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Determination of Fat Content and Fatty Acid Composition through 1-min Transesterification in the Food Sample

II. Solubilization of the Fat, Results

Key words: Fatty acid methyl esters, Fat content in foods, Solubilization of fat

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Introduction

Recently we described a method for determining fat content and/or fatty acid composition through base-catalyzed transesterification directly in the foodstuff (1). Since transesterification is faster than saponification, selection of suitable conditions enables the conversion of all the fatty acid esters into methyl esters (FAMEs) even in the presence of water. This in turn enables the formation of FAMEs directly in the foods, even if the latter mainly consist of water, such as milk. The FAMEs can be used for determining the fatty acid composition as well as the fat content of a food sample after adding up the components and calculation through the internal standard and a global response factor.

Direct transesterification in the foods circumvents extraction of the fat, thus avoiding the laborious procedures applied presently, yields a better defined result, i.e. the quantity of the fatty acids as opposed to the «fat extract» consisting of any apolar material, and avoids the stress on the fat material to be analyzed during hydrolysis of the food, i.e. preventing the formation of artifacts.

The previous paper described the transesterification that takes one min at ambient temperature. The large amount of methanol and methoxide added accelerates transesterification and favors it over saponification. Dioxane is added as a mediator, creating a one phase system of the fat, the methanol/methoxide solution and possibly water. With dioxane, the system remains in one phase also when the extraction solvent (e.g. heptane) is added and is split into two phases only upon addition of the aqueous hydrogen citrate solution. This results in a perfect extraction virtually without shaking.

A system of three or four internal standards enabled to check the completeness of transesterification, the extent of saponification, and the accuracy of the GC

analysis for each sample analyzed (1). The main internal standard, triundecanin, forms FAME-11, which is compared with a standard not participating in the reaction (a hydrocarbon). An insufficient amount of FAME-11 may be the result of incomplete transesterification or advanced saponification. The latter is recognized by the loss of a third internal standard, FAME-9. If a second hydrocarbon is added, also discrimination by the GC analysis can be monitored (primarily of interest with vaporizing injection). The hydrocarbon(s) were adjusted to the selectivity of the stationary phase.

Samples with enclosed fat, such as cheese, meat, milk powder, nuts, and cereals, require a pretreatment because the fat is not reached by the reagent within the short time available during transesterification otherwise. Complete access to the fat is essential not only for determining the fat content, but also for fatty acid composition.

In some dry foods, such as powdered milk, the fat becomes accessible after soaking with water. Water dissolves part of the sample (e.g. sugar) and swells the structure, facilitating diffusion. Other samples are heated in dimethyl formamide (DMF). DMF was chosen because it dissolves a wide range of components and well swells others. Its relatively high boiling point (150 °C) might be even more important: refluxing causes the temperature to rapidly exceed the boiling point of water. Water evaporates within the food sample and the resulting vapors disrupt the structure.

This paper discusses to which samples these pretreatments should be applied. It compares the fat contents thus determined with results obtained by the various conventional methods previously applied to the given sample. Obviously, results are not necessarily the same, since the conventional methods may include materials of low polarity other than fat, whereas the FAME method may include fatty acids from polar compounds which are not included in the material extracted as fat by the conventional methods.

Experimental

Materials

Gaschromatograph Mod. 8000 with on-column injector and FID, autosampler AS800, integration system ChromCard, all from C.E. Instruments (Milan, Italy), Blender (Büchi B-400), magnetic stirrer/heating plate, 50 ml Erlenmeyer flasks with glass stoppers.

Triundecanin (tri-11), 1-tetradecene (C14:1), methyl nonanoate (FAME-9), heptane purum, dimethylformamide (DMF) puriss, disodium hydrogen citrate purum, all from Fluka; 30% sodium methoxide in methanol, methanol p.A., 1,4-dioxane p.A., all from Merck.

Solutions: disodium hydrogen citrate in water, 15 g/100 ml; sodium methoxide in methanol, 5 g/100 ml; internal standards (tri-11, FAME-9, C14:1) in dioxane, 100 mg/100 ml. These solutions should be stored at ambient temperature, since the transesterification is conceived to occur at this temperature (reaction rates).

