Synthesis and Characterization of Mixed Short Chain Fatty Acid Triacylglycerols as a Potential Dietary Food Lipid Source

Ronald P. D'Amelia[*]**, Alexander E. Bleich, Lee Honig, William F. Nirode**

Department of Chemistry, Hofstra University, Hempstead, New York, USA
*Corresponding author: Ronald.P.Damelia@hofstra.edu

Abstract SCFA(Short Chain Fatty Acids) are readily absorbed in the gastrointestinal tract and play an important role in the maintenance of gut health. The deficiency of SCFA (particularly acetic, propionic and butyric) may affect the pathogenesis of a diverse range of diseases ranging from allergies to asthma to cancers. The potential of having three different SCFA on one molecule for direct ingestion and metabolism can be of significant importance to maintaining gut health. SCFA triglycerides have not been characterized for use in clinical nutrition therefore it is important to understand their specific structure and composition. We synthesized ten reaction products of various triacylglycerols containing different ratios of acetic, propionic and butyric SCFA by interesterification. The reaction products were characterized by Gas Chromatography (GC), GC/MS and H^1 NMR. The weight % of the triesters found were compared to the amount predicted using the statistical randomized interesterification reaction model. We found that the difference in fatty acid size and the reduced steric demands of the SCFA did not give rise to positional specificity and found no observable deviations from the random interesterification model. By interesterification we have synthesized a molecule that contains SCFA essential to gut health. This molecule could potentially be used for direct ingestion possibly eliminating the need for other means of daily fiber intake.

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

1. Introduction

Lipids are a large category of compounds that include fats, oils, waxes, and others. Fats and oils belong to a general class of compounds called triglycerides or triacylglycerols because of the three ester groups attached to the glycerin backbone not always only triglycerides, but di- or monoacylglycerols are in fats and oils. The hydrolysis of fats and oils produces glycerol and fatty acids which are classified according to their length (short, medium, or long). Short chain fatty acids (SCFA) have been identified as the key end products of colonic fermentation of dietary fiber [1-5]. SCFA are readily absorbed in the gut and play an important role in the maintenance of health [6,7,8]. The deficiency of the SCFA (particularly acetic, propionic and butyric) is associated with the pathogenesis of a diverse range of diseases ranging from allergies to asthma to cancers [8]. SCFA containing lipids are used in clinical nutrition to maintain gastrointestinal integrity and function in patients [9].

Interesterification is an important industrial fat modification process used to produce lipids with specific functionalities. The rearrangement of fatty acids by interesterification leads 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. These ester-exchange reactions have been studied and mathematical models have been developed that can be used to predict the distribution of the fatty acids in the products [10,11]. The interesterification of SCFA and long chain fatty acids (LCFA) triglycerides has been reported [12,13]; however, the nature of the product mixtures have not been studied in detail. It seems reasonable to question whether the significantly different size and reduced steric demands of the short chain fatty acids could give rise to positional specificity and observable deviations from the random interesterification chemistry.

We synthesized ten triacylglycerols containing SCFA using inter and intra randomization ester exchange reactions. Characterization of these reaction products are done using GC, GC-MS and ^{1}H NMR to compare the analytical data to the compositional model predicted by the random interesterification theory. Being able to synthesize a triacyglycerols having a known composition of three different SCFA on one molecule can be of significant importance for gut health.

2. Materials and Methods

2.1. Synthesis of Novel Short Chain Fatty Acid Triacylglycerols (s-TAGs)

The s-TAGs were prepared by the $NaOCH_3$ catalyzed interesterification of model reactions performed with different mole ratios of Triacetin, Tripropionin, and Tributyrin. All reagents were reagent grade having greater than 98% purity and all were purchased from Sigma-Aldrich Milwaukee, WI. The mole ratios of each reactant (see Table 1) were weighed in a 3 neck round bottom flask and reacted under the following conditions (1) stirred and heated to 120-130°C under slight vacuum for 30 minutes (2) a 2% (w/w) of $NaOCH_3$ catalyst was added and the reaction mixture was heated at 88-90°C for 3 hours (3) added 0.3% of 85% H_3PO_4 to neutralize the catalyst after the reaction went to completion and (4) liquid products were then purified by filtration for analysis. The reaction products were then characterized by GC, GC/MS and H^1 NMR. The weight % of the s-TAGs found were compared to the amount predicted using the statistical randomized interesterification reaction model. A typical reaction scheme for the interesterification of SCFA is shown in Reaction Scheme 1(modified from [13]) is shown below:

