2. pH Measurement Results

The pH is an important value that influences the particle aggregation since it is effective in the absorption and scattering of electromagnetic energy mechanism (Al-Gebory and Mengüç, 2018). Also it has crucial impact on the electrostatic charge so it affects the dielectric properties (Ahmed et al., 2008). The pH results of the vegan milk samples before and after treatment are given in Table 6. The soy milk results were compatible with the study reported by Bai et al. (1998). The pH values for almond milk are higher than the studies in the literature (Dhakal et al., 2014; Manzoor, 2017). The most probable reason is due to the process or ingredients. There is significant change only in almond milk pH after the heat treatments ($p < 0.05$). Manzoor (2017) stated that there was an important increase after the heat treatment. It is compatible with the results given in the Table 5.

Table 6. The pH measurement results

	Soy milk	Almond milk	Oat milk
U	7.14 ± 0.14^{ab}	7.28 ± 0.09^{b}	6.69 ± 0.07^{a}
MWP	7.21 ± 0.04^{a}	7.40 ± 0.05^{a}	6.68 ± 0.04^{a}
CP	7.04 ± 0.1^{b}	7.46 ± 0.07^{a}	6.73 ± 0.09^{a}
CP-H	7.16 ± 0.09^{ab}	7.54 ± 0.09^{a}	6.72 ± 0.07^{a}

Results were given as "means ± standard error". The least significant difference was determined by Tukey's comparison test. Means that have different superscripts indicate significantly different results ($p < 0.05$).

4.4.3. Zeta Potential Results

Figure 11 represents the zeta potential values of control, microwave, and conventional treated soy milk, almond milk and oat milk, respectively. Zeta potential value is key value related to evaluate particle stability. When zeta potential value increases, the stability increases due to increase of electrostatic repulsion (Gaikwad et al., 2019). Also zeta potential value in the range between ±10 mV and ±30 mV indicates incipient stability (Kumar et al., 2017). Given results showed that the soy milk affected significatly after the treatments ($p < 0.05$). Microwave pasteurized samples had the closest zeta potential value, so they had better stability than pasteurized samples. Extra holding time did not affect the stability. Conversely, almond milk was not affected from the different pasteurization process significantly ($p > 0.05$). Lastly, in oat milk samples, in only holding process zeta potential magnitude was decreased, so its stability was lower than others. pH affects the surface charge of the nanoparticles, so it can be effectual on the zeta potential magnitude (Selvamani, 2019). Comparision of pH and zeta potential values demostrate that there isn't corrrelation between the values. Treatments have different mechanisms, herewith stability could be affected from the other conditions.

Several studies have investigated the zeta potential values of vegan milk samples treated with different process. Dhakal et al. (2016) have stated that low temperature (85°C, 30 min) and high temperature (121 °C, 15 min) did not change the zeta potential values significantly. Even the values were lower than of these result, it could be stated that compatible results were observed. Shi and Guo (2016) highlighted that soy milk's dilution rate changed the zeta potential value significantly. Therefore, the values could not demonstrate the actual values. For this reason, dilution rate was selected as minimum as possible. The values had similar magnitude with the study reported by Shi and Guo (2016).

