GRASAS Y ACEITES, 60 (2),
ABRIL-JUNIO, 168-176, 2009,
ISSN: 0017-3495
DOI: 10.3989/gya.074708

Incorporation of medium chain fatty acids into fish oil triglycerides by chemical and enzymatic interesterification

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RESUMEN

Incorporación de ácidos grasos de cadena media a triglicéridos de aceite de pescado por interesterificación química y enzimática.

Triglicéridos estructurados (SL) conteniendo ácidos grasos de cadena media (MCFA) y ácidos grasos poliinsaturados (PUFA) en la misma molécula de glicerol tienen ventajas nutricionales y terapéuticas. Se establece la incorporación de MCFA a los triglicéridos (TAGs) de aceite de pescado, conservando un contenido considerable de ácidos docosahexaenóico (DHA) y eicosapentaenóico (EPA). El efecto de diferentes acil donadores (éster metílico de ácido cáprico/MeC_{10} o triglicéridos de cadena media/TCM) y de catalizador (químico o enzimático) sobre la composición del producto de las reacciones fue estudiado. La composición de ácidos grasos de los TAGs del aceite de pescado fue modificada después de las reacciones para contener MCFA y dependió del catalizador y de los substratos. Los termogramas obtenidos por Calorimetría Diferencial de Barrido (DSC) indicaron que la interesterificación provocó alteraciones considerables de los perfiles de fusión de las muestras. Fueron producidos STs de interés en nutrición clínica conteniendo EPA y DHA, además de MCFA.

PALABRAS-CLAVE: Aceite de pescado – Ácidos grasos de cadena media – Interesterificación – Triglicéridos estructurados.

SUMMARY

Incorporation of medium chain fatty acids into fish oil triglycerides by chemical and enzymatic interesterification.

Structured triglycerides (STs) containing both medium-chain fatty acids (MCFA) and polyunsaturated fatty acids (PUFA) in the same molecule offer nutritional and therapeutic benefits. The aim of this work was to establish the incorporation of MCFA into fish oil triglycerides (TAGs), while maintaining substantial levels of docosahexaenoic and eicosapentaenoic acids. The effects of different acyl donors (capric acid methyl ester/MeC_{10} or medium chain triglyceride/TCM) and of the catalyst (chemical or enzymatic) on the fatty acid composition of the reaction product were studied. The fatty acid composition of the fish oil TAG was modified after interesterification to contain MCFA, and it depended on the catalyst and on the substrates. Thermograms obtained by Differential Scanning Calorimetry (DSC) showed that interesterification promoted noteworthy changes in the melting profile of the samples. STs of clinical nutrition interest containing both EPA and DHA obtained from fish oil along with MCFA were successfully produced.

KEY-WORDS: Fish oil – Interesterification – Medium-chain fatty acid – Structured triglycerides.

1. INTRODUCTION

Medium-chain triglycerides (MCTs) containing medium-chain saturated fatty acids (MCFA, C6:0 to C12:0), show higher mobility and solubility than long-chain fatty acids (LCFA), and they are rapidly and almost completely oxidized (Bell and Bradley, 1997; Dunford, 2004; Jones *et al*., 2006; St-Onge and Borsarge, 2008). Therefore, MCTs are largely applied as lipid substrates in enteral and parenteral nutrition, providing energy for patients suffering from a number of disorders (Goldberg, 1994; Galante and Tenore, 2006; Nielsen *et al*., 2005; Mu and Porsgaard, 2005). MCT fats, however, have reduced palatability and lower digestibility than other fatty acids (Nielsen *et al*., 2005). Although MCFAs, mainly caprylic and capric acids, seem to be neutral with respect to their low-density lipoproteins-cholesterol (LDL-C) raising properties (Nicolosi, 1997), there are some concerns regarding the possible effects of MCFA consumption on plasma lipid concentrations (Cater *et al*., 1997; Kubow, 1996; Osborn and Akoh, 2002; St-Onge, 2005). Therefore, caprylic and capric acids are more useful in combination with long-chain polyunsaturated fatty acids (PUFAs) in formulas to provide essential fatty acids via the PUFAs (Mu and Hoy, 2000; Osborn and Akoh, 2002; Hartvigsen *et al*., 2003).

