Pre-treatments of Milk and their Effect on the Food Safety of Cheese

Elisabeth Eugster[1], Ernst Jakob[2]

[1] School of Agricultural, Forest and Food Sciences, Bern University of Applied Sciences, Länggasse 85, 3052 Zollikofen, Switzerland
[2] Food Microbial Systems, Agroscope, Schwarzenburgstrasse 161, 3003 Bern, Switzerland

Date submitted: 26/4/2019 *Date accepted: 20/08/2019* *Volume/Page(s): 45-52*

Abstract

In the manufacture of traditional cheese varieties, processing fresh milk that has been treated as little as possible is crucial. Preserving the microbiome and the activity of the original enzymes in the raw milk to the greatest extent possible allows these cheeses to retain their original character. This objective conflicts with the growing demands placed on products in terms of food safety. The present literature search addresses the influence of the pre-treatment of cheesemaking milk on the food safety and quality of ripened cheeses, with particular focus on heat treatment, bactofugation, and microfiltration.

Keywords: cheesemaking, food safety, milk treatment, pasteurization, thermization, bactofugation, microfiltration

1. Introduction

Many of Europe's traditional cheese varieties are made from raw milk. In France, Italy and Switzerland alone, over 600,000 tonnes of raw-milk cheese are produced each year, which corresponds to 10% to 40% of total cheese production, depending on the country [1, 2]. Among them are many cheeses with a protected designation of origin (PDO), whose production must be in accordance with the requirements of a PDO specification. Several PDO specifications allow the option of processing heat-treated milk, so that under the same label both raw-milk cheese and cheese from thermized or even pasteurized milk can be found. Examples are the French cheeses Cantal AOP [3] and Fourme d'Ambert AOP [4] and the Italian Taleggio DOP [5]. Only a few PDO specifications mandate a heat treatment of the milk. Examples are the Swiss Vacherin Mont d'Or AOP (thermization) [6] and the Greek Feta PDO (pasteurization) [7].

In France, where many raw-milk cheese varieties are produced, a fundamental debate is underway as to whether AOP specifications should be revised to allow the germ-reducing treatment of raw milk [8, 9]. The desire for approval of such processes comes particularly from the industrial producers of PDO cheeses with the aim of increasing the food safety of the products. Artisanal producers of PDO cheeses, on the other hand, insist on traditional production and the sensory superiority of raw-milk cheeses [10].

According to Hartmann & Maubois (2018) [11], microfiltration was temporarily authorised for the production of Camembert de Normandie PDO in 2002 because of various *L. monocytogenes* outbreaks. In Switzerland, too, there have been discussions about the authorization of certain methods of milk treatment in the production of traditional PDO cheeses in order to increase food safety. The main focus here is on the thermization of milk, and in some cases also on mechanical germ-reduction technologies such as bactofugation.

Food business operators are legally obliged to apply procedures based on the concept of Hazard Analytical Critical Control Point (HACCP) [12] to ensure the food safety of their products. European Regulation (EC) No 852/2004 [13] promotes the development of guidelines for good hygiene practices and the application of HACCP principles by the food industry. A major difficulty in developing an HACCP-based guideline for the production of traditional foods is often the lack of uniform production methods for the individual products. This also applies to foodstuffs with a protected designation of origin. PDO specifications are primarily meant to conserve a cultural heritage, i.e. to maintain the identity and the unique character of traditional food products. They were not established on the basis of HACCP studies or food safety considerations. Therefore, process parameters relevant for food safety such as milk storage conditions, thermization parameters and scalding temperature are not defined precisely. In practice, procedures complying with the PDO specifications but compromising on the food safety of the ripened cheese are found.

The aim of the present publication is to assess the methods of milk pre-treatment used in commercial cheese production regarding their contribution to the food safety of cheese, with a focus on cheese production in Switzerland. These methods include not only pasteurization but also thermization, bactofugation and microfiltration.

2. The Microbiome of Raw Milk

Due to the composition of raw milk, it represents the ideal environment for the growth of a wide range of different microorganisms. The totality of all microorganisms present in raw milk is known as the "raw milk microbiome". It is a very complex community, whose specific composition has a direct influence on the processability of raw milk into dairy products, and on its quality and safety [14, 15]. Recent studies identified up to 256 different species in raw milk [16, 17]. Among them are a number of unexpected genera and species not previously described in raw milk.

Lactic acid bacteria (LAB: genera *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Enterococcus*) and commensal staphylococci dominate in the microbiome of freshly milked raw milk. Other microorganisms are also present (*Propionibacterium* spp.,

Table 1: Pathogenic or toxin-forming microorganisms: Frequency in raw milk and behaviour in semi-hard cheese [28, 34-39]

Hazard	Prevalence in raw milk	Growth in semi-hard cheese	Inactivation during cheese ripening at 10–15°C	Importance
Listeria monocytogenes	0.1–1.0% (1)	On the surface of red smear cheeses	Decrease in cheese <0.5 log/month	High
Salmonella spp.	<0.10% (1)	No (no lactose fermentation)	Decrease of about 1 log/month	Low
Shiga toxin-producing *Escherichia coli*	0.1–1.0% (1)	Strong reproduction in the first 24 hours (lactose fermentation)	Decrease of about 1 log/month	High
Staphylococcus aureus	23–32% (2)	Strong reproduction in the first 24 hours, at levels >10^5 cfu/g, toxin formation possible	Decrease of about 2–3 log/month, toxins are not inactivated	High
Histamine-forming lactobacilli	14–25% (3)	In the manufacturing process and during maturation	Slow deactivation after 30–60 days with ongoing histamine formation	High

(1) Farm bulk milk samples (N = 601) delivered to cheese factories producing raw-milk cheese. Proportion of samples with positive result in 25 g milk [38]
(2) Percentage of samples (N = 403) with more than 100 cfu/mL [34]
(3) Percentage of samples (N = 199) with more than 1 cfu/mL [39]

Corynebacterium spp., *Arthrobacter* spp., *Brevibacterium* spp., *Carnobacterium* spp., *Bifidobacterium* spp., and yeasts), which, like LAB, are capable of growth in certain phases of cheese production and ripening, and through the fermentation of lactose, citrate, and lactic acid (as well as through proteolysis and lipolysis), contribute significantly to typical quality characteristics of cheese, such as flavour, taste, and texture. Due to the technological and nutritional function of these microorganisms, most of them are desirable in raw milk.

