Effect of various starters on the quality of Kefalotyri cheese

E.M. Anifantakis, S.E. Kaminarides

Le Lait, 1987, *67* (4), 527-536

Effect of various starters on the quality of Kefalotyri cheese

E.M. ANIFANTAKIS, S.E. KAMINARIDES

Agricultural College of Athens, Votanikos, Athens, Greece.

Summary

The effect of 3 combinaisons of lactic acid bacteria, namely A = *Streptococcus thermophilus* + *Lactobacillus bulgaricus*, B = *Streptococcus thermophilus* + *Lactobacillus bulgaricus* + *Lactobacillus casei*, C = *Streptococcus lactis* + *Lactobacillus bulgaricus* + *Lactobacillus casei*, on the chemical composition, microflora and organoleptic characteristics of Kefalotyri cheese was studied here in. Significant differences in their pH and water soluble nitrogen during all stages of ripening were determined. Some differences were also observed in the size and the tendency of the total counts and the lactobacilli of the various cheeses, during their ripening. All combinations of bacteria gave good quality of cheese, with type A combination surpassing all others.

Key words : Kefalotyri cheese - Lactic acid cultures - Maturation.

Résumé

Influence des différents ferments lactiques sur la qualité du fromage Kefalotyri

L'influence des levains mixtes, A = *Streptococcus thermophilus* + *Lactobacillus bulgaricus*, B = *Streptococcus thermophilus* + *Lactobacillus bulgaricus* + *Lactobacillus casei*, C = *Streptococcus lactis* + *Lactobacillus bulgaricus* + *Lactobacillus casei*, sur la composition chimique, l'évolution de la flore totale ainsi que les caractères organoleptiques a été étudiée. Nous avons constaté des différences significatives de pH, et de teneur en azote soluble pendant la maturation avec les différents ferments.

Les fromages faits avec le levain A, avaient un pH plus haut et une quantité plus faible d'azote soluble en comparaison avec ceux qui avaient été faits avec les ferments B et C, ces derniers ne présentant aucune différence entre eux. Nous avons aussi constaté des différences pendant la maturation de la population des microorganismes ainsi que du nombre des lactobacilles. Tous les fromages étaient de bonne qualité, mais les meilleurs étaient ceux qui avaient été faits avec les ferments A.

Mots clés : Fromage Kefalotyri - Ferment lactique - Maturation.

Introduction

Kefalotyri is a heavy salted hard Greek cheese traditionally made of sheep milk or mixture of sheep and goat milk without using starters. Last years it is also made from cow's milk using 1-2 % yogurt as starter but the quality of this cheese is lower than that of the traditional. It has strong flavour and small holes throughout its body.

Several research papers have been published, concerning the technology, the chemical composition and the organoleptic characteristics of Kefalotyri cheese (ZYGOURIS, 1952 ; BALTADJIEVA, 1967 ; ANIFANTAKIS, 1976 ; VEINOGLOU *et al.*, 1981).

This paper is dealing with the effect of 3 combinations of lactic acid bacteria to the composition, microflora and quality of Kefalotyri cheese.

I. Material and methods

A. *Milk*

Pasteurised sheep milk without antibiotics and with fat fixed at 5.7 % was used in the experiments.

B. *Starters*

Three combinations of lactic acid bacteria were used, which were obtained from Chr. Hansen's Laboratory, Denmark, namely A = *Streptococcus thermophilus* 0.5 % + *Lactobacillus bulgaricus* 0.5 %, B = *Streptococcus thermophilus* 0.5 % + *Lactobacillus bulgaricus* 0.25 % + *Lactobacillus casei* 0.25 %, C = *Streptococcus lactis* 1.0 % + *Lactobacillus bulgaricus 0.25* % + *Lactobacillus casei* 0.25 %. The average acidities of *Streptococcus thermophilus, Streptococcus lactis, Lactobacillus bulgaricus* and *Lactobacillus casei* were 0.76 %, 0.83 %, 1.12 % and 1.14 % as lactic acid respectively.

C. *Cheese preparation*

The technology described by ZYGOURIS (1952) was followed. Fifteen cheese preparations were made in total, at 5 different dates. Each date the cheeses were made of the same milk by using the 3 combinations of bacteria as mentioned above. They are called A, B and C-cheeses in reference to the combination of bacteria used for their preparation.

Chemical analysis of all cheeses was made during their various stages of ripening, namely 5, 15, 30 and 90 days after preparation.

Their total viable counts, lactobacilli and coliforms were also determined.

