An introduction to commercial sterility.

Index.

Introduction.

The purpose of this booklet is to provide a basic understanding of the microbiological aspects of aseptic technology with the focus on the processing part. We do not aim to cover everything in detail, but instead try to give you a good overview of the importance of commercial sterility and how to achieve it. If you need more detailed information, do not hesitate to contact us at Tetra Pak. We are happy to assist you with our competence and experience.

All quality is based on competence.

History and challenge.

The destruction of microorganisms.
Even before Louis Pasteur proved over a century ago that microorganisms caused fermentation and disease, Nicolas Appert, a Parisian confectioner, first succeeded in preserving certain foods in glass bottles that had been kept in boiling water for varying lengths of time. This happened in the first decade of the 1800's and by 1839, tincoated steel containers were widely in use. Thus began the birth of high heat treatment and canning as a means of preserving food.

Today, a combination of continuous heat-treatment and aseptic packaging produces high quality liquid food in a cost-effective way.

Commonly used temperature and time combinations for milk:
## Pasteurization 72°C for 15 s
Sterilization 140°C for 4 s

Single-cell friends and enemies.
Spores pose the major challenge to UHT (ultra high temperature) processing and determine the sterilization parameters.

Microorganisms carry out innumerable functions that are essential to life on earth. However, as regards food products, they are public enemy number one. Many bacteria, some of which are pathogenic to man, are responsible for the rapid spoilage of food. Applying a suitable temperature degrades proteins, essential components of living cells, and so it is quite easy to kill vegetative bacteria.

