Analytical methods for the determination of the geographic origin of Emmental cheese. Parameters of proteolysis and rheology

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Abstract

Nitrogen fractions, biogenic amines, free amino acids, casein fractions, HPLC-peptide profiles and rheological properties were investigated in 20 Emmental cheeses from six European regions (Switzerland, Vorarlberg (A), Allgäu (D), Finland, Bretagne (F) and Savoie (F)). The ripening time of the samples were different, according to what is found on the market in each region. The non-protein nitrogen and the water-soluble nitrogen fractions showed significant inter-regional differences due mostly to the different ripening times. "Allgäu" and "Savoie" showed the highest proteolysis rate and "Bretagne" had the lowest. Biogenic amines and rheological properties were of less significance for discriminating geographic origin. The relative amounts of five free amino acids allowed "Switzerland" to be separated from the others. The X3 (unknown structure) and αs1 casein fractions combined with one peak from the peptide profile allowed three distinct groups to be differentiated: "Finland", "Bretagne" and "Savoie".

Riassunto

Le frazioni azotate, le ammine biogene, gli amminoacidi liberi, le frazioni caseiniche, i profili dei peptidi-HPLC e le proprietà reologiche sono stati studiati in 20 formaggi Emmental provenienti da sei diverse regioni europee (Svizzera, Vorarlberg (A), Allgäu (D), Finlandia, Bretagne (F) and Savoie (F)). Le frazioni dell'azoto non proteico e dell'azoto solubile in acqua hanno mostrato differenze inter-regionali significative. "Allgäu" e "Savoie" hanno rivelato il più alto tasso di proteolisi mentre "Bretagne" quello più basso. Le ammine di provenienza biologica e le proprietà reologiche si sono mostrate meno importanti nel determinare l'origine geografica dei formaggi. I contenuti relativi di cinque amminoacidi hanno permesso di separare "Switzerland" dagli altri. Le frazioni della caseina X3 (struttura sconosciuta) e αs1, assieme a uno picco del profilo dei peptidi, hanno permesso di differenziare tre gruppi diversi: rispettivamente quello "Finland", quello "Bretagne" e quello "Savoie".

-Key words: authenticity, traceability, Emmental cheese, free amino acid, peptide, proteolysis

INTRODUCTION

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The enzymatic degradation of casein into peptides and amino acids is a very complex process because of the various origins of the proteases and peptidases involved. They may come from milk itself (plasmin, cathepsin), coagulant (chymosin, pepsin and further fungal proteases), starter, as well as non-starter micro-organisms, secondary cultures (e.g., *Propionibacterium freudenreichii*) and exogeneous proteinases or peptidases. Proteolysis occurs to a great extent during ripening. The degree of protein breakdown can be expressed by factors such as the content of water soluble nitrogen (WSN), non-protein nitrogen (NPN) or total free amino acids. Proteolysis has been considered as the basis for classifying different cheese types (MARCOS *et al.*, 1979; SMITH and NAKAI, 1990; AISHIMA and NAKAI, 1987, McGOLDRICK and FOX, 1999, FOX, 1993). The evolution of proteolysis, based on peptide mapping at various stages of ripening, has already been studied for Emmental (BICAN and SPAHNI, 1993, BÜTIKOFER *et al.*, 1997), Gruyère, Appenzell (BICAN and SPAHNI, 1993), Parmigiano-Reggiano (ADDEO *et al.*, 1994), Blue cheese (GONZALEZ DE LLANO *et al.*, 1991) and for several artisanal cheeses (GONZALEZ DE LLANO *et al.*, 1995). The influence of specific Lactobacilli strains on the peptide profile has been studied in cheeses such as Emmental (BÜTIKOFER *et al.*, 1997, CHOPARD *et al.*, 2001) and Cheddar (McSWEENEY *et al.*, 1994, PRIPP *et al.*, 1999). Considerable differences were found between peptide profiles of Cheddar cheese made from raw or pasteurised milk (McSWEENEY *et al.*, 1993).

Further degradation of peptides by peptidases leads to small peptides and free amino acids (FAA). The total concentration of FAA is also an indicator of age. RESMINI *et al.* (1985, 1993) identified genuine Parmigiano-Reggiano and Grana Padano cheese by their free amino acid composition. BÜTIKOFER and FUCHS (1997) were able to correctly classify Emmental, Gruyère and Sbrinz with more than 93% accuracy from their relative amounts of free amino acids. Appenzeller and Tilsiter could be correctly classified to 70-75%.

The degradation of the free amino acids through enzymatic decarboxylation may lead to the formation of biogenic amines. In cheese, non-starter bacteria such as enterococci, salt tolerant lactobacilli and enterobacteriaceae may be responsible for the formation of biogenic amines (JOOSTEN and NORTHOLT, 1987). Some of these amines may provoke allergies or illness. The best known amines in cheese are histamine, tyramine, cadaverine, putrescine, tryptamine and β-phenylethylamine (SIEBER and LAVANCHY, 1990).

Rheological characteristics are often correlated with proteolysis (BOSSET *et al.*, 1993; EBERHARD, 1985). Large peptides are important for the development of the correct texture. PIRISI *et al.* (2000) described large differences in mechanical properties of similar PDO ewe's milk cheeses from three different countries. Cheeses from milk produced on mountain pastures exhibited different rheological properties than those produced with milk from valley pastures; they were less elastic and less deformable (BUGAUD *et al*., 2001).

The present work is part of a broad screening test, which is the first step in a 3-year study on the authenticity of Emmental cheese and its geographic traceability (PILLONEL *et al.*, 2002a, BOSSET, 2001). To date, a large number of analytical methods have been tested for their discriminating potential (PILLONEL *et al*., 2002a-e) while the number of cheese samples for each region has been limited. Obviously, the analytical results obtained from a modest number of cheese samples from a region can only give trends which will need to be confirmed later if they appear to be of value for discriminating cheeses produced in different countries. The objective of the present paper was to determine whether chemical and physical parameters linked to proteolysis, such as nitrogen fractions, biogenic amines, free amino acids, casein fractions, peptide pattern and rheological analyses make it possible to discriminate between the different geographic origins of Emmental cheese samples. The goal was not to build a detailed model for predicting the origin but rather to observe the trends.

