

Non-Thermal Milk and Milk Products Processing

Hesham A. Ismail

Dairy Science Department, Faculty of Agriculture New Valley University, Egypt.

Correspondence: Hesham A. Ismail

(E-mail: h-alshnety@agr.nvu.edu.eg)

Received: 16/09/2021

Revised: 28/09/2021

Accepted: 29/09/2021

Published: 30/09/2021

Abstract

Manufacture of virtually all milk and dairy products involves heat treatment, with the goal of killing bacteria and inactivating enzymes, among other things. Thermal reactions, on the other hand, might result in undesired changes such as protein denaturation, non-enzymatic browning, the creation of a cooked flavour, nutritional quality loss (vitamins and volatile chemicals), bacterial inhibitor inactivation, and rennet ability deterioration. Over and above the thermal processes are characterized as the most energy-consuming technologies in the food industry. As a result, a number of contemporary developments and non-thermal technologies have been investigated and found to have no negative impact on milk quality; for example, High hydrostatic pressure (HHP), Ionization Radiation, Ultrasounds (US), Pulsed electric fields (PEF), carbon dioxide, High Voltage Arc Discharge (HVAD), Cold Plasma (CP), Bio-control cultures, Non-conventional chemical reagents, Ozonation, and Protective and preserving packaging technologies for processing milk have devised to replace heat treatment. The focus of this review is on certain non-thermal processing innovation as an emerging technology and its application on dairy products.

Keywords: Non-thermal processing, milk, milk products.

Introduction

Milk is the only diet that is specifically "intended" for mammalian newborns, including humans. Milk is a favorable growth medium for a variety of spoilage and pathogenic bacteria due to its high water activity (0.995) and water content (88 percent), almost neutral pH, and abundant supply of essential nutrients (Claeys et al., 2013). Because of these characteristics, raw milk is a highly perishable food that is quickly contaminated by microbes. Heat treatment is a traditional way of extending the shelf life of milk. The heat treatment is used to kill pathogens and inactivate spoilage bacteria and enzymes that could shorten the shelf life of the product. Heat processing, on the other hand, has some negative effects on milk. For example, depending on the strength and duration of the heating process, the colour of milk may change. Furthermore, there is a loss of nutritional value, an increase in heat-generated fragrance compounds such as lactulose, furosine, or hydroxyl methyl furfural, and a loss in sensory quality, as well as some protein deterioration (Calligaris et al. 2004). Thermal processing also has some limitations, consumers have objected to the "cooked flavour" of milk treated at high temperatures, as well as resulting in denaturation of protein and loss of vitamins and destruction of natural antimicrobial milk components and immunoglobulins (Pereda et al., 2009). The need for high-quality, convenient products with natural flavour and taste that are also safe, as well as natural products those are free of additives, preservatives, and humectants. These demands, combined with the detrimental effects of traditional food processing technologies, have prompted food scientists and technologists to seek out alternatives to thermal processing. The new technological approaches for food preservation make it possible to reduce the negative effects of heat on food quality, give foods fresh-like, and less affected on color, flavor, and

nutrients, energy savings, environment-friendly, reducing processing costs and improving the added-value of the products (Jan et al., 2017).

The objective of this review was to showcase certain non-thermal technology applications for milk and dairy products preservation.

1-High-pressure

HPP (high-pressure processing), also known as HHP (high hydrostatic pressure) or UHP (ultra-high pressure), is a type of high-pressure processing (UHP), entails applied it high pressures with or without external heating to accomplish microbial inactivation or change food characteristics (Anon 2008). Several HPP-treated food products are available around the world, including juices, jams, jellies, yoghurts, dips, guacamole, ready-to-eat meat, and oysters. It's the most widely used alternative technology since it may be utilized at room temperature, eliminating thermally caused cooked off-flavors (Fig.1). Currently, more than 200 enterprises use cold HPP to produce more than 600,000 tons of products each year (Wilches et al., 2015). Finally, this method can be employed in batch and semi-continuous equipment to process liquid and solid foods (both packed and unpacked). Pressure is applied to food samples very quickly and uniformly during HHP processing; hence pressure application is not reliant on the size and shape of the product, allowing for homogeneous processing of irregularly shaped food products (Balasubramaniam et al. 2008). At room temperature, high-pressure processing kills harmful organisms. Microbial inactivation is usually accomplished at pressures in the limit from 100 to 800 MPa for brief periods of time (from a few seconds to several minutes). Pressures low as 100 MPa can cause partial denaturalization of cellular proteins, when the pressure is increased to 200 MPa, the microbiological structure is damaged internally, and the cellular membrane is damaged externally. When,

