The Pixel Area Scanning Using Total Lab Software Version 1.11 for The Evaluation of Lipid Components on TLC Plates

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ABSTRACT

In case there is no availability for photoelectric scanners for measuring the area spot of any lipid material on TLC plates, pixel area scanning technique with Total Lab software version 1.11 can replace the former. In order to test this argument, different lipid blends were interestrified with different lipases and the degree of synthesis was detected by determining the remaining triglycerides by TLC and pixel area scanning and Total Lab software version 1.11 as compared with column chromatography and gravimetry.

Key words:

INTRODUCTION

Gravimetric technique was used early to determine the percentage any lipid fraction in a lipid mixture after being isolated by column chromatography using silica gel according to Homer & Virtanen (1967) based on Hirch & Ahrens (1959) as modified by Tahoun & Franzke (1976). After solvent evaporation, the residue of each fraction was gravimetrically determined and calculated as a percentage of the total. Barret *et al*. (1963) developed a densitometric scanning technique of a lipid blend on TLC plates, sprayed with 50% phosphoric acid in distilled water, followed by heating the plates on an aluminum block at 340° C for 5 min. When the spots turned black, the intensity of the black colour was measured with photodensitometer (Chromo Scan Fa. Joyce, Loebl & Co. Gateshead England). Up to that time quantitative TLC was fraught with experimental error. However, the introduction of commercial spectrodensitometric scanners enabled the quantification of analytes directly on the TLC layer. Initially peak areas were manually, but later inter integrators achieved this automatically.

Further modification of above technique was extended to the introduction of reflectance mode to the measurement of area spot on TLC plates as a function of reflectance in UV region using TLC scanners.

MATERIALS AND METHODS

Immobilized enzymes used in the present study are silica gel granulated lipases sn - 1,3 TUM from Thermomyces Icmginosus, lipozyme sn - 1,3 RMIM from Rhizopus miehei and Novozyme 435 from Candida sp. The enzymes were supplied by Novozyme ALS, krogshoejvei 36, Denmark, Olive oil was used as a substrate for the measurements of lipase activity. Silica gel G type 60 for Thin Layer Chromatography is a product of E. Merck AG, Darmstadt, Germany. Silica gel for column chromatography is a product of Fluka - Aldrich Chemie GmbH, Germany. Triton X -100 is a product of Fluka Chemie GmbH, Switzerland. All other chemicals were of reagent grade.

Measurement of lipase activity

Lipase activity was measured according to Dole & Meinertz (1960) and defined as the amount of enzyme that liberates 1 pM of fatty acids per min.

Interestrification reactions

The interestrification reaction mixtures consisted of 0.05 g of the immobilized lipase (Novozymes A/S. Krogshoejvej 36.2880 Bagsvaerd, Denmark) and 5 g of the following lipid blends (2.5g of each):

Palm oil - palm kernel oil / palm kernel oil - sunflower oil / palm oil - sunflower oil / palm kernel oil- sunflower oil.

The reaction mixtures each was incubated in a double cell wall, where circulation was performed by passing water heated to 50 °C on using the lipozyme TLIM, 60 °C when RMIM was used as a catalyst, while the reaction was performed at 37.5°C when Novozyme 435 was used. The interestrification reactions were conducted for 5, 10, 20, 30, 40 and 60hr with agitation at 200 rpm/min.

Isolation of Triglycerides

Isolation of triglycerides was carried out according to the basics of Homer & Virtanen (1967) that is based on the fundamentals of Hirch and Ahrens (1959) modified byTahoun & Franzke (1976).

Quantification of Interestrified reaction products into different lipid classes.

Activated TLC plates coated with silica gel G type 60 plates were developed in a solvent system consisting of petroleum ether: diethyl ether: acetic acid 70: 30: 2. Dried plates were sprayed with 50% H_2SO_4 and visualized by charring at 180 °C until the appearance of lipid spots. The concentration of each component on the TLC plates was calculated on the basis of a pixel area scanning technique using Total Lab Program, version 1.11.

Determination of the percentage of trisaturated triglycerides GS3 was carried out according to Barret *et al.*

Glass plates coated silica gel G type 60 Merck containing 12.5% $AgNO_3$ were used. The plates were dried in an oven at 110°C for l hr and developed with a solvent consisting of 60 volume carbon tetra chloride, 40 volume chloroform and 2 volume acetic acid and 0.3 volume ethyl alcohol. The plates were visualized by spraying with 50% H_2SO_4 and charring in an oven at 180 °C. The percentage of each glyceride category including GS3, GS2U, GSU2, GU3 and binary classes was calculated on the basis of a pixel area scanning technique using Total Lab Program, version 1.11.