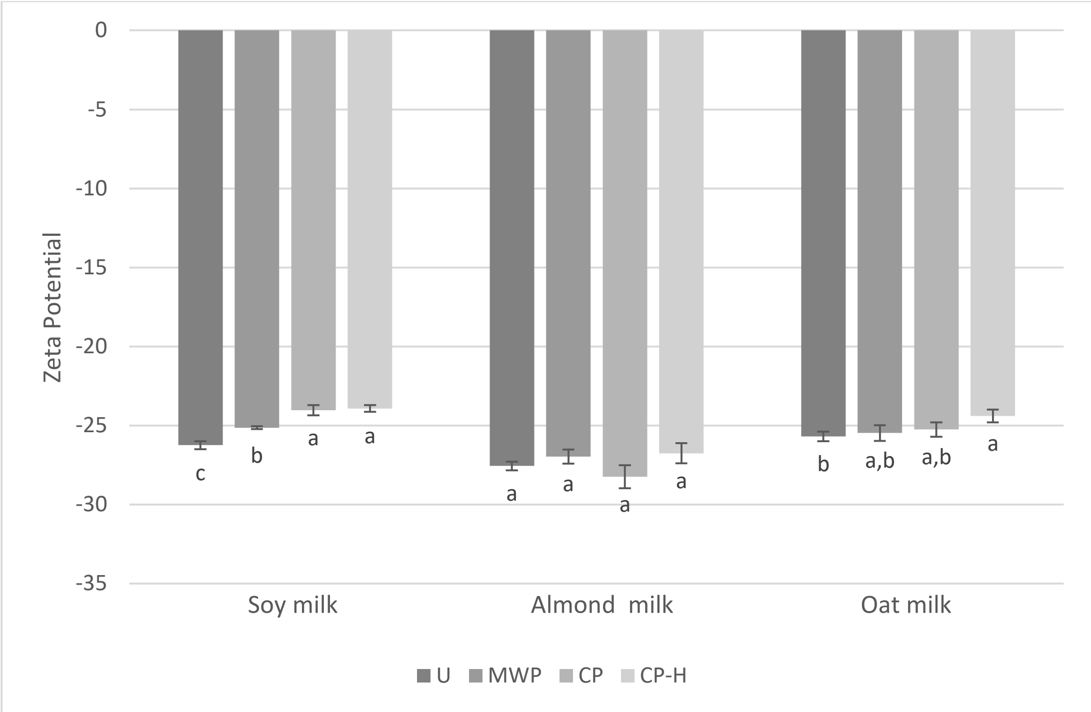


Figure 11. Zeta potential results of vegan milk samples

Results were given as "means ± standard error". The least significant difference was determined by Tukey comparison test. Means that have different superscripts indicate significantly different results ($p < 0.05$).

4.4.4. Particle Size Distribution

Tables 7, 8 and 9 represent the effect of different treatments on the particle size distribution of soy, almond and oat milk, respectively. Firstly, particle diameters were smaller than from the literature since the materials were shelf-stable products and have already been exposed to homogenization. Homogenization reduces the particle size and it brings about the increase in the number of solid or liquid particles of the dispersed phase (Innocente et al., 2009).

Vegan milks contain oil droplets, proteins and starch granules beside solid particles from raw materials. These dispersed particles form to colloidal systems (Briviba et al., 2016). In these products, biggest particles compose of the fat droplest, cellular tissues, while finest particles compose of the proteins (Bernat et al., 2015). Heat treatments are effective on aggregation of protein gloubules due to protein denaturation (Raikos et al., 2009; Briviba et al., 2016; Manzoor et al., 2020). Since these treatments can also lead to flocculation and coalescence, there can be increase on the size of fat globules (Bernat et al., 2015).

According to the results, all particle size distributions showed monomodal model. Both microwave processing and holding process lead to increase in particle diameters in almond milk. Also it is expected that the protein coagulation decreased the particle size but in the contact point, the particles started to aggregate and can interact the other particles such as protein-fat globules. There was noticable increase in the particle size diameters of soy milk subjected to more heating process. Therefore, it is difficult to evaluate what kind of disperse particles were analyzed. In holding process most probably proteins were affected from heating process. As same as the zeta potential value, there was a significant change in the particle size distribution of soy milk in holding process. In oat milk, particle sizes was decreased after the treatments. Most probable decrease was observed due to the protein denaturation especially in the finest particle distribution. Further studies are needed to understand the microstructural behavior of the vegan milk samples during different process.

Table 7. Effect of different treatments on particle size distribution of soy milk

	D_{10} (µm)	**D_{50} (µm)**	**D_{90} (µm)**	**$D_{3,2}$ (µm)**	**$D_{4,3}$ (µm)**	**span**
U	0.104± 0.001^{b}	0.126± 0.002^{b}	0.176± 0.002^{b}	0.138± 0.004^{b}	0.201± 0.005^{b}	0.373± 0.009^{a}
MWP	0.103± 0.005^{b}	0.124± 0.006^{b}	0.173± 0.008^{b}	0.138± 0.006^{b}	0.199± 0.009^{b}	0.357± 0.015^{a}
CP	0.129± 0.025^{a,b}	0.156± 0.030^{ab}	0.222± 0.044^{ab}	0.175± 0.034^{ab}	0.252± 0.041^{ab}	0.344± 0.029^{ab}
CP-H	0.147± 0.042^{a}	0.177± 0.052^{a}	0.256± 0.076^{a}	0.199± 0.058^{a}	0.284± 0.069^{a}	0.283± 0.072^{b}

Results were given as “means ± standard error”. The least significant difference was determined by Tukey’s comparison test. Means that have different superscripts indicate significantly different results ($p < 0.05$).

