

Effect of starters on gross and microbiological composition and organoleptic characteristics of Graviera Kritis cheese

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Abstract — The use of different starter cultures in the manufacture of Graviera Kritis cheese for improving its overall quality was investigated. Mixtures of *Streptococcus thermophilus*, *Lactobacillus helveticus* (1:1) and *Propionibacterium freundreichii* subsp. *shermanii* (cheese A); *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *S. thermophilus*, *L. helveticus* (1:1:10:2) and *P. freundreichii* subsp. *shermanii* (cheese B); and *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *S. thermophilus*, *L. helveticus* (2.5:2.5:1:1) and *P. freundreichii* subsp. *shermanii* (cheese C) were used and compared with cheese D made without starter cultures. The gross composition of the matured cheeses A, B and C was not significantly ($P < 0.05$) affected, although their pH values and their ash and salt contents were significantly ($P < 0.05$) lower than that of cheese D at the beginning of ripening. Regarding the cheese microflora, coliforms were not found in cheeses A, B and C after 60 d of ripening, while they were still detectable in matured (90 d) cheese D. Enterococci counts in matured cheeses A, B and C were significantly ($P < 0.05$) lower than in cheese D, while the other groups of microorganisms were not significantly affected. Finally, no significant difference ($P < 0.05$) was detected in the flavour, body and texture of the cheeses made with the starter cultures. © Inra/Elsevier, Paris.

Graviera Kritis cheese / starter culture / gross composition / microbiological composition

Résumé — Influence des levains sur la composition grossière et microbiologique et les caractéristiques sensorielles du fromage Graviera Kritis. Le Graviera Kritis est un fromage grec d'« appellation d'origine contrôlée » (AOC) qui traditionnellement subit une fermentation propionique limitée sans ajout de levains au lait de fabrication. L'utilisation de différents levains en production de fromage Graviera Kritis a été examinée afin d'améliorer sa qualité globale. Des combinaisons de *Streptococcus thermophilus*, *Lactobacillus helveticus* (1:1) et *Propionibacterium freundreichii* subsp. *shermanii* (fromage A) ; *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*,

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S. thermophilus, *L. helveticus* (1:1:10:2) et *P. freundreichii* subsp. *shermanii* (fromage B) ; et *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *S. thermophilus*, *L. helveticus* (2.5:2.5:1:1) et *P. freundreichii* subsp. *shermanii* (fromage C) ont été utilisées et comparées au fromage D fabriqué sans levains. La composition grossière des fromages affinés n'a pas été significativement influencée par l'emploi des levains. En revanche, en début d'affinage le pH et la concentration en cendres et en sel des fromages A, B et C étaient significativement ($p < 0,05$) inférieurs à ceux du fromage D. En ce qui concerne les caractéristiques microbiologiques, aucun coliforme n'a été détecté dans les fromages A, B et C pendant les 60 jours d'affinage alors que ces bactéries étaient encore détectables dans le fromage D affiné (90 jours). De même, le nombre des entérocoques était significativement ($p < 0,05$) inférieur dans les fromages A, B et C par rapport au fromage D. En revanche, les autres groupes de microorganismes n'étaient pas significativement influencés. Aucune différence significative ($p < 0,05$) n'a été constatée quant aux caractéristiques organoleptiques des fromages. © Inra/Elsevier, Paris.

fromage Graviera Kritis / levain / composition / microbiologie

1. INTRODUCTION

Graviera Kritis is a Controlled Denomination of Origin cheese [8] which is produced in Crete island of Greece. It is a Gruyère-type cheese which, however, undergoes a limited propionic acid fermentation and so differs from the Swiss-type cheeses in many ways.

The typical Swiss-type cheeses such as Emmentaler, Gruyère and Appenzeller are made from cows' milk using starter cultures which contain thermophilic lactic acid bacteria such as *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *lactis* and/or *Lactobacillus bulgaricus*. Propionibacteria (mainly *Propionibacterium freundenreichii* subsp. *shermanii*) are also used. They give to these cheeses their characteristic sweet flavour and eyes in the cheese body [27].

