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Composition of fatty acids in cow's milk fat produced in the lowlands, mountains and highlands of Switzerland using high-resolution gas chromatography

Marius Collomb[a,*], Ueli Bütikofer[a], Robert Sieber[a], Bernard Jeangros[b], Jacques-Olivier Bosset[a]

[a] *Federal Dairy Research Station, Liebefeld, CH-3003 Berne, Switzerland*
[b] *Federal Research Station for Plant Production, CH-1260 Nyon, Switzerland*

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Abstract

The composition of fatty acids (approx. 70 acids) in 44 summer milk samples from three geographical sites was determined using high-resolution gas chromatography. We observed large differences between Lowlands (600–650 m), Mountains (900–1210 m) and Highlands (1275–2120 m) which are analogous to those observed between winter and summer fats. The largest relative increases as a function of the altitude of these three sites were those of the concentration of conjugated linoleic acids (0.87, 1.61 and 2.36 g $100\,g^{-1}$), especially of the *cis* (c) 9 *trans* (t) 11 isomer (0.81, 1.50 and 2.18 g $100\,g^{-1}$), and the fatty acids C18:1 t10 + t11 (2.11, 3.66 and 5.10 g $100\,g^{-1}$). There were significant differences in the concentration of fatty acids between the three geographical sites. Some fatty acids could also be interesting potential indicators for the origin of cream and also of the Protected Designated Origine "mountain" cheeses.

Keywords: Milk fat; Fatty acid; Low- and highland pasture; Conjugated linoleic acid (CLA); *trans*-fatty acid

1. Introduction

The relationship between the characteristics of the fodder plants and those of the hard cheeses such as Gruyère type produced in the Lowlands (600–650 m), the Mountains (900–1210 m) and the Highlands (1275–2120 m) of Switzerland has been studied within a large-scale multidisciplinary investigation (Bosset et al., 1997, 1999; Jeangros et al., 1997; Jeangros, Scehovic, Troxler, Bachmann, & Bosset, 1999). One of the hypotheses to be tested consisted of determining whether under natural conditions a relationship exists between the botanical composition of fodder or grassland and the corresponding fatty acids in the milk fat, and if so, what type. Such a study was justified by (i) the socio-economic impact of the pastures in the Alpine Arc especially from a PDO perspective (Bosset, Bütikofer, Gauch, & Sieber, 1994; Bosset et al., 1998; Mariaca et al., 1997); (ii) the increasing interest of nutritionists in polyunsaturated fatty acids and (iii) the interest of technologists in the rheological characteristics of fatty products. Natural conditions imply that this study was conducted *in natura* under the usual conditions of pasture, and management of herds rather than under strictly defined and controlled experimental conditions.

Several publications have already shown that milk and cheese from Low- and Highland regions have a different composition (Bianca & Puhan, 1974; Bovolenta, Ventura, Piasentier, & Malossini, 1998; Bugaud, Buchin, Coulon, & Hauwuy, 2001a; Bugaud, Buchin, Coulon, Hauwuy, & Dupont, 2001b; Tschager et al., 1994). However, only few studies have analysed the fatty acid composition of milk (Bugaud et al., 2001b; Tschager et al., 1994).

The preliminary results on the global composition of fatty acids and triglycerides in milk fat in the Lowland and Highland regions of Switzerland have already been published (Collomb, Bütikofer, Spahni, Jeangros, & Bosset, 1999). The classical method for the determination of the composition of fatty acids (i.e. gas liquid

*Corresponding author. Tel.: +41-31-323-8133; fax: +41-31-323-8227.

E-mail address: marius.collomb@fam.admin.ch (M. Collomb).

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chromatography of methylester derivaties) previously used (ISO, 1997a, b) allowed us to determine only 18 fatty acids. The aim of the current study is to determine once more the fatty acid composition of milk fat in the 44 milk samples from the three vegetation sites in Switzerland using a new high-resolution gas chromatographic method, which makes it possible to quantify about 70 fatty acids and to discuss these new results compared to those previously obtained using the classical method.

2. Materials and methods

2.1. Aim and approach

The study deals with four vegetation sites in Switzerland: two in the Highlands, a Mountain and a Lowland zone. The cows were in the middle of the lactation period and were in excellent health. The botanical composition of the plants was determined as a function of the size and diversity (16 botanical records in the Lowlands, 31 in the Mountains and 55 in the Highlands). The phenological state of the plants was also noted on a scale of 1 to 8.

On the Highlands and Mountains, the botanical composition was generally performed as follows: a surface as homogenous as possible containing sufficient vegetation to feed the herd for at least 3 days was closed off. Before the animals were allowed access, the botanical composition of the area was determined and samples of the vegetation were taken. After the cows had access for 2–3 days, a 3-L sample of mixed evening milk was taken.

On the Lowlands the herd was mainly fed in the barn with grass with the addition of whole zea maize plants from the middle of the trial. The botanical composition was visually determined. The vegetation was composed mainly of standard mixtures of grasses and white or purple clover. The cows which produced more than 28 kg of milk per day (less than one-third of the cows) received a complementary mixture of cereals (<2 kg per day). A 3-L sample of mixed evening milk was also taken.

On the three vegetation sites, 12 observations per site were carried out: two sites in Highlands (pooled into a single zone since their fatty acid composition did not differ significantly), one site in Mountains and one site in Lowlands ($=48$ mixtures of milk of which 44 were analysed in this work) over a period of 3.5 months (from June to mid-September 1995).

Table 1 summarises the principal characteristics of the milk herd and feeding modes. The complete table, which includes the botanical composition of all the vegetation sites during the trial, is available from the authors.

2.2. Fodder composition

A summary of the botanical composition of the grassland of the three vegetation sites is described by Collomb, Bütikofer, Sieber, Jeangros, and Bosset (2002).

