Seasonal and Regional Variation in Triglyceride Composition of French Butterfat

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In the present study, high-performance liquid chromatography analysis of butterfat allowed separation of 46 peaks at 32°C. Knowing the theoretical carbon number value of each triglyceride (TG), 32 peaks of the butterfat chromatogram were identified. These TGs were determined by extrapolation of their capacity factor values, and their identifications were confirmed with some standard TGs. Analysis of winter and summer butterfat from five different French areas showed significant seasonal and regional variation in the TG composition. However, the most important contribution to this variation was provided by TG groups represented by only four peaks. To approximately select the predominant TGs in these four peaks, a random distribution hypothesis was used to predict the amount of each TG. This hypothesis allowed the prediction of the TG components that seem to provide the most important contribution to both seasonal and regional variation.

KEY WORDS: Butterfat, contribution to variation, cow milkfat, high-performance liquid chromatography, regional variation, seasonal variation, triglycerides, triglyceride composition, triglyceride identification.

Many authors have investigated the seasonal fluctuation of the fatty acid (FA) composition of butter (1,2). The variation in FA composition explains the regular seasonal fluctuations of iodine values observed by Cox and McDowall (3) and of solid fat content of milk observed by Norris *et al.* (4). However, the triglyceride (TG) structure, *i.e.*, arrangement and distribution of FAs in TGs, seems to have more influence on the physical characteristics of fats. In fact, Deman (5) and Pitas *et al.* (6) showed that interesterification of milkfat, which transforms a highly selective arrangement of FAs into a random distribution, markedly increased hardness, solid fat content and proportion of high melting TGs.

More accurate studies (7) showed that, in the case of milkfat, butyric acid (Bu) and caproic acid (Co) are mostly esterified in position 3, while myristic acid (My) and palmitic acid are in position 2 and in position 1 or 2, respectively. Stearic acid (S) and oleic acid (O) partitions depend on the TG molecular weight, mainly in positions 1 and 3 for high-molecular weight and in position 1 for low-molecular weight TG (8). To separate TGs, many scientists have exploited the advantages of reverse-phase high-performance liquid chromatography (HPLC). But because of animal fat complexity, few papers dealt with the TG separation.

It was important to find the proper parameters, *i.e.*, column, eluent, temperature or adequate temperature gradient, and detector to obtain the best peak separation. Several eluents were used to attempt to separate TGs, such as mixtures of methanol/water (9:1) (9), methanol/chloroform (9:1) (10) and methanol/acetone (11). However, according to Deffense (12), the most efficient way was to use an acetone/acetonitrile mobile phase at 50°C. Frede and Thiele (13) confirmed its efficiency by using the same mixture as the mobile phase (35:65), but they set the temperature of the column (Nucleosil C18-5 μm, 15 cm; Macherey and Nagel, Duren, Germany, + Microspher C18-3 μm, 10 cm, in series; chrompack, Mülhein, Germany) at 30°C.

In HPLC analysis of TGs, most investigators used a differential refractometer as a detector except for Marini and Balestieni (14), who worked on butter solutions with an ultraviolet (UV) detector operated at 210 nm.

The studies listed above were carried out under various chromatographic conditions (solvent, column, temperature) and are difficult to compare. Bouteiller and Maurin (15) developed an equation to correlate such different results. It takes into account the free energy of distribution increments of triacylglycerol structural units. The reported models have been checked successfully by plotting log a_1 *vs.* log a_2, (a_1 and a_2 are the relative retention times of a series of triacylglycerols, with triolein being the reference compound). The correlation coefficients are higher than 0.99 in all cases.

The purpose of the present study was to improve the separation of TGs from butterfat by reverse-phase HPLC, to identify TGs and, finally, to investigate changes in the composition of cow butterfat TGs from five different French areas throughout the dairy season (winter, summer). This investigation was undertaken to predict the TG components that seem to contribute the most to seasonal and regional variation in the TG composition.