AAA+PPP+BBB----------->AAP+AAB+PPA

  (6)   (9)   (12)   (7)   (8)   (8)

+PPB+BBA+BBP+APB

  (10) (10) (11) (9)
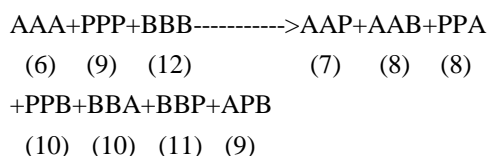
AAA = Triacetatin
PPP = Tripropionin
BBB = Tributyrin
(#) = corresponds to Acyl Carbon Number

2.2. Description of the Random Interesterification Model

The interesterification of s-TAGs is the random removal and replacement of SCFAs. As the reaction proceeds, a complex mixture of s-TAGs is formed. The reaction is complete when all possible combinations have taken place and then randomization of the SCFA is achieved (See Reaction Scheme 1). The composition of the randomized reorganized s-TAG can be calculated from probability theory. If X,Y,Z are the molar % of acetic, propionic, and butyric acid respectively, then the molar % of s-TAGs containing only one acid (A) is: $\%AAA = X^3 / 10{,}000$; molar % of s-tags containing two different fatty acids (A,P) is $\% AAP = 3X^2 * Y / 10{,}000$; and the molar % of s-TAGs containing three different (A,P, B) acids is $\% APB = 6X*Y*Z / 10{,}000$.

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 tripropionin is as follows:

  % moles of A = 3A/(3A + 3P) * 100 = 50% A
  %moles of P = 3P/(3A+3P) *100 = 50% P
  After interesterification:
  %AAA = (50)(50)(50) / 10,000 = 12.5%;
  %PPP = (50)(50)(50) / 10,000 = 12.5%;
  %AAP = 3(50)(50)(50) / 10, 000 = 37.5% and the same for PPA.

2.3. Characterization

2.3.1. Gas Chromatography

The GC profiles of each of the reaction products were obtained using a HP 5890E series II plus gas chromatograph with an on column injector and flame ionization detector (FID). The column used was an Alltech EC-1 capillary column 30 m x .53 mm ID x 1.0 um dimethylpolysiloxane stationary phase. Injection port temperature 280°C, Detector temperature 280°C, oven temperature was controlled by temperature program of 150°C initial for 3.0 min then increased at a rate of 10°C/min until a final temperature of 250°C was reached. Carrier gas was Helium at a flow rate of 3.0 mL/min. Quantitation was done by area % by developing a method in HP Chem station software.

2.3.2. GC-MS

GC-MS was performed on a HP 5890a GC-MS series II containing a HP 5971 mass selective detector using the same column and conditions as described above.

2.3.3. Proton NMR

Proton NMR spectra were obtained on a JEOL 400 ECS spectrometer as a single pulse single scan with a relaxation delay of 5 sec, at 20.7°C. Samples were run neat. The integrated areas of the SCFA were used to calculate the molar amounts of the individual short chain acids present. The relative molar acid compositions were determined by integration of the methyl peaks, CH_3, for the SCFA at 2.08 ppm (acetic), 1.14 ppm (propionic) and .95 ppm (butyric).

2.4. 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 0.2% - 0.8%.

3. Results and Discussion

Ten model interesterification reactions were performed using different molar ratios of Triacetin, Tripropionin, and Tributyrin according to Reaction Scheme 1. The products and their corresponding calculated weight percent for each interesterification reaction as predicted using the random interesterification model are shown in Table 1. Each reaction mixture was first characterized by GC in order to verify the correct number of products obtained from each reaction mixture. The GC chromatogram for the 1:1:1 Triacetin: Tripropionin: Tributyrin reaction mixture is shown in Figure 1. The seven peaks shown in the chromatogram elute based on their acyl carbon number. Three triacylglycerols with acyl carbon numbers 8, 9, and 10 respectively co-elute (peaks 3, 4, 5 shown in Figure 1) which is why there are only 7 peaks shown and not 10 as predicted. GC/MS was used to verify the elution order by obtaining the molar mass of each product to identify the corresponding peak by the acyl carbon number. Using the GC data, the experimental percent ratios of each sTAG in each reaction product as predicted by the random

interesterification model are shown in Table 2. The experimental values are in excellent agreement with the calculated values (see Table 1) indicating that these reactions can be described by the statistical random interesterification model. The sum of the weight percents of all the sTAGs for each reaction product is approximately 100% indicating the reaction completion. The sum of the experimental weight percent of each fatty acid found in each sTAG product reaction mixture as determined by GC is found at the bottom of Table 1. These fatty acid ratios correspond to the initial mole ratio of the starting triacylglycerols. Figure 2 shows a linear regression analysis (LRA) of the experimental weight percent vs calculated weight percent of the sTAGs. The

correlation coefficient, R^2, is 0.99 shows an excellent agreement between the experimental and calculated values.

