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Non-Thermal Processes Used in Milk Treatment

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Abstract: Renewed technology created a demand for natural foods or foods with more natural properties. Therefore, the processes used in food production necessiated taking measures to establish innovations over time. It takes a long time and a lot of practice to change the processing steps of the products in the food sector. Two of the most important technology used in dairy technology involve pasteurization and sterilization processes to ensure the inactivation of microorganisms. Although the currently used processes are able to inactivate microorganisms at high temperatures, they change the structural properties of milk components and decrease its nutritional value. This has led to the search for new methods. To protect milk and not to damage its nutritional value, it is also possible to apply non-thermal technological processes, which provide the desired microorganism inactivation. Non-thermal technological processes are microfiltration (MF), impact electric field, high pressure application, ultrasound, ultraviolet and X rays. Microfiltration is applied by considering the membrane system used and are classified according to pore size. Separation of bacteria and spores in milk are carried out by filtration through membranes. In the pulsed electric field and high pressure applications, inactivation is achieved by damaging the cell membranes of microorganisms.. In this review, these processes are explained and their advantages and disadvantages are discussed.

Keywors: microfiltration, ultrasound, impact electric field, ultraviolet, X rays

Süte Uygulanan Termal Olmayan İşlemler

Öz: *Yenilenen teknoloji beraberinde doğal ya da doğala daha yakın gıdaların talep edilmesini gündeme getirmiştir. Bu nedenle gıdalara uygulanan işlemler de zamanla yeniliklere kapılarını açmış ve açmaya da devam etmektedir. Gıda sektöründeki ürünlerin işlem basamaklarında değişiklere gidilmesi oldukça uzun zaman ve çokça uygulama gerektirmektedir. Süt teknolojisinde kullanılan en önemli iki teknoloji mikroorganizmaların inaktivasyonunu sağlamak için pastörizasyon ve sterilizasyon işlemlerini içerir. Yapılan çalışmalar yüksek sıcaklıklarda mikroorganizma inaktivasyonu sağlarken sütte var olan bileşenlerin yapısal özelliklerin değişmesine ve besin değerlerinin azalmasına sebep olmaktadır. Bu nedenledir ki yeni yöntemler denenmeye gidilmiştir. Sütü korumak besin değerlerine zarar vermemek amacıyla sütte yine istenilen mikroorganizma inaktivasyonu sağlamak amacıyla ısıl olmayan teknolojik işlemlerin uygulanabilmesi söz konusudur. Isıl olmayan teknolojik işlemler; mikrofiltrasyon (MF), darbeli elektrik alan, yüksek basınç uygulaması, ultrason, ultraviole ve X ışınlarıdır. Mikrofiltrasyon, memran sistemine göre uygulanmakta olup gözenek büyüklüklerine göre sınıflandırılmaktadır. Membranlardan geçirilen sütte bakterilerin ve sporlarının ayrılması söz konudur. Darbeli elektrik alan ve Yüksek basınç uygulamasında mikroorganizmaların hücre zarlarına zarar verilerek inaktivasyon sağlanmaktadır. Bu derlemede uygulanan bu işlemler anlatılmakta etkinlikleri avantajları ve dezavantajlarından bahsedilmektedir.*

Anahtar Kelimeler: *microfiltration, darbeli elektrik alan, ultrason, ultraviolet, X rays*

INTRODUCTION

Raw milk is a mammary gland secretion, except for the colostrum (mother's milk), which is not heated above 40 °C, obtained from one or more cows, goats, sheep or buffalo (Anonim, 2000). In dairy technology, raw milk is regarded as milk that is cooled after milking from the animal's mammary at regular intervals. No components are removed from its composition and no other ingredients are added. It is not previously subjected to other processes and it is accepted to dairy plants for processing (Metin, 2001). Milk and dairy products can lead to the emergence of various diseases as an ideal environment for the development of microorganisms. Thermal or non-thermal processes are applied to inhibit microbial activity and inhibit enzyme activation in drinking milk or the product to be produced from milk.

