Zeitschrift: Mitteilungen aus Lebensmitteluntersuchungen und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 93 (2002)

Heft: 3

Artikel: Analytical methods for the determination of the geographic origin of Emmental cheese : free fatty acids, triglycerides and fatty acid composition of cheese fat

Autor: Pillonel, Laurent / Collomb, Marius / Tabacchi, Raphael

DOI: https://doi.org/10.5169/seals-981726

Analytical Methods for the Determination of the Geographic Origin of Emmental Cheese. Free Fatty Acids, Triglycerides and Fatty Acid Composition of Cheese Fat*

Laurent Pillonel[1], Marius Collomb[1], Raphael Tabacchi[2] and Jacques O. Bosset[1]

[1]Federal Dairy Research Station (FAM) Liebefeld, Berne

[2]University of Neuchâtel, Neuchâtel

Received 3 April 2002, accepted 24 April 2002

Introduction

Cow's milk fat is composed of triglycerides consisting of approx. 400 different fatty acids whose composition in milk and milk products is mainly influenced by the forage (1). During summer or by addition of vegetable fats to the fodder, the concentration of long chain unsaturated fatty acids is increased and that of short and medium chain fatty acids (C6-C16) is decreased (2–4). In the case of triglycerides, the group C50-C54 increased as the fodder changed from hay to grass (4) or by addition of vegetable fats (2, 5). Similar results were obtained for the composition of fatty acids and triglycerides by comparing milk fats from cows grazing in the lowlands and highlands (6). In the highlands, the level of unsaturated long chain fatty acids (C18:1, C18:2 and C18:3) was higher, and that of the saturated short and medium chain fatty acids lower (6, 7). Also, the level of short (C28-C36) and medium chain triglycerides (C42-C46) decreased from lowlands to highlands whereas that of long chain triglycerides (C50-C54) increased. The stage of lactation (8, 9) also influenced the composition of milk fat. The content of free fatty acids depended on the lipolytic activity of the microbial flora during cheese making and ripening. Lactic acid bacteria have a very low lipolytic activity (10) whereas the dif-

* Part of the Ph.D. work of Laurent Pillonel

ferent propionic acid bacteria used in the manufacture of Emmental have much higher and differentiated activities (11, 12).

The fatty acid and triglyceride composition could therefore help in discriminating dairy products manufactured in regions of different altitudes or to determine what typical fodder compositions were fed to the cows. The level of free fatty acids may also make it possible to discriminate Emmental cheese making according to the lipolytic activity of the propionic acid bacteria used.

The current work reports an attempt to use free fatty acids and composition of triglycerides and fatty acid to determine the geographic origin of Emmental cheese. It forms the first part of a broad screening test in a 3-year study on the authenticity of Emmental cheese and its geographic traceability (13, 14). During this step, a great number of analytical methods have been tested with respect to their discriminating potential using only two to three cheese samples per region (14–18). It is therefore obvious that the analytical results obtained from such a modest number of cheese samples per region can only give trends which should be confirmed later if they appear valuable for discriminating cheeses produced in Switzerland from those produced in other countries.

Materials and methods

Origin and selection of the cheese samples

The main framework of this study and the sampling have been described in detail in (14). Table 1 summarises the origin, the date of manufacture and the ripening time of these winter Emmental samples as well as the average altitude of the production zones.

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

Sample	*Region (country)*	*Date of manufacture*	*Ripening time (months)*	*Average altitude (m) of the production sites*
AL 1-3	Allgäu (D)	20.12.2000	4	800
BR 1-3	Bretagne (F)	16.02.2001	2.5	100
CH 1-6	Switzerland (CH)	26.12.2000	4	570
FI 1-2	Middle Finland (FI)	05.02.2001	3	150
SA 1-3	Savoie (F)	08.02.2001	3	470
VO 1-3	Vorarlberg (A)	02.02.2001	3	780

Methods used for the determination of free fatty acid, triglyceride and fatty acid composition of milk fat

The gas chromatographic (GC) determination of the free fatty acids (FFA) was performed according to a method developed by *De Jong and Badings* (19), modified at the FAM. The FFA were extracted in sulfuric acid medium by a solution of diethylether/heptane 1:1 (v/v). They were retained on an anion-exchange (aminopropyl-) column, the neutral lipids were eluted with hexane/2-propanol 3:2 (v:v) and the FFA with diethyl ether containing 2% of formic acid. For the triglycerides and the composition of fatty acids, the fat was extracted according to an IDF standard (20). For the determination of the triglycerides, the isolated fat was dissolved in heptane and directly injected into the GC according to *Collomb et al.* (21). The fatty acid composition of fat was determined by GC after transesterification to methylesters according to *Collomb and Bühler* (1).

Statistical methods

The averages and standard deviations were calculated for each value. Descriptive statistics, analysis of variance (ANOVA) and pairwise comparisons of mean values with Fisher's LSD test were performed using Systat for Windows version 9.0 (SPSS Inc., Chicago, IL).

