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Interesterificação química e enzimática de misturas de estearina de palma, óleo de coco e óleo de canola para formulação de margarinas com baixa concentração de isômeros *trans*

Fabiana Andreia Schäfer De Martini Soares

Tese para obtenção do grau de

DOUTOR

Orientador:

Prof. Dr. Luiz Antonio Gioielli

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Comissão Julgadora

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O bravo não é quem não sente medo,
mas quem vence esse medo.

Nelson Mandela

A mente que a uma nova ideia
jamais volta ao seu tamanho normal

Albert Einstein

RESUMO

SOARES, F.A.S.D.M; GIOIELLI, L.A. Interesterificação química e enzimática de misturas de estearina de palma, óleo de coco e óleo de canola para formulação de margarinas com baixa concentração de isômeros *trans*.. São Paulo, 2014. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo.

O consumidor está cada vez mais consciente da relação entre dieta e doença, que tem impulsionado as pesquisas sobre alimentos funcionais e seus efeitos sobre o corpo. O papel dos óleos e gorduras na nutrição humana tem sido intensamente estudado e discutido por décadas. Tem sido enfatizada a importância da ingestão de ômega-3, ômega-6 e ômega-9 ácidos graxos redução de ácidos graxos saturados e, mais recentemente, controle da ingestão de ácidos graxos *trans*. Através da mistura e interesterificação química e enzimática de óleos e gorduras, gorduras *trans*-livre pode ser produzido. Mistura de gordura, foram formuladas por misturas ternárias de estearina de palma, uma gordura láurica (óleo de coco ou óleo de palmiste) e um óleo poliinsaturado (óleo de canola ou azeite de oliva) em diferentes proporções que foram interesterificadas. Neste trabalho, foram produzidos lipídios estruturados por interesterificação química e enzimática. A interesterificação química foi realizada nas seguintes condições: a 88 °C, 60 minutos de reação, 0,4% de catalisador metóxido de sódio, sob agitação e vácuo. A interesterificação enzimática, sendo realizada com duas lipases comerciais *Thermomyces lanuginosa* e *Rhizomucor miehei*, com seletividade *sn*-1,3. A interesterificação enzimática por batelada foi realizado seguindo um planejamento matriz central compósito rotativo em função da temperatura e da composição do meio, estearina de palma, óleo de palmiste e azeite de oliva e catalisado pelas lipases comerciais. O decréscimo do conteúdo de gordura sólida foi observado a 10 e 35 °C após a interesterificação. O biorreator contínuo foi operado nas seguintes condições: mistura de estearina de palma, óleo de palmiste, azeite de oliva (45:30:25), 10 gr de biocatalisador, 65 °C, com tempo de residência de 7 min e por 226 h para *Thermomyces lanuginosa* e 188 h para *Rhizomucor miehei*. A atividade do biocatalisador foi avaliada em termos da diminuição do conteúdo de gordura sólida a 35 °C, o qual é um parâmetro chave na produção de margarinas. O perfil de inativação do biocatalisador pode ser bem descrita pelo modelo de desativação de primeira ordem: meia-vida de 88 e 60 h foram estimados quando *Thermomyces lanuginosa* e *Rhizomucor miehei*, respectivamente, foram utilizados. Os óleos puros, as misturas originais e interesterificadas foram avaliados quanto à composição de ácidos graxos e triacilgliceróis, distribuição regioespecífica dos ácidos graxos nos triacilgliceróis, ponto de fusão e amolecimento, consistência, conteúdo de gordura sólida, comportamento de fusão e cristalização, estabilidade oxidativa, estrutura cristalina e polimorfismo. A interesterificação química e enzimática promoveram diminuição de triacilgliceróis trissaturados e triinsaturados e aumento dos monossaturados-diinsaturados e dissaturados-monoinsaturados, o que resultou no respectivo decréscimo dos pontos de fusão e amolecimento, consistência e conteúdo de gordura sólida, aumentando a plasticidade das gorduras. As curvas de fusão e cristalização das misturas foram modificadas pela alteração da composição dos triacilgliceróis pela interesterificação química e enzimática. Estabilidade térmica e a temperatura de oxidação da estearina de palma, óleo de coco e óleo de canola e suas misturas foram dependente da composição de ácidos graxos e independente da interesterificação química. Os resultados mostram que a interesterificação química e enzimática oferecem uma ferramenta útil para a concepção de gorduras com sintonizáveis propriedades físico-químicas, melhorando em relação a esse das gorduras de partida.

Palavras-chaves: Composição de triacilgliceróis, propriedades físico-químicas; comportamento de fusão e cristalização; microestrutura cristalina, polimorfismo, interesterificação enzimática batelada, interesterificação enzimática continua, estabilidade operacional, metodologia de superfície de resposta.

ABSTRACT

The consumer is becoming more aware of the relationship between diet and disease, which has driven the research on functional foods and their effects on the body. The role of fats and oils in human nutrition has been intensively studied and discussed for decades. It has been emphasized the importance of intake of omega-3, omega-6 and omega-9 fatty acids, reduction of saturated fatty acids and, more recently, control of intake of *trans* fatty acids. Through the blend and interesterification of oils and fats, *trans*-free fats can be produced. Fat blends, formulated by ternary blends of palm stearin, lauric fat (coconut oil and palm kernel oil) and polyunsaturated oils (canola oil and olive oil) were done in different ratios. In this work, were produced by chemical and enzymatic interesterification. Chemical interesterification was performed under the following conditions: at 88°C, 60 minutes reaction times, 0.4% sodium methoxide, under agitation and vacuum. For enzymatic interesterification being carried out with two commercial lipases *Thermomyces lanuginosa* e *Rhizomucor miehei*, with selectivity *sn*-1,3. Batch enzymatic interesterification were performed, following central composite rotatable designs (CCRDs) as a function temperature and media of palm stearin, palm kernel oil and olive oil formulation and catalyzed by a commercial immobilized lipase. A decrease in all SFC values of the blends at 10 °C and 35°C was observed upon interesterification. The bioreactor operated continuously: mixture of palm stearin, palm kernel oil and olive oil (45:30:25, wt %), at 65 °C, at a residence time of 7 min and for 226 h to *Thermomyces lanuginosa* and 188 h to *Rhizomucor miehei*.. Biocatalyst activity was evaluated in terms of the decrease of the solid fat content at 35 °C of the blends, which is a key parameter in margarine manufacture. The inactivation profile of the biocatalyst could be well described by the first-order deactivation model: Half-lives of 88 and 60 h were estimated when *Thermomyces lanuginose* and *Rhizomucor miehei*, respectively, were used. Pure oil, the original and interesterified blends were examined for fatty acids and triacylglycerols composition, regiospecific distribution of fatty acids in triacylglycerols, melting and softening points, consistency, solid fat content, thermal behavior, oxidation stability, crystalline microstructure and polymorphism. Chemical and enzymatic interesterification caused reduction of trisaturated and triunsaturated and increase in monosaturated-diunsaturated and disaturated-monounsaturated, lowering the initial melting and softening points, consistency and solid fat content, increasing plasticity of fats. Melting and crystallization curves were significantly modified by changing the composition of triacylglycerols by chemical and enzymatic interesterification. The thermal stability and oxidation temperature of palm stearin, coconut oil and canola oil and their blends were dependent on fatty acid composition and independent on chemical interesterification. The results show that the chemical and enzymatic interesterification provides a useful tool to design fats with tunable physicochemical properties, improved compared to that of the starting fats.

KEYSWORDS: Triacylglycerol composition, physicochemical properties, melting and crystallization behavior, crystalline microstructure, polymorphism, batch enzymatic interesterification, continuous enzymatic interesterification, operational stability, response surface methodology

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INTRODUÇÃO

As mudanças do estilo de vida das populações em decorrência dos impactos da vida moderna e da economia, muito presentes nos grandes centros urbanos, nos quais vive e trabalha o maior contingente de pessoas da nação, são consideradas importantes e influentes para o fenômeno da transição nutricional. Esta se caracteriza pela adoção de uma dieta rica em gorduras e açúcares e deficiente em fibras, aspectos que causam impacto negativo sobre os comportamentos, padrões de hábitos alimentares e mudanças no perfil epidemiológico, com aumento na prevalência de doenças crônicas, como diabetes mellitus tipo II, doenças cardiovasculares, hipertensão arterial sistêmica e dislipidemias (BALBINOT *et al*., 2009; HELMAN, 2003; PROENÇA; HISSANAGA, 2008).

Surgiram as refeições prontas, as cadeias de *fast-food* e produtos de confeitaria industrializados, entre outros. Junto a isso, surgiu a gordura vegetal parcialmente hidrogenada, que foi rapidamente aplicada a vários produtos e processos. Para Santos (2003), as facilidades da alimentação do tipo *fast-food* agradam à população, principalmente, pela sua praticidade e seu cardápio variado, voltado para a produção e o consumo em massa. Além do elevado consumo desse tipo de alimento, quase sempre não estão nele contempladas as necessidades nutricionais de cada pessoa e nem a oferta de uma alimentação equilibrada.

As gorduras vegetais hidrogenadas estão presentes nos mais variados produtos alimentícios, desde as margarinas e cremes vegetais, até sorvetes e biscoitos. A hidrogenação permite a produção de gorduras com características plásticas que melhoram a consistência e palatabilidade do produto e aumentam sua vida de prateleira.

Os sucessivos avanços em pesquisas relacionando o consumo de isômeros *trans* das gorduras hidrogenadas com as doenças coronarianas e os riscos para a saúde humana foram amplamente difundidos entre a população mundial. Surgiu, então, a necessidade de se buscar produtos alternativos para substituir as gorduras vegetais hidrogenadas.

Uma das alternativas encontrada pela indústria de alimentos para substituir as gorduras vegetais parcialmente hidrogenadas foi o desenvolvimento dos óleos vegetais interesterificados, que produzem gorduras com boas características plásticas e teores reduzidos de isômeros *trans* em sua composição.

O Capítulo 1 consiste de uma revisão bibliográfica sobre as pesquisas relativas à obtenção de gorduras zero *trans* por interesterificação química e enzimática, para produção de margarinas.

Também aborda questões como as progressivas modificações da legislação brasileira com respeito à presença dos ácidos *trans* nos alimentos processados e nas matérias-primas.

Os Capítulos 2, 3, 4 e 5 avaliam a influência da interesterificação química nas propriedades físico-químicas de misturas de estearina de palma, óleo de coco e óleo de canola.

Os Capítulos 6 e 7 apresentam a influência da interesterificação enzimática nas propriedades físico-químicas de misturas a base de estearina de palma, óleo de palmiste e azeite de oliva.

OBJETIVO GERAL

Analisar a possibilidade da utilização de estearina de palma, óleo de coco e óleo de canola na formulação de lipídios estruturados para a fabricação de margarinas livres de ácidos graxos *trans*.

OBJETIVOS ESPECÍIFICOS

Avaliar a influencia da interesterificação química sobre as propriedades físico-químicas sobre misturas de estearina de palma, óleo de coco e óleo de canola procurando desenvolver gorduras para aplicação em margarinas.

Analisar o efeito da interesterificação química no comportamento térmico da estearina de palma, óleo de coco e óleo de canola e de suas misturas.

Investigar o desempenho de enzimas imobilizadas comerciais para a interesterificação enzimática de misturas de estearina de palma, óleo de palmiste e azeite de oliva para ser incorporada em margarinas, por batelada ou em modo continuo, em condições de reações optimizadas previamente por metodologia de superfície de resposta.

Estudar as propriedades físico-químicas da mistura de estearina de palma, óleo de palmiste e azeite de oliva e dos lipídios estruturados obtidos por interesterificação enzimática continuam.

1. REVISÃO BIBLIOGRÁFICA

Os óleos e gorduras são indiscutivelmente fontes de energia essenciais para a vida humana. A grande discussão nas últimas décadas em relação aos óleos e gorduras tem sido sobre os diferentes tipos de gorduras e, consequentemente, suas diferentes formas de metabolização pelo organismo. Como resultado, vem sendo enfatizada a importância da ingestão de ácidos graxos ω-3, a redução de ácidos saturados e, mais recentemente, o controle da ingestão de ácidos graxos *trans* (CHANG; CHOW, 2008, MARTIN; MATSHUSHITA; SOUZA, 2004).

A formação dos ácidos graxos *trans* durante o processo de hidrogenação parcial é conveniente do ponto de vista tecnológico, pois estes conferem às gorduras hidrogenadas características semelhantes às gorduras animais, ou seja, alto ponto de fusão e consequente alta estabilidade oxidativa, além da modificação das características sensoriais (O'BRIEN, 2004a).

Apesar de suas indiscutíveis vantagens tecnológicas, os efeitos prejudiciais do consumo de ácidos graxos *trans* para a saúde, presentes em alimentos industrializados, têm sido objeto de muitos estudos. A discussão sobre os efeitos da ingestão de gorduras e alimentos com alto conteúdo de ácidos graxos *trans* ganhou força quando, pela primeira vez, estudos mostraram que os ácidos graxos *trans* promoviam o aumento dos níveis de lipoproteínas de baixa densidade (LDL-c) e reduziam os níveis de lipoproteínas de alta densidade (HDL-c), alterando significativamente a razão entre LDL e HDL (MENSINK; KATAN, 1990).

No início da década de 2000, os ácidos graxos *trans*, presentes nos óleos parcialmente hidrogenados, contribuíam com cerca de 80 a 90% de todos os isômeros *trans* provenientes da dieta, sendo encontrados em produtos industrializados como chocolates, bolos, *snacks*, margarinas e *fast foods* (LARQUÉ; ZAMORA; GIL, 2001; PADOVESE; MANCINI FILHO, 2002).

Muitos países têm mostrado preocupação no que diz respeito às informações nutricionais presentes nos rótulos das embalagens de alimentos processados. No Brasil, a Agência Nacional de Vigilância Sanitária (ANVISA) colocou em vigor a resolução RDC n° 360 (BRASIL, 2003), que obrigou as indústrias instaladas a informarem no rótulo de seus produtos a quantidade de ácidos graxos *trans* contida nos alimentos (GIBON; DE GREYT; KELLENS, 2007; KELLENS *et al.*, 2007; RIBEIRO *et al.*, 2007; ZALIHA *et al.*, 2004). São considerados como zero *trans* os alimentos que apresentem teor de gordura *trans* menor ou igual a 0,2 g/porção (BRASIL, 2003).

Segundo Block (2009), pesquisas realizadas no mercado brasileiro indicaram que 65 % dos produtos alimentícios são rotulados como "livre de *trans*", o que mostra uma grande adequação da indústria alimentícia brasileira para o segmento de gordura zero *trans*.

Com as novas legislações em vigor, a indústria foi motivada a procurar novas alternativas de processos que permitissem a eliminação ou redução das gorduras *trans* em seus produtos. Neste momento, dois grandes desafios técnicos devem ser superados: o de desenvolver, formular e produzir gorduras livres dos ácidos graxos *trans* a um custo competitivo, e o de garantir que este novo produto mantenha as características estruturais e de palatabilidade do alimento, como sensação na boca, plasticidade e sabor (TARRAGO-TRANI *et al*., 2006).

Cada país, através de seus centros de pesquisa, universidades e empresas, vem desenvolvendo alternativas mais saudáveis de gorduras conforme sua disponibilidade de matéria-prima, conhecimento, experiência e possibilidade de instalação de novos processos. No Brasil, as técnicas que vem determinando o desenvolvimento de gorduras mais saudáveis são novas alternativas de matérias-primas e os processos de mistura e interesterificação. Estas duas técnicas podem ser aplicadas combinadas ou não.

A combinação das técnicas possibilita imensa quantidade de opções para o desenvolvimento de gorduras baixo *trans*, mas também traz grande quantidade de possibilidades de novas propriedades físico-químicas, como perfis de sólidos, plasticidade, consistência, pontos de fusão, comportamentos de cristalização e polimorfismos, até então nunca estudados. Este conhecimento é importante uma vez que, quando uma gordura com baixo teor de ácidos graxos *trans* é desenvolvida, espera-se que ela apresente a mesma funcionalidade, como cremosidade, velocidade de cristalização e plasticidade, anteriormente fornecida pelas gorduras com altos teores de *trans* (WASSELL; YOUNG, 2007).

Logo, o estudo e delineamento do uso de gorduras modificadas em alimentos devem contemplar a compreensão conjunta de suas propriedades físico-químicas fundamentais e de importantes elementos que determinam sua aplicabilidade, como total compatibilidade da base oleosa com o produto a que se destina, além de estabilidade durante e após o processamento.

1.1.MATÉRIAS-PRIMAS

Vários tipos de óleos e gorduras vegetais podem ser utilizados na produção de gorduras zero *trans*. A escolha das fontes oleosas que irão compor a mistura é dependente de diversos fatores, sendo que a disponibilidade da matéria-prima, a viabilidade econômica e a funcionalidade são os critérios mais relevantes.

Óleo de palma e suas frações, assim como óleos de palmiste e de coco, são consideradas boas alternativas para substituir gorduras parcialmente hidrogenadas por seu alto teor de ácidos graxos saturados e adequados pontos de fusão.

1.1.1. ESTEARINA DE PALMA

O óleo de palma está em situação de destaque no mercado mundial de óleos e gorduras, tendo ocupado em 2010 o 1º lugar, com participação de 38,2 %, sendo que o óleo de soja participou com 27,8 % (OIL WORLD, 2011). Nos próximos dez anos acredita-se que a produção de óleo de palma aumentará cerca de 15 %, isto porque a maioria dos óleos vegetais está com produção estável e o óleo de palma é o único em plena expansão. O óleo de palma vem suprindo as necessidades de consumo em função da sua versatilidade, com qualidade e características benéficas na elaboração de produtos, além da tendência mundial da substituição das gorduras *trans* (BASIRON, 2007; GEE, 2007).

O óleo de palma é extraído do fruto da palmeira *Elaeis guineensis* Jacq., originaria do África do Sul. Devido à sua composição (cerca de 50 % de ácidos graxos saturados e 50 % de ácidos graxos insaturados), ele pode ser fracionado naturalmente, sem o uso de produtos químicos, após o resfriamento em condições controladas de cristalização. A separação por filtração separa os cristais (estearina de palma) da fase líquida (oleína de palma). Desta separação obtém-se cerca de 70-80 % de oleína e 20-30 % de estearina (AINI; MISKANDAR, 2007; DIAN; KALYANA; IDRIS, 2007; GEE, 2007; HAYATI *et al.*, 2000; KALLIO *et al.*, 2001; KELLENS *et al.*, 2007; LAI *et al.*, 2000; ZALIHA *et al.*, 2004).

A estearina de palma apresenta a seguinte composição em ácidos graxos: palmítico 41 a 65 %, esteárico 3 a 5 %, oléico 23 a 42 % e linoléico 5 a 12 % (SOARES *et al.*, 2009). Em virtude de sua composição peculiar, rica em ácido palmítico, destaca-se o comportamento do óleo nas transições e coexistência de fases sólidas e líquidas, que lhe confere consistência

semi-sólida, permitindo flexibilidade para produzir grande variedade de produtos alimentícios (SAMBANTHAMURTHI; SUNDRAM; TAN, 2000).

A estearina de palma tem em sua composição cerca de 30 % de triacilgliceróis trissaturados, 45 % de dissaturados-monoinsaturados, 20 % de monossaturados-diinsaturados e 4 % de triinsaturados. Os principais triacilgliceróis presentes são PPP, PPO e POO, sendo P = palmítico e O = oléico (KELLENS *et al.*, 2007).

Devido sua versátil composição em ácidos graxos e triacilgliceróis, a estearina de palma presta-se para a fabricação de grande variedade de produtos, como margarinas, *shortenings*, biscoitos, gorduras para sorvetes, chocolates e bolos (AINI; MISKANDAR, 2007, BRAIPSON-DANTHINE; GIBON, 2007; CHONG *et al.*, 2007, KELLENS *et al.*, 2007, SAMBANTHWMURTH *et al.*, 2000).

A Tabela 1.1 apresenta as principais propriedades físico-químicas da estearina de palma. Ela não é usada diretamente para produção de margarina ou *shortenings*, devido ao seu elevado ponto de fusão, por conferir baixa plasticidade e fusão incompleta na temperatura do corpo ao produto final, mas contribui para a dureza desejável (SOARES *et al.*, 2009). Contudo, produz gorduras com desejável plasticidade quando interesterificada com óleos vegetais, como óleos de canola, soja, milho e girassol ou azeite de oliva.

A espalhabilidade é significativamente influenciada pela firmeza da estearina de palma, que é determinada, em parte, pelo seu conteúdo de gordura sólida e pela composição em ácidos graxos. Quanto maiores forem o conteúdo de gordura sólida e o teor de ácidos graxos saturados da estearina de palma, mais firme ela será, reduzindo sua espalhabilidade (KAWANARI, 1996).

Em misturas de estearina de palma com outros óleos vegetais, é preciso considerar a eventual formação de misturas eutéticas e a influência das mesmas na consistência do produto ou em outras propriedades físico-químicas. Como regra geral, misturas de óleos e gorduras vegetais não apresentam consistência linearmente proporcional às dos componentes (RITTNER, 1996).

A estearina de palma se diferencia dos óleos vegetais usuais por apresentar alto teor de ácidos graxos saturados e também por ter quantidade significativa desses ácidos graxos na posição *sn*-2 dos triacilgliceróis (SAMBANTHWMURTH *et al.*, 2000).

Tabela 1.1. Propriedades físico-químicas da estearina de palma, óleo de coco, óleo de palmiste, óleo de canola e azeite de oliva.

Gordura	Ponto fusão[1]	Pamolecimento[1]	Índice iodo	Acidez[2]	Índice de peróxido[3]	SFC 10 °C	SFC 15 °C	SFC 20 °C	SFC 25 °C	SFC 30 °C	SFC 35 °C	SFC 40 °C	SFC 45°C	SFC 50°C
Estearina de palma	46,6-53,8	42,0-48,0	24,4-45,1	< 0,05	< 1,0	76,5	71,3	64,5	52,0	40,2	28,6	22,6	15,6	8,1
Óleo de coco	25,0-28,0	22,0-27,0	8,0-9,0	< 0,5	< 1,0	80,0	56,0	36,0	0,0	0,0	0,0	0,0	0,0	0,0
Óleo de palmiste	25,9-28,0	22,0-27,0	16,2-19,2	< 0,5	< 1,0	67,6	55,7	40,1	17,1	0,0	0,0	0,0	0,0	0,0
Azeite de oliva	0,0	0,0	80,0-88,0	<0,5	< 10,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Óleo de canola	-6,00	-6,00	110,0-126,0	<0,5	< 10,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

[1], (°C) [2], (% em ácido oléico); [3], ($mEqO_2/kg$). (CANAPI *et al.*, 2005; GEE, 2007; GUNSTONE; 2002; SOARES *et al*., 2009)

Os óleos e gorduras apresentam tendência para a cristalização nas formas *β* ou *β'*. Esta tendência está relacionada à percentagem de ácido palmítico que, nas posições *sn*-1,3, forma triacilgliceróis assimétricos. Óleos e gorduras com 10 % ou menos de ácido palmítico têm tendência β, enquanto aqueles com 20 % ou mais tendem à forma *β'* (WIEDERMANN, 1978).

Gorduras solidificadas na forma *β'* formam malhas de cristais pequenos, de textura suave, capazes de imobilizar grandes quantidades de óleo líquido e gotículas de água. Gorduras solidificadas na forma *β* formam cristais de maior tamanho com textura arenosa e menor capacidade de retenção de líquidos (CHRYSAM, 2002). As gorduras que cristalizam na forma *β'* possuem características desejáveis para a formulação de margarinas (KIM *et al*., 2008).

1.1.2. GORDURAS LÁURICAS

As gorduras láuricas são muito usadas na indústria cosmética e alimentícia onde, em virtude de suas propriedades físicas e resistência à oxidação, são muito empregadas no preparo de gorduras especiais para confeitaria, sorvetes, margarinas e substitutos de manteiga de cacau. As principais gorduras láuricas são o óleo de coco e o óleo de palmiste.

1.1.2.1. ÓLEO DE COCO

O óleo de coco é extraído do fruto da palmeira *Cocos nucifera* L., *p*odendo ser prensado a frio, não sendo nesse caso submetido aos processos de refino e desodorização (de LEON e DELORES, 2005).

Apresenta em sua composição cerca de 90 % de ácidos graxos saturados, principalmente o ácido láurico, que varia a sua proporção entre 45 a 50%. Também apresenta ácido caprílico (4 a 10 %), cáprico (5 a 8 %), mirístico (16 a 22 %), palmítico (8 a 10 %), esteárico (2 a 4 %), oléico (5 a 10 %) e linoléico (1 a 3 %) (GUNSTONE, 2006).

O óleo de coco apresenta cerca de 84 % de triacilgliceróis trissaturados, 12 % de dissaturados-monoinsaturados, 3,8 % de diinsaturados-monossaturados e 0,2 % de triinsaturados, sendo os principais triacilgliceróis LaLaLa, LaMM, LaLaM e CCLa, sendo La = láurico, M = mirístico e C = cáprico (O´BRIEN, 2004, REENA, RENDDY; LOKESH, 2009).

Resultados relatados por Caro *et al.* (2004) mostram que a posição *sn*-2 é rica em ácidos láurico e oléico, e que os ácidos saturados (capróico, caprílico e capríco) estão nas posições *sn*-1,3.

Segundo de Leon e Delores (2005) desde 1920 utiliza-se óleo de coco para a produção de margarina, devido suas características semelhantes à gordura do leite. O óleo de coco tem características únicas: sabor suave, odor agradável, alta resistência à oxidação, estreita faixa de temperatura da fusão, que varia de 25 a 28 ºC, e fácil digestibilidade, sendo frequentemente usado na indústria de panificação em países ocidentais (BHATNAGAR *et al*., 2009).

Ao contrário de outros óleos, o óleo de coco passa abruptamente do estado sólido a líquido, dentro de um estreito intervalo de temperatura, ao invés de exibir um abrandamento gradual com o aumento da temperatura (Tabela 1.1).

A morfologia típica do cristal de óleo de coco é esferulítica, consistindo em cristais em forma de agulha (CHALEEPA; SZEPES; ULRICH, 2010). Segundo Timms (1984) o óleo de coco apresenta apenas uma forma polimórfica estável na forma β'_2.

Alguns dos produtos fabricados a partir de óleo de coco incluem óleo de fritura, *shortenings*, margarina e produtos de confeitaria (CHE MAN; MANFAF, 2006). Devido conter em sua composição ácidos graxos de cadeia média, é utilizado como fonte de gordura em fórmulas infantis e alimentos destinados a pessoas com dificuldade de absorver ácidos graxos de cadeia longa (O´BRIEN, 2004).

1.1.2.2. ÓLEO DE PALMISTE

O óleo de palmiste, também designado por gordura de palmiste, é extraído da amêndoa do fruto da palmeira *Elaieis guineensis*, e devido à elevada porcentagem de ácido láurico que possui integra-se no grupo das gorduras láuricas. Em climas temperados, é solido a temperatura ambiente, mas funde-se completamente a temperaturas inferiores à temperatura corporal (KELLENS *et al*., 2007).

A composição em ácidos graxos e propriedades físico-químicas são muito semelhantes ao óleo de coco (Tabela 1.1). A diferença principal é que o óleo de palmiste tem um pouco menos de ácidos graxos de cadeia mais curta, como o ácido caprílico, e superior insaturação, com índice de iodo típico de 18,5 contra 8,5 do óleo de coco (GUNSTONE, 2006).

Os principais ácidos graxos são o ácido láurico, com cerca de 48 %, o ácido mirístico, ao redor de 16 % e o ácido oléico, com cerca de 15 % (Codex, 2004). A preponderância de um ácido graxo saturado, combinado com os baixos níveis de insaturação, dá ao óleo de palmiste seu perfil de fusão típico (GUNSTONE, 2006).

Segundo D'Agostini e Gioielli (2002) o óleo de palmiste apresenta cerca de 52,4 % de triacilgliceróis trissaturados, 37,7 % de dissaturados-monoinsaturados, 9,2 % de diinsaturados-monossaturados e 0,7 % de triinsaturados, contendo grande variedade de espécies de triacilgliceróis de cadeia média, que variam de C_{28} a C_{36} (GUNSTONE, 2006) . Os principais triacilgliceróis são: LaLaLa, LaLaM, CaLaLa, CLaLa, LaLaP, LaMM e LaLaO. Também contém quantidades apreciáveis de outros triacilgliceróis de cadeia média como LaOM, LaPM, LaOP/MMO, LaPP, MOO e MOP, onde La = láurico, M = mirístico, Ca = caprílico, C = cáprico, P = palmítico e O = oléico (LIDA *et al*., 2002).

O óleo de palmiste cristaliza predominantemente na forma β'. Isto ocorre devido a alta diversidade na sua composicão em ácidos graxos e triacilgliceróis (GOLD; UKHUN; AKOH, 2011; KIM et al., 2008). Suas propriedades de rápida fusão e formação de eutético também ajudam a contrabalançar o alto ponto de fusão da estearina de palma (AINI; MISKANDAR, 2007).

Entre os numerosos óleos vegetais, o óleo de palmiste tem sido amplamente aplicado em margarinas e *shortenings* devido às suas propriedades vantajosas, tais como elevada produtividade, baixo preço, elevada estabilidades térmica e oxidativa e plasticidade à temperatura ambiente (LIU *et al*., 2010).

1.1.3 .ÓLEOS INSATURADOS

Recomendações dietéticas sugerem a diminuição da ingestão e/ou substituição dos ácidos graxos saturados e aumento da ingestão dos ácidos graxos insaturados, principalmente os poli-insaturados (PUFAs). A principal fonte de ácidos graxos insaturados são os óleos vegetais. Como exemplo, pode ser mencionado o óleo de canola como um dos mais ricos em ácidos graxos monoinsaturados ω-9 (MUFAs) e poli-insaturados (PUFAs) do tipo ω-3 (GUNSTONE; HARWOOD; DIJKSTRA, 2007).

1.1.3.1 ÓLEO DE CANOLA

O óleo de canola foi desenvolvido por modificações genéticas da colza, uma planta cujas sementes apresentavam alto conteúdo de ácido erúcico, considerado tóxico, o que reduzia o valor nutricional de seus produtos. Com seu baixo teor de ácidos graxos saturados, alto teor de ácido oléico e a proporção de ácidos linoléico:α-linolênico de 2:1, o óleo de canola passou a ter grande apelo como

óleo saudável. Apresenta alto conteúdo de vitamina E (α-tocoferol), ao redor de 45 mg/100 g. Apresenta 3,7-7,3 g de fitosteróis/1000 g de óleo bruto (GUSTONE, 2006).

Por esses motivos, o óleo de canola é classificado por médicos e nutricionistas como sendo um óleo de composição equilibrada em ácidos graxos, indicado para integrar uma dieta saudável (KIM *et al.*, 2008; SOUZA; GIOIELLI, SAAD, 2011).

É produzido principalmente no Leste da Europa, China, Índia e Canadá e sua composição aproximada em ácidos graxos é a seguinte: palmítico 4 %, esteárico 2 %, oléico 62 %, linoléico 22 % e α-linolênico 10 % (GUNSTONE, 2006).

Apresenta em sua composição cerca de 0,4 % de triacilgliceróis trissaturados, 3,5 % de dissaturados-monoinsaturados, 20 % de diinsaturados-monossaturados e 76 % de triinsaturados, sendo os principais OOO, OLO e OLnO, sendo O = oléico, L = linoléico e Ln = linolênico (O´BRIEN, 2004; RIBEIRO *et al.*, 2009). Os ácidos saturados estão localizados nas posições *sn*-1,3, enquanto que os ácidos oléico, linoléico e linolênico encontram-se em maior proporção na posição *sn*-2 (GUNSTONE, 2006).

1.1.3.2 AZEITE DE OLIVA

O azeite de oliva tem estado em alta como um fator positivo para uma saúde excelente, ilustrado na baixa incidência de doença cardíaca em pessoas que consomem dieta mediterrânica, onde o azeite é a principal fonte de lipídios (VISSERS *et al*., 2002). O consumo de azeite de oliva tem a tendência de aumento em todo o mundo, com a diminuiçao do consumo de gorduras animais em favor de óleos vegetais, porque satisfaz os requisitos dietéticos de ácidos graxos insaturados (TAN; CHE MAN, 2000). Os benefícios para a saúde do azeite têm sido atribuídos a seu elevado teor do ácido oléico. Além disso, os níveis elevados de antioxidantes naturais encontrados fornecem benefícios para a saúde (VISSERS *et al.*, 2002).

A composição de ácidos graxos do azeite de oliva varia de 7,5-20 % de palmítico, 0,5-5 % de esteárico, 0,3-3,5 % de palmitoléico, 55-85 % de oléico, 7,5-20 % de linoléico e 0,0-1,5 % de linolênico (O"BRIEN, 2004).

Apresenta em sua composição cerca de 5,6 % de triacilgliceróis dissaturados-monoinsaturados, 38,3 % de diinsaturados-monossaturados e 56,7 % de triinsaturados. Os triacilgliceróis encontrados em proporções significativas no azeite de oliva são OOO (40-59 %), POO (12-20 %), OOL (12,5-20 %), POL (5,5-7 %) e SOO (3-7 %) (BOSKOU, 1996). Pequenas quantidades de POP, POSt, OLnL, LOL, OLnO, PLL, PLnO e LLL também são encontradas, onde P = palmítico, O = oléico, St = esteárico, L = linoléico e Ln = linolênico (O'BRIEN, 2004).

Como a maioria dos óleos vegetais, o azeite de oliva apresenta na posição *sn*-2 principalmente ácidos insaturados, como o oléico e poliinsaturados C18, enquanto os ácidos saturados estão principalmente nas posições *sn*-1,3 (DOURTOGLOU *et al*., 2003).

Segundo Criado *et al*. (2007a, 2008), a produção de gorduras interesterificadas enzimaticamente com azeite de oliva é uma perspectiva atraente para a indústria alimentícia, tendo em conta a elevada estabilidade oxidativa do produto em situações de fritura e como reforço à saúde da população.

1.2.. PROCESSOS DE MODIFICAÇÃO DE ÓLEOS E GORDURAS

Alguns óleos e gorduras naturais tem aplicação limitada em suas formas originais devido a sua composição em ácidos graxos e em triacilgliceróis, bem como suas propriedades físico-químicas. Para ampliar seu uso, são modificados por métodos industriais como mistura, fracionamento, interesterificação e hidrogenação ou pela combinação desses processos. Estas técnicas propiciam a modificação de propriedades físico-químicas, pela interação entre os triacilgliceróis (mistura), redução do grau de insaturação dos seus grupos acil (hidrogenação), separação física dos triacilgliceróis (fracionamento) e pela redistribuição dos ácidos graxos nas cadeias dos triacilgliceróis (interesterificação) (AINI; MISKANDAR, 2007; GIBON; DE GREYT; KELLENS, 2007; KARUPAIAH; SUNDRAM, 2007).

A hidrogenação e interesterificação (química ou enzimática) são baseadas em mudanças químicas irreversíveis na composição das gorduras, enquanto que no fracionamento a composição é modificada pela separação física dos diferentes grupos de componentes, sendo reversível (GIBON; DE GREYT; KELLENS, 2007; KELLENS *et al.*, 2007; ZALIHA *et al.*, 2004).

1.2.1. MISTURA DE ÓLEOS E GORDURAS

Na formulação de produtos gordurosos, é comum a mistura de óleos e gorduras, para se alcançar as especificações do produto final (SMALLWOOD, 1989). As interações que ocorrem entre os triacilgliceróis nas misturas promovem alterações nas propriedades físicas das gorduras. Desse modo, a mistura pode ser considerada como um método de modificação de óleos e gorduras, em um nível de intensidade ainda menor que o fracionamento.

A interação entre triacilgliceróis é um dos principais fatores que influenciam o comportamento das gorduras e seus comportamentos de fusão e cristalização. No caso de sistemas

com dois componentes, TIMMS (1984), GIOIELLI (1996); GRIMALDI (1999) e D´AGOSTINI (2001) citaram três casos de sistemas binários de triacilgliceróis que podem ser observados:

Soluções sólidas contínuas – formadas por triacilgliceróis muito similares com compatibilidade total entre os componentes. A similaridade é em relação ao ponto de fusão, volume molecular e forma polimórfica. Como exemplo, pode ser citado o sistema POSt/StOSt, sendo P = palmítico, St = esteárico e O = oléico. Os termos solução sólida ou cristais mistos referem-se a uma mistura, no estado molecular, de dois ou mais componentes. Devido à solubilidade no estado sólido, a separação desses componentes é muito difícil.

Sistema eutético – é o mais comum dos sistemas, ocorrendo quando os componentes da mistura diferem em volume molecular e forma polimórfica, sem diferença acentuada do ponto de fusão. Nesse caso, a solubilidade no estado sólido não é completa. O termo eutético, de origem grega "eu tektos" significa fusão fácil, sendo que a mistura de componentes possui menor temperatura de cristalização no sistema. Podem ser citados como exemplos as misturas PPP/StStSt, POSt/POP, StOSt/StStO, POSt/PStO e PPP/StOSt. Formação de compostos – neste caso de sistema binário, é favorecido o empacotamento dos componentes, formando compostos de maior ponto de fusão. Como exemplos, podem ser citadas as misturas PPO/POP, StPO/POP e POP/OPO.

Timms (1984) citou que, em princípio, se a composição molecular exata de uma gordura ou mistura de gorduras é conhecida, seria possível prever suas propriedades físico-químicas. Entretanto, há dificuldades que necessitam ser superadas. Primeiramente, as gorduras são misturas complexas de triacilgliceróis. Em segundo lugar, o conhecimento teórico sobre misturas sólido-líquido não é adequado para prever as propriedades físico-químicas, mesmo para misturas relativamente simples.

Uma alternativa, frequentemente utilizada por indústrias e pesquisadores, é adotar um tratamento empírico usando métodos de regressão múltipla, que pode ser útil para faixas limitadas de ponto de fusão e conteúdo de gordura sólida.

Segundo Smallwood (1989), se o resultado da interação de óleos e gorduras de características diferentes é linear, a formulação do produto final é relativamente simples. Contudo, frequentemente, propriedades como conteúdo de gordura sólida, consistência e ponto de fusão não são lineares, quando bases diferentes são combinadas. O fenômeno não linear envolvendo as propriedades físico-químicas pode ser devido ao efeito eutético.

O efeito da diminuição do valor das propriedades físico-químicas provocado pela formação de eutéticos pode ser útil, como em margarinas formuladas com misturas de óleo de palma e/ou suas frações e gorduras láuricas (YOUNG, 1985).

Apesar de ser possível balancear a composição em ácidos graxos em proporções desejadas pela simples mistura de óleos, nem sempre é possível resultar em um produto com as características físico-químicas e nutricionais desejadas, já que as características físicas individuais de cada óleo são mantidas na mistura (REENA; LOKESH, 2007). Portanto, a mistura de óleos tem sido utilizada como processo preliminar no desenvolvimento de lipídios estruturados, como pode ser observado em diversos trabalhos publicados nesta área (SAFRA; VILLAMIL, 2008).

1.2.2. INTERESTERIFICAÇÃO

A interesterificação representa a redistribuição dos ácidos graxos nos triacilgliceróis, até a obtenção do equilíbrio. A redistribuição ou rearranjo dos ácidos graxos pode ocorrer intra ou intermoléculas de triacilgliceróis, sem alterar sua composição química e sem a formação de isômeros *trans*, alterando, entretanto, as propriedades físicas e químicas dos óleos e gorduras. Este rearranjo molecular pode ocorrer em apenas uma gordura ou em misturas de duas ou mais gorduras (GIOIELLI, 1998; O'BRIEN, 2004, ROUSSEAU; MARANGONI, 1999).

O produto resultante da reação de interesterificação apresenta a mesma composição em ácidos graxos totais do material inicial, mas a composição em triacilgliceróis, as propriedades físico-químicas e o comportamento de cristalização são alterados (O'BRIEN, 2004).

Ao contrário do que ocorre na hidrogenação, a interesterificação não promove a isomerização dos ácidos graxos de *cis* para *trans*, pois os ácidos graxos não são modificados, mas sim redistribuídos nas ligações éster do glicerol, criando novas estruturas. Desta forma, a interesterificação é uma alternativa à hidrogenação parcial para obter produtos livres de ácidos graxos *trans*, com aplicações em margarinas e substitutos da manteiga de cacau (LIU; LAMPERT, 1999; SONNTAG, 1982).

A interesterificação de óleos e gorduras pode ser aplicada por diversas razões: para influenciar o comportamento de fusão, fornecendo consistência desejada a temperaturas ambiente e de refrigeração; para melhorar ou modificar o comportamento cristalino, de forma a facilitar os processos de produção; e para diminuir a tendência à recristalização durante a vida útil do produto (ROZENAAL, 1992). Portanto, a interesterificação é um processo fundamental para o desenvolvimento de gorduras livres de ácidos graxos *trans* e que tenham características especificas para aplicação em alimentos.

A proposta da interesterificação não é apenas obter propriedades físico-químicas satisfatórias, mas também atingir um comportamento cristalino desejável (normalmente,

cristais β'), de modo a produzir margarinas de alta qualidade sem exsudação de óleo ou sensação de arenosidade na boca (ZHANG; SMITH; ADLER-NISSEN, 2004).

Consequentemente, a estabilidade e as características inerentes de produtos interesterificados podem ser preditas. Na maioria dos casos, a interesterificação acarreta o aumento do ponto de fusão do produto, mediante a introdução de ácidos graxos saturados na posição *sn-2* dos triacilgliceróis e resultante aumento nos níveis de triacilgliceróis dissaturados-monoinsaturados e diinsaturados-monossaturados. Assim, é possível a obtenção de produtos plásticos com consistência característica de *shortenings* (RIBEIRO *et al.*, 2007).

1.2.2.1.INTERESTERIFICAÇÃO QUÍMICA

A interesterificação química é uma reação ao acaso que produz completa randomização dos ácidos graxos nos triacilgliceróis (WILLIS; MARANGONI, 1999). Sob a perspectiva de custo e aplicação em larga escala, a interesterificação química parece ser o método mais atrativo. Contudo, sob a perspectiva de produzir lipídios com composições muito específicas para aplicações funcionais e medicinais, os métodos de interesterificação enzimática são mais interessantes (RIBEIRO *et al.*, 2007, WILLIS; LENCKI; MARANGONI, 1998).

A interesterificação química tem sido utilizada comercialmente desde a década de 1940 com o objetivo de modificar as propriedades físicas da banha. Entretanto, sua aplicação está mais voltada para a produção de margarinas livres de ácidos graxos *trans* (ROUSSEAU; MARANGONI, 1998).

O mecanismo da reação de interesterificação química (Figura 1.1) envolve, inicialmente, a formação de um ânion diglicerinato, a partir do catalisador metóxido de sódio. Em função da polaridade, o ânion diglicerinato se aproxima da carbonila de uma ligação éster de um triacilglicerol, formando um complexo instável com cinco ácidos graxos. Este complexo, ao regenerar o triacilglicerol e o ânion diglicerinato, pode promover a troca ao acaso dos radicais acil presentes, provocando, então, a interesterificação. Este rearranjo prossegue até que seja atingido o equilíbrio químico, quando a distribuição dos ácidos graxos nos triacilgliceróis é totalmente ao acaso (MARANGONI; ROUSSEAU, 1995).

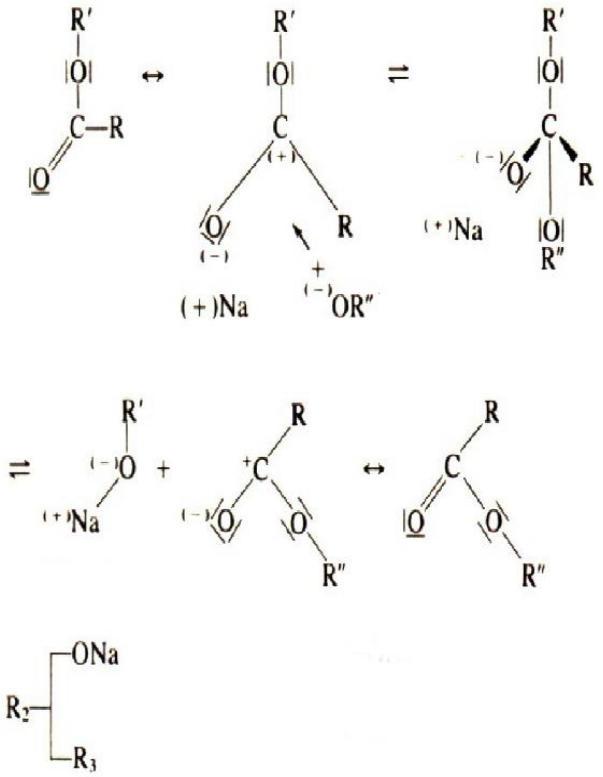


Figura 1.1. Mecanismo de interesterificação química (SHAHIDI, 2005).

No processo químico, óleos e gorduras isentos de umidade são aquecidos e o catalisador é adicionado em proporções apropriadas (0,1 a 0,5 %), de forma a ocorrer sua rápida e completa dispersão na matéria-prima. A reação é conduzida por intervalo de tempo predeterminado e finalizada mediante a adição de água ou ácido, que promove a inativação do catalisador. Fatores que podem influenciar a reação incluem intensidade de agitação, temperatura e tamanho de partícula do catalisador (AKOH, 1998; LEE; AKOH,1998; RIBEIRO *et al*., 2007).

As reações são realizadas em temperaturas acima do ponto de fusão da mistura (MARANGONI; ROUSSEAU, 1995). A tecnologia melhorou muito ao longo das últimas décadas: menor consumo de catalisadores, menos reações paralelas e menores perdas de óleo podem ser garantidas (COSTALES-RODRIGUEZ *et al.*, 2009).

As propriedades utilizadas para comprovar a ocorrência da reação e detectar o ponto final são descritas a seguir (MARANGONI; ROUSSEAU, 1995; GIOIELLI, 1998):

- Alteração na cor: a mudança visual que ocorre é o desenvolvimento de coloração marrom que se intensifica com o progresso da reação. Normalmente a reação é processada por período de tempo fixo (0,5-1 h) após o aparecimento da cor escura.

- Ponto de fusão: é uma das mais rápidas e simples técnicas. Entretanto, em alguns casos, as mudanças são tão pequenas que podem estar na faixa do erro experimental.
- Conteúdo de gordura sólida: as mudanças nos triacilgliceróis dos tipos trissaturados e dissaturados-monoinsaturados provocadas pela interesterificação são refletidas nas curvas de sólidos antes e após a reação.
- Análise da composição em triacilgliceróis: são utilizadas as técnicas de cromatografia em camada delgada, cromatografia em fase gasosa, cromatografia líquida, hidrólise por lipase pancreática e ressonância nuclear magnética do ^{13}C para comprovar as alterações que ocorrem na composição em triacilgliceróis das gorduras rearranjadas.

Vários autores mostraram que a interesterificação química altera a composição em triacilgliceróis e consequentemente as propriedades físico-químicas, como conteúdo de gordura sólida, consistência e tendência de cristalização, de misturas de gorduras sólidas (estearina de palma, óleo de coco, óleo de palmiste, sebo bovino e gordura totalmente hidrogenada) com óleos vegetais como de girassol, arroz, soja, canola, cártamo e azeite de oliva (AZADMARD-DAMIRCHI; DUTTA, 2008; COSTALES-RODRIGUEZ *et al.*, 2009; DIAN; KALYANA; IDRIS, 2007; FARMANI; SAFARI; HAMEDI, 2010; LIU *et al*.; 2010; 2011; MAYAMOL *et al.*, 2008; MENG *et al*., 2010; MENG *et al*.; NORIZZAH *et al.*, 2004; NASIRULLAH; UMESHA; REDDY, 2010; RIBEIRO *et al.*; 2009a).

1.2.2.2. INTERESTERIFICAÇÃO ENZIMÁTICA

A interesterificação por via enzimática é uma alternativa possível à interesterificação por via química. Sob a perspectiva de produzir lipídios com composições específicas para aplicações funcionais e nutricionais, como na produção de sucedâneos da gordura do leite materno, os métodos de interesterificação enzimática são mais interessantes (CRIADO; *et al.*, 2007a; SILVA; GIOIELLI, 2009; WILLIS; LENCKI; MARANGONI, 1998).

A Tabela 1.2 apresenta a comparação entre os processos e produtos resultantes de interesterificação química e enzimática, salientando-se as principais diferenças.

Tabela 1.2. Comparação entre os processos e produtos resultantes de interesterificação química e enzimática.

	Interesterificação química	Interesterificação enzimática
	Processo	
Temperatura	Alta	Moderada
Velocidade de reação	Alta	Normal
Preço do catalisador	Baixo	Elevado
Operações unitárias	Muitas	Poucas
Equipamentos	Há disponibilidade industrial de equipamentos e procedimentos operacionais prontos para a implantação	Necessita o desenvolvimento e otimização de biorreatores adequados
Formação de produtos secundários	Grande produção de produtos secundários	Reduzida produção de produtos secundários
Separação do catalisador	Demorada, com maiores custos	Fácil
	Produtos	
Ácidos graxos na posição *sn*-2	Impossível de controlar	Possível de preservar

Adaptado de PORTE (1999).

As lipases ocorrem na natureza e são ativas na interface óleo-água de emulsões. São enzimas obtidas predominantemente de bactérias, leveduras e fungos e atuam sobre ligações éster presentes em acilgliceróis, liberando ácidos graxos e glicerol. Como exemplo, temos a "*Lipozyme*™ RM IM", uma preparação enzimática isolada do fungo *Rhizomucor miehei* e a "*Lipozyme*™ TL IM", uma preparação enzimática isolada do fungo *Thermomyces lanuginosa*, comercializadas pela empresa Novozymes (DALLA VECCHI; NASCIMENTO; SOLDI, 2004; GIOIELLI *et al*., 1995; WILLIS, 2002).As razões para o enorme potencial biotecnológico de lipases incluem os fatos de que elas são estáveis em solventes orgânicos, não necessitam de co-fatores, possuem grande especificidade de substrato e exibem alta regioseletividade (JAEGER e REETZ, 1998, XU, 2000).

Visando reações com maior uniformidade tecnológica e viabilidade econômica, a utilização de enzimas na forma imobilizada permite considerável aumento na estabilidade e maior diversidade de aplicação, de fundamental importância para o desenvolvimento de processos

associados à bioconversão.A utilização de enzimas imobilizadas confere maior produtividade, facilidade na automação de processos e operações contínuas, controle preciso da extensão das reações, facilidade de separação dos produtos obtidos, estabilização da atividade enzimática, facilidade de recuperação e reutilização das enzimas (SILVA; GIOIELLI, 2009).

A interesterificação enzimática tem a vantagem de permitir grande controle sobre a distribuição posicional dos ácidos graxos no produto final, devido à seletividade e regioespecificidade das lipases. Pela utilização de lipases com seletividade *sn*-1,3, as trocas de grupos acil dão-se apenas entre as posições 1 e 3 dos triacilgliceróis, produzindo-se uma mistura que não se consegue obter por interesterificação química (SIL ROY; BHATTACHATYYA, 1993). De fato, os óleos vegetais caracterizam-se por apresentarem predominantemente ácidos graxos insaturados na posição *sn-2* dos triacilgliceróis. As propriedades físicas, reológicas, organolépticas e nutricionais destes óleos dependem fundamentalmente da sua composição em ácidos graxos, assim como da distribuição dos ácidos graxos nos triacilgliceróis.

A Figura 1.2 mostra o mecanismo para interesterificação enzimática utilizando uma lipase com seletividade *sn*-1,3 para produção de triacilgliceróis com ácidos graxos de cadeias média e longa.

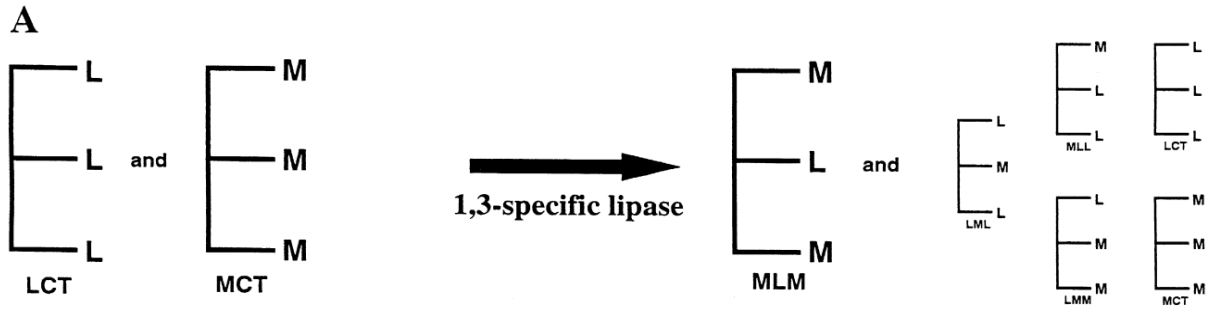


Figura 1.2. Mecanismo de interesterificação enzimática utilizando uma lipase com seletividade *sn*-1,3 (IWASAKI; YAMANE, 2000).

Esta habilidade de produzir novos tipos de misturas de triacilgliceróis utilizando lipases regioespecíficas é uma das características mais interessantes para a aplicação no setor de óleos e gorduras (de CASTRO *et al.*, 2004).

Existem vários fatores que influenciam a atividade e a estabilidade das enzimas destacando-se a temperatura, a atividade de água (a_w), a agitação, a pressão, a composição do meio reacional, a presença de solventes orgânicos, de agentes oxidantes e de compostos inibidores da atividade enzimática (produtos de oxidação lipídica, ácidos graxos livres,

pigmentos e íons metálicos) (CORREIA; FERREIRA-DIAS, 1998; XU *et al*., 1998 a,b). Estes fatores podem levar à ocorrência de migração acil durante a reação de interesterificação.

A migração acil é um sério problema na interesterificação. A razão para esta migração é a existência de acilgliceróis parciais, especialmente diacilgliceróis, que são intermediários necessários e inevitáveis. Ela ocorre pela formação de um intermediário cíclico instável, sendo iniciada pelo ataque nucleofílico de um par de elétrons e resultando em anel intermediário de cinco membros. Este anel abre e resulta em dois produtos, o diacilglicerol original e um que apresentou migração. A migração acil da posição *sn*-2 para as posições *sn*-1 ou *sn*-3, ou o oposto, ocorre do mesmo modo e continua até que o equilíbrio dinâmico seja alcançado (MU *et al*., 2000; XU *et al*., 1998 a,b).

A maioria das reações enzimáticas é realizada em reatores por bateladas, apresentando alguns inconvenientes como altos custos operacionais, baixa produtividade, alta variação na qualidade dos produtos obtidos e problemas de remoção da enzima (SILVA; GIOIELLI, 2009; WILLIS; MARANGONI, 2008).

Reatores contínuos (Figura 1.3) são utilizados associados a enzimas imobilizadas para produção em larga escala de lipídios estruturados. O sistema permite a produção em escala comercial, minimiza os custos e facilita o controle do processo, além de produzir poucos subprodutos e apresentar facilidade de operação. Em reatores contínuos, como o substrato entra em contato com grande quantidade de enzima, o tempo de reação é menor quando comparado com o reator descontínuo, resultando em menor migração acil (SILVA; GIOIELLI, 2009; TEIXEIRA, da FONSECA, VICENTE, 2007).

De acordo com Xu (2003) a utilização de enzimas regioespecificas é essencial para este sistema na maioria das aplicações. O tempo de reação deve ser tão curto quanto possível, uma vez que a migração acil durante o processo é proporcional ao tempo de residência. Por esta consideração, os sistemas contínuos têm sido considerados os melhores reatores para as reação de interesterificação, mostrando menor migração acil quando comparados aos sistemas descontínuos.

