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COMPARATIVE ASSESSMENT OF THE EFFICIENCY OF METHODS FOR DETECTING THE QUANTITY OF MAFANM AND COLIFORMS IN COW'S, GOAT'S MILK AND CHEESE

T. Ryzhkova
State Biotechnological University, Ukraine

Research was carried out with the aim of developing a national standard of Ukraine with an explanation of the (new for Ukraine) methodology of determining the number of bacteria of the group of Escherichia coli in milk and dairy products using plates with a nutrient medium applied to their surface. At the same time, a higher efficiency of the "plate" method of microbiological diagnosis of milk and dairy products compared to the "cup" method was established.

Key words: *milk, cheese, microbiological control, Escherichia coli bacteria, mesophilic aerobic and facultatively anaerobic microorganisms, Petri dishes, 3M "Petrifilm TM" plates.*

INTRODUCTION

One of the main issues of nowadays, the solution of which is entrusted to scientists and specialists of processing enterprises of Ukraine, is the development of standards for food products.
The first and most important requirement for food products in the World Trade Organization (WTO) is safety, that is, the absence of a threat to human health.
Due to the fact that Ukraine became a member of the WTO, the issue of the organization of proper control of food products and raw materials for its production according to safety indicators has acquired special importance.

THE RELEVANCE OF THE RESEARCH

Topic lies in the solution of the issue of the organization of proper control of dairy raw materials and food products at agricultural and milk processing enterprises of Ukraine.
The task for the coming years in Ukraine is the implementation of the national Program for the development of state and industry standards for milk processing products and their quality control, harmonized with International European Standards.
At the same time, the choice of a modern method of determining the microbiological purity of milk and dairy products, in the production conditions of agricultural and milk processing enterprises, is an important condition for the production and release of microbiologically high-quality milk and dairy products.
Due to this, scientific and practical work was carried out aimed at the development of the national standard of Ukraine with an explanation of the modern methodology for determining the content of bacteria of the BGEC group of Escherichia coli (E. coli and coli-forms) in milk and milk products.

EXISTING INFORMATION ON THIS ISSUE

It is known that the quality and safety of food products (from a microbiological point of view) are assessed by four groups of microorganisms: sanitary-indicative, potentially pathogenic, pathogenic and indicators of microbiological stability of the product.
In milk and dairy products, the maximum permissible content of sanitary indicator microorganisms - mesophilic aerobic and facultatively anaerobic microorganisms (MAFAnM) and bacteria of the group of coliforms (BGEC, coliforms) is regulated [1, 2].
The amount of MAFAnM in milk should not exceed:

- in raw (depending on the variety) from 300 to 3000 thousand colony-forming units (CFU) in 1 cm3;
- in pasteurized, depending on the group, from 5·104 to ·105 CFU/cm3.

The presence of BGEC 0.1–1.0 cm3 of pasteurized milk is not allowed (depending on the group).

The presence of coliforms in cheeses and cottage cheese is not allowed:

- in rennet solids - 0.01 g;
- in soft dietary, Liman brine - 0.001 g,
- in soft - 0.00001 g,
- in cow's milk cheese - 0.0001 g.

According to the international nomenclature, aerobic and facultative-anaerobic, gram-negative, non-spore-forming bacilli that ferment lactose with the formation of acid and gas at a temperature of 37 ˚C for 24-48 hours are classified as bacteria of the Escherichia coli group (BGEC).

Basically, these are representatives of the genera Escherichia, Citrodacter, Enterodacter, Klebsiella of the Enterobacteriaceae family, both citrate negative and citrate positive. Among them are both representatives of normal (resistant) microflora of the digestive tract, and pathogenic ones that cause human diseases.

BGEC ferment sugars to lactic, acetic, succinic and formic acids. At the same time, CO2, ethanol and a large amount of 2,3-butylene glycol are formed, which deteriorate the quality of products, in particular, cause deviations in organoleptic indicators and consistency.

Coliforms in cheesemaking are the main indicator of compliance with proper sanitary and hygienic standards during the technological process and are considered the main reason for the early swelling of cheese [3,4].

