HANDBOOK

The role of raw milk quality in UHT production

CONTENTS

Learning how milk quality affects your products

Who is this booklet for?

This booklet is for dairy management and those responsible for quality management who want to improve the quality of their UHT dairy products, while optimizing their plant operations and holding costs down. It gives you practical information and guidance in managing UHT milk products – from farm to shelf.

Why is milk quality so important?

► The quality of incoming raw milk – and its bacterial content – affects more than just the processability of your dairy products. It also has an effect on the quality of your products when they reach stores, and their shelf life.

Milk quality affects the efficiency and cost-effectiveness of your plant's processing machinery, in particular your heating units. Poor quality milk can clog heat exchangers, causing processing interruptions and requiring extra cleaning time – with all the costs associated with these two events.

Getting a good grip on milk quality means you control your reputation with your customers – and protect and build your business.

What can I do to improve my dairy operations?

The first step you can take is to **understand where your milk supply might be vulnerable**. Variations in the quality of incoming raw milk can create problems. Do you know enough about the raw milk coming in to your plant? This handbook will point out the major sources of quality problems.

The second step is to **monitor the quality by measuring it at key points**. We point out many simple measurements you can perform without advanced laboratory equipment. Of course if you have access to more advanced lab equipment, you can test even more.

The third step is to **provide feedback to your milk suppliers**. They need to know whether they have to take steps to deliver higher quality milk to you, or else risk being excluded from your list of suppliers.

Where can milk quality go wrong?

Milk – more than meets the eye

Milk is a highly complex biological system, containing microorganisms, fats, sugars, proteins, minerals, vitamins and other elements. The milk composition and microbiological quality determine the milk's suitability for processing and human consumption. Exactly what kinds of microorganisms are present and what the temperature conditions are, will determine the final quality of the milk.

Milk quality strongly affects the processability of milk. Poor milk quality will cause increased fouling in heat exchangers and thus reduce production time. The increased fouling also makes it harder to clean – cleaning must be done more often and the time for cleaning is prolonged. Poor milk quality can also affect the UHT product shelf life, resulting in:

► Fat separation
► Sedimentation
► Gelation
► Off flavours and off smell

Example of UHT milk with fat separation produced from raw milk with several million bacteria after storage one month at 22-23 °C

Dairy farm operations

The contamination of milk with microorganisms during milk production on the farm will have the greatest influence on the milk quality and the quality of the finished products. The health of the individual cows, barn maintenance, good hygiene at milking and proper cleaning of milking equipment and milk storage vessels are of utmost importance to minimise the contamination of the milk with microorganisms.

Milk from the mammary cells in the udder of a healthy cow is free from microorganisms. But as soon as the milk passes out through the teat channel it starts getting contaminated by microorganisms. In order to minimize the risk of bacterial growth it is important to cool down the milk as soon as possible to a low temperature, preferably 4°C or lower.

Transportation

There are many different ways of transporting milk in the world, and how it is transported and stored has an impact on quality. Using clean milk containers and keeping the milk properly refrigerated at low temperatures (2-4°C) will limit its destruction. But there are some microorganisms that can grow even at refrigerated temperatures.

Handling and processing at the plant

If the milk cannot be cooled fast and stored at refrigerator temperatures until processed the microorganisms in the milk will start growing rapidly. But even if the milk is cooled to refrigerator temperature, bacterial contamination on the farm should be avoided. Some microorganisms can grow even at refrigerated temperatures, forming heat-resistant enzymes that may challenge the quality and shelf life of the filled product.

We recommend thorough testing of incoming milk before beginning UHT processing. Some milk may not be appropriate for UHT, but may be appropriate for other uses.

The fastest-growing microorganisms are the bacteria that can feed on the milk sugar (lactose) and ferment the sugar to lactic acid. Milk has a good buffering system and can handle some acidification without causing quality problems. But if a lot of lactic acid forms, the acidity of the milk increases and it will reduce the milk's ability to be heat-treated especially at UHT temperatures; the low heat stability causes deposition in the heat exchanger and shortens production time. Already pH changes from 6.70 to 6.55 causes a very negative impact. In extreme cases it will be impossible to process the milk at UHT temperatures at all, since the milk might precipitate in the heat exchanger or in the package.

There is a group of microorganisms, Gram-negative psychrotrophs, that can grow even at refrigerated temperatures, and that can form heat-resistant enzymes. Enzymes are substances which can attack fat or proteins in milk and can change the taste and stability of the UHT product during storage. They are produced and released by the bacteria into the milk and are extremely heat-stable. They even survive heat treatments that kill all microorganisms. After a few months of storage, the activities of the enzymes will cause either a bitter or rancid taste, fat separation, sediment in the package or gelation of the UHT product. The higher the number of Gram-negative psychrotrophic bacteria in the raw milk – and the longer they have been present in the milk – the shorter the shelf life of the product will be.
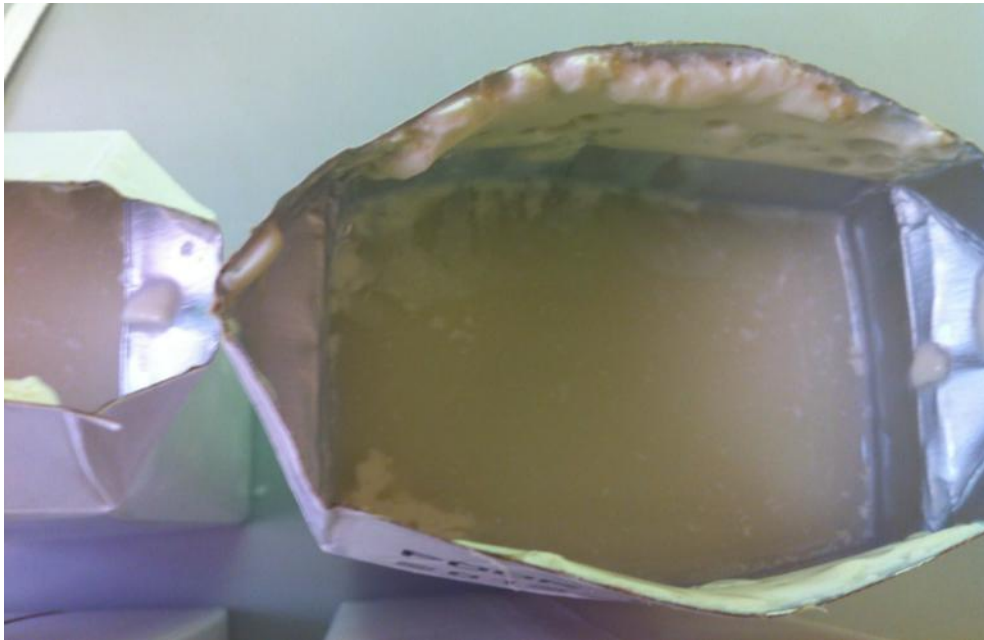
Sample of UHT milk suffering from strong enzymatic activity.