Results and discussions

Because of the limited number of cheese samples analysed per region, only compounds showing highly significant inter-region differences (ANOVA, $p \leq 0.001$) have been considered. Consequently, out of the compounds analysed, only two free fatty acids, five triglycerides and 29 fatty acids are discussed. Five groups of fatty acids (medium chain, polyunsaturated, C18:2, conjugated linoleic acids (CLA) and ω-3) showed the required significant differences. We also did not use trained classification techniques such a linear discriminant analysis as they require a large data set in order to be reliable.

Significant differences between the regions of lower and higher altitude

Highly significant differences ($p \leq 0.001$) were found between winter Emmental cheeses produced in the regions of lower altitude Bretagne (BR) and Finland (FI) (100–150 m) compared to those produced in the four other regions of higher altitude (470–800 m). Furthermore, silage was fed only in BR and FI. The concentrations of different fatty acids and triglycerides from cheeses produced in regions of lower altitudes were significantly higher (table 2) or lower (table 3) than those found in cheese from the four regions of higher altitude. Such observations agree with the literature quoted in the introduction.

The concentrations of only one triglyceride (C38) and of six minor C18:1 fatty acids were significantly higher in cheeses produced in BR and FI than in the four other regions of higher altitude (table 2). The concentration of the triglyceride C38 was the highest in cheese produced in BR (13.16 g/100 g), and that from FI (12.93 g/100 g) overlapped with that found in Switzerland (CH) (12.68 g/100 g), BR and Vorarlberg (VO) (12.71 g/100 g). *Precht and Heine* (22) found a mean value of this triglyceride generally higher in winter fat (12.7 g/100 g; $s_x = 0.32$; $n = 283$) than in summer fat (12.13; $s_x = 0.27$; $n = 309$). *Collomb et al.* (6) found similar values to those

Table 2

Compounds where the two lowland regions BR and FI showed significantly (P≤0.001) higher values than the regions bordering the Alps

Region[1] *(n =)*	*Overlapping*[2]	*AL (3)* x	s_x	*BR (3)* x	s_x	*CH (6)* x	s_x	*FI (2)* x	s_x	*SA (3)* x	s_x	*VO (3)* x	s_x
Triglyceride (g/100 g triglyceride)													
Triglyceride 38	fi	*12.42*^{D}	0.27	*13.16*^{A}	0.09	*12.68*^{BCD}	0.25	*12.93*^{AB}	0.05	*12.36*^{CD}	0.10	*12.71*^{BCD}	0.07
Fatty acids composition (g/100 g fat)													
C18:1 t12	fi/br	*0.163*^{AB}	0.005	*0.193*^{A}	0.006	*0.136*^{BC}	0.023	*0.197*^{A}	0.006	*0.116*^{BC}	0.025	*0.111*^{C}	0.027
C18:1 t13-14+c6-8	fi	*0.411*^{BC}	0.002	*0.547*^{A}	0.086	*0.393*^{C}	0.053	*0.539*^{AB}	0.015	*0.307*^{C}	0.051	*0.340*^{C}	0.030
C18:1 c11	fi	*0.467*^{BC}	0.029	*0.545*^{A}	0.012	*0.444*^{C}	0.032	*0.505*^{AB}	0.006	*0.455*^{BC}	0.015	*0.458*^{BC}	0.019
C18:1 c12	–	*0.143*^{B}	0.013	*0.208*^{A}	0.006	*0.123*^{B}	0.019	*0.181*^{A}	0.008	*0.124*^{B}	0.002	*0.116*^{B}	0.011
C18:1 c13	fi	*0.049*^{BC}	0.004	*0.064*^{A}	0.002	*0.048*^{C}	0.004	*0.058*^{AB}	0.004	*0.040*^{D}	0.002	*0.040*^{D}	0.005
C18:1 t16+c14	fi	*0.225*^{C}	0.010	*0.338*^{A}	0.027	*0.258*^{BC}	0.017	*0.291*^{AB}	0.004	*0.248*^{BC}	0.008	*0.232*^{C}	0.039

Caption: x = mean value; s_x = standard deviation

Production sites: A>B>C>D (=significantly different contents p≤0.001) or AB=A and B overlap by using an univariate discriminant analysis

[1] AL=Allgäu, BR=Bretagne, CH=Switzerland, FI=Finland, SA=Savoie, VO=Vorarlberg

[2] fi/br=the group "Finland"/"Bretagne" overlaps with at least one other group from the regions bordering the Alps

Table 3

Compounds where the two lowland regions BR and FI showed significantly ($P \leq 0.001$) lower values than the regions bordering the Alps