The major problem is bacterial spores. These are diffi cult to kill because they have thick protective walls which increase their resistance to heat, drying, freezing, chemical agents and radiation. This is why spores pose the biggest challenge to UHT processing and determine the parameters used for sterilization.
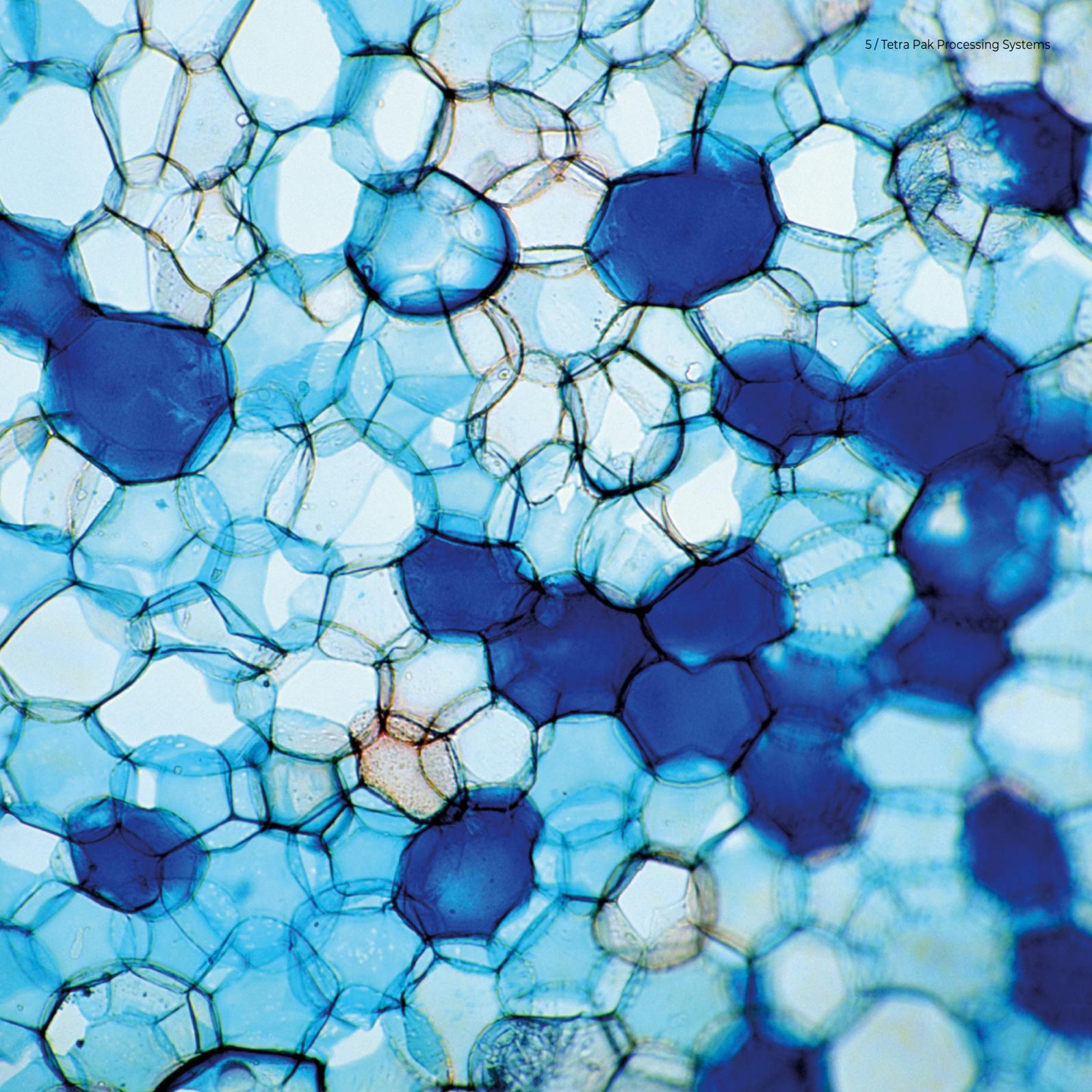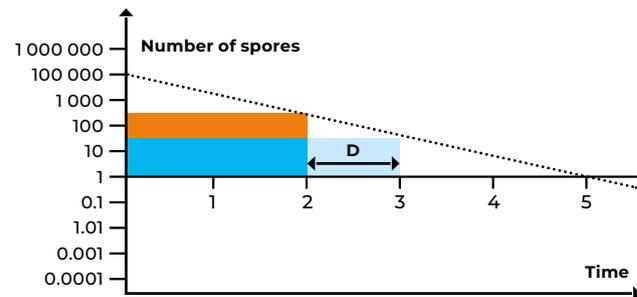
The logarithmic order.

A never-ending story.
Aseptic processing involves one or more separate sterilization steps aimed at killing all microorganisms. When applying a sterilization procedure, e.g. using heat, not all bacterial spores are killed at the same time. A certain number will be killed within a given unit of time as shown in the table here. The principle behind the killing process is independent of the sterilization temperature.

When the number of surviving spores is plotted against their logarithmic reduction, we obtain a straight line as shown in Figure 1. This straight line is commonly known as the logarithmic order of death, and the slope of the line indicates the rate of destruction.

Fig. 1. A typical graph of the logarithmic order of death for bacterial spores during sterilization. The steeper the slope, the higher the rate of killing efficiency. The initial microbial load is here assumed to be 100 000 bacterial spores of equal resistance. D is the decimal reduction time.

| Unit of time	Survivors	Spores killed per unit time	Total accumulated	Logarithmic reduction
0	100 000	0	0	0
1	10 000	90 000	90 000	1
2	1 000	9 000	99 000	2
3	100	900	99 900	3
4	10	90	99 990	4
5	1	9	99 999	5
6	0.1	0.9	99 999.9	6
7	0.01	0.09	99 999.99	7
8	0.001	0.009	99 999.999	8

Approaching absolute sterility.

As a logarithmic function never can reach zero but only approach it, absolute sterility never can be reached or guaranteed. The efficiency of a sterilization process is expressed by the number of decimal reductions in the count of resistant spores. The D-value (decimal reduction time) is normally used to indicate the killing rate, i.e. the time required at a certain temperature to kill 90% of the spores, which is the same as reducing their number by one logarithm unit. Measured under the same conditions, the D-value will be affected by the species of bacteria.

