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EVALUATION OF MICROBIOLOGICAL QUALITY OF SELECTED CHEESES DURING STORAGE

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ABSTRACT

The aim of this article was to evaluate and compare the microbiological quality of selected types of cheeses immediately after opening and after 5 days storage in the refrigerator. Total viable counts (TVC), coliform bacteria (CB) and microscopic filamentous fungi (MFF) were determined by microbiological analysis. We analyzed 8 samples of cheese of Slovak origin. Plate dilution method was used for microbiological analysis. The Codex Alimentarius of Slovak republic (2006) just indicates number of coliforms bacteria (10^2) and microscopic fungi (5×10^2). The TVC values after opening of cheeses ranged from 1.68×10^3 CFU.g^{-1} (3.22 log CFU.g^{-1}) in the sample no. 1 to 1.71×10^5 KTJ.g^{-1} (5.23 log CFU.g^{-1}) in the sample no. 4 after storage in the refrigerator. All samples were negative for the presence of coliform bacteria after opening. The values of CB were 1.18×10^2 CFU.g^{-1} (2.07 log CFU.g^{-1}) in sample no. 7 and 1.90×10^2 CFU.g^{-1} (2.27 log CFU.g^{-1}) in the sample no. 8 after storage in refrigerator. These values are not in accordance with Codex Alimentarius of Slovak Republic (2006). Other samples were negative for presence of CB after storage at 4 °C. The values of MFF in samplesranged from 1.81×10^1 CFU.g^{-1} (1.25 log CFU.g^{-1}) in the sample no. 1 after opening to 1.68×10^2 CFU.g^{-1} (2.22 log CFU.g^{-1}) in sample no. 7 after storage of samples. All analysed samples were in accordance with Codex Alimentarius of Slovak republic (2006).

Keywords: cheese; microbiological quality; total viable counts; coliform bacteria; microscopic filamentous fungi

INTRODUCTION

Dairy products are characterized by reduced shelf life because they are an excellent growth medium for a wide range of microorganisms. For this reason, it is important to monitor the microbiological quality of dairy products and, in particular, the total viable count and concentration of *Escherichia coli*, as they are indicators of the hygienic state of these products **(Losito et al., 2014)**.

Cheeses, although they have been characterized as one of the safest food products by some authors **(Little et al., 2008)**, in 2006 the consumption of contaminated cheese accounted for the 0.4% of the total foodborne outbreaks in Europe (**EFSA, 2008**). Furthermore, the scientific literature has reported several food poisoning outbreaks associated with various types of cheeses.

Cheeses are ready-to-eat (RTE) food products that do not undergo any further treatment to ensure their safety before consumption. Contamination of cheese with foodborne pathogens may occur at several stages. Thus, if contamination of any type of cheese is to be prevented or controlled information on the major sources of pathogens and the mechanisms by which these contaminate the dairy chain are required. Moreover, the need for knowledge about the vectors and the routes of contamination into RTE food and quantitative data on recontamination to accurately establish Microbial Risk Assessment has been also addressed **(Reij and Den Aantrekker, 2004)**.

Microbial contamination of cheese may originate from various sources. Such sources during cheese production might be: starter culture, brine, floor and packaging material, cheese cloth and curd cutting knife, cold room and production room air **(Temelli et al., 2006)**. Storage coolers have been also demonstrated to be the source of *Listeria monocytogenes* contamination of cheese made from pasteurized milk **(Brito et al., 2008)**. Moreover, humans have been found to serve as contamination source of cheese with pathogenic bacteria like *Staphylococcus aureus* **(Callon et al., 2008)**. However, in this section an attempt is made to review literature on two main sources of cheese contamination with pathogenic bacteria, i.e. the raw milk and routes of contamination at cheese processing plants.

The aim of the present study was to evaluate the microbiological stability of selected types of cheeses during storage.

MATERIAL AND METHODOLOGY

Microbiological analysis of 8 Slovak cheeses was performed immediately after opening and after 5 days of storage of samples in the refrigerator. Samples of evaluated cheeses:

Samples no. 1 and no. 2 spreadable processed dairy products, samples no. 3 and no. 4 melted slices with emental, sample no. 5. Steamed cheese – smoked, sample no. 6 steamed cheese – unsmoked, samples no. 7 and no. 8 Edam cheeses.