GC analysis was either performed on a 3 m × 0.25 mm i.d. column coated (in the laboratory) with an 0.15 µm film of PS-255 (a methyl silicone from Petrarch Systems through Fluka) or on a 25 m × 0.25 mm i.d. column designed for the FAME-method, coated with an immobilized Carbowax of 0.15 µm film thickness (BGB-Analytik, Adliswil, Switzerland). Both columns were equipped with a 10 cm section of 0.53 mm i.d. deactivated precolumn to enable automatic on-column injection. Inlet pressures were 10 and 100 kPa, respectively (hydrogen). Injection occurred at 80 °C column temperature (0.2 min). For the 25 m column, temperature was then programmed at 20 °/min to 180 °C and at 15 °C to 250 °C (3 min).

Methods

Direct transesterification

50–500 mg of homogenized food (containing a maximum of 50 mg of fat) were accurately weighed into a 50 ml Erlenmeyer flask and transesterified as desceribed below.

Slurry with water

50–500 mg of homogenized food (containing a maximum of 50 mg of fat) were accurately weighed into a 50 ml Erlenmeyer flask. 0.5 ml of water were added, the sample was shaken and allowed to stand for 5 min before transesterification.

Heating in dimethyl formamide (DMF)

50–500 mg of homogenized food (containing a maximum of 50 mg of fat) were accurately weighed into a 50 ml Erlenmeyer flask. 2.5 ml of DMF were added and the sample refluxed 15 min (unless stated otherwise) under stirring. Samples were cooled to ambient temperature before transesterification.

For many samples, a much shorter heat treatment was sufficient. However, the 15 min were maintained in the interest of simplicity and also because shortening was not considered as an important advantage.

Transesterification

5 ml of dioxane were added, containing 5 mg each of the internal standards tri-11, FAME-9, and C14:1. After mixing, dispersion and partial dissolution, 5 ml of 5% methoxide/methanol were added (Vortex, 3 s). 60–90 s later, 25 ml of heptane were admixed and the reaction stopped by adding 10 ml of the hydrogen citrate solution. Samples without DMF remained in one phase with the extraction solvent until the aqueous solution was added, i.e. extraction was complete with hardly any shaking. With samples containing DMF, however, heptane is not miscible and the phases

must be thoroughly mixed in order to extract the FAMEs. The supernatant was analyzed by GC-FID after 1:10 dilution of the FAME extract in heptane. Heptane was chosen as solvent in order to enable on-column injection at 80 °C.

Conventional methods for comparison

Results of the proposed FAME-method were compared with those obtained by the conventional methods previously used for the same sample. Methods are named as in the headings of tables 1–6.

Acidic hydrolysis followed a Swiss official method (2), similar to the method of Weibull-Stoldt (3), and involved heating in 4 M hydrochloric acid for 20 min, filtration through paper, washing of the residue, drying of the residue on the filter, Soxhlet extraction with petroleum ether, removal of the solvent, and weighing of the residue thus obtained.

Enzymatic hydrolysis followed the Swiss official method (4) and involved enzymatic cleavage of starch and proteins, extraction with methanol/chloroform, evaporation of the solvent, and weighing of the residue.

The gravimetric determination of fat in cheese according to *Schmid-Bondzynski* involves hydrolysis in 25% hydrochloric acid, addition of ethanol, extraction, and removal of the solvent (5).

The butyrometric fat determination in cheese according to *Gerber-van Gulik* hydrolyzes the sample with sulphuric acid and reads the amount of supernatant fat on the scale (6).

The fat determination in milk according to *Röse-Gottlieb* involves a pretreatment with ethanol and ammonia, then extraction with diethyl ether/petroleum ether, evaporation of the solvent, and gravimetry (7).

The method called *SLMB* in table 3 is designed for the determination of the fat content in margarines. The fat is mixed with sodium sulphate, then extracted with hexane, the hexane removed and the residue weighed (8).

The *Büchi/Caviezel* method (Büchi, Flawil, Switzerland, B815/820/821) was introduced recently. The fat is saponified in the food sample, the free fatty acids are extracted and analyzed by a specialized GC-FID system. The results listed in tables 1–6 were obtained at Büchi.

