Lactose fermentation at Camembert, made by classic and stabilised technology

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Summary

In our experiments the fermentation of lactose at Camembert type cheese by classic and stabilised technology was monitored. In each of the two technologies two experiments were made. The difference between these two technologies is in pH level, which drops below 5 by classic technology and remains above 5 by stabilised technology at all times. To achieve the criteria of stabilised technology the fermentation was stopped at a desired level of pH, by dropping the cheese in brine at 14 °C. After salting and moulding cheese was transferred from the first three experiments into a ripening chamber at 11 °C. With the last experiment (stabilised technology) the cheese ripened for 3 days at 5 °C. During ripening process pH dropped below 5 in all experiments. The process of fermentation was performed by the following lactic acid bacteria: ***Streptococcus thermophilus, Lactococcus lactis subsp. lactis*** *and* ***Lactococcus lactis subsp. cremoris****. As these lactic acid bacteria ferment differently D-galactose during manufacture and ripening process, the content of lactose and D-galactose was measured. Based on the results of our research the following conclusion can be made: the action of lactic acid bacteria can not be stropped even at 5 °C. The native microflora, which remains in the milk after thermisation, might be responsible for the fermentation of D-galactose. It could be possible that the* ***Streptococcus thermophilus*** *enzymes were not inactivated, causing continuation of lactose fermentation even at low temperature. Mesophilic lactococci were inhibited and for that reason D-galactose accumulated in cheese. Only after cheese were transferred into a ripening chamber at 11 °C and with low lactose concentration in medium left, mesophilic lactococci started to ferment D-galactose.*

Key words: cheese, Camembert, lactose fermentation, lactic acid bacteria, technology, lactic acid, D-galactose, lactose

Introduction

Fermentation by lactic acid bacteria (LAB) play a critical and pivotal role in the development of the unique characteristics of all cheese variety as it is the basis for the proper course of biochemical processes during cheese-making and ripening (Fox and McSweeney, 1998). In the production of Camembert type cheeses, two different technology are known regarding the course and extend of acidification, so called classic and stabilised. Using the classic technology of Camembert cheese making, the pH value drops below 5. The ideal value is between 4.5 and 4.7. With this pH value, the growth of most pathogenic bacteria is inhibited, including *Escherichia coli*. Acidification affects retention of calcium which affects cheese texture. Cheeses, produced by classic technology have longer duration period ranging from 12 to 30 days and they ripen according to secondary ripening. The course of ripening is from surface to interior. The aerobic moulds start to grow and metabolise proteins and lipids on the surface of the cheese. At the beginning, Camembert ripens slowly, due to low pH value while during the ripening, neutral (calcium lactate) and alkali substances are produced which induce the rise of the pH value (Moser, 1984).

The essence of the stabilised technology is to keep the pH value during the fermentation and ripening above 5. The most appropriate pH value before salting in brine is between 5.1 and 5.2. By intensive washing of the curd and intensive syneresis we can prevent further dropping of the pH value. Owing to lesser acidification, smaller amount of calcium migrate from cheese curd. Cheese produced in this way has elastic texture from the beginning of the ripening. In case the raw milk is used, in stabilised technology, the growth of contaminating bacteria is favoured because of higher pH value (Moser, 1984)

Microorganisms play important roles during both cheese manufacture and ripening. They can be divided into two main groups, starter bacteria and secondary flora. The starter bacteria are responsible for acid development during cheese production while during cheese ripening promote, together with the secondary flora, different biochemical reactions which are vital for the development of flavour and texture. The secondary microflora comprise of non-starter lactic acid bacteria (NSLAB) and other bacteria, yeast and/or moulds (Beresford et al., 2001). When starter bacteria are not capable to ferment all of the lactose present in cheese, it can be fermented by non-starter cultures. In this case, different defects in flavour and/or texture, which affect the quality of the cheese, may occur. Some strains of NSLAB for example

members of *Lactobacillus* spp., *Leuconostoc* spp. and *Pediococcus* spp. are capable to convert L-lactate to D-lactate. This contributes to precipitation of calcium lactate crystals and is therefore the reason for untypical taste and other textural changes (Rogelj, 1995). That is the reason, why the author O'Connor claims that the quality of the cheese is influenced by the quantity of the remaining lactose (Fox et al., 1990).