A estabilidade operacional das enzimas imobilizadas é um aspecto fundamental a considerar quando se pretende implementar sistemas contínuos ou descontínuos com reutilização sucessiva do biocatalisador, que utilizam preparações enzimáticas cujo elevado preço constitui um fator importante na economia do processo (PAULA, 2011).

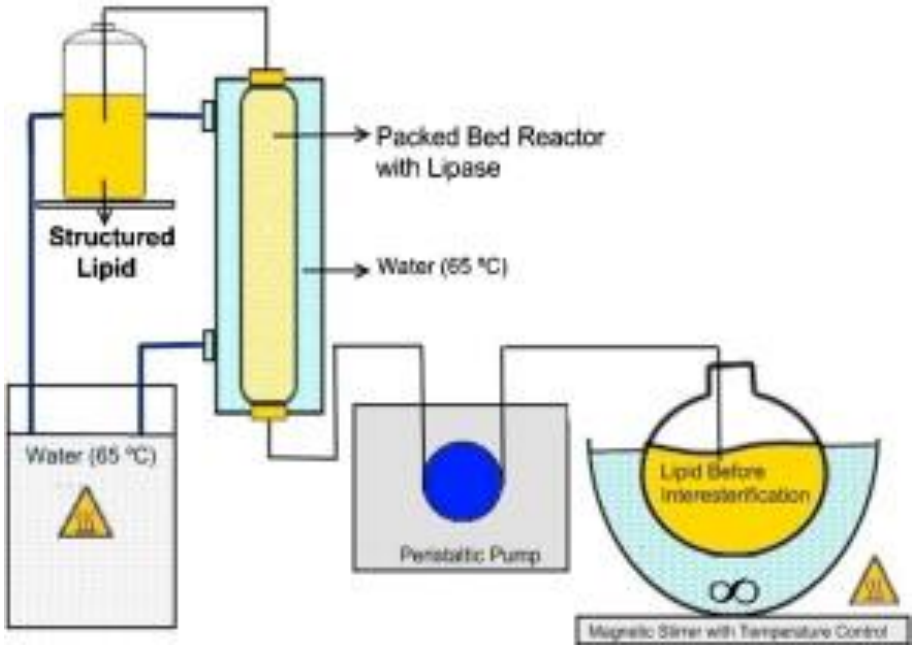


Figura 1.3. Reator enzimático contínuo (SILVA *et al*., 2012)

Num dado sistema operacional, é fundamental que o biocatalisador selecionado apresente atividade catalítica elevada e que essa seja mantida durante longos períodos de utilização. Só assim é possível tornar os processos enzimáticos competitivos com os processos químicos, particularmente nas reações catalisadas por lipases imobilizadas para produção de gorduras modificadas para incorporação em produtos alimentares, de valor agregado inferior ao de produtos destinados à indústria farmacêutica (OSÓRIO, 2008).

Vários trabalhos mostraram que a interesterificação enzimática com lipases imobilizadas comerciais possibilitou modificar as propriedades físico-químicas de misturas de gorduras (ADHIKARI *et al*., 2010; CHU *et al.*; 2002; CRIADO *et al.*, 2007a; CRIADO; *et al.*, 2008; HAYATI *et al.*; 2000; LONG *et al.*; 2003; LEE; AKOH; LEE, 2008; NASCIMENTO *et al*.; 2004; OSÓRIO *et al*., 2005; OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006; OSÓRIO *et al.*, 2008; OSÓRIO *et al.*, 2009; SHIN; AKOH; LEE, 2010a; SHIN; AKOH; LEE, 2010b; SIEW; CHEAH; TANG, 2007; SILVA *et al*., 2009; SILVA *et al*., 2011).

1.3. *SPREADS*

O termo "spread" utilizado neste trabalho engloba diversos produtos disponíveis no mercado que são utilizados para consumo juntamente com pães e biscoitos, como manteigas, margarinas, cremes vegetais e outros produtos com a mesma função (PAVAN, 2008).

A grande produção brasileira de sementes oleaginosas, bem como os melhoramentos na tecnologia de extração de óleos vegetais e as infinitas possibilidades de aplicação das gorduras parcialmente hidrogenadas fizeram com que as margarinas brasileiras fossem tradicionalmente fabricadas, durante muito tempo, pelo processo de hidrogenação (SEMMA, 2002).

O crescimento da demanda do consumidor por *spreads* (margarinas ou cremes vegetais) que associem boas propriedades físicas e sensoriais, como textura, cremosidade e sabor agradável, tem servido como um impulso para a produção industrial de manteigas modificadas ou produtos à base de manteiga. Muitos produtos são comercialmente disponíveis em todo o mundo e novos produtos estão surgindo constantemente (ROUSSEAU *et al*., 1996a; RODRIGUES; GIOIELLI; ANTON, 2003; SOUZA; GOLD; SAAD, 2011).

Desta maneira, o desenvolvimento de *spreads* contendo menor teor de lipídios, utilizando-se misturas de óleos e gorduras isentas de isômeros *trans*, como misturas de estearina de palma, gorduras láuricas e óleo insaturados parece promissor, uma vez que a procura por alimentos considerados mais saudáveis vem aumentando consideravelmente.

O primeiro registro do desenvolvimento comercial de um *spread* feito a partir da mistura de gordura do leite e óleo vegetal data do ano de 1963, na Suécia. O produto, chamado *Bregott*, continha 80 % de gordura, formada por 80 % de gordura do leite e 20 % de óleo de soja. No início dos anos 1980, misturas de manteiga e óleos vegetais surgiram no mercado americano. Estas misturas geralmente eram formadas por 40 % de manteiga e 60 % de óleo vegetal, para um teor de gordura de 80 %. Com a crescente popularidade dos *spreads* com teor reduzido de gordura (menos de 80 %), a partir de meados dos anos 1980, outras misturas com teores de gordura do leite de 2 a 25 % foram introduzidas no mercado (HUI, 1996).

Outra tendência na produção de *spreads* é o aumento do uso da interesterificação, que se apresenta como uma opção na indústria de gorduras para alterar propriedades de espalhabilidade, pois pode modificar substancialmente as propriedades físicas das gorduras sem, no entanto, produzir ácidos graxos *trans* (DE GREYT; KELLENS, 2001). Na indústria, é comum a substituição da hidrogenação parcial de lipídios pelos métodos combinados de fracionamento e interesterificação ou hidrogenação total e interesterificação com óleo líquido para a fabricação de margarinas e bases gordurosas com diversas aplicações (DE GREYT; KELLENS, 2001; GIOIELLI, 2002).

1.3.1. MARGARINA

A margarina foi inicialmente desenvolvida em 1869, como substituto da manteiga, pelo químico francês Hippolyte Mège Mouriés, que ganhou um concurso patrocinado pelo imperador Napoleão III, que objetivava desenvolver um produto similar à manteiga e com baixo custo, para as classes sociais menos favorecidas e para o exército (OLIVER; MAcGILL, 1987).

Hippolyte Mège-Mouriés criou uma substância a que chamou oleomargarina (mais tarde margarina), que preparou com gordura bovina, da qual extraía a porção líquida sob pressão. Em combinação com uma fase aquosa, resultou no substituto para a manteiga, com sabor similar. Desde então a margarina vem sendo comercializada e formulada com várias misturas de óleos e gorduras, animais e vegetais (GRASEFE, 1992).

Os responsáveis pela difusão da margarina na Europa foram duas famílias holandesas que, em 1871, criaram em Oss, uma província ao sul da Holanda, a primeira fábrica para industrializar a margarina (GRASEFE, 1992). Atualmente, é um produto de alta tecnologia, com características próprias e muitas variações (AINI; MISKANDAR, 2007; RODRIGUES; GIOIELLI; ANTON, 2003).

Em 1973, a Unilever lançou a margarina Becel®: o nome do produto vem da ideia original do projeto da empresa - "*Blood Cholesterol Lowering*" - ou redução do nível de colesterol no sangue. Entre 1975 e 1980, as concentrações de lipídios nos produtos tradicionais disponíveis para comercialização começaram a ser reduzidos. Surgiam assim, os chamados “produtos com teor reduzido de lipídios” (contendo cerca de 60 % de lipídios). No fim da década de 1970, as halvarinas, contendo cerca de 40% de lipídios, foram apresentadas ao mercado consumidor, pela primeira vez (PAVAN, 2008).

Em 1981, produtos formulados à base de mistura de margarina e manteiga foram lançados no mercado. Tais produtos continham concentrações de 5 a 40 % de manteiga e preço variável entre os valores da manteiga e da margarina. Além disso, apresentavam aroma de manteiga bem definido, porém com benefícios à saúde e propriedades de espalhabilidade características de margarinas. No final dessa mesma década, surgiram os produtos com teores de lipídios ainda mais reduzidos: 20 % (SOUZA; GIOIELLI, SAAD, 2011).

Nas últimas décadas, o consumo de margarina vem se elevando no Brasil, através da substituição da manteiga e crescente aumento da manufatura e de ingestão de produtos manufaturados industrializados (CHIARA; SICHIERI; CARVALHO, 2003). O consumo internacional de margarina é de cerca de cinco milhões de toneladas, sendo que na Europa o

consumo é ao redor de 1,8 milhão de toneladas e no Brasil de cerca de 500.000 toneladas. De acordo com Barros (2008) as margarinas estão presentes no dia-a-dia de mais de 50% da população adulta e idosa na cidade de São Paulo.

Segundo a legislação brasileira, de acordo com a Portaria 372 da DIPOA, entende-se por margarina o produto gorduroso em emulsão estável com leite ou seus constituintes ou derivados, e outros ingredientes, destinado à alimentação humana com cheiro e sabor característicos. A gordura láctea, quando presente, não deverá exceder a 3,0 % (m/m) do teor de lipídios totais. O teor máximo de gorduras é de 95 % (Brasil, 1997).

Na composição da margarina há ingredientes obrigatórios como: leite, seus constituintes ou derivados, óleos e/ou gorduras de origem animal ou vegetal e água. Os ingredientes opcionais são: culturas de fermentação, gema de ovo, sal, amidos e/ou amidos modificados, açúcares (excetos poli-álcoois), proteínas comestíveis, maltodextrina, vitamina A em quantidade mínima de 1500 UI, vitaminas e/ou sais minerais e/ou outros nutrientes (PAVAN, 2008).

As margarinas são classificadas como emulsão do tipo água em óleo. As gorduras podem consistir de mistura de gorduras modificadas (parcial ou totalmente hidrogenadas e/ou interesterificadas) e óleos líquidos. A tendência é incorporar óleos líquidos tanto quanto possível, para atingir a menor quantidade de ácidos graxos saturados, que é tida como sendo desejável nutricionalmente, além de ser uma vantagem econômica (DEMAN; DEMAN; BLACKMAN, 1989).

Os principais tipos de margarinas e produtos assemelhados são:

- Margarinas duras

Foram lançadas no Brasil, em nível de consumo de massa, no início da década de 1960. São mais adequadas para fritura, cozimento e panificação.

- Margarinas cremosas

As primeiras surgiram no Brasil no princípio dos anos 1970. Apresentam alto poder de espalhabilidade, mesmo a temperatura de refrigeração.

- Margarinas “aeradas”

São produtos em que há incorporação de 10 a 40 % de nitrogênio no resfriamento, provocando volume de cerca de 50 % maior. Como a densidade é menor, diminuiu a quantidade de calorias recebidas em cada porção.

- Margarinas líquidas

São usadas em frituras, uso direto sobre alimentos cozidos, pratos que serão congelados, ou mesmo para passar facilmente sobre o pão. São constituídas por misturas de

óleos líquidos, ou levemente hidrogenados com cerca de 5% de gordura dura. O conteúdo de gordura sólida é baixo e quase o mesmo em todas as temperaturas, o que garante a sua fluidez.

- Margarinas de uso comercial (confeitaria e cozinha profissional)

As margarinas de uso comercial são normalmente preparadas misturando uma base gordurosa padrão para margarinas duras com 4-8 % de gordura dura e/ou monoacilgliceróis.

As margarinas para massa folhada exigem etapas especiais de cristalização e temperagem para desenvolver alto nível de maleabilidade. Normalmente contém cerca de 90 % de gordura, que é emulsificada com água ao invés de leite ou outra proteína.

- Creme vegetal

No Brasil, em 1983 foi lançado um produto semelhante à margarina, mas com menor teor de gordura, chamado de creme vegetal. Apresentava na sua composição de 60 a 65 % de gordura. O creme vegetal é comercializado juntamente com a margarina, porém o consumidor normalmente não tem consciência de que há diferença entre os dois produtos. De acordo com a Portaria 270 da ANVISA, creme vegetal é o alimento em forma de emulsão plástica, cremoso ou líquido, do tipo água/óleo, produzido a partir de óleos e/ou gorduras vegetais comestíveis, água e outros ingredientes, contendo, no máximo, 95 % (m/m) e, no mínimo, 10 % (m/m) de lipídios totais (Brasil, 2005).

Margarinas e cremes vegetais com menores teores de lipídios não devem ser utilizados em cozimentos e frituras. A fase aquosa normalmente não contém leite porque a sua proteína prejudica a emulsão água em óleo. É necessário um sistema emulsificante mais forte, com 0,5-1,0 % de estabilizantes. São produzidos apenas na forma cremosa, pois bases gordurosas mais duras resultam na separação da água, com possíveis problemas microbiológicos. As vantagens destes produtos residem em poder ser mais econômicos e no menor valor calórico. Por outro lado, a fusão da gordura e a quebra da emulsão são mais lentas em relação às margarinas (MASSIELLO, 1978).

A adequação das gorduras para produção de margarinas depende das propriedades físicas, de cristalização e do ponto de fusão, que são dependentes da composição em triacilgliceróis (LUMOR *et al*., 2007).

Para a produção de margarinas, o óleo de palma e suas frações são componentes muito adequados, uma vez que são naturalmente semissólidos à temperatura ambiente, devido à sua composição equilibrada em ácidos graxos. Outra função importante do óleo de palma nas margarinas é fornecer consistência, textura e estrutura para os produtos (AINI;

MISKANDAR, 2007). Contudo, a propriedade de lenta cristalização do óleo de palma pode levar a pós-endurecimento das margarinas, sendo que a interesterificação com outros óleos vegetais melhora este problema (IDRIS; DIAN, 2005).

1.3.1.1. TECNOLOGIA DE MARGARINAS

A tecnologia de produção de margarinas evoluiu muito nos últimos anos. A produção da fase aquosa consiste na adição de ingredientes necessários à sua obtenção como água, sal e conservantes. A fase lipídica consiste na adição de corantes, aromatizantes, lecitina de soja e emulsificantes monoacilgliceróis, solubilizados em mistura de óleos e/ou gorduras vegetais modificadas. Em alguns casos, proteínas do leite e ingredientes espessantes também são utilizados como componentes emulsificantes (OSÓRIO, 2008).

O processo de produção margarinas é constituído pelas seguintes etapas (FAUR, 1996; GIOIELLI, 2002):

- Preparação da fase lipídica;
- Preparação da fase aquosa;
- Preparação da emulsão, resfriamento e cristalização;
- Embalagem e armazenamento

A Figura 1.4 apresenta esquema geral para produção de margarina e creme vegetal.

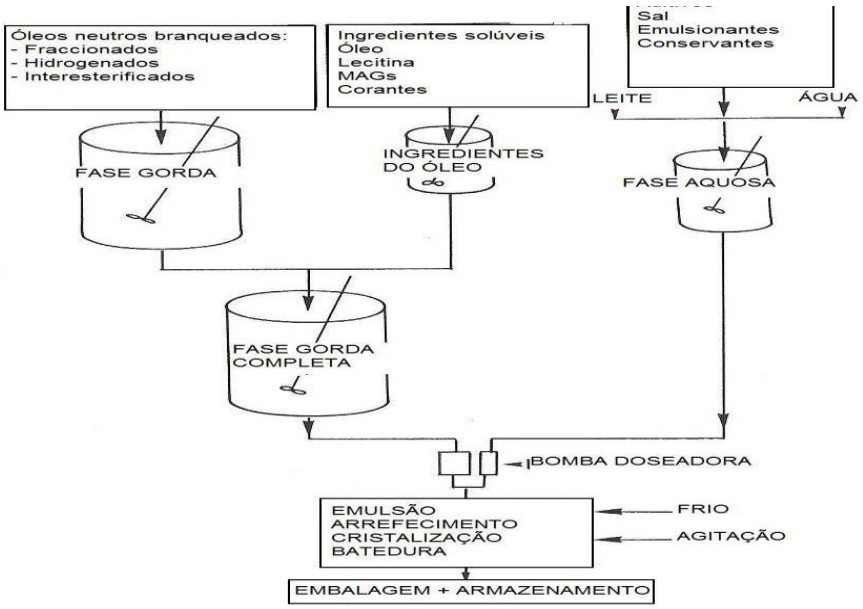


Figura 1.4. Esquema geral da produção de margarina (Fonte: FAUR, 1996).

A preparação da fase lipídica é extremamente importante, já que dela vai depender as características fundamentais do produto. Ao escolherem-se os óleos e gorduras que entram na composição da fase lipídica deve-se ter em atenção as propriedades desejadas para o produto final, como o ponto de fusão, a consistência, a plasticidade e a composição em ácidos graxos, que vai condicionar a resistência à oxidação (RANJITH, 2002).

Na preparação da fase aquosa pode haver problemas de contaminação por microrganismos. Assim, a água utilizada deve ser previamente analisada e tratada. O leite usado é normalmente em pó, reconstituído com água potável. Segue a pasteurização desta mistura. Após resfriamento, adicionam-se os restantes ingredientes solúveis na fase aquosa (YOUNG, 1985).

Os processos de emulsificação e cristalização promovem a formação de um produto gorduroso estável, no qual a fase aquosa se distribui. Além disso, a quantidade de cristais de gordura sólida influencia a estrutura, textura e consistência do produto (MISKANDAR *et al.*, 2002; SOUZA; GIOIELLI; SAAD, 2011; UPRITCHARD *et al.*, 2005).

Uma emulsão é definida pela mistura formada por dois líquidos imiscíveis, estabilizada pela ação de emulsificantes, onde um é uniformemente distribuído no outro sem separação de fases. Óleo e água são os dois principais componentes das emulsões em alimentos, com ingredientes sólidos ou semissólidos nas fases dispersa ou contínua. Esses sistemas são chamados de emulsão óleo em água ou água em óleo (Figura 1.5). As substâncias sólidas ou semi-sólidas presentes podem estar na forma de cristais, como ocorre, por exemplo, em sorvetes, manteigas e margarinas (RANJITH, 2002).

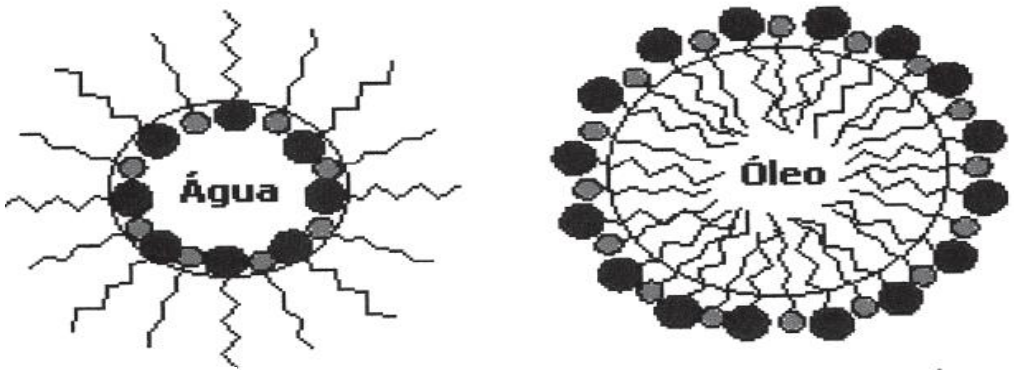


Figura 1.5. Representação da estrutura de emulsões água em óleo (a) e óleo em água (b). (Fonte: de OLIVEIRA *et al*., 2004).

Durante a preparação da emulsão água em óleo, deve-se criar condições que permitam a sua estabilidade. É este o papel dos emulsificantes, que diminuem a tensão superficial e possibilitam boa dispersão da água na fase lipídica (STUIJVENBERG, 1969). Os emulsificantes mais utilizados são os monoacilgliceróis e os diacilgliceróis. Ambos possuem baixos valores de equilíbrio hidrofílico-lipofílico (HLB - *hydrophilic-lipophilic balance*), o que auxilia a estabilizar emulsões água em óleo, como a margarina (RANJITH, 2002; SOUZA; GIOIELLI; SAAD, 2011).

A etapa de cristalização da gordura, realizada após a emulsificação, contribui para a formação de uma rede sólida de cristais, que estabiliza a emulsão, uma vez que essa rede retém os glóbulos de água e o óleo líquido, que constituem o produto (Figura 1.6).

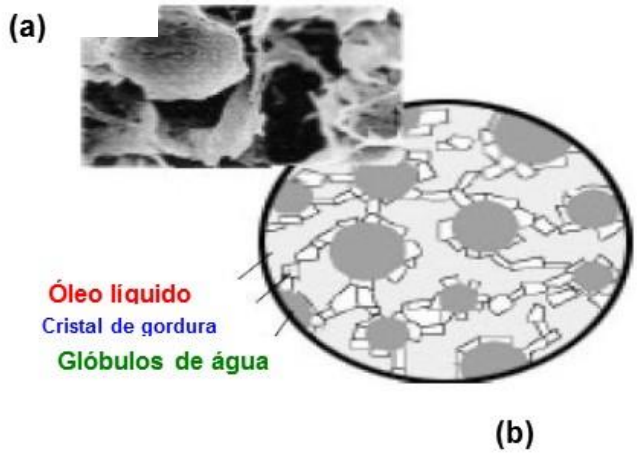


Figura 1.6. (a) Micrografia ilustrando a estrutura da gordura cristalizada. (b) Representação da estrutura da margarina (Fonte: UPRITCHARD *et al.*, 2005; SOUZA; GIOIELLI, SAAD, 2011).

Normalmente, nas indústrias esse processo é realizado rapidamente, sob refrigeração, em trocadores de calor de superfície raspada, para que se obtenha uma rede estruturada de cristais de gordura (AINI; MISKANDAR, 2007). A consistência é um aspecto funcional importante dessas gorduras: a relação entre as fases sólida e líquida e o caráter cristalino da fase sólida determinam a consistência e a firmeza dos produtos (SIMÕES; GIOIELLI; OLIVEIRA, 1998; RANJITH, 2002; SOUZA; GIOIELLI; SAAD, 2011).

Parte da estabilização física da margarina ocorre depois dela ter saído do trocador de calor de superfície raspada, ao longo e/ou depois de ter passado pela máquina de embalagem. Durante este período, os cristais de gordura se estabilizam, o que contribui de forma essencial para a consistência do produto. No entanto, se o processo de estabilização se prolongar por mais do que

algumas horas, podem formar-se progressivamente cristais de maiores dimensões, como consequência da transição β' → β, o que acarreta alterações indesejáveis (VAN der HOCK, 1983). O desenvolvimento de textura grosseira por aglomeração de cristais de maiores dimensões, promovida por condições que favorecem a fusão e a recristalização, pode ser evitado ao se reduzir a temperatura de armazenamento para 0 °C (CHRYSAM, 2002).

CHEMICAL INTERESTERIFICATION OF BLENDS OF PALM STEARIN, COCONUT OIL, AND CANOLA OIL: CHEMICAL COMPOSITION AND REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS IN THE TRIACYLGLYCEROLS

ABSTRACT

The role of fats and oils in human nutrition has been intensively studied and discussed for decades, emphasizing the importance of intake of omega-3, omega-6 and omega-9 fatty acids, reduction of saturated fatty acids and, more recently, control of intake of *trans* fatty acids. Binary and ternary blends of palm stearin, coconut oil and canola oil were done in different ratios and modified by chemical interesterification. The effect of chemical interesterification process was determined by comparing the fatty acids and triacylglycerols compositions and regiospecific distribution of fatty acids in triacylglycerols. Saturated fatty acids were found mainly in the *sn*-1,3 positions, while polyunsaturated fatty acids were found mainly in the *sn*-2 position. Interesterification caused considerable rearrangement of triacylglycerol species, reduction of trisaturated triacylglycerol content and increase in disaturated–monounsaturated and monosaturated-diunsaturated triacylglycerols. The changes of the chemical interesterification are associated to the increase of technological functionality and to a greater potential of these interesterified bases for food application.

KEYWORDS: Low *trans* fats, Blending, Triacylglycerol composition, Fatty acid composition, *sn*-1,3 positions, *sn*-2 position.

2.1. INTRODUCTION

The functional, nutritional and organoleptic properties of natural fats are determined by their fatty acid composition and by their organized fatty acid distribution in the triacylglycerols. By interesterification among triacylglycerols, it is possible to modify physicochemical characteristics of fats, without modifying the fatty acid composition. These changes, resulting from the rearrangement of the acyl residues on the triacylglycerol backbone, are important for margarine manufacture.

Interesterification has received increasing interest as an alternative to partial hydrogenation for the production of ‘‘low *trans*’’ hard fat suitable for shortenings, margarines and confectionary fat production (AHMADI; WHRIGHT; MARANGONI, 2008; COSTALE-RODRIGUES *et al*. 2009). Reducing the dietary intake of *trans* fatty acid has been recommended by various scientific studies, as well as public and regulatory policy (BERGER; IDRIS, 2005; ECKEL, *et al*., 2007). Interesterification, either chemical or enzymatic, can improve the functionality and physical properties of fats and oils (LIST *et al*., 1995).

Numerous efforts have been made to use palm stearin in margarine formulations. Palm stearin is not used directly for edible purposes due to its high melting point, ranging from 44 to 56 °C, giving the product low plasticity and uncompleted melting at body temperature, but contributes to desirable hardness in margarine (ADHIKARI *et al*., 2010; AINI; MISKANDAR, 2007; SOARES *et al*., 2009). Coconut oil may improve the nutritional aspect due to its relatively high medium chain fatty acids content, principally the lauric acid (ADHIKARI *et al*., 2010, ASSUNÇÃO et al. 2009). Canola oil has gained an excellent reputation for its fatty acid composition, which assures its oxidative stability and nutritional qualities in the human diet. However, due to its liquid state, it needs to be blended with hard stocks for production of margarine and shortening fats (FARMANI; SAFARI; HAMEDI, 2009).

Therefore, the aim of this study was to investigate the effect of blending and chemical interesterification on the fatty acids and triacylglycerols compositions and regiospecific distribution of fatty acids in triacylglycerols of the blends of palm stearin, coconut oil and canola and the corresponding interesterified fats.

2.2. MATERIALS AND METHODS

2.2.1. MATERIALS

Palm stearin was obtained from Agropalma S/A (Pará, Brazil), coconut oil from Copra Alimentos Ltda. (Alagoas, Brazil), and canola oil from Bunge Alimentos S.A. (São Paulo, Brazil). The fats were stored at 0 °C prior to use. All chemicals used were either of analytical or chromatographical grades.

2.2.2. BLEND PREPARATION

Fat blends, formulated with palm stearin, coconut oil and canola oil were done in different ratios, according to Table 2.1. Three blends represent the original components, three are binary blends and four are ternary blends. The blends were prepared after complete melting of the fats at 70 °C and were stored under refrigeration.

Table 2.1. Compositional design of the blends.

Blends	Palm stearin (%)	Coconut oil (%)	Canola oil (%)
1	100	0	0
2	0	100	0
3	0	0	100
4	50	50	0
5	50	0	50
6	0	50	50
7	33.3	33.3	33.3
8	66.6	16.7	16.7
9	16.7	66.6	16.7
10	16.7	16.7	66.6

2.2.3. CHEMICAL INTERESTERIFICATION

Chemical interesterification was performed according to Ahmadi, Wright and Marangoni (2008) with modifications. Two hundred grams of each blend were melted in a glass jar at 85°C, under reduced pressure to limit moisture and air. The chemical reaction was started by the addition of 0.3 % (w/w) sodium methoxide (Merck Co.) as a catalyst. The blends were interesterified under reduced pressure for 60 min at 88±2°C. The start of the reaction was

associated with the appearance of a red-brown color. To terminate the reaction, 5 mL of distilled water were added. The presence of water inactivates the catalyst by converting it to methanol (SREENIVASAN, 1978). Kieselghur and anhydrous sodium sulfate were added to minimize the darkening caused by the presence of a diacylglycerol metal derivative - the active catalyst - and to remove residual water, respectively. The reagents were removed through filtering the samples with a filter paper. The fat was poured into a glass jar and stored at 5°C prior to use (Figure 10.1 Annex 1). Non-interesterified oil is abbreviated to NIE and chemical interesterified oil to CIE.

2.2.4. FREE FATTY ACIDS

The free fatty acid content of the fats was determined according to the method described in the AOCS official method Ca 5a-4 (AOCS, 2009a). All samples were analyzed in triplicate and the reported values are the average of the three analyses. Its percentage (wt/wt) was calculated on the basis of the molecular weight of oleic acid (MM= 282 g).

$$\% \text{ Free fatty acids (as oleic acid)} = \frac{V \times M \times 28.2}{W} \qquad \textbf{Eq. 2.1}$$

Where:

V = volume of sodium hydroxide (mL),
M = normality of sodium hydroxide
W = weight of sample (g).

2.2.5. PEROXIDE VALUE

The peroxide value was determined in terms of milliequivalents of peroxide per kilogram of sample that oxidizes KI under the test conditions by following the method described in the AOCS official method Cd 8-53 (AOCS, 2009b). All samples were analyzed in triplicate and the reported values are the average of the three analyses. Results are expressed in mequiv oxygen/kg oil.

$$\text{Peroxide value} = \frac{(S - B) \times M \times 1000}{W} \qquad \textbf{Eq.2.2}$$

Where:

S = volume of titration of sample (mL),

B = volume of titration of blank (mL),

M = normality of the thiosulfate solution and

W = weight of sample (g).

2.2.6. FATTY ACID COMPOSITION

Fatty acid composition was determined after conversion of fatty acids into their corresponding methyl esters (FAMES) by the method described by Hartman and Lago (1973) for blend 1 and by ISO method 5509 (2000) for blends 2 to 10. Analyses of FAMEs were carried out in a Varian GC gas chromatograph (model 430 GC, Varian Chromatograph Systems, Walnut Creek, California, USA), equipped with a CP 8412 auto injector. The Galaxie software was used for quantification and identification of peaks. Injections were performed in a 100-m fused silica capillary column (ID = 0.25 mm) coated with 0.2 μm of polyethylene glycol (SP-2560, Supelco, USA) using helium as carrier gas at isobaric pressure of 37 psi; linear velocity of 20 cm/s; make-up gas: helium at 29 mL/min at split ratio of 1:50; volume injected: 1.0 μL. The injector temperature was set at 250 ºC and the detector temperature was set at 280 ºC. The oven temperature was initially held at 140 ºC for 5 min, then programmed to 240 ºC at rate of 4 ºC/min and held isothermally for 30 min. All samples were analyzed in triplicate and the reported values are the average of the three runs.

Medium-chain saturated fatty acids (MCSFAs) are expressed as the sum of the amounts of caprylic, capric and lauric acids.

Long-chain saturated fatty acids (LCSFAs) are expressed as the sum of the amounts of myristic, palmitic and stearic acids.

Saturated fatty acids (SFAs) are expressed as the sum of the amounts of caprylic, capric, lauric, myristic, palmitic and stearic acids.

Unsaturated acids (USFAs) are expressed as the sum of the amounts of oleic, linoleic and linolenic acids.

Monounsaturated fatty acids (MUFA) are expressed as amounts of oleic acid.

Polyunsaturated fatty acids (PUFAs) are expressed as the sum of the amounts of linoleic and linolenic acids.

2.2.7. IODINE VALUE (IV)

Iodine value was calculated from the fatty acid composition, according to the procedure described in the AOCS official method Cd 1c-85 (AOCS, 2009c). Results are expressed in g iodine/100 g fat.

$$IV = (\%\ C_{18:1} \times 0.860) + (\%\ C_{18:2} \times 1.732) + (\%\ C_{18:3} \times 2.616) \quad \textbf{Eq. 2.3}$$

Where:

$C_{18:1}$ = oleic acid
$C_{18:2}$ = linoleic acid
$C_{18:3}$ = linolenic acid.

2.2.8. ATHEROGENIC INDEX (AI)

Atherogenic index was calculated according to Kim, Lumor and Akoh (2008), by the following equation:

$$AI = [C_{12:0}\ (w/w, \%) + 4 \times C_{14:0}\ (w/w, \%) + C_{16:0}\ (w/w, \%)]/USFA\ (w/w, \%) \quad \textbf{Eq. 2.4}$$

Where:

USFA = total amount of unsaturated fatty acids,
$C_{12:0}$ = lauric acid
$C_{14:0}$ = myristic acid
$C_{16:0}$ =palmitic acid.

2.2.9. TRIACYLGLYCEROL COMPOSITION

2.2.9.1. EXPERIMENTAL VALUE

Triacylglycerol composition was analyzed in a Varian gas chromatograph (model 3400CX, Varian Ind. Com. Ltda., São Paulo, Brazil). A DB-17HT Agilent (Catalog: 122-1811) capillary column (50%-phenyl-methylpolysiloxane, 15 m in length x 0.25 mm bore and containing 0.15 µm film). The conditions were: split injection, ratio 1:100; column temperature: 250 °C, programmed up to 350 °C at 5°C/min; carrier gas: helium, at 1.0 mL/min flow rate; injector temperature: 360 °C; detector temperature: 375 °C; injection volume: 1.0 µL; sample concentration: 100 mg/5 mL of hexane (RIBEIRO *et al*., 2009a). All samples were analyzed in triplicate and the reported values are the average of three runs. TAG profiles were followed throughout the reactions by plotting the percents of the areas of Carbon Number peaks groups.

2.9.2. CALCULATED VALUE

2.2.9.2.1. TRIACYLGLYCEROL COMPOSITION (SATURATED AND UNSATURATED)

For these calculations, it was considered the fatty acid composition obtained experimentally and the experimental results of the fatty acids located at the *sn*-2 position. For the *sn*-1,3 positions was considered that the fatty acids are present in these positions in equivalent amounts, according to the formula:

$$C_{1,3} = [3 \times (C_{1,2,3}) - C_2] / 2 \qquad \textbf{Eq. 2.5}$$

Where:

$C_{1,3}$ = fatty acids in *sn*-1,3 positions
$C_{1,2,3}$ = fatty acid composition of total fat
C_2 = fatty acids in *sn*-2 position

According to the 1,3-random, 2-random theory, the levels of possible triacylglycerols, according to the saturation and unsaturation of fatty acids are:

For natural fats:

$$\% SSS = (\%S1)x(\%S2)x(\%S3)/10000 \quad \textbf{Eq. 2.6}$$
$$\% SUS = (\%S1)x(\%U2)x(\%S3)/10000 \quad \textbf{Eq. 2.7}$$
$$\% SSU = 2x(\%S1)x(\%S2)x(\%U3)/10000 \quad \textbf{Eq. 2.8}$$
$$\% USU = (\%U1)x(\%S2)x(\%U3)/10000 \quad \textbf{Eq. 2.9}$$
$$\% UUS = 2x(\%U1)x(\%U2)x(\%S3)/10000 \quad \textbf{Eq. 2.10}$$
$$\% UUU = (\%U1)x(\%U2)x(\%U3)/10000 \quad \textbf{Eq. 2.11}$$

After chemical interesterification was used the 1,2,3-random distribution theory:

$$\% SSS = (\%S)x(\%S)x(\%S)/10000 \quad \textbf{Eq. 2.12}$$
$$\% SUS/SSU = 3x(\%S)x(\%U)x(\%S)/10000 \quad \textbf{Eq. 2.13}$$
$$\% USU/UUS = 3x(\%U)x(\%S)x(\%U)/10000 \quad \textbf{Eq. 2.14}$$
$$\% UUU = (\%U)x(\%U)x(\%U)/10000 \quad \textbf{Eq. 2.15}$$

Where:

%S = % of saturated fatty acids
%U = % of unsaturated fatty acids

2.2.10. REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS

A proton-decoupled ^{13}C NMR was used to analyze the positional distribution of fatty acids on the triacylglycerol backbone. Lipid samples (250 mg) were dissolved in $CDCL_3$ (0.5 mL) in 5 mm NMR tubes, and NMR spectra were recorded on a Bruker Advance DPX spectrometer operating at 300 MHz. The ^{13}C spectra of the lipid samples were acquired with a spectral width of 2332.090 Hz, pulse of 10.2 μs, and a relaxation delay of 30s. Determination of ^{13}C was performed at a frequency of 75.8 MHz with a multinuclear probe of 5 mm operating at 30 °C, using method described by Vlahov (2005). The results showed the compositions of saturated fatty acids, oleic

acid and linoleic + linolenic acids in *sn*-2 and *sn*-1,3 positions. All samples were analyzed in triplicate and the reported values are the average of three analyses.

2.2.11. STATISTICAL ANALYSIS

Statistical analysis was performed for fatty and triacylglycerol composition and regiospecific distribution of fatty acids. Analysis of variance for multiple comparisons using Statistica 9.0, Statsoft (TULSA, USA, 2010), samples with homogenous variance were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test in order to identify contrasts among samples ($P < 0.05$).

2.3. RESULTS AND DISCUSSION

2.3.1. QUALITY PARAMETERS OF THE BLENDED AND INTERESTERIFIED OILS

The quality of the oil used in the chemical interesterification is essential. Fats and oils used in chemical interesterification must have low acidity, once the free fatty acids, as well as water and peroxide act as catalyst poison of sodium methoxide (RIBEIRO *et al*., 2009b). The peroxide values and free fatty acid contents of the native and blended oils were monitored before and after chemical interesterification. The quality characteristics of the blends are shown in Table 2.2 and meet the quality parameters described by the Codex standard (2004).

The free fatty acids should be less than 0.05% oleic acid, for use of 0.1-0.2% sodium methoxide as catalyst. If these levels are exceeded, it becomes a need for greater amount of catalyst, because it is initially used for the neutralization of free fatty acids (ROZENAAL, 1992).

Free fatty acids are responsible for undesirable flavor and aromas in fats. Free fatty acids are formed by hydrolytic rancidity, which is the hydrolysis of an ester by lipase or moisture. The free fatty acid values for palm stearin and coconut oil were 0.2 and 0.4 (oleic acid %), respectively. The canola oil had the lowest free fatty acid value.

The peroxide value is a measure of the concentration of peroxides and hydroperoxide forms in the initial stage of lipid oxidation. The number of peroxides present in vegetable oils reflects its oxidative level and thus its tendency to become rancid. Theoretically, palm stearin and coconut oil should exhibit a low rate of oxidation due to its low content of unsaturated

fatty acids. Unsaturated fatty acids easily react with oxygen to form peroxides. Oils with high peroxide values are unstable and easily become rancid.

The peroxide values obtained for palm stearin and coconut oil were relatively low, indicating that the samples were highly stable against oxidation. According to the Codex standard (2004), the maximum peroxide value for virgin oils is 15 meq oxygen/kg oil, while the maximum peroxide value was found for canola oil (2.5 meq oxygen/kg oil), which was far below the maximum limit.

The chemical interesterification didn't promote increase both at peroxide value and free fatty acids content.

Table 2.2. Parameters of quality of oils

Blend	Peroxide value (meq O_2/kg oil)		Free fatty acids (oleic acid %)	
	NIE	CIE	NIE	CIE
1	0.3±0.0	0.4±0.1	0.2±0.0	0.2±0.0
2	0.1±0.1	0.0±0.0	0.4±0.0	0.1±0.0
3	2.5±0.5	0.8±0.1	0.1±0.0	0.1±0.0
4	0.4±0.0	0.8±0.1	0.3±0.0	0.1±0.0
5	1.8±0.1	0.6±0.1	0.1±0.0	0.1±0.0
6	1.5±0.2	0.9±0.1	0.3±0.0	0.1±0.0
7	1.0±0.0	0.8±0.1	0.2±0.0	0.1±0.0
8	0.9±0.0	0.7±0.1	0.2±0.0	0.1±0.0
9	1.3±0.0	0.9±0.1	0.3±0.0	0.1±0.0
10	1.5±0.1	0.4±0.1	0.2±0.0	0.1±0.0

Values are means ± SD of three replications.

2.3.2. FATTY ACID COMPOSITION

The fatty acid composition of the blends is shown in Table 2.3 a and b. Medium-chain saturated fatty acids (MCSFAs) are represented by the sum of the amounts of caprylic, capric and lauric acids. Long-chain saturated fatty acids (LCSFAs) are represented by the sum of the amounts of myristic, palmitic and stearic acids.

Fatty acid composition and iodine value are among the most important characteristics of margarines and shortenings. Composition of palm stearin, coconut oil and canola oil are in agreement with the results published in literature (ADHIKARI *et al*., 2010; AINI; MISKANDAR, 2007; FARMANI; SAFARI; HAMEDI, 2009; RIBEIRO *et al*., 2009c). Saturated fatty acids are predominant in palm stearin and coconut oil ($p < 0.05$), mainly

palmitic acid and lauric acid, respectively. Canola oil showed significantly ($p < 0.05$) higher content of oleic, linoleic and linolenic acids.

Coconut oil showed higher ($p < 0.05$) content of MCSFAs. LCSFAs content was significantly ($p < 0.05$) higher in palm stearin. On the other hand, canola oil showed the highest content ($p < 0.05$) of USFAs.

The raw materials and, therefore, the blends, were free of *trans* fatty acids, which is of considerable importance from the nutritional point of view. The iodine values calculated for palm stearin, coconut oil and canola oil were within the ranges cited in the literature and, for the blends, are proportional to the amounts of each component (FARMANI; SAFARI; HAMEDI, 2009; SHIN; AKOH; LEE, 2010a,b; TAN; CHE MAN, 2002).

The risk of dietary lipid consumption for cardiovascular diseases can be evaluated by an Atherogenic Index (AI), which is calculated by the contents of atherogenic SFAs (lauric, myristic and palmitic acids) and USFAs present in the lipids (KIM; LUMOR; AKOH, 2008). Dietary *trans* fatty acids can affect more negatively than SFAs (12:0, 14:0 and 16:0) on plasma lipoprotein in human owing to decreased HDL cholesterol and elevated LDL cholesterol (SHIN; AKOH; LEE, 2010b).

The AI of substrates used was 1.7 for palm stearin, 7.3 for coconut oil and 0.1 for canola oil. Blends with smaller amount of coconut oil or greater amount of canola oil had lower AI values.

The benefits derived through blending of palm stearin and coconut oil with canola oil for the consumer were the improved amounts USFAs. It is important to emphasize that, according to Zhao *et al*. (2007), a higher dietetic intake of USFAs promotes reduction in lipids, lipoproteins and in the inflammatory markers C-reactive protein and cell adhesion molecules, which leads to a decreased risk of cardiovascular disease. So, based on the results of the present study the blends can contribute to an increase of USFAs dietary intake.

Interesterification did not affect the degree of saturation, and does not cause isomerization (SILVA *et al*., 2009). The results obtained in this research effort clearly suggest that interesterification did not cause a significant alteration in the fatty acid profile of the starting blends.

Table 2.3a. Fatty acid composition (g / 100 g) of palm stearin, coconut oil, canola oil and their blends before and after interesterification.

Fatty acids[a]	1		2		3		4		5	
(g/100g)	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE
$C_{8:0}$	0.0±0.0[a]	0.0±0.0	4.9±0.0[g]	5.0±0.0	0.0±0.0[a]	0.0±0.0	2.2±0.0[d]	2.5±0.0	0.0±0.0[a]	0.0±0.0
$C_{10:0}$	0.0±0.0[a]	0.0±0.0	3.9±0.0[f]	4.0±0.0	0.0±0.0[a]	0.0±0.0	1.8±0.0[c]	1.9±0.0	0.0±0.0[a]	0.0±0.0
$C_{12:0}$	0.0±0.0[a]	0.0±0.0	38.7±0.0[g]	39.3±0.0	0.0±0.0[a]	0.0±0.0	18.5±0.1[d]	19.4±0.1	0.0±0.0[a]	0.0±0.0
$C_{14:0}$	1.1±0.0[c]	1.1±0.0	19.8±0.0[j]	19.7±0.0	0.0±0.0[a]	0.0±0.0	10.1±0.0[h]	10.3±0.0	0.5±0.0[b]	0.5±0.0[b]
$C_{16:0}$	57.3±0.0[j]	57.3±0.0	12.7±0.0[c]	12.4±0.0	4.9±0.0[a]	4.4±0.0	36.4±0.1[h]	35.7±0.1	32.1±0.1[g]	31.3±0.1
$C_{18:0}$	5.2±0.0[j]	5.1±0.0	2.2±0.0[a]	2.1±0.0	2.5±0.0[c]	2.2±0.0	3.8±0.0[g]	3.7±0.0	3.9±0.0[h]	3.8±0.0
$C_{18:1}$	30.8±0.0[d]	31.1±0.0	12.5±0.0[a]	12.0±0.0	63.6±0.0[j]	65.4±0.0	22.1±0.1[b]	21.8±0.1	48.1±0.1[h]	47.9±0.1
$C_{18:2}$	5.4±0.0[a]	5.3±0.0	5.5±0.0[a]	5.3±0.0	21.2±0.0[g]	20.7±0.0	5.2±0.2[a]	4.7±0.2	11.5±0.2[d]	12.6±0.2
$C_{18:3}$	0.0±0.0[a]	0.0±0.0	0.0±0.0[a]	0.0±0.0	7.9±0.0[g]	7.4±0.0	0.0±0.0[a]	0.0±0.0	3.8±0.0[d]	3.9±0.0
IV[b]	26.5±0.0[c]	35.9±0.0	10.7±0.0[a]	19.6±0.0	112.0±0.1[j]	111.4±0.0	19.0±0.1[b]	26.9±0.1	71.3±0.3[h]	74.1±0.0
MCSFA[c]	0.0±0.0[a]	0.0±0.0	47.5±0.1[h]	48.5±0.1	0.0±0.0[a]	0.0±0.0	22.5±0.1[e]	23.9±0.1	0.0±0.0[a]	0.0±0.0
LCSFA[d]	63.6±0.0[i]	63.4±0.0	34.6±0.0[e]	34.1±0.0	7.4±0.0[b]	6.6±0.0	50.2±0.1[a]	49.6±0.1	36.5±0.1[h]	35.6±0.1
SFA[e]	63.6±0.0[g]	63.4±0.0	82.0±0.0[j]	82.6±0.0	7.4±0.0[a]	6.6±0.0	72.7±0.2[i]	73.5±0.2	36.5±0.1[c]	35.6±0.1
USFA[f]	36.2±0.0[d]	36.4±0.0	18.0±0.0[a]	17.4±0.0	92.6±0.0[j]	93.4±0.0	27.3±0.2[b]	26.5±0.2	63.5±0.1[h]	64.4±0.1
MUFA[g]	30.8±0.0[d]	30.8±0.0	12.5±0.0[a]	12.0±0.0	63.6±0.0[j]	65.4±0.0	22.1±0.1[b]	21.8±0.1	48.1±0.1[h]	46.9±0.1
PUFA[h]	5.4±0.0[a]	5.4±0.0	5.5±0.0[a]	5.3±0.0	7.4±0.0[h]	7.4±0.0	5.2±0.2[a]	4.7±0.2	15.3±0.2[e]	17.5±0.2
AI[g]	1.7±0.0[c]	1.7±0.0	7.3±0.0[f]	7.5±0.0	0.1±0.0[a]	0.0±0.0	3.5±0.0[e]	3.6±0.0	0.5±0.0[a]	0.5±0.0

Values are shown as means ± SD of three replications. [a] $C_{8:0}$, caprylic acid; $C_{10:0}$, capric acid; $C_{12:0}$, lauric acid; $C_{14:0}$, myristic acid; $C_{16:0}$, palmitic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid; $C_{18:3}$ linolenic acid, [b]IV, Iodine value (g iodine/100g); [c]MCSFA, Medium-chain saturated fatty acids; [d]LCSFA, Long-chain saturated fatty acids; [e]SFA, Saturated fatty acids; [f]USFA, unsaturated fatty acids; [g]MUFA, Monounsaturated fatty acids; [h]PUFA, Polyunsaturated fatty acids; [g]AI Atherogenic Index.

Table 2.3b. Fatty acid composition (g / 100 g) of palm stearin, coconut oil, canola oil and their blends before and after interesterification.

Fatty acids[a]	6		7		8		9		10	
(g/100g)	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE
$C_{8:0}$	2.4±0.0^{e}	2.6±0.0	1.5±0.0^{c}	1.7±0.0	0.7±0.0^{b}	0.8±0.0	3.1±0.0^{f}	3.4±0.0	0.7±0.0^{b}	0.8±0.0
$C_{10:0}$	1.9±0.0^{c}	2.0±0.0	1.2±0.0^{d}	1.3±0.0	0.6±0.0^{b}	0.6±0.0	2.5±0.0^{e}	2.6±0.0	0.5±0.0^{b}	0.6±0.0
$C_{12:0}$	19.0±0.1^{e}	19.3±0.1	12.4±0.0c	12.8±0.1	6.1±0.0^{b}	6.3±0.0	25.4±0.1^{f}	25.7±0.1	6.0±0.0^{b}	6.3±0.2
$C_{14:0}$	9.7±0.0^{g}	9.7±0.0	6.8±0.2^{f}	6.8±0.0	3.9±0.0^{e}	3.9±0.0	13.2±0.0^{i}	13.2±0.0	3.3±0.0^{d}	3.3±0.1
$C_{16:0}$	8.7±0.0^{b}	8.6±0.0	25.9±0.1^{f}	25.1±0.0	42.7±0.2^{i}	41.2±0.0	19.2±0.0^{e}	18.9±0.0	15.2±0.0^{d}	15.4±0.4
$C_{18:0}$	2.3±0.0^{b}	2.3±0.0	3.3±0.0^{f}	3.2±0.0	4.3±0.0^{i}	4.2±0.0	2.7±0.0^{d}	2.7±0.0	2.9±0.0^{e}	2.9±0.0
$C_{18:1}$	38.5±0.1^{g}	38.1±0.1	36.4±0.3^{f}	35.7±0.1	33.7±0.2^{e}	33.1±0.0	24.6±0.0^{c}	24.2±0.0	50.3±0.0^{i}	50.0±0.7
$C_{18:2}$	13.5±0.0^{e}	13.4±0.0	10.0±0.7^{c}	10.9±0.0	7.5±0.4^{b}	8.5±0.0	8.2±0.0^{b}	8.1±0.0	16.1±0.0^{f}	16.3±0.4
$C_{18:3}$	3.9±0.0^{e}	4.0±0.0	2.6±0.1^{c}	2.6±0.0	1.2±0.0^{b}	1.3±0.0	1.2±0.0^{b}	1.2±0.0	5.2±0.0^{f}	5.3±0.0
IV[b]	66.9±0.1^{g}	66.4±0.1	55.4±0.8^{f}	56.3±0.0	45.1±0.5^{e}	46.6±0.0	38.5±0.1^{d}	37.9±0.1	84.6±0.0^{i}	85.0±0.0
MCSFA[c]	23.3±0.1^{f}	23.9±0.1	15.1±0.0^{d}	15.8±0.1	7.4±0.0^{c}	7.8±0.0	31.0±0.1^{g}	31.8±0.1	7.2±0.0^{b}	7.8±0.2
LCSFA[d]	20.7±0.0^{c}	20.6±0.0	35.9±0.8^{g}	35.1±0.0	50.2±0.0^{a}	49.3±0.0	35.1±0.0^{f}	34.8±0.0	21.3±0.0^{d}	21.8±0.5
SFA[e]	44.0±0.1^{d}	44.5±0.1	51.0±0.3^{e}	50.9±0.1	57.6±0.2^{f}	57.1±0.0	66.0±0.1^{h}	66.5±0.1	28.5±0.0^{b}	29.6±0.0
USFA[f]	56.0±0.1^{g}	55.5±0.1	49.0±0.3^{f}	49.1±0.1	42.4±0.2^{e}	42.9±0.0	34.0±0.1^{c}	33.5±0.1	71.5±0.0^{i}	71.5±1.1
MUFA[g]	38.5±0.1^{g}	38.1±0.1	36.4±0.3^{f}	35.7±0.1	33.7±0.2^{e}	33.1±0.0	24.6±0.0^{c}	24.2±0.0	50.3±0.0^{i}	50.0±0.7
PUFA[h]	18.5±0.0^{f}	17.4±0.0	12.6±0.7^{d}	13.5±0.0	8.7±0.4^{b}	9.8±0.0	9.4±0.0^{c}	9.3±0.0	21.2±0.0^{g}	21.6±0.4
AI[g]	1.2±0.0^{b}	1.2±0.0	1.3±0.0^{b}	1.3±0.0	1.5±0.0^{bc}	1.5±0.0	2.9±0.0^{d}	2.9±0.0	0.5±0.0^{a}	0.5±0.0

Values are shown as means ± SD of three replications. [a] $C_{8:0}$, caprylic acid; $C_{10:0}$, capric acid; $C_{12:0}$, lauric acid; $C_{14:0}$, myristic acid; $C_{16:0}$, palmitic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid; $C_{18:3}$ linolenic acid, [b]IV, Iodine value (g iodine/100g); [c]MCSFA, Medium-chain saturated fatty acids; [d]LCSFA, Long-chain saturated fatty acids; [e]SFA, Saturated fatty acids; [f]USFA, unsaturated fatty acids; [g]MUFA, Monounsaturated fatty acids; [h]PUFA, Polyunsaturated fatty acids; [g]AI Atherogenic Index.

2.3.3. TRIACYLGLYCEROL COMPOSITION

Analysis of triacylglycerol composition represents a true indication of randomization, and is extremely useful for monitoring modification of interesterified fats and outlining specific applications for them (O'BRIEN, 2004, RIBEIRO *et al*., 2009a).

From a technological point of view, the molecular triacylglycerol species profile is the key for the understanding of several physical properties of a given oil or fat (BUCHGRABER *et al*., 2004). In a processed food that contains significant fat content, the product's behavior may depend on the triacylglycerol composition of that fat (RIBEIRO *et al.*, 2009d).

Oils and fats are considered complex samples, due to the large number of different triacylglycerols that make up. Thus, the identification of triacylglycerols becomes a difficult process, in which the number of possible structural forms is very large compared to the number of fatty acids (RIBEIRO *et al*., 2009e). The composition of blends of triacylglycerols and interesterified lipids was expressed as the number of carbon atoms in the fatty acids chains due to the difficulty of identifying the specific types of triacylglycerols formed after the interesterification reaction.

Table 2.4 a and b and Figure 2.1 to 2.4 show the main individual triacylglycerols that make up the palm stearin, coconut oil and canola oil raw materials and their blends, before and after chemical interesterification.

In palm stearin, five triacylglycerol groups were identified and were similar to those reported by other authors (BRAIPSON-DANTHINE; GIBON, 2007; CHEN *et al*., 2007; TAN; CHE MAN, 2002). Palm stearin has triacylglycerols ranging from C_{46} to C_{54}, mainly C_{50} (40.5 %) and C_{52} (26.4 %). The main TAGs of these groups are POP/PPO, POO/OPO, PPP and PPL/PLP, where P = palmitic acid, O = oleic acid and L = linoleic acid (Aini; Miskandar, 2007). More than 50% of the triacylglycerols are contributed by disaturated-monounsaturated (SSU, 44.8 %) and trisaturated (SSS, 29.8 %) triacylglycerols that have melting point above room temperature, resulting in the solid nature of palm stearin at room temperature.

The very high diversity of TAGs of coconut oil spread from C_{26} to C_{54}. The triacylglycerol profile of coconut oil showed 15 triacylglycerol groups, as major TAGs C_{32} (CCLa, 12.8 %), C_{34} (CLaLa, 16.0 %), C_{36} (LaLaLa, 16.5 %), C_{38} (LaLaM, 12.9 %) and C_{40} (LaMM, 9.7 %), where C= capric acid, La = lauric acid, M = myristic acid, being mainly saturated TAGs (84.5%). The triunsaturated triacylglycerols in coconut oil were only 0.2%.