EXPERIMENTAL PROCEDURES

Materials. The following model triglycerides (purity 99%) were purchased from Sigma (St. Louis, MO): PPP, SSM, OOO, LLL, LnLnLn, LaLaM, PPO, OPO, POS and POP. Acetone, acetonitrile and chloroform (Prolabo, Rhone Pulenc, France, for HPLC analysis) were used without further purification. The mixture of acetone and acetonitrile (59:41, vol/vol) was ultrasonically degassed. Creams from five different specific areas in France were churned, and the resulting butters were melted at 60°C and centrifuged, and the fat was dissolved in chloroform [1 g/1.9 mL (wt/vol)]. A subsequent solution (50 mg/mL) was prepared in a mixture of acetone/acetonitrile (59:41).

High-performance liquid chromatography (HPLC). The instruments used were a 110A solvent metering pump (Altex Instrument, Berkeley, CA), a Rheodyne loop (20 μL) injector (model 7125), a refractive index detector (LKB 2142; LKB, Bromma, Sweden) and an SP 4270 Integrator (Spectra-Physics, San Jose, CA). Two 150 mm × 4.6 mm packed columns were used in this study—Supelcosil LC-18 column with 5-μm octadecyl-bonded spherical silica (Supelco, Bellefonte, PA). Twenty μL of the preheated working solution was injected onto the column. (each assay was done in triplicate with an error<5%). The mobile-phase flow was set at 0.8 mL/min (constant flow mode), and the column temperature at 32°C. The speed of the chart paper was 0.5 cm/min.

Gas chromatography (GC). A Girdel Model 30 gas chromatograph (Girdel, Louisville, KY) equipped with a

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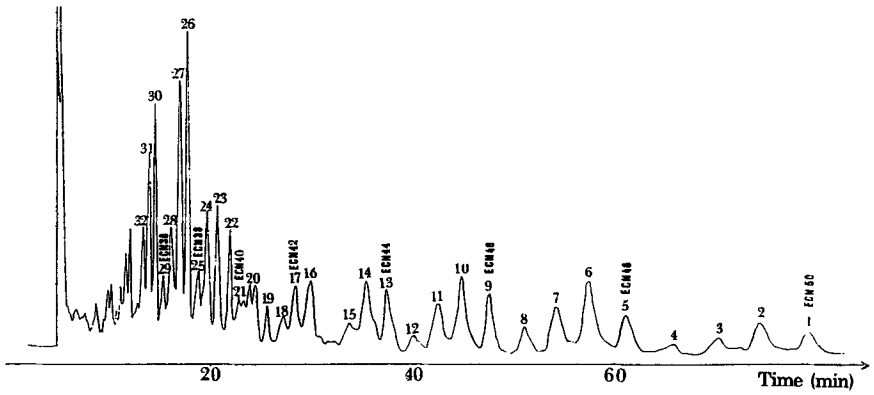

FIG. 1. Separation of butterfat triglycerides at 32°C on two 250 × 4.6 mm Supelcosil LC-18 columns (Supelco, Bellefonte, PA), with acetone/acetonitrile (59:41) as the mobile phase at a flow rate of 0.8 mL/min.

flame ionization detector (FID) and an Amilabo System electronic integrator (Lyon, France) was used.

A fused-silica capillary column 20 M (0.32 mm × 20 m), Carbowax, was used at a column temperature varying from 110 to 190°C at 5°C/min, and the injector and detector temperatures were set at 230 and 250°C, respectively. The carrier gas was helium at 0.6 bar.

For FA analysis, the fractions were transesterified with petroleum ether/sodium methylate by the following micro procedure: one gram of fat was dissolved in 10 mL of petroleum ether; 1.9 mL of this solution was treated by 100 μL of sodium methylate (2N). After clarification, 1 μL of this mixture was injected (unpublished method).

