Regioisomerism of Triacylglycerols in Lard, Tallow, Yolk, Chicken Skin, Palm Oil, Palm Olein, Palm Stearin, and a Transesterified Blend of Palm Stearin and Coconut Oil Analyzed by Tandem Mass Spectrometry

Heikki Kallio,* Kaisa Yli-Jokipii, Juha-Pekka Kurvinen, Olli Sjövall, and Raija Tahvonen

Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 Turku, Finland

Triacylglycerols (TAG) of lard, tallow, egg yolk, chicken skin, palm oil, palm olein, palm stearin, and a transesterified blend of palm stearin and coconut oil (82:18) were investigated by chemical ionization and collision-induced dissociation tandem mass spectrometry. Accurate molecular level information of the regioisomeric structures of individual TAGs was achieved. When existing in a TAG molecule of lard, palmitic acid occupied 90−100% of the *sn*-2 position. Within the major fatty acid combinations in tallow TAGs, the secondary position *sn*-2 was preferentially occupied in the decreasing order by oleoyl > palmitoyl > stearoyl residues, the order in saturated TAGs being myristoyl > stearoyl = palmitoyl. TAGs in egg yolk were more asymmetric than in chicken skin, with linoleic acid highly specifically attached in the yolk *sn*-2 carbon. Nearly 50% of yolk TAGs contained 52 carbon atoms with two or three double bonds. Linoleic, oleic, and palmitic acids were in the *sn*-2 location in decreasing quantities in palm oil and its fractions. Triacylglycerols of equal molecular weight behaved similarly in the fractionation process. Randomization of the parent oil TAGs was seen in the transesterified oil. The tandem mass spectrometric analysis applied provided detailed information of the distribution of fatty acids in individual combinations in TAGs.

Keywords: *Positional distribution; lard; tallow; yolk; chicken skin; palm oil; triacylglycerol; transesterification; MS/MS*

INTRODUCTION

Knowledge of triacylglycerol (TAG) structure is important with regard to nutritional functions, quality control, technological characteristics, and authenticity establishment. As the use of tailored TAGs increases, there is a growing need to know the structures of TAGs both in raw materials and in processed fats and oils.

As early as 1958 Mattson and Lutton concluded that no general pattern of distribution of fatty acids in TAGs prevails among animal fats, although nonrandom distribution is evident. There is a high variation in stereoisomers in oils and fats of different biological origin with typically strong species specificity. Lard is unique among animal depot fats, because it has a strong predominance of saturated fatty acids in the *sn*-2 position (*1*−*5*). In lard, most stearic acid is found in the *sn*-1 position, whereas position *sn*-3 is rich in oleic acid (*2*). In beef tallow and other bovine adipose tissues, nearly 50% of the fatty acids in the *sn*-2 position are oleic acid, whereas palmitic, stearic, and oleic acids are the major fatty acids in the *sn*-1/3 positions (*6*, *7*). The positional distribution, although characteristic for each animal species, can be modified to some extent by feeding (*6*).

Very similar structures of TAGs have been found in chicken plasma, in ovarian follicles, and in eggs (*3*). In these fats, a high degree of asymmetry between positions *sn*-1 and *sn*-3 is found. Palmitic acid is mainly in the *sn*-1 position, whereas >60% of *sn*-2 fatty acids and *sn*-3 fatty acids are oleic acid. Linoleic acid predominates in the *sn*-2 position (*3*, *8*−*10*). The positional distribution of fatty acids in hen's egg TAGs is not affected by variation in the dietary fats of hens (*9*).

Seed oils tend to have polyunsaturated fatty acids in the *sn*-2 position, but relatively little difference can be found between the primary positions, although less abundant fatty acids are often concentrated in the *sn*-3 position. The 1,3-random-2-random distribution of fatty acids in seed oils is commonly accepted, even though this model should be regarded as an approximation (*3*). The absence of palmitate in the *sn*-2 position is characteristic of plant TAGs in general (*11*). In palm oil, oleic acid comprises >60% and linoleic acid >20% of the fatty acids in the *sn*-2 position. In the fractionation process the amount of palmitic acid in the *sn*-2 position reflects the changes in fatty acid composition so that the amount of palmitic acid in the *sn*-2 position is decreased in palm olein and increased in palm stearin when compared to palm oil (*12*).

Transesterification produces fats with new characteristics. Because of the random arrangement of the fatty acids of the parent oil, the process is commonly referred to as randomization (*13*). Transesterification of an oil rich in palmitic acid together with an oil poor in palmitic acid decreases the formation of TAGs with three saturated fatty acids. Such TAGs may have melting points above body temperature, and they have been noted to be poorly absorbed (*14*).

