The physico-chemical peculiarities of Tilsit cheese obtained in a milk processing unit from Transilvania

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Abstract. The study material consisted of 48 Tilsit cheese samples taken in the January 2019- December 2021 timeframe from a milk processing unit in Transilvania. The pursued goals in our study were: the evaluation of the physico-chemical composition, the evaluation of the freshness parameters and the evaluation of the microbiological risk represented by *Escherichia coli* and *Staphylococcus aureus* for the Tilsit cheese during the ripening process. All samples were subjected to the standard analysis methods and the interpretation of the results was completed with the help of the Origin 8.5 program. The pH and titratable acidity values showed a consistent increase during the entire ripening process. Some non-conformities were highlighted regarding the following parameters: fat reported to dry substance and NaCl. The risk represented by *E. coli* and *Staphylococcus aureus* is low during the first stages of the ripening process, and completely absent after 28 months of ripening.
Key Words: microbiological risk, physico-chemical parameters, ripening process.

Introduction. The cheese microbiota, whose community structure evolves through a succession of different microbial groups, plays a central role in cheese-making. The subtleties of cheese character, as well as cheese shelf-life and safety, are largely determined by the composition and evolution of this microbiota. The interactions between bacteria and fungi within these communities determine their structure and function. Yeasts play a key role in the establishment of ripening bacteria. These interactions enhance cheese flavor formation and can control and/or prevent the growth of pathogens and spoilage microorganisms in cheese. There are over 1000 varieties of cheeses produced on artisanal and industrial scales. The associated microbiota contributes to the biopreservation and the development of organoleptic properties of cheeses. Therefore, unravelling the microbial diversity and the functioning of these ecosystems is an extraordinary challenge to more effectively control the quality and safety of cheeses. A higher degree of microbiological safety in cheeses is yet to be achieved. A recent study revealed that in 2% of marketed cheeses made from raw, thermised and pasteurized cheeses, counts of *Escherichia coli*, *Staphylococcus aureus* and/or *Listeria monocytogenes* were above those allowed by the European Commission Recommendations 2004/24/EC and 2005/175/EC (Irlinger & Mounier 2009). Because of these considerations, in this study we intend to assess the microbiological risk represented by *E. coli, S. aureus, Staphylococcal enterotoxin,* yeasts and molds for the ripened Tilsit cheese.

Material and Method. For the dynamic evaluation of the microbiological parameters, safety and hygiene criteria of the technological process of ripened Tilsit cheese, samples were taken from the ripening spaces of the processing unit in the January 2019-December 2021 timeframe. The 3 lots contained samples from different periods of the ripening process: the first day after obtaining the cheese, after 8 months, 16, 28 and 36 months of ripening. In order to obtain conclusive results, 6 samples (n=6) were taken from each lot. The samples were taken from three different lots, packed in sterile

polyethylene bags and transported in isotherm bags at 4-6ºC to the laboratory of the Food Inspection and Control discipline, within the Faculty of Veterinary Medicine, UASVM Cluj Napoca, where the samples were immediately processed.

The processing of the samples for the evaluation of the freshness during the ripening process. The determination of the pH was done with the help of the electronic Metler Toledo FiveGo (Mettle Toledo AG/CH) pH-meter. The pH electrode was inserted in the cheese samples, establishing the pH and temperature of the product.

The determination of the titratable acidity was carried out as follows. 10 g of cheese from the studied samples were weighted in a porcelain capsule with a precision of 0.01 g. 20 mL of water and 1 mL of 2% phenolphthalein solution were added. Afterwards, these were homogenized until a homogeneous paste was obtained. Next, these were titrated with NaOH 0.1 N solution, mixing until pink coloration appeared. This should persist for 1 minute. For each sample, two measurements were taken and their arithmetic average was calculated.

