The Pharma Innovation Journal 2022; SP-11(3): 502-512

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(3): 502-512

www.thepharmajournal.com
Received: 06-01-2022
Accepted: 09-02-2022

Gursharn Singh
Assistant Professor, Department of Dairy Engineering, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Davinder Singh
B.Tech. Scholar, Dairy Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Lovepreet Singh
B.Tech. Scholar, Dairy Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Tikanpreet Singh
B.Tech. Scholar, Dairy Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Narendar Kumar
Assistant Professor, Department of Dairy Engineering, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Corresponding Author
Gursharn Singh
Assistant Professor, Department of Dairy Engineering, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Application of emerging technologies for replacing heat treatment in milk: A review

Gursharn Singh, Davinder Singh, Lovepreet Singh, Tikanpreet Singh and Narendar Kumar

Abstract
Milk is a liquid food product which is secreted by the mammary glands of healthy milch animals. Primarily milk contains nutrients along with numerous enzymes and micro-organisms - which may later on enter milk, enact to destroy the quality of milk. Conventionally milk is heated for the purpose of killing the pathogenic and harmful bacteria along with inactivation of some of enzymes which deteriorates milk quality. However, heating lowers the quality attributes (sensory and nutritional) as well as requires large quantity of energy and water (steam or hot water). Different emerging potential technologies have revolutionized the dairy industry, they are used for various purposes like increasing the shelf life of milk without exposure to heat treatment, preserving natural fresh characteristics, standardization of the major components of milk for tailoring new products, increasing yield and quality of dairy products, concentrating specific components, separation and purification of individual milk components in their natural state etc.

Keywords: Milk, food preservation, emerging technologies, sensory, nutritional, quality

1. Introduction

1.1 Constituents of milk

Milk is a natural, biological and complete food which is secreted by the mammary glands of healthy mammals, primarily for the nutrition and well being of their young ones. Milk of few mammals i.e. cow, buffalo, goat and sheep are used for human consumption, either as such or in the form of vast range of dairy products. Average major compositional constituents (macronutrients) of milk are water (85.3-88.7%), solids not fat-SNF (7.9-10.0%), lactose (3.8-5.3%), fat (2.5-5.5%), protein (2.4-4.4%), casein (1.7-3.5%), minerals (0.57-0.83%) and organic acids (0.12-0.21%) respectively (Walstra *et al*. 2006) [57]. Among the minerals milk contains calcium, magnesium, selenium, riboflavin, zinc, vitamin B-12 and pantothenic acid respectively (Bhat & Bhat 2011, Raza & Kim 2018, Martin CPC *et al*. 2019) [43, 34]. Apart from major macronutrients milk contains bioactive peptides, antioxidants, oligosaccharides, organic acids, highly absorbable calcium, lactoferrin, lactoperoxidase, glycomacropeptides, sphingolipids, and conjugated linoleic acid (CLA) which have large health and pharmaceutical effect. Some of these are identified to act as bioactive substances responsible for benefits to human health, including protection against cardiovascular diseases, cancer, improved immunity and control of the action of pathogenic microorganisms especially those present in the gastrointestinal tract (Bhat & Bhat, 2011, Pardo *et al*. 2013, Martin *et al*. 2019) [39, 34]. Antioxidant activity of milk is contributed by naturally occurring vitamins (E and C), β-carotene and enzymatic systems, mainly glutathione peroxidise. Milk whey protein also has an anti-oxidant activity which is contributed by α-tocopherol, carotenoids, conjugated linoleic acid, casein and lactoferrin. While, the pro-oxidant activity of milk is due to presence of transition metals (e.g. copper and iron) and hydrogen peroxide. (Lanmark M & Akesson 2000, Calligaris S 2004) [9].

1.2 Contamination of raw milk

Raw milk immediately after milking of healthy milch animals contains very few micro-organisms. The total number is less than 103 / mL (Banat 2017) [29]. Raw milk may be contaminated by microorganism mainly during and after milking, which may occur through diverse sources and routes, including the interior of teats (mastitis, bovine tuberculosis and other organisms), vectors such as flies, milking utensils, milking machine, human carriers, soil, faeces, equipments, surroundings, pipelines and tanks used for storing milk.

Milk may also be contaminated by chemical residues i.e. pesticide spray residues on fodder crops, and risk factors i.e. residues of therapeutic drugs given to animals and residues of preventive agents mainly sanitizing agents used in dairy plants (Munoz *et al*. 2018, Raza & Kim 2018, Martin *et al*., 2019, Hamann, 2010) [43, 34, 25]. Thus ruminants are at danger from the various types and sources of anthropogenic contaminants and it is possible that these components are subsequently transferred to humans (Raza & Kim, 2018) [43]. Safety, shelf life and quality of milk are mainly related to the elimination of contamination of raw milk by spoilage & pathogenic microorganism and various chemical residues.

1.3 Effect of heat treatment on the components of milk

Conventional thermal processing is the oldest treatment capable of destroying or inactivating micro-organisms and enzymes that impair or modify the functionalities of milk ingredients thus reducing the risk of food poisoning, development of undesired colour and flavour, modification of nutrient bioavailability, natural antioxidant depletion, increase in anti-oxidant activity of milk, maillard reactions and thermal degradation of volatile and thermo-sensitive compounds resulting in loss of sensory and nutritional value of milk (Mishra & Ramchandran 2015, Muniz at el 2018, Calligaris *et al*. 2003) [36, 37]. To ensure that pathogenic microorganism and endogenous enzymes are inactivated, the food material is subjected to treatment at a certain target temperature for a specific period of time. In this conventional thermal processing generally milk pasteurization, is achieved through different time-temperature combinations, for example 63°C for 30 minutes, 72 °C for 15 seconds, 88°C for 1 second, 94°C for 0.1 second and 100 °C for 0.01 second or other suitable high-temp short time treatments (HTST), whereas milk sterilization is carried out at much higher temperature commonly with an ultra-high temperature (UHT) process, which involves heating milk at 144°C for 1.9 seconds or 115 °C for 20 minutes respectively (FAO, 2004) [19].

It has been found that when cow's milk is heated at 77°C for 30 minutes the soluble CaO decreases by 4.6% (Bell, 1925) [6]. Heat treatment can result in irreversible changes in milk protein structure. When milk is heated at temperature above 65°C whey proteins unfold and expose previously hidden hydrophobic groups (Croguennec *et al*. 2004) [12]. Following unfolding whey proteins are capable to interact with themselves and κ-casein to form heat-induced protein aggregates (Jang & Swaisgood, 1990) [28]. These changes at molecular level may have an impact on protein functionality which sometimes is desirable and other times can be detrimental (Singh & Creamer, 1992) [48]. By controlling the method of heating, the protein concentration and pH of the system degree of heat induced denaturation between the denatured whey proteins and the casein micelles may be manipulated. This in turn may be directly linked to the improvement or impairment of milk protein functionality. Heat treatment of sodium caseinate obtained from milk near the temperature of 50-100°C for 5 minutes resulted in improved emulsifying ability and capacity which was found to be attributed by heat induced exposure of previously hidden hydrophobic domains on the protein backbone. Whereas whey proteins subjected to temperature of 60-90°C for up to 1000 seconds exhibit significant loss of emulsifying ability due to protein denaturation and formation of large protein aggregates which are unable to cover efficiently fat droplets, leading to emulsion instability. Droplet size of milk protein stabilized emulsion increases with increasing the heating treatment. Furthermore proteins become less surface active and this has a negative impact on their interfacial properties as compared to the untreated proteins. Thus heating results in reduced emulsifying efficiency which is due to the degree of protein unfolding (Raikos, 2010) [41].