The triacylglycerol species identified in the coconut oil samples were similar to those reported by Reena, Reddy and Lokesh (2009) and Tan and Che Man (2002).

Three different triacylglycerol group species were present in canola oil, with the high predominance of C_{54} (83.5 %). Main TAGs are OOO, OLO/LOO, and OLnO/LnOO, where O = oleic acid, L = linoleic acid and Ln = linolenic acid. This significant predominance of triacylglycerols with C_{54} is directly related to the fatty acid composition of canola oil, composed mainly by oleic acid, linoleic acid, and linolenic acid. Concerning triacylglycerols with C_{52}, the POO and PLO species show a higher percentage. These results are in agreement with those presented by Andrikopoulos (2002), Ribeiro *et al* (2009d), Tan and Che Man (2000) and, for canola oil triacylglycerols. The major triacylglycerols in canola oil were triunsaturated (UUU, 81.6 %) and monosaturated-diunsaturated (SUU, 17.2 %).

Blending did not alter the composition of the triacylglycerol species of the parent oils, but their amounts varied according to the ratio of oils used for the blending. The blend containing 50% palm stearin and 50 % coconut oil (4 NIE) showed 47.6 % triacylglycerols having medium-chain fatty acids (C_{26}–C_{44}) and 52.4 % high melting triacylglycerols with C_{46} and C_{54}. This result is similar to that obtained by Jeyarani, Khan and Khatoon (2009).

Fats containing both medium and long-chain fatty acids, because of the fairly complex packing at the molecular level, produce smaller crystals and are more suitable as plastic fats (FOUBERT *et al*., 2007).

Blending of 50 % coconut oil and 50 % canola oil (6 NIE) decreased the proportion of the trisaturated triacylglycerols by 50.0 % compared to native coconut oil and caused the emergence of 38.1 % triunsaturated triacylglycerols.

Blending of palm stearin, coconut oil and canola oil (7, 8, 9 and 10 NIE) decreased the proportion of C_{26} to C_{46} trisaturated triacylglycerols and increased of C_{52} and C_{54} compared to native oils (REENA *et al*., 2009).

Minor changes were observed after chemical interesterification for the triacylglycerol composition of palm stearin, coconut oil and canola oil. On the other hand, interesterification produced significant alteration in the triacylglycerol composition of the blends. Randomization resulted in the increased or decrease in the quantity of existing triacylglycerol groups, suggesting that several species may be produced during chemical interesterification. This indicates that fatty acid chains on the glycerol backbone were changed during the reaction.

Table 2.4a. Triacylglycerol composition (g/100g) of palm stearin, coconut oil, canola oil and their blends, before and after chemical interesterification.

TAGs[a]	1		2		3		4		5	
(g/100g)	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE
C_{26}	0.0±0.0	0.0±0.0	0.4±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C_{28}	0.0±0.0	0.0±0.0	1.3±0.1	0.5±0.0	0.0±0.0	0.0±0.0	0.7±0.1	0.7±0.1	0.0±0.0	0.0±0.0
C_{30}	0.0±0.0	0.0±0.0	8.4±0.2	7.0±0.1	0.0±0.0	0.0±0.0	4.1±0.0	1.0±0.1	0.0±0.0	0.0±0.0
C_{32}	0.0±0.0	0.0±0.0	12.8±0.5	10.2±0.4	0.0±0.0	0.0±0.0	6.4±0.1	1.8±0.1	0.0±0.0	0.0±0.0
C_{34}	0.0±0.0	0.0±0.0	16.0±0.1	14.3±0.3	0.0±0.0	0.0±0.0	8.1±0.1	1.1±0.2	0.0±0.0	0.0±0.0
C_{36}	0.0±0.0	0.0±0.0	16.5±0.5	17.5±0.3	0.0±0.0	0.0±0.0	9.5±0.1	6.2±0.2	0.0±0.0	0.0±0.0
C_{38}	0.0±0.0	0.0±0.0	12.9±0.2	15.0±0.1	0.0±0.0	0.0±0.0	7.4±0.0	6.2±0.1	0.0±0.0	0.0±0.0
C_{40}	0.0±0.0	0.0±0.0	9.7±0.1	15.7±0.6	0.0±0.0	0.0±0.0	5.3±0.1	10.3±0.1	0.0±0.0	0.0±0.0
C_{42}	0.0±0.0	0.0±0.0	6.5±0.4	6.2±0.3	0.0±0.0	0.0±0.0	3.6±0.1	13.1±0.3	0.0±0.0	0.0±0.0
C_{44}	0.0±0.0	0.0±0.0	4.3±0.3	4.2±0.1	0.0±0.0	0.0±0.0	2.3±0.0	14.4±0.2	0.0±0.0	0.0±0.0
C_{46}	1.9±0.0	2.3±0.3	3.6±0.6	3.1±0.1	0.0±0.0	0.0±0.0	2.1±0.1	14.8±0.5	0.9±0.1	0.1±0.0
C_{48}	24.6±0.8	25.0±0.7	3.3±0.2	3.1±0.1	0.0±0.0	0.0±0.0	13.7±0.1	13.2±0.2	13.5±0.5	6.3±0.1
C_{50}	40.5±0.9	42.0±0.7	2.6±0.3	2.2±0.0	1.2±0.1	0.0±0.0	20.3±0.2	10.0±0.4	22.6±0.3	27.2±0.8
C_{52}	26.4±0.8	25.9±1.8	1.5±0.0	1.2±0.1	15.4±0.3	18.2±0.2	13.1±0.1	5.9±0.4	18.7±0.4	40.8±1.7
C_{54}	6.5±1.0	4.5±1.1	0.2±0.0	0.0±0.0	83.5±0.2	81.8±0.2	3.1±0.1	1.3±0.1	44.2±0.3	25.6±1.1
SSS[b]	29.8±0.0	25.7±0.1	84.0±0.1	56.4±0.1	0.4±0.0	0.0±0.0	56.9±0.0	39.7±0.0	15.1±0.0	4.5±0.0
SSU[c]	44.8±0.1	42.1±0.0	12.0±0.0	35.6±0.0	3.5±0.0	1.2±0.0	28.4±0.0	43.0±0.0	24.2±0.0	24.5±0.0
SUU[d]	21.8±0.0	29.3±0.0	3.8±0.0	7.5±0.0	20.0±0.1	17.2±0.0	12.8±0.0	15.5±0.0	20.9±0.0	44.3±0.0
UUU[e]	3.6±0.0	6.9±0.0	0.2±0.0	0.5±0.0	76.0±0.01	81.6±0.0	1.9±0.0	1.9±0.0	39.8±0.0	26.7±0.0

Values are shown as means ± SD of three replications. [a]Number of carbon derived from the triacylglycerol less glycerol. [b]SSS, trisaturated; [c]SSU, disaturated–monounsaturated; [d]SUU, monosaturated-diunsaturated; [e]UUU, triunsaturated.

Table 2.4b. Triacylglycerol composition (g/100g) of palm stearin, coconut oil, canola oil and their blends, before and after chemical interesterification.

TAGs[a]	6		7		8		9		10	
(g/100g)	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE
C_{26}	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.3±0.0	0.6±0.0	0.0±0.0	0.0±0.0
C_{28}	0.5±0.0	0.2±0.0	0.5±0.1	0.1±0.0	0.2±0.0	0.0±0.0	1.0±0.0	0.9±0.1	0.1±0.0	0.0±0.0
C_{30}	4.2±0.0	1.1±0.0	2.5±0.0	0.8±0.1	1.0±0.0	0.3±0.0	5.7±0.0	1.7±0.0	0.8±0.1	0.3±0.0
C_{32}	5.4±0.3	0.9±0.1	4.3±0.1	1.2±0.1	2.0±0.1	0.6±0.3	8.8±0.1	2.8±0.1	1.2±0.1	0.2±0.1
C_{34}	7.6±0.2	3.6±0.1	5.5±0.2	1.6±0.1	3.1±0.0	0.7±0.2	11.3±0.1	2.9±0.2	1.6±0.1	0.2±0.0
C_{36}	9.7±0.4	7.6±0.3	6.3±0.2	3.6±0.3	3.7±0.2	1.7±0.4	13.1±0.3	7.4±0.2	3.6±0.3	0.4±0.2
C_{38}	7.6±0.2	7.4±0.1	3.8±0.3	3.5±0.2	2.5±0.1	1.2±0.2	9.5±0.1	5.7±0.3	3.5±0.2	0.1±0.1
C_{40}	5.3±0.0	9.3±0.0	3.2±0.1	2.5±0.4	1.5±0.1	2.5±0.0	7.3±0.0	11.9±0.1	2.5±0.4	2.9±0.1
C_{42}	3.4±0.3	9.5±0.1	2.3±0.0	8.7±0.0	1.2±0.1	4.8±0.3	4.3±0.1	16.7±0.0	8.7±0.0	3.2±0.1
C_{44}	3.2±0.1	10.2±0.3	1.2±0.0	10.7±0.5	0.4±0.1	6.6±0.1	2.7±0.3	13.7±0.0	10.7±0.5	5.3±0.1
C_{46}	2.5±0.0	10.4±0.5	1.8±0.3	15.4±0.2	1.7±0.1	12.3±0.0	2.6±0.5	12.4±0.3	15.4±0.2	6.2±0.1
C_{48}	1.5±0.0	10.3±0.1	9.7±0.2	17.5±0.5	17.2±0.6	16.2±0.0	6.5±0.1	10.8±0.2	17.5±0.5	13.6±0.6
C_{50}	1.5±0.1	8.5±0.1	14.4±0.1	11.7±0.4	27.6±0.4	24.8±0.1	8.3±0.1	4.0±0.1	11.7±0.4	10.0±0.4
C_{52}	5.6±0.2	5.4±0.1	14.3±0.3	16.5±1.2	19.5±0.5	19.7±0.2	7.1±0.1	3.4±0.3	16.5±1.2	25.5±0.5
C_{54}	42.0±0.9	15.4±0.1	30.3±0.2	6.4±0.3	18.3±0.2	8.7±0.9	11.5±0.1	5.3±0.2	6.4±0.3	31.8±0.2
SSS[b]	42.2±0.0	8.7±0.0	37.7±0.0	13.2±0.0	34.0±0.1	29.5±0.0	61.1±0.0	3.7±0.1	19.3±0.0	2.6±0.2
SSU[c]	7.8±0.0	32.9±0.0	19.9±0.1	38.1±0.0	32.2±0.1	44.4±0.0	15.8±0.0	22.3±0.0	11.6±0.0	18.8±1.1
SUU[d]	11.9±0.0	41.2±0.0	15.2±0.0	36.8±0.0	18.4±0.1	22.3±0.0	9.4±0.0	44.4±0.0	17.5±0.0	45.4±2.2
UUU[e]	38.1±0.0	17.2±0.0	27.1±0.1	11.9±0.0	15.3±0.1	3.7±0.0	13.6±0.0	29.5±0.0	51.5±0.1	36.6±1.4

Values are shown as means ± SD of three replications. [a]Number of carbon derived from the triacylglycerol less glycerol. [b]SSS, trisaturated; [c]SSU, disaturated–monounsaturated; [d]SUU, monosaturated-diunsaturated; [e]UUU, triunsaturated.

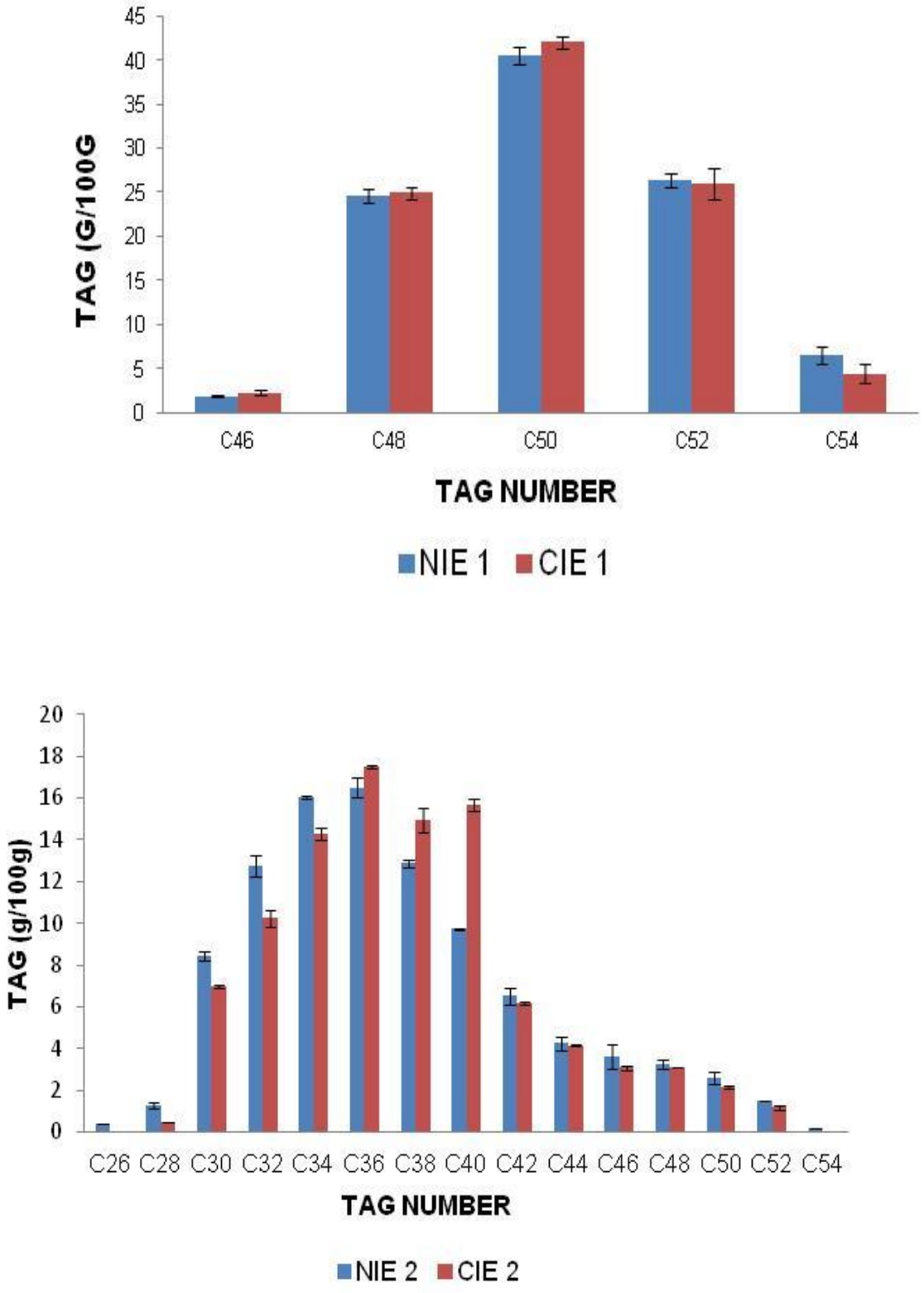


Figure 2.1. Triacylglycerol composition for palm stearin (1) and coconut oil (2) before (NIE) and after (CIE) chemical interesterification.

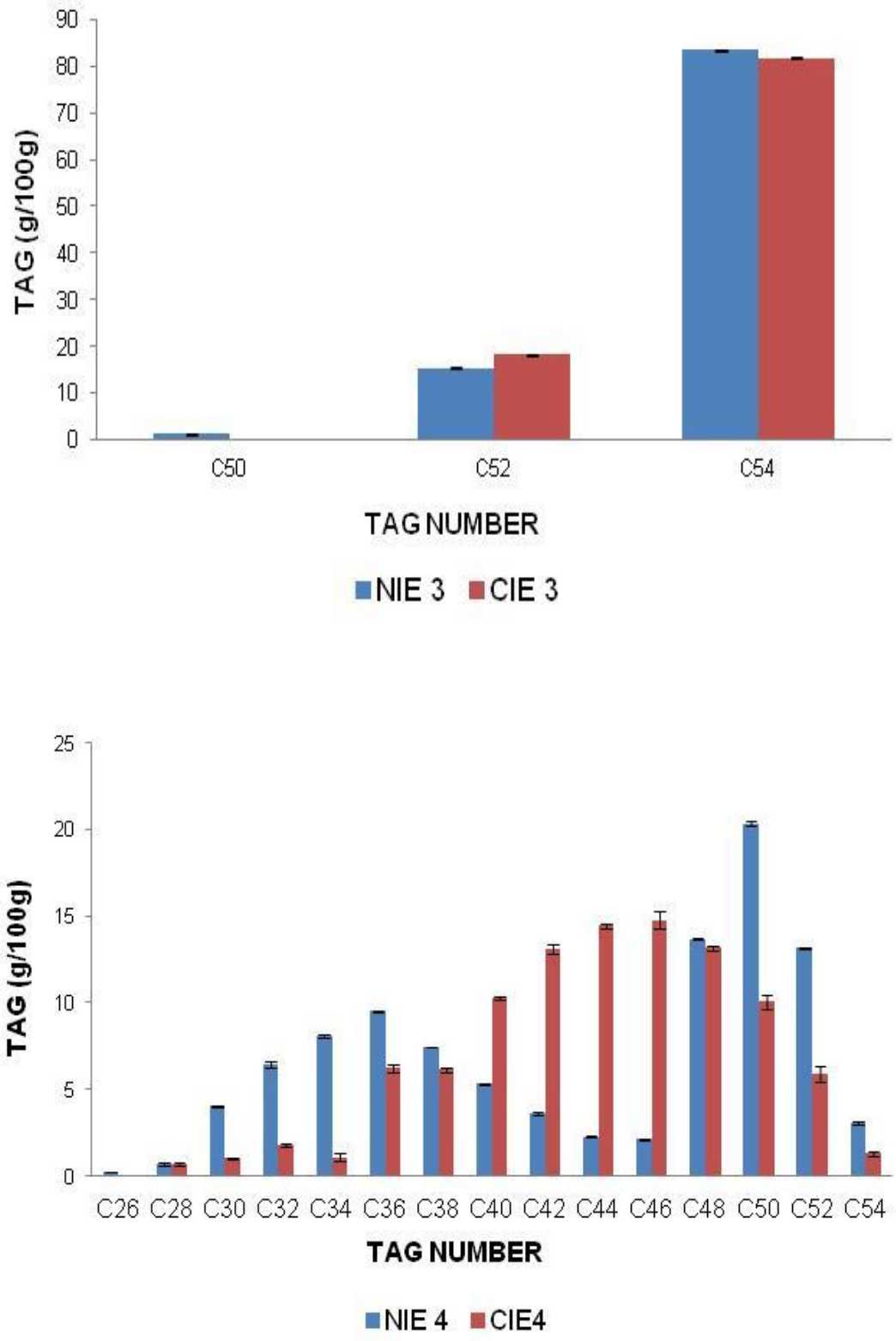


Figure 2.2. Triacylglycerol composition for canola oil (3) and the blend constituted by 50 % palm stearin and 50 % coconut oil (4) before (NIE) and after (CIE) chemical interesterification.

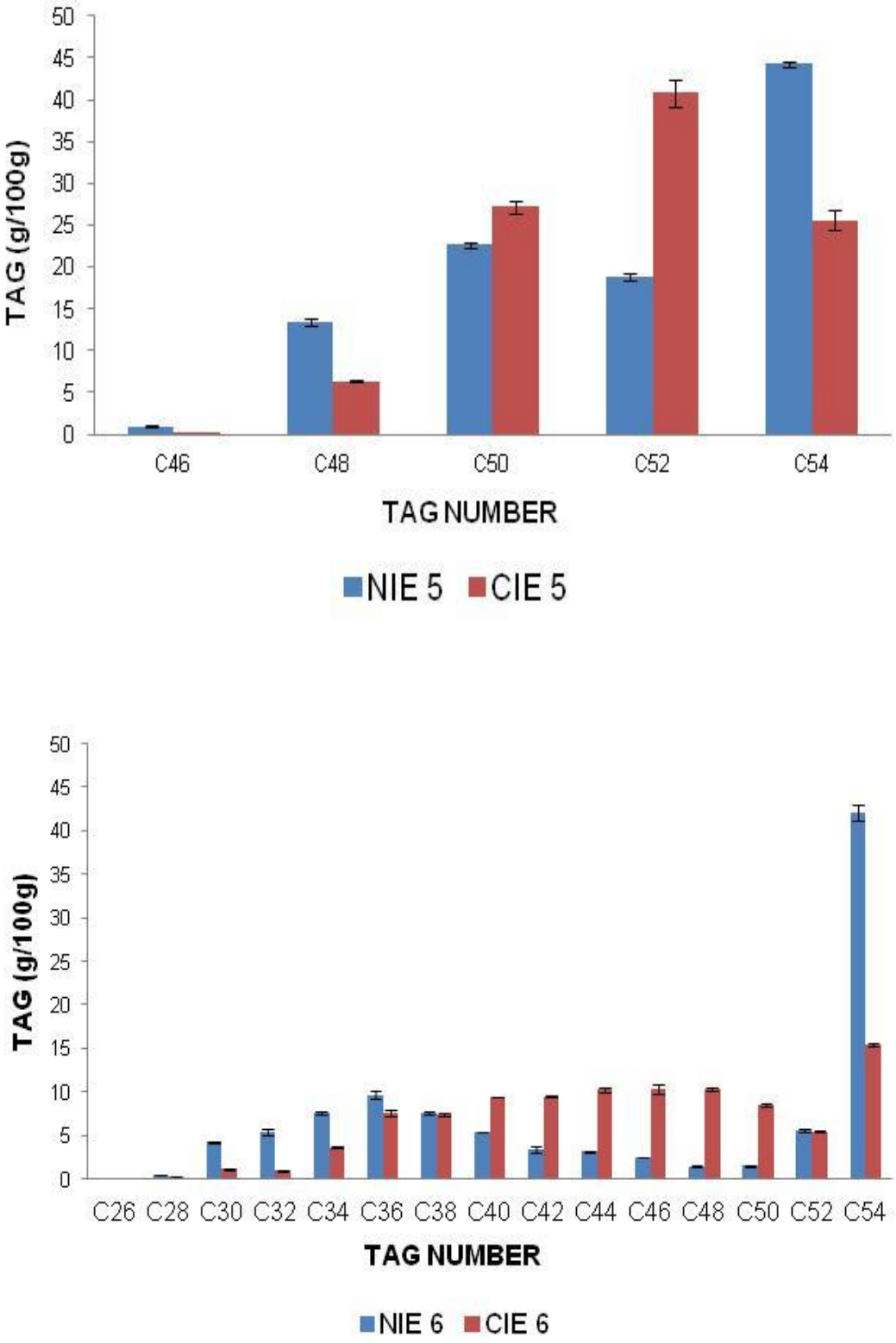
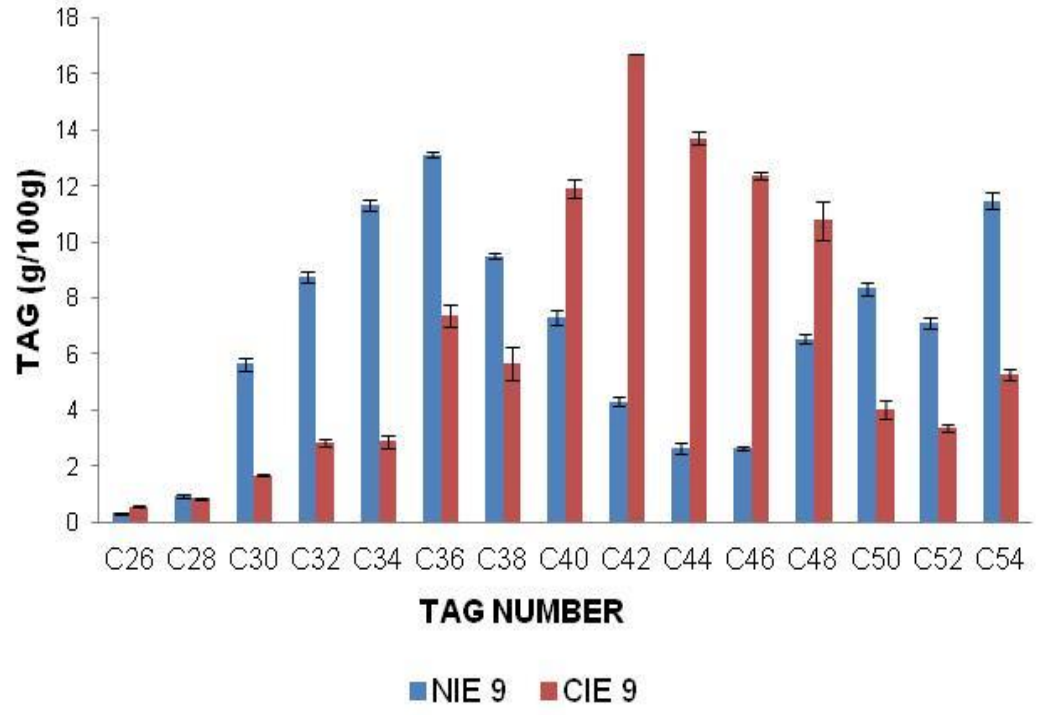


Figure 2.3. Triacylglycerol composition for the blends constituted by 50% palm stearin and 50 % canola oil (5) and 50 % coconut oil and 50 % canola oil (6) before (NIE) and after (CIE) chemical interesterification.



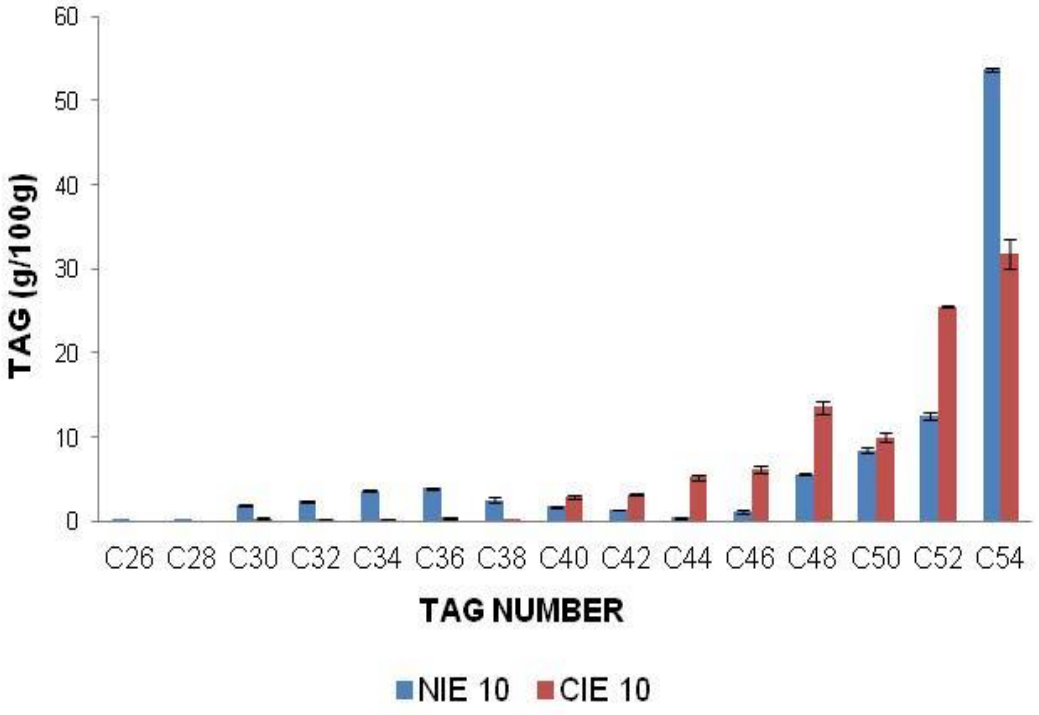


Figure 2.4. Triacylglycerol composition for the blends constituted by 16.7 % palm stearin, 66.6 % coconut oil and 16.7 % canola oil (9) and 16.7 % palm stearin, 16.7 % coconut oil and 66.7 % canola oil (10) before (NIE) and after (CIE) chemical interesterification.

Interesterification of the blends resulted in a decrease in C_{26}, C_{28}, C_{30}, C_{32}, C_{34}, C_{36} and C_{38} triacylglycerols. Reena, Reddy and Lokesh (2009) also observed a reduction in these TAGs in the interesterification of coconut oil and rice bran oil.

Interesterification also resulted in an increase in C_{40}, C_{42}, C_{44}, C_{46}, C_{48}, C_{50}, C_{52} and C_{54}, mostly of TAGs with intermediate carbon number, like C_{40}, C_{42}, and C_{44} in the blends 4, 6, 7, 8, 9 and 10 CIE. In particular, the percentage increase and the formation of C_{54} triacylglycerols endow the interesterified blends with properties of lubricity, aeration and gloss; while the presence of C_{50} and C_{52} triacylglycerols ensures important qualities as regards structure and moisture barrier, contributing to the functionality of the randomized fatty bases (GHOTRA; DYAL; NARINE, 2002; RIBEIRO *et al*., 2009a).

Chemical interesterification decreased the amount of SSS triacylglycerols (all samples), while the levels of SSU (with exception of blends 1, 3 and 5) and SUU (with the exception of blend 3) increased. UUU triacylglycerols decreased in the blends 5, 6, 8 and 10 and increased in the blends 1, 2, 3 and 9. After random rearrangement, triacylglycerols UUS presented the highest concentration among the triacylglycerol classes mainly as a result of the increase of C_{54}. Triacylglycerols UUS with melting points from 1 to 23 °C are important for sensory properties and related to fat functionality at ambient temperature (RIBEIRO *et al*., 2009d; RODRIGUES; GIOIELLI; ANTON, 2003).

Thus, addition of canola oil, that contains higher amounts of unsaturated fatty acids, resulted in the reduction of trisaturated triacylglycerols from palm stearin and coconut oil. Interesterification caused the emergence of new triacylglycerol molecules in the blends, which were present in less amounts in the native oils or in the blends.

Differences in the triacylglycerol composition of blends, resulting from random rearrangement, can promote important alterations in the physical properties of fats, such consistency, solid fat content and melting and softening points (SOARES *et al*., 2012).

Calculated TAG compositions of the individual fats and their blends before and after chemical interesterification are presented in Table 2.5 a and b.

These results are very similar with that obtained by gas chromatography. Largest differences were obtained in the TAGs with medium chain fatty acids derived from coconut oil. General error was between 0.0 and 12.0 % and the average between the differences was 2.0 %.

Table 2.5a. Calculated triacylglycerol composition (g/100g) of palm stearin, coconut oil, canola oil and their blends, before and after chemical interesterification.

TAGs[a] (g/100g)	1		2		3		4		5	
	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE
C_{26}	0.0±0.0	0.0±0.0	0.4±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C_{28}	0.0±0.0	0.0±0.0	1.3±0.1	0.3±0.0	0.0±0.0	0.0±0.0	0.6±0.1	0.0±0.1	0.0±0.0	0.0±0.0
C_{30}	0.0±0.0	0.0±0.0	8.4±0.2	0.6±0.0	0.0±0.0	0.0±0.0	4.2±0.0	0.1±0.1	0.0±0.0	0.0±0.0
C_{32}	0.0±0.0	0.0±0.0	12.8±0.5	2.7±0.1	0.0±0.0	0.0±0.0	6.4±0.1	0.3±0.1	0.0±0.0	0.0±0.0
C_{34}	0.0±0.0	0.0±0.0	16.0±0.1	4.4±0.0	0.0±0.0	0.0±0.0	8.0±0.1	0.3±0.2	0.0±0.0	0.0±0.0
C_{36}	0.0±0.0	0.0±0.0	16.5±0.5	9.8±0.0	0.0±0.0	0.0±0.0	8.2±0.1	1.9±0.2	0.0±0.0	0.0±0.0
C_{38}	0.0±0.0	0.0±0.0	12.9±0.2	13.6±0.1	0.0±0.0	0.0±0.0	6.4±0.0	3.1±0.1	0.0±0.0	0.0±0.0
C_{40}	0.0±0.0	0.0±0.0	9.7±0.1	14.0±0.1	0.0±0.0	0.0±0.0	4.9±0.1	6.6±0.1	0.0±0.0	0.0±0.0
C_{42}	0.0±0.0	0.0±0.0	6.5±0.4	16.7±0.1	0.0±0.0	0.0±0.0	3.3±0.1	9.8±0.3	0.0±0.0	0.0±0.0
C_{44}	0.0±0.0	0.0±0.0	4.3±0.3	13.8±0.1	0.0±0.0	0.0±0.0	2.1±0.0	13.8±0.2	0.0±0.0	0.0±0.0
C_{46}	1.9±0.0	1.1±0.1	3.6±0.6	9.7±0.1	0.0±0.0	0.0±0.0	2.8±0.1	18.0±0.5	1.0±0.1	0.2±0.0
C_{48}	24.6±0.8	21.2±0.2	3.3±0.2	7.9±0.0	0.0±0.0	0.0±0.0	13.9±0.1	17.0±0.2	12.3±0.5	4.0±0.1
C_{50}	40.5±0.9	42.1±0.1	2.6±0.3	3.4±0.0	1.2±0.1	0.7±0.0	21.6±0.2	15.2±0.4	20.8±0.3	21.5±0.8
C_{52}	26.4±0.8	29.3±0.2	1.5±0.0	1.5±0.0	15.4±0.3	13.3±0.2	14.0±0.1	10.5±0.4	20.9±0.4	43.8±1.7
C_{54}	6.5±1.0	6.9±0.1	0.2±0.0	0.8±0.0	83.5±0.2	86.0±0.2	3.4±0.1	3.0±0.1	45.0±0.3	30.8±1.1
SSS[b]	29.8±0.0	25.7±0.1	84.0±0.1	56.4±0.1	0.4±0.0	0.0±0.0	56.9±0.0	39.7±0.0	15.1±0.0	4.5±0.0
SSU[c]	44.8±0.1	42.1±0.0	12.0±0.0	35.6±0.0	3.5±0.0	1.2±0.0	28.4±0.0	43.0±0.0	24.2±0.0	24.5±0.0
SUU[d]	21.8±0.0	29.3±0.0	3.8±0.0	7.5±0.0	20.0±0.1	17.2±0.0	12.8±0.0	15.5±0.0	20.9±0.0	44.3±0.0
UUU[e]	3.6±0.0	6.9±0.0	0.2±0.0	0.5±0.0	76.0±0.01	81.6±0.0	1.9±0.0	1.9±0.0	39.8±0.0	26.7±0.0

Values are shown as means ± SD of three replications. [a]Number of carbon derived from the triacylglycerol less glycerol. [b]SSS, trisaturated; [c]SSU, disaturated–monounsaturated; [d]SUU, monosaturated-diunsaturated; [e]UUU, triunsaturated.

Table 2.5b. Calculated triacylglycerol composition (g/100g) of palm stearin, coconut oil, canola oil and their blends, before and after chemical interesterification.

TAGs[a] (g/100g)	6		7		8		9		10	
	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE
C_{26}	0.2±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.3±0.0	0.0±0.0	0.1±0.0	0.0±0.0
C_{28}	0.6±0.0	0.0±0.0	0.4±0.1	0.0±0.0	0.2±0.0	0.0±0.0	0.9±0.0	0.1±0.1	0.2±0.1	0.0±0.0
C_{30}	4.2±0.0	0.1±0.0	2.9±0.0	0.0±0.1	1.4±0.0	0.0±0.0	5.6±0.0	0.2±0.0	1.4±0.0	0.0±0.0
C_{32}	6.4±0.3	0.3±0.1	4.3±0.1	0.1±0.1	2.2±0.1	0.0±0.1	8.6±0.1	0.8±0.1	2.2±0.1	0.0±0.0
C_{34}	8.0±0.2	0.6±0.1	5.5±0.2	0.2±0.1	2.7±0.0	0.0±0.0	10.7±0.1	1.3±0.0	2.7±0.0	0.0±0.0
C_{36}	8.2±0.4	1.4±0.3	5.6±0.2	0.6±0.0	2.8±0.2	0.2±0.1	11.1±0.3	3.4±0.0	2.8±0.0	0.1±0.0
C_{38}	6.4±0.2	3.1±0.1	4.4±0.3	1.3±0.0	2.2±0.1	0.3±0.0	8.6±0.1	5.7±0.0	2.2±0.0	0.3±0.1
C_{40}	4.9±0.0	3.7±0.0	3.3±0.1	2.5±0.1	1.7±0.1	1.1±0.0	6.5±0.0	8.1±0.1	1.7±0.1	0.5±0.0
C_{42}	3.3±0.3	8.7±0.0	2.2±0.0	5.4±0.0	1.1±0.1	2.3±0.1	4.4±0.1	13.3±0.0	1.1±0.0	1.5±0.1
C_{44}	2.1±0.1	10.1±0.0	1.4±0.0	7.6±0.0	0.7±0.1	5.3±0.0	2.8±0.3	13.5±0.0	0.7±0.0	2.8±0.0
C_{46}	1.8±0.0	9.6±0.0	1.9±0.3	13.0±0.1	1.9±0.1	9.8±0.0	2.7±0.5	15.1±0.1	0.9±0.0	5.4±0.1
C_{48}	1.6±0.0	22.4±0.1	9.2±0.2	17.4±0.0	16.8±0.6	16.1±0.0	6.1±0.1	16.5±0.0	4.5±0.0	12.5±0.0
C_{50}	1.9±0.1	11.2±0.0	14.6±0.1	15.9±0.0	27.4±0.4	27.4±0.0	8.4±0.1	9.3±0.1	7.7±0.1	10.6±0.1
C_{52}	8.4±0.2	8.9±0.0	14.5±0.3	21.3±0.2	20.3±0.5	27.4±0.2	7.8±0.1	7.7±0.0	14.8±0.0	25.2±0.2
C_{54}	41.8±0.9	19.8±0.0	30.6±0.2	14.6±0.1	18.5±0.2	10.0±0.1	15.4±0.1	4.9±0.0	57.0±0.0	41,2±0.0
SSS[b]	42.2±0.0	8.7±0.0	37.7±0.0	13.2±0.0	34.0±0.1	29.5±0.0	61.1±0.0	3.7±0.1	19.3±0.0	2.6±0.2
SSU[c]	7.8±0.0	32.9±0.0	19.9±0.1	38.1±0.0	32.2±0.1	44.4±0.0	15.8±0.0	22.3±0.0	11.6±0.0	18.8±1.1
SUU[d]	11.9±0.0	41.2±0.0	15.2±0.0	36.8±0.0	18.4±0.1	22.3±0.0	9.4±0.0	44.4±0.0	17.5±0.0	45.4±2.2
UUU[e]	38.1±0.0	17.2±0.0	27.1±0.1	11.9±0.0	15.3±0.1	3.7±0.0	13.6±0.0	29.5±0.0	51.5±0.1	36.6±1.4

Values are shown as means ± SD of three replications. [a]Number of carbon derived from the triacylglycerol less glycerol. [b]SSS, trisaturated; [c]SSU, disaturated–monounsaturated; [d]SUU, monosaturated-diunsaturated; [e]UUU, triunsaturated.

2.3.4. REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS

Regiospecific distribution of fatty acids in triacylglycerols has implications for the nutritional quality and technology of oils and fats. The interesterification reaction produces fats with new features, due to randomization of fatty acids in triacylglycerols. With the increasing use of structured lipids in the preparation of food, pharmaceuticals and cosmetics, it is important to know the distribution of fatty acids in triacylglycerols (KALLIO *et al*., 2001; KARABULUT *et al*., 2007).

Analysis of the regiospecific distribution of fatty acids in triacylglycerols by NMR is desirable, as it does not require hydrolysis by pancreatic lipase, further separation of partial acylglycerols by thin layer chromatography and finally analysis of fatty acids by gas chromatography (D'AGOSTINI; GIOIELLI, 2002). However, it cannot discriminate between saturated fatty acids, and cannot discriminate between linoleic and linolenic acids, which are considered together. Another point that the NMR technique shows is that the signal of the spectra corresponding to the *sn*-2 position is always equivalent to around 33.3 g/100g (an acyl group of three), while the signal corresponding to *sn*-1,3 positions is always equivalent to around 66.6 g/100g (two acyl groups of three).

The technique of analysis by NMR also allows the determination of the fatty acid composition, with the limitations described above for the distinction of saturated fatty acids and linoleic acid/linolenic acid. The results for the fatty acid composition (saturated, oleic acid and linoleic acid + linolenic acid) by NMR were similar to those obtained by gas chromatography of all samples of this study (Figure 2.5).

Saturated fatty acids were found mainly ($p < 0.05$) in the *sn*-1,3 positions and polyunsaturated fatty acids were found mainly ($p < 0.05$) in the *sn*-2 position, typical feature of vegetable oils (Figure 2.6 a and b).

Palm stearin showed 79.2 g/100g saturated fatty acids, 17.6 g/100g oleic acid and 3.1 g/100g linoleic/linoleic acids in *sn*-1,3 positions of the triacylglycerols (Figure 2.6a), while for the *sn*-2 position were found 37.6 g/100g of saturated fatty acids, 50.7 g/100g oleic acid and 11.7 g/100g linoleic/linolenic acids (Figure 2.6b). According to Rossell, King and Downes (1985) palm stearin presents at the *sn*-2 position from 10 to 59 g/100g of palmitic acid.

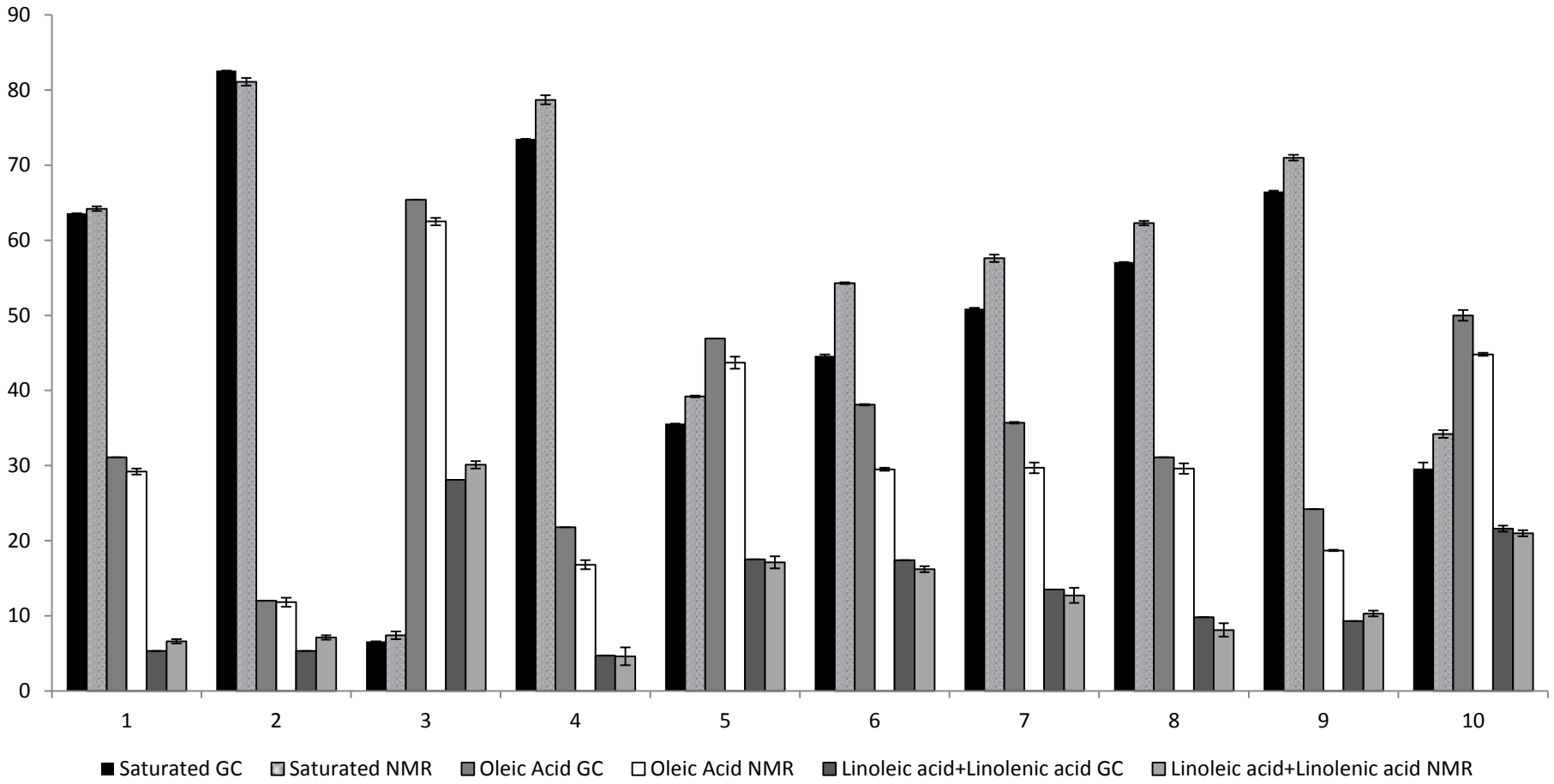


Figure 2.5. Fatty acid composition (saturated, oleic acid and linoleic acid + linolenic acid) obtained by gas chromatography and nuclear magnetic resonance of palm stearin, coconut oil, canola oil and their blends.

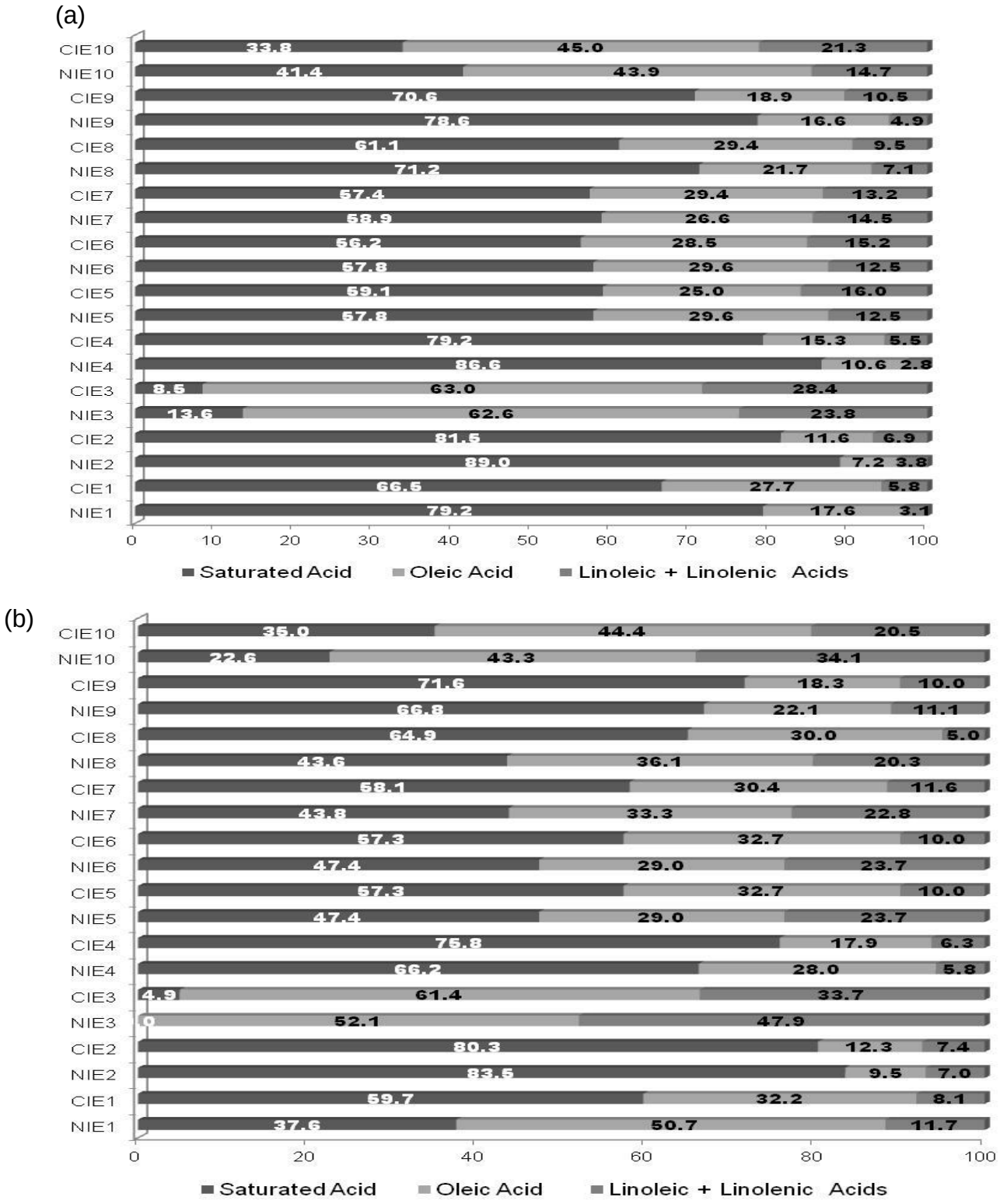


Figure 2.6. Regiospecific distribution of fatty acids in *sn*-1,3 positions (a) and *sn*-2 position (b) in triacylglycerols of palm stearin, coconut oil, canola oil and their blends before and after chemical interesterification

Coconut oil showed more than 80.0 g/100g of saturated fatty acids ($p < 0.05$) in the *sn*-1,3 and *sn*-2 positions. According to Caro *et al*. (2004) saturated fatty acids are in the *sn*-1,3 position and lauric acid is in *sn*-2 positions.

Moreover, canola oil showed more than 90.0 g/100g of polyunsaturated fatty acids ($p < 0.05$) in the *sn*-1,3 and *sn*-2 positions (Figure 2.6 a and b). According to Kim and Akoh (2005) canola oil presents in the *sn*-2 position 99.3 g/100g of polyunsaturated fatty acids and 0.7 g/100g of saturated fatty acids.

According Karabulut, Turan and Ergin (2004) and Xie and Qi (2013), during chemical interesterification fatty acids are exchanged within and among triacylglycerols until a thermodynamic equilibrium is reached, producing fats with different physical and chemical properties of the original.

Importantly, after chemical interesterification compositions in saturated fatty acids, oleic acid and linoleic acid / linolenic acid should be similar for the *sn*-2 and *sn*-1,3 positions, and in turn, close to the total fatty acid composition, confirming a completely random rearrangement.

Palm stearin, coconut oil, canola oil and blends 4, 6, 7, 8, 9 and 10 showed a decrease in saturated fatty acids at the *sn*-1,3 positions after chemical interesterification, while for blend 5 saturated fatty acids increased. At the *sn*-2 position after chemical interesterification, the oleic acid decreased in palm stearin, coconut oil, canola oil and blends 6, 7, 8, 9 and 10 (figure 2.6 a and b).

2.4. CONCLUSIONS

This study indicates that chemical interesterification is a viable tool to modify the compositional properties of palm stearin, coconut oil, canola oil and their blends with significant modification of the triacylglycerol composition. Hence, the increase of the SU_2 contents of palm stearin, coconut oil and canola oil blends, promoted by chemical interesterification, is associated to the increase of technological functionality, the betterment of sensorial characteristics, and, therefore, to a greater potential of these interesterified bases for food application.

CHEMICAL INTERESTERIFICATION OF BLENDS OF PALM STEARIN, COCONUT OIL, AND CANOLA OIL: PHYSICOCHEMICAL PROPERTIES

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ABSTRACT

Trans-free interesterified fat was produced for possible usage as a margarine. Palm stearin, coconut oil and canola oil were used as substrates for chemical interesterification. The main aim of the present study was to evaluate the physicochemical properties of blends of palm stearin, coconut oil and canola oil submitted to chemical interesterification using sodium methoxide as the catalyst. The original and interesterified blends were examined for fatty acid composition, softening and melting points, solid fat content and consistency. Chemical interesterification reduced softening and melting points, consistency and solid fat content. The interesterified fats showed desirable physicochemical properties for possible use as a margarine. Therefore, our result suggested that the interesterified fat without *trans* fatty acids could be used as an alternative to partially hydrogenated fat.

KEYWORDS: Eutectic behavior, validation, multiple regressions, consistency, solid fat content, melting point.

3.1. INTRODUCTION

The challenge faced by food industries to replace *trans* fat in numerous products lies in the development of formulations and processes which have equivalent functionality and economic viability (SOARES *et al*., 2009). To decrease or eliminate *trans* fat in food products, alternative technological approaches such as interesterification, fractionation, and blending have been developed to replace the conventional hydrogenation process.

In order to achieve similar functionality in fat products like hydrogenated fat, a saturated hard stock such as palm stearin rich in palmitic acid can be used. This is added to improve tolerance to high temperatures and stability (COSTALE-RODRIGUES et al., 2009). The production of edible fats, however, requires fat blends that are able to impart plasticity and body to the end product. This necessitates the enrichment of polyunsaturated oils, such as canola oil, into palm stearin, which basically lacks the ability to impart the required plasticity to the end product and must therefore be modified.

More recently, the concept of balancing fatty acids in oils and fats to promote health has been advocated by nutritionists and medical practitioners (REENA; REDDY; LOKESH, 2009). Coconut oil can improve the nutritional aspect of oils and fats due to its relatively high medium-chain fatty acids triacylglycerols content. Coconut oil is rich of saturated fatty acids as lauric acid. Due to the high medium-chain fatty acids triacylglycerols content, coconut oil is a major component of infant formulas and medical foods for people who cannot absorb longer chain fatty acids (ASSUNÇÃO et al., 2009; ADHIKARI et al., 2010; O'BRIEN, 2004).

The effect of blending and interesterification (chemical or enzymatic) on the physicochemical characteristics of blends of hard fat with various oils and fats has been reported (ADHIKARI et al., 2010; NORIZZAH et al., 2004; RIBEIRO et al., 2009a). However, reports describing the effect of blending and chemical interesterification on the physicochemical characteristics of ternary blends are scarce (KIM; LUMOR; AKOH, 2008; MAT DIAN; SUNDRAN; IDRIS, 2007).

The purpose of this study was to evaluate the chemical interesterification of palm stearin, coconut oil, canola oil and their blends, with a view to study fat bases for application in food products. Preparation of shortenings through chemical interesterification of palm stearin, coconut oil and canola oil is reported in the present investigation. The functionality of the finished product was based on the softening and melting points, consistency and solid fat

content of the blends and interesterified fats. The interaction of these oils and fats and their compatibility are also discussed.

3.2. MATERIALS AND METHODS

3.2.1. MATERIALS

Palm stearin was obtained from Agropalma S/A (Pará, Brazil), coconut oil from Copra Alimentos Ltda. (Alagoas, Brazil) and canola oil from Bunge Alimentos S.A. (São Paulo, Brazil). The fats were stored at 0 °C prior to use. All chemicals used were either of analytical or chromatographical grades.

3.2.2. BLEND PREPARATION

Fat blends, formulated with palm stearin, coconut oil and canola oil were mixed at different ratios, according to Table 2.1. Three blends represented the original components, three were binary blends and four were ternary blends. The blends were prepared after complete melting of the fats at 70 °C and stored under refrigeration. Blends 8, 9 and 10 were used to validate the statistical model.

3.2.3. CHEMICAL INTERESTERIFICATION

Chemical interesterification was performed according to Ahmadi, Wright and Marangoni (2008) with modifications. Two hundred grams of each blend were melted in a glass jar at 85°C under reduced pressure to limit moisture and air. The chemical reaction was started by the addition of 0.3 % (w/w) sodium methoxide (Merck Co.) as the catalyst. The blends were interesterified under reduced pressure for 60 min at 88±2°C. The start of the reaction was associated with the appearance of a reddish-brown color. To terminate the reaction, 5 mL of distilled water was added. The presence of water inactivates the catalyst by converting it to methanol (Sreenivasan, 1978). Kieselghur and anhydrous sodium sulfate were added to minimize the darkening caused by the presence of a diacylglycerol metal derivative (active catalyst) and to remove residual water, respectively. The reagents were removed by filtering the samples with filter paper. The fat was poured into a glass jar and stored at 5°C

prior to use. Non-interesterified oil is abbreviated to NIE and chemical interesterified blends to CIE.