The cheese samples, with different ripening times, were chosen according to what is sold by retailers in the corresponding regions.

MATERIALS AND METHODS

Origin and selection of the cheese samples

The main framework of this study and the sampling methods are described in detail in a previous work (PILLONEL *et al.*, 2002a). Table 1 summarises origin, date of production and ripening time of the samples.

Table 1

Analysis of the compounds produced by proteolysis

The samples were deep-frozen prior to the following analyses: total nitrogen (TN), water-soluble nitrogen (WSN) and non-protein nitrogen (NPN) according to Kjeldahl (COLLOMB *et al.*, 1990); free amino acids (FAA) using HPLC after pre-column derivatisation with o-phthalaldehyde and fluorenylmethyl chloroformate (FMOC) (BÜTIKOFER and ARDÖ, 1999); biogenic amines with HPLC after precolumn derivatisation with dansylchlorid (BÜTIKOFER *et al.*, 1990); o-phthalaldehyd-value (OPA) photometrically (FRISTER *et al.*, 1989). Casein fractions were determined by SDS electrophoresis (COLLIN *et al.*, 1987). The peptide pattern of the water-soluble peptide fraction was investigated by RP-HPLC (CHOPARD *et al.*, 2001).

Rheological analyses

The following three rheological properties were measured with a universal testing machine (Zwick 1435, Zwick GmbH, Germany) at 15±0.5°C according to the procedure of PESENTI and LUGINBUEHL (1999): i) the stress at 33% deformation, which characterises the elasticity of the body, ii) the deformation at fracture, which is a measure of the consistency of the body and iii) the stress at fracture, which corresponds, more or less, to the sensation of hardness in the mouth. The measurements were carried out on a cylindrical, holeless cheese sample (diameter: 12 mm, height: 15 mm).

The penetration depth was determined using a constant force penetrometer (Petrotest PNR-10, Petrolab, USA) under the following conditions: force, 0.63 N; time, 0.5 s; standard needle.

Statistical analyses

The averages and standard deviations were calculated for each parameter (Table 2-6). Descriptive statistics, analysis of variance (ANOVA), pairwise comparisons of mean values with Fisher's LSD test, principal component analyses of the correlation matrix and correlation test were performed with Systat for Windows version 9.0 (SPSS Inc., Chicago, IL). The correlation coefficients were calculated using the individual values for cheeses from each region.

RESULTS AND DISCUSSIONS

Since the number of samples analysed was very limited, we restricted the discussion of the results to the tests of the mean value differences and untrained classification techniques, such as the principal component analysis (PCA). Trained classification techniques were not used because they require larger data sets in order to be reliable.

Nitrogen fractions, OPA-values and biogenic amines

The results for the nitrogen fractions, the OPA-values and the biogenic amines are given in Table 2. The total nitrogen concentration values were only slightly different whereas the differences between the NPN and WSN values were highly significant. The NPN and the OPA-values were strongly correlated (r = 0.94). The OPA-values were less discriminating due to the higher relative standard deviations of the results. "Bretagne" had the lowest NPN and WSN values, as expected, for a cheese with such a short ripening period. It was followed by "Finland". "Allgäu" and "Savoie", ripened 4 and 3.5 months respectively, had the highest NPN and WSN values. The values for "Switzerland" laid in the middle. This Emmental, aged for 4 months, is still considered to be very young in Switzerland. In fact, the proteolysis of "Switzerland" is deliberately slowed down to allow a ripening time of up to 12 months and more, leading to a very characteristic and mature cheese. In the other regions, a fast maturation is preferred for economic reasons. The two ratios, WSN/TN and NPN/WSN, are indices of protein and peptide hydrolyses, respectively. The WSN/TN ratio showed exactly the same trend as that of WSN. For the NPN/WSN ratio, the differences were less significant. A point of interest is that the peptide hydrolysis in "Finland" was significantly higher.

Table 2

The lowest total concentration of biogenic amines was found in "Switzerland" and the highest in "Allgäu" and "Savoie". These differences were largely due to the degree of proteolysis. Significant differences were found for cadaverine, histamine, isopentylamine, putrescine, tryptamine and tyramine. "Switzerland" had the lowest cadaverine, histamine and putrescine values and slightly higher isopentylamine values. Spermine and spermidine could not be detected in the samples. It should be noted that the biogenic amine concentrations encountered did not present any danger to health.

The microbial flora was probably the main factor influencing the amount of biogenic amines. Propionibacterium and thermophilic *Lactobacillus (the latter being added specifically for this experiment)* may account for the formation of histamine in Maasdamer (JOOSTEN, 1987). The influence of Propionibacterium on the formation of histamine and tyramine in Emmental has been reported previously (ANTILA *et al*., 1984). Non-starter lactic acid bacteria (lactobacilli, enterococci) may cause the formation of tyramine and histamine in Gouda (JOOSTEN AND NORTHOLT, 1987). According to the latter authors, salt-tolerant lactobacilli account for a massive formation of putrescine and cadaverine.

Furthermore a concentration gradient was found in Gouda, with the central part of the block containing higher amounts of the investigated amines (tyramine, histamine, putrescine and cadaverine) than samples taken close to the rind (JOOSTEN and STADHOUDERS, 1987). Many chemical, physical and microbial factors also influence the formation of biogenic amines within a cheese variety (SIEBER and BILIC, 1992), leading to very scattered concentrations in samples which come from the same region of production. This was confirmed in "Switzerland" in earlier studies (SIEBER *et al*., 1988, BOSSET *et al*., 1992).

