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Original Scientific Article

MICROBIOLOGICAL QUALITY OF SOFT, SEMI-HARD AND HARD CHEESES DURING THE SHELF-LIFE

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ABSTRACT

Cheeses as ready-to-eat food should be considered as a potential source of foodborne pathogens, primarily *Listeria monocytogenes*. The aim of present study was to determine the microbiological quality of soft, semi-hard and hard cheeses during the shelf-life, with particular reference to *L. monocytogenes*. Five types of cheeses were sampled at different time-points during the cold storage and analyzed for presence of *Salmonella* and *L. monocytogenes,* as well as lactic acid bacteria, *Escherichia coli,* coagulase-positive staphylococci, yeasts, molds, sulfite-reducing clostridia and *L. monocytogenes* counts. Water activity, pH and NaCl content were monitored in order to evaluate the possibility of *L. monocytogenes* growth. Challenge test for *L. monocytogenes* was performed in soft whey cheese, to determine the growth potential of pathogen during the shelf-life of product. All analyzed cheeses were compliant with microbiological criteria during the shelf-life. In soft cheeses, lactic acid bacteria increased in the course of the shelf-life period (1.2-2.6 log increase), while in semi-hard and hard cheeses it decreased (1.6 and 5.2 log decrease, respectively). Soft cheeses support the growth of *L. monocytogenes* according to determined pH values (5.8-6.5), water activity (0.99-0.94), and NaCl content (0.3-1.2%). Challenge test showed that *L. monocytogenes* growth potential in selected soft cheese was 0.43 $\log_{10}$ cfu/g during 8 days at 4°C. Water activity in semi-hard and hard cheeses was a limiting factor for *Listeria* growth during the shelf-life. Soft, semi-hard and hard cheeses were microbiologically stable during their defined shelf-life. Good manufacturing and hygienic practices must be strictly followed in the production of soft cheeses as *Listeria*-supporting food and be focused on preventing (re)contamination.

Key words: cheese, shelf-life, *L. monocytogenes*

INTRODUCTION

Microbiological stability and safety of food during storage is related to many factors. Ready-to-eat food products, including cheeses, are intended for consumption without any treatment between final production step and consumption. The course of microbiological changes in different cheeses during storage and shelf-life depends on the production technology and cheese type (pasteurization, starters, acidity, ripening, etc.), physico-chemical properties (acidity, moisture content, salt content), storage conditions (microclimate, handling, packaging). *Listeria monocytogenes* is the most important foodborne pathogen in cheese microbiology, mainly in the post-processing phase (5). European Regulation on microbiological criteria of food (EC 2073/2005) categorizes foods into two groups, supporting or not supporting the growth of *L. monocytogenes*. Foods with pH ≤4.4 or water activity (a_w) ≤0.92, or products with pH ≤5.0 and a_w ≤0.94, as well as products with shelf-life less than 5 days are considered as safe regarding *Listeria* outgrowth (4). Recent survey conducted by EFSA showed that 0.06% of soft and semi-soft cheeses (n=3452) are exceeding the level of 100 cfu/g at the end of shelf-life. The occurrence of *L. monocytogenes* in cheese samples was 0.47% (6). In Croatia, microbiological surveys in recent years were mainly performed on traditional dairy products from non-pasteurized milk, and *L. monocytogenes* was frequently present (7, 12). Thus, the aim of the

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present study was to evaluate the microbiological quality of pasteurized-milk cheeses during the shelf-life with particular reference to *L. monocytogenes* prevalence and counts. The growth potential of *L. monocytogenes* was also evaluated in soft whey cheese during the shelf-life.

MATERIAL AND METHODS

Soft, semi-hard and hard cheeses were produced according to standard procedure in a local cheese producing plant and sampled at the end of production. Ten units of each cheese were sampled, stored in laboratory at 4°C and periodically analyzed during defined shelf-life (Table 1).

Table 1. Sampling scheme and parameters monitored in cheese during shelf-life

Cheese type	Cheese name	Shelf-life	Time-point sampling	Microbiological parameters	Chemical parameters
Soft cheese	Whey cheese Skuta	8 days	Day 5, 7, 8	*Salmonella, L.monocytogenes, E.coli, S. aureus, yeasts, molds, lactic acid bacteria*	Water activity, pH, NaCl
	Škripavac	15 days	Day 6, 9, 12, 15		
Semi-hard cheese	Trappist	9 months	Month 3,4,6,9	*Salmonella, L.monocytogenes, E.coli, S. aureus, Sulfite- reducing clostridia, lactic acid bacteria*	
Hard cheese	Livanjski	15 months	Month 3,4,6,11,12,15	*Salmonella, L.monocytogenes, E.coli, S. aureus, Sulfite- reducing clostridia, lactic acid bacteria*	
	Hard cheese in olive oil	9 months	Month 3,5,8,9		

For microbiological analyses, 25 g of sample was diluted in 225 ml of appropriate media (Buffered Peptone Water, Half-Fraser Broth or Peptone salt water) and homogenized for 2 min (Stomacher, Sedward, UK). Serial decimal dilutions were prepared and 0.1 ml or 1 ml of selected dilution were used for evaluation of lactic acid bacteria count (MRS, Merck, Darmstadt, Germany) at 30°C for 48 h, *Staphylococcus aureus* (Baird Parker agar, Merck, Germany) at 37°C for 48 h, *Escherichia coli* (Rapid E. coli, Bio-Rad, France) 37/44°C for 24 h, and sulfite-reducing clostridia (Iron Sulphyte Agar, bioMerieux, Marcy l'Etoile, France). *Salmonella* spp. was determined following a standard ISO 6579 method and Molecular Detection System (MDS, 3M, USA). *L. monocytogenes* presence and count was determined using ISO 11290-1 and 11290-2, respectively.

