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Raw milk flora affects composition and quality of Bergkäse. 2. Chemical composition

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Abstract — The effects of the indigenous raw milk flora on the chemical composition of Austrian Bergkäse was evaluated by means of repeated cheesemaking experiments from raw or pasteurised milk. Generally, changes caused by the pasteurisation of raw milk did not significantly affect the gross composition of the resulting cheeses but, as monitored by sampling during maturation, systematically showed effects on proteolysis. Whereas degradation of α_{s1}-casein proved to be delayed in cheeses made from pasteurised milk due to effects caused by heat treatment and partial elimination of the raw milk flora, an enhanced cleavage of β-casein was found in cheeses from pasteurised milk. These differences were also evident in the water-soluble fraction and, partly, in fractions containing smaller breakdown products and were presumably caused by heat-induced changes in the plasmin/plasminogen complex. Additionally, effects of heat treatment and of indigenous raw milk flora on alkaline phosphatase and lipolysis were observed. © Inra/Elsevier, Paris.

Bergkäse / raw milk flora / pasteurisation / composition / proteolysis

Résumé — La flore du lait cru influe sur la composition et la qualité du fromage Bergkäse. 2. Composition chimique. Les effets de la flore indigène du lait cru sur la composition chimique du fromage autrichien Bergkäse ont été évalués au moyen d'essais répétés de fabrication à partir de lait cru ou de lait pasteurisé. En général, la pasteurisation de lait cru n'affectait pas significativement la composition brute des fromages obtenus mais, comme contrôlé sur les échantillons prélevés au cours de l'affinage, montrait des effets systématiques sur la protéolyse. Dans les fromages au lait pasteurisé, alors que la dégradation de la caséine α_{s1} était retardée à cause du traitement thermique et de l'élimination partielle de la flore du lait cru, une hydrolyse accrue de la caséine β était observée. Ces différences étaient également évidentes dans la fraction soluble dans l'eau, et partiellement dans les fractions contenant des produits de dégradation plus petits et étaient manifestement causées par

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des changements dans le complexe plasmine/plasminogène dus au chauffage. De plus des effets du traitement thermique et de la flore indigène du lait cru sur la phosphatase alcaline et sur la lipolyse étaient observés. © Inra/Elsevier, Paris.

fromage Bergkäse / flore du lait cru / pasteurisation / composition / protéolyse

1. INTRODUCTION

Austrian Bergkäse represents a raw milk-base Gruyère-type hard cheese variety which was originally produced only in the summer season in very small cheese plants with basic equipment located on remote alpine sites in the western part of Austria. According to the Codex Alimentarius Austriacus [6], neither the application of any heat treatment nor the use of bactofugal equipment is allowed to affect and reduce the microflora of the raw milk. The application of the specific technology described previously [39] results in wheel-shaped cheeses (diameter approximately 35–50 cm, height ~ 12–15 cm) with a yellowish-brownish surface covered with dry smear and a light-yellowish cheese body with smooth texture and a few pea-sized holes, which exhibits a mild aromatic to piquant aroma [1]. The use of raw milk as well as differing technological conditions in the various production sites can be allocated as responsible for the wide variation in the properties of Bergkäse regarding microbiology [39], texture and appearance [26], sensory properties [27] as well as chemical composition [18, 20].

Within the framework of a European research project work was performed in order to gain information on the importance of the indigenous raw milk microflora for the characteristics of Austrian Bergkäse. In a preceding publication, the effects of milk pasteurisation on microbiology and fermentation compounds were described [15]. This paper focuses mainly on differences in cheese composition and proteolysis as well as in enzymatic activities, which are attributable to pasteurisation.

2. MATERIALS AND METHODS

2.1. Cheese production and sampling

As described in a previous paper [15], a set of 16 Bergkäse cheeses (two wheels each) was produced either from raw or pasteurised milk in parallel during a period of 1 year. Cylindrical samples (diameter ~10 cm) were cut from one cheese wheel along a circumferential line of 18 cm in radius at the predetermined ages of 1, 4, 8, 12, 16, 20 and 24 weeks. After each sampling procedure the void spaces were filled with paraffin. At 24 weeks, control samples were taken from the second cheese wheel, which was unaffected by any sampling procedure. This procedure was applied because a large amount of cheese was needed for sensory analysis [21], and to obtain evidence whether repeated sampling and the treatment with hot paraffin did affect the composition of the cheeses. Additional samples were taken from the young cheeses after pressing and before brining in order to evaluate the effects of milk pasteurisation (72 °C × 40 s) on basic compositional properties.

2.2. Chemical analyses

Grated cheese was subjected to chemical analyses comprising gross composition and proteolysis parameters as well as enzymatic activity. All measurements were performed in duplicate. Dry matter was evaluated by the oven method [3]. pH was measured by probing a glass electrode into the compressed grated cheese. Total fat content was evaluated butyrometrically, and total nitrogen (N) by the Kjeldahl method [4]. After air-drying and defatting with petroleum ether, an appropriate portion served as substrate for alkaline urea-polyacrylamide gel electrophoresis (PAGE) [30].

