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Catalyzed Interesterification of Mixed Short & Medium Chain Fatty Acid Triacylglycerols as a Potential Dietary Food Lipid Source: Synthesis and Characterization

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Abstract Interesterifcation reactions, more commonly called ester-exchange, are a process by which the arrangement of fatty acids in the triacylglycerol molecule is changed. Ester-exchange reactions lead to a random distribution of fatty acids resulting in a triacylglycerol composition that is predictable from the overall fatty acid composition of the starting reaction mixture. In this report, we synthesized various triacylglycerols containing different ratios of saturated short chain fatty acids (SCFA) such as: acetic, propionic and butyric, with a saturated medium chain fatty acid (MCFA) of octanoic acid. The interesterification of these triacylglycerols was prepared by the $NaOCH_3$ catalyzed interesterification of model reactions performed with different molar ratios of Triacetin, Tripropionin, Tributyrin and Trioctanoin. The reaction products were characterized by Gas Chromatography (GC) and Proton NMR. We found that the difference in fatty acid size and the reduced steric demands of the S/MCFA did not give rise to positional specificity and we found no observable deviations from the random interesterification chemistry model. The potential of having different short and medium fatty acids triacylglycerol on one molecule for direct ingestion and metabolism can be of significant importance to maintaining gut health.

Keywords: *interesterification, triacylglycerols, triglycerides, short chain fatty acids, medium chain fatty acids, ester-exchange, randomization theory*

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1. Introduction

Lipids are a large group of organic compounds that include fats, oils, waxes, sterols, and vitamins. Fats and oils are, in particular, classified under the name triglyceride or triacylglycerol and are named so because they are comprised of three ester groups attached to a glycerol backbone. Upon hydrolysis of these substances, the products are glycerol and three fatty acids that fall under three categories based on size. Short chain fatty acids (SCFA) have carbon chains ranging from 2-5 carbons, medium chain fatty acids (MCFA) have 6-12 carbons and long chain fatty acids (LCFA) have more than 12 carbons. Short chain fatty acids (SCFA) have been identified as the key end products of colonic fermentation of dietary fiber [1,2,3,4,5]. SCFA are readily absorbed in the gut and play an important role in the maintenance of health [6,7,8]. The deficiency of SCFA (particularly acetic, propionic, and butyric) is associated with the pathogenesis of a diverse range of diseases ranging from allergies to asthma to cancer [8]. SCFA containing lipids are used in clinical nutrition to maintain gastrointestinal integrity and function in patients [9]. Short chain fatty acids have been known to enhance the gut microflora and promote colon health. Both short and medium chain fatty acids (S/MCFA) are more easily absorbed into the bloodstream from the gastrointestinal tract and can easily cross the blood-brain barrier [10]. Medium chain fatty acids are, in particular, found in several substances, such as coconut and palm kernel oils, whole milk and yogurt. Compared to longer-chain fatty acids triglycerides, MCFA are digested and utilized differently. Instead of being combined into lipoproteins they are absorbed directly into the bloodstream and are then transported to the liver. Because MCFA don't need bile or pancreatic enzymes to break down, they are easier to digest, and because they are easier to digest, they provide the body with quick energy.

Medium chain triacylglycerols (MCT's) have been shown to provide a wide range of positive health benefits ranging from improved cardiovascular health [11], support the body immune system [12], stimulate the body metabolism, possible leading to weight loss [13], and reduce gastrointestinal disorders [14]. Both SCFA and MCFA as part of a triacylglycerol molecule can provide a significant dietary food lipid source that has the potential of many health benefits.

Interesterification is a process that can create new fatty acid triacylglycerols. It is an ester exchange reaction that leads to the random distribution of fatty acids and can therefore lead to several products of different isomers just from reacting two triacylglycerols. It is because of this that several mathematical models were made to account for the randomization of products [15,16,17]. The random nature of triacylglycerols produced by the catalyzed interesterification of short and long -chain fatty acid triglycerides has been reported [18]. A recent study of interesterification of different short chain fatty acid triacylglycerols has also been reported which follows the random mathematical model developed [19]. It seems reasonable to question whether the different size and increased steric demands of medium chain fatty acids could give rise to positional specificity and observable deviations from the random interesterification chemistry.