Commercial lipid emulsions containing a physical mixture of long-chain triglycerides (LCT) together with MCT have been used for years in

clinical nutrition (Osborn and Akoh, 2002; Carpentier, 2008; Simoens *et al.*, 2008). The inclusion of e docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) from marine origin in lipid emulsions could supply eicosanoids derived from EPA (Bell and Bradley, 1997; Stein, 1999), which offer physiological benefits (Pigott and Tucker, 1990; Hasselmann and Kummerlen, 1998; Kris-Etherton *et al.*, 2002; Hartvigsen *et al.*, 2003; Soccol and Oetterer, 2003), especially in acutely ill patients and in patients receiving long-term parenteral nutrition (Bourque *et al.*, 2003; Qi *et al.*, 2006).

In recent years, extensive research has been focused on interesterification reactions for the synthesis of specific triglycerides known as structured triglycerides (STs), with optimal properties as a result of modifications either to the fatty acid (FA) composition or a positional redistribution of the acyl groups bonded to the glycerol backbone of the original TAGs (Akoh, 1995; Willis *et al.*, 1998; Xu, 2004).

Based on these considerations, randomized or specific STs containing a mixture of MCFA and LCFA in the same molecule have been designed for medical purposes (Stein, 1999; Mu and Porsgaard, 2005; Carpentier, 2008). Lipid emulsions containing specific STs demonstrate improved hydrolysis and absorption in comparison with a randomized ST or a physical mixture of MCT and LCT (Bistrian, 1997; Stein, 1999; Straarup and Hoy, 2000; Xu, 2004). During digestion, a fat containing MCFAs located in the *sn*-1,3 positions of the glycerol backbone is rapidly hydrolyzed mainly *via* the portal vein; while the PUFA located in the position 2 of the same molecule is delivered as 2-monoglyceride, which will be absorbed efficiently *via* the lymphatic route and therefore will be incorporated into different tissues (Jandacek *et al.*, 1987; Mu and Hoy, 2000; Mu and Hoy, 2001; Versleijen *et al.*, 2005; Nielsen *et al.*, 2005; Carpentier, 2008). A diet rich in such a specific ST may reduce the incorporation and storage of dietary fats and oils into adipose tissue, increase the total carcass protein amount and significantly reduce serum cholesterol levels, with no genotoxic potential (Matulka *et al.*, 2006).

In interesterification reactions, the exchange of fatty acids within (rearrangement) or among molecules may occur in a random or in a specific way, depending on the catalyst (Macrae 1983; Willis *et al.*, 1998). Chemical catalysis has been extensively applied to ST synthesis especially because of the low cost of the catalyst and because of its efficiency even at low concentrations (Marangoni and Rousseau, 1995; Klinkesorn *et al.*, 2004). The chemical reaction, however, cannot be performed in an acid environment (Christie, 1982). A good alternative for overcoming the problem of the presence of the free fatty acid in the environment is its conversion into its alkyl ester (Klinkesorn *et al.*, 2004). Moreover, chemical interesterification produces a complete randomization of acyl groups in TAGs (Akoh, 1995; Osborn and Akoh, 2002).

The transformation of oils mediated by lipases has been extensively studied (Macrae, 1983; Gunstone, 1999; Osborn and Akoh, 2002). Biocatalysis takes advantage of the specificity of some lipases thus allowing the production of useful molecules which cannot be obtained either chemically or naturally (Akoh, 1995; Marangoni and Rousseau, 1995; Shimada, 2006). The *sn*-1,3-specific lipases have been used in the range of 5 to 10% by weight of reactants to promote the incorporation, for example, of capric acid into the end positions of a TAG molecule whose central position is preferentially occupied by fatty acids of nutritional interest (Gunstone *et al.*, 1994).

The aim of the present paper was to establish the interesterification reaction that could lead to the design of STs of clinical nutrition interest, containing MCFA along with a high n-3 EPA and DHA content. This report compares the results obtained from the fatty acid composition of the interesterified oils when using chemical (with sodium methoxide/$NaOCH_3$ as catalyst) and enzymatic interesterification (with a commercial immobilized *sn*-1,3-specific lipase). In both cases, a commercial fish oil was used as substrate and two substances were evaluated as acyl donors (capric acid methyl ester/MeC_{10} and medium chain triglycerides/TCM).

