A C T A A L I M E N T A R I A P O L O N I C A
Vol. V (XXIX), No. 4 1979

BOŻENA CZARNOCKA-ROCZNIAKOWA
ZOFIA DREWEK
ELŻBIETA KOPCZYŃSKA

CHANGES IN FOLACIN CONTENT IN THE NON-RIPENING CHEESE (TVAROG) PRODUCTION PROCESS

Department of Food Engineering and Biotechnology, Agricultural and Technical University, Olsztyn

Key words: folacin, synthesis lactic acid streptococci, milk starters.

The lactic acid streptococci used in starters were found to synthetise folacine during the logarthimic phase of growth both in single cultures and in the mixed ones. Folacin content increases by 119% during the acidification of milk to be used for the production of tvarog. Some 45% of this vitamin remains in the cheese and the rest is removed together with the whey.

INTRODUCTION

While assessing the values of the dairy products the basic nutritive components are usually taken into account, the content of many other valuable nutritive ingredients being neglected. These ingredients, however, constitute an important source of certain group B vitamins; this fact has been emphasised by many authors [1, 2, 4, 5, 14]. Technological processes esentially affect the content of vitamins. In the cheese production process the vitamins are partly destroyed by heat treatment, and as many as 50% of what remains of them are later disposed off with thewhey. The microflora active in the dairy products cartainly plays an important (sometimes even decisive) role in shaping the content of group B vitamins. The process es of the synthesis and decomposition of vitamins as affected by lactic acid bacteria are not sufficiently known. So far the greatest amount of information has been collected about vitamin B_{12} whose content in milk decreases under the effect of the lactic acid bacteria [7]. Folacin is another vitamin, besides cyanocobalamin, that displays hematopoietic activity. Information about the content of this vitamin in milk and in the dairy products lacks of agreement and is

rather scare. Gulko [4] and later Reddy and Shahani [12] as well as Reif, Shahani, Vakil and Crowe [13] have indicated that the level of the folic acid in products subjected to fermentation increases; they think this to be the result of biosynthesis by the lactic acid bacteria.

The purpose of this study was to collect information about changes in folacin content in the non-ripening cheese (tvarog) production process, with special emphasis put on the effect of starter microflora. The authors' interest in the tvarog results from the growing consumption of that product and the role it plays in dietetric nutrition, especially in the case of elderly people with liver diseases. Thus the nutritive value of this cheese including the content of folacin displaying a multi-directional activity in the organism, is of a great importance.

MATERIALS AND METHODS

Three successive processes of production of the skimmed milk tvarog produced according to the Polish Standard PN-68/A-86300, were investigated.

Folacin content was determined in:

— raw milk designed for the production of tvarog,

— pasteurised milk (72-73°C for 15-17 seconds) — before starter addition,

— milk after finished fermentation,

— fresh curd,

— whey left from the production of curds,

— tvarog stored for 10 days at room temperature,

— tvarog stored for 10 days at refrigeration temperature (+3°C).

Changes in folacin content in milk due to the development of single and mixed lacitic acid streptococci cultures in the starter were also investigated. Test included a mixed culture used in industrial production and strains contained in it, i.e.: *Streptococcus lactis* 190, *Streptococcus cremoris* 331 and *Streptococcus diacetylactis* 209; 265; 297. These cultures were taken from from the collection of the Dairy Biopreparations Production Laboratory (Zakład Produkcji Biopreparatów Mleczarskich) in Olsztyn, Poland.

The procedure was as follows: Milk was inocculated with a 18-24-hour culture in an amount of 1%, and it was then incubated at 25°C for 48 hours. The content of folacin was determined after 6, 12, 18, 24 and 48 hours. At the same time, the acidity of milk was determined by titration with 0.1 n NaOH and expressed as the per cent of lacit acid; the development of cultures was controlled by the reductase test with resazurin after Löber [8].