3.2.4. FATTY ACID COMPOSITION

Fatty acid composition was determined after conversion of fatty acids into their corresponding methyl esters (FAMES) using the method described by Hartman and Lago (1973) for blend 1 and by ISO method 5509 (2000) for blends 2 to 10. Analyses of FAMES were carried out in a Varian GC gas chromatograph (model 430 GC, Varian Chromatograph Systems, Walnut Creek, California, USA), equipped with a CP 8412 auto injector. The Galaxie software was used for quantification and identification of peaks. Injections were performed into a 100-m fused silica capillary column (ID = 0.25 mm) coated with 0.2 μm of polyethylene glycol (SP-2560, Supelco, USA) using helium as the carrier gas at an isobaric pressure of 37 psi; linear velocity of 20 cm/s; make-up gas: helium at 29 mL/min at a split ratio of 1:50; volume injected: 1.0 μL. The injector temperature was set at 250 ºC and detector temperature at 280 ºC. The oven temperature was initially held at 140 ºC for 5 min, then stepped to 240 ºC at a rate of 4 ºC/min, and held isothermally for 30 min. Pure oils and theirs blends were analyzed in triplicate and reported values represent the average of the three runs.

Medium-chain saturated fatty acids (MCSFAs) are expressed as the sum of the amounts of caprylic, capric and lauric acids.

Long-chain saturated fatty acids (LCSFAs) are expressed as the sum of the amounts of myristic, palmitic and stearic acids.

Saturated fatty acids (SFAs) are expressed as the sum of the amounts of caprylic, capric, lauric, myristic, palmitic and stearic acids.

Unsaturated acids (USFAs) are expressed as the sum of the amounts of oleic, linoleic and linolenic acids.

Monounsaturated fatty acids (MUFA) are expressed as amounts of oleic acid.

Polyunsaturated fatty acids (PUFAs) are expressed as the sum of the amounts of linoleic and linolenic acids.

3.3.5. IODINE VALUE (IV)

Iodine value was calculated from the fatty acid composition, according to the procedure described in the AOCS official method Cd 1c-85 (AOCS, 2009c). Results are expressed in g iodine/100 g fat.

$$IV = (\% C_{18:1} \times 0.860) + (\% C_{18:2} \times 1.732) + (\% C_{18:3} \times 2.616) \quad \textbf{Eq. 3.1}$$

Where:

$C_{18:1}$ = oleic acid
$C_{18:2}$ = linoleic acid
$C_{18:3}$ = linolenic acid.

3.2.6. ATHEROGENIC INDEX (AI)

Atherogenic index was calculated according to Kim, Lumor and Akoh (2008), by the following equation:

$$AI = [C_{12:0}\ (w/w, \%) + 4 \times C_{14:0}\ (w/w, \%) + C_{16:0}\ (w/w, \%)]/USFA\ (w/w, \%)$$ **Eq.3.2**

Where:

USFA = total amount of unsaturated fatty acids,
$C_{12:0}$ = lauric acid
$C_{14:0}$ = myristic acid
$C_{16:0}$ =palmitic acid.

3.2.7. SOFTENING POINT

Softening point was determined by the open tube melting point method, according to the AOCS official method Cc 3-25 (AOCS, 2009d). Three replicates of this analysis were performed.

3.2.8. MELTING POINT

Melting point was determined by the closed tube melting point method, according to the AOCS official method Cc 1-25 (AOCS, 2009e). Three replicates of this analysis were performed.

3.2.9. CONSISTENCY

Consistency was determined via the penetration test using a 45° acrylic cone fitted to a constant speed model TA-XT2 Texture Analyzer, Stable Micro Systems, UK. Fat samples were heated to 70 °C in a microwave oven for complete melting of the crystals, and stored in 50 mL glass beakers (Pyrex, USA). Tempering was allowed to occur for 24 h in a standard refrigerator (5–8 °C) and then for 24 h in an oven with controlled temperature (5, 10, 15, 20, 25, 30, 35, 40 and 45 °C ± 0.5 °C). The tests were conducted under the following conditions: determination of force in compression; distance, 10.0 mm; speed, 2.0 mm/s and time, 5s (GAMBOA; GIOIELLI, 2003 a,b). Measurements were performed in duplicate and the reported values are the simple average of the two values. Consistency was calculated as a ‘‘yield value” (kgf/cm^2), according to the equation proposed by Haighton (1959).

$$C = \frac{K \times W}{p^{1,6}} \qquad \textbf{Eq.3.3}$$

Where:

C = the yield value (kgf/cm^2),
K = constant depending on the cone angle (4700-undimensional),
W = the compression force (kgf), and
p = the penetration depth (mm/10).

3.2.10. SOLID FAT CONTENT

Solid fat content (SFC) was determined with a DSC 4000 differential scanning calorimeter (Perkin Elmer Corp., Norwalk, CT, USA). The data processing system used was the Pyris Series Thermal Analysis System software. An empty aluminium pan was used as a

reference, and each sample was accurately weighed (5–10 ± 0.1 mg) for DSC analysis. The sample was heated to 80 °C and held for 10 min. Thereafter, the temperature was decreased at 10 °C/min to −60 °C. After holding for 10 min at −60 °C, the melting curve was obtained by heating to 80 °C at 5 °C/min. The temperature and heat of fusion were calibrated with indium (onset temperature 156.6°C).

SFC was obtained from the melting curve. The solid fat content as a function of temperature was calculated from the partial areas at different temperatures (-25 to 60 °C, intervals of 5 °C) (ADHIKARI et al., 2010).

3.2.11. STATISTICAL ANALYSIS

Results were expressed as mean ± SD. Differences between the samples (fatty acid composition, softening and melting point) during the experimental period were statistically analyzed using repeated measures ANOVA, followed by post-hoc Tukey test, taking on $P<0.05$.

Experimental results for softening and melting points, consistency and solid fat content were applied to obtain the regression models, as a function of the proportions of each ingredient: x_1=palm stearin; x_2=coconut oi; x_3=canola oil,

present in the blends 1 to 7:

$$\hat{y}_i = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3, \quad \textbf{Eq. 3.4}$$

where

$\hat{y}_i$ = estimated response;

β_i = coefficients estimated by the least square method;

x_i = dependent variables.

Quality of the models was evaluated by ANOVA and Adjusted Coefficient of Determination (r^2), with the optimization obtained by the Barros Netto; Scarminio; Bruns (2001). Statistical analysis was performed using STATISTICA 9.0 software (TULSA; EUA, 2010).

Validation was performed based on three points (blends 8, 9, and 10) in conditions of interest within the surface and applying the same experimental procedures used to construct the models.

3.3. RESULTS AND DISCUSSION

3.3.1. FATTY ACID COMPOSITION

The fatty acid composition of the pure oil, their blends and interesterified fats are shown in Table 2.3 a and b. All blends were *trans-free* fatty acids. The total saturated fatty acid of the blends with higher content of palm stearin and coconut oil were greater than 35.6 %, a value that renders them highly resistant to oxidative rancidity (JAYADAS; PRABHAKARAN NAIR, 2006).

Blends prepared using palm stearin have higher concentrations of palmitic acid, which imparts a desirable smooth consistency required for application such as margarines and shortenings. The β′-crystal form is more stable in shortenings with higher palmitic acid contents (JEYARANI; YELLA REDDY, 2003). Blends with higher content of coconut oil contain predominantly lauric acid.

According to Zhang, Smith and Adler-Nissen (2004), fats that have a high content of lauric and myristic acids exhibit very sharp melting points. The sharp melt, low melting point and low unsaturated fatty acids content make coconut oil particularly suited as fats for low-moisture food products for applications such as confectionery fats, candy centers, cookie fillers, nut roasting, coffee whiteners and spray oils (O'BRIEN, 2004).

All blends were found to contain oleic acid (greater than 12.5 %) as their major unsaturated fatty acid. The content of linoleic acid in all blends was less than 22.0 % and linolenic acid was below 8.0 %.

3.3.2. SOFTENING AND MELTING POINTS

Softening and melting points are parameter of significant importance for characterizing and developing interesterified fats (RIBEIRO *et al*., 2009a). According to Karabulut, Turan and Ergin (2004), fat slips down the capillary tube when containing approximately 4–5 % solid fat, thus enabling melting point to be characterized when solid content reaches this range. The softening and melting points of the NIE and CIE blends are shown in Table 3.1. Adding coconut oil and canola oil to palm stearin decreased the melting point of the blends to values ranging from 51.7 to 17.0 °C.

According to Lumor *et al*. (2007), the melting point of canola oil is - 6 °C. The capillary tube method used in this study does not work in this temperature range, as it is performed in a water bath.

Interesterification lowered the softening and melting points of all binary and ternary blends by reducing the proportion of high melting point trisaturated triacylglycerols and increasing the percentages of disaturated–monounsaturated and monosaturated–diunsaturated triacylglycerols, which have intermediate melting points (RIBEIRO *et al*., 2009a). Other researchers have reported similar results (KIM; LUMOR; AKOH, 2008; PETRAUSKAITE *et al*., 1998). Rousseau and Marangoni (2002) found a directly proportional relationship between trisaturated triacylglycerols content and melting point.

Table 3.1. Softening and melting points of palm stearin, coconut oil, canola oil and their blends before and after chemical interesterification.

Blends	Softening Point (°C)		Melting Point (°C)	
	NIE	CIE	NIE	CIE
1	52.3 ± 0.1^{jA}	51.9 ± 0.1^{iB}	53.9 ± 0.2^{jC}	52.4 ± 0.0^{jD}
2	25.3 ± 0.0^{cA}	27.5 ± 0.2^{eB}	26.6 ± 0.2^{cC}	28.3 ± 0.0^{fD}
3	-6.0 ± 0.0^{aA}	2.0 ± 0.0^{aB}	-6.0 ± 0.0^{aC}	2.0 ± 0.0^{aD}
4	45.6 ± 0.3^{gA}	34.8 ± 0.0^{fB}	49.8 ± 0.2^{hC}	35.4 ± 0.1^{gD}
5	46.2 ± 0.2^{hA}	35.7 ± 0.1^{gB}	49.2 ± 0.2^{gC}	36.3 ± 0.1^{hD}
6	21.3 ± 0.3^{bA}	$17,0\pm0.0^{bB}$	23.6 ± 0.1^{bC}	18.8 ± 0.0^{bD}
7	44.4 ± 0.1^{fA}	27.5 ± 0.1^{eB}	46.2 ± 0.1^{fC}	28.7 ± 0.0^{eD}
8	48.6 ± 0.1^{iA}	37.1 ± 0.0^{hB}	51.7 ± 0.0^{iC}	38.8 ± 0.2^{iD}
9	32.7 ± 0.3^{dA}	25.9 ± 0.1^{dB}	35.1 ± 0.1^{dC}	27.0 ± 0.1^{dD}
10	36.3 ± 0.1^{eA}	19.0 ± 0.0^{cB}	41.6 ± 0.4^{eC}	21.0 ± 0.0^{cD}

Values are shown as means ± SD of three replications. Means (n = 3) values with different lower case letters superscript in the same column are significantly different ($P < 0.05$). Means (n = 3) values with different capital letters superscript in the same line are significantly different ($P < 0.05$).

No significant changes in softening and melting points of palm stearin resulting from chemical interesterification were observed (Table 3.1). Petrauskaite *t al*. (1998) stated that chemical interesterification of blends with high proportions of hard fat, such as palm stearin, causes only slight changes in melting point. These results are similar to those obtained by Soares *et al*. (2009).

Table 3.2. Coefficients calculated by multiple regression from the experimental results of softening and melting points (P <0.05).

	Coefficients of multiple regression															
	[a]β_1		[b]β_2		[c]β_3		[d]β_{12}		[e]β_{13}		[f]β_{23}		[g]β_{123}		[h]r^2	
	NIE	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**
Softening point	52.3	51.2	25.3	28.5	-6.0	2.2	+27.4	-20.1	+92.3	+36.1	+46.5	+11.5	0.0	0.0	0.99	0.99
Melting point	53.9	53.1	26.6	28.7	-6.0	2.2	+38.1	-15.8	+100.9	+42.7	+53.3	+14.7	0.0	0.0	0.99	0.99

[a]β_1, palm stearin; [b]β_2, coconut oil; [c]β_3, canola oil; [d]β_{12}, interaction between palm stearin and coconut oil; [e]β_{13}, interaction between palm stearin and canola oil; [f]β_{23}, interaction between coconut oil and canola oil; [g]β_{123}, interaction between palm stearin, coconut oil and canola oil; [g]r^2, coefficient of determination.

Interesterified blends displayed a wide range of melting points (2.0–52.4 °C). Fats with melting points lower than body temperature can be applied directly as shortenings, because they melt completely in the mouth and produce no waxy sensation during consumption (RIBEIRO *et al*., 2009a; KARABULUT; TURAN; ERGIN, 2004). Thus, the interesterified blends 2, 3, 4, 5, 6, 7 and 9, with melting points of between 2.0 and 36.3 °C, fall within this group. In addition, blends 3 and 6 display the characteristics of liquid shortenings, which can be readily pumped and bottled at low temperatures and have melting points of between 10 and 19 °C.

Interesterified blend 8, whose melting point was 38.8°C, lies within the range of most fatty bases for producing solid and semi-solid shortenings, generally represented by the all-purpose shortenings, used mainly in confectionery and bakery products and whose representative melting point is 42 °C. However, interesterified blend 1 was found to have an excessively high melting point (52.4 °C) for food applications.

The coefficients for the responses determined by applying multiple regressions to the experimental data are shown in Table 3.2. Softening and melting points NIE blends were dependent on palm stearin, coconut oil and canola oil, and on the positive interactions between palm stearin and coconut oil, palm stearin and canola oil, and coconut oil and canola oil ($P<0.05$).

After chemical interesterification, softening and melting points were dependent on palm stearin, coconut oil and canola oil, on the negative interaction between palm stearin and coconut oil, and on the positive interactions between palm stearin and canola oil, and coconut oil and canola oil ($P<0.05$).

3.3.3. CONSISTENCY

Figure 3.1 shows the consistency profiles of palm stearin, coconut oil, canola oil and their NIE and CIE blends as a function of temperature. It was not feasible to ascertain the consistency of blend 3 because of its low melting point.

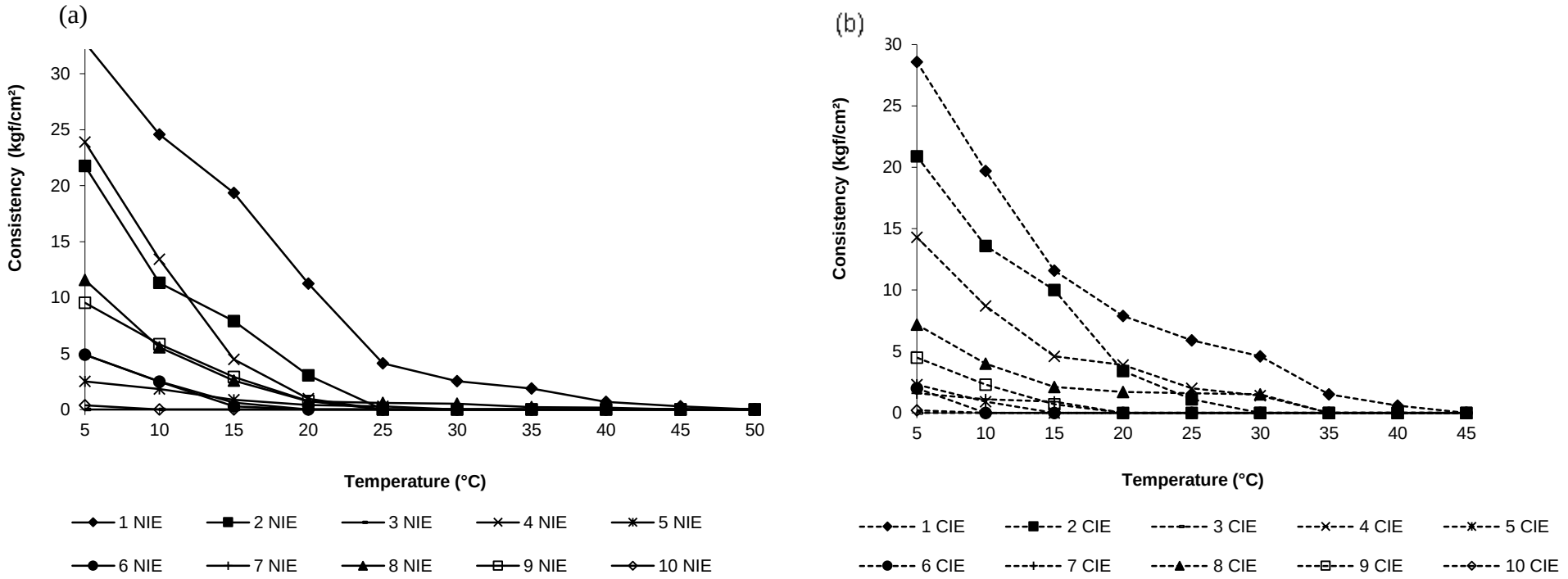

Figure 3.1. Consistency of palm stearin, coconut oil, canola oil and their blends before (a) and after (b) chemical interesterification.

The consistency of the blends decreased as a function of temperature. This decrease may be due to the gradual melting of the crystals, leading to a structurally weaker network, which is in turn responsible for the plasticity of fats (HAIGHTON, 1959; SOARES *et al*., 2009; Rodrigues *et al*., 2007. At higher temperatures (20 to 45 °C), blends 2, 3, 4, 5, 6, 7 and 9 showed no significant differences ($P < 0.05$). None of the blends exhibited measurable consistency at 50 °C NIE or at 45 °C CIE.

Consistency proved to be proportionally dependent on palm stearin concentration in the blends, before and after randomization. Increase in saturated fatty acids content of a sample strongly influences its consistency, due to their high melting points.

Overall, interesterification led to a reduction in yield values for all blends at all temperatures examined, with the sole exception of blend 2, whose consistency increased at 10, 15, 20 and 25 °C. Consistency of the interesterified blends decreased with increasing temperature, which causes the gradual melting of the crystals and consequent destruction of the crystalline network which endows the fat with plasticity (SOARES *et al.*, 2009). None of the interesterified blends exhibited measurable consistency at 45 °C.

According to Haighton (1959), a fat is plastic and spreadable at yield values ranging from 0.2 to 0.8 kgf/cm^2. Blends 5 and 8 NIE, and the interesterified blend 5, with a yield value of 0.8 kgf/cm^2 at 10 °C, had satisfactory plasticity and spreadability properties for use at refrigeration temperatures, in addition to melting point requisites for use in margarines, as mentioned earlier. However, there are other factors influencing the texture of a spread, such as the crystallization procedure including the variables cooling rate, degree of supercooling, mechanical working, tempering, solid fat content of fat used, and presence of nonfat materials (LUMOR et al., 2007).

Between 25 and 30 °C, the interesterified blend 2 was satisfactorily spreadable (yield values between 0.8 and 1.0 kgf/cm^2), but exhibited ideal plasticity at 35 °C, which is important for sensorial properties such as mouthfeel sensation and lack of adhesiveness.

Thus, its consistency profile from 25 to 35 °C corroborates its suitability for application in bakery and confectionery products. The interesterified blends 4 and 8 can be classified as hard at room temperature, with a yield value of between 1.6 and 2.0 kgf/cm^2 in the 25 to 30 °C interval; which is just above the spreadability limit (1.5 kgf/cm^2) at 35°C. According to Jeyarani and Yella Reddy (2003), fats considered hard at room temperature are suitable for firmer food products, where deformations must not occur during handling or stocking.

Table 3.3. Coefficients calculated by multiple regression from the experimental results for consistency and solid fat content (P< 0.05).

Temperature (°C)	Coefficients of multiple regression of consistency															
	$^{a}\beta_1$		$^{b}\beta_2$		$^{c}\beta_3$		$^{d}\beta_{12}$		$^{e}\beta_{13}$		$^{f}\beta_{23}$		$^{g}\beta_{123}$		$^{h}r^2$	
	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE
5	33.0	28.6	22.0	21.0	0.0	0.0	-17.0	-43.1	-59.1	-49.4	-27.6	-35.2	0.0	0.0	0.99	0.99
10	24.6	19.6	11.4	13.5	0.0	0.0	-19.7	-23.6	-43.5	-21.3	-14.5	-18.9	0.0	0.0	0.99	0.94
15	19.3	11.5	7.8	9.9	0.0	0.0	-34.9	-7.3	-33.6	-15.9	-12.9	-7.0	0.0	0.0	0.95	0.94
20	11.1	7.9	2.9	3.4	0.0	0.0	-23.3	-7.3	-19.5	-15.9	-4.7	-7.0	0.0	0.0	0.98	0.99
25	4.7	6.0	0.0	1.2	0.0	0.0	-7.8	-3.9	-6.5	-13.8	0.0	-4.1	0.0	0.0	0.98	0.95
30	2.5	2.5	0.0	0.0	0.0	0.0	-4.6	-4.7	-4.7	-4.7	0.0	0.0	0.0	0.0	0.96	0.96
35	1.8	1.9	0.0	0.0	0.0	0.0	-3.4	-3.5	-3.4	-3.5	0.0	0.0	0.0	0.0	0.97	0.97
40	0.6	0.7	0.0	0.0	0.0	0.0	-1.2	-1.2	-1.2	-1.2	0.0	0.0	0.0	0.0	0.98	0.99
Temperature (°C)	**Coefficients of multiple regression of solid fat content**															
	$^{a}\beta_1$		**$^{b}\beta_2$**		**$^{c}\beta_3$**		**$^{d}\beta_{12}$**		**$^{e}\beta_{13}$**		**$^{f}\beta_{23}$**		**$^{g}\beta_{123}$**		**$^{h}r^2$**	
	NIE	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**	**NIE**	**CIE**
5	73.9	76.7	99.8	84.1	0.0	17.4	+41.0	0.0	+60.0	+5.7	+29.9	0.0	0.0	0.0	0.99	0.96
10	65.9	77.2	94.4	75.9	0.0	15.7	+29.6	-41.5	+84.6	0.0	-13.6	-59.5	0.0	0.0	0.99	0.96
15	70.1	79.8	81.0	34.4	0.0	11.9	0.0	-67.4	+74.0	-70.8	-46.3	-88.7	0.0	0.0	0.99	0.97
20	80.7	82.3	56.7	34.	0.0	5.5	-32.1	-89.1	+31.1	-108.3	-69.3	-68.2	0.0	0.0	0.99	0.99
25	82.2	73.2	16.1	3.5	0.0	0.0	-19.0	-66.5	0.0	-109.9	-27.5	-6.3	0.0	0.0	0.99	0.99
30	71.2	59.7	0.0	0.0	0.0	0.0	-11.7	-84.5	-7.8	-102.6	0.0	0.0	0.0	0.0	0.99	0.99
35	59.9	45.7	0.0	0.0	0.0	0.0	-23.2	-84.3	-16.0	-84.1	0.0	0.0	0.0	0.0	0.99	0.99
40	46.8	26.9	0.0	0.0	0.0	0.0	-40.5	-50.1	-26.0	-50.0	0.0	0.0	0.0	0.0	0.99	0.99
45	30.5	2.7	0.0	0.0	0.0	0.0	-53.8	-5.1	-17.6	-5.1	0.0	0.0	0.0	0.0	0.99	0.98

$^{a}\beta_1$, palm stearin; $^{b}\beta_2$, coconut oil; $^{c}\beta_3$, canola oil; $^{d}\beta_{12}$, interactions between palm stearin and coconut oil; $^{e}\beta_{13}$, interactions between palm stearin and canola oil; $^{f}\beta_{23}$, interactions between coconut oil and canola oil; $^{g}\beta_{123}$, interactions between palm stearin, coconut oil and canola oil; $^{g}r^2$, coefficient of determination.

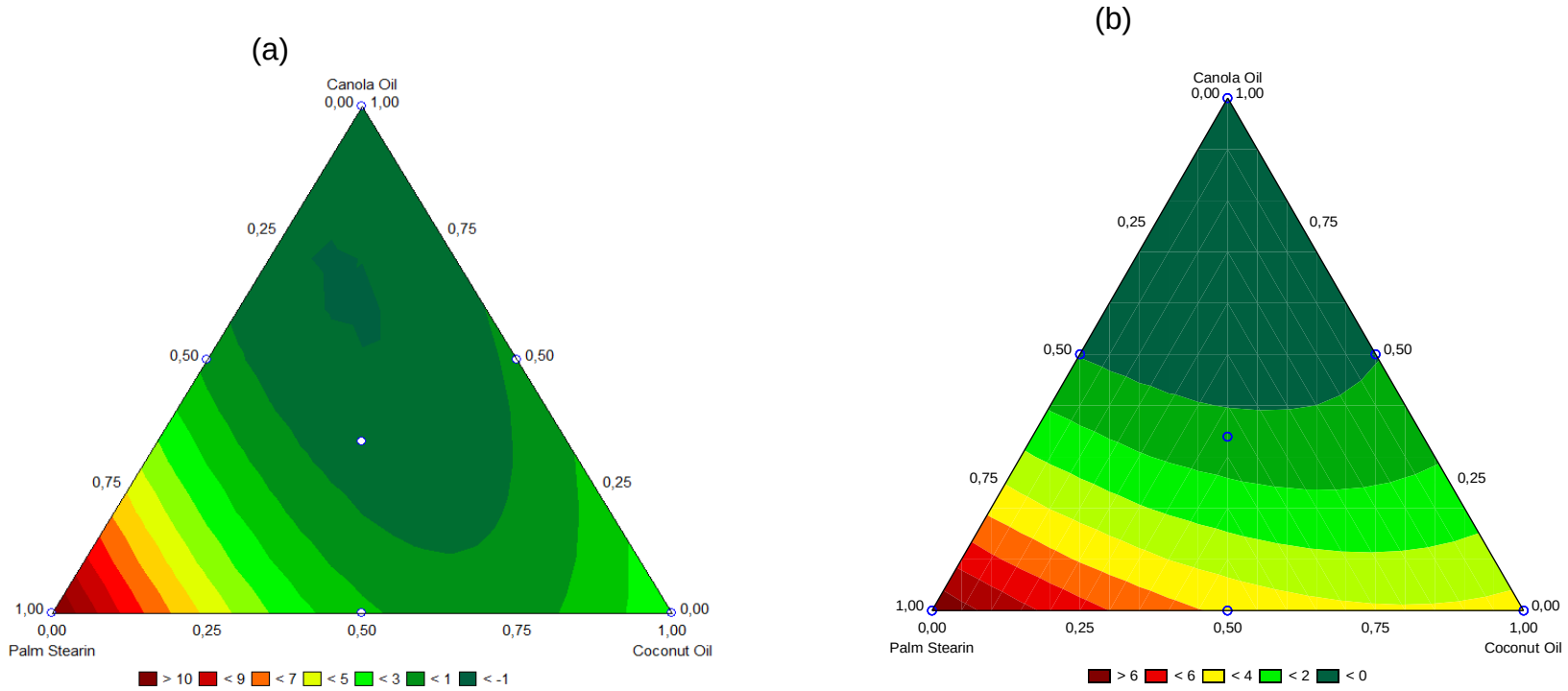

Figure 3.2. Triangular diagram of consistency (kgf/cm^2) at 20° C of blends of palm stearin, coconut oil and canola oil before (a) and after (b) chemical interesterification

The coefficients for the responses determined by applying multiple regressions to the experimental data are shown in Table 3.3. For non-interesterified and interesterified blends, consistency was dependent on palm stearin, on interactions between palm stearin and coconut oil, and between palm stearin and canola oil at all temperatures, and on coconut oil and on interaction between coconut oil and canola oil from 5 to 20 ºC for non-interesterified blends and from 5 to 25 °C for interesterified blends ($P<0.05$). The interaction coefficients were negative for all blends, showing a eutectic interaction between palm stearin, coconut oil and canola oil.

Eutectic behavior occurs in the blends due to the differences in the molecular size of the fatty acids and the shape and polymorph of the crystals among the three types of fat (RODRIGUES et al., 2007). This shows that palm stearin rich in palmitic acid, coconut oil rich in lauric acid, and canola oil rich in oleic acid, are incompatible with each other.Statistical models for consistency NIE and CIE blends are shown in Figure 3.2 using triangular diagrams. The three vertices correspond to the responses of palm stearin, coconut oil and canola oil. The points on the sides of the equilateral triangle represent the results of binary mixtures. The inner values indicate the responses for ternary mixtures.

The eutectic effect is clearly evidenced by depressions in the curves of consistency diagrams at 20 °C (Figure 3.2 a and b). The higher the eutectic effect, the lower the consistency because the incompatibility among fats in solid state hinders crystallization.

3.3.4. SOLID FAT CONTENT

Solid fat content is responsible for many product characteristics in margarines, shortenings and spreads, including their general appearance, ease of packing, spreadability, oil exudation and organoleptic properties (JEYARANI; KHAN; KHATOON, 2009).

DSC is an easy and fast technique, which is highly practical and useful for determination of solid fat content. At the application temperature, solid fat content of fats can be determined from the DSC melting curves by partial integration (NASSU; GONÇALVES, 1995). Partial areas are obtained according to the procedures described by Menard and Sichina (2000).Solid fat content of palm stearin, coconut oil, canola oil and their blends at different temperatures calculated from the DSC data are given in Figure 3.3 a and b.

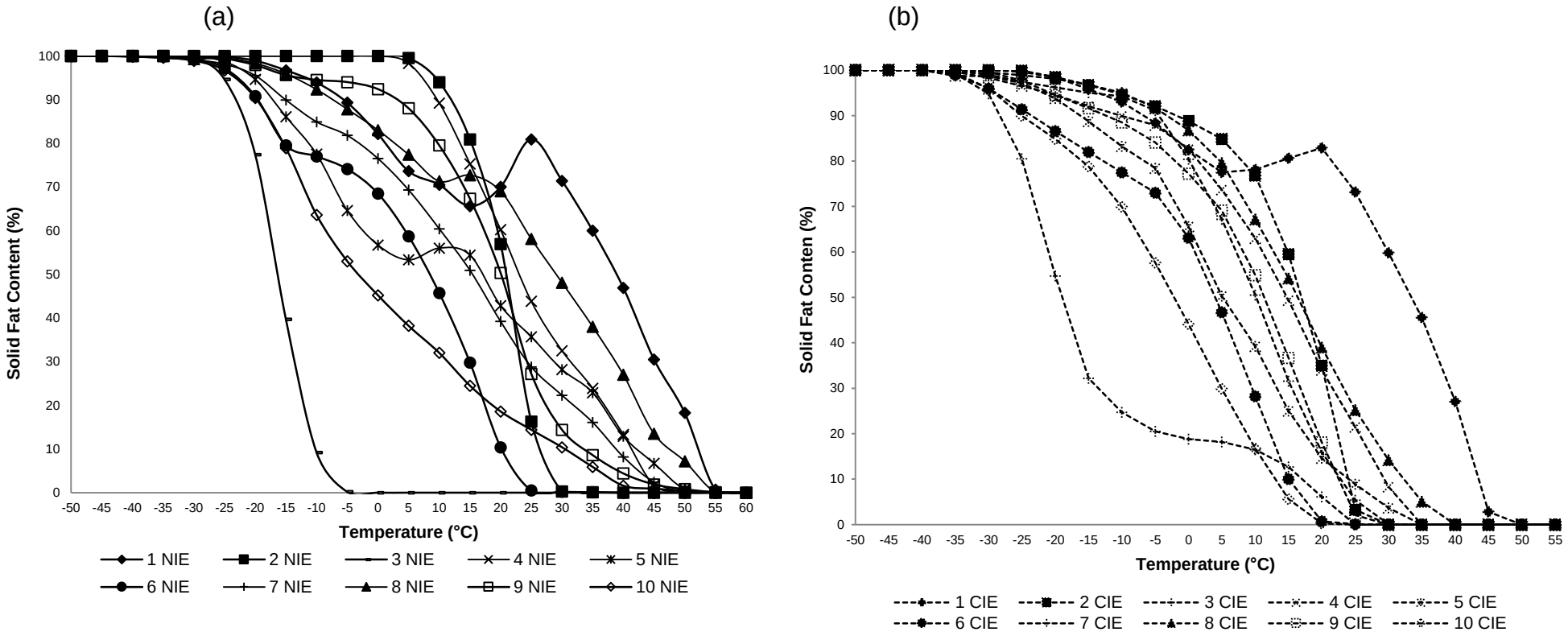

Figure 3.3. Solid fat content of palm stearin, coconut oil, canola oil and their blends before (a) and after (b) chemical interesterification.

The curves show that the blends have different profiles, covering large ranges of solid fat content versus temperature. The solid fat content of the blends were proportional to the addition of coconut oil and canola oil to palm stearin, at all the temperatures analysed.

Solid fat content of palm stearin was 93.9% at −10 °C, 82.3% at room temperature (25 °C), decreasing to 46.9% when temperature increased to 40 °C. Solid fat content obtained by DSC for palm stearin was higher than the value obtained by NMR provided by the supplier. This can be explained by the fact that the determination of solid fat content by DSC is done after crystallization of the samples at -60 ºC, while the determination by NMR precedes crystallization at 0 °C.

Solid fat content of palm stearin increased in the 15 to 25 °C range and subsequently decreased. This behavior is the result of the recrystallization observed in the heating curve obtained by DSC. The results obtained were in accordance with those described by Reshma *et al*. (2008). However, Alim, Lee, and Lee (2009) did not report this same behavior in their samples. The recrystallization behavior was also observed in blends 8 and 5 NIE, with higher proportions of palm stearin.

The solid fat content of the interesterified fats was lower than their corresponding initial blends, with the exception of palm stearin between 10 to 20 °C, where the recrystallization phenomenon occurred.

Under ambient temperature, coconut oil is a heterogeneous slurry of crystals admixed in liquid oil. The solid fat content of coconut oil was 56.9% at 20 °C, 16.1% at 25 °C, but at 30 °C the solid fat content was found to be only 0.3%, indicating that the triacylglycerols of coconut oil melted at 25-30 °C. However, the solid fat content increased after blending with palm stearin and decreased after blending with canola oil.

The non-interesterified blend 4 had solid fat content similar to that found by Jeyarani, Khan, Khatoon (2009) for a blend constituting 50% coconut oil and 50% palm stearin at 35 °C. Blends 1, 4, 5 and 8 NIE were not suitable as plastic fats, because they contained excessive solids at this temperature, which may affect the mouthfeel of the product (KARABULUT; TURAN; ERGIN, 2004).

Solid fat content at room temperature (25 °C) should be 15–35% for desirable spreadability as plastic fats (NORIZZAH *et al*., 2004). In the case of the non-interesterified blends 7 and 9 and interesterified blends 4 and 8, solid fat content at room temperature (25 °C) was within the scope of the above criteria, suggesting that blends in this study were suitable for spreadable fat or margarine stock.

Generally, solid fat content at 20 °C corresponds to a tendency towards oil exudation and more than 10% SFC is essential to avoid oiling off (*8*). All blends, except blend 6 NIE, had solid fat content higher than 10% at 20 °C Blends 3, 6 and 10 CIE had solid fat content lower than 10%.

According to Kim, Lumor and Akoh (2008) solid fat content should be < 32% at 10 °C to impart good spreadability at refrigerator temperature. The interesterified blend 10 had solid fat content below 32% at 10 °C. Blends 3, 5, 6, 9 and 10 CIE contained solid fat content of 16.5 %, 14.6 %, 0.7 %, 18.1 % and 0.3%, respectively.

For canola oil, solid fat content at −15 °C was 39.7 % and at 0 °C was 0.0 %. After chemical interesterification, solid fat content was 32.2 % at −15 °C and 18.8 % at 0 °C. These results are typical of liquid oils.

The coefficients for solid fat content after applying multiple regressions to the experimental data are shown in Table 3.3. Solid fat content before chemical interesterification was dependent on palm stearin at all temperatures and on coconut oil from 5 to 25 °C, on interactions between palm stearin and coconut oil and between palm stearin and canola oil at all temperatures, and between coconut oil and canola oil from 5 to 25 °C ($P<0.05$). After chemical interesterification, solid fat content was dependent on palm stearin from 5 to 45 °C, on coconut oil from 5 to 25 °C, and on canola oil from 5 to 20 °C, on interactions between palm stearin and coconut oil and between palm stearin and canola oil from 5 to 45 °C, and between coconut oil and canola oil from 5 to 25 °C ($P<0.05$). Negative interaction coefficients indicate that a eutectic interaction had occurred.

Eutectic behavior is due to the presence of residual amounts of triacylglycerols with long- and medium-chain fatty acids, respectively. This result is consistent with the findings of Norizzah *et al*. (2004) whereby interesterification eliminates or reduces eutectic interactions in a eutectic mixture. This eutectic effect is desirable if the blend is destined for use in the production of margarine and shortening.

3.3.5. VALIDATION

ANOVA was used to verify that the proposed model adequately expresses the responses of softening and melting points, consistency, and solid fat content, of the NIE and CIE blends.

The percentage variations explained by the models were between 90.00 and 99.99% confirming the good fit of the regression. The low rates of residuals showed that the

experimental errors were controlled and random in nature. In addition, a low rate of lack of fit and pure error was also found.

Blends 8, 9 and 10 were used to validate the model obtained by multiple regression for the parameters softening and melting points, consistency and solid fat content. The proportion of error found for softening and melting points, NIE and CIE blends, was lower than 20%. Proportion of errors found for solid fat content, before and CIE was lower than 10% in the 5 – 40 °C temperature range. Errors for the consistency variable, before and after chemical interesterification, were lower than 15% at some temperatures. These percentages confirmed the predictability of the multiple regression models.

3.4. CONCLUSIONS

A comprehensive understanding of the functions and properties of fats or oil bases produced by interesterification is essential to outlining applications for them and obtaining food products with the desired final attributes. Chemical interesterification of palm stearin, coconut oil and canola oil blends produced fats with different physicochemical properties, lower consistency, solid fat content and softening and melting points compared with the starting blends. Therefore palm stearin, coconut oil and canola oil interesterified blends can be used for the production of margarine and shortenings, non-temper type confectionery fats, whipping creams and similar products, representing an alternative to partially hydrogenated fats.

INFLUENCE OF CHEMICAL INTERESTERIFICATION ON THERMAL BEHAVIOR, CRYSTALLINE MICROSTRUCTURE AND POLYMORPHISM OF PALM STEARIN, COCONUT OIL AND CANOLA OIL BLENDS

ABSTRACT

Blends of palm stearin, coconut oil and canola oil in different rations were interesterified under the following conditions: 88 °C, 60 min reaction time, 0.3 % sodium methoxide catalyst. The original and interesterified blends were examined for triacylglycerol composition, crystalline microstructure and polymorphism. Chemical interesterification produced substantial rearrangement of the triacylglycerol species in all blends, reduction of trisaturated triacylglycerol content and increase in monounsaturated–disaturated and diunsaturated–monosaturated triacylglycerols. X-ray diffraction analyses revealed that interesterification altered crystalline polymorphism. The interesterified blends showed a predominance of the β′ polymorph, which is of more interest for food applications. It was verified reduction in crystal diameter in all blends, besides crystal morphology modification.

KEYSWORDS: Low *trans* fats, Polymorphism, Triacylglycerol composition, Melting and crystallization behavior, Crystalline microstructure.

4.1. INTRODUCTION

Chemical interesterification of blends of solid vegetable fats with liquid oils is currently the most versatile option for producing low *trans* fats for a diversity of industrial purposes (RIBEIRO *et al*., 2007). Even though chemical interesterification is extremely functional from the technological point of view, replacing partially hydrogenated fats in food products, particularly in shortenings and confectionery products, there are some challenges, because suitable solid fat content curves, plasticity, crystallization properties and texture are difficult to obtain in the absence of *trans* fatty acids (O'BRIEN, 2004; REYES-HERNANDEZ *et al*., 2007).

Palm stearin is not used directly for edible purposes due to its high melting point, ranging from 44 to 56 °C, giving the product low plasticity and uncompleted melting at body temperature, but contributes to desirable hardness in margarine (ADHIKARI *et al*., 2010; AINI; MISKANDAR, 2007; SOARES *et al*., 2009). Coconut oil may improve the nutritional aspect due to its relatively high medium chain fatty acids content, principally the lauric acid (ADHIKARI *et al*., 2010, ASSUNÇÃO *et al*., 2009). Canola oil has gained an excellent reputation for its fatty acid composition, which assures its oxidative stability and nutritional qualities in the human diet. However, due to its liquid state, it needs to be blended with hard stocks for production of margarine and shortening fats (FARMANI; SAFARI; HAMEDI, 2009).

Lipid composition and crystallization conditions influence the crystal habit: different polymorphic forms and crystal morphologies are possible. Natural lipids are composed of a variety of triacylglycerol groups with specific requirements, such as activation energy for molecular diffusion and formation of stable crystalline nuclei (FOUBERT *et al*., 2006). When these groups are modified by interesterification, the related energy requirements are altered, and changes occur in crystal growth velocity and size (NARINE; HUMPHREY; BOUZIDI, 2006). Interesterification thus produces significant modifications in the crystallization properties of oils and fats by increasing the number of triacylglycerol species present and altering the intersolubility among the triacylglycerol molecules (LIDA *et al*., 2007; PISKA *et al*., 2006).

The characteristic polymorph type of a fat or fat base is dependent on the distribution of fatty acids in the triacylglycerol molecules, being the degree of randomization particularly important. The microstructure concept, on its turn, encompasses information on the state, quantity, shape, size, spatial relation, and interaction among all components of a crystal network and presents an enormous influence on the macroscopic properties of fats (SHI;

LIANG; HARTEL, 2005). Chemical interesterification alters the triacylglycerol composition, causing modifications in the crystal morphology of fats and promoting changes of types and/or polymorph contents of a natural fat (MARANGONI, 2005). Hence, the chemical interesterification process consist in an important means for the stabilization of β' form, given that it can promote, according to the raw material composition characteristics, the formation of triacylglycerols with more varied network sizes. This results in a more disordered packing of terminal methyl groups, which is associated to the formation of crystal lattices of lower density. Yet, interesterification promotes significant alterations in the microstructure of fats, given that it modifies the crystal lattice's morphology and density. In general, the formation of smaller spherulites and/or the modification in the crystal's halo-nucleus ratio take place, affecting the texture and functionality of interesterified bases (ROUSSEAU; MARANGONI; JEFFREY, 1998; TANG; MARANGONI, 2006).

The crystals of plastic fats exhibit polymorphism, with large differences in physical behavior. The current nomenclature classifies the crystals in three main ways: α, β' and β, according to the structure of the sub-cell (cross section of the mode of packing of the carbon chain). Most fats shows monotropic polymorphism, i.e. when a transition occurs (for example, β' to β) the molecular chains packing of triacylglycerol becomes more compact, resulting in higher melting point (NARINE; MARANGONI, 1999a).

The crystallization behavior of lipids has implications for the processing of industrial products whose physical characteristics depend largely on the structure of the crystallized fat, like chocolates, margarines and shortenings (SILVA *et al*., 2008). The study of microstructure of fat systems has become increasingly important since the functional property of many foods depends on knowledge of their fine structure. This microstructure is dependent on the molecular composition, as well as its crystallization behavior, including polymorphism. Crystallization kinetics and polymorphism are also dependent on processing conditions, which in turn influence the resultant microstructure (NARINE; MARANGONI, 1999b).

According to Narine and Marangoni (2005), the microstructural level, or mesoscale, of a fat crystal network can be defined as the group of structures with dimensions ranging from 0.5 μm to 200 μm. The levels of a structure in a typical network are defined when the fat crystallizes from its completely melted state. Its quantification is mainly attained through the visualization of its geometry.

The objective of this work was to evaluate the effect of chemical interesterification on the thermal behavior and crystallization properties of blends of palm stearin, coconut oil and

canola oil, aiming at the study of interesterified bases for food product application. The modifications resulting from randomization were evaluated by triacylglycerol composition, differential scanning calorimetry, polarized light microscopy, and X-ray diffraction.

4.2. MATERIALS AND METHODS

4.2.1. MATERIALS

Palm stearin was obtained from Agropalma S/A (Pará, Brazil), coconut oil from Copra Alimentos Ltda. (Alagoas, Brazil), and canola oil from Bunge Alimentos S.A. (São Paulo, Brazil). The fats were stored at 0 °C prior to use. All chemicals used were either of analytical or chromatographical grades.

4.2.2. BLEND PREPARATION

Fat blends, formulated with palm stearin, coconut oil and canola oil were done in different ratios, according to Table 2.1. Three blends represent the original components, three are binary blends and four are ternary blends. The blends were prepared after complete melting of the fats at 70 °C and were stored under refrigeration.

4.2.3. CHEMICAL INTERESTERIFICATION

Chemical interesterification was performed according to Ahmadi, Wright and Marangoni (2008) with modifications. Two hundred grams of each blend were melted in a glass jar at 85 °C, under reduced pressure to limit moisture and air. The chemical reaction was started by the addition of 0.3 % (w/w) sodium methoxide (Merck Co.) as a catalyst. The blends were interesterified under reduced pressure for 60 min at 88 ± 2 °C. The start of the reaction was associated with the appearance of a red-brown color. To terminate the reaction, 5 mL of distilled water were added. The presence of water inactivates the catalyst by converting it to methanol. Kieselghur and anhydrous sodium sulfate were added to minimize the darkening caused by the presence of a diacylglycerol metal derivative - the active catalyst - and to remove residual water, respectively. The reagents were removed through filtering the

samples with a filter paper. The fat was poured into a glass jar and stored at 5 °C prior to use. Non-interesterified oil is abbreviated to NIE and chemical interesterified oil to CIE.

4.2.4. FATTY ACID COMPOSITION

Fatty acid composition was determined after conversion of fatty acids into their corresponding methyl esters (FAMES) by the methods described by Hartman, Lago (1973) for blend 1 and by ISO 5509 (2000) for blends 2 to 10. Analyses of FAMEs were carried out in a Varian GC gas chromatograph (model 430 GC, Varian Chromatograph Systems, Walnut Creek, California, USA), equipped with a CP 8412 auto injector. The Galaxie software was used for quantification and identification of peaks. Injections were performed in a 100-m fused silica capillary column (ID = 0.25 mm) coated with 0.2 µm of polyethylene glycol (SP-2560, Supelco, USA) using helium as carrier gas at isobaric pressure of 37 psi; linear velocity of 20 cm/s; make-up gas: helium at 29 mL/min at split ratio of 1:50; volume injected: 1.0 µL. The injector temperature was set at 250 ºC and the detector temperature was set at 280 ºC. The oven temperature was initially held at 140 ºC for 5 min, then programmed to 240 ºC at rate of 4 ºC/min and held isothermally for 30 min. All samples were analyzed in triplicate and the reported values are the average of the three runs.

Medium-chain saturated fatty acids (MCSFAs) are expressed as the sum of the amounts of caprylic, capric and lauric acids.

Long-chain saturated fatty acids (LCSFAs) are expressed as the sum of the amounts of myristic, palmitic and stearic acids.

Saturated fatty acids (SFAs) are expressed as the sum of the amounts of caprylic, capric, lauric, myristic, palmitic and stearic acids.

Unsaturated acids (USFAs) are expressed as the sum of the amounts of oleic, linoleic and linolenic acids.

Monounsaturated fatty acids (MUFA) are expressed as amounts of oleic acid.

Polyunsaturated fatty acids (PUFAs) are expressed as the sum of the amounts of linoleic and linolenic acids.

4.2.5. IODINE VALUE (IV)

Iodine value was calculated from the fatty acid composition, according to the procedure described in the AOCS official method Cd 1c-85 (AOCS, 2009c).

$$IV = (\%\ C_{18:1} \times 0.860) + (\%\ C_{18:2} \times 1.732) + (\%\ C_{18:3} \times 2.616) \quad \textbf{Eq. 4.1}$$

Where:

$C_{18:1}$ = oleic acid
$C_{18:2}$ = linoleic acid
$C_{18:3}$ = linolenic acid.

4.2.6. ATHEROGENIC INDEX (AI)

Atherogenic index was calculated according to Kim, Lumor and Akoh (2008), by the following equation:

$$AI = [C_{12:0}\ (w/w, \%) + 4 \times C_{14:0}\ (w/w, \%) + C_{16:0}\ (w/w, \%)]/USFA\ (w/w, \%) \quad \textbf{Eq. 4.2}$$

Where:

USFA = total amount of unsaturated fatty acids,
$C_{12:0}$ = lauric acid
$C_{14:0}$ = myristic acid
$C_{16:0}$ =palmitic acid.

4.2.7. REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS

A proton-decoupled ^{13}C NMR was used to analyze the positional distribution of fatty acids on the triacylglycerol backbone. Lipid samples (250 mg) were dissolved in $CDCL_3$ (0.5 mL) in 5 mm NMR tubes, and NMR spectra were recorded on a Bruker Advance DPX spectrometer operating at 300 MHz. The ^{13}C spectra of the lipid samples were acquired with a spectral width of

2332.090 Hz, pulse of 10.2 µs, and a relaxation delay of 30s. Determination of ^{13}C was performed at a frequency of 75.8 MHz with a multinuclear probe of 5 mm operating at 30 °C, using method described by Vlahov (2005). The results showed the compositions of saturated fatty acids, oleic acid and linoleic + linolenic acids in *sn*-2 and *sn*-1,3 positions. All samples were analyzed in triplicate and the reported values are the average of three analyses.

4.2.8. TRIACYLGLYCEROL COMPOSITION (SATURATED AND UNSATURATED)

For these calculations, it was considered the fatty acid composition obtained experimentally and the experimental results of the fatty acids located at the *sn*-2 position. For the *sn*-1,3 positions was considered that the fatty acids are present in these positions in equivalent amounts, according to the formula:

$$C_{1,3} = [3 \times (C_{1,2,3}) - C_2] / 2 \qquad \textbf{Eq. 4.3}$$

Where:

$C_{1,3}$ = fatty acids in *sn*-1,3 positions
$C_{1,2,3}$ = fatty acid composition of total fat
C_2 = fatty acids in *sn*-2 position

According to the 1,3-random, 2-random theory, the levels of possible triacylglycerols, according to the saturation and unsaturation of fatty acids are:

For natural fats:

$$\% SSS = (\%S1)x(\%S2)x(\%S3)/10000 \quad \textbf{Eq. 4.4}$$
$$\% SUS = (\%S1)x(\%U2)x(\%S3)/10000 \quad \textbf{Eq. 4.5}$$
$$\% SSU = 2x(\%S1)x(\%S2)x(\%U3)/10000 \quad \textbf{Eq. 4.6}$$
$$\% USU = (\%U1)x(\%S2)x(\%U3)/10000 \quad \textbf{Eq. 4.7}$$
$$\% UUS = 2x(\%U1)x(\%U2)x(\%S3)/10000 \quad \textbf{Eq. 4.8}$$
$$\% UUU = (\%U1)x(\%U2)x(\%U3)/10000 \quad \textbf{Eq. 4.9}$$

After chemical interesterification was used the 1,2,3-random distribution theory:

$$\% SSS = (\%S)x(\%S)x(\%S)/10000 \quad \textbf{Eq. 4.10}$$

$$\% SUS/SSU = 3x(\%S)x(\%U)x(\%S)/10000 \quad \textbf{Eq. 4.11}$$

$$\% USU/UUS = 3x(\%U)x(\%S)x(\%U)/10000 \quad \textbf{Eq. 4.12}$$

$$\% UUU = (\%U)x(\%U)x(\%U)/10000 \quad \textbf{Eq. 4.13}$$

Where:

%S = % of saturated fatty acids

%U = % of unsaturated fatty acids

4.2.9. X-RAY DIFFRACTION

The samples fat crystals' polymorphic form was determined according to the method AOCS Cj 2-95 (AOCS, 2009f). Analyses were carried out in a diffractometer Philips (PW 1710), using Bragg–Bretano (θ:2θ) geometry with radiation of Cu-Ka (λ = 1.54056 Å, tension of 40 kV and 30 mA). Measures were attained with 0.02° in 2θ steps and acquisition time of 2 s, with scans of 5–40° (2θ scale). The samples were melted in a microwave oven at approximately 80 °C and stabilized at 20 °C for 7 days. Analyses were carried out at -20 °C. Polymorphic form identification was performed with basis on the characteristic short spacings of crystals. Form α presents a single diffraction line at 4.15 Å. Form β' is characterized by two strong diffraction lines at 3.8 Å and 4.2 Å, whereas form β is associated to a series of diffraction lines, with a prominent line at 4.6 Å and lines of lesser intensity at 3.7 Å and 3.8 Å (AOCS, 2009; ROUSSEAU; MARANGONI, 2002).

4.2.10. DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The crystallization and melting behavior were obtained by differential scanning calorimetry (DSC) cell from the DSC 4000 Perkin Elmer (Perkin Elmer Corp.., Norwalk, CT, USA), under dynamic atmosphere of He (20 mL/min) and cooling rate of -10 °C/min and heating rate at 5 °C/min, at temperatures ranging from 80 to -60 ° C for cooling, with isothermal time of 10 min at 80 ° C and -60 to 80 °C for heating, with a isothermal of 10 min

at -60 °C, using sealed aluminum capsules closed containing sample mass between 5 to 10 mg. The temperature and heat of fusion were calibrated with indium (initial temperature of 156.6 °C). Before the trials were done white curves to assess the baseline equipment. Curves were processed by the software Pyris, melting and crystallization curves are analyzed for the onset (T_{onset} °C) and endset (T_{endset} °C) of melting and crystallization, melting and crystallization peak temperatures (T_{pf} and T_{pc} °C) and melting and crystallization enthalpies (ΔH_f and ΔH_c J/g) (RIBEIRO *et al*., 2009f).

4.2.11. POLARIZED LIGHT MICROSCOPY

Samples were melted at 70 °C in a stove and, with the aid of a capillary tube, a sample drop was placed on a glass slide preheated at controlled temperature (70 °C) and covered with a cover glass. Glass slides were prepared in duplicates for each sample. Samples were kept in the stove at the analysis temperature (25 °C) for 24 h. Crystal morphology was evaluated by means of the polarized light microscope (Olympus, model BX 51) coupled to a digital video camera (Media Cybernetics). The slide temperature was held constant by LTS 32 large heating and freezing stage operated by a TP93 temperature programmer (Linkam Scientific instruments Ltd., Surrey, England), kept at the same crystallization temperature. Images were captured by the Image Pro-Plus version 4.5.1.22 (Media Cybernetics) software, using polarized light and amplified up to 100 times. For each glass slide, three visual fields were focused, of which only one was chosen to represent the observed crystals. The evaluation parameters selected for quantitative image analysis were the mean diameter of crystals and the range between the mean diameter variations (GAMBOA; GIOIELLI, 2006).

4.3. RESULTS AND DISCUSSION

4.3.1 FATTY ACID COMPOSITION

The fatty acid composition of the blends is shown in Table 2.3 a and b. Palm stearin contains palmitic acid (57.3 g/100 g) and oleic acid (30.8 g/100 g) as major fatty acids. Coconut oil contains lauric acid (38.7 g/100 g) as major fatty acid, followed by myristic (19.8 g/100 g), palmitic (12.7g/100 g), and oleic (12.5 g/100 g) acids. Canola oil mainly contains

oleic acid (63.6 g/100 g), followed by linoleic (21.2 g/100 g), and linolenic (7.9 g/100 g) acids.

4.3.2. REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS

The fatty acid profile at the *sn*-2 position of palm stearin, coconut oil, canola oil and their blends, before and after chemical interesterification, is given in Figure 2.6 b. These results confirm a random distribution of fatty acids after the chemical interesterification.

According Karabulut, Turan and Ergin (2004) and Xie and Qi (2013), during chemical interesterification fatty acids are exchanged within and among triacylglycerols until a thermodynamic equilibrium is reached, producing fats with different physical and chemical properties of the original.