Region (n=)	*Overlapping*	*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
Triglyceride / Free fatty acids													
Triglyceride 48 (g/100 g triglyceride)	br	9.38^{A}	0.15	8.96^{AB}	0.08	9.17^{A}	0.27	8.53^{B}	0.08	9.35^{A}	0.12	9.35^{A}	0.02
Free linolenic acid (mg/kg)	–	35.7^{B}	3.3	17.1^{C}	2.7	34.8^{B}	7.5	13.4^{C}	2.6	35.5^{B}	0.3	51.3^{A}	8.9
Fatty acids composition (g/100 g fat)													
C13 iso	–	0.137^{AB}	0.005	0.088^{C}	0.004	0.124^{B}	0.013	0.075^{C}	0.003	0.157^{A}	0.007	0.142^{AB}	0.003
C14	–	10.33^{AB}	0.15	9.68^{C}	0.07	10.13^{B}	0.23	9.56^{C}	0.04	10.55^{A}	0.18	10.24^{AB}	0.19
C14 iso	–	0.240^{B}	0.005	0.203^{C}	0.001	0.240^{B}	0.018	0.185^{C}	0.004	0.304^{A}	0.008	0.245^{B}	0.006
C14 aiso	–	0.460^{AB}	0.020	0.384^{C}	0.006	0.419^{BC}	0.038	0.363^{C}	0.007	0.479^{A}	0.017	0.461^{AB}	0.013
C14:1 c	fi	0.913^{AB}	0.024	0.806^{D}	0.015	0.917^{A}	0.040	0.838^{BCD}	0.023	0.824^{CD}	0.021	0.905^{ABC}	0.033
C15	–	1.057^{A}	0.047	0.906^{B}	0.014	1.060^{A}	0.060	0.846^{B}	0.003	1.077^{A}	0.016	1.071^{A}	0.038
C15 iso	fi/br	0.262^{BC}	0.019	0.240^{BCD}	0.011	0.242^{CD}	0.023	0.213^{D}	0.003	0.322^{A}	0.010	0.279^{B}	0.013
C16 iso	br	0.331^{AB}	0.001	0.291^{CD}	0.007	0.326^{B}	0.017	0.277^{D}	0.004	0.339^{AB}	0.008	0.313^{BC}	0.006
C18:1 t10–11	fi	2.05^{A}	0.04	1.17^{D}	0.04	1.56^{BC}	0.29	1.20^{CD}	0.00	1.44^{BCD}	0.18	1.76^{AB}	0.05
C18:2 c9t13+(t8c12)	fi/br	0.172^{A}	0.016	0.175^{A}	0.009	0.146^{AB}	0.019	0.180^{A}	0.013	0.118^{B}	0.008	0.126^{B}	0.010
C18:3 c9c12c15	–	0.884^{A}	0.063	0.280^{C}	0.053	0.781^{AB}	0.162	0.372^{C}	0.032	0.673^{AB}	0.043	0.915^{AB}	0.075
C18:2 c9t11	fi	0.935^{A}	0.020	0.410^{D}	0.010	0.660^{B}	0.131	0.411^{CD}	0.010	0.623^{BC}	0.061	0.771^{AB}	0.020
C18:2 c9c11	fi/br	0.054^{A}	0.009	0.013^{C}	0.004	0.036^{B}	0.011	0.017^{C}	0.001	0.029^{BC}	0.005	0.045^{AB}	0.003
C22:5 (DPA) (n-3)	–	0.104^{AB}	0.006	0.051^{C}	0.003	0.101^{AB}	0.008	0.046^{C}	0.001	0.087^{B}	0.009	0.108^{A}	0.014
Groups of fatty acids (g/100 g fat)													
Sum C18:2	fi	3.29^{A}	0.03	2.44^{D}	0.05	2.75^{BC}	0.16	2.49^{CD}	0.16	2.67^{C}	0.07	2.97^{B}	0.08
Sum polyunsaturated FA[1]	–	4.61^{A}	0.09	3.06^{D}	0.11	3.97^{BC}	0.31	3.13^{D}	0.14	3.76^{C}	0.14	4.32^{AB}	0.17

Region (n=)	Overlapping	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
Sum CLA[2]	fi	*1.01*^{A}	0.03	*0.44*^{D}	0.01	*0.72*^{B}	0.15	*0.45*^{CD}	0.01	*0.67*^{BC}	0.06	*0.85*^{AB}	0.03
Sum ω-3[3]	fi	*1.42*^{A}	0.11	*0.61*^{D}	0.09	*1.25*^{AB}	0.22	*0.66*^{CD}	0.01	*1.04*^{BC}	0.08	*1.40*^{AB}	0.11

Caption: see table 2; FA=fatty acids; DPA=cis 7, cis 10, cis 13, cis 16, cis 19-docosapentaenoic acid according to reference (1).

[1] C18:2 -ttNMID to -c9c15, C18:3 -c6c9c12+-c9c12c15, C18:2 -c9t11 à C20:2 cc, C20:3 à C22:6

[2] CLA total (sum C18:2 -c9t11, -c9c11, -t9t11)

[3] C18:2 -t11c15+c9c15, C18:3 c9c12c15, C20:3 n-3, C20:5, C22:5 and C22:6

found in BR and FI in summer milk fat (13.4 g/100 g; $n=13$) produced in Switzerland at an altitude of 600–650 m and much lower values (12.0–12.5 g/100 g; $n=36$) at higher altitudes (900–2120 m). However, the concentration of this compound which ranged normally from 11.39 to 13.95 g/100 g ($n=592$) in milk fat (22), is very dependent on the fodder.