The LAB usually used as starter culture for the Camembert production are *Lactococcus lactis* subsp. lactis, *Lac. lactis* subsp. *cremoris* and occasionally *Streptococcus thermophilus*. They differ in the pathway used to metabolise lactose. Both *Lac. lactis* subsp. translocate lactose via a phosphotransferase system. In these organisms, lactose enters the cytoplasm as lactose-phosphate and is hydrolysed by phospho-β-galactosidase. Transfer of lactose into the *Streptococcus thermophilus* cell is made possible, with help of lactose permease. In the cell cytoplasm, the first step is hydrolysis with β-galactosidase. The glucose is then fermented by glycolytic pathway through L-lactate; whereas galactose is excreted into the medium (cheese). When in the media only limit concentration of lactose is present, galactose-positive strains of *Streptococcus thermophilus* are capable to ferment galactose by Leloir pathway (Ono et al. 1992, Axelsson, 1998).

Only 12 out of 30 strains of *Streptococcus thermophilus* bacteria are capable of fermenting all three sugars: lactose, glucose and D-galactose. 10 out of 12 strains have long adaptive phase (at least 7 hours) before they can uptake and ferment glucose and D-galactose and that occurs only if in the media limit concentration of lactose is present.

If the lactic acid bacteria have the choice to ferment several sugars, it is not clear which sugar they uptake first. *Lactococcus lactis* in the media containing lactose, glucose and D-galactose will at first ferment glucose and lactose and when no other sugars are present, the fermentation of D-galactose will take place. This phenomenon when the glucose prevents the synthesis of other enzymes for sugar fermentation is called catabolic repression (Ono et al., 1992).

Almost all *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* strains ferment galactose into lactate, formiate, acetate and ethanol if it is the only source of energy in the media. *Streptococcus thermophilus* grows better in media with lactose than in media with glucose and D-galactose,

which means that the catabolic repression has no effect on it (Ono et al., 1992).

The purpose of the presented work was to find out the differences in lactose fermentation between Camembert made by classic and stabilised technology.

Materials and methods

Following the basic procedure for Camembert production 4 batches of cheese were manufactured, 2 of them by classic (designated as cheese 1 and cheese 2) and 2 of them by stabilised method (designated as cheese 3 and cheese 4). Cheese making was performed in an experimental vat from 30 L of milk. Milk was heat-treated at 65 °C for 30 min. After rapid cooling to 32 °C milk was inoculated with 1 g of Wisby Cheese Mix1 (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Streptococcus thermophilus*) and.chymosin Maxiren® (Gist-Brocades, Holland) was added at a level sufficient to coagulate milk in 30 min. After cutting, the curd was resting for 30 min and at classic technology moulding and self-pressing for 24 hours when cheese reached on average the pH of 4.9. At stabilised technology the pressing was finished after 8 hours when cheeses reached on average the pH of 5.35. After pressing cheeses were immersed in brine (20 %, wt/vol) for 1.5 hours at 14 °C and drying. Surface of dry cheeses was inoculated with the mould *Penicillium candidum* NR (Wisby, Germany). The cheeses were ripened at 11 °C (80-85 % of relative humidity) for 16 days. The mould appeared on the surface of the cheeses after 5 days of ripening. The pH of cheeses manufactured according the stabilised technology dropped below the pH in the first day of ripening therefore in the second trial we tried to prevent the drop of the pH value by low temperature. We kept the cheeses for 3 days at 5 ° and after that the cheeses were ripened for 27 days at 11 °C and 80-85 % of relative humidity. We used low temperature in the first 3 days of ripening because the results of previous experiments showed that curd washing and reduction of the starter culture used for inoculation were ineffective.

pH value, acidity (SH) and the amount of fat, protein, lactose, D-galactose and dry matter were determined in raw milk. Cheese samples were aseptically collected immediately after moulding, after fermentation (self-pressing), and during the ripening (days of sampling are presented in the tables 3-6), and analysed for the pH value and quantity of lactose and D-galactose.