Results

The results listed in tables 1–6 were selected from over a thousand analyses. Selection occurred such that acceptable and inacceptable results were equally represented. Some particularly difficult samples were included to illustrate possible problems. Analyses were usually run as douplicates from the same homogenate.

Milk and milk products

Table 1 compares fat contents determined in milk and milk products by various methods. The first three columns report results by the proposed FAME-method, either without pretreatment («direct»), through preparation of a slurry in water, or heat treatment with DMF. The other methods are shortly described in the experimental section and results usually represent means of two analyses (except for Büchi).

For milk, the FAME as well as the Büchi method gave higher fat contents than labelled. However, the label indicates a guaranteed minimum fat content. Generally, the direct method applied to yoghurt yielded results in good agreement with those obtained through acidic hydrolysis or the method of Röse-Gottlieb. Some exceptional samples gave lower results after heat treatment with DMF than with direct transesterification (line 6), which was probably the effect of precipitating lumps. DMF pretreatment is meaningful if the fat in flavoring additives, e.g. nuts, should be included. Thus, milk, yoghurt, curd, and cream can normally be transesterified without pretreatment. Cheese, however, must be pretreated by the DMF procedure, as low and poorly reproducible results were obtained otherwise (line 16). The same applies to cheese products, such as cheese spread and cheese fondue.

Evaporated milk can be transesterified directly. For sweetened evaporated milk, however, it was observed that the direct method provided far too low fat contents (line 25). Addition of water, dissolving the sugar, gave satisfactory results. They were better than those obtained upon treatment with DMF, presumably because the particles tended to agglomerate to lumps in DMF (lines 26, 27). Samples 26 and 27 were used in ring tests organized by Nestlé and the results under «Label» obtained by the methods of Mojonnier and Gerber are means of 37 and 7 laboratories, respectively.

Powdered milk and products for bottle feeding of babies based on powdered milk again yielded far too low results when analyzed directly (lines 28 and 30). After addition of water, fat contents determined by the FAME method were in good agreement with the results obtained by the method of Röse-Gottlieb and acid hydrolysis. The cocoa milk (line 33) was a ready-to-drink preparation of milk, cocoa and sugar.

The analysis of butter is technically easy. However, the conventional methods are preferred, because they are well established and the FAME-method does not provide sufficient precision: in routine analysis, relative standard deviations may reach 2% (1), which is too much for this determination, particularly in view of the high profit achievable by a slight reduction of the fat content.

Meat products

The determination of fat contents in meat and meat products by direct transesterification usually resulted in too low values (line 8 in table 2). Heating to 60 °C in dioxane sometimes extracted the fat well (line 9), but not for all samples (line 10).

Table 1. Fat concentrations (%) in milk and milk products as determined by various methods. Label, fat content declared on the label or (for ring test samples) obtained by other laboratories. Conventional methods, (1) acidic hydrolysis; (2) Gerber; (3) Schmid-Bondzynski; (4) Röse-Gottlieb. dm, fat in dry matter; RT, sample from ring test. Values in italic, means of results of at least 4 results with standard deviations mostly given in table 7. Values given as range: poorly reproducible results

	Sample	Label % fat	FAME-method			Conventional Method	Büchi
			Direct	Water	DMF		
1	Milk	3.8	4.06/4.08			3.87 (1)	4.00/4.06
2	Milk	3.9	4.09/4.09			3.66 (1)	3.98/3.93
3	Milk		4.1/4.0			4.0 (2)	
4	Yoghurt	3.0	2.83/2.89			2.84 (1)	3.29/3.19
5	Yoghurt	2.8	2.86/2.92			2.83 (1)	3.27/3.23
6	Yoghurt	4.0	4.2/4.2		3.2/4.0	3.9 (4)	
7	Yoghurt	1.0	2.9/2.9			2.8 (4)	
8	Yoghurt	3.5			4.6/4.6	4.3 (4)	
9	Curd	0.1	0.49/0.47			0.38 (4)	
10	Curd	0.2	0.19/0.19				
11	Curd	16	16.4/16.7			17.0 (4)	
12	Kefir	2	*2.01*				
13	Cream	15.0	*15.3*			15.0 (4)	
14	Cream	15.0	*15.2*			15.0 (4)	
15	Cream	35.0	35.7/36.9			34.0 (1), 33.8 (2)	35.4/34.7
16	Cheese	45 dm	14.8–21.9			32.3 (1), 31.5 (2), 31.6 (3)	32.2/31.9
17	Cheese	19			20.3/20.4	18.2 (1), 19.0 (2), 18.3 (3)	19.0/19.0