Packaging

UHT milk that has been produced aseptically must be packaged aseptically (without any microorganisms entering the product) to ensure quality all the way to the retail shelves. Hermetically sealed containers made of paper board and plastic are leak-proofed and protect against air, light and bacteria entering.

Know your bacteria

Measuring bacteria

Bacteria are counted in terms of **colony forming units**, or CFUs per millilitre. Top-quality cow's milk will have less than 100,000 cfu/ml and a pH of 6.7 – 6.9.

Problems in producing UHT milk with a long shelf life start when the bacterial count reaches 1 million cfu/ml. If the milk contains more than 5 million cfu/ml, even if the pH is in the normal range, there is a large risk that the milk contains too high numbers of bacteria that have produced heat-resistant enzymes, making the milk unsuitable for UHT production, due to risk of short shelf life.

Lactic acid bacteria
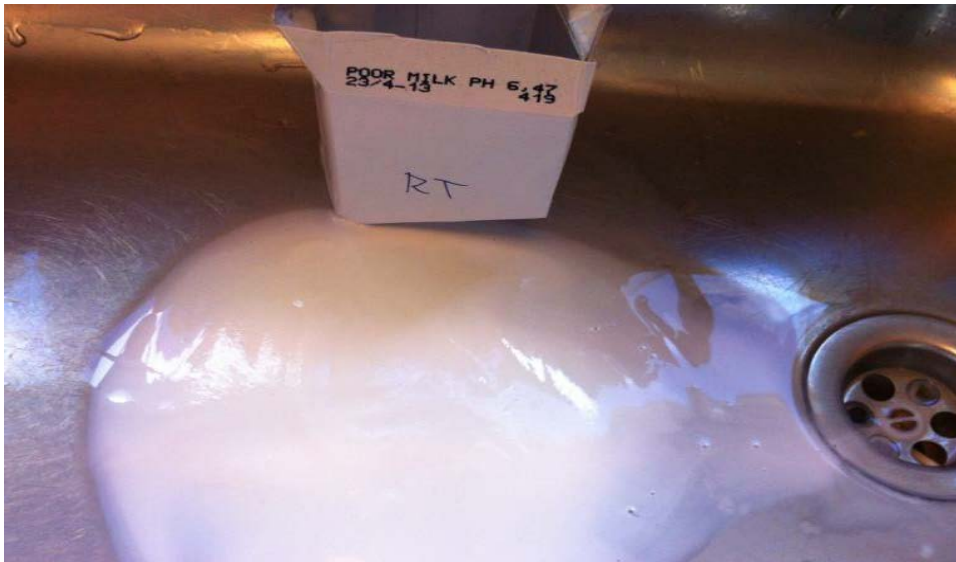
Content from UHT milk package based on raw milk of pH 6.47 on the day after production. Lactic acid bacteria are present on the skin of the cow, on bedding material and feed. They do not grow at low temperature and do not survive pasteurization of milk. Lactic acid bacteria do not produce heat-stable toxins or enzymes. Selected strains of lactic acid bacteria are added to milk on purpose to produce fermented milk products and cheese.

Lactic acid bacteria will ferment lactose to lactic acid. This will cause acidification of the milk and a decrease in pH value. Even a small change downwards in pH (0.1-0.2 pH units) can cause problems with fouling in the heat exchanger and shortened production time. A larger drop in pH will make the milk impossible to process at UHT temperatures since the milk can coagulate during the heat treatment or in the package.
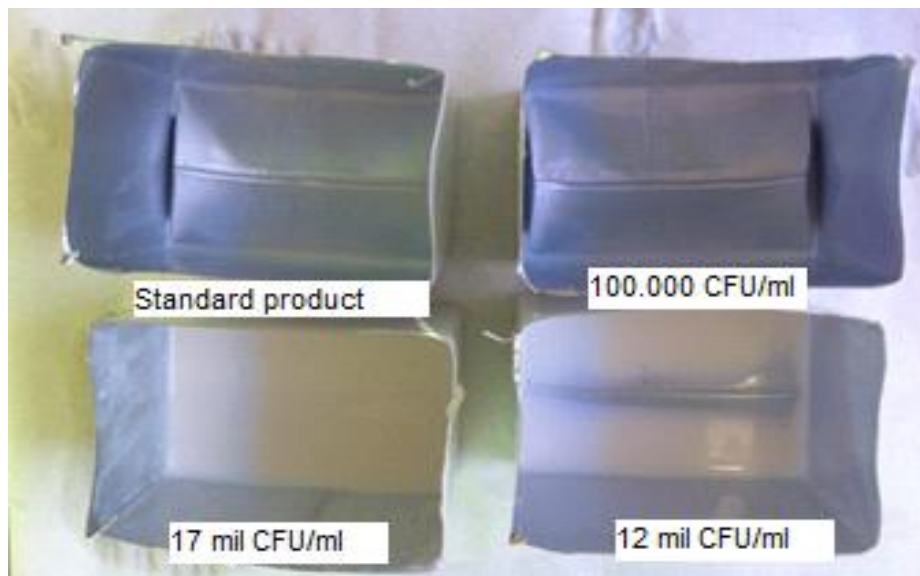
Gram-negative psychrotrophic bacteria

Gram-negative psychrotrophic bacteria can grow at refrigerator temperatures, but grow optimally at a higher temperature. Originally these microorganisms are present in dirty water. Since these bacteria can grow at low temperature they contribute heavily to the total count in refrigerated raw milk. The longer the milk is stored at low temperature, the larger the fraction of psychrotrophic bacteria will be.

Gram-negative psychrotrophic bacteria may attach to the surface of milking equipment and form very thin layers, called biofilms. If the milking machines are not cleaned properly the bacteria will grow there. The presence of biofilms elevates the number of these bacteria in the raw material, as they probably entered the raw material during milking. Most of them do not ferment lactose to lactic acid, and thus do not lower the pH of milk. They do not survive the pasteurization process but they can, however, produce heat-stable enzymes that are not inactivated in UHT processes, thus causing off-flavours, fat separation and early gelation formation, as described earlier.

To minimize the problem with psychrotrophic bacteria it is important to have a good cleaning routine for milking equipment and storage tanks/containers, and to use clean water.

In order to maintain good raw milk quality, keep the milk properly refrigerated but limit the time at refrigerated temperature before processing at the dairy.

UHT milk based on raw material with different number of psychrotropic bacteria after 2.5 month storage at ambient temperature 22-23 °C.

Sporeforming bacteria

Some microorganisms, when placed in what should be inhospitable environments, can gather the most important parts of their cell into a nucleus – a spore – and in doing so survive in very uninhabitable conditions for extremely long periods of time – even for years. Spores are very heat-resistant. Their count and resistance are critical to choosing the right UHT conditions in which to eliminate them.

