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EVALUATION OF THE MICROBIOLOGICAL QUALITY OF DAIRY PRODUCTS USING TEMPO SYSTEM

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A b s t r a c t

In order to ensure microbiological safety of foodstuffs a comprehensive and integrated approach needs to be applied to production process, comply with all obligatory quality standards at each step. In present work a microbiological quality of selected ripened cheeses (5) and liquid dairy products (5) was analyzed using following tests available in TEMPO system: YM – yeasts and molds, STA – the number of *Staphylococcus*, LAB – lactic acid bacteria, EC – *Escherichia coli*, CC – the number of coliforms, TC – total number of coliforms, EB – the number of bacteria belonging to *Enterobacteriaceae*family, TVC – total number of mesophilic microorganisms. Performed analysis using TEMPO system indicated that microbiological quality of selected ripened cheeses and liquid dairy products is satisfactory. TEMPO system turned out to be an useful tool in establishing parameters that define microbiological purity of analyzed food products.

OCENA JAKOŚCI MIKROBIOLOGICZNEJ PRODUKTÓW MLECZARSKICH Z WYKORZYSTANIEM URZĄDZENIA TEMPO

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S ł o w a k l u c z o w e: produkty mleczarskie, jakość mikrobiologiczna, TEMPO.

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Abstrakt

W celu zapewnienia bezpieczeństwa zdrowotnego produktów spożywczych należy zastosować wszechstronne i zintegrowane podejście do procesu produkcji, spełniając wymagane normy jakości na każdym jego etapie. W ramach pracy przeanalizowano jakość mikrobiologiczną wybranych serów dojrzewających (5) oraz płynnych produktów mleczarskich (5) z wykorzystaniem testów dostępnych w systemie TEMPO: YM – drożdże i pleśnie, STA – liczba bakterii *Staphylococcus*, LAB – liczba bakterii mlekowych, EC – liczba *Escherichia coli*, CC – liczba bakterii z grupy *coli*, TC – ogólna liczba bakterii z grupy *coli*, EB – liczba bakterii z rodziny *Enterobacteriaceae*, TVC – ogólna liczba drobnoustrojów mezofilnych. Analizy wykonane za pomocą urządzenia TEMPO wykazały, iż jakość mikrobiologiczna wybranych serów dojrzewających oraz płynnych produktów mleczarskich jest zadowalająca. System TEMPO okazał się przydatnym urządzeniem do określenia wskaźników stanowiących o czystości mikrobiologicznej badanych produktów.

Introduction

Assurance of microbiological safety of food is one of the aim of the food policy which has particular meaning in public health protection. According to Food Hygiene Basic Texts food safety denotes that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use (CODEX ALIMENTARIUS 2009). In order to guarantee high microbiological quality of the final product, one needs to comply with the safety standards at raw material production step, assuring appropriate quality of fodder and conditions for animal husbandry, through production process of foodstuffs control, as well as their distribution and storage. Each step has significant meaning to obtain high quality of food product, influencing also their commercial quality.

Requirements regarding microbiological quality of food products is defined by Regulation EC 2073/2005, together with later modifications (1441/2007 and 365/2010). This Regulation introduces following definitions:

– **microbiological criterion**: means a criterion allowing the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of microorganisms and/or their toxins or metabolites, per unit of mass, volume, area or batch,

– **food safety criterion**: means a criterion defining the acceptability of a product or a batch of foodstuffs applicable to products placed on the market,

– **process hygiene criterion**: a criterion indicating the acceptable functioning of the production process; it sets an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law (Regulation EC 2073/2005).

Above regulations sets microbiological criteria for dairy products present in Tables 1 and 2. Regulation 2073/2005 additionally defines safety criteria for ready-to-eat foods for infants and ready-to-eat foods for special medical purposes.