Gas chromatographic identification of fatty acids liberated by the action of different lipases on different lipid blends during interestrification.

The fatty acids liberated from the action of different lipases used in the interestrification reactions were qualitatively and quantitatively measured according to Tahoun & Ali (1981) , Ak/here a GC Shimadzu - 8A equipped with dual FID detector and a glass column 2.5 m X 3 mm inner diameter was used under the following conditions: column: 5% EGS on 80/100 mesh chromosorb W/AW/DMCS. Hydrogen flow rate: 75 ml/ min. Air flow rate: 0.5 ml/min. Nitrogen flow rate: 20 ml/min. Injection port: 100°C, Detector temp. 270°C with a chart speed of 2.5 mm/min and a sensitivity of 16 × 100.

RESULTS AND DISCUSSION

Early, Kaufman & Grothues (1960) observed that during the interestrification process, a drop in the melting points of the interestrified products were detected. Lai *et al*. (1998) stated that stearin melting point transestrified plam stearin - sun flower oil (40: 60) mixtures were generally decreased after transestrification catalyzed by *pseudomonos* and *Rhizopus* miehei lipases. The decrease of SMP of any lipid mixture during interestrification was accused to the decrease of the GS3 percentage of triglycerides as reported early by Täufel *et al*. (1958). To investigate this hypothesis the remaining triglycerides of different interestrificaltion products prepared during this work were isolated by column chromatography, followed by fractionation into triglyceride categories including GS_3, GS_2U, GSU_2, GU_3 and binary classes between on TLC plates coated with silica gel G type 60 impregnated with 12.5% $AgNO_3$ in 60: 40: 2, CCl_4: $CHCl_3$: C_2H_5COOH. After developing the chromatograms, the plates were sprayed with 50% H_2SO_4 and charred at 180°C till the appearance of the lipid spots as brown on a white background. The percentage of each category was calculated as a fraction of the total multiplied by hundred. To fulfill that aim, a photograph of the TLC plate was captured using Nikon digital camera (4.1 Mega pixels) and the captured photo was analzed using Total Lap software version. 1.11. All interestrified reaction products, Table (1), revealed decreased GS_3 values than starting lipid blend. The reason for above finding is explained by the fact that free fatty acids released by different lipases used during interetrification will be attached randomly or specifically to the free positions of glycerol backbone to form new TG. As an example of acyl radical exchange between triglycerides, the liberated fatty acids from a 1:1 pko-so lipid blend was measured by gas chromatomatography as shown in Fig. (1). All liberated fatty acids appeared on the chromatograms directly

Table 1: The GS3 percentage released from the interestrification of different lipid blends by different immobilized lipases for different reaction periods: 0, 5, 10, 30, 40, 60 hr.

Interestrification reaction time hr	Pko- so blend inter-estrified with sn-1,3 TL IM lipase		Pko-op blend inter-estrified with sn-1,3 TL IM lipase		Pko-so blend inter-estrified With Novo-zyme 435 lipase		Po-so blend inter-estrified With sn-1,3 RM IM lipase	
	Relative Remaining TG%	GS3%	Relative Remaining TG%	GS3%	Relative Remaining TG%	GS3%	Relative Remaining TG%	GS3%
0	100.00	36.08	100.00	43.71	100.00	36.08	100.00	43.03
5	56.00	29.73	76.06	26.09	56.10	19.50	69.60	39.33
10	58.00	25.64	80.10	26.93	62.09	20.80	75.50	39.85
20	53.00	25.35	79.20	26.73	57.40	24.90	90.20	40.04
30	61.60	25.18	82.10	22.13	63.90	19.80	87.02	39.68
40	48.80	25.16	81.80	23.60	73.10	13.50	74.40	35.00
60	45.00	19.60	71.80	21.76	70.50	11.50	67.60	38.44

PKo -50 blend : Palm kernel -Sunflower oil blend
Pko- po blend : palm kernel - palm oil blend
Po-so blend: palm oil - Sunburn oil blend