The Greek Gruyère-type cheeses are little studied and so the available literature for their characteristics is very scarce [28, 1, 29]. A typical composition of Greek Gruyère-type cheese made from ewes' milk, scalded at 48–50 °C and ripened for 90 days is usually the following: pH 5.7, dry matter 64.9%, fat in dry matter 54.6% and salt in moisture 3.6% [18]. The gross composition and the native microflora of Graviera Kritis cheese have never been studied before.

Graviera Kritis is traditionally made from ewes' or a mixture of ewes' milk with low percentage of goats' milk heated up to 68–70 °C. None starter cultures are added and the cheese curd is heated to 50–52 °C. The cheese wheel is salted in brine 16–20% for 4 to 5 d, ripens at 15–16 °C for 90 d and has a rind with surface smear. The matured cheese wheel usually has a diameter of 40 cm and a weight of 14–16 kg. Finally, because of the type of milk and the technology used, Graviera Kritis cheese has a distinctive but not sweet flavour, non-elastic texture and usually lacks openness.

Unfortunately, the overall quality of Graviera Kritis cheese is not always the desired one mainly because of the poor microbiological quality of the raw milk used. Raw milk is usually heavily contaminated by microorganisms, as it is collected without milking machines in faraway farms of a mountainous region and under high temperatures, especially during spring. Thus, the milk combined with the insufficient pasteurization and the absence of starter cultures yield cheeses without standardized overall quality.

The aim of this work was to study the gross composition and the microflora of Graviera Kritis cheese and to improve its characteristics by using starter cultures without, however, changing its typical flavour.

2. MATERIALS AND METHODS

2.1. Cheesemaking

In the spring time, three trials of cheesemakings were carried out in a cheese factory at Rethimnos of Crete, according to the experimental protocol shown in figure 1.

On average, the raw milk contained in colony-forming units (cfu)/mL: aerobic mesophilic microflora 2.5×10^8 , thermophilic streptococci 3.7×10^8 , mesophilic lactobacilli 1.0×10^8 , enterococci 0.3×10^8 , coliforms 1.6×10^7 and propionibacteria 2.0×10^3 . After heating, the cheese milk used had the following average counts of microorganisms: aerobic mesophilic microflora 1.4×10^5 , thermophilic streptococci 0.9×10^5 , mesophilic lactobacilli 0.3×10^5 , enterococci 0.1×10^4 , coliforms 1.4×10^3 and propionibacteria 0.5×10^3 cfu/mL.

Mesophilic and thermophilic lactic acid starters as well as propionic acid starter cultures were used. The mesophilic lactococci *L. lactis*, which produce only L-lactate, were selected to promote the propionibacteria growth, because propionibacteria use L-lactate in preference to D-lactate [10]. The cultures added to cheeses A, B and C were freeze-dried cultures for direct vat inoculation and were obtained from Wiesby (Wiesby GmbH & Co, Niebull, Germany), EZAL- Rhone Poulenc (Dange Saint-Romain, France) and Hansen (Horsholm, Denmark), respectively.

The rennet used was mixture (1:1) of calf rennet 'Hipoprotopoula' (A. Tsakanikas, Greece) and Fermentation Produced Chymosin 'MAXIREN' (Gist-Brocades, France). The pH of milk at which rennet was added was 6.35.

2.2. Microbiological, chemical and organoleptic analyses

All cheeses were analyzed at 1 (after pressing), 13, 30, 60, 90 and 180 d of ripening.

Microflora was measured by the means of the pour plate method using the following differential media:

- Mesophilic aerobic microflora: plate count agar (Biokar, France), at 30 °C for 3 d;
- Thermophilic streptococci: M-17 agar (Biokar) at 37 °C for 2 d;
- Mesophilic lactobacilli: MRS agar (Oxoid, UK), with pH 5.4-5.6, incubated anaerobically at 37 °C for 2 d;

- Propionibacteria: sodium lactate agar with cloxacillin (SLAC) as it is described by Drinan and Cogan [12];

- Coliforms: according to the IDF method [17];

- Enterococci: Kanamycin Aesculin Azide agar (Oxoid, UK) according to Oxoid manual instructions;

- Anaerobic spore-forming microorganisms: with the most probable number method in RCM broth (Biokar, France) incubated anaerobically at 37 °C for 14 d.

