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Physicochemical Properties of Enzymatically Synthesised Medium-Chain Triacylglycerols-based Enhancer Cream

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Abstract*: Structured lipids (SLs) containing medium-chain triacylglycerols (MCTs) were produced by lipase catalysed acidolysis of both octanoic acid and the virgin coconut oil (VCO). The production of SLs, namely structured virgin coconut oil (SVCO), was previously optimised using the central composite design (CCD) based on the percentage of octanoic acid incorporated in the reaction products. The fatty acids and triacylglycerols composition and their corresponding rheological properties of the formulated SVCO incorporated α-tocopherol cream were also determined. The parameters that were suggested for the highest incorporation of octanoic acid (68.07%) are octanoic acid to VCO ratio of 1.70 (w/w); an enzyme load of 22.60%; at 63.4°C; a water content of 3.53%; and at 96 h. The amount of octanoic acid (carbon-8), the medium-chain fatty acids present in the structured lipid after enzymatic esterification was increased to 60.1% as compared to the natural VCO with only contains 5.45%. Based on the calculated equivalent carbon number (ECN), the most probable MCTs found in the SVCO were CpCpCp, CpCCp, and CpLaCp. Based on the rheological analysis, the SVCO creams were less viscoelastic as compared to the VCO. It shows that the decrease of the saturated fatty acids composition (carbon-12, 14, and 16) in the SVCO creams had significantly decreased the elasticity of the cream.

Keywords* : Acidolysis, enzymatic modification, medium-chain triacylglycerols, structured lipids.

I. INTRODUCTION

The modification of fats and oils through interesterification reaction catalysed by lipases has been progressively studied since the 1980s due to their technical advantages over the traditional direct methods of chemical interesterification. Among the advantages offered are the synthesis of novel product and incorporation of desirable fatty acids at specific positions of the lipid to boost practicality, absorption, metabolism, nutrition, and for clinical use [1]. Additionally, the enzyme-mediated reaction has become the popular methodology for small scale modified lipid production as a result of reaction conditions tend to be milder and fewer side reactions might occur [2].

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In recent years, medium-chain triacylglycerols (MCTs) have been used as permeation enhancers for the delivery of highly lipophilic drugs across the skin. MCTs also act as carriers for fat-soluble vitamins and other actives as well as the oily core of nanocapsules as they work efficiently in drug delivery. MCTs have been reported to possess a higher solvent capacity for drugs than long-chain triglycerides (LCT). MCTs belong to the family of triacylglycerols (TAGs) that consists of caproic acid (carbon-6), octanoic acid (carbon-8), capric acid (carbon-10) and lauric acid (carbon-12), with minor amounts of carbon-6 and carbon-8 [3], [4]. Typical sources of MCT are coconut oil, palm kernel oil (PKO), and dairy milk.

Nowadays, structured lipids (SLs) containing MCTs are used primarily as emulsifiers in cosmetics and numerous human and veterinary pharmaceutical preparations [5], [6]. On the other hand, SLs have been used widely in the medical field as fat or nutritional feeding [7]. SLs that contain medium-chain fatty acids (MCFA) are often a helpful vehicle for fast hydrolysis and absorption because of their smaller molecular size and better water solubility compared to long-chain triacylglycerols (LCTs) [8]. SLs could be produced using lipase as the enzyme catalysts the incorporation of particular fatty acids at specific positions of the triacylglycerol molecules.

In this study, SLs containing MCTs were synthesise by lipase catalysed acidolysis of octanoic acid and the virgin coconut oil (VCO), to give oil with shorter carbon chain length to enhance the skin permeation of active ingredients in cosmetics and pharmaceutical formulations. The 1,3-specific lipase from *Rhizomucor miehei* (RM 1M) was used using hexane as a solvent. The SLs produced was analysed to determine its physicochemical properties such as fatty acids and triacylglycerols composition. The SLs creams containing vitamin E as the model antioxidant were produced and the rheological properties of the creams were tested and compared with the natural VCO-based creams.

