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Secondary standards of the industry, trade for enterobacteria in cheese

- Requirements, feasibility and limits

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Coliforms/E.coli situation in the production of cheese

- Limit value discussions -

At the end of the 1970s, microbiological criteria and limit values for coliforms/E.coli in cheese were discussed for the first time by Canada and the United States. These values had to be adhered to when exporting from Europe to Canada.

In order to test which enterobacteria/coliforms/E.coli standards are feasible or realistic in soft and semi-hard cheese production, status surveys on the coliform load of cheeses and stage controls were carried out in various companies/institutes at the beginning of the 1980s, including at the then South German Experimental and Research Institute of the Technical University of Munich-Weihenstephan under Prof. Dr Martin Busse. (e.g. dissertation: Dr J. Hübner: "Origin and development of coliforms in the production of soft cheese").

Results: The initial situation was relatively sobering. The coliform content of the soft cheeses averaged 100,000-1,000,000 cfu/g, the E. coli content was ~ 10,000 cfu/g (100-1,000,000 cfu/g).

Coliforms and E. coli enter the cheese dairy milk, the curd and then the cheese via milk/water residues from the plant in > 90% of cases. The personnel themselves play virtually no role in natural cheese production. If this were the case, it would also be possible to detect increased levels of Staph. aureus in cheese made from pasteurised milk. This has not yet been the case in the past 30 years (as part of the regular marketability tests for cheese from self-marketing and raw milk cheese dairies) - and if it was, then it turned out that the milk was insufficiently heated. Staphylococci are skin/udder germs and primarily enter the (raw milk) cheese via the milk or the milk of udderly sick animals.

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As already mentioned, enterobacteria/coliforms are primarily contamination germs, whereby the cleanability of the system itself plays a major role. Depending on the technical equipment (coagulator, vats, tube portioning, etc.) or pre-maturing technology (continuous or batch pre-maturing), it was or is possible to reduce the coliform and E. coli levels in cheese milk by 2-3 log levels (power of 10) (in the 1980s, as a result of the dissertation). This reduced the coliform content to < 10,000 cfu/g cheese and the E. coli content to < 1000 cfu/g, especially in new "vat cheese dairies" or with coagulator technology.

As already mentioned, lactose-negative enterobacteria do not play a role in curd, whey and cheese. The investigations therefore focussed exclusively on the parameters coliforms and E. coli.

Based on the knowledge gained in various soft cheese dairies, the following guide values were recommended for soft cheese production:

Milk - heated neg. in 100 ml

Milk (for renneting) neg. in 10 ml (prod. end: < 1/ml)

Whey (portioning) neg. in 10 ml (prod. end: < 1/ml)

Other institutions (TU Munich, in co-operation with Edelweiß Käsewerke in Kempten im Allgäu) came to similar conclusions.

Ultimately, these test results were used as a basis for discussion for the guideline values recommended within the IDF (International Dairy Federation).

Standards for cheese under discussion at the IDF (cfu/g)

	SOFT cheese				white cheese in BRINE			
	n	c	m	M	n	c	m	M
Coliformes	no	Norm		5	2	100	1000	
E.coli	5	2	100	1000	5	2	10	100
S.aureus	5	2	100	1000	5	2	1000	10.000
Yeasts			no	Norm		5	2	100 1000

m : of n=5 samples, only 2 samples (c) may exceed this value

M : Limit value that no sample (of n=5) may exceed

These values under discussion at the IDF - without the enterobacteria/coliforms parameter - were then the basis for the limit values issued within Regulation (EC) No. 2073/2005, among others.

Discussion of limit values with regard to the occurrence of pathogenic *E. coli* (STEC)

There is no doubt that the hygienic and, above all, technical cleaning effort is greater today than in the past - both for the farmer and the processor.

However, it is often overlooked that the occurrence and proliferation of undesirable germs is not exclusively a contamination or hygiene problem. In the production of natural cheese, the cultivated bacteria - the acidifying and ripening flora used - play an equally important role as "protective flora".

In many cases, the cultures are not adapted to the respective cheese-making technology. The culture bacteria should not only acidify quickly, but also ensure as far as possible that the cheeses contain as little residual sugar as possible. If this is not the case, a proliferation of spoilage flora can hardly be avoided. In this context, phage-induced acidification disorders, the processing of acidification carriers, overaged milk or inactive cultures play a major role.

