



MICROBIOLOGICAL QUALITY OF MACEDONIAN WHITE BRINED CHEESE

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ABSTRACT

This scientific paper determined the microbiological quality of Macedonian white brined cheese. Four best variants of Macedonian white brined cheeses were analysed for their microbiological parameters. From pathogenic microflora the following parameters were examined: a total number of: *Coliforms*, *E. coli*, pathogenic *staphylococci*, *Molds*, *Yeast*, *Listeria* and *Salmonella*. From lactic acid bacteria the following parameters were examined: the total number of *Lactococcus* bacteria and the total number of *Lactobacillus* bacteria. In all examined cheese samples pathogenic bacteria were not detected. *Lactococcus* bacteria was dominant bacteria in all four cheese variants at the beginning of ripening period till day 20. After that and at the end of ripening period (60 day) the *Lactobacillus* bacteria were predominant.

Key words: cheese variants, Lactic-acid bacteria, pathogenic bacteria, quality.

INTRODUCTION

White brined cheese belongs to the group of cheeses that ferment in brine solution in anaerobic conditions. This product is characterized with acid-salty flavour, no rind, usually white colour, but sometimes with yellowish tint, anaerobic brine fermented in plastic cans and pieces which are usually in form of cubes with dimensions 10x10x10 cm, (Veleski, 2015).

The microbiological quality and safety of white brine cheese largely depends on the quality of raw milk, the type of heat treatment applied during milk processing, the degree of microbiological contamination during processing, the salt absorption and its final concentration in water content and also the pH dynamics during ripening period. These factors control the type and the number of microorganisms in the cheese, and play an important role in the safety of white brine cheese, and also affect the metabolic processes that lead to the development of the taste of the final product (Abd El-Salam & Alichanidis, 2004).

The dynamics of the ripening process of white brine cheese are greatly influenced by microorganisms, including bacteria, yeasts and moulds. Their effect can be direct (through their metabolic activity) or indirect (through

the release of enzymes into the cheese matrix through autolysis). Pathogens that can be found in cheese during ripening have a negative effect on the quality of the final product, so the used technology should contain precautions to remove or prevent pathogen microorganisms to enter the cheese, (Beresford & Williams, 2004).

In white brined cheese except for non-dairy microflora (yeasts and moulds), micrococci and coliform bacteria can be found. Coliform bacteria usually disappear during the cheese ripening process and participate in the formation of CO₂ and H₂. Yeasts also form CO₂ from lactose, which is responsible for the early gas bloating that usually occurs in cheeses made from unpasteurized milk with poor hygiene, and also in cheeses exposed to high temperatures, (Alichanidis, 2007).

If we want to produce white brined cheese that fulfils all demanded microbiological parameters, we need to use high quality milk, but also hygiene maintenance in the premises where the production process takes place, the personal hygiene of the people involved in the production, the microbiological correctness of the ingredients used, the bins, the water, the salt, the sludge and all items that in any way come into direct contact with the cheese (Makarijoski, 2019).

MATERIAL AND METHODS

In order to make these examinations, cheese samples of four variants were provided from the producers, and were transported in the certified laboratory for testing milk and dairy product quality-LB Lact in Plovdiv, R. Bulgaria. Cheese

samples for microbiological quality parameters were analysed at 8th and 60th day. Methods that were used for microbiological analysis of white brined cheese were as follow:

- Determination of total number of *Lactobacillus*, ISO 7889, IDF 117;
- Determination of total number of *Lactococcus*, ISO 7889, IDF 117;
- Determination of total number of *Escherichia coli* (BDS ISO 16649-2:2014);
- Determination of total number of *Staphylococcus* (BDS EN ISO 6888-1:2005/A1:2005);
- Determination of total number of *Listeria monocytogenes* (BDS EN ISO 11290-1:2000/A1:2005);
- Determination of total number of *Salmonella* spp. (BDS EN ISO 6579-1/2017);
- Determination of total number of Coliforms (ISO 4832:2006); и
- Determination of total number of yeasts and molds (BDS ISO 6611:2006).