Fat, protein, lactose and dry matter content in milk were determined by using MilkoScan-133 (N.N.Foos Electric, Denmark).Acidity of milk (SH) was determined by titration with 0.25 M NaOH (VDLUFA, Metodenbuch, VI, 1985, C 8.3.).

pH value was determined with combined electrode, connected to pH meter (Mettler Toledo, AG. MP 120).

Amount of lactose and D-galactose was determined by using UV method (VDLUFA, Metodenbuch VI, 1993, C.20.2.3).

Results and discussion

Table 1: Composition of milk used in the experiments
Tablica 1: Sastav mlijeka korištenog u eksperimentima

Sample Uzorak	Fat Mast (%)	Proteins Proteini (%)	Lactose Laktoza (g/100 g)	D-galactose D-galaktoza (g/100 g)	Dry matter Suha tvar (%)
milk 1	4.42	3.28	4.58	0.04	13.03
milk 2	4.41	3.28	4.53	0.04	13.01
milk 3	4.35	3.27	4.52	0.04	12.93
milk 4	4.49	3.18	4.59	0.09	12.80

The chemical composition of milk used in the experiments is presented in the table 1. On average milk contained 4.4 % of fat, 3.2 % of proteins, 4.5 g/100 g of lactose, 0.05 g/100 g of D-galactose and 12.9 % of dry matter. As we can see, the small quantities of galactose were presented in milk.

Average pH value of fresh cheeses measured immediately after moulding was 6.5 and did not differ between the cheeses made by classic (cheeses 1 and 2) and stabilised (cheeses 3 and 4) technology. Evident differences can be observed between cheeses after fermentation in pH values as well as content of lactose and D-galactose. As we can see from the table 2 cheeses made by stabilised technology had higher pH values (fermentation was cut off after 8 hours) and also higher amount of lactose and D-galactose compared to cheeses made by classic technology which were fermented 24 hours.

Table 2: Amount of lactose and D-galactose and pH value of fresh cheese after moulding and after fermentation
Tablica 2: Količina laktoze, D-galaktoze i pH vrijednost svježeg sira nakon inokulacije s plijesni i fermentacije

	Sample Uzorak	Lactose Laktoza (g/100g)	D-galactose D-galaktoza (g/100g)	pH
After moulding Nakon inokulacije s plijesni	cheese 1	3.14	0.03	6.55
	cheese 2	3.23	0.03	6.54
	cheese 3	3.06	0.19	6.56
	cheese 4	2.63	0.09	6.53
After fermentation Nakon fermentacije	cheese 1	1.61	0.05	4.96
	cheese 2	1.68	0.12	4.89
	cheese 3	1.90	0.60	5.35
	cheese 4	1.99	0.36	5.35

Tables 3 and 4 as well as figures 1 and 2 represent the course of pH values and amounts of lactose and D-galactose during the ripening of cheese made by classic technology.

Table 3: Amount of lactose and D-galactose and pH values during ripening of cheese 1, manufactured at classic technology
Tablica 3: Količina laktoze, D-galaktoze i pH vrijednosti tijekom zrenja sira 1, proizvedenog klasičnom tehnologijom

Ripening time/days Vrijeme zrenja/dani	Lactose Laktoza (g/100g)	D-galactose D-galaktoza (g/100 g)	pH
0	3.14	0.03	6.55
1	1.29	0.34	4.95
2	0.54	0.57	4.77
3	0.52	0.60	4.73
4	0.52	0.50	4.68
5	0.42	0.49	4.67
6	0.36	0.48	4.64
7	0.24	0.50	4.64
8	0.12	0.49	4.65
16	0.07	0.08	4.78