To avoid a high numbers of spores in the milk it is important to have a good teat cleaning routine at milking. Keep the cows as clean as possible by having clean cubicles, and as dry and hard alleys as possible where the cows pass every day. For anaerobic spores – spores that grow in the product without the presence of oxygen – the quality of feed such as silage is the most important factor for the amount of spores in the milk.

There are also some psychrotrophic Gram-positive sporeforming bacteria that survive pasteurization and can limit the shelf life of pasteurized milk. But none of these will survive UHT processing conditions. These bacteria are mainly present in soil.

Thermoduric bacteria

This type of bacteria is part of the skin flora of the cow and can also be found in bedding material and feed. They may cause biofilms in milking equipment if cleaning is bad, but not extremely bad. Thermoduric bacteria do not influence the total CFU count very much, but since they survive pasteurization they influence the count after pasteurization and are important for the total count in milk powder.

Some thermoduric bacteria are psychrotrophic, which means they can survive pasteurization and grow in pasteurized milk. They can also form biofilms in the processing equipment if cleaning is not optimized.

Pathogenic bacteria

Pathogenic bacteria may cause illness in humans. All known pathogenic bacteria are killed at pasteurization temperature, but some can produce toxins that are extremely heat-resistant and that are not inactivated in UHT processes.

Most pathogens cannot grow at low temperature. An exception is *Listeria monocytogenes*, but this bacterium grows at a slower rate than psychrotrophic Gram-negatives due to competition.

Some human pathogens may also cause mastitis – udder infection in cows.

To minimize the risk of pathogenic bacteria in milk, the cows should be as clean as possible at milking and the milking equipment and storage tank/containers should be cleaned properly. The milk should be kept at low temperature until processed.

Mastitis bacteria

Mastitis bacteria cause udder infections in cows. Mastitis will cause an increase of somatic cells, white blood cells, in the milk. The somatic cells ally themselves with heat-resistant enzymes in the milk called plasmin, which can degrade proteins in a similar way to bacterial enzymes. This can cause bitterness, gelation and sedimentation of UHT milk.

Milk from cows with mastitis also has an altered composition of proteins and salts. More whey proteins and higher salt concentration make the milk more prone to fouling. Some of the bacteria causing mastitis can also be human pathogens and form heat-resistant toxins, for example *Staphylococcus aureus*.

The maximum amounts of somatic cells allowed are: 400,000 cells/ml in the EU and 750,000 cells/ml in the US.

Yeast and mould

Yeast and moulds can be present in raw milk. They grow slowly at low temperature and relevant species are inactivated by pasteurization at 72°C/15 s. Presence of mould toxins in milk is a feed-related problem. Aflatoxin B1 is a liver toxic and carcinogenic mould toxin that can be present in high amounts in feed. The cow metabolizes the aflatoxin B1 into aflatoxin M1 and secretes it into the milk. Aflatoxin M1 is also liver toxic and carcinogenic and many countries have legal limits for the presence of aflatoxin M1 in milk. Aflatoxin M1 is not inactivated by UHT processing conditions.

Dairy farm operations

The quality of your milk products is affected by the operations of the dairy farmers who deliver your raw milk. Dairy farming is one of the oldest agriculture professions, and its goals are timeless – having a healthy herd of animals that produce as much high quality milk as possible. There are many preventive actions that contribute to successful dairy farming, as we outline below. You need to be aware of them, because contamination of milk with microorganisms during milk production on the farm will have the greatest influence on milk quality and the quality of the finished products.

Milk from the udder of a healthy cow is free from microorganisms. But as soon as the milk passes out through the teat channel it starts getting contaminated, as milk is a very good environment for microorganisms to multiply in. The level of contamination is dependent on the teat cleaning procedure at milking and how dirty the cow is. Cleaning the teats with a moist cloth followed by a dry cloth gives the best result. Hand milking also requires good hand hygiene of the person doing the milking.

Another large source of microorganisms is mastitis – udder infection in the cow. Mastitis also changes the composition of raw milk, causing a reduction in milk sugar lactose and changes to the salt balance of the milk. The fat in milk is also affected, leading to the production of Free Fatty Acids and causing rancid flavours.

After milking, rapid and efficient milk cooling is essential for preserving milk quality. Milk leaves the udder at approximately 35 °C and the heat in fresh milk must be quickly removed. Milk retains a natural resistance to bacteria immediately after extraction, but only rapid cooling to a low storage temperature, preferably around 4 °C, prevents or minimises further micro-organism growth. A high level of hygiene is also required on all storage and transportation vessels for the raw milk.

A good **barn environment** is essential for optimum cow comfort, health and productivity, and to protect the quality of the milk delivered to your plant. This is especially true of modern, high density, dairy housing, and even more so when cows are indoors most or all of the time. A good barn environment must have adequate ventilation, lighting and pest control. Depending on regional climates, this usually requires a combination of air inlets, fans, control boxes and biosecurity products to control insects and rodents. Good barn hygiene reduces hoof problems and veterinary costs.

Cleaning and disinfecting the animals' environment is vital, and this involves maintaining hygiene on many surfaces, such as floors, walls, grooming equipment, halters and collars, cow mats, fences, gates and partitions. Efficient manure handling is a key point for day-to-day operations. Clean animals reduce preparation time for cleaning the udders of the cows, making the milker's time more efficient.

Equipment must be maintained and replaced. Older stalls might not meet modern cow traffic standards, cow size or milking demands. Outdated milking equipment will cause problems with milk quality and udder health. Since milking equipment is in operation for 1,500 to 2,000 hours a year, it must be up-to-date and in good condition to perform well.

Cows should have their body condition evaluated regularly, where the farmer uses sight and touch to evaluate the amount of fat covering the loin, rump and tail head with a score from one to five. Body condition is an indication of how much energy a cow has stored for future use, and it can help understand the past nutritional status of the cow, the reasons for good or poor milk production and reproductive performance, and the challenges to come. The monthly changes in body condition tend to be more highly correlated with health, productivity, and reproduction than a cow's actual body condition on any particular day.

A cow has to ruminate for seven to ten hours a day. 50 percent of cows lying down must be ruminating – otherwise there is not enough effective fibre in the ration. A diet low in fibre and high in starch may result in lameness caused by diet-induced laminitis. Increased time spent lying down in a clean dry comfortable stall can contribute to cow health. Cattle housed in wet, manure contaminated conditions are more likely to suffer infectious diseases of the hoof, such as interdigital necrobacillosis (foot rot), heel horn erosion (HHE) and papillomatous digital dermatitis (PDD or heel warts).

Cleaning water tanks at least once a week can have an impact on milk production. Cows like to drink clean, fresh water, and are even more sensitive than humans to poor quality water. Water quality can be compromised by high levels of bacteria, chemicals, organic matter and minerals. When testing water quality, the farmer should take samples from the cow's water tank or water bowl; not from the well. Samples to be analysed for bacteria should be kept cool (on ice) and delivered to the lab within six hours.