Water-soluble nitrogen (WSN) was determined by the Kjeldahl method [11, 42] from aqueous extracts prepared by ten-fold dilution

of grated cheese and subsequent homogenisation, centrifugation in the cold and filtration. Twelve percent trichloroacetic acid (TCA)-soluble nitrogen and 5 % phosphotungstic acid (PTA)-soluble N were determined after 1:1 dilution of aqueous extracts with 24 % TCA and 10 % PTA, respectively, followed by filtration. Free amino groups were determined in the water-soluble and in the 12 % TCA-soluble fraction by the photometric ortho-phtaldialdehyde (OPA) method and expressed as free Glu [17, 43]. Ammonia and D(–)- and L(+)-lactic acid were measured by enzymatic methods [7, 8], and lipolysis was estimated by a titration method [12]. Calcium and chloride contents were evaluated potentiometrically [2, 44]. A high performance liquid chromatography (HPLC) method was used for the quantification of selected organic acids [15]. After the preparation of cheese extracts according to Richardson and Pearce [37], plasmin and plasminogen activities were determined photometrically using Ollikainen and Nyberg's [34] modification of the method originally described by Rollema et al. [40]. The activity of alkaline phosphatase was measured by a spectrophotometric assay [5]. After freeze-drying of a defined amount of WSN and resuspending in a defined volume of an appropriate buffer solution, peptide mapping was performed by reverse-phase HPLC according to a method described by Rohm et al. [38].

2.3. Statistical evaluation

Various univariate and multivariate procedures of the SAS 6.12 HP-UX software package [41] were used for statistical evaluation.

3. RESULTS AND DISCUSSION

3.1. Effects of raw milk treatment on cheesemaking

It is well known that, due to heat-induced denaturation of β-lactoglobulin and its subsequent binding to casein, pasteurisation reduces rennetability and syneresis of milk, which may partly be balanced out by enhanced acidification as well as by the addition of calcium chloride [25, 36]. After the addition of 50 g $CaCl_2$ to 500 L pasteurised vat milk [15], the mean coagulation time was found to be 30.8 ± 1.85 min, which proved to be insignificantly different from the average coagulation time of raw vat bulk milk.

Effects on some compositional properties of the young cheeses after pressing and before brining were evaluated by analysis of variance (ANOVA) considering milk treatment, seasonal replication and within-season repetition. Generally, significancies of the ANOVA model were mainly attributable to variations between production seasons, whereas neither repeated manufacture within seasons nor milk treatment proved to be of significance (*table I*). Only in the case of alkaline phosphatase as an indicator of the heat treatment effect did milk pasteurisation significantly ($P < 0.001$) reduce enzyme activity from the equivalent of 9.88 mg *p*-nitrophenol (NP)·g^{-1} to 0.047 *p*-NP·g^{-1}.

3.2. Effects of continuous sampling on cheese properties and repeatability of cheesemaking

In order to monitor possible effects of the sampling procedure, selected parameters of the mature Bergkäse were analysed from both of the cheese wheels, which served as sample source, as well as from the unsampled cheeses. We found evidence that the significant model *F*-values had their origin either in the milk treatment or in the season effect, whereas the influence of sampling proved to be insignificant in all parameters evaluated. Selected mean values of these two sample subgroups are compared in *table II*.

For a thorough verification of the repeatability of the cheesemaking procedure, mixed ANOVAs comprising milk pasteurisation as a main effect and repetition as a sub-effect within each treatment were applied to selected chemical measures. Furthermore, the model considered the influence of maturation (cheese age) as well as the interaction between treatment and maturation to cover any influence of milk pasteurisation on

Table I. Influence of milk pasteurisation on physicochemical composition of 1-d-old Bergkäse as estimated by analysis of variance (ANOVA) ($n = 16$).

Tableau I. Influence de la pasteurisation du lait sur la composition physico-chimique du fromage Bergkäse à 1 j estimée par analyse de variance ($n = 16$).

Parameter[a]	ANOVA results[b]				Mean values[c]	
	Model	Milk treatment	Season	Repetition	Raw	Pasteurised
FAT ($g \cdot kg^{-1}$)	< 0.001	0.12	< 0.001	0 34	347[a]	345[a]
CA ($g \cdot kg^{-1}$)	< 0.001	0.96	< 0.001	0.21	8.98[a]	8.98[a]
PH (–)	< 0.001	0.84	< 0.001	0.31	5.24[a]	5.24[a]
PHOS (mg *p*-NP·g^{-1})	< 0.001	< 0.001	< 0.001	0.21	9.88[a]	0.047[b]
LLACT ($mmol \cdot kg^{-1}$)	< 0.001	0.63	< 0.001	0.36	70.6[a]	70.2[a]
DLACT ($mmol \cdot kg^{-1}$)	< 0.001	0.74	< 0.001	0.11	68.0[a]	67.7[a]

[a] FAT: fat content; CA: calcium; PH: pH value; PHOS: phosphatase activity; NP : nitrophenol; LLACT: L(+)-lactic acid; DLACT: D(–)-lactic acid. [b] Numerical values, probabilities $P(H_0)$: $F > F_{obs}$. [c] Mean values marked by different superscripts differ significantly ($P < 0.01$).

[a] FAT : teneur en matière grasse ; CA : calcium ; PH : valeur de pH ; PHOS : activité phosphatase ; NP : nitrophenol ; LLACT : acide lactique L(+) ; DLACT : acide lactique D (–). [b] Valeurs numériques, probabilités $P(H_0)$, $F > F_{obs}$. [c] Les valeurs moyennes marquées de lettres différentes sont significativement différentes ($p < 0{,}01$).

Table II. Effects of the sampling procedure on physicochemical properties of 24-week-old Bergkäse.

Tableau II. Effets des procédures d'échantillonnage sur les propriétés physico-chimiques de fromage Bergkäse de 24 semaines.

Parameter[a]	Mean values ± standard deviations	
	Continuous sampling	Unsampled cheese wheel
DM ($g \cdot kg^{-1}$)	670 ± 5.15	668 ± 4.88
WSN TN^{-1} (%)	22.9 ± 2.91	22.1 ± 2.68
TS-N TN^{-1} (%)	12.7 ± 1.81	12.1 ± 1.47
PS-N TN^{-1} (%)	6.05 ± 1.39	5.84 ± 1.24
PH (–)	5.70 ± 0.105	5.67 ± 0.068
LACT ($mmol \cdot kg^{-1}$)	93.0 ± 23.5	98.2 ± 26.1

[a] DM: dry matter; WSN: water-soluble nitrogen; TS-N: N soluble in 12 % trichloroacetic acid; PS-N: N soluble in 5 % phosphotungstic acid; PH: pH value; LACT: lactic acid.