Folacin was determined by the microbiological method [9] with the *Streptococcus faecalis* ATCC 8043 used as the test organism. The results

obtained with this strain are slightly lower than those obtained with the use of *Lbc. casei* ATCC 7469 strain, because they do not embrace the methylic derivatives of the folic acid. Neverthelles the employment of the method with the *Str. faecalis* strain, applied widely in analytical laboratories [10] has made it possible to compare the authors' results with the investigations of other researches. Determination was carried out on the Difco No 0318 medium [3]. The growth of the test strain was determined by the turbidimetric method measuring the degree of turbidity with a SPECOL photocolorimeter, the wawelength being 560 nm.

To liberate the bounded forms of the folic acid, enzymatic hydrolysis was used with the coniugase obtained from the chicken's pancreas [9]. The procedure was as follows: 100 ml of milk or 10 mg of tvarog were emulsified with 5 ml of phosphate buffer of pH 7 and, in the case of cheese, the emulsion was topped up with redistilled water to a volume of 100 ml. Then the samples were heated in a water bath of temperature of 100°C for a period of 3 minutes. After cooling, 2 ml of fresh chicken pancreas extract was added and the whole was subjected to digestion at 37°C for 24 hours. Afterwards the hydrolysate was heated for 5 minutes at 100°C to inactivate the enzymes. Before the determination of folacin the hydrolysate was filtered and its pH was brought to 6.8 with the use of sodium hydroxide.

RESULTS AND DISCUSSION

The results of three successive tvarog production processes investigated were similar, which is illustrated in Fig. 1. Raw skimmed milk contained 26.1 μg folacin/l on the average — its content varied from 25.2 to 28.0 μg/l.

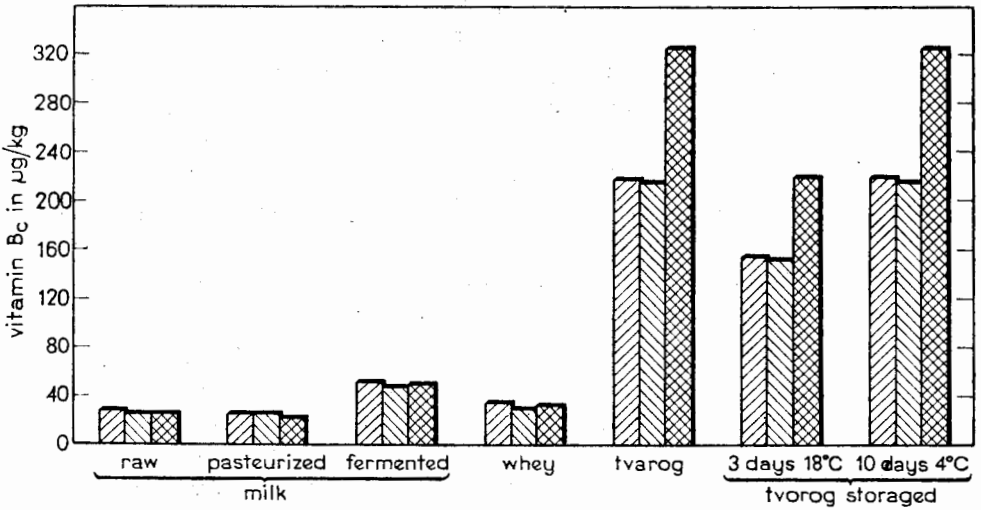

Fig. 1. Changes in the content of folacin in the course of production and storage of the tvarog

The content of folacin in pasteurised milk was 22.6 μg/l, on the average. Thus the heat treatment of milk was responsible for a 13.5% decrease in the content of this vitamin. This is confirmed by the observations of Karlin [6]. A high increase in the content of folacine — by 89.6% in relation to raw milk and by 119% in relation to pasteurised milk was observed in samples taken at the moment when the milk acidification process came to an end. The samples tested contained 49.5 μg/l of folacin on the average.

The average content of folacin in fresh curd was 255 μg/l per 1 kg (varying from 218 to 328 μg/l), folacine content in whey amounted to 31.5 μg/l.