In accordance with the Directive of the Security Council (92/46/EEC dated 16.06.1992) to ensure a high level of public health protection from the insemination of raw milk intended for the manufacture of milk-based products, the number of mesophilic aerobic and facultatively anaerobic microorganisms (MAFAnM) should not exceed:

- in cow's milk (since January 1, 1998) – 100,000 microbial cells (MC) in 1 cm3,
- in goat’s milk (since 01.12.99) - 500,000 MC/cm3.

In cheese made from raw or heat-treated milk, the amount of E. coli should not exceed 10,000 MK/g.

The content of E. coli from 10,000 to 100,000 MK/g is allowed in 2 out of 5 product samples of one batch.

In soft cheese (made from heat-treated milk), the amount of E. coli should not exceed 100 MK/g.

The content of E. coli from 100 to 1000 MK/g is allowed in 2 out of 5 tested samples of the product of one batch.

With the strengthening of control over the safety of food products, the volume of work of microbiologists of production laboratories of enterprises that produce food products will significantly increase.

Until now, in most production laboratories, microbiological research of products is carried out according to the classical scheme by inoculation on dense (agarized) nutrient media in Petri dishes (PP) or liquid nutrient media in test tubes [5–8].

Conducting a microbiological study in this case involves considerable time spent on preparing and sterilizing appropriate nutrient media, as well as preparing and sterilizing dishes, in the first case – a large number of Petri dishes, and subsequent disinfection of crops. That is, the main disadvantages of this classical method are its laboriousness, and even the need for a sufficiently large working space and the availability of a sufficient amount of equipment for cultivation and disinfection of crops.

The lack of proper quality control of the prepared nutrient media leads to inadequate results, which can cause losses already in production, in case of low-quality products.

An alternative to the cup method is 3M "Petrifilm TM" (PPF) plates with nutrient medium applied to them.

However, the use of this method is hindered by the lack of a regulatory framework: national standards of Ukraine (SSTU) with a description of methods for determining the number of mesophilic aerobic and facultatively anaerobic microorganisms; bacteria of the group of coliforms (E. coli) and coliforms.

Plates are ready-made systems containing a lyophilized nutrient composition, a cold water-soluble gelling agent, and an indicator that facilitates colony counting. This technological technique in the cultivation and identification of microorganisms has clear advantages over classical methods. The main advantages of 3M "Petrifilm TM" plates, compared to the classic cup method, are compact size, ease of use, reduction of research time, improvement of conditions and improvement of work efficiency and, as a result, reduction of costs [9, 10].

3M "Petrifilm TM" plates are used in France, USA, Belgium, Germany, Finland, Canada, Romania, Czech Republic, Japan, Australia, Russia [11]. With their help, the number of MAFAnM, yeast and mold fungi, BGKP (coliform), and the presence of pathogenic microorganisms are determined. However, the use of this method is hindered by the lack of a regulatory framework: national standards of Ukraine (SSTU) with a description of methods for determining the number of mesophilic aerobic and facultatively anaerobic microorganisms; bacteria of the group of coliforms (E. coli) and coliforms.

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The purpose of the research was to conduct a comparative assessment of the effectiveness of methods for detecting the number of MAFAnM and coliforms in cow's, goat's milk, and salted cheese from them by sowing dilutions of the studied samples on nutrient media contained in Petri dishes (PD) and (in parallel) on plates - 3M "Petrifilm TM" (PPF).

For this purpose, samples of goat and cow's milk were taken in private yards of the Kharkiv region. Milk obtained from two (at least 10 cows and goats) groups of animals was processed into cheese. In milk and cheese, the content of MAFAnM and coliforms (KF) was determined by means of parallel cultures in PP and on PPF. We took into account the information that the confidence interval for the probability level of 95%, when repeating the microbiological examination of the sample using the same method, the same reagents, materials and equipment by the same specialist in the same laboratory through a short period of time is: ± 0.25 log10 from the originally obtained results of these studies. And the confidence interval of the probability level of 95% when reproducing the microbiological examination of the sample using the same method, similar reagents, materials and equipment, in different laboratories by different specialists is: ± 0.45 log10 from the originally obtained result of these studies. Since during the study, different nutrient media from different manufacturers were compared, it seems more correct to use (when evaluating the results) a confidence interval (±0.45 log10 of the number of microorganisms or coliforms cultivated on traditional media grown in Petri dishes).

RESEARCH METHODS

The amount of MAFAnM was determined in accordance with SSTU 7357:2013 [7]. Detection and determination of the number of coliforms - in accordance with the interstate standard STST 30518 [8] by sowing dilutions of the studied products in NP on Endo medium.