Heat treatment also affects the rheology and structure of milk protein emulsions and which determines consumer acceptability. Heating has an impact on the particle size of emulsions stabilized with milk proteins. The particle size distribution profile of emulsions (pH 6.8) shifted from below 1 µm prior to heating to 1-10 µm when heated at 140 °C for 80 sec. This increase in particle size distribution was attributed to fat globules aggregation which resulted from interactions between non-adsorbed protein molecules in the serum phase and proteins adsorbed at the interface of fat globules. The same observation was found to enhance the creaming rates when whey protein stabilized emulsion were heated (Euston *et al*., 2000) [18].

The colloidal interactions between droplets from heat induced unfolding of protein molecules adsorbed at the oil water interface at pH of 7.0 resulted in increase in particle size when heated between 65-80 °C for 30 minutes, however when heated above (80-90°C) resulted in decrease in particle size which is attributed to extent of protein denaturation at the oil water interface. Adsorbed whey protein are only partially unfolded when heated at temperature as high as 80 °C which promotes surface hydrophobicity and droplet flocculation. At higher temperature proteins become fully unfolded and are able to rearrange effectively all non-polar amino acids towards oil phase thus reducing the tendency for aggregation. Most likely at higher temperature proteins at the interface possibly may form a more compact layer covering the oil droplet which increases the density of the droplet and lowers the susceptibility to creaming.

Heat treatment has also been found to increase the rate permeate flow in the ultra filtration process of milk. The research work on the same has been conducted for milk which has been prior heat treated at different levels *viz*., 63°C for 30 minutes, 70°C for 15 minutes and 80°C for 5 minutes respectively. This has been found due to formation of enlarged complexes between denatured whey proteins and casein micelles which are retained on the membrane surface and protect the pores from fouling. The large particles accumulated on the pore (channel) of membrane play a sieving role and because of this the flow of permeate accelerates (Erdem & Yuksel, 2005) [17].

In order to minimize the deleterious effects of conventional thermal processes, alternative dairy processing technologies have been studied.

2. Alternate dairy processing techniques to thermal treatment

2.1 Microfiltration

Microfiltration membranes are made from a variety of materials, including organic polymers (e.g. polyethersulfone, polyethylene, polytetrafluroethylene, polyvinylidene fluoride, nylon, polyster, polycarbonate, cellulose acetate and regenerated cellulose), and inorganic ceramics (aluminium and zirconium oxide), glasses (borosilicate glass fiber), & metals (silver and Stainless steel). Organic membranes work in certain ranges of temperatures, pH and TMP (trans-membrane pressure). Organic membranes are more sensitive to washing chemicals too (Cheryan, 1998) [11]. While

inorganic membranes (mostly ceramic material) can be operated in more extreme conditions have longer service life but they are more expensive. The underlying pore morphology of the membranes depends on the techniques used to prepare them. Most polymeric microfiltration membranes consist of an isotropic network of polymer fibres resulting in a highly interconnected pore structure. Metallic membranes generally consist of an array of sintered metal particles or spheroids, giving an isotropic structure with more uniform pores in the interstices between the metal particles. Many ultra filtration membranes have an asymmetric structure consisting of an ultrathin skin (approximately 0.5 µm thick) which determines the sieving characteristics of the membrane, a porous substructure and a porous matrix which provides the membrane with its structural integrity. Most ultra filtration membranes are rated by nominal molecular weight cut-offs indicating the molar mass of the species with a specified retention coefficient. The exact retention coefficient used to define the pore size rating of membranes varies throughout the membrane industry.

Microfiltration is a form of membrane processing technique that can be used to improve dairy product quality, extend their shelf lives and to tailor functionality ingredients in the development of new products or improvement of the existing products (kosikowski and Mistry, 1990; Eckner and Zottola, 1991; Pafylias *et al*., 1996) [31, 14, 38]. Microfiltration provides a low temperature processing alternative to thermal methods (e.g. ultra-pasteurization) for reducing the number of bacteria in fluid milk or in whey (Eckner and Zottola, 1991; Bargeman, 2003) [14, 5]. In dairy industry ceramic membranes are commonly used in tubular modules and are available for MR and UF treatments respectively. As per the claims of producers, microfiltration is preferred over UHT because (a) it tastes comparable or surprisingly better than condensed milk, (b) the taste stays consistent for the entire shelf life (c) it is more economical. The following schematic diagram shows co-current permeate flow (CPF) mode of operation. For conventional cross flow MF, the permeate loop would not be used and permeate would be drawn off the system from the module's permeate outlet.

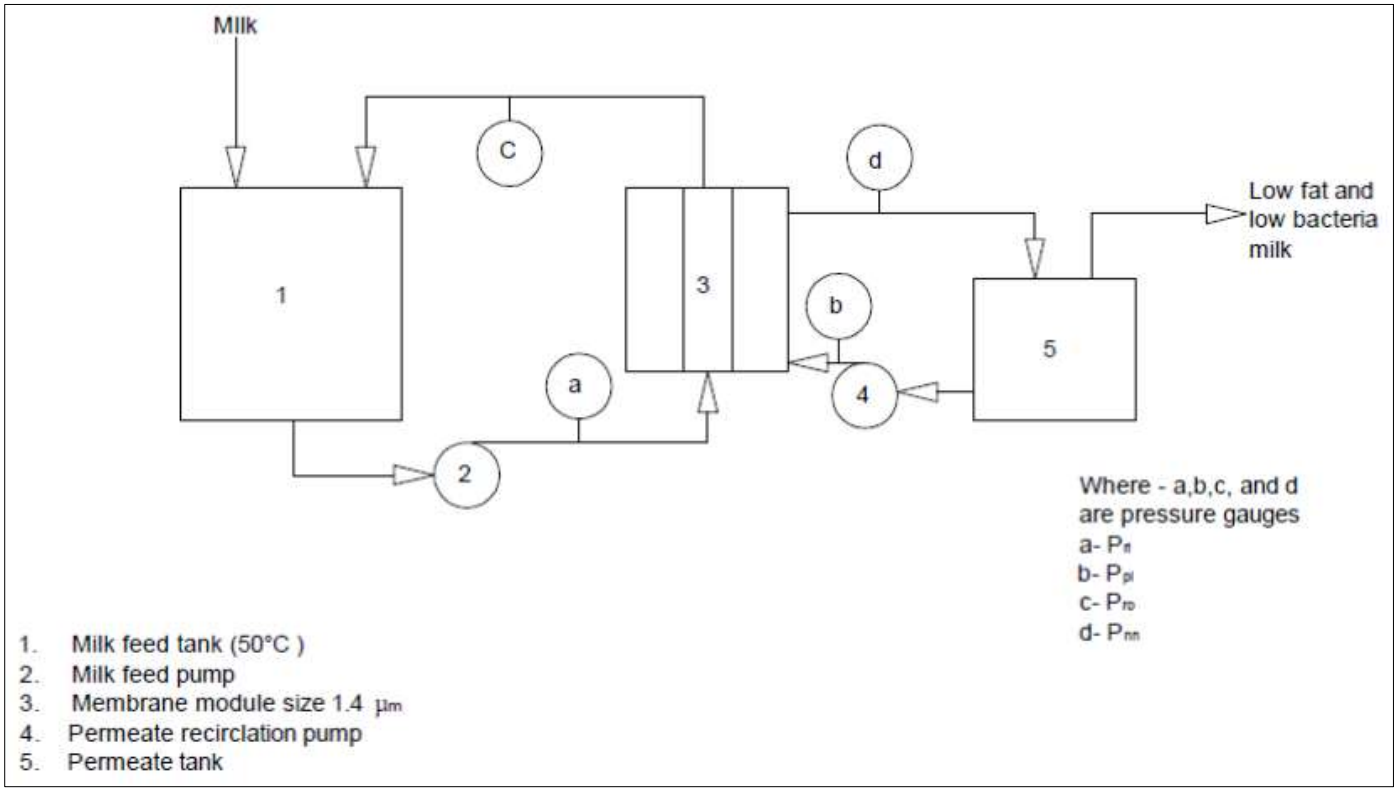

Fig 1: Microfiltration of milk with ceramic membrane

Above set up was used by Pafylias *et al*., 1996 [38] to conduct Microfiltration of milk with ceramic membranes. In this co-current permeate flow (CPF) method was developed. The permeate side of the membrane is pressurized by pumping permeate in a closed loop parallel to the direction of flow of the retentate. Uniform trans-membrane pressure (1 bar) was maintained along the entire length of membrane module. Skim milk was used for the experiment at the temperature of 50 °C. Ceramic was the material of construction of membrane. Membrane module was 85 cm in length with 4 mm diameter channel 0.2m^2 of active area and a rated pore size of 1.4 µm. It was fitted in Stainless steel housing and placed in the retentate loop of the system. To maintain a cross flow velocity of 5 m/s and transmembarne pressure of 1 bar (14.5 psi), the system was operated at P_{ri} = 2.07 bar. P_{ro} = 0.41 bar and P_p = 0.24 bar. In the CPF mode