2.3. Sampling and sample treatment

A total of 44 milk samples were collected from the three sites from the beginning of June to mid-September 1995 (Jeangros et al., 1997), centrifuged and the resulting creams deep frozen at -18°C. Prior to analysis, these creams were thawed in a water bath at $\sim 40^{\circ}$C and churned at $\sim 5^{\circ}$C. After filtering of the resulting molten butter on a hydrophobic filter (Schleicher Schuell no. 597 hy 1/2), the pure milk fat was collected and stored deep frozen until analysis.

Table 1
Characteristics of dairy herds and type of feedstuffs for each vegetation site

Vegetation sites/ altitude (m)	Characteristics of dairy herds			Fodder[a]	Milking system	Collected milk
	Breed	Number of cows	Milk yield (kg per lactation)			
Lowlands (one site)/600–650	Simmental × Red Holstein	45–50	7500	Mixed (grass in the barn or whole zea maize, pasture, concentrates)	By hand	In a steel vat
Mountains (one site)/900–1210	Simmental × Red Holstein	4–6 herds of 10–30 cows	4500	Only pasture	Bucket milking machine	In a steel vat
Highlands (two sites)/1275–2120	Simmental × Red Holstein	1st site: 30–48[b] 2nd site: 27–40[b]	4500	Only pasture	Bucket milking machine	In an Alpine copper vat

[a] During the project time (from June to the half of September).
[b] The number of cows has changed during the grazing period.

2.4. Methods of analysis

The qualitative and quantitative determination of the fatty acid composition was carried out by high-resolution gas chromatography with flame ionisation detection according to Collomb and Bühler (2000). After dissolution of the pure milk fat in hexane, the glycerides were transesterified to the corresponding methyl esters of fatty acids by a solution of potassium hydroxide in methanol. The fatty acids were then separated on a capillary column CP-Sil 88 (100 m × 0.25 mm i.d. × 0.20 μm) and quantified using nonanoic acid as internal standard. The results are expressed in absolute values, in g fatty acids (and not as esters) per 100 g fat.

For the classical method used (ISO, 1997a, b) in the previous study (Collomb et al., 1999), the glycerides were also transesterified by a solution of potassium hydroxide in methanol. The fatty acids were then separated on a capillary column BPX 70 (50 m × 0.22 mm i.d. × 0.25 μm) and the results were expressed in percent of the total measured methyl esters of fatty acids.

2.5. Statistical analysis

Descriptive statistics, Pearson correlations, principal component analysis (PCA), analysis of variance (ANOVA) and pairwise comparisons of mean values with Fisher's LSD test were performed with Systat for Windows version 9.0 (Anonymous, 1999).

3. Results

Fatty acids can be named according to both the systematic or trivial nomenclature. Because of the high number of fatty acids analysed, the systematic terminology was used in this study.

The results obtained with the new method, including the comparison with those obtained with the classical method are presented in Table 2.

The significant differences in the concentrations of fatty acids between the three vegetation sites previously obtained (Collomb et al., 1999) using the classical method (ISO, 1997a, b) have been confirmed using the new method except for octadecanoic (C18) and eicosanoic acids (C20). With the classical method, the concentration of C18 in milks from the Lowlands and Mountains was similar and significantly higher than that in the Highlands. The concentration of C20 in milks from the Lowlands and Highlands was similar and significantly lower than that in the Mountains.

3.1. Composition of the individual fatty acids of milk fat

The different fatty acids in milk fats from the three vegetation sites were divided into six classes according to their highest concentrations found firstly in Lowlands, secondly in Mountains and finally in Highlands (Table 2).

Thirteen fatty acids, principally saturated compounds from C4 to C16, were present at significantly higher levels in milks from the Lowlands than in those from the other two sites. With the exception of butanoic acid, the concentrations of fatty acids in this class were statistically similar in milks from the Mountains and the Highlands.

Only two fatty acids (C20:1 c11 and C22), present in low concentration in milk fat, showed significantly higher concentrations in milks from the Lowlands and the Mountains than in those from the Highlands. The content of docosanoic acid (C22) in Lowland milks was not significantly different from that found in milks from the other two vegetation sites.

The concentrations of three fatty acids (C17 aiso, C18:1 t13−14+c6−8 and C18:1 c14+t16) were significantly higher in milks from the Lowlands and the Highlands than in milks from the Mountains. However, the contents of the C17 aiso in milks from the Highlands and that of the C18:1 c14+t16 from the Lowlands were not significantly different from the concentrations found in milks from the two other vegetation sites.

The concentrations of seven fatty acids were highest in milks from the Mountains. The levels of three compounds (C18, C20 and C20:3) were lowest in milks from the Highlands and that of t9 octadecanoic acid (C18:1 t9) was lowest in milks from the Lowlands. The concentrations of the other fatty acids (C18:1 t4, C18:1 c9 and C20:1 c9) were comparable in milks from the Lowlands and the Highlands.

The contents of 14 fatty acids, principally iso and anteiso compounds from C13 to C17, the saturated fatty acid C15, linoleic acid and minor fatty acids (C18:1, C18:2, C20:1 and C20:2), were highest in milks from the Mountains and the Highlands. The concentrations of only three fatty acids in milks from the Mountains (C16 aiso, C17 and C20:4) were similar to those found in milks from the Lowlands and the Highlands.

The concentrations of 14 fatty acids were highest in milks from the Highlands. Some fatty acids, principally monounsaturated trans isomers of C16, C17, C18 (except C18:1 t12) and many C18:2 trans compounds, increased with elevation from the Lowlands to the Mountains to the Highlands. The highest increases were observed for the conjugated C18:2 c9t11 and C18:1 t10+t11 fatty acids (Figs. 1 and 2). Other fatty acids were present at similar levels in milks from the Lowlands and the Mountains, especially unsaturated C18 fatty acids with two or three double bonds.

