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ACCELERATION OF CHEESE RIPENING BY THE USE OF LAC$^-$ MUTANTS OF GROUP N-STREPTOCOCCI

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The paper presents the results indicating the possibility of reduction of the ripening period of cheese by one month using starter based on Lac$^-$-mutants of group N-3 treptococci in addition to ordinary cheese starter. Schemes for preparation of Lac$^-$ -starters are given.

Cheese ripening includes a great number of complicated biochemical transformations of the various components in the cheese. The transformations are enzymatically regulated and include:

— enzymes originally present in the milk,
— enzymes produced by the microbial flora of the milk,
— coagulating enzymes added for cheesemaking,
— enzymes from starter-organisms or other cultures of microorganisms added to the milk or to the cheese.

A regulated ripening should result in a cheese with texture, consistency, flavour and aroma characteristic for the various cheese varieties.

For semi-hard rennet cheese varities the ripening needs a considerable number of weeks. Ripening periodes of 3-6, months, or even longer, is necessary before the cheese is ready for sale. This long storage period may represent a significant cost because of necessary handling of the cheese in the store-room and rather great capital-costs.

For a number of years dairy research institutes all over the world have shown great interests in the development of techniques which could make acceleration of cheese ripening practically applicable. Various methods or systems have been investigated with variable success. In order to be of any professional interest, a system for the acceleration of cheese ripening has to satisfy the following requirements:

— a ripening typical for the quality characteristics for the various cheese varieties,
— results in cheese with a reasonable keeping quality,

— easy to perform in the dairy plant,
— economical beneficial for the dairy company,
— not beinjurious to the health.

The most common problem in accelerated cheese ripening seems to be a rather frequent occurence of the flavour defects "bitter" and "atypical".

Various methods or systems for accelerated cheese ripening which have been under investigation in dairy research institutes all over the world are listed below. Such a list will however never be complete. One should also realize that some methods can easily be combined:

A. USE OF MODIFIED STARTERS IN ADDITION TO ORDINARY STARTERS

1. Lactose-negative (Lac^-) mutants
2. Mutants induced by ultraviolet- or ionizing irradiation
3. Heat- or freeze shocked starten
4. Addition of dead cells, autolyzed cells, cell-walls or cell free extracts to the milk.

B. ADDITION OF PURE ENZYMES OR ENZYME MIXTURES

1. Addition to the milk
2. Addition to the cheese after whey drainage
3. Addition of enzymes in liposomes.

C. OTHER METHODS

1. Increased storage temperature
2. Increased amount of ordinary starter
3. Addition of "cheese slurries" to the milk or to the cheese after whey drainage.
4. Cheese-making from lactose-hydrolyzed milk.

Generally speaking the ripening of the cheese may be accelerated either by increasing the content of ripening enzymes in the cheese or by causing an increased activity of the enzymes in the cheese. A combination of the two principles is of course also possible. The well known factors in cheese technology as for instance the pH of the cheese, the water content, the salt content and the temperature of the storage room, will thus be factors of importance also for the acceleration of cheese ripening.

USE OF LACTOSE-NEGATIVE (LAC^-) MUTANTS OF GROUP N-STREPTOCOCCI

From most mesophilic starters of lactic acid streptococci, it is possible to isolate some individual bacteria which have lost their ability to produce lactic acid from lactose. These genetic variants are considered to be lactose negative mutants. It is also possible to produce Lac^--mutants by exposing the starter

culture to certain chemical comounds, mutagens, as for instance ethidiumbromide.

It is known, in most cases, that the ability of the bacteria to metabolize lactose and their ability to hydrolize the proteins by means of proteinases are localized in the extrachromosomal elements called plasmids. In some cases these two abilities are localized on the same plasmid and in other cases on different plasmids.