The processing of the samples for the evaluation of the microbiological quality of ripened cheeses. For the isolation and identification of the microorganisms classified into the hygiene criteria category of the technological process, standardized methods were used, as follows: β-glucuronidase positive *E. coli* (SR EN ISO 16649-1/2018); coagulase-positive staphylococci (SR ISO 6888-1:1999/Amd. 2:2018). To avoid the contamination of the environment and of the samples, the processing took place on the laminar flow bench and the samples were handled in a manner meant to avoid any risk of contamination. From the collected samples, 10 g were taken and then homogenized in an Easy mix machine for 90 seconds in the presence of 90 mL of buffered peptone water, resulting in the initial suspension (10^{-1}). 1 mL of the base dilution was taken with a sterile pipette, which was then inserted into a test tube with 9 mL of dilution liquid. After closing the test tube, the suspension was homogenized in a Vortex shaker. This resulted in a 10^{-2} dilution. This operation was repeated to obtain the decimal dilutions (10^{-6}), a sterile pipette being used for each dilution. 1 mL of each obtained dilution was placed in the center of 2 Petri dishes using a sterile pipette, before adding approximately 15 mL TBX culture medium (*E. coli*), Baird-Parker agar (coagulase-positive staphylococci), and DRBC (yeasts and molds), which was melted and cooled at 47±1ºC, to obtain a continuous surface. The inoculum and the culture medium were homogenized using rotational movements in a horizontal plane: clockwise movements and counterclockwise movements, and also forward and backward movements. Afterward, the mixture was left to solidify on a cold, flat surface. After complete solidification of the medium, the Petri dishes were placed and kept at 44ºC for the identification of *E. coli*, and at 37°C for coagulase-positive staphylococci, for 18-24 hours.

Mathematical methods of statistics. All obtained results were carried out and interpreted using the Origin 8.5 software. The results were interpreted based on the determination of the individual mediums, expressed depending on the standard deviation (n=6). The obtained results were analyzed depending on the following indicators and statistics: arithmetical average, the standard deviation and the probability index. The statistical interpretation of the results depending on the value of the probability index p was carried out as such: $p>0.05$ - statistically insignificant results/differences; $p\leq0.05$ - statistically significant results/differences, (level of reliability 95%).

Results and Discussion

The dynamic evaluation of the freshness parameters found in the ripened Tilsit cheese. During the ripening process, complex enzymatic processes and the dehydration of the Tilsit cheese take place. This dehydration is accentuated as the cheese ripens. As a result of water loss from the cheeses' mass, the components of the cheese concentrate.

The determination of the pH value represents one of the assessment methods of the cheeses' ripening process. Its value depends on the quantity of lactic cultures used

for the milk's ripening, on the sodium chloride quantity, the treatment of the obtained curd and on the pH of the whey in the production process. The results obtained in this study show a slight increase in the average value of the pH from day 0. This increase continues during the whole ripening process, starting from 5.22±0.6 and ending at 6.06±0.26 pH value after 36 months of ripening (Figure 1). This slight increase is due to the biochemical reactions conducted by the activity of the enzymes specific to milk, such as phosphatase, lipases and proteinases, but also the activity of the microorganisms, which finalize the organoleptic characteristics during the ripening. Such a similar pH value was obtained by Fox et al (2004), when analyzing the freshness parameters of Tilsit cheese. In the first days after obtaining the cheese, the pH was 5.5, it increased to 7.5 pH after the first two weeks of the ripening process, and afterwards it remained relatively stable.

