

# The Count of Lactic Acid Bacteria in White Cheese

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**Abstract:** The aim of the study was to investigate the effect of pH and the temperature on the lactic acid bacteria during 45 day cheese making time because they have the most important effect in taste and aroma as final product. The determination of the presence of lactic acid bacteria counted in milk and cheese is important because they have the most important effect in taste and aroma as final product. Raw material for white soft cheese production was bulk cow milk (summer lactation) from the terrain of the whole Pcinja region in Serbia. The samples for microbial and physical-chemical testing were monitored from: raw milk, milk before making cheese, whey, coagulate before production, after self pressure and pressure, cheese after moulding and dry salting and cheese after the 1<sup>st</sup>, 10<sup>th</sup>, 20<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day of brine. During the cheese making, the number of lactic acid bacteria in the milk prepared for making cheese was decreasing in comparison to their number in the raw milk, which proves that the total number of microorganisms is less after pasteurization. During the further production, the number of lactic acid bacteria came up the maximum in the phase of pressing or self-pressing, forming end salting. The number of lactic acid bacteria decrease during the cheese ripening in the pickle. The biggest fails of number cover with the biggest fail of pH. At the end of salting, the number of lactic acid bacteria moves from  $2.4 \times 10^5$  –  $5.8 \times 10^5$ /g of cheese.

**Keywords:** Milk, White Soft Cheese, Pickle, Lactic Acid Bacteria

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## 1. Introduction

Conversion of carbohydrate to lactic acid by lactic acid bacteria (LAB) may be considered as the most important fermentation in food industry, [1].

Lactic acid bacteria (LAB) with different ecological niches are widely seen in fermented meat, vegetables, dairy products and cereals as well as in fermented beverages. Lactic acid bacteria are the most important group of bacteria in dairy industry due to their probiotic characteristics and fermentation agents as starter culture, [2].

Species of lactic acid bacteria (LAB) belong to numerous genus under the family of *Lactobacillaceae*, [3]. Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major product during fermentation of carbohydrates. LAB includes: *Lactococcus*, *Lactobacillus*, *Streptococcus* and *Leuconostoc* species. The presence of LAB in milk fermentation to produce acid which is important as preservative agents and generating flavour of the products. The main reasons for the fermentation practice using LAB

are to increase milk palatability and improve the quality of milk by increasing the availability of proteins and vitamins. Furthermore, LAB confers preservative and detoxifying effects on milk as well. When it is used regularly, LAB fermented milks boost the immune system and strengthen body in the fight against pathogen bacterial infection, [4].

The application of antimicrobial-producing lactic acid bacteria (LAB) or food-grade ferments in the manufacture of dairy products, which can be incorporated into fermented or nonfermented dairy products, implies a processing additional advantage to improve the safety and increase the quality of dairy products, providing an additional hurdle to reduce the likelihood of food-borne diseases, [5].

The creation of lactic acid has several functions: it helps spread the activities and reduces the moisture content of cheese's weight, which contributes to the secretion of whey from the curd of milk after cheesemaking [6]. On the other hand, lactic acid bacteria contribute to cheese ripening, because their enzymes are essential in getting the important ingredients to taste.

The lactic acid bacteria can be added to the milk at the

start of production or be present as natural contaminants of milk as is the case with many traditional cheeses made from raw milk. Lactic acid bacteria in cheese production can reach the number of  $10^8/\text{g}$  -  $10^9/\text{g}$  of cheese [7]. In terms of the optimum temperature they can be mesophilic and thermophilic depending on the technology of making cheese. Nine isolates of lactic acid bacteria (LAB) obtained from the predominant microbiota of different camel milk samples collected in South-West Algeria, were selected in accordance with their growth ability in (cow) milk. The isolates were phenotypically and genotypically assigned to the following species: 4 *Leuconostoc mesenteroides* subsp. *dextranicum*; 2 *Lactobacillus brevis*; 2 *Lb. plantarum*; and 1 *Lactococcus lactis* subsp. *lactis*, [8].

The purpose of work of [9] was isolation and identification of LAB from traditional Carpathian cheeses made from ewe's milk and the study of their technological properties. Three samples of cheese were selected for our research – one sample of brine cheese bryndza and one sample of budz (bryndza before salting), produced in the highlands of the Carpathians and one sample of buts, produced in the foothills zone. 106 cultures were isolated from the samples of cheese: *Lactococcus* spp. (26 cultures), including *L. lactis* (13 cultures) and *L. garvieae* (13 cultures); *Lactobacillus* spp. – *L. plantarum* (31 cultures); *Enterococcus* spp. – *E. faecium* (25 cultures); *Leuconostoc* spp. – *L. mesenteroides* (24 cultures).