pressures greater than 300 MPa cause irreparable harm to the microorganism, eventual cellular death (Yang et al., 2012). All enzymes are inactivated only at pressures of 800 MPa. Microbial cellular membranes suffer irreversible damage under high pressure, such as denaturation of membrane protein, resulting in microbial inactivation (Datta and Deeth, 1999). Bacterial spores are the environmentally resistant form of some Gram-positive bacteria, whereas yeasts and fungi are more susceptible to high pressure than vegetative bacteria (Table 1). One study found that treating milk with pressures of 400–600 MPa for 10 minutes at 25°C can obtain a similar outcome to heat pasteurisation (72.8°C, 15 s) in terms of pathogenic and spoilage bacteria inactivation (Buffa et al. 2000). High-pressure-treated milk yogurts are coagulated at a higher pH and have similar physical qualities (firmness and water holding capacity) to heat-treated milk yogurts (Harte et al. 2002). In comparison to UHP-treated only samples, the combination of UHP with thermal treatment boosts yoghurt viscosity and reduces gelation periods. The counts of *L. monocytogenes*, total aerobic mesophilic bacteria, moulds and yeasts, *Lactococcus* spp., and *Lactobacillus* spp. decreased after treatment of Turkish white cheese with high pressure from 50 to 600 MPa for 5 or 10 minutes at 25°C (Evrendilek et al. 2008). Generally, proteolysis, lipolysis, textural, and rheological qualities of Edam, Mozzarella, Cheddar, goat cheese, sheep cheeses, and Reggianito Argentino cheese were all affected by an HHP processing level of 400 MPa for 5–20 minutes (Costabel et al., 2015). Because the cellular viability of *L. acidophilus* and *Lb. casei* is maintained, utilizing HPH, it is enhancing of making probiotic cheeses with functional and sensory qualities.

2-Pulsed electric field (PEF)

Short pulses of a strong electric field at low temperature are applied to food (fluid or semi-fluid) put between two electrodes for a

including internal component leakage into the surrounding media and very short duration (usually less than 1 second) (Fig.2). Where the generation of pulsed light gradually increasing from low to high energy and then releasing the highly concentrated energy as broad-spectrum bursts, to assure microbiological decontamination on the surface of foods and packaging foods (Fig. 3) (Proulx et al., 2015). The pulsed electric field process is more energy-efficient than thermal processing because microbial or enzymatic inactivation is accomplished at ambient or mild temperatures by applying short bursts of high-intensity electric fields to liquid food flowing between two electrodes for a few microseconds (Picart and Cheftel 2003). Microorganisms exposed to PEF become inactivated as a result of electromechanical destabilization of the cell membrane, which causes a breakdown of the microbial cell membrane, resulting in cell deformation and destruction, and eventually microbial cell death (Abinaya et al., 2017). PEF inactivation impact can be boosted by combining it with other stressors such as the presence of antimicrobial chemicals like organic acids and nisin, increment of water activity, pH, and mild heat treatments (Devlieghere et al., 2004). Cheddar cheese created with PEF-treated milk had a better flavour profile than cheese manufactured with milk pasteurised at 63 degrees Celsius for 30 minutes. The hardness and springiness of PEF-treated milk cheese increased, whereas the other textural properties (adhesiveness and cohesiveness) remained unaltered (Sepulveda-Ahumada et al. 2000).