THE RESULTS OF THE STUDY OF MILK AND CHEESE SAMPLES

The results of testing samples of milk and ready-made cheese for the presence of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in milk and cheese, as well as the number of coliforms in them, are presented in Tables 1 and 2, accordingly.

Table 1. Number of mesophilic aerobic and facultatively anaerobic microorganisms (MAFAnM) in milk and cheese

№	Sample	Number of microorganisms (MAFAnM)			
		Petri dishes, HRM-agar	Confidence interval for the probability level 95 %		PPF, Aerobic Count Plate
			±0,25 log10 1	±0,45 log10 2	
Milk					
1	2	3	4	5	6
1	Pasteurized goat № 1	2,0 · 104	1,1 · 104–3,6 · 104	7,1 · 103–5,6 · 104	2,0 · 104
2	Goat № 2	4,4 · 106	2,5 · 106–7,8 · 106	1,6 · 106–1,2 · 107	4,0 · 106
3	Goat № 3	<105	<5,6·104–<1,8·105	<3,6·104–<2,8·105	3,0 · 105
4	Goat № 4	7,5 · 105	4,2 · 105–1,3 · 106	2,7 · 105–2,1 · 106	6,0 · 105
5	Goat № 5	1,2 · 107	6,7 · 106–2,1 · 107	4,3 · 106–3,4 · 107	1,4 · 107
6	Pasteurized cow № 6	>1,0 · 107	>5,6·106–>1,8·107	>3,6·106–>2,8·107	1,6 · 105
Cheese					
7	Cheese № 1	4,5 · 106	2,5 · 106–8,0 · 106	1,6 · 106–1,3 · 107	3,6 · 106
8	Cheese № 2	5,2 · 103	2,9 · 103–9,3 · 103	1,9 · 103–1,5 · 104	2,4 · 103
9	Cheese № 3	6,0 · 106	3,4 · 106–1,1 · 107	2,1 · 106–1,7 · 107	5,6 · 103
10	Cheese № 4	2,8 · 107	1,6 · 107–5,0 · 107	1,0 · 107–7,8 · 108	7,2 · 105
11	Cheese № 5	8,3 · 107	4,7 · 107–1,5 · 108	3,0 · 107–2,3 · 108	1,7 · 106

Notes. 1. Confidence interval for a probability level of 95% when repeating the microbiological examination of the sample using the same method, the same reagents, materials and equipment, by the same specialist in the same laboratory after a short period of time (according to SSTU ISO 4833).
2. Confidence interval for the probability level of 95% when reproducing the microbiological examination of the sample using the same method, similar reagents, materials and equipment in different laboratories by different specialists (according to SSTU ISO 4833).
3. Cheese No. 1 - from unpasteurized goat's milk; cheese No. 2 - from pasteurized goat's milk; cheese No. 3 – made from pasteurized goat's milk with 3 types of leaven: SMS, acidophilic and propionic acid bacteria; cheese No. 4 - from unpasteurized goat's milk with SMS leaven; cheese No. 5 – from pasteurized cow's milk with SMS leaven.

From the data in Table 1, it can be seen that when determining the amount of MAFAnM in 4 out of 6 milk samples (66.7%), the number of microorganisms determined by sowing on PPF was not only within the confidence interval (±0.45 log10 of the amount of MAFAnM in Petri dishes on HRM - agar), but also within the confidence interval (±0.25 log10). That is, it corresponded to the probability level of 95%.

Out of 5 cheese samples, in 2 samples (40.0%) the amount of MAFAnM, determined by sowing on PPF, was within the confidence interval (±0.45 log10 of the amount of MAFAnM in Petri dishes on HRM-agar).

At the same time, in one of them, the number of microorganisms was within the confidence interval (±0.25 log10).

Thus, out of 11 samples of milk and cheese, in 6 samples (54.5%) the amount of MAFAnM, determined by sowing on PPF, was in the range (±0.45 log10 of the amount of MAFAnM in Petri dishes on HRM - agar), i.e. h hours in 5 samples (45.5%) - in the interval (±0.25 log10).

At the same time, in 5 samples (Nos. 1, 2, 4, 5, 7), the amount of MAFAnM in PP on GRM-agar and PPF either coincided or differed by no more than 27%.