There were no significant differences in the concentrations of the following fatty acids between

Table 2
Fatty acid composition (g $100\,g^{-1}$ fat) of the milk fat on the three vegetation sites

Fatty acids	Systematic name	Lowlands		Mountains		Highlands		Comparison of current and previous results[a]
		$\bar{X}$	S_x	$\bar{X}$	S_x	$\bar{X}$	S_x	
n		11		12		21		
The most present in Lowlands								
C4	Butanoic	3.48^{A}	0.19	3.30^{B}	0.09	3.14^{C}	0.18	Confirmed
C6	Hexanoic	2.07^{A}	0.10	1.80^{B}	0.06	1.74^{B}	0.12	Confirmed
C7	Heptanoic	0.02^{A}	0.01	0.01^{B}	0.00	0.01^{B}	0.00	New
C8	Octanoic	1.16^{A}	0.08	0.96^{B}	0.06	0.95^{B}	0.09	Confirmed
C10	Decanoic	2.42^{A}	0.26	1.93^{B}	0.18	1.96^{B}	0.21	Confirmed
C10:1	Decenoic	0.31^{A}	0.04	0.24^{B}	0.02	0.24^{B}	0.03	Confirmed
C12	Dodecanoic	2.63^{A}	0.33	2.12^{B}	0.19	2.13^{B}	0.22	Confirmed
C12 aiso	Aiso-dodecanoic	0.08^{A}	0.01	0.07^{B}	0.01	0.07^{B}	0.01	New
C12:1 + C13	Dodecenoic + tridecanoic	0.14^{A}	0.03	0.12^{B}	0.01	0.12^{B}	0.01	New
C14	Tetradecanoic	8.97^{A}	0.54	7.86^{B}	0.35	7.92^{B}	0.51	Confirmed
C14:1 c	c-Tetradecenoic	0.87^{A}	0.09	0.74^{B}	0.06	0.71^{B}	0.08	New
C16	Hexadecanoic	24.15^{A}	1.01	21.35^{B}	0.50	20.83^{B}	1.09	Confirmed
C16:1 c	c-Hexadecenoic	1.20^{A}	0.08	1.03^{B}	0.06	1.04^{B}	0.09	Confirmed
The most present in Lowlands and Mountains								
C20:1 c11	c11-Eicosenoic	0.082^{A}	0.014	0.083^{A}	0.013	0.041^{B}	0.004	New
C22	Docosanoic	0.103^{AB}	0.032	0.113^{A}	0.029	0.082^{B}	0.030	New
The most present in Low- and Highlands								
C17 aiso	Aiso-heptadecanoic	0.26^{A}	0.04	0.23^{B}	0.01	0.25^{AB}	0.03	New
C18:1 t13−14 + c6−8	t13-14 + c6-8-octadecenoic	0.73^{A}	0.09	0.60^{B}	0.06	0.70^{A}	0.09	New
C18:1 c14 + t16	c14 + t16-octadecenoic	0.37^{AB}	0.05	0.36^{B}	0.02	0.40^{A}	0.04	New
The most present in Mountains								
C18	Octadecanoic	9.60^{B}	0.78	10.47^{A}	0.59	9.02^{C}	0.80	o.p.c.
C18:1 t4	t4-Octadecenoic	0.014^{B}	0.003	0.019^{A}	0.001	0.014^{B}	0.004	New
C18:1 c9	c9-Octadecenoic	16.69^{B}	1.55	19.25^{A}	0.88	17.42^{B}	1.40	New
C18:1 t9	t9-Octadecenoic	0.23^{C}	0.03	0.33^{A}	0.07	0.28^{B}	0.03	New
C20	Eicosanoic	0.19^{B}	0.02	0.21^{A}	0.02	0.16^{C}	0.02	o.p.c.
C20:1 c9	c9-Eicosenoic	0.153^{B}	0.016	0.192^{A}	0.018	0.145^{B}	0.019	New
C20:3 (ω−6)	Eicosatrienoic	0.056^{B}	0.007	0.064^{A}	0.009	0.051^{C}	0.005	New