The specific distribution or regioisomerism of fatty acids in TAGs is traditionally determined by enzymatic

* Author to whom correspondence should be addressed (e-mail heikki.kallio@utu.fi; fax + 358 2 333 6860; telephone + 358 2 333 6870).

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hydrolysis procedures. Several chromatographic methods can also be applied to the analysis of TAGs as such. However, these methods, if applicable to discriminating between different regioisomers, fail to show how the fatty acids are distributed in individual combinations of fatty acids in TAGs. Furthermore, achieving such information is often difficult, extremely slow, or impossible with traditional chromatographic or enzymatic methods.

Tandem mass spectrometry, instead, has been proved to be a useful tool in determining the regioisomerism of fatty acids in TAGs. It provides information of regioisomerism in individual molecular species (*15*). In this study ammonia negative-ion collision-induced mass spectrometry (NICI-MS) was used to determine the molecular weight distributions of triacylglycerols of lard, tallow, yolk, chicken skin, palm oil, palm olein, palm stearin, and a transesterified blend (82:18) of palm stearin and coconut oil. The fatty acids in each molecular weight species and the regioisomerism of these acids were determined from the collision activation spectra using a triple-quadrupole mass spectrometer. The method has been described previously and applied to, for example, seed oils and human milk fat (*15*−*17*).

MATERIALS AND METHODS

Oil Samples. Eggs and tallow orginated from the Agricultural Research Centre of Finland. Chicken skin came from Broilertalo (Säkylä, Finland). Lard and vegetable fats were obtained from Raisio Group (Raisio, Finland). Palm oil and its derivates originated from the 1997 crop in Malaysia.

Extraction of Triacylglycerols. The lipids of animal origin were extracted with a mixture of chloroform and methanol (2:1 v/v) (*18*). The TAGs of the extracted animal fats were purified as described elsewhere (*19*). The neutral lipids of plant origin were purified by elution with hexane (10 mL) from a short Florisil column.

Fatty Acid Composition. Fatty acid methyl esters were prepared by sodium methoxide-catalyzed transesterification in duplicate (*20*). Fatty acid methyl esters were dissolved in hexane, diluted, and analyzed twice by gas chromatography (Perkin-Elmer AutoSystem, Norwalk, CT) on a WCOT column (NB-351, 25 m × 0.32 mm i.d., 0.20 μm film thickness, Nordion, Finland). The temperature program began with a 2 min hold at 120 °C and was raised at 3 °C min^{-1} to 230 °C, followed by a 20 min hold at 230 °C. The temperature of the flame ionization detector was 270 °C. Fatty acid methyl ester sample was introduced via a programmable split/splitless injector (170 °C, 200 °C min^{-1} to 250 °C, 58 min at 250 °C). The split was opened after 1 min. Helium was used as the carrier gas (linear flow rate = 30 cm s^{-1}). Peaks were identified by comparisons to retention of fatty acid methyl ester standards (Nu-Chek Prep, Inc., Elysian, MN).

Molecular Weight Distribution. The molecular weight distributions of TAGs were determined by ammonia negative ion chemical ionization with a triple-quadrupole tandem mass spectrometer (TSQ-700, Finnigan MAT, San Jose, CA) (*15*). The sample (0.2−0.5 μg) was introduced directly to the ion source on a rhenium wire with a direct exposure probe. Chemical ionization with ammonia resulted in the formation of deprotonated TAG ions ($[M - H]^-$), which were analyzed by scanning the mass range from *m*/*z* 500 to 1000. The combined number of acyl carbons and double bonds in the acyl chains of TAGs was calculated according to the *m*/*z* values of $[M - H]^-$ ions. Relative molar proportions of different molecular weight species were calculated using the $[M - H]^-$ quantities. The amount of naturally occurring ^{13}C was taken into account when the proportions of TAGs were calculated. The analytical parameters were set in accordance with the optimization conducted earlier in our laboratory (*21*). The samples of animal origin were analyzed three times and the samples of plant origin four times each, and the results were averaged.

Table 1. Fatty Acid Compositions of Lard, Tallow, Chicken Skin, Yolk, Palm Oil, Palm Olein, Palm Stearin, and a Transesterified Blend of Palm Stearin and Coconut Oil (82:18) (Values Are Relative Molar Percentages)