Through the process of interesterification, we synthesized nine different triacylglycerols of short and medium chain fatty acids. These products were then characterized through the use of GC and H^1 NMR and the results were compared to the calculated data from the mathematical randomization model.

2. Materials and Methods

2.1. Synthesis of New Fatty Acid Triacylglycerols

The new triacylglycerols were prepared through interesterification reactions and were catalyzed by $NaOCH_3$. The reaction was performed using different mole ratios of the short chain fatty acid triacylglycerols (Triacetin, Tripropionin, and Tributyrin), which were combined with the medium chain triacylglycerol Trioctanoin. All of the reactants used were reagent grade and had 98% purity or better. The reactants were measured in a 3 neck round bottom flask and put through the following conditions with stirring: heated to 120-130°C under vacuum for 30 minutes, introduced to 2% (w/w) of $NaOCH_3$ catalyst followed by reducing the heat to 88-90°C and kept under heat for 3 hours, neutralized by 0.3% of 85% H_3PO_4 after the reaction was complete. The products were characterized using GC and H^1 NMR. The weight % of the products were obtained and compared to the weight % calculated from the random interesterification reaction model. The scheme for the reaction between short chain and medium chain triacylglycerols is shown below as reaction scheme 1:

AAA + PPP + BBB + OOO →→
(6) (9) (12) (24)

AAO+OOA+PPO+OOP+BBO+OOB +AAP+
(12) (18) (14) (19) (16) (20) (7)

AAB + PPA + PPB + BBA + BBP + APB+
(8) (8) (10) (10) (11) (9)

ABO+PBO+APO
(14) (15) (13)

AAA = Triacetin
PPP = Tripropionin
BBB = Tributyrin
OOO = Trioctanoin
(#) = Corresponds to acyl carbon number

REACTION SCHEME 1

2.2. Description of the Random Interesterification Model

The interesterification of SCFA and MCFA triacylglycerols is essentially the removal and replacement of fatty acids at random. Throughout the course of the reaction, an intricate mixture of all possible interesterification products are formed and randomized. The reaction ultimately obtains equilibrium when all possible combinations have taken place. This process is referred to as randomization. The equilibrium maybe simplified and represented as shown in reaction scheme 1. The composition of such a randomized reorganization can be calculated from probability theory. For example, If X, Y, and Z are the molar % of acetic, propionic, and butyric acid respectively, then the molar % of Triacylglycerols containing only one acid (A) is: $\%AAA = X^3/10{,}000$; molar % of the triacylglycerols containing two different fatty acids (A,P) is $\%\ AAP = 3X^2*Y/10{,}000$; and the molar % of the triacylglycerols containing three different (A,P,B) acids is $\%\ APB = 6X*Y*Z/10{,}000$. A more generalized mathematical description is shown in Table 1.

Table 1. Theoretical Triacylglycerol Composition after Interesterification for a Fatty Acids (A, P B, O) with mole fraction a, b, c, d

Type	Number	Amounts
Mono-acid AAA, PPP,	n	a^3, b^3, c^3
Di-acid AAP, AAC, etc.	n(n-1)	$3a^2b$, $3ab^2$, $3a^2c$
Tri-acid APB, PBO, ABO, etc.	1/6 n(n-1) (n-2)	6abc, 6acd, etc.
Total	$1/6n^3+1/2n^2+1/3n$	--

For example: the theoretical calculation of the % composition of a reaction mixture based on the interesterification of a 1:1 molar ratio of triacetin and trioctanoin is as follows:

$$\%\text{ moles of A} = 3A/(3A + 3O)*100 = 50\%\ A$$
$$\%\text{ moles of O} = 3O/(3A + 3O)*100 = 50\%\ O$$

After interesterification:

$$\%\ AAA = (50)(50)(50)/10,\ 000 = 12.5\%$$
$$\%OOO = (50)(50)(50)/10{,}000 = 12.5\%$$
$$\%\ AAO = 3(50)(50)(50)/10{,}000 = 37.5$$

and the same for OOA.