	Sample	Label % fat	FAME-method			Conventional Method	Büchi
			Direct	Water	DMF		
18	Cheese	45 dm			24.6/25.1	23.3 (1), 23.3 (2), 23.8 (3)	24.7/24.8
19	Cheese spread	18			19.6/19.9	19.2 (1)	19.5/19.4
20	Cheese spread	22			20.3/21.3	20.5 (1)	21.1/21.5
21	Cheese fondue	16			17.2/17.3		
22	Evaporated milk	7.5	8.1/8.1			7.9 (4)	
23	Evaporated milk	7.5	8.7/8.4			8.5 (1), 7.8 (4)	
24	Evaporated milk	8.0	8.0/8.2			8.6 (1), 8.8 (4)	
25	Sweet evap. milk	8.0	0.4–2.2		7.6/7.9	8.0 (4)	
26	Sweet evap. milk	8.1 RT		7.8	6.2–9.4	8.2 (2)	
27	Sweet evap. milk	9.1 RT		9.1	9.8–11.1	9.2 (2)	
28	Powdered milk	0.5	0.05/0.04	0.77/0.77		0.80 (4)	
29	Powdered milk	0.5		0.96/1.03		1.00 (4)	
30	Infant formula	ca. 24	10.1/11.3	27.5/25.8		24.4 (1)	24.6/24.6
31	Infant formula	26		26.6/26.6	28.1/28.1	26.4 (1)	
32	Cocoa-milk	1.2	1.29/1.28			1.23 (1)	1.58/1.64
33	Cream liqueur		4.6/4.7			5.1 (1)	
34	Cream liqueur		16.2/15.9			15.6 (1)	

Table 2. Fat concentrations (%) in meat and meat products, with the options of a 15 min (standard) or 30 min DMF treatment. Direct, 60°, samples warmed in dioxane to 60 °C for 15 min prior to transesterification

	Sample	Label % fat	Fat by FAME method				Acid hydr.	Büchi
			direct	direct, 60°	DMF, 15 min	30 min		
1	Meat FAPAS	15.1 RT			15.2/15.0/15.2		14.9	
2	Meat				13.9/14.0		14.0	
3	Ham				4.3/4.3			
4	Liver sausage				31.4/30.9		31.4	
5	Liver sausage				29.2/29.4		29.3	
6	Liver sausage				43.6/43.5/43.6		45.5	
7	Sausage	24.9 RT			25.0/25.3/25.0		25.2	
8	Sausage	24	16.9/17.7				25.2	26.5/25.8
9	Sausage	26		22.6/22.6			22.1	22.8/23.6
10	Sausage	3.0	4.2/4.6	4.6/4.9			6.2	7.6/7.7
11	Sausage				19.6/19.7		19.8	
12	Sausage				27.6/27.6		26.6	
13	Sausage				20.9/20.6		20.7	
14	Sausage				23.1/22.9		22.9	
15	Sausage				43.4/43.4	43.2/43.5	44.1	
16	Collared pork				11.6/11.8		11.7	
17	Collared pork				9.6/9.6		9.6	
18	Collared pork				7.1/7.0		7.3	
19	Collared pork				11.0/10.9		10.7	

	Sample	Label % fat	Fat by FAME method				Acid hydr.	Büchi
			direct	direct, 60°	DMF, 15 min	30 min		
20	Bacon	15.0 RT			14.7/14.6	14.7/14.9	14.9	
21	Salami	30.4 RT			28.9/27.8		30.7	
22	Salami				30.4/31.5/31.6	34.4	36.6	
23	Salami				30.3/29.1		31.0	
24	Salami				31.3/31.1/33.7	36.6	35.4	

After treatment with DMF, however, the structures enclosing the fat were opened and the amounts of fat then determined corresponded well with the alternative methods. A homogenized meat sample from FAPAS®(CSL Food Science Laboratory, Norwich, UK) confirmed this (see line 1). The 15.1% fat content given under «Label» is the mean result of over 200 laboratories using acid hydrolysis. The same is observed for meat products, such as sausages, cold meat, collared pork. The results in lines 7 and 19 are from a sausage («servelat») and homogenized bacon serving as materials for ring tests organized by Migros-Genossenschafts-Bund. The mean fat content found by 6 laboratories was 24.9 and 15.0%, respectively.