fatty acid	lard	tallow	chicken skin	yolk	palm oil	palm olein	palm stearin	transesterified blend
8:0								1.3
10:0								1.1
12:0					0.5	0.3	0.2	8.8
14:0	1.5	3.7	0.5	0.4	1.1	1.0	1.1	4.3
14:1(*n*−5)		0.2						
16:0	28.4	28.1	19.9	21.3	43.8	34.9	51.9	44.8
16:1(*n*−7)	1.5	1.4	4.3	2.4	0.2	0.2	0.1	
18:0	21.6	37.7	5.4	5.6	4.3	3.8	5.1	4.9
18:1(*n*−9)	31.4	23.3	38.0	48.7	40.7	47.0	33.9	28.2
18:1(*n*−7)	2.0	3.1	2.3	2.2				
18:2(*n*−6)	11.1	1.4	26.0	15.5	8.8	12.0	6.9	6.0
18:3(*n*−3)	0.9	0.6	2.9	1.9	0.2	0.3	0.2	
20:0	0.2	0.4			0.4	0.4	0.4	0.3
20:1(*n*−9)	0.7		0.4	0.6	0.1	0.2	0.1	0.3
20:2(*n*−6)	0.4		0.2	0.1				
20:4(*n*−6)	0.2		0.3	0.4				
20:5(*n*−3)				0.3				
22:6(*n*−3)				0.6				

Regioisomerism of Selected Molecular Weight Species. Tandem mass spectrometric analysis (TSQ-700, Finnigan MAT) based on negative ion chemical ionization and collision-induced dissociation with argon gas was applied to the regioisomer analysis in which the primary (*sn*-1/3) versus secondary (*sn*-2) positions of fatty acids in TAGs were determined (Europatent 0566599) (*16*, *17*). Only the most frequently occurring molecular weight species were selected for the analysis. The results were calculated with the TAGS-100 program (Nutrifen, Turku, Finland) (*16*). The chosen species contained from 42 to 90% of all TAGs in each fat or oil. The analyses were repeated four times each.

Statistical Analysis. The proportions of major molecular weight fractions of the transesterified blend of palm stearin and coconut oil (82:18) were compared to the proportions of corresponding fractions in theoretically randomized distribution by using Student's *t* test. The randomized distribution was calculated from the analyzed fatty acid composition with the Randtags program (Nutrifen). Proportions of chosen molecular weight fractions were compared within the animal fats and the vegetable fats by analysis of variance (ANOVA) and Tukey's test. The regioisomerism within each fatty acid combination was compared to random distribution with Student's *t* test. All statistical procedures were carried out using the SPSS-PC statistical package (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Fatty Acid Composition. The fatty acid compositions of the analyzed oils and fats are displayed in Table 1. The fatty acid compositions of the animal fats analyzed were characteristic to each species. The fatty acid compositions of palm oil, palm olein, and palm stearin were in agreement with the compositions reported elsewhere, although the palm oleins and palm stearins show wide variations due to the different manufacturing processes (*12*).

Molecular Weight Distribution. All fats analyzed contained a large variety of TAGs of different molecular weights (Table 2). In lard, more than one-fourth of the TAGs contained 52 acyl carbons and 2 double bonds (ACN:DB 52:2). In addition, two other molecular weight species, 52:1 and 52:3, exceeded the 10% level in lard. Of the analyzed fats, tallow showed the widest variation of molecular weight species. Only one of the molecular

Table 2. Molecular Weight Distributions of Triacylglycerols of the Analyzed Oils and Fats Presented as Molar Percentage

ACN:DB[a]	lard	tallow	chicken skin	yolk	palm oil	palm olein	palm stearin	transesterified blend
38:0								0.5
40:0								1.9
42:1								1.4
42:0		0.1						1.4
44:1		0.1						1.1
44:0		0.4						3.4
46:2			0.2					1.4
46:1		0.5	0.2					4.8
46:0		1.8			0.1		0.2	2.0
48:3			0.5					1.0
48:2	0.5	0.4	0.9		0.1	0.3		2.7
48:1	1.0	2.8	1.1	0.2	0.8	0.7	0.6	3.2
48:0	0.8	5.9	0.7		3.0		8.2	8.0
50:4			1.0					
50:3	1.1	0.5	3.3	1.3	0.3	0.2	0.2	0.8
50:2	4.2	2.2	7.6	2.1	7.0	6.0	6.1	5.5
50:1	8.0	9.5	4.8	3.0	27.3	15.0	35.8	23.1
50:0	3.1	7.8	0.4			1.2	0.4	0.7
52:5	0.4		2.5	0.8				
52:4	2.6	0.4	9.1	3.3	2.1	2.6	1.7	0.7
52:3	10.3	1.4	18.0	24.3	10.5	12.0	6.8	5.9
52:2	26.5	8.1	10.4	32.0	32.4	39.7	20.8	13.4
52:1	18.8	15.8	2.0	3.0	2.8	1.1	7.0	5.4
52:0	3.5	6.4		0.7	0.1	0.9	1.1	0.4
54:7	0.2	0.7	0.8	0.5				
54:6	0.5		3.3	0.7				
54:5	1.4	0.2	8.5	2.8	0.6	0.8		0.5
54:4	2.8	0.6	12.3	8.1	2.4	4.2	2.3	2.0
54:3	5.1	2.3	7.2	9.9	7.4	8.4	4.2	3.7
54:2	3.6	7.1	1.7	3.2	2.5	6.1	3.3	2.1
54:1	1.7	8.7	0.3	0.6	0.5	0.7	1.2	0.8
54:0	0.3	1.0					0.2	
others[b]	3.4	15.5	3.4	3.5	0.0	0.0	0.0	2.1