In 2 samples (Nos. 3, 8) the amount of MAFAnM differed by no more than 3 times. In 3 samples (No. 6, 10, 11), the number of microorganisms in the NP was 39–63 times greater than in the PPF, in 1 sample (No. 9) – more than 1000 times.

In 9 (81.8%) of the 11 studied milk and cheese samples, the number of MAFAnM in PP on HRM-agar exceeded the number of microorganisms on PPF, and in 2 samples it was 1.2–3 times less.

Table 2. The number of coliforms in milk and cheese

№	Sample	Number of coliforms			
		Petri dishes, Endo environment	Confidence interval for the probability level 95 %		PPF, Coliform Count Plate
			±0,25 log10 1	±0,45 log10 2	
Milk					
1	Pasteurized goat	1,0 · 105	5,6·104–1,8·105	3,6·104–2,8·105	3,1 · 102
2	Goat №1	6,1 · 104	3,4·104–1,1·105	2,2·104–1,7·105	6,2 · 105
3	Goat №2	2,0 · 106	1,1·106–3,6·106	7,1·105–5,6·105	1,7 · 106
4	Goat №3	1,6 · 106	9,0·105–2,8·106	5,7·105–4,5·106	4,8 · 104
5	Goat №4	>1,3 · 107	>7,3·106–>2,3·107	>4,6·106–>3,6·107	1,1 · 105
6	Goat №5	1,2 · 104	6,7·103–2,1·104	4,3·103–3,4·104	5,6 · 103
7	Goat №6	4,4 · 104	2,5·104–7,8·104	1,6·104–1,2·105	1,3 · 103
8	Goat №7	6,7 · 104	3,8·104–1,2·105	2,4·104–1,9·105	5,9 · 104
9	Goat №8	6,7 · 105	3,8·105–1,2·106	2,4·105–1,9·106	1,3 · 106
10	Goat №9	3,1 · 105	1,7·105–5,5·105	1,1·105–8,7·105	2,6 · 104
11	Pasteurized cow	6,0 · 104	3,4·104–1,1·105	2,1·104–1,7·105	2,2 · 103
Cheese					
12	Cheese №1	2,3 · 104	1,3·104–4,1·104	8,2·103–6,4·104	6,9 · 106
13	Cheese №2	1,9 · 106	1,1·106–3,4·106	6,8·105–5,3·106	1,4 · 104
14	Cheese №3	3,3 · 106	1,8·106–5,9·106	1,2·106–9,2·106	7,2 · 106
15	Cheese №4	1,7 · 107	9,6·106–3,0·107	6,1·106–4,8·107	3,7 · 106
16	Cheese №5	4,1 · 107	2,3·107–7,3·107	1,5·107–1,2·108	2,2 · 107

From the data in Table 2, it can be seen that the amount of BGEC in 4 out of 11 studied milk samples on PPF was in the interval (±0.45 log10 of the number in PP on Endo environment).

Samples No. 3, 6, 8, 9: of them, in 2 samples (No. 3, 8) - in the interval (±0.25 log10 of the amount in NP on Endo environment).From the data in Table 2, it can be seen that the amount of BGEC in 4 out of 11 studied milk samples on PPF was in the interval (±0.45 log10 of the number in PP on Endo environment).

Samples No. 3, 6, 8, 9: of them, in 2 samples (No. 3, 8) - in the interval (±0.25 log10 of the amount in NP on Endo environment).

In 2 out of 5 examined samples of cheese, the amount of BGEC on PPF was in the interval (±0.45 log10 of the amount in NP on Endo environment), of them in 1 sample - in the interval (±0.25 log10 of the amount in NP on Endo environment). That is, out of 16 samples of milk and cheese examined, in 6 samples (37.5%) the amount of BGEC on PPF was in the interval (±0.45 log10 of the amount in PP on Endo environment), including in 3 samples - in the interval (±0.25 log10 of the amount in NP on Endo environment).In 2 out of 5 examined samples of cheese, the amount of BGEC on PPF was in the interval (±0.45 log10 of the amount in NP on Endo environment), of them in 1 sample - in the interval (±0.25 log10 of the amount in NP on Endo environment). That is, out of 16 samples of milk and cheese examined, in 6 samples (37.5%) the amount of BGEC on PPF was in the interval (±0.45 log10 of the amount in PP on Endo environment), including in 3 samples - in the interval (±0.25 log10 of the amount in NP on Endo environment).

