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Study on the effects of different sterilization methods and storage conditions on milk quality

Mars KHAYRULLIN[1], Maksim REBEZOV[2]*

Abstract

Milk is processed in different ways. Milk may be heated, frozen, or condensed as it is excreted in raw milk or in order to kill pathogenic microorganisms and increase shelf life. Its protein and fat content may change. Lactose is hydrolyzed, homogenized, cholesterol is removed, or fermented cultures are added to it, and its calcium levels are increased. Some special processing operations are performed to meet the consumer's needs and the specific market situation. Due to its characteristics such as relatively long shelf life, the use of UHT sterilized milk has always been associated with the suspicion among consumers that this type of milk contains preservatives or its nutritional value is greatly reduced by applying high temperatures. In this article, while briefly referring to the nutritional value of milk, the effect of pasteurization and sterilization heat on the milk components is compared. Milk is the only food known in nature that can meet the body's basic needs for nutrients in a balanced way. Milk contains a very valuable set of proteins, sugars, fats, various minerals, especially calcium and phosphorus, and a variety of vitamins. Due to its special composition, this valuable nutrient is a very suitable environment for the multiplication of pathogenic microorganisms and the transmission of diseases from animals to humans.

Keywords: nutritional value; pasteurization; high temperatures; microorganisms.

Practical Application: Consuming healthier milk.

1 Introduction

Milk is a complete and nutritious drink that is used in various forms in daily meals and snacks. Today, per capita milk consumption in any society is one of the most important indicators of development. Cow's milk is the main milk consumed; however, in our country, sheep, goat, and camel milk are also common in some places (Brick et al., 2020; Marangoni et al., 2019).

Milk is the only nutrient known in nature that provides the body with the most nutrients it needs in a balanced way. Important components of milk include protein, calcium, phosphorus, some types of B vitamins, and vitamin A. Cow's milk protein is a high-quality protein because it contains essential amino acids that our bodies are unable to make and must supply through food. When taken at appropriate levels, dairy products can lower the risk of heart attack, boost immune system function, protect against free radical damage, and mitigate the possibility of bone loss (Verruck et al., 2019). Milk proteins are classified into two main groups: casein and whey soluble proteins, each of which consists of smaller groups (James et al., 2019; Yuan et al., 2020). Milk is a rich source of calcium, which, if consumed in sufficient quantities, provides most of the body's daily calcium needs. Also, the rate of calcium absorption increases due to the presence of vitamin D in milk. Milk has eight water-soluble vitamins and four fat-soluble vitamins and is a very good source of vitamins B2 and B12 (Scholz-Ahrens et al., 2020).

Due to its special composition, this valuable food is a very suitable environment for the multiplication of pathogenic microorganisms and the transmission of diseases from animals to humans. Therefore, due to the dangers of consuming raw milk and unpasteurized products, thermal methods are used to make it healthier. In the usual method of boiling milk, although the pathogenic microbes are killed, the nutritional value of milk, including proteins, minerals, and vitamins, is damaged; For this reason, with industrial methods of milk sanitization such as pasteurization and sterilization with UHT (Ultra High Temperature) method, in addition to sanitization, minimal changes are made in the quality and composition of milk and its nutritional value is almost completely preserved (Lamberti et al., 2018; László et al., 2018).

1.1. Pasteurization

The purpose of the pasteurization process is to kill all living pathogenic microorganisms. This industrial method involves applying 72 degrees Celsius of heat for 15 seconds, after which the milk cools rapidly to less than 5 degrees Celsius. This type of milk can be stored in the refrigerator for several days (Figure 1) (Panchal et al., 2018; Pitino et al., 2021).

