Raw milk flora affects composition and quality of Bergkäse.

1. Microbiology and fermentation compounds

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Abstract — Experimental samples of Bergkäse, an Austrian regional hard cheese variety usually made from raw milk, were produced from raw or pasteurised milks in parallel to evaluate the influence of the indigenous microflora on microbiological composition and selected fermentation compounds. Facultatively heterofermentative lactobacilli (FHL) dominated the non-starter flora in cheeses made from raw milk. Viable numbers increased from 10^4 – 10^6 cfu.g⁻¹ at the age of 1 week to reach their final level of 10^8 cfu.g⁻¹ within the first month of ripening. In contrast, cheeses made from pasteurised milk exhibited FHL counts which were at least three orders of magnitude lower or even undetectable. In the raw milk cheeses, the development of organic acids during maturation differed from that in the pasteurised ones, and was dependent on the number and the metabolic activity of the microorganisms. Differences in the citrate metabolism and in propionic acid fermentation, which occurred in raw milk cheeses only, can be attributed to the occurrence of FHL and propionic acid bacteria, respectively. © Inra/Elsevier, Paris.

Bergkäse / raw milk flora / pasteurisation / microbiology / organic acid

Résumé — La flore du lait cru influe sur la composition et la qualité du fromage Bergkäse. **1. Microbiologie et produits de fermentation.** Des échantillons de Bergkäse, fromage régional autrichien à pâte dure, habituellement fabriqué à partir de lait cru, ont été produits à la fois à partir de lait cru et de lait pasteurisé pour évaluer l'influence de la microflore indigène sur la composition microbiologique et les produits de fermentation sélectionnés. Les lactobacilles hétérofermentaires facultatifs (FHL) étaient dominants dans la flore non-levain des fromages au lait cru. Les nombres de cellules viables augmentaient de 10^4 – 10^6 ufc.g⁻¹ à une semaine pour atteindre leur niveau final de 10^8 ufc.g⁻¹ au cours du premier mois d'affinage. Par contre les fromages au lait pasteurisé présentaient

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des nombres de FHL au moins 3 log plus bas ou même indétectables. Dans les fromages au lait cru, le développement des acides organiques pendant l'affinage diffèrait de celui obtenu dans les fromages au lait pasteurisé, et il était dépendant du nombre de microorganismes et de leur activité métabolique. Des différences dans le métabolisme du citrate et dans la fermentation en acide propionique, apparue uniquement dans les fromages au lait cru, peuvent de toute évidence être attribuées respectivement à la présence de FHL et de bactéries propioniques respectivement. © Inra/Elsevier, Paris.

fromage Bergkäse / flore du lait cru / pasteurisation / microbiologie / acide organique

1. INTRODUCTION

Bergkäse is a Swiss-type hard cheese variety, which is manufactured in the alpine regions of the western part of Austria. Due to the different production conditions at the remote alpine sites and in the valley plants as well as to the partial use of natural whey starters, a wide range of microbiological and chemical compositions and sensory qualities occur [19–21, 25, 27, 33]. Raw milk produced without silage feeding may only be used in the manufacture of this traditional cheese.

Due to the technology typical of Swiss-type cheeses in general and, particularly, Bergkäse, only the part of the indigenous flora capable of surviving acidification and the specific temperature conditions during manufacture is able to develop during the ripening process, and to contribute to flavour and texture formation. Besides the thermophilic starter cultures added during the cheesemaking process, facultatively (FHL) and obligately (OHL) heterofermentative lactobacilli, enterococci, propionic acid bacteria and clostridia spores have to be considered in the context of partly surviving production.

The aim of this study was to evaluate the influence of the raw milk flora on microbiological, chemical, rheological and sensory characteristics of Bergkäse by considering both qualitative and quantitative aspects. The indigenous flora of the milk was partially eliminated by pasteurisation, and dif-

ferences due to heat treatment (e.g. changes in rennetability) were, as far as possible, compensated by adaptation of the technology. Generally, pasteurisation eliminates the heat-sensitive raw milk flora such as coliforms and psychrotrophs and, due to their higher heat resistance, a certain amount of lactic acid bacteria, propionibacteria, coryneforms and others. Further, it attenuates the activity of many indigenous milk enzymes, e.g. lipases and proteases but, on the other hand, causes some activation of plasminogen into plasmin [39].