RESULTS AND DISCUSSION

In order to determine the safety of the product that will be consumed by the final consumer, it was necessary to make a microbiological analysis according to the Rulebook on special requirements for food safety in terms of microbiological criteria (Official Gazette No. 100/2013). The microbiological analyses of the four varieties of white brine

cheese were made in two time intervals, on the 8th day after production process (Tab. 1) and on the 60th day after the ripening process was already finalised (Tab. 2). It is of great importance to meet the microbiological criteria for a particular product, in order for it to be safe for people's consumption.

Table 1. Microbiological parameters of examined cheese variants (8 day).

Microbiological parameter	Coliforms Cfu/g	E. coli Cfu/g	Pathogenic staphylococci Cfu/g	Molds	Yeast	Listeria Cfu/g	Salmonella Cfu/g
Variant							
Variant No. 1	8.5x10 ²	3.1x10 ²	< 10	< 10	9.9x10 ⁴	Absence in 25 g	Absence in 25 g
Variant No. 2	3.9x10 ²	6.3x10 ²	< 10	< 10	4.8x10 ³	Absence in 25 g	Absence in 25 g
Variant No. 3	< 10	< 10	< 10	< 10	6.9x10 ⁴	Absence in 25 g	Absence in 25 g
Variant No. 4	< 10	< 10	< 10	< 10	1.2x10 ⁵	Absence in 25 g	Absence in 25 g

Table 2. Microbiological parameters of examined cheese variants (60 day)

Microbiological parameter	Coliforms Cfu/g	E. coli Cfu/g	Pathogenic staphylococci Cfu/g	Molds	Yeast	Listeria Cfu/g	Salmonella Cfu/g
Variant							
Variant No. 1	< 10	< 10	< 10	< 10	< 10	Absence in 25 g	Absence in 25 g
Variant No. 2	< 10	< 10	< 10	< 10	< 10	Absence in 25 g	Absence in 25 g
Variant No. 3	< 10	< 10	< 10	< 10	< 10	Absence in 25 g	Absence in 25 g
Variant No. 4	< 10	< 10	< 10	< 10	< 10	Absence in 25 g	Absence in 25 g

A total of nine parameters were examined to determine the microbiological quality of the four varieties of white brine cheese. Seven of the examined parameters referred to microorganisms that are undesirable in the cheese during and at the end of the ripening period (*Coliforms*, *E. coli*, *pathogenic staphylococci*, *moulds*, *yeasts*, *Listeria monocytogenes*, *Salmonella*). The other two parameters referred to the beneficial microflora, *Lactococcus* and *Lactobacillus*, which were found in the product.

From the point of view of obtaining a quality microbiological product, it is important to note that in both examined periods (day 8 and day 60) in none of the examined variants of white brined cheese was detected the presence of *Listeria monocytogenes* and *Salmonella* (absence in 25 g cheese).

Poor hygiene is the most common reason for the presence of coliform bacteria in white brine cheese. These bacteria have a wide range of distribution in soil, water, digestive tract of humans and animals. They usually cause premature bloating and cavities in cheeses because they produce gases such as hydrogen and carbon dioxide.

On the 8-th day of ripening process, coliform bacteria were observed in two variants Variant No. 1: 8.5×10^2 cfu/g and Variant No. 2: 3.9×10^2 cfu/g. In the other two variants Variant No. 3 and Variant No. 4 the number of coliform bacteria was insignificant <10 . On the 60th day when the ripening process was already finalised, the number of coliform bacteria in all variants was reduced to a minimum number <10 , which is a good hygienic indicator.

Our results for the total number of coliform bacteria were in accordance with the data obtained by Stojiljkovic (2007), who found that in the industrial production of white brine cheese, the coliform bacterial species completely disappear or are present in minimal numbers.

