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PRODUCTION AND CHARACTERISTICS OF TWAROG CHEESES CONTAINING STRAINS OF PROBIOTIC BACTERIA

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Advance of knowledge on both proper nutrition as well as effects of probiotic bacteria strains on human health result in ever increasing interest of consumers in food products containing that microflora. The Polish market of dairy food products offers yoghurts, cream cheese and milks being a carrier of bacterial strains evoking a beneficial effect in consumer health. Twarog cheese (acidic fresh white cheese) – a popular product in Poland – might extend the assortment of such products on the market. The reported research was aimed at designing a production technology of twarog cheeses containing *Lactobacillus acidophilus* LA5 and *Bifidobacterium* BB12.

Experimental products were prepared by means of a modified technology and with the use of various mixtures of cultures, including probiotic strains of bacteria. During storage, the cheeses were monitored for changes in acidity, hardness and water content. Additional analyses were carried out for the survivability of introduced non-technological microflora and for the total bacteria count.

The study demonstrated satisfactory survivability of probiotic bacteria strains in four out of the five products examined. Acidity of twarog cheeses was observed to decrease during storage, except for product D, where values of pH were gradually increasing. An appropriate selection of strains and production technology results in the elongation of the shelf life of a product, with simultaneous preservation of probiotic properties, from 14 to even over 28 days of chill storage. The results described were confirmed in industrial-scale production.

INTRODUCTION

Today, of increasing significance to contemporary consumers is the quality of consumed food. Facing requirements imposed by the market, both producers and scientists elaborate new production technologies or enrich traditional products with novel nutritional or functional components. Recently, increasing popularity has been observed for food with probiotic properties. Most often, they are products of the dairy industry – milks, yoghurts and cream cheese. Yet, not all consumers accept the available assortment of probiotic foodstuffs, while others seek for some other products to add variety to their diet.

Taking into account the popularity of twarog cheese in Poland and all countries of Eastern Europe as well, an attempt was made to supplement that product with probiotic bacteria. It might be a real challenge due to high susceptibility of probiotic microflora to acidity of the product and oxygen content. The presence of other microorganisms and availability of nutrients in the products are also of significance to the survivability of probiotic microflora. It should be emphasized that not all probiotic strains are capable of surviving the processes of production and storage. The are also likely to negatively affect the sensory quality and shelf life of the finished product.

Therefore an attempt was undertaken to produce twarog cheese containing probiotic strains at a level over $1x10^6$ cfu/g in the last day of its shelf life with simultaneous preservation of sensory attributes of the product known to a consumer.

MATERIAL AND METHODS

Twarog cheese were produced with mixtures of mesophilic lactic bacteria and probiotic strains of *Lactobacillus acidophilus* LA5 and/orb *Bifidobacterium* BB12 and coded with letter A, B, and C, respectively. Two products were additionally prepared with the use of thermophilic bacteria *Lactobacillus delbruecki* ssp. *termophilus* and *Streptococcus termophilus*; they were coded with letters D and E (Table 1). Both cultures of probiotic bacteria and yoghurt starters were introduced

TABLE 1. Letter codes of twarog cheeses.

Twarog cheese code	Introduced non-technological microflora
K	-
A	*Lactobacillus acidophilus* LA 5
B	*Bifidobacterium animals* ssp. *lactis* BB 12
C	*Lactobacillus acidophilus* LA 5, *Bifidobacterium animals* ssp. *lactis* BB 12
D	*Lactobacillus acidophilus* LA 5, *Bifidobacterium animals* ssp. *lactis* BB 12, *Streptococcus termophilus*
E	*Lactobacillus acidophilus* LA 5, *Bifidobacterium animals* ssp. *lactis* BB 12, *Streptococcus termophilus, Lactobacillus delbruecki* ssp. *bulgaricus*

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to twarog cheeses in appropriate proportions providing the numbers of *Lactobacillus acidophilus* LA5 and/or *Bifidobacterium* BB12 in the processing milk at a level of $1x10^7$ cfu/mL. The cheesemaking process required modifying the production technology. A control sample (K) was made of twarog cheese produced according to traditional technology with a starter of mesophilic lactic fermentation bacteria. The bacteria used in the study originated from a collection of commercial starters of Chr. Hansen company.