A critical factor - especially with thermophilic/mesophilic cheeses - is excessive or rapid proliferation of thermophilic streptococci if the mesophilic lactococci do not have sufficient acidification activity. A common cause is a weakening of the culture by bacteriophages. However, excessively high ($> 38^{\circ}\text{C}$) cheese temperatures can also be critical.

An increased input of thermophilic streptococci via the heater (exchanger, cooler) can also lead to increased coliform proliferation in the cheese. *Streptococcus thermophilus* splits the lactose - into glucose and galactose. Indirectly, this leads to an inhibition of the lactose-loving lactococci. This can result in incorrect fermentation and increased acidification (including coliform proliferation).

The "wisdom" from older textbooks, where milk microbiology is roughly divided into 2 areas, is still probably justified. On the one hand, we are dealing with the more beneficial milk/intestinal microorganisms, and on the other with an increasing number of harmful microorganisms from the "aqueous" (investment) environment.

Many gram-negative germs prefer to grow in an aqueous environment, on moist surfaces. Gram-negative germs have a different cell wall structure (here: presence of lipopolysaccharides) than gram-positive bacteria (streptococci, lactobacilli, etc.) and are therefore often more difficult to combat with the usual oxidative disinfectants. This means that disinfection measures are useful, but care should be taken to ensure that the systems are free of product residues before disinfection and that the disinfectant component is sufficiently active (correct dosages!). The "wet vapour disinfection method" used in the past is therefore still likely to be the most effective.

In this context, it is interesting to note that the number of food-borne infections in certain groups of germs has increased rather than decreased in recent years. (see overview, compiled by Prof Dr Bülte at a seminar in Frankfurt, May 2014). An increase in food-borne infections can still be observed with listeria, although listeriosis in livestock, especially in sheep, has decreased significantly in recent years.

According to official statistics, there has also been an increase in STEC (shigatoxin-forming enterohaemorrhagic *E. coli*). In contrast, the number of cases of the classic pathogenic intestinal germs from the group of cholera and typhoid bacteria has fallen significantly in recent years - this is probably mainly due to the implementation of successful vaccination measures.

The fact that the situation with pathogenic *E. coli* germs and LM diseases caused by EHEC/STEC in cheese production is different is not surprising. *E. coli* germs are often part of the plant, even in closed systems. This means that the detection of *E. coli* - especially in milk processing - does not necessarily allow conclusions to be drawn about faecal contamination and thus the introduction of potentially pathogenic (heat-sensitive) *E. coli* intestinal germs.

As far as cheese is concerned, the all-clear can be given - at least for cheese made from pasteurised milk. To date, there have only been positive STEC findings from raw milk cheeses - mostly detected by PCR analysis.

According to current knowledge, heating and pasteurisation can be regarded as a sufficient safety criterion. At least there are no documented cases of STEC in pasteurised milk cheeses.

Microbiological standards - legal requirements

- Regulation (EC) No. 2073/2005

As enterobacteria, especially *E. coli*, are obligate potential intestinal inhabitants and can be directly or indirectly pathogenic (indication of faecal contamination when detected in drinking water), the legislator has only set microbiological limits for the enterobacteria species *Escherichia coli* in cheese. As enterobacteria or *E. coli* themselves do not necessarily have to be pathogenic, but do indicate secondary contamination, these germs do not act as a safety criterion according to Regulation (EC) No. 2075/2005, such as salmonella and listeria, but as a process hygiene criterion.

Limit values for *E. coli* (in cheese from pasteurised or heat-treated milk) based on a 5-sample plan:

"m" : 100 cfu/g

"M" : 1000 cfu/g

This means that the *E. coli* limit value of 1000 cfu/g should not be exceeded in any cheese sample. Of n=5 samples, c=2 samples may exceed a value of 100 cfu/g.

Exceeding the respective limit values does not de facto imply that the cheese products are immediately "not marketable". According to Regulation (EC) No. 2075, "measures, improvements in production hygiene and in the selection of raw materials must be taken". We therefore recommend that the necessary stage/product controls be carried out immediately - otherwise the product may actually be classified as "not marketable".

However, these regulations according to Regulation (EC) No. 2073/2005 do not apply to private law requirements, such as export regulations (e.g. Russia, TR 88) or requirements of the processing industry (convenience, frozen food) and trade. For exports in particular, it is important not to exceed the respective limits for process hygiene criteria such as *E. coli* - otherwise the goods will not be accepted or will be returned.