For the pH determination, 10 g of cheese was first homogenized with 10 mL distilled water. The dry matter content was determined by heating at 102 °C to constant weight, the ash content by charring at 550 °C to constant weight, the fat content by the Gerber method and the salt content as suggested by Anifantakis [2].

Finally, all cheeses were assessed organoleptically by a ten member panel for the flavour (scale 0-45) and body and texture characteristics (scale 0-35) at 90 and 180 d of ripening.

2.3. Statistical analyses

The microbiological data, expressed as \log_{10} cfu, organoleptical and chemical data were subjected to one way analysis of variance at a 95% level of significance and to multiple range test using the LSD-test (LSD: least significant difference). Furthermore, regression analysis for the data from chemical analyses was applied at the same level of significance. For all data manipulations the statistical software STATGRAPHICS v.6. was used.

3. RESULTS AND DISCUSSION

3.1. Gross composition

It has been shown that the level of *S. thermophilus* and the strain of lactobacillus used in Swiss-type cheeses affect the pH of curd before and after draining, respectively [24] and that high quality Swiss cheeses usually have pH value 5.64-5.7 [21]. In this experiment, the pH value of cheeses A, B and C made with starter cultures was significantly ($P < 0.05$) lower than the pH of cheese D made without starters at the first day of

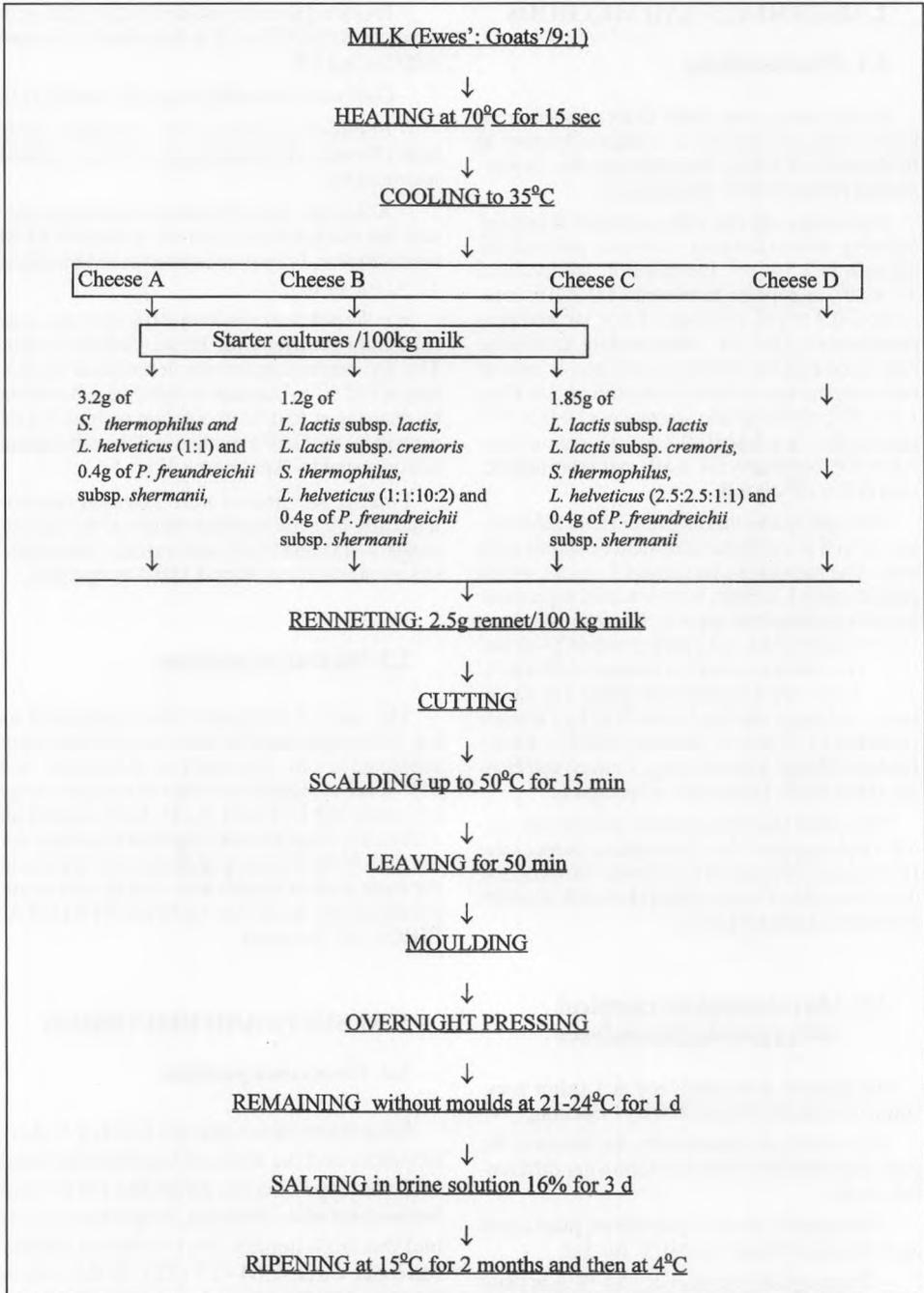


Figure 1. Flow diagram of the manufacture of Graviera Kritis cheese with different mixtures of starter cultures.

Figure 1. Diagramme de fabrication du fromage Graviera Kritis avec différents levains.

Table I. pH, dry matter and ash contents¹ during ripening of Graviera Kritis cheese made with different starter cultures.**Tableau I.** Valeurs¹ du pH, de l'extrait sec et des cendres pendant l'affinage du fromage Graviera Kritis fabriqué avec différents levains.