RESULTS AND DISCUSSION

Qualitative considerations and peak identification. Under HPLC conditions, butterfat samples are separated into TG classes differing by the acyl carbon number (CN) and the double-bond number (ND) simultaneously. According to El Hamdy and Perkins (16), as well as Plattner (17) and Podlaha (18), the criteria of separation are the equivalent carbon number (ECN) and the theoretical carbon number (TCN). Using another definition of ECN, Frede (19) pointed out that the latter seemed to be dependent on the distribution of the double bonds of the FAs.

In the present study, two calculation terms were used to identify individual TGs occurring in butterfat: ECN = CN $-2 \times$ ND was employed to characterize each class; and TCN = CN $-f_i$. ND allowed individual peak identification in each class. Figure 1 shows an HPLC chromatogram as one typical example. Forty-five different peaks can be distinguished. Most of them, however, seem to have a uniform pattern in their retention times and intensities, so that association with certain TG classes might be well facilitated, especially in peaks 1–32, which can be classified visually as eight quartets. Each TG class is characterized by different ECNs, as was found by Frede and Thiele (13).

To be able to extract more information on the TG composition of butter, it is necessary to know the retention time for some saturated TGs (PPP, SSM, LaLaM, *etc.*). In Figure 2, logarithms of the capacity factors of these TGs are graphically drawn against their TCN values, which are consistent with their carbon number. It is important to emphasize that only FAs occurring at a percentage higher than 1% with respect to the overall butterfat are considered in this work (Bu, Co, Cy, C, La, M, P, S, O, L, Ln). The unsaturated FAs are oleic (O), linoleic (L) and linolenic (Ln) acids.

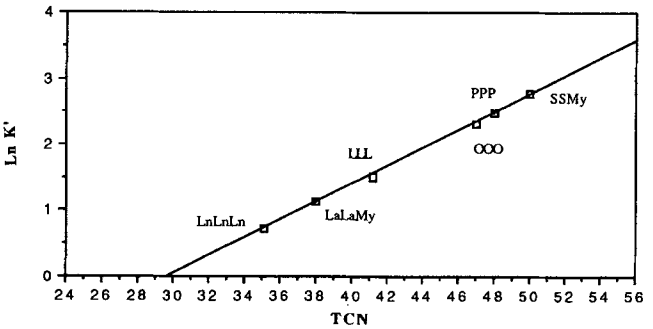

FIG. 2. Capacity factor values (K') of butterfat triglycerides drawn on a logarithmic scale *vs.* the theoretical carbon number (TNC) of some standard triglycerides.

To determine the TCN of each TG, the major homogeneous unsaturated TGs (OOO, LL, LnLnLn) are added to the samples. Their retention times, as well as their TCN values, are determined. The calculation gives 46.6, 40.5 and 34.92 for TCN of OOO, LLL and LnLnLn, respectively, and 2.47, 2.25 and 2.12 for f_i. The retention times of all TGs can be predicted from Table 1. These calculations are confirmed by injection of some standard TGs (*e.g.*, PPO, OPO, POS, POP). As mentioned above, these TGs shown in Table 1 are only the combination of 11 major FAs.

Seasonal and regional variation in the triglyceride composition. The TG composition of butter from five different areas (types 1 to 5) was analyzed in January (winter) and in July (summer). The selected months represent the periods of maximum change of milk fat composition in France. The winter and summer average values for each region are presented in Table 2.