Absolute sterility can never be reached or guaranteed.

A heat sterilization process result depends on:
- the initial number of microorganisms;
- the decimal reduction time (heat-resistance) of microorganisms;
- the time and temperature of heat exposure.

Another important parameter is the Z-value, which is the change in temperature needed to alter the D-value by a factor of one logarithm unit.
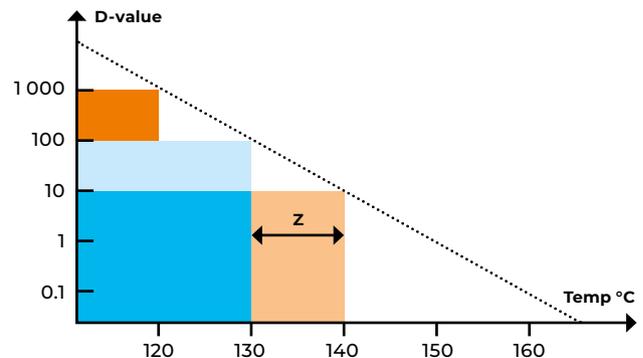
Fig. 2. *The Z-value is the increase in temperature needed to achieve the same lethal action or the same effect in one-tenth of the time.*

A weak link breaks the chain.

Aseptic processing and packaging is rather complex because a minimum of three different sterilization steps are required to achieve aseptic filling of the liquid food product. Sterilization is carried out:
- on the equipment prior to production;
- on the liquid food product;
- on the packaging material surface.

The performance level of an aseptic plant must be regarded as the performance level of the entire process rather than that of a single component of the production line. Each link in the processing and packaging chain must be equally strong.

Each link in the processing and packaging chain must be equally strong.

Raw material

Pre-processing

UHT treatment

Packaging material

Sterilization

Aseptic packaging
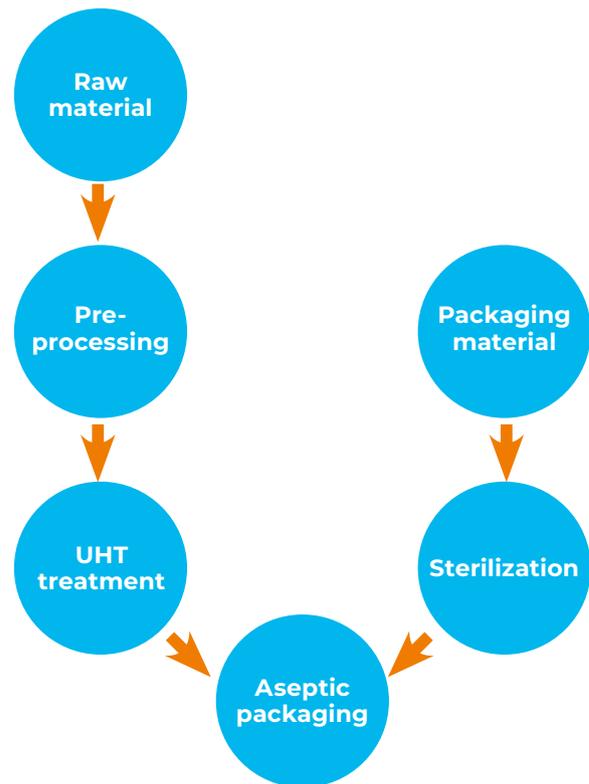
Meeting market requirements.

The processing result is not determined by the processing factors alone but also by the quality of raw material, pre-processing procedures, environmental and operative parameters as well as maintenance. Absolute sterility – defined as the total absence of microorganisms – is not possible for the reason mentioned above and thus cannot be guaranteed.