1.2. UHT sterilization

In the UHT sterilization process, the goal is to kill all the microorganisms in the milk, whether vegetative or sporulated. In this method, using hot water vapor, the milk is exposed to

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[1] *K.G. Razumovsky Moscow State University of Technologies and Management, The First Cossack University, Moscow, Russia*
[2] *V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia*
*Corresponding author: rebezov@yandex.ru

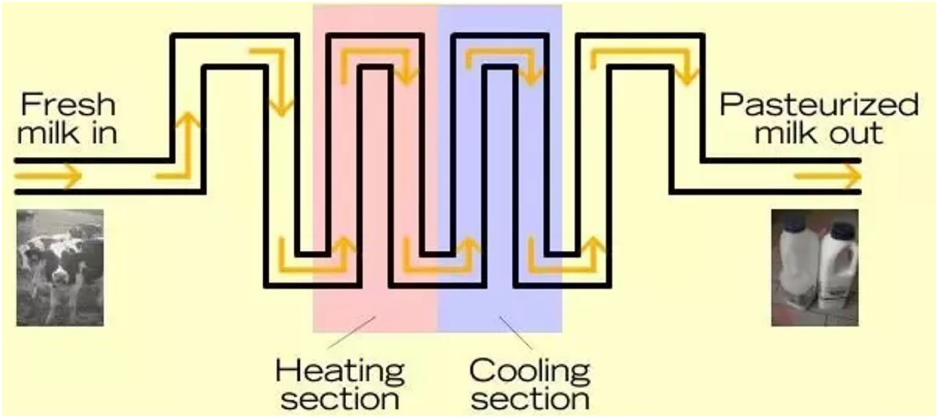

Figure 1. Pasteurization procedure.

a temperature of 135 to 150 degrees Celsius for 2 to 4 seconds (Bottiroli et al., 2020; Irfan et al., 2019). Depending on the type of method chosen, the temperature may be applied directly, i.e., by contact with water vapor, or indirectly by using heat exchangers. The milk is then cooled to a temperature of 20 ° C and packed in sterile and suitable containers under completely aseptic conditions. Direct boiling of milk for a long time damages the nutritional value of proteins due to the reduction of lysine in it, but the UHT process has little or no effect on milk proteins. Serum proteins are denatured by 50 to 90% in the UHT process according to the applied method and between 5 to 15% in the rapid pasteurization method, which has no adverse effect on the nutritional value of milk. Studies show that denaturation of cow's milk serum proteins does not cause problems in human and even infant nutrition, while children fed UHT milk gain weight faster than infants fed pasteurized milk. The UHT process has no chemical or physical changes and no negative effect on the nutritional value of fats. To prevent the separation of milk fat during the shelf life, it is necessary that the milk be homogeneous. During the UHT process and during the storage period, a reversible flow of calcium, magnesium, and phosphate ions may occur between casein and serum micelles, but there will be no mineral depletion (Cattaneo et al., 2008; Morales et al., 2000). Studies show that the amount of calcium and potassium intake of infants fed UHT milk is higher than pasteurized milk, and the phosphorus uptake is the same in both (Elbagermi et al., 2020; Qi et al., 2009; Masotti et al., 2020). The loss of milk vitamins in the UHT process compared to HTST pasteurization is negligible. There's a lot of data that conventional HTST pasteurization doesn't always result in heat-treated milk that's devoid of live Mycobacterium avium subsp. paratuberculosis cells (MAP) (Mullan, 2019). Vitamins A, D, E, and beta-carotene do not change significantly (Lorenzen et al., 2011). Some water-soluble vitamins such as riboflavin, nicotinic acid, pantothenic acid (also known as vitamin B5), and biotin are heat resistant. Vitamins B1, B6, B12, and C are slightly affected by heat. In fact, UHT heat has very little effect on the nutritional value of milk compared to its shelf life. The effect of storage on the nutritional value of milk the effect of storage on the nutritional value of milk depends on its temperature and shelf life. The cause of the changes is mainly due to heat-resistant enzymes or dissolved oxygen in milk (Akyilmaz & Kozgus, 2009; Barrett et al., 1999). Prolonged storage reduces lysine and reduces its nutritional value. For example, storing milk for six months at 30 to 37 °C reduces milk lysine by 10%. After six months of storage at 37 °C, 50% of the protein becomes a polymer, but storage under normal conditions does not notice these changes. The residue of the heat-resistant lipase enzyme, which is naturally present in milk or produced by cold-loving microbes, increases the free fatty acids in UHT milk and causes an unpleasant taste in milk. This phenomenon is significantly reduced by improving the quality of raw milk and reducing the shelf life. Fat-soluble vitamins are stored for at least three months. Water-soluble vitamins also remain stable in the absence of light. The greatest change is related to folic acid and vitamin C, the decrease of these vitamins depends on the presence of dissolved oxygen in milk. Dissolved oxygen in raw milk is 10 to 11 mg/l and in the direct method UHT process, it is 1 mg/l, in which case vitamin C and folic acid.