Table 1

Food safety criteria for dairy products

Food category	Micro-organisms/their toxins, metabolites	Sampling plan		Limits	
		n	c	m	M
1.2. Ready-to-eat foods able to support the growth of L. monocytogenes, other than those intended for infants and foe special medical purposes	*Listeria monocytogenes*	5	0	100 cfu/g	
		5	0	Absence in 25 g	
1.3. Ready-to-eat foods unable to support the growth of L. monocytogenes, other than those intended for infants and foe special medical purposes	*Listeria monocytogenes*	5	0	100 cfu/g	
1.11. Cheeses, butter and cream made from raw milk or milk that has undergone a lower heat treatment than pasteurisation	*Salmonella*	5	0	Absence in 25 g	
1.12. Milk powder and whey powder	*Salmonella*	5	0	Absence in 25 g	
1.13 Ice cream, excluding products where the manufacturing process or the composition of the product will eliminate the salmonella risk	*Salmonella*	5	0	Absence in 25 g	
1.21. Cheeses, milk powder and whey powder, as referred to in the coagulase-positive staphylococci criteria	Staphylococcal enterotoxins	5	0	Not detected in 25 g	

Explanations to Table 1: n – number of units comprising the sample, c – number of sample units giving values between m and M , m=M, cfu – colonies forming units. References: Regulations 2073 (2005), 1441 (2007), 365 (2010)

Evaluation of microbiological quality of food products based on traditional plate counts method is time-consuming, labor- and material-intensive, and in consequencemuch higher costs of analysis are generated. Another essential issue is waiting time for the results of analysis, especially where one deals with perishable foods. Food safety management systems demand fast evaluation of microbiological quality in order to make a decision about actions which eliminate hazard to the health of consumers. One of the methods which significantly shortens the waiting time for the results of analysis, and uses much less materials and work, is a method based on fluorescence phenomena used in TEMPO device (Biomerieux). The mechanism of reading is based on the measurements of fluorescence signal as a result of fluorescent compound light induction which is obtained through reaction performed by microorganisms present in analyzed product (NOWAK and CHARLIŃSKI 2012). TEMPO is an automated system that enables the quantitative analysis of such microbiological indicators as total number of microorganisms, *Enterobacteriaceae*, the

Table 2

Process hygiene criteria for dairy products

Food category	Micro-organisms/their toxins, metabolites	Sampling plan		Limits (cfu/g or ml)	
		n	c	m	M
2.2.1. Pasteurised milk and other pasteurized liquid dairy products	*Enterobacteriaceae*	5	0	10 cfu/ml	
2.2.2. Cheesed made from milk or whey that has undergone heat treatment	*E. coli*	5	2	100 cfu/g	1000 cfu/g
2.2.3. Cheeses made from raw milk	Coagulase-positive staphylococci	5	2	10^4 cfu/g	10^5 cfu/g
2.2.4. Cheeses made from milk that has undergone a lower heat treatment than pasteurisation and ripened cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment	Coagulase-positive staphylococci	5	2	100 cfu/g	1000 cfu/g
2.2.5. Unripened soft cheeses made from milk or whey that has undergone pasteurization or a stronger heat treatment	Coagulase-positive staphylococci	5	2	10 cfu/g	100 cfu/g
2.2.6. Butter and cream made from raw milk or milk that has undergone a lower heat treatment than pasteurisation	*E. coli*	5	2	10 cfu/g	100 cfu/g
2.2.7. Milk powder and whey powder	*Enterobacteriaceae*	5	0	10 cfu/g	
	Coagulase-positive staphylococci	5	2	10 cfu/g	100 cfu/g
2.2.8. Ice cream and frozen dairy desserts	*Enterobacteriaceae*	5	2	10 cfu/g	100 cfu/g

Explanations to Table 2: n – number of units comprising the sample, c – number of sample units giving values between m and M , m=M, cfu – colonies forming units. References: Regulations 2073 (2005), 1441 (2007), 365 (2010)

number of *coliforms*, *E. coli*, coagulase positive *S. aureus*, yeasts and molds, lactic acid bacteria (CHARLIŃSKI 2012).

The aim of present work was to (i) evaluate the microbiological quality of selected dairy products using TEMPO system, as well as (ii) to analyze the possibilities of using TEMPO system to check the compliance with the hygienic and safety criteria of tested food products according to Regulation EC 2073/2005 with further modifications.

Materials and Method

Analyzed dairy products

Following dairy products were bought in a local supermarket and tested microbiologically:

– ripened cheeses: Gouda, Ementaler, Edam, Brie, Camembert,

– liquid products: pasteurized milk, buttermilk, kefir, cranberry yoghurt, plain yoghurt.

Microbiological experiment was performed in three replicates (three items of the same product from different batches were purchased).