D. *Methods of analysis*

The sodium chloride, moisture and total nitrogen content of the cheeses was determined, respectively, according to the International Dairy Federation Standards n° 4 (1958), 17 (1960) and 25 (1964), their soluble nitrogen using Kosikowski's method (1977) and their fat content using Gerber - Van Gulik's method (SCHNEIDER, 1954).

The total counts and the lactobacilli were determined using the methods of the American Public Health Association (1967), and the coliforms according to the International Dairy Federation, Standard n° 73 (1974).

The following media, incubation temperatures and times were used.

— Milk agar (Oxoid) at 30 °C/72 h for the total counts.

— M.R.S. agar at pH 5.7 (Oxoid) and 37 °C/48 h for the lactobacilli.

— Mac Conkey broth (Oxoid) at 37 °C/48 h for the coliforms.

The statistical analysis of the experimental data (randomized complete block design, F test and Duncan's new multiple range test) was done according to STEEL and TORRIE (1960).

E. *Quality assessment*

The ripe cheeses were subjected to quality panel assessment according to LARMOND (1977). A comparison of the cheeses made using the three combinations of lactic acid bacteria was based on 45 points to include the indices, taste, 0-15 ; texture, 0-10 ; consistency, 0-10 ; colour, 0-5 ; and external appearance, 0-5.

The statistical significance of the differences observed of the various quality characteristics of the cheeses produced was evaluated by using the multiple comparisons test (LARMOND, 1977).

II. Results and discussion

Table 1 presents the results of the chemical analysis of all kind of cheeses that have been prepared using the 3 different bacteria combinations, during their various stages of ripening. In the same table the results of their comparison are also given from which the following are concluded.

— There was no statistical significant difference in the cheese yield, at 5 % level, among the three treatments in all their stages of ripening. All combinations of bacteria cultures produced very similar cheese quantities.

— There was statistical significant difference in the pH among the various cheeses. The A-cheeses had in all cases statistically higher pH values than the B and C-cheeses (table 1 and fig. 1). There was no statistical significant difference among the pH value of B and C-cheeses.

TABLE 1

Chemical composition of Kefalotyri cheeses made using the following bacterial cultures :

Composition chimique des fromages Kefalotyri obtenus à l'aide des levains suivants :

A = Streptococcus thermophilus + Lactobacillus bulgaricus, B = Streptococcus thermophilus + Lactobacillus bulgaricus + Lactobacillus casei, C = Streptococcus lactis + Lactobacillus bulgaricus + Lactobacillus casei

Treatment	Ripening time (days)															
	5				15				30				90			
	A	B	C	Significance	A	B	C	Significance	A	B	C	Significance	A	B	C	Significance
pH	5.15 ± 0.03	4.81 ± 0.04	4.83 ± 0.05	S	5.13 ± 0.03	4.75 ± 0.03	4.75 ± 0.05	S	4.96 ± 0.03	4.70 ± 0.04	4.70 ± 0.02	S	4.95 ± 0.04	4.89 ± 0.04	4.78 ± 0.03	S
Total N %	3.85 ± 0.01	3.80 ± 0.03	3.85 ± 0.03	N/S	3.91 ± 0.02	3.88 ± 0.01	3.78 ± 0.01	N/S	4.00 ± 0.03	3.98 ± 0.02	3.99 ± 0.02	N/S	4.06 ± 0.05	4.10 ± 0.02	4.11 ± 0.02	N/S
Soluble N as % of total N	7.53 ± 0.72	11.58 ± 1.07	11.95 ± 0.71	S	10.23 ± 0.76	14.95 ± 0.30	14.47 ± 0.19	S	13.00 ± 1.24	16.58 ± 0.32	16.79 ± 0.70	S	15.52 ± 0.85	19.27 ± 1.34	18.73 ± 0.74	S
Yield %	18.70 ± 0.21	19.13 ± 0.25	19.13 ± 0.27	N/S	18.03 ± 0.22	18.52 ± 0.28	18.65 ± 0.24	N/S	17.83 ± 0.55	17.74 ± 0.27	17.84 ± 0.23	N/S	15.86 ± 0.19	16.03 ± 0.30	16.01 ± 0.32	N/S
Moisture %	—	—	—	—	—	—	—	—	—	—	—	—	36.53 ± 0.37	34.99 ± 0.67	34.77 ± 0.56	N/S
Fat	—	—	—	—	—	—	—	—	—	—	—	—	29.87 ± 0.33	30.98 ± 0.49	30.91 ± 0.63	N/S
Fat on dry matter %	—	—	—	—	—	—	—	—	—	—	—	—	47.06 ± 0.39	47.66 ± 0.57	47.37 ± 0.60	N/S
NaCl %	—	—	—	—	—	—	—	—	—	—	—	—	3.86 ± 0.09	3.60 ± 0.31	3.81 ± 0.16	N/S

Means ± Standard error, S : Significant difference ($P > 0.05$), N/S : No significant difference.