Modern milking and animal care solutions are designed to give excellent milking hygiene, improve udder health, reduce labour costs, meet other current needs and allow for future expansion. For more information on all aspects of dairy farming equipment and animal care, please see www.delaval.com/en/Dairy-knowledge-and-advice.

Transport to the plant

There are many different ways of transporting milk in the world, and how it is transported and stored has an impact on quality. Keeping the milk in containers that have been badly cleaned will increase the number of microorganisms and that can influence the quality of the milk.

If jerry cans are used for transporting milk it is important that they are clean and that no chemicals or gasoline have been stored in them before.

As we said earlier, storage temperature is of utmost importance. Keeping the milk properly refrigerated at low temperatures (2-4°C) will limit its destruction. However, there is a group of microorganisms that can grow even at refrigerated temperatures. These can influence quality in a way that dramatically shortens the final product shelf life.

Detailed information on how to cool milk in an efficient way is available at www.delaval.com/en/Dairy-knowledge-and-advice.

Different ways of collecting milk.

Handling and testing milk before processing

Handling at cool temperatures

We recommend that you measure the temperature of incoming milk. It can indicate if it can be chilled for storage, or must be heat treated more immediately.

When milk arrives at the dairy, it should be either chilled down to 2°C as quickly as possible in order to prevent the growth of microorganisms, or else thermised prior to cooling down. Thermisation – heating up to 63 - 65°C for 15 seconds – kills most of the vegetative microorganisms but not all pathogens. It is a heating process which eliminates most of the microorganisms that can grow at low temperature and thus enable cold storage of milk for another 1 - 2 days without major deterioration of the quality. But as the process does not kill ALL pathogens, it is not allowed to use thermised milk for consumption directly.

In general terms raw milk should be heat-treated as quickly as possible, in order to maintain good quality of the final products.

Silo tanks

The number and size of silo tanks are determined by the raw milk delivery schedules and volume of each delivery. In order to operate the plant continuously without stoppages due to lack of raw material, a sufficient supply of raw milk must be available.

Preferably, the milk should have been stored for at least one hour before being processed, as natural degassing of the milk takes place during that period of time. Short periods of agitation are acceptable, but agitation is not really needed until about 5 – 10 minutes before the silo is to be emptied, to equalise the overall quality. This avoids interference with the natural degassing process.

Why test milk?

Testing milk can help you improve your dairy operations in a number of ways:

► You can divide the milk you receive into **different quality grades**, which can be suitable for different purposes.
► You can **avoid quality issues**, health problems and damage to your dairy's reputation.
► You can **save on resources and processing costs** by only using milk that will stand up to processing, transport and storage.
► You can **provide valuable feedback to your raw milk suppliers**, and encourage them to improve their milking and transport operations – and thus improve the efficiency, quality and profitability of your own operations.

What can testing tell you about milk quality?

A variety of tests are available to illuminate different aspects of milk quality and help you sort milk into different quality grades. Some of them may be more relevant to your dairy operations than others, depending on your location, climate, equipment, supply chain, etc.

Physical and chemical tests

No.	TEST NAME	BENEFIT
1	pH value	Identify microbiological spoilage as well as chemical contamination.
2	Milk acidity by titration	Identify the result of an intense microbiological metabolism in the sample and obtain a rough estimate of the milk quality.
3	Alcohol	You can assess the stability of milk proteins
4	Freezing point	Helps you detect milk that has been diluted by water, as well as judge milk stability.
5	Density	Allows you to estimate the solid content.

Microbiological tests

No.	TEST NAME	BENEFIT
6	Methylene blue reduction (Resazurin)	Rough identification of the total amount of microorganisms present in raw milk.
7	Total Aerobic Plate Count for mesophilic aerobes	Determining the risk of spoilage and lower quality products from organisms growing at temperatures between 30-40°C. Relevant to milk heat sensitivity.
8	Total Spore count and Heat-resistant spore count	To enumerate total spores and heat-resistant spores associated with spoilage. Important for choosing right conditions for high temperature treatment.
9	Total counting of psychotropic aerobes	Detecting organisms growing at temperatures between 0-30°C. Relevant to enzymatic spoilage during storage.

In the following sections, we explain these tests in detail.

Physical and chemical tests

Test 1 - pH value

Measuring the pH value of milk is a preliminary quality control step to identify microbiological spoilage as well as chemical contamination.

Milk normally has a pH of about 6.7 (6.6-6.8) at 20°C. Small decreases in pH (0.1-0.25 units) have a large impact on the running time of the heat exchanger.

Of course, there may be other factors than pH that can influence running time or product stability, such as a high total bacterial count, or incorrect salt balance.

Testing the pH of milk at 20°C yields the following gradation categories.

pH	Milk quality judgment for further processing
< 6.45	Not recommended for UHT processing due to very short running time and unstable product after heat treatment
6.45-6.55	Risk for very short running time and unstable product after heat treatment
6.55-6.65	Risk for shortened running time
6.65-6.80	Normal pH for milk is this range
> 6.80	Potential risk for mastitis. Check somatic cell count

If your raw milk quality ranges from a pH of 6.45 to 6.65, please contact Tetra Pak in order to determine the most suitable design of your UHT plant.

What you will need

► Beaker (50 ml)
► Buffer solution (pH 4.0 and 7.0)
► Potassium chloride solution (saturated or specified by the manufacturer)
► Distilled water
► pH meter (Potentiometer)
► Paper to dry the electrode

Procedures

1. Calibrate the pH meter (potentiometer), first in a pH 7.0 buffer solution, then in a buffer solution pH 4.0 (wash the electrode with distilled water between solutions).
2. After calibration, wash the electrode with distilled water, dry it by lightly blotting and dip it into the beaker containing the sample at 20 ±2°C.
3. Proceed to read the pH value
4. Wash, dry and store the electrode in potassium chloride solution

Guidelines

The pH value is dependent on the temperature (decreases with increased temperature) and should preferably be measured at 20 ±2°C. If the pH is measured at low temperature it should always be measured at the same temperature and be recalculated to the pH at 20°C. The accuracy of pH measurement is about ± 0.05 pH units.

Test 2 - Milk acidity

To evaluate the acidity of milk samples submitted to normal solution of NaOH, in order to identify the result of an intense microbiological metabolism in the sample and obtain a rough estimate of the milk quality.

Potential acidity can be expressed in several units: Soxhlet-Henkel degrees (°SH), Dornic degrees (°D), Thorner degrees (°Th) and lactic acid percentage (% l.a.) are the most common. A Soxhlet-Henkel degree is obtained by titrating 100 ml of milk with a sodium hydroxide solution N/4, every millilitre corresponds to 1°SH. The newly formed milk must have 6.4 to 7.2°SH.

The Dornic degree is obtained by titrating 100 ml of milk with a sodium hydroxide solution N/9, every millilitre corresponds to 1°D. The normal value of the potential acidity of milk is between 15-22 Dornic degrees.