Partially dried meat is more difficult to analyze, presumably because it lacks the water to form the vapors disrupting the structure. For a sample of salami from the above-mentioned ring test, with a mean fat content of 30.4% (line 20), the FAME-method gave slightly lower results (mean of 28.4%). The same tendency was observed for other salami samples when compared with the fat content determined after acidic hydrolysis (lines 20–23). Prolongation of the heat treatment to 30 min provided a satisfactory result for sample 23, but for salami 21 it appeared that still more time would have been needed.

Fatty products

Table 3 summarizes results from a wide variety of products characterized by a high fat concentration. The determination of the fat content in margarines and analogous products is, of course, easy, because the fat is readily accessible. For mayonnaise, direct transesterification provided satisfactory results. Often they were slightly higher than those labelled. This could be explained by labelled fat contents being calculated from composition, whereas additional fatty acids, e.g. from emulgators, were not included. In fact, the results better agreed with those of the enzymatic method. With DMF treatment, fatty acid contents were still higher, probably because again more fatty acids from sources other than the oil were included. Also the analysis of salad sauce (lines 8 and 9) is quick and precise.

For some samples of ice cream, direct transesterification provided satisfactory results (line 12), but for others, the fat concentrations found were far too low (see lines 10 and 11). After heat treatment with DMF, however, the values reached those obtained by acid hydrolysis (line 14). Line 13 points to a possible problem: the result of the second determination with DMF treatment was 5.6% instead of the around 8.3% expected. This was due to poor dispersion or the formation of lumps enclosing fat during the DMF treatment, which is not reproducible, but can easily be observed by visual control.

In general, chocolate can be analyzed by direct transesterification. The results in line 16 are from a ring test sample of milk chocolate from Nestlé and the fat content under «Label» was obtained by the method of Weibull-Stoldt. The sample of line 18, a plain milk chocolate, was especially made by Halba AG, Wallisellen, for quality control of the fat analysis. The fat content listed under «Label» was calculated from the accurate mixture and the fat contents of the ingredients. The

Table 3. Fat concentrations (%) in fatty products. QC, sample for quality control. Method 5, SLMB; 6, enzymatic release

	Sample	Label % fat	Fat by FAME method		Convent. methods	Büchi
			direct	DMF		
1	Margarine	83	85.2/84.4		82.3 (5)	83.3/82.3
2	Margarine	40	41.0/41.0		41.4 (5)	40.1/40.4
3	Margarine	40	42.3/41.1		40.3 (5)	42.3/41.3
4	Margarine	35	36.3/36.7		36.2 (5)	34.9/35.2
5	Mayonnaise	36.7	37.3	40.3/40.7	37.1 (6)	
6	Mayonnaise	81.6	83.2	84.1/83.5	83.9 (6)	
7	Mayonnaise	76.2	75.7	78.1/77.5	75.5 (6)	
8	Salad-sauce	28	29.8/29.4		28.7 (6)	30.5/29.9
9	Salad-sauce	35	*34.6*		36.4 (6)	
10	Ice cream	10	13.9/13.7		18.1 (1)	17.7/17.6
11	Ice cream		4.7/7.0	15.1/15.2		
12	Ice cream		7.9/8.3	8.3/8.2		
13	Ice cream		7.3/7.5	8.5/5.6		
14	Ice cream	9.8		15.7/16.1	15.7 (1)	16.6/17.2
15	Chocolate		*32.5*		32.8 (1)	
16	Chocolate	34.2 RT	34.0/34.0		34.2 (1)	
17	Chocolate		32.4/31.7		31.7 (1)	32.7/34.2
18	Chocolate	34	34.9/34.8		34.8 (1)	36.7/35.6
19	Chocolate		30.3/29.7		30.8 (1)	
20	Chocolate diabetic	39	39.2/37.3	41.1/39.2	39.4 (1)	
21	Chocolate diabetic	37	35.8/36.3	36.8/38.2	35.2 (1)	

data shows good agreement, but also that DMF-pretreatment is unnecessary. DMF pretreatment would only be necessary if chocolate contained a substantial proportion of ground nuts, as, e.g., the diabetic chocolates in lines 20–21, and the fat of these should really be included.

