Staphylococcus aureus in unripened ewes' lump cheese. Part 1: Exposure assessment after first 24 h of fermentation

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Summary

The exposure assessment to *Staphylococcus aureus* in unripened ewes' lump cheese manufactured on the farm level after first 24 hours fermentation was performed in this study. The first scenario dealt with the assumption that there was slow and insufficient acid production and the presence of lactic acid bacteria (LAB) culture in ewes' milk had practically no inhibitory effect on the *Staph. aureus* growth. The second and the third scenario took into account also the initial LAB culture density in milk. Within the second scenario, it was assumed that LAB culture was able to suppress the *Staph. aureus* growth to some extent represented by a beta function with the mean probability of 0.40. The third scenario was based on the assumption that LAB population had the potential to inhibit the population under study. This represented the effect of active starter culture added to milk prior to fermentation. When the initial numbers of *Staph. aureus* in ewes' milk were up to 4 log CFU·ml^{-1}, the exposure assessment indicated that about 11.6%, 3.0% and 0.2% of ewes' lump cheese samples could contain more than 10^5 CFU·g^{-1} *Staph. aureus* within the first, second and third scenario, respectively.

Keywords

ewes' lump cheese; *Staphylococcus aureus*; predictive microbial models; exposure assessment; Monte Carlo simulations

The safety and quality of fermented raw foods are generally determined by the presence of pathogenic and spoilage microorganisms, their interaction with lactic acid bacteria (LAB), intrinsic, extrinsic and technological factors [1]. These factors are particularly important for cheeses made from raw milk with a short ripening time, such as ewes' lump cheese traditionally produced in Slovakian upland cottages immediately after milking. The cheese is curdled with rennet, fermented by native mesophilic lactic acid bacteria, briefly ripened for 1 day, and consumed at a regional level as a fresh cheese within three days. LAB microflora of the cheese usually comprises *Lactococcus lactis* subsp. *lactis*, *Enterococcus faecalis*, *Pediococcus pentosaceus*, *Lactobacillus casei*, *Lb. paracasei*, *Lb. lactis*, *Lb. plantarum*, *Lb. fermentum*, *Lb. brevis* and other minor lactobacilli [1–3]. During ripening, the essential role is played by the milk yeast-like moulds *Geotrichum candidum*, as well as oxidative yeasts of the genera *Torulopsis*, *Candida* and *Kluyveromyces* [1, 3]. Most of the production is ripened for 7 to 10 days and usually sent to a cheese factory for production of the soft Slovakian "Bryndza" cheese [4].

Staph. aureus is considered ubiquitous in raw milk. It is a Gram-positive, facultative anaerobic, non-motile coccus of 0.5–1.5 μm in diameter, occurring singly or in irregular three-dimensional bunch of grapes-like clusters [5]. The optimal growth temperature of *Staph. aureus* is in the range from 37 °C to 40 °C [6, 7] with a minimum of 7 °C and a maximum of about 47–48 °C [8, 9]. Staphylococcal enterotoxins (SE) production is expected in the range of 10–46 °C, with the optimum of 40–45 °C [5, 9, 10]. The bacteria can be killed through heat treatment of the food, but the enterotoxins will still remain in the heat-treated food and can cause staphylococcal food poisoning [5, 7]. *Staph. aureus* is able to grow in the pH range

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of 4.0–9.8, with the optimum of pH 6–7 [5, 9]. The minimum pH for growth in cheese appears to be close to 5.0, with raw milk having a pH of about 6.5. Growth of *Staph. aureus* is likely to occur in early stages of cheese manufacture. Generally, *Staph. aureus* is considered to be sensitive to acidification. Inability of *Staph. aureus* to compete with LAB due to the inhibitory effect of lower pH can be amplified by lactic acid, acetic acid, H_2O_2 or bacteriocin production, together with a decrease in the nutrient availability during fermentation, as well as with salting (lowering water activity) [11, 12]. Complete inhibition of *Staph. aureus* growth was observed at pH 4.5–4.4 when adjusted with lactic acid [11]. SE production is inhibited at pH below 5 [12].

