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RESEARCH ARTICLE

LITERATURE REVIEW ON STRUCTURED LIPIDS AS A HEALTHY ALTERNATE FOR THE CLASSICAL COOKING OILS.

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Abstract

Structured triacylglycerolsLipids differ from conventional triacylglycerols, because the medium-chain fatty acids (MCT) esterified to the glycerol skeleton of Long chain triglycerides (LCT) to yield a new oil called medium –long chain tri glycerides (MLCT). MLCT oils are absorbed in the intestines via portal blood as free fatty acids; absorption pathways are also conventional with long-chain triacylglycerols.

Medium- and long-chain triacylglycerol (MLCT) is a modified lipid containing medium- chain (C6-C12) and long-chain fatty acids (C14-C24) in the same triacylglycerol (TAG) molecule. It can be produced either through enzymatic (with 1,3 specific or nonspecific enzyme) or chemical methods. The specialty of this structured lipid is that it is metabolized differently compared to conventional fats and oils, which can lead to a reduction of fat accumulation in the body. Therefore, it can be used for obesity management. It also contains nutritional properties that can be used to treat metabolic problems. (1) This review will discuss on the previous research and applied work done on the synthesis and application of MLCT, its production methods especially via enzymatic inter-esterification processes and its applications in food industries. The MLCT .oil which is addressed in this thesis is coming under the edible oils & fats categories called **structured lipids** .

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Introduction:- Structured lipids

Structured lipid is a lipid that has been modified from its native form either biologically with enzymes such as lipase or chemically with sodium methoxide as catalyst. These modifications will result in changes in fatty acid composition, fatty acid position in a TAG molecule, physicochemical properties such as melting properties, solid fat content (SFC), oxidative stability, iodine value, viscosity, and saponification number to enhance its functionality. Sometimes, structured lipid with nutritional value can also be obtained through these modifications. Modification of lipid can also be done to produce either zero or low calories lipid to cater for the growing consumers' interest for healthier food and to control the worldwide obesity problem.

In other context (7) ,Memorial University of Newfoundland The term “structured lipid” (SL), very broadly defined, refers to acylglycerols whose fatty acid composition or distribution has been altered by enzymatic or nonenzymatic catalysis, or any of a number of biological or physical methods (8). The products of acidolysis and

transesterification are thus structured lipids. More specifically, this term is often applied to acylglycerols in which some of the long-chain fatty acids at the sn-1 and -3 positions have been replaced by medium-chain length ones, generally caprylic acid (8:0) or capric acid (10:0). In these positions, medium chain fatty acids are readily released from fats and oils in the gut by the action of (1,3-positionally specific) pancreatic lipase and absorbed. Medium-chain fatty acids are directly metabolized for energy rather than deposited as depot fat. Thus, structured lipids containing them provide readily available energy and have a reduced tendency to foster obesity. They are desirable dietary components for those requiring high-density energy sources, such as athletes and individuals recuperating from burns. The presence of some degree of long-chain fatty acid content in SLs is desirable to provide a source of essential fatty acids, especially linoleic acid (18:2). Substantial work has been conducted to investigate and optimize SL production from vegetable oils. Animal fats have also been shown to be substrates for SL production. Thus, the introduction of caprylic acid into the sn-1 and sn-3 positions of an unsaturate-rich fraction of tallow (9),(10) and of unfractionated chicken fat have been described(11)

A structured, lipid-containing dairy fat is covered by a U.S. patent (12) .

The invention relates to a trans-esterification product of a mixture of fatty acids and triglycerides, including milk fat, in the form of cream or butter as the main component. The product has nutritional applications and may also be used as an enteral or parenteral supplement.

Other than MCT oils as an example of structured lipids , the common scientific terminology of Structured lipids means to any TAG in which specific fatty acids have been placed at specific positions on the glycerol backbone of the TAG.(6). This is done in order to confer specific functional, nutritional or medical Properties.

FAO/WHO definition of structured lipids:

Structured lipids (SL) or structured triacylglycerols (ST) may be broadly defined as triacylglycerols that have been altered or restructured using natural oils and fats. The earliest example of ST is the development of medium chain triglycerides (MCT). Using coconut and palm kernel oil, caprylic acid C8:0 and capric C10:0 are liberated. MCT are produced by esterification of these fatty acids with glycerol. The most widely available MCT have a C8:0:C10:0 ratio of 10:30. MCT also have the trade name Captrin®. One of the earliest uses of SL was in enteral and parenteral nutrition, followed by its application in a range of clinical settings including prevention of thrombosis, improved nitrogen balance, and enhanced immune (19) as per FAO foods and nutrition .

How structured lipids (SL) are manufactured ?

Structured lipids can be produced by interesterifying a mixture of conventional fats and oils of interest using chemical or enzymatic methods. Chemical methods provide random distribution of different fatty acids on the glycerol backbone, whereas enzymatic reactions could be position specific, affording controlled production of triacylglycerols with desired configuration (6)

Interesterification involves rearrangement or randomization of acyl residues in triacylglycerols with the fats and oils taking on new properties. ‘Tailored fats’ (fats with specific nutritional or textural properties) are easily obtained during this phase. The raw materials and processing conditions can be controlled or manipulated to produce a fat that has specific desired characteristics. The most widely used class of interesterification in the food industry is trans-esterification, where the ester bonds linking the fatty acids to the glycerol molecule are broken to release the fatty acids. The liberated fatty acids are then randomly shuffled in a fatty acid pool and re-esterified in new positions, either in the same or in a different glycerol molecule.(8)

Applications of structured lipids :

(Geoff Talbot-Oxford brookes university – School of life science) (6)

- Functional uses
- Margarine fats
- Plastic fats for margarine bases
- Hardstocks to structure margarine
- Cocoa butter equivalents
- StOSt TAG
- Nutritional uses
- Enteral and parenteral nutrition
- Combinations of medium chain and long chain fatty acids

- Infant nutrition
- TAG with C16:0 in the sn-2 position to mimic breast milk
- Weight loss – or weight management(2)

Medium chain fatty acids (MCFA):

Medium-chain fatty acids (MCFA) with C6-C12 carbon chain length is more rapidly metabolized than LCFA. Due to its small size and greater solubility compared to long-chain fatty acid (LCFA), MCFA is transported directly to the liver via portal vein to undergo beta oxidation process producing ketones, thus providing a rapid source of energy. MCFA also causes an increase in the diet-induced thermogenesis and satiety(2), (23).