Commercial sterility.
According to the WHO/FAO, commercial sterility of low-acid food is defined as follows:

"Commercial sterility means the absence of microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during manufacture, distribution and storage."

(Ref: Codex Alimentarius Commission (WHO/FAO) CAC/RCP 40-1993)

The EU defines the heat treatment necessary to achieving commercial sterility.

"UHT treatment is achieved by a treatment: (i) involving a continuous flow of heat at a high temperature for a short time (not less than 135°C in combination with a suitable holding time) such that there are no viable microorganisms or spores capable of growing in the treated product when kept in an aseptic container at ambient temperature, and (ii) sufficient to ensure that the products remain microbiologically stable after incubating for 15 days at 30°C in closed containers or for seven days at 55°C in closed containers or after any method demonstrating that the appropriate heat treatment has been applied."

Ref: Commission Regulation (EC) No 1662/2006 (amending Regulation (EC) No 853/2004)).

Specifying quality levels.
The practical consequences of absolute sterility being unobtainable means establishing microbiological specifications for the end-product. This is necessary in order to develop a viable quality control programme. Such specifications or acceptance quality levels (AQL) form an integral part of a company's quality policy.

A microbiological AQL for long-life products should be expressed as a maximal acceptable defect rate. A clear distinction has to be made between the maximal acceptable defective rates and the goal of production. Regardless of established maximal acceptable defect rates, the goal of production must always be perfection.

Sampling procedure.
A maximal acceptable defect rate of around 1 per 1 000 to 10 000 samples is generally approved. In order to detect such defect rates, sampling statistics must be introduced. This includes defining the level of probability (confidence level) to be used when checking for defect rates. Usually, a 90–95% confidence level of achieving a correct result is used.

How many samples?

More samples will improve the detection level. To determine how many samples we need to take to detect a defective product, let's look at a typical example. How many samples are required to detect a defect rate of 1 per 1 000 with 90% probability?

This problem is solved with the help of the random sampling curve for 90% probability shown in Figure 3. If we follow the 1% figure up to where the curve intersects the y-axis we get the result 240. For 0.1% (1 per 1 000) the answer to the question is 2 400. The statistical results are affected only by the sampling size and not by the total number of units produced.

Let's take another example using the same 90% probability curve. If we take 50 samples from a batch and none of them are defective, what is the defect rate?

In this case we take a horizontal line from 50 samples on the y-axis, and where it intersects

the 90% curve is the defect rate at this level of probability: less than 5%, or about 5 samples per 100 units. More samples will improve the detection level.

To detect a defect rate of 1 per 1 000 with 95% probability, we would need to take 3 000 samples.
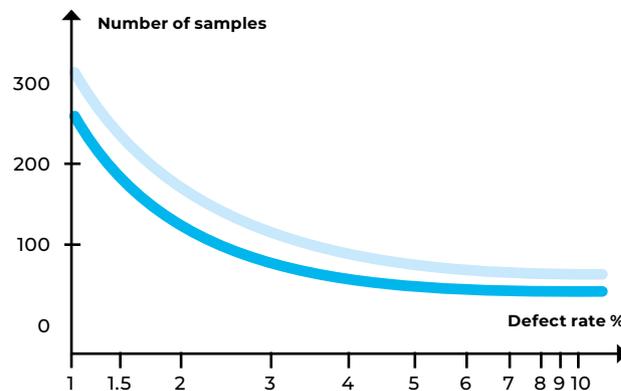
Fig. 3. he relation between defective product samples in percent and the total number of samples to be analysed for bacteria needed to find one or more defects in samples at 90 and 95% levels of probability.
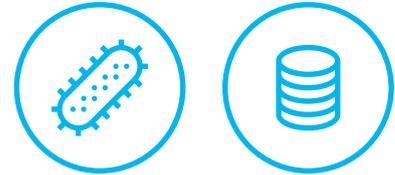
Analytical methods

There are many methods available for detecting bacteria and estimating their number. The most common ones are given in the table below.