HEAT TREATMENTS AND THEIR EFFECTS

The methods that can be used in the production of drinking milk as determined by The Ministry of Agriculture, Food and Rural Affairs, and Turkish Standards Institute (TSE) are; Pasteurization (processes applied to milk below 100 °C), and sterilization; Ultra High Temperature (UHT) (processes applied over 100 °C) (Anonim, 2000).

Pasteurization

Pasteurized milk, according to TS 1018, is obtained by heating and cooling the raw milk in special facilities and devices and destroying the vegetative forms of pathogenic microorganisms completely and the vast majority of other microorganisms without causing any changes in the natural and biological properties (Söylemez, 2005). In other words, pasteurized milk is the heat treatment of raw milk under 100 °C in a certain duration that will provide the total inhibition of all pathogens and 95-99% inhibition of the total bacterial content. The target microorganism in pasteurization is *Coxiella burnetii* (Anonim, 2004). Low-

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Temperature-Long-Time (LTLT) pasteurization norm is conducted at 62–65 °C for 30 minutes and High-Temperature-Short-Time (HTST) norm is conducted at 71–74 °C for 40-45 s (Anonim, 1994). Pasteurized milks should show negative reaction to alkaline phosphatase test and positive reaction to peroxidase test (Anonim, 2000).

Sterilization and UHT Application

Milks subjected to proper pasteurization process contain heat-resistant microorganisms. Therefore, it should be stored at low temperatures. The shelf life of pasteurized milk is limited to a few days. Long-life milk (UHT milk) is produced by sterilization, which is another process based on higher temperature principle for producing suaitable milk for storage at ambient temperature. Sterilization is defined as the heat treatment applied to milk above 100 °C. In sterilization process, the aim is to destroy *Bacillus stearothermophilus*, which is the most resistant microorganism to heat. This milk is also called "Long Life Milk" (Anonim, 2000). Milk obtained by the UHT method, which is a kind of sterilization, is obtained by applying indirect steam or spraying the milk onto the steam at 135-150 °C for 2-6 s using plate or tubular systems in special mechanisms. It is defined as homogenized milk, free of all kinds of pathogenic microorganisms in normal taste and consistency, which can be kept under room temperature for approximately four months while maintaining all of its nutritional value as long as the package is not opened or damaged (Anonim, 2000)

Milk and Heat Treatment

Heat treatment is applied to prevent the generation of microorganisms and to inactivate the enzymes that affect the quality of milk. Therefore, for a healthy milk consumption, the production must be carried out under hygienic conditions, and time and temperature during processing, packaging and storage must be well controlled (Fox et al., 2003; Walstra et al., 1999). The physical and chemical changes occurring during the heat treatment process vary depending on the temperature and duration of the process. The most suitable time and temperature parameters are selected by considering changes under factory conditions (Anonim, 2000). In terms of the nutritional value, heat application at 60-90 ° C causes the reversible denaturation of proteins and an easier digestion. In a study investigating the effect of temperature and duration on the nutritional elements of milk, it was determined in samples taken from UHT milk, duration had no effect on the whey protein content and the proteolytic activity was higher in the milk stored in the room temperature (Ordolff, 2001). Heat treatment plays an important role in obtaining the unique structure of fermented dairy products (Lucey et al., 1998). In ayran production, heat treatment at 87 °C for 30 min yielded the highest viscosity value and it was viewed as the most appreciated product in terms of sensory properties. The ayran samples produced from milk that was subjected to the UHT method (145 °C for 5 s) had the lowest viscosity values and they were the least appreciated product in terms of sensory properties (Salji et al.,1984) The milks used in ayran production were heat treated for 5 min. at 75 °C, 85 °C or 95 °C and then cooled to 45 °C. They were inoculated with 0.04% starter cultures and incubated at 43 °C. It has been reported that, in ayran samples, acetaldehyde levels increased with increasing heat treatment temperatures. For viscosity and serum separation, the best result was determined in the sample subjected to high temperature (5 min. at 95 °C) (Özünlü and Koçak, 2010). It was determined that pasteurization, brine salting and consequently vacuum packaging had positive effects on the preservation of physical, chemical and sensory properties in Çeçil cheese produced from pasteurized milk by using a starter culture, compared to Çeçil cheese produced from raw milk by using no starter cultures (Yardımcıer and Güven, 2011).