2.3. Characterization

2.3.1. Gas Chromatography

The reaction products were run through Agilent 7820A gas chromatograph with an on column injector and flame ionization detector to obtain the GC profiles. The column used was an Altech EC-1 capillary column 30m x .53mm ID x 1.0µm dimethylpolysiloxane stationary phase. The following conditions were applied to the sample: 280°C injection port temperature, 280°C detector temperature, oven temperature controlled by program of 150°C initial temperature increased to 250°C at a rate of 10°C/min. Helium was used as a carrier gas at a flow rate of

3.0ml/min. Quantitation was done by area % by developing a method in HP Chem station software

2.3.2. Proton NMR

The H^1 NMR spectra were obtained by running the products on a JEOL 400 ECS spectrometer as a single pulse scan with a 5 second relaxation delay at 20.7°C. The samples were run neat. The integrated spectra of the reaction products were used to calculate the amount of the short and medium chain fatty acids present in the product. The relative molar compositions of the fatty acids were determined by the integration of the methyl peaks, CH_3 for the SCFA at 1.38 ppm (acetic), .39 ppm (propionic) .28 ppm (butyric) and for the octanoic, the CH_3 peak was .15 ppm.

2.3.3. Statistical Analysis

GC analysis was done by performing multiple runs on each sample. The data reported is an average of the percent area obtained. The average percent relative standard deviation for the samples analyzed ranged from 0.3% - 0.8%.

3. Results and Discussion

Nine model interesterification reactions were performed by reacting different molar ratios of one of the short chain fatty acid triacylglycerols with the Trioctanoin. The different products and their relative weight percentages were calculated using the random interesterification model and the results of those calculations are shown in Table 2. These calculated percentages were verified using GC characterization of the reaction products. The GC profile for the reaction of 1:1 Tributyrin: Trioctanoin is shown in Figure 1. The peaks in the chromatogram are shown to elute in accordance to their acyl carbon number and polarity of the triacylglycerol. The triacylglycerols with the same acyl carbon number, such as BOB and BBO, were originally believed to co-elute, but the extra peaks on the chromatogram seem to imply differently, resulting in separate labels for the two isomers due to the difference in polarity of dipole moment of the triacylglycerol. The experimental percent ratios for the triacylglycerols in each of the reaction products were obtained from the GC profiles and tabulated as shown in Table 3. The experimental values are in very good agreement with the calculated data. The experimental and calculated weight percentages were compared using linear regression analysis, the results of which are shown in Figure 2. The correlation coefficient, R^2, at lower concentrations of Trioctanoin is 0.99, which shows an excellent agreement between the calculated and experimental weight percent,. Figure 3 shows the H^1 NMR of the reaction product of 1:1 Triacetin to Trioctanoin with the chemical shifts of the protons identified. The integrated areas of the methyl protons of the particular fatty acids correspond to the molar ratios of the reactants.

Table 2. Calculated Weight Percent Composition of Interesterification Reaction Mixture of Triacetin (AAA), Tripropionin (PPP), Tributyrin (BBB), and Trioctanoin (OOO)

S/MTags	Acyl Carbon #	Molar Mass	A:O 6:1	P:O 6:1	B:O 6:1	A:O 2:1	P:O 2:1	B:O 2:1	A:O 1:1	P:O 1:1	B:O 1:1
AAA	6	218	63.6			29.6			12.5		
PPP	9	260		63.6			29.6			12.5	
BBB	12	302			63.6			29.6			12.5
OOO	24	470	0.3	0.3	0.3	3.7	3.7	3.7	12.5	12.5	12.5
AAO/AOA	12	302	31.1			44.4			37.5		
OOA/OAO	18	386	5.1			22.2			37.5		
PPO/POP	14	330		31.1			44.4			37.5	
OOP/OPO	19	400		5.1			22.2			37.5	
BBO/BOB	16	358			31.1			44.4			37.5
OOB/OBO	20	414			5.1			22.2			37.5
TOTALS			100	100	100	99.9	99.9	99.9	100	100	100

Table 3. Experimental Weight Percent Composition of Interesterification Products from Reaction Mixture of Triacetin (AAA), Tripropionin (PPP), Tributyrin (BBB), and Trioctanoin (OOO)