Analytical method	Accuracy	Cost
Bacteriological plating	● ● ●	● ●
Bacterial ATP	● ● ●	● ● ●
Sensory	● ●	●
pH	●	●

Highly sensitive methods detect the most bacteria but are also the most expensive. In bacteriological plating, a sample of liquid food is spread out on nutrient agar plates and any colonies that arise from individual bacteria after incubation are counted to estimate the concentration of bacteria in the product. Several methods are available that disrupt the bacterial wall so that a compound called adenosine triphosphate (ATP) present in vegetative cells can leak out and be measured to estimate the number of viable bacteria.

Sensory and pH methods are the cheapest but have poor accuracy.

Which method is chosen depends on a balance between the maximal acceptable defect rate, analytical cost and the competence of company personnel. According to the European Council Directive, random samples of UHT milk must be checked using the bacteriological plate count technique, although the number of samples is not stated.

The statistical results are affected only by the sampling size and not by the total number of units produced.

Increasing sterilization efficiency.

Sterilization efficiency can be expressed as the number of decimal reductions in microbial population. The efficiency of a sterilization process can be expressed as the number of decimal reductions in microbial population achieved by the process.

This is mainly determined by two factors:
1. The temperature and the length of time it is applied.
2. The heat-resistance of bacterial spores present, D-value.

Some other factors, e.g. the composition, viscosity, uniformity and pH of the liquid food product, will also affect the sterilization efficiency. For typical UHT processing equipment it can be assumed that an average of 9 – 10 decimal reductions can be achieved with bacterial spores growing at ambient temperatures.

Initial spore load /ml	Spore load/l	UHT decimal reduction	Spores/l in product	Defect rate
$10\ 000 = 10^4$	10^7	9D	0.01	1:100
$100 = 10^2$	10^5	9D	0.0001	1:10 000

More spores, more problems.

In addition to the factors mentioned above, the efficiency of the UHT process is influenced by the spore content of the liquid food, i.e. the total spore load, fed into the final heat-treatment section of the UHT plant. What is the effect of different initial spore loads on end-product quality, assuming identical processing conditions? If the product is filled into 1-litre packages, the defect rate can be determined from the information given in the table.

Figure 4 illustrates the effect of increased sterilization temperature on initial spore load, and the effect of different spore loads on the same temperature.
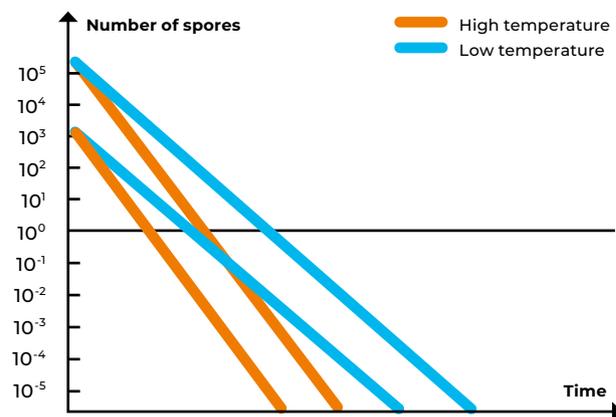
Fig. 4. *Increasing the temperature for a particular initial spore load achieves a quicker killing rate. Increasing the initial spore load for a particular temperature leads to a longer time required to achieve the same spore reduction as for a lower spore load.*

A mixture of remedies.

The importance of initial spore load on sterilization efficiency, and hence end-product quality, is obvious. But what can be done to improve the situation? The most important methods of achieving this are given below and summarised in the table.

Improved hygiene.
A high concentration of spores may already be present in milk at the farm, or be introduced into raw materials as a result of poor preprocessing routines. The answer here is to improve the level of hygiene both at the farm and the processing plant. However, it is difficult to control what happens on a farm and it is not always possible to change milk suppliers.