Plate dilution method was used for the microbiological analysis of cheeses. Basic dilutions (10^{-1}) was obtained by

mixing 5 g of the sample (cheese) and 45 ml of physiological solution (0.85% NaCl), followed by homogenization of the sample for 30 minutes.

Plate dilution method was applied for quantitative cfu counts of respective groups of microorganisms in 1 g of cheese. Gelatinous nutritive substrate in petri dishes was inoculated with 1 ml of honey samples by flushing and on surface in three replications.

Determination of TVC

Plate Count Agar was used for determine of Total Viable Counts in samples. Dilutions of 10^{-3} and 10^{-4} were used to determine of TVC. Petri dishes were cultivated upside-down in a thermostat at 30 °C for 48-72 hours under aerobic conditions.

Determination of CB

Violet red bile agar was used for determine of Coliform Bacteria in samples.

Dilutions of 10^{-3} and 10^{-4} were used to determine of Coliform Bacteria. Petri dishes were cultivated upside-down in a thermostat at 37 °C for 24 – 48 hours.

Determination of MFF

Chloramfenicol yeast glucose agar was used for determine of Microscopic Filamentous Fungi.

Dilutions of 10^{-1} and 10^{-2} were used to determine of MFF. Petri dishes were cultivated upside-down in a thermostat at 25 °C for 5 – 7 days under aerobic conditions.

Calculation of microorganisms

The number of microorganisms in1 g samples (N) were calculated using the following formula:

$$\mathbf{N = \Sigma C / [(n_1 + 0{,}1n_2) .d]}$$

ΣC – sum of characteristic colonies on selected plates,

$\mathbf{n_1}$ – number of dishes from 1. dilutions used to calculate,

$\mathbf{n_2}$– number of dishes from 2. dilutions used to calculate,

d – dilution factor identical with 1. used dilution.

Statistics

Mathematical and statistical analyzes are processed in the tables. Arithmetic mean, standard deviation, coefficient of variation were performed using MS Excel.

RESULTS AND DISCUSSION

Microbiological quality of selected kinds of cheeses were performed. Total Viable Count (TVC), Coliform Bacteria (CB) and Microscopic Filamentous fungi (MFF) were determined immediately after opening of products and after 5 days of storage of products at 4 °C.

The factors that contribute to the safety of cheese with respect to pathogenic bacteria include milk quality, starter culture or native lactic acid bacterial growth during cheese making, pH, salt, control of aging conditions and chemical changes that occur in cheese during aging. Other technologies may provide opportunities to add additional barriers to the growth of bacterial pathogens. It is particularly important for the producers of raw milk cheeses to have a documented and systematic approach to ensure product safety **(Donnelly, 2004)**.

Values of Total Viable Counts (TVC) in samples of Slovak cheeses after opening ranged from 1.68×10^3 CFU.g^{-1} (3.22 log CFU.g^{-1}) in sample no. 1 to 2.91×10^3 CFU.g^{-1} (3.46 log CFU.g^{-1}) in sample no. 7 (Figure 1). Average number of TVC after opening was 3.33 log CFU.g^{-1} (Table 1).

Dermes-Mathieu et al. (2013) studied The efficacy of an anionic peptides-enriched extract (APEE), produced by nanofiltration of a tryptic hydrolysate from whey proteins, to inhibit the growth of *Listeria innocua* and *Listeria monocytogenes* in reconstituted Cheddar cheese was studied. The antimicrobial activity of APEE in reconstituted cheese was greater against *L. monocytogenes* than *L. innocua* and was higher in storage at 30 °C than at 4 °C. The combination of 20 mg.g^{-1} of APEE and 1.75% salt/moisture (S/M) in cheeses incubated for 7 days at 30 °C was the most efficient condition to inhibit the growth of *Listeria*. Using these conditions, *L. monocytogenes* counts were significantly reduced by 1.1 and 1.5 log CFU.g^{-1}, compared with cheeses without APEE and prepared with lactococci at 1.75 and 3.5% S/M, respectively. These results suggest that antimicrobial anionic peptides from whey proteins can contribute to control pathogen in reduced-salt Cheddar cheeses.