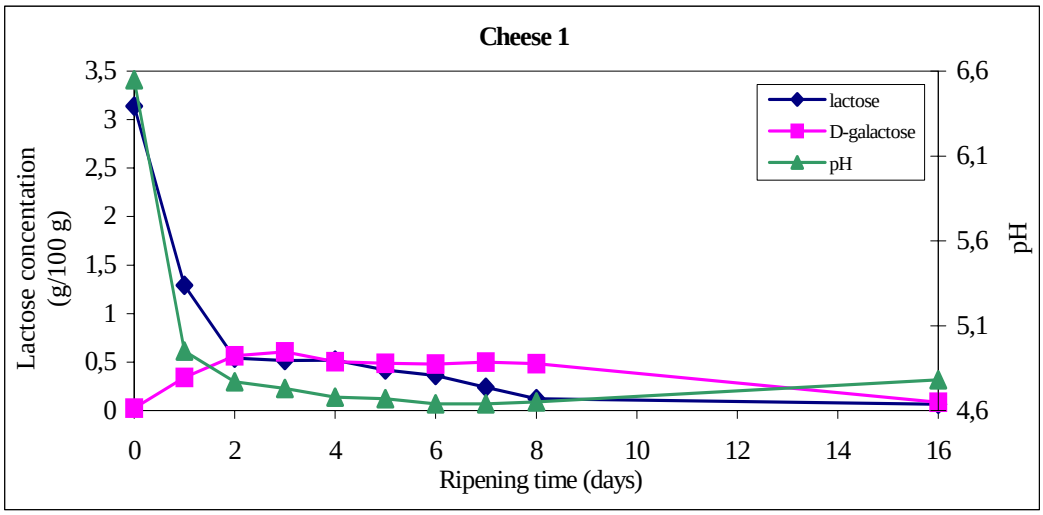

Figure 1: Changes of the amount of lactose, D-galactose and pH value during ripening of cheese 1
Slika 1: Promjena količine laktoze, D-galaktoze i pH vrijednosti tijekom zrenja sira 1

As it is possible to see in the figures 1 and 2, most of the lactose is fermented in first two days. Then the slower rate of fermentation can be observed until the 7th day when pH value starts to rise due to activity of Penicillium candidum mould. After 28 days, only limit amount of lactose is still present in cheese. Inversely, the amount of D-galactose is increasing in the first two days of fermentation than after remains on the same quantity approximately 7 days when it starts to decrease. The decreasing of D-galactose is a consequence of its fermentation by mesophilic lactic acid bacteria which starts in the moment when only limit concentrations of glucose and lactose are still present. This phenomenon, when one sugar inhibits synthesis of the enzymes for degradation of other sugars is called catabolic repression.

The course of pH values and amounts of lactose and D-galactose during the ripening of cheese made by stabilised technology are summarised in the tables 5-6 and figures 3-4.

Table 4: Amount of lactose and D-galactose and pH value during, ripening of cheese 2, manufactured by classic technology
Tablica 4: Količina laktoze, D-galaktoze i pH vrijednost tijekom zrenja sira 2, proizvedenog klasičnom tehnologijom

Ripening time/days Vrijeme zrenja/dani	Lactose Laktoza (g/100g)	D-galactose D-galaktoza (g/100 g)	pH
0	3.13	0.03	6.54
1	1.65	0.69	4.86
2	0.59	0.60	4.79
3	0.58	0.59	4.73
4	0.56	0.52	4.70
5	0.49	0.49	4.70
6	0.36	0.49	4.69
7	0.17	0.51	4.64
15	0.08	0.18	4.72
22	0.02	0.17	4.81
28	0.01	0.16	5.04