$$P_T = (a - b) = P_{ri} - P_{Pi} = (c - d) = P_{ro} - P_{po} \quad \text{. eq. 1}$$

Where

P_T represents trans-membrane pressure

(P_{ri} – P_{pi}) represents pressure difference at inlet side of module that is pressure difference between milk feed inlet and permeate inlet from permeate tank as shown in fig.1

(P_{ro} – P_{po}) represents pressure difference at outlet side of module that is pressure difference between retentate outlet and permeate outlet as shown in fig.1

It was found that cross flow MF is an effective tool for the reduction of the microbial population. Bacterial counts and spore count in micro-filtered milk was reduced up to 99.99%

(Eckner and Zottola, 1991; Pafylias *et al*., 1996) [14, 38]. Because spores of psychro-trophic strains of Bacillus spp. can survive pasteurization, elimination of these spores by microfiltration can reduce spoilage in milk products. Applications of microfiltration that have been used in the dairy industry include the processing of extended shelf life fluid milk products and the productions of milk for cheese manufacturing (Van der Horst, 2001) [53].

Combination of microfiltration and high temperature short time (HTST) pasteurization was tried to increase the shelf life of milk. Milk was heated to 50°C followed by MF through ceramic membrane having pore size of 1.4 µm and surface area of 2.31 m^2. Followed by HTST pasteurization at 72°C for 15 seconds, and then cooled to 6°C. These MF and pasteurized milk samples were stored at 4 different temperatures i.e. 0.1, 2.0, 4.2 and 6.1°C respectively. End of shelf life was based on microbial count greater than 20,000 cfu/per ml based on pasteurized milk ordinance. Across 3 replicates total bacterial counts of the raw milk were reduced from 2400, 3600 and 1475 cfu/ml to 0.240, 0.918 and 0.240 cfu/ml respectively by MF. Bacterial count in MF and pasteurized skim milk (combinations) for the three replicates were 0.005, 0.008 and 0.005 cfu/ml respectively. MF alone achieved an average 3.79 log reductions and MF in combination with pasteurization of the skim milk achieved an additional 1.84 log reduction producing an average total of 5.6 log reduction from the raw milk count. End of shelf life with respect to proteolysis was defined as the time to decrease in CN%TP >4.76%, a level that corresponds to sensory detection of off-flavours due to proteolysis. Considering proteolysis MF in combination with pasteurization of skim milk stored at 0.1°C exceeded 92 days of microbial shelf life, at 2.0 °C - 78 days, 4.2°C - 46 days and milk stored at 6°C have shelf life of 32 days due to proteolysis respectively (Elwell and Barbano, 2006) [15]. Microfiltration can reduce the amount of viable bacteria for a lower energy cost than a heat treatment and without affecting the taste of the milk. A commercial process, called the 'bactocatch system" is in use since, 1992. In order to reduce fouling, high cross-flow velocities are used (typically 6-8 m/s). It is possible to filter for at least 6 h at a permeate flux of 1.4 X 10^{-4} m/s at a concentration factor of 10 by use of reversed asymmetric ceramic membranes (0.87 µm) and optimizing back-pulsing system.

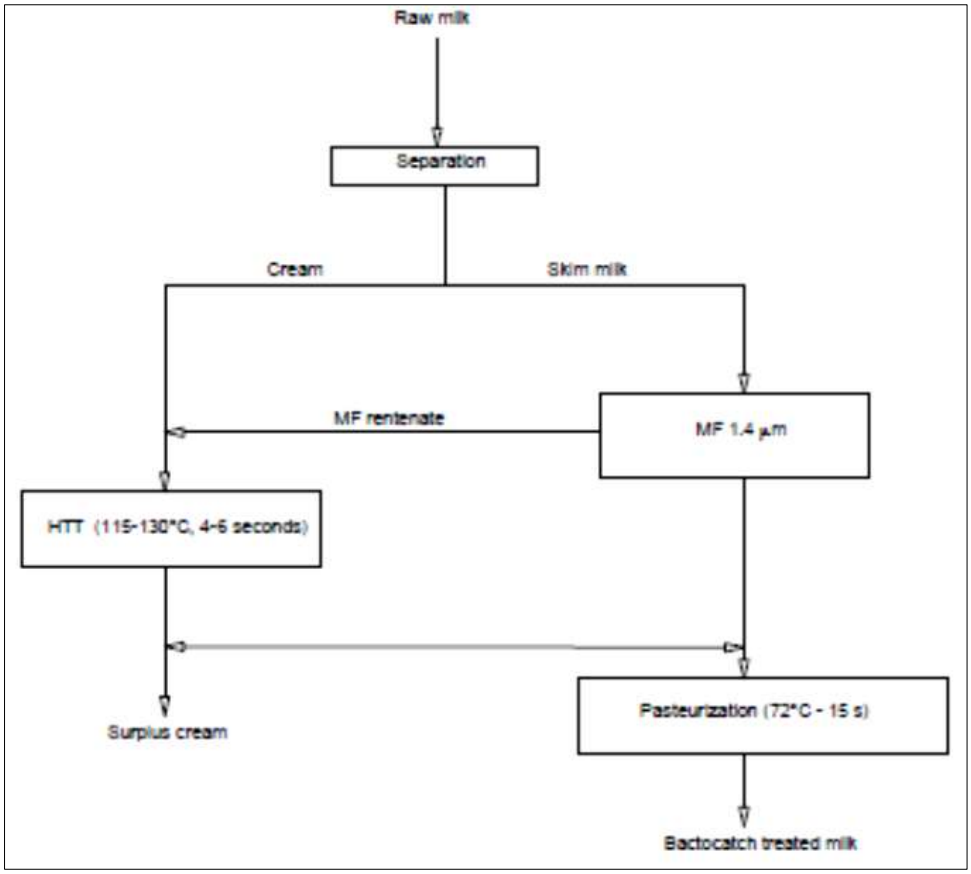

Fig 2: The "Bactocatch" process for removal of organisms from milk (Elwell and Barbano 2006, Madec *et al*. 1992) [15, 33]

Fat is predominantly present in spherical globules varying in diameter from 0.1 to 15 µm. Globules below 1 µm in diameter account for 80% or more of the total globules in numbers, but they contain little of the total volume of the milk fat. Globules between 1 to 8 µm in diameter contain 90% or more of the total volume of milk fat. MFGM comprises from about 2 to more than 6% of the mass of the globules. Special patented design of ceramic Microfiltration membranes was developed by Henri *et al*., (2000) to fractionate milk fat globules from whole milk and creams. Fat globules were separated into fractions of small fat globules having diameter lower than 2 µm and large fat globules having diameter more than 2 µm. Then the results were observed on different dairy products (such as liquid pasteurized milk, yoghurt, fresh cheese, camembert cheese, Swiss cheese and butter were made by suitable combination of small fat globules and large fat globules respectively. A significant difference was found by the use of small and large size fat globules in the texture of different dairy products.