Time (d)	pH				Dry matter %				Ash %			
	A ²	B ³	C ⁴	D ⁵	A	B	C	D	A	B	C	D
1	5.07 ^{a*}	5.08 ^a	5.07 ^a	5.32 ^b	59.92 ^{a,b}	60.08 ^{a,b}	60.70 ^b	57.69 ^a	2.67 ^a	2.73 ^{a,b}	2.64 ^a	2.83 ^b
13	5.21	5.21	5.21	5.22	63.21 ^{a,b}	62.99 ^{a,b}	63.59 ^b	61.65 ^a	3.63	3.64	3.54	3.83
30	5.35	5.27	5.29	5.33	63.78	64.47	63.41	62.97	3.90	4.15	3.91	4.21
60	5.39	5.33	5.37	5.32	65.60 ^b	63.51 ^{a,b}	63.96 ^{a,b}	62.55 ^a	4.08	3.83	3.86	4.00
90	5.53	5.44	5.49	5.52	65.42	64.31	65.31	64.30	4.33	4.16	4.14	4.19
180	5.50	5.49	5.51	5.51	66.20 ^b	65.61 ^{a,b}	65.28 ^{a,b}	64.77 ^a	4.28	4.24	4.36	4.53

¹ Mean values from three trials. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁵ Without starters. *Means in the same row, followed by different superscript differ significantly ($P < 0.05$).

¹ Valeurs moyennes de trois essais. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁵ Sans levains. * Les valeurs moyennes d'une même ligne suivies de lettres différentes diffèrent significativement.

ripening (*table I*) and this difference is attributed to the activity of the thermophilic lactic acid starters added. At the end of ripening, however, pH values of all cheeses were similar to each other, but they were lower than pH of Greek Gruyère made from cows' milk [29] and higher than pH of Gruyère-type cheese made by Anifantakis et al. [1]. Furthermore, the pH changes in cheeses A, B and C were more significantly correlated with the age of the cheese (multiplicative model $r = 0.94$, $r = 0.95$, and $r = 0.95$, respectively) than in cheese D (linear model $r = 0.74$). Thus, pH of cheeses A, B and C increased rapidly during the first 30 d of ripening, while pH of traditional cheese D increased gradually, because lactose was fermented by the native microflora. According to Martley and Crow [25], the non-starter lactic acid bacteria (NSLAB) in a cheese ferment the lactose slowly, increase in numbers and then maintain constant throughout ripening.