However, there are also a number of undesirable microorganisms in raw milk that can affect the quality and safety of cheese. These undesirable microorganisms include, in particular, spores of *Clostridium tyrobutyricum*, which cause the undesired butyric acid fermentation in cheese [18]. Gram-negative bacteria play a subordinate role in freshly milked milk, but can dominate after cold storage of the milk [19]. Many of these microorganisms form lipases and proteases, which may lead to flavour defects in mature cheese products [20].

Enterococci, in particular, *E. faecalis* and *E. faecium*, belong to the LAB group, and are found in many ready-to-eat foods [21, 22]. Enterococci are discussed very controversially in literature. They are important nosocomial pathogens [23]. Moreover, they are known for their ability to easily acquire and transfer antibiotic resistance genes [24]. Although enterococci occur in high numbers in certain types of cheese and fermented sausages, they are usually not deliberately added as starter cultures. Some strains are, however, used as probiotics [25]. At present, enterococci are not considered food-borne pathogens and are therefore not a legal food safety criterion.

Fermented foods may contain large amounts of biogenic amines, which can be problematic. The amines histamine and tyramine are particularly undesirable; both can trigger a broad spectrum of health disorders. Contents of more than 300–500 mg of histamine and tyramine in 1 kg of cheese are in line with cheese defects, such as pungency and undesired eye formation, as well as poor maturation properties [26, 27]. The absence of biogenic amine-forming microorganisms (*Lactobacillus parabuchneri*, Enterococci, and Enterobacteria) in raw milk is an important prerequisite for the production of high-quality cheese from unpasteurized milk.

3. Food Safety and Quality of Raw-milk cheese

In Switzerland, about 45% of the milk produced is processed into cheese. Around half of that cheese production is raw-milk cheese and cheese made from thermized milk (subpasteurization conditions). Matured semi-hard and hard cheeses are regarded as relatively safe foods, because most pathogenic microorganisms are continuously inactivated during cheese ripening. In semi-hard cheese, however, the inactivation rate is lower than in hard cheese. *Listeria monocytogenes* and *Mycobacterium avium* ssp. *paratuberculosis* stand out, in particular, due to inactivation rates of 0.5 log or less per month [28, 29]. Due to the higher water content, semi-hard cheeses are ripenng faster, and are usually consumed earlier. Therefore, they represent higher risk to human health than hard cheeses. In the United States, cheese made from non-pasteurized milk must be matured for at least 60 days before sale, in accordance with the FDA Code of Federal Regulation 21, part 133 [30]. This so-called 60-days rule has been adopted in other countries, although maturation for 60 days may not be enough to eliminate or reduce pathogenic bacteria in cheese to an acceptable level [31-33]. The microbial hazards that must be addressed in the context of an HACCP study for semi-hard and hard cheeses include microorganisms that occur frequently in the microbiome of raw milk, and have a good survival capacity in the cheese. Or they are able to multiply during certain phases of the cheese production and ripening process and therefore may reach problematic bacterial counts, even with low initial contamination (Table 1). In addition, the microorganisms present in raw milk largely pass into the cheese during cheese manufacturing, and are physically enriched about tenfold.

Within the framework of the HACCP concept, microbiological end product controls mainly have the task of checking its functioning, and they are not carried out closely for semi-hard and hard cheeses. This lack of close monitoring makes it all the more important to inhibit the microbial hazards described in Table 1 through a well-controlled production process.

4. Milk Treatment to Improve the Food Safety of Cheese

4.1. Storage of Raw Milk

If raw milk is not subjected to a germicidal process before being processed into cheese, the microbiological quality of the raw milk plays a crucial role in food safety and the sensory quality of the cheese. According to European Regulation (EC) No 853/2004 [40], milk for cheese production may be stored at temperatures >8°C with the approval of the competent authority. This holds true for Switzerland, but the Swiss ordinance on hygiene in milk production limits the storage time to 24 hours if the milk is stored >8°C.

As Figure 1 shows, the growth of *Escherichia coli* at temperatures above 12°C accelerates to such an extent that unacceptable contamination of the processing milk may occur if the milk is stored for only 12 hours. Below 10°C, however, there is no significant increase in pathogenic germs in the milk for 24 hours. An exception is *Listeria monocytogenes*, which is still able to multiply even at 0°C (Figure 2).

Producers of traditional cheeses do not like to store raw milk at

Milk Science International (72) 2019 P. 45-52
ISSN 2567-9538

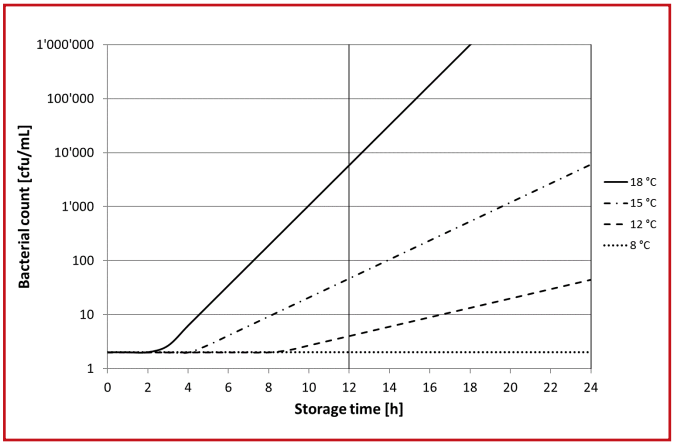

Figure 1: Propagation of *Escherichia coli* in milk at different temperatures (Simulation with Sym'previus®, [41])