In contrast, LCFA needs to be cycled back into the intestinal lymphatic ducts and transported as chylomicron to the thoracic ducts into the systemic circulation and deposited in the body as fat. As such, medium-chain triglyceride (MCT) has been used for years to treat patients with malabsorption of fat problems and provide instant energy to the athletes. However, as MCT contain solely of MCFA, it lacks essential fatty acid. Besides, MCT is also not suitable to be used for cooking purposes such as frying oil due to foam formation.

Hence, LCFA is incorporated in the MCT molecule to overcome these weaknesses. This has led to the development of a new type of structured lipid, called medium-and long-chain triacylglycerol (MLCT).

In MLCT, each individual triacylglycerol (TAG) molecule contains both the MCFA and LCFA. This can either be produced via lipase-catalyzed reaction of acidolysis, esterification, or interesterification(3), (20), (33).

Lipase, which is nonspecific, will give randomly structured MLCT consisting of 6 configurations, which are MLM, MML, LLM, LML, LLL, and MMM (Figure 1). However, when 1,3 specific enzyme is used, it will give the desired structured MLCT (4).

In the individual MLCT TAG molecule, the LCFA is usually contributed by the common vegetable oil (such as canola, soybean, cottonseed, sunflower, peanut, olive, corn, safflower seed, rice bran, and sesame seed oil) while MCFA is contributed either by medium chain triglyceride (MCT) (such as coconut or palm kernel) or fatty acids such as caprylic acid, respectively. The presence of LCFA helps to increase the smoke point of the MCT, making it feasible for frying purposes (5).

Medium-chain fatty acids (MCFA), mainly C8 and C10, obtained by hydrolysis of coconut oil, is re-esterified with glycerol to form a mixture of randomized medium-chain triglyceride (MCT) through a method developed by Babayan (13). MCTs are absorbed and oxidized rapidly with an energy rated at 34.7 kJ/g. A number of medical and infant food formulations have MCTs as the principal source of fat supplemented with polyunsaturates(14)

In a field study by Intengan et al. (15).

A structured (interesterified) 75 coconut oil–25 corn oil preparation gave better weight gain and nutritional recovery, *vis-a-vis* a polyunsaturated vegetable oil, when given to malnourished children as supplemental fat source in their diet.

Another area of current research is development of structured lipids where GLA is combined with a fatty acid of omega-3 family, preferably EPA or DHA, into one triacylglycerol molecule. Structured lipids can be produced by interesterifying a mixture of conventional fats and oils of interest using chemical or enzymatic methods. Chemical methods provide random distribution of different fatty acids on the glycerol backbone, whereas enzymatic reactions could be position specific, affording controlled production of triacylglycerols with desired configuration (16).

Intesterification using the chemical method usually involves a reaction between two oils using metal alkoxide (sodium methoxide) as a catalyst. The unreacted fatty acids are removed by vacuum distillation. The alternative and more researched process involves acidolysis using lipases. In this process, either pure fatty acid is reacted with a triacylglycerol molecule or relatively rich fraction of fatty acid of interest is taken/prepared before acidolysis reaction. The structured lipids have unique chemical, physical, or physiologic properties that are not observed by simply blending mixtures of the starting fats and oils (17).

Structured lipids containing both omega-3 long-chain PUFAs, possibly from seal blubber oil, or their omega-3 concentrates, and medium-chain fatty acids (MCFAs), which are saturated fatty acids with 6–12 carbon atoms, have been produced. Enzyme-catalyzed synthesis of structured lipids has been proposed, with commercial lipase

preparations. The final products, with reduced calorie, exhibit the combined health benefits of long-chain PUFAs and MCFAs, which are believed to possess many unique nutritional and metabolic characteristics (18).

Reduced-calorie structured lipids are functioning as fat substitutes. For several years, structured lipids with less than 9 kcal/g have been on the market. These engineered lipid molecules contain fatty acids that are less digestible to create a reduced-calorie content. These low-calorie TAGs are characterized by the presence of short-chain fatty acids (SCFAs) or medium-chain fatty acids (MCFAs) and long-chain fatty acids (LCFAs) in a single TAG structure. The caloric content of constituent SCFAs is lower compared with that of their LCFA counterparts.

The products have all the functional properties of full-calorie fats, including their ability to act as carriers for fat-soluble ingredients. Reduced-calorie structured lipids are intended for use in baking chips, coatings, dips, bakery and dairy products, or as cocoa butter substitutes.

Medium-Chain Triacylglycerols (MCTs)