The production of each variant of twarog cheese was performed in three replications in a quarter-technical scale at the Technological Hall, University of Warmia and Mazury in Olsztyn. The products packed on a Multivac A 300 packing machine (subatmospheric pressure of 600 bar) were stored under chill conditions until sensory unacceptability. The twarog cheeses were determined for: changes in acidity expressed by pH (with Portamess 913 pH-meter by Knick) according to the Polish Standard [PN-91/A-86300]; content of dry matter – on a WPS 110 scale-dryer; and for penetration degree with an AP4/2 conical penetrometer. The products were also subjected to microbiological analyses. They were assayed for changes in the total bacteria count on a culture medium by Merck (the medium was prepared following producer's instructions). To this end, inoculation was conducted with the surface method and incubation was carried out at a temperature of 37°C for 72 h. The presence of probiotic microflora was detected following methodology recommended by the producer of starters, *Lactobacillus acidophilus* on MRS medium – maltose – agar and *Bifidobacterium* on MRS medium – agar with the addition of NNLP. Plates were stored under anaerobic conditions in anaerostates at a temperature of 37°C for 72 h. Anaerobic atmosphere was achieved by means of a reagent for the production of anaerobic atmosphere Anaerocult A by Merck. All results were confirmed in microscopic analyses.

The statistical analysis of the results obtained was carried out by means of Microsoft Excel 2007. Figures present mean values of the results obtained.

RESULTS AND DISCUSSION

The study demonstrated elongation of the shelf life of twarog cheese containing strains of probiotic bacteria, except for product C whose shelf life corresponded to that of the control sample. The longest shelf life, *i.e.* 28 days, was observed for product B.

During storage of the twarog cheese, changes were observed in acidity between the products (Figure 1). All twarog cheeses, except for product B, were characterised by lower pH values than the product K. Of all the products examined, the highest acidity was reported for product D. That cheese was also characterised by different dynamics of changes of that parameter. After the first week of storage, values of pH were observed to decrease from 4.13 to 3.94, whereas in the subsequent weeks they appeared to increase to 4.20 in the 21st day of storage. That phenomenon may be due to peptonization of casein [Bohdziewicz *et al*., 2004]. In the other twarog cheeses, the dynamics of changes in acidity was similar to that of the control sample. In the consecutive experimental periods,

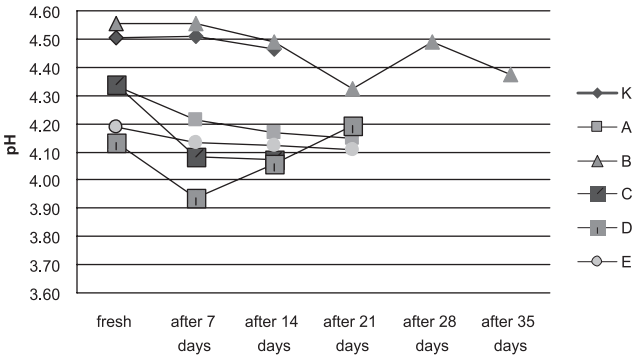

FIGURE 1. Changes in pH value during storage of twarog cheeses.

those products were characterised by a gradual decrease of pH, yet in sample B after 28 days the acidity diminished by only 0.16 pH.

Also Bohdziewicz *et al.* [2004] reported a decrease in pH of twarog cheese in the subsequent weeks of storage, though sometimes the phenomenon of alkalization was observed that resulted in an increase in pH value of the stored product.

The content of dry matter (dm) in the produced twarog cheeses ranged from 25 to 30% (Figure 2). The highest dry matter content was observed in the control sample and product A. Cottage cheeses C and E were characterised by a subsequent increase of dry matter during storage, from 25.54% to 28.97% for C and from 27.12% to 29.62% for E. Analogous dynamics of changes in dry matter content of classic twarog cheese was reported by Bohdziewicz *et al.* [2004].

In the other twarog cheese, values of that parameters were observed to fluctuate negligibly. Differences in water content of twarog cheese may be due to the extent of pressing during the production process, whereas changes observed during storage result from shrinkage of cheese mass and whey leakage to the packaging.

During storage of the products, a step-wise increase was observed in their hardness, except for cheeses D. (Figure 3). They were characterised by the most delicate structure and the penetration of a fresh product reached 321.28°p. After 7 days of storage, cheese hardness increased rapidly to 246°p, yet after the subsequent weeks a gradual increase was noted in the penetration degree. In respect of literature data [Bohdziewicz *et al*., 2004], the twarog cheeses obtained were characterised by a very delicate structure.

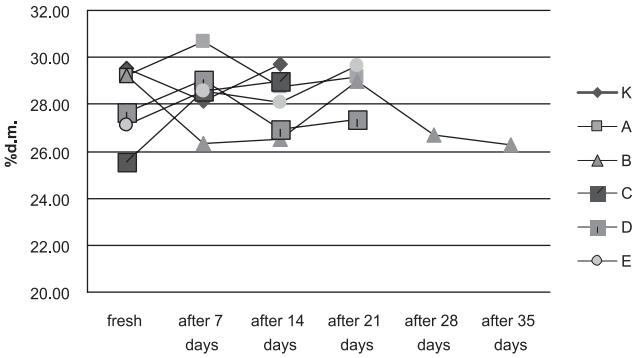

FIGURE 2. Changes in dry matter content of twarog cheeses during storage.