By supplementing the ordinary starter with a Lac^--mutant of group N-streptococci in the milk for cheese making, a considerable increase in the number of bacteria in the milk without any abnormal change in the development of lactic acid during the cheese-making procedure or in the unripened cheese would be expected. The Lac^--mutant must then have been maintained and cultivated on a substrate with another source of carbohydrate than lactose. The mutant-strain ought to be concentrated before the addition to the milk. Addition of Lac^--mutants supply the milk with whole cells. Cell wall bound proteinases may speed up the breakdown of proteins, while later autolysis of the cells will liberate the intracellulare proteinases, peptide hyrolases and lipases also contributing to the acceleration of the cheese ripening.

Acceleration of cheese ripening by addition of Lac^--mutants to the milk has been investigated only to a limited extent in the dairy research institutes throughout the world. Australian scientists however, have published a number of papers from research projects in which this method has been investigated. One of the main advantages connected to the use of Lac^--mutans are that the enzymes which accelerates the ripening of the cheese are evenly distributed in the cheese. Another advantage is that the enzymes liberated from the Lac^--bacteria are identical with the ones supplied to the milk and the cheese by the ordinary starter bacteria. Use of Lac^--strains in addition to the ordinary starter organisms will thus mainly increase the amount of wanted enzymes.

Our department received in 1984 economical support from the Norwegian Agricultural Research Council in order to do a project in the field of accelerated cheese ripening for a 3 years period. This period ended last summer. From the Norwegian Milk Producers Association, we have received economic support for another year.

Based on the positive results from Australia on the use of Lac^--mutants and on the assumed advantages of the method, we decided to concentrate our activities on further investigations in the use of this method for the accelerated cheese ripening.

The first part of the project included use of Lac^--strains in addition to ordinary starters in the production of Norwegian Saint Paulin cheese. In this first trial cheese were produced from 18 vats, each of 400 liters of milk. Four different Lac^--mutants were tested, each in three separate productions, while 6 of the vats were without any Lac^--strains (controls). The Lac^--mutants were added in quantities corresponding to $3.3\text{-}3.6 \times 10^{10}$ cells pr. liter of milk. The mutants were grown and cultivated on modified M 17—broth in which the lactose was replaced by glucose. In order to avoid addition of broth to the milk, the outgrown cultures

were concentrated by means of centrifugation. Before addition to the milk for cheese-making the concentrated cultures were suspended in a small quantity of sterile milk.

In Fig. 1 some typical results from protein degradation of the cheese are given. The figure shows that a faster formation of soluble nitrogen and amino nitrogen were obtained in cheeses to which *S. cremoris* C 12 Lac$^-$ and *S. lactis* L2 Lac$^-$ were added than in control cheeses with only regular starter. This first experiment gave inspiring results. The use of Lac$^-$-strains did not not influence the development of acidity in the milk, in the curd or in the cheese. We also realized that the four mutants under investigation influenced the cheese characteristics in different ways, indicating that the search for a mutant-strain which will influence the characteristic properties of the cheese in a very positive way should be given a certain priority. Most of the cheeses with Lac$^-$-mutants were graded to the same quality or better than control cheeses.