The Thorner degree is obtained by titrating 100 ml of milk with a sodium hydroxide solution 0.1N, every millilitre corresponds to 1°Th.

Lactic acid percentage is obtained by dividing the Dornic degree by 100.

Conversion table

Acidity degree	°SH	°Th	°D
°SH	1	2.5	2.25
°Th	0.4	1	0.9
°D	0.444 (= 4/9)	1.111 (= 10/9)	1

What you will need

► Volumetric pipettes (10 ml)
► Erlenmeyer flask (125 ml)
► Graduated burette (10 ml)
► Sodium hydroxide solution NaOH N/4 or 0.1N or (N/9)
► Alcoholic solution of phenolphthalein: 2 g of indicator in 75 ml of 95% ethanol plus 20 ml of water.

Procedures

1. Pipette 10 ml of the sample into a 125 ml Erlenmeyer flask.
2. Add about two drops of the 1% phenolphthalein alcoholic solution.
3. Proceed with titration using the sodium hydroxide until a pinkish colour appears.
4. Read and record the result in millilitres of alkaline solution.

Guidelines

► Use dry glassware since the presence of water during the indicator process interferes in the titration.

► It is recommended to use approximately two drops of the indicator (about 0.1 ml) to evaluate acidity. A difference of up to 3º D can be detected when larger quantities of the indicator are used (ten drops).

► When powdered milk is tested, an appropriate reconstitution must be done before analysis. For example:

► Whole milk powder = 1:7 powder to distilled water

► Skimmed milk powder = 1:10 powder to distilled water

► Generally 5 g of SNF (Solid Not Fat) reconstituted with water. The final number of millilitre used in the titration must be multiplied by the factor 2 (it is recognized as a standard to refer to 10 g of SNF)

► Follow the sample titration with a control until the dye turning point is achieved (a weak pink colour will develop).

Test 3 – Alcohol test (alcohol stability)

You can assess the stability of milk proteins by their precipitation with alcohol under different concentrations.

What you will need

- ► Petri dishes
- ► Graduated pipettes (2 ml)
- ► Neutralized ethyl alcohol solutions (pH 7.0), 75% alcohol concentration (v/v) or others.

Procedures

1. With graduated pipettes (2 ml), stir onto a Petri dish 2 ml of milk and 2 ml of ethyl alcohol 75% and move the Petri dish carefully in a circular movement.
2. If neither clots nor flakes form, carry out a new analysis by increasing the alcohol concentration.
3. Repeat these analyses until clots or flakes are seen.
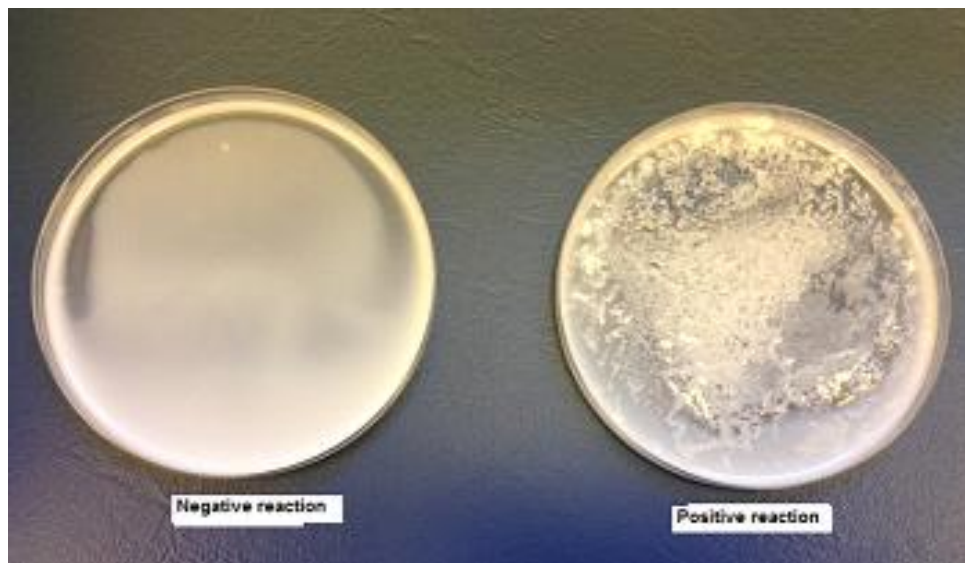
Guidelines

► The "alcohol number" is the highest concentration of alcohol mixed with the same amount of milk, which will not lead to clot formation or precipitation (gives negative reaction)..

► Note that Tetra Pak recommends a product stable to 75% alcohol without precipitation (negative) as a minimum. (The International Dairy Federation ,IDF, suggests 72% alcohol as a minimum, but our practical experience shows that this will increase fouling and reduce the running time, and may even lead to reduced stability of the final stored product.)

► Use only pure ethanol free from additives for alcohol stability tests of raw milk. Ethanol with additives, so called denatured ethanol, gives false results with raw milk. Raw milk of good quality may precipitate (clot) if denatured ethanol is added, regardless of the concentration.

Test 4 - Freezing point

Evaluating the freezing point of milk helps you detect milk that has been diluted by water.

The addition of water to milk not only reduces its quality, but also leads to spoilage or contamination that can present a health hazard. Raw milk has an average freezing point of -0.54°C. When milk is mixed with water, its freezing point moves closer to 0°C. The milk freezing point is measured using the Digital Cryoscope.

What you will need

► Thermistor cryoscope
► Cryoscopy tubes
► Graduated pipette (2 ml)
► Calibration solution for the machine and anti-freeze solution:
► Standard "A" solution: distilled water (-0.000°C freezing point)
► Standard "B" solution: sodium chloride solution (-0.600°C freezing point). Put approximately 12 g of sodium chloride into an oven at 300°C for 5 hours or at 130°C for at least 24 hours. Cool down the sample in a desiccator. Weigh exactly 10.161 g and dissolve into distilled water, bringing the volume up to 1,000 ml. Let the solution stabilize for 24 hours.
► A suitable cooling liquid for the cryoscope is 33% aqueous solution of propylene glycol
► Absorbent paper
► Tube rack

Procedure

1. Test the sample and the sodium chloride solution after they have reached the same temperature.
2. If the sample total acidity exceeds 20 ml of 0.1mol/l sodium hydroxide solution per 10g of non-fat solids , the final result of the test will not be representative of the original milk

Guidelines

Proceed according to the instructions supplied by the equipment manufacturer.

Test 5 - Density

Measuring milk density allows you to estimate the solid content.

The milk density varies between 1.028 and 1.033 g/ml at 15.5°C. The density changes according to the milk temperature, washing and skimming.