There appear a restricted number of studies in literature which focused on a similar subject. A French group intensively studied the effects of raw milk flora on Comté cheeses by eliminating the indigenous flora either by pasteurisation or by microfiltration, but, applied these techniques to so-called 'mini-cheeses' being approximately 1 kg in weight [13–16]. The influence of milk pasteurisation on the semi-hard surface-ripened Swiss variety, Raclette, has also been studied by Gallmann and Puhani [17, 18], who concluded that the distinctive sensory and chemical characteristics of the raw milk cheeses were largely attributable to the presence of undesired microorganisms such as *Escherichia coli*, *Streptococcus faecalis* and *Pseudomonas fluorescens*.

In part 1 of this series of papers we focus mainly on the development of the microflora during the ripening process and its influence on the development of the content of organic acids. The forthcoming contribu-

tions will refer to chemical composition as well as to physical properties and sensory quality [22, 23].

2. MATERIALS AND METHODS

2.1. Cheesemaking

Experimental cheeses were produced during 1 year at 3-month intervals. Within each season (winter: December, spring: March, summer: June, autumn: September), cheeses from raw or pasteurised milk were produced in parallel on a small scale in stainless steel vats (500 L) of the same type in four repetitions carried out within 2 weeks.

The raw cheese milk was selected from herds that were not fed silage, and the total aerobic mesophilic count had to be below 10^5 cfu·mL⁻¹. The fat content was standardised (3.7 %) and the milk was separated into two parts. After pasteurisation in a small-scale technology plant (72 °C × 40 s), CaCl₂ was added to compensate for the heat-induced changes in coagulability.

The cheeses were produced according to a standard Bergkäse protocol (figure 1). From each vat two wheels of approximately 25 kg were moulded, pressed for 20 h and salted in brine for 3 d. During the ripening period of 6 months, the cheeses were turned and rubbed with brine regularly to develop a smear surface.

Samples of milk were taken from the vat after culture addition and after pre-ripening at 32 °C for 45 min, and samples of the curd before cooking at 52 °C. Samples of cheese were taken from young Bergkäse after pressing and before brining, and from all the cheeses at 1, 4, 8, 12, 16, 20 and 24 weeks. Cylinders with a diameter of approximately 10 cm were cut from one cheese wheel along a circumferential line with a radius of 18 cm. After each sample was withdrawn, the void spaces were filled with paraffin. At an age of 24 weeks, control samples were taken from the second cheese wheel that had not been used for sampling to provide a sufficient and unaffected amount of cheese for sensory analysis.

2.2. Microbiological analyses

The milk samples were directly diluted in Ringer's solution. Cheese curd or cheese samples (10 g) were dissolved in 90 mL tri-sodium

Figure 1. Manufacture of Bergkäse from raw or pasteurised milk.

Figure 1. Fabrication du fromage Bergkäse à partir de lait cru ou de lait pasteurisé.

citrate (2 %) using a Stomacher 400 peristaltic lab blender (Seward Medical Inc., London, UK). Further decimal dilutions were made with quarter-strength Ringer's solution, and all counts were performed in duplicate [11]. The following methods were used to differentiate the microflora:

– mesophilic aerobic microflora: plate count skim milk agar (Merck 15338, Merck AG, Darmstadt, Germany), pour plate method, aerobic incubation at 30 °C for 3 d [4];

– *Staphylococcus aureus*: Baird Parker agar + egg yolk tellurite supplement (Oxoid CM275 + SR 054C, Unipath Ltd., Basingstoke, UK), surface method, aerobic incubation at 37 °C for 2 d, colonies were selected and their catalase reaction, thermonuclease reaction, clumping factor and coagulase reaction determined [2];

– coliforms: violet red bile agar (Merck 1406), pour plate method with overlayer, incubation at 30 °C for 24 h [1];

– *Escherichia coli*: flourocult laurylsuphate broth (Merck 12588), aerobic incubation at 37 °C for 24 h, most-probable-number (MPN) quantification by visual detection of gas and fluorescence [10];

– total lactobacilli: *Lactobacillus* selective medium (Becton-Dickinson 43113, Becton-Dickinson GmbH, Heidelberg, Germany), pour plate method with overlayer, incubation at 30 °C for 3 d;

– FHL: FH-agar with vancomycin [24], surface method, anaerobic incubation at 37 °C for 3 d;

– OHL: OH-medium [24], aerobic incubation at 37 °C for 3 d, MPN method (gas);

– contaminating microorganisms as defined by the International Dairy Federation, i.e. non-lactic acid bacteria capable of forming colonies on: sugar-free agar (Merck 19878), surface method, aerobic incubation at 30 °C for 3 d [3];

– enterococci: kanamycin esculin azide agar (Oxoid CM591 + SR 92), surface method, aerobic incubation at 37 °C for 24 h [5].