The data, which were statistically analyzed, showed a significant variation. It is now well known that the FA composition depends on the origin of the butterfat. Thus the regional variation in TG composition found in this

TABLE 1

Identification of High-Performance Liquid Chromatographic Butterfat Chromatogram Peaks

No. of peak	TG[a]	ECN[b]	TCN[c]	Ln K'[d]	RT[e]	No. of peak	TG	ECN	TCN	Ln K'	RT	No. of peak	TG	ECN	TCN	Ln K'	RT
1	SSM	50	50	2.76	71.81	17	CoSS		42	1.67	27.2	26	CLaO		37.53	1.07	16.88
	PPS		50	2.76	67.4	18	SLnLn		41.28	1.58	25.09		CyMO		37.53	1.07	16.88
2	POS		49.53	2.7	67.4		MML		41.5	1.61	25.71		CoPO		37.53	1.07	16.88
	SSL		49.53	2.7	67.4		LaPL		41.5	1.61	25.71		BuSO		37.53	1.07	16.88
3	SOO		49.06	2.63	63.68		CSL		41.5	1.61	25.71	27	LLnLn		36.76	0.96	15.66
5	PPP	48	48	2.49	56.02		LaMO		41.53	1.61	25.71		CLL		37	0.99	16.02
	MPS		48	2.49	56.02		CPO		41.53	1.61	25.71		CPLn		37.64	0.96	15.66
	LaSS		48	2.49	56.02		CySO		41.53	1.61	25.71		CyOL		37.03	1	16.12
6	POP		47.53	2.42	52.38	19	MLL		41	1.54	24.4		CoCO		37.06	1	16.12
	MSO		47.53	2.42	52.38		LaOL		41.03	1.54	24.4	29	LaLaLa	36	36	0.86	14.57
	SSLn		47.53	2.42	52.38		COO		41.06	1.55	24.48		CLaM		36	0.86	14.57
	PSL		47.53	2.42	52.38	20	LLL		40.5	1.47	23.02		CCP		36	0.86	14.57
7	POO		47.06	2.36	49.61		OLLn		40.67	1.49	23.45		CyMM		36	0.86	14.57
	SOL		47.06	2.36	49.61	21	LaMM	40	40	1.4	21.81		CyLaP		36	0.86	14.57
8	OOO		46.6	2.3	47.01		CMP		40	1.4	21.81		CyCS		36	0.86	14.57
9	MPP	46	46	2.22	43.56		CLaS		40	1.4	21.81		CoMP		36	0.86	14.57
10	LaSO		45.53	2.15	41.15		CyPP		40	1.4	21.81		CoLaS		36	0.86	14.57
	MPO		45.53	2.15	41.15		CyMS		40	1.4	21.81		BuPP		36	0.86	14.57
	PSLn		45.64	2.17	41.7		CoPS		40	1.4	21.81		BuMS		36	0.86	14.57
	PPL		45.5	2.15	41.15		BuSS		40	1.4	21.81	30	BuPO		35.53	0.8	13.94
	MSL		45.5	2.15	41.15	22	CoSO		39.53	1.34	20.74		CoMO		35.53	0.8	13.94
11	MOO		45.06	2.09	38.88		CyPO		39.53	1.34	20.74		CyLaO		35.53	0.8	13.94
	SLL		45	2.08	38.6		CMO		39.53	1.34	20.74		CCO		35.53	0.8	13.94