[a] ACN, acyl carbon number; DB double bond. [b] ACN:DB species consisting of <0.5% of total species in all of the analyzed fats are summarized in this category.

weight species contained >10% of the TAGs (ACN:DB 52:1, 16%). Chicken skin had a wider range of TAGs than egg yolk, but the most abundant species were the same in both fats. Egg yolk TAGs with ACN:DB 52:2 and 52:3 contained 32 and 24% of all TAGs, respectively. In chicken skin, the corresponding proportions were 10 and 18%.

The animal fats studied can be distinguished from one another by the proportions of certain molecular weight fractions. For example, the amounts of ACN:DB 50:1, 52:3, 54:4, and 54:3 were statistically significantly different in all of the analyzed animal fats (Table 3).

Palm oil, palm olein, and palm stearin were less complex than the animal fats. The large proportions of TAGs of ACN:DB 50:1, 52:3, and 52:2 were due to the large proportions of palmitic and oleic acids. The fractionation of palm oil concentrated some fractions, which is characteristic of the process. Several molecular weight species that were not present in palm stearin were detected in the transesterified blend due to the special fatty acid composition of coconut and random distribution of the fatty acids (Table 2). The proportions of TAGs of ACN:DB 50:2, 52:3, 52:2, and 52:1 in the transesterified oil did not differ statistically significantly from the theoretically randomized distribution. The amount of 48:0 was smaller in the analyzed oil ($p = 0.010$) and the amount of 50:1 larger in the analyzed oil ($p = 0.030$) than in the theoretically randomized distribution.

Regiospecific Structures of Triacylglycerols. If fatty acids (A−C) are distributed in a random manner in a fatty acid combination, the TAGs *sn*-1(3)A-2A-3(1)B and *sn*-1A-2B-3A should be found in a 2:1 ratio. Similarly, the combinations *sn*-1(3)A-2B-3(1)C, *sn*-1(3)B-2C-3(1)A, and *sn*-1(3)C-2A-3(1)B should be found in the ratio of 1:1:1 in the regioisomer studies.

In lard TAGs, the fatty acid positional distribution differed from the random distribution in most fatty acid combinations studied. It is well-known that palmitic acid is situated mainly in the *sn*-2 position in pig tissues (*2*, *5*). As we were able to look at individual molecular species, we noted that palmitic acid was virtually absent from the *sn*-1/3 positions when the molecule contained one palmitic acid residue but was situated in the *sn*-1/3 positions at 1(3),2-dipalmitoyl-3(1)-oleoyl-*sn*-glycerols (7.3% of all TAGs). The two most abundant regioisomers in lard were 1(3)-oleoyl-2-palmitoyl-3(1)-stearoyl-*sn*-glycerol (*sn*-18:1-16:0-18:0 + *sn*-18:0-16:0-18:1) (18.1% of all TAGs) and 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol (*sn*-18:1-16:0-18:1) (17.3% of all TAGs) (Table 3). By Al-Rashood et al. (*4*) 19.3% of lard TAGs have dioleoyl and palmitoyl and 13.2% oleoyl, palmitoyl, and stearoyl residues, respectively; the corresponding figures in our study were 17.9 and 18.8%. These differences may result from differences in sample material and partially also of the methods used. Al-Rashood et al. used an HPLC method to determine the fatty acids in individual TAGs. However, most of the peaks were not resolved, and only three TAGs were identified. The special TAG composition of lard may be of nutritional significance because saturated fatty acids in the *sn*-2 position are absorbed more efficiently than saturated acids from the *sn*-1/3 positions (*14*, *22*−*24*), and they are claimed to slow the cleareance of chylomicrons (*25*−*27*). The positional distribution of TAG fatty acids in lard resembles the composition of human milk. This fatty acid distribution, whether from mother's milk or formula TAG, is of great importance to the infant (*28*−*31*). Increased absorption of dietary fat and delayed clearance of chylomicrons may lead to attenuated postprandial lipemia, an independent risk factor for coronary artery disease (*32*−*34*). It has been suggested that the atherogenicity of fats would, in part, be a function of the level of palmitic acid at the *sn*-2 position (*35*, *36*).