NON-THERMAL TECHNOLOGICAL PROCESSES APPLIED TO MILK

Processes applied in good productin have changes with the developing technology. Alternative methods to heat treatment have been used for controlling microorganisms, separating desired molecules from liquids and preserving the sensory and chemical properties of food.

Microfiltration

Membranes used in milk and dairy products are structures that separate two different phases from each other. According to the pore diameters, they are grouped as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) MF is used to reduce bacterial content, while UF is used to separate protein molecules from fluid, and RO is used to concentrate the solutions by removing water and to demineralize the fluids (Brans et al., 2004). MF is based on the principles involvingretaining molecules of size 0.1 to 20 µm by membranes (Rosenberg,1995). MF is used to separate biologically-derived materials such as colloidal particles, casein micelles, serum protein aggregates and milk fat globules, somatic cells, microorganisms (Yetişmeyen and Yıldız, 2006). MF is a purification process usually used in the concentration of suspensions. Along with the changing and developing technology, MF has a great advantage in the membrane process in terms of reducing the bacterial content in milk at lower temperatures without damaging its properties (El-Shibiny et al., 1994).

It was determined that MF application on a suspension containing microorganisms (approximately 10^8 cfu/mL) using a membrane with a pore diameter of 0.2 µm retained the microorganisms in the suspension (Madec et al., 1992). In a study on retaining the bacteria in skimmed milk, it was determined that the total bacteria in the milk were retained at 99.10-99.90%, *Bacillus cereus* (*B. cereus*) spores were retained at > 99.95% and the spores of bacteria that can ferment lactate were retained at >98.40% (Pedersen, 1992). Compared to bactofugation, MF was generally found to be better at separating the bacteria and their spores (Giffel and Horst, 2004; Brans et al., 2004). It has been reported that MF, applied to increase the content of casein, increases thequality of functional and sensory properties of serum proteins (Saboya and Maubois, 2000).

Pulsed Electric Field

There is also an increasing interest in non-thermal food preservation and processing techniques, with consumers increasingly demanding less-processed products that have not been damaged in terms of nutritional value (Knorr et al., 2002). Pulsed electric field application, as one of the non-thermal food preservation technologies, is used for pasteurization and sterilization of food. It has been observed that, since the PEF process do not significantly increase the temperature of the product, along with microorganism inactivation, food properties such as color, taste, odor, protein, and vitamin C contents have been well preserved in food (Yeom et al., 2000). Bacteria are thought to be inactivated by the disintegration of cell wall and cell membrane by high voltage impulses. It has been shown that PEF applications are effective on microorganisms such as, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* (*E. coli*, *S. aureus*, *B. subtilis*), *B. cereus*, *L. monocytogenes* and *S. cerevisia* (Cserhalmi et al., 2002). Initial studies on this subject have proved the inhibitive effect on microorganisms by applying a high 3000-4000V voltage (Bendicho et al., 2002). In UHT skimmed milk, 2 log reduction was achieved with 90 µs application on *B. cereus* vegetative cells at 35 kV/cm electric field. In UHT milk, *B. stearothermophilus* had a 3 log reduction at 50 ° C by 210 µs treatment at 60 kV/cm. It has been reported that PEF-applied milks can be stored under refrigerator conditions for more than two weeks without altering their physical, chemical, and sensory properties (Qin et al., 1995). The researchers have demonstrated that applying PEF with a light heat treatment was a more effective protection method (Sepulveda et al., 2003). In another study, it was detected that as a result of PEF application using 18.8 kV/cm electric field force and 70 pulses on skimmed milk, 2% fat milk and whole fat milk, alkaline phosphatase values were reduced by 65% in skimmed milk and by 59% in 2%-fat and whole fat milks (Castro et al., 2001). Cheddar cheese was produced from heat-treated and PEF-applied (35 kV / cm electric field strength and 30 pulse counts) milk and untreated milk. The researchers have stated that cheese made from pasteurized milk were harder than cheese made from unprocessed milk. However, it has been reported that that the PEF process kept the protein structures and fat globules within the processed milk unchanged, although it did not prove to be a better option than the HTST process (Sepulveda et al., 2000). In a study, PEF was applied to milks at 20 kV/cm or 30 kV/cm electric area force at 18-50 °C and the rennet coagulation time of the milk samples were compared with that of pasteurized milk. The researchers detected that rennet coagulation time of the milk samples subjected to 20 kV/cm electric area force were shorter than that of the pasteurized milk at all temperatures, whereas the rennet coagulation time of the milk samples subjected to 30 kV/cm electric area force were shorter than that of the pasteurized milk at temperatures below 45 °C (Yu et al., 2009).