Table 8. Effect of different treatments on particle size distribution of almond milk

	D_{10} (µm)	**D_{50} (µm)**	**D_{90} (µm)**	**$D_{3,2}$ (µm)**	**$D_{4,3}$ (µm)**	**span**
U	0.27± 0.02^{b}	0.33± 0.02^{b}	0.47± 0.04^{b}	0.37± 0.03^{b}	0.55± 0.04^{a}	0.42± 0.02^{a}
MWP	0.38± 0.05^{a}	0.47± 0.07^{a}	0.66± 0.09^{a}	0.52± 0.07^{a}	0.67± 0.05^{a}	0.18± 0.72^{b}
CP	0.26± 0.03^{b}	0.31± 0.04^{b}	0.45± 0.06^{b}	0.35± 0.04^{b}	0.52± 0.05^{a}	0.41± 0.02^{a}
CP-H	0.35± 0.11^{ab}	0.43± 0.14^{ab}	0.61± 0.20^{ab}	0.48± 0.15^{ab}	0.66± 0.16^{a}	0.31± 0.12^{a}

Results were given as "means ± standard error". The least significant difference was determined by Tukey's comparison test. Means that have different superscripts indicate significantly different results ($p < 0.05$).

Table 9. Effect of different treatments on particle size distribution of oat milk

	D_{10} (µm)	**D_{50} (µm)**	**D_{90} (µm)**	**$D_{3,2}$ (µm)**	**$D_{4,3}$ (µm)**	**span**
U	0.162 ±0.037^{a}	0.196± 0.046^{a}	0.287± 0.069^{a}	0.222± 0.051^{a}	0.319± 0.068^{a}	0.292± 0.049^{b}
MWP	0.112 ±0.003^{b}	0.135± 0.003^{b}	0.192± 0.003^{b}	0.152± 0.003^{b}	0.223± 0.007^{b}	0.346± 0.015^{a}
CP	0.103 ±0.005^{b}	0.125± 0.005^{b}	0.176± 0.008^{b}	0.140± 0.006^{b}	0.202± 0.008^{b}	0.374± 0.014^{a}
CP-H	0.105 ±0.016^{b}	0.128± 0.002^{b}	0.167± 0.007^{b}	0.112± 0.012^{b}	0.190± 0.009^{b}	0.356± 0.013^{a}

Results were given as "means ± standard error". The least significant difference was determined by Tukey's comparison test. Means that have different superscripts indicate significantly different results ($p < 0.05$).

CHAPTER 5

CONCLUSION

In this study, microwave heating was applied to three different vegan milk samples (soy, almond, and oat milks) to evaluate their microbiological and physicochemical quality attributes. For this approach, microwave pasteurization results were compared to conventional pasteurization results. Samonella inhibition, pH, color, zeta potential, and particle size distributions were evaluated as quality attributes. Also, dielectric properties at two main microwave frequencies of industrial interest (915 MHz and 2450 MHz) were investigated.

Since conventional methods consume time and energy, microwave heating has started to be preferred as a novel technique for pasteurization of food products. In this study, an inverter microwave oven was used to pasteurize vegan milk samples. Process conditions were determined as 38 s and 360 W. To compare, a water bath system (at 70°C) was used. Come-up times were calculated and also 30 s holding was applied. *Salmonella* Enteritidis was chosen as the target bacterium in the treatments. All treatments were provided 5 log reduction of *Salmonella* Enteritidis. According to the results, holding time is not required at 70 °C for the inhibition of target bacteria.

Dielectric properties explained the heating profile of the vegan milk samples during microwave heating. These properties were affected both by the temperature and frequency values. Color differences of vegan milk samples were not perceptible or perceptible through close observation. The pH values generally were not affected by the heat treatments for soy and oat milk. Only there was a significant increase in the almond milk pH value with the heat treatments. Zeta potential values were generally close to -30 mV (the limit for the stability) which means the threshold of delicate dispersion. Only soy milk was significantly affected by the different processes. But microwave pasteurized samples gave the closest value to untreated samples. Also holding influenced the stability of oat milk. Particle size diameters were changed after the different processes. Vegan milk is a colloidal system, so with further investigations, these mechanisms could be explained in details.

As a result, microwave heating could take the place of conventional methods. The study can be developed with the use of raw vegan milk samples. Also during the treatments, understanding its effects on the microstructures of the vegan milk samples could be investigated with further studies. For industrial usage, microwave systems for the large volume of vegan milk products could be designed with the lights of the results of this study.

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