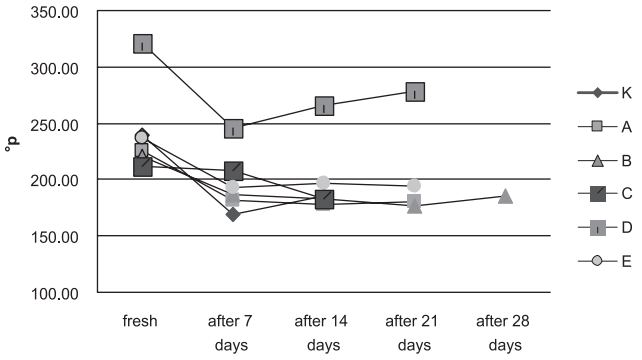

FIGURE 3. Changes of penetration degree in twarog cheese during storage.

Analyses demonstrated a greater decrease of cheeses penetration after the first week of storage as compared to the consecutive analytical periods. Such a dependency was not observed only for cheese C in which the increase of hardness proceeded less dynamically.

Microbiological analyses conducted in the study showed tangible differences in both the number and dynamics of changes in the total bacteria count (TBC) between particular products during storage period. Out of the twarog cheese examined, product E was characterised be the highest TBC in the subsequent weeks of storage, *i.e.* from 8.20 to 9.45 log cfu/g) (Figure 4). Values of TBC were found to decrease during storage in products supplemented with bacteria displaying probiotic properties. Cheeses B were characterised by the lowest TBC (6.40 log cfu/g) out of all discussed cheeses; their TBC was subject to a subsequent decrease in the consecutive weeks of analyses. The greatest reduction in TBC after the first week of storage was reported for twarog cheese D (from 7.13 to 4.87 log cfu/g, respectively).

Also Kornacki *et al*. [2002] reported that the application of yoghurt inoculum, ABT and *Streptococcus thetrmophilus* enabled obtaining twarog cheese with better microbiological quality as compared to traditional products.

In all twarog cheeses the count of *Bifidobacterium animails* ssp. *lactis* BB12 appeared to be higher than $1x10^7$ cfu/g. During storage, only in product E the count of those bacteria dropped below 10^6 cfu/g already after the first week (Figure 5). A gradual decrease in the count of the discussed strain of *Bifidobacterium* was noted in products B and C, yet those changes proceed earlier in the product containing only *Bifidobacterium animalis* ssp. *lactis* BB12. Boylston *et al.* [2004] reported a reduction in the number of *Bifidobacterium* in Fresco cheese only by 1 log cycle as late as after 60 days (preserving the count over 10^6 cfu/g).

All the discussed products were characterised by the presence of *Lactobacillus acidophilus* LA5 whose number exceeded $1x10^6$ cfu/g (Figure 6) Over the entire storage period the highest count of that bacteria was recorded in products C, *i.e.* from 8.94 to 8.85 log cfu/g. Lourens-Hattingh & Viljoen [2001] report on mutual symbiosis of *Lactobacillus acidophilus* and *Bifidobacterium*, that provide one another with indispensable growth stimulants. This is likely to be the cause of such a high number of the discussed strain *L. acidophilus* as well as of a high stability of *Bifidobacetrium animals* ssp. *lactis* BB12 in twarog cheese C. In contrast, product E was characterised by the greatest reduction in the count of *L. acidophilus* LA5 during storage, which was at a level of 0.84 log cycle cfu/g within 21 days. Interesting results have also been obtained by Kasimoglu *et al*. [2004], in the produced probiotic "white cheese" the number of *L. acidophilus* bacteria in the last day of shelf life was higher than 10^6 cfu/g.

A research conducted by Boylston *et al*. [2004] demonstrated the presence of *B. lactis* BB-12 and *L. acidophilus* LA-5 at a level of 10^6 cfu/g in Talaga cheese even after 28 days of storage. It suggests that those bacteria may maintain their count in the cheese bulk for a longer period than that examined by those authors.