Milk was taken separated in centrifugal cream separator.

Small globules creams were obtained by separation of the MF permeate. To adjust texture and may be flavour of dairy products. Acting on fat globule size without damage of the MFGM offers a way to understand deeper how fat globules play a role in textural characteristics of the different cheese varieties. Performance of membrane was well in a ceramic membrane of 2 µm average diameter pore size. Results revealed that drinking pasteurized milk prepared with MF permeate (SG milks) was significantly more onctuous and more creamy than reference milk. Yoghurt was prepared using MF fractions. It was found that full fat yoghurt shear stress was 507 Pa for reference, 607 Pa for LG and 404 Pa for SG. The same rheological measurements for the low fat yoghurts were respectively 669 Pa for the reference, 810 Pa for LG and 601 Pa for SG. Testing panel also qualified SG yoghurt smooth and finer in texture then reference products. Fresh cheese assay – three lots of fresh un-ripened cheeses with the same total solids (186 g/kg) and fat (78g/kg) contents were then rheologically characterized and tasted. The same shear stress (450 Pa) was determined on reference and on LG product but SG product had a lower shear stress (387 Pa). No taste differences were detected by the tasting panel between 3 products. However in mouth SG fresh cheese was appreciated as smoother as and finer than reference and LG products. Sour cream cheese – after preparation it was cooled to 4 °C and characterized rheologically. Shear stress was 3020 Pa for cream, 3590 Pa for LG cream and 258 Pa for SG cream. Camembert cheese assay – SG cheeses TS content were significantly lower (39.3 g/100) than that of reference cheeses (40.1 g/100). This TS content difference corresponds to 2% increase in cheese yield. This TS difference was reflected by the rheological characteristics. Firmness and shear stress of reference camembert were respectively 36.0 N and 5838 Pa versus 31.6 n and 5130 Pa for SG products. Mini Swiss cheese assay – cheese milk were prepared with reference LG and SG creams mixed with skim milk. TS content was different for all. TS content was 61.2 g/100 g for the reference product, 58.6 g/100g for the SG product and 60.6 g/100 g for the LG minni cheese. A 3% increase in cheese yield was observed for the SG cheese versus reference or the LG cheese. Butter making assay – no difference in churning abilities was noticed. The composition of reference LG butter was similar in terms of fat and water. The tasting panel judged with a slightly higher score for LG butter versus the reference one.

2.2 Microwave heating

Microwave heating is a form of dielectric heating which is used industrially for the processing of food and also used domestically for cooking or thawing of food (Song & Kang, 2016) [49]. MW are electromagnetic radiations with wavelengths ranging from about 1mm to 1 m and are within a frequency band of 300 MHz to 300 GHz (Chandrasekaran *et al*., 2013) [10], however MW heating applications have been limited to a few narrow frequency bands for industrial, scientific and medical use to avoid interference with radio frequencies used for telecommunication purposes. The typical bands are 915± 25 Mz and 2450±50 MHz with penetration depth ranging from 8 to 22 cm at 915 MHz and from 3 to 8 cm at 2450 MHz depending on the moisture content are used. The domestic MW ovens operate at 2450 MHz whereas both frequencies are used for industrial purposes. It is a worthwhile to note that outside of the United States, frequencies of 433.92, 896, and 2375 MHz are used for MW heating (Ahmed & Ramaswamy 2007, Guo *et al*. 2017) [2, 24]. Microwaves have some similarities to visible light i.e. can be focused into beams, transmitted through hollow tubes, transmit through materials without absorption, depending on the materials dielectric properties it may be reflected or absorbed by the material, some materials like glass, ceramics and thermoplastics allow microwaves to pass through with little or no absorption.

Food materials possess properties of dielectric medium and absorb microwaves. The absorption of microwaves results in giving up their energy to the food material, thus raising its temperature. Heat dissipation is governed by basic heat and mass transfer mechanisms (Salazar *et al*. 2012) [45]. The heating of a food material by MWs is the result of two main mechanisms i.e. ionic polarization and dipole interactions (Franco *et al*. 2015) [21]. Firstly, when an electrical field is applied to a food solution containing ions, the ions move at an accelerated pace due to their charge, collisions between the ions cause the conversion of kinetic energy to thermal energy this is known as Ionic polarization. Secondly, food materials contains polar molecules such as water and others, they have random orientation when electrical field is applied these molecules orient themselves according to the polarity of the field, this is known as dipole rotation. The polarity alternates rapidly (for 2450 MHz polarity changes at the rate of 2.54 billion cycle/second). These polar molecules rotate to maintain alignment with the rapid change in polarity. Rotation of molecules leads to friction with surrounding medium thus heat is generated, further at high temperature the molecules try to align more rapidly. There is phase lag between the cause (electric field) and its effect (polarization) results in loss of energy in the system. This loss of energy, known as loss factor contributes to heating. Therefore, a food with a high concentration of ions would present a higher temperature rise than a food with a lower concentration of ions (Datta & Davidson, 2000) [13]. As heat is generated, it flows through the food components, spreading in all the directions by conduction or convection mechanisms (Chandrasekaran *et al*., 2013) [10]. This result in higher energy efficiency, reduced heating times, and allows the production of products with better sensory and nutritional qualities when compared to conventional heating methods (Salazar-Gonzalez *et al*., 2012) [45].

2.3 Microwave operating system

MW equipment basically consists of a magnetron, responsible for converting electric energy into an oscillating electromagnetic field and conductors of waves that reflect the electric field internally (wave guides), transferring it to the heating chamber (FDA, 2000). The heating chamber can be designed for batch (Fig – 3a) or continuous flow operations (Fig – 3b) and have appropriate mechanisms to prevent the leakage of MWs. Batch and continuous systems can be applied for solid or liquid foods depending on the equipment configuration. In batch equipment, a rotating antenna or a propeller is used to distribute the energy, or the food is placed on the turntable that rotates during the cooking operation. For liquid foods, a stirrer can be used to homogenize the MW absorption and heat distribution. However continuous systems have advantages over batch processing with higher efficiency and easier cleaning and automation (Ahmad & Ramaswamy, 2007, Salazar-Gonzakez *et al*., 2012) [2, 45]. Continuous MW heating of solid foods normally occurs with the transportation of the solid material in trays or pouches inside the chamber

where the food material receives MW radiation with the first patent published by Kenyon *et al*., 1976. In the case of liquid foods, the fluid is pumped through helical coils into a MW oven (alternately, more MW ovens can be connected in series for heating, or several magnetrons can be arranged in an oven, Ahmed and Ramaswamy 2007 [2] fig 3b). After MW heating, the fluid passes through a retention section to allow a predefined holding time followed by cooling and thermocouples are used to collect sample temperatures in the inlet and outlet sections, and fibre optic probes are used to monitor the temperature inside the MW cavity (Ahmed & Ramaswamy, 2017) [2].