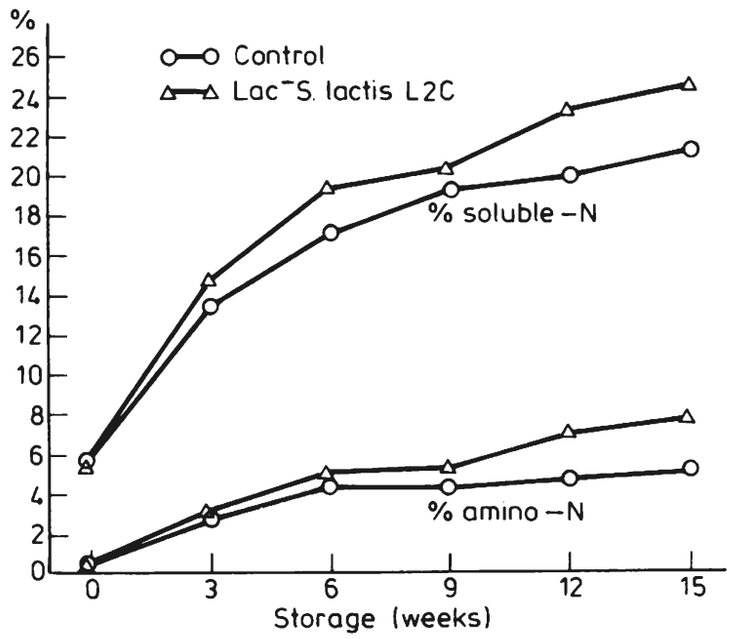

Fig. 1. Amount of soluble — and amino nitrogen in cheese with Lac$^-$-strains (Values calculated as percent of total nitrogen content)

We continued to search for good Lac$^-$-strains. In the course of the project period 8 different Lac$^-$-strains have been used in cheese-making experiments. All strains have been mutated by treatment of strains of *S. lactis*, *S. cremoris* and *S. lactis* subsp. *diacetylactis* by ethidiumbromide.

From the making of Norwegian Saint Paulin cheese we turned to production of a Norwegian Gouda type cheese called Norvegia. The cheese was moulded in order to give 5 kg's cheeses with rind. Approximately 80 vats, each of 400 liters of milk have been produced to this type of cheese. In the first series of this cheese-making experiments, the mutants were again cultivated on M 17-broth and concentrated as mentioned earlier.

In this series of experiments, the mutant strains were added in two quantity levels corresponding to 10 and 20 times the amount of cells of ordinary starter cultures. The ordinary starter was the same for all vats namely a Redi-Set,

mesophilic mixed strain starter from Chr. Hansens laboratory, consisting of *S. lactis*, *S. cremoris*, *S. lactis* subsp. *diacetylactis* and *Leuconostoc cremoris*.

Cheese from each vat vere analyzed every third week during a 15 week period after the cheese-making and after 30 weeks. A normal ripening period for this type of cheese is aproximately 12 weeks. The purpose of analyzing the cheese after a 30 weeks storage was to observe the keeping quality of cheeses which had undergone an accelerated cheese ripening.

In addition to sensoric evalution, the cheese was analyzed for number of Lac^- cells, extent of bacterial autolysis during ripening, amount of soluble-nitrogen calculated in per cent of total nitrogen content in the cheese. A more detailed investigation of the breakdown of the proteins during the ripening was undertaken by the application of polyacrylamide gel electrophoresis. The production of peptides was registered by means of fast protein liquid chromatography.