Medium-chain triacylglycerols (MCTs) contain predominantly 8-carbon (caprylic) and 10-carbon (capric) saturated fatty acids esterified to the glycerol backbone of TAGs. The primary sources for MCTs are fractions of coconut and palm kernel oils. Hence, MCTs are manufactured from these oils via hydrolysis followed by fractionation of the resulting fatty acids to concentrate caprylic and capric acids, and re-esterification with glycerol to form new TAGs (73, 74). Caprylic and capric acids comprise more than 96% of the fatty acids in MCT preparations. MCTs are readily hydrolyzed by digestive enzymes, to produce MCFAs. The fatty acid end products are rapidly absorbed into the bloodstream (75). MCFAs do not require carnitine to cross the double mitochondrial membrane of the hepatocyte, thus they quickly enter the mitochondria and undergo rapid β -oxidation. Hence, MCFAs are used as immediate sources of energy by the liver, yielding fewer calories per gram than LCFAs. The gross energy content of MCTs is 8.3 kcal/g vs 9 kcal/g for fat or LCTs. However, the net caloric energy value of MCT preparation is 6.8 kcal/g (76). MCTs are relatively stable at high temperatures and are less susceptible to oxidation (77). MCTs are much more soluble in water than LCTs. MCTs are a translucent and odorless liquid at room temperature. Although completely saturated, it is not solid in consistency like other saturated fats, because of the shorter chain lengths of the fatty acids within the oil. MCTs were originally developed for therapeutic purposes to provide a source of energy for individuals with compromised gastrointestinal systems; thus, they are beneficial for AIDS and cancer patients, premature infants, burn victims, and individuals with shortened bowel syndrome. MCTs have been used extensively in the manufacture of parenteral and enteral nutrition formulations. MCTs are beneficial for such applications because they are more readily hydrolyzed and metabolized much more rapidly than LCTs. MCTs have specific nutritional applications such as infant formulas, energy bars and drinks, geriatric preparations, and sports nutrition products. In addition, they are used as carriers for colors, flavors, and vitamins and provide gloss and prevent sticking on confectionery products. It is reported that feeding diets, wherein a fat source of LCTs was replaced with MCTs, to laboratory animals and humans resulted in decreased body weight gain and reduced fat deposition (78, 79). Such results have led to the suggestion that replacing conventional source of dietary fat, which composed primarily of LCTs, with MCTs may yield food with lower caloric content. In the sports world, MCTs have been positioned as an easily absorbed and oxidized fuel source and have been marketed to bodybuilders and athletes as a fat source that is less likely to deposit as body fat.

Safety of MCTs in direct food application:

The safety of medium-chain triacylglycerol (MCTs) in dietary oil has been debated, and associated effects on cholesterol metabolism remain unclear. Although some studies have shown that MCTs are essentially nontoxic, noncarcinogenic, and nonmutagenic for human consumption with a safety level up to 1 g/kg (58), other studies have indicated that MCT oil-containing diets can increase blood cholesterol levels (59). MCTs, on a percent energy basis, have half the potency of palmitic acid (C16:0) in raising plasma cholesterol (59). Palmitic acid (C16:0) can lead to increases in blood cholesterol levels; however, when ingested in a diet that contains a recommended intake of C18:2, n-6, the effect on both total and LDL cholesterol levels are minimized (60). This has been shown with fat blends, such that hypercholesterolemia was not observed in animals fed either butter or tallow fat sources that were blended with soybean oil in a low-cholesterol-containing diet (61, 62). In gerbils and monkeys, the relative ratio between C14:0 to C18:2 n-6 fatty acids as well as dietary cholesterol are important factors in modulating increases in serum cholesterol levels (63, 64).

Intake of saturated fat sources has also been associated with insulin resistance, leading to altered glucose metabolism, type II diabetes, and impaired glucosetolerance(65). Comparatively, saturated fat has a more deleterious effect on fat-induced insulin sensitivity than both mono- and polyunsaturated fat sources (65). Higher intakes of saturated fat and trans-fat adversely affect glucose metabolism and insulin resistance, whereas higher intakes of polyunsaturated fat and possibly long-chain n-3 fatty acids are beneficial (66). Within the category of saturated fats, dietary saturated, short-chain, and $\omega 6$ fatty acids have been found to have the most deleterious effects on insulin action associated with insulin sensitivity, as opposed to medium- and long-chain fatty acids and $\omega 3$ fatty acids (67). Intramuscular triacylglycerol (MTG) and elevated plasma free fatty acid (FFA) levels also have roles in insulin-mediated glucose uptake, reflecting a pivotal role of the high saturated fatty acid content in the MTG (68). Changing dietary fat quality by substituting saturated for monounsaturated fat can impair insulin sensitivity, as saturated fat has a greater deleterious impact on insulin sensitivity (69). For example, substituting a monounsaturated fatty acid diet (MUFA diet) for a saturated

fatty acid diet (SAFA diet) has been shown to be favorable for only those subjects that had a lower-than-average total fat intake. This intervention improved insulin sensitivity, but had no effect on insulin secretion. Notably, the addition of n-3 fatty acids to MUFA and SAFA diets affected neither insulin secretion nor insulin sensitivity (69).

Food applications of MCFA/MCT :

Medical and Infant Food Formulations:

Medium-chain fatty acids (MCFA), mainly C8 and C10, obtained by hydrolysis of coconut oil, is re-esterified with glycerol to form a mixture of randomized medium chain triglyceride (MCT) through a method developed by Babayan(70). MCTs are absorbed and oxidized rapidly with an energy rated at 34.7 kJ/g. A number of medical and infant food formulations have MCTs as the principal source of fat supplemented with polyunsaturates(71). In a field study by Intengan et al. (72) a structured (interesterified) 75 coconut oil–25 corn oil preparation gave better weight gain and nutritional recovery, vis-à-vis a polyunsaturated vegetable oil, when given to malnourished children as supplemental fat source in their diet.

Medium –long chain triglycerides (MLCT)Oils :

MLCT oils is an abbreviation for medium long chains fatty acids triglycerides .Medium-chain fatty acids (MCFA) with C6-C12 carbon chain length is more rapidly metabolized than long chain fatty acids LCFA ,due to its small size and greater solubility compared to LCFA , MCFA is transported directly to the liver via portal vein to undergo beta oxidation process producing ketones, thus providing a rapid source of energy. MCFA also causes an increase in diet-induced thermogenesis and satiety (2). In contrast, LCFA needs to be recycled back into the intestinal lymphatic ducts and transported as chylomicron to the thoracic ducts into the systematic circulation and deposited in the body as fat. As such, medium-chain triglyceride (MCT) has been used for years to treat patients with malabsorption of fat problems and provide instant energy to the athletes. However, as MCT contain solely of MCFA, it lacks essential fatty acid. Besides, MCT is also not suitable to be used for cooking purposes such as frying oil due to foam formation.

Hence, LCFA is incorporated in the MCT molecule to overcome these weaknesses. This has led to the development of a new type of structured lipid, called **medium-and long-chain triacylglycerol (MLCT)**.