Staph. aureus belongs to ubiquitous micro-flora of ewes' milk. In small ruminants, it is a major cause of mastitis. Infected mammary glands, the mucous membranes of vaginas of mammals are considered as the main sources of raw milk and cheese products' contamination [13, 14]. However, in contrast to cattle, the nose of small ruminants has been indicated as a primary reservoir [15]. Depending on the dairy farm management of hygiene practices and herd characteristics, significant differences in *Staph. aureus* counts were observed [14]. Precessing of raw milk without any standardized thermal treatment, coupled with different milking and handling protocols, lead to extensive and unpredictable variability in the growth of pathogens in the produced cheese [16]. *Staph. aureus* is able to multiply rapidly in milk in particular during the initial phase of cheese preparation when natural lactic acid bacteria are in lag phase and lactic acid is not produced in a sufficient amount to suppress the growth of *Staph. aureus*. As observed previously [17], the first 24 hours of the process of making raw milk cheese is critical, with the most troublesome period being the first 6 h, during which the exponential growth of contaminating bacteria mainly occurs. *Staph. aureus* can reach the density higher than 10^6 CFU·g^{-1}, which is generally considered as the level at which SE production is highly expected [7, 18, 19]. Heat-stable enterotoxins, as the most notable virulence factor associated with this organism, represent actual threat to public health resulting in food poisoning outbreaks. EFSA reported a total of 5648 food-borne outbreaks in 2011, with more than 69500 human cases and 93 deaths. Within EU, the second largest number 730 of reported outbreaks (12.9%), and one death, were caused by bacterial toxins produced by *Bacillus*, *Clostridium* and *Staphylococcus*. For example in Slovakia, an outbreak of SE poisoning from ewes' cheese involving 9 cases was confirmed in 2011 [20]. Since the prevalence of coagulase-positive staphylococci in ewes' cheese was 65.8% in Slovakia in 2011 [21], the need to prevent the presence of *Staph. aureus*, growth and SE production during the fermentation of young raw milk cheese, should be emphasized.

This article deals with the exposure assessment to *Staph. aureus* in the unripened ewes' lump cheese just after its fermentation. It is important to know the distribution of *Staph. aureus* concentrations in the cheese in this phase in terms of evaluation the fermentation process, and also with regard to safety of unripened ewes' lump cheese, which is a favourite seasonal food. This work presents three exposure assessment scenarios from the point of *Staph. aureus* growth and activity of lactic acid bacteria.

MATERIALS AND METHODS

Staph. aureus exposure assessment methodology

Predictive microbiological models were applied to the three exposure assessment scenarios where effects of temperature, the initial *S. aureus* and LAB culture densities and the duration of pH lag phase on *Staph. aureus* counts were investigated. The primary predictive model by Baranyi and Roberts [22] was used to fit the experimental growth curves [17]. Estimated maximum specific growth rates were modelled as a function of temperature using the Ratkowsky secondary predictive model [23]. Dependences of the specific growth rate and the duration of pH lag phase on temperature and initial concentration of LAB culture density, resulting from the linear regression analysis, were expressed by relationships adopted from Medveďová and Valík [17]. The microbiological predictive models were applied for probability calculation of *Staph. aureus* density thorough Monte Carlo simulations using ModelRisk software (Vose software, Gent, Belgium).

Mathematical model

For an exposure assessment study on *Staph. aureus* presence in the unripened ewes' lump cheese within milking and draining, Monte Carlo simulation model was constructed. The exponential growth model was assumed:

$$\log(N) = log(N_0) + k \cdot t \quad (1)$$

where N is the cells density at time t, N_0 is the initial cells density (in colony forming units per millilitre), $k = \mu_{max}/\ln 10$ is the growth rate parameter

(logarithm of colony forming units per hour), and t is the time (in hours).

