MICROBIOLOGICAL INVESTIGATION OF EDIBLE ACID CASEIN

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Summary. The number of microorganisms in edible acid casein might increase if the hygiene is not observed during packing or storing casein products in inadequate conditions. Microbiological investigations of casein covered the following: total bacteria count, thermophylic bacteria, coliforms, *Salmonella* spp., *Listeria monocytogenes*. One hundred and sixty eight edible acid casein samples (each sample of 200 g) were randomly selected in the carried out experiment. The experiment included two stages, where samples were taken 16 hours after production and 6 days after production of casein, respectively. The results of our investigations show, that the enlarged bacterial contamination, which exceed the limit value of 3.0×10^{-4} CFU/g was identified in 3% of the analysed casein samples. Decrease in total bacteria count was observed in casein, when the samples were taken 6 days after production of the investigated casein. The mean value of casein total bacteria count in the first stage of investigations was increased and thermophylic bacteria in the analysed samples amounted to 0.62×10^{-3} CFU/g. Percentage of this group of bacteria was approximately 24.2% of total bacteria count. The mean value of thermophylic bacteria in total bacteria count was statistically significant ($p<0.001$). After the repeated investigations to 80% of samples, total bacterial contamination was decreased. The results of repeated samples and related to the initial results were statistically significant ($p<0.001$). The experiment proved that 20% of casein samples had decreased presumable number of coliforms microorganisms.

Key words: edible acid casein, microorganisms.

VALGOMOJO RŪGŠTINIO KAZEINO MIKROBIOLOGINIAI TYRIMAI

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Santrauka. Mikroorganizmų skaičius valgomajame kazeine gali padidėti nesilaikant higienos reikalavimų fasavimo metu ar laikant produktus netinkamose sąlygose. Savo darbe tyrėme valgomojo rūgštinio kazeino bendrą bakterinį užterštumą, termofilinių bakterijų skaičių, koliformines bakterijas, iš patogeninių mikroorganizmų – *Salmonella* spp., *Listeria monocytogenes*. Tirti 168 valgomojo kazeino mėginiai (po 200 g). Pakartotini tyrimai buvo atlikti praėjus 6 dienoms po kazeino pagaminimo dienos. Padidėjęs bakterinis užterštumas nustatytas 3% kazeino tirtų mėginių, kuris viršijo $3{,}0\text{x}10^{-4}$ KSV/g nustatytą ribą. Atlikus kazeino pakartotinius tyrimus, pastebėtas bendro bakterinio skaičiaus sumažėjimas valgomajame rūgštiniame kazeine ($p<0{,}001$). Tirtuose mėginiuose termofilinių bakterijų skaičius buvo $0{,}62\text{x}10^{-3}$ KSV/g. Šios grupės bakerijos sudarė 24,2% bendro bakterinio užterštumo skaičiaus ($p<0{,}001$). Ištyrus koliformines bakterijas nustatyta 20% valgomojo rūgštinio kazeino mėginių su sumažėjusiu koliforminių bakterijų tikėtinu skaičiumi.

Raktažodžiai: valgomasis rūgštinis kazeinas, mikroorganizmai.

Introduction. Casein is the main protein found in milk and contains twenty one amino acid. Acid casein is produced by controlled acid precipitation from skim milk. There are two varieties of casein: edible acid casein and technical acid casein. Edible acid casein is a low fat milk protein, free of the carbohydrate, has a good flavour profile and excellent nutritional properties making it ideal for medical and nutritional application. According to the data concerning Lithuanian milk and milk products market in 2004, 111 tons of casein and its products were produced in Lithuanian milk dairies. As much as 78 tons were delivered to the customers in the EU Member States (Visackas, 2000). Because casein is used as an important component of many food products, there are strong needs to meet some standards given in obligatory legal acts. The most often used indicators of biological impurity, which characterise edible acid casein are: total bacteria count (TBC), number of thermophylic bacteria, coliforms and such pathogenic microorganisms as *Listeria monocytogenes* and *Salmonella* spp. (Al-Haddad and Robinson, 2003; Olsen et al., 2004; Šarkinas, 2000). Sanitary research object is not only pathogenic, comparatively pathogenic and sanitary indicator microorganisms, but also physical, chemical conditions in the environment, which suppress or stimulate the spread of above mentioned microorganisms in the environment. The principles of evaluation of microbial parameters of edible acid casein are described in the national standards. However, beside the mentioned standards, other types of sources are sometimes taken into account too. The increased level of total bacteria count and coliforms in casein constitute the source of total product contamination, including a group of pathogenic microorganisms (Inayat et al., 2003). During the edible acid casein production processes it is very important to control the temperature of pasteurization (Novella – Rodriguez et al., 2004). The number of thermophylic bacteria, which survive the pasteurization process and