II. MATERIALS AND METHODS

A. Materials

Three types of oils used in this study were VCO, structured virgin coconut oil 30% (30SVCO) and structured virgin coconut oil 60% (60SVCO). The 60SVCO was produced by enzymatic acidolysis using the optimised parameters that was previously studied.

The 30% (30SVCO) and 60% (60SCVO) refer to the total amount of octanoic acid in the oils.

The solvents and chemicals used in the study were obtained from different companies in different they were of analytical or HPLC grade. α-Tocopherol (Covitol F-1370) and butylated hydroxyanisole (BHA) were purchased from American Companies namely Cognis Corporation and Sigma Chemical Co., respectively. A German-based company, Merck, supplied methanol, hexane, and tetrahydrofuran while emulsifying wax was provided by Zulat Pharmacy Sdn. Bhd. From Selangor, Malaysia. As for water, deionised water was obtained from Water Reservoir® (Elga Water System, UK).

B. Production of Structured Lipids

The acidolysis of octanoic acid and VCO was performed using the optimised parameters from the CCD results: octanoic acid to VCO ratio of 1.70 (w/w); an enzyme load of 22.60%; at 63.4°C; a water content of 3.53%; and at 96 h. The 1,3 immobilised *Rhizomucor meihei* was used as the biocatalyst. The mixture was incubated at 60°C in an orbital shaking water bath at 200 rpm. All reactions were performed in triplicate.

C. Analysis of Products

First of all, the enzyme used in the acidolysis reaction was cleared after being filtered through a filter paper. An amount of 20 ml of a mixture of acetone/ethanol, 1:1 (v/v) was then added to the reaction products which were already pit into 250 ml conical flasks. This was followed by adding 0.5 N sodium hydroxide (NaOH) to the mixture before titrating it to a phenolphthalein endpoint. In a separating funnel, 25 ml hexane was added to the mixture before mixing them thoroughly. To obtain acylglycerol fraction, the upper hexane layer containing acylglycerols went through a bed of anhydrous sodium sulphate after separating the mixture from the lower aqueous layer. Acylglycerol fraction was obtained after removing hexane at 45℃ using a rotary evaporator. A gas chromatography was used to determine the fatty acid composition of the acylglycerols.

D. Production of Creams

The oil in water (o/w) cream was prepared using the basic formulation which consisted of oil, emulsifying wax and deionized water. For formulations containing the model active ingredient, 5% (w/w) of α-tocopherol was added to the oil phase. Emulsions were prepared by melting the emulsifying wax with the aid of gentle heat (70-75°C). After the wax was completely melted, the oil was added and stirred. Butylated hydroxyl anisole (0.05% w/v) was added to the donor and receptor phases to inhibit oxidation. Then, water at 70°C was added slowly and stirred. The emulsion was stirred using a homogenizer starting from a low rpm to 2000 rpm, until completely homogeneous and cold.

E. Determination of Fatty Acids

To find out the fatty acids profiles, gas chromatography (Agilent Technologies 6890N, Santa Clara, CA) equipped with a mass spectrophotometry detector was employed. A polar capillary column of Zebron ZB-FFAP with an internal diameter measuring 0.25 mm, a length of 30 m and film thickness of 0.25 µm from Phenomenex, Bellefonte, PA, USA was utilized at a split ratio of 1:50 and at a column pressure of 15 psi. The column temperature was increased twice at a different rate and retained for different times. The temperature was set at 50℃ and retained for 2 minutes before increasing the temperature to 180℃ at a rate of 5℃/min and retaining it for another 2 minutes. Finally the temperature was raised to 200℃ at a rate of 8℃ and held for 5 minutes. After the solvent was evaporated, the TAGs species collected were methylated in 0.8 ml of hexane and 0.2 ml of 1 M sodium methoxide. Peak identification process was carried out by looking at the different relative retention times of the standard FAMEs (Sigma Aldrich, St Louis, MO).