Determination of the safety and hygiene criteria for analyzed dairy products according to Regulation EC 2073/2005 with further modifications

Table 3

Food safety and process hygiene criteria for analyzed dairy products according to Regulation 2073/2005 with later modifications

Product	Food safety criteria		Process hygiene criteria		
	Listeria monocytogenes	Staphylococcal enterotoxins (*St. aureus*)	*Enterobactericeae*	*E. coli*	Coagulase-positive staphylococci
Gouda	–	+	+	+	+
Ementaler	–	+	+	+	+
Edam	–	+	+	+	+
Brie	–	+	+	+	+
Camembert	–	+	+	+	+
Pasteurised milk	–	+	+	+	+
Buttermilk	–	+	+	+	+
Kefir	–	+	+	+	+
Cranberry yoghurt	–	+	+	+	+
Plain yoghurt	–	+	+	+	+

Explanations to Table 3: – no possibility to detect in Tempo system, + possibility to detect in Tempo system

Tested dairy products can be qualified to following food categories by analyzing the Regulation 2073/2005:

– ripened cheeses:
  - food safety criteria: 1.2, 1.2.1
  - process hygiene criteria: 2.2.2, 2.2.4
– pasteurised milk:
  - food safety criteria: 1.2

- process hygiene criteria: 2.2.1

– fermented dairy products:
  - food safety criteria: 1.3

Above qualification enabled to define particular microorganisms for analyzed dairy products on the basis of food safety and process hygiene criteria (Table 3).

Microbiological analyses using Tempo system

Depending on analyzed dairy product a 1/4, 1/40 or 1/400 dilution was prepared. TEMPO test requires to hydrate the selective media by adding 3 ml of sterile water. After adding a 1 ml of appropriately diluted product, a scan was made. Prepared samples were placed in the filler. After reading the data and closing the cards, a stands containing cards were transferred to incubators and stored in the following conditions:

– TEMPO EC (*Eschierichia coli)*: 37°C /24h
– TEMPO EB (*Enterobacteriaceae):* 35°C/24h
– TEMPO TC (total number of *coliforms*): 30°C/24h
– TEMPO LAB (lactic acid bacteria): 30°C/48h
– TEMPO TVC (total number of mesophilic microorganisms): 30°C/48h
– TEMPO STA (the number of *Staphylococcus aureus)*: 37°C/24h
– TEMPO YM (yeasts and molds): 25°C/72h.

From the cards filler station data were sent to reading station. After incubation period the cards were placed in TEMPO Reader station, where the data was saved. The last stage of work was validation and printing.

Results and Discussion

In present paper the microbiological quality of selected ripened cheeses and liquid dairy products using TEMPO system is analyzed. The main disadvantage of the device is lack of microbiological selective tests against *Listeria monocytogenes*, which makes unable to check the accordance with food safety criteria for this pathogen. Table 4 presents the results of microbiological analysis performed in soft, semi-hard and hard cheeses.

Tests performed in order to determine the number of lactic acid bacteria (LAB) indicated their level ca. > 4.9×10^4 cfu/g in each of the analyzed cheese. This bacteria ferment lactose to lactic acid and they are essential in dairy products production processes, e.g. they are responsible for cheese maturation. The evaluation of total number of mesophilic microorganisms (TVC) revealed their level > 4.9×10^4 cfu/g. This number is in accordance with the polish

Table 4

Results of microbiological analysis performed in selected ripened cheeses (cfu/g)

Test/Product	Gouda	Ementaler	Edam	Brie	Camembert
LAB	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$
TVC	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$
STA	< 10	< 10	< 10	< 10	< 10
EB	< 10	< 10	< 10	< 10	< 10
EC	< 10	< 10	< 10	< 10	< 10
TC	< 10	< 10	< 10	< 10	< 10
YM	< 100	< 100	< 100	> $4.9x10^4$	> $4.9x10^4$

Explanations to Table 4: LAB – lactic acid bacteria, TVC – total number of mesophilic microorganisms, STA – *Staphylococcus aureus,* EB – *Enterobacteriaceae*, EC – *Escherichia coli*, TC – total number of coliforms ISO30°C, YM – yeasts and molds

Ministry of Health Regulation from 13th of January year where the maximum number of microorganisms should not exceed $5x10^5$ cfu/g. Obtained results for *S. aureus* (STA), *Enterobacteriaceae* (EB) and *E. coli* (EC) in ripened cheeses showed their level < 10 cfu/g. It suggests that analyzed cheeses do not contain pathogenic microorganisms that can be harmful for consumers. Similar results were obtained for coliforms (TC) – their level did not exceed 10 cfu/g. In case of mold cheeses Brie and Camembert, the number of molds and yeasts was > $4.9x10^4$cfu/g.