Free amino acids (FAA)

The sum of FAA showed the same trend as the WSN and the NPN values but with smaller significant differences between the groups. To produce a discrimination that was less age-dependant, relative amounts were considered (normalisation by the total free amino acids). Out of the 23 free amino acids analysed (Table 3), 16 had a significant difference between at least two groups. No phosphoserine was found. The highest significant differences were found for asparagine, glutamine, methionine, phenylalanine, proline and valine.

"Bretagne" could easily be differentiated using the values of methionine and glutamine. With five parameters, asparagine, glycine, lysine, phenylalanine and proline (selection made with the help of a trained classification technique), it was possible to isolate "Switzerland" by PCA (Figure 1). The other regions could not be clearly separated.

Table 3

Figure 1

HPLC-peptide profile and casein fractions

Figure 2 is a typical peptide chromatogram. The 18 peptide peaks which showed significant differences are listed in Table 4. The highest values for peaks 10.1, 11.3, 12.1, 14, 22 and 24 were recorded for "Switzerland". The use of *Lb. helveticus* in starter cultures is known to lead to a smaller peak 14 (CHOPARD *et al.*, 2001). These findings were confirmed in this study; the highest peak 14 values were found in "Switzerland", the only sample that did not contained *Lb. helveticus* (PILLONEL *et al*., 2002a). Peaks 2 and 3 were only found in "Finland" and peaks 4, 7, 12 and 14.1 had much higher values in "Finland" than in cheeses from the other regions.

Table 4

Figure 2

The relative proportions of α-, β-, and indirectly γ-caseins in milk are subject to genetic variations between cows within a same breed (VARNAM AND SUTHERLAND, 1994). In cheese however, the differences between two samples are more likely to be due to different intensities of proteolysis than to the milk used. The results of the casein fractions are listed in Table 5. All casein fractions showed significant differences except for X1-2, X2-1, X2-2 and β degraded. The fractions coded with a "X" are of unknown structure. X1 and X1-2 migrate after β, X2-1 and X2-2 after αS and X3 before αs_1. The αs1 fraction was highest in "Bretagne" and "Finland" and lowest in "Switzerland", followed by "Allgäu". In soft cheeses, the αs1 fraction is typically degraded to αs1-I by the rennet enzyme chymosin. In cooked cheeses such as Emmental, the chymosin is extensively denatured during manufacturing and contributes little to ripening (COLLIN *et al*., 1988; BOUDJELLAB *et al*., 1994). It is more likely that the αs1 degradation is due to the enzymes of the lactobacilli present in the cheese (OLSON, 1990, CHAMBA, 2000). This type of proteolysis however is relatively slow. The low αs1 level in "Switzerland" and "Allgäu" can therefore be explained by the longer ripening time in these regions and/or by a higher protease activity of the lactobacilli. "Bretagne" showed the highest value because of the very short ripening time and probably because of the thermisation applied to the milk (ITFF, not published). The significantly higher αs1 level in "Finland" compared with "Savoie" and "Vorarlberg", though all three were ripened for approximately three months, may be explained by the higher cooking temperature in the former (54°C *vs*. 52-54°C) and by the milk thermisation process applied in "Finland" and not in the other two countries (information held by the producers).

Unlike chymosin, the endogenous milk enzyme, plasmin, is not damaged by the high temperature used in cooked cheese. The plasmin activity even elevated in "high cook" cheese varieties is due to thermal inactivation of inhibitors of plasminogen activators. This results in the increased conversion of plasminogen to the active form, plasmin (LU AND NIELSEN, 1993; FARKYE AND FOX, 1990). Plasmin degrades αs2 and β casein. αs2 commonly disappears in Emmental after 45 days of ripening (CHAILLET, 2002). In the current study, two samples from "Bretagne" still contained αs2, probably due to the very short ripening time. The degradation of the β casein leads to the γ1 to γ3 fractions. The same tendency was

observed in β as in αs1. “Bretagne” had the highest value followed by “Finland”, whereas “Allgäu” and “Switzerland” showed the lowest values. The likely explanation is once again the longer ripening time. The X1-2 fraction was only found in two Swiss samples. The parameters αs1 and X3, which gave the best discrimination, combined with peak 14 from the HPLC-peptide profile, gave an interesting separation profile (Figure 3). The ratio of casein fractions to TN gave a similar separation profile.

Table 5

Figure 3

Rheology

No significant differences were found between the cheeses from any of the regions except for the penetration depth (Table 6). “Switzerland” showed the highest penetration depth, while “Savoie”, “Bretagne”, and “Finland” had the lowest. The penetration depth is supposed to be correlated with the water and fat content (BOSSET *et al.*, 1993). This was not observed in this case with water and the correlation with the fat content was poor ($r = 0.40$). The penetration depth was negatively correlated with the stress at 33% deformation ($r = -0.873$), which corresponds to the firmness of the body. The stress at fracture was positively correlated with the strain at fracture ($r = 0.704$), which corresponds to the length of the body.

Table 6

CONCLUSION

Compounds linked to the proteolysis allowed one to separate some of the Emmental cheese samples according to their geographic origin. The NPN, WSN and WSN/TN fractions showed highly significant differences which were partly due to differences in ripening times. These differences are useful because each region produces Emmental with a typical ripening time before being put on the market. The high standard deviations of the biogenic amine concentrations made them unusable as markers of origin. The relative amounts of the free amino acids asparagine, glycine, lysine, phenylalanine and proline made it possible to separate “Switzerland”. A clear differentiation of “Bretagne” was achieved using the methionine and glutamine values. The casein fractions (αs1 and X3) and the peptide pattern (peak 14) provided a good separation of the “Finland”, “Bretagne” and “Savoie” groups. The rheological results were of less interest for the geographic discrimination of Emmental cheese.