3-Ionizing Radiation

Radiation is a type of energy that travels through space as radiant energy in a wave pattern. Such an energy form can be produced by man-made objects, such as various electrical household gadgets, or it can arise naturally (e.g., from the sun or rocks) (Fig. 4). Ionizing radiation is defined as radiation with a sufficiently high

frequency, such as UV, gamma, beta, and X-rays, or electron beams (Jan, et al., 2017), as a result, it produces charged particles or ions in the material with which it comes into contact. Irradiation of over 100 food items has been permitted in more than 40 nations. It has been proposed for pre-packaged meals to be exposed to an ionizing energy source, such as Cobalt-60 or Cesium137, X-rays from electron beam accelerators, or ultraviolet (UV) sources (Zhang, et al., 2021) (Fig. 5). While non-ionizing radiation, such as microwaves or infrared light, does not make ions, it can generate heat in moist environments and is commonly employed for cooking and warming foods (Ortega-Rivas, 2012). Irradiation is often regarded as the most effective method of removing diseases and spoilage germs from the food supply. Microorganisms may become inactivated as a result of radiation absorbed by DNA, which causes it to split its links, halting cell growth and leading to cell death (Liltved and Landfald 2000). The process of food irradiation has two key advantages: i) Food microbe annihilation, resulting in the manufacture of safer foods (ii) Extending the shelf-life of food by killing pests and delaying the deterioration process, resulting in less waste and an increase in the food supply. Irradiating plain yogurt has the potential to improve shelf life and deliver a safer product without losing chemical or sensory properties, according to researchers. Applying UV light to the surface of the cheese shortly before packaging can be a useful way to avoid mold growth. Lacivita et al., (2016) found that applying UV radiation to the surface of cheese reduced *Pseudomonas* spp. and *Enterobacteriaceae* by 1–2 logs without affecting the color, texture, or surface of the cheese. Using UV radiation (continuous) on solid surfaces to all types of dairy packaging, including tubs, bottles, cans, lids, covers, and foils for yoghurt, milk, butter, cheese, and other dairy products with the appropriate dose and duration prior to filling with dairy products reduces spoilage microorganisms, extending the shelf life of

the product and lowering the risk of contamination (Adams and Moss, 2007).

4-High Voltage Arc Discharge (HVAD):

High Voltage Arc Discharge is the creation of intense dynamic shock waves by an electrical arc (Boussetta et al., 2012). High-pressure shock waves can cause bubble cavitations, which can result in powerful secondary shocks that last only a few seconds. These shocks have the potential to interact with cell structures. Mechanical rupture of cell membranes occurs as a result of the phenomenon, which speeds up the extraction of intracellular chemicals (Liu et al., 2011), via indirect arc discharge, energy from the electric field can be converted to plasma, which can subsequently be transformed into shock waves, which generate free radicals and oxidizing agents within the product. The irreversible loss of membrane function as a semipermeable barrier between the bacterial cell and the environment was from mainly the generation of free radicals (toxic compounds such as oxygen radicals) and oxidizing agents within the product, that inactivate certain intracellular components needed for cellular metabolic

5-Ultrasound

Ultrasound (US) is a term for sound waves with a frequency greater than the higher limit of the human hearing range (Fig.6). Sound waves with a frequency of 20,000 Hz or higher cause gas bubbles in the liquid medium, which produce a high temperature of up to 5500 °C and a pressure increase of up to 50,000 kPa before they explode, lead to shocks can interact with the structures of the cells. As a result of the occurrence, cell membranes are mechanically disrupted, allowing intracellular molecules to be extracted more quickly (Liu et al., 2011). Ultrasound is a viable alternative to a traditional homogenizer for lowering fat globule size. As a result, ultrasonic food processing and preservation is a viable alternative. Milk gels

and yogurt manufacture from milk treated by the high-intensity US have superior physical features and a high value for textural characteristics (firmness, cohesiveness), as well as yogurt's water-holding capacity, increased viscosity, and reduced syneresis (Wu, et al., 2009).