The mesophilic aerobic microflora was counted in the raw milk before cheesemaking. In both vat milk and curd, *S. aureus*, coliforms, *E. coli*, contaminating microorganisms, FHL, OHL and enterococci were evaluated. Out of the cheese samples, we investigated contaminating microorganisms, FHL and OHL, total lactobacilli as well as enterococci.

2.3. Chemical analyses

Grated cheese taken from the cylindrical samples was subjected to chemical analyses. Lactose and galactose, D-glucose, D(-)- and L(+)-lactate as well as citrate [6–9] were determined by enzymatic methods. In the high performance liq-

uid chromatography (HPLC) determination of acetate, formate and propionate, organic acids were extracted by iso-butyric acid (internal standard) with H₂O/NaOH (pH 7.0–7.5) at 65 °C. After pre-separation on a AG 1 × 8 ion exchanger (Bio-Rad, Hercules, California USA), separation was performed by using a 9095 autosampler (Varian Corp., Walnut Creek, Mitchell, California USA) and a 880-PU pump (Jasco Ltd., Tokyo, Japan) on two Nucleogel ION 300 OA columns (300 × 7.7 mm) mounted in parallel (Macherey-Nagel, Düren, Germany) at 64 °C [38]; 0.003 mol·L⁻¹ sulphuric acid was used as mobile phase, and detection was carried out at 215 nm with a SPD-6A detector (Shimadzu Ltd., Kyoto, Japan).

2.4. Data treatment

Selected univariate and multivariate procedures of the SAS 6.12 HP-UX software package [34] were used for statistical evaluation of the data.

3. RESULTS

3.1. Effects of pasteurisation on vat milk and curd

The pasteurisation process eliminated the heat-sensitive raw milk flora such as coliforms, *S. aureus* and, to a lesser extent, lactic acid bacteria (table 1). In pasteurised milk the levels of contaminating microorganisms growing on sugar-free medium, which represent the total of non-lactic acid bacteria, were approximately two orders of magnitude lower than in raw milk. In the raw vat milk, enterococci counts ranged from 1.9×10^2 to 1.0×10^3 cfu·mL⁻¹ and FHL from 4×10^1 to 9.7×10^3 cfu·mL⁻¹. OHL were less than 10 cfu·mL⁻¹. FHL counts always remained below the detection limit of 10 and 100 cfu·mL⁻¹ for pasteurised milk and curd, respectively, even in those samples where growth was detected during ripening. Similar differences were found for the curd sampled prior to cooking.

Further growth of the microorganisms in the cheese mass was strongly reduced by acidification and curd cooking temperature,

Table I. Microbial counts in raw and pasteurised vat milks (cfu·mL⁻¹) and curds (cfu·g⁻¹).**Tableau I.** Dénombrements microbiens dans les laits (ufc·mL⁻¹) et les caillés (ufc·g⁻¹) crus et pasteurisés.