The growth function of BARANYI and ROBERTS [22] expressed in the explicit form, was applied to fit the growth curves with the data observed [17]:

$$y(t) = y_0 + \mu_{\max} A(t) - \frac{1}{m} \ln\left(1 + \frac{e^{m\mu_{\max} A(t)} - 1}{e^{m(y_{\max} - y_0)}}\right) \quad (2)$$

where $y(t)$ is the natural logarithm of the cell concentration, y_0 is the natural logarithm of the cell concentration at $t = t_0$, $\mu_{\max}$ is the maximum specific growth rate, m is the curvature parameter to characterize the transition from the exponential phase, $y_{\max}$ is the natural logarithm of the maximum cell concentration, $A(t)$ is the function that plays the role of a gradual delay in time:

$$A(t) = t + \frac{\ln(e^{-m\mu_{\max} t} + e^{-h_0} - e^{-\nu t - h_0})}{\mu_{\max}} \quad (3)$$

where t is the time, h_0 is the dimensionless parameter quantifying the initial physiological state of the cells, ν is the curvature parameter to characterize the transition to the exponential phase.

The lag time λ, can be calculated as:

$$\lambda = \frac{h_0}{\mu_{\max}} \quad (4)$$

For the curvature parameters, BARANYI and ROBERTS [22] suggested $\nu = \mu_{max}$ and $m = 1$. This decreased the number of parameters by 2, so that model had four parameters: $\mu_{\max}$, h_0, y_0, $y_{\max}$.

The effect of temperature on the growth rate of *Staph. aureus* was described by using the RATKOWSKY model [23]:

$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}) + q \quad (5)$$

where parameter b is the slope and depends on additional growth conditions and the microorganism involved, q is the intercept, T is the temperature, T_{min} = 7 °C [8, 9] is the theoretical minimum growth temperature.

Temperature influence on the length of lag phase for *Staph. aureus* was calculated by means of the model developed by DAUGHTRY et al. [24] expressed in a re-arranged form as follows:

$$\lambda_T = e^{\left(a + \frac{b_T}{T} + \frac{c_T}{T^2}\right)} \quad (6)$$

where λ_T is the lag phase duration depending on temperature (in hours), a_T (in hours), b_T (in hours-degrees Celsius) and c_T (in hours-quadratic degrees Celsius) are the equation parameters.

Dependences of the maximum specific growth rate and the duration of pH lag phase on temperature and initial concentration of LAB culture density were expressed by the following relationships adopted from [17]:

$$\sqrt{\mu_{\max}} = -0.2111 + 0.0478\,T - 0.0541 \log(N_{\mathrm{LAB},0}) \quad (7)$$

$$\ln(t_{\mathrm{L,pH}}) = 6.494 - 0.129\,T - 0.23 \log(N_{\mathrm{LAB},0}) \quad (8)$$

where $N_{\mathrm{LAB},0}$ is the initial LAB culture density and $t_{\mathrm{L,pH}}$ is the duration of pH lag phase.

For the temperature drop from 37 °C to 30 °C within the first hour after milking, and from 18 °C to about 7 °C, in a refrigerator, within the last two hours of the first 24 h fermentation, the relation as follows was assumed:

$$T = e^{(-k_T t)} \quad (9)$$

where k_T is the respective value calculated under the above mentioned conditions.

Temperature decrease from 30 °C to 18 °C, during the next about 21 hours of cheese fermentation, was described by the relationship derived on the basis of experimental data adopted from [25]:

$$T = a_{\mathrm{F}} t^3 + b_{\mathrm{F}} t^2 + c_{\mathrm{F}} t + d_{\mathrm{F}} \quad (10)$$

where a_{F}, b_{F}, c_{F} , d_{F} are the estimated parameters.

Eq. 1 can be re-written by inserting Eq. 5–10 to a simplified form:

$$\log(N) = \log(N_0) + c \quad (11)$$

where c is the growth parameter (in colony forming units per millilitre):

$$c = \frac{\mu_{\max}}{\ln 10} \cdot t_{\exp} \quad (12)$$

where $t_{\exp} = t - \lambda_T$ is the real time for the pathogen growth.