6-Cold Plasma (CP)

Plasma is the fourth matter state after solid, liquid, and gas which is a partially or fully ionized state of gas and consists of electrically charged or ionized atoms and molecules such as electrons, positive and negative ions, free radicals, electrons, and gas atoms, photons (Fig.7). Plasma is divided into two types: thermal (equilibrium) and non-thermal (nonequilibrium) or cold plasma. Thermal plasma is formed when a gas is heated to a high temperature of around 20000 K in order to ionize it. In this state, all ions, electrons, and chemical species are in thermodynamic equilibrium (Misra et al., 2016). While cold Plasma is formed when an inert gas is exposed to electricity; it produces reactive substances such as charged particles, free radicals, photons, and other radiations, it is formed at room temperature, which has a temperature of 30-60 °C and is most commonly employed in the food processing sectors. The gases, such as N₂, O₂, or noble gases (He, Ar, or Ne) or combinations thereof that could either are at decreased or ambient pressure (Ekezie et al., 2017). Cold plasma is useful for decontaminating food and packaging materials, creating packaging materials, active packaging, and preventing browning reactions. Gurol et al. (2012) used low-temperature plasma to destroy *E. coli* in raw milk, and they found a 54 percent reduction in *E. coli* after 3 minutes of treatment. The buildup of charged particles on the surface of fresh and processed milk can cause cell membrane bacteria to rupture. Furthermore, the cold plasma approach can be used to sterilize a widely of liquid food products because it is an ultra-fast procedure that operates at ambient temperatures (perfect for thermolabile products), has a low

running cost, and is environmentally friendly (Yakup, 2016).

7-Non-conventional Chemical Reagents

Chemical preservatives are commonly utilized in the food industry to ensure that foods have a long shelf life and are free of harmful microorganisms that might cause foodborne illness. Despite this, customers are increasingly rejecting foods prepared with chemical preservatives, owing to legitimate or irrational fears about the various undesirable side effects that chemicals in foods may produce. As a result, Natural antimicrobial compounds derived from many sources have been employed as a substitute for conventional chemicals used in food processing, and they are referred to as "on-conventional Chemical Reagents" in general (Ortega-Rivas, 2012). Organic acids present in fruits and vegetables, such as citric, succinic, malic, and tartaric acids, have antibacterial effects and are thought to target cell walls, cell membranes, and metabolic processes (According to Stratford and Eklund, 2003). Phenolic acids are plant-derived metabolites that are wide range found all over the kingdom of plants. Some phenols are germicidal and are used in formulating disinfectants. Polyphenols include flavonoids and tannins, capsaicin, the pungent compound of chili peppers, and the amino acid tyrosine (Nychas 1995). Essential oils, such as patchouli, orange, and lemongrass, are amber or yellow liquids distilled from the leaves, stems, blossoms, bark, roots, or other parts of a plant. They're made up of a combination of esters, aldehydes, ketones, and terpenes. Plant essential oils such as thymol from thyme and oregano, cinnamaldehyde from cinnamon, and eugenol from clove have antibacterial effects against key food-borne diseases (Table 2). Gouvea et al., (2017), tested 21 different essential oils against the activity of *Salmonella enteritidis*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*. Some plant extracts and essential oils have

been proved to be effective natural preservatives in cheeses, with considerable inhibitory activity against key pathogens and spoilage microbes. According to Shan et al. (2011), garlic extract inhibited *L. monocytogenes* more effectively in Cheddar cheese than in the control cheese Chitosan, because of its outstanding biodegradability, biocompatibility, antibacterial activity, nontoxicity, and economic benefits, it is thought to be the most promising material for future uses. Chitosan can suppress pathogens and some unwanted microbes while also extending the shelf life of dairy products as *S. aureus*, *Clostridium botulinum*, and *Bacillus cereus* (El-Dahma et al (2017)).