The dry matter content of cheese C was significantly ($P < 0.05$) higher than the dry matter content of cheese D at 1 and 13 d of ripening (*table I*). The difference at 1 d is the most important and can be attributed to the higher pH value of cheese D at that time. The pH value affects the ability of casein micelles to absorb water and as pH increases up to the maximum level 5.5 the water absorption also increases [9, 24]. Compared with other Greek Gruyère-type cheese from ewes' milk [28], all matured cheeses of this experiment had slightly lower dry matter content due probably to goats' milk used. Finally, the changes in the dry matter content of all cheeses were significantly correlated with the age of the cheeses (multiplicative model $r = 0.94$ in cheese A, $r = 0.91$ in cheese B, $r = 0.92$ in cheese C and $r = 0.94$ in cheese D).

Significant ($P < 0.05$) differences in the ash content of all cheeses were observed only at 1 d of ripening (*table I*). Cheeses A, B and C had lower ash content than cheese D. This was expected because the rapid pH

decrease in a cheese curd during the stage of draining causes intense removal of colloidal calcium phosphate from the casein micelles and consequently high mineral loss from the curd [6, 24]. The ash contents of cheeses A, B and C were significantly correlated ($r = 0.89$ in cheese A, $r = 0.80$ in cheese B, $r = 0.85$ in cheese C and $r = 0.38$ in cheese D) with the respective pH values, confirming thus the effect of the curd acidification on the mineral removal. The results for the ash content are similar to those obtained by Veinoglou et al. [28], concerning Greek Gruyère-type cheese from ewes' milk. Finally, like the dry matter content, the ash content of all cheeses was significantly correlated with the age of the cheeses (multiplicative model $r = 0.96$ in cheese A, $r = 0.89$ in cheese B, $r = 0.91$ in cheese C and $r = 0.91$ in cheese D).

Cheeses A and B had significantly ($P < 0.05$) lower salt in moisture (S/M) content than cheese D at 13 d of ripening (*table II*). Since no other significant difference was observed in the S/M contents of all cheeses throughout ripening, this difference is rather due to the fact that the salt absorption in the cheese mass was not still equilibrated at that time. Compared with other Swiss-type cheeses, Gravière Kritis cheese has higher S/M content than Gruyère de Comté and Emmental which have about 2.0% and 1.2% S/M, respectively [4, 16]. The S/M content of all cheeses was highly correlated with their age (multiplicative model, $r = 0.93$ in cheese A, $r = 0.93$ in cheese B, $r = 0.92$ in cheese C and $r = 0.88$ in cheese D) and with the respective values of their dry matter contents ($r = 0.75$). This was expected as generally the increase of salt content of a cheese causes moisture removal from it, limits the extent of proteolysis and consequently affects the pH increase in the matured cheese [13, 14, 22, 24].

Finally, the starter cultures did not affect, as expected, the fat in dry matter (FDM) content of the matured Gravière Kritis

Table II. Fat in dry matter and salt in moisture contents¹ during ripening of Graviera Kritis cheese made with different starter cultures.**Tableau II.** Teneurs en matière grasse dans l'extrait sec et en sel dans l'humidité pendant l'affinage du fromage Graviera Kritis fabriqué avec différents levains.

Time (d)	Fat in dry matter %				Salt in moisture %			
	A ²	B ³	C ⁴	D ⁵	A	B	C	D
1	60.90	60.10	58.27	56.36	1.14	1.30	1.11	1.31
13	61.97	61.28	62.32	61.72	2.38 ^{a*}	2.54 ^a	3.30 ^{ab}	4.43 ^b
30	60.19 ^a	61.46 ^{ab}	62.12 ^b	60.20 ^a	4.99	5.36	5.68	6.06
60	57.20	58.50	59.20	59.96	5.57	4.62	5.33	5.02
90	61.14	61.10	63.56	63.75	5.34	5.17	4.94	5.00
180	56.17	55.87	59.42	58.00	5.18	5.23	5.48	5.78

¹ Mean values from three trials. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁵ Without starters. * Means in the same row with different superscript differ significantly ($P < 0.05$).