Milk components:

- ► Water: d = 1.000 g/cm^3
- ► Fat: d = 0.930 g/cm^3
- ► Protein: d = 1.346 g/cm^3
- ► Lactose: d = 1.666 g/cm^3

What you will need

- ► Graduated cylinder
- ► Lacto-density meter

Procedure

1. Slowly pour approximately 250 ml of milk into the graduated cylinder; avoid producing foam.
2. Lay down the Lacto-density meter carefully.
3. After its stabilization, record the temperature (T) and density (Dt). The density is generally referred to at the temperature of 20°C (sometimes 27°C in tropical countries).

Guidelines

Apply one of the following formulae:

$D = Dt + (T - 20) \times 0.25$ (temperature of 25°C)
$D = Dt + (T - 27) \times 0.3$ (temperature of 30°C)

D = density at 15°C
Dt = density read from the hydrometer
T = temperature of the reading

Microbiological tests

For milk in the normal quality range of pH 6.65-6.8, there is a scale of acceptability for how many Gram-negative psychrotrophic bacteria can be present. This is one of the major determinants of shelf life of UHT products.

Total count (CFU/ml)	Comment
≤ 300 000	Milk of good quality
300 000 – 1 million	Risk for organoleptic problems
1 million – 5 million	Risk for organoleptic problems Shortened shelf life stability due to stability problems
>5 million	Not suitable for UHT processing Product not stable

Test 6 - Methylene blue reduction (Resazurin test)

The total amount of microorganisms present in raw milk can be evaluated through direct or indirect methods.

The indirect processes are more commonly applied due to speed and low cost; they are, however, not as precise as the direct methods. One of the indirect methods most often used to evaluate the total amount of microorganisms present in raw milk is methylene blue reduction.

Basically, methylene blue is added to milk and the time required to discolour the blue mixture is verified. Other indicators, such as resazurin, are also applied. The precision of this test decreases the longer the raw milk was stored, since over time the traditional mesophilic flora is replaced by psychrotrophic flora. Most psychrotrophic bacteria will not reduce methylene blue and will not be detected by this method (see test 9 - Total counting of psychotropic aerobes).

What you will need

► Water bath at 37°C
► Sterile test tubes
► Graduated cylinder
► Sterile water
► Methylene blue solution (saturated in 96% (v/v) ethanol)
► Timer or clock

Procedure

In this methylene blue test, approximately 10 ml of milk are poured into a graduated cylinder. A small amount of methylene blue solution (add 1 ml of a solution prepared by diluting 5 ml of a saturated solution of methylene blue in ethanol 96% in 95 ml of sterile water) is added to the milk, which makes the solution a bluish colour. This mixture is incubated in a 37°C water bath. The time required to turn the mixture to a white colour again is measured as the Methylene Blue Reduction Time (MBRT).

The longest discolouration time indicates the lowest level of metabolic activity and, consequently, the lowest Total Bacterial Count (TBC) was present in the milk.

(Note that the methylene blue solution shouldn't be exposed to light and should be discarded two months after preparation.)

Guidelines

Based on the determined discolouring time, milk is commonly divided into three quality classes.

Class	MBRT (minutes)
1	> 120
2	30 - 120
3	< 30

Indirectly a certain reduction time indicates roughly the probable Total bacterial Count that was present in the raw milk and based on that the raw milk quality can be judged:

| MBRT (minutes)	TBC / ml	Raw milk quality
420	35,000	Very good
300	350,000	Good
240	600,000	
180	850,000	
120	1,500,000	Bad
60	3,450,000	
30	10,000,000	
15	15,000,000	

Test 7 – Total aerobic plate count for mesophilic aerobes

This test counts the total number of mesophilic aerobes. These organisms grow at temperatures between 30 and 40°C, with optimum temperatures between 30 and 35°C.

The counting method of microorganisms on plates is a general method used to count many different types, such as the mesophilic aerobes, thermophilic aerobes, psychrotrophic aerobes, mould, yeast and others.

This versatility comes from the theory that each microbial cell present in a sample will form a visible and isolated colony when the cell is fixed in an appropriate solid culture medium.

Much of this same procedure will be used in tests 8 and 9, in particular preparation of dilutions, which we explain only once in this booklet.
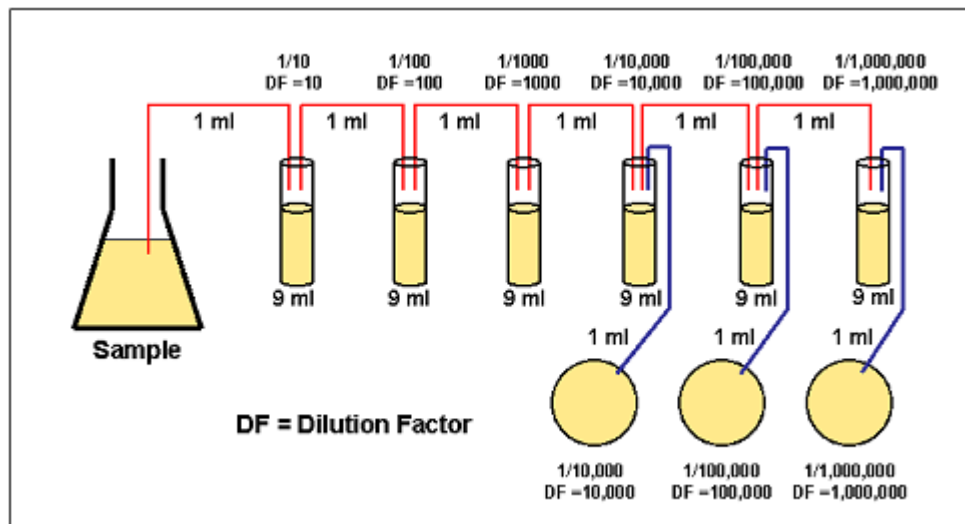
Dilution of samples for microbiological analysis.

Preparation of the series dilutions

Many tests require a series of sample dilutions before beginning. Just to review the concepts and notations here, a sample that is undiluted is said to be in proportion 1:1. If something is diluted to 1/10 or 10% of its strength, it is in a proportion of 1:10. Another way of writing this is 10^{-1}. Diluting it further 10 times – so that it is a 1% solution or 1:100 – would be 10^{-2}. The following series we describe includes dilutions to 10^{-7}.

1. Dilution 10^{-1}: Aseptically transfer 1 ml of the sample into 9 ml of the diluent (in a test tube), or 10 ml of the sample into 90 ml of the diluent (in a dilution flask), and stir.
2. Dilution 10^{-2}: Aseptically transfer 1 ml of the dilution 10^{-1} into 9 ml of diluent, or 10 ml of the dilution 10^{-1} into 90 ml of diluent, and stir.
3. The subsequent dilutions are obtained in a similar way, by transferring either 1 or 10 ml of the previous dilution into 9 or 90 ml of the diluent, respectively.
4. The diluent used to prepare the dilutions 10^{-2} and above must be the same as used to prepare the first dilution 10^{-1}.
5. The amount of required dilutions depends on the contamination level expected. For instance, if the expected counting is around 2,500-25,000/g per sample, the dilutions recommended for dish counting are 10^{-1}, 10^{-2} and 10^{-3}, so that dishes with 25-250 colonies are found. In case there is no way to previously evaluate the sample contamination level, a larger amount of dilutions must be prepared and inoculated (10^{-1} and 10^{-7}).