High Pressure Application

High pressure has the ability to inactivate pathogenic and saprophytic microorganisms. In this method, pressure is used instead of temperature (Özcan and Kurtuldu, 2011). The high pressure method, in particular, applies a pressure between 100 and 600 MPa. Pressure can be applied to packaged and non-packaged foods (Hajos et al., 2004). The application of 100-600 MPa of HP leads and increase in the the ionic calcium concentration in milk (Zobrist et al., 2005). It was stated that 300 MPa pressure application increases the content of phosphate, magnesium and calcium in milk serum (Lopez-Fandino, 2006). It has been reported that 100–200 MPa pressure application at room temperature had a very low effect on the changes in casein micelle sizes, whereas the casein micelle sizes were reduced almost by half under a pressure higher than 300 Mpa (Anema et al., 2005; Merel-Rausch, 2006). The dissolution of the casein fractions by HP is in β-casein κ-casein αs_1-casein αs_2-casein direction, based on the abundance of fractions. Pressure application at 250-300 MPa caused a maximal increase in the levels of dissolved αs1 and β-casein (Huppertz et al., 2004). It has been reported that the denaturation of pressure-applied milk protein increases with the increasing pressure duration (Considine et al., 2007). It has been reported that, 200–500 MPa pressure application after the 1st and the 15th day of semi-hard ewe's milk cheese increased the pH value and water holding capacity of the cheese and the sensory properties of the cheese became softer, less elastic and less brittle, which was more appreciated, as a result of high pressure application on the 15th day (Juan et al., 2007). It has been reported that 483 or 676 MPa pressure application at 10 °C on the milk used in Cheddar cheese production increased the protein and moisture contents of the cheese, resulting an increase in the yield. However, it has also been found that 483 MP pressure application at 40 °C does not allow proper gel formation in cheese production (San Martin-Gonzalez et al., 2007). When the yogurt produced from HP-applied milk is compared to non-pressurized milk, it was determined that HP-applied milk coagulated at a higher pH, exhibited lower syneresis, and that the coalescence, gelation and water holding capacity of whey proteins increased (Huppertz et al., 2006).

Ultrasound

Ultrasound is a form of energy with mechanical proerties at frequencies above 20 kHz, which can be heard by people. The application of this technique with other techniques increases the efficacy (Aydoğan and Kılıçkan 2012). These applications are classified as thermosonication (sound wave combined with heat treatment), monosonication (sound wave combined with high pressure) and monothermosonication (sound wave process combined with both heat treatment and high pressure) (Yüksel, 2013). The sonic wave forms longitudinal waves when it encounters a liquid medium and the changes in the pressure in the relaxation zones leads to cavitation, which cause gas bubbles in the liquid. While these bubbles cause the surface area to expand, the condensing molecules collide with each other, forming shock waves. These pressure changes constitute the bactericidal effect of ultrasonic sound (Piyasena et al., 2003). An ultrasound application was conducted on the *B. subtilis* spores in milk at a temperature range of 70-95 °C and ultrasound reduced the spore population by 63-73% when applied with heat treatment. In another study, ultrasound method was applied to *S. aureus* in UHT milk with the previous norms (Liu et al., 2014). Another, ultrasound application showed that reconstitution of dry milk with use of ultrasound improves further accumulation of biologically active compounds and rises the nutritional quality of the fermented product (Potoroco et al., 2018).