[a] DM : matière sèche ; WSN : azote soluble dans l'eau ; TS-N : N soluble dans l'acide trichloroacétique à 12 % ; PS-N : N soluble dans l'acide phosphotungstique ; PH : valeur de pH ; LACT : acide lactique.

the behaviour during ripening. In the case of significant interactions, the corresponding mean square was used to calculate the *F*-values of the effects treatment and repetition [35].

In all parameters evaluated, both the model *F*-values and the maturation time proved to be significant ($P < 0.01$). Interactions appeared in the cases of some measures of proteolysis and for the contents of

Table III. Effects on composition of Bergkäse produced during the winter period as estimated by three-factor analysis of variance (ANOVA).

Tableau III. Effets sur la composition du fromage Bergkäse produit au cours de l'hiver estimés par analyse de variance à trois facteurs.

Parameter[a]	Milk treatment	Repetition	Maturation	Treatment × Maturation
DM ($g \cdot kg^{-1}$)	0.26[b]	0.37	< 0.001	0.031[c]
TN ($g \cdot kg^{-1}$)	0.81	0.43	< 0.001	0.20
WSN TN^{-1} (%)	0.019	0.79	< 0.001	< 0.001[c]
TS-N TN^{-1} (%)	< 0.001	0.006	< 0.001	< 0.001[c]
PS-N TN^{-1} (%)	< 0.001	0.062	< 0.001	0.28
AMM ($mg \cdot kg^{-1}$)	0.12	0.50	< 0.001	< 0.001[c]
ADEG (%)	0.017	0.78	< 0.001	< 0.001[c]
BDEG (%)	< 0.001	0.65	< 0.001	< 0.001[c]
PH (–)	0.46	< 0.001	< 0.001	0.38
LACT ($mmol \cdot kg^{-1}$)	0.017	0.49	< 0.001	< 0.001[c]
SUCC ($mmol \cdot kg^{-1}$)	0.14	0.78	< 0.001	< 0.001[c]
ACET ($mmol \cdot kg^{-1}$)	0.009	0.98	< 0.001	< 0.001[c]
PROP ($mmol \cdot kg^{-1}$)	0.031	0.71	0.001	< 0.001[c]
FORM ($mmol \cdot kg^{-1}$)	0.003	0.99	0.006	< 0.001[c]
CIT ($mmol \cdot kg^{-1}$)	0.004	0.99	0.002	< 0.001[c]

[a] DM: dry matter; TN: total nitrogen; WSN: water-soluble nitrogen; TS-N: 12 % trichloroacetic acid soluble nitrogen; PS-N: 5 % phosphotungstic acid soluble nitrogen; AMM: ammonia; ADEG: degradation of α_{s1}-casein; BDEG: degradation of β casein; PH: pH value; LACT: lactic acid; SUCC: succinic acid; ACET: acetic acid; PROP: propionic acid; FORM: formic acid; CIT: citric acid. [b] Numerical values, probabilities $P(H_0)$: $F > F_{obs}$. [c] Interaction sum-of-squares were used for the calculation of the F-values of the factors milk and repetition.

[a] DM : matière sèche ; TN : azote total ; WS-N : azote soluble dans l'eau ; TS-N : azote soluble dans l'acide trichloroacétique à 12 % ; PS-N : azote soluble dans l'acide phosphotungstique à 5 % ; AMM : ammoniaque ; ADEG : dégradation de la caséine α ; BDEG : dégradation de la caséine β ; PH : valeur du pH ; LACT : acide lactique ; SUCC : acide succinique ; ACET : acide acétique ; PROP : acide propionique ; FORM : acide formique ; CIT : acide citrique. [b] Valeurs numériques, probabilités $P(H_0)$, $F > F_{obs}$. [c] Les sommes des carrés de l'interaction étaient utilisées pour calculer traitement lait et répétition.

certain organic acids (*table III*). Effects of milk treatment were evident for the low molecular-weight soluble nitrogen fractions, the relative degradation of β-casein calculated from PAGE densitogram peak areas by:

$$\beta\text{-casein breakdown} = 100\ \gamma\text{-casein} / (\beta\text{-casein} + \gamma\text{-casein})$$

as well as for acetic, formic and citric acid. It is evident from the F-values for the repetition factor that the production day within a relatively short period of time of 2 weeks and related changes in bulk milk composition cannot be regarded as a systematic source of variation. Regarding cheese pH, multiple mean comparison did not show systematic effects on production day at an error probability level of $P < 0.01$.

3.3. Effects of milk pasteurisation on the chemical composition of Bergkäse

For the chemical data of mature Bergkäse samples, mean values were calculated after appropriate grouping with respect to production period and milk treatment (*table IV*). For gross composition parameters compris-

Table IV. Composition of mature Bergkäse as affected by production period and milk treatment.
Tableau IV. Composition du fromage Bergkäse affiné en fonction de la période de production et du traitement du lait.