About pipettes used in preparing dilutions:

► Avoid dipping pipettes deeper than 2.5 cm as the sample content is poured with the pipette.
► Always choose pipettes that have a capacity 10 times larger in volume than the amount to be collected. For instance, when pouring amounts of 1 ml, use at least 10 ml pipettes.
► During the transference of amounts between the dilutions, always use a different pipette for each dilution. Before removing the amount to be transferred, vigorously shake the tube or the flask. The pipette must be thoroughly filled and the amount discharged from the upper mark, even though a smaller amount than the pipette is to be released.
► Avoid releasing amounts from the last or next-to-last lower marks of the pipettes. The amount must be released with the pipette tip touching the internal wall of the tube or flask, so that the liquid is poured down the wall. Pipettes should not be buckled. In case the tip touches any non-sterile surface, such as the external area of the pipette-rack, the tip of the other pipettes or the external tube/dilution flask walls, for instance, the pipette must be discharged and replaced by another.

Procedure

1. Shake the sample carefully.
2. Open the sample container aseptically and carry out the appropriate series of dilutions, as explained at the beginning of this test.
3. If the container is a carton package, cleanse the external surface of the package with cotton soaked in 70% ethanol to remove any contaminant (cleanse the counter surface as well). Open the package with cleansed and sterilized scissors and carry out the appropriate series of dilutions, as explained at the beginning of this test.
4. Pipette1 ml of each dilution into separate, labelled empty Petri dishes (this procedure will ease the subsequent mixing with the culture medium), opening the dishes only enough to let the pipette in, close to the Bunsen burner. To increase the counting precision, it is recommended to inoculate two or more dishes per dilution (duplicates or triplicates).
5. Add to the dishes approximately 15-20 ml of the PCA (previously melted and cooled down to 45 - 50°C, which is cool enough to touch). This is the pour plating method. Smoothly and slowly move the dishes in figure eight movements; make sure the mixture does not touch the dish lids and wait for the agar to solidify.
6. Invert the dishes; incubate them for 48 hours at 30 ± 1°C for the total aerobic plate count.

Result assessment and interpretation

1. With a magnifying glass or colony counter, count all colonies developed on the agar dish, and which present an amount between 25 and 250 colonies.
2. Proceed with the calculation, multiplying the amount of colonies by the inverse of the inoculated dilution. In case more than one dish is used for dilution (duplicate or triplicate), consider as colony number the arithmetic average of the counting obtained in each of the dishes.
3. After all the results are read, the microorganism can be identified through different methods.
4. Express the results in number of spores/ml or g. When presenting the results, use exponential notation and only one digit after the decimal point.
5. Autoclave all the dishes at 121°C for 30 minutes before the material is discharged.

Guidelines

Media must be sterilized in the autoclave at 121ºC for 15 minutes. All the glassware must be sterilized in an oven at a minimum temperature of 170°C for over 2 hours. Graduated glassware must be sterilized in an autoclave at 121°C for 30 minutes.

Test 8 - Total spore count and heat resistance spore count

This test enumerates total spores and heat resistance spores. It differs from the aerobic test on two steps: the pre-heat treatment before dilution and plating, and the incubation temperatures.

Spores

Bacterial spores are highly resistant structures created when spore forming organisms experience environmental stress. Spores do not multiply as such. If favourable conditions are present (for instance, the correct oxygen supply, temperature, etc.) each spore creates a vegetative cell (viable cell), which is able to multiply.

The sporogenic bacteria commonly present in food belongs to the genera Bacillus, Clostridium and Desulfotomaculun. These, due to the heat resistance of the spores, are generally associated with the spoilage of thermally processed products that are packed into hermetic packages (commercially sterile products). The introduction of such spores in this kind of food occurs mainly because of raw material used during the formulation procedures, such as spices, sugar, flours and powdered milk. According to the heat resistance of the spores and the optimum growth temperature, the sporogenic bacteria are divided into two groups:

Thermophilic sporogenic bacteria

These include species whose optimum growth temperature is around 55°C and whose spores are highly heat-resistant.

There are two thermophilic sporogenic types associated with the deterioration of commercially sterile products: aerobic and anaerobic thermophilic.

Mesophilic sporogenic bacteria

These include species whose optimum temperature is around 30-35°C and whose spores are highly heat-resistant and which are also able to survive after severe thermal treatments are applied to low acidity food.

Even here there are two types of mesophilic sporogenic bacteria associated with the deterioration of commercially sterile food: aerobic and anaerobic mesophilic.

What you will need

1. All materials used in test 7
2. Incubators at 35 and 55 ± 1°C
3. Thermostatic bath at boiling temperature
4. Semi-analytical scale

Procedure

For a general introduction to plate count technique and preparation of the dilutions, see the previous test. Many of the steps are the same as in test 7. Please note that the **steps in boldface type are unique for test 8**.

1. Shake the sample carefully.
2. Open the sample container aseptically.
3. If the container is a carton package, cleanse the external surface of the package with cotton soaked in 70% ethanol to remove any contaminant (cleanse the counter surface as well). Open the package with cleansed and sterilized scissors.
4. **Aseptically pour 3-5 ml of the sample with a pipette onto a sterile screw-thread test tube.**
5. **Place the tube in a thermostatic bath at 80°C (total spores) and at boiling temperature100°C (heat-resistant spores) for 10 minutes. An additional tube (with a thermometer inside) with 10.0 ml of product must also be placed in the bath (the time counting is only started after the additional tube reaches the specified temperature).**
6. Carry out appropriate series dilutions, as explained at the beginning of test 7.
7. Inoculate, out of the dish centre,1 ml of each dilution into separate, sterile and empty Petri dishes (this procedure will ease the subsequent mixing with the culture medium), opening the dishes only enough to let the pipette in, close to the Bunsen burner. To increase the counting precision, it is recommended to inoculate two or more dishes per dilution (duplicates or triplicates).
8. Add approximately 15-20 ml of PCA (previously melted and cooled to at 45-48°C, harmless to skin touch) on the dishes (This is the depth plating method).
9. Smoothly revolve the dishes in eight movements; making sure the mixture does not touch the dish lids and wait for the agar to solidify.
10. **Invert the dishes, incubate them at 35-37°C (total spores) for a period of 48hrs, 35-37°C (thermoresistant mesophilic spores), and 55°C (thermoresistant thermophilic spores) for 5 days.**

Result assessment and interpretation

1. With a magnifying glass or colony counter, count all colonies developed on the agar dish, and which present an amount between 25 and 250 colonies.
2. Proceed with the calculation, multiplying the amount of colonies by the inverse of the inoculated dilution. In case more than one dish is used for dilution (duplicate or triplicate), consider as colony number the arithmetic average of the counting obtained in each of the dishes.
3. Express the results in number of spores/ml or g. When presenting the results, use exponential notation and only one digit after the decimal point.
4. Autoclave all the dishes at 121°C for 30 minutes before the material is discharged.