¹ Valeurs moyennes de trois essais. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. Sans levains. * Les valeurs moyennes d'une même ligne suivies de lettres différentes diffèrent significativement.

cheese (table II). However, the FDM values of all cheeses were high and varied with the cheeses because the fat content of the cheese milk used was not standardized.

3.2. Microbiological characteristics

No anaerobic spore-forming microorganism was found in any cheese and this is rather due to the fact that silage food is not used for sheep and goats breeding on Crete island.

On the other hand, as mentioned in the materials and methods section, the raw milk was heavily contaminated, thus the heated cheese milk contained high numbers of NSLAB and contaminants, i.e., coliforms. In addition, a great part of the NSLAB seemed to survive the scalding at 50 °C as seen in the mesophilic microflora counts of cheese D made without starters (table III). Thus, there was no significant ($P < 0.05$) difference in

the mesophilic aerobic microflora counts of cheeses A, B and C made with the starters and cheese D at 1 d of ripening. This result seems to be in contrast with the pH difference at the same time (table I), but this happened because the thermophilic starters, which are mainly responsible for the pH decrease in this cheese at the beginning of ripening, did not grow as rapidly under the conditions used for the enumeration of the mesophilic aerobic microflora. Also, this group possibly contains the survived mesophilic starters of cheeses A, B and C and an heterofermentative mesophilic microflora of cheese D. Finally, from the 13 d of ripening and after, the 'wild' mesophilic microflora count was significantly higher in cheese D than in the other cheeses.

With regard to presumptive thermophilic streptococci counts, cheeses A, B and C were also significantly differentiated from cheese D at 13 d of ripening (table III). This is probably due to the fact that the 'wild'

Table III. Log₁₀ cfu·g⁻¹ of aerobic mesophilic microflora, thermophilic streptococci and mesophilic lactobacilli counts¹ during ripening of Graviera Kritis cheese made with different starter cultures.

Tableau III. Dénombrements (en Log₁₀ ufc·g⁻¹) de la flore aérobie mésophile, streptocoques thermophiles et lactobacilles mésophiles pendant l'affinage du fromage Graviera Kritis fabriqué avec différents levains.

Time (d)	Aerobic mesophilic microflora				Presumptive thermophilic streptococci				Presumptive mesophilic lactobacilli			
	A ²	B ³	C ⁴	D ⁵	A	B	C	D	A	B	C	D
1	8.30	8.64	8.35	8.61	8.12	8.67	8.14	8.60	7.13 ^a	8.06 ^b	7.62 ^{a,b}	8.40 ^b
13	8.00 ^{a*}	8.17 ^a	7.88 ^a	8.68 ^b	8.06 ^a	8.04 ^a	7.92 ^a	8.71 ^b	7.77 ^a	8.19 ^a	7.86 ^a	8.71 ^b
30	8.02 ^a	8.17 ^a	7.97 ^a	8.47 ^b	8.24 ^{b,c}	8.21 ^{a,b}	7.97 ^a	8.45 ^c	8.14 ^a	8.14 ^a	7.96 ^a	8.53 ^b
60	7.79 ^a	8.22 ^{a,b}	7.98 ^{a,b}	8.46 ^b	8.04	8.23	7.96	8.31	7.85 ^a	8.25 ^{a,b}	8.05 ^{a,b}	8.47 ^b
90	7.91 ^{a,b}	7.97 ^{a,b}	7.72 ^a	8.30 ^b	8.13 ^{a,b}	8.01 ^{a,b}	7.96 ^a	8.35 ^b	8.00	8.01	7.97	8.30
180	7.67	8.06	7.77	7.96	8.00	8.25	7.67	8.03	7.94	8.07	7.71	8.06

¹ Mean values from three trials. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁵ Without starters. * Means in the same row, followed by different superscript differ significantly ($P < 0.05$).