	Substrate: raw milk (n = 16)			Substrate: pasteurised milk (n = 16)		
	Geometric mean	Minimum	Maximum	Geometric mean	Minimum	Maximum
Vat milk						
Coliforms	2.0×10^3	$< 1.0 \times 10^2$	1.1×10^5	$< 1.0 \times 10^1$	—	—
<i>E. coli</i>	$< 3.0 \times 10^{2a}$	$< 1.0 \times 10^2$	2.3×10^3	$< 1.0 \times 10^1$	—	—
<i>S. aureus</i>	6.2×10^2	4.0×10^1	2.6×10^3	n.i. ^b	n.i.	n.i.
Enterococci	3.5×10^2	1.9×10^2	1.0×10^3	$< 1.0 \times 10^1$	—	—
Contaminating microorganisms	7.7×10^4	9.0×10^3	9.5×10^5	2.5×10^3	3.5×10^2	9.8×10^3
FHL ^c	4.0×10^2	4.0×10^1	9.7×10^3	$< 1.0 \times 10^1$	—	—
Curd						
Coliforms	2.2×10^4	9.2×10^2	1.1×10^5	$< 1.0 \times 10^1$	—	—
<i>E. coli</i>	7.9×10^2	3.0×10^2	1.5×10^4	$< 1.0 \times 10^1$	—	—
<i>S. aureus</i>	2.2×10^4	2.0×10^3	1.3×10^5	n.i.	n.i.	n.i.
Enterococci	9.2×10^3	3.0×10^3	4.7×10^4	$< 1.0 \times 10^2$	—	—
Contaminating microorganisms	5.9×10^5	1.2×10^5	2.5×10^6	1.1×10^4	8.0×10^2	9.0×10^4
FHL	1.5×10^3	2.0×10^2	9.5×10^3	$< 1.0 \times 10^2$	—	—

^a $< 3.0 \times 10^2$, 12 out of 16 samples were $< 3.0 \times 10^2$ cfu·g⁻¹. ^b n.i.: not investigated. ^c FHL: facultatively heterofermentative lactobacilli.

^a $< 3.0 \times 10^2$, 12 échantillons sur 16 étaient $< 3.0 \times 10^2$ ufc·g⁻¹. ^b n.i., non recherché. ^c FHL, lactobacilles hétérofermentaires facultatifs.

which eliminated both acid- and heat-sensitive contributors to the raw milk flora. Generally, < 100 cfu·g⁻¹ coliforms, *E. coli* or *S. aureus* were detectable in 1-week-old cheeses, whereas OHL and FHL started to develop (data not shown).

3.2. Microflora evolution during ripening

The development of FHL and OHL viable counts in the cheeses during the ripening period of 6 months is shown in figure 2. FHL predominated the non-starter flora in cheeses made from raw milk with numbers increasing from approximately 10^4 to 10^6 cfu·g⁻¹ at a cheese age of 1 week to

above 10^7 cfu·g⁻¹ within the first month and then remaining at this level. In cheeses made from pasteurised milk, < 100 cfu·g⁻¹ FHL occurred in the spring and autumn productions whereas, in the case of the three cheeses of the winter productions and in two cheeses of the summer productions, a level of 10^3 cfu·g⁻¹ at a cheese age of 1 week was found, which might be attributed to recontamination. As compared to raw milk cheeses, the maximum level of 10^5 cfu·g⁻¹ remained at least three orders of magnitude lower than in the raw milk cheeses. The OHL viable counts proved to range between 10^2 and 10^4 cfu·g⁻¹ for the 1-week-old raw milk cheeses and showed a slight increase up to, typically, 10^5 cfu·g⁻¹ during maturation.

Figure 2. Evolution of obligately (OHL) and facultatively (FHL) heterofermentative lactobacilli in individual Bergkäse cheeses made from raw milk or pasteurised milk in different production periods. OHL: ○, raw; ●, pasteurised. FHL: □, raw; ■, pasteurised.

Figure 2. Évolution des lactobacilles hétérofermentaires stricts (OHL) ou facultatifs (FHL) dans les fromages Bergkäse individuels produits à partir de lait cru ou de lait pasteurisé au cours de différentes périodes de production. OHL : ○, cru ; ●, pasteurisé. FHL : □, cru ; ■, pasteurisé.

In contrast, $< 100 \text{ cfu} \cdot \text{g}^{-1}$ OHL were found in Bergkäse made from pasteurised milk.

As an example, *figure 3* shows the development of the total lactobacilli flora observed for the summer productions. In the cheeses made from pasteurised milk, these counts represent the starter lactobacilli only, whereas the numbers of the raw milk cheeses also include the FHL group. During maturation, the viable counts decreased by not more than one order of magnitude for both groups of cheeses.

In the raw milk cheeses, enterococci reached their maximum in the curd, with viable counts of approximately $10^4 \text{ cfu} \cdot \text{g}^{-1}$, and showed a tendency to decline during the ripening. No enterococci were found in Bergkäse made from pasteurised milk (*table II*). The maximum level of contaminating microorganisms found in the curd was below 10^6 and $10^4 \text{ cfu} \cdot \text{g}^{-1}$ for the raw and pasteurised milk cheeses, respectively. Independent of the raw milk treatment, numbers below $10^4 \text{ cfu} \cdot \text{g}^{-1}$ were found during ripening.