2. MATERIALS AND METHODS

2.1. Materials

Capric acid was obtained from Sigma Chemical Co. ROPUFA n-3 "30" Food Oil was supplied by DSM Nutritional Product Ltd. Trigliceril CM® was obtained from Support Produtos Alimenticios Ltda, Brazil. Lipozyme® RMIM was gift from Novozymes. This lipase is sn-1, 3-specific.

2.2. Interesterification reactions

Capric acid (C10:0) was esterified according to Klinkesorn *et al.* (2004). The MeC_{10} was stored at −20 °C in a nitrogen atmosphere until use. For the reactions, the substrates were mixed as follows: ROPUFA®:MeC_{10} (1:2, mol/mol) and ROPUFA®:Trigliceril CM® (1:1, w/w). Before the reaction, the samples were dried using a vacuum rotary evaporator at 90-95 °C for 1 h. Reactions were performed as described below.

For the chemical reaction, sodium methoxide ($NaOCH_3$) was prepared by heating the mixture NaOH:MeOH (1:25, w/v) to 60 °C while stirring until the complete dissolution of the reactants. The solvent was removed at 60 °C using a vacuum rotary evaporator. The catalyst was stored in a dark flask in a desiccator until use. The chemical interesterification was performed according to Díaz Gamboa and Gioielli (2003a). The reaction was carried out with magnetic stirring in a water bath. After the mixture of reactants had reached the

reaction temperature (60 °C), $NaOCH_3$ in fine powder was added at a mass ratio of 0.4% of reactants. After 1 h under a vacuum of 56 mmHg the reaction was stopped with the addition of 0.5 mL of an aqueous solution of 4% (m/v) citric acid (Klinkesorn *et al.*, 2004).

For the enzymatic interesterification, the temperature was kept at 60 °C (Moura *et al.*, 2006). The lipase Lipozyme RM IM® was added at a mass ratio of 5% of reactants. The reaction took place for 6 h with magnetic stirring under a vacuum of 56 mmHg (Díaz Gamboa and Gioielli, 2003a) and it was stopped by the removal of the immobilized enzyme through centrifugation (3,000 rpm for 15 min) and filtration.

The interesterified oils were stored at −20 °C in nitrogen until analysis.

2.3. Analytical Methodology

Determination of the quality of the samples

The acid value (Method Ca 5a-40) and the peroxide value (Method Cd 8-53) of the ROPUFA n-3 '30' Food Oil® (DSM Nutritional Products Ltd) were determined according to the Official Methodology of the American Oil Chemists' Society (AOCS, 1990). The iodine value (I.V.) of the original mixture of the oils and the interesterified oils was calculated according to the expanded formula from the American Oil Chemists' Society Official Method Cd 1c-85 (AOCS, 1997).

Fatty acid composition

After interesterification reactions, TAG fractions were isolated from interesterified oils by thin-layer chromatography (TLC) on 20 cm silica gel 60G plates containing a fluorescent reactant (Macherey-Nagel, Germany) using the elution system hexane/ethyl ether/formic acid (80:20:2, v/v/v) (Christie, 1982).

The fatty acid composition of the samples (Trigliceril CM®, ROPUFA® fish oil and the isolated TAGs from the interesterified oils) was determined by GC after the conversion of fatty acids into their fatty acid methyl esters (Hartman and Lago, 1973). The gas chromatograph was a Shimadzu (Shimadzu Co., Japan) 17A model, equipped with a flame-ionization detector and an SP 2340 fused-silica capillary column (60 m x 0.25 mm, 0.2 μm of polyethyleneglycol, Supelco). The injector and detector temperatures were 240 and 260 °C, respectively. The column temperature was held at 120 °C for 5 min, then programmed to 240 °C at 4 °C.min^{-1} and held for 5 min. The carrier gas used was helium set at a flow rate of 0,67 mL.min^{-1} (the linear speed was 17 cm.s^{-1}). The qualitative fatty acid composition was determined by comparing the retention times of the sample peaks with respective fatty acid methyl ester standards (Supelco 37 components FAMEs Mix, ref. 47885-U). The quantitative fatty acid determination was accomplished by area normalization and expressed as mass percent.