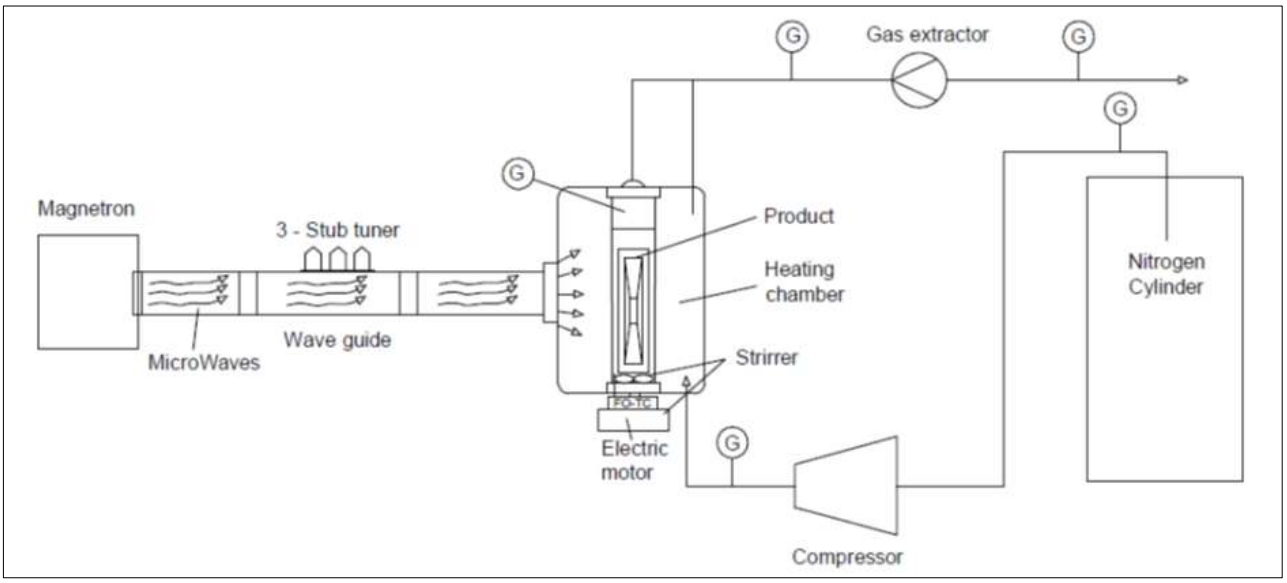

Fig 3a: Microwave heating of liquid foods applied in batch system. Thermocouple (TC) Pressure gauge (G), fiber optic thermocouple (FO-TC)

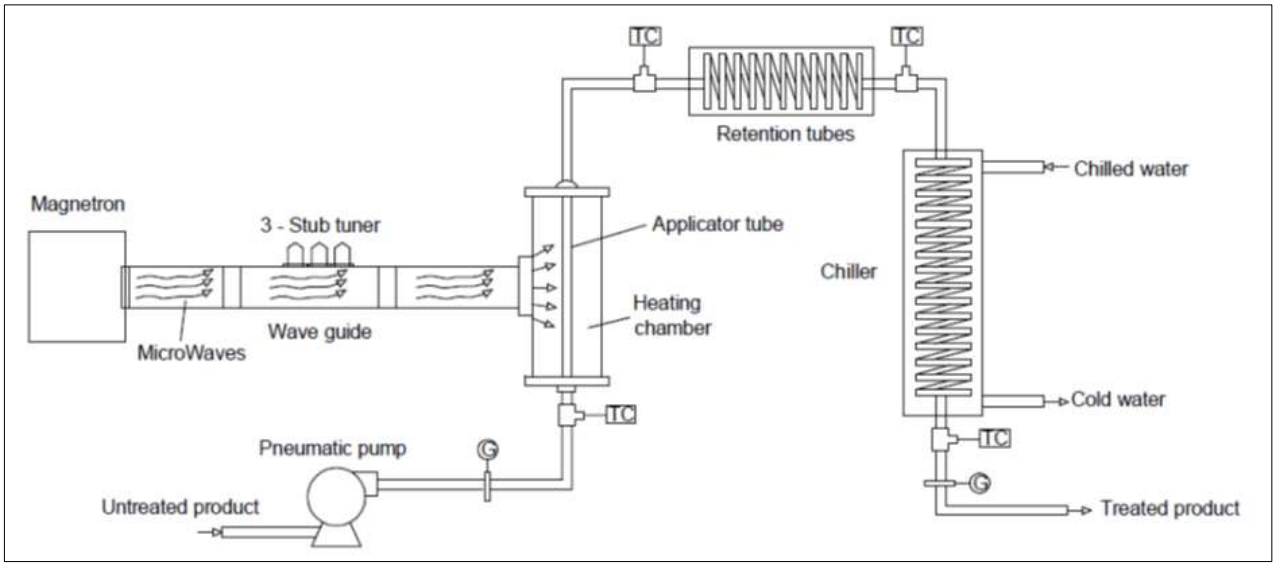

Fig 3b: Microwave heating of liquid foods applied in continuous system. Thermocouple (TC) Pressure gauge (G), fiber optic thermocouple (FO-TC)

According to Stanley and Peterson (2017) [51], several continuous flow systems have been developed along the years. For non-flowable foods, MW tunnels have been used to develop continuous MW assisted thermal processes, mostly applied for industrial continuous in pack pasteurization/sterilization of ready to eat meals, such as Sairem Labotron 8000 in France (Sairem Company Website, 2015) and Mic Vac in Sweden (MicVac, 2015) [35] both operating at 2450 MHz. For flowable foods, MW systems such as UHT/HTST Microthermics (Microthermics, Ralleigh, NC, USA) and Enbiojet (Enbio Technology, Kosakowo-k-Gdyni, Poland), have been developed and industrial applied. The Microthermics pilot scale unit can process pasteurization/sterilization of liquid foods at flow rates between 48 and 180 L/hr. The system uses a focussed MW applicator operating at nominal power of 6000 W and frequency of 2450 MHz and with maximum temperature of 150° C. No other commercial MW system industrially used is the Enbiojet (Enbio Technology; Stanley & Petersen, 2017) [51].

2.4 Industrial application

According to review done by Carlina PC, Martin *et al*. 2018, the major industrial applications of MW heating in the food industry are:

- Tempering/thawing of frozen food products
- Precooking /cooking of various products for foodservice
- Drying of food materials

- Baking of various foods
- Extraction of food compounds
- Blanching of vegetables
- Heating and sterilizing of fast food, cooked meals and cereals
- Pasteurization and sterilization of various foods.

Microwave heating can be used a good and superior alternate to conventional heat treatment of milk. The main drawbacks are non-uniform temperature distribution resulting in hot and cold spots mainly in solid and semi solid products but MW heating had been found suitable for liquid foods, especially a continuous fluid system.

3. Ultrasound Technology

Ultrasound (US) refers to sound waves of frequency above 20 kHz, which is undetectable by human ear and is divided into two categories, *viz*., power and diagnostic ultrasound. The power ultrasound, ranging from 20 kHz to ~1MHz, has recently been explored in food processing. (Ashokkumar & Mason, 2009; Patel *et al*., 2008, Shanmugam *et al*., 2012) [4, 40, 46]. The inactivation of bacteria using ultrasound was first initiated in 1920 by (Harvey & Loomis, 1929) [27]. This technology relies on the application of ultrasonic waves (frequency ranging from 20 to 100 kHz) to the liquid food, causing microbial cell death. The effect of ultrasound alone has been considered ineffective for the inactivation of bacterial spores (Butz & Tauscher, 2002) [8]. Hence, the combination with other treatments such as temperature, pressure, or both heat and pressure to increase the lethal effect has been investigated (Ramteke *et al*., 2020) [42]. The major advantages of ultrasound is being non-toxic and environmentally friendly nature compared to other processing techniques like microwaves, gamma radiation and pulsed electric field which are considered cautiously by the general population (Shanmugam *et al*. 2012) [46].