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Table 1 Origin and ripening time of the 20 cheese samples investigated.

Abbreviation	Region (country)	Number of samples	Date of manufacture	Ripening time (months)
AL	Allgäu (D)	3	25.12.2000	4
BR	Bretagne (F)	3	20.02.2001	2.5
CH	Switzerland (CH)	6	26.12.2000	4
FI	Middle Finland (FI)	2	04.02.2001	3
SA	Savoie (F)	3	05.02.2001	3
VO	Vorarlberg (A)	3	02.02.2001	3

Table 2 Nitrogen fractions and biogenic amines in the 20 Emmental cheese samples investigated.

Analytes	ANOVA	Region (n=) AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
		x	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x
TN (g/kg)	*	**44.1**^{AB}	1.7	**45.03**^{AB}	0.72	**44.42**^{AB}	0.85	**45.5**^{AB}	1.3	**46.20**^{A}	0.77	**42.9**^{B}	1.6
WSN (g/kg)	***	**11.2**^{A}	1.1	**6.4**^{C}	1.7	**9.35**^{B}	0.41	**8.31**^{BC}	0.51	**10.72**^{AB}	0.99	**8.77**^{B}	0.38
WSN / TN (%)	***	**25.5**^{A}	1.8	**14.2**^{C}	4.0	**21.1**^{B}	1.0	**18.3**^{BC}	0.6	**23.2**^{AB}	1.8	**20.4**^{B}	0.6
NPN (g/kg)	***	**7.37**^{A}	0.65	**3.98**^{D}	0.90	**5.86**^{BC}	0.55	**6.19**^{ABC}	0.58	**7.20**^{AB}	1.07	**5.42**^{CD}	0.37
NPN / WSN (%)	ns	**65.6**	0.8	**62.9**	8.2	**62.6**	4.3	**74.4**	2.4	**66.9**	4.3	**61.7**	2.6
OPA (mmol/kg)	*	**246**^{A}	63	**136**^{B}	39	**184**^{AB}	20	**222**^{AB}	34	**255**^{A}	61	**187**^{AB}	34
Cadaverine (mg/kg)	**	**44**^{A}	35	**5.6**^{B}	7.0	**0.22**^{B}	0.27	**25**^{AB}	15	**8.6**^{B}	6.7	**1.13**^{B}	0.75
Histamine (mg/kg)	*	**672**^{A}	376	**123**^{B}	149	**12.3**^{B}	7.4	**59**^{B}	26	**478**^{A}	434	**205**^{A}	105
Isopentylamine (mg/kg)	**	**1.80**^{AB}	0.66	**0.93**^{B}	0.55	**2.78**^{A}	1.12	**1.05**^{AB}	0.21	**0.53**^{B}	0.15	**0.17**^{B}	0.29
Putrescine (mg/kg)	*	**15**^{AB}	21	**2.3**^{AB}	1.2	**<2**^{B}	--	**<2**^{B}	--	**24**^{A}	19	**<2**^{B}	--
β-Phenylethylamine (mg/kg)	ns	**6.0**	4.0	**1.1**	1.9	**11**	16	**15**	14	**34**	27	**1.2**	2.1
Tryptamine (mg/kg)	ns	**<2**	--	**<2**	--	**<2**	--	**3.0**	2.2	**<2**	--	**<2**	--
Tyramine (mg/kg)	*	**178**^{AB}	121	**8.6**^{B}	8.4	**54**^{B}	123	**220**^{AB}	45	**403**^{A}	269	**117**^{AB}	91
Total biogenic amines (mg/kg)	*	**920**^{A}	420	**142**^{B}	165	**81**^{B}	134	**325**^{AB}	14	**948**^{A}	709	**325**^{AB}	195

Caption: x = mean value; s_x = standard deviation; ANOVA: ns = not significant, *) $p \leq 0.05$, **) $p \leq 0.01$, ***) $p \leq 0.001$
Production sites: A>B>C>D (=significantly different contents $p \leq 0.01$) or AB = A and B overlap by using an univariate discriminant analysis
AL = Allgäu, BR = Bretagne, CH = Switzerland, FI = Finland, SA = Savoie, VO = Vorarlberg

Table 3 Free amino acid ratios in the 20 Emmental cheese samples investigated.