Iurlina and Fritz (2004) studied the microbiological quality of Port Salut Argentino cheese during 10 days (after ripening) at two storage temperature treatments: (a) 4 °C and (b) a temperature combination of both 4 and 20 °C (4/20 °C), which implied 12 h at 4 °C and 12 h at 20 °C. Total coliforms were not higher than 10^3 CFU.g^{-1} among samples. *E. coli* was detected at both treatments. Thirty three percent of the cheese contained *Staphylococcus aureus*. *Listeria* spp. and *Salmonella* spp. were not detected in any treatment. *Bacillus* spp. incidence was 50% of the cheese, being *B. cereus*, *B. cereus* variety mycoides and *B. pumilus*. *Bacillus cereus* and *Staphylococcus aureus* grew at 4/20 °C. Mesophilic aerobic bacteria were between 10^4 and 10^7 CFU.g^{-1}. At 4/20 °C counts decreased. At 4 °C the behaviour was variable. Moulds were lower than 10^4 CFU.g^{-1} and yeasts were between 10^4 and 10^5 CFU.g^{-1}. pH, moisture content and tritatable acidity ranges of samples were 5.5 – 6, 51 – 52.3% and 1.215 – 1.935 g.100g^{-1} of lactic acid, respectively. Manufacturing of this cheese includes a short heat treatment and starter culture addition; consequently, our results indicate that this processing may be insufficient for achieving hygienic cheese production. The storage at refrigeration temperature will not always guarantee the cheese safety and quality.

All our samples of cheeses were negative for presence of Coliform Bacteria, all samples are in accordance with Codex Alimentarius of Slovak Republic (2006) (Figure 2).

Melanie and Siegfried (2001) determined the incidence of *Listeria* and *Listeria monocytogenes* in European red smear cheese in order to assess whether the lack of recent outbreaks of listeriosis associated with cheese is due to improved hygenic conditions in the dairies. Out of European red-smear cheese samples of various types, 15.8% contained organisms of the genus *Listeria*, 6.4% of the samples were contaminated with *L. monocytogenes*, 10.6% with *L. innocua*, and 1.2% with *L. seeligeri*. Six cheese samples contained two or more *Listeria* species, including at least one *L. monocytogenes* isolate. The incidences of *L. monocytogenes* in cheeses from various

countries were: Italy 17.4%, Germany 9.2%, Austria 10%, and France 3.3%. *Listeria* was found most frequently in soft and semi-soft cheese. Eight samples contained more than 100 *L. monocytogenes* CFU.cm^{-2} cheese surface, 2 samples had counts above 10^4 CFU.cm^{-2} cheese surface. Surprisingly, a higher incidence of *L. monocytogenes* was observed in cheeses made from pasteurized milk (8.0%) than in cheeses manufactured from raw milk (4.8%).

The values of CB samples after storage in refrigerator were 1.18×10^2 CFU.g^{-1} (2.07 log CFU.g^{-1}) in sample no. 7 and 1.90×10^2 CFU.g^{-1} (2.27 log CFU.g^{-1}) in sample no. 8 (Figure 2). These values are not in accordance with Codex Alimentarius of Slovak Republic (2006). Other samples were negative for presence of CB after storage at 4 °C (Figure 2). Average number of CB after storage was 0.54 log CFU.g^{-1} (Table 2).

Yucel and Ulusoy (2006) studied a total of 200 dairy (raw milk, cheese) samples obtained from Ankara, for the presence of *Yersinia* spp., total coliform and *Escherichia coli*. As expected, raw milk 55% (55/100) were significantly contaminated with *Yersinia* spp., than cheese samples 14% (14/100). *Y. enterocolitica* was the most commonly isolated species, and was recovered from 47.3% in raw milk 35.7% in cheese samples. The other *Yersinia* spp. were identified as *Y. frederiksenii* (31.0%, 21.4%), *Y. kristensenii* (12.7%), *Y. intermedia* (7.2%, 7.1%) and atypical *Yersinia* spp. (1.8%, 35.7%) in raw milk and cheese samples, respectively. All the samples of cheese examined were negative for *Y. kristensenii*. All *Y. enterocolitica* strains tested gave negative results in the autoagglutination tests and crystal violet binding test.