White cheese is the traditional product on the whole Balkan peninsula, and the Serbian Republic too. The specific geographic and the climate conditions of Pcinja region, the particularity of technological operations are all connected with the white soft cheese characteristics.

Milk is a highly perishable food raw material. Its transformation into stable milk products provides an ideal vehicle to preserve its valuable nutrients, and making them available throughout the year. It is known that while unprocessed milk can be stored for only a few hours at room temperatures, cheeses may reach a shelf-life up to 5 years (depending on variety), [10].

Microflora which is present in milk in certain number is not static during the cheese production; it is changing constantly. The causes of these changes are: the change of temperature, pH, the presence of microb's products etc. Active acidity and temperature of milk and cheese mass during the production of white soft cheese is followed and kept under control, in order to prevent its enormous rising which would bring to final product but with bad characteristics.

## 2. Materials and Methods

Researches which are the subject of this project are made in the "Diary Han" DOO Vladicin Han, in a microbiological

and physical-chemical laboratory which are both in the diary. As raw for producing white soft cheese is used collective cow milk from the terrain of the whole Pcinja region (Vladicin Han, Vranje, Surdulica, Bujanovac, Trgoviste) in the Serbian republic.

Researching in the dairy are made on the three separate production processes in production of white soft cheese, realized in three summer months (July, August and September), with the milk from summer lactation.

### 2.1. Preparation of Samples for Analysis

During the production of white soft cheese, samples for microbiological testing are taken in a diary from:

1. Raw collective milk, milk before making cheese, whey, coagulate before production and from it after selfpressure and pressure on the first day;
2. Cheese after moulding and salting, on the second day;
3. Cheese on the first, tenth, twentieth, thirtieth and forty-fifth day of pickling.

Samples are taken steril on the following way:

1. Milk and whey samples are taken with steril pipette of 10mL.
2. Coagulate samples before production, after selfpressure and from cheese are taken with the pearlitic knife with sharp spire which is sterilized by flaming.

### 2.2. Enumeration of Bacteria

After taking, samples are put in steril glass dishes. The number of the lactic acid bacteria is determined due to classic cultural method, based on the number of colonies grown on the hard stratum. The researches of the lactic acid bacteria are done on the standard lactosa agar (substratum with 10% lactosa) and MRS (Man, Sharp and Rogosa) agar.