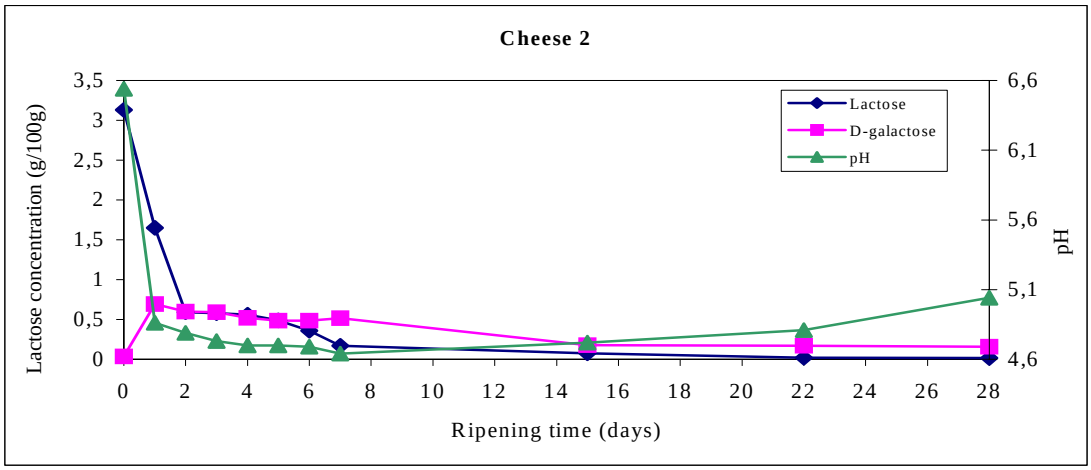

Figure 2: Changes of the amount of lactose, D-galactose and pH value during ripening of cheese 2, manufactured by classic technology
Slika 2: Promjene količine laktoze, D-galaktoze i pH vrijednosti tijekom zrenja sira 2, proizvedenog klasičnom tehnologijom

Table 5: Amount of lactose, D-galactose and pH value during ripening of cheese 3, manufactured by stabilised technology
Tablica 5: Količina laktoze, D-galaktoze i pH vrijednosti tijekom zrenja sira 3, proizvedenog stabilizacijskom tehnologijom

Ripening time/days Vrijeme zrenja/dani	Lactose Laktoza (g/100g)	D-galactose D-galaktoza (g/100 g)	pH
0	3.06	0.19	6.56
1	1.59	0.47	5.12
3	1.42	0.07	4.87
5	0.80	0.05	4.76
7	1.12	0.06	4.68
9	1.15	0.11	4.68
11	1.03	0.08	4.70
17	1.94	0.07	4.81
22	1.03	0.04	4.85
27	1.02	0.02	4.91

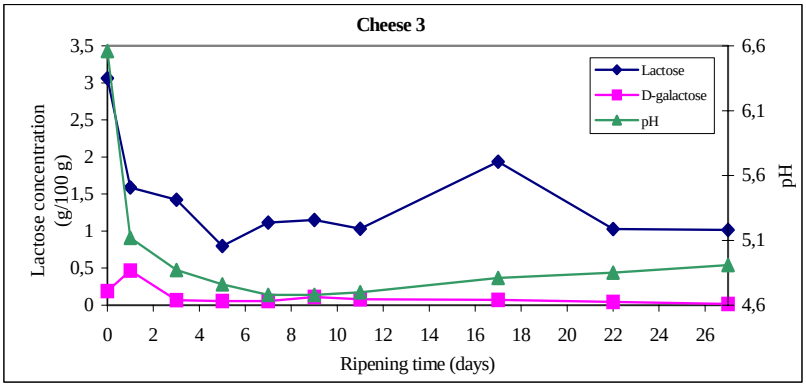

Figure 3: Changes of amount of lactose, D-galactose and pH value during ripening of cheese 3, manufactured by stabilised technology
Slika 3: Promjene količine laktoze, D-galaktoze i pH vrijednosti tijekom zrenja sira 3, proizvedenog stabilizacijskom tehnologijom