The most present in Mountains and Highlands								
C13 iso	Iso-tridecanoic	0.10^{B}	0.01	0.15^{A}	0.01	0.15^{A}	0.02	New
C14 iso	Iso-tetradecanoic	0.24^{B}	0.02	0.32^{A}	0.02	0.30^{A}	0.04	New
C15	Pentadecanoic	1.00^{B}	0.09	1.08^{A}	0.04	1.14^{A}	0.11	Confirmed
C15 iso	Iso-pentadecanoic	0.22^{B}	0.01	0.28^{A}	0.01	0.29^{A}	0.03	New
C16 iso	Iso-hexadecanoic	0.39^{B}	0.03	0.41^{A}	0.02	0.42^{A}	0.03	New
C16 aiso	Aiso-hexadecanoic	0.53^{B}	0.15	0.59^{AB}	0.03	0.61^{A}	0.05	New
C17	Heptadecanoic	0.60^{B}	0.06	0.65^{AB}	0.06	0.66^{A}	0.08	Confirmed
C17iso	Iso-heptadecanoic	0.054^{B}	0.007	0.064^{A}	0.007	0.068^{A}	0.011	New
C18:2 c9c12 ($\omega-6$)	c9c12-Octadecadienoic	1.14^{B}	0.16	1.35^{A}	0.09	1.33^{A}	0.12	New
C18:1 t6 + 8	t6-8-Octadecenoic	0.13^{B}	0.02	0.20^{A}	0.03	0.19^{A}	0.02	New
C18:1 t9t11	t9t11-Octadecadienoic	0.017^{B}	0.003	0.024^{A}	0.004	0.022^{A}	0.003	New
C20:1 t	t-Eicosenoic	0.032^{B}	0.005	0.038^{A}	0.003	0.035^{AB}	0.007	New
C20:2 cc ($\omega-6$)	Eicosadienoic	0.019^{B}	0.003	0.028^{A}	0.003	0.027^{A}	0.004	New
C20:4 ($\omega-6$)	Eicosatetraenoic	0.104^{B}	0.011	0.113^{AB}	0.007	0.113^{A}	0.010	New
The most present in Highlands								
C14aiso	Anteiso-tetradecanoic	0.44^{C}	0.05	0.57^{B}	0.02	0.63^{A}	0.06	New
C16:1 t	t-Hexadecenoic	0.11^{C}	0.01	0.21^{B}	0.02	0.29^{A}	0.03	New
C17:1 t	t-Heptadecenoic	0.009^{C}	0.004	0.013^{B}	0.003	0.026^{A}	0.005	New
C18:1 t10 + 11	t10-11-Octadecenoic	2.11^{C}	0.26	3.66^{B}	0.32	5.10^{A}	0.46	New
C18:1 c11	c11-Octadecenoic	0.38^{C}	0.05	0.44^{B}	0.02	0.49^{A}	0.04	New
C18:2 $\sum$ ttNMID	ttNMID-Octadecadienoic	0.115^{C}	0.017	0.166^{B}	0.016	0.218^{A}	0.031	New
C18:2 t11c15 ($\omega-3$) + t9c12	t11c15 + t9c12-Octadecadienoic	0.33^{C}	0.04	0.42^{B}	0.04	0.70^{A}	0.14	New
C18:2 c9t11 (CLA)	c9t11-Octadecadienoic	0.81^{C}	0.11	1.50^{B}	0.18	2.18^{A}	0.21	New
C18:2 c9c11 (t11c13) (CLA)	c9c11 + t11c13-Octadecadienoic	0.043^{C}	0.008	0.083^{B}	0.011	0.158^{A}	0.043	New
C18:1 t12 ($\omega-6$)	t12-Octadecenoic	0.22^{B}	0.03	0.23^{B}	0.02	0.25^{A}	0.04	New
C18:2 t9t12 ($\omega-6$)	t9t12-Octadecadienoic	0.013^{B}	0.004	0.016^{B}	0.004	0.021^{A}	0.005	New
C18:2 c9t13 + (t8c12)	c9t13-Octadecadienoic	0.24^{B}	0.03	0.23^{B}	0.02	0.30^{A}	0.03	New
C18:2 c9t12 ($\omega-6$) + (ccMID + t8c13)	c9t12-Octadecadienoic	0.27^{B}	0.03	0.27^{B}	0.01	0.30^{A}	0.02	New
C18:3 c9c12c15 ($\omega-3$)	c9c12c15-Octadecatrienoic	0.79^{B}	0.17	0.82^{B}	0.05	1.15^{A}	0.15	New

[a] Previous results (Collomb et al., 1999): new: new compound identified using the new method applied in this study (Collomb & Bühler, 2000); confirmed = significant differences between the three vegetation sites, as observed in previous study, confirmed; o.p.c.: differences between the three vegetation sites, as observed in previous study, only partly confirmed; $\sum$ = sum of the concentrations; c = *cis*; t = *trans*; tt NMID = *trans*, *trans*-nonmethylene interrupted diene; cc MID = *cis*, *cis*-methylene interrupted diene; n = number of samples analysed; $\bar{X}$ = mean value; S_x = standard deviation; values within a row not showing a common superscript (A, B, C) differ, $P \leqslant 0.05$.

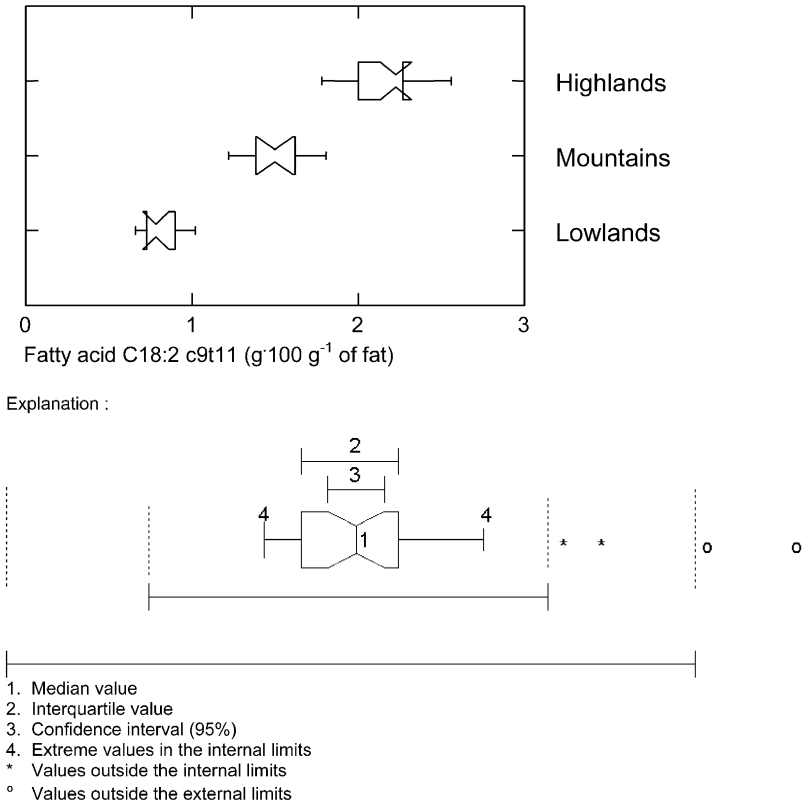

Fig. 1. Schematic comparison of the concentration of the fatty acid C18:2 c9t11 in milk fat from cows fed in Lowlands, Mountains, and Highlands in Switzerland.