In **MLCT**, each individual triacylglycerol (TAG) molecule contains both the MCFA and LCFA. This can either be produced via lipase-catalyzed reaction of acidolysis, esterification, or interesterification(3);(21); (20). Lipase, which is nonspecific, will give randomly structured MLCT consisting of 6 configurations, which are MLM, MML, LLM, LML, LLL, and MMM.

However, when 1,3 specific enzyme is used, it will give the desired structured MLCT (4).

In the individual **MLCT** TAG molecule, the LCFA is usually contributed by the common vegetable oil (such as canola, soybean, cottonseed, sunflower, peanut, olive, corn, safflower seed, rice bran, and sesame seed oil) while MCFA is contributed either by medium chain triglyceride (MCT) (such as coconut or palm kernel) or fatty acids such as caprylic acid, respectively.

The presence of LCFA help to increase the smoke point of the MCT, making it feasible for frying purposes .

Numerous studies reported that this MLCT can restrain the accumulation of body fat, reduce cholesterol, and blood triglyceride upon consumption (22);(36);(37);(38).

MLCT oil also has myriads of applications in the food industry as home cooking oil, salad dressing, vegetable-oil spreads, dietary supplement, and frozen dinner.(1)

Synthesis of MLCT oil as a structured food:

MLCT is synthesized through an enzymatic reaction or chemical inter-esterification .

Various lipase-catalyzed enzymatic approaches have been studied to determine the best method of getting high MLCT yield. MLCT can be produced via enzymatic process in 3 routes:

(1) Interesterification

(2) Acidolysis

(3) Esterification

Small scale and large scale production of it have also been studied to find out the optimum conditions for producing best yield of MLCT, thus providing useful information for MLCT production to the industries.

Interesterification

Interesterification is the reaction between esters or TAG molecules. Not much research has been reported on MLCT production via interesterification reaction. For MLCT production, interesterification often involved the coconut oil or palm kernel oil or saturated TAG such as tricaprylin that will provide the MCFA. LCFA part in MLCT is contributed by the vegetable oil such as soybean oil, rapeseed oil (Fomuso and Akoh 1998; Lopez-Hernandez and others 2005; Adhikari and others 2011b)(24&3 & 25).

The progress of the interesterification reaction is measured by the changes in the TAG composition before and after the reaction .

For interesterification, substrate ratio is an important parameter that will affect the desirable yield. For example: substrate molar ratio of trilinolein to tricaproin from 1 : 1 to 1 : 4, the mole ratio of 1 : 2 gave the highest yield of 50.7% dicaproyllinolein (ECN 33) and 23.6% monocaproyldilinolein (ECN 45) (Fomuso and Akoh 1998)(24).

Acidolysis:

Acidolysis involved the exchange reaction between acyl moiety of acylglycerol and a free carboxylic acid. For MLCT production via acidolysis reaction, the acylglycerol mostly came from native oils such as soybean oil, canola oil, lard, fish oil, sesame oil, borage oil, menhaden oil, and chicken fat (Lee and others 1999(4) Xu and others 2000, Kawashima and others 2001, 2002; Kim and Akoh 2006; Zhao and others 2007; Li and others 2008; Shuang and others 2009)(27-33). These native oils were used in acidolysis as it contained essential fatty acids like, linoleic acid (LA), alpha-linolenic acid (ALA), eicosapentanoic acid (EPA), docosahexanoic acid (DHA), and gamma-linolenic acid (GLA) that impart good health to our body. However, tripalmitin, tristearin, and triolein also can be used as the acylglycerol for this reaction (Sellappan and Akoh 2000; Yankah and Akoh 2000).(34);(39).

As for the free carboxylic acid, the most commonly used is the caprylic acid (CA). Little has been done on capric or lauric acid (Sellappan and Akoh 2000;(34) Nunes and others 2011)(35). Summary of research done on MLCT production via acidolysis is summarized by Yee-Ying Lee, Teck-Kim Tang, and Oi-Ming Lai.(1) , in their excellent review summary of MLCT in food applications .

Literature shows that parameters such as substrate ratio, residence time, temperature, enzyme load, water content have to be taken into consideration when producing MLCT -Kim and Akoh 2006;(30), Zhao and others 2007;(31), Li and others 2008;(32) Shuang and others 2009;(33) Nunes and others 2011)(35). These parameters are not only important to be considered when running in flasks for small scale experimental purposes, but also for large scale production in either packed bed reactor or stirred tank reactor (Xu and others 2000;(27) Kawashima and others 2002)(29).

Besides, choosing the right type of enzyme is important when running acidolysis reaction as different types of enzyme will affect the yield of MLCT. For acidolysis reaction to produce MLCT, 1,3 specific immobilized enzyme Rhizomucor meihei (RM IM) (Novozyme, Bagsvard, Denmark) lipase is commonly used compared to 1,3 specific enzyme TLIM though sometimes nonspecific enzymes such as Rhizopus oryzae and Rhizopus

delemar lipase may be used. 1, 3 specific enzymes will make sure that the essential fatty acid is maintained in the sn2 position, creating the desired structured type of MLCT (Sellappan and Akoh 2000; (34) Yankah and Akoh 2000; (39) Foresti and Ferreira 2010; (40) Nunes and others 2011) (35).

When RMIM enzyme is utilized for acidolysis reaction, it normally gives around 40 to 50 mol% of CA incorporation at reaction times of around 20 to 24 h (Akoh and Moussata 2001; (41) Kim and Akoh 2006; (30) Li and others 2008; (32) Shuang and others 2009) (33).

As for *Thermomyces languninosus* (TLIM) (Novozyme, Bagsvard, Denmark) enzyme at the same duration time, 20 h, and only 27.01 mol% of CA can be incorporated into soybean oil. However, when organic solvents such as hexane and isooctane are added in the reaction, TLIM will give a 50.14% of CA incorporation (Zhao and others 2007) (31).