### 2.3. pH Measurement

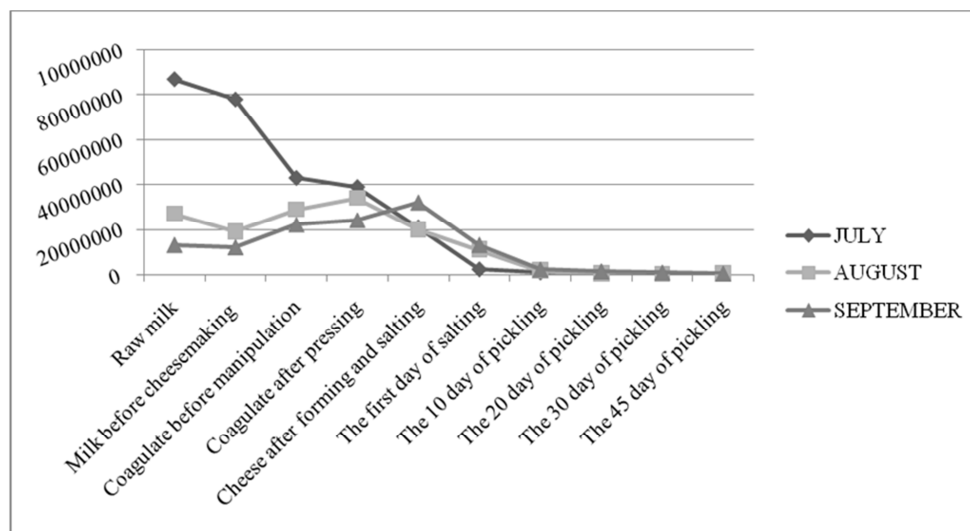
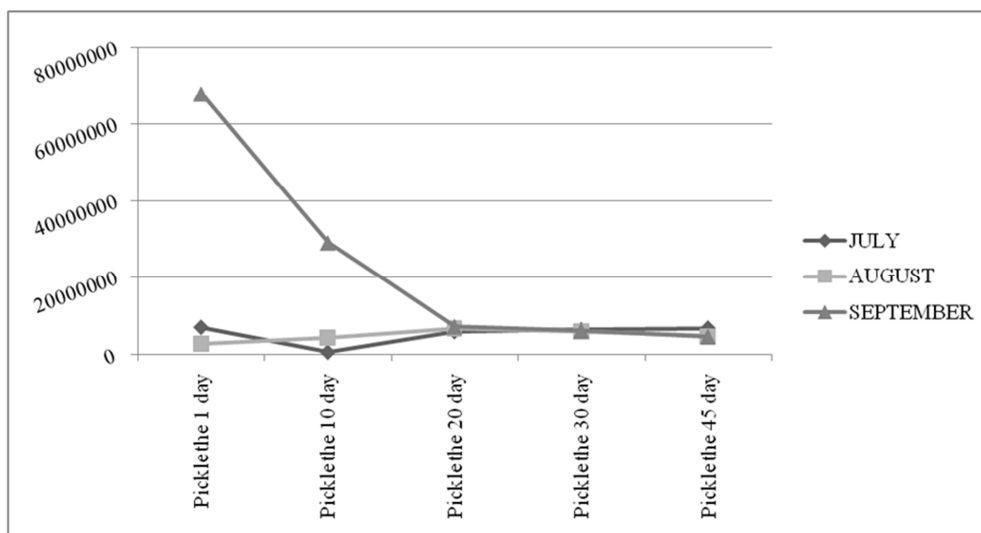
In a physical-chemical laboratory is followed pH (active acidity) and temperature of: raw milk, milk before making cheese, whey, coagulate before production, after selfpressure and pressure, cheese after moulding and dry salting and white soft cheese after the first, the tenth, the twentieth, the thirtieth and the forty-fifth day of pickling. Measuring of pH is done with digital pH-meter HANNA HI199161 FOOD CARE, and temperature with digital thermometer HANNA HI 145.

## 3. Results and Discussion

Taking into account the role of lactic acid bacteria cheesemaking, literature data show that the most common group of bacteria during production and ripening [11, 12]. Changes in the number of lactic acid bacteria in cheesemaking is given in Table 1.