Assuming that 9 kg of tvarog is obtained from 100 l skimmed milk [11], the folacin balance sheet can be presented as follows:

raw milk, 100 l	2,610 mg of folacin
milk after fermentation (100 l)	4,950 mg of folacin
tvarog, 9 kg	2,295 mg of folacin
whey, 91 l	2,867 mg of folacin
total	5,162 mg of folacin

This balance-sheet shows a high concordance of the authors' results with those calculated theoretically. From the figures quoted it results that 44.4% of vitamin remains in the curd, the rest being removed with the whey. This is why the accumulation of this vitamin during fermentation is so important, In spite of the losses of vitamin removed with the whey, the tvarog contains only 12% less folacin (88%) than milk from which it has been produced.

A similar content of folacin in the cottage cheese produced by a different technology was indicated by Reif and coll. [13].

Since a human organism needs 0.4 mg of folacine per day (0.1 mg in case of children aged 1-3 and 0.8 mg for pregnant women), the tvarog should be considered as an important supplementary source of this vitamin, especially in the wintertime diet.

The storage of the tvarog for 10 days at the refrigerator temperature (+4°C) did not result ina decrease of the folacin content, whereas after 3 days of storage at the room temperature its content decreased by 29%, which might have been i.a. the result of increased acidity of the product.

In the course of further investigations the starter streptococci were found to be able synthetise folacin in the milk. Individual species and strains were found to greatly differ in the yield of this process (Fig. 2c). The highest content of folacin — 116 μg/l after 18 hours of incubation — was found in milk fermented with the use of a mixed culture (Fig. 3). The productivity of the monocultures varied within a wide range, from 57.0 (*Str. diacetylactis* 209) to 110 μg/l (*Str. diacetylactis* 265).

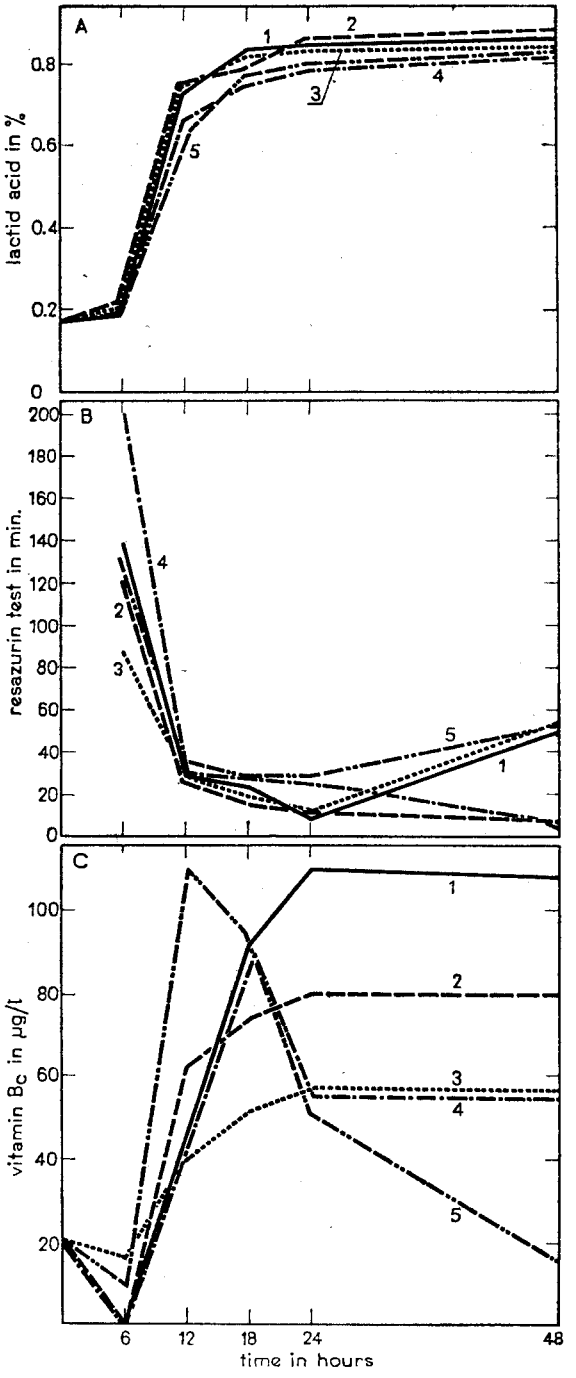

Fig. 2. Activity of ferment streptococci in milk and falacin biosynthesis; A — growth of acidity, B — reduction of resazurin, C — biosynthesis of folacin; 1 — *Str. diacetilactis* 265, 2 — *Str. diacetilactis* 297, 3 — *Str. diacetilactis* 209, 4 — *Str. lactis* 190, 5 — *Str. cremoris* 331