Spore reduction.
A good way of improving the end-product is to reduce the spore content of raw materials before the liquid food is heat-treated. Processes such as bactofugation and microfiltration are specially designed to greatly reduce the spore content of milk.

Effective heat treatment.
An increase in temperature and/or prolonging the product holding time during heat treatment will result in a more effective sterilization process. As a consequence, the number of decimal reductions will increase and the defect level will decrease.

Spore reduction remedies.

Process	Remedial action to reduce spore count	Effectiveness
Milking	Improve farm hygiene Change suppliers	Helpful but hard to control
Preprocessing	Improve plant hygiene	Helpful
Spore reduction	Bactofugation, microfiltration or other measure	Effective
UHT treatment	Increase temperature Prolong holding time	Very effective

Spore reduction and improved UHT treatment are the most effective means of increasing sterilizing efficiency.
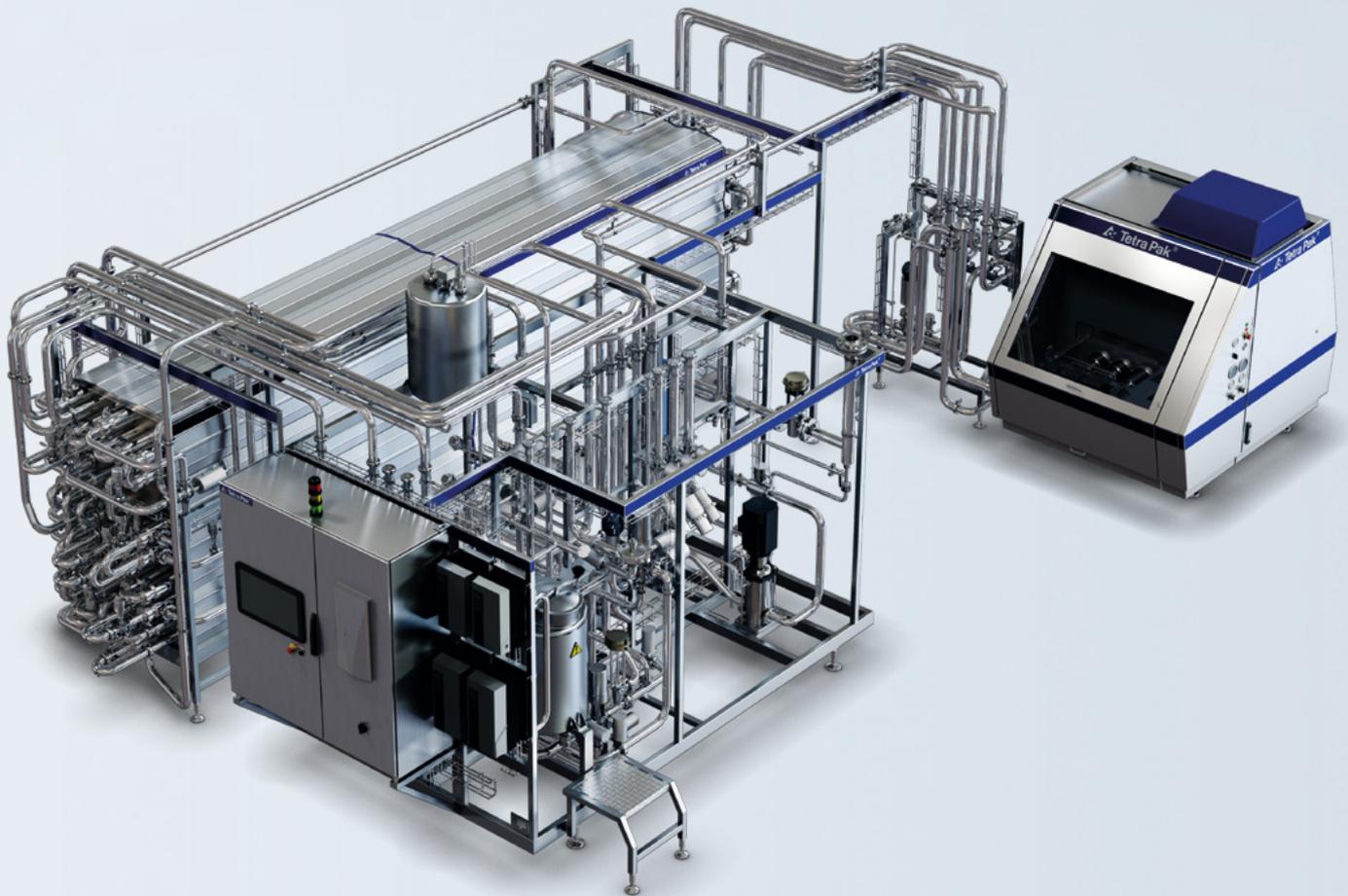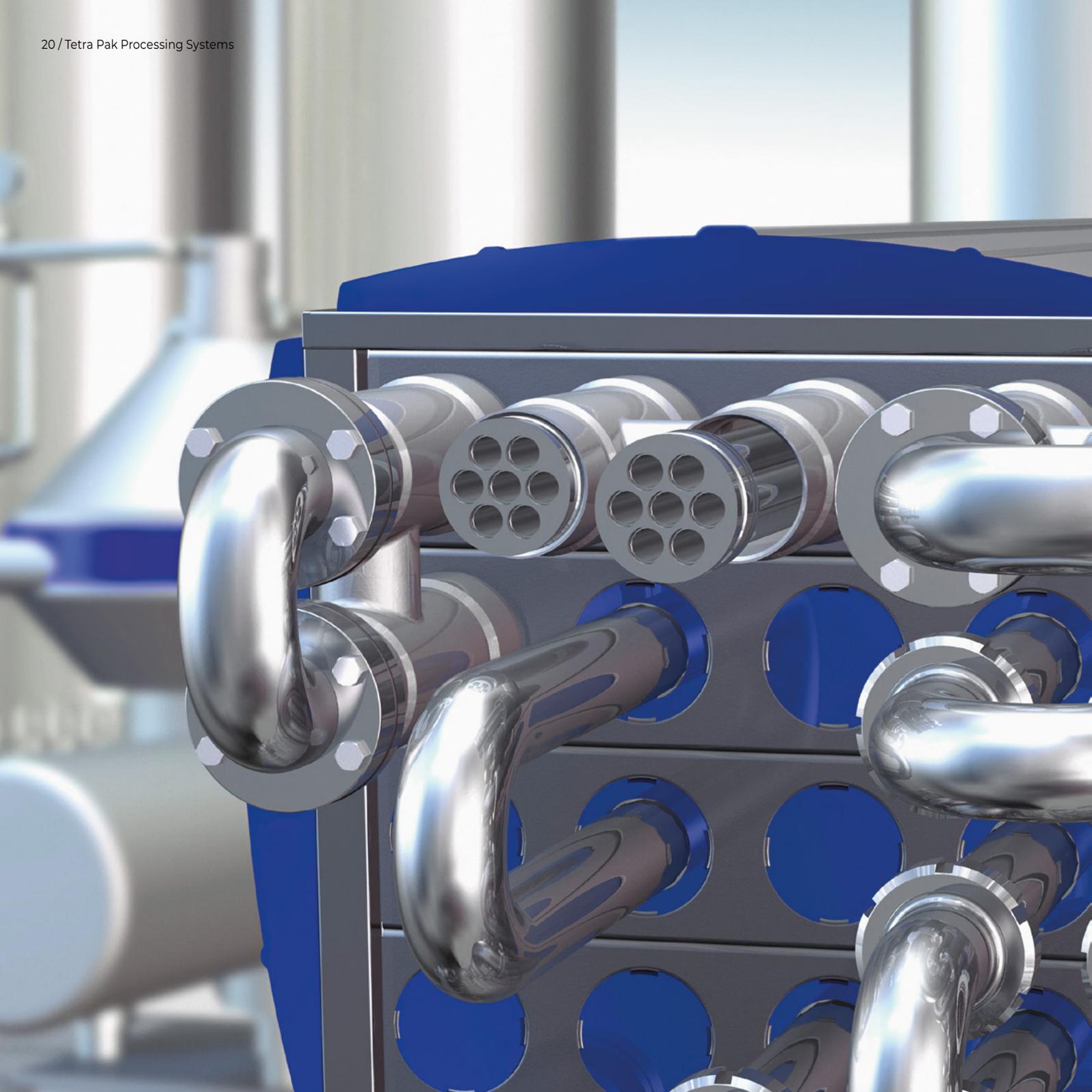
UHT treatment.