4.3.3. TRIACYLGLYCEROL COMPOSITION

The trisaturated (S_3), disaturated-monounsaturated (S_2U), monosaturated-diunsaturated (SU_2) and triunsaturated (U_3) contents of triacylglycerols for the noninteresterified (NIE) and interesterified (IE) blends are shown in Table 2.4 a and b and Figure 4.1.

Triacylglycerol composition of palm stearin was dominated by the SSU group, while coconut oil showed a predominance of SSS group. The trisaturated triacylglycerols, with melting points from 54 to 65 °C, and some disaturated-monounsaturated triacylglycerols, with melting points from 27 to 42 °C, are responsible for the solid structure of fat products (RODRIGUES; GIOIELLI, 2003).

Canola oil (before and after chemical interesterification) showed more than 76 g/100 g of triacylglycerols of the UUU group and therefore did not present solid fat content even in refrigeration temperature (5 ºC), because the melting point of this group is in the range of -13 to 1 °C, remaining liquid at 5 °C.

Chemical interesterification distributes fatty acids equally through in the three positions of the glycerol backbone (GUNSTONE, HARWOOD; PADLEY, 1986; RODRIGUES; GIOIELLI, 2003). Chemical interesterification decreased the amount of SSS triacylglycerols (all samples), while the levels of SSU (with exception of blends 1, 3 and 5) and SUU (with the exception of blend 3) increased. UUU triacylglycerols decreased in the

blends 5, 6, 8 and 10 and increased in the blends 1, 2, 3 and 9. These results indicated that there were exchanges of fatty acids between triacylglycerols.

The triacylglycerol fraction of a fat is responsible for most of its physical properties that affect lubricity (pourability or melting in the mouth to give a pleasant cooling effect). Lubricity is dependent on melting temperature, solid **fat** content and texture. Fats that have predominantly TAGs SUU melt in temperatures between 6-23 ºC. These products provide appropriate lubricity at 25 ºC and are easy to handle and pour over temperatures ranging from 5 to 25 ºC. The functional properties of margarine oils, such as holding together at room temperature and mouth melting characteristics, are all influenced by disaturated-monounsaturated and trisaturated TAGs. Research has been directed towards improvement of both TAG composition and structure of vegetable oils, which may then be used as base-stocks or components of base-stocks (LIST et al., 1995; LIU; WHITE, 1992; MOUNTS et al., 1988; RODRIGUES; GIOIELLI, 2003 SLABAS, SIMON; ELBOROUGH, 1995; WILSON, 1993; ZEITOUN et al., 1993).

Hence, the increase of the SU_2 contents of palm stearin, coconut oil and canola oil blends, promoted by chemical interesterification, is associated to the increase of technological functionality, the betterment of sensorial characteristics, and, therefore, to a greater potential of these interesterified bases for food application (SOARES *et al*., 2009).

4.3.4. POLYMORPHISM

X-ray diffraction has been frequently used as a chemical interesterification evaluation technique, helping in the application outlining of fat bases produced (OMAR *et al*., 2005). A fundamental aspect in fat processing techniques is the crystallization tendency of fats.

Diffractograms of blends attained at - 20 °C, before and after chemical interesterification, are shown in Figure 4.2. The corresponding short spacings and polymorphic form presents are shown in Table 4.1.

Silva *et al*. (2013) showed that samples subjected to crystallization at - 20 °C tend to produce less stable polymorphic forms.

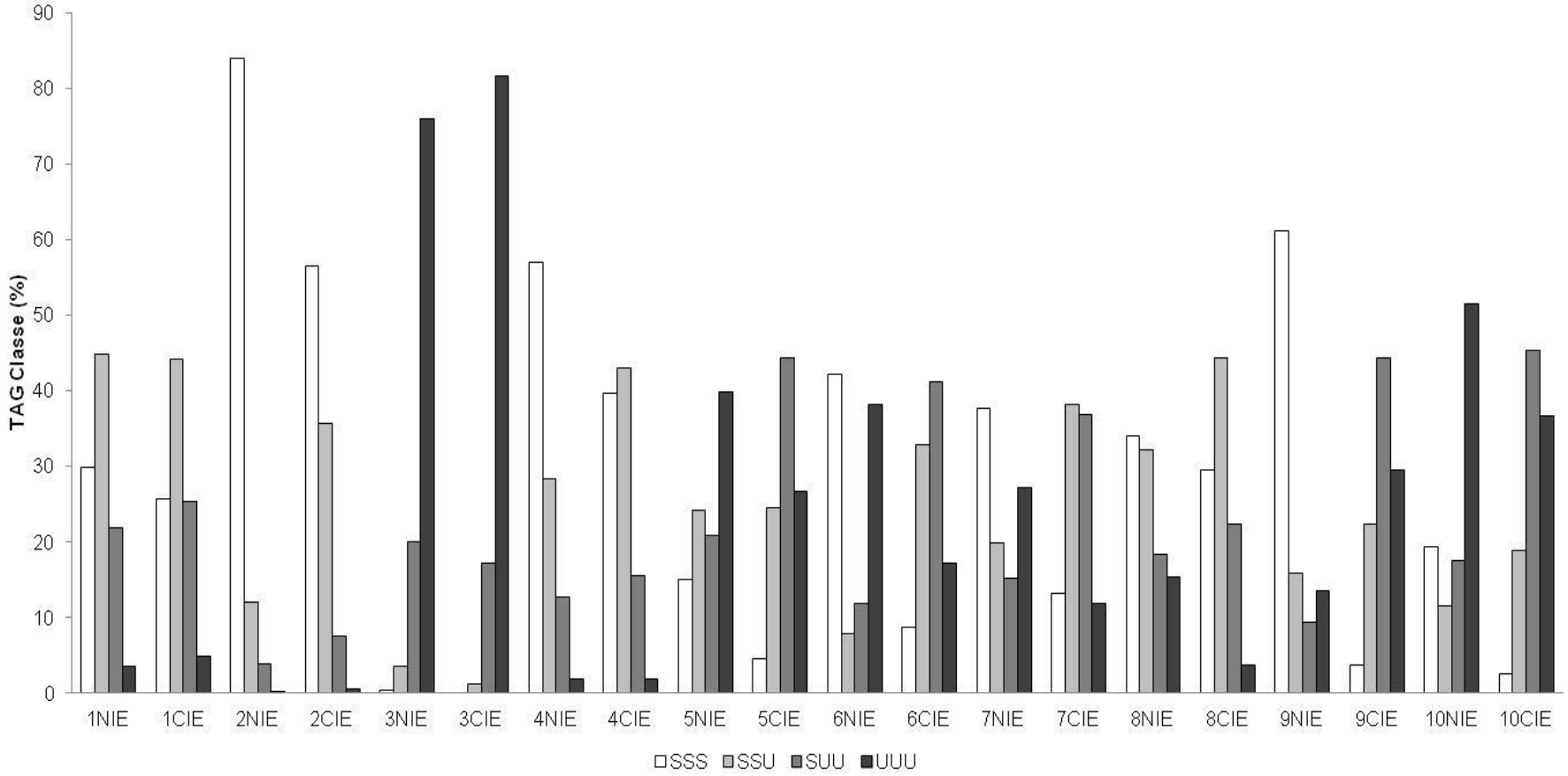

Figure 4.1. Triacylglycerol classes (TAG) (%) of palm stearin, coconut oil and canola oil blends, before and after interesterification. SSS (trisaturated), SSU (disaturated–monounsaturated), SUU (monosaturated-diunsaturated) and UUU (triunsaturated).

Sample 1 NIE (palm stearin) demonstrates polymorphism in the β-prime form. According to Akoh and Pande (2013), palm stearin, due to its high content of C_{48} triacylglycerols, has a tendency to crystallize in the β-prime form. According to Ghotra, Dyal and Narine (2002), palmitic acid contents above 20% are critical to promote stability of the polymorphic β-prime form in oils and fats.

Sample 2 NIE (coconut oil) demonstrates the polymorphic forms α + β-prime. According to Lawler and Dimick (2002) the form α is fleeting and quickly becomes as β-prime. This is not surprising, given the variation in size of the fatty acid chains and asymmetry of the triacylglycerols of coconut oil. Sample 3 NIE (canola oil) presents polymorphic form β, related to the low diversity in fatty acid composition, which provides relatively homogeneous triacylglycerol composition (FOUBERT *et al*., 2007; WIEDERMANN, 1978). This result is similar to that observed by Ribeiro *et al*. (2009g) for canola oil.

All samples after chemical interesterification showed crystals in β-prime form, due to the greater diversity of triacylglycerols formed after the chemical rearrangement. Randomized blends kept crystallization tendency in β-prime form, which is probably associated to the effect that is characteristic of the interesterification process, which causes the formation of triacylglycerols with higher network size variation, resulting in a more disordered packing of the terminal methyl groups and the formation of lower-density crystal structures (ROUSSEAU; MARANGONI, 2002).

These results are similar to that obtained by Adhikari *et al*. (2010) in the enzymatic interesterification of rice bran oil, palm stearin and coconut oil. These authors didn't found β crystals before interesterification of the blends.

Shin, Akoh and Lee (2010) also found that the interesterification modified the polymorphic form to β-prime, in blends of palm stearin, butter fat and linseed oil. Silva *et al.* (2013) also observed that the samples after the continuous enzymatic interesterification of lard and soybean oil exhibited β-prime form.

Table 4.1. Polymorphic forms at -20 °C and short spacings of palm stearin, coconut oil, canola oil and their blends before and after chemical interesterification

Blends	*Short spacings* (Å)						Polymorphic form	Predominant form
	4.6	4.2	4.1	3.9	3.8	3.7		
NIE1		4.21 (s^3)				3.77 (s)	β’	β’
CIE 1	4.62 (s)	4.26 (s)			3.80 (s)		β + β’	β = β’
NIE 2		4.25 (s)	4.16 (s)		3.86 (m^2)		α + β’	α = β’
CIE 2		4.22 (s)			3.87 (m)	3.79 (s)	β’	β’
NIE 3	4.61 (s)				3.87 (m)	3.71 (m)	β	β
CIE 3	4.68 (w)	4.26 (s)			3.84 (m)		β + β’	β’
NIE 4	4.6 (m)	4.25 (s)			3.85 (s)	3.73 (w)	β + β’	β’
CIE 4	4.61 (w^1)	4.21 (s)			3.81 (m)		β + β’	β’
NIE 5	4.65 (w)	4.20 (s)		3.95 (w)		3.75 (m)	β + β’	β’
CIE 5	4.63 (w)	4.21 (s)				3.77 (m)	β + β’	β’
NIE 6		4.28 (m)	4.15 (s)		3.87 (m)		α + β’	α
CIE 6		4.25 (s)			3.87 (m)		β’	β’
NIE 7			4.16 (s)	3.90 (m)			α	α
CIE 7		4.21 (s)			3.89 (m)	3.77 (w)	β’	β’
NIE 8	4.63 (w)	4.22 (s)		3.92 (w)		3.78 (m)	β + β’	β’
CIE 8	4.62 (m)	4.20 (s)			3.89 (m)	3.77 (m)	β + β’	β’
NIE 9	4.63 (w)		4.16 (s)		3.89 (m)	3.73 (w)	α + β	α
CIE 9		4.26 (s)			3.87 (m)		β’	β’
NIE 10			4.18 (s)		3.88 (m)	3.77 (w)	α	α
CIE 10		4.21 (s)			3.88 (m)		β’	β’

*Intensities: [1]w, weak; [2]m, medium; [3]s, strong;

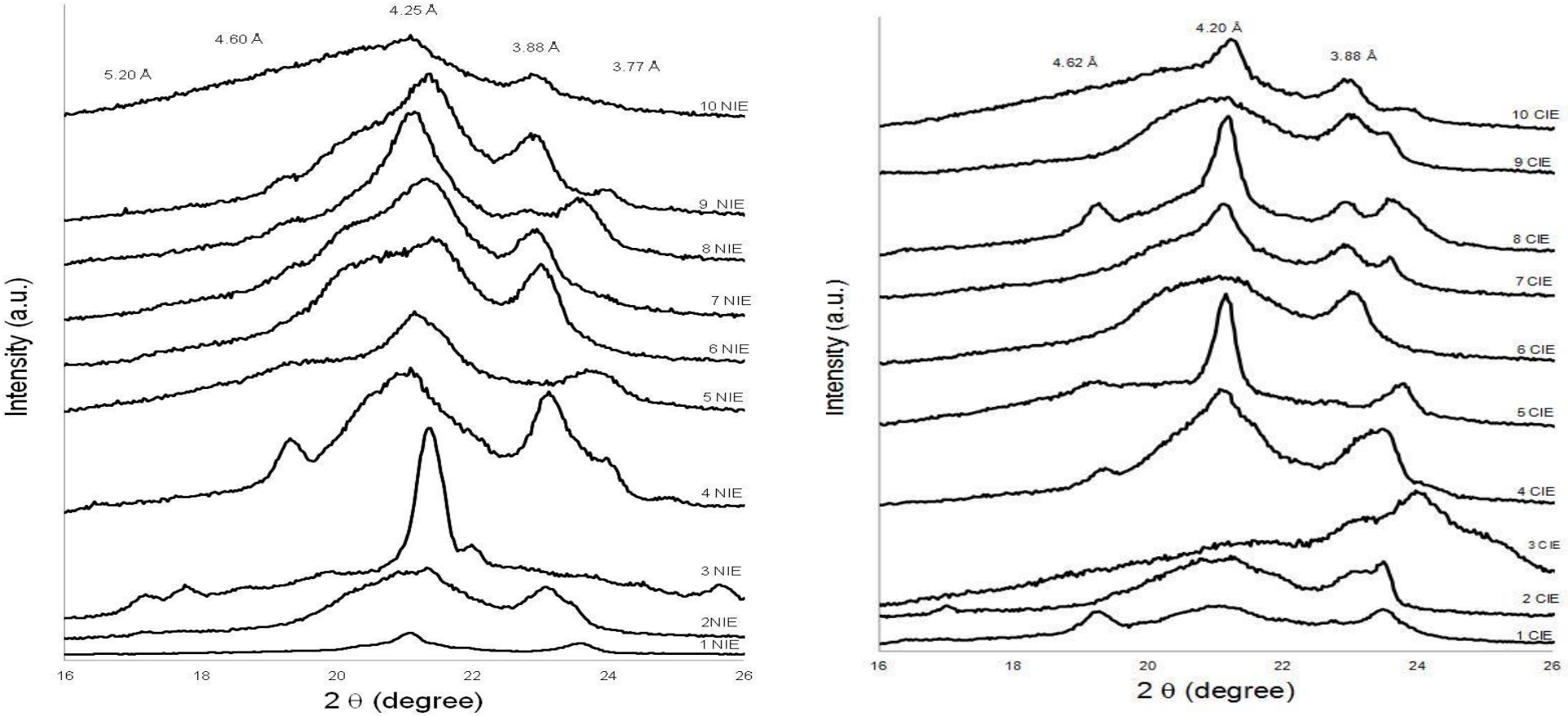

Figure 4.2. X-ray diffraction patterns for palm stearin, coconut oil, canola oil and their blends crystallized at -20 °C before (a) and after (b) chemical interesterification.

Crystals β-prime are small and present morphology that is suitable for the plasticity characteristics which are desirable in products such as margarines, shortenings, and fat for baking and pastry. Conversely, polymorphic form β tends to produce wide granular crystals, generating sandy products with low potential for aeration, which may compromise the macroscopic properties of some kinds of food (ROUSSEAU, MARANGONI, 2002).

4.3.5. THERMAL PROPERTIES

Differential scanning calorimetry is the thermo-analytical technique most employed in studying oils and fats. It is considered also an important tool for characterizing interesterified products. Evaluation by differential scanning calorimetry yields direct measurements of the energy involved in the processes of melting and crystallization of oils and fats. Crystallization of oils results in shrinking volume, associated with an exothermic effect. Conversely, when fats melt, their volume expands, characterizing an endothermic effect (RIBEIRO *et al*., 2009f; TAN, CHEN MAN, 2002).

As observed from the melting and crystallization processes, the curves of the blends differ, depending on the amount of palm stearin, coconut oil and canola oil in the blends. Palm stearin, coconut oil and canola oil for both NIE and CIE showed only small changes in peaks and shoulders distributions rather than in binary and ternary blends, due to interesterification effects which involves rearrangement of fatty acid within the TAG molecules and hence the melting and crystallization profiles also were hardly altered by CIE.

Figure 4.3 shows the changes of melting profiles in palm stearin, coconut oil, canola oil and their blends before and after interesterification. All blends showed endothermic heat flow over a wide range during the scanning from -60 °C to 80 °C, indicating the presence of low melting (< 0 °C), medium-melting (about 5 °C) and high-melting (> 20 °C) regions.

Figure 4.3 shows that blends with the high proportion of palm stearin (1 and 8 NIE) had the highest final complete melting temperature among all blends, at 55.3 and 53.9 °C, respectively, and displayed melting curves with well defined endotherm regions which are related to the low, medium and high melting components due to presence of large and broad amount of saturated TAGs in the blends. These results are similar of that obtained by Fauzi, Norizzah and Zaliha (2013).

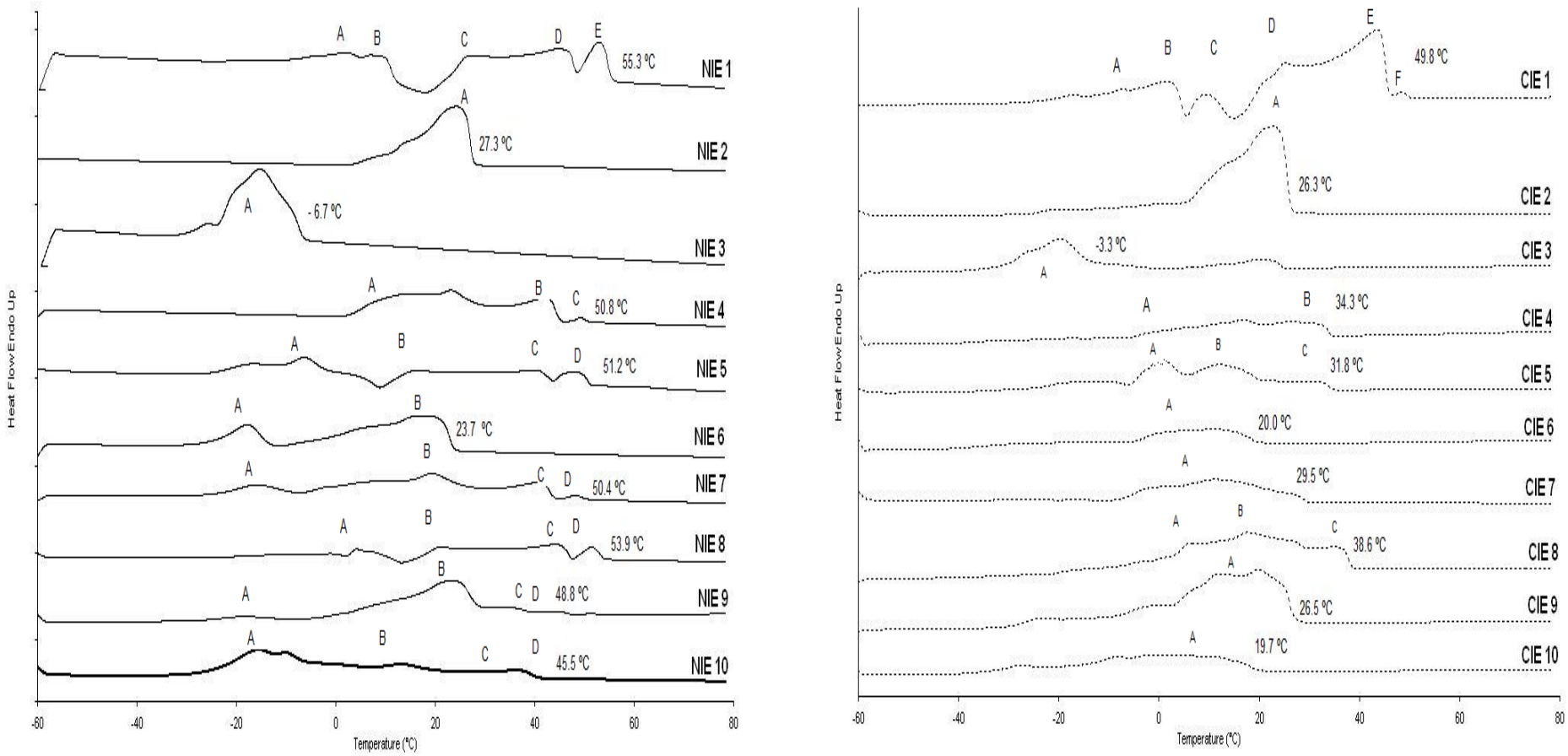

Figure 4.3. DSC melting curves of palm stearin, coconut oil, canola oil and their blends before (NIE) and after (CIE) chemical interesterification.

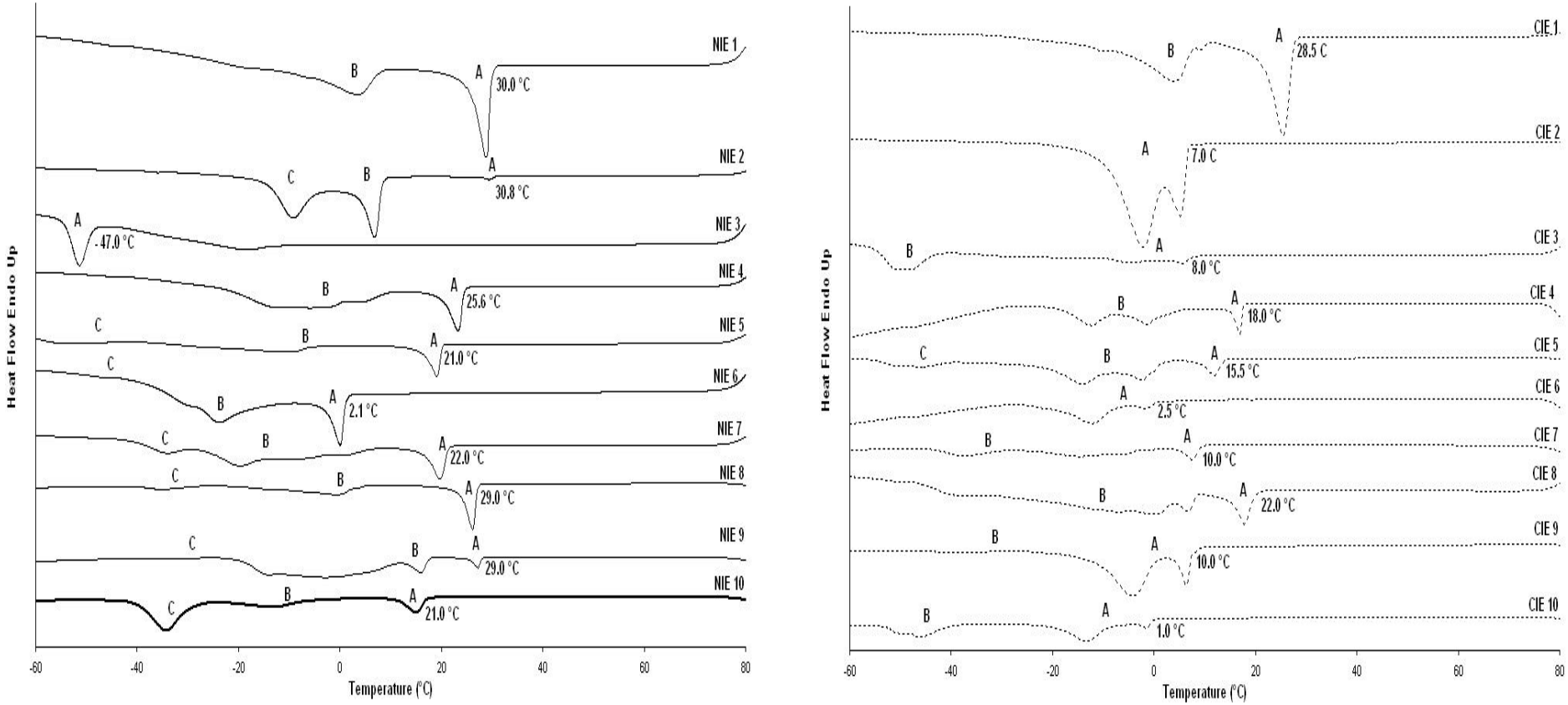

Figure 4.4. DSC crystallization curves of palm stearin, coconut oil, canola oil and their blends before (NIE) and after (CIE) chemical interesterification.

Coconut oil was observed to have an endotherm with the relatively narrowest temperature range of 10–30 °C with final melting at 27.3 °C (NIE) and at 26.3 °C (CIE) as related to its high proportion of medium chain saturated fatty acids.

The final melting temperature of canola oil was – 6.7 °C, attributed to the high presence of monounsaturated and polyunsaturated fatty acids.

The binary and ternary blends of palm stearin, coconut oil and canola oil before and after interesterification showed more complex DSC melting patterns. The higher numbers of peaks and shoulders can be attributed to the complicated interactions among the blend constituents.

As the concentration of canola oil in blends with palm stearin and coconut oil before interesterification increased, the melting regions shifted to lower temperatures due to the dilution effects of canola oil on the blends. Interestingly, the CIE samples showed more uniformity in reducing the melting regions toward the lower temperature rather than its NIE counterparts. This could be explained due to decreased proportion of high and medium melting temperature TAGs, simultaneously with the formation of several low-melting temperature TAGs owing by canola oil.

In contrast, a gradual displacement of melting peaks towards the higher temperature regions coincide with creation of new endotherm in higher temperature region when concentration of palm stearin increased in blends with coconut oil and canola oil.

The blend in equivalent amounts of palm stearin, coconut oil and canola oil (7 NIE and CIE) displayed a melting curve with an interaction of all three-blend components.

As observed from Figure 4.3, all the CIE blends had low final melting temperature when compared to the NIE blends. This could be resulted from redistribution and interaction of the original TAGs and formation of new TAGs that arise among the molecular species in the mixture constituents during the interesterification process (Figure 4.1). The changes in DSC melting profiles can be used to show the physical interaction of fats blends due to changes in their thermal characteristics.

The crystallization curve of an oil or fat can be subdivided into different exothermic regions reflecting different types of triacylglycerols. The palm stearin and coconut oil showed a prominent peak in the crystallization curves (Figure 4.4), which characterizes the trisaturated fraction.

Before the chemical interesterification, the T_{onset}, T_{endset} and T_{peak} values are function of the palm stearin content in the blends, proving that the crystallization process was accelerated by the increase of this hardfat content. The crystallization enthalpy values were between - 24.3 and - 110.3 J/g. All parameters evaluated in relation to the crystallization

curves showed a positive linear relation with the increasing of palm stearin concentration in the original blends. A similar relationship among these parameters and hardfats concentration was observed by Humphrey, Moquin and Narine (2003), by Humphrey and Narine (2004) and by Ribeiro *et al.* (2009f,g) in studying blends containing various liquid and fully hydrogenated oils.

The onset crystallization temperature ($T_{onset\ c}$) represents the beginning of the transition phase, that is, the temperature at which the first crystals are formed (ARONHIME, 1988). The addition of palm stearin to liquid oils produced blends with crystallization onset between 20.0 to 30.0 °C.

Peak crystallization temperature (T_{peak}), on the other hand, refers to the temperature at which the biggest proportion of lipid species crystallizes with maximum thermal effect (CAMPOS, 2005). Palm stearin showed $T_{peak\ c1}$ at 29.1 °C. At the beginning of the crystallization process of the blends, the components coming from hardfats crystallize immediately. Therefore, the higher the hardfat concentration in the blends, the higher the contents of high melting point components to be crystallized. Consequently, the increase in the number of molecules with simultaneous crystallization leads to a larger energy release by the system (CAMPOS, 2005; HUMPHREY, NARINE, 2004).

4.3.6. CRYSTALLINE MICROSTRUCTURE

The study of crystalline microstructure of fat systems has become increasingly important since the functional properties of many foods depend on knowledge of their fine structure. Microstructure is dependent on the composition of a fat, as well as its crystallization behavior, including polymorphism, and the characteristics of the microstructure in turn determine the physical properties of the fat. Success in the measurements requires several stages including obtaining a truly representative image of material, analyzing that image properly, and interpreting the resulting data (GIOIELLI; SIMÕES; RODRIGUES, 2003).

The crystallization of the samples can be analyzed from the viewpoints of the influence of blending of coconut oil and canola oil with palm stearin, of the influence of chemical interesterification in the formation of crystal lattice and of the influence of the temperature of crystallization.

Figure 4.5 a and b shows the crystalline microstructure of palm stearin, coconut oil, canola oil and their blends, before and after interesterification by slow crystallization at 25 °C. Crystal

mean diameter of blends and the range of crystalline distribution, before and after randomization, are shown in Table 4.2. The high standard deviation values with relation to crystal mean diameter, that is, the high variation coefficients, are characteristic of crystallized fats when observed in PLM (SILVA *et al*., 2008; RIBEIRO *et al*., 2009f,g). Samples 2NIE, 2CIE, 3NIE, 3CIE, 6NIE, 6CIE, 10NIE and 10CIE didn't show crystalline microstructure at 25 °C.

Table 4.2. Number of crystals, diameter and crystallized area of palm stearin and its blends with coconut oil and canola oil before and after chemical interesterification, at 25 ºC in static process.

Blend	25 °C 3600 min		
	Cristal number[a]	Diameter ± SD (μm)[b]	Area (%)[c]
1 NIE	5434	10.4±3.5	20.3±3.5
1 CIE	2239	4.5±2.5	16.6±4.5
4 NIE	1245	12.5±4.2	15.6±3.5
4 CIE	543	4.2±2.8	12.5±2.5
5 NIE	4685	10.3±2.3	11.3±3.8
5 CIE	17	107.8±31.2	6.5±1.5
7 NIE	350	120.3±55.6	25.6±9.8
7 CIE	2	84.1±52.6	3.6±1.5
8 NIE	1053	12.2±2.8	15.6±7.5
8 CIE	500	4.3±1.8	13.3±2.6
9 NIE	4	75.0±23.6	10.5±2.5
9 CIE	1	303.3	1.07±0.5

[a]Number of crystals, total number of crystals in an image; [b]Diameter ± SD (μm), mean ± standard deviation of the diameters of the crystals; [c]Area (%), ratio of the sum of the areas of all crystals and the total area of the image, multiplied by 100.

Palm stearin (1NIE) consists of crystal aggregates with fine plate-like form and orderly packed structure. After interesterification, sample 1CIE shows a complete alteration in the microstructure. The formation of finer, smaller crystals and decreasing of crystal diameters with loosely packed structure was observed. Moreover, CIE also promotes an increasing on the number of crystals in palm stearin. Blends with the high proportion of palm stearin (1NIE and 8NIE) have the highly dense crystal network and closely packed structure.

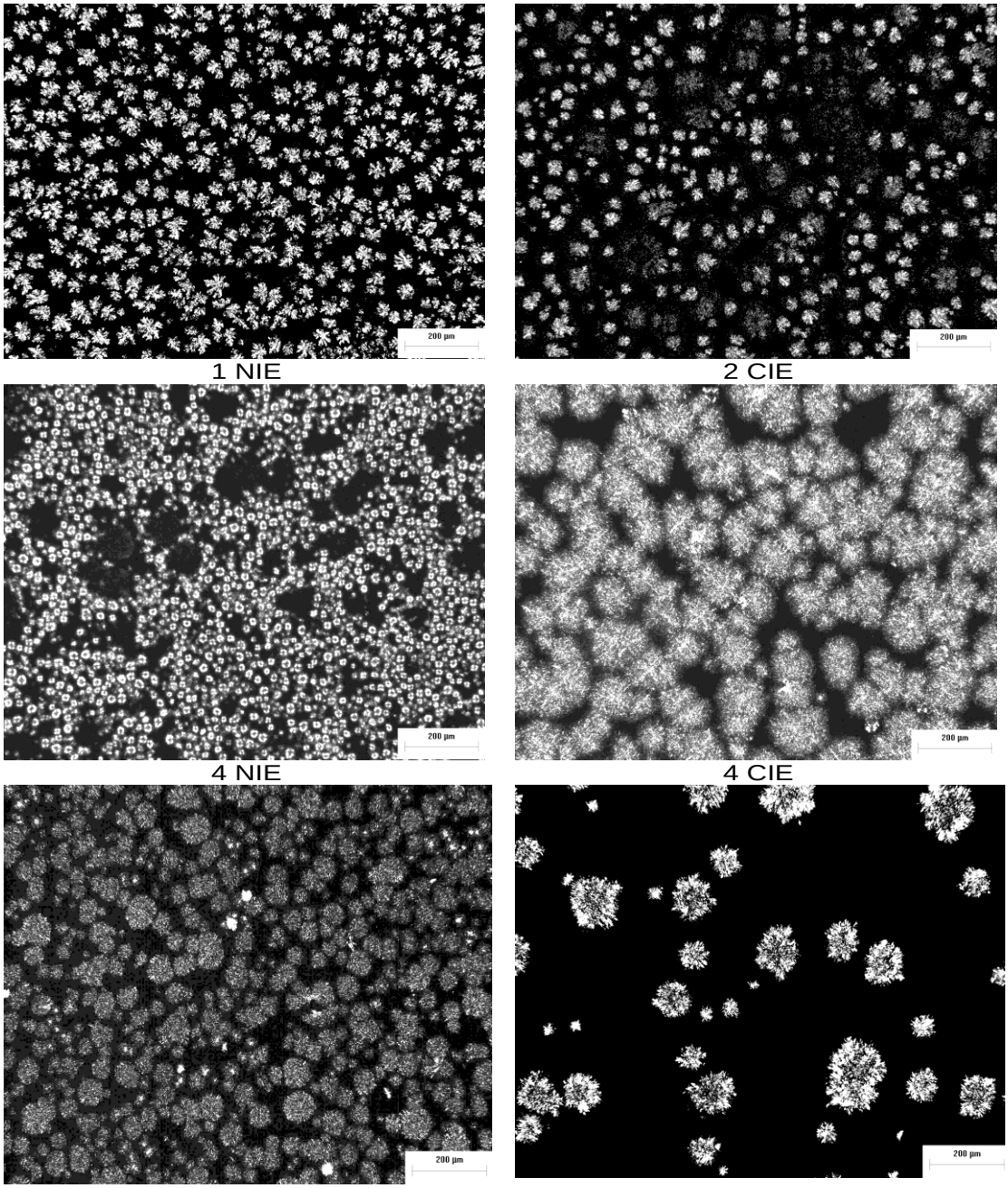

Figure 4.5a. Images of the crystallization of palm stearin, coconut oil and canola oil blends, before and after chemical interesterification at 25 ºC in static process. The bar represents 200 µm.

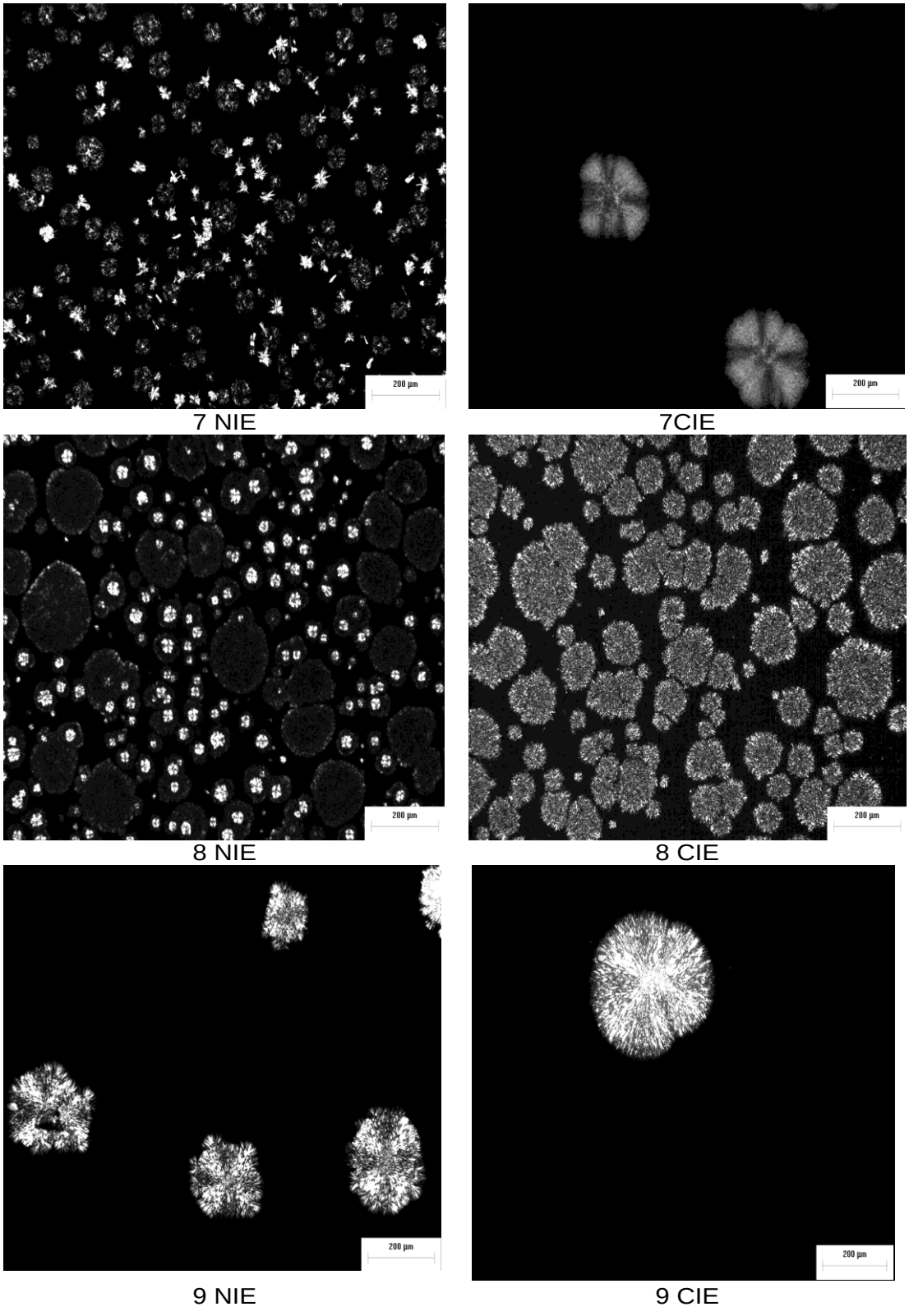

Figure 4.5b. Images of the crystallization of palm stearin, coconut oil and canola oil blends, before and after chemical interesterification at 25 ºC in static process. The bar represents 200 µm.

Shi, Liang and Hartel (2005) who stated that the highest-melting point triacylglycerol species of blend dominates crystal morphology explained this behavior. The presence of large spherulites or spherulite clusters gives the fat an undesirable grainy texture. The palm stearin consists of high amount of saturated TAGs, with melting points of 55–60 °C. After CIE, smaller crystals with decreasing diameters were observed in palm stearin and its blends (1CIE and 8 CIE).

The blend with 50% of palm stearin and 50% of coconut oil (4NIE) resulted in the mixture of plate-like and spherulitic shape with orderly packed structures, respectively. After interesterification (4CIE), the crystals forms are smaller and with loosely packed structure, with the mixtures of spherulite and plate-like shape. An incremental addition of canola oil to the binary and ternary blends with palm stearin and coconut oil before and after interesterification exhibited noticeable changes in the crystal morphologies. The crystal diameter decreased as the canola oil concentration increased in the blends, with formation of loosely and freely packed crystals.

According to Reyes-Hernández *et al*. (2007) the incorporation of saturated medium chain TAG (i.e., LaLaLa) into the crystals seemed to further decrease the level of structural organization.

Regarding the effect of the blend of coconut oil and canola oil with palm stearin in forming the crystal lattice, the results indicate that the tendency of crystallization at 25 °C of palm stearin is maintained in the blends, but the crystallization is hindered by the effect of dilution. Thus, there was a tendency to decrease the crystallized area and the number of crystals with increasing participation of the liquid oils in the blend.Interesterification promotes significant alterations in the microstructure of fats, since it modifies the morphology and density of the crystalline network. In general, smaller spherulites are formed and/or halo/nucleus ratio of the crystal is modified, affecting the properties of texture and functionality of the interesterified fats (NARINE, MARANGONI, 1999a,b; NORIZZAH *et al*., 2004; ROUSSEAU, MARANGONI, JEFFREY; 1998; TANG, MARANGONI, 2006).

Interesterification produced significant reductions in crystal diameter in the blends 1 CIE, 4 CIE and 8 CIE. It is interesting to note that these blends show more regular and homogeneous crystallization than the corresponding non-interesterified blends.

The microstructural level (or mesoscale) of a fat crystalline network can be defined as the structures with dimensions between approximately 0.5 and 200 μm (NARINE, MARANGONI, 2005).

According to Herrera *et al.* (1998), fats for food product applications should have crystal diameters of less than 30 μm in order to prevent grainy mouthfeel. For all blends

evaluated, except samples 4 CIE, 7 NIE and 7 CIE, chemical interesterification was effective in attaining fat bases which are compatible with food application.

According to Rousseau, Marangoni and Jeffrey (1998), the alterations in fat microstructure caused by interesterification result from modifications of morphology and the density of the crystalline network and affect the texture and functionality of the interesterified bases. In addition, the change in solid fat content intrinsic to the process of interesterification influences how the crystalline network is structured. When solid fat content decreases, as observed in this study, changes occur towards greater molecular area and mobility for crystal formation (HIMAWAN *et al*., 2006). Thus, according to our results, interesterification of palm stearin with coconut oil and canola oil tends to avoid sandy mouthfeel caused by large crystals.

Increased canola oil concentration in the randomized blends was associated with increased medium crystal diameter. This effect may be related to the longer time required for this sample to crystallize, due to its low melting point (RIBEIRO *et al*., 2009g).

According to Kloek, Walstra and Van Vliet (2000), a dispersion of fat crystals with a large number of small crystals may represent desirable properties, such as good spreadability. A large number of small crystals make harder fat than a smaller number of large crystals, which is also associated with undesirable characteristics, such as grainy mouthfeel. Besides, they are suitable for bakery products, because crystals of small dimensions can enclose and stabilize air bubbles during the cream-formation stage, providing these products with a soft and aerated texture.

4.4. CONCLUSIONS

A comprehensive understanding of the functions and properties of fats or fat bases produced by interesterification is essential for the outlining of their applications and the attainment of food products with desirable final attributes. This study made possible to verify that triacylglycerol composition, thermal behavior and microstructure of palm stearin, coconut oil and canola oil blends were significantly altered by both increasing hardfat concentration and chemical interesterification process. In addition, these properties proved to be directly related to the triacylglycerol composition of the blends before and after randomization and, taken together, can determine their applicability.

THERMAL BEHAVIOR OF STRUCTURED LIPIDS PRODUCED BY CHEMICAL INTERESTERIFICATION OF BLENDS OF PALM STEARIN, COCONUT OIL AND CANOLA OIL

ABSTRACT

Blends with palm stearin, coconut oil and canola oil were interesterified under the following conditions: 60 min reaction time, 0.3% sodium methoxide catalyst, under agitation, at 88 °C. The original and interesterified blends were examined for triacylglycerol composition, thermal behavior, oxidation temperature and thermal stability. Interesterification produced rearrangement of the triacylglycerol species in all the blends, reduction of trisaturated and triunsaturated triacylglycerols content and increase in monosaturated-diunsaturated and disaturated-monounsaturated triacylglycerols. Melting and crystallization curves were significantly modified by randomization. The thermal stability and oxidation temperature of palm stearin, coconut oil and canola oil and their blends were dependent on fatty acid composition and independent on chemical interesterification.

KEYWORDS: Melting and crystallization curves, Thermal stability, Oxidation temperature, Fatty acid composition, Triacylglycerol composition.

5.1. INTRODUCTION

In recent decades, thermal analysis has gained increasing importance in all areas of knowledge in basic and applied chemistry. Thermal analysis is defined as "a group of techniques by which a physical property of a substance and / or its reaction products is measured as a function of temperature and / or time, while the substance is subjected to a controlled temperature program (SILVA; de PAOLA; MATOS, 2007).

Thermal analysis is a method of choice to investigate the phase transitions and the changes in heat capacity of the materials used and plays an important role in the process development and formulation of fat food (MATOS; MACHADO, 2004).

There are several thermoanalytical techniques, the most common that can be used in the development of new fat or oil are: Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). These techniques provide information concerning important effects or properties like polymorphism, stability, interactions, and purity, as well as properties of packaging materials. In the literature are investigations of the melting point, crystallization and oxidation of edible oils and fats by Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG).

Differential Scanning Calorimetry is the most applicable technique in the research and development process. A great number of applications are referred, concerning the study of effects of materials, such as melting point and range, heat of fusion, polymorphism, interaction, compatibility, oxidation stability and crystallization kinetics.

Differential Scanning Calorimetry (DSC) is the most used thermo analytical technique for the study of oils and fats. The numerous thermal phenomena related to these raw materials are verified by monitoring the enthalpy and phase transition changes of the various triacylglycerols blends (RIBEIRO *et al*., 2009f; TAN, CHE MAN, 2002).

Generally, the thermal behavior of fats is commonly described by melting and crystallization curves. The thermal melting and crystallization characteristics of each sample in a DSC scan can be indicated by various temperatures. Oil crystallization results in volume contraction, which is associated to an exothermic effect. Fat melting, on the other hand, contributes for volume expansion, characterizing an endothermic effect. In general, oils and fats may show an extremely complex thermal behavior, which will be highly dependent on the chemical composition and on the protocol for the DSC experiment (DIAN, SUNDRAM, IDRIS, 2007).

Marangoni *et al*. (1998) and Tan and Chen Man (2002) showed that fats crystallize and melt in several steps that correspond to separated group of triacylglycerols. The complex DSC recordings result from: the broad distribution of triacylglycerols composition; compound crystallization meaning that the composition of the crystals is changing during melting; and the existence of a polymorphism of monotropic type for each triacylglycerols group constituting the crystal.

Thermogravimetric Analysis provides information regarding mass changes as a function of time and/or temperature under certain weather conditions. The experiments are performed using a Thermal balance of high sensitivity, reproducibility and rapid response to variations in mass. The curves provide information on the composition and thermal stability of the sample, intermediate products and the residue formed. TGA can be used to estimate the quality of oils and fats from the determination of oxidative induction period, analysis of thermal stability and oxidation temperature (MATOS, MERCURI, ARAÚJO, 2009).

The relationship between fatty acid composition and Differential Scanning Calorimetry properties of a fat has been pointed out in the literature (MARIKKAR *et al*., 2001). Therefore, physical characteristics of fats such as their melting temperatures should be investigated by interrelating them with their fatty acid compositions. In addition, the chemical and thermal data to be obtained in this way may be a beneficial basis for future researches to develop to food production purposes. So far, very little information regarding chemical composition and thermal properties of palm stearin, coconut oil and canola oil has been reported.

Thermogravimetry (TG) is a thermoanalytical technique in which the change (loss or acquisition) weight of sample, solid or liquid, is determined by the temperature and / or time, while the sample is subjected to a controlled temperature programming. This technique makes it possible to know the changes that warming may cause in the mass of material and thus allows establishing the range in which they acquire mass fixed, constant and defined. It allows determine the temperature at which the material begins to decompose (thermal stability) and also track the progress of reactions of dehydration, oxidation, combustion, decomposition, among other applications (MATOS, MACHADO, 2004).

The derivative thermogravimetry (DTG) is the first derivative of TG curve. DTG curve presents information that is more accessible visually: the peak area has direct relation to the mass variation and also allows ready determination of the maximum temperature peak where the mass change occurs more quickly (ARAUJO *et al*., 2010).

Edibles oils are oxidized by metal traces, oxygen and temperature, and their stabilities are also influenced by the fatty acid composition, mainly unsaturated fatty acid. Generally, the rate of oxidation of fatty compounds depends on the number of double bonds and their position. The oxidation chain reaction is usually initiated at the positions allylic to double bounds. Thus, fatty acids with methylene interrupted double bonds, for example, linoleic acid, are more susceptible to oxidation because they contain methylene groups that are allylic to two-double bonds. Fatty acids with conjugated double bonds, for example, linolenic acid, are even more susceptible to oxidation.

The purpose of this study was to evaluate the thermal behavior of palm stearin, coconut oil, canola oil and their blends, with a view to studying fat bases for application in food products. Preparation of shortenings through chemical interesterification of palm stearin, coconut oil and canola oil is reported here. The functionality of the finished product was related to the chemical composition and triacylglycerols of the blends and interesterified fats.

5.2. MATERIAL AND METHODS

5.2.1. MATERIAL

Palm stearin was obtained from Agropalma S/A (Pará, Brazil), coconut oil from Copra Alimentos Ltda. (Alagoas, Brazil) and canola oil from Bunge Alimentos S.A. (São Paulo, Brazil). The fats were stored at 0 °C prior to use. All chemicals used were either of analytical or chromatographical grades.

5.2.2. BLEND PREPARATION

Fat blends, formulated with palm stearin, coconut oil and canola oil were mixed at different ratios, according to Table 2.1. Three blends represented the original components, three were binary blends and four were ternary blends. The blends were prepared after complete melting of the fats at 70 °C and stored under refrigeration.

5.2.3. CHEMICAL INTERESTERIFICATION

Chemical interesterification was performed according to Ahmadi; Wright; Marangoni (2008) with modifications. Two hundred grams of each blend were melted in a glass jar at 85°C under reduced pressure to limit moisture and air. The chemical reaction was started by the addition of 0.3 % (w/w) sodium methoxide (Merck Co.) as the catalyst. The blends were interesterified under reduced pressure for 60 min at 88±2°C. The start of the reaction was associated with the appearance of a reddish-brown color. To terminate the reaction, 5 mL of distilled water was added. The presence of water inactivates the catalyst by converting it to methanol (SREENIVASAN, 1978). Kieselghur and anhydrous sodium sulfate were added to minimize the darkening caused by the presence of a diacylglycerol metal derivative (active catalyst) and to remove residual water, respectively. The reagents were removed by filtering the samples with filter paper. The fat was poured into a glass jar and stored at 5°C prior to use. Non-interesterified oil is abbreviated to NIE and chemical interesterified blends to CIE.

5.2.4. FATTY ACID COMPOSITION

Fatty acid composition was determined after conversion of fatty acids into their corresponding methyl esters ($FAME_S$) by the method described by Hartman and Lago (1973) for blend 1 and by ISO method 5509 (2000) for blends 2 to 10. Analyses of $FAME_S$ were carried out in a Varian GC gas chromatograph (model 430 GC, Varian Chromatograph Systems, Walnut Creek, California, USA), equipped with a CP 8412 auto injector. The Galaxie software was used for quantification and identification of peaks. Injections were performed into a 100-m fused silica capillary column (ID = 0.25 mm) coated with 0.2 µm of polyethylene glycol (SP-2560, Supelco, USA) using helium as the carrier gas at an isobaric pressure of 37 psi; linear velocity of 20 cm/s; make-up gas: helium at 29 mL/min at a split ratio of 1:50; volume injected: 1.0 µL. The injector temperature was set at 250 ºC and detector temperature at 280 ºC. The oven temperature was initially held at 140 ºC for 5 min, then stepped to 240 ºC at a rate of 4 ºC/min, and held isothermally for 30 min. All samples were analyzed in triplicate and reported values represent the average of the three runs.

Long-chain saturated fatty acids (LCSFAs) are expressed as the sum of the amounts of myristic, palmitic and stearic acids.

Saturated fatty acids (SFAs) are expressed as the sum of the amounts of caprylic, capric, lauric, myristic, palmitic and stearic acids.

Unsaturated acids (USFAs) are expressed as the sum of the amounts of oleic, linoleic and linolenic acids.

Monounsaturated fatty acids (MUFA) are expressed as amounts of oleic acid.

Polyunsaturated fatty acids (PUFAs) are expressed as the sum of the amounts of linoleic and linolenic acids.

5.2.5. IODINE VALUE (IV)

Iodine value was calculated from the fatty acid composition, according to the procedure described in the AOCS official method Cd 1c-85 (AOCS, 2009c).

$$IV = (\%\ C_{18:1} \times 0.860) + (\%\ C_{18:2} \times 1.732) + (\%\ C_{18:3} \times 2.616) \quad \textbf{Eq. 5.1}$$

Where:

$C_{18:1}$ = oleic acid
$C_{18:2}$ = linoleic acid
$C_{18:3}$ = linolenic acid.

5.2.6. ATHEROGENIC INDEX (AI)

Atherogenic index was calculated according to Kim, Lumor and Akoh (2008), by the following equation:

$$AI = [C_{12:0}\ (w/w, \%) + 4 \times C_{14:0}\ (w/w, \%) + C_{16:0}\ (w/w, \%)]/USFA\ (w/w, \%) \quad \textbf{Eq. 5.2}$$

Where:

USFA = total amount of unsaturated fatty acids,
$C_{12:0}$ = lauric acid
$C_{14:0}$ = myristic acid
$C_{16:0}$ =palmitic acid.

5.2.7. REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS

A proton-decoupled ^{13}C NMR was used to analyze the positional distribution of fatty acids on the triacylglycerol backbone. Lipid samples (250 mg) were dissolved in $CDCL_3$ (0.5 mL) in 5 mm NMR tubes, and NMR spectra were recorded on a Bruker Advance DPX spectrometer operating at 300 MHz. The ^{13}C spectra of the lipid samples were acquired with a spectral width of 2332.090 Hz, pulse of 10.2 μs, and a relaxation delay of 30s. Determination of ^{13}C was performed at a frequency of 75.8 MHz with a multinuclear probe of 5 mm operating at 30 °C, using method described by Vlahov (2005). The results showed the compositions of saturated fatty acids, oleic acid and linoleic + linolenic acids in *sn*-2 and *sn*-1,3 positions. All samples were analyzed in triplicate and the reported values are the average of three analyses.

5.2.8. TRIACYLGLYCEROL COMPOSITION

For these calculations, it was considered the fatty acid composition obtained experimentally and the experimental results of the fatty acids located at the *sn*-2 position. For the sn-1,3 positions was considered that the fatty acids are present in these positions in equivalent amounts, according to the formula:

$$C1,3 = [3 \times (C1,2,3) - C2] / 2 \qquad \textbf{Eq. 5.3}$$

Where:

C1,3 = fatty acids in *sn*-1,3 positions
C1,2,3 = fatty acid composition of total fat
C2 = fatty acids in *sn*-2 position

According to the 1,3-random, 2-random theory, the levels of possible triacylglycerols, according to the saturation and unsaturation of fatty acids are:

For natural fats:

$$\% SSS = (\%S1)x(\%S2)x(\%S3)/10000 \quad \textbf{Eq. 5.4}$$
$$\% SUS = (\%S1)x(\%U2)x(\%S3)/10000 \quad \textbf{Eq. 5.5}$$
$$\% SSU = 2x(\%S1)x(\%S2)x(\%U3)/10000 \quad \textbf{Eq. 5.6}$$
$$\% USU = (\%U1)x(\%S2)x(\%U3)/10000 \quad \textbf{Eq. 5.7}$$
$$\% UUS = 2x(\%U1)x(\%U2)x(\%S3)/10000 \quad \textbf{Eq. 5.8}$$
$$\% UUU = (\%U1)x(\%U2)x(\%U3)/10000 \quad \textbf{Eq. 5.9}$$

After chemical interesterification was used the 1,2,3-random distribution theory:

$$\% SSS = (\%S)x(\%S)x(\%S)/10000 \quad \textbf{Eq. 5.10}$$
$$\% SUS/SSU = 3x(\%S)x(\%U)x(\%S)/10000 \quad \textbf{Eq. 5.11}$$
$$\% USU/UUS = 3x(\%U)x(\%S)x(\%U)/10000 \quad \textbf{Eq. 5.12}$$
$$\% UUU = (\%U)x(\%U)x(\%U)/10000 \quad \textbf{Eq. 5.13}$$

Where:

%S = % of saturated fatty acids
%U = % of unsaturated fatty acids

5.2.9. DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC curves are obtained by differential scanning calorimetry (DSC) cell from the DSC 4000 Perkin Elmer (Perkin Elmer Corp.., Norwalk, CT, USA), under dynamic atmosphere of He (20 mL / min) and cooling rate of -10 ° C / min and heating at 5 ° C / min, at temperatures ranging from 80 to -60 ° C for cooling with isothermal time of 10 min at 80 ° C and -60 to 80 ° C for heating with a isothermal of 10 min at -60 ° C, using sealed aluminum capsules partially closed containing sample mass between 5 to 10 mg. The temperature and heat of fusion were calibrated with indium (initial temperature of 156.6 ° C). Before the trials were white curves to assess the baseline equipment. Curves will be processed in the program Pyris, melting and crystallization curves are analyzed for the onset (T_{onset} °C) and endset (T_{endset} °C) of melting and

crystallization , peak crystallization and melting temperatures (T_{pf} and T_{pc} ° C) and crystallization and melting enthalpies (ΔH_f and ΔH_c J / g) (RIBEIRO et al., 2009f).