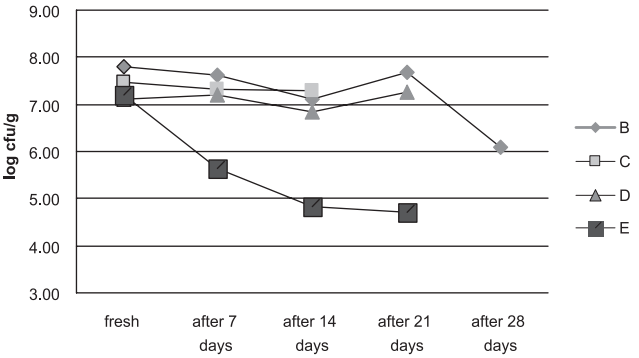

FIGURE 5. Changes in the count of *Bifidobacterium animalis* ssp. *Lactis* BB12 (log cfu/g) in twarog cheeses during storage.

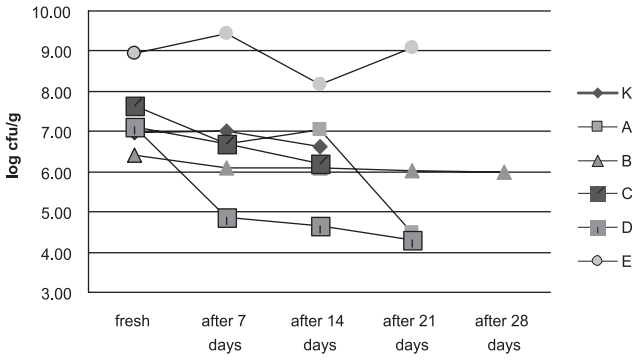

FIGURE 4. Changes in total bacteria count (TBC, log cfu/g) in twarog cheeses during storage.

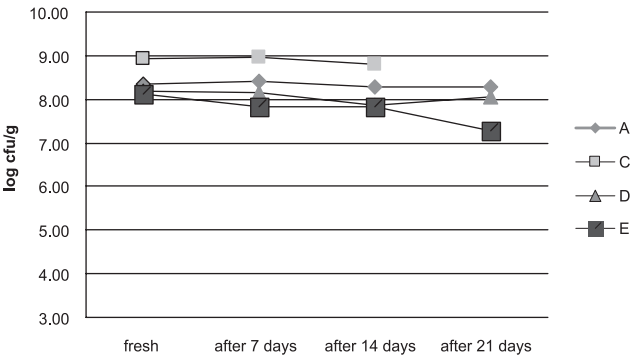

FIGURE 6. Changes in the count of *Lactobacillus acidophilus* LA5 (log cfu/g) in twarog cheese during storage.

CONCLUSIONS

1. It is possible to produce twarog cheese containing the required numbers of probiotic microflora over the entire storage period.

2. An appropriate selected of microflora, including probiotic strains, enables extending the shelf life of twarog cheese up to 28 days.

3. Introduction of *Lactobacillus acidophilus* LA5 into the inoculum's composition resulted in a decrease of the acidity of finished product.

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OTRZYMYWANIE I CHARAKTERYSTYKA TWAROGÓW KWASOWYCH ZAWIERAJĄCYCH SZCZEPY BAKTERII PROBIOTYCZNYCH

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Postęp wiedzy w zakresie dobrego odżywiania się jak i wpływu szczepów bakterii probiotycznych na zdrowie człowieka skutkują coraz większym zainteresowaniem konsumentów żywnością zawierającą wspomnianą mikroflorę. Na Polskim rynku mleczarskich produktów spożywczych dostępne są jogurty, serki homogenizowane i mleka będące nośnikiem szczepów bakterii wpływających korzystnie na zdrowie konsumenta. Twaróg kwasowy będący bardzo popularnym produktem w Polsce mógłby poszerzyć asortyment tego typu produktów na rynku. Celem przeprowadzonych badań było zaprojektowanie technologii produkcji twarogów kwasowych zawierających *Lactobacillus acidophilus* LA5 i *Bifidobacterium* BB12.

Produkty wytworzono przy zastosowaniu zmodyfikowanej technologii oraz przy użyciu różnych mieszanin kultur w tym probiotycznych szczepów bakterii. W czasie przechowywania kontrolowano zmiany kwasowości, twardości oraz zawartości wody w twarogach. Monitorowano również przeżywalność wprowadzonej mikroflory nie technologicznej oraz ogólną liczbę drobnoustrojów.

W wyniku przeprowadzonych analiz stwierdzono zadowalającą przeżywalność szczepów bakterii probiotycznych w czterech z pięciu przebadanych produktów (rys. 5 i 6). Kwasowość twarogów zwiększała się w czasie przechowywania za wyjątkiem produktu D, gdzie stwierdzono stopniowy wzrost pH (rys. 1). Odpowiedni dobór szczepów oraz technologii skutkuje wydłużeniem okresu trwałości produktu, przy zachowaniu właściwości probiotycznych, z 14 do nawet ponad 28 dni chłodniczego przechowywania. Przytoczone wyniki zostały potwierdzone podczas produkcji w skali przemysłowej.