Figure 3. Ranges of total lactobacilli counts in Bergkäse made from raw milk (light shaded area) or pasteurised milk (dark shaded area) produced during summer as compared to the average count of facultatively heterofermentative lactobacilli in raw milk cheese (○).

Figure 3. Amplitude des dénombrements de lactobacilles totaux dans le fromage Bergkäse au lait cru (aire claire) ou au lait pasteurisé (aire sombre) produit durant l'été comparés à la moyenne des nombres de lactobacilles hétérofermentaires facultatifs dans le fromage au lait cru (○).

Table II. Viable count ranges of enterococci and contaminating microorganisms (cfu·g⁻¹) in Bergkäse made from raw or pasteurised milk (*n* = 16).**Tableau II.** Amplitude de variation des dénombrements de cellules viables d'entérocoques et de microorganismes contaminants (ufc·g⁻¹) dans le fromage Bergkäse produit à partir de lait cru ou de lait pasteurisé (*n* = 16).

Cheese age (weeks)	Enterococci		Contaminating microorganisms	
	Raw milk	Pasteurised milk	Raw milk	Pasteurised milk
Curd	$3.0 \times 10^3 - 4.7 \times 10^4$	$< 1.0 \times 10^2$	$9.0 \times 10^3 - 9.5 \times 10^5$	$3.5 \times 10^2 - 9.8 \times 10^3$
1	$1.0 \times 10^2 - 6.5 \times 10^3$	$< 1.0 \times 10^2$	$1.2 \times 10^5 - 2.5 \times 10^6$	$8.0 \times 10^2 - 9.0 \times 10^4$
4	$1.0 \times 10^2 - 3.8 \times 10^3$	$< 1.0 \times 10^2$	$3.5 \times 10^3 - 8.1 \times 10^4$	$1.0 \times 10^2 - 5.8 \times 10^4$
8	$1.0 \times 10^2 - 4.5 \times 10^3$	$< 1.0 \times 10^2$	$1.2 \times 10^3 - 1.2 \times 10^5$	$2.5 \times 10^2 - 3.0 \times 10^5$
12	$1.0 \times 10^2 - 5.0 \times 10^3$	$< 1.0 \times 10^2$	$3.0 \times 10^2 - 1.8 \times 10^5$	$2.0 \times 10^2 - 1.9 \times 10^5$
16	$1.0 \times 10^2 - 1.4 \times 10^3$	$< 1.0 \times 10^2$	$2.0 \times 10^2 - 7.0 \times 10^4$	$1.0 \times 10^2 - 5.0 \times 10^4$
20	$1.0 \times 10^2 - 4.1 \times 10^3$	$< 1.0 \times 10^2$	$4.0 \times 10^2 - 3.2 \times 10^4$	$1.0 \times 10^2 - 1.1 \times 10^4$
24	$1.0 \times 10^2 - 2.8 \times 10^3$	$< 1.0 \times 10^2$	$1.0 \times 10^2 - 6.4 \times 10^4$	$2.0 \times 10^2 - 1.8 \times 10^4$

3.3. Organic acids

In the cheese mass sampled after brining, the contents of residual sugars, i.e. lactose and galactose, were always less than 0.2 mmol·kg⁻¹. Organic acid contents of mature cheeses are presented in *table III* as influenced by both production period and raw milk treatment. As compared to raw milk cheeses, we observed a trend towards higher contents of lactic and citric acids but lower amounts of succinic, acetic, propionic and formic acids for cheeses made from pasteurised milk, which is reflected by the corresponding *P*-values.

The development of the organic acids during maturation (*figure 4*) presumably depends on the numbers and the metabolic activity of the microorganisms and reflects differences between the manufacturing periods, but also indicates that tendencies were similar for cheeses out of all productions. Lactic acid content decreased steadily with partial differences between raw and pasteurised milk cheeses being attributable to propionic acid fermentation. In Bergkäse made from raw milk during winter, summer and autumn, a slight propionic acid fer-

mentation occurred whereas, in the case of the spring cheeses, no significant propionic acid fermentation was observed. In the raw milk cheeses of the winter period the mean propionic acid content, which is presumably related to the number of viable propionibacteria [26, 35], was 40 mmol·kg⁻¹. Accordingly, acetic acid as a metabolism product of propionic acid fermentation [35] reached levels of 40 mmol·kg⁻¹ in these cheeses. No significant propionic acid fermentation and, consequently, eye formation [22] was observed in any of the cheeses made from pasteurised milk.