Eight tallow ACN:DB species covered 5−10% of all TAGs each, and no regioisomer reached the 10% level of the total TAGs. The five most abundant TAGs were 1(3)-palmitoyl-2-oleoyl-3(1)-stearoyl-*sn*-glycerol (*sn*-16:0-18:1-18:0 + *sn*-18:0-18:1-16:0) (9.6%), 1(3),2-dipalmitoyl-3(1)-stearoyl-*sn*-glycerol (*sn*-16:0-16:0-18:0 + *sn*-18:0-16:0-16:0) (7.8%), 1(3),2-dioleoyl-3(1)-stearoyl-*sn*-glycerol (*sn*-18:1-18:1-18:0 + *sn*-18:0-18:1-18:1) (6.3%), 1(3),2-dioleoyl-3(1)-palmitoyl-*sn*-glycerol (*sn*-18:1-18:1-16:0 + *sn*-16:0-18:1-18:1) (6.0%), and 1(3)-palmitoyl-2-myristoyl-3(1)-stearoyl-*sn*-glycerol (*sn*-16:0-14:0-18:0 + *sn*-18:0-14:0-16:0) (5.0%) (Table 3). Nonrandom distribution was seen in a major proportion of TAGs. As described before (*6*, *7*), oleic acid was found to be the most predominant fatty acid in the *sn*-2 position. It is worth mentioning that the myristoyl residue predominated in the secondary position over the palmitoyl residue in ACN:DB 48:0 species analogously to human milk (*17*).

Enzymatically determined fatty acid distribution suggests that the TAGs of chicken skin and egg yolk closely resemble each other. However, the differences in molecular weight distribution point to clear differences in the individual TAG species between these two fats. When the molecular level composition was studied,

Table 3. Regioisomers of Lard, Tallow, Chicken Skin, and Yolk (Values Are Molar Percentages ± Standard Deviation)[a]