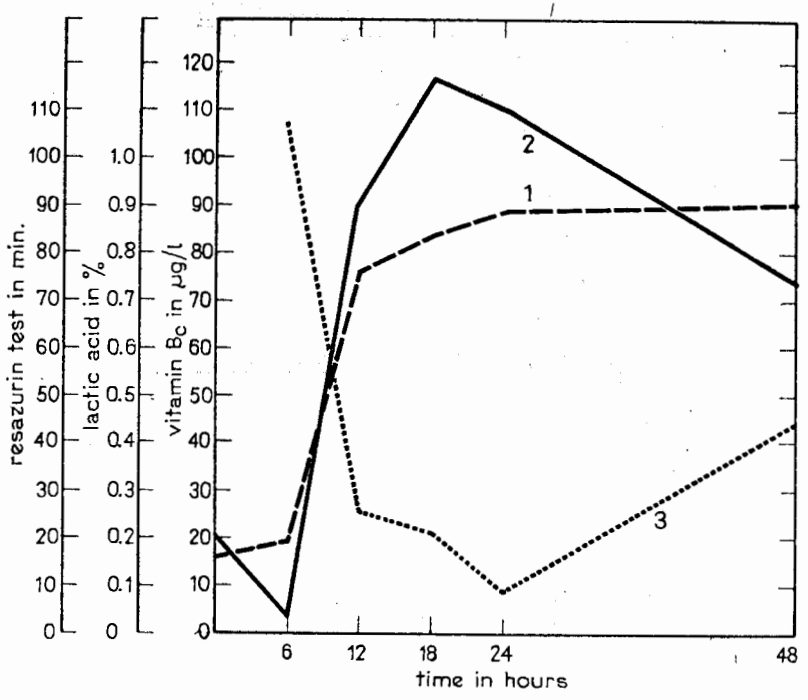

Fig. 3. The biosynthesis of folacin and the activity of the mixed culture in milk; 1 — acidity, 2 — vit. B_c, 3 — resazurin test

In the lag-phase of growth, during the first six hours of incubation, the streprococci utilised the folacin present in the milk, and therefore its content dropped drastically or was completely depleted. During that period no increase in acidity was found, in general (Fig. 2). The synthesis of folacin took place in the logarithmic phase, when the growth of acidity was the highest. The maximum content of synthetised felacin was found after 18-24 hours of incubation (in exceptional cases after 12 hours) after the accumulation of cells but with the culture still maintaining its full activity. This is confirmed by the shortest resazurin reduction time in the reductase test (Fig. 2b). Extension of the time of incubation to 48 hours resulted in a decrease of folacin content or had no effect at all on the content of this vitamin.

The information concerning the biosynthesis of folacin by the streptococci is of a preliminary character, but even so it explains the role of these bacteria in the cumulation of folacin in the fermented dairy products. The information collected also indicates to a possibility of increasing the folacin content in some fermented products ba appropriate selection of strains featuring a high ability to carry out biosynthesis. From the practical point of view it is important that the product be not over acidified, because this may lead to a loss of this vitamin.

GENERAL CONCLUSIONS

1. Microbiological processes play a decisive part as regards the content of folacin in tvarog; during fermentation the content of this vitamin in milk grows by more than 100%.

2. The lactic acid streptococci present in the starters synthetise folacin in the logarithmic phase of growth; individual speciec and strains greatly differ by the yield of the biosynthesis.