Thermosonication is one of the methods of ultrasound technology, which combines moderate heat of 37 to 75 °C with low frequency ultrasound waves (20 kHz) treatment to enhance inactivation of enzymes and pathogenic microorganisms (Lee *et al*., 2013) [32]. When heat and ultrasound are used together, the process temperature and time is considerably reduced (16 to 55%) compared to the conventional heating process. The sonication process described by Altaf *et al*., (2018) [3] consists of creating longitudinal waves with a frequency higher than 20 kHz. When a sonic wave passed through a liquid food material (medium), regions of alternating compression and expansion of the medium particles are created. These regions of pressure change because cavitation forces in the medium. Cavitation is the process whereby micro bubbles or cavities filled with vapours are formed. Sonication causes tiny bubbles to expand and contract thousands of times every second. A point is reached where the ultrasonic energy provided is not sufficient to retain the vapour phase in the bubble, therefore, rapid condensation occurs. The condensed molecules collide violently, creating shock waves. These shock waves create regions of very high temperature and pressure, reaching 5,000 degrees Kelvin and 2,000 bar pressure and finally bubble collapses. Cavitation can result in the occurrence of micro-streaming which is able to enhance heat and mass transfer in the milk, because of streaming; the bulk milk is treated results in a localized pasteurization effect. Destruction of microorganisms and inactivation of enzymes can be induced by one or more of these consequences of sonication. Microbial killing involves the thinning of the cell membranes and localized heating. Cavitation depends on ultrasound characteristics (e.g. frequency, intensity), product properties (e.g. viscocity, surface tension) and ambient conditions (e.g. temperature, pressure) (Abdulla and Nyuk, 2014) [1]. Gram positive bacteria are more resistant to ultrasound than gram negative bacteria because of their thicker calls wall that give them a better protection against ultrasound effects. Differences in cell sensitivity could be caused by more tightly adherent layer of peptidoglycans in gram positive cells. Bacterial spores and fungi are more resistant to ultrasound than vegetative bacteria. Spores are more difficult to be destroyed than vegetative cells which are in phase of log growth.

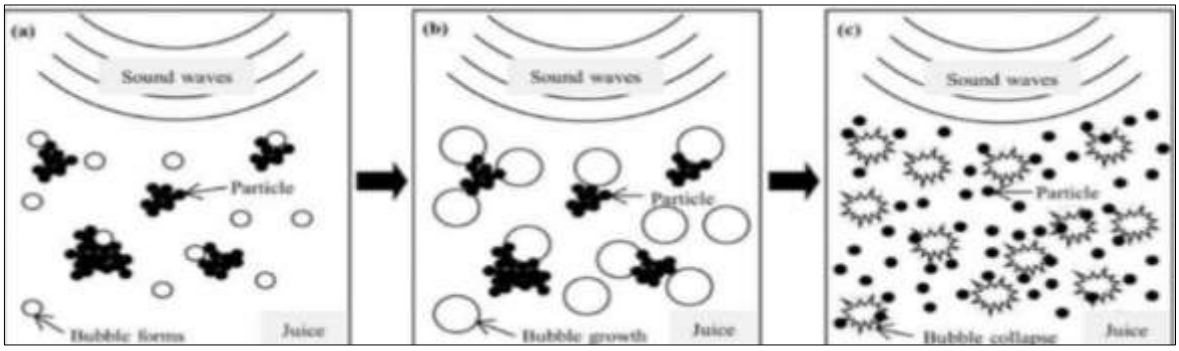

Fig 4: Cavitation phenomenon (a) bubbles formation by sound waves 9b) bubbles growth to the maximum size (c) bubble collapse and particle dispersion and cell disruption (Abdulla and Nyuk Ling Chin 2014) [1].

Shanmugam *et al*. 2012 [47], sonicated pasteurized homogenized skim milk using a 20 kHz, 450W ultrasonic horn (12mm diameter, Branson Sonifier, Model 102 (CE)) at 90 and 180W of applied powers for 15, 30, 45 and 60 minutes respectively. The actual powers delivered to the solution were determined to be 20 and 41W by calorimeter, respectively. During sonication water with temperature maintained at 22.5± 2 °C was circulated continuously. The solution temperatures during US treatment at 20 and 41W were maintained between 22 to 30 °C and 22 to 37 °C, respectively.

Engine and Yuceer (2012) [16] used Bovine milk (3.32% fat). US application was done by using the US system (Sonics & Materials Inc., Newtown, CT, USA) comprised a 20 kHz frequency acoustic power unit (VibraCell500 W), a transducer and a sonotrode. In order to prevent an increase in temperature during US application, the treatment was applied at a processing temperature of 5 °C, maintained by cooling water bath. The sonotrode was inserted in amber coloured

glass jar of 500 ml capacity filled with milk and US was applied at 75 W for 15 min. The power density of the US treatment was 150 W L^{-1}. The US intensity was 135 J mL^{-1}. Total aerobic mesophilic bacteria, *S. aureus*, total coliforms, *Escherichia coli* and yeasts/moulds were counted in microbial analysis. Three independent heating/UV/US experiments were performed. The pour plate method was used for inoculation of most samples. Only for *Staphylococcus* spp. counts was the spread plate method used. Fresh raw milk had 5.17 log colony-forming units (CFU) mL^{-1} of mesophilic aerobic counts. Heat treatment reduced the number of these bacteria by 4 log cycles. Application of US was less effective than heat and UV treatments with regard to mesophilic aerobic counts. US treatment led to a significant reduction in numbers of total coliforms (by about 2 log cycles) and E. coli (by 1 log cycle) in milk. No significant difference was determined between raw milk and US milk in terms of yeast/mould counts ($P \geq 0.05$). Even though the energy intensity applied to the milk was higher in US treatment than in UV treatment, the microbial reduction was higher in UV milk than in US milk. The National Advisory Committee on Microbiologist Criteria for Foods concluded that the effect of US treatment is limited in the pasteurization of foods. However combinations of US and some other food preservation technologies may be used in a more effective manner for commercial applications.

Table 1: Microbiological counts (log colony forming units/ml) obtained in raw, pasteurized (65°C, 30 min), ultraviolet light treated (UV) (13.87 J/ml), Ultrasound treated (US) (150 W/L) milk samples

Milk	Mesophilic counts	Total coliforms	Escherichia coli	Staphylococcus spp.	Yeasts/moulds
Raw	5.17 ± 0.02 a	4.18 ± 0.15 a	2.85 ± 0.15 a	2.96 ± 0.14 a	4.43 ± 0.13 a
Pasteurized	1.32 ± 0.31 d	<1c	<1b	<1c	0.70 ± 0.01 c
UV treated	3.16 ± 0.09 c	<1c	<1b	<1c	3.78 ± 0.04 b
US treated	4.56 ± 0.01 b	2.95 ± 0.03 b	1.70 ± 0.01 b	2.19 ± 0.12 b	4.19 ± 0.03 a

Values are mean ± standard error. Means in the same column followed by different letters are significantly different (P<0.05)

Bermúdez-Aguirre (2009) [7] studied the inactivation of Listeria inocula and mesophilic bacteria in raw whole milk. Five systems were evaluated in an ultrasonic processor (24 kHz, 120 µm, 400 W). Tested amplitudes of ultrasonic waves were 0, 40, 72, 108 and 120 µm, with a constant temperature of 63 °C and treatment time of 30 min. After 10 min of treatment, thermal pasteurization achieved a 0.69 log and a 5.3 log-reduction after 30 min. However, after using ultrasound at 60, 90 or 100% in combination with temperature, a 5 log-reduction was obtained after 10 min.

Vijaykumar (2012) studied the effects of thermosonication on proteases and characteristics of milk and cream. He observed that thermosonication at 133 and 152 µm for 1 and 3 min completely destroyed coliforms and destroyed over 99% of the total aerobic bacteria. The total aerobic bacteria count of thermosonicated skim milk and cream samples were less than 20,000 cfu/mL on day 30.

Table 2: Lethal effect of thermosonication on different species of micro-organisms

Microorganism	Shape	Cell wall structure	Frequency (kHz)	Time (min)	Temperature (°C)	Microbial reduction
Salmonellea spp.	Bacill	Gram Negative	24	2-10	52-58	5 log
S. boydii	Bacill	Gram Negative	22.3	10	37	5 log
L.monocytogenes	Bacill	Gram Positive	22.3	2.5	65	5 log
S.aureus	Cocci	Gram Positive	30	20	55	3.3 log
E.coli	Bacill	Gram Negative	20	3.8	59	5 log

Engine and Yuceer (2012) [16] studied a total of 41 aroma-active compounds in the various samples, but FD factors of the compounds varied. In general, aldehydes, ketones, esters and acids were major volatiles in milk samples. They revealed that US treatment of milk increased the intensity of acidic aroma components.