Analytes	ANOVA	Region (n=) AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
		x	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x
Total free amino acids (g/kg)	*	**28.2**^{A}	7.1	**17.2**^{B}	5.2	**19.8**^{AB}	3.0	**22.5**^{AB}	7.3	**28.7**^{A}	4.4	**19.3**^{AB}	2.0
Alanine (%)	ns	**3.08**	0.58	**3.84**	1.3	**3.34**	0.17	**3.71**	0.84	**3.26**	0.49	**3.03**	0.21
α-Amino butyric acid (%)	ns	**1.49**	2.10	**0.13**	0.01	**0.18**	0.12	**0.06**	0.02	**0.20**	0.11	**0.06**	0.06
Arginine (%)	ns	**0.10**	0.04	**0.04**	0.01	**0.84**	0.83	**0.86**	0.15	**0.08**	0.03	**0.08**	0.03
Asparagine (%)	***	**3.08**^{BC}	1.17	**4.63**^{AB}	1.95	**7.06**^{A}	0.62	**1.35**^{C}	0.11	**2.51**^{BC}	1.22	**4.77**^{AB}	1.8
Aspartic acid (%)	*	**2.91**^{A}	1.54	**1.27**^{AB}	0.80	**1.78**^{AB}	0.42	**0.16**^{B}	0.00	**0.96**^{B}	0.16	**1.35**^{AB}	1.00
Citrulline (%)	*	**0.89**^{B}	0.39	**2.14**^{AB}	0.52	**3.31**^{A}	0.83	**2.43**^{AB}	0.92	**2.10**^{AB}	1.10	**2.45**^{AB}	0.55
γ-Amino butyric acid (%)	*	**0.40**^{A}	0.34	**0.30**^{AB}	0.28	**0.05**^{B}	0.04	**0.01**^{AB}	0.01	**0.05**^{AB}	0.04	**0.03**^{AB}	0.02
Glutamine (%)	***	**2.30**^{B}	0.55	**4.25**^{A}	0.67	**1.94**^{B}	0.58	**3.20**^{AB}	0.74	**2.81**^{B}	0.26	**2.43**^{B}	0.52
Glutamic acid (%)	ns	**18.38**	0.54	**16.99**	0.24	**18.19**	1.1	**17.63**	0.62	**18.23**	0.41	**19.03**	0.64
Glycine (%)	*	**2.03**^{AB}	0.07	**2.03**^{AB}	0.14	**1.90**^{B}	0.15	**1.83**^{B}	0.01	**1.97**^{AB}	0.02	**2.16**^{A}	0.01
Histidine (%)	**	**0.35**^{B}	0.36	**2.81**^{A}	0.09	**1.84**^{A}	0.32	**3.24**^{A}	0.17	**2.20**^{A}	1.8	**1.87**^{AB}	0.41
Isoleucine (%)	**	**5.12**^{A}	0.72	**3.87**^{ABC}	0.65	**2.86**^{C}	0.28	**3.77**^{ABC}	0.66	**4.50**^{AB}	0.78	**3.53**^{BC}	0.73
Leucine (%)	**	**12.14**^{B}	1.2	**12.19**^{B}	0.84	**13.90**^{A}	0.53	**11.87**^{B}	1.3	**11.94**^{B}	0.59	**13.87**^{A}	0.55
Lysine (%)	*	**12.58**^{A}	0.34	**10.82**^{B}	1.6	**11.34**^{AB}	0.43	**12.69**^{A}	0.26	**12.69**^{A}	0.07	**11.60**^{AB}	0.35
Methionine (%)	***	**2.50**^{A}	0.11	**1.95**^{B}	0.09	**2.44**^{A}	0.07	**2.57**^{A}	0.03	**2.28**^{A}	0.22	**2.45**^{A}	0.09
Ornithine (%)	*	**5.09**^{A}	0.31	**3.63**^{AB}	0.49	**2.47**^{B}	1.0	**2.99**^{AB}	1.7	**4.33**^{AB}	1.8	**3.73**^{AB}	0.82
Phenyalanine (%)	***	**6.26**^{AB}	0.72	**5.33**^{C}	0.18	**6.96**^{A}	0.35	**6.06**^{BC}	0.34	**5.95**^{BC}	0.09	**6.53**^{AB}	0.29
Proline (%)	***	**6.28**^{BC}	2.74	**9.44**^{AB}	1.00	**4.05**^{C}	0.35	**9.98**^{AB}	0.78	**10.21**^{A}	0.74	**5.27**^{C}	2.4
Serine (%)	ns	**2.10**	0.10	**2.20**	0.47	**1.82**	0.20	**2.35**	0.38	**1.80**	0.37	**1.98**	0.28
Threonine (%)	ns	**2.90**	0.07	**2.57**	0.44	**2.77**	0.08	**3.16**	0.34	**2.83**	0.28	**2.97**	0.07
Tryptohphan (%)	*	**0.34**^{B}	0.05	**0.37**^{B}	0.10	**0.41**^{AB}	0.04	**0.53**^{A}	0.07	**0.45**^{AB}	0.09	**0.39**^{AB}	0.00
Tyrosine (%)	ns	**2.21**	0.65	**2.42**	0.37	**2.76**	1.0	**2.48**	0.99	**1.30**	0.57	**2.63**	0.72
Valine (%)	***	**7.49**^{AB}	0.20	**6.80**^{C}	0.16	**7.78**^{A}	0.19	**7.08**^{BC}	0.00	**7.34**^{AB}	0.40	**7.77**^{A}	0.13

Caption: x = mean value; s_x = standard deviation; ANOVA: ns = not significant, *) $p \leq 0.05$, **) $p \leq 0.01$, ***) $p \leq 0.001$
Production sites: A>B>C>D (=significantly different contents $p \leq 0.01$) or AB = A and B overlap by using an univariate discriminant analysis
AL = Allgäu, BR = Bretagne, CH = Switzerland, FI = Finland, SA = Savoie, VO = Vorarlberg

Table 4 Peptide pattern of the 20 Emmental cheese samples investigated using HPLC (peak area, arbitrary units).