Figure 3 shows the proton NMR of the reaction product of the interesterification of 1:1:1 Triacetin: Tripropionin: Tributyrin. The chemical shifts of the appropriate protons are assigned as shown. The integrated values of the assigned methyl protons for the specific fatty acids correspond to the corresponding mole reaction ratios of the starting triacylglycerols are shown in the bottom of Table 1. The fatty acid weight percent ratio determined by GC and the fatty acid ratio determined by NMR are in excellent agreement with the mole ratio of the starting triacylglycerols for each reaction mixture studied.

Table 1. Calculated Weight Percent Composition of Interesterification Reaction Mixture of Triacetin (AAA), Tripropionin (PPP) and Tributyrin (BBB)

sTags	Acyl C#	M.M.	1:1:0 A:P	0:1:1 P:B	1:0:1 A:B	2:1:0 A:P	2:0:1 A:B	0:2:1 P:B	1:2:0 A:P	1:0:2 A:B	0:1:2 P:B	1:1:1 A:P:B
			Calculated Area % Found for AAA, PPP, BBB Reaction Ratios									
AAA	6	218	12.5		12.5	29.5	29.5		3.7	3.7		3.7
AAP	7	232	37.5			44.3			22.2			11.1
APP	8	246	37.5			22.2			44.3			11.1
AAB	8	246			37.5		44.3			22.2		11.1
PPP	9	260	12.5	12.5		3.7		29.5	29.5		3.7	3.7
APB	9	260										22.2
ABB	10	274			37.5		22.2			44.3		11.1
PPB	10	274		37.5				44.3			22.2	11.1
PBB	11	288		37.5				22.2			44.3	11.1
BBB	12	302		12.5	12.5		3.7	3.7		29.5	29.5	3.7
totals			100	100	100	100	100	100	100	100	100	100

Table 2. Experimental Area % Composition of Interesterification Products from Reaction Mixture of Triacetin (AAA), Tripropionin (PPP, Tributyrin (BBB)

sTags	Acyl C#	M.M.	1:1:0 A:P	0:1:1 P:B	1:0:1 A:B	2:1:0 A:P	2:0:1 A:B	0:2:1 P:B	1:2:0 A:P	1:0:2 A:B	0:1:2 P:B	1:1:1 A:P:B
			Experimental Area % Found for AAA, PPP, BBB Mole Reaction Ratios									
AAA	6	218	13.0		11.9	29.4	28.5		3.6	3.9		3.9
AAP	7	232	36.6			43.1			21.9			11.1
APP	8	246	35.9			21.1			44.6			21.6
AAB	8	246			36.8		44.6			22.0		
PPP	9	260	13.4	13.0		4.8		29.7	30.1		3.6	26.6
APB	9	260										
ABB	10	274			36.9		22.9			44.3		22.2
PPB	10	274		36.1				43.7			22.8	
PBB	11	288		36.6				22.8			43.3	11.3
BBB	12	302		12.8	13.9		3.3	3.9		30.7	29.6	4.0
totals			98.9	98.5	99.6	98.4	99.3	100.1	100.3	100.9	99.4	100.7
GC Wt % FA ratio[a]			49.3 To 49.6 A:P	49.3 To 49.2 P:B	48.8 To 50.8 A:B	65.2 To 33.2 2A:P	65.9 To 33.4 2A:B	66.4 To 33.7 2P:B	33.1 To 67.2 A:2P	33.3 To 67.6 A:2B	33.3 To 66.1 P:2B	33.2 To 33.4 To 33.5 A:P:B
NMR Fatty Acid Product Ratio[b]			.97 To 1.0	1.0 To 1.1	.92 To 1.0	2.0 To 1.04	2.0 To 1.06	1.5 To 1.0	.98 To 2.0	.92 To 2.0	1.0 To 2.2	.91 To .98 To 1.0