Water activity (a_w) was determined by means of HygroPalm AW1 (Rotronic, Switzerland), pH with pH-meter (pH 510, Eutech instruments, Netherlands) and NaCl following Mohr's method. Challenge test was performed in whey cheese "skuta" (shelf-life 8 days) inoculated with mixed culture of *L. monocytogenes* (two strains of cheese origin and *L. monocytogenes* ATCC 7644). The strains were grown in Brain Hearth Infusion Broth (bioMerieux, France) at 37ºC for 24 h. One ml of each culture was centrifuged at 10000 *g* for 10 min, followed by supernatant removal and washing the cells with sterile saline water. Cells were diluted in 1 ml of sterile saline water and serially diluted to determine the cell number, using PALCAM agar (Merck, Germany) incubated at 37ºC for 24-48 h. Appropriate dilutions were taken for cheese portion inoculation to obtain an inoculation level of 30-50 cfu/g. Cheese samples were inoculated in triplicate, vacuum-packed and stored at 4ºC for 8 days. *L. monocytogenes* count, pH and water activity were determined at day 0 and at the end of shelf-life.

RESULTS

The results of the microbiological and chemical analyses of cheeses during the storage are summarized in Table 2. Foodborne pathogens *Salmonella* spp. and *L. monocytogenes* were absent in all cheese samples analyzed during the shelf-life. *Escherichia coli, L. monocytogenes,* sulfite-reducing clostridia and *S. aureus* were below the detection limits of the methods used at all sampling points.

L. monocytogenes counts were below 10 cfu/g in all cheese samples during storage. Population of lactic acid bacteria increased during the storage of soft cheeses, reaching 7-8 log cfu/g at the end of shelf-life. The pH values, water activity and NaCl content in soft cheeses were in the range of *Listeria*-supporting values. Semi-hard and hard cheeses were characterized by decrease of lactic acid bacteria counts during the storage and a slight pH increase. The pH values and NaCl content found in semi-hard and hard cheeses were not a limiting factor for *Listeria* growth. However, it is evident that water activity was below values that enables the growth of most foodborne pathogens.

Since soft cheeses showed to be supporting of *Listeria* growth according to their physico-chemical characteristics, the challenge test was performed with *L. monocytogenes*. Results of *L. monocytogenes* growth potential in whey cheese skuta are shown in Table 3, as well as pH and a_w values (Table 4). Despite high water activity and optimal pH, *L. monocytogenes* inoculated in low numbers (30-50 cfu/g) didn't reach a critical limit of 100 cfu/g in cheese stored at 4 ºC for 8 days.

DISCUSSION

Microbiological quality and interpretation of microbiological findings of different kind of cheeses depends on the sampling points during the production or retail phase. In present study, the

Table 2. Results of microbiological and physico-chemical analyses of cheeses during the shelf-life

Cheese type	Cheese name	Microbiological parameter and sampling day	Microbial counts (log_{10} cfu/g)	Results of physico-chemical analyses		
				pH	a_w	NaCl (%)
Soft cheese	Whey cheese Skuta	Lactic acid bacteria				
		Day 5	4.20±0.07	6.21±0.02	0.945±0.01	0.12±0.02
		Day 7	4.60±0.05	6.53±0.01	0.960±0.01	0.23±0.03
		Day 8	6.80±0.02	5.80±0.02	0.990±0.01	0.29±0.04
		Yeasts				
		Day 5	2.47±0.01			
		Day 7	2.77±0.11			
		Day 8	2.95±0.12			
	Škripavac	Lactic acid bacteria				
		Day 6	6.62±0.15	6.28±0.02	0.950±0.02	1.10±0.02
		Day 9	6.50±0.07	6.40±0.03	0.960±0.01	1.10±0.02
		Day 12	7.25±0.12	6.55±0.03	0.978±0.02	1.20±0.01
		Day 15	7.82±0.14	6.20±0.01	0.990±0.02	1.22±0.02
		Yeasts				
		Day 6	2.30±0.11			
		Day 9	2.54±0.06			
		Day 12	2.58±0.07			
		Day 15	2.77±0.10			
Semi-hard cheese	Trappist	Lactic acid bacteria				
		Day 90	6.20±0.12	5.40±0.04	0.910±0.01	1.51±0.02
		Day 120	5.04±0.10	5.48±0.02	0.900±0.04	1.68±0.03
		Day 180	4.80±0.08	5.80±0.04	0.866±0.03	2.12±0.06
		Day 270	4.62±0.10	5.96±0.02	0.806±0.02	2.85±0.03
Hard cheese	Livanjski	Lactic acid bacteria				
		Day 90	7.20±0.15	5.52±0.03	0.900±0.02	1.85±0.02
		Day 120	5.83±0.10	5.60±0.02	0.907±0.05	2.20±0.02
		Day 180	4.60±0.12	5.72±0.05	0.880±0.03	2.28±0.04
		Day 330	3.40±0.12	5.80±0.02	0.795±0.03	3.30±0.01
		Day 360	2.20±0.05	5.92±0.02	0.791±0.06	3.35±0.02
		Day 450	2.00±0.02	5.97±0.02	0.650±0.04	3.62±0.03
	Hard cheese in olive oil	Lactic acid bacteria				
		Day 90	6.80±0.11	5.72±0.02	0.890±0.02	1.74±0.02
		Day 150	6.78±0.14	5.80±0.04	0.870±0.03	1.75±0.02
		Day 240	6.75±0.05	5.84±0.03	0.842±0.04	1.80±0.04
		Day 270	6.42±0.04	5.90±0.03	0.760±0.04	1.92±0.03