In order to separate factors of potential influence statistically, analysis of variance (ANOVA) was applied. In the selected model, milk treatment and maturation effects were considered as main effects, and the seasonal factor served as a sub-effect within milk treatment. In the case of significant interactions between treatment and maturation, *F*-values of the main effects were calculated by using the interaction mean square [32]. The ANOVA results clearly indicate that, besides effects of maturation and season, the raw milk treatment proved to be of significant influence on the content of

Table III. Organic acids (mmol·kg⁻¹) in mature Bergkäse as affected by production period and milk treatment.**Tableau III.** Acides organiques (mmol·kg⁻¹) dans le fromage Bergkäse affiné en fonction de la période de production et du traitement du lait.

Parameter	Winter ^a			Spring			Summer			Autumn		
	Raw ^b	Pasteurised	$P_{t=0}$ ^c	Raw	Pasteurised	$P_{t=0}$ ^c	Raw	Pasteurised	$P_{t=0}$ ^c	Raw	Pasteurised	$P_{t=0}$ ^c
Organic acid:												
Lactic	49.7	112	0.042	99.3	98.3	0.94	97.1	121	0.002	63.4	100	0.008
Succinic	5.91	1.63	0.027	1.48	1.09	0.28	2.29	1.03	0.058	5.52	1.72	0.003
Acetic	41.2	6.46	0.002	22.8	4.44	< 0.001	30.0	3.31	< 0.001	34.2	4.40	< 0.001
Propionic	38.1	1.97	0.028	8.30	0.447	0.31	19.2	0.495	0.014	27.6	1.46	< 0.001
Formic	4.71	1.83	< 0.001	4.04	1.04	< 0.005	9.19	4.51	0.004	6.03	1.92	< 0.001
Citric	0.207	10.2	< 0.001	1.83	10.97	< 0.001	1.09	10.9	< 0.001	0.217	11.1	< 0.001

^a Production period. ^b Arithmetic mean ($n = 4$). ^c $P_{t=0}$, significance in t -test.

^a Période de production. ^b Moyenne arithmétique ($n = 4$). ^c $P_{t=0}$, seuil de signification.

Figure 4. Development of the organic acids mean concentrations during maturation of Bergkäse made from raw milk or pasteurised milk in different production periods. Upper figures: Lactic acid: ○, raw; ●, pasteurised. Propionic acid: □, raw; ■, pasteurised. Acetic acid: △, raw; ▴, pasteurised. Lower figures: Formic acid: ○, raw; ●, pasteurised. Citric acid: □, raw; ■, pasteurised.

Figure 4. Développement des concentrations moyennes d'acides organiques durant l'affinage du fromage Bergkäse au lait cru ou au lait pasteurisé au cours de différentes périodes de production. En haut : acide lactique (○, cru ; ●, pasteurisé. Acide propionique : □, cru ; ■, pasteurisé. Acide acétique : △, cru ; ▴, pasteurisé. En bas : Acide formique : ○, cru ; ●, pasteurisé. Acide citrique : □, cru ; ■, pasteurisé.

organic acids. For all of the organic acids, the $P(H_0): F > F_{\text{obs}}$ -values were found to be < 0.01 .

In the raw milk cheeses the citric acid content decreased from 9–10 mmol·kg⁻¹ to levels of about 0.2–2 mmol·kg⁻¹ cheese for the mature Bergkäse and, therefore, proved to be related to FHL growth. Prior to any observable propionic acid fermentation, both formic and acetic acid contents increased from approximately 1–2 to 4–7 mmol·kg⁻¹ cheese and from 2–3 to 15–37 mmol·kg⁻¹ cheese during the first 4 months of ripening, respectively. In contrast, the levels of acetate, citrate and formate remained almost unchanged in cheeses made from pasteurised

milk, even in those in which FHL recontamination occurred.