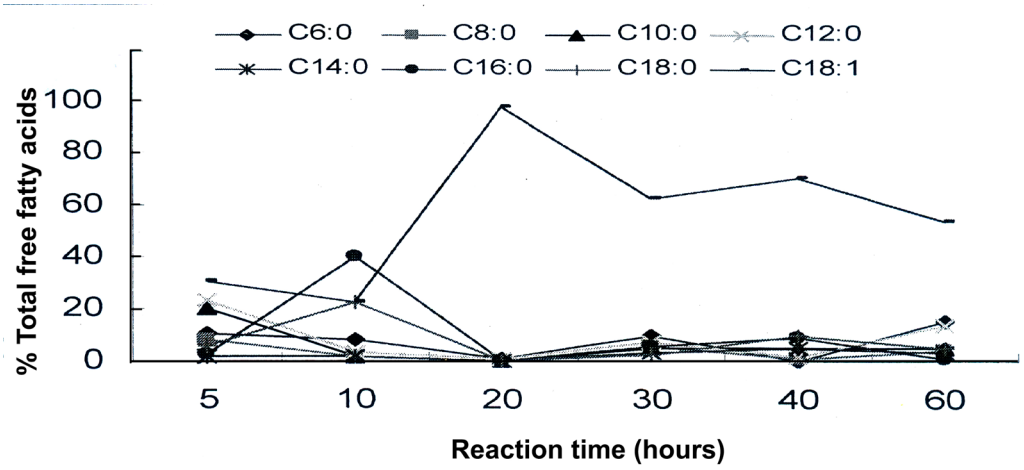

Fig. 1. Changes of liberated free fatty acids concentration during the interestrification of pko-so catalyzed by lipozyme sn-1, 3 TL IM lipase for different reaction times

after the injection of the samples, then recoded decreases after 5 and 10 hr to reach 1% after 20 hr. On the other hand, oleic acid was the only fatty acid that accumulated at high percentage ~ 98% after 20 hr of interestrification, then decreased to reach 53% after 60 hr.

To check the accuracy of the Total Lab software as a tool for the measurement of the percentage of interestrification products on TLC plates, the results shown in Table (2) were compared with those obtained regarding remaining triglycerides from column chromatography on silica gel measured by gravimetry. The data obtained were found quite similar, where later technique facilitated the application of lipases in the interestrification of different lipid blends as a matter of ease and quick obtaining the final result. In that context 1,3- specific RMIM lipase revealed ~ 87% remaining triglycerides from the interestrification of (1: 1) po-so lipid blend, whereas 1,3 specific TLIM lipase gave ~ 82% and ~ 61% remaining triglycerides from the interestrification of (1:1) pko-po and pko-so lipid blends after 30 hr reaction time, respectively. Where, Novozyme 435 recorded ~ 64% remaining TG during the interestrification of (1:1) pko-so blend interestrified for 30 hr.

Table 2: Changes of the percentage of remaining TG during the interestrification of different lipid blends as measured either by pixel area scanning and Total Lab software version 1.11 or by column chromatography on silica gel and gravimetry.

Substrate plus enzyme	Reaction time hr	% TG measured by gravimetric after isolation by column chromatography	% TG measured using TLC and pixel area scanning technique
Palm Kernel oil- palm oil, Lipozyme sn-1, 3 TL IM lipase	30	70.00	82.10
	40	77.80	81.80
	60	93.40	71.80
Palm kernel oil-sun flower oil, Lipozyme sn-1, 3 TL IM lipase	30	78.90	61.60
	40	66.70	46.80
	60	61.10	45.00
Palm kernel oil-sun flower oil, Novozyme 435 lipase	30	44.40	63.90
	40	47.80	73.10
	60	70.60	70.50
Palm oil - Sun flower oil, Lipozime sn-1,3 RM IM lipase	30	68.90	87.02
	40	66.70	74.40
	60	62.20	67.60

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مسح مساحة البيكسيل باستخدام برنامج الحاسوب Total Lab Software Version 1.11 لتقدير مكونات الليبيدات على ألواح كروماتوجرافيا الطبقة الرقيقة

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في حالة عدم وجود أجهزة ماسحات كهروضوئية لتقدير مساحات البقع للمواد الدهنية المفصولة على ألواح كروماتوجرافيا الطبقة الرقيقة، فإنه يمكن استخدام برنامج حاسوب آلي هو «Total lab software version ١,١١» ولكي تختبر هذه الطريقة تم إجراء أسترة داخلية لمخاليط مختلفة من الليبيدات باستخدام إنزيمات الليباز، وتم تقدير درجة التخليق عن طريق تقدير الجلسريدات الثلاثية المتبقية باستخدام هذا البرنامج ومقارنته بطرق كروماتوجرافيا العامود والجاذبية الأرضية.