The enzyme load that was normally used is in between 5% and 10% (w/w). At least 24 h of residence time were required for a maximum CA incorporation. Besides 1-step acidolysis reaction discussed previously, there is a 2-step enzymatic process for MLCT production. For this method, the 1st step involved the production of TriPUFA from EPA, DHA, and AA via esterification with glycerol at 3 : 1 mol/mol using *Candida antarctica* (Novozyme, Bagsvard, Denmark) enzyme for 24 h. The TriPUFA of the following (1) 89% of γ -linolenic acid (GLA), (2) 89% of arachidonic acid (AA), (3) 88% of eicosapentaenoic acid (EPA), and (4) 83% of docosahexanoic acid (DHA) then undergone acidolysis with caprylic acid via *Rhizopus delemar* lipase (Ta-lipase Tanabe Seiyala Co. Ltd., Osaka, Japan) for 48 h producing acylglycerol containing CA. This 24-h acidolysis step was carried out consecutively for 3 cycles to increase the incorporation of CA. The repeated acidolysis reaction managed to increase the amount of CA incorporated in each of the TriPUFA with TriE 66%, TriA 63.8%, TriG 52.6%, and TriD 31.1%. MLM type structured lipid also increased with the 3 repeating acidolysis steps (diCA 86.5%, diCE 85.7%, diCD 62.6%, and diCE 32.3%).

This process gives the highest yield of MLCT among all the other acidolysis methods carried out by other researchers. In this study, the enzyme can be reused up to 10 cycles in esterification step and 20 cycles in acidolysis step, respectively, reducing the cost of operation (Kawashima and others 2001) (28). However, the concern about this method is that it is time consuming compared to the normal 1-step acidolysis method in which the first esterification step needed 24 h and the 3 consecutive acidolysis steps each required 48 h to be carried out. Substrate mole ratio of fatty acid/glycerol also significantly affects the CA incorporation. Increase in the amount of fatty acid will lead to an increase in the CA incorporation (Li and others 2008) (32). However, too much of the fatty acids will make the medium too acidic, thus inactivating the enzyme. Furthermore, the use of higher amounts of fatty acids will also incur higher cost of production. Thus, it is important to get the optimum substrate mole ratio for acidolysis reaction to obtain a high yield of MLCT. Temperature is a crucial parameter as it will affect the activity of enzyme, solubility and viscosity of the substrate. Temperature for acidolysis reaction normally falls in the range of 40 to 65 °C. However, recently, novel cold active lipase from *Pichia pastoris* NRRL Y-7223, (culture collection of National Centre for Agricultural Utilization Research, Peoria IL, USA) was found to have comparable activity with RMIM at 20 °C. This *Pichia pastoris* NRRL Y-7223 cold lipase gives 47.5% of CA incorporation on the borage oil at 20 °C, which is comparable to the RMIM with 45.7% incorporation at 40 °C (Kim and others 2010) (42). Lower temperature for acidolysis reaction is preferable especially for structured lipid that contained PUFA such as fish oil, borage oil, GLA from evening primrose oil, which is susceptible to oxidation. Hence, it is necessary to maintain the temperature as low as possible during storage or reaction. Besides, lower temperature uses less energy consumption. However, this is not suitable to be applied to TAG production that has higher melting points such as palm kernel olein as this TAG will crystallize during the reaction.

The drawback of this acidolysis reaction is that a high ratio of fatty acids especially short chain and MCFA, which are easily soluble will create acidic condition in the reaction. This will inactivate the enzyme and restrict its reaction, leading to a low yield of MLCT.

Esterification

Esterification is another alternative in the synthesis of MLCT. However, not much research has been done on the esterification reaction of MLCT production due to the high cost of fatty acids and glycerol. In esterification process, the desired fatty acid (such as oleic acid, stearic acid, capric acid, and so on) is made to react with glycerol in the presence of the enzyme lipase. To drive the reaction forward towards synthesis, vacuum pump or nitrogen gas purging is used to remove the water formed during the esterification reaction. Esterification process is a much more preferable method to produce a higher concentration of MLCT as it gives a high purity of MLCT and few

unnecessary TAG, FFA, and MAG as compared to acidolysis. However, the drawback from this method is that it does not contain any residual natural antioxidants. Thus, natural antioxidants such as rosemary extract, sage extract, or chemical antioxidant such as tert-butyl hydroquinone (TBHQ), butylated hydroxyanisole (BHA) has to be added to increase the oxidative stability of this oil (Koh and others 2009)(43).

Koh and others (2010)(20) and Arifin and others (2011a, 2012)(44-46) managed to produce MLCT oil via esterification reaction. Both studies use the same RMIM enzyme and same MCFA (capric acid) but different LCFA, with the former using oleic acid and the latter using stearic acid as their substrate. Both studies optimized the conditions for MLCT production using response surface methodology. Koh and others (2010)(20) managed to get 58 wt% of MLCT oil under optimized conditions of 13.6 to 14 h reaction time, 7.9% to 8% for enzyme load, and 3 : 1 for fatty acid/glycerol molar ratio. This is comparable to that reported by (Arifin and others 2011a, 2012)(44-46) who obtained 59.76 wt% of MLCT oil with 10% enzyme load, 70 °C reaction temperature, 14 h reaction time, and 3.5 : 1 substrate molar ratio. Purity of MLCT increases after undergoing refining process. Refining will remove the unnecessary matters like free fatty acids, and unsaponified matter (Kawashima and others 2001; Koh and others 2010; (20) Arifin and others 2011a)(44). For example, after refined, bleached, and deodorization (RBD) processes, the MLCT content increased from 59.76% to 76% (Koh and others 2010)(20). Hereafter, the schematic illustration of Lipase reaction with triacylglycerols in different reaction mechanisms.