	SOLn		45.17	2.11	39.4		LaLaO		39.53	1.34	20.74		BuSL		35.5	0.79	13.91
	POL		45.03	2.09	38.88		CySL		39.5	1.33	20.67		CoPL		35.5	0.79	13.91
12	OOL		44.56	2.02	36.63		CPL		39.5	1.33	20.67		CyML		35.5	0.79	13.91
13	MMP	44	44	1.95	34.27		LaML		39.5	1.33	20.67		CLaL		35.5	0.79	13.91
	LaPP		44	1.95	34.27		MMLn		39.64	1.35	20.98		CyOLn		35.17	0.75	13.5
	LaMS		44	1.95	34.27		laPLn		39.64	1.35	20.98		LaLnLn		35.28	0.76	13.63
	CPS		44	1.95	34.27		CSLn		39.64	1.35	20.98	31	LnLnLn		34.92	0.71	13.2
	CySS		44	1.95	34.27	23	CyOO		39.06	1.27	19.67		CyLL		35	0.72	13.29
14	MMO		43.53	1.88	32.32		COL		39.03	1.27	19.67		CoOL		35.03	0.73	13.33
	LaPO		43.53	1.88	32.32		LaLL		39	1.27	19.67		BuOO		35.06	0.73	13.33
	CSO		43.53	1.88	32.32		OLnLn		38.81	1.24	19.23	33	CLaLa	34	34	0.59	12.18
	PPLn		43.64	1.9	32.85	24	LLLn		38.64	1.22	18.89		CCM		34	0.59	12.18
	MSLn		43.64	1.9	32.85	25	LaLaM	38	38	1.13	17.7		CoLaM		34	0.59	12.18
	MPL		43.5	1.88	32.32		CMM		38	1.13	17.7		CyCP		34	0.59	12.18
15	LaOO		43.06	1.82	30.71		CLaP		38	1.13	17.7		CyCyS		34	0.59	12.18
	SLLn		43.14	1.83	30.99		CCS		38	1.13	17.7		CoMM		34	0.59	12.18
	PLL		43	1.81	30.6		MMP		38	1.13	17.7		CoLaP		34	0.59	12.18
	POLn		43.17	1.83	30.99		CyLaS		38	1.13	17.7		CoCS		34	0.59	12.18
	MOL		43.03	1.81	30.6		CoPP		38	1.13	17.7		BuMP		34	0.59	12.18
16	OLL		42.53	1.75	28.89		CoMS		38	1.13	17.7		BuLaS		34	0.59	12.18
	OOLn		42.7	1.77	29.46		BuPS		38	1.13	17.7	34	BuMO		33.54	0.52	11.71
17	MMM	42	42	1.67	27.2	26	MLnLn		37.28	1.03	16.47		CoLaO		33.53	0.52	11.71
	LaLaS	42	42	1.67	27.2		COLn		37.17	1.02	16.29		CyCO		33.53	0.52	11.71
	LaMP		42	1.67	27.2		LaMLn		37.64	1.08	17.07		BuPL		33.5	0.52	11.71
	CPP		42	1.67	27.2		CySLn		37.64	1.08	17.07		CoML		33.5	0.52	11.71
	CMS		42	1.67	27.2		LaLaL	38	37.5	1.06	16.83		CyLaL		33.5	0.52	11.71
	CyPS		42	1.67	27.2		CMl		37.5	1.06	16.83		CCL		33.5	0.52	11.71
							CyPL		37.5	1.06	16.83		CoOLn		33.17	0.47	11.34
							CoSL		37.5	1.06	16.83		CLnLn		33.28	0.49	11.47
													BuOL		33.29	0.49	11.47