The concentrations of the six minor unsaturated fatty acids were the highest in BR and those of the fatty acids C18:1 -t12 and -c12 the highest in BR and FI. These results agreed better with the values generally measured in Switzerland for summer than for winter milk fat (1) and could be due to the influence on milk fat of the silage fed to the cows in FI and BR.

The concentrations of one triglyceride (C48), one free fatty acid (linolenic acid), fourteen fatty acids and four groups of fatty acids (C18:2, polyunsaturated, conjugated linoleic acid and ω-3) were significantly lower in cheese from BR and FI than in samples from the four other regions of higher altitude (table 3). The concentration of triglyceride C48 was the lowest in cheese from FI, and that from BR overlapped with that found in VO, Savoie (SA) and Allgäu (AL). *Precht and Heine* (22) found a mean value for this compound generally higher in winter fat (9.13 g/100 g; $s_x=0.38$; $n=283$) than in summer fat (9.07; $s_x=0.39$; $n=309$). *Collomb et al.* (6) found a trend towards higher values (8.15 to 8.60 g/100 g, $n=49$) as a function of the altitude but the results were not significantly different. In this study, the lower concentration found in BR (8.96 g/100 g) and FI (8.53 g/100 g) compared to that found in other countries (9.17–9.38 g/100 g) could be attributed to the influence of silage and/or to the altitude (lower diversity of plant species in hay (23)). Here again, the concentration of the triglyceride C48 in milk fat normally lies in a much broader range (7.91 to 10.65 g/100 g; $n=592$) (22) than that found in this study.

Except for the C18:2 c9t13+(t8c12), the fatty acids present in a lower concentration in cheeses from BR and FI than in those from other countries were essentially iso, anteiso and unsaturated long chain fatty acids. The concentration of myristic acid (C14) is generally higher in winter fat than in summer fat. That of the iso and anteiso compounds gives generally similar values in the two seasons. That of the unsaturated long chain fatty acids is generally higher in summer milk fat than in winter milk fat (1) and increased with the altitude (6, 7, 23). However, the concentration of fatty acids normally varies within a broader range than that found in this study. For example, *Precht and Molkentin* (24) found in 100 milk fats values ranging from 8.90 to 13.57 g/100 g for the myristic acid and from 0.34 to 1.06 for linolenic acid (C18:3 c9c12c15). For these two compounds, the values found within the six regions considered ranged from 9.56 to 10.55 g/100 g fat for the myristic acid and from 0.28 to 0.88 g/100 g fat for the second compound. The low concentration of esterified as well as free linolenic acid in cheeses from BR and FI can be attributed to a low concentration of this compound in silage fat. The lowest concentration of free linolenic acid in BR (17.1 mg/kg) and FI (13.4 mg/kg) compared to that found in other countries (34.8–51.3 mg/kg) may have the same cause. Another explanation may be the different lipolytic activity of the propionic acid bacteria used for cheese making. In a study carried out at the FAM, the concentrations of free linolenic acid ranged from 23 to 57 mg/kg Emmental cheese depending on the bacteria used (FAM, unpublished results). However in the current study, the sum of the thirteen free fatty acids analysed (without butyric acid) did not show any significant differences.

The concentrations of groups of fatty acids (Σ C18:2, Σ polyunsaturated, Σ CLA and Σ ω-3) were the highest in AL and VO and the lowest in BR and FI. Table 4 presents the sum of the CLA and the content of the fatty acids C18:1 t10+t11 as a function of the altitude.

Table 4
Influence of altitude on the concentration of CLA and C18:1 t10-11 in Emmental cheese

Forage type	*Region (altitude)*	*CLA (g/100 g fat)*	*C18:1 t10-11 (g/100 g fat)*
Maize and grass silage, hay	Bretagne (100 m)	0.44	1.17
Grass silage, hay	Finland (100–150 m)	0.45	1.20
Hay	Savoie (470 m)	0.67	1.44
Hay	Switzerland (570 m)	0.72	1.56
Hay	Vorarlberg (780 m)	0.85	1.76
Hay	Allgäu (800 m)	1.01	2.05

The contents of both sums of fatty acids increased as a function of the altitude. The elevated concentration of the sum of the t10 and t11 fatty acids is likely to be mainly due to that of the trans vaccenic acid (C18:1 t11) from which the CLA is endogenously synthesised (25). According to *Precht and Molkentin* (26), trans vaccenic acid normally represents approximately 90 % of the total of the two acids t10 and t11, which cannot be separated using our chromatographic conditions. These authors showed in feeding tests – where cows stayed in the barn or on pasture or were fed with rape oil, rapeseed wholemeal or pellets – that the increase in the CLA concentration in milk fat usually correlates with the concentration of trans fatty acids. *Collomb et al.* (23, 27) found significantly different values for the sum of CLA and C18:1 t10+t11 fatty acids in milk fat produced from cows grazing in lowlands (600–650 m), mountains (900–1210 m) and highlands (1275–2120 m). The concentrations of CLA, compounds well-known for their positive effects on health (28), increased from 0.87 to 1.61 and to 2.36 g/100 g fat (maximum value: 2.87 g/100 g fat) as a function of the altitude. The concentration of the fatty acids C18:1 t10+t11 increased similarly from 2.11 to 3.66 and to 5.10 g/100 g fat (maximum value: 5.67 g/100 g fat). In the current study, the values obtained were relatively low compared to those of summer milk fats in high altitude but corresponded to values for summer milk fats from AL and VO.