Until now, the existence of a functioning HACCP concept (e.g. as part of the IFS standard) was considered sufficient - especially by the industry and trade - especially as this ensured that the microbiological limits specified in Regulation (EC) No. 2073 were met.

Microbiological standards - Secondary/trade standards

It can be observed that, in deviation from the limit values of Regulation (EC) 2073, the processing industry and, in some cases, the trade are setting significantly lower product standards for enterobacteria (previously not regulated at all) and *E. coli*. Yeasts represent a separate category - here only shelf-life aspects are likely to be of significance.

The parameter enterobacteria - in cheese > 99% are coliforms, i.e. lactose-degrading enterobacteria - was deliberately no longer regulated in Regulation (EC) No. 2073, in contrast to the old German Milk Regulation of 1992. On the one hand, the hygienic relevance of this group of bacteria - at least in cheese production - is relatively questionable; on the other hand, it is common practice to deliberately add some of these bacteria to cheese milk

For example, in the production of typical French cheeses (such as Coloumbier), some companies also use *Hafnia alvei* bacteria, which are close to enterobacteria (in terms of nomenclature, these bacteria were previously also categorised as Enterobacteriaceae), primarily as ripening/flavouring bacteria. These bacteria are sometimes added in very high concentrations (> 10⁸ cfu/g) in order to achieve a slight raw milk flavour or "farmhouse taste" even in soft cheeses made from heat-treated milk.

The reason for the very high product standards - increasingly from retailers and the processing industry - is the growing pressure from "outside", i.e. from politics, consumer protection and from the "responsible" or unsettled consumers themselves. According to Prof Andreas Hensel from the Federal Institute for Risk Assessment in Berlin (BfR), "many of these risks are perceived rather than actual dangers. Setting such standards often has nothing to do with statistics and toxicology" (press release from 2 June 2014).

Press releases, in whatever form, should be avoided. Ultimately, the aim is to avert potential (image) damage to the company. Today, it is not uncommon for cheese not to be approved internally even if the limits set for the process hygiene parameter *E. coli* ("m" = 100 cfu/g, "M" = 1000 cfu/g) are slightly exceeded. But, strictly speaking, this product would still be marketable (if the limit values were slightly exceeded). Only measures to improve production hygiene and the selection of raw materials" would be necessary.

In this context, the regularly published so-called RASFF (Rapid Alert System for Food and Feed, EU rapid alert system) notifications certainly play a role. Originally, warnings were to be issued about food or its consumption where there was a potential health risk. However, this is not always the case. For example, warnings are also issued for products with discolouration (such as mozzarella), products with organoleptic changes, *E. coli* (not EHEC), normal moulds (without specification), *Bac. subtilis* in milk, etc.. It is therefore more than understandable that both the press and the consumer are unsettled.

Microbiological (secondary standards) standards - feasibility and limits

Food scandals, "publicising" them and the resulting costly damage to the company's image have resulted in existing standards being set even higher, primarily by retailers. At least the perception is that the food is therefore even "safer".

There is no doubt that the hygienic standards practised in industrial food production today are many times higher than they were 20-30 years ago.

Today, it is possible to produce even larger batches of sliced cheese and soft cheese in such a way that even the limit values of 100 cfu/g (in young cheese) set for "m" are not exceeded, even in multi-batch production.

Depending on the type of cheese and its composition, coliform proliferation is theoretically still possible. To date, however, no proliferation of these germs, especially *E. coli*, has been detected in technologically flawlessly manufactured semi-hard cheese (MIH, Milk-/Dairy Institute Dr.Hüfner)

Tab.1:

		Coliforme-/E.coli - Germ count development in semi-hard cheese (storage at 8°C)					
		factory A (n=4 batches)		factory B (n=2 batches)		Factory C (n=2 batches)	
Time of investigation	unit	large industry		commercial		commercial	
		Coliforms*)	<i>E.coli</i>	Coliforms*)	<i>E.coli</i>	Coliforms*)	<i>E.coli</i>
Before BRINE	cfU/g	---	---	39.000	1.000	190.000	3.000
After BRINE	cfU/g	18	14	110.000	4.000	220.000	3.000
After 2 weeks	cfU/g	8	6	28.500	1.700	81.200	3.000
After 4 weeks	cfU/g	8	1	19.400	500	30.000	3.000
After 6 weeks	cfU/g	3	1	19.200	300	15.000	2.000
After 8 weeks	cfU/g	1	1	16.200	200	---	---

*) identical with enterobacteria content

Against this background, it is therefore neither justified nor sensible (costs!) to further reduce the E. coli limit values, for example to a level of "m" = 10 cfu/g or "M" = 100 cfu/g, in deviation from the requirements of Regulation (EC) No. 2073/2005.