	lard	tallow	chicken skin	yolk
48:0	0.8 ± 0.1 a	5.9 ± 0.2 b	0.7 ± 0.1 a	0.0 ± 0.0 c
14:0/16:0/18:0				
sn-16:0-14:0-18:0 + *sn*-18:0-14:0-16:0		85.2 ± 3.9 **		
sn-14:0-18:-16:0 + *sn*-16:0-18:0-14:0		2.7 ± 3.8 **		
16:0/16:0/16:0				
sn-16:0-16:0-16:0		12.2 ± 2.6		
50:2	4.2 ± 0.6 a	2.2 ± 0.2 a c	7.6 ± 0.9 b	2.1 ± 1.2 c
16:1/16:0/18:1				
sn-16:1-16:0-18:1 + *sn*-18:1-16:0-16:1			14.2 ± 11.2	
sn-16:0-16:1-18:1 + *sn*-18:1-16:1-16:0			22.2 ± 16.3	
sn-16:1-18:1-16:0 + *sn*-16:0-18:1-16:1			26.2 ± 21.4	
16:0/16:0/18:2				
sn-16:0-16:0-18:2 + *sn*-18:2-16:0-16:0			13.3 ± 10.1	
sn-16:0-18:2-16:0			24.2 ± 17.1	
50:1	8.0 ± 0.9 a	9.5 ± 0.5 b	4.8 ± 0.4 c	3.0 ± 0.1 d
14:0/18:1/18:0				
sn-14:0-18:1-18:0 + *sn*-18:0-18:1-14:0	0.0 ± 0.0 a	2.8 ± 2.0 b **	0.0 ± 0.0 a	
sn-18:1-14:0-18:0 + *sn*-18:0-14:0-18:1	0.0 ± 0.0 a	28.6 ± 1.8 b **	0.0 ± 0.0 a	
16:1/16:0/18:0				
sn-16:1-16:0-18:0 + *sn*-18:0-16:0-16:1	3.7 ± 4.4	0.0 ± 0.0	0.0 ± 0.0	
sn-16:0-16:1-18:0 + *sn*-18:0-16:1-16:0	5.0 ± 5.8	0.0 ± 0.0	0.0 ± 0.0	
16:0/16:0/18:1				
sn-16:0-16:0-18:1 + *sn*-18:1-16:0-16:0	91.3 ± 10.1 a *	25.1 ± 9.1 b *	40.4 ± 9.8 b *	
sn-16:0-18:1-16:0	0.0 ± 0.0 a	43.5 ± 9.4 b *	59.6 ± 9.8 c *	
50:0	3.1 ± 0.3 a	7.8 ± 0.5 b	0.4 ± 0.1 c	0.0 ± 0.1 c
16:0/16:0/18:0				
sn-16:0-16:0-18:0 + *sn*-18:0-16:0-16:0		100.0 ± 0.0 **		
52:4	2.6 ± 0.1 a	0.4 ± 0.0 a	9.1 ± 0.6 b	3.3 ± 2.8 a
16:1/18:2/18:1				
sn-16:1-18:2-18:1 + *sn*-18:1-18:2-16:1			16.0 ± 7.8	
sn-18:2-16:1-18:1 + *sn*-18:1-16:1-18:2			13.1 ± 10.9	
sn-16:1-18:1-18:2 + *sn*-18:2-18:1-16:1			11.3 ± 1.2 *	
16:0/18:2/18:2				
sn-16:0-18:2-18:2 + *sn*-18:2-18:2-16:0			40.8 ± 10.2	
sn-18:2-16:0-18:2			6.2 ± 6.2	
16:0/18:3/18:1				
sn-18:3-16:0-18:1 + *sn*-18:1-16:0-18:3			4.6 ± 5.3	
sn-16:0-18:1-18:3 + *sn*-18:3-18:1-16:0			2.0 ± 4.0	
sn-16:0-18:3-18:1 + *sn*-18:1-18:3-16:0			6.1 ± 4.3	
52:3	10.3 ± 0.7 a	1.4 ± 0.9 b	18.0 ± 1.2 c	24.2 ± 1.5 d
16:1/18:2/18:0				
sn-16:1-18:2-18:0 + *sn*-18:0-18:2-16:1	2.4 ± 4.8		0.0 ± 0.0	0.0 ± 0.0
16:1/18:1/18:1				
sn-16:1-18:1-18:1 + *sn*-18:1-18:1-16:1	1.6 ± 3.1 a		13.5 ± 3.9 b	14.3 ± 1.6 b *
sn-18:1-16:1-18:1	2.3 ± 2.9		6.1 ± 4.5	1.2 ± 1.4 *
16:0/18:3/18:0				
sn-18:3-16:0-18:0 + *sn*-18:0-16:0-18:3	3.5 ± 4.0		0.0 ± 0.0	0.0 ± 0.0
16:0/18:2/18:1				
sn-16:0-18:2-18:1 + *sn*-18:1-18:2-16:0	9.3 ± 18.7 a		41.7 ± 6.1 b *	74.3 ± 6.8 c **
sn-18:2-16:0-18:1 + *sn*-18:1-16:0+18:2	79.5 ± 23.5 a *		4.4 ± 5.5 b **	5.7 ± 6.5 b *
sn-16:0-18:1-18:2 + *sn*-18:2-18:1-16:0	1.5 ± 3.0 a **		34.3 ± 7.9 b	4.5 ± 5.5 a **
52:2	26.5 ± 1.2 a	8.1 ± 0.3 b	10.4 ± 0.7 b	32.0 ± 2.4 c
16:0/18:1/18:1				
*sn*16:0-18:1-18:1 + *sn*-18:1-18:1-16:0	2.2 ± 1.9 a **	74.4 ± 20.9 b	74.2 ± 20.0 b	96.2 ± 4.6 b **
sn-18:1-16:0-18:1	65.2 ± 1.2 a **	12.110.2 b *	11.4 ± 12.2 b	3.9 ± 4.6 b **
16:0/18:2/18:0				
sn-16:0-18:2-18:0 + *sn*-18:0-18:2-16:0	0.0 ± 0.0 **	9.4 ± 12.1	7.8 ± 5.6	0.0 ± 0.0
sn-18:2-16:0-18:0 + *sn*-18:0-16:0-18:2	31.5 ± 0.7 a **	3.5 ± 5.7 b	1.9 ± 2.2 b	0.0 ± 0.0 b
sn-16:0-18:0-18:2 + *sn*-18:2-18:0-16:0	1.2 ± 2.3 **	0.7 ± 1.5 *	4.7 ± 4.7	0.0 ± 0.0
52:1	18.8 ± 1.3 a	15.8 ± 0.6 a	2.0 ± 0.3 b	3.0 ± 2.9 b
16:1/18:0/18:0				
sn-16:1-18:0-18:0 + *sn*-18:0-18:0-16:1	0.0 ± 0.0	1.1 ± 2.2		
16:0/18:1/18:0				
sn-16:0-18:1-18:0 + *sn*18:0-18:1-16:0	2.2 ± 2.4 a **	60.5 ± 8.8 b *		
sn-18:1-16:0-18:0 + *sn*-18:0-16:0-18:1	96.3 ± 3.1 a **	23.2 ± 13.8 b		
sn-16:0-18:0-18:1 + *sn*-18:1-18:0-16:0	1.7 ± 2.1 a **	15.3 ± 4.5 b **		
52:0	3.5 ± 3.0 a b	6.4 ± 0.6 a	0.0 ± 0.1 b	0.7 ± 0.2 b
16:0/18:0-18:0				
sn-16:0-18:0-18:0 + *sn*-18:0-18:0-16:0		45.9 ± 3.0 **		

[a] Data in a row marked with different letters are statistically significantly different ($p < 0.05$). An asterisk indicates a difference between defined regioisomerism and random placement in each fatty acid combination (*, $p < 0.05$; **, $p < 0.005$).