The lower concentration of the long chain polyunsaturated fatty acids found in BR and FI can be correlated with a silage fodder fat poor in polyunsaturated fatty acids (23). The concentration of the ω-3 compounds ranged from 0.61 to 1.42 g/100 g cheese fat. *Collomb et al.* (23) found increasing values as a function of the altitude (1.39 to 2.09 g/100 g fat). The low value found in BR (0.61 g/100 g fat) and FI (0.66 g/100 g) is due to the low level of linolenic acid (C18:3 c9c12c15), the main ω-3 compound of milk fat.

Significant differences between Finland and other countries

Table 5 presents the concentrations of compounds which were the highest or the lowest in FI cheeses compared to those produced in other countries.

The concentrations of the triglycerides C40 and C54 and those of the fatty acids C18, C18:1 t9, C20 and C20:1 c11 were the highest in FI cheeses. *Precht and Heine* (22) found lower mean values for the triglycerides C40 and C54 in winter fat (C40:9.78 g/100 g; s_x=0.31; n=283; C54:3.99 g/100 g; s_x=0.87; n=283) than in summer fat (C40:10.01; s_x=0.28; n=309; C54: 6.58; s_x=0.82; n=309). For the triglyceride C40, *Collomb et al.* (6) found significantly higher values in lowlands (10.4 g/100 g; n=13) compared to the highlands 9.84–10.0 g/100 g; n=24) and much lower values for the triglyceride C54 in the lowlands (4.83 g/100 g) compared to the highlands (7.48–7.78 g/100 g). In this study, the high concentrations of the triglycerides C40 (10.34 g/100 g) and C54 (5.01 g/100 g) in FI cheeses were rather in the range of the values generally obtained for summer fats. These two triglycerides probably consist on a high extent of oleic acid (C18:1 c9) as confirmed by the highest level of this acid (results not tabulated) found in cheese fat from FI (16.72 g/100 g fat) compared to that found in cheese from the other regions (14.22–15.44 g/100 g fat). The concentration of stearic acid (C18) was the highest in cheese from FI (10.20 g/100 g fat) compared to that from the other regions (7.31–8.42 g/100 g fat). This result could be attributed to the high level of oleic acid in grass silage combined with a high biohydrogenation activity in the rumen of the cow (23). The normal range in milk fat lies between 6.12 and 12.50 g/100 g (24).

The concentration of the triglyceride C46 and that of palmitic acid (C16) as well as of other minor fatty acids difficult to interpret were the lowest in FI cheeses. *Precht and Heine* (22) found higher mean values for the triglyceride C46 in winter fat (7.55 g/100 g; s_x=0.51; n=283) than in summer fat (6.82 g/100 g; s_x=0.47; n=309). For this triglyceride *Collomb et al.* (6) found significantly higher values in lowlands (6.67 g/100 g) than in the highlands (6.08–6.31 g/100 g). In this study, the low concentration of the C46 triglyceride (7.07 g/100 g) in FI cheese were once again rather in the range of summer fat and could be due to the silage fodder based on grass. The lower content of the fatty acid C16 found in FI cheese (24.66 g/100 g) supports this

Table 5
Compounds where only FI was significantly different (P≤0.001) from all other regions

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
Higher value												
Triglyceride 40 (g/100 g triglyceride)	9.68^{B}	0.12	9.90^{B}	0.10	9.77^{B}	0.24	10.34^{A}	0.04	9.56^{B}	0.05	9.62^{B}	0.06
Triglyceride 54 (g/100 g triglyceride)	3.92^{B}	0.14	3.70^{B}	0.20	3.73^{B}	0.37	5.01^{A}	0.13	3.64^{B}	0.12	3.49^{B}	0.15
C18 (g/100 g fat)	7.81^{BC}	0.16	8.42^{B}	0.25	7.55^{BC}	0.62	10.20^{A}	0.55	7.31^{C}	0.16	7.33^{BC}	0.28
C18:1 t9 (g/100 g fat)	0.213^{B}	0.016	0.231^{B}	0.024	0.199^{B}	0.025	0.292^{A}	0.013	0.209^{B}	0.017	0.192^{B}	0.008
C20 (g/100 g fat)	0.141^{B}	0.008	0.122^{C}	0.006	0.141^{B}	0.009	0.171^{A}	0.009	0.145^{B}	0.008	0.134^{B}	0.002
C20:1 c11[1]	0.052^{AB}	0.003	0.038^{C}	0.002	0.047^{B}	0.003	0.057^{A}	0.002	0.044^{BC}	0.002	0.039^{C}	0.007
Lower value												
Triglyceride 46 (g/100 g triglyceride)	7.74^{AB}	0.18	7.46^{B}	0.06	7.72^{AB}	0.18	7.07^{C}	0.13	7.81^{A}	0.12	7.68^{AB}	0.01
C16 (g/100 g fat)	27.17^{A}	0.49	27.91^{A}	0.78	27.78^{A}	0.75	24.66^{B}	0.26	27.05^{A}	0.27	27.73^{A}	0.92
C17 (g/100 g fat)	0.540^{A}	0.014	0.519^{A}	0.070	0.581^{A}	0.072	0.265^{B}	0.163	0.600^{A}	0.073	0.667^{A}	0.063
C17 iso (g/100 g fat)	0.062^{A}	0.007	0.068^{A}	0.009	0.068^{A}	0.007	0.036^{B}	0.003	0.079^{A}	0.001	0.074^{A}	0.014
C17 aiso (g/100 g fat)	0.212^{A}	0.015	0.207^{A}	0.003	0.221^{A}	0.010	0.16^{B}	0.006	0.222^{A}	0.006	0.217^{A}	0.007
Sum medium chain FA[2]	46.00^{A}	0.79	45.59^{A}	0.87	46.29^{A}	1.11	41.66^{B}	0.22	46.27^{A}	0.54	46.27^{A}	1.23