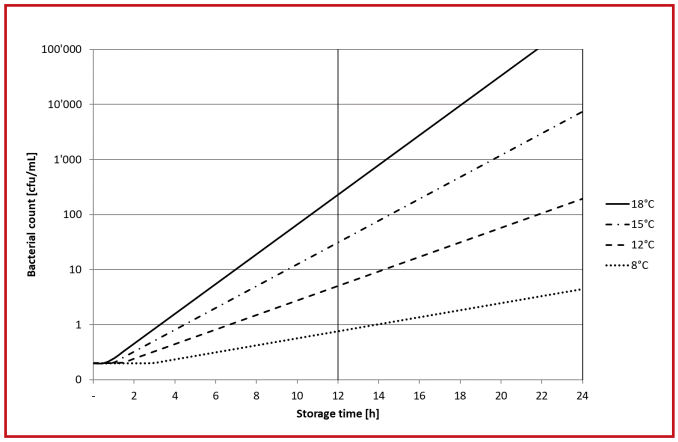

Figure 2: Propagation of *Listeria monocytogenes* in milk at different temperatures (Simulation with Sym'previus®, [41])

temperatures below 12°C for various reasons. Cold storage leads to an increase in pH and dissociation of casein micelles, which has a negative effect on rennet coagulation and cheese yield [42]. Moreover, cold storage of the milk binds the milk's own lipoprotein lipase to the fat globules, and initiates spontaneous lipolysis [43]. Finally, cold storage of the milk allows only the psychrotrophic bacteria, most of which are Gram-negative, to grow, while lactic acid bacteria do not multiply. This can also lead to off-flavor in cheese. Based on Figure 1 and experience, it can be said that overnight storage of milk milked in the evening at 12–13°C does not call into question the quality and safety of cheeses matured for 60 days, provided that the risk of contamination with listeria is addressed with targeted control measures. In the production of smear-ripened cheeses, the regular examination for listeria of the brine used for cheese care represents such a control measure.

Table 2: D- and z-values for heat inactivation of different bacterial species

Bacterial species	Medium	D-value 65°C [s]	z-value [°C]	Source
Campylobacter jejuni/coli	Various media	1.3	6.4	[47]
Coxiella burnetii	Milk	156.1	4.4	[46]
Enterococcus faecalis	Various media	123.2	9.5	[47]
Escherichia coli [1]	Milk	3.4	4.3	[33]
Listeria monocytogenes [2]	Milk	21.6	6.7	[46]
Mycobacterium avium ssp. *paratuberculosis*	Milk	68.5	7.1	[48]
Mycobacterium bovis/caprae [3]	Milk	6.6	5.3	[49]
Salmonella spp.	Various media	2.6	5.2	[47]
Staphylococcus aureus	Milk	15.4	9.5	[50]
Yersinia enterocolitica	Milk and other media	5.4	6.7	[50]

[1] Average of six strains without pronounced heat resistance

[2] Mean values calculated using Sörqvist's regression equations for experiments with capillary tubes or a slug flow heat exchanger [47]

[3] Mean values calculated using the D values at 60°C, 62°C, and 65°C of two strains of *M. caprae* and one strain of *M. bovis*

4.2. Heat Treatment

Heat treatment of milk is the most common method of eliminating unwanted microorganisms from raw cheese milk. A distinction is made between pasteurization and thermization. The conditions for the pasteurization of milk are specified precisely by law as a heat treatment at 72°C for at least 15 seconds, or a temperature-time combination with the same effect which leads to a negative phosphatase test [44, 45]. The temperature-time combination of 63°C and 30 minutes is considered equivalent to 72°C and 15 seconds. Under these conditions, the bacterial count of pathogenic microorganisms, such as *Coxiella burnetii*, is reduced by ≥ 7 log [46].

Table 2 shows for different bacterial species typical decimal reduction times at 65°C (D-values) and the number of degrees the temperature has to be increased to achieve a reduction in the D-value by 1 log (z-values). D- and z-values vary from strain to strain. Some pathogenic bacteria show a significantly higher z-value than *C. burnetii*, which means that their D-value is less influenced by temperature changes. For example, *Salmonella enterica* serovar *Senftenberg* is considerably more heat resistant than other salmonella. The medium and test conditions also have an influence on the values [47].

In contrast to pasteurization, thermization is not precisely defined by law, except that it is a heat treatment of milk below 72°C, which does not lead to complete inactivation of alkaline phosphatase. In general, however, a temperature in the range 57°C to 68°C for 10–20 seconds is used [51]. The primary purpose of thermization of cheese milk is to reduce the risk of undesired fermentation. Due to the lower heat load, enzymes (such as lipoprotein lipase) and thermoduric bacteria (such as pediococci and enterococci) are less inactivated, which affects the ripening and aroma development of the cheese [52-55].

According to the specifications for certain Swiss PDO cheeses, only thermization at 57°C to 68°C for 15 seconds, if at all, is permitted as heat treatment. However, the figures in Table 3 show that listeria and salmonella are only slightly inactivated at 57°C and a holding time of 15 seconds. Even at temperatures of 62°C and 65°C, *Listeria monocytogenes* is not sufficiently inactivated in 15 seconds (reduction by 0.2 log = 40% and by 0.7 log = 80%, respectively).

If the thermization of the cheese milk in the context of an HACCP study is regarded as one of the measures for controlling microbial hazards

Table 3: Reduction of the bacterial count of *Listeria monocytogenes* and Salmonella at different thermization conditions calculated on the basis of average D- and z-values

Conditions for thermization		Reduction of viable counts	
Temperature [°C]	Holding time [sec]	Listeria monocytogenes[1]	Salmonella spp.[2]
57	15	< 0.1 log	0.2 log
62	15	0.2 log	1.5 log
65	15	0.7 log	5.7 log
68	15	2.0 log	> 7 log

[1] Basis for calculation: D-value at 65°C in milk: 21.6 seconds, z-value: 6.7°C [47]
[2] Basis for calculation: D-value at 65°C in various media: 2.6 s, z-value 5.2°C for *Salmonella* spp. without *S. senftenberg* [47]

in a particular cheese variety, it is necessary to define acceptable temperature-time combinations. Such a definition can be found in the Swiss guideline for milk processing in alpine dairies [56]: A temperature-time combination of 65°C/15 seconds corresponds to 60°C/5 minutes (calculated with a z-value of 4.3°C).

5. Bactofugation

In the early 1960s, special centrifuges known as Bactofuge units came on the market. Since then, they have been widely used in the cheese industry to remove the heat-resistant spores of *Clostridium tyrobutyricum* from milk, which cause the undesired late blowing in cheese [18, 57, 58]. Bactofugation (BF) made it possible to produce cheese from milk originating from cows fed with silage without the addition of nitrate, which was controversial at the time [59].