Guidelines

All sterile glassware must be sterilized in an oven at the minimum temperature of 170ºC for over 2 hours. Graduated glassware must be sterilized in autoclave at 121°C for 30 minutes.

Test 9 - Total counting of psychotropic aerobes

This test counts the total amount of psychrotrophic aerobes. These organisms develop under temperatures between 0 and 30°C, with optimum temperatures below 25°C.

The test differs from the total aerobic count in the details of surface plating, as well as the incubation temperature and time.

Dairy products are prone to contamination of psychrotrophic bacteria, which can lead to spoilage and lower quality products. The surface plating method is recommended to estimate the total amount of psychrotrophic aerobic microorganisms. This procedure avoids exposing the cell to the heat from the melted agar, since psychrotrophic microorganisms are vulnerable to high temperatures.

What you will need

- ► All materials used in test 7
- ► Refrigerator at 6.5 ± 0.5°C or incubators at 18 ± 1°C / 24 ± 1°C
- ► Oven at 35 ± 1°C
- ► Drigalski loop

Procedure

Many of the steps are the same as in tests 7 and 8. Please note that the **steps in boldface type are unique for test 9.**

1. Shake the sample carefully. Shake the package carefully.
2. Open the sample container aseptically and carry out the appropriate series of dilutions, as explained at the beginning of test 7.
3. If the container is a carton package, cleanse the external surface of the package with cotton soaked in 70% ethanol to remove any contaminant (cleanse the counter surface as well). Open the package with cleansed and sterilized scissors and carry out the appropriate series of dilutions, as explained at the beginning of test 7.
4. **For the surface plating procedure, the dishes must be previously prepared with 15-20 ml of the Plate Count Agar (PCA), melted and then solidified. Before using, the medium surface must be dried (this procedure can be done in an oven at 50°C for 2 hours or 30-35°C for one night, lids closed) or in a layer-flux chamber, 0.5-1 hour, with lids partly open.**

5. Inoculate 0.1 ml of each dilution on the previously prepared dish surfaces, one or more dishes for each dilution. Spread the inoculate over the whole surface of the medium with a Drigalski loop until the excessive liquid is absorbed. Pipettes of 1 ml must be used to transfer the inoculate of 0.1 ml. Do not blow the pipette and do not change the direction of the pipette tip when applying the last drop.

6. Spread the material from the higher dilution dish to the lower dilution dish, sterilizing the Drigalski loop with 70% ethyl alcohol between applications.

7. **Wait until the dishes dry (minimum of 15 minutes), invert them and incubate them at 6.5°C for 10 days.**

Note: The reference time/temperature for the total counting psychrotrophic microorganism is 6.5°C/10 days, but there are several other incubation conditions, which can be used in certain situations:

► Surface plating: 7°C/7-8 days
► Milk analysis: 18°C/45 hours
► Milk analysis and milk cream analysis: 23-25°C /24-28 hours

Note: As the inoculate amount used in the surface plating is 10 times smaller than the one used in the pour plating, the method detection limit is over 100 CFU/g for solid samples and 10 CFU/ml for liquid samples. In case the contamination level expected for the sample is below that range, a larger amount of the first dilution must be inoculated and distributed into several dishes. The commonly applied distribution is: three dishes with 0.3 ml and one dish with 0.1 ml. The required time for the liquid absorption is larger in spreading on 0.3 ml dishes and special care must be taken so that moist films do not remain in the surface.

Result assessment and interpretation

1. Proceed for the plate reading using the same procedure as described for the spore count in test 8.

2. After all the results are read, the microorganism can be identified through different methods.

3. Autoclave all the dishes at 121°C for 30 minutes before the material is thrown away.

Guidelines

All the glassware must be sterilized in an oven at a minimum temperature of 170°C for over 2 hours. Graduated glassware must be sterilized in an autoclave at 121°C for 30 minutes.

Summary

To summarize the principles in this booklet, milk quality starts at the farm with animal health and good hygiene – including barns, equipment and containers. Because as soon as milk leaves the animal, it is subject to contamination by microorganisms that will start to multiply and that will greatly affect its suitability for production of UHT milk and other milk products.

Milk quality affects the efficiency and cost-effectiveness of your plant's processing machinery, in particular your heating units. Poor quality milk can clog heat exchangers, causing processing interruptions and requiring extra cleaning time – and thus extra costs.

In the section on dairy farm operations, we indicated where milk supplies can become vulnerable. Systematically testing incoming milk, monitoring its quality, and only selecting milk that is fit for UHT treatment will mean your dairy operations can be run more efficiently and more profitably. Packaged UHT milk should have a shelf life of several months, but as we have mentioned, this can vary depending on the quality of the raw materials used.

Some of the major test parameters to keep in mind:

► The pH of milk should be between 6.65 and 6.8 to ensure trouble-free processing and high quality of the final product. A lower pH will risk product stability and cause fouling. A higher pH will increase the risk of mastitis-fouled milk.
► To avoid the effects of mastitis, the maximum amounts of somatic cells allowed according to legislation are: 400,000 cells/ml in the EU and 750,000 cells/ml in the US. You should become familiar with any specific requirements or regulatory standards in your region. High somatic cell counts speed up enzymatic reactions and reduce shelf life of the packaged UHT milk.
► For milk in the normal quality range of pH 6.65-6.8, there is a scale of acceptability for how many Gram-negative psychrotrophic bacteria can be present. Less than 300,000 CFU/ml indicates good quality. Between 300,000 – 1,000,000 CFU/ml there is the risk for processing and organoleptic problems, and over 1,000,000 CFU/ml additionally reduces stability and shelf life.

If milk does not meet these criteria, then it is not suitable for UHT processing. If the quality of your raw milk supply repeatedly deviates from the recommendation, then you have the basis for being more selective about your suppliers. They may need some encouragement or training themselves in modern dairy farm operations in order to bring their milk up to the desired level of quality.

Running your dairy operations according to our recommendations should lead to longer running times, fewer maintenance problems due to machine fouling, and higher product quality with the desired shelf life for your UHT products.

Tetra Pak – your dairy advisor

Tetra Pak and its network of partners stand ready to assist you in many ways with your business and technical questions regarding milk processing and safety issues. Feel free to relay on these sources, or contact your Tetra Pak representative.

Dairy farm-related questions – from DeLaval
www.delaval.com/en/Dairy-knowledge-and-advice

Dairy processing and packaging-related questions

www.tetrapak.com

More related information

http://www.tetrapak.com/about-tetra-pak/cases/how-milk-quality-affects-your-uht-dairy-products