The possibility of various pathogens in raw milk led to extensive heat treatment on the milk (Ford et al., 1977; Muir, 1996). The use of thermal methods at the time and temperature of pasteurization eliminates all pathogens and most of the organisms that cause milk spoilage, and as a result, milk is suitable and healthy for consumption, and its shelf life will be increased. The use of more intense heat, along with aseptic packaging of milk, increases the shelf life of milk to a greater extent. Other methods of milk processing include concentrating it, adding some chemical compounds to the milk, and even freezing it. UHT processing of milk in an aseptic plant can be broken down into specific unit operations (Figure 2).

Usually, to make sterilized milk better in terms of taste and protein stability during storage, special chemical compounds called dioctyl sodium sulfosuccinates and sodium hexametaphosphate have been added to the milk the amounts of 0.015 and 0.016%.

The pasteurizer line consists of 5 units, of which part 1 is the coolant, parts 2 and 3 are the heat reducer, 4 are the preservatives, and finally, part 5 is the heater until the pasteurization temperature. The milk is directed by centrifugal pumps from the balancing tank into the pasteurizer and reaches a temperature of 45 degrees in the heat-reducing part, and then it is creamed towards the mechanical creamer there, and in terms of fat, after standardization, it goes back to the next part of the heat reducing agent. It reaches