Vijaykumar, (2012) evaluated the effects of combined heat and ultrasound on the activity of Staphylococcus aureus protease and total plasmin, as well as the impact on sensory properties of milk and cream. The samples were heated at 60 °C and Sonication at 133 µm for 2.5 min was applied, results showed that ultrasound, when combined with heat, is capable of decreasing the activity of *Staphylococcus aureus* protease in skim (by approx. 72%), reduced-fat (by approx. 92%) and whole milk (by approx. 92%) and total plasmin activity in fresh skim milk (by approx. 81 to 94%) and cream (by approx. 96%). He concluded that Ultra-high temperature processing inactivates proteases but detrimentally affects milk's sensory quality, thermosonication did not induce off-aromas or viscosity changes, but inactivated microorganisms and protease enzymes, thermosonication may be an appropriate alternative to pasteurization (Ramteke *et al*. 2020) [42].

2.4 Bactofugation

Bactofugation is a mechanical centrifugal process is which micro-organisms are removed by centrifugal force due to density difference between the milk constituents and micro-organisms. This process was first done and practiced by Prof. Paul Simonart, who along with his associates developed this process to be used in commercial dairy plants. In general the density of milk is 1028-1032 kg/cm^3 (1.028-1.038 g/ml). Since micro-organisms as well as their spores are heavier than milk, thus are separated by the high speed centrifugal bactofuge machines. It removes 90% of the bacteria. Usually two bactofuge machines are used in series operating at 20,000 rpm (approx.). First machine removes 90% of the bacteria; second machine removes another 9% of the total count resulting in 99% removal of bacteria (Banat, 2017) [29].

Milk is heated to 77 °C for reducing the viscosity of milk, then fed to bactofugation machine. Two streams are obtained major being the bactofugated bacteria free milk and the other being bacterial rich bactofugate which is further sterilized before being discharged into main stream. Bactofugate generally accounts for 0.15% of the total volume of the milk being taken. Bactofugation is specially efficient against spores as the spores have relatively high density (1.30.- 1.32 g/ml)

and quite small size 1.0 -1.5 µm, than that of vegetative bacteria bacteria (in liquids approx. 1.07 – 1.12 g/ml) and at temperature of 60-65° C, a significant portion typically 90-95% is removed (Stack and Sillen, 1998) [50]. It is very quick process takes less than second for entry to discharge of milk. It also removes dirt, cells and even other heavy particles from milk (Valstra *et al.*, 2011) [52]. Raw milk with different number of microbial population were taken, following are the resultant decrease in bacterial population by two stage centrifugal bactofugation process.

Table 3: Effect of two stage bactofugation process on the microbial population of milk with different initial count

Milk sample	Trail 1		Trail 2		Trail 3	
	Enterobacteriaceae (cfu/ml)	Aerobic mesophilic bacteria (cfu/ml)	Enterobacteriaceae (cfu/ml)	Aerobic mesophilic bacteria (cfu/ml)	Enterobacteriaceae (cfu/ml)	Aerobic mesophilic bacteria (cfu/ml)
Raw milk	2.8	13200	10000	12000000	-	15200000
Bactofugated milk	-	100	500	80000	-	200000

2.5 Design of bactofuge machine

Currently two main categories of bactofuges are used in dairies. The first type is one phase type bactofuge and is like a normal clarifier. It has only one outlet at the top of the bactofuge for the bacteria reduced milk, while the portion containing particles (bactofugate) is collected as a sludge in the bowl and discharged continuously or by intervals through a part in the bowl body. In some equipment, a continuous centrifugate stream (about 3% of the milk feed) can be recycled through the centrifuge and a discontinuous bacteria rich portion making up around 0.2% of the milk, is ejected periodically (every 15-20 minutes). The second type is a two phase type bactofuge and is more like a cream separator. It has two outlets at the top i.e. one for the bacteria reduced milk and one for the continuous discharge of bactofugate (about 3% of the total liquid flow) via a special top disc. In such a bactofugate the milk to be treated enters the machine along the central axis. It enters a stack of conical discs and the flow is divided over the numerous slit towards the periphery of the bowl, and the bacteria reduced milk moves towards the central axis of the centrifuge, both streams then move up and remain separated, before being discharged from the bactofuge (Guiziou GG, 2010) [23]. Waes and Heddeghem (1990) [56] suggested that several factors affect the performance of bactofugation machine. Some of these factors are related to microorganisms and some are related to constructional features of the centrifuge as enlisted:

- Size, form and characteristics of the outer surface of microorganisms
- Density of the microorganisms
- Ability to agglomerate between bacteria themselves or between bacteria and milk ingredients
- Bacteriological quality of the milk, which may lead to chemical or physical changes.
- Temperature of milk (50-68 °C) which plays a role in viscosity of milk
- Rate of milk flow in machine at rated capacity
- Centrifugal force or rpm of the machine
- Space between the disc stack
- Design of the bactofuge which should avoid recontamination of the centrifugated milk.

3. References

1. Abdullah N, Nyuk LC. Application of Thermosonication treatment in processing and production of high quality and safe to drink fruit juices., Agriculture and Agricultural Science Procedia. 2014;2:320-327.
2. Ahmed J, Ramaswamy HS, Raghavan VGS. Dielectric properties of butter in the MW frequency range as affected by salt and temperature. Journal of Food Engineering. 2007;82:351-358.
3. Altaf U, Rouf A, Varsha K, Qudsiya A, Imtiyaz Z. Ultrasound treatment: A novel processing technique for food preservation, The Pharma Innovation Journal. 2018;7(2):234-241.
4. Ashokkumar M, Lee J, Zisu B, Palmer M, Kentish S. Hot topic: Sonication increases the heat stability of whey proteins. Journal of Dairy Science. 2009;92(11):5353-5356.
5. Bargeman G. Separation technologies to produce dairy ingredients. Dairy processing: improving quality, Woodhead publishing ltd. 2003, 366-390.
6. Bell RW. The effect of heat on the solubility of the calcium and phosphorous compounds in milk, Journal of Biological Chemistry, ASBMB. 1925, 391-400.
7. Bermúdez-Aguirre D, Mawson R, Barbosa-C'ANovas. Microstructure of fat globules in whole milk after thermosonication treatment, Journal of Food Science. 2009;73(7):325-332.
8. Butz P, Tauscher B. Emerging technologies: Chemical aspects. Food Research International. 2002;35(2-3):279-284.
9. Calligaris S, Manzocco L, Anese M, Nicoli MC. Effect of heat treatment on the anti-oxidant activity of milk, International Dairy Journal. 2004;14:421-427.
10. Chandrasekaran S, Ramanathan S, Basak T. Microwave food processing: A review. Food Research International. 2013;52:243-261.
11. Cheryan M. Ultrafiltration and microfiltration handbook CRC Press. 1998.
12. Croguennec T, Kennedy BTO, Mehra R. Heat-induced denaturation/aggregation of β-lactoglobulin A and B: Kinetics of the first intermediates formed. International Dairy Journal. 2004;14(5):399-409.
13. Datta AK, Davidson PM. Microwave and radio frequency processing. Journal of Food Science. 2000;65:32-41.
14. Eckner KF, Zottola EA. Potential for the low temperature pasteurization of dairy fluids using membrane processing. Journal of food protection. 1991;54(10):793-797.
15. Elwell MW, Barbano DM. Use of microfiltration to improve fluid milk quality. Journal of Dairy Science. 2006;89:E20-E30.
16. Engin B, Yuceer YK. Effects of ultraviolet light and ultrasound on microbial and aroma-active components of milk. Journal of Science, Food and Agriculture. 2012;92:1245-1252.
17. Erdem YK, Yuksel Z. Sieving effect of heat denatured milk proteins during ultrafiltyration of skim milk I, The