Bakery products and cereals

The determination of fat in bread and bakers' ware always requires pretreatment with DMF. Then, however, reproducible results are obtained which agree well with

the fat content determined by other methods (table 4). For cookies with cheese, a method was successful that was no longer applied later: heating of the sample in dioxane to 60 °C for 15 min before cooling and addition of the transesterification reagent. The samples of müesli consisted of a wide variety of cereals, nuts, and other dry components and required heat treatment in DMF. The samples in lines 12 and 13 consisted of infant cereales with milk, ring test samples from Nestlé, and the labelled fat content was determined by the method of Mojonnier.

Ready meals

The ready meals listed in the first lines of table 5 contained all the main components, such as meat, vegetables, and noodles. After heat treatment in DMF, good results were obtained. The vegetable pastes consistently yielded somewhat lower results than the conventional acid hydrolysis method. This included the ring test samples from Nestlé of lines 8–9, with mean fat contents of 18.5 and 18.6%, respectively, obtained by Weibull-Stoldt. The FAME-method yielded 17.1 and 18.0% fat only. Fat extracts obtained by acid hydrolysis from the product of line 10 contained, in fact, just 88.2 and 88.0% of fat as determined by the FAME method, i.e. it contained more than 10% apolar components other than fatty acid esters, maybe from surfaces of the plant materials. Corrected in this way, the deviation

Table 4. Fat concentrations (%) in bakery products and cereals. Results marked by an asterisk: samples heated in dioxane prior to transesterification

	Sample	Label % fat	Fat by FAME method		Acid hydr.	Büchi
			direct	DMF		
1	Bread			*1.8*	1.9	
2	Bread	1		0.9/1.1		
3	Cookies	2.7		3.4/3.4		
4	Cookies	18		18.2/18.2		
5	Gingerbread	12		11.6/11.2	11.4	
6	Gingerbread	4		5.4/5.5	5.8	
7	Cookies/cheese		23.8/23.5*		23.5	23.7/23.2
8	Fried cookies	34		34.6/34.1		
9	Noodles	3	2.76/2.96	2.90/3.11	3.14	2.73/2.58
10	Müesli	5.7		5.7/5.8		
11	Müesli	20		20.7/19.8	19.5	19.8/20.3
12	Infant formula	8.73 RT		*8.2*		
13	Semolina		0.30/0.27	*0.67*		

Table 5. Fat concentrations (%) in ready meals as well as puddings and dessert creams. Results marked by an asterisk: samples heated in dioxane prior to transesterification

	Sample	Label % fat	Fat by FAME method		Acidic hydrol.	Büchi
			direct	DMF		
1	Ready meal	3.1		5.2/5.6	4.9	
2	Ready meal	4.1		5.6/5.8	5.1	
3	Ready meal	8.5		9.0/8.6	8.7	
4	Ready meal	7		8.3/8.3	8.3	
5	Ready meal	6		5.1/5.2	5.0	
6	Ready meal	6		3.9/4.0	3.7	
7	Cooked vegetables	4	2.68/2.52		2.81	2.93/3.03
8	Vegetable paste	18.5 RT		*17.1*		
9	Vegetable paste	18.6 RT		*18*		
10	Vegetable paste		12.6/11.5	20.0/20.2	21.1	
11	Soyburger	6	3.1	6.6/6.7		
12	Pudding	4	3.5	4.1/4.0		
13	Pudding		4.2/4.1/3.9	3.8/3.4/3.3		
14	Pudding	4	4.41/4.46*		4.03	4.46/4.44
15	Soybean cream dessert	1.8	1.8	1.9/1.9		

turns to the other side: the fat extract of 21.1% yielded 18.6% fat only, and the difference to the 20.1% obtained by the FAME method would have to be explained by fatty acids not included into the fat extract. As shown in line 11, the soyburger required the DMF pretreatment for solubilizing the fat.