The situation with soft cheeses is somewhat more differentiated. Traditionally, these cheeses had very high salt contents (Camembert > 2.00%; red smear cheese: > 2.2%). This is often no longer the case with white mould cheeses (Camembert, Brie). In these - less salted cheeses - enterobacteria/coliforms can actually still multiply during cold storage (8°C). E. coli multiplies somewhat less during cold storage. Storage tests have shown that bacteria multiply mainly in the alkaline edge zone.

Abb.2:

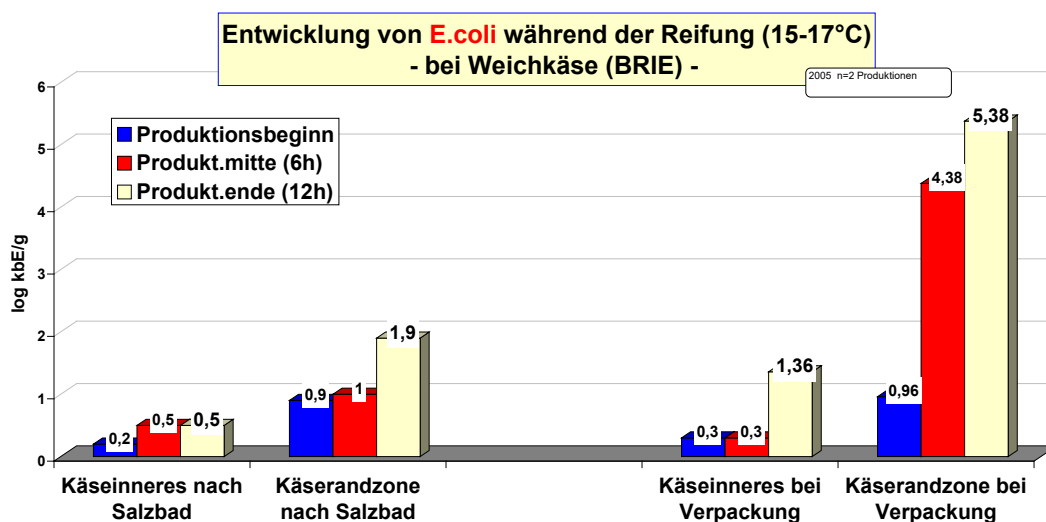


Abb.3:

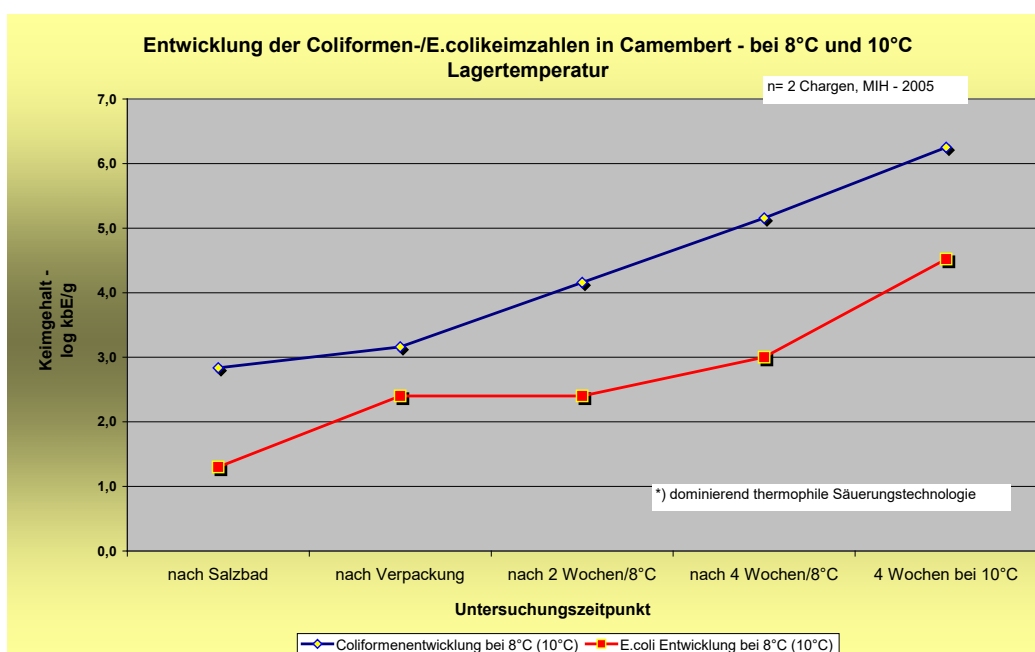


Abb.4:

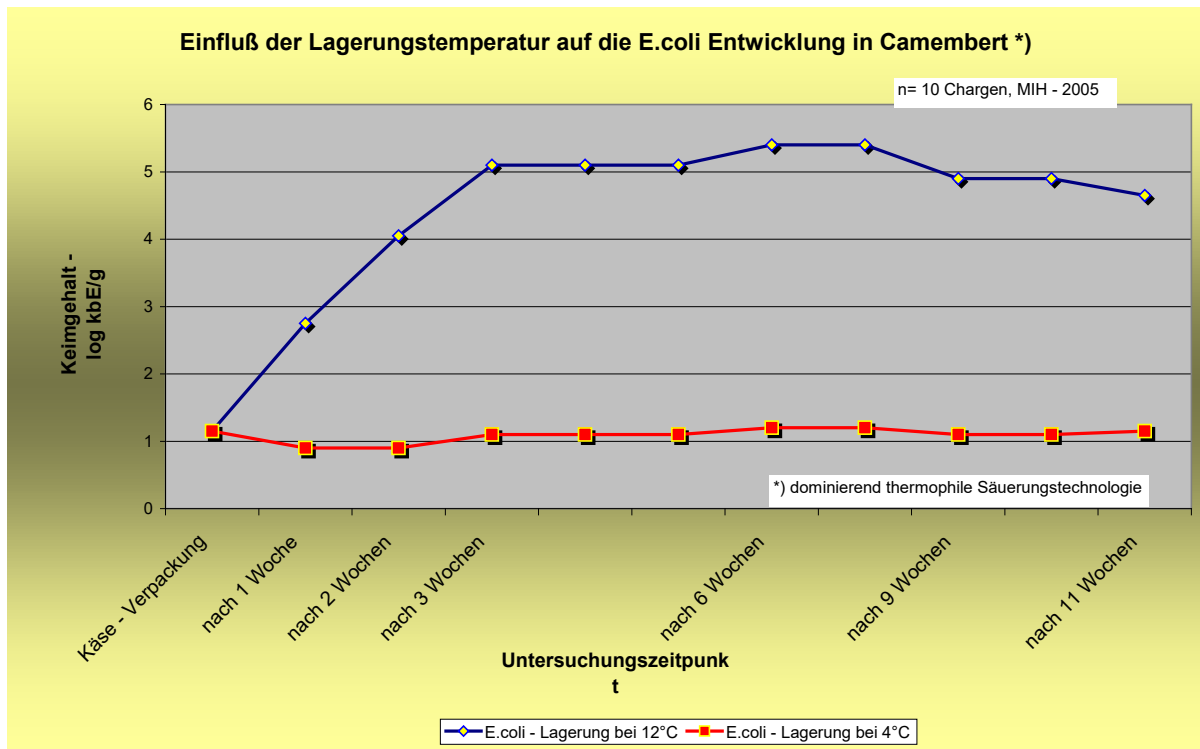
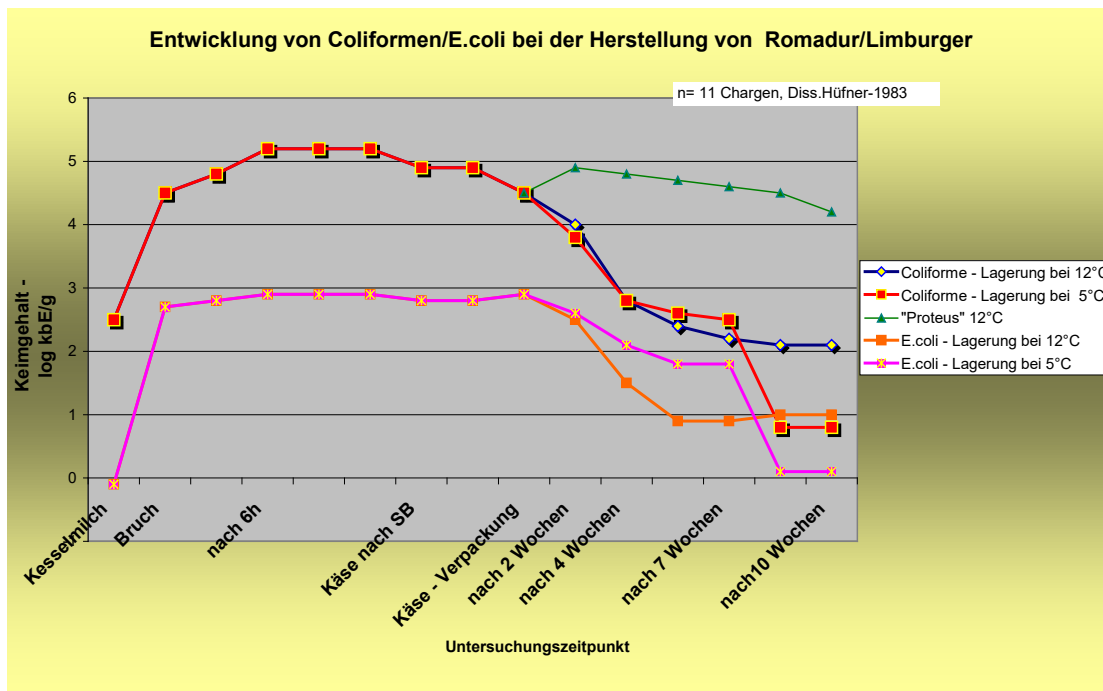