3. Mixed streptococci cultures show a higher folacin biosynthesis activity compared with the monocultures.

4. Some 44% of folacin remains in fresh curd, the rest is removed with the whey.

5. No folacin losses were found to occur during the storage of the tvarog under refrigerating conditions.

LITERATURE

1. Černa J., Hrabowa H.: Milchwiss., 1977, 32 (5), 274.
2. Dawidow R. B., Gulko L. E., Krugłowa L. A., Fajgar B. I.: Mołoko i mołocznyje produkty kak istocznik witaminow. Izdatielstwo "Piszczewaja Promyszlennost", Moskwa 1972.
3. Difco supplementary literature — Difco Laboratories, Detroit, Michigan 1964, 166.
4. Gulko L. E.: Dokłady TSCHA Zoot., 1971, 167, 94.
5. Hoppner K., Lampi F., Perrin D.E.: Can. Inst. Food Technol. J., 1972, 5 (2), 60.
6. Karlin R.: Vitaminforsch. 1964, 39 (4), 359.
7. Karlin R.: Prum. Potravin 1964, 15, 529.
8. Löber H.: Milk Plant Monthly 1950, 39, 40.
9. Myszkowska K., Tautt J., Tuszyńska St., Woźnika W.: Mikrobiologiczne metody badania witamin grupy B, WPLiS, Warszawa, 1963.
10. Official Methods of Analysis of the Association of Official Analytical Chemists, W. Horwitz ed. 10th Ass. Offic. Anal. Chemists, Washington, D. C., 1965, 770.
11. Pijanowski E.: Zarys chemii i technologii mleczarstwa, 2, PWRiL, Warszawa 1974, 492.
12. Reddy K. P., Shahani K. M., Kulkarni S. M.: J. Dairy Sci., 1976, 59 (2), 191.
13. Reif G. D., Shahani K. M., Valil J. R., Crowe L. K.: J. Dairy Sci., 1976, 59 (3), 410.
14. Shahani K. M., Hathaway I. L., Kelly P. L.: J. Dairy Sci., 1962, 45 (7), 833.

Manuscript received: July, 1978
Author address: 10-537 Olsztyn-Kortowo

B. Czarnocka-Roczniakowa, Z. Drewek, E. Kopczyńska

ZMIANY ZAWARTOŚCI FOLACYNY W TOKU PRODUKCJI NIE DOJRZEWAJĄCYCH SERÓW TWAROGOWYCH

Instytut Inżynierii i Biotechnologii Żywności, AR-T, Olsztyn

Streszczenie

Badano zmiany zawartości folacyny w toku produkcji twarogu otrzymywanego metodą kwasową. Mleko przeznaczone na twarogi zawierało średnio 26.1 μg folacyny w 1 l. Pasteryzacja w temp. 72-73°C przez 15 s powodowała obniżenie poziomu tej witaminy średnio o 13,5%.

Podczas fermentacji zawartość folacyny wzrastała o 119%, co miało decydujący wpływ na jej poziom w gotowym produkcie. Przeprowadzony bilans wykazał, że w świeżym twarogu pozostaje 44.4% folacyny, a reszta jest usuwana z serwatką. Przechowywanie twarogu przez 10 dni w temperaturze chłodniczej praktycznie nie wpłynęło na poziom badanej witaminy, natomiast już po 3 dniach przetrzymywania w temperaturze pokojowej straty wynosiły 29%.

W celu wyjaśnienia roli paciorkowców zakwasu w kształtowaniu zawartości folacyny w mleku poddano badaniom mieszaną hodowlę stosowaną w produkcji twarogów oraz monokultury wchodzące w jej skład: *Streptococcus lactis* 190, *Streptococcus cremoris* 331 oraz *Streptococcus diacetylactis* 209, 265, i 297. Wykazano, że paciorkowce mlekowe zakwasu syntetyzują folacynę w logarytmicznej fazie wzrostu, przy czym poszczególne gatunki i szczepy znacznie różnią się wydajnością biosyntezy. Mieszana hodowla wykazała wyższą aktywność biosyntezy w porównaniu z monokulturami wchodzącymi w jej skład.