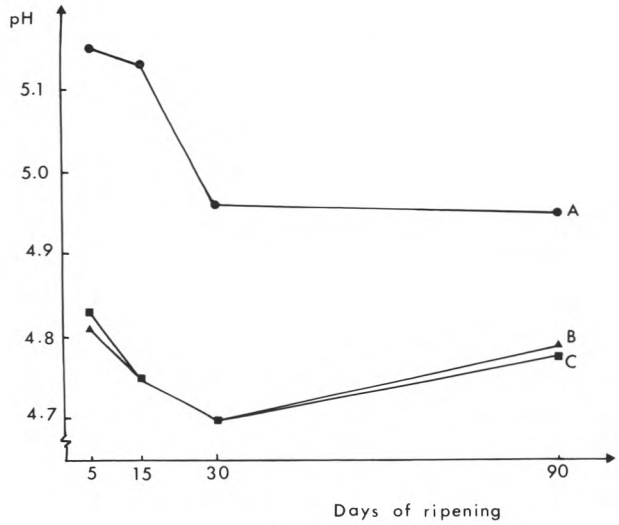

Fig. 1

pH of Kefalotyri cheese made with different starters.

pH du fromage Kefalotyri fabriqué avec différents levains :

A = Streptococcus thermophilus + Lactobacillus bulgaricus, B = Streptococcus thermophilus+ Lactobacillus bulgaricus + Lactobacillus casei, C = Streptococcus lactis + Lactobacillus bulgaricus + Lactobacillus casei.

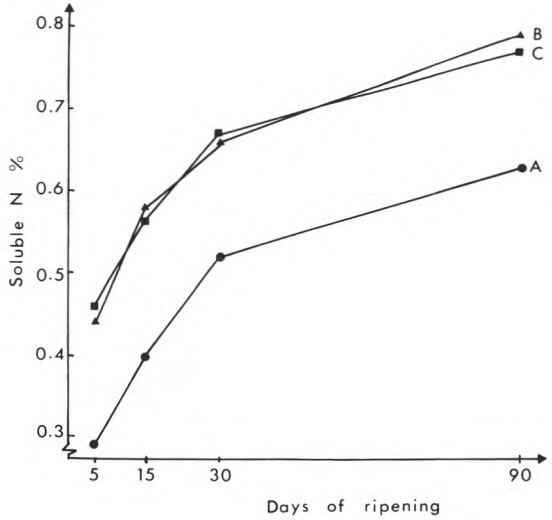

Fig. 2

Soluble nitrogen of Kefalotyri cheeses made by using different starters.

Proportion d'azote soluble des fromages Kefalotyri obtenus avec différents levains.

A = Streptococcus thermophilus + Lactobacillus bulgaricus, B = Streptococcus thermophilus+ Lactobacillus bulgaricus + Lactobacillus casei, C = Streptococcus lactis + Lactobacillus bulgaricus + Lactobacillus casei.

— Statistical significant difference was observed in the water soluble nitrogen of the cheeses, while their total nitrogen does not exhibit such differences (table 1 and fig. 2). The A-cheeses, have less water soluble nitrogen than B and C-cheeses. Among B and C-cheeses no differences have been observed.

It means that in the first case the proteolysis of the casein into water soluble constituents was done at a slower rate.

This difference could be attributed to the presence of *Lactobacillus casei* which is known to have a strong proteolytic system (NAGUIB *et al.*, 1980). The contribution of the pH to this proteolytic action must be also important. In the case of B and C-cheeses, pH is lower, approaching more the optimum value for proteolytic action of the rennet, a fact that favours the general proteolysis in cheese.

— The ripening coefficients, (% water soluble nitrogen x 100/ % total nitrogen), of the B and C-cheeses were significantly higher than that of A-cheeses and this in all stages of the ripening.

— The mature cheeses that have been made with the three bacteria combinations are classified after the Greek Food Regulations (Greek Ministry of Finance, 1972) in the first class, according to their moisture and fat content.