[a]Triglyceride.
[b]Equivalent carbon number.
[c]Theoretical carbon number.
[d]Logarithm of capacity factor.
[e]Retention time.

TABLE 2

Triglyceride Composition (%) of Winter and Summer Butterfat from Five French Areas and Their Contribution to Variation

	Type 1			Type 2			Type 3			Type 4			Type 5		
1	1.39	1.31	0.01	1.40	1.69	0.18	1.50	0.84	0.10	1.57	1.83	0.05	1.03	1.50	0.30
2	1.69	2.98	2.59	1.69	2.80	3.89	2.12	1.98	1.14	2.37	3.96	3.52	1.86	1.44	0.34
3	0.95	1.84	0.74	1.11	1.13	0.00	0.83	0.90	0.03	0.76	2.71	2.92	0.69	1.42	0.63
4	0.35	0.80	0.08	0.45	0.76	0.08	0.78	0.51	0.31	0.42	1.28	0.28	0.57	0.78	0.03
5	3.04	2.02	1.77	2.49	2.55	0.01	2.32	1.90	2.43	2.46	2.95	0.29	2.38	2.85	0.65
6	5.79	5.40	0.57	5.89	6.09	0.35	5.16	4.07	3.64	4.73	6.21	5.27	5.31	6.60	11.05
7	3.40	5.07	7.98	4.21	5.49	11.18	3.68	3.72	4.18	3.09	6.67	27.72	3.20	5.35	22.05
8	1.29	2.17	0.89	1.68	1.81	0.04	1.19	1.95	1.88	1.11	2.46	1.44	1.49	2.24	1.18
9	3.63	2.36	3.28	3.58	3.05	1.32	3.10	2.97	4.34	3.36	3.37	0.00	3.80	2.95	2.73
10	5.10	4.76	0.39	5.57	5.59	0.01	5.62	5.05	3.15	5.71	5.48	0.14	5.14	5.60	1.26
11	2.48	4.27	7.26	2.86	4.56	15.04	3.39	4.37	2.32	2.62	4.57	6.05	2.56	3.95	6.95
12	0.74	1.51	0.46	0.99	1.49	0.44	1.16	2.14	2.05	0.71	1.67	0.49	0.81	1.01	0.04
13	3.52	2.17	3.49	3.27	2.44	2.75	3.24	2.98	1.24	3.95	2.45	3.18	3.20	2.52	1.46
14	3.76	3.28	0.55	3.50	3.36	0.10	3.90	4.26	1.02	3.83	3.33	0.39	3.05	2.98	0.02
15	1.23	2.28	1.31	1.42	2.16	1.39	1.95	3.09	1.59	1.41	2.19	0.49	1.06	1.22	0.03
16	3.58	2.13	4.08	3.74	2.07	11.45	2.96	3.24	0.05	3.49	2.59	1.10	3.28	2.49	2.00
17	2.35	2.63	0.13	2.78	2.36	0.65	2.41	3.16	0.25	2.80	2.18	0.42	2.53	1.97	0.79
18	0.79	2.16	1.88	1.32	1.31	0.00	1.92	2.89	0.00	1.12	1.63	0.16	0.99	1.55	0.44
19	1.17	1.71	0.29	1.33	1.35	0.00	1.20	1.96	3.64	1.02	1.11	0.00	1.13	1.16	0.00
20	4.18	2.91	3.90	3.88	2.87	4.92	3.61	3.67	0.62	3.67	2.44	2.07	3.93	3.25	1.85
21	2.42	2.56	0.03	2.50	2.38	0.05	2.50	2.94	0.40	2.91	2.05	0.83	2.60	2.55	0.01
22	3.00	3.47	0.49	3.14	3.32	0.14	3.02	3.12	1.26	2.94	2.81	0.02	3.14	3.21	0.02
23	4.66	4.22	0.58	4.36	3.94	1.05	4.32	4.06	0.43	4.22	3.20	1.73	4.66	3.96	2.36
24	4.51	3.63	2.13	4.18	3.72	1.17	4.23	3.80	2.91	4.41	3.04	3.09	4.67	4.07	1.76
25	2.37	2.85	0.40	2.35	2.66	0.34	2.41	2.84	6.46	2.45	2.21	0.06	2.78	2.56	0.14
26	8.42	5.24	46.49	7.31	5.37	34.03	7.26	4.96	3.63	7.59	4.85	20.73	8.18	6.18	31.81
27	7.10	7.01	0.04	6.98	7.22	0.59	7.28	6.01	34.09	7.87	6.08	9.97	7.34	7.60	0.57
28	3.65	4.15	0.66	3.54	3.81	0.37	3.71	3.59	3.70	3.96	3.27	0.77	3.97	4.07	0.04
29	1.65	1.93	0.10	1.58	1.76	0.07	1.68	2.07	3.05	1.68	1.48	0.03	1.99	1.72	0.15
30	5.11	3.62	6.56	4.57	3.69	4.47	4.85	3.75	0.22	4.98	3.15	6.03	5.36	4.22	6.93
31	4.39	4.73	0.37	4.17	4.94	3.89	4.46	4.48	5.06	4.66	5.17	0.57	4.58	5.03	1.07
32	2.29	2.84	0.52	2.14	2.26	0.04	2.26	2.73	4.81	2.11	1.63	0.20	2.69	1.97	1.36