A commercial process engineering software Athena Visual Workbench (Stewart & Associates Engineering Software, Madison, Wisconsin, USA) was used for parameters estimation in Eq. 2, Eq. 5 and Eq. 6. To assess the probability of *Staph. aureus* exposure, the implementation of probability distributions of the input model parameters had to be done. Log-normal frequency distributions of the initial pathogen and LAB culture densities in milk were used. For *Staph. aureus*, the mean of 2.9 log CFU·ml^{-1} and standard deviation of 0.185 log CFU·ml^{-1} within the range of 1.0–3.5 log CFU·ml^{-1} were applied. Analogically, the log-normal distribution of the initial numbers of LAB was characterized with the mean of 3.5 log CFU·ml^{-1}, standard deviation of

0.4 log CFU·ml^{-1} and range from 2.6 log CFU·ml^{-1} to 5.0 log CFU·ml^{-1}. Temperature data were specifically linked to the ranges of values found in field experiments in each step of ewes' cheese manufacture [17]. ModelRisk software was used for probability calculation of *Staph. aureus* density in the short ripened ewes' lump cheese produced on the farm level in Slovakian mountain areas, 24 hours after milking.

RESULTS AND DISCUSSION

Exposure assessment to *Staph. aureus* in ewes' lump cheese after 24 h of fermentation was determined at three scenarios, where effects of input factors (temperature, initial *Staph. aureus* and LAB culture densities, and the duration of pH lag phase) were investigated. The first scenario dealt with the assumption of a slow and insufficient acid production, when the LAB culture present in ewes' milk had practically no inhibitive effect on *Staph. aureus* growth (a probable scenario in the beginning of the season). Only temperature and the initial *Staph. aureus* counts were taken into account in this case. Eq. 11 was used for probability calculation of *Staph. aureus* density with the suitable formulation of the growth parameter c for the given scenario. The second and third scenarios counted also with the influence of initial content of LAB naturally present in raw milk. Additionally, the second model was based on the assumption that LAB naturally present in milk were able to inhibit *Staph. aureus* growth to some extent, which was described by a beta function with the mean probability 0.45 (expert's opinion). The third scenario represented the situation when an active LAB culture was added to the milk, and it had a potential to efficiently compete with the growth of *Staph. aureus*.

Tab. 1. Estimated parameters μ_{max}, h_0 (Eq. 2, Eq. 3) for growth of *Staph. aureus* in ewes' milk with their standard errors and λ values of respective lag phases (Eq. 4).

T [°C]	μ_{max} [h^{-1}]	h_0	λ [h]
7	0.0134 ± 0.0054	2.438 ± 1.643	182.2
8	0.0296 ± 0.0039	3.319 ± 0.976	112.2
10	0.0556 ± 0.0031	2.955 ± 0.654	53.1
12	0.0822 ± 0.0062	2.789 ± 0.923	33.9
15	0.1337 ± 0.0262	1.401 ± 1.623	10.5
18	0.2750 ± 0.0200	1.438 ± 0.693	5.2
21	0.5064 ± 0.0431	2.830 ± 1.034	5.6
25	0.7525 ± 0.0581	2.799 ± 0.744	3.7
30	1.2254 ± 0.0973	5.230 ± 0.975	4.3
35	1.7119 ± 0.1537	1.587 ± 0.902	0.9
39	2.0473 ± 0.2796	2.018 ± 1.325	1.0

Standard errors were calculated with asymptotic 95% confidence intervals.

Parameter estimation from the predictive microbiological models

For the first *Staph. aureus* exposure assessment scenario, the growth parameter c (Eq. 12) was expressed as:

$$c = [b^2(T - T_{\min})^2 + 2b(T - T_{\min})q + q^2] \cdot t_{\exp} \quad (13)$$

In order to estimate the exposure to *Staph. aureus* 24 hours after milking, parameters μ_{max} and h_0 were needed. These were obtained from Eq. 2 and Eq. 3, the values of respective lag phases via Eq. 4, parameters b and q by applying the square root model of Ratkowsky (Eq. 5) and the temperature influence on lag phase by means of the model developed by Daughtry et al. [24] expressed in a re-arranged form (Eq. 6).

Results of the parameter estimation using the growth function of Baranyi and Roberts [22], (Eq. 2 and Eq. 3) obtained by fitting of experimental data of pure *Staph. aureus* culture, adopted from [6], and values of respective lag phases (Eq. 4) are shown in Tab. 1. Comparing the determined maximum specific growth rates with the estimated values to [17], it can be concluded that values of parameters were very close and corresponded with those generated by the Combase Predictor (Institute of Food Research, Norwich, United Kingdom) or the Pathogen Modeling Program ver. 7.0 (USDA-ARS Eastern Regional Research Center, Wyndmoor, Pennsylvania, USA).