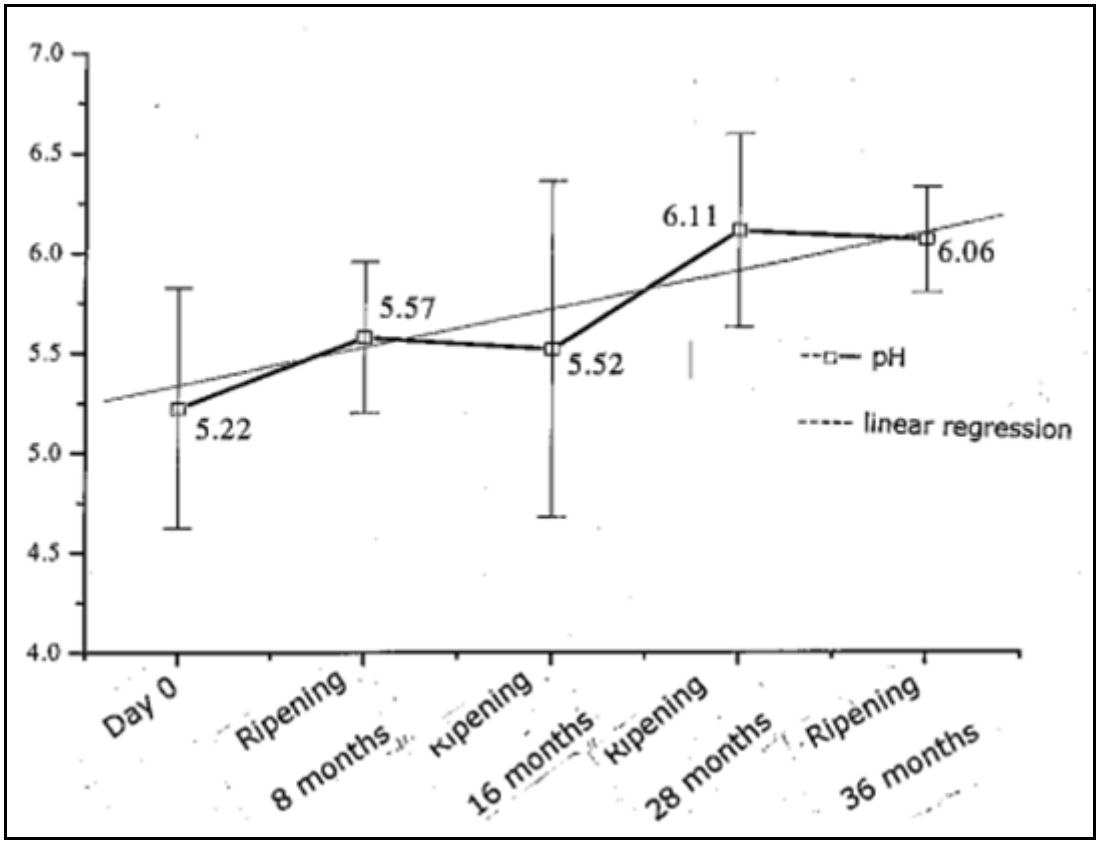

Figure 1. The average value of the pH during the production process of the ripened Tilsit cheese (n=48).

The average value of the titratable acidity showed an increase from the moment of the Tilsit cheeses' production and during the whole ripening process, showing a significant difference from a statistical point of view ($p<0.05$). This aspect is also revealed by the simple linear regression (Figure 2). The 59°T value from day 0 of the product increased to 260°T over the 36 months of ripening.

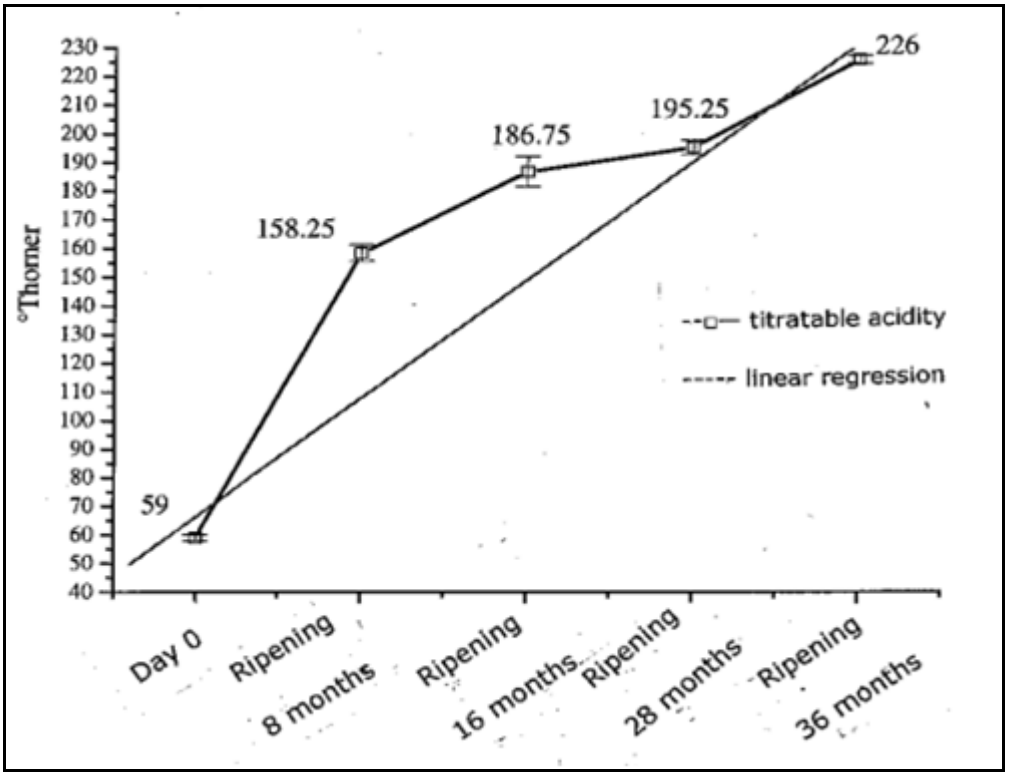

Figure 2. The average value of the titratable acidity during the production process of the ripened Tilsit cheese (n=48).