TABLE 3

Fatty Acid Composition (%) of Five Types of Winter and Summer Butterfat

		Type 1		Type 2		Type 3		Type 4		Type 5	
No. of peak	Name of fatty acid	Winter (%)	Summer (%)	Winter (%)	Summer (%)	Winter (%)	Summer (%)	Winter (%)	Summer (%)	Winter (%)	Summer (%)
1	C_6	1.62	0.98	2.22	1.11	2.13	1.25	1.72	1.33	1.89	1.20
2	C_8	1.74	1.00	2.08	1.10	1.87	1.32	1.71	1.15	1.90	1.27
3	C_{10}	4.86	2.46	5.43	2.75	4.71	3.36	4.73	2.76	5.16	3.24
4	C_{11}	0.44	0.25	0.52	0.27	0.44	0.30	0.46	0.28	0.52	0.29
5	C_{12}	6.24	2.92	6.43	3.32	5.72	3.94	5.99	3.29	6.41	3.86
6	iC_{14}	0.03	0.06	0.00	0.08	0.11	0.04	0.03	0.09	0.09	0.07
7	C_{14}	19.63	10.89	19.58	11.47	19.36	13.11	19.60	11.67	20.52	12.84
8	C_{15}	0.87	0.66	0.85	0.72	0.78	0.65	0.85	0.70	0.88	0.66
9	$C_{15:1}$	0.76	0.41	0.76	0.41	0.79	0.43	0.82	0.45	0.88	0.43
10	iC_{16}	0.13	0.22	0.07	0.25	0.25	0.27	0.09	0.26	0.07	0.22
11	C_{16}	32.49	28.85	28.83	28.53	28.53	29.67	30.74	29.88	30.96	31.58
12	$C_{16:1}$	2.94	1.95	2.81	1.85	2.43	1.58	2.58	1.91	2.70	1.61
13	iC_{17}	1.07	0.20	0.91	0.40	1.02	0.19	0.84	0.44	0.87	0.00
14	IC_{18}	0.30	0.40	0.27	0.38	0.39	0.00	0.15	0.39	0.30	0.36
15	C_{18}	8.70	12.87	9.75	13.05	9.35	11.88	9.62	12.39	8.69	11.42
16	$C_{18:1}$	15.48	32.48	16.74	30.09	18.46	27.70	17.49	29.47	15.86	27.73
17	$C_{18:2}$	1.02	1.20	1.31	1.48	1.39	1.58	1.04	1.45	0.96	1.34
18	$C_{18:3}$	1.29	2.21	1.22	2.46	1.76	2.76	1.16	2.12	0.89	1.88

work can be easily explained. The seasonal variation in TG composition of butterfat also was studied, and the contribution of each group of TG to this variation is expressed by the term V_i/V_t, which is equivalent to a variance and defined as:

$$V_i = [(TG_{is}-TG_{iw})^2/100] \times [(TG_{is}+TG_{iw})/2] \quad [1]$$

$$V_t = \Sigma V_i \quad [2]$$

where TG_{is} is the intensity of peak i (%) of a summer butterfat, and TG_{iw} is the intensity of peak i (%) of a winter butterfat.

The results are summarized in Table 2. It appears that TG groups that contribute the most to the variation are represented by peaks 6,7,11,16,26,27 and 30. On the other hand, Table 2 shows that with all types, peaks 7 and 11 are larger in summer fat than in winter fat. However, peaks 26 and 30 are larger in winter. The other peaks do not display a defined pattern, or their differences in intensity are not significant.