Differential scanning calorimetry analysis

The differential scanning calorimetry (DSC) analysis was performed according to the Method Cj 1-94 (AOCS, 1997) using a power compensation differential calorimeter (Model Delta Series DSC 7, PERKIN ELMER). The DSC melting and crystallization curves were obtained for the samples before and after interesterification. Analysis conditions: weight of samples: ~10 mg; melting curves: 10 min (80 °C); 80 °C to −40 °C (10 °C.min^{-1}); 30 min at −40 °C; −40 °C to 80 °C (5 °C.min^{-1}). The following parameters were obtained from thermograms: melting enthalpy (J.g^{-1}), initial and final melting temperatures (°C), onset melting temperature (°C), and maximum peaks (°C).

3. RESULTS AND DISCUSSION

The acid and the peroxide values of the ROPUFA® fish oil were 0.44% of oleic acid and 1.46 meq O_2.kg^{-1}, respectively. These results agree with supplier specifications and match international regulations whose criteria of quality for refined oil include a peroxide value lower than 10 meq O_2.kg^{-1} (FAO-WHO, 1981, revision 1999) or 5 meq O_2.kg^{-1} in the case of oil extracted from herring (FDA, 1995, revision 2003).

The fatty acid composition of the samples as determined by GC is shown in Table 1. The total n-3 fatty acid content in the original ROPUFA® fish oil was 31.92 %, with a predominance of EPA and DHA, and no MCFA was identified in this sample. Such a composition is a characteristic feature of marine oils (Pigott and Tucker, 1990; Aro *et al.*, 2000). Capric and caprylic acids were responsible for more than 98% of the Trigliceril CM® fatty acid composition.

The choice of the reaction conditions during synthesis of the STs was based on a previous study concerning the chemical and the enzymatic synthesis of STs containing MCFAs from palm kernel fat and n-3 PUFAs from fish oil (Díaz Gamboa and Gioielli, 2003a). The conditions applied by these authors for the enzymatic reaction are in agreement with other results found in the literature for similar substrates (Jennings and Akoh, 1999; Jennings and Akoh, 2001). In the latter work, these authors have observed that a high incorporation of capric acid into fish oil was achieved at an enzyme load of 5 % (by mass of substrates) for the solvent-free reaction using the lipase Lipozyme® RM IM. The enzymatic reaction temperature was established in accordance with another work involving the reaction between n-3 PUFA ethyl esters and MCT (Moura *et al.*, 2006). In the present study, the substrates were mixed in a ratio that allowed the inclusion of the capric acid

Table 1
Fatty acid composition (%, w/w) of ROPUFA® food oil, trigliceril CM® (TCM) and their interesterified triglycerides

			Trigliceril CM® + ROPUFA®		MeC_{10} + ROPUFA®	
Fatty acid	ROPUFA®	Trigliceril CM®	After CI[a]	After EI[b]	After CI	After EI
C6:0	–	1.23	–	–	–	–
C8:0 (Caprylic)	–	63.13	14.04	18.68	0.10	0.70
C10:0 (Capric)	–	34.99	10.35	11.22	4.44	9.04
C12:0	–	0.61	–	–	–	–
C14:0	6.49	–	5.56	4.75	6.22	6.05
C14:1	0.30	–	–	–	–	–
C15:0	1.05	–	–	–	0.68	0.66
C16:0	19.58	–	19.42	15.50	18.98	17.44
C16:1	5.97	–	4.39	3.59	6.75	6.52
C17:0	0.72	–	–	–	0.55	0.61
C17:1	0.54	–	–	–	–	–
C18:0	3.65	–	3.42	2.80	3.66	3.26
C18:1n9t	2.36	–	–	–	–	–
C18:1n9c	17.38	–	19.38	15.83	20.01	18.03
C18:2n6c	2.99	–	2.51	2.15	3.39	0.12
C20:0	0.62	–	–	–	1.91	0.40
C18:3n6	0.25	–	–	–	–	–
C18:4n3	1.94	–	–	–	–	–
C20:1	1.10	–	–	–	1.86	1.95
C18:3n3	1.26	–	–	–	1.78	2.11
C20:2	0.39	–	–	–	0.45	0.39
C22:0	0.27	–	–	–	–	0.18
C20:3n6	–	–	–	–	1.51	1.21
C20:3n3	–	–	–	–	0.78	0.71
C22:2	0.24	–	–	–	–	–
C20:4n6	0.97	–	–	–	–	0.74
C20:5n3 (EPA)	11.17	–	7.51	12.33	11.06	11.85
C22:5n3	1.07	–	–	–	0.68	0.53
C22:6n3 (DHA)	19.68	–	13.42	13.17	15.19	17.52
Total	100.00	100.00	100.00	100.00	100.00	100.00
Saturated	32.38	100.00	52.79	52.95	36.54	38.34
Monounsaturated	27.65	–	23.77	19.42	28.62	26.50
Polyunsaturated	39.96	–	23.44	27.65	34.84	35.18
Total	100.00	100.00	100.00	100.00	100.00	100.00
EPA+DHA	30.85	–	20.93	25.50	26.25	29.37
EPA:DHA	0.57	–	0.56	0.94	0.73	0.68
n3:n6	8.34	–	8.34	11.86	6.02	15.81
Iodine value	187	–	120	134	157	166