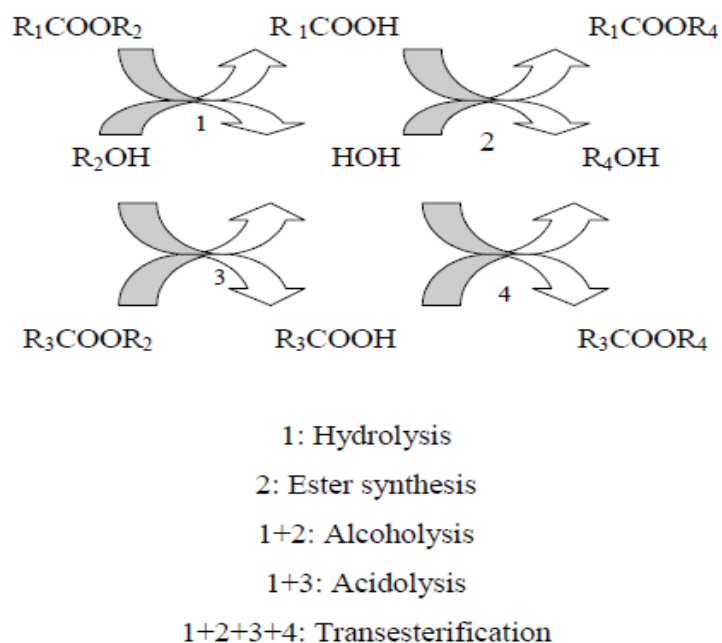


Figure 2.3. Schematic illustration of lipase reactions (Balcao et al., 1996).

Structured oils based on MLCT application in Food Industries

Fats and oils give palatability to a food product and cannot be totally eliminated in our food. As some foods are high in fat, consumption of it can be detrimental to health. As such, MLCT is seen to have potential to prevent body weight gain and body fat accumulation when used to replace the conventional fats/oils for food production especially those that required high amount of fat. There are several studies that tested on the application of MLCT in food products.

Cooking oil

The first commercialized MLCT was produced by Nisshin Oil Group Ltd. (Japan) and are sold widely as cooking oil in Japan and United States with the name Resetta. It is stable for 30 min in 200 °C. Koh and others (2009)(43) found that MLCT when blended with soybean or palm olein, can be used for cooking purposes especially frying. This

is because, the presence of long chain fatty acid from soybean oil and palm olein such as oleic acid (C18), linoleic acid (C18:1), and linolenic acid (C18:3) increases the blended MLCT oil's smoke point. For example, the smoke point of MLCT blended with palm olein (225 ± 1.41 °C) and soybean oil (229 ± 1.41 °C) at ratio 1 : 1 were much higher compared to the control, which is the unblended MLCT oil (210 ± 0 °C). Apart from increasing the smoke point, blending with soybean or palm olein also helped to reduce the production cost of MLCT. Koh and others (2011) (5) also demonstrated that the MLCT added with antioxidants has a higher thermal resistant oxidative strength (above 180 °C) than RBD palm olein, lighter in color and lower free fatty acid content, thus having the characteristic required for deep frying oil. Sensory test showed that there is no difference in terms of taste and rancidity assessment in potato chip fried with MLCT oil and those with palm olein. Jennings and others (2010) (47) reported that rice bran oil structured lipid (RBOSL) consisting primarily of CA at the sn-1,3 position and oleic and linoleic acid at the sn-2 position can be used in frying sweet potato chip (SPC) at 165 to 185 °C for 20 to 60 s. The color variable, smoke point, foaming ability, and γ -oryzanol concentration showed no significant difference between RBOSL and RBO after frying. However, RBOSL tends to have a lower viscosity and oil uptake compared to RBO after frying.

Energy bars

Jennings and others (2010) (47) also used 13.27% of the RBOSL to make energy bar (EB), which was baked at 176 °C for 30 to 40 min. Sensory evaluation showed a significant difference in energy bar prepared with RBOSL and RBO. RBO EB has a softer texture compared to RBOSL EB. In addition, the willingness to purchase (WTP) of EB made from RBOSL was 60% indicating that 60% of the consumers accepted the RBOSL EB. This indicates that the product prepared with SL has a bright future to be commercialized (Jennings and others 2010). (47)

Butter fat

MLCT made via Rhizomucor meihei lipase trans-esterification of 57.7% of capric acid and canola oil when blended with butterfat improved the cold spreadability of the pure butter. Triangle test showed that 23 out of the 30 panelists were able to detect the difference between the pure butter and blended butter due to the cold spreadability between samples. In terms of flavor, there is not much difference between both pure butter and butter blended with structured blended butter fat. Structured blended butter fat also has a higher atherogenic index than pure butter (0.07), which can counterbalance the attribute of hypercholesterolemic of pure butter. As such, MLCT showed potential to be used as substitute for canola oil in making cold spreadable butter (Kim and Akoh 2006). (30).

Margarine and shortening

Both margarine and shortening are visco-elastic semi solid food products. Margarine is water in fat emulsion, which consists of 80% fat and 20% water. In contrast, shortening is any fat that is solid at room temperature and used to shorten baked products such as making crumbly pastry. Palm stearin is hard stock that is usually blended with soft oils such as soybean oil, canola oil, sunflower oil, and cotton seed oil through enzymatic interesterification process to produce trans free plastic fats as well as to improve the SFC of the product. The presence of palm stearin helps to enhance the plasticity and maintain the shape and structure of the product to withstand temperature fluctuation (Nor Aini and Miskandar 2007). (48) MLCT can be used to prepare shortening and margarine. Arifin and others (2011b) (45) showed that MLCT produced from lipase esterification of stearic acid, capric acid, and glycerol at 3 : 1 for fatty acid/glycerol molar ratio is suitable to be a functional hard stock in shortening and **margarine that can be used for obesity management** purposes as it has a high SFC at 25 °C. Besides, MLCT produced from interesterification between a hard stock (fully hydrogenated soybean oil) and soft oil (rice bran oil, coconut oil) give the newly formed oil plasticity property suitable to be made into margarine and shortening (Adhikari and others 2011a). (26) Similarly, (Zhang and others 2010) (38) studied the production of margarine using Lipozyme IM lipase-catalyzed interesterification of palm stearin and coconut oil (75/25, w/w) in 1 kg batch stirred tank reactor. For baking purposes, shortening should have a SFC of 15% to 25% at ambient temperature (25 °C). Arifin and others (2011a) (44) carried out a study on the binary (MLCT: palm stearin) and ternary (MLCT: palm stearin: palm olein) blends of MLCT to be used as shortening for baking. Blending with other oils will help to reduce the production cost of MLCT. From the study, increasing the MLCT from 40% to 90% causes a reduction in the SFC of the MLCT-enriched formulation. All these shortenings have melting points of 55 °C. Binary blends of MLCT and palm stearin in the ratio 70 : 30, 80 : 20, 90 : 10 and ternary blends of MLCT, palm stearin, and palm olein with ratio 40 : 40 : 20, 50 : 40 : 10, 50 : 30 : 20 fulfill the 15% to 25% SFC requirement for shortening at 25 °C. Therefore, they are suitable to be used as shortening for baking purposes. Quantitative descriptive analysis (QAD) showed that Madeira cakes made from