Within quantitative predictive microbiology, secondary models are used to characterize the influence of intrinsic or extrinsic food factors on specific growth parameters. The effect of temperature on *Staph. aureus* growth parameters μ_{max} and λ can be described by the square model of Ratkowsky [23] (Eq. 5) and Daughtry et al. [24] (Eq. 6), respectively. The maximum specific growth rates and the lag phases of *Staph. aureus* at a suboptimal temperature range 7–39 °C were analysed. Comparison of experimental and predicted data is shown in Fig. 1 and Fig. 2. The results in Fig. 1 showed high linearity with a correlation coefficient $R^2 = 0.995$. Estimated parameters b, q, a_T, b_T, c_T with their standard errors are presented in Tab. 2. The coefficient b was in a good

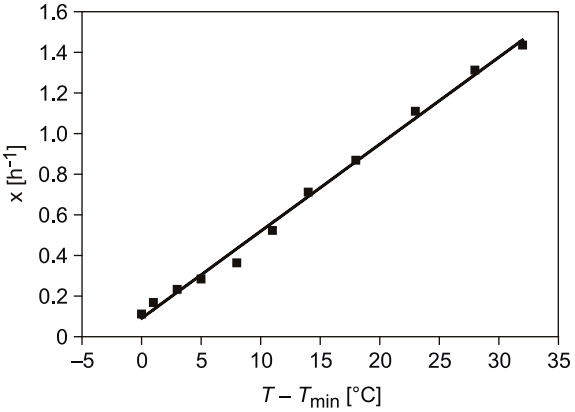

Fig. 1. The RATKOWSKY model [17] as applied to the specific growth rates of *Staph. aureus* in the suboptimal temperature range of 7–39 °C.

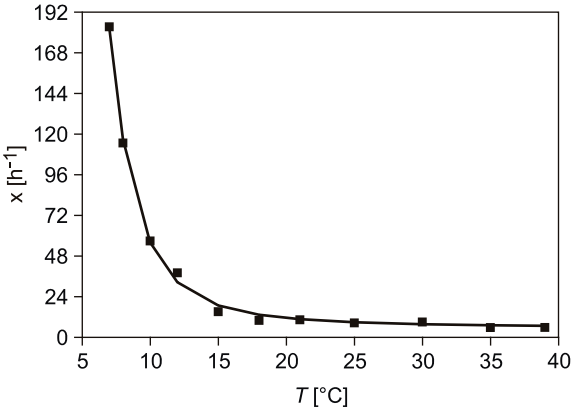

Fig. 2. Model of DAUGHTRY et al. [18] as applied to the lag phases of *Staph. aureus* in the suboptimal temperature range of 7–39 °C.

Tab. 2. Estimated parameters from the models of RATKOWSKY et al. [17] and DAUGHTRY et al. [18].

Model	b [h^{-1}]	q [CFU·ml^{-1}]	a_T [CFU·ml^{-1}]	b_T	c_T [h]
RATKOWSKY et al. [17]	0.0426 ± 0.0023	0.0946 ± 0.0385	–	–	–
DAUGHTRY et al. [18]	–	–	0.826 ± 1.084	–60.91 ± 18.57	131.1 ± 78.9

Standard errors were calculated with asymptotic 95% confidence intervals.

agreement with the coefficient of Combase Predictor line $b = 0.048$ or $b = 0{,}042$ found by FUJIKAWA and MOROZUMI [19]. Lag phases obtained from Eq. 6 together with Eq. 9, describing the temperature drop from milking to curd fermentation during the first 24 hours, was applied for calculation of the real time for *Staph. aureus* growth, t_{exp}. Under the condition that ewes' milk temperature dropped from 37 °C to 30 °C within the first hour after milking, $k_T = 0.210$ h^{-1} (Eq. 9) and the values of estimated parameters in the relationship describing the temperature decrease from 30 °C to 18 °C during the next 23 hours of cheese fermentation (Eq. 10) were calculated by Microsoft Excel (Microsoft, Redmond, Washington, USA) as follows: $a_F = –0.0019$, $b_F = 0.1011$, $c_F = –1.873$, $d_F = 30$ ($R^2 = 0.9952$).