BF is based on the difference in density between the milk (d_4^{20}= 1.034 g/mL) and the microorganisms. Bacterial spores, in particular, have a high density of 1.30–1.32 g/mL. Vegetative cells have a density of only 1.07–1.12 g/mL, and therefore, are eliminated to a lower extent in BF than spores [60]. In addition to density, cell size is important. Large cells and cell clusters are better separated than small ones. Because the efficiency of the BF strongly depends on the viscosity, the milk must be heated. In cheese dairy practice, bactofugation is usually carried out at 55°C to 60°C [61].

Numerous studies have shown that BF of milk can eliminate 90.0% to 99.5% of bacterial spores, depending on the conditions, which corresponds to a reduction of 1–2.3 log (Table 4). Anaerobic spores (e.g., *C. tyrobutyricum*) are better separated than aerobic spores, for example, *Bacillus* spp. [60, 62, 63]. Milk from farms feeding silage is often bactofugated twice, as a single treatment may not be sufficient to reduce the number of clostridial spores below the damage threshold, that is, below 25 spores per liter, depending on the cheese variety.

Table 5: Elimination of vegetative cells of microorganisms by bactofugation of milk

	Volume flow rate [L/h]	Temperature [°C]	Reduction in viable counts [%]	Source
Aerobic mesophilic bacteria	25,000	55.0–65.0	86.0–92.0	[62]
Aerobic mesophilic bacteria	30,000	55.0	10.0	[65]
Enterobacteria	30,000	55.0	72.0	[65]
Escherichia coli [1]	2950 (50%)	54.4	95.3 (double BF)	[66]
Enterococci	30,000	55.0	7.00	[65]
Yeasts	30,000	55.0	55.0	[65]
Lactobacilli	not specified	50.0	90.0	[69]
Lactobacilli	30,000	55.0	33.0	[65]
Mycobacterium avium* spp. *paratuberculosis	not specified	60.0	74.0–93.0	[67]

[1] Two serial bactofuges were operated with 50% of the nominal output (L/h). The reduction of the germ count by 95.3% after two BFs corresponds to a reduction of approximately 78.0% per treatment.

Various authors have investigated the effect of BF on vegetative bacteria in milk. However, the findings are very inconsistent (Table 5). According to Te Giffel and Van der Horst [62], the total bacterial count of raw milk can be reduced by 86% to 92% by BF at 55°C to 65°C. This corresponds to a reduction of about 1 log. Faccia et al. [65] found a much lower reduction rate under real conditions, and large differences between the germ groups. Enterobacteria were reduced by 72%, enterococci by 7%, and aerobic mesophilic germs by 10%. The significant reduction of enterobacteria is consistent with the results of Kosikowski and Fox [66], who achieved a reduction of 95% with double-BF. This result corresponds to a reduction of approximately 78% per treatment.

Table 4: Efficacy of bactofugation of milk to reduce spore content

Microorganism	Acceleration	Temp.	Volume flow rate	Spore removal	Source
Bacillus subtilis	9000 g	71°C	5400 L/h	98.80%	[64]
Bacillus subtilis	9000 g	71°C	1800 L/h	99.20–99.80%	[64]
Bacillus cereus	9000 g	71°C	5400 L/h	90.30%	[64]
Bacillus cereus	9000 g	82°C	5400 L/h	97.10%	[64]
C. tyrobutyricum	not specified	60°C	6000 L/h	95.80%	[18]
C. tyrobutyricum	not specified	65°C	6000 L/h	96.40%	[18]
C. tyrobutyricum	not specified	65°C	4000 L/h	97.60%	[18]
C. tyrobutyricum	not specified	70°C	6000 L/h	97.50%	[18]
Anaerobic spores	not specified	48°C	n.a.	97.40–98.70%	[64]
Aerobic spores	not specified	48°C	n.a.	94.10–97.70%	[64]
Anaerobic spores	not specified	50°C	48,000 L/h	99.40%	[63]

Milk Science International (72) 2019 P. 45-52
ISSN 2567-9538

The fact that enterobacteria and enterococci differ primarily in heat resistance (Table 2) indicates that the heat load during BF contributes significantly to the reduction of enterobacteria and other thermolabile microorganisms. The residence time of the milk in the Bactofuge unit lies in the range of only 5–7 seconds [67]. The transportation time from the milk heater to the Bactofuge unit and back must also be considered. Agroscope investigated industrially bactofugated milk (holding time 30 seconds at 62°C). No significant reduction in enterococci was observed in comparison to only thermized milk [68].

The results in Table 5 confirm Kessler's statement [70] that pathogenic microorganisms cannot be reliably eliminated by BF of milk under subpasteurization conditions.

6. Microfiltration

In the 1980s, microfiltration with the use of membrane separation processes for the elimination of microorganisms appeared on the market [71, 72]. The process later became known mainly through the Bactocatch process patented by Tetra Pak [73].

As a rule, ceramic membranes with a pore size of 1.4 µm are used. This pore size makes it possible to retain microorganisms in the so-called retentate without losing too much micellar casein [62]. In contrast to BF, microfiltration (MF) always treats skimmed milk. The cream must be subjected to Ultra High Temperature (UHT) treatment together with the retentate, to inactivate the bacterial spores. MF is usually carried out at 50°C, to reduce the viscosity of the milk and to counteract the growth of microorganisms. However, microfiltration at 6°C using tubular ceramic membrane with a nominal pore size of 1.4 µm has been shown to be feasible too [74, 75].

Numerous studies have shown that MF reduces germs in skim milk by 2-4 log [76-79]. In contrast to the BF, the reduction rates of spores and vegetative cells are not significantly different. Trouvé et al. [76] inoculated skimmed milk with various Gram-negative and Gram-positive bacterial species, as well as spores of *Clostridium tyrobutyricum*, and microfiltered the milk at 50°C using a ceramic filter with a pore size of 1.4 µm. It has been shown, that about 99.90% to 99.98% of the cells of all species were eliminated, which corresponds to a reduction of 3-4 log. However, in experiments with cold microfiltration (pore size 1.4 µm), Griep et al. found that the reduction of bacterial spores is species-dependent [75]. Spores of *Bacillus licheniformis* were reduced by only 2.17 ± 0.64 log while spores of *Geobacillus* sp. were reduced by >6 log.