these MLCT have better taste and aroma than the commercial shortening. The acceptability test in terms of taste, texture, and overall acceptability showed a higher degree of liking for Madeira cakes made of 50 : 30 : 20 ratio of MLCT, palm stearin, and palmolein. MLCT-enriched shortening also has a higher SFC compared to local commercial shortenings made from soybean, sunflower, and hydrogenated palm oil.

Beverages

Canola oil is commonly used in preparing beverages. Canola oil-based structured lipid is of importance so that the manufacturer does not need to change the formulation when replacing canola oil with canola oil-structured lipid. Triangle test showed that 23 out of 38 participants managed to distinguish the difference between the chocolate beverages made of canola oil-based structured lipid and chocolate beverages made of canola oil. Structured lipid beverage is sweeter (2 times sweetness intensity) and has less bubble formation than the canola oil beverages. However, not much difference was observed in terms of other attributes such as texture, aroma, aftertaste, and color (Osborn and others 2003). (49) The presence of CA acts as a flavor carrier that helps in transporting the flavor component. This increases the sweet taste in the SL containing chocolate beverages. This beverage is useful for those who require a rapid source of energy. It can also provide essential fatty acids.

Coating lipid

In the food industry, coating a layer of polysaccharides, protein, or lipid on to a food product is important to prolong the storage lifespan and maintain the quality of the products as it prevents the diffusion of moisture, air, and aroma to enter or escape from the food. Lipid with its hydrophobic property and giving a glossy appearance is most preferred compared to protein and polysaccharides as coating material. Acidolysis of the tristearin with lauric acid and oleic acid in substrate ratio of 1 : 4 : 1 using RMIM lipase was better in inhibiting moisture than cocoa butter when applied on cracker. This may be due to the compactness and rigidity of the structured lipids. This structured lipid has a sharp melting point of 31.4 °C. Addition of lauric acid and oleic acid will help to regulate the melting profile of tristearin to be in the range of 30 to 37 °C, which is suitable for coating applications (Sellappan and Akoh 2000). (34)

Nutrient admixtures

MLCT oil can be used in nutrient admixtures. It helps to increase the storage stability of the nutrient admixtures. When 20% soybean oil was replaced with 20% MLCT oil in nutrient admixtures, kinetic stability test showed that the mean droplet size (300 to 400 nm), surface tension value remained unchanged for MLCT oil throughout the 10 d of storage period at both 2 to 7 °C and 37 °C. However, soybean oil nutrient admixtures stability starts to deteriorate starting Day 4 (Balogh and others 2005). (50).

Parenteral nutrition

Structolipid 20% is a parenteral type structured fat emulsion that was produced from Pharmacia/Upjohn, Uppsala, Sweden. It is made up of soybean oil and purified coconut oil in a ratio of 36 : 64, w/w, that was interesterified. Study revealed that this structolipid has similar properties as the control, which was LCT (Intralipid 20%) of soybean oil triglyceride. For example, no difference was observed in the plasma lipid (triglyceride, total and free cholesterol, phospholipid, free fatty acid) between those subjects consuming LCT and structolipid as well as on its clinical safety. Besides, structolipid 20% showed possible reduction in liver dysfunction as the liver problem in 2 patients resolved after switching to the structolipid 20%. The presence of soybean oil in the structured lipid will provide essential fatty acids, not seen in the commonly used MCT parenteral (Rubin and others 2000). (52)

Health benefits of MCT & MLCT in dietary food application

Medium-chain fatty acids (MCFAs) comprise saturated fatty acids with 6–10 carbons. Besides synthetic medium-chain triglyceride (MCT) oils there are natural sources, like coconut oil and dairy fat. Compared with long-chain fatty acids (LCFAs), the chemical and physical properties of MCFAs show substantial metabolic differences. MCFAs do not require binding to proteins such as fatty-acid binding protein, fatty acid transport protein, and/or fatty acid translocase (FAT, homolog to human platelet CD36). MCFAs are a preferred source of energy (β-oxidation). MCFAs are also incorporated into adipose tissue triglycerides, and may influence adipose tissue and other systemic functions more substantially than previously assumed. MCTs reduce fat mass, through down-regulation of adipogenic genes as well as peroxisome proliferator-activated receptor-γ. Recent studies confirmed the potential of MCFAs to reduce body weight and particularly body fat. This effect was not transient. MCFAs reduce lipoprotein secretion and attenuate postprandial triglyceride response. It was, however, frequently observed that MCTs increase fasting cholesterol and triglyceride levels. But, given in moderate amounts, in diets with moderate fat supply,

MCFA's may actually reduce fasting lipid levels more than oils rich in mono- or polyunsaturated fatty acids. The same is true for glucose levels. MCT's improved several features contributing to enhanced insulin sensitivity. Under certain in vitro conditions, MCT's exert proinflammatory effects, but in vivo MCT's may reduce intestinal injury and protect from hepatotoxicity. Berit Marten, et al. (80)