S/MTags	**Acyl Carbon #**	**Molar Mass**	**A:O 6:1**	**P:O 6:1**	**B:O 6:1**	**A:O 2:1**	**P:O 2:1**	**B:O 2:1**	**A:O 1:1**	**P:O 1:1**	**B:O 1:1**
AAA	6	218	62.6			29.8			11.9		
PPP	9	260		61.7			28.7			12.5	
BBB	12	302			62.3			29.6			11.9
OOO	24	470	0.5	0.5	0.6	3.3	2.9	3.7	12.9	12.5	12.7
AAO/AOA	12	302	32.1			43.5			36.5		
OOA/OAO	18	386	5.5			21.3			38.5		
PPO/POP	14	330		31.4			42.6			37.5	
OOP/OPO	19	400		6.5			22.7			37.5	
BBO/BOB	16	358			32.4			44.4			37.9
OOB/OBO	20	414			6.1			22.2			38.3
TOTALS			100.7	100.1	101.4	97.9	97.9	97.4	99.9	101.7	100.8
GC Wt. % Fatty Acid Ratio			85.8 To 14.9 A:O 6:1	84.8 To 15.3 P:O 6:1	85.9 To 15.5 B:O 6:1	65.9 To 32 A:O 2:1	64.7 To 32.2 P:O 2:1	66.6 To 32.2 B:O 2:1	48.8 To 50.0 A:O 1:1	50.0 To 50.0 P:O 1:1	50.0 To 50.8 B:O 1:1
NMR Fatty Acid Product Ratio			5.9 To 1	6.0 To 1	5.9 To 1	2.1 To 1	1.9 To 1	2.0 To 1	1.1 To 1	1.0 To 1.1	1.0 To 1

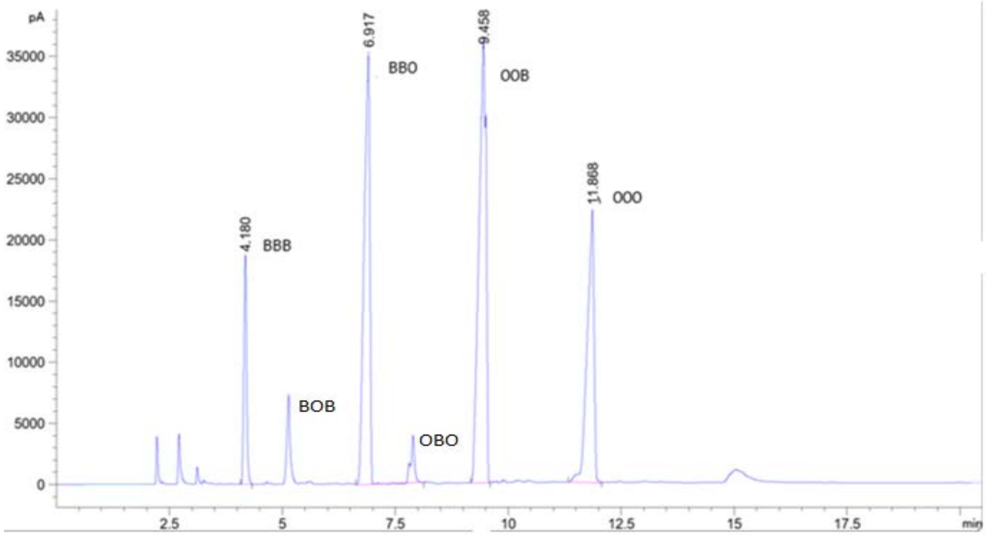

Figure 1. GC Profile of Reaction Product of Interesterification of 1:1 Tributyrin: Trioctanoin. Elution order is by increasing acyl carbon number and polarity on the triacylglycerols