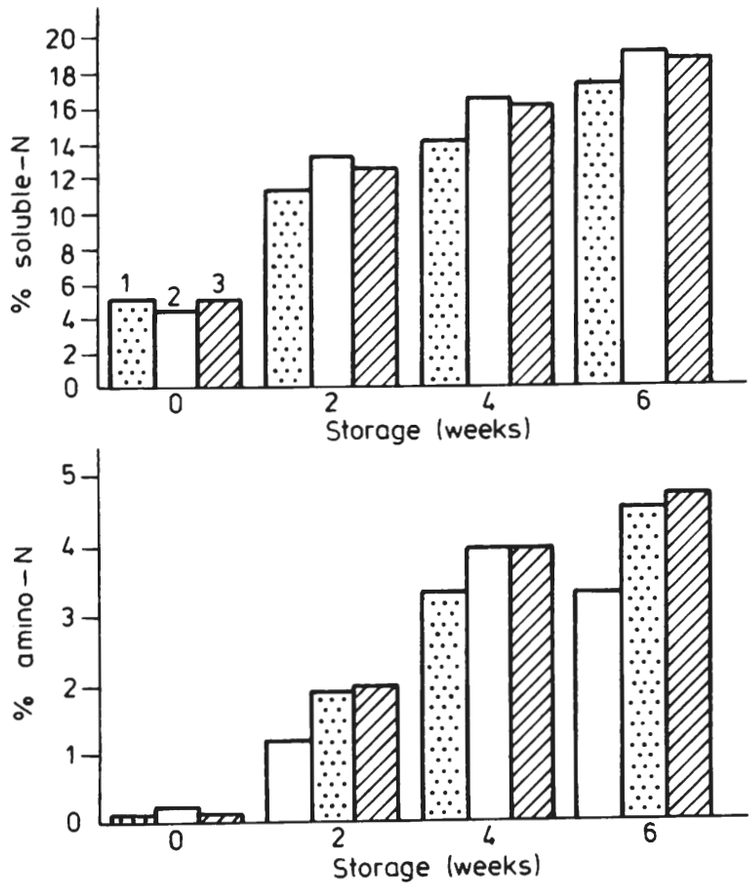

Fig. 2. Amount of soluble — and amino nitrogen in cheese with Lac^--strains (Values calculated as per cent of total nitrogen content)

In Fig. 2 we have shown some characteristic results from the anylysis of the amount soluble- and amino nitrogen calculated in per cent of the content of total nitrogen in the cheese. The results referred to, originates from the analysis of Norvegia F 45+ — cheese made from milk to which was added Lac^--cells in a number of 20 times the number of cells added from the ordinary starter. The figure shows the results obtained every third week in a 15 weeks ripening periode. From this trial, the general conclusion are that the cheese with Lac^--cells had undergone a faster and more profound proteolysis than the control cheeses. Some

of the mutant-strains resulted in cheese which reached the same level of protein breakdown approximately one month earlier than the control cheese. The cheese with Lac$^-$-strains were graded as better than the control cheese trough the whole ripening period, also after 30 weeks of storage. A careful conclusion from this investigation may be that none of the mutants used, influenced the cheese quality in a negative way.

Modified M 17-broth is an excellent medic for the cultivation of Lac$^-$-strains. M 17-broth is however, very expensive. The use of the broth makes it also necessary to remove the cells from the media before addition of relatively great quantities of broth to the cheese milk. Work has therefore been undertaken, and are still under way, in order to look for other media for the cultivation of Lac$^-$-strains. If the Lac$^-$-strains can be cultivated on lactose-hydrolyzed milk or lactose-hydrolyzed whey, they can be added directly to the milk for cheese-making. If desiderable or necessary the culture can of course be concentrated before additon to the milk.

Based on this approach to the problem, a cheesemaking experiment has been carried out in which Lac$^-$-strains cultivated on lactose-hydrolyzed milk were added to milk for cheesemaking. The cultures were added directly to the milk in an amount of 3% of the milk quantity. Cheese of the Norvegia-type was made from 44 vats of 400 litres of milk and 8 different Lac$^-$-strains were tested separately. If addition of unconcentrated Lac$^-$-cultures, cultivated on lactose--hydrolyzed milk could shown sufficient acceleration of the ripening, the method would be very easy to adept in cheese-making plants.

The results from this experiment supported our conclusions from the earlier cheese-making experiments. The cheese made with Lac$^-$-strains obtained flavour and aroma characteristic for the Norvegia-cheese. Of the 8 different Lac$^-$-strains used, 7 gave cheese graded better than the control cheese during the whole storage periode of 30 weeks. In some cultures the number of cells had, to our opinion, not reached a satisfactory level when the outgrown culture had to be mixed with the cheese milk. For some of the mutants, a concentration procedure before transfer to the milk would have been advantageous. Nevertheless, the analysis of the protein breakdown in the cheese showed, that cheeses with Lac$^-$-strains, in general, had achieved a faster ripening than control cheese. For some of the mutant sretains the ripening time of the cheese seemed to be reduced by approximately 4 weeks.

In we succeed in finding a Lac$^-$-strains which are able to give high cell-numbers after incubation in lactose-hydrolyzed milk, direct addition of unconcentrated cultures to the cheese-milk may be a practically applicable method for the acceleration of cheese ripening..