Abb.5:



Secondary industry/trade standards for enterobacteria in cheese
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As a proliferation of enterobacteria, especially in soft cheese, is often unavoidable, the enterobacteria content of soft cheese was or is assessed in a more differentiated manner in some countries. For example, the enterobacteria content of soft cheese was only to be objected to if the enterobacteria content exceeded a value of ~ 1,000,000 cfu/g and sensory changes were perceptible, in line with the Swiss "Ordinance on the hygienic-microbiological requirements for foodstuffs, consumer goods and articles of daily use" (version dated 25 February 1988). This determination was probably also made against the background that enterobacteria/coliforms are part of the natural ripening flora of raw milk cheeses. In the meantime, however, the E. coli values in Switzerland have also been adapted to the requirements of Regulation (EC) No. 2073/2005.

Needless to say, the E. coli limits laid down in Regulation (EC) No. 2073 are difficult to comply with, especially for white mould cheeses (at the end of their shelf life) - at least for cheeses that were manufactured at the end of production.

Irrespective of the fact that the "enterobacteria/E.coli" parameter is not a safety criterion, but merely a process hygiene criterion according to Regulation (EC) No. 2073 - and a correlation to pathogenic bacteria and pathogenic E.coli strains has not yet been proven - it will be necessary to at least prevent excessive bacterial proliferation in the product.

Soft cheeses that are traditionally produced with a predominantly mesophilic technology - using phage-stable multi-strain cultures - pose fewer problems. The residual galactose content of these cheeses (when packaged) is usually < 0.10%. Stronger outliers usually occur if there is also a phage infestation in the culture and the Leuconostoc bacteria, which are important for galactose degradation, are not present and active in sufficient numbers.

A critical factor - especially with thermophilic/mesophilic cheeses - is excessive or rapid proliferation of thermophilic streptococci if the mesophilic lactococci do not have sufficient acidification activity. A common cause is a weakening of the culture by bacteriophages. However, excessively high (> 38°C) cheese temperatures can also be critical.

An increased input of thermotolerant thermophilic streptococci via the heater (exchanger, cooler) can also lead to increased coliform proliferation in the cheese. Streptococcus thermophilus splits the lactose - into glucose and galactose. Indirectly, this leads to an inhibition of the lactose-loving lactococci. This can result in incorrect fermentation and increased acidification (including coliform proliferation).

However, most enterobacteria/E.coli are good galactose utilisers. Enterobacteriaceae are less acid-tolerant than the common lactic acid bacteria (cultures). Rapid and complete acidification is therefore still a very effective means of preventing a strong increase in enterobacteria/E.coli in milk, curd and cheese. It should also be understood that coliform growth stagnates in portioning systems and buffer tanks (before portioning), provided there are no (phage-related) acidification disorders.