¹ Valeurs moyennes de trois essais. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁵ Sans levains. * Les valeurs moyennes d'une même ligne suivies de lettres différentes diffèrent significativement.

streptococci of cheese D grew slowly up to 13 d of ripening, while the streptococci starters of cheeses A, B and C grew quickly in 1 d of ripening. According to Mocquot [26], in Swiss-type cheeses the thermophilic streptococci usually reach their highest numbers after 3 h of the cheese curd pressing. Zerfiridis et al. [29] found that in Greek Gruyère cheese made with cows' milk and ripened at 12 °C streptococci reached their maximum numbers at 15 d of ripening.

Mesophilic lactobacilli, which constitute the main part of the NSLAB [11, 25], grew more rapidly in cheese D than in cheeses A, B and C (table III). The mesophilic lactobacilli in cheese D reached their maximum count at 13 d of ripening, while in cheeses A, B and C at 30 and/or 60 d of ripening. Therefore, significant ($P < 0.05$) differences in the mesophilic lactobacilli counts among cheese D and the others were observed up to 60 d of ripening.

Taking into consideration the above results, it seems that, the growth of the starters in cheeses A, B and C was partially suppressed by the 'wild' microflora, i.e., NSLAB and coliforms. According to Langsrud and Reinbold [19], even if the milk is sufficiently pasteurized, the initial microbiological quality of the raw milk and the types of microorganisms present in it severely affect the ripening processing and finally the Swiss cheese quality. The high numbers of coliforms in the cheese milk generally inhibit the lactic acid bacteria growth during the manufacture of Gruyère cheese, while *Enterobacter aerogenes* and *E. coli* specifically inhibit the lactobacilli and streptococci growth, respectively. On the other hand, coliforms are sensitive to acid conditions caused in a cheese by lactic acid starter cultures [7]. Therefore, the low pH of cheeses A, B and C at 1 d of ripening (table I) inhibited the coliforms growth and thus, their counts decreased gradually up to 30 d of ripening (table IV). On the contrary, the coliforms counts in cheese D were significantly higher and they were detected up

to 90 d of ripening. Zerfiridis et al. [29] have also reported that the coliforms counts in a Greek Gruyère cheese made with starters decreased dramatically after 4 d of ripening.

Enterococci, which are expected to be a great part of the NSLAB in these cheese types, were present in all cheeses throughout ripening. However, their counts in cheese D were significantly ($P < 0.05$) higher than in cheeses A, B and C (table IV). It is also noteworthy that in cheeses A, B and C enterococci counts decreased after 60 d of ripening, while in traditional cheese D they remained constant throughout ripening. On the contrary, in the Greek Gruyère cheese studied by Zerfiridis et al. [29] enterococci almost disappeared at the end of ripening.

As far as the propionibacteria are concerned, cheese D had significantly ($P < 0.05$) lower counts than cheeses B and C throughout ripening (table IV). The propionibacteria of cheese D were from the native microflora of the raw milk, because many of them survived the heat treatment. According to Gautier et al. [15], propionibacteria can survive the HTST pasteurization, 72 °C for 15 s (HTST: high temperature short time). In a Swiss-type cheese, however, the propionibacteria growth is dependent on several combined factors. Propionibacteria are very sensitive to salt and they need a salt in moisture content less than 2% in order to grow [5, 20, 23]. Moreover, their growth rate and the CO₂ production are greatly affected by the pH of cheese which should be comprised between 5.2 and 5.4 at the first d of ripening [20, 23]. The propionibacteria growth is also dependent on the ripening temperature. They can grow at numbers up to 10⁸–10⁹cfu.g⁻¹ within two months in a 'warm' room with a temperature of 20–22 °C for Emmental cheese and 16–18 °C for Comté cheese [7]. Therefore, propionibacteria in cheeses A, B and C, all made with propionic starter culture, did not grow at levels as high as expected because of, firstly, the high salt in moisture contents and, secondly, the low pH at the first d (table I)

Table IV. Log₁₀-cfu·g⁻¹ of coliforms, enterococci and propionibacteria counts¹ during ripening of Graviera Kritis cheese made with different starter cultures.

Tableau IV. Dénombrements (en Log₁₀-cfu·g⁻¹) des coliformes, entérocoques et bactéries propioniques pendant l'affinage du fromage Graviera Kritis fabriqué avec différents levains.