Table 4. Regioisomers of Palm Oil, Palm Olein, Palm Stearin, and a Transesterified Blend of Palm Stearin and Coconut Oil (82:18) (Values Are Molar Percentages ± Standard Deviation)[a]

	palm oil	palm olein	palm stearin	transesterified blend
48:0	3.0 ± 0.1 a	0.0 ± 0.0 b	8.2 ± 2.0 c	8.0 ± 0.7 c *
16:0/16:0/16:0				
sn-16:0-16:0-16:0			100.0 ± 0.0	100.0 ± 0.0
50:2	7.0 ± 0.9	6.0 ± 0.4	6.1 ± 0.5	5.5 ± 0.9
14:0/18:1/18:1				
sn-14:0-18:1-18:1 + *sn*-18:1-18:1-14:0	4.6 ± 5.4	0.0 ± 0.0	0.0 ± 0.0	
sn-18:1-14:0-18:1	2.5 ± 5.4	0.0 ± 0.0	0.0 ± 0.0	
16:0/16:0/18:2				
sn-16:0-16:0-18:2 + *sn*-18:2-16:0-16:0	11.1 ± 16.7 *	13.3 ± 6.5 **	23.3 ± 9.0 **	
sn-16:0-18:2-16:0	81.8 ± 12.6 **	86.7 ± 6.5 **	76.8 ± 9.1 **	
50:1	27.4 ± 3.7 a	15.1 ± 2.0 b	35.9 ± 2.3 c	23.0 ± 2.1 a *
16:0/16:0/18:1				
sn-16:0-16:0-18:1 + *sn*-18:1-16:0-16:0	22.1 ± 5.6 a **	23.3 ± 8.7 a **	25.0 ± 3.2 a **	72.9 ± 14.6 b
sn-16:0-18:1-16:0	78.0 ± 5.6 a **	76.7 ± 8.7 a **	75.0 ± 3.2 a **	27.1 ± 13.8 b
52:3	10.5 ± 0.6 a	12.0 ± 0.4 a	6.8 ± 0.9 b	5.9 ± 1.4 b
16:1/18:1/18:1	trace	trace		trace
16:0/18:2/18:1				
sn-16:0-18:2-18:1 + *sn*-18:1-18:2-16:0	54.6 ± 5.1 **	64.4 ± 13.6	53.6 ± 14.5	36.4 ± 15.4
sn-16:0-18:1-18:2 + *sn*-18:2-18:1-16:0	31.8 ± 3.9	26.9 ± 17.3	26.0 ± 14.6	38.1 ± 5.6
sn-18:2-16:0-18:1 + *sn*-18:1-16:0-18:2	13.7 ± 2.0 a b **	8.8 ± 8.0 a *	20.5 ± 4.4 a b *	25.6 ± 10.1 b
52:2	32.4 ± 6.6 a	39.7 ± 3.5 a	20.8 ± 3.7 b	13.4 ± 2.3 b
16:0/18:1/18:1				
sn-16:0-18:1-18:1 + *sn*-18:1-18:1-16:0	100.0 ± 0.0 a **	100.0 ± 0.0 a **	94.9 ± 9.7 a *	68.8 ± 7.6 b
sn-18:1-16:0-18:1	0.0 ± 0.0 a **	0.0 ± 0.0 a **	5.2 ± 7.9 a *	31.2 ± 7.6 b
16:0/18:2/18:0		trace	trace	trace
52:1	2.8 ± 2.2 a b	1.1 ± 1.8 a	7.0 ± 1.4 b	5.4 ± 4.2 a b
16:0/18:1/18:0				
sn-16:0-18:1-18:0 + *sn*-18:0-18:1-16:0			83.9 ± 19.4 *	
sn-18:1-16:0-18:0 + *sn*-18:0-16:0-18:1			6.1 ± 10.0 *	
sn-16:0-18:0-18:1 + *sn*-18:1-18:0-16:0			10:1 ± 11.7	
54:3	7.4 ± 1.2 a b	8.4 ± 3.3 a	4.2 ± 0.5 b c	3.7 ± 0.8 c
18:2/18:1/18:0				
sn-18:1-18:2-18:0 + *sn*-18:0-18:2-18:1		6.4 ± 7.9		
sn-18:2-18:1-18:0 + *sn*-18:0-18:1-18:2		0.3 ± 0.6 **		
sn-18:2-18:0-18:1 + *sn*-18:1-18:0-18:2		15.0 ± 10.1		
18:1/18:1/18:1				
sn-18:1-18:1-18:1		78.4 ± 8.6		

[a] Data in a row marked with different letter are statistically significantly different ($p < 0.05$). An asterisk indicates a difference between defined regioisomerism and random placement in each fatty acid combination and in transesterified blend molecular weight species (*, $p < 0.05$; **, $p < 0.005$).