preliminary approach, Journal of Dairy Science. 2005;88:1941-1946.
18. Euston S, Finnigan S, Hirst R. Aggregation kinetics of heated whey protein – stabilized emulsion. Food hydrocolloids. 2000;14(2):155-161.
19. FAO. Worldwide regulations for mycotoxins in food and feed in 2003. Food and agricultural organization of the United Nations. 2004.
20. FDA. Kinetics of microbial inactivation for alternative food processing technologies: Microwave and radio frequency processing food and drug administration. Department of Health and Human Services. USA. Journal of Food Science. 2000;65:32-41.
21. Franco AP, Yamamoto LY, Tadini CC, Gut JAW. Dielectric properties of green coconut water relevant to microwave processing: Effect of temperature and field frequency. Journal of Food Engineering. 2015;155:69-78
22. Goudedranche H, Fauquant J, Maubis JL. Fractionaltion of globular milk fat by membrane microfiltration. Le Lait, INRA editions. 2000;80(1):93-98. 10.1051/lait: 2000110hal-00895390.
23. Guiziou GG. Removal of bacteria, spores and somatic cells from milk by centrifugation and microfiltration techniques. Wood head Publishing Limited. 2010, 350-371.
24. Guo Q, Sun DW, Cheng JH, Han Z. Microwave processing techniques and their recent applications in the food industry. Trends in Food Science & Technology. 2017;67:236-247.
25. Hamann J. Mastitis and raw milk quality, safety and yield. Improving the safety and Quality of Milk. 2010, 246-263.
26. Hamann J. Mastitis and raw milk quality, safety and yield. Improving the safety and quality of milk. Woodhead publishing limited. 2010, 246-263.
27. Harvey EN, Loomis AL. The destruction of luminous bacteria by high frequency sound waves. Loomis laboratory Tuzedo Park and the Physiological laboratory, Princeton University. 1929, 373-376.
28. Jang HD, Swaisgood HE. Disulfide bond formation between thermally denatured β-lactoglobulin and κ-casein in Casein micelles. Journal of Dairy Science. 1990;73(4):900-904.
29. Jovanovska V, Sovreski Z, Arapceska M, Dimitrovska G, Bojkovska K, Joshevska E, *et al*. Devices for bactofugation in the function of providing quality long life milk. Banat's Journal of Biotechnology. 2017;8(15):12-17.
30. Kenyon EM, Berkowitz D, Ayoub JA. Apparatus for continuous microwave sterilization of food in pouches. Patent. 1976, US3961569.
31. Kosikowski FV, Mistry VV. Microfiltration, Ultrafiltration and Centrifugation separation and Sterilization processes for sterilization processes for improving milk and cheese quality. Journal of Dairy Science. 1990;73(6):1411-1419.
32. Lee H, Lim H, Cadwallader KR, Feng H, Martin SE. Sonication in combination with Heat and Low pressure as an Alternative pasteurization treatment- effect on Escherichia Coli K12 Inactivation and Quality of Apple cider. Ultrasonics Sonochemistry. 2013;20(4):1131-38.
33. Madec MN, Mejean S, Manbois JL. Retention of Listeria and Salmonella cells contaminating skim milk by tangential membrane microfiltration (Bactocatch process) Lait. 1992;72:327-32.
34. Martin CPC, Cavalcanti RN, Couto SM, Moraes J, Esmerino EA, Silva MC, *et al*. Comprehensive reviews in Food science and safety. 2019, 18.
35. MicVac. Quality without compromising on safety. 2021. Retrieved from https://www.micvac.com/method/equipment/
36. Mishra VK, Ramchandran L. Novel methods in Dairy processing, Book Emerging Dairy Processing technologies: Opportunities for the Dairy Industry Ch-2. 2015, 33-50.
37. Muniz I, Gou P, Picouet PA, Barlabe A, Felipe X. Dielectric properties of milk during ultra-heat treatment. Journal of Food Engineering. 2018;219:137-146.
38. Pafylias I, Cheryan M, Mehaia MA, Saglam N. Microfiltration of milk with ceramic membranes. Food research international. 1996;29(2):141-146.
39. Pardo RP, Altahona L, Palencia JP. Milk as functional food: a review. Livestock research for rural development. 2013, 25(8).
40. Patel HA, Caroll T, Kelly AL. Dairy Processing & Quality Assurance: Nonthermal preservation technologies for Dairy applications Ch 21. 2008. https://doi.org/10.1002/9780813804033.ch21
41. Raikos V. Effect of heat treatment on milk protein functionality at emulsion interfaces. A review Food Hydrocolloids. 2010;24(4):259-265.
42. Ramteke SP, Desale RJ, Kankhare DH Fulpagare YG. Thermosonication technology in the dairy industry: A review. International Journal of advanced research in biological sciences, ISSN: 2348-8069. 2020;7(1):82-89.
43. Raza N, Kim HK. Quantification techniques for important environmental contaminants in milk and dairy products. TrAC trends in analytical chemistry. 2018;98:79-94.
44. Sairem Company Website. SAIREM SAS. 2021. Retrieved from https://www.sairem.com/solutions-for-food-and-industry/rapid-cooking-of-food/
45. Salazar Gonzalez C, Martin Gonzalez MFS, Lopez Malo A, Sosa Morales ME. Recent studies related to microwave processing of fluid foods. Food and Bioprocess Technology. 2012;5:31-46.
46. Shanmugam A, Chandrapala J, Ashokkumar M. The effect of ultrasound on the physical and functional properties of skim milk. Innovative Food Sci & emerging technologies. 2012;16:251-258.
47. Shanmugam A, Chandrapala J, Ashokkumar M. The effect of ultrasound on the physical and functional properties of skim milk. Innovative Food Science & emerging technologies. 2012;16:251-258.
48. Singh H, Creamer LK. Heat stability of milk. Advanced dairy chemistry -1 Proteins, cabdirect.org. 1992.
49. Song WJ, Kang DH. Influence of water activity on inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium and Listeria monocytogenes* in peanut butter by microwave heating. Food Microbiology. 2016;60:104-111.
50. Stack A, Sillen G. Bactofugation of liquid milks. Nutrition & Food Science, MCB University Press. 1998, 280-281.
51. Stanley RA, Petersen P. Microwave assisted pasteurization and sterilization: Commercial perspective. In M. Regier K, Knoerzer & H. Schubert (Eds.), The microwave processing of foods. United Kingdom:

Elsevier. 2017, 200-219.
52. Valstra P, Jan Vouters TM, Gerts Tom. Journal of Science and Technology in the milk, Ars Lamina. 2011, 3-4, 11-12, 242-245, 276-277, 226-227.
53. Vander Horst HC. Membrane processing – Mechanization and automation in dairy technology, Sheffield academic press. 2001, 296-317.
54. Vassilios Raikos. Effect of heat treatment on milk protein functionality at emulsion interface - A review. Food Hydrocolloids. 2010;24:259-265.
55. Vjaykumar S. Effects of thermosonication on proteases and characteristics of milk and cream, M.Sc. (Food Sci and Tech.) Thesis submitted to Iowa State University, Ames Iowa. 2012.
56. Waes G, Heddeghem van A. Prevention of butyric acid fermentation by bacterial centrifugation of the cheese milk. Bulletin of the International Dairy Federation. 1990;251(16):47-50.
57. Walstra P, Wouters JTM, Geurts TJ. Dairy Science and Technology, CRC Press: Boca Raton, FL, USA. 2006.