Our section of food industrial economics are now preparing an economical calculation in order to elucidaee the economic effects of accelerated cheese ripening for the dairy industry in Norway. The calculations are based on the use of Lac$^-$-strains in two different ways; either by the use of eunconcentrated

cultures of Lac$^-$-strains cultivated at the dairy plants, or the use of concentrated cultures produced on and distributed from a central laboratory.

The first alternative is illustrated in Fig. 3. Each cheese-making plant will have to recombine lactose-hydrolyzed milk powder or to hydrolyze the milk itself. After incubation, the Lac$^-$-strains will be transferred to the cheese vat and mixed with the milk in an appropriate amount, for instance 3%, in addition to the ordinary starter which will be responsible for acidification. This method will require a special production line for the Lac$^-$-mutants in each cheesemaking plant.

In Fig. 4 the steps in the second alternative are illustrated. The system is based on a central production of Lac$^-$-cultures. No special investments or new

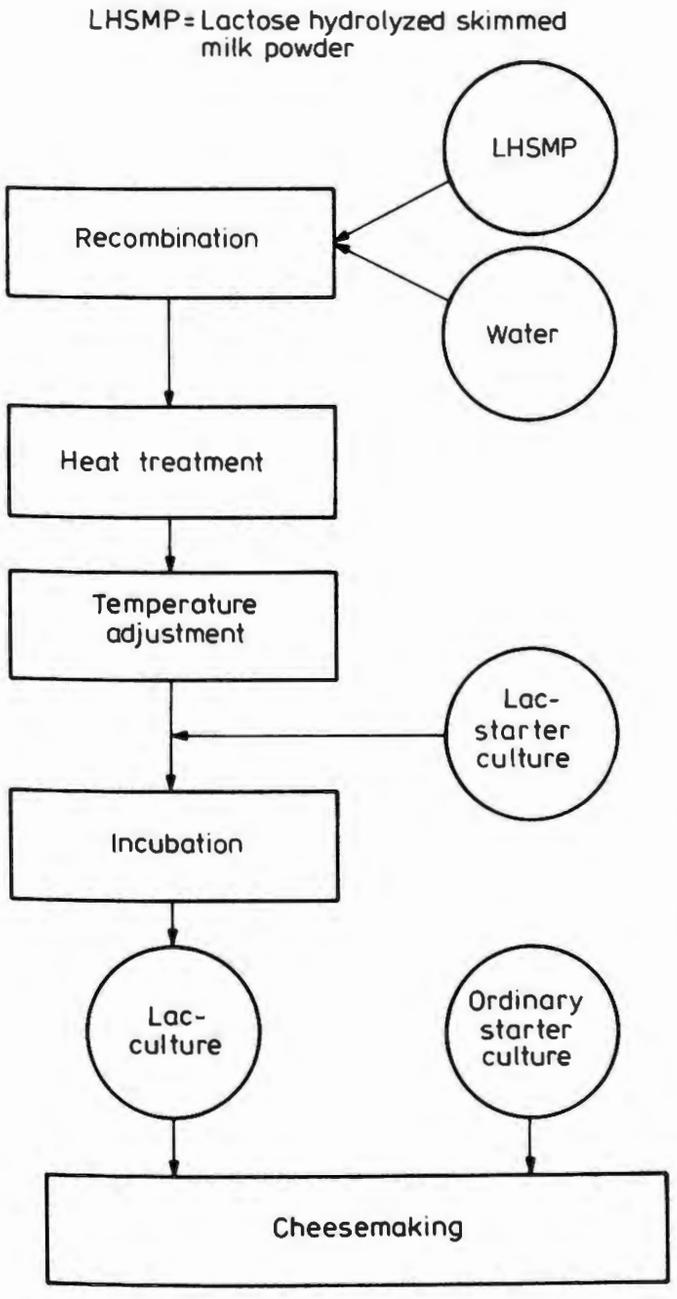

Fig. 3. Cultivation of Lac$^-$-mutant culture at the dairy plant and direct use of unconcentrated Lac$^-$-culture