Caption: see table 2

[1] FI not significantly different from AL

[2] C12 to C16:1 c according to reference (1)

interpretation and corresponds to a concentration found for summer milk fat in lowlands regions (23).

Significant differences between Bretagne and other countries

Table 6 presents the concentrations of compounds which were the highest or the lowest in BR cheeses compared to those produced in other countries.

The concentration of free butyric acid, and minor fatty acids difficult to interpret, were respectively the highest or the lowest in BR. Butyric acid is produced by butyric acid bacteria which are commonly found in silage.

Conclusion

Free fatty acid, triglyceride and fatty acid (FA) composition of Emmental cheese fat were investigated to find potential markers of geographic origin. The fat composition of milk and milk products is strongly correlated with the forage fed to the herd. The latter depends on the season, on regional parameters such as altitude, geology, type of agriculture and on further non-regional parameters such as type of concentrates used and addition of vegetable oils. The cheeses investigated in this study all originated from winter productions. In "Bretagne" and "Finland", feeding with silage is permitted, whereas it is prohibited in the other regions. The differences in altitude and silage feeding are the most likely explanation for the highly significant differences ($P \leq 0.001$) found between these two regions and the others. For groups of fatty acids such as the C18:2, polyunsaturated FA, conjugated linoleic acid and ω-3 FA, "Bretagne" and "Finland" showed the lowest values. The butyric acid content was the highest in those regions. It is thereafter only possible to discriminate between the three groups FI, BR and the remaining regions.

Moreover, these differences may partly disappear during summer production while fodder is based mainly on fresh grass. Consequently, it will not be possible to interpret any fatty acids composition during the transition phases between summer and winter production, which represents almost half a year's production. Finally, the composition of the forage changes during a season. Any addition of a new type of cereal or vegetable fat will modify the fatty acid profile of the milk. Thereafter, apart for the higher content of trans vaccenic acid and conjugated linoleic acids in summer Emmental cheese produced at higher altitude, the fat composition is not adequate for discriminating between the various geographic origins of Emmental cheese.

Acknowledgements

The authors thank the Swiss Federal Office of Public Health for its financial and logistical support to the project, *G. Urbach* and *G. Gremaud* for their reviewing of the publication as well as *J.F. Chamba* (ITFF, France), *M. Carl* (MUVA Germany), *H. Spring* (Emmentaler Switzerland™), *H. Jatila* (Valio Ltd, Finland) and *H. Schmallegger* (Alma GmbH, Austria) for collecting the samples.

Table 6
Compounds where BR was significantly different (P≤0.001) from all other regions

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
Higher value												
Free butyric acid (mg/kg)	87.2^{B}	16.3	425.3^{A}	41.4	85.9^{B}	17.9	169.5^{B}	175.0	83.3^{B}	5.1	88.5^{B}	3.0
C16:1 c (g/100 g fat)	1.21^{B}	0.03	1.37^{A}	0.04	1.24^{B}	0.06	1.07^{C}	0.01	1.19^{BC}	0.06	1.16^{BC}	0.05
Lower value												
C20:1 c9 (g/100 g fat)	0.119^{A}	0.007	0.091^{B}	0.003	0.119^{A}	0.007	0.125^{A}	0.006	0.118^{A}	0.008	0.112^{A}	0.008
C20 (g/100 g fat)	0.141^{B}	0.008	0.122^{C}	0.006	0.141^{B}	0.009	0.171^{A}	0.009	0.145^{B}	0.008	0.134^{B}	0.002

Caption: see table 2

Summary

Free fatty acids, triglycerides and fatty acid composition in cheese fat were investigated on 20 Emmental cheese samples from six regions in Europe to find the best parameters for discriminating between their different geographic origins. These samples originated from a winter production. "Bretagne" and "Finland", where silage feeding is permitted and which are located close to sea level, were easily differentiated from the other regions. The concentration of some groups of polyunsaturated fatty acids such as C18:2 , conjugated linoleic acids and ω-3 was significantly lower. However, the ranges of values obtained in this study were always smaller than those found in broader studies. The butyric acid content was higher in the two regions with silage feeding. These differences may however partially disappear during summer production. The other regions showed almost no significant differences. The high variability of the fatty acid composition due to both seasonal and feeding effects leads to the conclusion that theses methods are not adaquate for discriminating between the geographic origins of Emmental cheeses.