F. Triacylglycerols Analysis

HPLC analysis was carried out to separate TAGs species of the oils by using reversed-phased liquid chromatography (RP-HPLC) consisting of an Agilent 2000 controller coupled with a 2414 refractive index (RI) detector. The column used was XDB-18, 4.6 mm x 100 mm x 5 micron. The injection volume was 20 µl of 20% (w/v) oil in chloroform. As the mobile phase, acetone/acetonitrile (63.6:36.5, v/v) at the flow rate of 1 ml/min was used to isocratically elute the samples. The eluting TAGs species emerging from the RI detector were collected automatically for fatty acid analysis by Gas Chromatography-Mass Spectrophotometer (GC-MS). The individual TAGs species were methyl esterified with hexane (0.8 ml) and 1 M sodium methoxide (0.2 ml). For data quantification, TAGs data were calculated as percentage areas. TAGs peaks were identified based on the retention time of TAGs standard purchased.

G. Rheological Measurement

Changes in the rheological properties of o/w cream differing in the type of oil used were studied. Several creams were produced consisting of several MCTs rich oils, namely VCO, 30% structured virgin coconut oil (30SVCO) and 60% SVCO (60SVCO). α-Tocopherol (5% w/w) was added to the three formulations containing active compound while the other three creams were without the active ingredient. Rheological properties of creams were determined by dynamic small-amplitude oscillatory rheology. A rheometer (Rheometer Physica MCR 301, Germany) at 25 ± 0.1°C was used with a 25 mm serrated parallel plate.

In order to measure the rheological properties of the creams, two tests namely the oscillation frequency sweep and complex viscosity were conducted. The oscillation frequency sweep test was done at a low frequency to prevent any alterations in the microstructure during the ensuing evaluations. The angular frequency, ω, in the range of 0.1 to 100 rad/s and 1% strain of a constant deformation were used for the oscillation frequency sweep test. Before the complex viscosity tests were conducted, a survey of the size of the viscoelastic regions at a regular frequency was already done by applying and renewing samples of roughly 1 g to the proximate measurement. This is done as the complex viscosity tests are used to measure the viscosity and elasticity of the straight viscoelastic regions of the formulation. Elastic or storage modulus (G') and loss tangents (tan δ) were the parameters measured.

The loss tangent was estimated using this equation:

$$\text{Loss tangent } (\tan \delta) = \frac{\text{loss modulus } (G'')}{\text{storage modulus } (G')} \quad (1)$$

How elastic the material is measured by the storage modulus while the loss tangent is based on the stickiness of the material. The higher the loss tangent value is, the lesser elastic the material. The measurement started with 1 Pa for a duration of 10 s and this was proceeded by an equilibrium period of 15 s. The inclusion of a pre-shear period prior to each measurement was done to ensure standardised testing.

III. RESULTS AND DISCUSSION

A. Fatty Acids Composition

Table- I shows the fatty acid profiles of VCO before and after modification with octanoic acid by *Rhizhomucor meihei* lipase. The primary fatty acids found in VCO before enzymatic incorporation were dodecanoic/lauric acid, C12:0 (48.21%) and myristic acid, 14:0 (21.07%). The percentages of these acids were comparable with those reported by [11]. After enzymatic incorporation, 12:0 and 14:0 decreased by 29.27 and 11.88%, respectively. The amount of octanoic acid, 8:0 incorporated into VCO was 60.10%.