affect the quality of the pasteurized product can be minimized if manufacturing process is started with raw milk characterised by good hygienic quality. The presence of *Escherichia coli* means that pathogenic strains of these microorganisms might be present in milk or its products (Nair et al., 2004). The number of microorganisms in edible acid casein might increase if the hygiene is not observed during packing or storing the products in inadequate conditions (high humidity) (Ramanauskas and Alenčikienė, 2002).

The goal of this work was to investigate the microorganisms of edible acid casein in different producing stage. In the study a special attention was paid to the analysis of conditions deciding about decrease in total bacteria count and coliforms in edible acid casein samples.

Materials and methods. One hundred and sixty eight edible acid casein samples (each sample of 200 g) were randomly selected in the carried out experiment. Microbiological investigations included the following group of elements: total bacterial count, thermophylic bacteria, coliforms (including *Escherichia coli*), *Salmonella* spp. and *Listeria monocytogenes* (LST ISO 7251, 1996; LST ISO 10560+Cor.1, 1998; LST ISO 4831, 1999; LST EN ISO 4833, 2003; LST EN ISO 6579, 2003). The samples used to determine the total bacteria count and coliforms were taken 16 hours after the time of casein production. Randomly selected 50 casein samples were again investigated for the same parameters 6 days after casein production. Bags with casein were stored in required standard conditions and temperature, i.e. 5–25°C within 24 months period.

One gramme of casein was weighed from 200 g casein sample (LST EN ISO 707, 1999). Dilutions (10^{-2}, 10^{-3}) were made (LST EN ISO 8261, 2002) and inoculated on Plate Count Agar (Liofilchem, Italy) to evaluate the total bacteria count (LST EN ISO 4833, 2003). The samples were incubated at 30°C for 72 hours. Petri plates evaluated for total bacteria count amounted to between 15 and 300 colonies on the incubated plate. According to the suitable formula the colony forming unit per gram (CFU/g) was counted (LST EN ISO 4833, 2003). Edible acid casein samples for thermophylic bacteria analysis were prepared using the same procedures as for total bacteria count. Petri plates were incubated at 55±1°C for 48 hours.

To detect coliforms in casein samples selective media like Lauryl tryoptose soy broth, E.C. broth, tryoptone water, Endo agar, E. M. B., Levine agar, violet red bile lactose agar and lactose brilliant green bile broth were used (Liofilchem, Italy), (LST EN ISO 4833, 2003). Presumable number of coliforms was determined using 0.1 g of casein sample. The inoculated plates were incubated at 30°C, 35°C, and 37°C for 48 hours.

Pathogenic microorganisms (*Listeria* spp., *Salmonella* spp.) were evaluated in 25 grams of casein sample (LST ISO 10560+Cor.1, 1998; LST EN ISO 6579, 2003). Casein samples for *Salmonella* spp. were inoculated on different culture media. The enrichment broth (Rappaport Vassiliadis) was plated onto agar media which have found the following wide application: brilliant green agar, xylose lysine desoxycholate agar, Muller Kauffman tetrathionate neomycin broth base, buffered peptone water (Liofilchem, Italy). The samples were incubated at 41.5°C in Rappaport Vassiliadis broth for 24 hours and at 37±1°C for 24 hours on brilliant green agar. Selective media – *Listeria* Oxford Agar Base, and Tryptic Soy Yeast Extract Agar were used to isolate the *Listeria monocytogenes* (Liofilchem, Italy).