Peak number	ANOVA	Region (n=) AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
		x	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x
1	*	**158**^{AB}	138	**459**^{A}	59	**190**^{B}	51	**251**^{AB}	8	**262**^{AB}	58	**154**^{B}	176
2	***	**n.d.**^{B}	--	**n.d.**^{B}	--	**n.d.**^{B}	--	**107**^{A}	20	**n.d.**^{B}	--	**n.d.**^{B}	--
3	***	**n.d.**^{B}	--	**n.d.**^{B}	--	**n.d.**^{B}	--	**122**^{A}	18	**n.d.**^{B}	--	**n.d.**^{B}	--
4	*	**n.d.**^{B}	--	**26**^{B}	45	**29**^{B}	71	**193**^{A}	83	**32**^{B}	55	**n.d.**^{B}	--
7	***	**n.d.**^{B}	--	**n.d.**^{B}	--	**12**^{B}	29	**528**^{A}	32	**30**^{B}	53	**n.d.**^{B}	--
8	**	**212**^{B}	120	**269**^{B}	60	**434**^{A}	51	**466**^{A}	8	**279**^{B}	90	**351**^{AB}	38
10.1	**	**74**^{BC}	69	**27**^{C}	47	**184**^{A}	67	**177**^{AB}	13	**24**^{C}	42	**129**^{ABC}	18
11	*	**1276**^{A}	73	**595**^{AB}	587	**1144**^{A}	429	**554**^{AB}	297	**322**^{B}	398	**687**^{AB}	248
11.1	*	**367**^{A}	227	**55**^{B}	96	**91**^{B}	115	**283**^{AB}	91	**31**^{B}	53	**278**^{AB}	206
11.3	**	**215**^{AB}	19	**79**^{BC}	138	**309**^{A}	110	**101**^{ABC}	143	**n.d.**^{C}	--	**145**^{ABC}	13
12	*	**n.d.**^{B}	--	**447**^{AB}	496	**n.d.**^{B}	--	**783**^{A}	696	**263**^{AB}	77	**n.d.**^{B}	--
12.1	***	**8827**^{AB}	8275	**1537**^{BC}	1347	**12380**^{A}	2285	**1178**^{BC}	1666	**n.d.**^{C}	--	**8122**^{ABC}	667
13.1	*	**1402**^{AB}	340	**908**^{B}	399	**1745**^{AB}	379	**2187**^{A}	249	**829**^{B}	823	**1722**^{AB}	419
14	***	**5707**^{BC}	3774	**2554**^{C}	1246	**14755**^{A}	3873	**955**^{C}	585	**683**^{C}	58	**9649**^{AB}	1641
14.1	**	**268**^{B}	326	**558**^{B}	401	**202**^{B}	170	**2020**^{A}	480	**1043**^{AB}	866	**227**^{B}	242
22	*	**997**^{B}	818	**589**^{B}	300	**2791**^{A}	1069	**2219**^{AB}	1937	**536**^{B}	292	**1135**^{AB}	387
24	*	**970**^{AB}	984	**234**^{AB}	17	**1832**^{A}	816	**492**^{AB}	188	**98**^{B}	22	**819**^{AB}	709
37	*	**n.d.**^{B}	--	**53**^{A}	47	**n.d.**^{B}	--	**n.d.**^{B}	--	**n.d.**^{B}	--	**n.d.**^{B}	--
Total area	***	**70092**^{A}	8108	**37076**^{B}	2338	**81370**^{A}	12527	**73532**^{A}	1769	**35576**^{B}	24376	**68974**^{A}	15313

Caption: x = mean value; s_x = standard deviation; ANOVA: ns = not significant, *) $p \leq 0.05$, **) $p \leq 0.01$, ***) $p \leq 0.001$
Production sites: A>B>C>D (=significantly different contents $p \leq 0.01$) or AB = A and B overlap by using an univariate discriminant analysis
AL = Allgäu, BR = Bretagne, CH = Switzerland, FI = Finland, SA = Savoie, VO = Vorarlberg
n.d.: below the detection limit

Table 5 Casein fractions using SDS-PAGE-electrophoresis in the 20 Emmental cheese samples investigated (peak area, arbitrary units).

Fraction name	ANOVA	Region (n=) AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
		x	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x
Para*K*	*	**8.87**^{A}	0.85	**6.60**^{B}	0.56	**8.02**^{AB}	0.83	**7.05**^{AB}	0.92	**7.33**^{AB}	0.58	**7.87**^{AB}	0.55
β degraded	ns	**3.40**	0.66	**1.93**	0.71	**2.72**	0.58	**1.85**	0.07	**3.87**	0.32	**3.8**	1.2
γ1	*	**12.40**^{A}	0.96	**8.4**^{B}	1.7	**11.1**^{AB}	1.4	**9.6**^{AB}	2.6	**10.13**^{AB}	0.87	**9.57**^{AB}	0.23
γ2	**	**7.1**^{A}	1.0	**3.73**^{B}	0.80	**6.53**^{A}	0.69	**6.0**^{A}	1.2	**5.83**^{A}	0.32	**5.93**^{A}	0.81
γ3	*	**3.46**^{ABC}	0.76	**2.00**^{C}	0.10	**4.23**^{A}	0.88	**3.05**^{ABC}	0.78	**3.93**^{AB}	0.72	**4.0**^{AB}	1.2
X1	ns	**3.53**	0.38	**2.40**	0.50	**3.50**	0.42	**3.00**	0.57	**3.37**	0.15	**2.87**	0.86
X1-2	ns	**n.d.**	--	**n.d.**	--	**0.40**	0.67	**n.d.**	--	**n.d.**	--	**n.d.**	--
β	**	**14.4**^{C}	1.4	**21.5**^{A}	1.6	**16.8**^{B}	1.5	**17.6**^{AB}	2.7	**17.1**^{B}	1.6	**18.1**^{AB}	1.4
X2-1	ns	**1.87**	0.57	**2.37**	0.72	**1.97**	0.27	**1.90**	0.85	**2.43**	0.25	**2.73**	0.90
X2-2	ns	**8.67**	0.83	**7.77**	1.21	**8.68**	0.37	**7.70**	0.28	**8.30**	0.92	**7.80**	0.61
α_{s2}	**	**n.d.**^{B}	--	**2.7**^{A}	2.4	**n.d.**^{B}	--	**n.d.**^{B}	--	**n.d.**^{B}	--	**n.d.**^{B}	--
α_{s1}	***	**8.7**^{BC}	1.5	**20.6**^{A}	1.4	**6.68**^{C}	0.96	**19.70**^{A}	0.28	**11.7**^{B}	1.8	**10.5**^{B}	1.7
α_{s1}-I	**	**19.5**^{ABC}	1.8	**15.7**^{C}	1.8	**22.3**^{A}	1.8	**16.9**^{BC}	2.2	**19.47**^{ABC}	0.92	**21.1**^{AB}	2.1
X3	***	**8.00**^{A}	0.66	**4.23**^{C}	0.60	**7.1**^{AB}	1.2	**5.70**^{BC}	0.14	**6.60**^{AB}	0.17	**5.53**^{BC}	0.23

Caption: x = mean value; s_x = standard deviation; ANOVA: ns = not significant, *) $p \leq 0.05$, **) $p \leq 0.01$, ***) $p \leq 0.001$
Production sites: A>B>C>D (=significantly different contents $p \leq 0.01$) or AB = A and B overlap by using an univariate discriminant analysis
AL = Allgäu, BR = Bretagne, CH = Switzerland, FI = Finland, SA = Savoie, VO = Vorarlberg
X1, X1-2: fractions with unknown constitution migrating after β
X2-1, X2-2: fractions with unknown constitution migrating after αs_1
X3: fractions with unknown constitution migrating before αs_1
n.d.: below the detection limit

Table 6 Rheological analyses of the 20 Emmental cheese samples investigated.