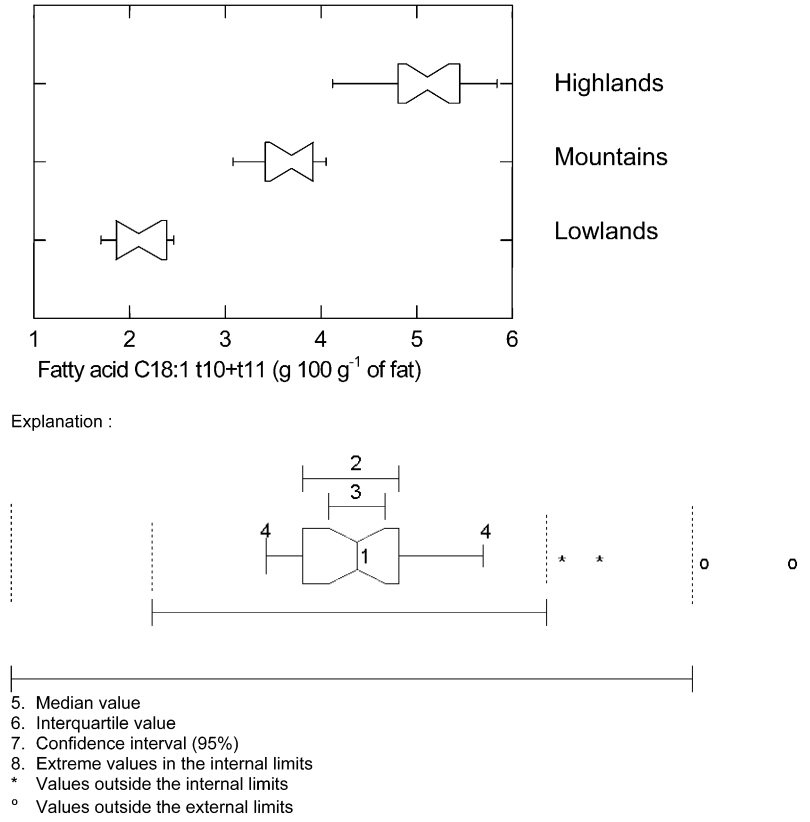

Fig. 2. Schematic comparison of the concentration of the fatty acids C18:1 t10+t11 in milk fat from cows fed in Lowlands, Mountains, and Highlands in Switzerland.

Table 3
Composition (g $100\,g^{-1}$ fat) of pools of fatty acids in the milk fat from the three vegetation sites

Fatty acids	Lowlands		Mountains		Highlands	
	$\bar{X}$	S_x	$\bar{X}$	S_x	$\bar{X}$	S_x
n	11		12		21	
The most present in Lowlands						
$\sum$ saturated[1]	58.90^{A}	1.34	54.70^{B}	0.88	52.71^{C}	1.40
$\sum\sum$ short chain[2]	9.53^{A}	0.46	8.30^{B}	0.32	8.11^{B}	0.54
$\sum\sum$ medium chain[3]	41.12^{A}	1.65	36. 94^{B}	0.90	36.71^{B}	1.81
Sat. C12, C14 and C16	35.75^{A}	1.63	31.33^{B}	0.92	30.88^{B}	1.70
The most present in Mountains and Highlands						
$\sum\sum$ long chain[4]	36.47^{B}	2.63	42.78^{A}	1.72	42.44^{A}	1.87
$\sum\sum$ C18:1	21.06^{B}	1.66	25.27^{A}	1.03	25.01^{A}	1.23
$\sum\sum$ monounsaturated[5]	23.84^{B}	1.66	27.83^{A}	1.00	27.56^{A}	1.19
$\sum\sum$ $\omega-6$[6]	1.96^{B}	0.22	2.21^{A}	0.12	2.23^{A}	0.16
The most present in Highlands						
$\sum\sum$ C18:2	3.01^{C}	0.28	4.11^{B}	0.22	5.27^{A}	0.43
$\sum\sum$ unsaturated[7]	28.08^{C}	1.98	33.20^{B}	1.17	34.42^{A}	1.24
$\sum\sum$ polyunsaturated[8]	4.24^{C}	0.43	5.37^{B}	0.24	6.86^{A}	0.51
$\sum\sum$ CLA[9]	0.87^{C}	0.12	1.61^{B}	0.19	2.36^{A}	0.24
$\sum\sum$ C18:1 t[10]	3.43^{C}	0.36	5.06^{B}	0.40	6.53^{A}	0.57
$\sum\sum$ C18:2 t without CLA t[11]	0.96^{C}	0.11	1.11^{B}	0.07	1.54^{A}	0.21
trans total without CLA t[12]	4.55^{C}	0.47	6.44^{B}	0.48	8.44^{A}	0.73
$\sum$ $\omega-3$[13]	1.39^{B}	0.20	1.49^{B}	0.07	2.09^{A}	0.22

$\sum$ = sum of the concentrations; CLA = conjugated linoleic acid; c = *cis*; t = *trans*; tt NMID = *trans*, *trans*-nonmethylene interrupted diene; cc MID = *cis*, *cis*-methylene interrupted diene; DHA = c4,c7,c10,c13,c16,c19-docosahexanoic acid; EPA = c5,c8,c11,c14,c17 eicosapentanoic acid; DPA = c7,c10,c13,c16,c19-docosapentaenoic acid; *n* = number of samples analysed; $\bar{X}$ = mean value; S_x = standard deviation; values within a row not showing a common superscript (A, B, C) differ, $P \leqslant 0.05$.

Ordered according to increasing retention time:

[1]C4, C5, C6, C7, C8, C10, C12, C12 iso, C12 aiso, C13 iso, C14, C14 iso, C14 aiso, C15, C15 iso, C16, C16 iso, C16 aiso; C17, C17 iso, C17 aiso, C18, C19, C20, C22.

[2]C4, C5, C6, C7, C8, C10, C10:1.

[3]C12, C12 iso, C12 aiso, C12:1 + C13, C13 iso, C14, C14 iso, C14:1 t, C14 aiso, C14:1 c, C15, C15 iso, C16, C16 iso, C16:1 t, C16 aiso, C16:1 c.