In contrast to BF, MF of cheese milk is less common. This is not only because MF is a relatively new technology. MF also entails higher investment and operating costs [63]. Furthermore, Bio Suisse, the federation of Swiss organic farmers, prohibits UHT treatment of cream used to make organic-labeled cheese, which also makes MF more difficult to use.

7. Conclusions

The microbiome and indigenous enzymes of raw milk have a significant influence on ripening and flavour development in cheese [10]. Indeed, many consumers attribute superior sensory quality to raw-milk cheeses. Most specifications for PDO cheeses require only minimal pre-treatment of the milk. These restrictions aim to preserve the original characteristics of the traditional cheeses. But this objective is in conflict with the growing requirements regarding food safety particularly faced by industrial cheesemakers.

Although there is still no official limit for histamine in cheese, it is now essential to define control measures to avoid high levels of histamine. Given the low effect level of histamine-forming *Lactobacillus parabuchneri,* however, this is one of the challenges for manufacturers of raw-milk cheese. Another challenge is Shiga toxin-producing strains of *Escherichia coli* (STEC). Their prevalence in raw milk is significantly higher than that of *Salmonella* spp. as shown in Table 1 and confirmed by Hartung et al. [80]. STEC in semi-hard and soft cheeses from raw milk can often be detected by PCR but rarely isolated [81]. Given the low infectious dose and potentially serious consequences of infection, the hazard must be addressed in every HACCP study for cheese from unpasteurized milk.

When the food safety of cheese is called into question, hurdle technology is often referred to. In the concept of hurdle technology, the interaction and combination of various processing measures leads to a safe foodstuff, not a single control measure (critical control point CCP) alone [82]. In the case of cheese made from unpasteurised milk, these factors are primarily animal health, milking hygiene, milk storage, milk treatment, scalding and acidification of the curd, as well as cheese ripening. The proper use of hurdle technology needs to include sound information on factors affecting the survival and growth of targeted microorganisms [83]. Unfortunately, in many PDO specifications, process parameters relevant to food safety (e.g. milk storage conditions) are often not defined precisely enough. In the production of raw-milk cheese, milk is stored preferably at temperatures above 10°C for various reasons as outlined in chapter 4.1 [42, 84]. This practice is in line with European Regulation (EC) No 853/2004 [40] if food safety can be guaranteed. In order to be able to establish HACCP studies on a scientific basis, it is important to know all process parameters exactly. As shown above, it makes a big difference, whether the milk is stored at 10° or 15° C. The same applies to the conditions under which milk is thermized. For unpasteurized cheeses ripened for fewer than 60 days, milk thermization at 65°C for at least 15 seconds, or equivalent temperature-time conditions, is recommended. Such thermization is suitable for largely controlling the pathogenic enterobacteria (STEC, salmonellae) as a hazard in mature cheeses. Moreover, *Staphylococcus aureus* is also reduced under these conditions to such an extent that toxin formation in the cheese, which requires a microorganism count of $> 10^5$ cfu/g, is highly unlikely. The comparatively heat-resistant listeria, however, cannot be safely controlled by milk thermization. Therefore, additional specific measures are needed, such as examination of the smear water after cheese treatment.

BF of milk cannot substantially reduce thermoduric microorganisms, with the exception of bacterial spores. Enterobacteria and other thermolabile germs are, however, thermally inactivated depending on the temperature applied for BF. Compared to thermization with the same temperature and residence time, BF hardly improves the food safety of cheese.

MF of the milk allows nearly complete removal of all microorganisms, and therefore makes a significant contribution to the food safety of cheese. However, the milk must be skimmed before MF. The cream is then usually UHT treated and re-added to the microfiltered skimmed milk. As Beuvier et al. [85] showed, cheese made from milk processed in this way is sensorially close to cheese made from pasteurized milk. In France, debate has emerged about whether microfiltration at temperatures below 40°C could be a technology for producing traditional raw-milk cheeses while complying with European food safety guidelines [86, 87]. However, the designation of a cheese made from microfiltered milk as raw-milk cheese is hardly consistent with the definition of raw milk in Regulation (EC) No 853/2004 [40].

There is still no method of milk treatment suitable for artisanal

cheese production that makes it possible to remove pathogenic microorganisms from the raw milk without significantly altering the organoleptic properties of the cheeses made from it. This makes it all the more important to create guidelines for good manufacturing practice for artisanal cheese production that are based on GHP and HACCP studies and that interpret scientific findings for practitioners. It is questionable whether the Community Guide by FACE network [88] is a real help to artisanal cheese dairies. The guideline leaves the interpretation of legal requirements largely to the individual food business operator, for example the interpretation of the requirements given by Regulation (EC) No. 2073/2005 [89] regarding monitoring of process hygiene criteria and food safety criteria.