5.2.10. THERMOGRAVIMETRIC ANALYSES

The TG / DTG curves were obtained in Shimadzu (equipment of mass, in the temperature range 25-900 ° C with a rate of 10 ° C / min under a synthetic air atmospheres and sample mass of approximately 20 to 30 mg in crucible platinum. Before the trials were blank curves to assess the baseline equipment. The calibration of equipment TGA-51 was performed according to standard ASTN (E1582-04), to verify the system used a standard calcium oxalate monoidratado (Merck) with purity of 99.9% Curves will be treated in the program TA60.

5.3. RESULTS AND DISCUSSION

5.3.1. FATTY ACID COMPOSITION

The fatty acid composition of the blends and interesterified fats are shown in Table 2.3 a anb b. Medium-chain fatty acids (MCSFAs) are represented by the sum of the amounts of caprylic acid, capric acid and lauric acid. Long-chain fatty acids (LCSFAs) are represented by the sum of the amounts of myristic, palmitic and stearic.

All the blends were *trans* fatty acid-free. The total saturated fatty acid of the blends with higher content of palm stearin and coconut oil were more than 35.6 %, a value that renders it a strong resistant to oxidative rancidity (JAYADAS; PRABHAKARAN NAIR, 2006; NEHDI *et al*., 2010).

Blends prepared by using palm stearin have higher concentrations of palmitic acid, which imparts a desirable smooth consistency required for purposes such as margarines and shortenings. Shortenings with higher palmitic acid content are reportedly more stable in β′-crystal form than those with less palmitic acid (JEYARANI; REDDY, 2003). Blends with higher content of coconut oil are predominant in lauric acid. According to Zhang, Smith and Adler-Nissen (2004), fats that have a high content of lauric and myristic acids show very sharp melting point.

All the blends contained oleic acid (more than 12.5 %) as their major unsaturated fatty acid. The content of linoleic acid in all blends was less than 22.0 % and linolenic acid was below than 8.0 % (Table 2). Oleic acid is very important in nervous cell construction. It

can be changed by organism into a set of compounds close to prostaglandins which have an important role at the vessel level and for blood coagulation. Oleic fatty acid has fundamental role in cardiovascular diseases prevention. Linoleic fatty acid is indispensable for the healthy growth of human skin (NEHDI *et al*., 2010). It can be transformed by the organism into a series of long fatty acids chains, which are the precursors of eicosanoids (NASRI *et al*., 2005; NEHDI *et al*., 2010).

5.3.2. REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS

The fatty acid profile at the *sn*-2 position of palm stearin, coconut oil, canola oil and their blends, before and after chemical interesterification, is given in Figure 2.6 b.

Polyunsaturated fatty acids were found mainly in the *sn*-2 position, typical feature of vegetable oils. These results confirm a random distribution of fatty acids after the chemical interesterification.

5.3.3. TRIACYLGLYCEROL COMPOSITION

Triacylglycerol fraction of a fat is responsible for most of its physical properties that affect lubricity (pourability, holding together at room temperature or melting in the mouth to give a pleasant cooling effect). Lubricity is dependent on melting temperature, solid fat content and texture. The relationship between triacylglycerol structure and lubricity has been reviewed by Bessler and Orthoefer (1983).

Analysis of triacylglycerol composition represents a true indication of randomization, and are extremely useful for monitoring modification of interesterified fats and defining specific applications for them (O'BRIEN, 2004).

For food product formulation, the physical properties of a fat are more easily interpreted when triacylglycerols are designated by their degree of unsaturation: SSS (trisaturated), SSU (disaturated–monounsaturated), SUU (monosaturated–diunsaturated) and UUU (triunsaturated), instead of by the individual triacylglycerol species (NEFF; BYRDWELL; LIST, 2001; PETRAUSKAITE *et al.*, 1998).

Table 2.4 a and b and Figure 4.1 show composition of triacylglycerols classes of palm stearin, coconut oil, canola oil and their blends before and after chemical interesterification.

Triacylglycerol composition of palm stearin was dominated by the SSU group, while coconut oil showed a predominance of SSS group. Canola oil (before and after chemical interesterification) showed more than 76 g/100 g of triacylglycerols of the UUU group and therefore did not present solid fat content even in refrigeration temperature (5 ºC), because the melting point of this group is in the range of -13 to 1 °C, remaining liquid at 5 °C.

Chemical interesterification distributes fatty acids equally through in the three positions of the glycerol backbone (GUNSTONE, HARWOOD; PADLEY, 1986; RODRIGUES; GIOIELLI, 2003). Chemical interesterification decreased the amount of SSS triacylglycerols (all samples), while the levels of SSU (with exception of blends 1, 3 and 5) and SUU (with the exception of blend 3) increased. UUU triacylglycerols decreased in the blends 5, 6, 8 and 10 and increased in the blends 1, 2, 3 and 9. These results indicated that there were exchanges of fatty acids between triacylglycerols.

Hence, the increase of the SUU and SSU contents of palm stearin, coconut oil and canola oil blends, promoted by chemical interesterification, is associated to the increase of technological functionality, the betterment of sensorial characteristics, and, therefore, to a greater potential of these interesterified bases for food application (SOARES *et al*., 2009).

5.3.3. DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC melting curves can be used to highlight the changes in melting and crystallization behavior resulting from the interesterification reaction. Melting and crystallization behavior of fats and oils depends on the fatty acid composition in their triacylglycerols backbone. For example, vegetable oils composed of various triacylglycerols species have broad endothermic peaks.

The melting and crystallization curves of the non-interesterified fats were distinctly different from those of the interesterified fats. The shape of the curves was modified by the reaction; chemical interesterification has caused smaller and broader peaks to be formed as compared to the non-interesterified blends, indicating different distribution of the low- and medium-melting triacylglycerols in the blends, which was consistent with the results of the triacylglycerols structure. Because the change in chemical composition of interesterified fats, endothermic and exothermic peaks in the blends were renamed.

Thermal curves, as determined by DSC after chemical interesterification, are given in Figures 5.1-5.6 and Tables 11.1-11.12. Crystallization curves of oil samples are illustrated in

Figures 5.1-5.6a and Tables 11.1-11.11, while the melting curves are displayed in Figures 5.1-5.6b and Tables 11.2-11.12. Table show the crystallization and e melting curves parameters for the blends before and after randomization are in Annex 2.

Figures 5.1 (a and b) and 5.2 (a and b) show the results for changes in the thermal properties of palm stearin and its blends with coconut oil and canola oil before and after chemical interesterification. Crystallization and melting behavior of coconut oil and their blends with palm stearin and canola oil before and after chemical interesterification, are shown in Figures 5.3 (a and b) and 5.4 (a and b). Results of the crystallization and melting behavior of canola oil and their blends with palm stearin and coconut oil before and after chemical interesterification, are shown in Figure 5.5 (a and b) and 5.6 (a and b).

The DSC crystallization curves of palm stearin showed two distinct peaks at 3.4 and 29.1 °C, indicating that heterogeneous types of triacylglycerols were crystallized at different temperatures. Peak C_1 represents crystallization of triacylglycerols with saturated fatty acids and peak C_2 represents crystallization of triacylglycerols with more unsaturated fatty acids (RESHMA *et al.*, 2008). According Tan and Che Man (2002) these peaks can be attributed to the transition from crystallization of the stearin fraction (major exotherm) and some small amount of olein fraction (minor exotherm).

All blends of palm stearin with coconut oil and canola oil (NIE4, NIE5, NIE7, NIE8, NIE9 and NIE10) showed peak C_1, and range from 26.6 to 15.8 °C. As palm stearin content is decreased, the peak heights and areas decreased and the peaks shifted slightly to a lower temperature.

Generally, in melting curves of oil samples, complex features that are not easily interpretable, such as shoulders not separable from peaks, are noticed. These results illustrate the complex nature of triacylglycerols in oil samples (TAN; CHE MAN, 2000).

The unique features of triacylglycerols present in vegetable oils are the main building block for their application in the food industry. Generally, the highly saturated triacylglycerols (SSS) melt at higher temperatures than the highly unsaturated triacylglycerols (UUU), with the SSU and SUU triacylglycerols melting in between these two extremes (TAN; CHE MAN, 2002).

Although these observations are of interest by themselves, they become particularly important when the endothermic peaks are being used to establish the thermodynamic properties of the initial fat crystallites. Nevertheless, there is no obvious way to determine the origin of the multiple peaks by just examining the melting curves.

Palm stearin showed six endothermic peaks H_1–H_6 and a broad melting peak was observed at 53.0 °C. It can be observed a peak of recrystallization stage between the peaks H_3 and H_4, ranging from 10.1 to 25.8 °C.

Triacylglycerols can exist in several crystalline structures or polymorphic forms. As the oil samples are heated, some of the less thermally-stable polymorphs melt; the remaining triacylglycerols rearrange, and recrystallize into more stable polymorphs that melt at higher temperatures (RESHMA *et al*., 2008).

The amount of recrystallization depends on the stability of the original crystal population, which will depend on its crystallization history, and on the amount of time that the sequences have for rearrangement and recrystallization, which will depend on the heating rate (TAN, CHE MAN, 2002).

According Zhang, Smith and Adler-Nissen (2004), on heating, palm stearin shows crystal transformation through three crystal forms.

The melting curves of the blends show that peak H6, which represents the high melting triacylglycerols of palm stearin, gradually disappeared, decreasing in size as the content of stearin in the blends decreased.

Palm stearin showed both endotherm regions; the higher region was distinguished by a tall peak (and two small fusion peaks) preceding the low region (consisting of three small merging peaks). The small low-temperature peak in the melting curve of palm stearin indicates that a small amount of olein was trapped in this oil sample after fractionation. These results agree with our earlier observation from the crystallization curves.

The melting and crystallization curves of the non-interesterified fats were distinctly different from those of the interesterified fats. The shape of the curves was modified by the reaction; chemical interesterification has caused smaller and broader peaks to be formed as compared to the non-interesterified blends, indicating different distribution of the low- and medium-melting triacylglycerols in the blends, which was consistent with the results of the triacylglycerols structure (Table 2.4 a and b). Because the change in chemical composition of interesterified fats, endothermic and exothermic peaks in the blends were renamed.

DSC crystallization curves of palm stearin after chemical interesterification showed two distinct peaks at 25.5 and 4.0 °C. Similarly, crystallization peaks of the non-interesterified palm stearin were observed at 29.1 and 3.1 °C. The broadening effect between the two peaks was due to the wide triacylglycerols compositions in palm stearin. In the position of Peak C_2, two small shoulders were observed and are labeled as C_{2a} and C_{2b} after

chemical interesterification, while these shoulders were not observed in the non-interesterified blend.

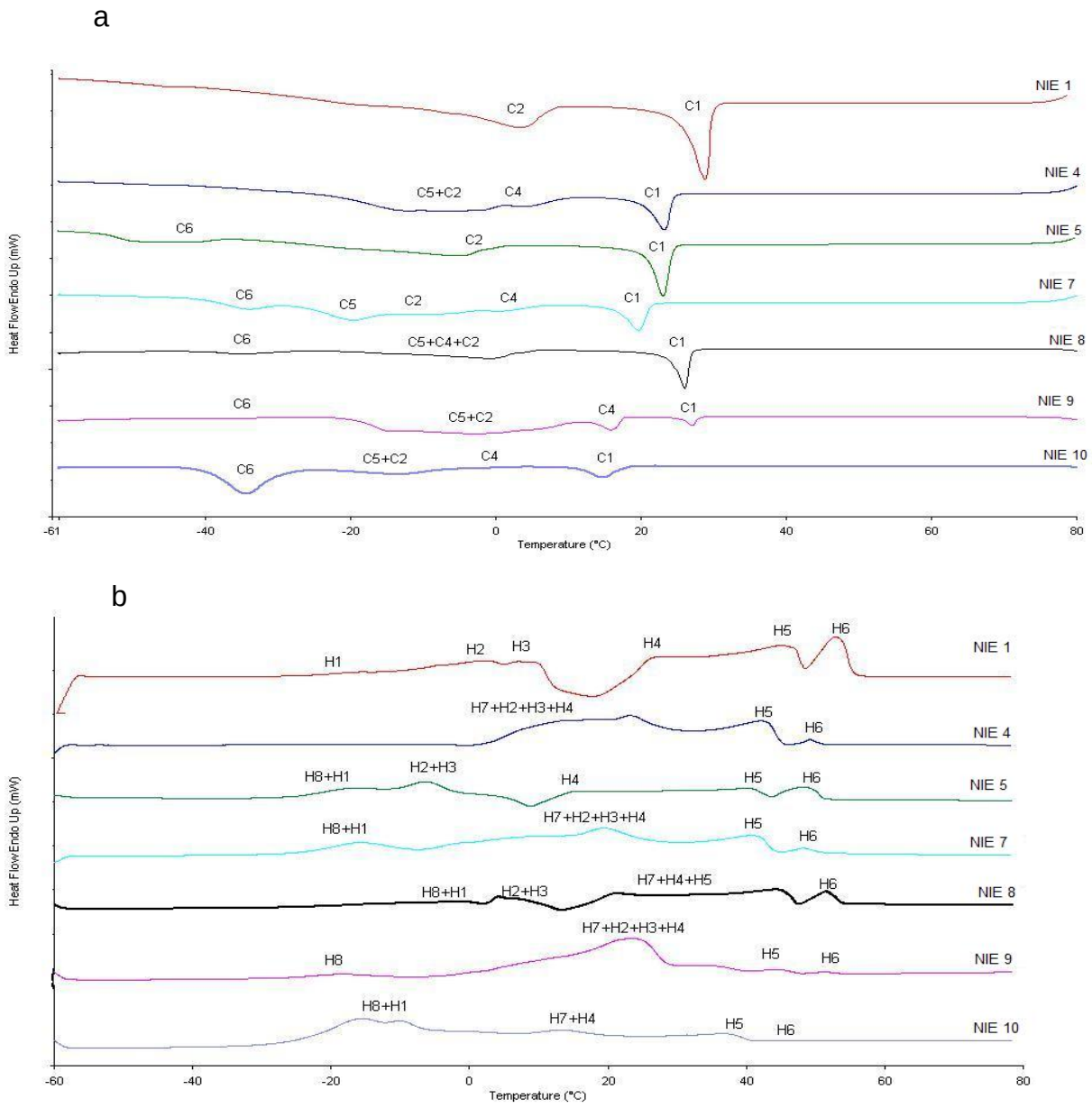

Figure 5.1. DSC curves of palm stearin and its blends with coconut oil and canola oil before chemical interesterification under dynamic atmosphere of He (a) crystallization obtained at 10 °C/min and (b) melting obtained at 5 °C/min.

After chemical interesterification palm stearin showed six melting peaks (H_1-H_6) and a broad melting peak was observed at 48.5 °C. It can be observed two recrystallization peaks between peaks H_3 and H_4, ranging from 3.50 to 21.9 °C. Before chemical interesterification

the broad melting peak was observed at 53.0 °C and was observed a recrystallization peak between peaks H_3 and H_4, ranging from 10.1 to 25.8 °C.

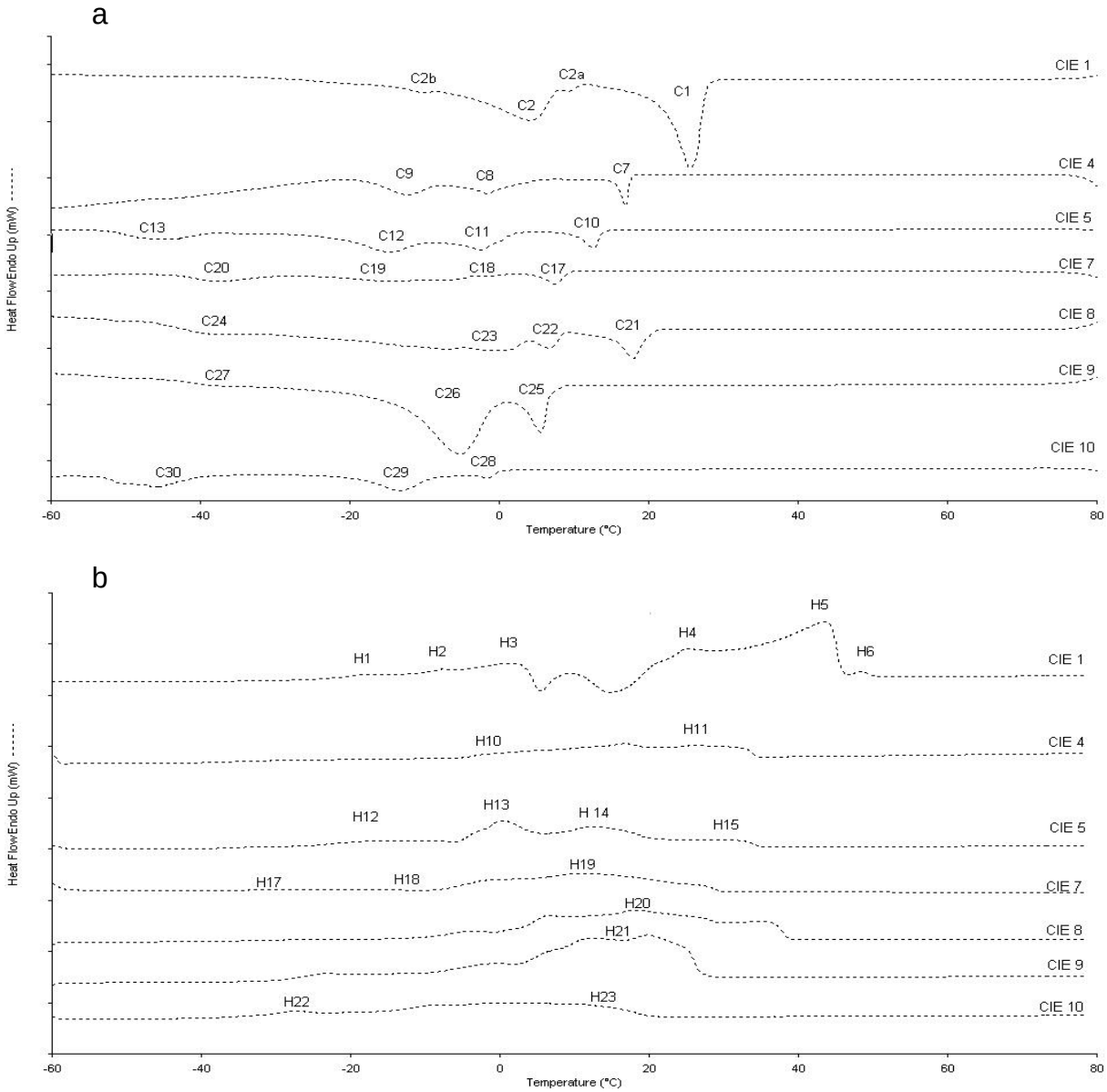

Figure 5.2. DSC curves of palm stearin and its blends with coconut oil and canola oil after chemical interesterification under dynamic atmosphere of He (a) crystallization obtained at 10 °C/min and (b) melting obtained at 5 °C/min.

According De Clercq *et al.* (2011), it can be assumed that the endothermic peak between -30 and 3°C corresponds to the melting of SUU and UUU triacylglycerols, the endothermic peak between 3 and 14 °C corresponds to the melting of SUS and SUU

triacylglycerols and the endothermic peak between 14 and 47°C corresponds to the melting of SSS and SUS triacylglycerols, taking into account the intersolubility of each one.

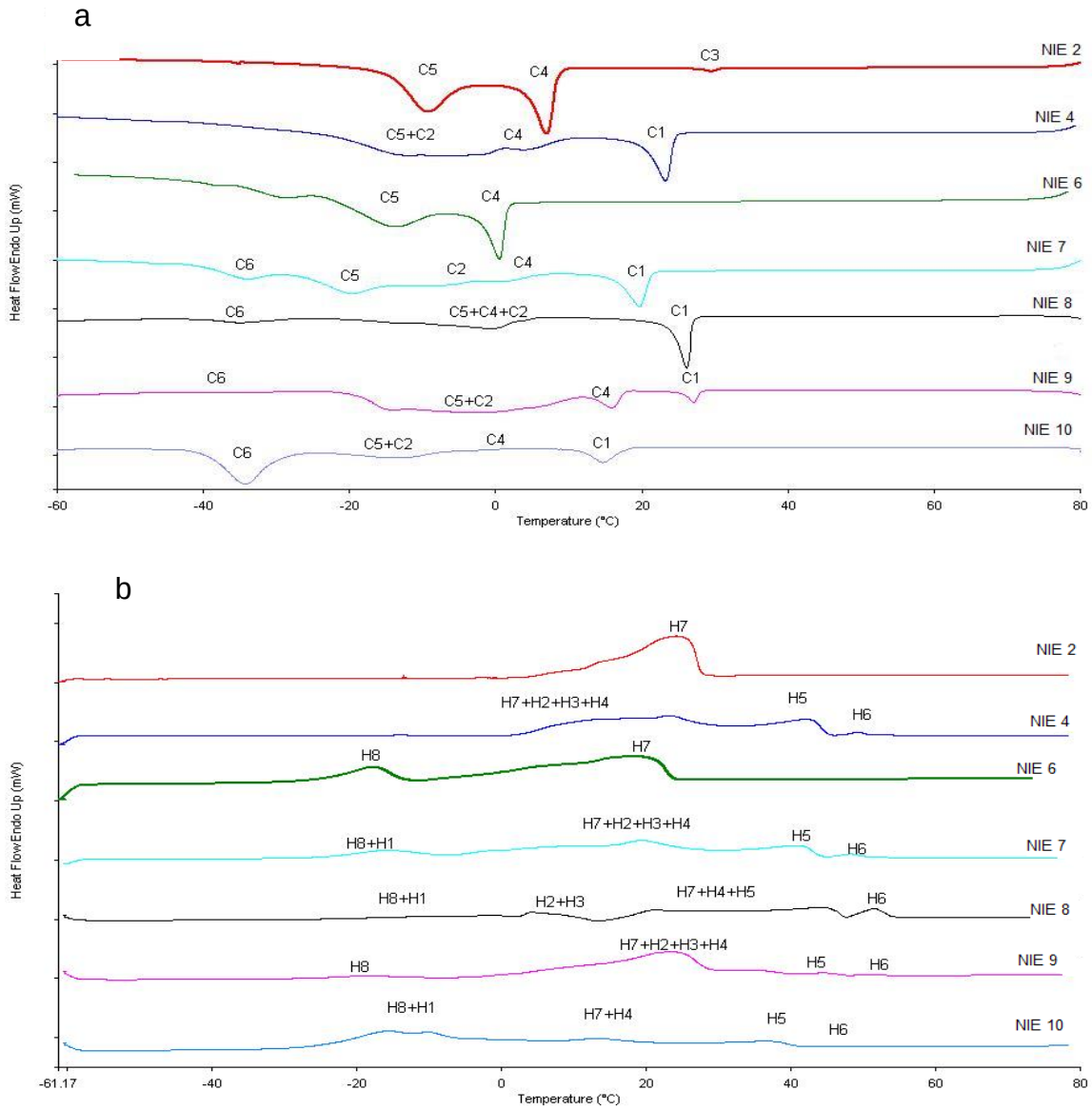

Figure 5.3. DSC curves of coconut oil and its blends with palm stearin and canola oil before chemical interesterification under dynamic atmosphere of He (a) crystallization obtained at 10 °C/min and (b) melting obtained at 5 °C/min.

The depressed areas between peaks H_3 and H_4 are recrystallization peaks, which probably occurred when a α polymorph transitioned to the β' form upon melting of the unstable α form. This recrystallization peak decreased as the amount of palm stearin decreased (JENNINGS, AKOH, 2010).

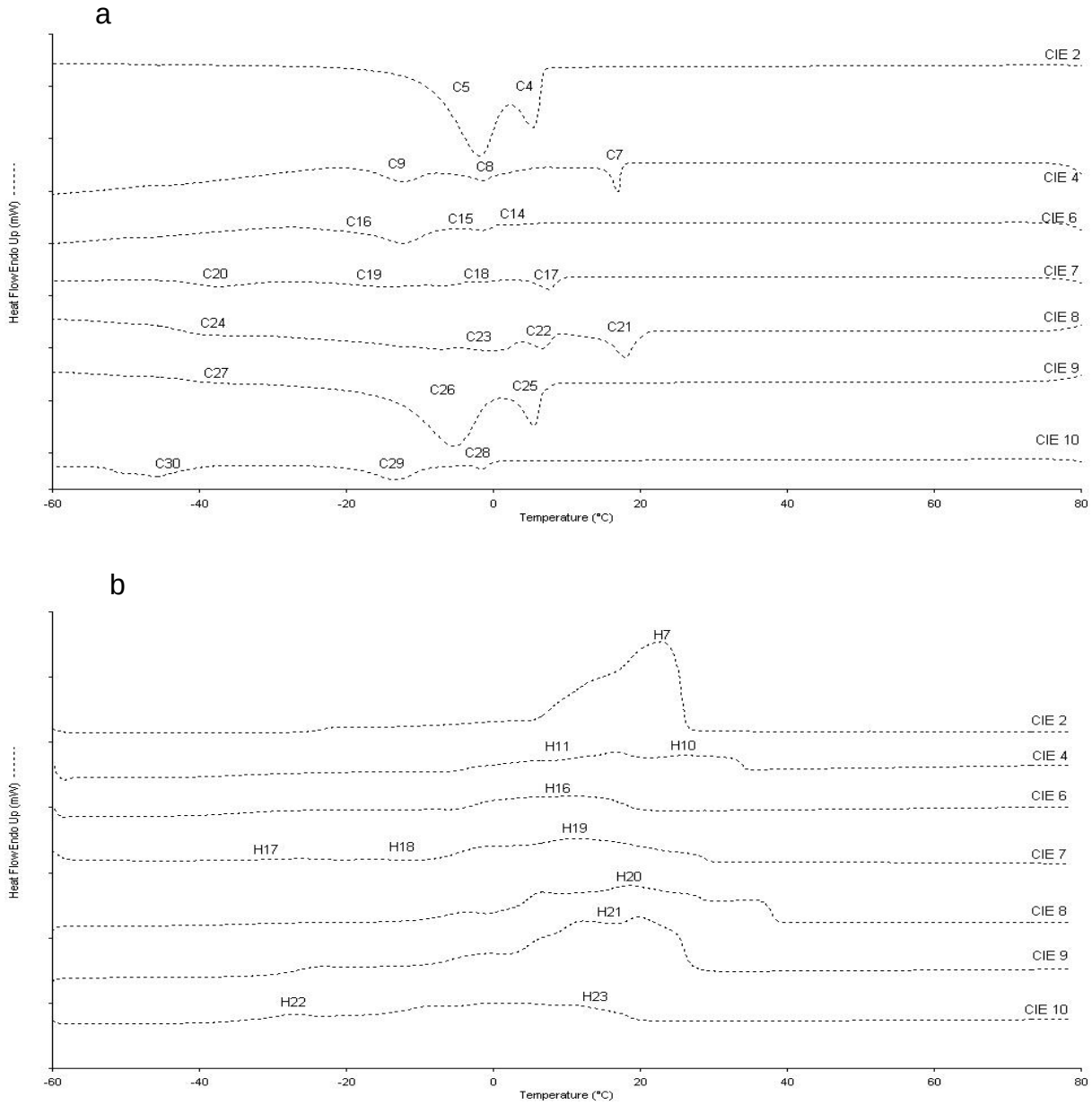

Figure 5.4. DSC curves of coconut oil and its blends with palm stearin and canola oil after chemical interesterification under dynamic atmosphere of He (a) crystallization obtained at 10 °C/min and (b) melting obtained at 5 °C/min.

Polymorphic behavior, with the α polymorph being formed by high cooling rates, apparent α to β′ transitions occurring at low cooling rates with recrystallization upon immediate melting, and β′ polymorphs occur exclusively in stored samples (LITWINENKO *et al*., 2002).

The melting curve of the blends show that peak H6, which represents the high melting triacylglycerols of palm stearin, gradually disappeared, decreasing in size as the content of

palm stearin in the interesterified blends decreased, indicating that chemical interesterification altered the melting profiles and induced the formation of a softer fat. This was similar to the findings of Chu *et al.* (2000), in their work with enzymatic interesterification of palm stearin and palm kernel olein.

Norizzah *et al.* (2004) evaluated melting curves of the interesterified and non interesterified palm stearin and palm kernel olein blends and confirmed a product of lower melting point being formed in interesterified blends with the disappearance of the high-melting triacylglycerols.

Khatoon and Reddy (2005) noticed the presence of three distinct endotherms peaks in blends containing palm stearin and mahua oil (1:1 and 1:2) indicating a heterogeneity of triacylglycerols, the proportions of which altered after chemical interesterification. They also concluded that changes in melting behavior are due to reduction in high-melting and increase in low-melting triacylglycerols after chemical interesterification.

The crystallization behavior of coconut oil, as shown in the cooling curve obtained by differential scanning calorimetry, showed three distinct exotherms peaks. This result is different from that found by Reena, Reddy and Lokesh (2009), that found a single exothermic peak at 0.97 °C. According Nasirullh, Umesh and Reddy (2010) coconut oil has two crystallization peaks, at 8.4 and -1.6 °C.

According to Tan and Che Man (2002), this major endothermic peak was the melting peak of five major trisaturated triacylglycerols present in coconut oil, namely CCLa, CLaLa, LaLaLa, LaLaM, and LaMM, which accounted for almost 80% of the total triacylglycerols.

The incorporation of saturated medium chain triacylglycerols (i.e., LaLaLa) into the crystals seemed to further decrease the level of structural organization and hence the heat released on crystallization (REYES-HERNANDEZ *et al*., 2007). This can be observed in blends 8, 9 and 10, where blend 9, which shows 67% of coconut oil, has the lowest energy value of enthalpy, -24.3 J/g.

When coconut oil was blended with canola oil, this blend showed two endothermic peaks at different temperatures. The high-melting peak, which accounts for 79.0% in terms of energy consumption, is in the range of 3.4 to 23.7 °C, while the other low-melting peak is in the range of -13.5 to -25.2 °C. Coconut oil showed a sharp and narrow crystallization exotherm with a crystallization onset at 8.4 °C associated with its high content of triacylglycerols with medium chain fatty acids (REYES-HERNANDEZ *et al*., 2007). In coconut oil, a major endothermic peak was observed at 24.2 °C.

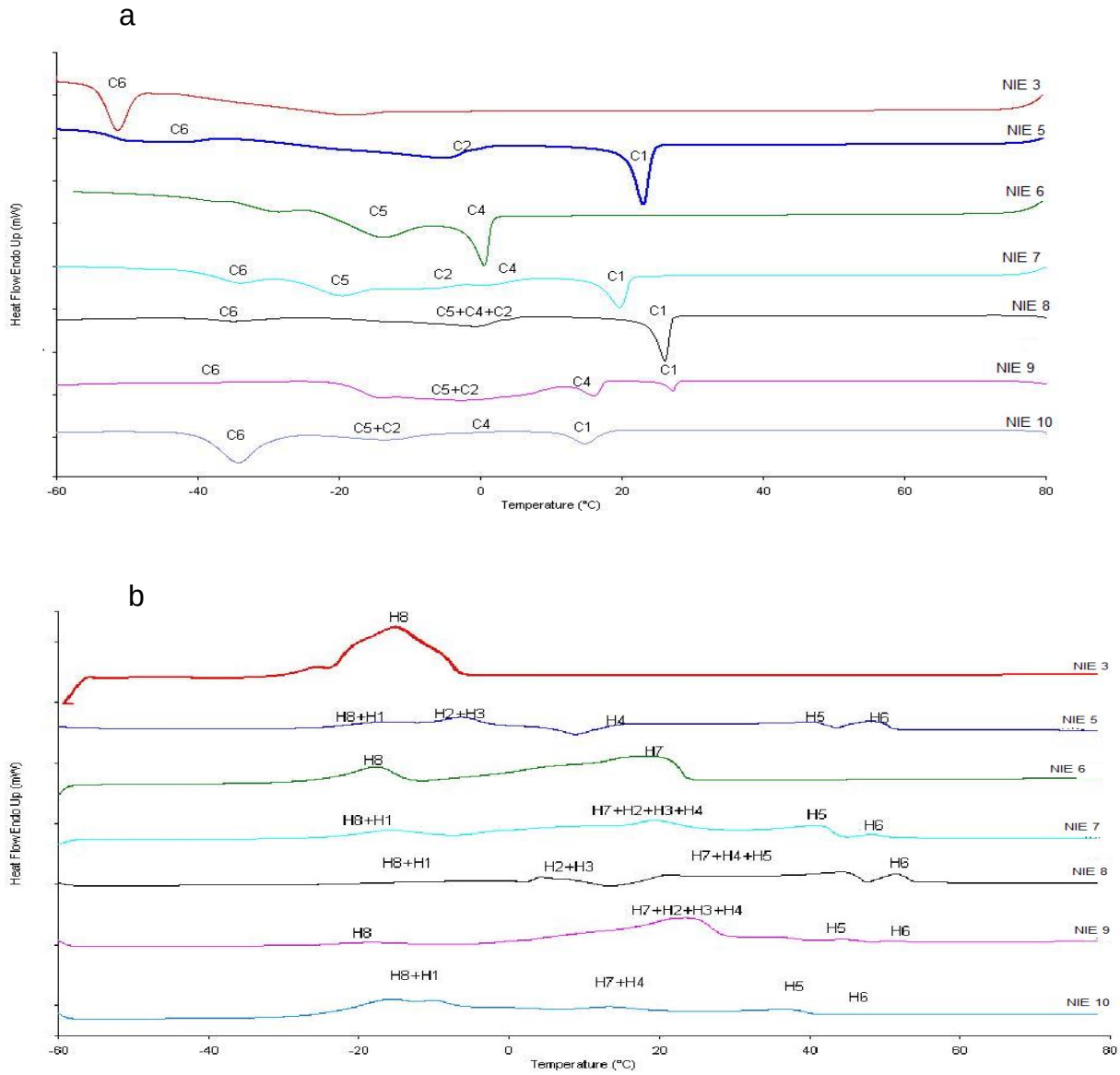

Figure 5.5. DSC curves of canola oil and its blends with palm stearin and coconut oil before chemical interesterification under dynamic atmosphere of He (a) crystallization obtained at 10 °C/min and (b) melting obtained at 5 °C/min.

According Zhang, Smith and Adler-Nissen (2004) coconut oil shows only form II (β-prime) crystals. After chemical interesterification, crystallization behavior of coconut oil was altered because of a change of composition. Overall, exothermic peaks were still mainly divided into two parts. However, with increasing conversion degree, the broadening effects became smaller than that of the original blends because of increasingly homogenized triacylglycerols distribution of different chain lengths. The second peak with a slight shoulder

at the beginning from -4.7 °C became one peak at 1.2 °C. The temperature difference of the two peaks decreased from 13.0 to 3.2 °C.

The thermal profiles of coconut oil showed a single endothermic melting peak in the range of 9.4 - 23.6 °C, showing the homogeneous nature of the triacylglycerols, which accounts for more than 80% of the triacylglycerols in coconut oil. After chemical interesterification, this peak became broader and moved toward a lower temperature.

Canola oil showed a major exothermic event, sharp and tall, with peak at -50.0 °C and one endothermic peak at -16.0 °C.

Similar cooling and heating profiles were reported in literature for canola oil, with the major event being related to the crystallization and melting of UUU triacylglycerols, especially triolein (TAN, CHE MAN, 2000; TAN, CHE MAN 2002).

In the canola oil blends, crystallization peak C_6 was moved towards high temperatures when the proportion of palm stearin and coconut oil increased. Similar profiles were observed by Adhikari *et al*. (2010) in the interesterified fats of palm stearin, rice bran oil and coconut oil, where crystallization peaks were moved towards high temperatures when the molar ratio of palm stearin increased.

Adding canola oil in the blends resulted in an increasing endothermic peak (H_8) and a shift to lower temperatures of the palm stearin (H_6) and coconut oil (H_7) main peaks temperatures.

Crystallization curves of canola oil after chemical interesterification showed three peaks. The first peak was at a low temperature, between approximately -60 and -30 °C. This peak was attributed to the liquid unsaturated triacylglycerols as dioleoyl-palmitoyl-glycerol, palmitoyl-oleoyl-linoleoyl-glycerol, and triolein (SAADI *et al.*, 2012).

According to the crystallization behavior of the interesterified blends with palm stearin, coconut oil and canola oil, peaks became broader and moved toward a lower temperature.

The major crystallization peaks were linked to the primary crystal formed due to the accumulation of highly saturated triacylglycerols as dipalmitoyl-oleoyl-glycerol, tripalmitin, dipalmitoyl-stearoyl-glycerol, and dipalmitoyl-linoleoyl-glycerol, while the small shoulder peaks were attributed to the content of unsaturated TAG as dioleoyl-palmitoyl-glycerol, triolein, and palmitoyl-oleoyl-linoleoyl-glycerol (TAN; CHE MAN, 2002).

Canola oil showed crystallization total enthalpy value of -99.8 J/g and melting total enthalpy value of 140.9 J/g. Melting onset temperature varied slightly with canola oil content in the blends.

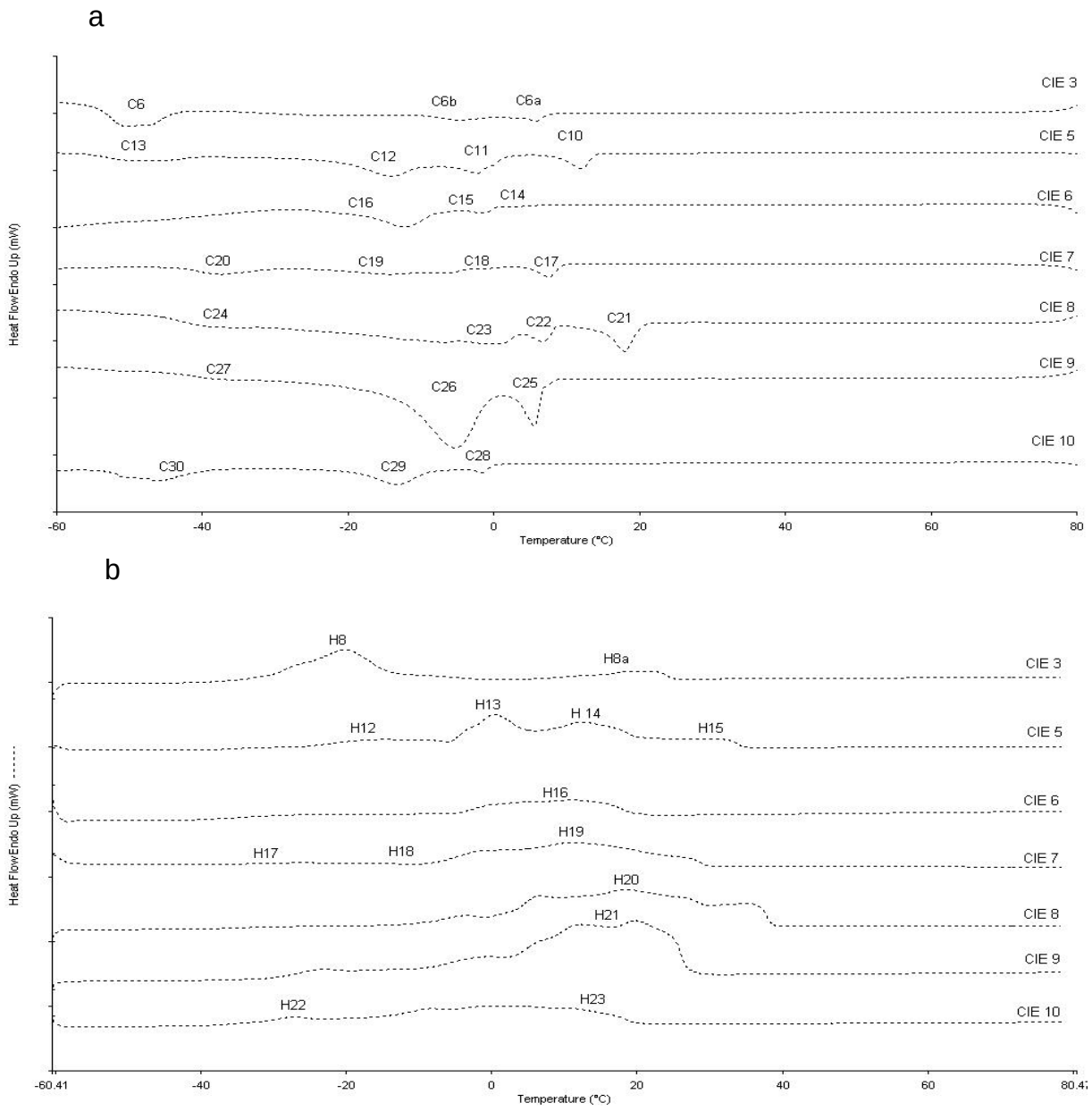

Figure 5.6. DSC curves of canola oil and its blends with palm stearin and coconut oil after chemical interesterification under dynamic atmosphere of He (a) crystallization obtained at 10 °C/min and (b) melting obtained at 5 °C/min.

Chemical interesterification of canola oil resulted in the emergence of two endothermic peaks in different temperature ranges. The low-melting peak was observed in the range of -31.6 to -12.9 °C. The high-melting peak of canola oil was observed at 12.1 to 24.8°C. The low and high-melting peaks of the interesterified canola oil were observed in the range of 18.7°C and 12.7 °C, respectively.

After chemical interesterification, blends with palm stearin, coconut oil and canola oil shifted the peak melting point of the triacylglycerols towards a lower temperature range from -32.5 to 49.8°C.The shift of the melting point towards lower temperatures after chemical interesterification may be due to the decrease in the disaturated and trisaturated triacylglycerols. Similar results were found by Debnath, Raghavarao and Lokesh (2011).

Chemical interesterification introduced more low-melting triacylglycerols, and this broadened the low-T peak and reduced the crystallization and melting temperature and total ΔH of the blends. This may be due to the decrease in trisaturated triacylglycerols in the interesterified blends as compared to their non-interesterified counterparts.

5.3.4 THERMOGRAVIMETRIC ANALYSES

Oxidative rancidity of edible oils and fats is of great practical importance, resulting in the development of unpleasant tastes and odors. To estimate the stability of oils and fats, the sample is usually subjected to an accelerated oxidation test under standardized conditions where heating is the most common means of accelerating the oxidation (SIMON, KOLMAN; 2001).

Polyunsaturated fatty acids are important constituent of human diet. Unsaturated lipids are considerably more sensitive to oxidation than saturated fatty acids and during the last thirty years the studies of stability of fats and fat containing foods have gained great attention (MUSIALI, LITWINENKO, 2007).

TG has been used by Hassel (1976) as an alternative method for measuring the stability of oil, thermo-oxidative behavior, activation energy, estimating the resistance of vegetable oil oxidation, action of antioxidants in oil thermal stability and the initial and final oxidation temperature.

Figures 5.7- 5.8 a and b show the decomposition of palm stearin, coconut oil, canola oil and their blends before chemical interesterification in a dynamic atmosphere of air (100 mL/min), heating at 10 °C/min.

The higher is the onset temperature of decomposition of edible oil, the higher is its thermal stability. This property is of great practical importance, especially when frying foods, to avoid the deterioration of the oils. On the other hand, their calorific power, which can be estimated from the heat of combustion, allows an evaluation of the corresponding calorific value during their metabolism in the human body (DWECK; SAMPAIO, 2004).

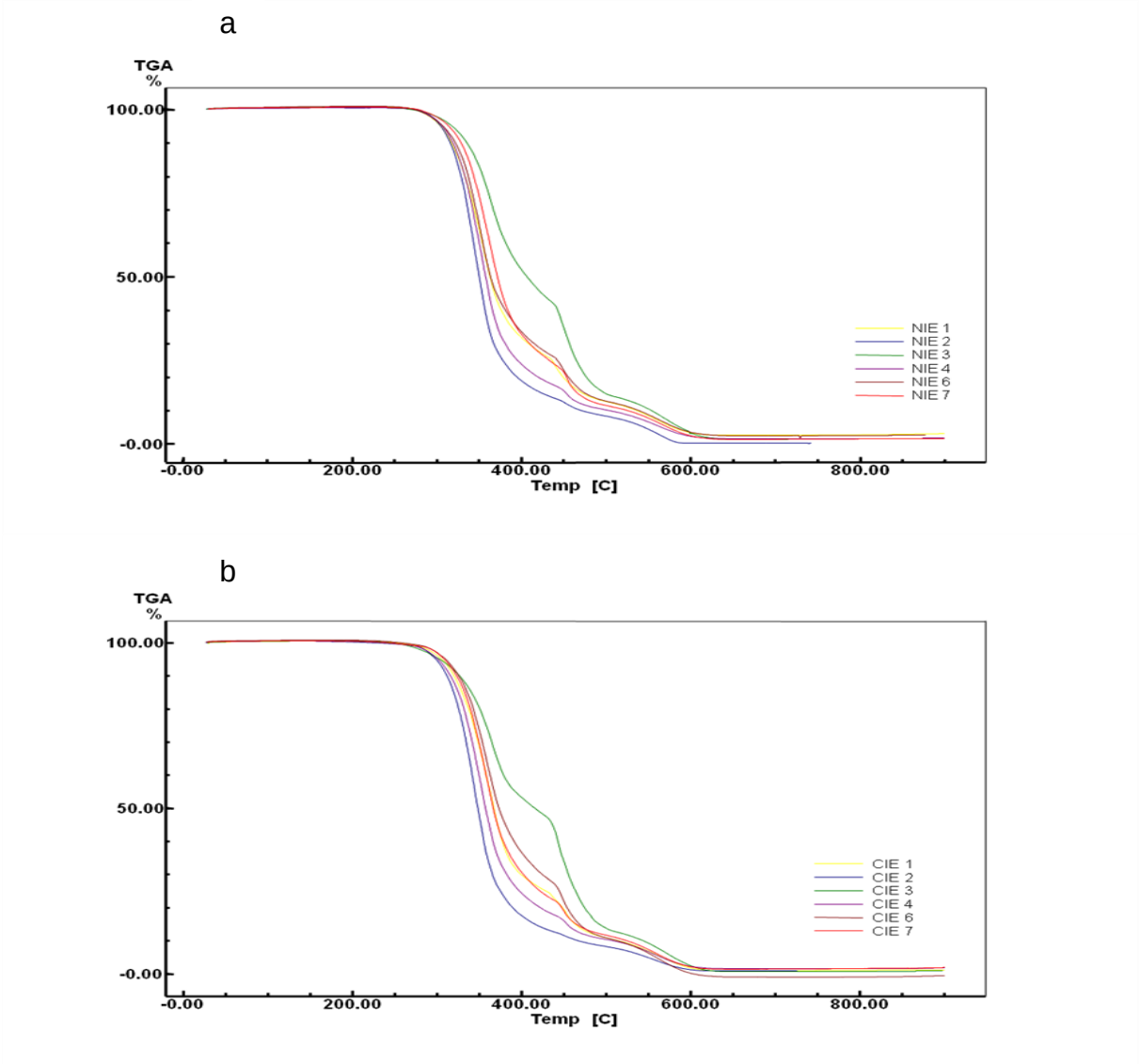

Figure 5.7. Curve decomposition of palm stearin, coconut oil and canola oil and blends (a) before chemical interesterification (b) after chemical interesterification in a dynamic atmosphere of air (100 mL / min), β 10 °C/min.

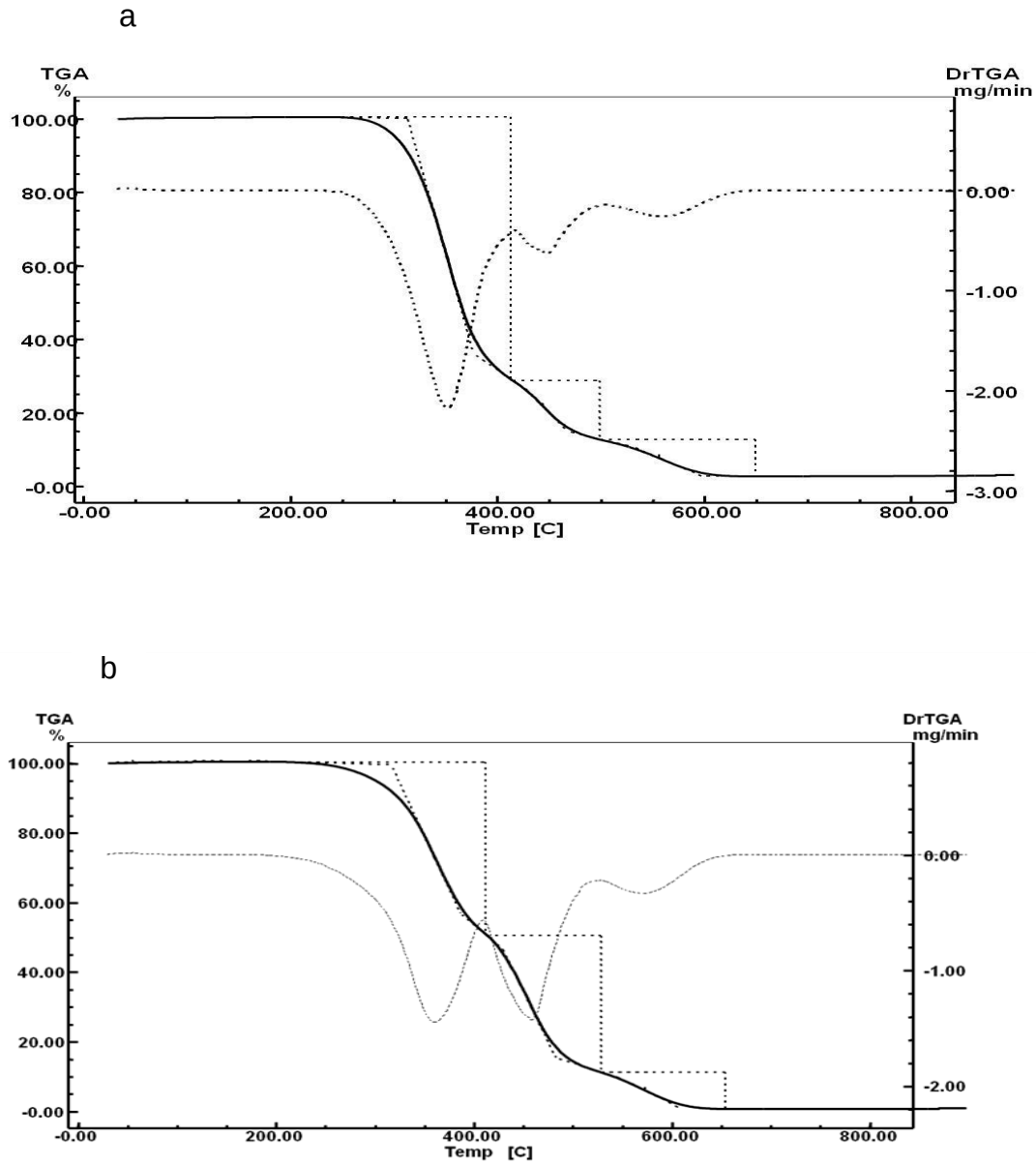

Figure 5.8. Curve decomposition of palm stearin (a) and canola (b) before chemical interesterification in a dynamic atmosphere of air (100 mL / min), β 10 °C/min.

The thermogravimetric curves of the palm stearin, coconut oil and their blends before and after chemical interesterification showed similar profiles presenting three mass loss steps. All steps were assigned to the combustion of organic compounds, as showed by the exothermic peaks in the DTA curves (Figure 5.8a).

The first step of 313.4 to 377.0 °C (Δm1 = 71.4%), the second step between 429.4 to 468.2 °C (Δm2 =15.9%) and the third step between 531.3 to 598.4 °C (Δm3 =10.1%).The

beginning of the oxidation of canola oil is characterized by the absorption of oxygen by chain fatty acids; oxidation products formed later called peroxides (Figure 5.8b).

This behavior is identified by an increase in the initial mass of the sample. It occurred between 164.2 to 198.1 °C with mass gain of 0.21%. Canola oil before chemical interesterification the second step of 316.7 to 386.9 °C (Δm1 = 49.5%), the third step between 429.5 to 482.6 °C (Δm2 =39.3%) and the four step between 545.7 to 600.9 °C (Δm3 =10.5%).

This different between oxidation temperature of palm stearin, coconut oil and canola oil was dependent of the fatty acid composition of the oils. Palm stearin and coconut oil are higher in saturated fatty acids, like lauric and palmitic acids, and canola oil is higher in unsaturated fatty acids, like oleic, linoleic and linolenic acids (Table 2.3 a anb b).

5.4. CONCLUSION

Chemical interesterification of palm stearin, coconut oil and canola oil and their blends causes a more balanced rearrangement of triacylglycerols. A narrower melting and crystallization temperature ranges were obtained mainly due to decreased of S_3 and U_3 triacylglycerols and increased SU_2 triacylglycerols after chemical interesterification.

A comprehensive understanding of the functions and properties of fats or fat bases produced by interesterification is essential for the outlining of their applications and the attainment of food products with desirable final attributes. This study made possible to verify that triacylglycerol composition and thermal behavior of palm stearin, coconut oil and canola oil and their blends were significantly altered by the chemical interesterification process.

The thermal stability and oxidation temperature of palm stearin, coconut oil and canola oil and their blends were dependent on fatty acid composition and independent on chemical interesterification.

BATCH AND CONTINUOUS LIPASE-CATALYZED INTERESTERIFICATION OF BLENDS CONTAINING OLIVE OIL FOR *TRANS*-FREE MARGARINES

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RUNNING TITLE:

Batch and continuous lipase-catalyzed interesterification

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SUMMARY

The interesterification of natural fats can improve certain physical and nutraceutical properties by modification of their acylglycerol profile. The aim of this study was the production of structured lipids by lipase-catalyzed interesterification of blends of palm stearin, palm kernel oil and olive oil, to be incorporated in margarines.

Interesterification activity was evaluated by the decrease of the solid fat content at 35°C ($SFC_{35°C}$) of the blends. The best interesterification conditions, found by response surface methodology (RSM), catalyzed by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", were 65 °C and a blend of 45 % (w/w) PS, 30 % (w/w) of PK and 25 % (w/w) of OO. Under these conditions, after 2 h of batch interesterification, the product had a $SFC_{35°C}$ of 3.0 %.