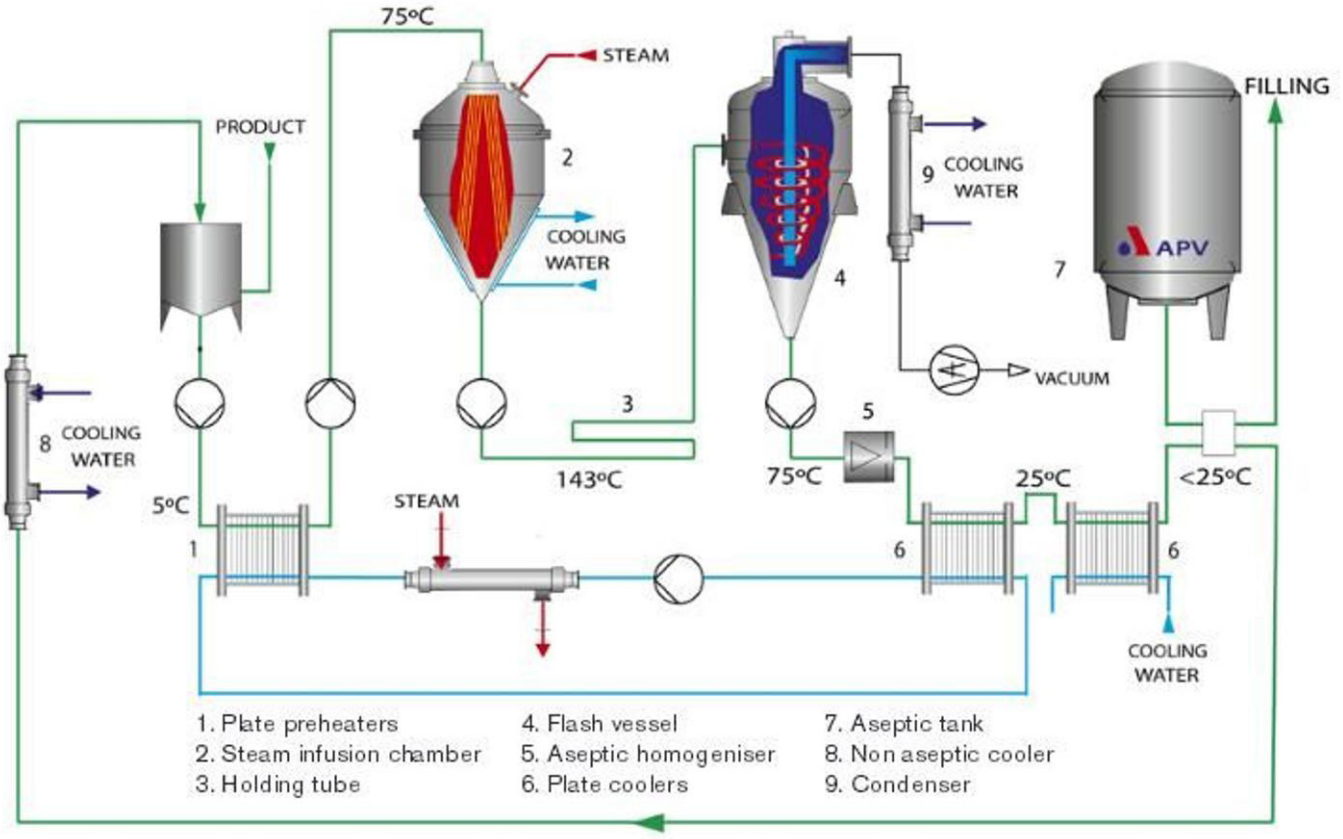

Figure 2. UHT Milk Processing in an Aseptic Plant.

a higher temperature and about 60 degrees which are prepared for homogenization, and after homogenization, it is returned to the pasteurizer, and in the 5th part, it is increased to the required temperature, and in the holding part, it is kept for 15 seconds, and the cheese is prepared for cooling (Millqvist-Fureby et al., 2001; Raikos, 2010). A tube holder can be used, and if the valve in this section has not reached a temperature of 75 degrees or less, it will be returned to the balance tank by a valve. The cream from this milk is passed through another route for use in low-fat milk or as a surplus.

2 Material and methods

In order to sterilize raw milk and keep it at room temperature for six months or more, the UHT method is usually used, where the heat source is water or steam under pressure and above 121 degrees. Of course, electric heat sources can also be used. In general, thermal methods are divided as follows (Mozaffarian, 2019; Özcan et al., 2020):

1-Indirect heat sterilization by steam or hot water

A- Heat exchange plates

B- Heat exchange pipes

C- Lathe surface heat exchange

2-Sterilize with direct heat with steam 145 °C

A. Direct injection of 145-degree steam into the VTIS or Injection valve

B- Direct injection of milk into the steam of 145 degrees, polarizers in indirect heating systems, plates, and pipes are designed in such a way that they can withstand steam pressure.

So that between some plates or around the pipes, steam temperature up to 150 degrees is applied, and therefore the valve enters the adjacent plates or pipes with a temperature of 72 to 85 degrees and is sterilized in less than 10 seconds and then into the plates or pipes. The refrigerants are transferred to bring the milk to room temperature and then to be packed aseptically.

In the direct method, 85 to 90 degrees of milk is injected into the hot steam of 145 degrees or steam into the milk in the polarizer chamber. The milk is immediately sterilized within 3 seconds and guided to cool (Coutinho et al., 2018).

Sterilization of milk in glass and autoclaves, which was common in the past, is not used today, but in general, sterilization in bottles and in the autoclave is done for 15 to 40 minutes at a temperature of 110 to 120 degrees Celsius.

Other methods of sterilization that are not very common are the hydrostatic method, which uses hydrostatic towers of boiling

water, steam, and cold water, and glass packages on the conveyor belt with chains are inserted into each tower respectively, and then the thermal process cools and evaporates. The continuous method with the steam injection of 145 degrees for 3 seconds is faster than UHT methods and maintains better milk quality (Bonislawska et al., 2013; Walowski, 2021).

2.1 Disadvantages of sterilized milk

1-Gypsum taste:

It is caused by the instability of proteins at high temperatures, and to prevent this defect in the sterilization line, a homogenization device with a pressure of 300 to 350 kg should be used to help stabilize the proteins.

2-Thermal methods:

The long thermal method produces a dark color and produces caramel and brown colors or a taste of ripeness in the milk, while in the aseptic and metadata methods, these defects are less or not present at all.