Table 6: Amount of lactose, D-galactose and pH value during ripening of cheese 4, manufactured by stabilised technology
Tablica 6: Količina laktoze, D-galaktoze i pH vrijednosti tijekom zrenja sira 4, proizvedenog stabilizacijskom tehnologijom

Ripening time/days Vrijeme zrenja/dani	Lactose Laktoza (g/100g)	D-galactose D-galaktoza (g/100 g)	pH
0	2.63	0.93	6.53
1	1.79	0.46	5.29
3	0.25	0.83	4.98
5	0.24	0.86	4.85
7	0.26	0.85	4.76
9	0.24	0.73	4.71
11	0.16	0.68	4.77
17	0.18	0.53	4.79
22	0.12	0.31	4.82
27	0.11	0.14	4.86

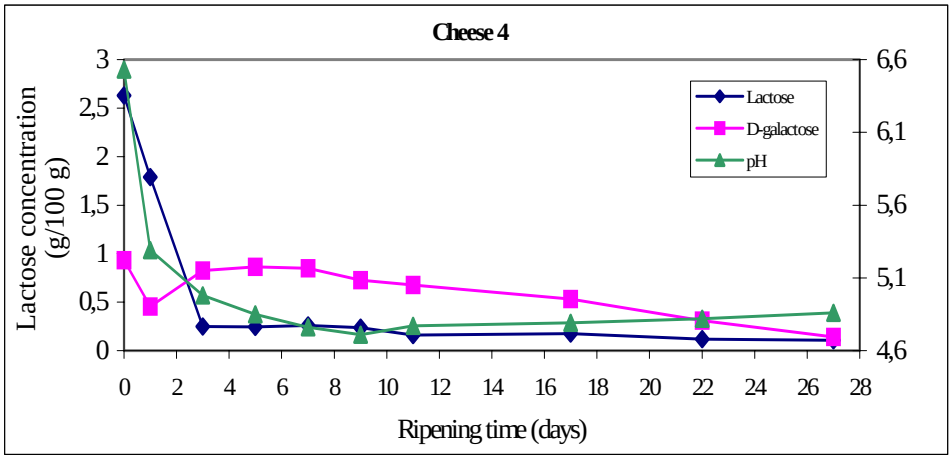

Figure 4: Changes of the amount of lactose, D-galactose and pH value during ripening of cheese 4, manufactured by stabilised technology
Slika 4: Promjene količine laktoze, D-galaktoze i pH vrijednosti tijekom zrenja sira 4, proizvedenog stabilizacijskom tehnologijom

In the first trial of stabilised technology the fermentation was interrupted at pH value 5.35 and cheeses were than transferred into the brine with the temperature of 14°C. After 1.5 hours the cheeses were transferred to the ripening chamber where the temperature was 11 °C and relative humidity of 80-85 %. After one day of ripening pH dropped below 5 and it kept falling until seventh day, when the final pH was 4.68. It is not known why the fermentation of D-galactose occurred at relatively high amount of lactose, but it is possible that native microflora, which remains in the milk after thermisation is responsible. As we could not prevent the over-acidification we lowered the ripening temperature of cheeses in the first 3 days from 11 °C to 5 °C (trial 4).

In spite of low temperature lactose was fermented and after three days the amount was 0.248 g/100g. This is, compared to other batches, the lowest amount obtained. The amount of D-galactose reached the highest value after 5 days of ripening.

In spite of low temperature during the first three days of ripening, the pH value dropped under 5 and thereafter it was still dropping until the day 9 of ripening. Because of the low temperature, in the first three days of ripening, mesophilic lactococci were inhibited and this is the reason why D-galactose is accumulated in cheese until day 7 of ripening, even though only limited concentration of lactose was present.

The rise of temperature after three days to 11 °C was essential for the favour of mould development. This led mesophilic lactococci to start with fermentation of D-galactose. At this time, only limited concentration of lactose was still present in cheese, so catabolic repression had no impact on their metabolism.