Dikalium hydrophosphate saline solutions were used for ten folded dilutions (Liofilchem, Italy).

The data of investigations were evaluated statistically by the statistic model "R. 1.7.1." and *WinExcel* program. Arithmetic means of the indicators (X), standard deviation (SD), coefficients of variation (C_v) and mean errors (m_x) were calculated. The reliability of the differences among arithmetic means (P) was evaluated according to Stjudent (Juozaitienė and Kerzienė, 2001). The results are considered to be reliable when $p<0.01$, $p<0.001$.

Results and discussion. In the study we examined the following elements: edible acid casein total bacteria count, the number of thermophylic bacteria, coliform and such pathogenic microorganisms as *Listeria monocytogenes*, *Salmonella* spp.

Our data show, that the enlarged bacterial contamination, which exceeded the limit value of 3.0×10^{-4} CFU/g, was identified in 3% of the analysed acid casein edible samples. The total bacterial contamination in the remaining samples did not exceed the acceptable limit. As observed by Ramanauskas and Alenčikienė (2003**)** the microorganisms gradually die in the edible acid casein powder during storage, when the casein humidity is approximately 8.4%.

In the carried out study, the decrease in total bacteria count and presumable number of coliforms microorganisms were estimated in edible casein samples during storage. Similar findings have been reported for Božanic (2003).

Measurements covering bacteria count constitute important activity because of their direct affect in milk spoilage. Moreover, results of the undertaken measurements are one of the indicators to assess poor hygienic production or ineffective pasteurization of milk. During the edible acid casein production process, the temperature of pasteurization was reached twice and as a result comparatively low pH was maintained. The mean of thermophylic bacteria in analysed edible acid casein samples was 0.62×10^{-3} CFU/g. Percentage of this group of bacteria was approximately 24.2% of total bacteria count. The mean of thermophylic bacteria in total bacteria count was statistically significant ($p<0.001$). Thermophylic bacteria can survive the process of pasteurization. Such results are comparable with some earlier observations (Vaitkus, 1999). In our investigations thermophylic bacteria count did not exceed the acceptable limit (EEB, 1990).

Further characterization of edible acid casein samples was performed after casein production. In this particular case decrease in total bacteria count was observed six days after the production of edible acid casein. The mean

value of casein total bacteria count in the first stage of investigations was higher, however in the second stage (i.e. six days after production) only 3.83×10^{-3} CFU/g. After the repeated experiment to 80% of samples, total bacteria count was decreased. The results of repeated 50 samples with the initial results, were statistically significant ($p<0.001$). Sixty eight edible acid samples were investigated in respect of *Escherichia coli. Escherichia coli* was determined in 23 analysed samples (20.2%). The presence of *Escherichia coli* means, that the pathogenic strains of these microorganisms might be present in milk or its products (Elhanafl et al., 2004; Nair et al., 2004; Parisi, 2003). Pathogenic *Listeria monocytogenes* and *Salmonella* spp. were not identified in analysed samples of edible acid casein.

Microorganisms multiply fractionally during edible acid casein production process. The number of microorganisms in casein might increase if the proper hygiene conditions are not observed during packaging (hygiene not kept by working staff) or storing the products in inadequate conditions, i.e. relatively high humidity. This is also in accordance with early studies (Sarli et al., 2004). The fatness of acid casein edible samples is rather low and the fatness of analysed acid casein edible samples accounted to 0.6% in average. The majority of microorganisms can not develop in these conditions as reported by Šarkinas (2000). If humidity of edible acid casein increases during storage, the fungi and putrescence bacteria can multiply, as secondary sources.

These investigations theorized that during storage, the microorganisms gradually die in the product.

Conclusion:

1. The total bacteria count of casein was increased in 3% percent of samples, while total bacterial contamination in 97% did not exceed the allowed limit.

2. After repeated research of 50 casein samples, the total bacteria count was decreased in 80% of edible acid casein samples, six days after production of casein. The results of repeated samples and related to the initial results were statistically significant ($p< 0.001$).

3. After repeated research of 50 casein samples the presumable index of coliform microorganims decreased by 20%.

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