References

1. Union Suisse des Paysans. Statistique laitière de la Suisse, 2017; 2018 [cited 2019 Oct 14]. Available from: URL: www.sbv-usp.ch/fr/medias/publications/statistique-laitiere/.
2. Ministère de l'Agriculture et de l'Alimentation Secrétariat général Service de la Statistique et de la Prospective. Enquête annuelle laitière 2017; 2018. Available from: URL: http://agreste.agriculture.gouv.fr/IMG/pdf/Cd191iaav3.pdf.
3. Hartmann K, Licitra G, Eugster-Meier E, Fröhlich-Wyder M-T, Jakob E, Wechsler D et al. Hard cheeses. In: Papademas P, Bintsis T, editors. Global cheesemaking technology. Chichester: John Wiley & Sons. Ltd.; 2017. p. 204–46.
4. López Morales MB, Ardö Y, Berthier F, Karatzas K-A, Bintis T. Blue-veined cheeses. In: Papademas P, Bintsis T, editors. Global cheesemaking technology. Chichester: John Wiley & Sons. Ltd.; 2017. p. 415–35.
5. Consorzio Tutela Taleggio: Official website for Taleggio PDO; 2019 [cited 2019 Aug 9]. Available from: URL: https://www.taleggio.it/il-taleggio/produzione/.
6. Ardö Y, Berthier F, Harmann K, Eugster-Meier E, Fröhlich-Wyder M-T, Jakob E et al. Bacterial surface-ripened (smear) cheeses. In: Papademas P, Bintsis T, editors. Global cheesemaking technology. Chichester: John Wiley & Sons. Ltd.; 2017. p. 397–414.
7. Bintsis T, Alichanidis E, Uzonoy I, Özer B, Papademas P, Radulovic Z et al. White-brined cheeses. In: Papademas P, Bintsis T, editors. Global cheesemaking technology. Chichester: John Wiley & Sons. Ltd.; 2017. p. 349–67.
8. Anne VI. Pourquoi la future AOP Camembert de Normandie est-elle critiquée par certains producteurs ? Le Monde [cited 2019 Oct 14]. Available from: URL: https://www.lemonde.fr/economie/article/2019/03/13/pourquoi-la-future-aop-camembert-de-normandie-est-elle-critiquee-par-certains-producteurs_5435488_3234.html.
9. Sperat-Czar A, Sainte Marie C de. Cru-pasteurisé, à qui profite la coexistence? Profession fromager [cited 2019 Oct 14]:27–8. Available from: URL: https://www.professionfromager.com/thematiques/dossiers/.
10. Licitra G, Caccamo M, Lortal S. Artisanal Products Made With Raw Milk. In: Nero LA, Carvalho AF de, editors. Raw Milk. London: Academic Press; 2019. p. 175–221.
11. Hartmann K, Maubois JL. Mould surface-ripened cheeses. In: Papademas P, Bintsis T, editors. Global cheesemaking technology. Chichester: John Wiley & Sons. Ltd.; 2017. p. 392–6.
12. Food and Agriculture Organization of the United Nations (FAO). Codex Alimentarius: General Principles of Food Hygiene [CXC 1-1969 ; CAC/RCP 1-1969]; 1969 [cited 2019 Oct 14]. Available from: URL: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B1-1969%252FCXP_001e.pdf.
13. European Union (EU). Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs; 2004 [cited 2019 Oct 14]. Available from: URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0852&from=GA.
14. Mayo B, Rachid CTCC, Alegría A, Leite AMO, Peixoto RS, Delgado S. Impact of next generation sequencing techniques in food microbiology. Curr Genomics 2014; 15(4):293–309.
15. Quigley L, O'Sullivan O, Stanton C, Beresford TP, Ross RP, Fitzgerald GF et al. The complex microbiota of raw milk. FEMS Microbiol Rev 2013; 37(5):664–98.
16. Masoud W, Vogensen FK, Lillevang S, Abu Al-Soud W, Sørensen SJ, Jakobsen M. The fate of indigenous microbiota, starter cultures, *Escherichia coli*, *Listeria innocua* and *Staphylococcus aureus* in Danish raw milk and cheeses determined by pyrosequencing and quantitative real time (qRT)-PCR. Int J Food Microbiol 2012; 153(1-2):192–202.
17. Quigley L, O'Sullivan O, Beresford TP, Ross RP, Fitzgerald GF, Cotter PD. High-Throughput Sequencing for Detection of Subpopulations of Bacteria Not Previously Associated with Artisanal Cheeses. Appl. Environ. Microbiol. 2012; 78(16):5717–23.
18. Bergère JL, Le Bars D, Commissaire J. La bactofugation du lait et l'élimination des spores de *Clostridium tyrobutyricum*. Lait 1969; 49(488):507–19.
19. Robinson RK, Scott R, Wilbey RA. Cheesemaking practice. 3. ed. Gaithersburg, Md: Aspen Publishers; 1998.
20. Richard J, Desmazeaud M. The microbial flora of milk intended for cheesemaking. In: Eck A, Gillis J-C, editors. Cheesemaking: From science to quality assurance. 2nd ed. London: Intercept; 2000. p. 189–98.
21. Baumgartner A, Kueffer M, Rohner P. Occurrence and antibiotic resistance of enterococci in various ready-to-eat foods. Archiv für Lebensmittelhygiene 2001; 52:1–24.
22. McAuley CM, Britz ML, Gobius KS, Craven HM. Prevalence, seasonality, and growth of enterococci in raw and pasteurized milk in Victoria, Australia. J Dairy Sci 2015; 98(12):8348–58.
23. Top J, Willems R, Bonten M. Emergence of CC17 *Enterococcus faecium*: from commensal to hospital-adapted pathogen. FEMS Immunol Med Microbiol 2008; 52(3):297–308.
24. Teuber M, Meile L, Schwarz F. Acquired antibiotic resistance in lactic acid bacteria from food. Antonie Van Leeuwenhoek 1999; 76(1-4):115–37.
25. Franz CMAP, Huch M, Abriouel H, Holzapfel W, Gálvez A. Enterococci as probiotics and their implications in food safety. Int J Food Microbiol 2011; 151(2):125–40.
26. Wechsler D, Walther B, Jakob E, Winkler H. Bedeutung biogener Amine in der Ernährung und deren Vorkommen in Schweizer Käsesorten [ALP forum]: Agroscope; 2009 [cited 2019 Oct 14]. Available from: URL: https://www.agroscope.admin.ch/agroscope/de/home/publikationen/suchen/reihen-bis-2013/alp-forum.html.
27. Leuschner RGK, Kurihara R, Hammes WP. Formation of biogenic amines by proteolytic enterococci during cheese ripening. J. Sci. Food Agric. 1999; 79(8):1141–4.
28. Bachmann HP, Spahr U. The fate of potentially pathogenic bacteria in Swiss hard and semihard cheeses made from raw milk.