Parameter[a]	Winter[b]			Spring			Summer			Autumn		
	Raw[c]	Pasteurised	$P_{t=0}$[d]	Raw[c]	Pasteurised	$P_{t=0}$[d]	Raw[c]	Pasteurised	$P_{t=0}$[d]	Raw[c]	Pasteurised	$P_{t=0}$[d]
FAT ($g \cdot kg^{-1}$)	372	372	0.96	369	365	0.15	370	366	0.082	351	347	0.29
DM ($g \cdot kg^{-1}$)	668	666	0.089	672	671	0.78	669	666	0.28	666	674	0.014
FDM (%)	55.7	55.1	0.41	55.0	54.4	0.071	55.3	55.0	0.54	52.5	51.7	0.076
WSNF (–)	1.12	1.07	0.064	1.08	1.07	0.49	1.11	1.12	0.62	1.06	1.00	0.005
TN ($g \cdot kg^{-1}$)	42.7	42.0	0.18	41.8	41.5	0.12	42.9	43.0	0.73	41.9	42.8	0.014
WS-N ($g \cdot kg^{-1}$)	8.73	10.3	0.008	8.22	9.03	0.041	8.93	9.62	0.046	10.5	12.2	< 0.001
TS-N ($g \cdot kg^{-1}$)	5.88	6.12	0.50	5.08	4.77	0.13	4.79	4.26	0.029	5.75	6.32	0.007
PS-N ($g \cdot kg^{-1}$)	3.38	3.26	0.54	2.80	2.49	0.022	1.93	1.81	0.35	2.37	2.43	0.81
AMM ($mg \cdot kg^{-1}$)	630	495	0.023	433	450	0.64	485	402	0.041	676	625	0.013
OPAW ($g \cdot kg^{-1}$)	46.9	66.4	0.006	49.4	59.0	0.016	52.3	61.8	0.002	62.2	74.6	0.001
OPAT ($g \cdot kg^{-1}$)	30.2	29.1	0.54	24.9	23.1	0.22	16.7	15.5	0.21	25.2	23.3	0.48
ADEG (%)	60.7	51.7	< 0.001	53.8	47.4	< 0.001	55.6	48.0	< 0.001	63.0	51.4	< 0.001
BDEG (%)	60.0	68.7	< 0.001	57.4	65.0	< 0.001	57.7	63.2	< 0.001	62.6	70.1	< 0.001
PLAS ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	0.713	2.90	< 0.001	0.674	1.90	0.003	0.773	2.04	< 0.001	0.754	2.00	0.009
GEN ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	1.43	0.400	< 0.001	1.08	0.469	0.002	1.31	0.553	0.007	1.04	0.735	0.068
P P ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	2.15	3.30	< 0.001	1.75	2.36	0.039	2.09	2.59	0.064	1.80	2.74	0.014
NACL ($g \cdot kg^{-1}$)	7.10	6.80	0.51	11.3	11.6	0.83	9.47	9.28	0.85	11.6	11.5	0.94
CA ($g \cdot kg^{-1}$)	8.48	8.59	0.55	8.51	8.57	0.72	8.72	8.68	0.83	9.02	8.88	0.64
PH (–)	5.79	5.75	0.60	5.66	5.69	0.58	5.61	5.57	0.039	5.80	5.76	0.49
LACT($mmol \cdot kg^{-1}$)	49.7	112	0.042	99.3	98.3	0.94	97.1	121	0.002	63.4	100	0.008
PHOS (mg *p*-NP$\cdot g^{-1}$)	3.80	0.017	< 0.001	4.37	< 0.015	< 0.001	3.80	0.01	< 0.001	4.10	0.052	< 0.001
LIP ($mequ \cdot kg^{-1}$)	4.90	3.08	0.044	5.85	3.95	0.003	–[e]	–	–	7.00	4.10	0.029

[a] FAT: fat content; DM: dry matter; FDM: fat in dry matter; WSNF: water to solid non-fat ratio; TN: total nitrogen (N); WSN: water-soluble N; TS-N: N soluble in 12 % trichloroacetic acid (TCA); PS-N: N soluble in 5 % phosphotungstic acid; AMM : ammonia; OPAW: glutamic acid equivalents (GAE) in the water-soluble fraction; OPAT: GAE in the 12 % TCA-soluble fraction; ADEG: degradation of α-casein; BDEG: degradation of β-casein; PLAS: plasmin acivity; GEN: plasminogen activity; PP: PLAS + GEN; NACL: salt content; CA: calcium; PH: pH value; LACT: lactic acid; PHOS: phosphatase activity; LIP: lipolysis. [b] Production period. [c] Arithmetic mean ($n = 4$). [d] $P_{t=0}$: significance in *t*-test. [e] Not determined.

[a] FAT : teneur en matière grasse ; DM : matière sèche ; FDM : gras/sec ; WSNF : eau/solides non gras ; TN : azote total ; WS-N : azote soluble dans l'eau ; TS-N : azote soluble dans l'acide trichloroacétique (TCA) à 12 % ; PS-N : azote soluble dans l'acide phosphotungstique à 5 % ; AMM : ammoniaque ; OPAW : équivalent acide glutamique (GAE) dans la fraction soluble dans l'eau ; OPAT : GAE dans la fraction soluble dans le TCA à 12 % ; ADEG : dégradation de la caséine α ; BDEG : dégradation de la caséine β ; PLAS : activité plasmine ; GEN : activité plasminogène ; PP : PLAS + GEN ; NACL : teneur en sel ; CA : calcium ; PH : valeur de pH ; LACT : acide lactique ; PHOS : activité phosphatase ; LIP : lipolyse. [b] Période de production. [c] Moyenne arithmétique ($n = 4$). [d] $P_{t=0}$, seuil de signification dans le *t*-test. [e] Non déterminé.

ing dry matter, fat content and derived measures as well as Ca, NaCl and pH, a few significant differences ($P < 0.05$) were only observed in the cheeses produced in autumn. For all production periods, a higher amount of casein breakdown products in the water-soluble fraction was found in the case of cheeses made from pasteurised milk. As far as the fractions covering smaller proteolysis products were concerned, however, the differences diminished and even showed an inverse direction in a very few cases.