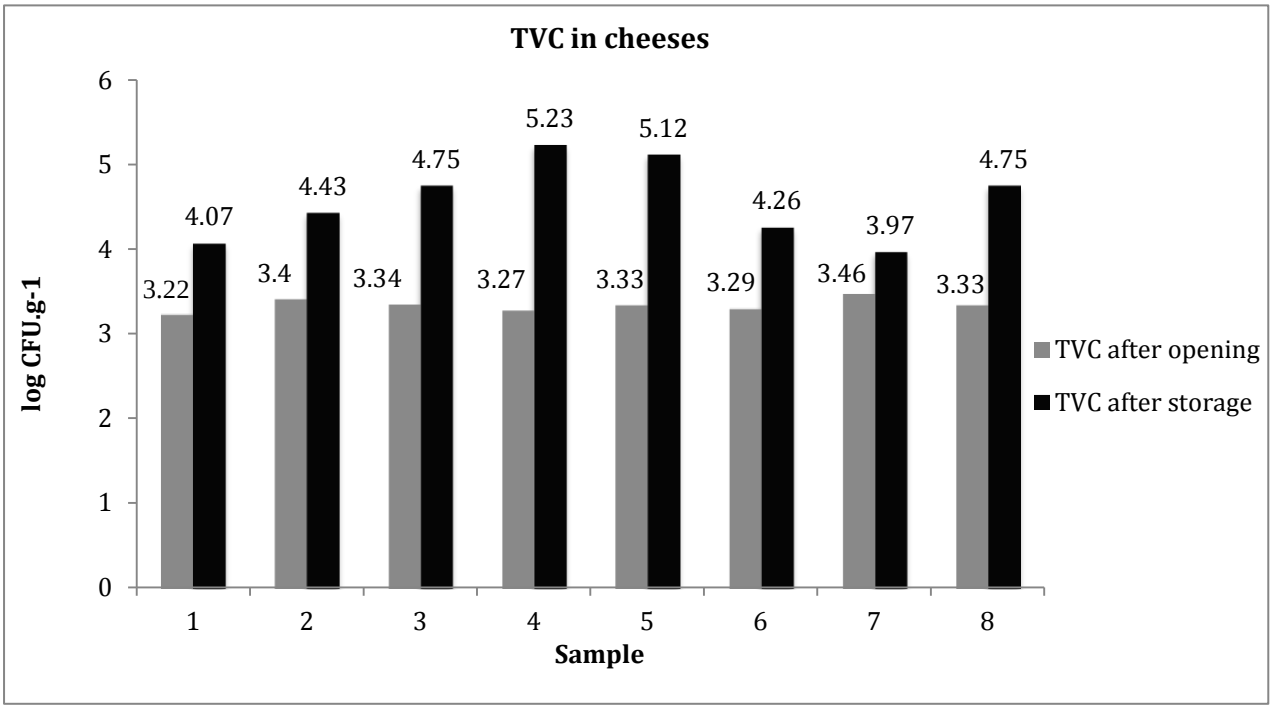

Figure 1 Values of TVC in cheeses after opening and after storage in refrigerator.

Values of TVC after 5 days of storage at temperature 4 °C were in range from 9.54×10^3 CFU.g^{-1} (3.97 log CFU.g^{-1}) in sample no. 7 to 1.71×10^5 CFU.g^{-1} (5.23 log CFU.g^{-1}) in sample no. 4 (figure 1). Average number of TVC after storage was 4.57 log CFU.g^{-1} (table 1).

Table 1 Basic statistical characteristics of TVC in cheeses after opening and after storage.