The greatest impact on the number of coliform bacteria during the ripening period of white brine cheese had the decrease of the active acidity, the increase of the concentration of lactic acid, the concentration of salt in the brine and also the anaerobic way of ripening process of white brine cheese.

Escherichia coli and *Coagulase positive staphylococci* according to the Rulebook on special requirements for food safety in terms

of microbiological criteria (Official Gazette No. 100/2013) should have values below 100 cfu/g cheese at the end of the ripening period. *Escherichia coli* in our examined variants were detected on the 8th day in Variant No.1: 3.1×10^2 cfu/g and in Variant no.2: 6.3×10^2 cfu/g, and in the other two variants No.3 and No.4 were detected in insignificant number <10 cfu/g cheese. After the completion of the ripening period in all variants the number of *Escherichia coli* bacteria was <10 cfu / g, which was a good hygienic indicator.

Pathogenic staphylococci in all variants were detected in insignificant numbers <10 cfu/g, (8th day and 60th day during ripening process).

The starter cultures that were used, play a significant role in preventing the development of harmful microorganisms. They successfully reduce the pH value to a level at which microorganisms cannot grow at all, so the created conditions do not suit them completely.

Bulajic & Mijacevic (2011) also confirm that the active acidity of the cheese is the main factor for the development of pathogenic microflora. The microorganisms listed in the Rulebook on microbiological criteria have optimal growth in the range of pH 6-7.5.

According to Cogan (2003), salt concentration also plays an important role in preventing the growth of harmful microorganisms, with the exception of *Staphylococcus aureus* which can grow in the presence of 6.5% salt and *Listeria monocytogenes* which grows in a concentration of 10% salt.

In all examined variants, the concentration of salt in the final product was between 3% and 4%, while the concentration of salt in the brine was about 10%, the presence of these bacteria was not detected.

Moulds were detected in the four examined variants in both study periods, but in insignificant numbers <10 cfu/g.

Yeasts were detected in all variants in high levels at 8th day, mostly in the Variant No.4: 1.2×10^5 cfu/g, then in the Variant No.1: 9.9×10^4 cfu/g, followed by the variants Variant No.3: 6.9×10^4 cfu/g and Variant No.2 with the lowest number of 4.8×10^3 cfu/g. At the end of the ripening period, the number of yeasts were insignificant in all examined cheese samples (<10 cfu/g).

Yeasts and moulds can enter the cheese

from a variety of sources. They can come from the starter culture, the air, the brine solution, the equipment used for production, as well as from workers (Mounier et al., 2006). Although yeast can have a positive effect on cheese (appearance, taste), some species can spoil it. If they are found on the surface of the cheese, they spoil it, create an unwanted aroma, taste, as well as contribute to the formation of some other metabolic products that reduce the quality of the cheese, (Bintsis & Papademas, 2002; Jakobsen & Narvhus, 1996).

Moulds as well as yeasts are added to some types of cheese in order to provide a characteristic appearance, consistency and taste, but also have the function of extending the shelf life of the product (Haasum & Nielsen, 1998). However, moulds that are found on the surface of the cheese under improper hygienic conditions can contaminate it with mycotoxins, and thus such a product is a potential risk to human health (Sengun et al., 2008).

From lactic acid bacteria, the total number of *Lactococcus* and *Lactobacillus* were observed. After 8 days, during ripening period, the number of *Lactococcus* bacteria was higher in all four variants compared to the number of *Lactobacillus* bacteria. The greatest number of *Lactococcus* bacteria were counted in Variant No.4: 10.49 log cfu/g, followed by the Variant No.3 with 10.38

cfu/g, then Variant No.2: 10.32 log cfu/g, and the lowest number was determined in Variant No.1: 10.27 log cfu/g (Fig. 1). In the same period, the total number of *Lactobacillus* lactic acid bacteria were from 9.28 to 9.74 log cfu/g. The highest number of *Lactobacillus* bacteria was counted in Variant No.3, and the lowest number was counted in Variant No.1, (Fig. 2). The highest number of *Lactococcus* bacteria was counted in Variant No.3, and the lowest number in Variant No.1, (Fig. 1).

