DETERMINATION OF FATTY ACID DISTRIBUTION IN THE *sn*-2 POSITION OF TRIACYLGLYCEROLS

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ABSTRACT

Fatty acid distributions in the *sn*-2 position of vegetable oil triacylglycerols (TGs) were determined by GC after pancreatic lipase hydrolysis and TLC isolation of monoacylglycerols (MGs). In rapeseed oil, the ratio between MUFAs and PUFAs in the *sn*-2 position was twice as high (1.0) as in total oil. The proportions of linoleic (LA) and α-linolenic acids (α-LLA) in the *sn*-2 position accounted for 33 and 16.5 %, respectively, having a similar LA / α-LLA ratio as that usually found in rapeseed oil TGs. Only traces of saturated fatty acids were detected (< 0.5 %). Fatty acid analyses of other oils also showed that LA and α-LLA were enriched in the *sn*-2 position.

INTRODUCTION

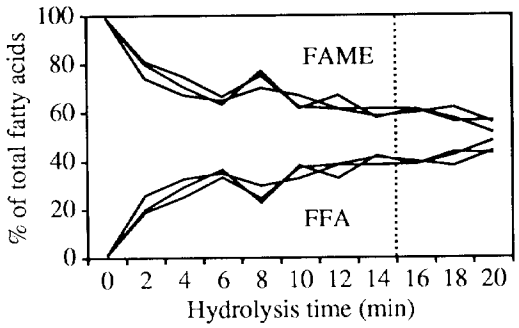
Fatty acids in the *sn*-2 position of TGs are important for human metabolism, since they can be used in the synthesis of phospholipids (Brindley, 1991). PUFAs from the *sn*-2 position of phosphatidylglycerols can further act as precursors of longer chain fatty acids and eicosanoids. This position is also of major interest when modifying the composition of TGs. Fatty acids in the *sn*-2 position can be determined by enzymatic or chemical methods (Brockenhoff, 1971). Pancreatic lipase hydrolyses the *sn*-1 and *sn*-3 positions only. The reaction products are usually isolated by TLC, since a complex mixture is always obtained. In this study, TGs of vegetable oils were partially hydrolysed with pancreatic lipase. The fatty acids originating from the *sn*-2 position of isolated MGs were determined by GC.

EXPERIMENTAL

Pancreatic lipase (10 mg in 2.5 ml tris buffer pH 8.0) was added by vortexing (10 s) into a mixture containing 5mg of fatty oil in 2.5 ml of tris buffer (10 % arabic gum) and 0.15 ml 45 % $CaCl_2$ (Myher and Kuksis, 1979). The enzyme had been purified twice with acetone and diethylether. The hydrolysis was performed at 37 °C for 15 min and stopped with 15 % HCl (1.5 ml). The products were extracted with diethylether (3 x 2 ml). MG, DG, free fatty acid (FFA) and TG fractions were separated on Kieselgel 60 HPTLC plates using petroleum ether : diethylether : acetic acid (65 : 35 : 1) as eluent.

After visualising with 2,7-dichlorfluorescein (1 % in EtOH), the MG, DG and FFA bands were scraped off and transesterified with NaOMe prior to GC analysis on an NB-351 silica capillary column (Seppänen-Laakso *et al.*, 1990).When following the stage of hydrolysis, the ether extract from the reaction mixture was directly transmethylated and the proportions of fatty acid methyl esters (FAMEs) and FFAs determined by a single GC run.

FIGURE 1. The proportions of FFAs (from *sn*-1 and *sn*-3 positions) and FAMEs in the hydrolysis mixture of rapeseed oil during lipase treatment (2 - 20 min, n = 3).



At 15 minutes 40 % of the fatty acids were in the form of FFAs. Although the proportions of FFAs and FAMEs changed during hydrolysis, the fatty acid composition of the MG fraction remained unchanged between 4 - 20 minutes. When hydrolysing for 2 hours more than 90 % of fatty acids had been cleaved. This may be due to the migration of the *sn*-2 fatty acid to one of the primary hydroxyl groups (Mattson and Volpenhein, 1961), since the compositions of 1- and 2-MGs isolated on boric acid TLC were identical.

TABLE 1. Reproducibility of the method in the determination of the isolated hydrolysis products of rapeseed oil (for each fraction, n = 6).

Fatty acids	MG		DG		FFA	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Palmitic	0.2	6.1	2.4	3.7	4.6	4.0
Palmitoleic	0.1	2.9	0.1	11.8	0.3	10.5
Stearic	0.1	11.4	1.3	3.6	2.1	3.5
Oleic	50.4	0.9	57.8	0.4	66.3	0.7
Linoleic	32.8	0.4	24.5	1.1	16.3	2.4
α-Linolenic	16.4	1.9	12.5	1.1	8.7	2.4
Arachidic			0.4	2.2	0.5	5.6
Eicosenoic			0.8	4.0	1.1	3.4
Eicosadienoic			0.1	12.4		
Behenic			0.2	3.7		
	Mean	3.9	Mean	4.4	Mean	4.1

The fatty acid compositions of the MG, DG and FFA fractions could be reliably determined (mean RSD values 3.9 - 4.4 %, Table 1) using this method including hydrolysis, TLC isolation, transesterification and GC analysis.

TABLE 2. Fatty acid compositions of the total oils and TLC isolated MG fractions.

Source of vegetable oil	SaFA (%)		MUFA (%)		n-6 PUFA (%)		n-3 PUFA (%)	
	Total	MG	Total	MG	Total	MG	Total	MG
Rapeseed	4.8	0.4	61.3	50.2	21.6	33.0	11.7	16.5
Soyabean	14.5	0.9	25.1	23.3	53.7	69.9	6.4	5.9
Olive	10.8	0.8	79.7	85.8	8.1	12.3	0.6	1.1
Sunflower	11.3	1.3	25.1	24.2	62.3	74.2	0.3	0.4
Corn	12.3	1.8	29.6	27.7	56.9	69.4	1.1	1.2
Peanut	18.9	2.4	44.4	39.5	35.2	57.3	0.6	0.8
Sesame	15.1	1.5	41.5	44.0	42.4	54.0	0.4	0.5
Palm	42.3	9.9	44.7	67.5	12.3	22.4	0.2	0.2
Linseed	8.4	1.1	19.8	24.5	14.5	20.3	56.3	54.1

In all the fatty oils studied, the *sn*-2 position of TGs was characterized by higher proportions of n-3 and n-6 PUFAs compared to total oils. In rapeseed oil only traces of SaFAs were found in the *sn*-2 position.

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