Figure 1 shows the electrophoretic patterns of casein of Bergkäse made from raw or pasteurised milk. As a result of the action of plasmin, β-casein is hydrolysed during maturation and the γ-caseins are accumulated as its major degradation products. α_{s1}-Casein represents the principal target for proteolysis during the early stages of ripening. Consequently, α_{s1}-I casein, as its primary degradation product, continues to increase and is subsequently hydrolysed to yield α_{s1}-II casein and other peptides. Both the differentiation and identification of these 'α_{s1}-I casein degradation products' (previously termed 'α_{s1}-II casein' [23, 31]) were not included in this study.

It is evident that the intensity of casein cleavage was heavily influenced by the heat treatment of the milk. Whereas the relative amount of β-casein breakdown appeared to be significantly ($P < 0.001$) higher in Bergkäse made from pasteurised milk, these cheeses showed a less pronounced degradation of α_{s1}-casein calculated from PAGE fractions by

$$\alpha_{s1}\text{-casein breakdown} = 100\ (\alpha_{s1}\text{-I} + \alpha_{s1}\text{-I-DP}) / (\alpha_{s1} + \alpha_{s1}\text{-I} + \alpha_{s1}\text{-I-DP})$$

and using α_{s1}-I degradation products (α_{s1}-I-DP) as defined earlier. For experimental Swiss-type mini-cheeses, Beuvier et al. [9] reported similar data with respect to β-casein degra-

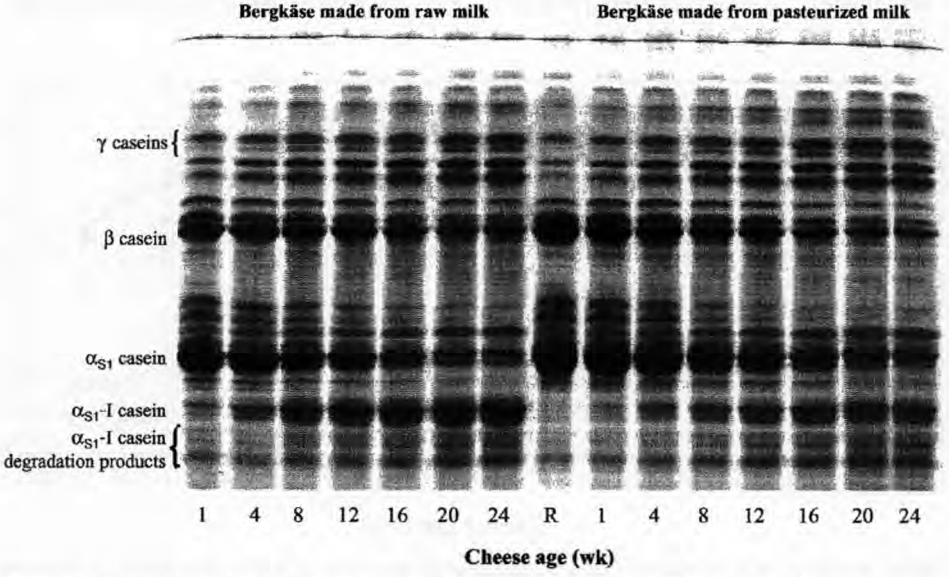

Figure 1. Casein degradation in Bergkäse made from raw or pasteurised milk as evaluated by polyacrylamide gel electrophoresis. Numbers refer to cheese age in weeks. R: native casein reference.

Figure 1. Dégradation de la caséine dans le fromage Bergkäse fabriqué à partir de lait cru ou de lait pasteurisé, évaluée par électrophorèse sur gel de polyacrylamide. Les nombres indiquent l'âge des fromages en semaines. R : caséine native.

dation but stated that the quantity of α_{s1}-I casein was not significantly different between cheeses made from raw or pasteurised milk. However, it must be taken into account that the herewith described measure to quantify the α_{s1}-casein breakdown is more dynamic in nature as it considers also degradation products of α_{s1}-I casein, which were included in the 'α_{s1}-I-DP' fraction.

Apart from the heat treatment effect on the activity of phosphatase, which is still present in mature raw milk cheeses and which might serve as a potential analytical indicator for distinguishing between cheeses made from raw or pasteurised milk, a certain influence on lipolysis was also observed. On total average, the plasmin content of raw milk Bergkäse decreased during maturation from 0.83 ± 0.11 µmol·g^{-1}·h^{-1} *p*-Nitroaniline released (1 week) to 0.73 ± 0.07 µmol·g^{-1}·h^{-1} (24 weeks). In Bergkäse from pasteurised milk, however, the plasmin content increased strongly from 1.18 ± 0.14 to 2.21 ± 0.28 µmol·g^{-1}·h^{-1}. The plasminogen levels (after activation) were found to be 2.06 ± 0.19 and 2.36 ± 0.15 µmol·g^{-1}·h^{-1} for young (1 week) Bergkäse made from raw and pasteurised milk, respectively, and declined during ripening to 1.21 ± 0.24 µmol·g^{-1}·h^{-1} (raw) and 0.54 ± 0.24 µmol·g^{-1}·h^{-1} (pasteurised). These findings are in line with the results of Beuvier et al. [9], who concluded that, due to similar plasminogen levels at the beginning of the ripening, pasteurisation probably showed a more pronounced influence on plasmin inhibitors than on inhibitors of plasmin activators.

Figure 2 depicts in detail changes in the plasmin and plasminogen contents during Bergkäse maturation for the series of four cheeses produced in autumn. In contrast to raw milk cheeses, the plasmin activity of Bergkäse from pasteurised milk increased continuously with ripening time. Additionally, total enzyme activity appeared to be significantly higher in pasteurised milk cheeses. The graphs also show that the variability between the cheeses was much higher for the cheeses made from pasteurised milk.