Exposure assessment of *Staph. aureus* via Monte Carlo simulations

The available modelling techniques in the field of quantitative microbiological risk assessment (QMRA) are generally based on Monte Carlo simulations that result in frequency distribution of the output of interest, providing not only extreme values but also the most likely outcome based on the combinations of input probability values that could occur. In the exposure assessment, as a part of QMRA, the likelihood that an individual or a population will be exposed to a microbial hazard, and the likely numbers of colony forming units per gram or per millilitre ingested are estimated [18].

The first case of *Staph. aureus* exposure assessment in unripened ewes' lump cheese assumed that only temperature and the initial *Staph. aureus* numbers had effect on the output probability distribution of pathogen 24 hours after milking. As mentioned above, this was a more or less improbable scenario that overestimated the *Staph. aureus* exposure.

The initial *Staph. aureus* distribution in ewes' milk was described by a log-normal distribution (ModelRisk software) that was determined by parameters for minimum of 1 log CFU·ml^{-1}, maximum of 3.5 log CFU·ml^{-1}, with the mean of 2.9 log CFU·ml^{-1}, and standard deviation 0.185 log CFU·ml^{-1}. The parameters were based on *Staph. aureus* prevalence data in bulk milk samples, ranging between 100 CFU·ml^{-1} and 200 CFU·ml^{-1}, that were found in properly drawn milk in the Slovakian farms [25, 26]. In case of a contaminated udder, we extended the upper limit of *Staph. aureus* content in milk to the maximum of 4 log CFU·ml^{-1} (the worst case) [7, 26].

The real time for *Staph. aureus* exponential growth ($t_{exp} = t – \lambda_T$) within the first 24 after

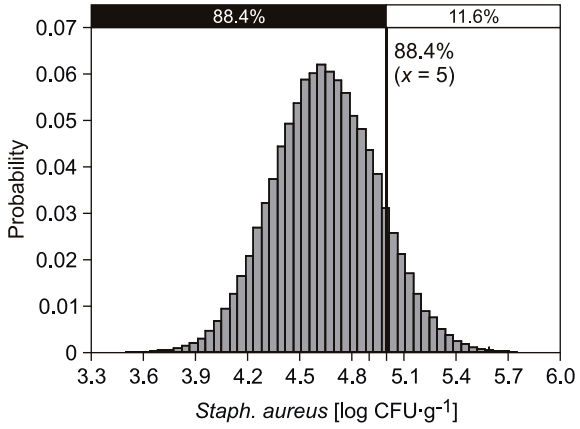

Fig. 3. The probability distribution of *Staph. aureus* content in cheese after 24 h of fermentation (the first scenario, no active LAB culture).

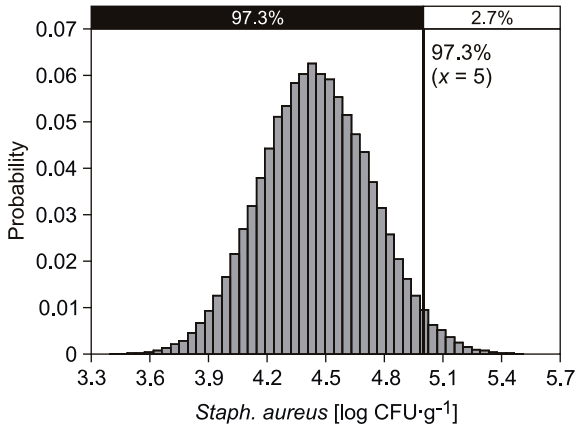

Fig. 4. The probability distribution of *Staph. aureus* after first 24 h of fermentation (the second scenario representing "normally occurring case" when acidification was performed only by LAB of indigenous flora of raw milk).