[a] After CI = After chemical interesterification.
[b] After EI = After enzymatic interesterification.

into two positions of the ROPUFA® triglycerides when the MeC_{10} was applied as substrate (1:2, mol:mol, ROPUFA®:MeC_{10}), as previously stated by Jennings and Akoh (1999) for the esterification of capric acid to fish oil TAG by means of an enzymatic reaction with the lipase Lipozyme® RM IM. In the case of the interesterification between the ROPUFA® and the Trigliceril CM®, the ratio 1:1 (w/w) was chosen because it is usually applied for the physical mixture of LCT and MCT applied in enteral and parenteral nutrition (Hasselmann and Kummerlen, 1998).

After both the chemical and the enzymatic reaction between the fish oil and the acyl donor (Trigliceril CM® or MeC_{10}), MCFAs were detected in the TAGs of the interesterified oils. This composition confirms the exchange of acyl groups among substrates during interesterification (Reyes and Hill, 1994; Marangoni and Rousseau, 1995; Rousseau and Marangoni, 2002; Xu, 2004).

In this study, the reaction conditions favored the occurrence of chemical interesterification: the concentration of the catalyst (0.4% by weight of reactants) was greater than concentrations commonly used (0.1-0.2% by weight) (Marangoni and Rousseau, 1995), while the media was extremely apolar, and there was also no solvent.

Despite this, MCFA incorporation was greater for the enzymatic reaction than for the chemical reaction regardless of the substrate. Furthermore, the EPA and DHA content of the enzymatically interesterified oils remained close to that of the

original values. These results agree with others which show that lipases discriminate strongly between MCFA and LCFA in favor of MCFA (Akoh, 1995; Kubow, 1996; Bell and Bradley, 1997; Osborn and Akoh, 2002).

The use of MeC_{10} as acyl donor led to a higher content of EPA and DHA after the reaction. The total EPA and DHA content in the interesterified Trigliceril CM® and ROPUFA® oils was 20.93% and 25.50% for the chemical and the enzymatic reactions, respectively. The total EPA and DHA content in the MeC_{10} and the ROPUFA® oil was 26.25% and 29.37% after chemical and enzymatic interesterification, respectively. The success of the use of methyl esters as substrates for interesterification reactions has been previously demonstrated in another study whose objective was the enrichment of tuna oil with EPA and DHA by chemical transesterification (Klinkesorn *et al*., 2004).

A previous study (Moura *et al*., 2006) also reported the efficiency of the incorporation of long-chain esters (of the ethyl type), obtained from fish oil, into MCT, at a molar ratio of 1:2.5, respectively, by using a *sn*-1,3-specific lipase (5% by weight of reactants), without previous conditioning. After 30 h at 60 °C with stirring and under vacuum, the highest incorporation of esters (66.66%) into the end position of the TAGs was reached, thus obtaining a specific ST with nutritional interest.

In another study, the incorporation of C10:0 into fish oil TAGs (Pronova Biocare, Noruega), at a molar ratio of 2:1, respectively, was performed over 24 h at 55 °C and 200 rpm by using an immobilized *sn*-1,3-specific lipase (10 % by weight of reactants). The authors obtained a final product with an average of 43 mol% of C10:0 in TAGs for the reaction in the presence of hexane, and 31.8 mol% for the solvent-free reaction. It was found that EPA and DHA were predominantly located in the central position of TAGs, thus confirming the ST synthesis, even with some acyl migration (Jennings and Akoh, 1999).