	TVC after opening	TVC after storage
n	8	8
x	3.33	4.57
s	0.07	0.44
v%	2.10	9.62

n – number of samples, x – average, s - standard deviation, v% - coefficient of variation

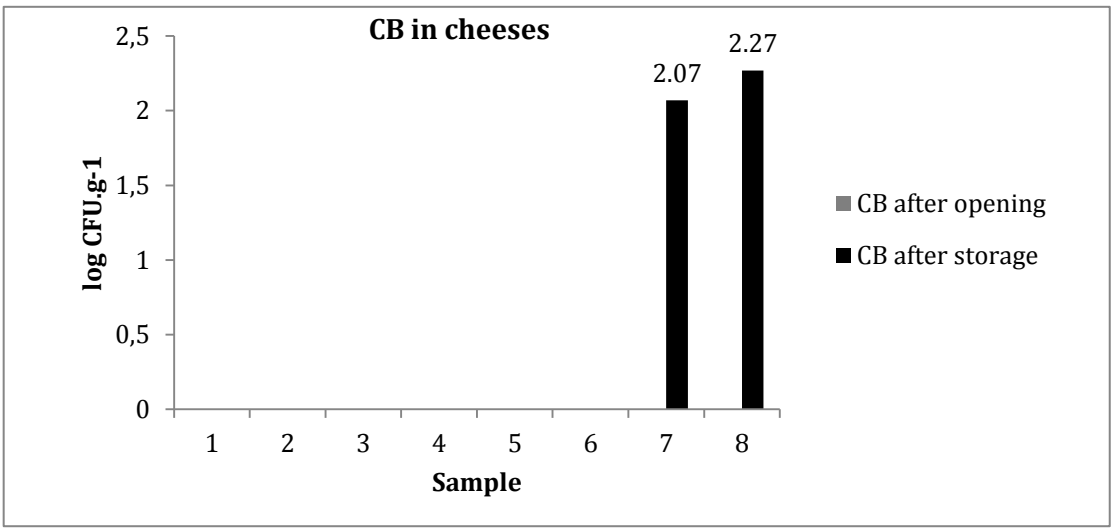

Figure 2 Values of CB in cheeses after opening and after storage in refrigerator.

The wide array of available dairy foods challenges the microbiologist, engineer, and technologist to find the best ways to prevent the entry of microorganisms, destroy those that do get in along with their enzymes, and prevent the growth and activities of those that escape processing treatments. Troublesome spoilage microorganisms include aerobic psychrotrophic Gram-negative bacteria, yeasts, molds, heterofermentative lactobacilli, and spore-forming bacteria. Psychrotrophic bacteria can produce large amounts of extracellular hydrolytic enzymes, and the extent of recontamination of pasteurized fluid milk products with these bacteria is a major determinant of their shelf life. Fungal spoilage of dairy foods is manifested by the presence of a wide variety of metabolic by-products, causing off-odors and flavors, in addition to visible changes in color or texture **(Ledenbach and Marshall, 2009)**. Samples no. 2, 5, 6 and 8 were negative for presence of MFF after opening. Values of MFF ranged from 1.81×10^1 CFU.g^{-1} (1.25 log CFU.g^{-1}) in sample no. 1 to 2.72×10^1 CFU.g^{-1} (1.43 log CFU.g^{-1}) in sample no. 7 (figure 3). Average number of MFF after storage was 0.67 log CFU.g^{-1} (Table 3).

Values of MFF after 5 days of storage ranged from 5.4×10^1 CFU.g^{-1} (1.73 log CFU.g^{-1}) in sample no. 4 to 1.68×10^2 CFU.g^{-1} (2.22 log CFU.g^{-1}) in sample no. 7. Samples no. 5, 6 and 8 were negative for presence MFF after storage (figure 3). Average number of MFF after storage was 1.20 log CFU.g^{-1} (Table 3). All anelysed samples meet the requirements of Codex Alimentarius of Slovak Republic (2006).