Conclusions

When the Camembert cheese was manufactured according to classic technology the expected results were obtained. During the first 24 hours of fermentation pH value dropped under 5 as a consequence of starter culture activity. The lowest pH value was reached after 6 or 7 days of ripening. D-galactose was in the first days accumulated in curd, because the catabolic repression prevented the mesophilic lactococci to induce enzyme for its

fermentation. The fermentation was possible, when limit amounts of lactose were present in cheese.

The main problem of stabilised technology is how to keep the pH value of cheese during manufacturing over the 5. In our experiments we tried to reach this goal by interrupting the fermentation during the self-pressing at pH of 5.35. This procedure was ineffective as the pH value during the ripening of Camembert at 11 °C could not be kept over 5. The over-acidification was in our opinion the result of fermentation by secondary microflora which survived the termisation of milk. Therefore, in the last trial the cheeses were kept during the first three days of ripening at 5 °C. Unexpected fermentation continued and after three days, pH value dropped under 5. The amount of lactose in cheese reached the lowest number of all experiments and D-galactose was accumulated in cheese. This phenomenon is difficult to explained by the activity of starter culture bacteria or secondary microflora. Usually, mesophilic lactococci are responsible for D-galactose fermentation, but they were inhibited at 5 °C. It could be possible that at 5 °C the enzymes of *Streptococcus thermophilus* were not inactivated and were responsible for the degradation of the lactose. Following the rise of temperature to 11 °C, fermentation of D-galactose was possible by lactococci.

FERMENTACIJA LAKTOZE U CAMEMBERT SIRU PROIZVEDENOM KLASIČNOM I STABILIZACIJSKOM TEHNOLOGIJOM

Sažetak

U našim eksperimentima provedena je fermentacija laktoze u Camembert siru klasičnom i stabilizacijskom tehnologijom. U obje primjenjene tehnologije načinjena su po dva eksperimenta. Razlika u ove dvije tehnologije je u pH vrijednosti koja kod klasične tehnologije pada ispod 5, a kod stabilizacijske se zadrži cijelo vrijeme iznad 5. Da bi se dostigli kriteriji stabilizacijske tehnologije fermentacija je zaustavljena kod željene pH vrijednosti, potapanjem sira u salamuru pri 14 °C. Nakon salamurenja i inokulacije s plijesni sir je, iz prva tri eksperimenta, prebačen u komoru za zrenje pri 11 °C. U posljednjem eksperimentu (stabilizacijska tehnologija) sir je podvrgnut zrenju kroz 3 dana pri 5 °C. Tijekom zrenja pH vrijednost sira je u svim eksperimentima pala ispod 5. Proces fermentacije proveden je pomoću

slijedećih bakterija mliječne kiseline: ***Streptococcus thermophilus, Lactococcus lactis subsp. lactis*** *i* ***Lactococcus lactis subsp. cremoris****. Kako ove bakterije različito fermentiraju D-galaktozu, tijekom proizvodnje i zrenja, provedeno je određivanje i laktoze i D-galaktoze. Na osnovi dobivenih rezultata mogu se izvući slijedeći zaključci: djelovanje bakterija mliječne kiseline ne može se zaustaviti čak niti na 5 °C. Prirodna mikroflora, koja zaostaje u mlijeku, mogla bi biti odgovorna za fermentaciju D-galaktoze. Moguće je da se* ***Streptococcus thermophilus*** *enzimi nisu aktivirali uzrokujući tako daljnju fermentaciju laktoze čak i na niskoj temperaturi. Mezofilni laktokoki su bili inhibirani zbog čega je došlo do nakupljanja D-galaktoze u siru. Nakon što je sir prebačen u komoru za zrenje, na 11 °C, i pri niskoj koncentraciji laktoze u mediju, mezofilni laktokoki započeli su fermentaciju D-galaktoze.*

Ključne riječi: sir, Camembert, fermentacija laktoze, bakterije mliječne kiseline, tehnologija, mliječna kiselina, D-galaktoza, laktoza

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