Knowing their FA composition, the amount of TGs predicted by a random distribution hypothesis would be estimated by the following equations: $\%A\cdot A\cdot A = A^3/10000$, $\%A\cdot A\cdot B = 3\cdot A^2\cdot B/10000$, and $\%A\cdot B\cdot C = 6\cdot A\cdot B\cdot C/10000$.

The FA compositions of winter and summer butter from the five different areas (types 1 to 5) are presented in

TABLE 4

Summer and Winter Triglyceride (TG) Averages or the Most Important Peaks (determination of predominant TG in each peak)

No. of peak	TG	Summer TG average[a] (%)	Winter TG average[a] (%)	% of Summer TG/peak	% of Winter TG/peak
Peak 7	POO	7.68	2.56	96.00	95.00
	SOL	0.32	0.12	4.00	5.00
Peak 11	MyOO	3.13	1.67	72.79	76.61
	SLL	0.05	0.00	1.16	0.00
	SOLn	0.54	0.14	12.56	6.42
	POL	0.71	0.37	16.51	16.97
Peak 26	MyLnLn	0.01	0.01	0.58	0.52
	COLn	0.10	0.06	5.83	3.13
	LaMyLn	0.04	0.10	2.33	5.21
	CySLn	0.01	0.01	0.58	0.52
	LaLaL	0.00	0.01	0.00	0.52
	CMyL	0.02	0.05	1.17	2.60
	CyPL	0.02	0.03	1.17	1.56
	CoSL	0.01	0.00	0.58	0.00
	CLaO	0.05	0.30	2.92	15.63
	CyMyO	0.24	0.37	13.99	19.27
	CoPO	0.58	0.61	33.82	31.77
	BuSO	0.68	0.37	39.65	19.27
Peak 30	BuPO	1.57	1.21	76.21	50.21
	CoMyO	0.24	0.39	11.65	16.18
	CyLaO	0.07	0.12	3.40	4.98
	CCO	0.07	0.13	3.40	5.39
	BuSL	0.03	0.12	1.46	4.98
	CoPL	0.02	0.30	0.97	12.45
	CyMyL	0.01	0.02	0.49	0.83
	CLaL	0.00	0.10	0.00	4.15
	CyOLn	0.04	0.02	1.94	0.83
	LaLnLn	0.01	0.00	0.49	0.00

[a]Amount of triglyceride is predicted by random distribution hypothesis.

Table 3. The proportions of butyric acid, considered in this work, are estimated at 3% in summer and 4% in winter (references 1,2). Table 4 shows the amounts of TGs corresponding to highly varying peaks, which present a uniform pattern between summer and winter butterfats (peaks 7, 11, 26 and 30).

Of course, the predicted TG amounts are probably different from the actual quantities in butterfat, but the random distribution hypothesis allows determination of predominant TGs that appear as a single peak. According to Table 4, peak 7 is formed by two TGs (POO, SOL) that represent 95% and 5%, respectively, of the total amount. Therefore, in the first approach, SOL looks negligible compared to POO. Consequently, peak 7 can be considered to be formed mainly by POO. From physiological characteristics, Frede and Thiele (13) reached the same conclusion (peak 7 corresponds to peak 36 in their study). In the same way, peak 11 is calculated to be MyOO (*ca.* 75%); peak 26 is formed primarily by CLaO (*ca.* 15%), CyMyO (*ca.* 19%), CoPO (*ca.* 31%) and BuSO (*ca.* 19%). Finally, three major TGs are calculated for peak 30: BuPO (*ca.* 50%), CoMyO (*ca.* 16%) and CoPL (*ca.* 12%); all other TGs can be neglected.

The random distribution hypothesis associated with a characterization of HPLC peaks by use of the TCN permits calculation of a more probable composition of butterfat triglycerides. It is used here to identify the seasonal effects of the variations of triglyceride composition and mainly to predict TG components that seem to provide the most important contribution to seasonal and regional variation.

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