Two principles.
There are two different UHT methods used in the liquid food industry – direct and indirect heating. Which one to use depends on a number of factors such as desired end product quality level and production economy.

Direct heating.
When using direct heating, the product is brought into direct contact with hot steam under strictly controlled conditions. The sterilization temperature (140°C for 4 sec) is rapidly reached and after holding, the temperature is lowered by flash cooling in a vacuum vessel. The rapid heating and cooling gives minimal heat-load on the product with a high-quality product as the result.

Indirect heating.
In this method a partition is placed between the product and the heating or cooling medium. The principle is that a hot medium is flowing on one side of the partition and the product on the other. The partition is heated up and transfers the heat over to the product flow without any direct contact between the hot medium and the product. The same principle applies to cooling.

This procedure is done in what is referred to as a heat exchanger. The following three types of heat exchangers are the most widely used:

- Plate heat exchangers (PHE) are mainly used for smooth low viscous liquid products.
- Tubular heat exchangers (THE) can also handle products with particles up to a certain size and offer longer running times. The maximum size of particles depends on the diameter of the tube.
- Scraped-surface heat exchangers (SSHE) are specially designed for heating and cooling of viscous, sticky and lumpy products, with or without particles.

Which one to choose depends on several factors, e.g. product flow rate, physical properties of the liquid, required running time, cleaning require-ments etc.

The efficiency and flexibility of UHT processing are continuously being improved. One type of UHT module incorporates both direct and indirect heating with tubular heat exchangers to provide different combinations of heat treatment in one system.

Microfiltration.

A combination of membrane filtration and UHT treatment is proven to be the most effective method of increasing sterilizing efficiency.

By using a microfiltration membrane, 99.5-99.95% of bacteria and spores are effectively removed in a cost-efficient way. This is achieved by letting the liquid substance pass over a membrane at high speed under pressure in a closed system.

The membrane has very specific properties that only allow the bacteria and spores of a "maximum size" to pass through the membrane. Since the

size and weight of the unwanted bacteria and spores are known, the filter used is constructed with appropriate pore sizes to remove these parts from the liquid and let the smaller protein, lactose, mineral and water molecules pass on to final UHT treatment.

The main benefits of this procedure are that the product will be more purified before UHT treatment and the final product will not contain any dead spores. This gives an end-product with higher quality, better taste and longer shelf-life.

Microfiltration minimises the bacterial and spore content of milk in a cost-effective way.

Summary.

- Spores pose the biggest challenge to UHT processing and determine the sterilization parameters.
- Absolute sterility can never be reached or guaranteed.
- Each link in the processing and packaging chain must be equally strong.
- Acceptance quality levels (AQL) form an integral part of a company's quality policy.
- Statistical results are affected only by the sampling size and not by the total number of units produced.
- More samples will improve the detection level.
- Sterilization efficiency can be expressed as the number of decimal reductions in microbial population.
- Spore reduction and improved UHT treatment are the most effective means of increasing sterilizing efficiency.

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PROTECTS WHAT'S GOOD