**Table 1.** The number of lactic acid bacteria in white soft cheese and pickle (in mL or g test).

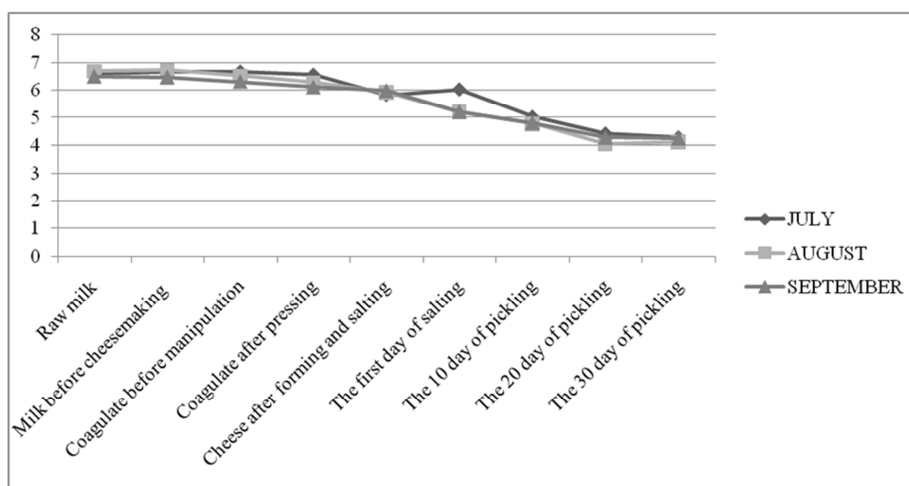
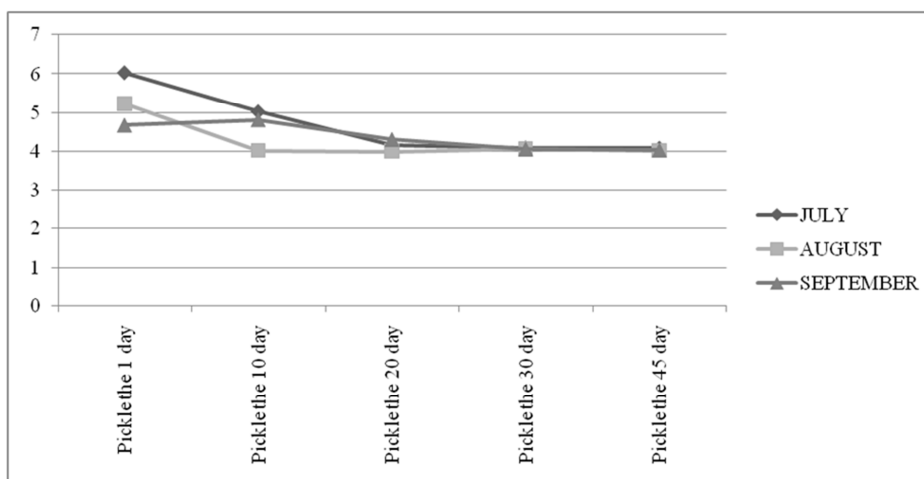
Test	JULY	AUGUST	SEPTEMBER
Raw milk	$8.7 \times 10^7$	$2.7 \times 10^7$	$1.3 \times 10^7$
Milk before cheesemaking	$7.8 \times 10^7$	$1.9 \times 10^7$	$1.2 \times 10^7$
Whey	$2.9 \times 10^6$	$2.7 \times 10^6$	$1.8 \times 10^6$
Coagulate before manipulation	$4.3 \times 10^7$	$2.9 \times 10^7$	$2.2 \times 10^7$
Coagulate after pressing	$3.9 \times 10^7$	$3.4 \times 10^7$	$2.4 \times 10^7$
Cheese after forming and salting	$2.1 \times 10^7$	$2.0 \times 10^7$	$3.2 \times 10^7$
The first day of salting	$2.4 \times 10^6$	$1.1 \times 10^7$	$1.3 \times 10^6$
Pickle the 1 day	$7.0 \times 10^6$	$2.8 \times 10^6$	$6.8 \times 10^6$
The 10 day of pickling	$1.1 \times 10^6$	$1.8 \times 10^6$	$2.1 \times 10^6$
Pickle the 10day	$5.6 \times 10^5$	$4.4 \times 10^6$	$2.9 \times 10^7$
The 20 day of pickling	$8.9 \times 10^5$	$2.9 \times 10^5$	$1.3 \times 10^6$
Pickle the20day	$5.9 \times 10^6$	$6.8 \times 10^6$	$7.1 \times 10^6$
The 30 day of pickling	$5.4 \times 10^5$	$2.1 \times 10^5$	$7.8 \times 10^5$
Pickle the30day	$6.4 \times 10^6$	$5.9 \times 10^6$	$6.1 \times 10^6$
The 45 day of pickling	$5.8 \times 10^5$	$2.4 \times 10^5$	$3.4 \times 10^5$
Pickle the45day	$6.8 \times 10^6$	$4.8 \times 10^6$	$4.5 \times 10^6$

**Figure 1.** Dynamic of lactic acid bacteria in milk and white soft cheese (in mL or g test).**Figure 2.** Dynamic of lactic acid bacteria in pickle (in mL test).

Moving of the active acidity and temperature of milk, cheese mass and pickle during the production of white soft cheese is represented in Table 2.

**Table 2.** Active acidity pH and temperature (°C) in milk, cheese and pickle during cheese making.

Test	JULY		AUGUST		SEPTEMBER	
	pH	°t	pH	°t	pH	°t
Raw milk	6.60	17.4	6.70	18.5	6.50	17.8
Milk before cheesemaking	6.67	33.6	6.73	32.5	6.47	33.8
Whey	6.60	32.4	6.64	32.2	6.46	32.6
Coagulate before manipulation	6.67	32.5	6.53	32.2	6.31	32.5
Coagulate after pressing	6.56	29.6	6.29	28.8	6.11	29.6
Cheese after forming and salting	5.83	18.0	5.93	6.80	5.98	18.8
The first day of salting	6.03	19.0	5.22	16.7	5.21	18.7
Pickle the 1 day	6.03	19.0	5.23	16.8	4.68	17.1
The 10 day of pickling	5.03	19.1	4.78	18.5	4.81	18.2
Pickle the 10 day	5.03	19.0	4.02	18.9	4.82	16.6
The 20 day of pickling	4.43	18.4	4.05	16.3	4.28	18.1
Pickle the 20 day	4.17	18.4	4.00	17.8	4.31	18.1
The 30 day of pickling	4.29	17.6	4.13	17.2	4.25	17.6
Pickle the 30 day	4.10	17.9	4.07	17.4	4.06	17.3
The 45 day of pickling	4.27	10.1	3.90	18.1	4.11	16.9
Pickle the 45 day	4.09	9.80	4.02	18.1	4.03	18.8

**Figure 3.** Dynamic of the pH in milk and cheese.**Figure 4.** Dynamic of the pH in pickle.

The highest value of number of lactic acid bacteria in milk for producing white soft cheese is marked on the first repetition (July), and the lowest value on the third repetition (September).