Table- I. Fatty Acids Composition (%) of Virgin Coconut Oil (VCO) and Structured Virgin Coconut Oil (SVCO) after Acidolysis Analyzed by Gas Chromatography-Mass Spectrophotometer

Fatty acids	Percentage of fatty acids (Mean ± standard deviation)	
	VCO	*SVCO after acidolysis*
Hexanoic acid, C6:0	0.29 ± 0.02	0.12 ± 0.01
Octanoic acid, C8:0	5.45 ± 0.18	60.10 ± 0.25
Decanoic acid, C10:0	4.94 ± 0.08	1.93 ± 0.05
Dodecanoic acid, C12:0	48.21 ± 0.09	18.94 ± 0.15
Tetradecanoic acid, C14:0	21.07 ± 0.05	9.19 ± 0.12
Hexadecanoic acid, C16:0	9.80 ± 0.35	4.52 ± 0.08
Octadecanoic acid, C18:0	3.34 ± 0.12	1.62 ± 0.45
cis-9-octadecenoic acid acid, C18:1	5.86 ± 0.07	3.05 ± 0.34
cis,cis,-9,12-octadecadienoic acid, C18:2	1.04 ± 0.01	0.52 ± 0.44
% Saturated FA	93.10 ± 0.26	96.42 ± 0.46
% Unsaturated FA	6.90 ± 0.11	3.58 0.14

B. Triacylglycerols Composition

To access the composition of TAGs, a deliberate and equivalent carbon number (ECN) was used with the equation ECN = CN-2n. CN corresponds with the number of carbon atoms in the fatty acids whereas n is similar to the number of double bonds in each molecule. The TAGs structure of structured virgin coconut oil is much more complex than that of VCO and some empirical observations can be noted. From a comparison of Table IIa and IIb, SVCO has only 6 fractions with some of the unidentified TAGs. While for VCO, it has 14 fractions with probable TAGs predicted. The prediction was based on the fatty acids determination by GC-MS, the peak of TAGs standards and comparing with the chromatograms in related papers. In VCO, the highest percent area counted was LaLaLa at ECN of 36, followed by LaLaM

Table- IIa. Probable TAGs Species, Calculated Equivalent Carbon Number (ECN) and Percent Area (%) of TAGs Fractions of SVCO Separated by RP-HPLC

Fraction no.	Cal. ECN[a]	Prob. TAGs[a]	Major fatty acids type	Mean Area (%)	Standard deviation
1	24	CpCpCp,	8, 16	30.32	1.33
2	26	CpCCp	8, 10, 12, 14, 16	20.49	0.49
3	28	CpLaCp	8, 12, 16, 18;1, 18:2	17.20	0.26
4	30	CpMCp	8, 12, 14, 18:1, 18:2	17.16	0.29
5	32	CpPCp, CpOCp,	8, 16, 18:1, 18:2	8.96	0.12
6	34	CpCpLa,	8, 12, 16, 18:1, 18:2	5.88	0.13

[a] Based on TAGs standards and literatures

Cp, 8 = caprylic/octanoic acid; C, 10 = capric/decanoic acid; La, 12 = lauric/dodecanoic acid; M, 14 = myristic acid/tetradecanoic; P, 16 = palmitic acid/hexadecanoic; O, 18:1 = oleic/ cis-9-octadecenoic acid; La, 18:2 = linoleic acid

Table- IIb. Probable TAGs Species, Calculated Equivalent Carbon Number (ECN) and Percent Area (%) of TAGs Fractions of VCO Separated by RP-HPLC

Fraction no.	Cal. ECN[a]	Prob. TAGs[a]	Major fatty acids type	Mean Area (%)	SD
1	28	CpCpLa	8, 12, 14, 16	1.67	0.13
2	30	CpCLa	8, 10, 12, 14, 16	4.09	0.10
3	32	CCLa	8, 10, 12, 16, 18:1	12.79	0.09
4	34	CLaLa	8, 10, 12, 14, 18;1	17.34	0.37
5	36	LaLaLa	12, 14, 16, 18:1	20.65	0.09
6	38	LaLaM	12, 14, 16, 18:1	17.59	0.03
7	40	LaLaO	12, 14, 18:1	2.61	0.08
8	42	LaMM	12, 14, 16, 18:1	10.11	0.04
9	42	LaMO	12, 14, 16, 18:1	2.29	0.13
10	42	LaMP	12, 14, 16, 18:0	5.16	0.03
11	44	LaOO	12, 14, 16, 18:1	1.20	0.51
12	44	LaPP	12, 14, 16, 18:1	1.51	0.10
13	44	MOO	14, 16, 18:1, 18:2	1.89	0.16
14	46	POO	14, 16, 18:1, 18:2	0.48	0.06