[a] A = Triacetin; FA = Acetic Acid, P = Tripropionin; FA = Propionic acid, B = Tributyrin ; FA = Butyric acid
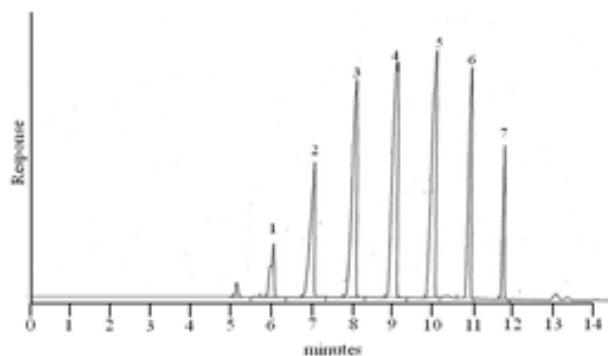[b] Obtained from the integration of pertinent CH_3 peaks in the NMR profile

Figure 1. GC Profile of Reaction Product of Interesterification of 1:1:1 Triacetin: Tripropionin: Tributyrin. Elution order is by increasing acyl carbon number of the triacylglycerols formed as follows: peak 1) AAA, 2) AAP, 3) APP/AAB, 4) PPP/APB, 5) ABB/PPB, 6) PBB and 7) BBB
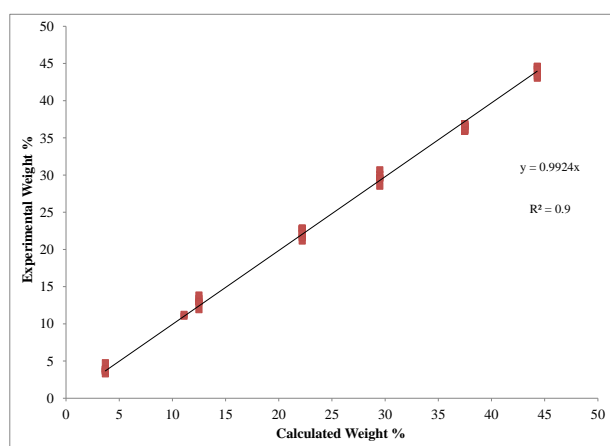

Figure 2: Plot of the Experimental vs Calculated Weight Percent Composition of the sTAG formed in each reaction mixture using Gas Chromatography Data
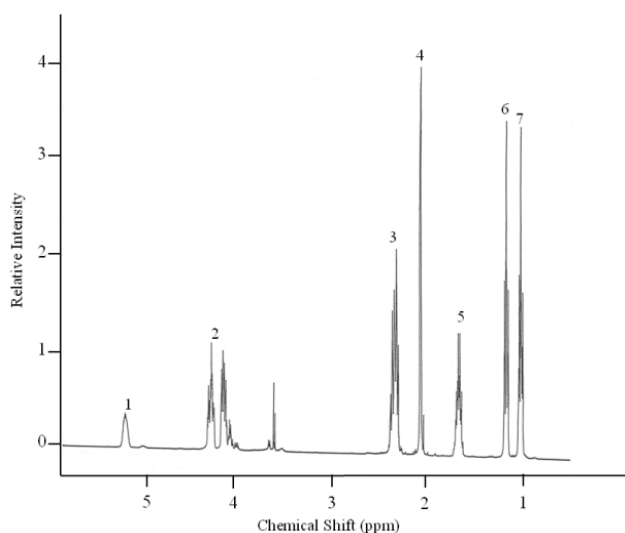

Figure 3. Proton NMR of Reaction Product of the Interesterification of 1:1:1 Triacetin: Tripropionin: Tributyrin. The corresponding protons and their chemical shifts are (1) Glycerol CH 5.28 ppm, (2) Glycerol CH_2 4.25 ppm, (3) Propyl/Butyl CH_2 2.35 ppm, (4) Acetyl CH_3unsplit 2.08 ppm, (5) Butyl CH_2 1.65 ppm, (6) Propyl CH_3 1.14 ppm, and (7) Butyl CH_3 0.95 ppm

The potential of having three different SCFA on one molecule for direct ingestion and metabolism to maintain gut health can be of significant importance and possibly eliminating the need for other means of daily fiber intake.

4. Conclusions

Ten interesterification reactions between Triacetin, Tripropionin, and Tributyrin produced mixed SCFA triglycerides. The statistical random interesterification model was used to predict the reaction products. The reaction products were characterized to determine the weight percent composition of each mixed SCFA triglyceride. Excellent agreement was found between the experimental and calculated weight percent compositions of the interesterification reaction products using GC and ^{1}H NMR. The difference in fatty acid size and the reduced steric demands of the SCFA did not give rise to positional specificity. There is a new need for further studies examining the potential effects of the combination of a nutraceutical including SCFA triacylglycerols and their effects on health. The combination of experimental, clinical and in vitro trials will help to guide future recommendations for the potential uses of SCFA triglycerides as a beneficial lipid source.

Acknowledgement

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