[4]C17, C17 iso, C17:1 t, C17 aiso, C18, C18:1 t4, C18:1 t5, C18:1 t6-8, C8:1 t9, C18:1 t10-11, C18:1 t12, C18:1 t13-14 + c6-8, C18:1 c9, C18:1 c11, C18:1 c12, C18:1 c13, C18:1 t16 + c14, C19, C18:2 ttNMID, C18:2 t9t12, C18:2 c9t13 + (t8c12), C18:2 c9t12 + (ccMID + t8c13), C18:2 t11c15 + t9c12, C18:2 c9c12, C18:2 c9c15, C20, C20:1 t, C18:3 c6c9c12, C20:1 c5, C20:1 c9, C20:1 c11, C18:3 c9c12c15, C18:2 c9t11, C18:2 c9c11 + t11c13, C18:2 t9t11, C20:2 c,c ($\omega-6$), C22, C20:3 ($\omega-6$), C20:3 ($\omega-3$), C20:4 ($\omega-6$), C20:5 (EPA) ($\omega-3$), C22:5 (DPA) ($\omega-3$), C22:6 (DHA) ($\omega-3$).

[5]C10:1, C14:1 ct, C16:1 ct, C17:1 t, C18:1 t4, C18:1 t5, C18:1 t6-8, C8:1 t9, C18:1 t10-11, C18:1 t12, C18:1 t13-14 + c6-8, C18:1 c9, C18:1 c11, C18:1 c12, C18:1 c13, C18:1 t16 + c14, C20:1 t, C20:1 c5, C20:1 c9, C20:1 c11.

[6]C18:1 t12, C18:1 c12, C18:2 t19t12, C18:2 c9t12, C18:2 c9c12, C18:3 c6c9c12, C20:2 cc, C20:3 ($\omega-6$), C20:4 ($\omega-6$).

[7]C10:1, C14:1 ct, C16:1 ct, C17:1 t, C18:1 t4, C18:1 t5, C18:1 t6-8, C8:1 t9, C18:1 t10-11, C18:1 t12, C18:1 t13-14 + c6-8, C18:1 c9, C18:1 c11, C18:1 c12, C18:1 c13, C18:1 t16 + c14, C18:2 ttNMID, C18:2 t9t12, C18:2 c9t13 + (t8c12), C18:2 c9t12 + (ccMID + t8c13), C18:2 t11c15 + t9c12, C18:2 c9c12, C18:2 c9c15, C20:1 t, C18:3 c6c9c12, C20:1 c5, C20:1 c9, C20:1 c11, C18:3 c9c12c15, C18:2 c9t11, C18:2 c9c11 + t11c13, C18:2 t9t11, C20:2 c,c ($\omega-6$), C20:3 ($\omega-6$), C20:3 ($\omega-3$), C20:4 ($\omega-6$), C20:5 (EPA) ($\omega-3$), C22:5 (DPA) ($\omega-3$), C22:6 (DHA) ($\omega-3$).

[8]C18:2 ttNMID, C18:2 t9t12, C18:2 c9t13 + (t8c12), C18:2 c9t12 + (ccMID + t8c13), C18:2 t11c15 + t9c12, C18:2 c9c12, C18:2 c9c15, C18:3 c6c9c12, C18:3 c9c12c15, C18:2 c9t11, C18:2 c9c11, C18:2 t9t11, C20:2 c,c ($\omega-6$), C20:3 ($\omega-6$), C20:3 ($\omega-3$), C20:4 ($\omega-6$), C20:5 (EPA) ($\omega-3$), C22:5 (DPA) ($\omega-3$), C22:6 (DHA) ($\omega-3$).

[9]CLA total ($\sum$ C18:2 –c9t11, -c9c11 + t11c13, -t9t11)

[10]C18:1 ($\sum$ -t4, - t5, -t6-8, -t9, -t10-11, -t12, -t13-14).

[11]C18:2 *trans* ($\sum$ -ttNMID, -t9t12, -c9t13 + (t8c12), -c9t12 + (ccMID + t8c13), -t11c15 + t9c12).

[12]C14:1t, C16:1t, C17:1 t, C20:1t, C18:1 *trans* + C18:2 *trans*.

[13]C18:2 t11c15, C18:2 c9c15, C18:3 c9c12c15, C20:3 ($\omega-3$), C20:5 (EPA) ($\omega-3$), C22:5 (DPA) ($\omega-3$), C22:6 (DHA) ($\omega-3$).

milk fats from the Lowlands, Mountains and Highlands: C5, C12 iso, C14:1 t, C18:1 -t5, -c12, -c13, C19, C18:2 c9c15, C18:3 c6c9c12, C20:1 c5, C20:3 ($\omega-3$), C20:5 ($\omega-3$), C22:5 ($\omega-3$) and C22:6 ($\omega-3$).

3.2. Composition of pools of fatty acids of milk fat

Table 3 lists pools of fatty acids (e.g. saturated, unsaturated) in three classes according to their presence in the different vegetation sites.

The concentrations of four pools of fatty acids (saturated, short chain, medium chain and saturated C12, C14, C16) were highest in milks from the Lowlands. Except for the content of saturated fatty acids, the concentrations of all other pools of fatty acids in the milks from the Mountains and the Highlands were similar.

The levels of four pools of fatty acids (long chain, C18:1, monounsaturated and ω 6) were highest in milks from the Highlands and the Mountains. The concentrations of all these pools of fatty acids were significantly different from those found in milk fats from the Lowlands.

The concentrations of eight pools of fatty acids were highest in milks from the Highlands. The total concentration of the $\omega-3$ fatty acids were present at similar levels in milks from the Lowlands and the Mountains. The contents of all the other pools of fatty acids increased with elevation of pasture, i.e. from Lowlands to Mountains to Highlands.

3.3. Correlations between different fatty acids of milk fat

The correlations between the main fatty acids in milk fat, obtained using PCA, are shown schematically in Fig. 3.

There were strong positive correlations between the concentrations of saturated C6–C16 fatty acids, and between the concentrations of the polyunsaturated linoleic, conjugated linoleic c9t11 and linolenic fatty acids. Butyric, stearic and oleic acids are clearly well separated from each other and from the above-mentioned saturated and polyunsaturated acids. This figure also shows the excellent discrimination between the fatty acids of milk fat in the three vegetation sites.