J Dairy Sci 1995; 78(3):476–83.
29. Spahr U, Schafroth K. Fate of *Mycobacterium avium* subsp. *paratuberculosis* in Swiss Hard and Semihard Cheese Manufactured from Raw Milk. Applied and Environmental Microbiology 2001; 67(9):4199–205.
30. Food and Drug Administration. Cheeses and related cheese products: Code of Federal Regulations 21 [Subchapter B - Food for Human Consumption]; 2015 [cited 2019 Oct 14]. Available from: URL: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=133&showFR=1.
31. Reitsma CJ, Henning DR. Survival of Enterohemorrhagic Escherichia coli O157:H7 During the Manufacture and Curing of Cheddar Cheese. J Food Prot 1996; 59(5):460–4.
32. Schlesser JE, Gerdes R, Ravishankar S, Madsen K, Mowbray J, Teo AYL. Survival of a five-strain cocktail of *Escherichia coli* O157:H7 during the 60-day aging period of cheddar cheese made from unpasteurized milk. J Food Prot 2006; 69(5):990–8.
33. Peng S, Schafroth K, Jakob E, Stephan R, Hummerjohann J. Behaviour of *Escherichia coli* strains during semi-hard and hard raw milk cheese production. International Dairy Journal 2013; 31(2):117–20.
34. Rusch M. Untersuchungen zu verschiedenen mikrobiologischen Parametern in Rohmilch und Rohmilchkäse einer Biokäsereigenossenschaft über den Zeitraum eines Jahres: Doctoral Thesis, University of Zurich; 2005 [cited 2019 Oct 16]. Available from: URL: https://nbn-resolving.org/urn/resolver.pl?urn=urn:nbn:ch:bel-295374.
35. Menéndez González S, Hartnack S, Berger T, Doherr M, Breidenbach E. A qualitative risk assessment approach for Swiss dairy products: opportunities and limitations. Zoonoses Public Health 2011; 58(3):209–19.
36. Verraes C, Vlaemynck G, van Weyenberg S, Zutter L de, Daube G, Sindic M et al. A review of the microbiological hazards of dairy products made from raw milk. International Dairy Journal 2015 [cited 2019 Oct 14]; 50:32–44. Available from: URL: http://www.afsca.be/comitescientifique/publications/articles/_documents/Verraesetal.2015a.pdf.
37. Fröhlich-Wyder MT. Bedeutung des Arginin Deiminase Stoffwechsels von Starterkulturen und Milchsäurebakterien aus der Rohmilch für die Qualität und Reifung von Käse. Ansbach; 2016. (Ansbacher Fachgespräche).
38. Imhof R, Schwendimann L. Prevalence of pathogenic bacteria in raw milk in Switzerland. Nantes; 2014. (International ICMFH conference).
39. Ascone P, Maurer J, Haldemann J, Irmler S, Berthoud H, Portmann R et al. Prevalence and diversity of histamine-forming Lactobacillus parabuchneri strains in raw milk and cheese – A case study. International Dairy Journal 2017; 70:26–33.
40. European Union (EU). Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules on the hygiene of foodstuffs.; 2004 [cited 2019 Oct 14]. Available from: URL: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:139:0055:0205:en:PDF.
41. Leporq B, Membré J-M, Dervin C, Buche P, Guyonnet JP. The "Sym'Previus" software, a tool to support decisions to the foodstuff safety. Int J Food Microbiol 2005; 100(1-3):231–7.
42. Lenoir J, Remeuf F, Schneid N. Rennetability of milk. In: Eck A, Gillis J-C, editors. Cheesemaking: From science to quality assurance. 2nd ed. London: Intercept; 2000. p. 213–38.
43. Deeth HC. Lipoprotein lipase and lipolysis in milk. International Dairy Journal 2006; 16(6):555–62.
44. Food and Drug Administration. Grade "A" Pasteurized Milk Ordinance: Includes provisions from the Grade "A" Condensed and Dry Milk Products and Condensed and Dry Whey--Supplement I to the Grade "A" PMO; 2017 [cited 2019 Oct 14]. Available from: URL: https://www.fda.gov/media/114169/download.
45. European Union (EU). Regulation (EC) No 1662/2006 amending Regulation (EC) No 853/2004 [Official Journal of the European Union]; 2006. Available from: URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1662&qid=1571080851939&from=EN.
46. Cerf O, Condron R. *Coxiella burnetii* and milk pasteurization: an early application of the precautionary principle? Epidemiol Infect 2006; 134(5):946–51.
47. Sörqvist S. Heat resistance in liquids of *Enterococcus* spp., *Listeria* spp., *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella* spp. and *Campylobacter* spp. Acta Vet Scand 2003; 44(1-2):1–19.
48. Sung N, Collins MT. Thermal tolerance of *Mycobacterium paratuberculosis*. Applied and Environmental Microbiology 1998; 64(3):999–1005.
49. Hammer P, Richter E, Rüsch-Gerdes S, Walte H-GC, Matzen S, Kiesner C. Inactivation of *Mycobacterium bovis* ssp. *caprae* in high-temperature, short-term pasteurized pilot-plant milk. J Dairy Sci 2015; 98(3):1634–9.
50. Firstenberg-Eden R, Rosen B, Mannheim CH. Death and injury of *Staphylococcus aureus* during thermal treatment of milk. Can. J. Microbiol. 1977; 23(8):1034–7.
51. Rukke EO, Sørhaug T, Stepaniak L. Heat treatment of milk: Thermization of milk. In: Fuquay JW, Fox FP, McSweeney PHL, editors. Encyclopedia of Dairy Sciences. 2nd ed. Oxford UK: Elsevier; 2011. p. 693–8.
52. Franklin JG, Sharpe ME. The incidence of bacteria in cheese milk and Cheddar cheese and their association with flavour. Journal of Dairy Research 1963; 30(1):87–99.
53. Grappin R, Beuvier E. Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese. International Dairy Journal 1997; 7(12):751–61.
54. Foulquié Moreno MR, Sarantinopoulos P, Tsakalidou E, Vuyst L de. The role and application of enterococci in food and health. Int J Food Microbiol 2006; 106(1):1–24.
55. Hickey DK, Kilcawley KN, Beresford TP, Wilkinson MG. Lipolysis in cheddar cheese made from raw, thermized, and pasteurized milks. J Dairy Sci 2007; 90(1):47–56.
56. Jakob E, Menéndez Gonzâlez S. Guideline for the milk production and dairy products on alpine farms [Leitlinie für die gute Verfahrenspraxis bei der Milchgewinnung und -verarbeitung in Sömmerungsbetrieben]. Bern: Schweizerischer Alpwirtschaftlicher Verband (SAV); 2015.
57. Jacobsson L, Thurell KE. Bactofugation of cheese milk. Svenska Mejeritidn 1970; 62(22):405–7.
58. Invernizzi GB. Esperienze Alfa Laval nell'applicazione della bactofugazione per la produzione di formaggio Grana. Latte 1984; 9:465–73.
59. Walstra P, Geurts TJ, Noomen A, Jellema A, van Boekel MAJS. Dairy Technology: Principles of Milk Properties and Processes: CRC Press; 1999.
60. Deeth HC, Datta N. Non-thermal Technologies: Introduction. In: Fuquay JW, Fox FP, McSweeney PHL, editors. Encyclopedia of