Parameters analysed	ANOVA	Region (n=) AL (3)		BR (3)		CH (6)		FI (2)		SA (3)		VO (3)	
		x	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x	**x**	s_x
Depth of penetration (mm)	*	**10.6**^{AB}	1.6	**9.4**^{B}	1.4	**12.0**^{A}	1.2	**9.5**^{AB}	1.3	**9.3**^{B}	0.77	**10.9**^{AB}	1.3
Stress at fracture (N)	ns	**68**	14	**74**	7.7	**74**	8.7	**65**	3.1	**64**	2.3	**71**	5.8
Strain at fracture (%)	ns	**56**	19	**104**	46	**68**	31	**79**	3.6	**56**	5.7	**60**	25
Stress at 33% deformation (N)	ns	**18**	5.4	**19**	4.8	**14**	1.4	**20**	3.1	**18**	3.7	**17**	2.4

Caption: x = mean value; s_x = standard deviation; ANOVA: ns = not significant, *) $p \leq 0.05$, **) $p \leq 0.01$, ***) $p \leq 0.001$
Production sites: A>B>C>D (=significantly different contents $p \leq 0.01$) or AB = A and B overlap by using an univariate discriminant analysis
AL = Allgäu, BR = Bretagne, CH = Switzerland, FI = Finland, SA = Savoie, VO = Vorarlberg

Figure 1 Principal component analysis using relative concentrations of asparagine, glycine, lysine, phenylalanine and proline. Separation of the region “Switzerland”.

Figure 2 Peptide chromatogram of an Emmental sample from Switzerland. Peptide E is a synthetic standard added.

Figure 3 Principal component analysis using values of αs1, X3 (unknown structure) and peak 14. Separation of the regions “Bretagne”, “Finland” and “Savoie”.

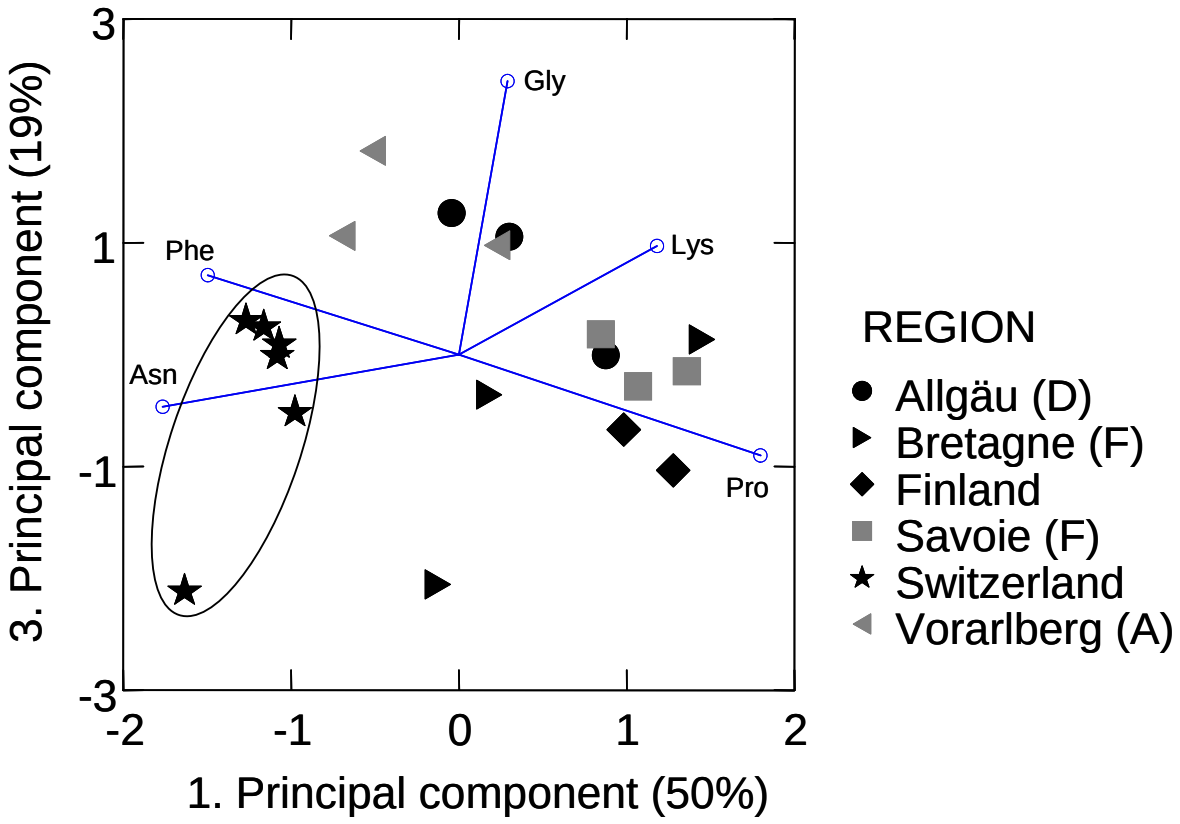
3
1
-1
-3
-2
-1
0
1
2
3. Principal component (19%)
1. Principal component (50%)
Gly
Phe
Lys
Asn
Pro
REGION
Allgäu (D)
Bretagne (F)
Finland
Savoie (F)
Switzerland
Vorarlberg (A)