Lactobacilli are normally present at the level of 10^9 bacteria/g, actively participating in the fermentation process during the ripening period. Their number decreases rapidly during the ripening period, and this depends on the sensitivity of the starter cultures to salt, active water, the power of autolysis of the species, (Coeuret et al., 2003). When the ripening process is finished, it is normal to expect a reduction in the number of lactic acid bacteria, and that was confirmed by our results. The total number of *Lactococcus* bacteria was ranged from 6.68 to 6.93 log cfu/g and *Lactobacillus* from 7.04 to 7.38 log cfu/g, which was in the same range as the results obtained by Mojsova et al., (2013). In their research the number of *Lactobacillus* bacteria after the ripening period was between 5.35 to 7.43 log cfu/g and the number of *Lactococcus* bacteria was from 4.11 to 7.10 log cfu/g.

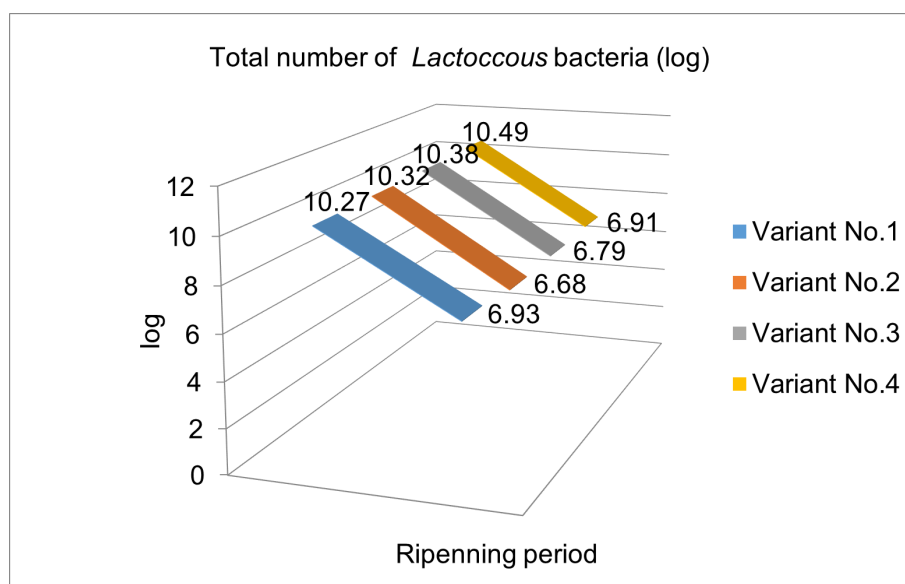


Figure 1. Dynamics of *Lactococcus* bacteria during ripening

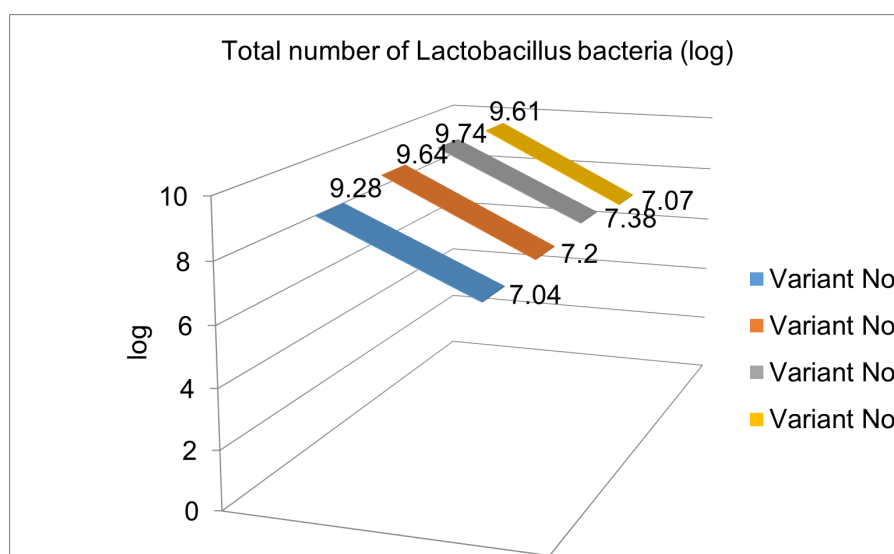


Figure 2. Dynamics of *Lactobacillus* bacteria during ripening

From the results obtained for lactic acid bacteria, it can be concluded that *Lactococcus* bacteria was dominant bacteria in all four cheese variants at the beginning of ripening period till day 20. After that and at the end of ripening period (60 day) the *Lactobacillus* bacteria were predominant.

The reason for this phenomenon is the greater resistance of *Lactobacillus* bacteria to increased salt concentration. The growth of *lactococci* is inhibited, the production of lactic acid is decreased due to the level of salt in aqueous phase (especially at the level of salt / water > 5.0 g/100g), and the inhibition of *Lactobacillus* bacteria growth that have a higher salt tolerance occurs when the aqueous salt level

is greater than 6 g/100 g, (Fox et al., 2004).

Our results for the number of *Lactococcus* and *Lactobacillus* bacteria were approximately equal to the results obtained by Balabanova (2015), which determined the total number of bacteria of these species 7.4 ± 0.5 log cfu/g and 7.1 ± 0.5 log cfu/g, respectively.