extreme differences between these two fats were observed. Twenty-seven ACN:DB combinations were found by ammonia NICI mass spectrometry in the chicken skin. The MS/MS analysis showed that no regioisomer pair contained >8% of all the TAGs. The most abundant compounds were 1(3),2-dioleoyl-3(1)-palmitoyl-*sn*-glycerol (*sn*-18:1-18:1-16:0 + *sn*-16:0-18:1-18:1) (7.7%), 1(3)-palmitoyl-2-linoleyl-3(1)-oleoyl-*sn*-glycerol (*sn*-16:0-18:2-18:1 + *sn*-18:1-18:2-16:0) (7.5%), 1(3),2-dioleoyl-3(1)-linoleyl-*sn*-glycerol (*sn*-18:1-18:1-18:2 + *sn*-18:2-18:1-18:1) (6.7%), and 1(3)-palmitoyl-2-oleoyl-3(1)-linoleyl-*sn*-glycerol (*sn*-16:0-18:1-18:2 + *sn*-18:2-18:1-16:0) (6.2%). With few exceptions, chicken skin regioisomers were distributed in a random manner in the fatty acid combinations of the analyzed TAGs. However, our results are in agreement with previous findings (*3*), where palmitic acid is found predominantly in the *sn*-1/3 positions and oleic and linoleic acids in the *sn*-2 position.

The two major isomer pairs of egg yolk were 1(3),2-dioleoyl-3(1)-palmitoyl-*sn*-glycerol (*sn*-18:1-18:1-16:0 + *sn*-16:0-18:1-18:1) (30.8%) and 1(3)-palmitoyl-2-linoleyl-3(1)-oleoyl-*sn*-glycerol (*sn*-16:0-18:2-18:1 + *sn*-18:1-18:2-16:0) (18.1%). These compounds were most abundant also in chicken skin fat, but in smaller proportions. Although relatively abundant in chicken skin, regioisomers *sn*-18:1-18:1-18:2 + *sn*-18:2-18:1-18:1 and *sn*-16:0-18:1-18:2 + *sn*-18:2-18:1-16:0 were present only in minor quantities (2.5 and 1.1%, respectively) in yolk. Similarly, the ratios of the other species analyzed varied greatly between these two fats. The TAG regioisomers in yolk were clearly distributed nonrandomly in fatty acid combinations (Table 3). Linoleic and oleic acids were found in the *sn*-2 position and palmitic acid in the *sn*-1/3 positions as described previously (*8*−*10*), but enzymatic analyses have failed to reveal the predominance of the two regioisomers containing almost half of all TAGs.

Five major ACN:DB species from palm oil, six from palm olein, and six from palm stearin representing 76−88% of all TAG molecules were chosen for MS/MS analysis. The selected species consisted primarily of palmitic acid, oleic acid, linoleic acid, and stearic acid (Table 4). The presence of TAGs with myristic acid and palmitoleic acid was detected in some species. Oleic and linoleic acids were found to be the most common fatty acids in the *sn*-2 position as described before (*12*), but in each of the analyzed molecular weight species, linoleic, oleic, and palmitic acids existed in the *sn*-2 position in decreasing abundance compared to one

another. The location of a fatty acid residue was clearly dependent on the two other residues present in the same molecule. The most abundant regioisomers in palm oil were 1(3),2-dioleoyl-3(1)-palmitoyl-*sn*-glycerol (*sn*-18:1-18:1-16:0 + *sn*-16:0-18:1-18:1) (32.4%) and 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol (*sn*-16:0-18:1-16:0) (21.9%) containing together more than half of all TAGs. The same two compounds were the most abundant ones also in palm olein (39.7 and 11.5%, respectively) and palm stearin (19.7 and 26.9%, respectively). The regioisomerism within each molecular weight species in palm oil, palm olein, and palm stearin did not differ statistically significantly from each other. Therefore, fractionation of palm oil changed the abundance of molecular weight species but did not cause statistically significant changes in the proportions of different regioisomers within individual ACN:DB species. These results indicate that TAGs within one ACN:DB species behave similarly in the fractionation process.

The analyzed regioisomers of transesterified blend of palm stearin and coconut oil (82:18) did not differ statistically significantly from the assumed random distribution (Table 4). Therefore, we conclude that the tandem mass spectrometric method for analysis of regioisomers is reliable for analysis of the transesterification process.

Finally, the present study indicates that there are significant differences in regioisomerism between the analyzed oils and fats. Some of the differences observed are impossible to determine with enzymatic hydrolysis procedures of unfractionated oil. The positional distribution of fatty acids in TAGs may be of importance with regard to, for example, nutritional and techological points of view.

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