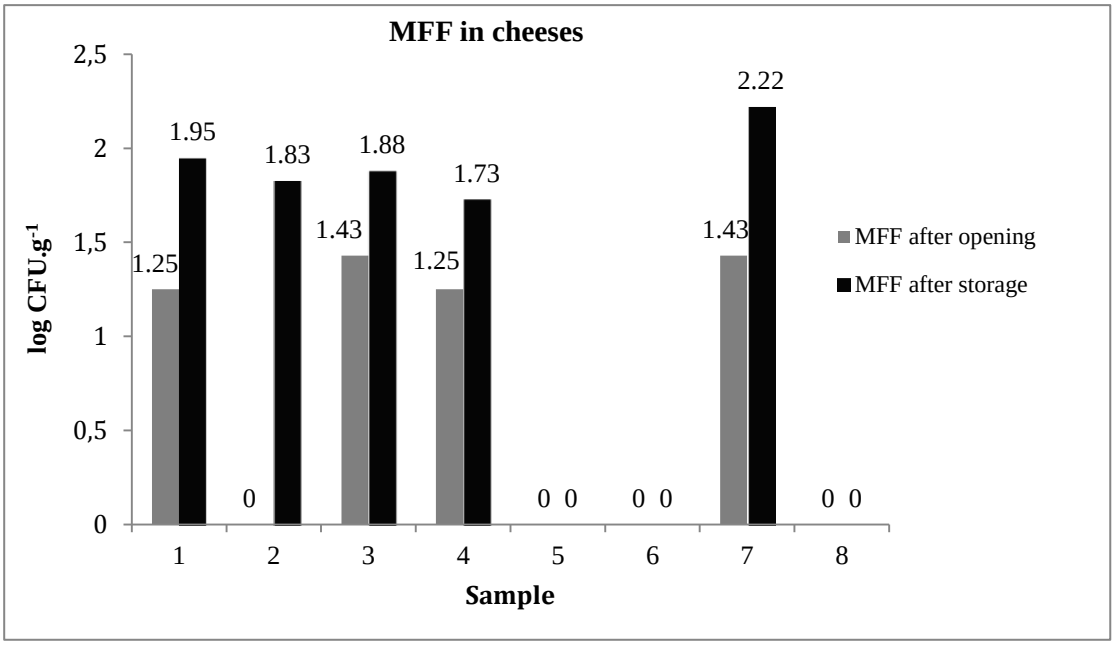

Figure 3 Values of MFF in cheeses after opening and after storage in refrigerator.

Table 2 Basic statistical characteristics of CB in cheeses after storage.

	CB after storage
n	8
x	0.54
s	0.94
v%	174.07

n – number of samples, x – average, s - standard deviation, v% - coefficient of variation

Table 3 Basic statistical characteristics of MFF in cheeses after opening and after storage.

	MFF after opening	MFF after storage
n	8	8
x	0.67	1.20
s	0.67	0.94
v%	100	78.33

n – number of samples, x – average, s - standard deviation, v% - coefficient of variation

Hayaloglu and Kirbag (2007) studied the chemical and microbial qualities, including microscopic filamentous fungi, of 30 samples of Kuflu cheese randomly purchased from different markets in Turkey. The levels of main microbial groups including total mesophilic and coliform bacteria, yeasts and moulds and the presence of some potentially pathogenic microorganisms (*E. coli*, *Salmonella* spp. and *Staphylococcus aureus*) were determined. The high numbers of all microbial groups and presence of potentially pathogenic organisms in the cheese samples suggested that the production and maturation of Kuflu cheese should be improved by better hygiene. Moulds at the cheese surface were isolated and identified. A total of 24 different mould species were detected and the genus most frequently isolated was *Penicillium* spp. which represented 70.25% of total isolates. *Penicillium commune*, *P. roqueforti* and *P. verrucosum* were the most abundant species in the cheeses sampled. The other dominant fungal groups were *Geotrichum candidum*, *Penicillium expansum* and *P. chrysogenum*. Other genera isolated from the cheese were *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Geotrichum*, *Mucor*, *Rhizopus* and *Trichoderma*. The potentially toxigenic species, including some *Penicillium* spp. and *Aspergillus flavus*, were also detected.

CONCLUSION

Microbiological quality of selected types of cheeses were evaluated after opening and after 5 days of storage in a refrigerator. The results were compared with the Codex Alimentarius of the Slovak Republic. The results show that the values of coliform bacteria in Edam cheeses after storage slightly exceeded the maximum limit established by legislation. Other samples had a good microbiological quality after opening and also during storage.