The dynamic evaluation of the microbiological parameters found in the ripened Tilsit cheese. The evaluation of hygiene during the technological process is achieved by determining the *E. coli* and coagulase-positive staphylococci load. The obtained results for the *S. aureus* microbial load ranged between the average values of 3.82±0.12 log ufc g^{-1} for the production day of the Tilsit cheese and 0.27±0.56 log ufc g^{-1} after 16 months of ripening, followed by a downward trend. We note that after 28 and 38 months of ripening, no coagulase-positive staphylococci were isolated from the examined samples (Figure 3).

From the analysis of this data, we determined that the maximum permissible limit was surpassed at the stage of forming the Tilsit blocks and after 8 months of ripening. These show shortcomings of the hygiene conditions during the technological process and of the hygienic quality of the milk (raw material). Beresford et al (2001) obtained different results in a study of microorganism identification on the cheeses' surface. The study was conducted on 400 samples, of which only 10 contained coagulase-negative staphylococci (*Staphylococcus equorum*, *Staphylococcus vitulus* and *Staphylococcus xylosus*). Similarly, Mariani & Battistotti (1999), after analysing over 100 ripened Grana Padano cheese samples, have obtained only negative results for the detection of the microorganism species: *Salmonella* spp., *S. aureus*, *L. monocytogenes*, coliforms and Enterobacteriaceae. The maximum permissible microbial load for cheeses is regulated by Reg. (CE) nr. 2073/2005 and should range between 10-1000 ufc g^{-1}. Because the staphylococci load did not surpass 100000 ufc g^{-1}, testing the samples for the screening of the staphylococcal enterotoxins was not required.

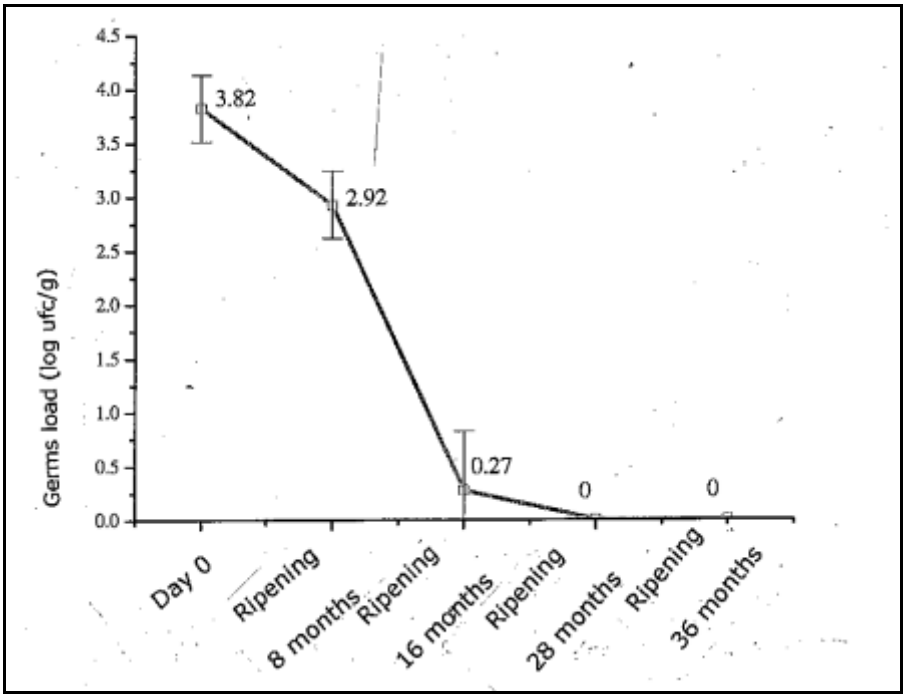

Figure 3. The coagulase-positive staphylococci load during the production process of the ripened Tilsit cheese (n=48).