That raw milk contains a higher number of lactic acid

bacteria, show trials other authors who have examined other types of milk, such as goat and sheep [13, 14].

Heat-treated cow's milk at the moment cheesemaking contains a small number of lactic acid bacteria than raw untreated milk. The highest value of number of lactic

acidbacteria in milk for producing white soft cheese is marked on the first repetition (July), and the lowest value on the third repetition (September).

Comparative analysis of the percentage of the lactic acid bacteria in whey and coagulate in the dairy "Han", show that in whey in the first, second and third repetition stay 6.31%, 8.52% and 7.56%, which brings to 93.69%, 91.48% and 92.44% in coagulate.

During the phase of self-pressing and pressing, the number of lactic acid bacteria is high and almost at the same level. For increasing the number of lactic acid bacteria is responsible added pollution the pressed mass during the handle manipulation of employees.

In the phase of molding and dry salting, the number of lactic acid bacteria in relation to the previous one, begins to decrease in the first two repetitions and 1.85 and 1.7 times, and in the third repetition increases 1.33 times. During the production of white soft cheese, after the cutting of pressed coagulate, masters moved the pieces by hand on frames where the cheese is salted with dry salt. It is possible that in this phase of producing cheese, as a result of dirty hands of workers and salting cheese with dirty salt in moulded cheese, to bring in additionally microorganisms.

Reducing the number of lactic acid bacteria was observed during ripening in brine.

The first day of curing of white soft cheese, the number of lactic acid bacteria in relation to the initial count in raw milk is reduced to 2.75% in the first repetition, 40.74% in the second repetition, and remains at the same level for the third repetition. In relation to the molding and dry-salting, after the 24-hour curing cheese, it remains 11.43% of lactic acid bacteria for the first repetition, 55% in the second and 40.63% in the third repetition.

On the tenth day of brining - percentage of these lactic acid bacteria in cheese's weight in relation to the initial number of microorganisms are greatly affected. The cheese remains 1.26% of the bacteria in the first repetition, with another 6.66% and 16.15% in the third repetition.

In relation to the stage of molding and dry salting in brine cheese tenth day, remains 5.24% of lactic acid bacteria for the first repetition, 9% in the second repetition, and 6.56% of lactic acid bacteria for the third repetition. The twentieth day of curing, the percentage distribution of lactic acid bacteria in relation to the initial number of increasingly smaller. They are: 1.02% for the first repetition, 1.07% for the second repetition and 10% for the third repetition.

In relation to the stage of molding and dry salting, the cheese is the twentieth day of brining rest 4.23% of lactic acid bacteria for the first repetition, 1.45% for the second repetition, and 4.06% for the third repetition. During brining the number of lactic acid bacteria and continues to fall. Thirtieth day brining for the first two repetitions percentage of lactic acid bacteria in the brine phase and reached the lowest value amounted 0.62% and 0.77%, while the third repetition percentage of lactic acid bacteria in relation to the initial number was 6%.

In relation to the stage of molding and dry salting, the

cheese on the thirtieth day brining remained 2.57% for the first repetition, 1.05% in the second repetition and 2.44% in the third repetition. This is explained by the pH value decreases at this stage of curing cheese.

The lowest percentage of lactic acid bacteria in relation to the initial number of third repetition occurs the forty-fifth days curing cheese is 2.62%. In the first and second repeat these percentages amount to 0.66% and 0.88%. The cheese to the phase of molding and dry salting remains 2.76% of lactic acid bacteria for the first repetition, 1.2% of lactic acid bacteria for the second repetition and 1.06% of lactic acid bacteria for the third repetition.