The total colony counts of the cheeses are presented in figure 3. All cheeses contained at the first day after their manufacture about the same number of bacteria, which progressively develops differently. In the A-cheeses the total colony counts increased slowly till the 30th day and decreased slowly afterwards. In the C-cheeses, continuous decrease of the total colony counts is observed while in the B-cheeses a slight increase till the 5th day and then slowly decrease occured.

In figure 4 are presented the viable number of lactobacilli recovered on M.R.S. from Kefalotyri cheeses made by using the three types of starters. All cheeses contained a high number of lactobacilli since the first day of their preparation, which increased till the 30th day and then was almost constant till their full ripening. The A-cheeses contained at the beginning of their ripening a smaller number of lactobacilli types which afterwards increased more rapidly than in the other type of cheeses.

The above mentioned results for the total colony counts and the viable number of lactobacilli of Kefalotyri cheese are similar to those obtained by ORDONEZ *et al.* (1978) for Manchego cheese.

The existence of coliforms in the various type of cheeses was also investigated. It was found that only few cheeses contained 10 to 10^3 coliforms at the beginning of their ripening, which can be considered as contaminants. Coliforms have never been observed in the ripe cheese.

Organoleptic examinations have shown that all combinations of lactic acid bacteria gave good quality of Kefalotyri cheese but among them there are significant differences (table 2). The best quality of cheese was obtained with the A combination, followed by B and C. The A-cheeses had better elasticity and texture than B and C-cheeses. The latter types of cheeses lack elasticity, brake down easily and are recommended only for grinding and using with macaronies.

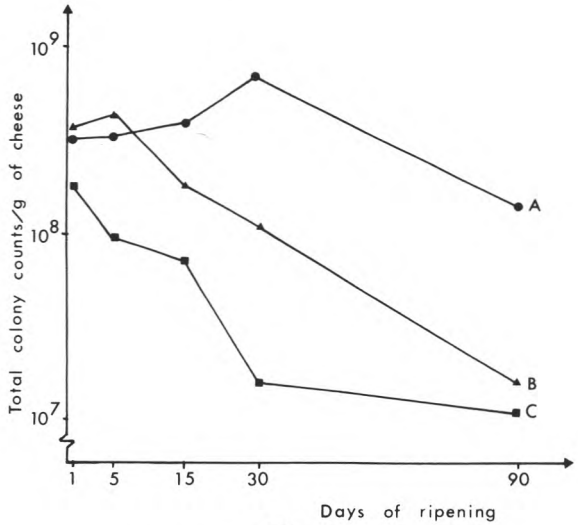

Fig. 3

Total colony counts of Kefalotyri cheeses made by using different starters.

Population bactérienne des fromages Kefalotyri fabriqués avec différents levains.

A = Streptococcus thermophilus+ Lactobacillus bulgaricus, B = Streptococcus thermophilus+ Lactobacillus bulgaricus + Lactobacillus casei, C = Streptococcus lactis + Lactobacillus bulgaricus + Lactobacillus casei.

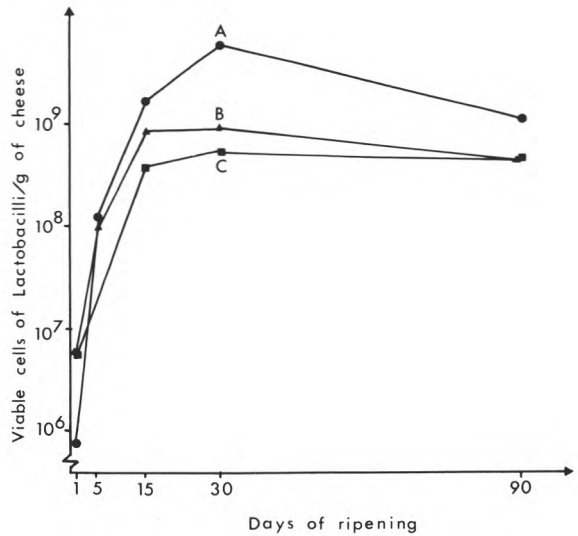

Fig. 4

Lactobacilli recovered on MRS from Kefalotyri cheeses made using different starters.

Dénombrement sur MRS de lactobacilles présents dans les fromages Kefalotyri fabriqués avec différents levains.

A = Streptococcus thermophilus + Lactobacillus bulgaricus, B = Streptococcus thermophilus+ Lactobacillus bulgaricus + Lactobacillus casei, C = Streptococcus lactis + Lactobacillus bulgaricus + Lactobacillus casei.