In general, in 11 (68.8%) of the 16 investigated samples, the number of enterobacteria on the Endo environment in the NP exceeded the number of coliforms on the PPF:

– in 4 samples (No. 3, 6, 8, 16) – 1.1–2.0 times; in 4 samples (No. 4, 7, 10, 11) – 12 – 34 times;

– in 3 samples (No. 1, 5, 13) – 118–323 times.

In 3 samples (No. 9, 14, 15), the number of enterobacteria on Endo environment was 1.9–4.6 times lower than on PPF; - in 2 samples (No. 2, 12) – 10–300 times.
That is, the number of mesophilic aerobic and facultatively anaerobic microorganisms, determined by sowing on 3M "Petrifilm TM" Aerobic Count Plates, in 6 (54.5%) of the 11 studied samples of milk and cheese, was in the interval (± 0.45 log10 of the amount in Petri dishes on HRM-agar), which corresponds to the level of probability of reproducibility of the results of 95%.
In 3 samples (No. 9, 14, 15), the number of enterobacteria on Endo environment was 1.9–4.6 times lower than on PPF; - in 2 samples (No. 2, 12) – 10–300 times.
That is, the number of mesophilic aerobic and facultatively anaerobic microorganisms, determined by sowing on 3M "Petrifilm TM" Aerobic Count Plates, in 6 (54.5%) of the 11 studied samples of milk and cheese, was in the interval (± 0.45 log10 of the amount in Petri dishes on HRM-agar), which corresponds to the level of probability of reproducibility of the results of 95%.
In 9 (81.8%) of 11 investigated samples, the number of microorganisms that grew in Petri dishes on HRM - agar was greater than on 3M "Petrifilm TM" plates.
The number of bacteria of the coliform group, detected by sowing on 3M "Petrifilm TM" Coliform Count Plates, in 6 (37.5%) of the 16 investigated milk and cheese samples, was in the range (±0.45 log10 in Petri dishes on Endo environment), which corresponds to a level of probability of reproducibility of the results of 95%.
In general, in 11 (68.8%) of 16 tested samples, the number of enterobacteria grown in Petri dishes on Endo environment was greater than the number of coliforms on 3M "Petrifilm TM" plates.

BASED ON THE ABOVE, THE FOLLOWING CONCLUSIONS CAN BE DRAWN:

1. The use of plates, in production laboratories, to determine the number of mesophilic aerobic and facultative anaerobic microorganisms, as well as coliforms in milk and cheese, is more effective, compared to Petri dishes.
2. The positive experience of using "Petrifilm TM" plates for determining the microbiological parameters of milk and dairy products in a number of European countries and the USA, as well as the research results obtained by us, testify to the feasibility of developing two national standards of Ukraine:
SSTU 7089:2009 "Milk and dairy products. Methodology for counting the number of mesophilic aerobic and facultatively anaerobic microorganisms, yeasts and molds using plates" and SSTU 7090:2009 "Milk and dairy products. The method of counting the number of coliforms and E. coli using plates", as well as their implementation in the production of milk processing enterprises and farms of Ukraine.

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ПОРІВНЯЛЬНА ОЦІНКА ЕФЕКТИВНОСТІ МЕТОДІВ ВИЯВЛЕННЯ КІЛЬКОСТІ МАФАнМ І КОЛІФОРМ У КОРОВ'ЯЧОМУ, КОЗИНОМУ МОЛОЦІ ТА РОЗСІЛЬНОМУ СИРІ

Т. Рижкова
Державний біотехнологічний університет

Проводились дослідження з метою розробки національного стандарту України з викладом у ньому (нової для України) методики визначення кількості бактерій групи кишкових паличок у молоці та молочних продуктах з використанням пластин з нанесеного на їх поверхню живильного середовища. При цьому встановлено більш високу ефективність «пластинкового» методу мікробіологічної діагностики молока та молочних продуктів порівняно з «чашковим».

Ключові слова: *молоко, сир, мікробіологічний контроль, бактерії групи кишкових паличок, мезофільні аеробні та факультативно-анаеробні мікроорганізми, пластини 3M «Petrifilm TM».*

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