The number of lactic acid bacteria in white pickled cheese is reduced immediately after its immersion in brine and from 8.81% to 0.05% compared to the initial count, and the particulars [15]. They followed the dynamics of technologically important microflora during ripening of camembert (*Camembert*) and found that the number of lactic acid bacteria according to the manufacturer ranged from  $10^7$  to  $10^9$ /g of cheese. The LAB counts ranged between 4.78 and 9.68 log CFU/g according to the different area and medium in traditional Urfa cheese made from sheep's milk, [16].

The diversity of non starter lactic acid bacteria in raw, pasteurised milk and matured Swiss type cheese was tested, [17]. The most frequently isolated lactobacilli were found at low level in raw milk (mean  $27.5 \times 10^4$  CFU/mL) and the most frequently identified species were *Lactobacillus brevis* and *Lactobacillus paracasei*. The microflora of raw and pasteurised milk is similar analysing lactic acid bacteria representatives in the samples. *Lactobacillus brevis* and *Lactobacillus paracasei* were detected in the same samples in raw milk, pasteurized milk and matured cheese. This study showed that lactic acid bacteria concentration was quite low in pasteurized milk (1-12 CFU/mL), but they grow rapidly in cheese during ripening reaching  $1.1-1.8 \times 10^6$  CFU/g.

Study of [18] was conducted to evaluate the growth of live lactic acid bacteria (LAB) on the flavored cheese. Results of the study revealed the microbial count to be generally high, exceeding 106 CFU/g, in the treatments incubated at 44°C for 15 hours and treatments with low concentrations of salt. The incubation period showed a direct relationship with the microbial count. Growth of LAB increased with the corresponding increase of the incubation period. Salt, however, offered an inverse relationship with the microbial count. At constant incubation time, treatment with 1.0% salt concentration produced higher bacterial growth than treatments with 2.5% and 4.0% salt.

The count (CFU/g) of the LAB ranged from  $2.9 \times 10^4$  to  $2.04 \times 10^8$  with ten of total fifteen treatments or technological phases obtaining counts over  $10^6$ , [18].

Dangke is a traditional cheese from South Sulawesi that has been developed by the people of the Enrekang district throughout history. The microbiota of this cheese consists of a wide variety of bacterial species. The majority of which belongs to lactic acid bacteria (LAB) genera. The viable counts of LAB among all sample of dangke in this study ranged from 4.03-4.45 log CFU/g, [19]. In similar studies,

[20] reported that the viable counts of LAB from Tarag (the traditional dairy product from Mongolia) ranged from 4.02-8.88 log CFU/mL. It is important to note that the LAB microorganisms must be viable, active and abundant, with a concentration of at least 6 log CFU/g or mL in the product throughout the specified shelf life to exert a positive effect.

The complexity of the biotechnological process in cheese making practice is characterized by several physical, chemical, biochemical and microbiological transformations [21]. During ripening of cheese, texture and flavor development take place. Three metabolisms are important for these developments, namely proteolysis, glycolysis and lipolysis. Proteolysis can be divided into two groups: Primary and secondary proteolysis. Primary proteolysis is influenced by the type of rennet added to the milk and defined as the breakdown of caseins into  $\gamma$ ,  $\beta$  and  $\alpha$ s-caseins [22]. Secondary proteolysis, which is the breakdown of proteins to low molecular weight peptides and amino acids, is influenced by the type of LAB. Two types of Lactic acid bacteria (LAB) are responsible for the acidification in cheese. Starter lactic acid bacteria (SLAB) are added to the cheese to speed up the acidification, non-starter lactic acid bacteria (NSLAB) are already present in raw milk or can enter processed milk through contamination during manufacture [23].

## 4. Conclusion

In raw milk had high number of lactic acid bacteria, which indicates the inadequate hygiene in the production of raw milk. Given that the technology of production of white soft cheese provides heat treatment of raw milk, the normal decrease in the number of lactic acid bacteria. In cheesemaking, can see a significant increase in the number of lactic acid bacteria in curd than in whey. Pressed cheese mass is extruded mainly residual whey and curd pressed in and still retains much of the microflora. Salting of cheese is a limiting factor for microbial growth. On the other hand, during the salting because of an additional salt contamination, the number of bacteria can be increased and what happened in this test under dry salting of cheese and salt in cheese ripening stage in brine. During ripening in brine, the number of lactic acid bacteria is drastically reduced. The largest decrease in the number of microorganisms coincides with the greatest reduction in pH. Lactic acid bacteria are the most significant for the entire course of the production of cheese and their positive effects depend on the characteristics of the final product, ie specific taste, flavor, aroma and texture.

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