The same researchers outlined the same reaction conditions for the enzymatic modification of another fish oil (menhaden fish oil from National Marine Fisheries Service, USA). TAGs were produced with an average of 28.8 mol% of C10:0, predominantly located in the end positions (Jennings and Akoh, 2001).

Others have described the possibility of the synthesis of STs containing MCFAs, EPA and DHA by both chemical and enzymatic interesterification of binary mixtures between palm kernel oil (rich in lauric acid) and fish oil. The chemical reaction was performed over 1 h at 60 °C with stirring and under reduced pressure, with sodium methoxide as catalyst (0.4 % by weight of reactants). A final product was obtained with plastic behavior and containing 5.0 to 23.4% of n-3 PUFAs (Díaz Gamboa and Gioielli, 2003a). The enzymatic interesterification was carried out over 6 h at 65 °C in a nitrogen atmosphere by using a commercial *sn*-1,3-specific lipase (5% by weight of reactants) (Díaz Gamboa and Gioielli, 2003b).

Table 1 shows that both the original fish oil and the interesterified oils exhibited a value DHA greater than that of EPA and all samples containing fish oil were important sources of n-3 fatty acids. The I.V. of the original fish oil was 187 g iodine/100g oil. This high I.V. is associated with the unsaturated fatty acids present in the sample, which is consistent with other studies involving fish oils (Pacheco and Barrera-Arellano, 1994; Wanasundara and Shahidi, 1998). As expected (Nassu and Gonçalves, 1999), the I.V. of the samples decreased as MCFA was incorporated, especially when using the Trigliceril CM® as substrate, which has a high oxidative stability (Akoh *et al*., 1998).

Such results indicated that the obtained STs are more stable in storage than the original fish oil, which is important considering the sensitivity of PUFAs to peroxidative damage (Carpentier, 2008). Also, their content of MCFAs may supply significant energy intake and their content of PUFAs may prevent a deficiency of essential fatty acids, thereby helping modulate important metabolic reactions (Lands, 2002; Carpentier, 2008).

The lipid emulsion formulas have to provide not only the nutrient composition necessary for the patients, but also the physical characteristic requirements for their administration in enteral or parenteral nutrition. Once lipid emulsions containing MCTs have low melting points and viscosity, they are liquid products that can be administered in their pure form or as a main vehicle from pharmaceutical formulas (Bach and Babayan, 1982; Goldberg, 1994; Akoh *et al*., 1998).

In mixed-acid triglycerides, not only the fatty acid composition, but also the position to which the fatty acids are esterified would have influence on the melting point and the fluidity of the oil, and therefore on its digestibility (Bockisch, 1993; Christophe, 1998; Rousseau and Marangoni, 2002; Nielsen *et al*., 2005). The differences in the chain lengths and a residue in an *sn* position on the glycerol molecule of ST would affect both the overall hidrophobicity, which in turn may influence its solubility in water, and the incorporation into TAGs and/or phospholipids (Stein, 1999).

DSC thermograms, correlating temperature and normalized heat flow, are shown in Figures 1 and 2. Both chemical and enzymatic interesterification produced noteworthy changes in the melting profile, as already reported in another study (Rousseau and Marangoni, 2002).

The melting behavior of the samples varied markedly after the reactions because of the incorporation of MCFA into fish oil TAG, which originally contained mainly unsaturated fatty acids. This behavior was observed when using either the MeC_{10} or the Trigliceril CM® as substrate and was also due to a rearrangement of TAGs after the interesterification reactions, resulting in a higher complexity in TAG composition, as already established (Rousseau *et al*., 1996; Grimaldi *et al*., 1998; Grimaldi *et al*., 2001).