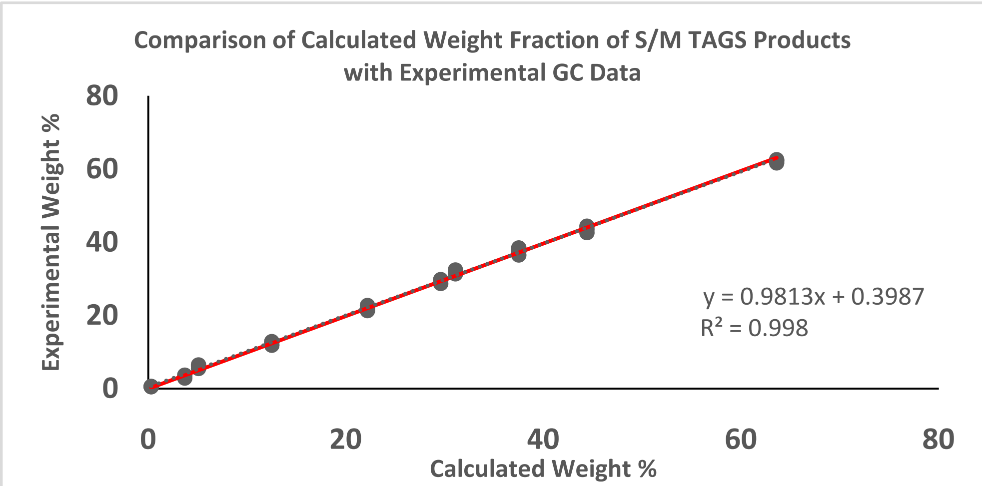

Figure 2. Plot of Experimental vs Calculated Weight Percent Composition of the products formed in each reaction mixture using Gas Chromatography Data

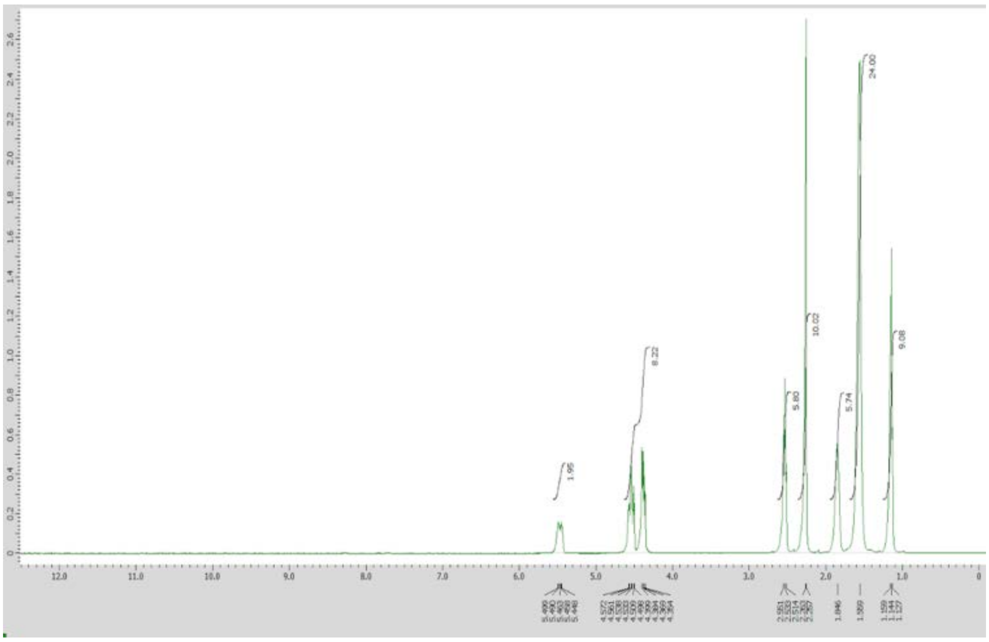

Figure 3. Proton NMR of Reaction Product of the Interesterification of 1:1 Triacetin: Trioctanoin. The corresponding chemical shifts for the methyl groups are: Acetyl CH_3 2.263ppm, Octyl CH_3 1.144ppm

4. Conclusions

The interesterification reactions of the short chain fatty acid triacylglycerols of Triacetin, Tripropionin, and Tributyrin, with the medium chain triacylglycerol Trioctanoin produced randomized mixed reaction products. The statistical randomized interesterification mathematical model was used to predict the relative amounts of the reaction products. Excellent agreement was found between the experimental and calculated weight percent compositions of the interesterifications reactions products using GC and 1H NMR.. The difference in fatty acid size and the reduced steric demands of the selected fatty acids did not give rise to any significant positional specificity. There is a need for further studies examining the potential effects of the combination of a nutraceutical including both SCFA and MCFA triacylglycerols and their effects on health. The combination of experimental, clinical and in vitro trails will help to guide future recommendations for the potential uses of S/MCFA triglycerides as a beneficial health source.

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