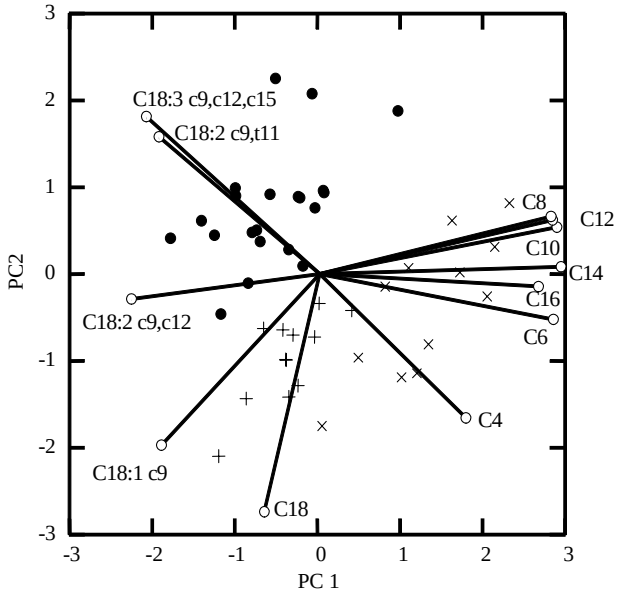

Fig. 3. Correlations between the principal fatty acids of milk fat from Lowland ($\times$), Mountain (+), and Highland (●) regions. The first principal component (PC1) explains 63% and the second principal component (PC2) explains 20% of the total variance.

4. Discussion

The application of the new method (high-resolution gas chromatography) enabled the measurement of nearly 70 fatty acids in milk fat. In a previous paper (Collomb et al., 1999), we used the classical method (gas liquid chromatographic analysis of methylester derivaties (ISO, 1997a, b) for the determination of fatty acids in milk fat from the three vegetation sites at different altitudes in Switzerland. Eighteen compounds were determined using this classical method and the results were expressed in g $100\,g^{-1}$ of the total measured fatty acids. All the isomers of C18:1 fatty acids were assigned to oleic acid (C18:1 c9) and those of the C18:2 compounds to linoleic acid (C18:2 c9c12) as specified in the method. The high content of the monounsaturated fatty acids C18:1 in milk fats from the Highlands (29.2 g $100\,g^{-1}$) and the Mountains (29.2 g $100\,g^{-1}$) compared with that from the Lowlands (24.1 g $100\,g^{-1}$) for example was attributed to oleic acid.

With the new method, about 50 other fatty acids were determined (e.g. 12 isomers of C18:1 and 10 isomers of C18:2 fatty acids, including four conjugated linoleic acids (CLA)). The differences in the concentrations of monounsaturated C18:1 fatty acids between milk fats from the Lowlands and Highlands, as observed previously (Collomb et al., 1999), was confirmed in this study and is mainly due to the high level of the C18:1 t11 (*trans*-vaccenic acid). The latter was not determined, and therefore, not reported by Collomb et al. (1999) using the classical method (ISO, 1997a, b). The same conclusions can be drawn with respect to other isomers of fatty acids (Table 2, e.g. C18:2 *trans*-fatty acids).

A previous study (Bosset et al., 1999; Bütikofer, Bosset, Sieber, & Jeangros, 2002) proved that milk from cows which grazed on different vegetation sites showed large differences in composition (e.g. fat, sodium, magnesium, zinc, manganese, iron) and colour which were attributable to altitude. In the present study, milk fats from the Highlands contained a smaller amount of saturated short chain-, medium chain-, and more polyunsaturated-fatty acids, especially CLA, than those from the Lowlands (Table 3). These results confirm the observations of Bugaud et al. (2001b) and Tschager et al. (1994) who found that milk from the Highlands had a lower content of saturated fatty acids with 4–16 C atoms and a higher content of stearic, oleic, elaidic and of polyunsaturated fatty acids (C18:2 + C18:2 conj + C18:3) compared to milk from the Lowlands. Classical

methods for the determination of fatty acids were used in the latter studies (Bugaud et al., 2001b; Tschager et al., 1994). The differences in composition of fatty acids of milk fats from the Highlands and the Lowlands are likely to be due to different plant species with a different fat composition and/or to the activity of the desaturases of the intestine and of the mammary gland of the cow (Jeangros et al., 1999). Milk fat from the Lowlands contained a significantly higher level of endogenous fatty acids (e.g. fatty acids synthesized in the mammary gland) than milk fat from the Highlands. In the Highlands, the feed of the cows obviously contains higher concentrations of fatty acids, which are incorporated, either directly, or after desaturation, into the milk fat.

Bugaud et al. (2001b) hypothesized that: (i) the environmental conditions of grazing, which are less favourable for cows in Highland pastures compared to cows in Lowland pastures, could explain the higher proportion of long-chain unsaturated fatty acids in milk fat from the Highlands and (ii) the decrease in temperature with elevation or greater degree of walking freely by the cows on Highlands pastures may induce an increase of the concentration of oleic acid. These authors concluded that the higher proportion of polyunsaturated fatty acids in milk fats from the Highlands compared to those from the Lowlands may be related to a lower rate of ruminal biohydrogenation in animals fed on the Highlands. This was not confirmed in the current study, where the increase in *trans*-vaccenic acid (C18:1 t11) and CLA as a function of the altitude, from the Lowlands to the Highlands, resulted in a higher degree of biohydrogenation in the rumen of the cows. Indeed, the *trans*-fatty acids (which are thermodynamically more stable than their *cis*- forms), and CLA are considered to be intermediate products in the biohydrogenation of linoleic acid and other polyunsaturated fatty acids from plants in the rumen of the cow by the bacteria *Butyrovibrio fibrisolvum* (Kepler, Hirons, McNeill, & Tove, 1966; Wu, Ohajuruka, & Palmquist, 1991). The increase in the combined concentrations of the *trans*-10 and *trans*-11 fatty acids as a function of altitude is probably due mainly to the concentration of the *trans*-vaccenic acid from which CLA is endogenously synthesized (Griinari et al., 2000). According to Precht and Molkentin (1996) the concentration of *trans*-vaccenic acid normally represents approximately 90% of the total concentration of the two isomers C18:1 *trans*-10 and *trans*-11 which cannot be separated using the chromatographic conditions in the current study.