According to Kayagil (2006) the number of *Lactobacillus* bacteria at the beginning of the ripening period (day 2) was between 1.8×10^7 to 5.2×10^{10} cfu/g. This number was reduced to 9.0×10^6 cfu/g at day 30. The number of *Lactococcus* at the beginning of the ripening period (day 2) was between 2.1×10^7 to 4.6×10^{10} cfu/g. This number was reduced to 1.8×10^6 cfu/g at day 30.

CONCLUSION

In all examined cheese samples pathogenic bacteria were not detected. *Lactococcus* bacteria was dominant bacteria in all four cheese variants at the beginning of ripening period till day 20. After that and at the end of ripening period (60 day) the *Lactobacillus* bacteria were predominant. In order to produce cheese that is microbiologically correct, we must use milk in production process with appropriate microbiological quality. Also, it is of great importance to maintain hygiene

in the premises where the production process takes place, personal hygiene of people involved in production, microbiological correctness of ingredients that were used, bins, water, salt, strainers and all items that in any way come in direct contact with the cheese. It is also necessary to keep regular records and appropriate technology logs which are mandatory under HACCP standards.

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МИКРОБИОЛОШКИ КВАЛИТЕТ НА МАКЕДОНСКОТО БЕЛО САЛАМУРЕНО СИРЕЊЕ

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Резиме

Во овој научен труд е испитуван микробиолошкиот квалитет на македонското бело саламурено сирење. Беа анализирани микробиолошките параметри на четири најдобри варијанти македонско бело саламурено сирење. Од патогената микрофлора беа испитувани: вкупен број на колиформни бактерии, *E. Coli*, патогени стафилококи, квасци, мувли, *Listeria* и *Salmonella*. Од млечнокиселинските бактерии беа испитувани следните параметри: вкупен број на бактерии од видот *Lactococcus* и вкупен број на бактерии од видот *Lactobacillus*. Во сите испитувани примероци не беа детектирани патогени микроорганизми. Бројот на бактериите од видот *Lactococcus* беа доминантни кај сите четири варијанти македонско бело саламурено сирење на почетокот од процесот на зреење до 20. ден. Потоа и до крајот на процесот на зреење (60. ден) доминантни беа бактериите од видот *Lactobacillus*.

Клучни зборови: варијанти сирење, млечнокиселински бактерии, патогени бактерии, квалитет.