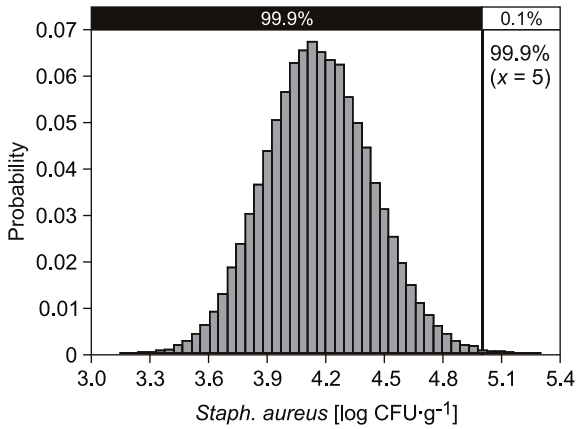

Fig. 5. The probability distribution of *Staph. aureus* after first 24 h of fermentation (the third scenario representing fermentation with LAB starter added).

milking was calculated by combining Eq. 6, Eq. 9 and Eq. 10, taking into account the lag phase. From this it followed that the time for *Staph. aureus* exponential growth t_{exp} was about 19.5 h.

The probability distribution of *Staph. aureus* in unripened ewes' lump cheese after first 24 h of fermentation in the first scenario (no active LAB culture) is shown in Fig. 3. The mean content of *Staph. aureus* was 4.65 log CFU·g^{-1} and the maximum content was 5.89 log CFU·g^{-1}. Simulations demonstrated that about 11.6% of unripened fresh lump cheeses could contain more than 10^5 CFU·g^{-1} of *Staph. aureus*, which is a European legislative criterion, according to the Commission Regulations No. 1441/2007 [27], indicating a potential to produce SE. On the other hand, the simulation showed that none of the cheeses should contain more than 10^6 CFU·g^{-1} of *Staph. aureus*. Taking into consideration also other intrinsic and extrinsic factors, e.g. pH decrease with organic acids produced by LAB, competition with LAB, temperature, the presence of SE at numbers of coagulase-positive staphylococci lower than 10^6 CFU·g^{-1} is not probable. However from the point of the legislative limit mentioned above, it is supposed that 11.6% of the cheeses would be taken as unacceptable regarding the food safety and process hygiene criteria [27]. For the worst considered case, when the initial maximum *Staph. aureus* density in milk reached 4 log CFU·ml^{-1}, the mean value in unripened ewes' cheese after first 24 h of fermentation was 4.65 log CFU·g^{-1} and the most probable density was 6.05 log CFU·g^{-1} (probability distribution not shown). Consequently, some of cheese samples could contain more than 10^6 CFU·g^{-1} of *Staph. aureus*, when the pathogen overcame the hurdles imposed by intrinsic or extrinsic factors of cheeses. However, as it was mentioned above, such a case was more or less improbable.

In dairy practice, the initial numbers of *Staph. aureus* play an important role in particular in the beginning of fermentation, within the first 6 h or 24 h. One of the effective ways how to inhibit *Staph. aureus* is the addition of bacteria capable of a fast production of the sufficient amounts of lactic acid. Linear regression analysis revealed strong relations between the specific growth rates of *Staph. aureus* and independent variables (temperature and initial number of LAB Fresco culture by Christian Hansen, Hørsholm, Denmark (Eq. 7) [17]. This suggests that it is important to use LAB starters, which are able to efficiently inhibit *Staph. aureus* growth, to guarantee cheese safety together with improving the sensorial quality of the final product. However on the other hand, the strong acidification may limit the activities of other

bacterial populations involved in the development of the sensorial properties of ewes' lump cheese [28]. The effectiveness of LAB is related to the rate at which can the culture produce sufficient amounts of lactic acid, predominantly in the first six hours of fermentation. This is connected with pH lag phase duration, which depends not only on the initial LAB density but on temperature as well: the higher the incubation temperature, the more intensive the metabolism of LAB, and the sooner a pH decrease will occur. The relation between the duration of pH lag phase, temperature and initial counts of LAB is expressed by Eq. 8. When the duration of pH lag phase is smaller than the time passing since milking, growth of *Staph. aureus* will cease and its population will become reduced. This period was found to be influenced by the amount of the starter culture that, at a specific temperature, should be higher than 10^5 CFU·ml^{-1} [17]. For the purpose of *Staph. aureus* exposure assessment in this study, by means of Eq. 11, the growth parameter c equaled zero, as this organism was able to grow only during the pH lag phase.