Some puddings and cream desserts proved to be difficult to analyze. As shown in line 12, direct transesterification does not pick up all the fat present. On the other hand, an example is shown in line 13 of results which are lower with DMF treatment than with direct transesterification. This product formed visible lumps during the DMF treatment. The sample in line 14 yielded good results with heating to 60 °C in the dioxane phase prior to addition of the methanol/methoxide reagent.

Various products

Table 6 lists results from a variety of foodstuffs. The analysis of the fat content of nuts presupposes heat treatment with DMF. The results obtained for the samples of hazelnuts and coconut reasonably agreed with acid hydrolysis. For a sample of walnuts, heating in DMF during 30 min slightly increased the results (68.7/68.8%),

Table 6. Fat concentrations (%) in a variety of foodstuffs.
Results marked by an asterisk: samples heated in dioxane prior to transesterification

	Sample	Label % fat	Fat by FAME method			Acidic hydr.	Büchi
			direct	water	DMF, 15 min		
1	Hazelnut				55.5/51.4	52.8	
2	Coconut				67.9/68.2	67.5	
3	Walnut				67.5/67.8	69.6	
4	Coffee				13.1/13.7	14.0	14.1/14.1
5	Egg		9.0/9.1		8.8/8.7	8.5	
6	Malt powder	3.3	0.5/0.5	1.6/1.7	2.8/2.8	2.7	
7	Malt powder				*2.71*	2.66	
8	Malt powder	6		8.0/7.5	6.8/6.9	6.7	8.25/8.37
9	Cocoa drink		0.6	3.8/3.9	5.4/5.4		
10	Cocoa drink	3.7 RT			*3.68*		
11	Cocoa drink	3.6 RT			*3.71*		
12	Cocoa powder	20.3			20.8/21.3	20.0	21.3/21.4
13	Cocoa powder	20.3	22.1/22.4*		22.3/21.9	20.5	24.3/24.5
14	Tofu	5.3	5.8		6.8/6.7		

whereas additional 30 min had no further effect (68.8/68.1%). For the egg (line 5), similar fat contents were observed with or without heat treatment in DMF.

The malt powder in line 6 only released its fat after DMF treatment. Among the many samples analyzed, there was, however, also one with too low results after DMF treatment (line 8) owing to formation of lumps. Dispersion in water was more successful for that sample. For the analysis of the powders of malt- and cocoa-based breakfast drinks, DMF-treatment was clearly superior (line 9). The two ring test samples (lines 10 and 11) were from Nestlé and the fat contents under «Label» were determined by the method of Mojonnier and by the IOCCC method (International Office of Cocoa, Chocolate and Sugar Confectionary). Cocoa powders (lines 12 and 13) do not well disperse in cold dioxane. They can either be analyzed through the DMF pretreatment or by warming the dioxane solution prior to transesterification. With DMF pretreatment, the analysis of Tofu was more reliable (line 14).

Reproducibility of results

Table 7 reports some statistical data on the results for samples analyzed at least four times. From a homogenate, two analyses were performed in parallel, i.e. results were obtained as pairs. Additional pairs of analyses were carried out one day later

Table 7. Reproducibility of results

Line / Table	Sample	*n*	Mean fat (%)	±	% St. dev.
12/1	Kefir	4	2.01	0.03	**1.3**
13/1	Cream	6	15.3	0.19	**1.3**
14/1	Cream	6	15.2	0.12	**0.8**
9/3	Salad-sauce	4	34.6	0.34	**1.0**
15/3	Chocolate	7	32.5	0.33	**1.0**
1/4	Bread	4	1.83	0.03	**1.6**
12/4	Infant formula	4	8.4	0.18	**2.2**
	Infant formula	4	8.2	0.19	**2.4**
13/4	Semolina	4	0.67	0.03	**4.2**
8/5	Vegetable paste	4	17.1	0.15	**0.9**
9/5	Vegetable paste	4	18.0	0.26	**1.4**
7/6	Malt powder	4	2.71	0.09	**3.2**
10/6	Cocoa drink	4	3.68	0.19	**5.1**
11/6	Cocoa drink	4	3.71	0.18	**4.8**
	Fatty glaze	51	35.0	0.95	**2.7**
	Meat loaf	10	26.7	0.30	**1.0**

n = number of analyses

at least. The sample of fatty glaze was used for internal quality control and was analyzed over a period of one year. The standard deviation is relatively high because it includes results from the very beginning of using this method. The sample of meat loaf was analyzed as 5 different cans over 3 weeks. For samples with more than 10% fat, relative standard deviations were below 1.5%.