The melting profiles of the feedstock mixtures of Trigliceril CM® and ROPUFA® (Figure 1) were quite

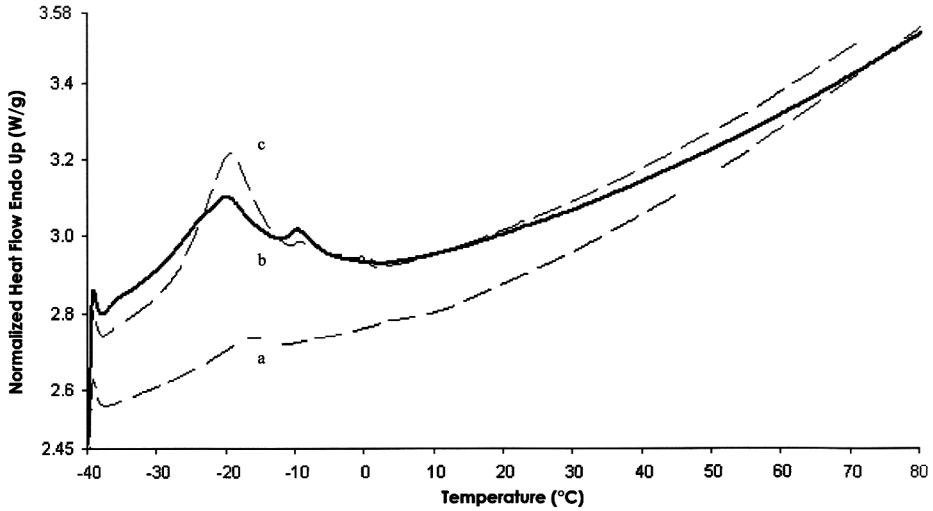

Figure 1.
DSC thermograms (melting curves) of the mixture Trigliceril CM® and ROPUFA® food oil (a) before interesterification; (b) after chemical interesterification; and (c) after enzymatic interesterification.

alike, with just one peak of similar shape, suggesting little difference between TAGs obtained by chemical and enzymatic interesterification.

The DSC thermograms of the fish oils interesterified with the MeC_{10} (Figure 2) exhibited a broad melting range, with two distinct peaks for the product of the chemical reaction, probably because of a random distribution of fatty acids in TAGs. The higher complexity of the thermograms shown in Figure 2 suggests a higher complexity of the STs obtained by the interesterification between the fish oil and the MeC_{10}. This result could also be associated with the formation of TAGs without intersolubilization, which is consistent with other studies involving TAG modification (Rousseau *et al.*, 1996; Nassu and Gonçalves, 1999).

Capric acid has a melting point greater than that of its methyl ester, thus leading to TAGs with a greater melting point. Further, capric acid has a melting point greater than that of caprylic acid, which in turn is the main fatty acid in the Trigliceril CM® (Bailey, 1979; ISEO, 2006). Hence, the thermograms shifted a few degrees higher when using MeC_{10} as acyl donor.

Information on the characteristic temperatures of the samples was obtained from thermograms and the values are shown in Table 2. The very low or negative melting temperatures obtained for the oils can be attributed to the presence of unsaturated fatty acids in all samples, provided from the fish oil. This behavior has been already observed for salmon oil (Sathivel, 2005) and is a special feature of fish oils. According to another ST containing MCFAs and n-3 PUFAs mentioned in the United States Patent 6.608.223 (Rao *et al.*, 2003), the STs obtained herein as a result of interesterification reactions

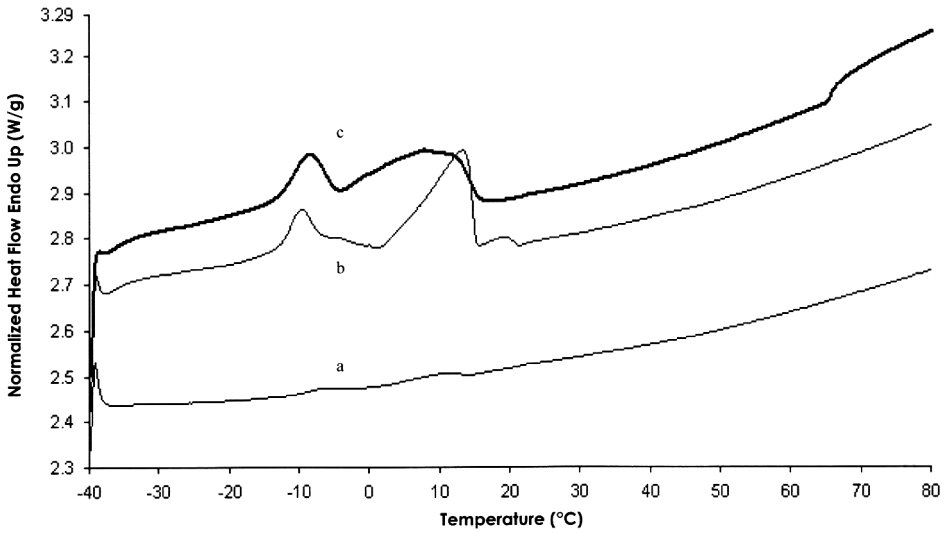

Figure 2.
DSC thermograms (melting curves) of the mixture MeC_{10} and ROPUFA® food oil (a) before interesterification; (b) after chemical interesterification; and (c) after enzymatic interesterification.