Figure 1

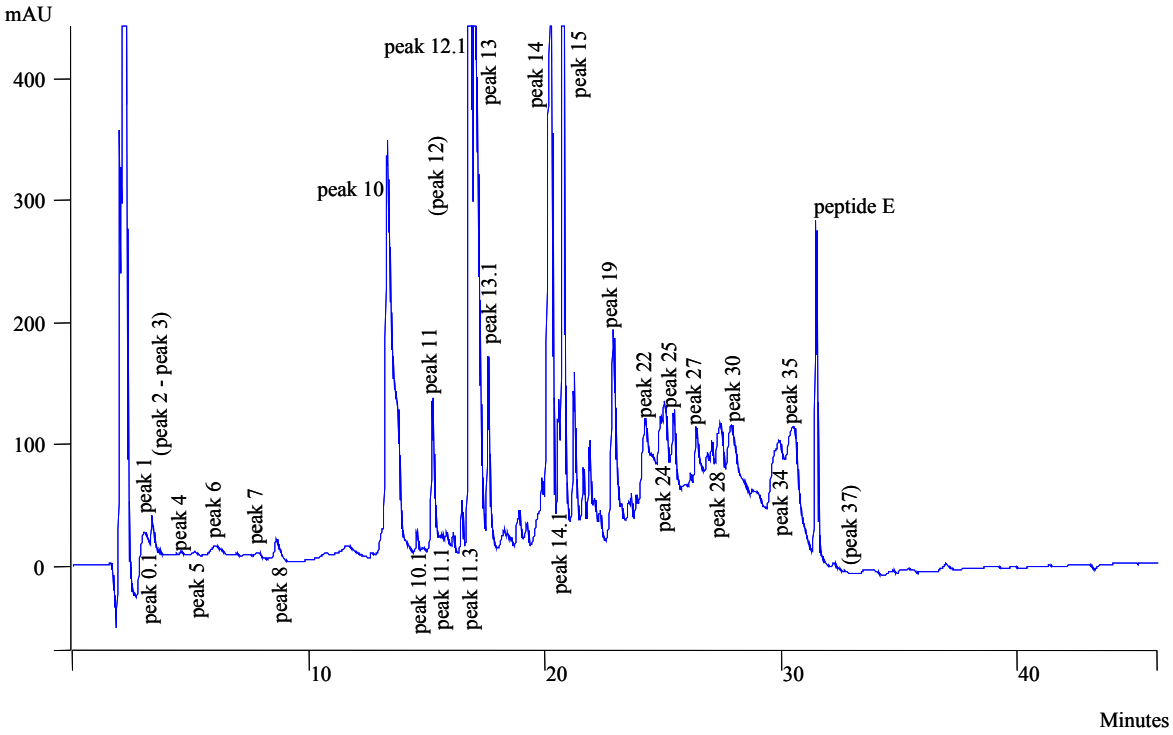
mAU
400
300
200
100
0
peak 12.1
peak 13
peak 14
peak 15
peak 10
(peak 12)
peptide E
peak 13.1
peak 19
peak 11
peak 22
peak 25
peak 27
peak 30
peak 35
(peak 2 - peak 3)
peak 1
peak 4
peak 6
peak 7
peak 24
peak 28
peak 34
(peak 37)
peak 0.1
peak 5
peak 8
peak 10.1
peak 11.1
peak 11.3
peak 14.1
10
20
30
40
Minutes

Figure 2

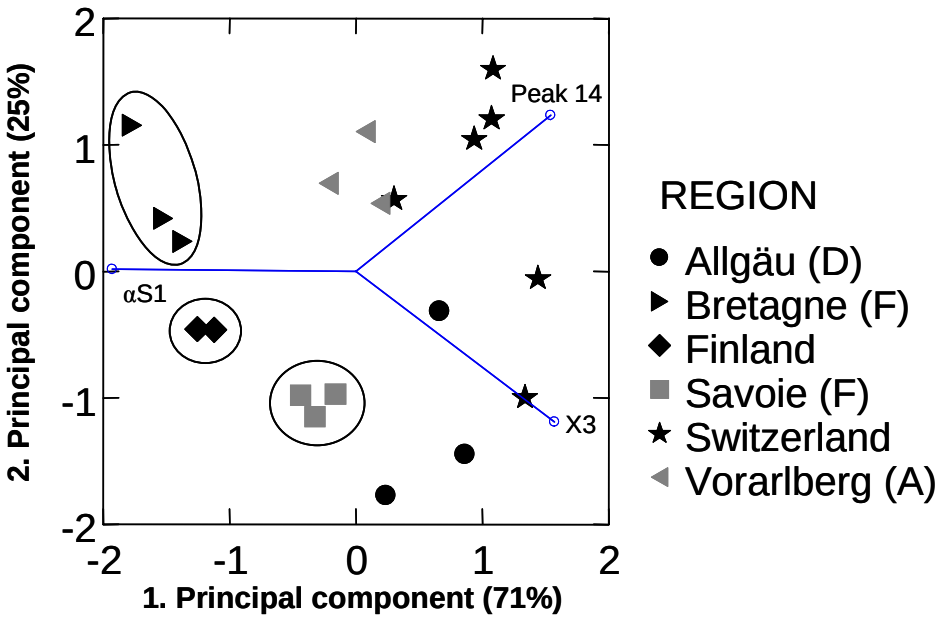
2. Principal component (25%)
1. Principal component (71%)
Peak 14
X3
αS1
REGION
Allgäu (D)
Bretagne (F)
Finland
Savoie (F)
Switzerland
Vorarlberg (A)

Figure 3