3-UHT method:

Some proteases, such as plasmin and the like, may remain in sterile milk, creating a bitter taste and unpleasant odor when the sterile milk remains in the package.

To prevent the above sulfur flavors, sterile oxygen gas is usually used in sterilized cans or packages of milk to prevent oxidation of the compounds, and therefore the taste or smell of oxidized hydrogen sulfide is eliminated.

3 Results

Milk is sterilized and packaged in accordance with the Ordinance of the Ministry of Milk, etc., based on the Food Sanitation Law. The sterilization method is stipulated by the Ordinance of the Ministry of Milk, etc., as "heat sterilize at 63 °C for 30 minutes by holding type, or heat sterilize by a method having a sterilization effect equal to or higher than this". The sterilization methods are roughly divided into five, as shown in Table 1.

In addition, as equipment for heat sterilization, there is an indirect heating method that heats milk without contacting the heating heat source and a direct heating method that brings heated steam into contact with milk. For the indirect heating method, a plate-type heat exchanger (a closed corrugated plate heat exchanger that connects the preheating part, the heating part, and the cooling part) is used, and the plate type that sterilizes milk when it passes between the plates, etc. Most of the continuous pasteurization (LTLT), high-temperature short-time sterilization (HTST), and ultra-high temperature instant sterilization (UHT) are performed by this method. On the other hand, the direct heating method includes a steam infusion method in which milk is blown into heated steam to sterilize it and a steam injection method in which heated steam is blown into milk to sterilize it.

Most of the milk sold on the market is sterilized milk, and whether it is the low or high temperature during sterilization, it will have a certain impact on the nutritional value of milk, and the real fresh milk should be unprocessed milk. The milk that advertises fresh milk in the market cannot actually be called real fresh milk. Heating has the greatest impact on the nutrition of milk is water-soluble vitamins and proteins. During the heating process, about 10% of the vitamin B complex and 25% of the vitamin C are lost, and the deeper the heating, the greater the nutrient loss. In addition, the most nutritious whey protein in milk has about 10% of whey protein denaturation during low-temperature sterilization. In UHT sterilized milk, 70% of whey protein may be denatured. In terms of sterilization methods, it is basically divided into two categories: pasteurized milk and normal temperature milk. They each have their own different characteristics:

Pasteurized milk usually means heating 75 °C~80 °C, which instantly kills harmful bacteria in the milk while preserving the pure taste. The advantage is that it contains more nutrients, but the disadvantage is that this method cannot eliminate all microorganisms in the milk. Therefore, refrigeration is required, and the shelf life is relatively short, usually only a few days. At present, milk pasteurization is the mainstream in European and American countries.

Table 1. Various sterilization methods and their effects.

Sterilization method	Overview	Bactericidal effect
Cold retention sterilization (LTLT)	How to sterilize raw milk by heating at 63-65 °C for 30 minutes	Not all bacteria can be killed, but bacteria that are harmful to humans are killed, so refrigerated storage allows you to drink with confidence for a certain period of time.
Continuous pasteurization (LTLT)	Method of continuously heating sterilizing raw milk at 65-68 °C for 30 minutes or more	
High temperature holding sterilization (HTLT)	A method of holding raw milk and sterilizing it by heating it at 75 °C or higher for 15 minutes or longer.	
High-temperature short-time sterilization (HTST)	A method of continuously heating sterilizing raw milk at 72 °C or higher for 15 seconds or longer.	
Ultra-High Temperature Instant Sterilization (UHT)	A method of heat sterilizing raw milk at 120 to 150 °C for 2 to 3 seconds. More than 90% of the milk sold in Japan is processed by this sterilization method.	This method is the only method that kills thermostable sporogenic bacteria and has a sterilizing ability that is 10,000 times higher than that of low-temperature holding sterilization (LTLT).

Room temperature milk (sterilized milk) uses an ultra-high temperature of 135 °C to 150 °C for 4 to 15 seconds of instantaneous sterilization to completely kill the bacteria in the milk; because there are no microorganisms in the milk, it can be It is stored at room temperature, and the shelf life is relatively long, generally up to three months, but it also destroys nutrients.