Dairy Sciences. 2nd ed. Oxford UK: Elsevier; 2011. p. 725–31.
61. Spreer E. Technologie der Milchverarbeitung. 10., vollst. überarb. Aufl. Hamburg: Behr; 2011.
62. Te Giffel MC, van der Horst, H. C. Comparison between bactofugation and microfiltration regarding efficiency of somatic cell and bacteria removal. Bulletin of the IDF 2004; (389):49–53.
63. GEA Westfalia Separator GmbH. Separators from GEA Westfalia: Separator for milk clarification and bacteria removal; 2015 [cited 2019 Oct 14]. Available from: URL: www.gea.com.
64. Torres-Anjel MJ, Hedrick TI. Spore removal by centrifugation and its effect on ultra-high temperature commercial sterilization of milk. J Dairy Sci 1971; 54(3):326–30.
65. Faccia M, Mastromatteo M, Conte A, Del Nobile MA. Influence of the Milk Bactofugation and Natural Whey Culture on the Microbiological and Physico-Chemical Characteristics of Mozzarella Cheese. J Food Process Technol 2013; 04(04):1–7.
66. Kosikowski FV, Fox PF. Low Heat, Hydrogen Peroxide, and Bactofugation Treatments of Milk to Control Coliforms in Cheddar Cheese. J Dairy Sci 1968; 51(7):1018–22.
67. Grant IR. Occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in foods and the impact of milk processing on its survival. In: Proceedings of the 8th International Colloquium on Paratuberculosis. Madison; 2005. p. 271–7.
68. Fragnière C, Bütikofer U. Comparaison de différents traitements du lait (bactofugation, thermisation) pour la fabrication de Vacherin Fribourgeois. Liebefeld Posieux; 2006. (ALPinterne 204; vol 204).
69. Mc Carthy OJ. Centrifuges and Separators: Applications in the Dairy Industry. Encyclopedia of Dairy Sciences 2011; 4:175–81.
70. Kessler HG. Lebensmittel- und Bioverfahrenstechnik: Molkereitechnologie. 3. neubearb. u. erw. Aufl. Freising: Verlag A. Kessler; 1988.
71. Gillot J, Brinkman G, Garcera D. Nouveaux médias filtrants céramiques pour la microfiltration tangentielle et ultrafiltration: Société Française de Filtration; 1984.
72. Holm S, Malmerg R, Svensson K, inventors. Method and plant for producing milk with a low bacterial count. WO1986001687A1.
73. Uhl M. Bactocatch – Ein neues Verfahren zur Verbesserung der Milchqualität. DMZ, Lebensmittelindustrie und Milchwirtschaft 1990; 110(40):1272–6.
74. Fritsch J, Moraru CI. Development and optimization of a carbon dioxide-aided cold microfiltration process for the physical removal of microorganisms and somatic cells from skim milk. J Dairy Sci 2008; 91(10):3744–60.
75. Griep ER, Cheng Y, Moraru CI. Efficient removal of spores from skim milk using cold microfiltration: Spore size and surface property considerations. J Dairy Sci 2018; 101(11):9703–13.
76. Trouvé E, Maubois JL, Piot M, Madec MN, Fauquant J, Rouault A et al. Rétention de différentes espèces microbiennes lors de l'épuration du lait par microfiltration en flux tangentiel. Lait 1991; 71(1):1–13.
77. Klantschitsch T. Influence of microfiltration on the quality of semi-hard cheese from raw milk with particular emphasis on *Clostridium tyrobutyricum* spores [Dissertation ETH Zürich Nr. 13233]; 1999.
78. Saboya LV, Maubois J-L. Current developments of microfiltration technology in the dairy industrie. Lait 2000; (80):541–53.
79. Elwell MW, Barbano DM. Use of microfiltration to improve fluid milk quality. J Dairy Sci 2006; 89:E20-30.
80. Hartung M, Tenhagen B-A, Käsbohrer A. Erreger von Zoonosen in Deutschland im Jahr 2013. Bundesinstitut für Risikobewertung BfR 2015 [cited 2019 Oct 14]; (2). Available from: URL: https://www.bfr.bund.de/cm/350/erreger-von-zoonosen-in-deutschland-im-jahr-2013.pdf.
81. Zweifel C, Giezendanner N, Corti S, Krause G, Beutin L, Danuser J et al. Characterisics of Shiga Toxin-Producing *Escherichia coli* isolated from Swiss raw-milk cheese within a 3-Year monitoring program. J Food Prot 2010; 73(1):88–91.
82. Jay JM, Loessner MJ, Golden DA. Modern food microbiology [Food Science Text Series]. 7. ed. New York, NY: Springer Science + Business Media Inc.; 2005.
83. Mukhopadhyay S, Gorris LGM. Hurdle Technology. In: Batt CA, Tortorello ML, editors. Encyclopedia of Food Microbiology. 2nd ed. London: Academic Press; 2014. p. 221–7 (vol. 2).
84. Raynal K, Remeuf F. Effect of storage at 4 degrees C on the physicochemical and renneting properties of milk: a comparison of caprine, ovine and bovine milks. J Dairy Res 2000; 67(2):199–207.
85. Beuvier E, Berthaud K, Cegarra S, Dasen A, Pochet S, Buchin S et al. Ripening and quality of Swiss-type cheese made from raw, pasteurized or microfiltered milk. International Dairy Journal 1997; 7(5):311–23.
86. Bérard L, Marchenay P. Les produits de terroir - Entre cultures et règlements. Paris: CNRS Éditions; 2004 [cited 2019 Oct 14]. Available from: URL: http://www.doabooks.org/doab?func=fulltext&rid=19346.
87. Majdi A. Séminaire sur les fromages AOP et IGP; 2009 [cited 2019 Oct 14]. Available from: URL: www.memoireonline.com/07/10/3635/m_Seminaire-sur-les-fromages-AOP-ET-IGP2.html.
88. FACE network. European Guide for Good Hygiene Practices in the production of artisanal cheese and dairy products; 2016. Available from: URL: https://ec.europa.eu/food/safety/biosafety/food_hygiene/guidance_en.
89. European Union (EU). Regulation (EC) No 2073/2005 of November 2005 on microbiological criteria for foodstuffs.; 2005 [cited 2019 Oct 14]. Available from: URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073&qid=1571081229917&from=EN.