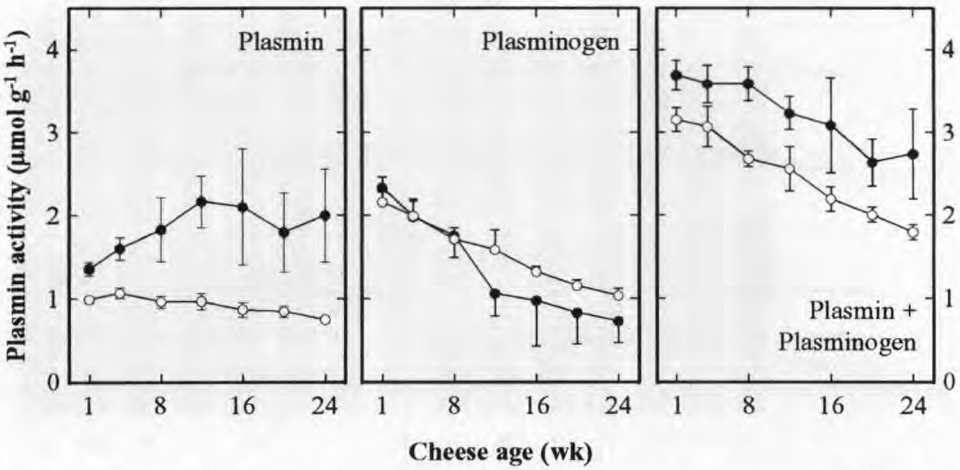

Figure 2. Changes in the content of plasmin and plasminogen activity during maturation of Bergkäse made from raw milk (○, $n = 4$) or pasteurised milk (●, $n = 4$) in autumn. Bars denote standard deviations.

Figure 2. Changements dans l'activité plasmine et plasminogène au cours de l'affinage de fromage Bergkäse fabriqué à partir de lait cru (○, $n = 4$) ou de lait pasteurisé (●, $n = 4$) en automne. Les barres indiquent l'écart type.

This indicates that, despite an almost similar plasmin level in the raw milk and, consequently, raw milk cheeses, the different milks subjected to pasteurisation varied in their reaction on the heat treatment procedure.

In order to cover the influence of maturation on the development of analytical measures, a mixed ANOVA model was applied. In this model, both milk treatment and maturation time were considered as main effects. The seasonal factor served as a subeffect within milk treatment. In the case of significant interactions between treatment and maturation time, which appeared in a number of parameters (*table V*), the corresponding mean square was used to calculate the *F*-values for the effects treatment and season. The ANOVA table clearly shows that, apart from maturation and seasonal effects, milk pasteurisation had a pronounced influence on the composition, proteolysis and enzymatic activities of the resulting cheeses. Together with variations in the content of the organic acids primarily related to the amount and development of

Table V. Effects on composition of Bergkäse as estimated by three-factor (ANOVA).
Tableau V. Effets sur la composition du fromage Bergkäse estimés par analyse de variance à trois facteurs.

Parameter[a]	Milk treatment	Repetition	Maturation	Treatment × Maturation
DM ($g \cdot kg^{-1}$)	0.39[b]	< 0.001	< 0.001	0.017
TN ($g \cdot kg^{-1}$)	0.19	< 0.001	< 0.001	0.54
WS-N TN^{-1} (%)	< 0.001	< 0.001	< 0.001	< 0.001[c]
TS-N TN^{-1} (%)	0.072	< 0.001	< 0.001	0.62
PS-N TN^{-1} (%)	0.49	< 0.001	< 0.001	0.26
AMM ($mg \cdot kg^{-1}$)	< 0.001	< 0.001	< 0.001	0.060
OPAW($mequ \cdot kg^{-1}$)	< 0.001	< 0.001	< 0.001	< 0.001[c]
OPAT ($mequ \cdot kg^{-1}$)	0.43	< 0.001	< 0.001	0.36
ADEG (%)	< 0.001	< 0.001	< 0.001	< 0.001[c]
BDEG (%)	< 0.001	< 0.001	< 0.001	< 0.001[c]
PLAS ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	< 0.001	< 0.001	< 0.001	< 0.001[c]
GEN ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	< 0.001	0.11	< 0.001	< 0.001[c]
PP ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	< 0.001	0.004	< 0.001	0.49
PH (–)	0.57	< 0.001	< 0.001	0.11
LACT ($mmol \cdot kg^{-1}$)	0.004	< 0.001	< 0.001	< 0.001[c]
PHOS (mg *p*-NA$\cdot g^{-1}$)	< 0.001	< 0.001	0.005	0.006[c]

[a] DM: dry matter; TN: total nitrogen; WS-N: water-soluble nitrogen; TS-N: 12 % trichloroacetic acid (TCA) soluble nitrogen; PS-N: 5 % phosphotungstic acid soluble nitrogen; AMM: ammonia; OPAW: glutamic acid equivalents (GAE) in the water-soluble fraction; OPAT: GAE in the 12 % TCA-soluble fraction; ADEG: degradation of α_{S1}-casein; BDEG degradations, of β-casein; PLAS: plasmin activity; GEN: plasminogen activity; PP: PLAS + GEN; PH: pH value; LACT: lactic acid; PHOS: phosphatase activity. [b] Numerical values, probabilities $P(H_0): F > F_{obs}$. [c] Interaction sum-of-squares were used for the calculation of the *F*-values of the factors milk and repetition.