Time (d)	Coliforms				Enterococci				Propionibacteria			
	A ²	B ³	C ⁴	D ⁵	A	B	C	D	A	B	C	D
1	2.16 ^{a*}	1.93 ^a	2.62 ^a	4.74 ^b	3.95 ^a	5.19 ^b	5.02 ^b	6.38 ^c	3.41 ^a	4.63 ^{a,b}	5.23 ^b	3.63 ^a
13	0.93 ^a	0.63 ^a	0.74 ^a	5.01 ^b	4.02 ^a	4.18 ^a	4.42 ^b	6.68 ^c	3.75 ^a	5.55 ^b	5.33 ^b	3.95 ^a
30	0	0	0.59 ^a	3.69 ^b	6.18 ^{a,b}	4.75 ^a	5.27 ^{a,b}	6.88 ^b	4.73 ^{a,b}	6.05 ^c	5.78 ^{b,c}	4.53 ^a
60			0	2.10	6.49 ^{a,b}	5.98 ^{a,b}	5.29 ^a	6.84 ^b	4.45	6.34	6.30	4.90
90				0.92	4.98	5.36	5.67	6.92	5.35 ^{a,b}	6.10 ^{a,b}	6.71 ^b	5.02 ^a
180				0	3.64 ^a	3.87 ^a	4.11 ^a	6.84 ^b	4.03	4.42	4.44	3.84

¹ Mean values from three trials. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁵ Without starters. * Means in the same row, followed by different superscript differ significantly ($P < 0.05$).

¹ Valeurs moyennes de trois essais. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁵ Sans levains. * Les valeurs moyennes d'une même ligne suivies de lettres différentes diffèrent significativement.

Table V. Flavour, body and texture¹ at 90 and 180 d of ripening of Graviera Kritis cheese made with different starter cultures.**Tableau V.** Flaveur et texture¹ à 90 et 180 j d'affinage du fromage Graviera Kritis fabriqué avec différents levains.

Time (d)	Flavour (0–45)				Body and texture (0–35)			
	A ²	B ³	C ⁴	D ⁵	A	B	C	D
90	37.90	36.30	35.70	34.80	28.20	25.40	26.50	26.90
180	36.33	35.66	32.33	34.75	27.83	26.58	25.00	26.83

¹ Mean values from three trials. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁵ Without starters.

¹ Valeurs moyennes de trois essais. ² *S. thermophilus* + *L. helveticus* (1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ³ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (1:1:10:2) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁴ *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *S. thermophilus* + *L. helveticus* (2.5:2.5:1:1) + *Propionibacterium freudenreichii* subsp. *shermanii*. ⁵ Sans levains.

and the low ripening temperature of these cheeses. In Greek Gruyère cheese, made with propionic starter culture, propionibacteria were also found to be up to 10⁵cfu·g⁻¹ at 45 d of ripening [29].

3.3. Organoleptic characteristics

The different starter cultures used in this experiment did not seem to affect significantly the flavour and the body and texture characteristics of Graviera Kritis cheese. All cheeses had the typical flavour of Graviera Kritis cheese at 90 and 180 d of ripening and thus scored similarly (table V). No sweet taste was observed in any case and this fact coincides with the limited growth of propionibacteria in all cheeses (table IV). The texture of all cheeses was not elastic and apart from a few slits there were no eyes. The texture characteristics could possibly be attributed to the high salt in moisture and dry matter contents and also to the low pH values of the cheeses at the end of ripening, since these parameters seem to affect the eye formation in a Gruyère-type cheese. According to Berdagué and Grappin [3], the eye formation in gruyère de Comté is nega-

tively correlated with the salt ($r = -0.36$) and dry matter ($r = -0.33$) contents, while the split defect intensity is positively correlated with the pH ($r = 0.50$) at the end of ripening.

4. CONCLUSIONS

This work showed that the starters used in the manufacture of Graviera Kritis cheese influenced only the raw milk flora growth and in particular the coliforms and enterococci growth. Consequently, the use of a certain inoculum level of selected starter cultures in the manufacture of Graviera Kritis cheese could be useful in order to control and improve the cheese quality without significantly changing the typical organoleptic characteristics of this cheese.

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