Antiobesity Effect of MLCT

The benefits of MLCT oil is that it can act as functional oil that can prevent fat accumulation in our body. Various clinical and preclinical studies have been carried out so far regarding the antiobesity effects of MLCT and this will be discussed here. Most animal and human trials on MLCT were based on the effect of Nisshin Ollio Group's MLCT oil, a randomly structured MLCT made by interesterification of MCT and soybean oil to suppress body fat and body weight (Matsuo and others 2001; (52) Kasai and others 2003; (22) Matsuo and Takeuchi 2004; (36), Shinohara and others 2005; Shinohara and others 2006). (53-54) Regarding the body composition, MLCT when tested on human, was shown to be able to reduce the body weight and body fat accumulation (Matsuo and others 2001; (52) Kasai and others 2003) (22). For example: consumption of 200 g of MLCT (10% w/w of MCFA) containing liquid diet twice daily for 12 wk in 13 healthy human male subjects aged 18 to 20 y caused a lower mean weight gain and body fat percentage (0.91 kg and 2.16%) compared to those consuming soybean (1.83 kg and 4.30%) (Matsuo and others 2001) (52). A thorough study demonstrated that reduction of body fat (either subcutaneous or visceral) can be seen particularly in the waist circumference (4.6% loss), hip circumference (2% loss), as well as body weight (6.1% loss) when MLCT is consumed. This study is performed under strict calories diet on 82 healthy human subjects of 21 to 59 y for 12 wk when 14 g MLCT (12%, w/w, of MCFA) is consumed at breakfast (Kasai and others 2003). (22) Overweight and obese Chinese human subjects when consumed 25 to 30 g/d of MLCT (13% w/w of MCFA) for 28 d under strict diet regime showed significant decrease in their body fat. Overweight people with body mass index (BMI) of 24 to 28 has more significant decrease compared to obese people with BMI more than 28 (Zhang and others 2010). (38) In another study, MLCT upon consumption for 8 wk at 25 to 30 g (13%, w/w, MCFA) daily in Chinese hypertriglyceridemic (1.7 to 4.5 mmol/L) subjects with ages less than 60, showed the ability to lower the body weight and body fat accumulation. However, MLCT was shown to have no effect on subjects that are more than 60 y old (Xue and others 2009). (55) As for rats, the outcome on the effect of MLCT in reducing body fat and weight vary among studies. When 48 male Wistar rats (Japan SLC Inc., Shizuoka) fed ad libitum with MLCT in the amount of 150 and 200 g/kg diet (20% of MCFA) for 8 wk, they were found to have a lower body weight and total intraabdominal adipose tissue. However, MLCT in the amount of 50 or 100 g/kg diet was not able to decrease the body weight gain or body fat (Matsuo and Takeuchi 2004). (36) Also, MLCT in 70 g/kg diet (12% MCFA) showed no effect on reducing both the body weight and total intra abdominal tissue in Sprague-Dawley rats (Japan SLC Inc., Shizuoka) for a duration of either 2 or 4 wk (Shinohara and others 2005). (53) The previous studies reported were tested using Nisshin Ollio Group's MLCT oil. Besides, the Nisshin Ollio Group's MLCT, MLCT that has been incorporated with fish oil was also shown to have lower body weight gain in ICR mice (Harlan Sprague Dawley, Indianapolis, IN) compared to control group fed with soybean oil with the former having gain of 5.8% and latter of 11.4%, respectively (Lee and others 1999). (4) Besides the previously mentioned studies, there were also studies related to the enzymes that are involved in the metabolism of fatty acids and these were conducted on animal models consuming MLCT. It is shown that 30 min after administration of MLCT and LCT, Wistar rats (Japan SLC Inc., Shizuoka) fed with MLCT have a higher activity of the hepatic fatty acid oxidation enzyme than those fed with LCT, demonstrating that MLCT is oxidized more rapidly than LCT in the liver (Shinohara and others 2002). Examples of such enzymes include short chain acyl coA dehydrogenase, medium chain acyl coA dehydrogenase (ACAD), citrate synthase, cytochrome oxidase, carnitine palmitoyltransferase (CPT). Meanwhile, the activity of lipogenic enzyme is not affected by MLCT (Lee and others 1999; Shinohara and others 2002, 2005, 2006; (57) (53-54) Matsuo and Takeuchi 2004). (36) It was found that no caprylic acid is detected in the liver of the group of mice fed with MLCT for 21 d, further supporting that the incorporation of MCFA will provide a quick source of energy as they are rapidly metabolized (Lee and others 1999) (4). Also, diet induced thermogenesis (DIT) of MLCT in 21 adults human showed that 6 h after taking a diet consisting of MLCT, energy expenditure increased by 14 kcal (Ogawa and others 2007). (57) Safety evaluation also showed that acute dosage of MLCT at 5000 mg/kg and subchronic study for 6 wk at 3500 mg/kg showed no signs of toxicity of MLCT in Wistar rats (Japan SLC Co.Ltd., Hammamatsu). MLCT is also non-mutagenic when tested (Matulka and others 2006). (37) Numerous studies have been done on the effect of MLCT on the blood composition of either rats or humans. (Table 1) To sum it up, most of the studies clearly showed that MLCT oil consumption can help to reduce body fat accumulation and body weight gain. Nonetheless, MLCT was found to be more effective as anti-obesity functional oil on humans as compared to animals. This may be due to the short duration of the preclinical studies that were unable to show the effect of MLCT. MLCT effect on blood parameters on human and animal's studies were also not consistent. Some were found

to improve the blood lipid and blood cholesterol levels while others did not. As such, the effect of MLCT on blood lipid and cholesterol level still remained unclear.

Conclusions:-

From the health benefits point of view, MLCT not only can provide us with nutritional properties from the essential fatty acids incorporated, but most importantly it can also help to reduce body weight and body fat accumulation in the body. However, at least 12% of MCFA must be present in the product to see the beneficial effects. As such, including MLCT into our diet is 1 way to curb the increasing rise of worldwide obesity. As for the enzymatic production, among the 3 enzymatic processes discussed, (interesterification, esterification, and acidolysis), esterification gave the highest yield of MLCT though it may be costly when produced in large scale due to the substrates used. More studies need to be carried out to find more MLCT application in the food and industries. MLCT as functional lipids is gaining its momentum in recent fats and oils industries. It may be the next generation "potion" to be included to our diet.

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