8-Bio- control cultures

Another example of a non-thermal alternative food processing technique is the use of biocontrol cultures to cause competitive development between harmful bacteria and a non-pathogenic culture. These bacteria can be employed as protective cultures (living microorganisms that inhibit infections and/or extend the shelf life of food goods) or probiotic cultures (live microorganisms that, if consumed by farm animals, can aid in the improvement of animal health) (Gaggia et al., 2011). The use of microorganisms as protective cultures could offer various advantages, as microorganisms can produce a wide range of molecules, including anti-microbial peptides, organic acids like propionic, acetic, and lactic acids as final products, carbon dioxide, ethanol, hydrogen peroxide, and diacetyl, all of which have antimicrobial properties, as microorganisms can produce a wide range of molecules, including anti-microbial peptides, organic acids like propionic, acetic, and lactic acids as final products, carbon dioxide, ethanol, hydrogen peroxide, and diacetyl, all of which have antimicrobial properties (Cotter et al., 2013) (show in Fig. 8). Nisin is a polycyclic peptide generated by *Lactococcus lactis* subsp *lactis* strains. It possesses antibacterial action against a widely of Gram-positive and Gram-negative

bacteria, especially pathogens. According to toxicological studies, Nisin is one of the most often utilized bacteriocins in the food industry as an antimicrobial action agent in cheese and liquid eggs, sauces, and canned foods against *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and other pathogens and LAB species (Rilla et al., 2004). This is achieved by a dual-action mechanism that includes interference with cell wall synthesis and cell membrane whole creation. It was also listed on the European food additive list as a bio preservative ingredient, with the code E234 allocated to it.

9- Utilization of carbon dioxide:

The non-thermal treatment of liquid foods or liquid model solutions using dense-phase carbon dioxide (DPCD) has sparked a lot of attention. Because of its safety, low cost, and great purity, it is widely used. After leaving the sample in the vessel for a while, the CO₂ exit valve is opened to let the gas out into the sample (Hong et al., 1999) (Fig.9). Direct inhibition of metabolism by molecular CO₂ and HCO₃, disturbance of intracellular electrolyte balance, extraction of essential elements from cells and cell membranes, and physical damage of the cell membrane (Jan, et al., 2017). The cells may not be able to remove all of the protons, and the internal pH begins to drop. Cell viability will be compromised if the internal pH is dropped too low, resulting in impairment of cell metabolism or denaturation of particular proteins and enzymes. Calvo et al. (1993) discovered that acidifying raw milk by used CO₂ to a pH range from 6.0 to 6.5 decreased the number of psychrotrophic bacteria, resulting in increased cheese yields. According to Vinderola et al. (2000), CO₂ modification shortened milk fermentation time in *Streptococcus thermophiles* / *Lactobacillus acidophilus* (AT) and *S. thermophiles* / *L. acidophilus* / *Bifidobacterium bifidum* (ABT)-fermented milk products with no detrimental impact on milk sensory qualities.

10-Ozonation (Ozone Treatment):

After fluorine, ozone (O₃) is the second most frequent oxidizing agent (Guzel-Seydim et al. 2004). It was first utilized commercially in 1839 in France to purify drinking water. Ozone, a bluish gas with a pungent odor, has a high oxidation potential of 2.07V and antibacterial capabilities that are broad-spectrum. It is created when free oxygen radicals react with O₂ molecules (Rice et al. 1981) (Fig.10). Among the many methods for producing ozone is electric corona discharge, ultraviolet light, thermal, chemical, electrolytic, and thermonuclear procedures (Patil and Bourke 2012). According to the Food and Drug Administration, ozone is usually considered safe for a wide range of food applications, making it a valuable product for use as a disinfectant or sanitizer in the food processing industry. A unique in-package ionization (plasma) technology that creates significant levels of ozone inside sealed food packaging using high-voltage, low-current electrodes located below and above the package must also be noted (Klockow and Keener 2009). The method of ozonation-induced microbial inactivation is complicated. Ozone can destroy cell membranes, cell walls, the cytoplasm, endospore coats, virus capsids, and viral envelopes, among other things (Patil and Bourke 2012). Furthermore, the high oxidation potential of unsaturated fatty acid double bonds (Guzel-Seydim et al. 2004). On dairy farms, ozone was applied to the air in the barn at very low concentrations to kill airborne bacteria and eradicate the smell of manure in the barn. The researchers concluded that ozone therapy is a safe, effective, and low-cost treatment for mastitis that does not leave antibiotic residues in raw milk (Ogata and Nagahata 2000). It's also worth noting that ozone has been successfully utilized to detoxify common mycotoxins by either entirely destroying them or producing chemical changes, lowering their bioactivity significantly (Patil and Bourke 2012). Several authors have