REFERENCES

Brito, J. R. F., Santos, E. M. P., Arcuri, E. F., Lange, C. C., Brito, M. A. V. P., Souza, G. N. et al. 2008. Retail survey of Brazilian milk and Minas frescal cheese and a contaminated dairy plant to establish prevalence, relatedness, and sources of *Listeria monocytogenes* isolates. *Applied and Environmental Microbiology*, vol. 74, no. 15, p. 4954-4961. http://dx.doi.org/10.1128/aem.01828-07

Callon, C., Gilbert, F. B., Cremoux, R. D., Montel, M. C. 2008. Application of variable number of tandem repeat analysis to determine the origin of *S. aureus* contamination from milk to cheese in goat cheese farms. *Food Control*, vol. 19, no. 2, p. 143-150. http://dx.doi.org/10.1016/j.foodcont.2007.02.014

Codex Alimentrius SR 2009. - Druhá časť, Štvrtá hlava – Mikrobiologické požiadavky na potraviny a na obaly na ich balenie. 2009.

Dermes-Mathieu, V., Gauthier, S. F., Britten, M., Fliss, I., Robitaille, G., Jean, J. 2013. Inhibition of *Listeria monocytogenes* growth in Cheddar cheese by an anionic peptides-enriched extract from whey proteins. *International Dairy Journal*, vol. 32, no. 1, p. 6-12. http://dx.doi.org/10.1016/j.idairyj.2013.03.008

Donnelly, C. 2004. Growth and survival of microbial pathogens in cheese. *Cheese: Chemistry, Physics and Micribiology*, vol. 1, 2004, no. 4, p. 541-559. http://dx.doi.org/10.1016/s1874-558x(04)80081-2

European Food Safety Authority (EFSA). (2008). Zoonoses data collection reports. [cit. 2015-03-03] Available at: http://www.efsa.europa.eu/en/science/monitoring_zoonoses/reports.html

Hayaloglu, A. A., Kirbag, S. 2007. Microbial quality and presence of moulds in Kuflu cheese. *International Journal of Food Microbiology*, vol. 115, no. 3, p. 376-380. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.12.002

Iurlina, M. O., Fritz, R. 2004. Microbiological quality of Port Salut Argentino cheese stored at two temperature treatments. *Food Science and Technology*, vol. 37, no. 7, p. 739-748. http://dx.doi.org/10.1016/j.lwt.2004.02.011

Ledenbach, L. H., Marshall, R. T. 2009. *Microbiological Spoilage of Dairy Products*. *Compendium of the Microbiological Spoilage of Foods and Beverages*, p. 41-67, ISBN 978-1-4419-0826-1.

Little, C. L., Rhoades, J. R., Sagoo, S. K., Harris, J., Greenwood, M., Mithani, V., Grant, K., McLauchlin, J. 2008. Microbial quality of retail cheese made from raw, thermised or pasteurized milk in UK. *Food Microbiology*, vol. 25, no. 2, p. 304-312. http://dx.doi.org/10.1016/j.fm.2007.10.007

Losito, F., Arienzo, A., Bottini, G., Priolisi, F. R., Mari, A., Antonini, G. 2014. Microbiological safety and quality of Mozzarella cheese assessed by the microbiological survey method. *Journal of Dairy Science*, vol. 97, no. 1, p. 46-55. http://dx.doi.org/10.3168/jds.2013-7026

Melanie, R., Siegfried, S. 2001. High incidence of *Listeria monocytogenes* in European red smear cheese. *Int. J. Food Microbiology*, vol. 63, no. 1/2, p. 91-98. http://dx.doi.org/10.1016/s0168-1605(00)00413-x

Reij, M. W., Den Aantrekker, E. D. 2004. Recontamination as a source of pathogens in processed foods. *International Journal of Food Microbiology*, vol. 91, no. 1, p. 1-11. http://dx.doi.org/10.1016/s0168-1605(03)00295-2

Temelli, S., Anar, S., Sen, C., Akyuva, P. 2006. Determination of microbiological contamination sources during Turkish white cheese production. *Food Control*, vol. 17, no. 11, p. 856-861. http://dx.doi.org/10.1016/j.foodcont.2005.05.012

Yucel, N., Ulusoy, H. 2006. A Turkey survey of hygiene indicator bacteria and *Yersinia enterocolitica* in raw milk and cheese samples. *Food Control*, vol. 17, no. 5, p. 383-388. http://dx.doi.org/10.1016/j.foodcont.2005.01.005

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