4. DISCUSSION

In this study – the object of which was to obtain information on the importance of the raw milk flora on the quality of Bergkäse – we found significant differences between cheeses produced from raw or pasteurised milk with respect to both microflora and the resulting fermentation compounds. In accordance with Bachmann and Puhani [12], who studied the behaviour of pathogens in Swiss-type cheeses, we found no incidence of the

ability of potential pathogens to survive the production process. *E. coli* and *S. aureus* were < 0.1 cfu·g⁻¹ cheese after 1 week of ripening.

Among the non-starter flora present in the raw milk cheeses, FHL played a major role. Besides Bergkäse, this group occurs in high numbers in Swiss-type cheese varieties [15, 28] and also contributes to the maturation of Cheddar cheese [31]. The FHL, which partly have their origin in recontaminations from the cheese plant environment, did not increase to the same level in the cheeses made from pasteurised milk as in the raw milk cheeses. This may be attributed to differences in the microflora between the raw and pasteurised milks but, moreover, the different quantities at contamination or inoculation time may also be of some importance for a balanced distribution of the FHL throughout the cheese matrix. Additionally, the log phase of growth may be restricted to the manufacture and beginning of ripening, and in later periods FHL may grow and develop only locally because of physical reasons. Nevertheless, the increase in the FHL count also depends on the cheese variety, the salt content of the cheeses and the general characteristics of the starter used.

It was not found to be typical for the experimental Bergkäse that the starter bacteria decreased pronouncedly as reported for Comté by Demarigny et al. [15], who, however, applied a ripening cycle with warm and cold room maturation typical for Comté. It is therefore not evident that the adventitious microflora, mainly FHL, generally competes with the starter bacteria for major growth factors essential for their survival. Furthermore, the development of enterococci in Bergkäse differs from that obtained by Demarigny et al. [15], who found *Enterococcus* numbers to increase to 10^6 cfu·g⁻¹. Viable counts of that magnitude (10^6 cfu·g⁻¹) were, however, observed in a previously published study on mature Vorarlberger Bergkäse from a large num-

ber of different production sites [33]. Therefore, the question arises whether enterococci capable of growing during maturation actually originate from the raw milk or from the cheese plant environment. Additionally, residual sugars which serve as an energy source for enterococci (i.e. lactose and galactose) were not detectable in our experimental Bergkäse.

The levels of the organic acids significantly differed from those of the cheeses made from pasteurised milk presumably due to the growth of the indigenous flora in the raw milk cheeses. As Bergkäse represents a surface-ripened cheese variety, the decrease in the content of lactate, which is primarily metabolised by the microorganisms of the smear surface, may be attributed to the migration of lactic acid from the centre to the surface layer [29]. After 3 months of ripening, however, we observed a more pronounced decrease in the lactate content for cheeses in which propionic acid fermentation occurred.

There were mainly two reasons why aspects of the surface flora were not included in the study. This is because all cheeses were similarly treated, i.e. regularly smeared with *Brevibacterium linens* and ripened in the same ripening room, so that we could assume that the surface flora was not different on cheeses made from raw or pasteurised milk. This assumption is based on results of a preceding study [33] where, for a large number of Bergkäse samples of different origin but ripened centrally, neither significant quantitative nor qualitative differences were found. Additionally, the surface flora is mainly influenced by factors such as temperature and humidity in the ripening room, frequency of the smear treatment, NaCl content and pH of the surface and the culture used [35].

Citrate has been frequently described as serving as one of the main energy sources for non-starter lactic acid bacteria in ripening cheeses [28, 36]. The initial phase of citrate breakdown was found to be in line

with a rapid growth of heterofermentative lactobacilli from about 10^5 cfu·g⁻¹ in the 1st week of ripening to a population maximum of about 10^8 cfu·g⁻¹ in the 4th week, which then remained stable during further maturation. Only in one series, i.e. the summer cheeses, did the FHL counts achieve their maximum in the 4th week, whereas no citrate degradation was observed prior to the 8th week. As lactose and galactose were metabolised completely before brine salting, the growth of FHL in these cheeses could not be explained by the occurrence of residual sugars. Therefore, our investigations indicate that citrate may appear to be an important energy source for the growth of FHL, but further investigations are obviously necessary to clarify the demands of non-starter lactic acid bacteria on a substrate in general. Additional energy sources may, for example, arise from proteolytic and lipolytic activities [30] as well as from autolysis of the starter bacteria [37].

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