Table 2
Data obtained from DSC melting curves of mixtures before and after chemical and enzymatic interesterification

Data	Peak	Triglyceril CM® + ROPUFA®			MeC_{10} + ROPUFA®		
		a	b	c	a	b	c
Peak temperature (°C)	1	−17.3	−19.5	−20.3	–	−9.7	7.7
	2	–	–	–	–	13.3	–
Onset temperature (°C)	1	−25.7	−37.1	−37.5	–	−13.1	−15.6
	2	–	–	–	–	1.3	–
Initial melting point (°C)	1	−27.0	−37.1	−37.7	–	−19.5	−15.6
	2	–	–	–	–	1.3	–
Final melting point (°C)	1	−9.4	2.1	2.1	13.5	0.8	16.1
	2	–	–	–	–	20.8	–
Melting enthalpy ($J.g^{-1}$)	1	5.1	65.1	52.4	~0	7.9	26.6
	2	–	–	–	–	17.8	–
Total melting enthalpy ($J.g^{-1}$)	–	5.1	65.1	52.4	~0	25.7	26.6

a) before interesterification; b) after chemical interesterification; c) after enzymatic interesterification.

displayed a melting behavior different from the one presented by original oil blends. In this present work, the STs had a melting temperature lower than the unmodified oil melting temperature, thus maintaining them in the liquid state even at low temperatures, without phase separation, which is important for their administration in enteral and parenteral nutrition (Jores, 2004). These STs have melting points below body temperature remaining liquid in the intestinal lumen, with a good digestion and absorption (Christophe, 1998). Some commercial STs such as Intralipid® (Férezou *et al*., 2001) and Captex® 810 (Bach and Babayan, 1982) applied for medical purposes are also available in the liquid state. The latter is a transesterified oil composed by linoleic acid along with caprylic and capric acids.

Melting enthalpy is the energy required for the complete melting of the sample (Nassu and Gonçalves, 1999; Grimaldi *et al*., 1998). The melting enthalpy values increased with decreasing I.V. of the samples (I.V. values already shown in Table 1), which in turn is related to a higher degree of saturated fatty acids in the sample, as already shown for other substrates (Nassu and Gonçalves, 1999). This result was evident in the case of the remaining products of both chemical and enzymatic interesterification of fish oil with Triglyceril CM®, whereas the diversified fatty acid composition of TAGs resulted in a small deviation from this rule for the product of the incorporation of MeC_{10} into fish oil TAGs.

From the results shown in Figures 1 and 2 as well as in Table 2, it can be seen that the observed differences between samples were due to reaction conditions involving both the substrate and the catalyst.

4. CONCLUSIONS

This work confirmed the feasibility of the synthesis of structured TAGs containing both n-3 EPA and DHA, obtained from fish oil, and MCFA. We have also shown that the composition of the feedstock mixtures as well as the catalyst play an important role in the establishment of the fatty acid composition of the interesterified oils. We described the incorporation of MCFA into fish oil TAGs with the maintenance of a high EPA and DHA content mainly when using MeC_{10} as acyl donor in the enzymatic interesterification. The STs herein obtained have relatively high amounts of PUFAs accompanying the MCFAs. The STs thus obtained have potential application in clinical nutrition as an energy-yielding substrate rich in essential fatty acids, particularly beneficial for patients with malabsorption syndromes.

ACKNOWLEDGMENTS

The authors thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for providing a Master's Fellowship, Novozymes, DSM Nutritional Products Ltd and Support Produtos Alimentícios Ltda. for donations, Prof. Francisco C. Deschamps (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A., Brazil) for his assistance in GC analysis and Prof. Luiz A. Gioielli (Universidade Estadual de São Paulo, Brazil) for his suggestions.

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Recibido: 3/7/08
Aceptado: 28/10/08