Ultraviolet

Ultraviolet (UV) radiation is an electromagnetic radiation, which has a shorter wavelength than that of the visible light and a longer wavelength (about 10-400 nm) than that of X-rays. UV radiation can inactivate any kind of microorganism as a result of its short wavelength and high energy. In a study, microbial inactivation of UV application was found to be not as effective as pasteurization in the case of independent pasteurization and ultraviolet application in raw milk. It was observed that ultraviolet application was more suitable for applications including the pasteurization of brine in cheese production plants, pasteurization of water that will be directly added to the product for various purposes (e.g. ayran production) and in the pasteurization of some liquid/semi-liquid dairy products (Tsiotsiaset al., 2002; Liu et al., 2014).

Irradiation

Gamma rays, X-rays and accelerated electron beams are the sources of ionizing radiation used for preservation of foods (WHO, 1991). Irradiation employed to dairy products demonstrated a great success on destroying pathogens or all microorganisms. In a study, irradiation dose of 45 kGy was required to kill 10^{12} C. botulinum spores in the environment. However, work having been reported that in some studies the efficiency of radiation on the reduction of pathogenic and toxin-producing microorganisms (considered in studies on irradiation of cheeses) are *L. monocytogenes*, pathogenic *E. coli*, *Salmonella*, *Clostridium*, *Staphylococcus* (mycotoxins), *Brucella* and *mycobacterium* for the treatment of Camembert cheeses with gamma irradiation at doses up to 2.5 kGy (from a health point of view) have found acceptable (Anonim 2003). The freshly produced soft whey cheese has been used effectively reducing doses of 2-4 kGy for inoculated *L. monocytogenes* (Tsiotsias et al., 2002). Also, the irradiation of fried-frozen cheese balls demonstrated that irradiation at dose of 3 kGy was a successful treatment to make sure microbiological safety (Ju-Woon et al., 2005). Abdel Baky et al., (1986) recoded a reduction in the total bacterial count and spore formers of gamma irradiated cheese milk by 98.98% and 95.77%, respectively. Dairy products were exposed to irradiation at a dose of 40 kGy at -78 °C in nitrogen-modified atmosphere to introduce microbiologically safe diet foods for the severely impaired immune system patients. No significant loss of riboflavin was noted in cheddar and mozzarella cheeses, yoghurt bars, ice cream, and nonfat dry milk. Mozzarella cheese and cheddar cheese, ice cream, thiamine levels were unaffected by irradiation (Dong et al., 1989). Adeil Pietranera et al., (2003) reported that 4 kGy-irradiated chocolate ice creams presented a very unpleasant taste, mostly rancid or burnt like. Kim et al., (2008a) also indicated that gamma irradiation caused unfavorable changes in flavor and taste of vanilla ice cream at doses above 3 kGy. Furthermore, Kim et al. (2007) demonstrated that the addition of mint or citrus flavor in the manufacturing ice cream with irradiation less than 3 kGy may enhance the safety and maintain proper sensory quality for sensitive consumer. However, low dose irradiation did not affect the proximate compositions of various cheese products (Konteles et al., 2009) and color, pH, lactose and lactic acid content of plain yogurt (Kim et al., 2008b). Hashisaka et al., (1990) discussed that exposing dairy products to 40 kGy of gamma irradiation at -78°C resulted in little change in color or texture, but generally decreased the overall acceptability and characteristic flavors due to increased levels of off-flavors and after taste. Therefore, it is suggested that the irradiation of food should be carried out in combination with other food preservation methods such as heat treatments, freezing or modified atmospheric packaging.

CONCLUSION

As a result, the increasing awareness infood should be supported with the developing technology. Milk and dairy products are of great importance in industrial production in Turkey. Therefore, the preservation of milk needs to be carefully considered in terms of health and economy. Researches have supported the advantages of technological processes applied to milk against heat treatment. As they do not cause changes in milk, they are considered as an alternative method that can be applied to these processes. It is possible to use them in combination with other methods when they are not effective while used alone.

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