Summary - Conclusion

In the Milk Regulation in force until 1992, a limit value of "M" = 100,000 cfu/g was set for the parameter *Escherichia coli* in raw milk cheese. In the meantime - according to Regulation (EC) No. 2073/2005 - there are no longer any *E. coli* limits for raw milk cheese. However, there is no doubt that past heat contaminations are to be assessed differently than an original *E. coli* entry via raw milk. Contaminants - especially coliforms, but also listeria - can multiply much better in cheese made from heated milk than in a germ-rich ("protective flora") environment. The legislator has therefore set very low ("m" = 100 cfu/g and "M" = 1000 cfu/g) *E. coli* limits specifically for cheese made from pasteurised milk. Compliance with these limits is not easy, especially in multi-batch production. Therefore, in today's industrial multi-batch production, cheese-making technology, especially the cleanability of curd processing and portioning systems, plays the decisive role.

Certain retail chains and processors are now demanding *E. coli* levels of < 10 kbEg cheese. The situation is comparable to the yeast limit of < 10 cfu/g for brine cheese. It is perfectly possible to produce semi-hard cheese (we are rather sceptical about soft cheese) with this high *E. coli* standard. However, this *E. coli* standard cannot be guaranteed by any party (cheese dairy plant manufacturer, milk processor) to the trade or industry with a clear conscience, as it is not possible to manufacture complete batches (> 95%) to this high standard.

It is essential that the *E. coli* levels of "m" = 100 cfu/g or "M" = 1000 cfu/g required on the basis of Regulation (EC) No. 2073/2005 are not significantly exceeded or that no further *E. coli* multiplication can take place in the cheese or the end product.

This is the case, as extensive storage tests have shown. (see Tab. 2).

1. in all cases/storage tests - for semi-hard and hard cheese - no further proliferation of enterobacteria, in particular of *E. coli*, was detectable.
2. the situation with soft cheeses must be assessed in a more differentiated manner. Here, especially in less salted white mould cheeses, enterobacteria/coliforms can still multiply, and to a certain extent also *E. coli*. On the other hand, in the more heavily salted red smear cheeses - similar to semi-hard cheeses - a reduction in enterobacteria/coliforms was observed throughout. However, it is also important to note here that *E. coli* levels of 1000 cfu/g were never exceeded in the cheese, provided that the milk in particular was largely free of coliforms (neg. in 10 ml).

How:

Milk - heated neg. in 100 ml

Milk (for renneting) neg. in 10 ml (prod. end: < 1/ml)

Whey (portioning) neg. in 10 ml (prod. end: < 1/ml)

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I.e.: The demands for even lower E. coli limit/guideline values (< 10 cfu/g cheese) by the trade and industry could only be justified in cases where a greater increase in E. coli (to values of $>100,000$ cfu/g) could actually be expected in cheese. However, according to Prof Andreas Hensel, President of the Institute for Risk Assessment (BfR-Berlin), even stricter specifications or limits - especially by the trade - would have no real basis. This means that such limit values ("secondary standards") no longer differentiate per se between "toxic" and "non-toxic" or "pathogenic" and "non-pathogenic". ,

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Ansbach Expert Talks 2014

Dr Josef Hübner, Milk & Dairy Institute Dr Hübner in Hergatz, took a critical look at the secondary standards defined by retailers and the processing industry. These standards sometimes regulate product characteristics much more strictly than state standards - and very often such standards miss the mark completely. For *E. coli*, the legislator has set the limit values for past. Milk and cheese with $m = 100\text{kbE/g}$ and $M = 1,000\text{ kbE/g}$. In the meantime, however, trade and industry are already demanding $< 10\text{ kbE/g}$ cheese for *E. coli*. Although individual batches of semi-hard cheese (but certainly not soft cheese) can be produced with such values, according to Hübner, this special standard cannot be guaranteed as it is impossible to produce complete batches ($> 95\%$) in this way. It is essential that the values required by the EU are not significantly exceeded and that *E. coli* cannot multiply further in the end product. At $< 8\text{ °C}$, *E. coli* has no relevance for semi-hard and hard cheese, explained the microbiologist. The trade and industry standards are in no way suitable for differentiating between "pathogenic" and "non-pathogenic", said Hübner.