The higher level of the stearic acid in the milk fats from the Mountains (10.47 g $100\,g^{-1}$) compared to that in the milk fats from the the Highlands (9.02 g $100\,g^{-1}$) or the Lowlands (9.60 g $100\,g^{-1}$) may be due to a higher activity of desaturase in the intestine and mammary gland of the cows fed on Mountains pastures.

The high concentrations (e.g. as high as 2.87 g $100\,g^{-1}$) of the CLA in the Highland milk fat could provide a positive opportunity for the promotion of alpine milk and meat as healthy products. The latter fatty acids have anticarcinogenic, antiatherogenic, immunomodulatory, growth-promoting and lean body mass-enhancing properties (MacDonald, 2000; Parodi, 1999). The concentration of CLA in milk fats is influenced by several factors such as the feeding of dietary oils, full fat rapeseeds, soybeans, fish meals, grazing, forage: concentrate ratio, animal factors (e.g. stage of lactation, breed: Lawson, Moss, & Givens, 2001). In our study, the content of the CLA isomer c9t11 obtained with pasture feeding was in the range from 1.8 to 2.6 g $100\,g^{-1}$. In comparison, Precht and Molkentin (1999) found a content of the CLA isomer c9t11 from 0.1 to 1.9 $100\,g^{-1}$ of milk fat when analysing 1756 milk fat samples from different regions of Germany. The c9t11-isomer of CLA, which is regarded as the most effective in cancer prevention, generally accounts for $>82\%$ of the total CLA concentration in milk fat (Chin, Liu, Storkson, Ha, & Pariza, 1992).

Some *trans*-fatty acids such as elaidic acid (18:1 t9), which are produced from hydrogenated oils, are associated with cardiovascular diseases (Willett et al., 1993), but in milk fat, *trans*-vaccenic acid (C18:1 t11) is predominant. Ip et al. (1999) suggested that the higher accumulation of total CLA in the tissues of rats fed CLA as in butter rather than chemically pure CLA, was probably due to the conversion of the *trans*-vaccenic acid to the C18:2 c9t11 CLA by the *cis*-9 desaturase in animals.

A maximal recommended ratio of $\omega-3$ to $\omega-6$ fatty acids of $<1{:}5$ is generally considered highly valuable from a nutritional point of view (Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, & Schweizerische Vereinigung für Ernährung, 2000). In this study, we obtained values of $\sim 1{:}2$, which were markedly lower than the recommended maximum values, in milk from the Highlands.

It is noteworthy that the chromatographic peak of the fatty acid C18:2 t9t12, which is an important inhibitor of the $\Delta 6$ desaturase, was well separated from the isomer C18:2 c9t13 under our chromatographic conditions. The concentration of the latter compound in milk fats from the three vegetation sites was very low (0.01–0.02 g $100\,g^{-1}$), lying below the values previously published for milk fat (Precht & Molkentin, 1997).

5. Conclusion

The high-resolution gas chromatographic method used in the current study enabled the separation of a high number of fatty acids in milks from cows grazing

on the Lowlands, Mountains or Highlands of Switzerland. About 70 fatty acids were separated and their concentrations were quantified (in absolute values) using C9 acid as an internal standard. The high-resolution gas chromatographic method is far more informative than the method used previously (ISO, 1997a, b). Using the new method, more fatty acids have been quantified ($\sim$50 fatty acids compared with the official ISO Norm) than in the previous study (Collomb et al., 1999); the concentrations of these fatty acids in milks from the three vegetation sites differed significantly. For example, the CLA isomers, the main *trans*-fatty acids C16:1, C18:1, C18:2, $\omega-3$ and $\omega-6$ fatty acids have been identified in the three vegetation sites using the new method. The results obtained with the new method demonstrated that the increase in the concentration of polyunsaturated fatty acids in milk fats from the Lowlands to the Highlands, as observed in an earlier study (Collomb et al., 1999), was mainly due to the increase in the concentration of conjugated linoleic acid c9t11.

The main conclusions of this study were that the concentrations of *trans*-vaccenic acid (*trans*-11 octadecenoic acid) and conjugated linoleic acid in bovine milk increased as a function of elevation of pasture. Similarly, the concentrations of unsaturated and polyunsaturated fatty acids generally increased as a function of altitude. The opposite was true for the saturated fatty acids.

Milk fat from Highlands appears interesting from the nutritional point of view because of the great reduction of saturated and the increase of polyunsaturated fatty acids, including the CLA. The results present an argument for milk production in Highlands. Also, the relatively high content of *trans*-vaccenic acid (C18:1 t11) in milk from the Highlands should be considered positively in future as evidence (Ip et al., 1999) indicates that it may be transformed endogenously into health-promoting CLA. In contrast, the other *trans*-fatty acids (e.g. C18:1 t9) are often associated with the occurrence of cardiovascular diseases (Willett et al., 1993). The higher content of unsaturated fatty acids in milk fat from the Highlands and Mountains, compared to milk fat from the Lowlands have also an effect on the rheological properties of milk products, and thereby improve the spreadability of butter and give a more desirable consistency (lower friability) to Mountain and Highland cheese (Bütikofer et al., 2002). The high levels of the CLAs could be also considered as an additional indicator for the authenticity of cheese from the Mountains and Highlands; currently the volatile mono- and sesquiterpenoids and other specific compounds such as polycyclic aromatic hydrocarbons (which are due to the use of an open log fire for smoking of cheese in the alpine cabins) are used as indicators of the origins of these cheeses. Other investigations should therefore be undertaken to prove the PDO indicator efficiency of the CLAs.

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