[a] Based on TAGs standards and literatures

Cp, 8 = caprylic/octanoic acid; C, 10 = capric/decanoic acid; La, 12 = lauric/dodecanoic acid; M, 14 = myristic acid/tetradecanoic; P, 16 = palmitic acid/hexadecanoic; O, 18:1 = oleic/ cis-9-octadecenoic acid; La, 18:2 = linoleic acid

C. Rheological Properties

Rheological measurements offer a simple and effective means for viscoelastic properties of creams. In general, VCO creams with and without α-tocopherol were more viscoelastic than other creams. The values of the storage modulus (G'), which indicate the

elastic behaviour of the material, showed a significant difference between formulations (Table III). The VCO cream with α-tocopherol had the highest value of storage modulus even at lowest frequencies followed by 60SVCO and VCO creams without the active. Similarly, the smaller values of loss tangent (G''/G') were found in 60SVCO and VCO cream without α-tocopherol. Surprisingly, 60SVCO cream with α-tocopherol which had small G' value was small of loss tangent value. According to Marten et. al. (2006), the smaller the loss tangent is the more elastic is the material and it characterises the material's viscous characteristics [9]

Table- III. Results of Oscillation Frequency Sweep Test Analyzed using Rheometer at Room Temperature, 25°C

Cream	Storage modulus, G' (Pa)	Loss modulus, G'' (Pa)	Loss tangent tan δ	Complex viscosity (η)
VCO	1545 ± 33[a]	581 ± 16[a]	0.38[a]	262.50 ± 7[a]
30SVCO	735 ± 17[b]	369 ± 19[b]	0.50[b]	106.60 ± 18[b]
60SVCO	1590 ± 36[a]	326 ± 17[c]	0.21[c]	209.00 ± 19[c]
VCO + α-tocopherol	2075 ± 56[c]	707 ± 26[d]	0.34[d]	282.00 ± 21[d]
30SVCO + α-tocopherol	956 ± 19[d]	415 ± 25[e]	0.43[e]	135.00 ± 19[e]
60SVCO + α-tocopherol	569 ± 16[e]	162 ± 11[f]	0.28[f]	76.10 ± 5[f]

VCO = Virgin coconut oil; 30SVCO = structured virgin coconut oil containing 30% of octanoic acid; and 60SVCO = structured virgin coconut oil containing 60% of octanoic acid

[a to f] Means ± standard deviation, within a column followed by different superscripts are significantly different ($p < 0.05$)