[a] DM : matière sèche ; TN : azote total ; WS-N : azote soluble dans l'eau ; TS-N : azote soluble dans l'acide trichloroactique (TCA) à 12 % ; PS-N : azote soluble dans l'acide phosphotungstique à 5 % ; AMM : ammoniaque ; OPAW : équivalents acide glutamique (GAE) dans la fraction soluble dans l'eau ; OPAT : GAE dans la fraction soluble dans le TCA à 12 % ; ADEG : dégradation de la caséine α ; BDEG : dégradation de la caséine β ; PLAS : activité plasmine ; GEN : activité plasminogène ; PP : PLAS + GEN ; PH : valeur de pH ; LACT : acide lactique ; PHOS : activité phosphatase. [b] Valeurs numériques, probabilites $P(H_0)$, $F > F_{obs}$. [c] Les sommes des carrés de l'interaction étaient utilisées pour calculer lait et répetition.

viable bacteria counts [15] and besides the effect on phosphatase activity, treatment-induced differences appeared in the case of casein degradation and the amount of WSN within the plasmin complex and in the ammonia content.

Both α_{s1}- and β-casein breakdown showed a distinct increase during ripening from approximately 15 % (1 week) to 55 % (24 weeks) and 30 % (1 week) to 65 % (24 weeks), respectively. As pointed out by Beuvier et al. [9], Grappin and Beuvier [22] and Visser [45], the more pronounced α_{s1}-casein breakdown in raw milk cheeses may be attributed to enzymes of the starter bacteria and of the indigenous raw milk flora, which were almost totally eliminated by milk pasteurisation [15]. The role of the indigenous microflora in cheese proteolysis has been demonstrated for some varieties including, e.g. Swiss-type cheeses [9, 10, 13, 14], Cheddar [32] and Manchego cheese made from ewes' milk [19]. Another possible explanation is that the indigenous acid milk protease (Cathepsin D), which is able to hydrolyse primarily α_{s1}-casein [29, 33], is partially inactivated by pasteurisation. Concerning Bergkäse made from pasteurised milk, the increased degradation of β- to γ-casein is obviously related to the observed heat-induced changes within the plasmin/plasminogen complex [16, 34], which cover both activation of plasminogen and the inactivation of plasminogen inhibitors.

In contrast to the enhanced cleavage of α_{s1}-casein in the raw milk cheeses (*table IV*), the differences in β-casein breakdown related to milk treatment are partly reflected by the development of the soluble nitrogen fractions. There appears to be a significant trend for a higher amount of WSN in Bergkäse produced from pasteurised milk ($P < 0.001$), with the absolute difference depending on maturation time (*figure 3*). Regarding the fractions containing smaller peptides and mainly amino acids, i.e. those soluble in 12 % TC and 5 % PTA, respectively [28, 46], the differences were less pronounced and insignificant in ANOVA

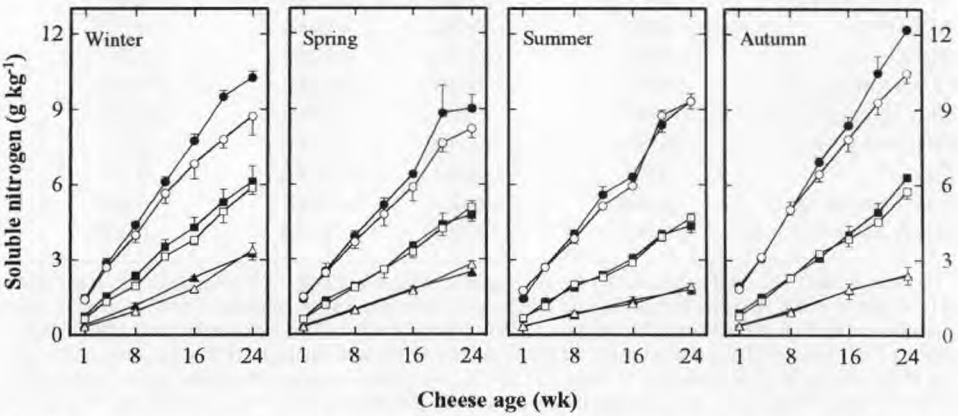

Figure 3. Development of soluble nitrogen (N) fractions in Bergkäse made from raw milk (○ □ △, $n = 4$) or pasteurised milk (● ■ ▲, $n = 4$) in different production periods. ○ ●, water-soluble N; □ ■, N soluble in 12 % trichloroacetic acid; △ ▲, N soluble in 5 % phosphotungstic acid.

Figure 3. Développement des fractions azote (N) soluble dans le fromage Bergkäse fabriqué à partir de lait cru (○ □ △, $n = 4$) ou de lait pasteurisé (● ■ ▲, $n = 4$) au cours de différentes périodes de production. ○ ●, N soluble dans l'eau ; □ ■, N soluble dans l'acide trichloroacétique à 12 % ; △ ▲, N soluble dans l'acide phosphotungstique à 5 %.

(*table V*). It is noticeable, however, that ammonia showed a significantly higher content in the cheeses made from raw milk. As all the cheeses were maintained equally with respect to ripening conditions and surface treatment it is obvious that deamination of amino acids is mainly induced by raw milk flora enzymes [24].

Some reverse-phase HPLC chromatograms of water-soluble extracts of selected cheeses depicted in *figure 4* point to quantitative and, to a minor extent, qualitative differences between elution profiles of cheeses made from raw and pasteurised milk. Generally, Bergkäse produced from pasteurised milk showed higher concentrations of peak 3 (containing tyrosine) and hydrophobic peptides, i.e. fractions 7–9 eluting between 2100 and 3600 s. In agreement with some work done on Swiss-type cheeses [9, 10], and due to the fact that propionibacteria were less relevant and even negligible in most of our raw milk cheeses [15], the lower concentration of these peptides may be attributed to an increased aminopeptidase activity in cheeses containing the indigenous microflora and, especially, facultatively heterofermentative lactobacilli.