Conclusion

In this study, emphasis was put on the determination of the fat content. The prerequisite for the analysis of the fatty acid composition is, however, the same: all fatty-acid-containing components in the food sample must be reached. Hence, the procedure is equally well suited if the FAMEs are prepared just for the analysis of the fatty acid composition.

The limitations of the methods are reached at the following three points: Complete solubilization of the fat is difficult in partially or fully dried meat and

sausages: owing to the lack of water, heating in DMF does not result in the disrupture of the structure. DMF nevertheless extracts the fat if more time is given; 30 min appears to be a minimum. The second problem concerns precipitation of lumps instead of formation of a homogeneous suspension, as observed in the instance of a malt powder, an ice cream, a yoghurt, and some puddings. Since the amounts of fat thus determined are mostly too low, the lumps seem to enclose some fat. The problem is easily detected by visual control. Usually it does not occur regularly and mostly a second attempt is more successful. The third limitation concerns the precision primarily of the GC analysis. In the routine of a non-specialized laboratory, long term reproducibility easily reach ± 2%, which may be too high for particular analyses, such as the determination of the fat content of butter.

Table 8 summarizes the recommended procedures for the various foodstuffs.

Table 8. Recommended sample pretreatment for the foodstuffs investigated. Direct, no pretreatment; DMF, heat treatment in DMF; water, forming a slurry with water

Bread	DMF
Cheese	DMF
Chocolate	direct
Chocolate with nuts	DMF
Cocoa powder	DMF
Cookies	DMF
Cream	direct
Cream liqueur	direct
Curd	direct
Evaporated milk	DMF
Eggs	direct
Ice cream	DMF
Malt powder	DMF or water
Margarines	direct
Mayonnaise	direct
Meat/fish	DMF
Milk	direct
Milk powder	water
Müesli	DMF
Noodles	DMF
Nuts	DMF, 30 min
Pudding	water or DMF
Ready meal	DMF
Salad sauce	direct
Salami	DMF, ≥ 30 min
Sausages	DMF
Semolina	DMF
Sweetened evaporated milk	water
Tofu	DMF
Yoghurt	direct

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Summary

For various products, direct transesterification of fatty acid esters in foodstuffs presupposes a short pretreatment, i.e. either the formation of a slurry in water (e.g. powdered milk) or a heat treatment in DMF (e.g. cheese, meat, bakers' ware or cereals). Fat contents determined by the new FAME method agree well with results obtained by conventional methods and are further confirmed by comparison with results from ring test samples.

Zusammenfassung

Die direkte Umesterung von Fettsäureestern in Lebensmitteln setzt für manche Produkte eine Vorbehandlung voraus: entweder eine Aufschlämmung in Wasser (z. B. Milchpulver) oder eine Erhitzung in DMF (z. B. Käse, Fleisch, Backwaren und Cerealien). Die nach der neuen FAME-Methode bestimmten Fettgehalte stimmen mit Resultaten verschiedener konventioneller Methoden akzeptabel überein und konnten über den Vergleich mit Proben von Ringversuchen weiter abgesichert werden.

Résumé

La transestérification des esters d'acides gras dans les denrées présuppose pour plusieurs produits un prétraitement, soit une suspension à l'eau (p.e. lait en poudre), soit un échauffement court dans DMF (p.e. fromage, viande, pâtisseries ou céréales). Les teneurs en matière grasse determinées d'après la nouvelle méthode FAME s'accordent avec les résultats de plusieurs méthodes conventionelles et ont été confirmées en comparaison avec des résultats d'essais interlaboratoires.

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