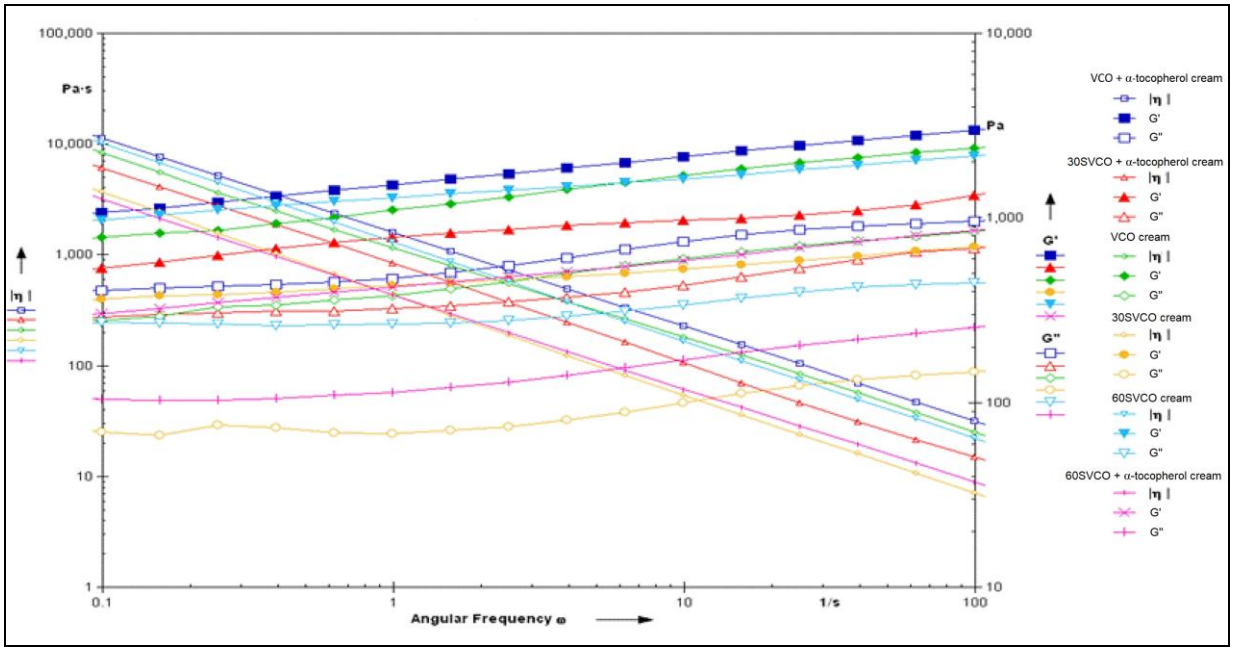

Fig. 1. Values of Storage Modulus (G'), Loss Modulus (G'') and Complex Viscosity (η) in the Oscillation Frequency Sweep Test

VCO = Virgin coconut oil; 30SVCO = structured virgin coconut oil containing 30% of octanoic acid; and 60SVCO = structured virgin coconut oil containing 60% of octanoic acid

The amount of storage modulus revealed a significant difference between VCO cream with α-tocopherol and other creams but there was no significant difference between VCO and 60SVCO creams without the active ingredient. The storage modulus mean level was 2075 ± 56 Pa for VCO cream with α-tocopherol while

for VCO and 60SVCO were 1545 ± 33 and 1590 ± 36 Pa, respectively. The corresponding mean levels of 30SVCO creams with and without α-tocopherol were 735 and 956 Pa, while for 60SVCO with α-tocopherol was 569 Pa.

The oscillation frequency sweep test gave the viscoelastic properties of the creams. The oscillation frequency sweep test showed that VCO creams with and without α-tocopherol and 60SVCO cream had the most viscoelastic structure (Fig. 1). This was due to the highest content of lauric and octanoic acids, the saturated fatty acids in the oils. The elastic characteristic of those creams was also observed at the minimum level. When the frequency increases, the 30SVCO cream was found to possess the most stable storage modulus value, proving its ability to retain its structural form even under external forces. In the oscillation frequency sweep test,

VCO creams with and without α-tocopherol had the highest value of complex viscosity among all creams.

It is important to notice that the behaviour of 30SVCO creams with and without α-tocopherol was not linearly viscoelastic. Therefore the results obtained from these creams were not dependent of the stress used.

The decrease of the saturated fatty acids composition (C12:0, C14:0, and C16:0) in 30SVCO and 60SVCO cream decreased the elasticity of the cream. However, 60SVCO cream without the active showed more elastic behaviour than VCO cream at a lower frequency based on the G' value. This might be due to the longer saturated fatty acids in the VCO cream.

Theoretically, the elasticity of creams increases with an increasing of carbon chain length. Thus the increase in chain length increased the consistency of creams up to a certain limit when the cream starts to revert back to the original two-phase system [13].

IV. CONCLUSION

The production of SLs with high octanoic acid was optimised, with the main fatty acids present were octanoic acid, carbon-8 (60.10%) and dodecanoic acid, carbon-12 (18.94%). Results of TAGs indicated that the most probably TAGs in the structured lipid were CpCpCp, CpCCp and CpLaCp.

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