According to the Regulation 2073/2005 the process hygiene criteria are determined by such microorganisms like *E. coli* and coagulase-positive *S. aureus*. Mentioned Regulation demands that in the case of cheeses made from milk or whey that has undergone heat treatment, the level of *E. coli* does not exceed 100 cfu/g. Performed analyses using TEMPO system confirmed that the cheese samples contain less than 10 cfu/g. The source of *E. coli* in ripened cheeses can be the raw material, as well as the reinfection of final product. Studies performed by BERTHOLD and STACHURA (2009) showed that *E. coli* and enterohemorrhagicstrain O157:H7 grow well in soft cheeses. Moreover, in cited paper it was stated that the microflora of hard cheese creates appropriate conditions for *E. coli* growth.

The Regulation 2073/2005 with later modifications says that cheeses made from milk that has undergone a heat treatment should contain < 100 cfu/g of coagulase-positive *S. aureus* (STA). Microbiological analyses performed in present paper showed that cheeses available on the market contain approximately >10 cfu/g of *S. aureus*. Total viable count (TVC) in each kind of cheese was around > $4.9x10^4$ cfu/g. Total number of microorganisms in cheese depends firstly on the microflora of raw material, production and ripening conditions, as well as treatment of cheeses after production (BERTHOLD 2009). Contamina-

tion of all samples of cheese by yeasts and molds varied between 10^2 – $4.9x10^4$ cfu/g. According to literature the number of yeasts and molds that has negative impact on cheese quality is around $>10^5$ cfu/g (URARTE 1999). The higher number can deteriorate the sensory properties of cheese, and can influence the taste defect – yeast and foreign off-flavour (BERTHOLD 2009).

Table 5 presents the results of microbiological analysis performed in liquid dairy products.

Table 5

Results of microbiological analysis performed in liquid dairy products (cfu/ml)

Test/Product	Pasteurised milk	Butter milk	Kefir	Cranberry yoghurt	Plain yoghurt
LAB	< 1	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$
TVC	< 1	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$	> $4.9x10^4$
STA	< 1	< 10	< 10	< 10	< 10
EB	< 1	< 1	< 1	< 1	< 1
EC	< 1	< 1	< 1	< 1	< 1
TC	< 1	< 1	< 10	< 10	< 1
YM	< 1	< 100	< 100	< 100	<100

Explanations to Table 5: LAB – lactic acid bacteria, TVC – total number of mesophilic microorganisms, STA – *Staphylococcus aureus,* EB – *Enterobacteriaceae*, EC – *Escherichia coli*, TC – total number of coliforms ISO30°C, YM – yeasts and molds

Total number of coliforms (TC) in analyzed liquid dairy products was from <1.0 cfu/ml to <10.0 cfu/ml, whereas *E. coli* (EC) for each product was <1.0 cfu/ml. According to the polish Ministry of Health Regulation from 13th of January 2003 coliforms cannot be present in foodstuffs. In studies performed by PLUTA et al. (2001) the presence of coliforms was determined in all samples of yoghurt and bio-yoghurt tested in 1998–2001. The level of *Enterobacteriaceae* (EB) and *E. coli* was less than 1.0 cfu/ml. The number of *S. aureus* (STA) was less than 1.0 cfu/ml in case of pasteurized milk, and less than 10 cfu/ml for other fermented dairy products. Microbiological tests performed in order to assess the number of spoilage microflora, such as STA, EB and EC determined their level in a range 1.0 – 10.0 cfu/ml. It suggests that analyzed dairy products does not contain foodborne pathogens that can be harmful to consumers. In performed studies an appropriate level of lactic acid bacteria (LAB) was obtained: for pasteurized milk – <1.0 cfu/ml, for buttermilk, kefir, cranberry yoghurt, plain yoghurt the number of LAB was approximately $>4.9x10^4$ cfu/ml. Lactic acid bacteria are typical microorganisms found in fermented dairy products. Total viable counts of mesophilic microorganisms (TVC) for milk was <1.0 cfu/ml, and for fermented dairy products $>4.9x10^4$