Zusammenfassung

In 20 Emmentalerproben (Winterproduktion) von sechs europäischen Regionen wurden die freien Fettsäuren, die Triglyzeride und die Zusammensetzung der Fettsäuren des Käsefettes studiert. Ziel dieser Untersuchung war, aussagekräftige Parameter zu finden, um diese Käse nach ihrer geographischen Herkunft zu unterscheiden. Die Käse der Regionen «Bretagne» und «Finnland», wo die Verfütterung von Silage erlaubt ist und die in der Nähe des Meeres liegen, sind von den anderen leicht zu unterscheiden. Dabei waren einige mehrfach ungesättigte Fettsäuren wie C18:2, konjugierte Linolsäuren und ω-3 in signifikant geringerer Konzentration vorhanden. Doch ist die Spannbreite der Werte in dieser Studie immer schmaler als in umfangreicheren Studien. Der Buttersäuregehalt war dort höher als in den Käsen der Regionen ohne Silageverfütterung. Diese Unterschiede können während der Sommerproduktion teilweise verschwinden. In den Käsen der anderen Regionen konnten nur geringe Unterschiede festgestellt werden. Die geographische Herkunft der Emmentaler Käse kann mit diesen Methoden aufgrund der grossen saisonal und fütterungsbedingten Variabilität der Fettsäurezusammensetzung nicht unterschieden werden.

Résumé

Les acides gras libres, les triglycérides et la composition en acides gras de la matière grasse du fromage ont été étudiés dans 20 échantillons d'emmental de productions hivernales provenant de six régions d'Europe pour trouver les meilleurs régresseurs susceptibles de discriminer leur origine géographique. Les régions de «Bretagne» et «Finlande», où l'affouragement au silo est autorisé et qui sont proches du niveau de la mer, sont facilement différenciées des autres. La concentration de quelques groupes d'acides gras polyinsaturés tels que C18:2, linoléiques conjugués

et ω-3 était significativement plus faible que dans les autres régions. Cependant les plages de valeurs trouvées étaient toujours moins étendues que celles publiées lors d'études plus larges. La teneur en acide butyrique y était plus haute que dans les régions sans ensilage. De telles différences peuvent pourtant s'atténuer durant les productions estivales. Les autres régions n'ont montré que peu de différences. La forte variabilité de la composition de ces acides, liée tant à la saison qu'à l'affouragement, rend ces méthodes inadéquates pour discriminer l'origine géographique de ces fromages.

Key words

Emmental, Fatty acid, Triglyceride, Authenticity, Traceability

References

1 *Collomb, M.* et *Bühler, T.:* Analyse de la composition en acides gras de la graisse de lait. I. Optimisation et validation d'une méthode générale à haute résolution. Trav. chim. aliment. hyg. **91**, 306–332 (2000).

2 *Kennelly, J.J.:* The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol. **60**, 137–152 (1996).

3 *Muuse, B.G., Werdmuller, G.A., Geerts, J.P.* and *Knegt, R.J.:* Fatty acid profile of Dutch butterfat. Neth. Milk Dairy J. **40**, 189–201 (1986).

4 *Precht, D., Frede, E., Hagemeister, H.* und *Timmen H.:* Zur fütterungsbedingten Variation des Milchfettes der Kuh unter besonderer Berücksichtigung von Regulationsmechanismen beim Fettstoffwechsel. Fette, Seifen, Anstrichmittel **87**, 117–126 (1985).

5 *Culliane, N., Aherne, S., Connolly, J.F.* and *Phelan, J.A.:* Seasonal variation in the triglyceride and fatty acid composition of Irish butter. Irish J. Food Sci. Technol. **8**, 1–12 (1984).

6 *Collomb, M., Bütikofer, U., Spahni, M., Jeangros, B.* et *Bosset, J.O.:* Composition en acides gras et en glycérides de la matière grasse du lait de vache en zones de montagne et de plaine. Sci. Aliments **19**, 97–110 (1999).

7 *Bugaud, C., Buchin, S., Coulon, J.B., Hauwuy, A.* and *Dupont, D.:* Influence of the nature of alpine pastures on plasmin activity, fatty acid and volatile compound composition of milk. Lait **81**, 401–414 (2001).

8 *Renner, E.* und *Kosmack, U.:* Genetische Aspekte zur Fettsäurezusammensetzung des Milchfettes. 1. Abgrenzung gegenüber nichtgenetische Faktoren. Züchtungskunde **46**, 90–99 (1974).

9 *Zegarska, Z.* and *Jaworski, J.:* Effect of lactation period and seasonal feeding system on quantitative triglyceride composition of milk fat. Milchwissenschaft **36**, 396–398 (1981).