advocated for the use of low-dose ozone to inhibit mould growth on cheese during ripening. Shiler et al. (1983) found that ozone levels of about 0.05 ppm and 5 ppm in the air of a cheese maturing chamber inhibited 80–90% and 99 percent of the bacteria, respectively. Recently, Lâszlô et al., (2009) show that ozone processing to be a very hopeful strategy for lowering the quantities of organic contaminants in dairy wastewaters. As a result, ozonation in combination with H₂O₂ could be a beneficial technology for the ultimate purification of cheese whey effluents following biological treatment with activated sludge, resulting in final streams that can be discharged into natural waterways.

11- Protective and Preserving packaging

The primary role of packaging is protection from climatic variables (oxygen, moisture, light, and temperature), transportation and storage damages, and biological factors (microorganisms, insects, and other pests) (Galikhanov et al., 2014). In recent years, have been developed packaging technologies, such as changing region packaging, active packaging, intelligent packaging, and Nano packaging (Patel et al., 2018).

A) Modified atmosphere packaging (MAP) is the technique of replacing air with a single gas or a mixture of gases during the packing process like carbon dioxide, ethanol vapour, and sulphur dioxide are all common gases, the presence of oxygen in the gas mixture is often avoided. Its goal is to slow down deteriorative chemical and biological reactions, as well as to inhibit or slow the growth of spoilage bacteria (Robertson, 2016).

B) Active packaging is a cutting-edge packaging technique that includes binding additives into packaging film or inside packaging containers, allowing the package, product, and environment to move to extend shelf life, improve safety, and maintain food quality (Priyanka and Anita, 2014). Now the

focus of research is the incorporation of antioxidant substances such as plant extracts or bacteriocins or natural antibacterial. As a result, prospective food applications include vacuum packaged products in particular and another option is to combine the antimicrobial component into an edible film or coating that may be applied to food by dipping or spraying.

c) Intelligent (smart) Packaging: Intelligent packaging refers to the use of the packaging as an intelligent messenger to monitor the state of packed meals and give consumers quality information. Fig. 11 shows that smart packaging can be classified into four categories: mechanical, chemical, electrical, or electronic (Sharma et al., 2017). Intelligent packaging can be defined as a packaging system capable of triggering intelligent functions (sensing, detecting, tracking, recording, and communicating) to aid higher cognitive processes such as increasing time, improving quality, increasing safety, providing information, and warning about potential issues (Ogles and Yalcin, 2008). Time-temperature indicators, oxygen indicators, carbon dioxide indicators, colour indicators, pathogen indicators, freshness indicators, and leak indicators are all included in intelligent packaging systems (Patel et al., 2018). It's also worth noting that improved packaging technologies can't increase the quality of the packaged products but, they can only slow down further degradation. It is critical that the product's initial quality must be excellent to achieve a significant shelf-life extension using an advanced packaging style.

Conclusions

Non-thermal techniques extend shelf life without using preservatives or additives while preserving color, flavor, texture, nutritional, and functional properties. Unfortunately, the implementation of novel preservation methods necessitates the approval of governmental authorities, which is frequently lacking as well as limited in practice and needs a large upfront

expenditure. However, further study is needed to demonstrate and explain the impact of new preservation technologies on food shelf life and safety.

Conflicts of Interest/ Competing interest

The author declares no conflict of interest.

Abbreviations

HHP	High Hydrostatic Pressure
HPP	High-Pressure Processing
UHP	Ultra-High Pressure
PEF	Pulsed Electric Field
UV	Ultraviolet
HVAD	High Voltage Arc Discharge
US	Ultrasounds
CP	Cold Plasma
LAB	Lactic Acid Bacteria
O ₃	Ozone
MAP	Modified Atmosphere Packaging

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