Before choosing milk or dairy products, most consumers will first watch manufacturing date and shelf-life packaging and storage of drinking as a basis, in general, in the processing of fresh milk will be through pasteurization (Pasteurization), Reduce the number of internal bacteria to a safe edible value. According to the method of sterilization, the milk sold in the market is divided into long-term low-temperature sterilization or high-temperature short-time sterilization of fresh milk and ultra-high-temperature sterilization long-term milk.

Although the process of milk sterilization can kill most of the bacteria, it cannot completely eliminate spore-forming bacteria such as Bacillus, Paenibacillus, and Viridibacillus. In the face of adversity, endospores are produced and dormant, waiting for the end of adversity to continue to multiply. The refrigerated storage after sterilization can inhibit the growth of most microorganisms. However, some Psychrotolerant spore-formers can multiply in low-temperature environments, which may cause lactate failure in cattle and cause gastrointestinal discomfort after drinking. These Bacillus-like bacteria can be ubiquitously present in the pasture, soil, cattle excrement, and even milking equipment and other dairy processing links. In addition to reducing the number of bacteria through microfiltration during processing, controlling the temperature of the processing and storage environment is also the key to slowing down the growth of bacteria.

A Monte Carlo simulation is established by observing the 30-day growth curve of Bacillales at six °C; the Monte Carlo simulation model is used to estimate the growth status of bacteria under different sterilization treatments and storage temperatures and to estimate the rancidity rate of milk. Actual experiments can verify the accuracy of the model. In addition, the team used the model to predict the results of 1.9 liters of milk that had been sterilized at a high temperature for a short time and stored at six °C and 4 °C for three weeks. 66% of the milk stored in a 6°C environment was rancid, the bacteria count is greater than 20,000 CFU/ml), and only 9% of fresh milk refrigerated at 4°C is rancid, indicating that low-temperature storage can extend the shelf life.

In the next 5 to 8 years, consumers may not find the expiration date on the milk carton but will use the barcode on the product to understand the actual expiration date in the production history. In the future, the temperature indicator can also be placed on the packaging to monitor the temperature in the logistics or storage process and accurately predict the shelf life (shelf life). The construction of this model will help consumers and logistics companies to monitor individually or in batches.

4 Discussion

Sterilization entails a high-temperature heat treatment followed by packing in airtight containers, either before or after the heat treatment. Aseptic packing is critical if heat treatment is performed thereafter. Sterilized milks are those that have been sterilized. Although sterilized milk is believed to be commercially sterile, it is not always devoid of germs. Those microorganisms that survive the heat treatment, on the other hand, are unlikely to grow during storage and ruin the milk. Sterilization methods for milk and milk products include continuous sterilization, in-container sterilization in autoclaves at 105 to 120 degrees Celsius for 10 to 40 minutes, and ultra-high temperature treatment (UHT). When the temperature is greater, the holding duration is cut in half. The goal of sterilization is to create a long-lasting product by destroying germs that are harmful to people's health and those that can degrade food. The US Food and Drug Administration specifies temperature-time combinations that guarantee a 12-log decrease in Clostridium botulinum spores. However, depending on the product, the thermal treatment parameters necessary to achieve a 12-log decrease of Clostridium botulinum must be expanded. For example, the criteria for sterile milk are based on a 9-log reduction of thermophilic spores. A variety of physical and chemical changes occur in the milk as a result of the high temperature employed (Table 2). Direct UHT-sterilized milks have a shelf life of 12 to 6 months at 4 and 20 degrees Celsius, respectively, but indirect UHT-sterilized milks have a shelf life of more than 12 months at 4 degrees Celsius and up to a year at 20 degrees Celsius. UHT and retort sterilization both have the same sporicidal effect. The UHT method has much fewer negative impacts on color, flavor, and nutritional value.

Table 2. During the manufacture and storage of sterilized milk products several physical, enzymatic, and chemical phenomena can occur.