cfu/ml. It is a proper level because maximum number of microorganisms is $5x10^5$ cfu/ml and above this number food product becomes disqualified and cannot be placed on the market (BERTHOLD 2009). The last microbiological test which was performed in order to determine the number of yeasts and molds (YM) revealed their level below 1 cfu/ml in case of pasteurized milk, and below 100 cfu/ml for analyzed fermented dairy products. PLUTA et al. (2001) studied the prevalence of yeasts and molds in 1995–1998 and 2000–2001 in food products. The researchers found the number of yeasts and molds above 10^4 cfu/g in yoghurts and bio-yoghurts in 10% of analyzed samples, whereas in 2000–2001 this level of microorganisms was in 7% of tested yoghurts and bio-yoghurts. In another studies conducted also in 2000–2001 by ORZECHOWSKA et al. (2001) yeasts and molds were absent in 0.1g of product in 85% of analyzed samples, and in a range of 10^1–10^3 cfu in 1g of product in 15% of analyzed products.

Conclusions

On the basis on performed studies it was stated that TEMPO system used in the following studies increased the cohesion of obtained results and facilitated their interpretation. Nevertheless the main disadvantage of this device is lack of test in order to determine the number of *Listeria monocytogenes*, which makes unable to check the accordance with obligatory Regulation 2073/2005 with later modifications. Performed analysis confirmed that tested dairy products were produced from raw material with appropriate microbiological quality; each step of dairy products production was made in adequate manner, in order to achieve a satisfactory quality of food product placed on the market.

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References

BERTHOLD A., STACHURA A. 2009. *Jakość mikrobiologiczna serów pochodzących z gospodarstw ekologicznych.* Postępy techniki przetwórstwa spożywczego, 1: 65–69.

CHARLIŃSKI J. 2012. *Ocena przydatności systemu Tempo® w oznaczaniu drożdży i pleśni oraz bakterii E. coli w smakowych serkach twarogowych oraz pleśniowych.* Aktualności bioMerieux , 14: 15–17.

Commission Regulation (EU) No 365/2010 of 28 April 2010 amending Regulation (EC) No 2073/2005 *on microbiological criteria for foodstuffs as regards Enterobacteriaceae in pasteurised milk and other pasteurised liquid dairy products and Listeria monocytogenes in food grade salt.*

Commission Regulation (EU) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 *on microbiological criteria for foodstuffs.*

Commission Regulation (EU) No 2073/2005 of 15 November 2005 *on microbiological criteria for foodstuffs.*

Food Hygiene, Basic Texts, Fourth Edition, Codex Alimentarius, Rome 2009.

NOWAK A., CHARLIŃSKI J. 2012. *Wpływ związków barwnych w mrożonych owocach oraz enzymów w mieszankach warzywnych z grzybami na dokładność ilościowego oznaczania ogólnej liczby mezofilnych bakterii tlenowych i E. coli z użyciem automatycznego systemu Tempo* . Aktualności bioMerieux, 13: 15–17.

ORZECHOWSKA K., MOLSKA I., FRELIK I. 2001. *Jakość mikrobiologiczna jogurtu i biojogurtu na rynku warszawskim, w latach 2000/2001.*Przegląd Mleczarski, 9: 404–406.

PLUTA A., BERTHOLD A., ZIARNO M., MOLSKA I., FRELIK I., NOWOSIELSKA R., ORZECHOWSKA K. 2001. *Jakość mikrobiologiczna krajowych jogurtów rynkowych w latach 1995–20.* Przemysł Spożywczy, 1: 55–57.

Rozporządzenie Ministra Zdrowia z dnia 13 stycznia 2003 r. *w sprawie maksymalnych poziomów zanieczyszczeń chemicznych i biologicznych, które mogą znajdować się w żywności, składnikach żywności, dozwolonych substancjach dodatkowych, substancjach pomagających w przetwarzaniu albo na powierzchni żywności.*

URARTE E., FERNANDEZ J., MOLINERO M. 1999. *Microbiological quality of fresh cheese marketed in the Basgue Antono-mous Region.* Alimentaria, 299: 37–47.