Log-normal distribution was applied for the initial LAB density distribution in ewes' milk by parameters for minimum (2.6 log CFU·ml^{-1}), maximum (5 log CFU·ml^{-1}) and mean count (3.5 log CFU·ml^{-1}), with the standard deviation 0.4 log CFU·ml^{-1}. The used values were estimated on the basis of experiments with LAB Fresco culture [17].

The effects of all input factors, namely, temperature, the initial *Staph. aureus* and LAB culture densities and the duration of pH lag phase, were involved in the other two scenarios. The assumption that the uncertainty in the estimation of LAB culture able to inhibit efficiently *Staph. aureus* growth was described by a beta function; beta($n-s+1,\ s+1$) [29], where n is the total number (or percentage) of LAB culture (n = 100 or n = 100%), for calculation purposes. Value s is the number (or percentage) of LAB culture samples with ability to efficiently inhibit *Staph. aureus* growth. In our case, s = 45 (expert's opinion) was implemented for the second scenario (realistic scenario). Then the beta value was sampled from the probability distribution described by beta(56, 46). The third scenario represented the case when an improvement in the safety of the product was attempted by the addition of active starter LAB culture that was able to inhibit the pathogen growth. Probability distributions of *Staph. aureus* counts for both scenarios and the normal case, when the maximum initial *Staph. aureus* density is 3.5 log CFU·ml^{-1}, are shown in Fig. 4 and Fig. 5, respectively. Monte Carlo simulations for above mentioned scenarios demonstrated that the density of *Staph. aureus* in none of ewes' lump cheeses exceeded 10^6 CFU·g^{-1}. The mean and the maximum densities for the both scenarios were 4.45 log CFU·g^{-1}, 5.62 log CFU·g^{-1}, and 4.17 log CFU·g^{-1}, 5.29 log CFU·g^{-1}, respectively.

Tab. 3. Probability of *Staph. aureus* counts in ewes' lump cheese after first 24 h of fermentation.

Scenario	Case	Probability [%]	
		Density ≤ 10^4 CFU g^{-1}	Density ≤ 10^5 CFU g^{-1}
1	Normal	1.2	88.4
	Worst	1.3	88.3
2	Normal	5.2	97.3
	Worst	5.3	97.0
3	Normal	27.0	99.9
	Worst	27.6	99.8

For the worst case of the second and third scenarios (the maximum initial *Staph. aureus* density in ewes' milk reached 4 log CFU·ml^{-1}; probability distributions not shown), the maximum of *Staph. aureus* densities of 5.69 log CFU·g^{-1} and 5.42 log CFU·g^{-1} were estimated, respectively. However, no case with *Staph. aureus* counts higher than 10^6 CFU·g^{-1} was recorded within the second and third scenarios, when naturally present LAB had an inhibitory potential and addition of LAB starter were applied, respectively. The legislative criterion of 10^5 CFU·g^{-1} was exceeded only in about 3% cases within the second scenario (usual situation; Tab. 3).

CONCLUSIONS

Exposure assessment to *Staph. aureus* was studied under the conditions relevant for initial phases of short ripened ewes' lump cheese after first 24 h of fermentation within the temperature range of 18–37 °C. The presence of LAB culture and the pH lag phase duration were taken into account. Monte Carlo simulations showed that *Staph. aureus* could reach the densities higher than 10^5 CFU·g^{-1}. Low initial counts of *Staph. aureus* in milk and high initial numbers of active LAB, producing sufficient amounts of lactic acid, can inhibit the growth of *Staph. aureus* and ensure an acceptable hygienic quality of the product. Implementation of preventive measures to reduce *Staph. aureus* prevalence in ewes' milk as well as use of LAB

starters in cheese production can be recommended to guarantee safety of short ripened ewes' lump cheese. Effects of such measures should be evaluated. Besides *Staph. aureus*, *L. monocytogenes* should be also involved in the studies, as this is a pathogen with high severity of adverse effects.

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