From the total of analyzed samples, the microbial load with *E. coli* germs showed a downward trend, with significant statistical differences ($p \leq 0.05$). We obtained average values of 2.2±0.40 logufc g^{-1} on day 0 of the product, an average value of 0.25±0.5 logufc g^{-1} after 8 months of ripening and starting with the 16th ripening month, *E. coli* was no longer isolated (Figure 4). This result is due to the low pH value as well as due to the increase in the NaCl concentration during the ripening process, which then causes the inhibition of microbial activity (Miszczycha et al 2013).

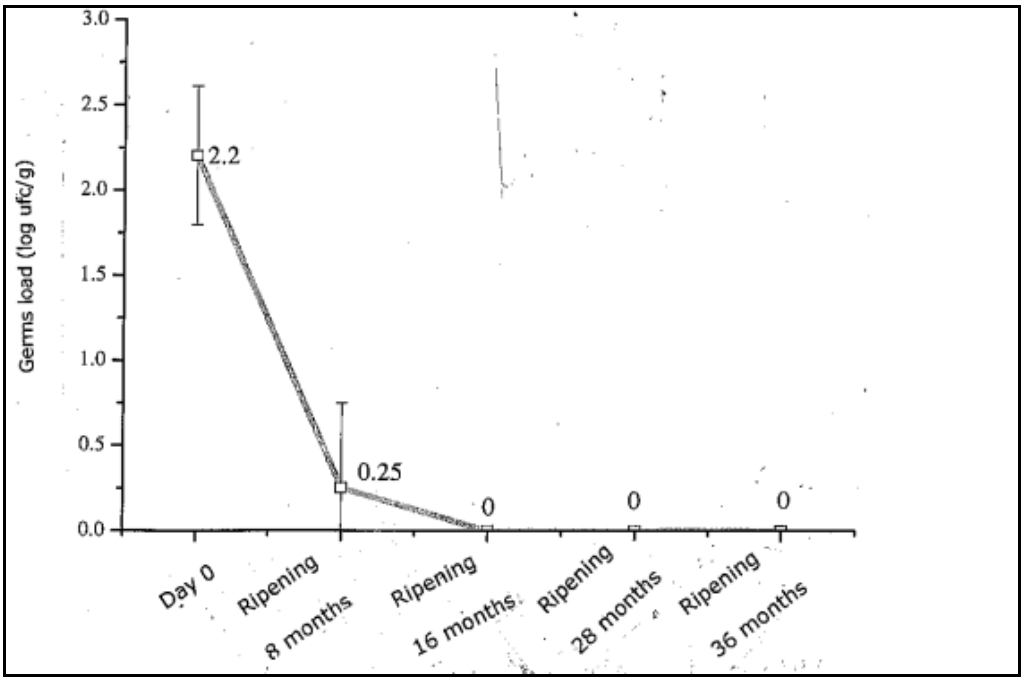

Figure 4. The E. coli load during the production process of the ripened Tilsit cheese (n=48).

Although the *E. coli* load surpassed the maximum limit permitted by Reg. 2073 (CE)/2005 immediately after forming the cheese, after 8 months, since the analyzed product was delivered for public consumption, the *E. coli* number was within the allowed limits. Similar results were recorded by Miszczycha et al (2013), where the bacterial *E. coli* O_{26}:H_{11} load reached values of 6 $\log_{10}$ufc g^{-1} in the first 24 hours after obtaining the cheese and *E. coli* O_{157}:H_7 reached a value of 4 $\log_{10}$ ufc g^{-1}. These values remained steady between the first and 60th day of the ripening process, showing decreasing values and after day 240 of ripening, the bacterial load decreasing below detectable level.

Conclusions. It can be concluded that during the ripening process of the Tilsit cheese, the microbiological risk is low. Although *E. coli* and *S. aureus* were found only in the first ripening stages, showing a relatively low microbial load, the presence of these microorganisms shows shortcomings regarding the strict enforcement of good hygiene practices in the studied unit. Taking into consideration the presence of *E. coli* and *S. aureus*, we recommend improving the hygiene conditions along with the technological process of Tilsit cheese production in the studied unit.

Conflict of Interest. The authors declare that there is no conflict of interest.

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*** ISO 6888-1:1999/Amd.2:2018 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) — Part 1.

*** ISO 16649-1:2018 Microbiology of the food chain — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* — Part 1.

*** Regulation (EC) no. 2073 of the Commission of November 15, 2005 regarding the microbiological criteria for food products.

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