10 *Khalid, N.M.* and *Marth, E.H.:* Lactobacilli – their enzymes and role in ripening and spoilage of cheese: a review. J. Dairy Sci. **73**, 2669–2684 (1990).

11 *Fessler, D.:* Characterisation of propioni-bacteria in Swiss raw milk by biochemical and molecular-biological methods. Diss. ETH No. 12328, Zürich 1997.

12 *Bachmann, H.P.:* Lipolyse im Käse: nicht zu viel – nicht zu wenig. Agrarforschung **5**, 293–295 (1998).

13 *Bosset, J.O.:* Authenticity of Emmental cheese Switzerland™. Mitt. Lebensm. Hyg. **92**, 328–332 (2001).

14 *Pillonel, L., Badertscher, R., Bütikofer, U., Casey, M., Dalla Torre, M., Lavanchy, P., Meyer, J., Tabacchi, R.* and *Bosset, J.O.:* Analytical methods for the determination of the geographic origin of Emmental cheese. Main framework of the project; chemical, biochemical, microbiological, colour and sensory analyses. Eur. Food Res. Technol. (In press, 2002).

15 *Pillonel, L., Albrecht, B., Badertscher, R., Chamba, J.F., Bütikofer, U., Tabacchi, R.* and *Bosset, J.O.:* Analytical methods for the determination of the geographic origin of Emmental cheese. Parameters of proteolysis and rheology. Italian J. Food Sci. Submitted (2002).
16 *Pillonel, L., Luginbühl, W., Picque, D., Schaller, E., Tabacchi, R.* and *Bosset, J.O.:* Analytical methods for the determination of the geographic origin of Emmental cheese. Mid- and Near-Infrared spectroscopy. Eur. Food Res. Technol. (submitted).
17 *Pillonel, L., Ampuero, S., Tabacchi, R.* and *Bosset, J.O.:* Analytical methods for the determination of the geographic origin of Emmental cheese. Volatile compounds by GC/MS-FID and electronic nose. Eur. Food Res. Technol. (submitted).
18 *Pillonel, L., Badertscher, R., Froideveaux, P., Haberhauer, G., Jakob, A., Pfammatter, E., Piantini, U., Rossmann, A., Tabacchi, R. and Bosset, J.O.:* Analytical methods for the determination of the geographic origin of Emmental cheese. Stable isotope ratios, major, trace and radioactive elements (in progress).
19 *De Jong, C.* and *Badings, H.T.:* Determination of free fatty acids in milk and cheese. Procedure for extraction, clean-up and capillary gas chromatographic analysis. J. High Res. Chromatogr. **13**, 94–98 (1990).
20 *Ulberth, F.:* Milkfat – Determination of the fatty acid composition by gas-liquid chromatography. Revised Draft-Standard (Group – Fat – E301). FIL/IDF (1994).
21 *Collomb, M., Spahni, M.* et *Bühler, T.:* Analyse des triglycérides. I. Optimisation et validation d'une méthode GC rapide et application à diverses graisses végétales et animales. Trav. chim. aliment. hyg. **89**, 59–74 (1998).
22 *Precht, D.* and *Heine, K.:* Nachweis von modifiziertem Milchfett mit der Triglyceridanalyse. 1. Nachweis von Fremdfetten im Milchfett aufgrund der Schwankungsbereiche von Triglyceriden und Triglyceridverhältnissen. Milchwissenschaft **41**, 329–334 (1986).
23 *Collomb, M., Bütikofer, U., Sieber, R., Jeangros, B.* and *Bosset, J.O.:* Composition of fatty acids in cow's milk fat produced in the lowlands, mountains and highlands of Switzerland using high resolution gas chromatography. Int. Dairy J. (In press, 2002).
24 *Precht, D.* und *Molkentin, J.:* Vergleich der Fettsäuren und der Isomerenverteilung der trans C18:1 Fettsäuren von Milchfett, margarine, Back-, Brat- und Diätfetten. Kieler Milchw. Forschungsber. **49**, 17–34 (1997).
25 *Griinari, J.M., Coril, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V.V.* and *Bauman, D.E.:* Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta-9-desaturase. J. Nutr. **130**, 2285–2291 (2000).
26 *Precht, D.* and *Molkentin, J.:* Rapid analysis of the isomers of trans-octadecenoic acid in milk fat. Int. Dairy J. **6**, 791–809 (1996).
27 *Collomb, M., Bütikofer, U., Sieber, R., Bosset, J.* and *Jeangros, B.:* Conjugated linoleic acid and trans fatty acid composition of cow's milk fat produced in lowlands and highlands. J. Dairy Res. **68**, 519–523 (2001).
28 *McDonald, H.B.:* Conjugated linoleic acid and disease prevention: A review of current knowledge. J. Amer. Coll. Nutr. **19**, 111S–118S (2000).

Corresponding author: Dr. Jacques Olivier Bosset, Federal Dairy Research Station, Liebefeld, CH-3003 Berne, E-mail: jacques-olivier.bosset@fam.admin.ch