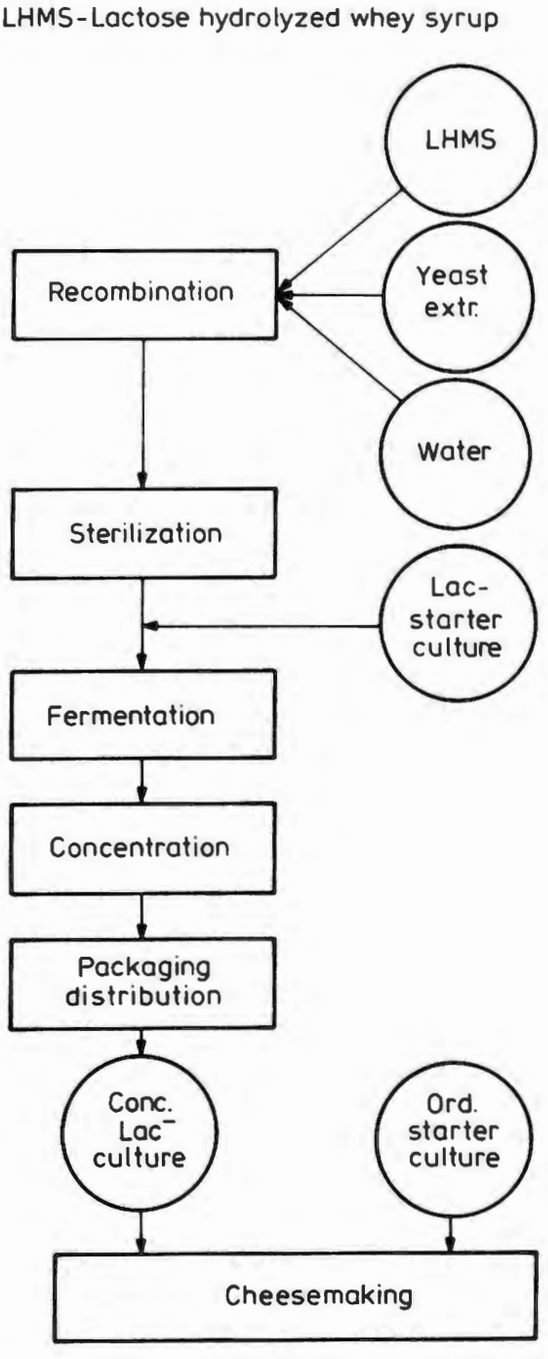

Fig. 4. Central cultivation and concentration of Lac$^-$-mutant culture for distribution to the dairy plants for direst vat use

operations in the cheesemaking plants will be necessary. In the central production of Lac$^-$-cultures the concentration will be an important step. In order to obtain a concentrate with as high a number of organisms as possible, experiments are under way in which lactose-hydrolyzed milk hase been replaced by lactose-hydrolyzed whey to which some yeast extract is added. If use of ultrafiltration, for the concentration of bacterial cells are feasible, growth on lactose-hydrolyzed whey will make it posible to avoid concentration of caseins from the milk. Bacterial cells will be the main source of the dry matter in the concentrate.

Our calculation so far indicates that introduction of a system for accelerated cheese ripening based on this idea, may be economically benefical for the dairy industry in Norway if we are able to shorten the ripening period of Norvegia-cheese with 4-8 days. Our results from the cheesemaking experiments indicate that the ripening period will be approximately one month shorer. The cheesemaking industry in Norway will, according to our calculations, save approximately 475 000 Nkr for each day of reduction in the ripening period if the reduction is greater than one week.