The peaks 4, 5 (containing phenylalanine) and 6 (containing tryptophane) increased more rapidly during the maturation of raw milk cheeses. In Bergkäse made from raw milk, tyramine observed in mature cheeses (data not shown) indicated an increased decarboxylation of amino acids by the enzymes of the raw milk flora.

The applied technique for sample preparation made it possible to interpret HPLC chromatograms semi-quantitatively. In line with the results of Kjeldahl analyses of the

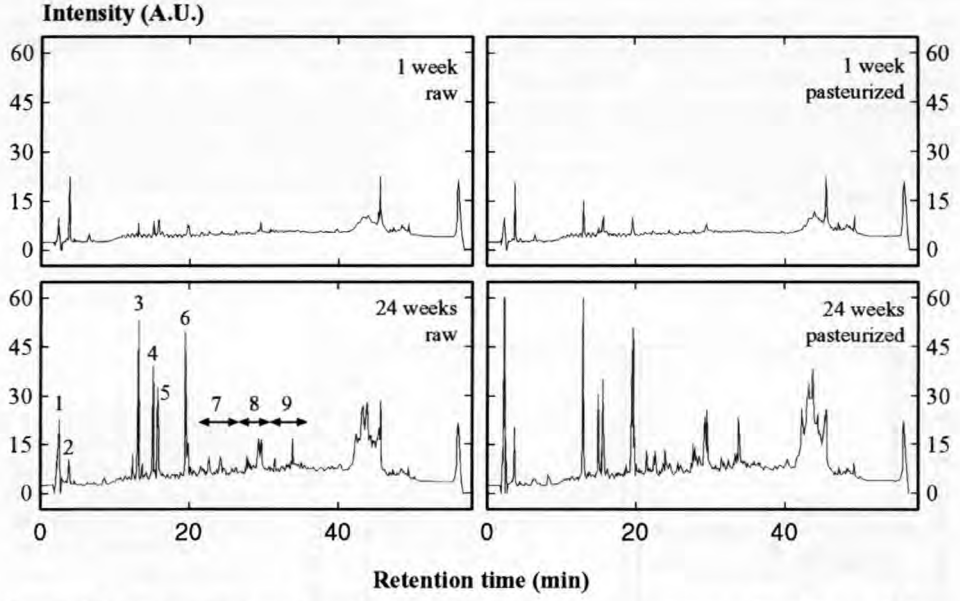

Figure 4. Peptide profiles of the water-soluble fractions of young and mature Bergkäse made from raw or pasteurised milk. Identification of peaks: 1–2, not identified; 3–6, hydrophilic peptides containing (3) tyrosine, (4, 5) phenylalanine and (6) tryptophane; 7–9, hydrophobic peptides.

Figure 4. Profil peptidique des fractions solubles dans l'eau de fromage Bergkäse jeune ou affiné fabriqué à partir de lait cru ou de lait pasteurisé. Identification des pics : 1–2, non identifiés ; 3–6, peptides hydrophiles contenant (3) la tyrosine, (4, 5) la phénylalanine et (6) le tryptophane ; 7–9, peptides hydrophobes.

soluble nitrogen fractions, a lower extent of proteolysis was evident from the peptide profiles for cheeses produced in spring and summer. Whereas hydrophilic fractions (peaks 3–6, including free amino acids) dominated in the cheeses produced in spring, extremely high proportions of hydrophobic peptides (peaks 7, 8) were observed in summer cheeses. Bergkäse produced in autumn showed high amounts of hydrophilic fractions (peaks 3–6) as well as hydrophobic peptides (peaks 7–9), whereas winter cheeses had high concentrations of peaks 3–6 only (results not shown).

Figure 5 depicts the development of selected enzymatic activities in Bergkäse during maturation. Evidently, raw milk cheeses showed increased lipolysis in line with a larger variability, which points to the importance of the raw milk flora for the development of aroma and flavour. Additionally, pasteurisation is known to reduce the activity of the indigenous lipoprotein lipase. It can also be seen from *figure 5* that phosphatase activity declines during maturation but even after 6 months remains on a level clearly distinguishable from the zero activities in Bergkäse produced from regularly pasteurised milk.

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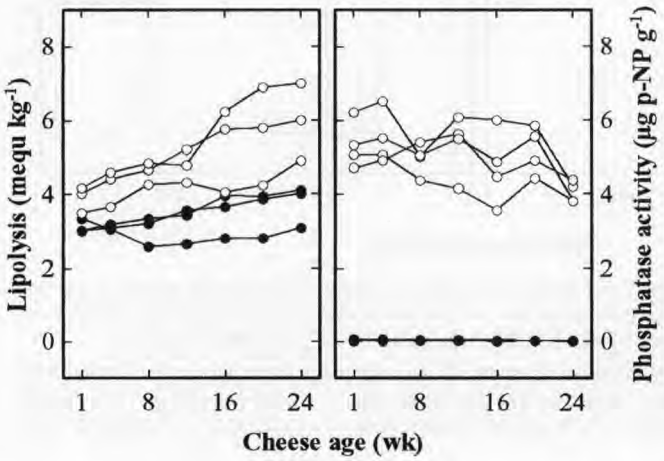

Figure 5. Lipolysis and activity of alkaline phosphatase in Bergkäse made from raw milk (○, $n = 4$) or pasteurised milk (●, $n = 4$) as affected by maturation time.

Figure 5. Lipolyse et activité de la phosphatase alcaline dans le fromage Bergkäse fabriqué à partir de lait cru (○, $n = 4$) ou de lait pasteurisé (●, $n = 4$) en fonction du temps d'affinage.

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