Property or component	Phenomena
Lactose	Maillard reaction, isomerization to lactulose
Lipids	Lipolysis by heat resistant or reactivated lipases during storage
Minerals	Decrease in proportion of ionic Ca and Mg due to precipitation as PO_4 during processing, partial reversal of the latter during storage
Proteins	Unfolding, denaturation (50-85%), formation of complexes with κ-casein, increase in proportion of non-sedimentable casein, proteolysis during storage, increase in proportion of NPN and noncasein N, polymerization during storage, formation of lactulosyl lysine and fructosyl lysine during storage
Rennet coagulation time	Increases with both UHT and retort sterilization
Sensitivity to alcohol	UHT milk: increases during storage Retort sterilized milk: unchanged during storage
Sensitivity to Ca	UHT: significant increase during storage Retort: some increase during storage

5 Conclusion

Milk is required to indicate the pasteurization temperature and pasteurization time on the container.

In recent years, heat-resistant spore-forming bacteria, bacteria against which antibiotics do not work, new pathogens, and bacteria that propagate even at low temperatures have been discovered one after another. In order to provide safer milk, dairy manufacturers and dairy farmers, distributors, and the government are working together to conduct food safety research and quality checks. At local health centers, on-site inspections of factories and sampling inspections of milk on the market are also conducted, and efforts are being made to improve the safety of milk.

Traditionally, fresh milk sterilization methods can be divided into low temperature and longtime sterilization (Low Temperature and Long Time, LTLT), standard high-temperature short-time sterilization (High-Temperature Short Time, HTST), loose high-temperature short-time sterilization (Higher- Heat Shorter Time (HHST), Ultra High Temperature (UHT).

(1) Low-temperature and long-term sterilization (LTLT) means that the sterilization temperature is controlled between 62 °C and 65 °C and kept for 30 minutes. This type of sterilization method can better retain nutrients such as vitamins and whey protein. According to Hualien County Agriculture Department, Data show that about 10% of the vitamin B complex and 25% of the vitamin C will be lost during the heating process of milk. This type of sterilization method is less economical and is only suitable for small-scale production. Although the low temperature has less effect on the function of whey protein and vitamins, it leaves more bacteria than other sterilization methods. Follow-up refrigeration must ensure that the entire process is below 7 °C, and the shelf life is only about 7 days.

(2) The standard high-temperature short-time sterilization method (HTST) means that the sterilization temperature is controlled between 72 °C and 75°C for 15 seconds. It is the main sterilization method for fresh milk in Europe, America, and other countries. The advantage of this sterilization method is that it can retain. There is more whey protein, but a few bacteria will remain after sterilization, so the whole process needs to be refrigerated below 7 °C. In order to ensure the safety of fresh milk, in addition to adopting the HTST sterilization method, the industry also uses membrane filtration equipment to filter out bacteria and reduce the number of bacteria. The storage period is approximately 12 days.

(3) The loose high temperature and short time sterilization method (HHST). In the milk sterilization process, the industry also uses HTST sterilization equipment, but in order to ensure the safety of fresh milk during transportation and sales, the sterilization temperature is increased to 80-90 °C. The time is more than 15 seconds to reduce the number of bacteria, but it is not as strong as the ultra-high temperature and short-time sterilization method.

(4) Ultra-high temperature short-time sterilization (UHT) means that the sterilization temperature is increased to between 125 °C and 135 °C, and the time is up to 2 to 3 seconds. This type of sterilization method can kill almost 99.9% of the bacteria. To reduce the deterioration of fresh milk due to changes in refrigeration temperature during transportation and sale and ensure fresh milk safety. Depending on the filling conditions, the fresh milk is refrigerated in an unopened state. The storage period can be up to 15 to 60 days. In fact, UHT-sterilized fresh milk can be preserved for longer if it is filled with aseptic. However, in order to emphasize freshness and locality, the packaging usually states that the freshness period is only 12-14 days.

In fact, imported long-lasting milk also adopts the UHT sterilization method. The UHT sterilization time of long-lasting milk will be longer, and the filling and packaging must be carried out under sterile conditions. This is to ensure that the milk is almost sterile so that it can be stored at room temperature for more than half a year. The shelf life and whether it needs to be kept under refrigeration is usually used to determine whether imported milk is “fresh milk” or “long-lasting milk” as indicated on the product packaging. It is one of the easiest ways to distinguish.

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