Table 3. Growth potential of *Listeria monocytogenes* in whey cheese skuta

Batch	Sample	*L. monocytogenes* number (log_{10} cfu/g) Day 0	*L. monocytogenes* number (log_{10} cfu/g) Day 8	Growth potential (log_{10} cfu/g)
I	1a	1	1.84	0.24
	1b	1.60	1.90	
	1c	1.69	1.69	
II	2a	1.47	1.90	0.43
	2b	1.30	1.95	
	2c	1.69	1.95	
III	3a	1.30	1.69	0.09
	3b	1.60	1.30	
	3c	1.69	1.90	

Table 4. pH and aw in whey cheese skuta during challenge test

Batch	Sample	Day 0		Day 8	
		pH	a_w	pH	a_w
I	1a	6.22	0.952	5.80	0.992
	1b	6.30	0.950	5.48	0.996
	1c	6.06	0.955	5.62	0.998
II	2a	6.42	0.960	5.45	0.993
	2b	6.23	0.958	5.50	0.997
	2c	6.30	0.950	5.62	0.998
III	3a	6.17	0.952	5.50	0.998
	3b	6.15	0.980	5.46	0.997
	3c	6.22	0.976	5.52	0.994

phase of post-processing was monitored in order to evaluate compliance with microbiological criteria, with particular reference to *L. monocytogenes* counts. In relation to overall microbiological quality, results showed that soft, semi-hard and hard cheeses were microbiologically stable during their defined shelf-life. Microbiological stability of semi-hard and hard cheeses relies on several hurdles including starter cultures competitiveness, increased salt content and, most importantly, low water activity (2, 9). Physico-chemical values and their changes during the storage of semi-hard and hard cheeses in our study are in line with previous reports (11, 13, 15). *L. monocytogenes* was not present in viable counts, however it should be stressed that secondary contamination is possible during the cutting of the cheese into quarters or halves, followed by packaging. Prevention of contamination with *Listeria* at this point should be based on Good Hygienic Practice and verification of sanitation programs.

Soft cheeses in general are of limited durability, because of high moisture content and high pH which support the proliferation of some spoilage microorganism like *Enterobacter* spp. or *Pseudomonas* spp. under cold storage (8). Whey cheese (skuta) used in our study showed physicochemical characteristics comparable with other similar products from the Mediterranean area (1, 10, 16). During the storage of whey cheese skuta the pH decreased and lactic acid bacteria count increased which is in accordance with other studies (10, 16). Lactic acid bacteria are known to be beneficial and functional microbes in many different dairy products (20), however their outgrowth in this kind of products (whey cheese) could contribute to spoilage (8). Soft cheeses are ready-to-eat products that support the growth of *L. monocytogenes* based on their physic-chemical characteristics (14, 18), which is also presented by the results in current survey (pH values 5.8-6.5, water activity 0.99-0.94 and NaCl content 0.3-1.2%). Challenge test showed that *L. monocytogenes* growth potential in whey cheese was 0.43 log_{10} cfu/g during 8 days at 4°C, meaning that the proposed shelf-life is acceptable for products stored under defined conditions. Many studies from last decades emphasized that *L. monocytogenes* represents a serious public-health problem due to high prevalence in soft cheeses (7, 17, 19). Recent studies are more focused on

bacterial kinetics in cheeses made from pasteurized milk during their shelf-life at different storage conditions, and related application of bio-protective strategies (3).

CONCLUSION

Compliance with microbiological criteria at the end of cheese production (final product) doesn't guarantee that microbiological hazards are excluded. *Listeria monocytogenes* is a ubiquitous bacteria and secondary contamination of products is possible under poor hygienic conditions. Despite the fact that the growth of the pathogen is limited in semi-hard and hard cheeses by low water activity, the secondary (surface) contamination could result in hazardous products. The significance of following strict hygienic procedures is evident even more in soft cheese production, since they support the growth of *L. monocytogenes*.

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