The reaction was implemented in a continuous packed-bed bioreactor, under optimized conditions, for 226 h ("*Lipozyme*® TL IM") or 188 h ("*Lipozyme*® RM IM"), at a residence time of 7 min. The inactivation profile of both biocatalysts followed the first-order deactivation model: half-lives of 88 h ("*Lipozyme*® TL IM") and 60 h ("*Lipozyme*® RM IM") were estimated, respectively. Free fatty acid (FFA) content of continuous interesterified blends (c.a. 1 %, w/w) was lower than that of batch-interesterified blends (c.a. 4-5 %, w/w).

PRACTICAL APPLICATIONS

The obtained structured lipids, with low $SFC_{35°C}$, are adequate for the production of *trans*-free table margarines. Using *sn*-1,3 specific lipases as catalysts, the original fatty acids at *sn*-2 position will be maintained with nutritional benefits. PS and PK are fats used as *trans*-free sources of solid fat, for the margarine industry, which are not very sensitive to oxidation. PK, a lauric fat, imparts a fast crystallization rate and confers plasticity to the final products.

The incorporation of OO will enrich the blends with natural antioxidants and oleic acid at *sn*-2 position in acylglycerols.

The development of response surface models, describing both final SFC values of interesterified blends and SFC decrease, will allow predicting results for novel proportions of fats and oils and/or a novel combination composition-temperature.

The implementation of the interesterification in continuous reactors promotes a faster reaction and lower acidity of interesterified blends, with benefits in terms of product separation and purification.

KEYWORDS: Batch interesterification, Continuous interesterification, Lipase, Modeling, Operational stability, Regiospecific distribution.

6.1. INTRODUCTION

The physical, nutritional and sensory properties of margarines are attained by appropriate processing of selected blends of fats and oils (HOUMÖLLER; KRISTENSEN; ROSAGER, 2007) Until the 1990s, hardstocks for margarines and shortenings were mainly obtained through hydrogenation of polyunsaturated oils (HOUMÖLLER; KRISTENSEN; ROSAGER, 2007; KARABULUT; TURAN, 2006; RASERA *et al*., 2012). Although this process is relatively inexpensive, it destroys essential polyunsaturated fatty acids (PUFAs) and creates non-natural isomers of saturated fats (KARABULUT; TURAN, 2006). Further, the partial hydrogenation of PUFAs results in the formation of undesirable *trans* fatty acids (HOUMÖLLER; KRISTENSEN; ROSAGER, 2007).

Natural fats and oils are not always ideal products for Food and Pharmaceutical Industries and the modification of their fatty acid composition and their regio-chemical and stereo-chemical structure can improve their properties and nutritional value (OTERO *et al*., 2006). However, it is possible to get the desired physical properties by blending different fats, which is a straight forward process, but it does not change the functionality of the fatty acids in the blend since they remain the same as in the individual oils (Reshma *et al*., 2008). The most appropriate modification is interesterification. The interesterification via enzyme or chemical catalysis can be used to modify the solid-to-liquid ratio of vegetable oils and solid fats, and produce various types of plastic fats free of *trans* fatty acids (AHMADI; WRIGH; MARANGONI, 2008).

Enzymatic interesterification based on the use of either *sn*-1,3 specific or nonspecific lipases are advantageous over chemical interesterification because of their enhanced selectivity (generation of fewer undesired byproducts compared to chemical interesterification), mild reaction conditions (temperatures lower than 100 °C), ease of product recovery when immobilized enzymes are used, reduced losses of oil/fat, fewer unit operations, lower requirements of investment capital, and decreased degradation of antioxidants such as tocopherols. In addition, when *sn*-1,3 lipases are used, the presence of unsaturated fatty acids at the original position *sn*-2 in acylglycerols of vegetable oils is maintained with nutritional benefits. The most important disadvantage of the enzymatic interesterification is the high cost of the biocatalyst. Nevertheless, this cost can be reduced if the biocatalyst can be reused (CRIADO *et al.*, 2007b).

Interesterification of blends of PS, PK and OO can produce healthy fats, free of *trans* fatty acids and with adequate physical properties, to incorporate in shortenings or margarines.

PS is relatively inexpensive, but due to its high content of saturated fatty acids (48.0-74.0 % of palmitic and 3.9-6.0 % of stearic acids) and high melting point (44–56 °C) it does not confer the required plasticity to the end-product (MING; GHAZALI; LET, 1999). PK has 40–50 % (w/w) of lauric acid, which provides for easy digestion and confers plasticity to the final product (NORIZZAH et al., 2004; SONNTAG, 1982). The health benefits of virgin OO have been attributed to its high content of the monounsaturated fatty acid, oleic acid, and high levels of natural antioxidants, namely tocopherols, providing health benefits (JANSEN; BIRCH, 2009; VISSERS *et al*., 2002). This oil is mainly produced in Mediterranean countries where it is traditionally used as dressing and as cooking oil. However, olive oil is not currently used in margarine formulations. Its incorporation in margarines will impart important functional properties to the final product, in addition to economical benefits for the olive oil extraction industry.

The blending of three different fats increases the heterogeneity of the TAGs. The crystallization of the fat phase in small needle-shape crystals (β prime), responsible for a smooth, pleasant mouthfeel and good spreadability of the margarine, is thus expected (NASCIMENTO et al., 2004).

Over the last decade, lipase-catalyzed interesterification processes have been developed for the production of margarines and shortenings (KARABULUT; TURAN, 2006; MU; XU; HOY, 1998; NASCIMENTO et al., 2004; OSÓRIO *et al*, 2001; OSÓRIO *et al*, 2005; OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006; OSÓRIO *et al*, 2009a; OSÓRIO *et al*, 2009b; RASERA *et al*., 2012; SERIBURI; AKOH, 199; XU *et al*., 1998) and some processes have already been implemented at industrial scale (HOLM; COWAN, 2009; OTERO *et al*., 2006; SCHORKEN; KEMPERS, 2009).

In the industry, the solid fat content (SFC) profile is used for the evaluation of the suitability of a fat blend for shortening or margarine formulation: the values of SFC at 10 ºC, 20 ºC and 35 ºC ($SFC_{10°C}$, $SFC_{20\ °C}$ and $SFC_{35°C}$) are important as related to the rheological behavior of fats at storage, packaging and utilization of margarines, respectively. The $SFC_{10°C}$ determines the hardness of the final product at refrigerator temperature. $SFC_{35°C}$ is particularly important in margarine manufacture, since it is related to the extent of melting in the mouth. In interesterified blends, this parameter must be as low as possible to prevent a sandy and coarse texture of the margarine (OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006).

Previous studies on lipase-catalyzed production of structured lipids have indicated that reaction time, substrate ratio, reaction temperature, enzyme load, and water content in the systems

are important factors in interesterification. The selection of parameters and their ranges for optimization depend not only on reaction systems but also on economical and practical factors (OSÓRIO *et al*, 2001). Usually, the longer the reaction time and the higher the enzyme loads, the higher will be the expected product yields. However, shorter reaction times and/or lower enzyme loads are preferred for economical and practical reasons. Higher temperature will increase the reaction rate according to the Arrhenius law. However, the half-lives of the enzyme will decrease with increasing temperatures. All of these factors, not only affect the yield of products, but also influence the content of byproducts. Therefore, compromises have to be made when one is choosing the ranges of parameters for optimization (OSÓRIO *et al*, 2009a).

The aim of this study was to evaluate the performance of the commercial immobilized *sn*-1,3 selective lipases from *Thermomyces lanuginosus* ("*Lipozyme*® TL IM") and *Rhizomucor miehei* ("*Lipozyme*® RM IM") as catalysts for the interesterification of blends of PS, PK and OO, to be incorporated in margarines, in solvent-free media, either batchwise or in continuous mode, under optimized reaction conditions previously established by response surface methodology (RSM).

6.2. MATERIALS AND METHODS

6.2.1. MATERIALS

PS and PK were supplied by FIMA/Unilever Jerónimo Martins, Lda., Santa Iria de Azóia, Portugal; a blend of virgin with refined olive oil from Gallo®, Portugal, with the commercial name of OO, was obtained from local market. The chemical properties of these fats are presented in Table 6.4. The immobilized thermostable lipases, from *Thermomyces lanuginosus* ("*Lipozyme*® TL IM") and *Rizomucor miehei* ("*Lipozyme*® RM IM") were kindly donated by *Novozymes A/S* (Bagsvaerd, Denmark). All chemicals used were either of analytical or chromatographic grades.

6.2.2. MODELING ENZYMATIC INTERESTERIFICATION EXPERIMENTS

The interesterification reactions were carried out in thermostated cylindrical glass reactors (100 mL) closed with rubber stoppers, under magnetic stirring. The biocatalyst was used with the original water activity (a_w = 0.12 for "*Lipozyme*® TL IM" and a_w = 0.43 for

"*Lipozyme*® RM IM", at 22 °C), measured in a Rotronic Hygroskop DT humidity sensor, DMS-100H, and its load was fixed at 5 % (w/w), according to previous experiments (OSÓRIO *et al*, 2001).

The best reaction conditions were established *via* RSM (HAALAND, 1989; MONTGOMER, 1991). For each biocatalyst, 18 experiments (Tables 6.1 and 6.2) were carried out following a central composite rotatable design (CCRD) as a function of reaction medium formulation and temperature (55–75 °C), for 2 h.

The reaction media consisted of blends of three fats (PS, PK and OO) in different proportions, according to the experimental design followed: PS (20-70 %, w/w), PK (1.5-43.5 %, w/w) and OO (7-58 %, w/w). PS and PK concentrations were dictated by the experimental design. The biocatalysts were used at their original water activity and its load was fixed at 5 % (w/w), according to previous experiments (OSÓRIO *et al*, 2001).

At the end of each experiment, 5 mL samples were taken and the enzyme was separated from the medium by paper filtration (Whatman nº 4, fast flow rate, coarse porosity, particle retention: 20–25 μm) in an oven at approximately 80 °C. All samples were stored at -18 °C for subsequent analysis (Figure 10.2 Annex 1).

6.2.3. VALIDATION OF THE INTERESTERIFICATION MODEL

After the selection of the most adequate model, four experiments were carried out at different values for each factor, inside the technological space considered in the model (Table 6.3).

Table 6.3. Reaction conditions of experiments carried out to investigate the applicability of the polynomial models describing SFC $Final_{35°C}$ values of interesterified fat blends.

Experiment nº	PS (%, w/w)	PK (%, w/w)	OO (%, w/w)
1	45.0	30.0	25.0
2	66.0	17.0	17.0
3	16.0	67.0	17.0
4	16.0	17.0	67.0

PS concentration ranged from 16 to 66 % (w/w) and PK and OO concentrations varied from 17 to 67 % (w/w). The temperature was 65 °C and 5 % (w/w) of biocatalyst was used. The obtained experimental results were compared to the theoretical values predicted by the model.

Table 6.1. Central composite rotatable design (CCRD) followed in the experiments as a function of PS (% w/w), PK (% w/w) and T (°C), respective values of $SFC_{10°C}$, and $SFC_{35°C}$ of fat blends, before (initial) and after (final) enzymatic interesterification catalyzed by "*Lipozyme*® TL IM" and corresponding percentage of SFC decrease ($\Delta SFC_{10°C}$; $\Delta SFC_{35°C}$).

Experiment	PS (%,	PK (%,	T	SFC	SFC	$\Delta SFC_{10°C}$	SFC	SFC Final$_{35}$	ΔSFC_{35}
1	35.0	10.0	60	29.3	20.6	29.8	7.9	0.0	100.0
2	35.0	10.0	70	29.3	22.6	22.8	7.9	0.0	100.0
3	35.0	35.0	60	43.7	35.8	18.1	7.9	0.0	100.0
4	35.0	35.0	70	43.7	34.9	20.1	7.9	0.0	100.0
5	65.0	10.0	60	55.2	51.8	6.1	19.5	6.1	68.6
6	65.0	10.0	70	55.2	53.7	2.6	19.5	8.1	58.6
7	65.0	35.0	60	69.6	66.4	4.7	19.8	8.6	56.5
8	65.0	35.0	70	69.6	63.5	8.7	19.8	6.7	66.0
9	20.0	22.5	65	22.3	12.5	44.1	3.7	0.0	100.0
10	70.0	22.5	65	65.1	63.6	2.8	22.0	10.7	51.3
11	45.0	1.5	65	36.0	35.1	2.5	11.7	0.2	98.5
12	45.0	43.5	65	59.4	30.8	48.2	11.5	1.7	85.1
13	45.0	22.5	57	43.8	40.4	7.9	11.7	2.3	80.5
14	45.0	22.5	73	43.8	41.3	5.9	11.7	0.8	92.8
15	45.0	22.5	65	43.8	40.6	7.4	11.7	0.9	92.5
16	45.0	22.5	65	43.8	40.0	8.7	11.7	0.7	93.7
17	45.0	22.5	65	43.8	41.3	5.8	11.7	0.9	92.6
18	45.0	22.5	65	43.8	40.2	8.2	11.7	0.7	93.6

Table 6.2. Central composite rotatable design (CCRD) followed in the experiments as a function of PS (% w/w), (PK (%, w/w) and T (°C), respective values of $SFC_{10°C}$, and $SFC_{35°C}$ of fat blends, before (initial) and after (final) interesterification catalyzed by "Lipozyme® RM IM" and corresponding percentage of SFC decrease ($\Delta SFC_{10°C}$; $\Delta SFC_{35°C}$).

Experiment nº	PS (%, w/w)	PK (%, w/w)	T (°C)	SFC Initial$_{10°C}$	SFC Final$_{10°C}$	ΔSFC$_{10°C}$	SFC Initial$_{35°C}$	SFC Final$_{35°C}$	ΔSFC$_{35°C}$
1	35.0	10.0	60	19.6	33.1	40.0	8.0	1.0	87.1
2	35.0	10.0	70	18.5	36.8	43.3	8.0	0.0	100.0
3	35.0	35.0	60	34.0	22.3	45.1	8.0	0.6	92.5
4	35.0	35.0	70	32.9	24.7	47.5	7.9	0.0	100.0
5	65.0	10.0	60	48.7	11.6	49.7	19.5	8.8	54.7
6	65.0	10.0	70	46.2	16.3	52.6	19.5	6.5	66.6
7	65.0	35.0	60	64.9	6.8	64.2	19.8	9.8	50.3
8	65.0	35.0	70	64.9	6.8	64.2	19.8	7.2	63.6
9	20.0	22.5	65	12.4	44.3	30.7	3.7	0.0	100.0
10	70.0	22.5	65	60.9	6.5	57.2	22.0	8.3	62.0
11	45.0	1.5	65	26.2	27.3	27.3	11.7	2.1	82.5
12	45.0	43.5	65	51.2	13.8	64.3	11.5	1.3	88.2
13	45.0	22.5	57	37.1	15.5	38.3	11.7	3.1	73.0
14	45.0	22.5	73	37.1	15.4	38.2	11.7	0.9	92.1
15	45.0	22.5	65	36.8	16.1	39.0	11.7	2.0	83.3
16	45.0	22.5	65	36.5	16.7	39.5	11.7	2.1	82.4
17	45.0	22.5	65	35.8	18.5	41.3	11.7	1.9	83.3
18	45.0	22.5	65	36.0	17.9	40.7	11.7	2.1	82.1

6.2.4. TIME-COURSE BATCH INTERESTERIFICATION REACTIONS

To investigate the maximum $SFC_{35\ °C}$ reduction that could be achieved by interesterification catalyzed by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", time-course batch experiments were carried out at 65 °C for 55 h for the fat blend of PS (45 %, w/w), PK (30 %, w/w) and OO (25 %, w/w). Interesterification reactions were performed as above described (Section 2.2.). During the time course of the reaction, 5 mL samples were taken, paper-filtered in an oven at approximately 80 °C to remove biocatalyst particles and stored at -18 ºC until analyses.

6.2.5. CONTINUOUS INTERESTERIFICATION EXPERIMENTS

A continuous packed-bed reactor consisting of a jacketed glass column (2 cm in internal diameter and 10 cm in height) was tested for the interesterification of blends of PS (45 %, w/w), PK (30 %, w/w) and OO (25 %, w/w). An amount of 10 g of immobilized lipase preparation was used. After its complete immersion in the substrate, a bed volume of 20.4 cm^3 of "*Lipozyme*® TL IM" and 29.8 cm^3 of "*Lipozyme*® RM IM" was achieved. Under these conditions, the biocatalyst load was about 45.1 % and 49.6 %, (w/v), respectively. The void fraction (ε) is a characteristic of the immobilized lipases. It is dependent on the packing method, particle size and form, support porosity and immobilization method (XU et al., 1998). A void fraction of 0.51 was assumed for "*Lipozyme*® TL IM" (XU et al., 1998) and 0.45 for "*Lipozyme*® RM IM" (XU; FOMUSO; AKOH, 2000). The bioreactor operated at a flow rate of 1.7 mL/min, which leads to a residence time of ca. 6 and 7 min, respectively.

The temperature in the reactor was maintained at 65 °C by circulating water in the jacket. The fat blend was continuously pumped upwards through the bioreactor column by a peristaltic pump, from a reservoir at 65 °C. To avoid solidification of the fat inside the silicone tubing, a coiled insulation strap with a thermostated electrical resistance was used (Figure 10.2 Annex 1).

6.2.6. LIPASE DEACTIVATION KINETICS

To describe the deactivation kinetics of the biocatalysts when used in continuous bioreactors, each experimental data point ($SFC_{35ºC}$ of the outlet fat stream at time *t*), was

converted to the fraction of the original activity, *i.e.* its residual activity. This residual activity, *a*, was defined as the ratio between the observed $SFC_{35°C}$ reduction at time *t* and the initial $SFC_{35°C}$ reduction. The latter was the reduction in the SFC value obtained 2 h after continuous reaction operation was started, *i.e.* when a "pseudo" steady-state had been reached (assumed as time 0).

The following first-order deactivation kinetics model was tested on the residual activity values:

$$a = Ae^{-k_d t} \qquad \textbf{Eq.6.1}$$

Where:

k_d = the deactivation rate constant (h^{-1}),
t = the operation time (h),
A = a constant.

The operational half-life time of the biocatalyst, *i.e.* the operation time needed to reduce its original activity to 50 %, was estimated by the models fitted to the deactivation profiles.

The fit of the model to experimental data was carried out using "solver" add-in from Excel for Windows, version 8.0 SR2, by minimizing the residual sum-of-squares between the experimental data points and those estimated by the respective model, using the following options: Newton method; 100 iterations, precision of 1025; 5 % of tolerance and 0.001 convergence.

6.2.7 ANALYTICAL METHODS

6.2.7.1 FATTY ACID COMPOSITION

Methylation of PS, PK, OO and blends after reaction was performed according to ISO, method 5509 (2000). Analyses of the fatty acid methyl esters (FAMEs) were carried out in a Varian gas chromatograph (model 430 GC, Varian Chromatograph Systems, Walnut Creek, California, USA), equipped with a CP 8412 auto injector. The Galaxie software was

used for quantification and identification of peaks. Injections were performed in a 100 m fused silica capillary column (ID = 0.25 mm) coated with 0.2 µm of polyethylene glycol (SP-2560, Supelco, USA) using helium as carrier gas at isobaric pressure of 37 psi; linear velocity of 20 cm/s; make-up gas: helium at 29 mL/min at split ratio of 1:50; volume injected: 1.0 µL. The injector temperature was set at 250 ºC and the detector temperature was set at 280 ºC. The oven temperature was initially held at 140 ºC for 5 min, then programmed to 240 ºC at a rate of 4 ºC/min and held isothermally for 30 min. All samples were analyzed in triplicate and the reported values are the average of the three runs.

6.2.7.2 REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS

A proton-decoupled ^{13}C NMR was used to analyze the positional distribution of fatty acids on the triacylglycerol backbone. Lipid samples (250 mg) were dissolved in deuthered chloroform (0.5 mL) in 5 mm NMR tubes, and NMR spectra were recorded on a Bruker Advance DPX spectrometer operating at 300 MHz. The ^{13}C spectra of the lipid samples were acquired with a spectral width of 2332.090 Hz, pulse of 10.2 µs, and a relaxation delay of 30s. The determination of ^{13}C was performed at a frequency of 75.8 MHz with a multinuclear probe of 5 mm operating at 30 °C, using the method described by Vlahov (2005). All samples were analyzed in triplicate and the reported values are the average of three analyses.

6.2.7.3 SOLID FAT CONTENT ASSAY

The reaction was indirectly followed by the variation in the SFC at 10 and 35 °C ($SFC_{10°C}$ and $SFC_{35°C}$) of the blend, assayed by NMR in a pulsed NMR spectrophotometer (Minispec P-20i; IBM). For NMR analysis, samples were melted at 60 °C, maintained at this temperature for about 10 min, then kept at 0 °C for 60 min and finally maintained for 30 min at the test temperature (10 ºC or 35 ºC) prior to the SFC measurement, according to the AOCS official method Cd-16b-93 (2009g).

6.2.7.4 FREE FATTY ACIDS ASSAY

The free fatty acid (FFA) content of the fats was determined according to the method described in the AOCS (2009a). The FFA content was assayed by titration with a 0.1 N sodium hydroxide aqueous solution. Its percentage (g/100 g of oil) was calculated on the basis of the molecular weight of lauric, palmitic or oleic acid, for PK, PS and OO, respectively. The acidity index (AI), defined by the amount of potassium hydroxide (mg) needed to neutralize the FFA present in 1 g of fat, was also calculated for the characterization of raw materials because this index is independent from the fatty acid composition of an oil or a fat. However, the FFA content is easier to interpret than the AI.

$$\% \text{ Free fatty acids (as oleic acid)} = \frac{V \times M \times a}{W} \qquad \textbf{Eq. 6.2}$$

Where:

V = volume of sodium hydroxide (mL),

M = normality of sodium hydroxide

a = molecular weight to fatty acid

W = weight of sample (g).

Since the fatty acid composition varies in the fat blends (Tables 6.1 and 6.2), it is difficult to decide which acid will be used to express the FFA contents. Therefore, to calculate the amount of FFA in every fat blend, prior to and after batch interesterification, we used the following average molecular weights of the fatty acids calculated from the composition of each fat blend: 270.3 (experiments 1 and 2), 258.1 (experiments 3 and 4), 266.3 (experiments 5 and 6), 254.1 (experiments 7 and 8), 266.3 (experiment 9), 259.6 (experiment 10), 273.3 (experiment 11), 252.6 (experiment 12) and 262.9 (experiments 13 to 18).

For continuous experiments, the average molecular weight of 259.1 was used for FFA calculation.

6.2.7.5 OXIDATION PRODUCTS

Thermal oxidation of the fat was indirectly evaluated by UV absorbance at 232 nm (Abs_{232nm}: related to the presence of initial products of oxidation, *i.e.* conjugated

hydroperoxides) and at 270 nm (Abs_{270nm}: related with the presence of final oxidation products, *i.e.* FFA, aldehydes, ketones and with conjugated trienes formed along oil refining) of 1 % (w/v) of fat blend in *iso*-octane (ISO 3656:2002). The Abs_{270nm} was assumed as the highest value of the measurements at 268, 270 and 272 nm.

6.2.7.6 PEROXIDE VALUE

The peroxide value was determined in terms of milliequivalents of peroxide per kilogram of sample that oxidizes KI under the test conditions by following the method described in the ISO, official method ISO 3960:2007 (2007). All samples were analyzed in triplicate and the reported values are the average of the three analyses.

$$\text{Peroxide value} = \frac{(S - B) \times M \times 1000}{W} \qquad \textbf{Eq.6.3}$$

Where:

S = volume of titration of sample (mL),

B = volume of titration of blank (mL),

M = normality of the thiosulfate solution and

W = weight of sample (g).

6.2.8 STATISTICAL ANALYSIS

The obtained results of SFC values of the interesterified fat blends, the observed variations on these parameters, as well as the contents of FFA and the values of Abs_{232nm} and Abs_{270nm} of these blends, were analyzed using the software "Statistica®", version 10, from *Statsoft*, (Tulsa, USA, 2012).

The linear and quadratic effects of each of the three factors under study, as well as their linear interactions, on interesterification, hydrolysis and oxidation kinetics were calculated. The significance of each effect was evaluated by analysis of variance.

A surface, described by a first or a second order polynomial equation, was fitted to each set of experimental data points ($SFC_{10°C}$, $SFC_{35°C}$, FFA, Abs_{232nm}, and Abs_{270nm}). First and second order coefficients were generated by regression analysis. The goodness of fit of the models was evaluated by the determination (R^2) and adjusted R^2 (R^2_{adj}) coefficients. High

values of both R^2 and R^2_{adj} suggest a good fit of the model to the experimental data points. In practice, R^2 should be at least 0.95 or greater. Values above 0.95 are considered to be very good, since they indicate that the polynomial model explains more than 95 % of the variability of the experimental data (HAALAND, 1989).

6.3. RESULTS AND DISCUSSION

6.3.1 FATTY ACID COMPOSITION AND OTHER CHEMICAL CHARACTERISTICS

The physical and functional aspects of a margarine product are primarily dependent upon the characteristics of the major ingredient: the margarine oil (CHRYSAM, 2002). Fatty acid composition is the most important characteristics of margarines and shortenings. The fatty acid composition of PS, PK and OO used in this study, is shown in Table 6.4.

Table 6.4. Chemical characterization of raw-materials

Fatty acids	PS	PK	OO
$C_{8:0}$	0.0±0.0	3.0±0.3	0.0±0.0
$C_{10:0}$	0.0±0.0	2.9±0.1	0.0±0.0
$C_{12:0}$	0.0±0.0	47.1±0.9	0.0±0.0
$C_{14:0}$	0.8±0.1	15.4±0.2	0.0±0.0
$C_{16:0}$	60.1±0.5	9.8±0.3	10.4±0.1
$C_{18:0}$	6.7±0.3	2.1±0.1	3.2±0.2
$C_{18:1}$	27.1±0.0	17.5±0.6	81.4±0.2
$C_{18:2}$	5.3±0.2	2.1±0.2	4.6±0.0
$C_{18:3}$	0.0±0.0	0.0±0.0	0.3±0.0
TFA[b]	0.0±0.0	0.0±0.0	0.0±0.0
MCSFAs	0.0±0.1	53.1±1.2	0.0±0.0
LCSFAs	67.7±0.2	27.3±0.5	13.6±0.3
SFAs	67.7±0.2	80.4±0.7	13.6±0.3
USFAs	32.3±0.2	19.6±0.7	86.0±0.3
MUFAs	27.1±0.0	17.5±0.6	81.4±0.2
PUFAs	5.3±0.2	2.1±0.2	4.9±0.0
FFA	0.13±0.0	0.11±0.0	0.73±0.0
AI	0.29±0.0	0.31±0.0	1.46±0.0
PV	0.1±0.0	0.1±0.0	4.5±0.2
Abs_{232nm}	1.7±0.1	1.6±0.1	2.7±0.1
Abs_{270nm}	0.2±0.0	0.2±0.0	0.5±0.0

Fatty acid composition (g/100 g), FFA (g/100 of oil), TFA, *trans* fatty acids; MCSFA, medium-chain saturated fatty acids; LCSFA, long-chain saturated fatty acids; SFA, saturated fatty acids; USFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AI, acidity index (mg KOH/ g of oil); PV, Peroxide Value (mequiv of oxygen/kg of oil); Abs_{232nm}; Abs_{270nm}. Values are shown as mean ± SD of three replications.

PS contains palmitic (60.1 g/100g) and oleic acid (27.1 g/100g) as major fatty acids. PK contains lauric acid (47.1 g/100g) as major fatty acid, followed by oleic (17.5 g/100g), myristic (15.4 g/100g) and palmitic (9.8 g/100g) acids. OO mainly contains oleic acid (81.4 g/100g) as major fatty acid, followed by palmitic (10.4 g/100g), linoleic (4.6 g/100g) and stearic (3.2 g/100g) acids. The fatty acid compositions of the blends of fats (before interesterification and after enzymatic interesterification) were similar because during interesterification, only rearrangement of fatty acids in the TAG backbone occurs (data not shown).

The quality of the oil used in the enzymatic interesterification is essential. Fats and oils used in enzymatic interesterification must have low acidity, once the FFAs, as well as water and peroxide act as catalyst poison of stability of lipases (XU; 2000). The PV, FFA contents and the Abs_{232nm}, and Abs_{270nm} values of PS, PK and OO, shown in Table 16, meet the quality parameters described by the Codex standard (2004).

6.3.2 BATCH INTERESTERIFICATION EXPERIMENTS

6.3.2.1 MODIFICATION OF SFC BY LIPASE-CATALYZED INTERESTERIFICATION

In all the experiments of both CCRDs, the interesterification of the fat blends, catalyzed by "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM", promoted a decrease in the SFC values of these fats at 10 °C and 35 °C (Tables 6.1 and 6.2). The SFC variation range of the interesterified fat blends at different temperatures was similar for both CCRDs. Also, the maximum reduction levels of $SFC_{10°C}$ and $SFC_{35°C}$ attained by interesterification catalyzed by "*Lipozyme*® TL IM" were similar to those achieved when "*Lipozyme*® RM IM" was used as biocatalyst.

The SFC of the fat blend is an indicator of several characteristics of margarines, shortenings, and fat spreads, including their general appearance, ease of packaging, spread ability, oil exudation, and organoleptic properties (NOOR LIDA; SUNDRAM; IDRIS, 2007). So, the measurement of SFC (*i.e.* the crystallized fat) over a range of temperatures is used by the industry to evaluate the plastic temperature interval of fats and oils.

Table 6.5. Effects and respective p-levels of PS, PK concentrations and T used in CCRD, and respective linear interactions on values of SFC Final$_{10\,°C}$, $\Delta SFC_{10°C}$, SFC Final$_{35°C}$ and $\Delta SFC_{35°C}$ of fat blends after enzymatic interesterification catalyzed by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM".

	"*Lipozyme*® TL IM"				"*Lipozyme*® RM IM"			
Factor	**SFC Final$_{10°C}$**	**$\Delta SFC_{10°C}$**	**SFC Final$_{35°C}$**	**$\Delta SFC_{35°C}$**	**SFC Final$_{10°C}$**	**$\Delta SFC_{10°C}$**	**SFC Final$_{35°C}$**	**$\Delta SFC_{35°C}$**
Intercept	45,49 (0.00)	4.06 (0.43)	2.03 (0.00)	87.60 (0.00)	2.94 (0.00)	13.87 (0.00)	41.08 (0.00)	78.85 (0.00)
(1) PS (L)	30.25 (0.00)	-17.55 (0.0)	7.76 (0.00)	-36.76 (0.00)	29.24 (0.00)	-18.09 (0.00)	7.16 (0.00)	-31.53 (0.00)
PS (Q)	-1.06 (0.70)	10.64 (0.07)	2.97 (0.00)	-11.45 (0.00)	-0.03 (0.94)	6.68 (0.00)	1.97 (0.00)	-3.66 (0.12)
(2) PK (L)	6.70 (0.04)	-9.94 (0.10)	0.57 (0.13)	-4.06 (0.13)	15.71 (0.00)	-8.53 (0.00)	0.08 (0.80)	-0.66 (0.77)
PK (Q)	-4.64 (0.14)	11.89 (0.06)	-0.00 (0.96)	0.11 (0.96)	1.25 (0.06)	3.37 (0.02)	0.04 (0.90)	0.94 (0.68)
(3) T (L)	-0.17 (0.95)	0.95 (0.86)	-0.32 (0.27)	2.80 (0.27)	-0.71 (0.24)	1.54 (0.21)	-1.62 (0.00)	11.52 (0.00)
T (Q)	0.89 (0.76)	-1.13 (0.84)	0.42 (0.19)	-3.50 (0.19)	0.09 (0.87)	-0.25 (0.83)	0.28 (0.42)	-1.06 (0.65)
1L by 2L	1.30 (0.72)	0.67 (0.92)	0.18 (0.93)	-0.24 (0.93)	1.60 (0.05)	1.86 (0.23)	0.59 (0.18)	-3.58 (0.23)
1L by 3L	-0.54 (0.87)	1.31 (0.85)	0.13 (0.72)	-1.09 (0.72)	-0.26 (0.72)	0.02 (0.98)	-0.83 (0.07)	1.16 (0.68)
2L by 3L	-1.92 (0.69)	4.14 (0.57)	-0.95 (0.15)	4.85 (0.15)	0.66 (0.39)	-1.51 (0.34)	0.03 (0.93)	-1.04 (0.72)

L- linear effect; Q- quadratic effect; p-levels values between brackets

For the he $SFC_{10ºC}$, values ranging from 18–48 are required for the production of bakery margarines, varying from cream to pastry usage, according to margarine manufacturers (NASCIMENTO et al., 2004). The majority of interesterified blends (except blends from experiments 5 to 8 and 10, catalyzed by "*Lipozyme*® TL IM", and 5, 7, 8, 10 and 12, catalyzed by "*Lipozyme*® RM IM") had SFC less than 48 %.

Concerning $SFC_{35ºC}$, margarine fat should have SFC lower than 3.5 % at temperatures above 35 °C or should completely melt at temperatures below body temperature to eliminate waxy mouthfeel, which is one of the important textural drawbacks of margarine (KIM; LUMOR; AKOH, 2008). The majority of interesterified samples (except blends from experiments 5 to 8 and 10) in both CCDRs had $SFC_{35\ °C}$ below 3.5 %. However, interesterified fat blends with SFC values higher than these indicative values are not a technical problem for the margarine industry. By mixing interesterified blends, in different proportions with natural liquid oils and fats, it is possible to formulate a wide range of consumer table margarines and spreads, bakery margarine and even frying shortenings (MOUSTAFA, 1995).

The results obtained in the present work are in agreement with those obtained by several authors who have dealt with enzymatic interesterification of blends of two or three different fats and oils in solvent-free media (CHO; DEMAN; ALLEN, 1994; NASCIMENTO et al., 2004; OSÓRIO *et al*, 2001; OSÓRIO *et al*, 2005; OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006; OSÓRIO *et al*, 2009a; OSÓRIO *et al*, 2009b; RASERA *et al*., 2012; ZAINAL; YUSOFF, 1999; ZHANG *et al*., 200).

The results are also consistent with those reported for the chemical interesterification of fat blends (NOOR LIDA; SUNDRAM; IDRIS, 2007; Norizzah *et al*., 2004). However, an increase of SFC for temperatures lower than 15 ºC was observed upon either batch or continuous interesterification of PS and soybean oil, catalyzed by "*Lipozyme*® TL IM" (CHU *et al*., 2001; COSTALES-RODRÍGUEZ *et al*., 2004).

In order to investigate the role of operation conditions and fat blend formulation on the interesterification kinetics catalyzed by "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM", linear and quadratic effects of each factor and of their linear interactions on the final SFC values, as well as on the corresponding variations promoted by interesterification (SFC $Final_{10ºC}$, $\Delta SFC_{10ºC}$, SFC $Final_{35ºC}$ and $\Delta SFC_{35ºC}$) were calculated (Table 6.5).

All the variables considered in this study showed significant effects on $SFC_{10ºC}$ or $SFC_{35ºC}$ and, thus, on the interesterification reaction catalyzed by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM".

PS content showed linear effects on final SFC at 10 and 35 ºC and on ΔSFC at 10 and 35 °C of the interesterified blends with "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM". However, the effects are positive on SFC Final and negative on ΔSFC, indicating that the increase of PS content promotes the increase of SFC Final at 10 and 35 ºC and a decrease of ΔSFC, at both testing temperatures. Similarly, PK content showed linear and positive effects on final SFC at 10 and 35 ºC and negative effects on ΔSFC at 10 and 35 ºC.

The linear negative effects of the temperature on the SFC $Final_{10°C}$ of fat blends interesterified by "*Lipozyme*® RM IM" and on SFC $Final_{35°C}$ values of blends obtained with both biocatalysts cannot be neglected. The effect of temperature on interesterification kinetics showed to be more important for "*Lipozyme*® RM IM", with a linear significant negative effect on SFC $Final_{35°C}$ and positive effect on $\Delta SFC_{35°C}$, than to "*Lipozyme*® TL IM". In fact, both biocatalysts are adequate to work under the temperature range tested.

The linear interactions were not significant for final $SFC_{10°C}$ and $\Delta SFC_{10°C}$ of blends interesterified by "*Lipozyme*® TL IM". However, the interaction PK x T on SFC $Final_{35°C}$ (negative effect) and $\Delta SFC_{35°C}$ (positive effect) of blends obtained by interesterification catalyzed by this lipase cannot be neglected. When "*Lipozyme*® RM IM" was used, the interaction PS x PK on final $SFC_{10°C}$ (positive effect), SFC $Final_{35°C}$ (positive effect), $\Delta SFC_{10°C}$ (positive effect) and $\Delta SFC_{35°C}$ (negative effect), as well as the interaction of PK x T on SFC $Final_{10°C}$ (positive effect) and $\Delta SFC_{10°C}$ (negative effect) of interesterified blends, are important enough to be neglected.

Response surfaces, described by polynomial equations, were fitted to the experimental results to visualize the dependence of the responses on the significant variables and, therefore, to identify the optimal operation conditions. The SFC $Final_{10°C}$ and SFC $Final_{35°C}$ of the interesterified fat blends, catalyzed by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", can be well described by second-order models as a function of PS, PK and T (Table 6.6).

The significant effects ($p < 0.05$) and those having a confidence range smaller than the value of the effect, or smaller than the standard deviation (data not shown), were included in the model equations of these surfaces. The high values of R^2 and R^2_{adj} indicate a good fit of these models to the experimental data points, which is confirmed by the linear relationships obtained between the observed and predicted SFC values (data not shown).

Table 6.6. Model equations for the response surfaces fitted to the values of SFC Final$_{10° C}$ and SFC Final$_{35 °C}$ of fat blends after interesterification catalyzed by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", as function of PS, PK concentrations and T and respective R^2 and R^2_{adj}.

Systems	Model equations	R^2	R^2_{adj}
"*Lipozyme*® TL IM"	SFC Final$_{10°C}$ = -18.85 + 1.02 (PS) + 0.925 (PK) - 0.015 (PK)^2 + 4.145 (PK x T)	0.93	0.92
	SFC Final$_{35°C}$ = 32.31 - 0.402 (PS) + 0.006 (PS)^2 + 0.517 (PK) -0.973 (T) + 0.008 (T)^2 - 0.007 (PK x T)	0.98	0.97
"*Lipozyme*® RM IM"	SFC Final$_{10°C}$ = -13.43 + 0.936 (PS) – 0.0006 (PS)^2 + 0.414 (PK) – 0.067 (T) + 0.0043 (PS x PK)	0.99	0.99
	SFC Final$_{35°C}$ = -5.72 + 0.171 (PS) + 0.004 (PS)^2 + 0.116 (PK) -0.005 (PS x T)	0.97	0.97

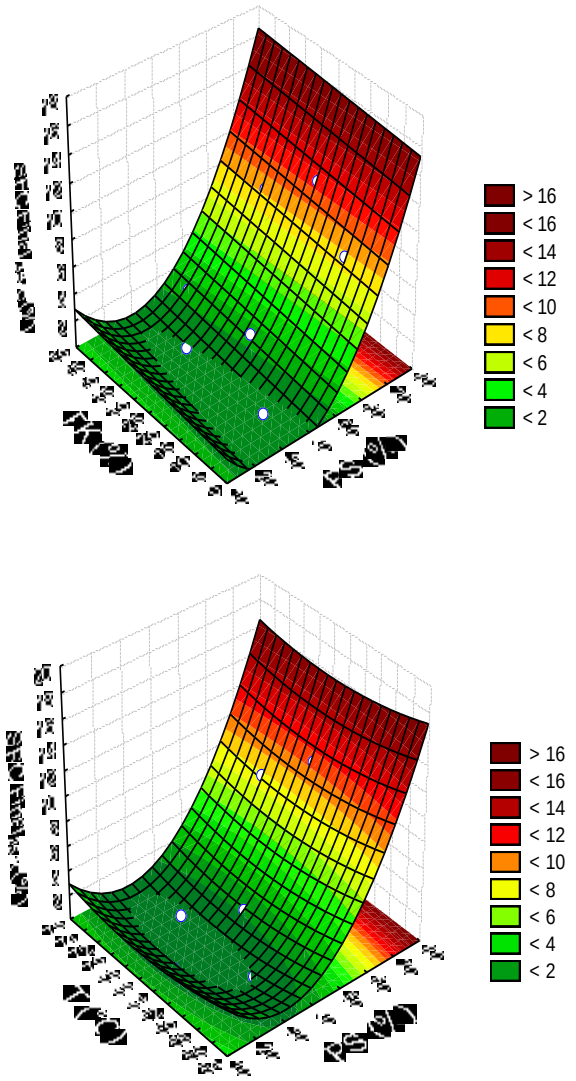


Figure 6.1. Response surface fitted to the SFC Final$_{35°C}$ values (%) of interesterified fat blends catalyzed by "*Lipozyme*® TL IM" as a function (a) of PS and PK concentrations and (b) of PS and T.

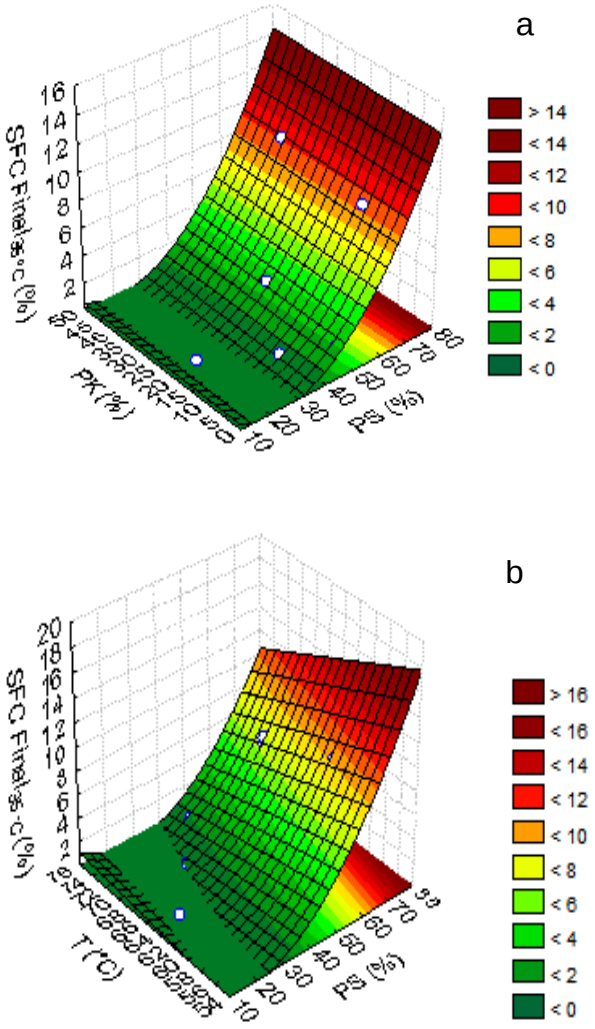


Figure 6.2. Response surface fitted to the SFC Final$_{35°C}$ values (%) of interesterified fat blends catalyzed by "*Lipozyme*® RM IM" as a function of (a) PS and PK concentrations and (b) PS and T.

The 4-dimensional surfaces obtained for SFC $Final_{35°C}$ (as a function of PS, PK and T) are illustrated by sets of two 3-dimensional surfaces, where only 2 of the 3 initial factors vary. The remaining factor is kept constant and equal to the value assumed in the central point. SFC $Final_{35°C}$ of the interesterified fats can be well fitted to a second-order model (Table 6.6), representing concave surfaces (Figures 6.1 and 6.2).

By visualization of these concave surfaces, it is possible to identify the best reaction conditions to obtain fat blends with low SFC at 35ºC. When "*Lipozyme*® TL IM" was replaced by "*Lipozyme*® RM IM", the SFC $Final_{35°C}$ surfaces obtained were not very different (Figures 6.1 and 6.2). According to these surfaces, blends with low SFC $Final_{35°C}$ values are obtained by interesterification catalyzed by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", when low PK and PS concentrations are used. With "*Lipozyme*® TL IM", slightly lower SFC $Final_{35°C}$ values are observed at the temperatures tested. This is an indication that the reaction proceeds at faster rate with this enzyme than with "*Lipozyme*® RM IM". However, the temperature had not a very pronounced effect on final SFC at 35 ºC. In fact, a comparison between the SFC $Final_{35°C}$ values obtained for blends, upon interesterification with "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", strongly suggests that similar SFC $Final_{35°C}$ results could be reached under similar reaction conditions and blend formulation. This may be due to the fact that both lipases are *sn*-1,3-selective.

The selected blend formulation to obtain interesterified fat blends with low SFC at 35ºC was 45 % (w/w) of PS, 30 % (w/w) of PK and 25 % (w/w) of OO. In order to avoid thermal inactivation of the biocatalysts, the temperature of 65ºC was selected for further experiments.

Response surfaces fitted to the SFC values are an invaluable tool to identify the most adequate conditions to obtain interesterified fat blends with the required properties. Through the models describing these surfaces, it is possible to predict the final SFC values, as well as the SFC decrease, of a given formulation interesterified under a set of fixed conditions.

Our results are also consistent with those reported by Osório *et al.* (2001) and Nascimento *et al.* (2004) for the enzymatic interesterification of blends of PS, PK and concentrate of TAGs enriched with ω-3 PUFA catalyzed by commercial immobilized lipases.

Arifin *et al*. (2012) demonstrated that RSM was successfully applied to model and optimize the conditions used of "*Lipozyme*® RM IM" catalyzed esterification of medium and long chain TAGs to obtain hard fats with physical and chemical characteristics adequate to produced margarine.

6.3.1.2. VALIDATION OF THE SECOND-ORDER MODELS FOR SFC FINAL$_{35\,°C}$

To investigate the applicability of the second-order model describing SFC Final$_{35°C}$ (Table 6.6) of blends obtained by interesterification catalyzed either by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", four additional interesterification experiments were carried out under the conditions previously described (*c.f.* 2.3).

The obtained values (Table 6.7) were compared with the theoretical SFC Final$_{35°C}$ values predicted by the models and a linear relationship was obtained (R^2=0.991). ANOVA test showed that there were no significant (p>0.05) differences between the predicted and the experimental SFC Final$_{35°C}$ values of interesterified fat blends (Table 6.7).

Table 6.7. Experimental results and predicted values of validation of the second-order model for SFC Final$_{35°C}$.

Experiment	Experimental results "*Lipozyme*® TL IM"	Predicted values "*Lipozyme*® TL IM"	Experimental results "*Lipozyme*® RM IM"	Predicted values "*Lipozyme*® RM IM"
1	0.2±0.0^a	0.1±0.0^a	3.0±0.0^b	3.1±0.0^b
2	4.0±0.0^a	3.6±0.0^a	9.0±0.0^b	9.2±0.0^b
3	2.5±0.0^a	2.2±0.0^a	0.3±0.0^b	0.5±0.0^b
4	0.1±0.0^a	0.1±0.0^a	0.1±0.0^b	0.2±0.0^b

Values are shown as means ± SD of three replications. Means (n = 3) with different letters in the same line are significantly different (p < 0.05).

Experiment 1 corresponds to the optimal reaction conditions while the other experiments were run under experimental conditions in the border zone of the technological space considered in the CCRD. Even though, only slight deviations between the theoretical and the experimental results were observed (Table 6.7).

Therefore, both models were adequate to predict SFC Final$_{35°C}$ of fat blends of PS, PK and OO, after interesterification catalyzed by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM". Our results are similar to those reported by Osório *et al*. (2001).

6.3.1.3. PRODUCTION OF FREE FATTY ACIDS AND OXIDATION PRODUCTS

The presence of FFA and partial acylglycerols may result from the hydrolysis reaction, which is promoted in high water activity (a_w) environments. Also, the FFA may be formed

during the first step of lipase-catalyzed interesterification in which fatty acids are released from acylglycerols to the reaction medium (OSÓRIO *et al*., 2001).

The initial content of FFAs in fat blends varied from 0.2 to 0.3 g/100 g of fat (Table 6.8). However, in all experiments a rise in FFA was observed upon interesterification (5.0 % and 4.4 % in average with "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", respectively), in spite of the low initial a_w value of the biocatalyst ("*Lipozyme*® TL IM", a_w = 0.12, and "*Lipozyme*® RM IM", a_w = 0.43, at 22 °C) (Table 6.8).

Values of FFA from 1.9–3.9 % were reported when "Novozyme® 435", at an initial a_w of 0.1, was used in the interesterification of PS with soybean oil and a concentrate of TAG rich in omega-3 PUFA ("EPAX 2050 TG") (OSÓRIO *et al*., 2001). Contents of FFA between 2.0 % and 2.9 % in interesterified blends of PS with PK, catalyzed by "*Lipozyme*® TL IM", were attained when dried molecular sieves were added to the reaction medium to reduce the water content (ZAINAL; YUSOFF, 1999).

The formation of FFA is not desirable since a lower yield of interesterified products is obtained and the formation of off-flavors (rancidity) by oxidation of FFA may also occur. However, the production of low levels of monoacylglycerols (MAG) and diacylglycerols (DAG) can be beneficial for margarine production (ZAINAL; YUSOFF, 1999). In fact, about 0.3 % MAG is usually added as emulsifiers in oil/water emulsions and DAG can be used as stabilizers of β-polymorphic crystals in margarines (SIEW; NG, 1999).

Concerning $Abs_{232\ nm}$ and $Abs_{270\ nm}$ values, related with the presence of first and final oxidation products, no significant increase in the final values of the interesterified fat blends was observed. This indicates that under the interesterification reaction conditions followed, the thermal oxidation of the fats can be neglected (Table 6.8). Linear and quadratic effects of each factor and of their linear interactions on the hydrolysis (FFA) and oxidation reactions (Abs_{232nm} and Abs_{270nm}), were calculated for both CCRDs (data not shown). For both FFA and oxidation products in interesterified fats, no significant effects of the variables considered in the experimental designs (medium composition and temperature) were detected either when "*Lipozyme*® TL IM" or "*Lipozyme* RM IM" were used.

These results are similar to those obtained by Nascimento *et al.* (2004) and Osório *et al.* (2001) relatively to FFA production and to the first stage of lipid oxidation ($Abs_{232\ nm}$). These authors observed that the formation of FFA was dependent on the initial a_w of the biocatalyst but independent from the other factors (medium composition and temperature).

Table 6.8. Free fatty acid (FFA) content and $Abs_{232\ nm}$ and $Abs_{270\ nm}$ (related with initial and final oxidation products) of the reaction media prior and after interesterification with "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM".

	"*Lipozyme*® TL IM"						"*Lipozyme*® RM IM"					
	FFA		$Abs_{232\ nm}$ (%)		$Abs_{270\ nm}$ (%)		FFA		$Abs_{232\ nm}$ (%)		$Abs_{270\ nm}$ (%)	
Experiment	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
1	0.2	5.2	2.4	2.6	0.7	0.9	0.2	4.5	2.4	2.5	0.7	0.9
2	0.2	5.1	2.4	2.3	0.7	1.0	0.2	4.8	2.4	2.4	0.7	0.9
3	0.3	4.8	2.5	1.9	0.5	0.7	0.3	4.3	2.5	2.4	0.5	0.7
4	0.3	5.1	2.5	2.4	0.5	1.2	0.3	4.4	2.5	2.6	0.5	1.0
5	0.3	4.7	2.0	2.6	1.0	1.3	0.3	4.4	2.0	2.5	1.0	1.1
6	0.3	4.8	2.0	2.6	1.0	1.3	0.3	4.4	2.0	2.6	1.0	1.2
7	0.2	4.9	1.9	2.0	1.1	1.2	0.2	4.2	1.9	3.2	1.1	1.3
8	0.2	5.0	1.9	2.6	1.1	1.3	0.2	4,3	1.9	2.3	1.1	1.0
9	0.3	5.2	1.8	2.3	0.6	1.0	0.3	4.6	1.8	2.5	0.6	0.7
10	0.3	4.8	2.1	2.2	1.0	1.2	0.3	4.3	2.1	2.4	1.0	1.0
11	0.3	5.3	1.9	2.5	0.9	1.2	0.3	4.4	1.9	2.3	0.9	1.0
12	0.2	4.7	2.1	2.6	0.8	0.9	0.2	4.3	2.1	2.4	0.8	0.9
13	0.3	5.0	1.7	2.6	0.9	1.0	0.3	4.5	1.7	2.8	0.9	1.0
14	0.3	5.2	1.8	2.6	0.9	1.0	0.3	4.4	1.8	2.5	0.9	1.0
15	0.3	5.1	1.8	1. 4	1.0	1.0	0.3	4.5	1.8	2.4	1.0	1.0
16	0.3	5.0	1.8	2.2	1.0	1.0	0.3	4.6	1.8	2.4	1.0	1.0
17	0.3	5.0	1. 8	2.8	0.9	1.1	0.3	4.6	1. 8	2.4	0.9	1.1
18	0.3	4.9	1.8	2.6	0.9	1.1	0.3	4.6	1.8	2.4	0.9	1.0

6.3.1.4. TIME COURSE INTERESTERIFICATION EXPERIMENTS

Using the selected blend formulation from RSM (45 % w/w of PS, 30 % w/w of PK and 25 % w/w of OO) and a biocatalyst load of 5 %, w/w, 55-h batch interesterification reactions, performed with "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", were carried out (Figure 6.3).

Again, interesterification was indirectly followed by the reduction of SFC values at 35 °C. With both lipases, a similar profile of decrease of SFC $Final_{35°C}$ was observed (Figure 6.3a). After 2 h reaction-time, a decrease of 75 and 60 % was observed for SFC at 35ºC, respectively for "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM" systems. Quasi-equilibrium was attained after 4 hours of reaction, corresponding to 93 and 90 % SFC $Final_{35°C}$ reduction, respectively when "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM" were used under completely mixed conditions (Figure 6.3b).

These results are analogous to those previously obtained by lipase-catalyzed interesterification of similar fat blends in solvent-free media (OSÓRIO et al., 2001; NASCIMENTO et al., 2004; OSÓRIO et al., 2005; OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006; OSÓRIO et al., 2009; Costales-RODRÍGUEZ et al., 20090). The decrease in SFC $Final_{35°C}$ values was accompanied by the release of FFA into the reaction medium during interesterification (Figure 6.3c). For the same reaction time, higher FFA contents were observed in blends obtained with "*Lipozyme*® TL IM", in spite of its lower a_w (a_w = 0.12) when compared to that of the other counterpart (a_w= 0.43). Also, even after 4h reaction time, the amounts of FFA continue to increase in reaction media, up to 7.1 % with "*Lipozyme*® TL IM" and 6.8 % with "*Lipozyme*® RM IM".

Thus, long reaction times are not adequate since a considerable increase in FFA content of interesterified blends will be observed, without any changes in SFC values. Lipase-catalyzed interesterification involves hydrolysis of ester bonds in TAGs species, followed by re-esterification with a FFA. FFA may be produced via either interesterification or hydrolysis reactions of triacylglycerol species.