TABLE 2

Quality assessment of Kefaloryri cheeses made using the bacteria culture (means of 25 values)

Qualité organoleptique des fromages Kefalotyri fabriqués avec différents levains (moyenne de 25 valeurs) : A = Streptococcus thermophilus + Lactobacillus bulgaricus, B = Streptococcus thermophilus + Lactobacillus bulgaricus + Lactobacillus casei, C = Streptococcus lactis + Lactobacillus bulgaricus + Lactobacillus casei

Assessment	Combinations of lactic acid bacteria			
	A	B	C	Significance
Taste	12.98 ± 0.13	12.66 ± 0.08	12.12 ± 0.13	N/S
Consistency	9.10 ± 0.05	8.26 ± 0.03	8.02 ± 0.11	S
Texture	9.12 ± 0.06	7.84 ± 0.09	7.28 ± 0.30	S
Colour	4.62 ± 0.05	4.36 ± 0.04	3.78 ± 0.25	N/S
Appearance	4.88 ± 0.03	4.86 ± 0.03	4.76 ± 0.05	N/S
Total	40.70 ± 0.16	37.98 ± 0.14	35.98 ± 0.77	S

Means ± Standard error.
S : Significant difference ($P < 0.05$).
N/S : No significant difference

Conclusion

From the results given it was shown that there were not statistical significant differences, at the level of 5 %, in the yield and the chemical composition -total proteins, fat, moisture and salt- among the cheeses made by using the three combinations of the lactic acid cultures, A, B, and C. On the contrary significant differences were found in pH and the ratio SN/TN throughout the ripening period among these cheeses. The A-cheeses had significant higher pH and lower SN/TN values than the B and C-cheeses between of which there were not significant differences. The higher proteolysis in B an C-cheeses could be attributed to the presence of *Lactobacillus casei* in these cheeses as well as their lower pH which approach more to the optimum for the rennet activity.

Some differences were also found in the size and the tendency of the total counts and the lactobacilli of cheeses during their ripening period. The organoleptic examinations of the cheeses have shown that all combinations of the lactic acid bacteria used, gave good quality of cheese with type A combination surpassing all the others.

Reçu le 24 octobre 1986.
Accepté pour publication le 7 mars 1987.

Acknowledgements

The authors thank Mrs Katsarou Kerkira for her technical assistance.

References

American public Health association, 1967. *Standard methods for the examination of dairy products,* 12th Ed, 34-52.

ANIFANTAKIS E.M., 1976. Influence d'une présure d'agneau sur la qualité du fromage Kefalotyri. *Lait, 56,* 76-83.

BALTADJIEVA M., 1967. Studies on the technology of Kefalotyri cheese produced in Bulgaria. Doctor thesis, Institute of Food Industries, Plovdid, Bulgaria.

Greek ministry of finance, 1972. *Code of foods, drinks and other subjects of common use*, 160.

International dairy federation, 1958. International Standard n° 4.

International dairy federation, 1960. International Standard n° 17.

International dairy federation, 1964. International Standard n° 25.

International dairy federation, 1974. International Standard n° 73.

KOSIKOWSKI F., 1977. *Cheese and fermented milk foods*, 2nd Ed, Edwards Brothers, Inc., Michigan, USA, 568.

LARMOND E., 1977. *Laboratory methods for sensory evaluation of food*, Canada Department of Agriculture, Research publication n° 1637, Ottawa, Canada, 31.

NAGUIB M.M., NOUR M.A., TOHAMY M.M., HOFI A.A., 1980. Justification of the use of lactic starter and micrococci for the dairy industry. *Egypt. J. Dairy Sci., 8*, 1-7.

ORDONEZ J.A., BARNETO R., RAMOS M., 1978. Studies on Manchego cheese ripened in olive oil. *Milchwissenchaft, 33,* 609-613.

SCHNEIDER C., 1954. *Traité pratique des essais du lait et du contrôle de produits laitiers.* Imp. Edit, Les Hoirs C.J., Wyss S.A., Berne, 140.

STEEL R.G.D., TORIIE J.H., 1960. *Principles and procedures of statistics with special reference to the biological sciences*. Mc Graw-Hill Book Company Inc, New York - Toronto-London, 107-109 and 132-139.

VEINOGLOU B., BALTADJIEVA M., ANIFANTAKIS E., EDGARYAN M., 1981. Kefalotyri cheese. *Dairy Ind. Int., 46*, 7-13.

ZYGOURIS N., 1952. *The milk industries,* 2nd Ed, Greek Ministry of Agriculture, 511-543.