In 1978 the Australian group: Dulley, Brooks and Grieve published results from cheesemaking experiments where the milk used for Cheddar cheese production had been added 100 times as high a number of Lac$^-$-cells of *S. lactis* C 2 as of the ordinary starter used. The results were very promising and the work was continued. Aston and Douglas found in 1983 that addition of rather limited number of Lac$^-$-cells, for instance 2-4 times the number of cells from the ordinary starter, gave a Cheddar cheese which, during the ripening periode, obtained higher contents of trichloroacetic acid solub nitrogen and of free amino acides than the control cheese. The experimental cheeses also appeared to have less occurence of the flavour defect "bitter" and an increased flavour intensity compared with the control cheese. The Australian scientists realized that cheeses with Lac$^-$strains were considered as more mature and had a better aroma and flavour throughout the whole ripening period than the control cheese. Later, in 1986, Australian scientists published, results which supported their earlier findings in this respect.

Work published in 1986 by Fedrick, Cromie, Dulley and Giles concluded that an addition of a number of Lac$^-$-cells corresponding to 100 times the number of cells of ordinary starter used, gave Cheddar cheese which after 3.2 months at 8°C had ripened to the same extent as the control cheese after 6 months at the same temperature.

In the selection of Lac$^-$-mutants used for acceleration of cheese ripening one should try to find a strain able to produce an aromaprofile in the cheese characteristic for the particular variety of cheese under investigation. Quite often the Lac$^-$-strains will have a reduced activity from cell wall bound proteinases and become Prt$^-$-strains as well. For this reason it is of particular interest to look for Lac$^-$-strains which easily will undergo autolysis in the cheese. In that way the autolyzed cells will probably liberate a greater arsenal of intracellular proteolytic

enzymes. The development of Lac$^-$-strains of group N streptococci, which also autolyze rapidly in the cheese, represent an area of research in which we by means of modern biotechnology could expect in the future to be able to create Lac$^-$-strains with the wanted properties for use in the acceleration of cheese ripening.

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PRZYSPIESZENIE PROCESU DOJRZEWANIA SERA PRZY UŻYCIU MUTANTÓW LAC PACIORKOWCÓW MLEKOWYCH Z GRUPY N

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Streszczenie

We wstępie artykułu podano informacje na temat różnych sposobów przyspieszania procesu dojrzewania sera. Należą tu 3 grupy metod: a) oparte na użyciu zmodyfikowanych zakwasów w połączeniu ze zwykłymi zakwasami, w tym: zakwasami modyfikowanymi zawierającymi mutanty bakterii mlekowych nie fermentujące laktozy (Lac$^-$), mutanty indukowane promieniami UV lub jonizującymi, bakterie poddane szokowi termicznemu (przez ogrzanie lub zamrożenie), martwe komórki bakteryjne, komórki autolizowane lub ekstrakty komórkowe, b) polegające na dodatku pojedynczych enzymów lub ich mieszanin do mleka lub ziarna serowego, c) inne metody, jak: podwyższona temperatura dojrzewania, zwiększona ilość zakwasu, dodatek homogenatu sera do mleka lub do ziarna serowego, użycie mleka z częściowo shydrolizowaną laktozą.

W dalszej części artykułu przedstawione są wyniki badań nad użyciem mutantów Lac$^-$ paciorkowców mlekowych do przyspieszenia procesu dojrzewania sera. Dodatek do mleka zakwasu zawierającego ww. mutanty w połączeniu ze zwykłym zakwasem pozwala na znaczny wzrost zawartości biomasy bakteryjnej jako źródła enzymów proteolitycznych biorących udział w dojrzewaniu sera. Nie występuje przy tym obawa zmiany tempa przyrostu zawartości kwasu mlekowego w mleku serowarskim podczas wyrobu sera i w pierwszym etapie jego dojrzewania. Zaproponowano dwa sposoby przygotowania i użycia mutantów Lac$^-$ paciorkowców mlekowych. Na przykładzie produkcji sera typu Saint Paulin i Gouda (Norvegia) wykazano możliwość skrócenia czasu dojrzewania o 1 miesiąc dzięki zastosowaniu ww. metody, co pozwala na znaczne obniżenie kosztów produkcji sera.