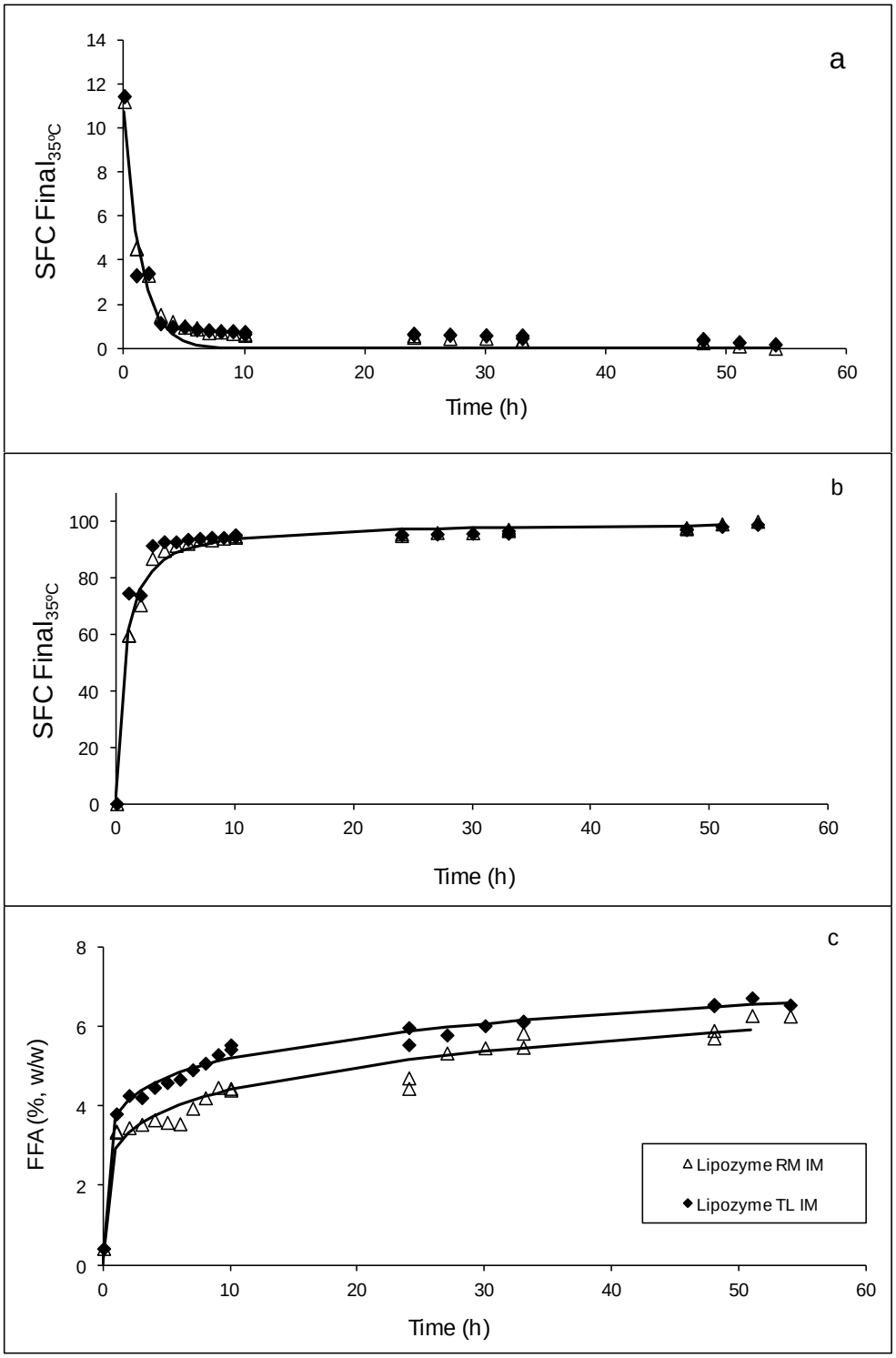


Figure 6.3. Time-course of batch interesterification of fat blends of PS (45 %, w/w), PK (30 %, w/w) and OO (25 %, w/w) , at 65 °C, catalyzed by (♦) "*Lipozyme*® TL IM" or (Δ) "*Lipozyme*® RM IM", followed by (a) the SFC at 35 °C (SFC Final$_{35°C}$), (b) the corresponding percentages of reduction (ΔSFC Final$_{35°C}$) and (c) the amount of FFA in the reaction medium.

6.3.2. CONTINUOUS INTERESTERIFICATION EXPERIMENTS

The interesterification was implemented in a continuous packed-bed bioreactor which operated continuously at 65 °C, for 226 h and 188 h, with "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM", respectively, for the interesterification of a blend of 45 % of PS (w/w), 30 % of PK (w/w) and 25 % of OO (w/w) with a SFC Initial$_{35°C}$ of 13 %.

During the operation of the bioreactors, the decrease of SFC at 10 and 35 °C, the presence of oxidation products and FFA were investigated. The evolution of the SFC values at 35 ºC and the FFA content of the outlet fat blends during the continuous operation of the reactor is shown in Figure 6.4 a and b. With both biocatalysts, a gradual increase in the SFC Final$_{10°C}$ (data not shown) and SFC Final$_{35°C}$ values of the outlet fat samples were observed, which indicates a progressive decrease in lipase interesterification activity along the operation.

Again, in continuous interesterification, the thermal oxidation of fats can be neglected, despite the considerable amounts of unsaturated fatty acids in the blends. It is worth to notice that the continuous reactors operated at residence times lower than 8 min. These results are similar to those obtained by (OSÓRIO et al., 2001; OSÓRIO et al., 2005; OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006; OSÓRIO et al., 2009).

For both blends, an initial value of about 3.8–4.9 % of FFA was observed in the interesterified blends during the first operation hour. A sharp decrease to about 1.0 % was observed during the subsequent 24 h of operation, after which the FFA content of the interesterified fat blends remained approximately constant in both bioreactors and below 1 % (Figure 6.4 b). These values were considerably lower than the amounts of FFA formed during batch interesterification, where the average values were 5.0 and 4.4 g/100g, when "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM" were used, respectively (Table 6.8).

The decrease in FFA content during continuous interesterification was also previously reported. The FFA content of interesterified fats using (i) "*Lipozyme*® TL IM" as biocatalyst, for the interesterification of blends of PS, PK and sunflower oil (blend A) and PS, PK and a concentrate of TAG rich in omega-3 PUFA (blend B), in the same continuous reactor, remained approximately constant at ca. 1 % (OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006) while (ii) a value of 0.8 % FFA was observed in interesterified blends of PS and soybean oil, catalyzed by "Novozyme 435", during the 21-day running period in a fluidized-bed reactor.

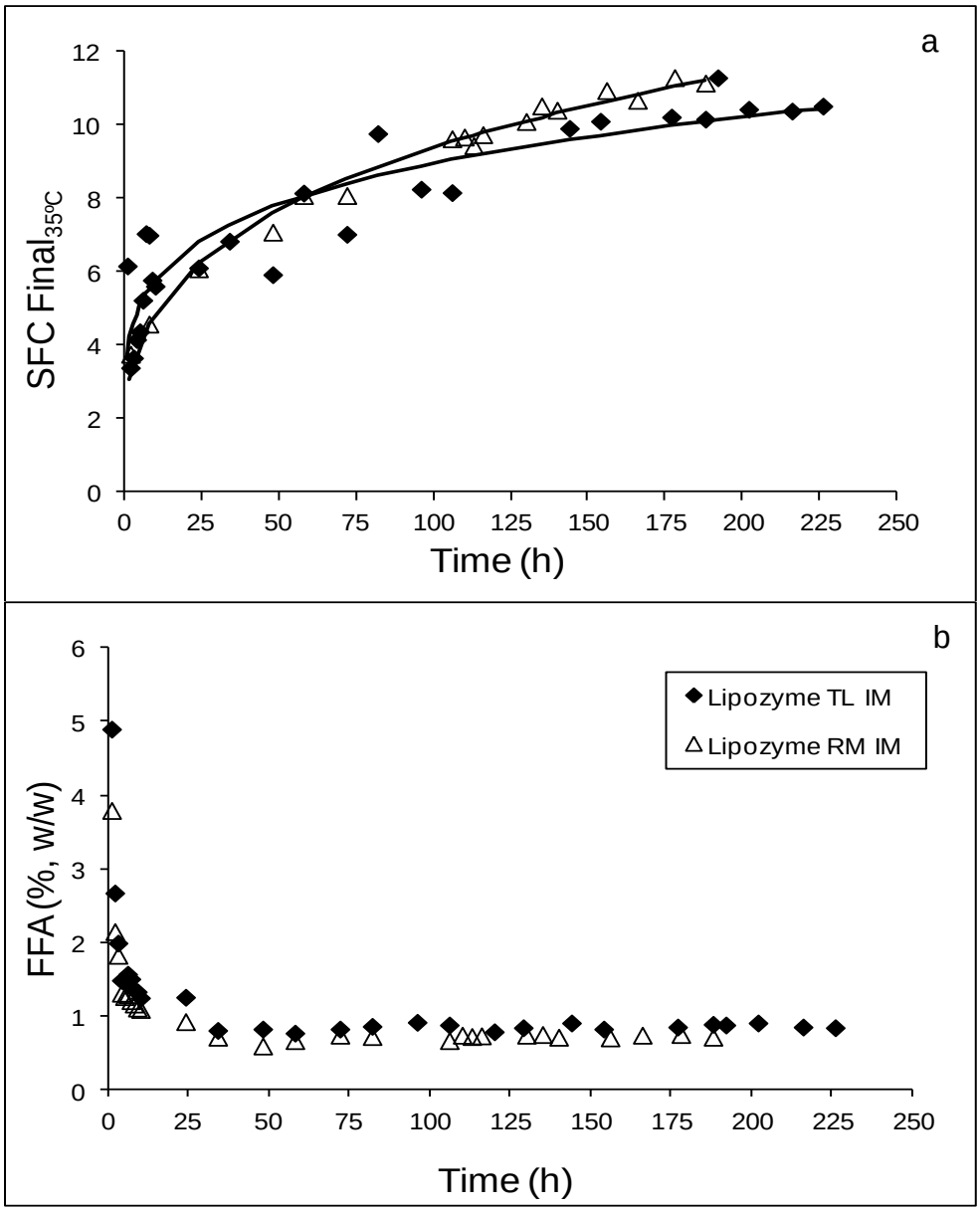


Figure 6.4. SFC Final$_{35°C}$ (a) and FFA (b) content of the fat blends of 45 % (w/w) PS, 30 % (w/w) PK and 25% (w/w) OO obtained by continuous interesterification at 65 °C, catalyzed by (♦) "*Lipozyme*® TL IM" or (Δ) "*Lipozyme*® RM IM" in a packed-bed reactor.

This value was lower than that of the fat obtained batchwise (2–6 %), (OSÓRIO et al., 2001). A similar behavior was observed when the immobilized lipase/acyltransferase from *Candida parapsilosis* was used in batch or in a fluidized-bed reactor as catalyst for the interesterification of blends of PS, PK and a concentrate of TAGs rich in omega-3 PUFA OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006; OSÓRIO et al., 2009).

A decrease in FFA content was also observed during the interesterification of fat blends, in solvent-free media, in continuous packed-bed column reactors, namely (i) after 2 days of interesterification of blends of hydrogenated canola oil, canola oil and PS, catalyzed by "*Lipozyme*® RM IM" (CHO; DEMAN; ALLEN, 1999); (ii) during the first day of interesterification between fish oil and medium-chain TAG, catalyzed by "*Lipozyme*® TL IM" (XU *et al*., 2002). Probably, the decrease in FFA content may be ascribed to the removal of water by the fat stream (XU *et al*., 2002).

Also, the presence of FFA in the interesterified fat blends may result mainly from the first step of lipase-catalysed interesterification mechanism (OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006). Thus, the observed decrease in catalytic activity of the lipase may be explained by a loss of its ability to catalyse the second step of interesterification.

6.3.3. LIPASE DEACTIVATION KINETICS

The experimental results presented in Figure 33 were converted to normalized data, *i.e.* to residual activity (*c.f.* 2.6.), to investigate which model of enzymatic inactivation kinetics fitted the inactivation of "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM".

For both lipases, a similar inactivation exponential profile was observed (Figure 6.5). The inactivation profile of the biocatalysts could be well described by a first-order deactivation model, given by Eqs. 6.4 and 6.5 when "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM" were used, respectively:

$$a = 84.74 \times e^{-0.006t} \qquad \textbf{Eq 6.4}$$

$$a = 91{,}22 \times e^{-0.01t} \qquad \textbf{Eq 6.5}$$

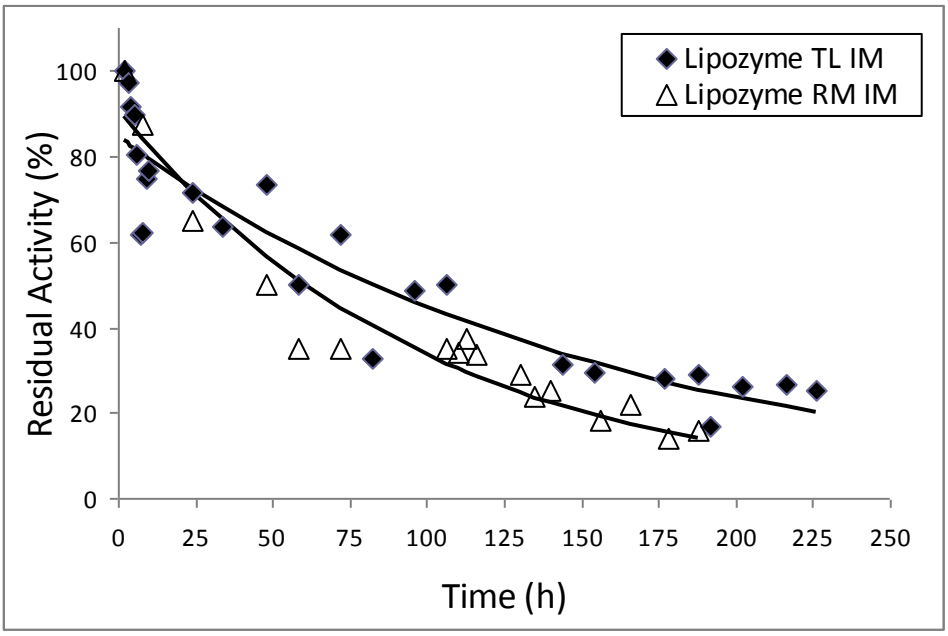


Figure 6.5 . Residual activity of (♦) "*Lipozyme*® TL IM" and (∆) "*Lipozyme*® RM IM" during the continuous interesterification of fat blends 45 % (w/w) PS, 30 %, (w/w) of PK and 25 % (w/w) OO, at 65 °C, in a packed-bed reactor.

Using these equations, half-lives of 88 h and 60 h were estimated for "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM", respectively. The operational stability of these biocatalysts is comparable to the values reported in literature for similar systems. "*Lipozyme*® TL IM" presented half-lives of 135 h and 77 h, when used in a packed-bed reactor for the interesterification of blends of PS, PK and sunflower oil or PS, PK and a concentrate of TAGs rich in omega-3 PUFA, respectively (OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006).

In the present study, a blend of refined and virgin olive oils was used. The lower activity presented by the biocatalysts may be ascribed to the presence of green pigments and oxidation products, coming from the virgin olive oil, with inhibitory effects on lipases (OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006; XU et al., 2002).

A lower operational stability was observed for the lipase/acyltransferase from *Candida parapsilosis* either in batch (half-life of 10 h) or in a continuous fluidized bed reactor (half-life of 9 h), in the interesterification of blends of PS, PK and a concentrate of TAGs rich in omega-3 PUFA (OSÓRIO *et al.*, 2009). Among other factors, the operational stability of a

biocatalyst depends on the bioreactor type, media composition and the operation mode used (OSÓRIO; da FONSECA; FERREIRA-DIAS, 2006).

6.3.4. FATTY ACID REGIOSPECIFIC DISTRIBUTION IN INTERESTERIFIED BLENDS

The analysis of the regiospecific distribution of fatty acids in TAG by NMR is desirable, since this method does not require hydrolysis of TAG by pancreatic lipase, with further separation of partial acylglycerols performed by thin layer chromatography and finally, the analysis of fatty acids as FAME by gas chromatography (GUNSTONE; HARWOOD, 2007; SILVA et al., 2011).

The fatty acid profile at the *sn*-1,3 and *sn*-2 positions of the interesterified blends of PS (45 %, w/w), PK (30 %, w/w) and OO (25 %, w/w), obtained along the continuous interesterification, catalyzed either by "*Lipozyme*® TL IM" or by "*Lipozyme*® RM IM", at 65 °C, are given in Table 6.9. Saturated fatty acids were found mainly at the *sn*-1,3 positions and unsaturated fatty acids were found mainly at the *sn*-2 position, which is the typical pattern for vegetable oils.

During the enzymatic continuous interesterification, the distribution of acyl residues at *sn*-2 position remained substantially unchanged. These results suggest that very little, if any, acyl migration occurred. Thus, the regiospecificity of both enzymes was maintained during the continuous reaction, although certain reduction in enzyme activity occurred.

Acyl migration is usually associated with such factors such as the water content of the lipase and the reaction medium, the chemical nature of the support material, reaction time and temperature, and the type of reactor used (PANDE; AKOH, 2013; XU et al., 1998).

The results obtained in the present study were similar to those reported by others (KIM et al., 2002; PANDE; AKOH, 2013; RØNNE *et al*., 2005, SILVA *et al*., 2013). After continuous enzymatic interesterification of PS with high stearate soybean oil, catalyzed by ''*Lipozyme*1 TL IM,'' changes in the composition of fatty acids in the sn-2 position were observed, which may be ascribed to acyl migration (PANDE; AKOH, 2013). Also, Kim *et al*. (2002) found that reaction conditions can induce acyl migration, when ''*Lipozyme*1 TL IM'' or ''*Lipozyme*1 RM IM'' were used.

Table 6.9. Regiospecific distribution of fatty acids at the *sn*-1,3 positions and *sn*-2 position of triacylglycerols of the blend palm stearin (45 %, w/w), palm kernel oil (30 %, w/w) and olive oil (25 %, w/w) obtained along the continuous interesterification catalyzed either by "*Lipozyme*® TL IM" and *"Lipozyme*® RM IM" at 65 °C.

	"*Lipozyme*® TL IM"			
	sn-1,3 positions		*sn*-2 position	
Time (h)	Saturated	Unsaturated	Saturated	Unsaturated
0	73.7±0.1	26.3±0.1	45.2±0.1	54.8±0.2
1	72.1±0.1	27.9±0.2	44.5±0.1	55.5±0.1
24	70.8±0.2	29.2±0.1	43.2±0.0	56.8±0.1
48	65.6±0.1	34.4±0.1	45.9±0.3	54.1±0.2
120	68.0±0.1	32.0±0.2	44.2±0.2	55.8±0.1
144	73.7±0.0	26.3±0.2	44.1±0.2	55.9±0.1
226	70.3±0.1	29.7±0.1	43.0±0.0	57.0±0.0
	"Lipozyme® RM IM"			
	sn-1,3 positions		*sn*-2 position	
Time (h)	Saturated	Unsaturated	Saturated	Unsaturated
0	73.4±0.1	26.6±0.1	47.0±0.1	53.0±0.2
1	73.2±0.2	28.8±0.1	47.1±0.2	52.9±0.2
24	68.7±0.3	31.3±0.1	46.5±0.3	53.5±0.2
72	73.7±0.5	26.7±0.1	45.2±0.3	54.8±0.1
120	71.6±0.0	28.4±0.1	48.7±0.2	51.3±0.2
188	71.6±0.0	28.4±0.1	48.4±0.0	51.6±0.2

Values are shown as means ± SD of three replications.

In continuous interesterification reactions, short residence times (high flow rates), together with the positional specificity of the lipases, lead to conditions under which minimal migration of acyl groups is expected (RØNNE *et al*., 2005, SILVA *et al*., 2013). Longer residence times give rise to larger changes in fatty acid composition at sn-2 position probably due to acyl migration (RØNNE *et al*., 2005).

6.4. CONCLUSIONS

In this study, the obtained structured lipids are adequate for the production of *trans*-free table margarines. Using *sn*-1,3 specific lipases as catalysts, the original fatty acids at *sn*-2 position will be maintained with nutritional benefits. The use of olive oil, rich in oleic acid and natural antioxidants, provides nutritional benefits.

RSM was successfully applied to model and optimize the conditions used in the interesterification of PS, PK and OO. The generated regression models could be used to predict the optimum conditions to apply in batch and continuous interesterification. Based on these models, blends with low SFC $Final_{35°C}$ are obtained by interesterification, catalyzed by "*Lipozyme*® TL IM" or "*Lipozyme*® RM IM", at 65.0 °C, and using a blend of PS (45 %, w/w), PK (30 %, w/w) and OO (25 %, w/w). Under these conditions, both biocatalysts were used in a continuous packed-bed bioreactor: a first-order deactivation model was observed and half-lives of 88 h and 60 h were estimated for "*Lipozyme*® TL IM" and "*Lipozyme*® RM IM", respectively. Along the continuous interesterification, the biocatalysts used maintained their *sn*-1,3 regioselectivity. Thus, as in the original fats, in the interesterified fat blends the unsaturated fatty acids are mainly located at position sn-2 of the TAG.

Also, the continuous interesterification has the advantages of (i) being faster than the batch mode and (ii) to produce interesterified blends with low acidity, with benefits in terms of product separation and purification.

INFLUENCE OF CONTINUOUS ENZYMATIC INTERESTERIFICATION ON PHYSICOCHEMICAL PROPERTIES OF PALM STEARIN, PALM KERNEL OIL AND OLIVE OIL BLEND

RUNNING TITLE: physicochemical properties

ABSTRACT

Blend of 45 % of palm stearin, 30 % of palm kernel oil and 25 % of olive oil was submitted to continuous enzymatic interesterification catalyzed by "*Lipozyme*® TL IM" or *"Lipozyme*® RM IM", at 65 °C for 10 days (*"Lipozyme*® TL IM") or 7 days (*"Lipozyme*® RM IM"), at a residence time of 7 min. The aim of this study was to evaluate the influence of continuous enzymatic interesterification on physico-chemical properties of palm stearin, palm kernel oil and olive oil blend. The effect of continuous enzymatic interesterification process was determined by comparing the fatty acids and triacylglycerols compositions, regiospecific distribution of fatty acids in triacylglycerols, consistency, solid fat content, thermal properties and crystallization behavior. Analysis of triacylglycerols (TAGs) of the interesterified blends showed a decrease in the concentration of high-melting TAGs and increase of TAGs having medium-chain fatty acids. The changes in the TAG profiles were reflected in the physico-chemical properties of the blend. The results indicated that it is possible to prepare a *trans*-free fat phase enriched in olive oil to produce margarines or other similar products.

PRACTICAL APPLICATIONS

Most natural oil and fat present limited application in their unaltered forms, which is imposed by their particular composition in fatty acids and triacylglycerols. Due to the increasing concern about the nutritional impact of *trans* fatty acids on health, continuous enzymatic interesterification has become the main method for the preparation of plastic fats. Dietary ingestion of olive oil has been reported to have physiological benefits such as lowering serum cholesterol levels, suppressing certain types of cancer, enhancing liver function, and reducing the effects of aging and heart disease. Preparation of semi-solid fats from enzyme-modified olive oil is an attractive alternative for the food industry, given the high oxidative stability.

KEYWORDS: Low *trans* fats, Triacylglycerol composition, Consistency, Solid fat content, Regiospecific distribution, Melting and crystallization behavior, Crystalline microstructure

7.1. INTRODUCTION

Structured lipids (SLs) are often referred to as a new generation of fats that can be considered as nutraceuticals: foods or parts of foods that provide medical or health benefits beyond basic nutrition, including the potential for prevention and/or treatment of certain diseases (AKOH, 2002). SLs are produced by chemical or enzymatic modification of triacylglycerols (TAGs) (WILLIS; MARANGONI, 1999). In the past few years, enzymatic modification of TAGs has gained preference over chemical modification (ALIM *et al*., 2008; FOMUSO; AKOH, 2002; HAMAN; SHAHIDI, 2005; LEE *et al.*, 2008; XU; FOMUSO; AKOH, 2002).

The use of lipases for the modification of fats and oils has many benefits when compared to chemical processes. Lipases are well known for their efficacy under mild reaction conditions (pH, T and P), leading to reduced costs and energy consumption. The use of lipases in a natural reaction system can also reduce environmental pollution by reducing production of side products, since these reactions mimic natural pathways. The availability of lipases from a wide range of biological sources offers a tremendous potential for a post-production modification in industrial application (YAMANE, 1987). However, the most important property of lipases that has led to their overwhelming interest remains their specificity. This property has been shown to be a versatile tool for the preparation of a wide variety of novel TAGs (GHAZALI; HAMIDAH; CHE MAN, 1995; MARANGONI; ROUSSEAU, 1995; WILLIS, LENCKI; MARANGONI, 1998).

Typical applications of SLs include (but are not limited to) margarines, modified fish oil products, cocoa butter, human milk fat replacer, and many other lipid products (HAMAN; SHAHIDI, 2005; JENSEN, 2001; NIELSEN *et al*., 2006).

Palm stearin have been used as solid substrates to produce semi-solid fats *via* interesterification with coconut oil, olive oil, sunflower oil, and palm kernel oil (CRIADO *et al.*, 2007a). Palm stearin, obtained by fractionation of palm oil after crystallization at a controlled temperature, can be used in formulation of margarines and shortenings as it provides strength and structure to the product (KHATOON; KHAN; JEYARANI, 2012). Palm kernel oil is rich in medium-chain fatty acids, which are transported mainly via the portal system and rapidly oxidised in the liver to provide instant energy. Because of its soft nature and higher content of lauric acid, palm kernel oil after blending with hard oils like palm stearin provides good lubricating action

during baking (AINI; MISKANDAR, 2007). It is also reported that the fats containing both medium- and long-chain fatty acids have fairly complex packing at the molecular level, which produce smaller crystals and are more suitable as plastic fats (FLOTER; van DUIJIN, 2006).

Several studies have investigated the influence of enzymatic interesterification on physicochemical properties of oils and fats or their blends (KHATOON; KHAN; JEYARANI, 2012; PANDE; AKOH, 2013; ZHAO *et al*., 2013). In particular, interesterification of blends of hard fats with liquid oils currently represents the most versatile option for the production of zero *trans* fats for many industrial purposes (KARABULUT *et al*., 2004; KHATOON; REDDY 2005). Systems that are composed of raw materials of widely varied compositions provide good heterogeneity with regard to triacylglycerol species and, consequently, can produce interesterified fats with substantially modified physicochemical properties. In this regard, olive oil stands out among vegetable oils mainly because of its nutritional qualities, given that it is characterized by low saturated fatty acid content and a high oleic acid content of over 80%, which has a neutral effect on the plasma levels of total cholesterol, hence being of particular interest for production of special fats (CRIADO *et al.*, 2007b).

A comprehensive understanding of the functions and properties of fats produced by interesterification is pre-eminent for outlining their applications and attaining food products with specific attributes, particularly when the replacement of partially hydrogenated fats is considered. The satisfactory performance of a given fat depends on important factors that determine its application. These include, first, stability during and after processing and total oil base compatibility with the product to which it is destined. Besides, functional characteristics such as plasticity and spreadability must be taken into account in the development of a new formulation. Therefore, the study of the application of interesterified fats in margarines, shortenings and other high fat products must be based mainly on the understanding of the relations among parameters such as triacylglycerol composition, melting point, solid profile and consistency (O'BRIEN, 2004).

The purpose of the present work was to produce zero-*trans* plastic fat by continuous enzymatic interesterification of palm stearin, palm kernel oil and olive oil by *"Lipozyme*® TL IM" and *"Lipozyme*® RM IM". The physicochemical properties (fatty acids profile, TAG composition, regiospecific distribution of fatty acids, solid fat content, consistency, thermal properties and microstructure) of the interesterified

product and its physical blend were evaluated to seek a desirable product for margarine production.

7.2. MATERIALS AND METHODS

7.2.1. CHEMICAL

Palm stearin and palm kernel oil were supplied by *FIMA/VG Produtos Alimentares*, Santa Iria de Azóia, Portugal; a blend of virgin with refined olive oil Galo® was obtained from local market. The fats were stored at 0 °C prior to use. The immobilized thermostable lipases, from *T. lanuginose* (*"Lipozyme*® TL IM") and ***R. miehei*** (*"Lipozyme*® RM IM") were kindly donated by *Novozymes A/S* (Bagsvaerd, Denmark). Fatty acid methyl ester (FAME) standards used for GC analysis were from Sigma–Aldrich. All chemicals used were either of analytical or chromatographical grades.

7.2.2. CONTINUOUS INTERESTERIFICATION EXPERIMENTS

A continuous packed-bed reactor consisting of a jacketed glass column (2 cm in internal diameter and 10 cm in height) was tested for the interesterification of blends of palm stearin (45 wt-%), palm kernel oil (30 wt-%) and olive oil (25 wt-%). An amount of 10 g of immobilized lipase preparation was used. After its complete immersion in the substrate, a bed volume of 20.4 cm^3 of *"Lipozyme*® TL IM" " and 29.8 cm^3 of *"Lipozyme*® RM IM" " was achieved. Under these conditions, the biocatalyst load was about 45.1% and 49.6 %, (wt/vol), respectively. The bioreactor operated at a flow rate of 1.7 mL/min, which leads to a residence time of ca. 6 and 7 min, respectively.

The temperature in the reactor was maintained at 65 °C by circulating water in the jacket. The fat blend was continuously pumped upwards through the bioreactor column by a peristaltic pump, from a reservoir at 65 °C. To avoid solidification of the fat inside the silicone tubing, a coiled insulation strap with a thermostated electrical resistance was used.

7.2.3. FREE FATTY ACIDS ASSAY

The free fatty acid content of the fats was determined according to the method described in the AOCS official method Ca 5a (AOCS, 2009a). All samples were analyzed in triplicate and the reported values are the average of the three analyses. Its percentage (wt/wt) was calculated on the basis of the molecular weight of oleic acid (MM= 282 g).

$$\% \text{ Free fatty acids (as oleic acid)} = \frac{V \times M \times 28.2}{W} \qquad \textbf{Eq. 7.1}$$

Where:

V = volume of sodium hydroxide (mL),
M = normality of sodium hydroxide
W = weight of sample (g).

7.2.4. PEROXIDE VALUE

The peroxide value was determined in terms of milliequivalents of peroxide per kilogram of sample that oxidizes KI under the test conditions by following the method described in the AOCS official method Cd 8b-90 (AOCS, 2009b). All samples were analyzed in triplicate and the reported values are the average of the three analyses. Results are expressed in mequiv oxygen/kg oil.

$$\text{Peroxide value} = \frac{(S - B) \times M \times 1000}{W} \qquad \textbf{Eq.7.2}$$

Where:

S = volume of titration of sample (mL),
B = volume of titration of blank (mL),
M = normality of the thiosulfate solution and
W = weight of sample (g).

7.2.5. OXIDATION PRODUCTS

Thermal oxidation of the fat was indirectly evaluated by UV absorbance at 232nm (Abs_{232nm}: related to the presence of initial products of oxidation, *i.e.* conjugated hydroperoxides) and at 268, 270 and 272nm (Abs_{268nm}, Abs_{270nm} and Abs_{272nm}: final oxidation products, *i.e.* FFA, aldehydes and ketones) of 1% (wt/vol) fat blend in *iso*-octane (ISO, 2002).

7.2.6. FATTY ACID COMPOSITION

Fatty acid composition was determined after conversion of fatty acids into their corresponding methyl esters (FAMES) by the method described by ISO method 5509 (2000). Analyses of FAMEs were carried out in a Varian GC gas chromatograph (model 430 GC, Varian Chromatograph Systems, Walnut Creek, California, USA), equipped with a CP 8412 auto injector. The Galaxie software was used for quantification and identification of peaks. Injections were performed in a 100-m fused silica capillary column (ID = 0.25 mm) coated with 0.2 µm of polyethylene glycol (SP-2560, Supelco, USA) using helium as carrier gas at isobaric pressure of 37 psi; linear velocity of 20 cm/s; make-up gas: helium at 29 mL/min at split ratio of 1:50; volume injected: 1.0 µL. The injector temperature was set at 250 ºC and the detector temperature was set at 280 ºC. The oven temperature was initially held at 140 ºC for 5 min, then programmed to 240 ºC at rate of 4 ºC/min and held isothermally for 30 min. All samples were analyzed in triplicate and the reported values are the average of the three runs.

Medium-chain saturated fatty acids (MCSFAs) are expressed as the sum of the amounts of caprylic, capric and lauric acids.

Long-chain saturated fatty acids (LCSFAs) are expressed as the sum of the amounts of myristic, palmitic and stearic acids.

Saturated fatty acids (SFAs) are expressed as the sum of the amounts of caprylic, capric, lauric, myristic, palmitic and stearic acids.

Unsaturated acids (USFAs) are expressed as the sum of the amounts of oleic, linoleic and linolenic acids.

Monounsaturated fatty acids (MUFA) are expressed as amounts of oleic acid.

Polyunsaturated fatty acids (PUFAs) are expressed as the sum of the amounts of linoleic and linolenic acids.

7.2.7. IODINE VALUE (IV)

Iodine value was calculated from the fatty acid composition, according to the procedure described in the AOCS official method Cd 1c-85 (AOCS, 2009c). Results are expressed in g iodine/100 g fat.

$$IV = (\%\ C_{18:1} \times 0.860) + (\%\ C_{18:2} \times 1.732) + (\%\ C_{18:3} \times 2.616) \quad \textbf{Eq. 7.3}$$

Where:

$C_{18:1}$ = oleic acid
$C_{18:2}$ = linoleic acid
$C_{18:3}$ = linolenic acid.

7.2.8. ATHEROGENIC INDEX (AI)

Atherogenic index was calculated according to Kim, Lumor and Akoh (2008), by the following equation:

$$AI = [C_{12:0}\ (w/w, \%) + 4 \times C_{14:0}\ (w/w, \%) + C_{16:0}\ (w/w, \%)]/USFA\ (w/w, \%) \quad \textbf{Eq. 7.4}$$

Where:

USFA = total amount of unsaturated fatty acids,
$C_{12:0}$ = lauric acid
$C_{14:0}$ = myristic acid
$C_{16:0}$ =palmitic acid.

7.2.9. REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS

A proton-decoupled ^{13}C NMR was used to analyze the positional distribution of fatty acids on the triacylglycerol backbone. Lipid samples (250 mg) were dissolved in deuthered chloroform (0.5 mL) in 5 mm NMR tubes, and NMR spectra were recorded on a Bruker Advance DPX spectrometer operating at 300 MHz. The ^{13}C spectra of the lipid samples were acquired with a spectral width of 2332.090 Hz, pulse of 10.2 μs, and a relaxation delay of 30s. Determination of ^{13}C was performed at a frequency of 75.8 MHz with a multinuclear probe of 5 mm operating at 30 °C, using method described by Vlahov (2005). The results showed the compositions of saturated fatty acids, oleic acid and linoleic + linolenic acids in *sn*-2 and *sn*-1,3 positions. All samples were analyzed in triplicate and the reported values are the average of three analyses.

7.2.10. TRIACYLGLYCEROL COMPOSITION

Triacylglycerol composition was analyzed in a Varian gas chromatograph (model 3400CX, Varian Ind. Com. Ltda., São Paulo, Brazil). A DB-17HT Agilent (Catalog: 122-1811) capillary column (50%-phenyl-methylpolysiloxane, 15 m in length x 0.25 mm bore and containing 0.15 μm film). The conditions were: split injection, ratio 1:100; column temperature: 250 °C, programmed up to 350 °C at 5°C/min; carrier gas: helium, at 1.0 mL/min flow rate; injector temperature: 360 °C; detector temperature: 375 °C; injection volume: 1.0 μL; sample concentration: 100 mg/5 mL of hexane (RIBEIRO *et al*., 2009a). All samples were analyzed in triplicate and the reported values are the average of three runs. TAG profiles were followed throughout the reactions by plotting the percents of the areas of Carbon Number peaks groups.

7.2.11. CONSISTENCY

Consistency was determined via the penetration test using a 45° acrylic cone fitted to a constant speed model TA-XT2 Texture Analyzer, Stable Micro Systems, UK. Fat samples were heated to 70 °C in a microwave oven for complete melting of the crystals, and stored in 50 mL glass beakers (Pyrex, USA). Tempering was allowed to occur for 24 h in a standard refrigerator (5–8 °C) and then for 24 h in an oven with

controlled temperature (5, 10, 15, 20, 25, 30, 35, 40 and 45 °C ± 0.5 °C). The tests were conducted under the following conditions: determination of force in compression; distance, 10.0 mm; speed, 2.0 mm/s and time, 5s (GAMBOA; GIOIELLI, 2003 a,b). Measurements were performed in duplicate and the reported values are the simple average of the two values. Consistency was calculated as a ‘‘yield value” (kgf/cm^2), according to the equation proposed by Haighton (1959).

$$C = \frac{K \times W}{p^{1,6}} \qquad \textbf{Eq.7.5}$$

Where:

C = the yield value (kgf/cm^2),
K = constant depending on the cone angle (4700-undimensional),
W = the compression force (kgf), and
p = the penetration depth (mm/10).

7.2.12 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The crystallization and melting behavior were obtained by differential scanning calorimetry (DSC) cell from the DSC 4000 Perkin Elmer (Perkin Elmer Corp., Norwalk, CT, USA), under dynamic atmosphere of He (20 mL/min) and cooling rate of -10 °C/min and heating rate at 5 °C/min, at temperatures ranging from 80 to -60 °C for cooling, with isothermal time of 10 min at 80 °C and -60 to 80 °C for heating, with a isothermal time of 10 min at -60 °C, using sealed aluminum capsules containing sample mass between 5 to 10 mg. The temperature and heat of fusion were calibrated with indium (initial temperature of 156.6 °C). Before the trials were done white curves to assess the baseline equipment. Curves were processed by the software Pyris, melting and crystallization curves are analyzed for the onset (T_{onset} °C) and endset (T_{endset} °C) of melting and crystallization, melting and crystallization peak temperatures (T_{pf} and T_{pc} °C) and melting and crystallization enthalpies (ΔH_f and ΔH_c J/g) (RIBEIRO *et al.*, 2009b).

7.2.13. SOLID FAT CONTENT

Solid fat content (SFC) was determined with a DSC 4000 differential scanning calorimeter (Perkin Elmer Corp., Norwalk, CT, USA). The data processing system used was the Pyris Series Thermal Analysis System software. An empty aluminium pan was used as a reference, and each sample was accurately weighed (5–10 ± 0.1 mg) for DSC analysis. The sample was heated to 80 °C and held for 10 min. Thereafter, the temperature was decreased at 10 °C/min to −60 °C. After holding for 10 min at −60 °C, the melting curve was obtained by heating to 80 °C at 5 °C/min. The temperature and heat of fusion were calibrated with indium (onset temperature 156.6°C).

7.2.14. POLARIZED LIGHT MICROSCOPY

Samples were melted at 70 °C in a stove and, with the aid of a capillary tube, a sample drop was placed on a glass slide preheated at controlled temperature (70 °C) and covered with a cover glass. Glass slides were prepared in duplicates for each sample. Samples were kept in the stove at the analysis temperature (25 °C) for 24 h. Crystal morphology was evaluated by means of the polarized light microscope (Olympus, model BX 51) coupled to a digital video camera (Media Cybernetics). The slide temperature was held constant by LTS 32 large heating and freezing stage operated by a TP93 temperature programmer (Linkam Scientific instruments Ltd., Surrey, England), kept at the same crystallization temperature. Images were captured by the Image Pro-Plus version 4.5.1.22 (Media Cybernetics) software, using polarized light and amplified up to 100 times. For each glass slide, three visual fields were focused, of which only one was chosen to represent the observed crystals. The evaluation parameters selected for quantitative image analysis were the mean diameter of crystals and the range between the mean diameter variations (GAMBOA; GIOIELLI, 2006).

7.3. RESULTS AND DISCUSSION

7.3.1. QUALITY PARAMETERS OF THE NATIVE AND BLENDED OILS

The quality of the oil used in the enzymatic interesterification is essential. Fats and oils used in enzymatic interesterification must have low acidity, once the free fatty acids, as well as water and peroxide act as catalyst poison of stability of lipases (XU, 2000). The peroxide values, free fatty acid contents and the oxidation products of the native and blended oils were monitored before and after enzymatic interesterification. The quality characteristics of the blend are shown in Table 22 and meet the quality parameters described by the Codex Standard (2004).

The stability of lipases also is affected by lipid quality. It was found that minor compounds in oils and fats, such as lipid hydroperoxides, phospholipids, emulsifiers, chlorophyll, carotenoids, lipid polymers, heavy metal ions, and even some antioxidants, had effects on the stability of lipases. Therefore, a high quality of starting oils and fats is necessary in favor of a better stability of lipases. However, the refining of oils and fats is also costly. A compromise has to be made between the quality of lipids and the stability of lipases (XU, 2000).

Free fatty acids are responsible for undesirable flavor and aromas in fats. Free fatty acids are formed by hydrolytic rancidity, which is the hydrolysis of an ester by lipase or moisture. The free fatty acid values for palm stearin and palm kernel oil were 0.2 g oleic acid/100g fat. Olive oil had the higher free fatty acid value. As part of olive oil had gone through the refining process, it was expected that its free fatty acid would be low.

The peroxide value is a measure of the concentration of peroxides and hydroperoxide forms in the initial stage of lipid oxidation. The number of peroxides present in vegetable oils reflects its oxidative level and thus its tendency to become rancid. Theoretically, palm stearin and palm kernel oil should exhibit a low rate of oxidation due to its low content of unsaturated fatty acids. Unsaturated fatty acids easily react with oxygen to form peroxides. Oils with high peroxide values are unstable and easily become rancid.

The peroxide values obtained for palm stearin and palm kernel oil were relatively low, indicating that the samples were highly stable against oxidation. According to the Codex Standard (2004), the maximum peroxide value for virgin oils is

15 mequiv oxygen/kg oil, while the peroxide value for olive oil was 4.5 mequiv oxygen/kg oil, which was far below the maximum limits.

7.3.2. FATTY ACID COMPOSITION

The fatty acid composition of the blend is shown in Table 7.1. The compositions of palm stearin, palm kernel oil and olive oil are in agreement with the results published in literature (BOSKOU, 2002; LIN, 2002; PANTZARIS; BASIRON, 2002).

The physical and functional aspects of a margarine product are primarily dependent upon the characteristics of the major ingredient: the margarine oil (CHRYSAM, 2002). Fatty acid composition and iodine value are among the most important characteristics of margarines and shortenings.

Palm oil and its fractions are suitable for margarine production. There are several advantages in using palm stearin as a component for interesterification with liquid oils to yield a good hard stock, such as availability of oil, cheap raw material, and the removal of the need for hydrogenation. By interesterification of ternary or binary blends, it is possible to obtain suitable formulations which are free of *trans* fatty acids (LIN, 2002). The studied blend was *trans*-free fatty acids. Saturated fatty acids are predominant in palm stearin and palm kernel oil ($P< 0.05$), mainly palmitic acid (60.1 %) and lauric acid (47.1 %), respectively.

Blends prepared using palm stearin have higher concentrations of palmitic acid, which imparts a desirable smooth consistency required for applications such as margarines and shortenings. Shortenings with higher palmitic acid content are reportedly more stable in β′-crystal form than those with less palmitic acid (AINI, MISKANDAR, 2007; JEYARANI; REDDY, 2003).

deMan and deMan (1994) stated that β' has relatively very small crystals, which enables it to incorporate relatively large amounts of liquid oil in the crystal network. This phenomenon leads to the production of smooth, continuous and homogeneous products. β' crystals do not only provide good texture to margarines but also contribute to good creaming properties (AINI, MISKANDAR, 2007). According to Nawar (1995), the small size of the β' crystals helps trap and hold air during the creaming process.

Lauric oils, like palm kernel oil, in margarines contribute short-chain fatty acids, which make the fatty acid composition of the blend more like that of butter and give a

cooler mouth feel. Their steep-melting properties and eutectic formation also help to counterbalance the high melting point of palm stearin which now feature prominently in most margarine formulations (PANTZARIS; BASIRON, 2002).

Palm kernel oil showed higher (P<0.05) content of MCSFAs (53.5 %). LCSFAs content was significantly (P<0.05) higher in palm stearin. On the other hand, olive oil showed the highest content (P < 0.05) of USFAs (86.0 %) and MUFAs (81.3 %).

Table 7.1. Fatty acid composition (g/100g), FFA (g oleic acid/100 g fat) and PV (mequiv oxygen/kg oil) of palm stearin, palm kernel oil, olive oil and their blend.

Fatty acids[a]	PS	PKO	OO	BIE[l]
$C_{8:0}$	0.0±0.0[a]	3.0±0.3[h]	0.0±0.0[a]	1.4±0.0f
$C_{10:0}$	0.0±0.0[a]	2.9±0.1[h]	0.0±0.0[a]	1.3±0.0f
$C_{12:0}$	0.0±0.0[a]	47.1±0.9[i]	0.0±0.0[a]	21.2±0.1[g]
$C_{14:0}$	0.8±0.1b	15.4±0.2[h]	0.0±0.0[a]	7.2±0.0[f]
$C_{16:0}$	60.1±0.5[l]	9.8±0.3[a]	10.4±0.1[b]	25.1±0.1[d]
$C_{18:0}$	6.7±0.3[l]	2.1±0.1[a]	3.2±0.2[b]	3.7±0.0[d]
$C_{18:1}$	27.1±0.0[d]	17.5±0.6[a]	81.4±0.2[l]	36.4±0.0[f]
$C_{18:2}$	5.3±0.2[j]	2.1±0.2[a]	4.6±0.0[g]	3.7±0.0[c]
$C_{18:3}$	0.0±0.0[a]	0.0±0.0[a]	0.3±0.0[b]	0.1±0.0[ab]
IV[b]	32.4±0.3[e]	18.7±0.8[a]	78.9±0.3[k]	37.9±0.2[f]
MCSFAs[c]	0.0±0.1[a]	53.1±1.2[j]	0.0±0.0[a]	23.9±0.1[h]
LCSFAs[d]	67.7±0.2[l]	27.3±0.5[b]	13.6±0.3[a]	36.0±0.1[e]
SFAs[e]	67.7±0.2[i]	80.4±0.7[l]	13.6±0.3[a]	59.9±0.3[g]
USFAs[f]	32.3±0.2[d]	19.6±0.7[a]	86.0±0.3[l]	40.1±0.2[g]
MUFAs[g]	27.1±0.0[d]	17.5±0.6[a]	81.4±0.2[k]	36.4±0.1[f]
PUFAs[h]	5.3±0.2[j]	2.1±0.2[a]	4.9±0.0[i]	3.9±0.0[cd]
AI[i]	2.0±0.0[f]	6.1±0.2[k]	0.1±0.0[a]	1.9±0.0[f]
FFA[j]	0.2±0.0	0.2±0.0	0.8±0.0	0.8±0.0
PV[k]	0.1±0.0	0.1±0.0	4.5±0.2	1.4±0.0
Abs_{232nm}	1.7±0.1	1.6±0.1	2.7±0.1	1.7±0.1
Abs_{270nm}	0.2±0.0	0.2±0.0	0.5±0.0	0.9±0.0

Values are shown as means ± SD of three replications. Means (n = 3) with different letters in the same line are significantly different (P < 0.05). [a] Fatty acids (g/100g) $C_{8:0}$ caprylic acid, $C_{10:0}$ capric acid, $C_{12:0}$ lauric acid, $C_{14:0}$, myristic acid; $C_{16:0}$, palmitic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid; $C_{18:3}$ linolenic acid, [b]IV, Iodine value (g iodine/100g); [c]MCSFA, Medium-chain saturated fatty acids; [d]LCSFA, Long-chain saturated fatty acids; [e]SFA, Saturated fatty acids; [f]USFA, unsaturated fatty acids; [g]MUFA, Monounsaturated fatty acids; [h]PUFA, Polyunsaturated fatty acids; [i]AI Atherogenic Index; [j]FFA Free Fatty Acid (g/100 g of oleic acid); [k]PV Peroxide Value (mequiv of oxygen/kg of oil); [l]BIE Blend Interesterified.

According to the study of Roos, Schouten and Katan (2001), consumption of solid fat rich in lauric acid could give a more favorable serum lipoprotein pattern than consumption of partially hydrogenated soybean oil rich in *trans* fatty acids.

Atherogenicity index (AI) calculated with hypercholesterolemic fatty acids (lauric acid, $C_{12:0}$; myristic acid, $C_{14:0}$; palmitic acid, $C_{16:0}$) illustrates hypercholesterolemic degree (ULBRICHT; SOUTHGATE, 1991). Dietary *trans* fatty acids can affect more negatively than SFAs ($C_{12:0}$, $C_{14:0}$ and $C_{16:0}$) on plasma lipoprotein in human owing to decreased HDL cholesterol and elevated LDL cholesterol (SUNDRAM *et al*., 1997). Therefore, AI was calculated using the modified calculation that considers the negative effect of *trans* fatty acids on hypercholesterolemic degree (KIM, LUMOR, AKOH, 2008).

The AI of the used substrates was 2.0 for palm stearin, 6.1 for palm kernel oil and 0.1 for olive oil. Oleic acid is very important in nervous cell construction and can be changed by the organism into a set of compounds closely resembling prostaglandins which play an important role at the vessel level and for blood coagulation. Oleic acid has a fundamental role in cardiovascular disease prevention. Linoleic acid is indispensable for the healthy growth of human skin. It can be transformed by the organism into a series of long fatty acids chains, which are the precursors of eicosanoids (NASRI *et al*., 2005; NEHDIA *et al*., 2010).

The benefits derived through blending of palm stearin and palm kernel oil with olive oil for the consumer were the improved amounts USFAs. It is important to emphasize that, according to Zhao *et al*. (2007), a higher dietetic intake of USFAs promotes reduction in lipids, lipoproteins and in the inflammatory markers C-reactive protein and cell adhesion molecules, which leads to a decreased risk of cardiovascular disease. So, based on the results of the present study the blend can contribute to an increase of USFA dietary intake.

7.3.3. **REGIOSPECIFIC DISTRIBUTION OF FATTY ACIDS**

Analysis of the regiospecific distribution of fatty acids in triacylglycerols by NMR is desirable, as the method does not require hydrolysis by pancreatic lipase, with further separation of partial acylglycerols performed by thin layer chromatography and finally, analysis of fatty acids by gas chromatography (GUNSTONE, HARWOOD, 2007; SILVA *et al*., 2011). However, the technique cannot discriminate all component fatty acids.

Using the NMR data for fatty acids in the *sn*-2 and *sn*-1,3 positions it is possible to calculate the fatty acid composition of the mixture. The fatty acid composition calculated by NMR and that obtained by gas liquid chromatography (GLC) showed similar results.

Saturated fatty acids were found mainly in the *sn*-1,3 positions and unsaturated fatty acids were found mainly in the *sn*-2 position, typical feature of vegetable oils (TAN; CHE MAN, 2002). According to Karupaiah and Sundram (2007) unsaturated fatty acids are better metabolized and utilized in human body when present at *sn*-2 position.

According to Pande, Akoh and Shewfelt (2013), contents of 40 to 45% of saturated fatty acids at *sn*-2 position may be more suitable for margarine formulation.

The fatty acid profiles at the *sn*-1,3 and *sn*-2 positions of the of the blend 45 % (w/w) PS, 30 % (w/w) PK and 25 % (w/w) OO before and after continuous enzymatic interesterification with *"Lipozyme*® TL IM" and "*Lipozyme*® RM IM", at different time of reaction are given in Table 6.9.

These results suggest that very little, if any, acyl migration have occurred. So, during enzymatic interesterification the distribution of residues at *sn*-2 position remains substantially unchanged. Thus, the regiospecificity of the enzyme was maintained during the continuous reaction, although certain reduction in enzyme activity has occurred. Acyl migration is usually associated with such factors as the water content of the lipase and the reaction medium, the chemical nature of the support material, reaction times and temperatures, and the type of reactor employed (XU *et al*., 1998; PANDE, AKOH, 2013).

Adhikari and Hu (2012) showed that batch enzymatic interesterified products presented higher level of unsaturated fatty acids at *sn*-2 position. It is due to the specific *sn*-1,3 lipase used (*"Lipozyme*® TL IM"), which can rearrange *sn*-1,3 positions of TAG molecules, where most of unsaturated fatty acids are naturally present at the *sn*-2 position.

Pande and Akoh (2013) observed changes in the composition of fatty acids in the *sn*-2 position after continuous enzymatic interesterification with *"Lipozyme*® TL IM" of palm stearin and high stearate soybean oil. This effect may be attributed to acyl migration that can occur during the reaction due temperature, reaction time, and substrates affecting the specificity of "*Lipozyme*® TL IM".

The lipases used in this study are reportedly *sn*-1,3 selective, although Kim *et al.* (2002) found that reaction conditions can induce acyl migration when these biocatalysts are used. The relatively short space times necessary to reach quasi-equilibrium conditions,

together with the positional specificity of the lipases, lead to conditions under which one expects to observe minimal migration of acyl groups, as found in the cited results.

According to Quinlan *et al*. (1995), unsaturated fatty acids initially located at the *sn*-2 position should largely remain in this position after using *sn*-1,3 specific lipase, although some degree of acyl migration to the *sn*-1,3 positions can occur.

Ronne *et al.* (2005) observed that using lower residence times leads to small changes in the fatty acid composition at *sn*-2 position. Longer residence times give rise to larger changes in fatty acid composition at *sn*-2 position due to most likely acyl migration.

According to Silva *et al.* (2012), when used at higher flow rates in continuous enzymatic interesterification and lower residence times, the acyl migration occurs to a lesser degree.

7.3.4. TRIACYLGLYCEROL COMPOSITION

Analysis of triacylglycerol composition represents a true indication of randomization or specific interesterification, and is extremely useful for monitoring modification of interesterified fats and outlining specific applications for them (O'BRIEN, 2004, RIBEIRO *et al*., 2009b).

From a technological point of view, the molecular triacylglycerol species profile is the key for the understanding of several physical properties of a given oil or fat (BUCHGRABER *et al*., 2004). In a processed food that contains significant fat content, the product's behavior may depend on the triacylglycerol composition of that fat (RIBEIRO *et al*., 2009c).

As the TAGs composition of fats and oils is complex, there is no perfect analytical technique to separate all of the TAG species in the fats and oils clearly (ADHIKARI *et al*., 2009; ZHAO *et al*., 2013). Interpretation of TAG profiles of the interesterified mixtures is a difficult task as slight shift in retention time is observed. Besides that, there are no reference standards corresponding to all the triacylglycerols.

The TAG compositions of physical blend and interesterified products with *"Lipozyme*® TL IM" during the 1, 2, 4, 6, 8 and 10 days and *"Lipozyme*® RM IM" during the 1, 3, 5, and 7 days are presented in Table 7.2 a and b and Figure 7.1 a and b.

Table 7.2a. Triacylglycerol composition (g/100g) of the blend, before and after continuous enzymatic interesterification with *"Lipozyme*® TL IM" during the 1, 2, 4, 6, 8 and 10 days.

TAGs[a] (g/100g)	Blend	1 Day	2 Day	4 Day	6 Day	8 Day	10 Day
C_{26}	0.4±0.1	0.4±0.0	0.4±0.0	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1
C_{28}	2.1±0.2	1.9±0.3	1.8±0.2	1.7±0.2	1.6±0.1	1.7±0.1	1.8±0.1
C_{30}	2.7±0.1	2.3±0.1	2.1±0.1	2.2±0.1	2.4±0.1	2.4±0.1	2.5±0.1
C_{32}	7.3±0.1	6.3±0.2	6.3±0.2	6.8±0.2	6.4±0.2	6.5±0.2	6.7±0.2
C_{34}	0.8±0.1	1.5±0.3	1.1±0.1	1.1±0.1	1.4±0.1	1.5±0.1	1.7±0.1
C_{36}	5.5±0.0	6.2±0.3	6.1±0.1	6.4±0.1	6.5±0.1	6.4±0.1	6.5±0.1
C_{38}	3.8±0.1	5.3±0.1	5.6±0.2	5.7±0.1	5.1±0.2	5.2±0.2	5.3±0.2
C_{40}	3.0±0.0	4.4±0.0	4.3±0.2	4.2±0.1	4.6±0.1	4.7±0.1	4.9±0.1
C_{42}	0.5±0.2	1.3±0.0	1.4±0.2	1.3±0.2	1.4±0.1	1.5±0.1	1.6±0.1
C_{44}	1.6±0.1	5.0±0.0	5.2±0.2	5.3±0.3	5.3±0.1	5.7±0.1	5.8±0.1
C_{46}	2.7±0.1	3.6±0.3	3.9±0.1	3.9±0.1	3.8±0.1	3.8±0.1	3.9±0.1
C_{48}	14.2±02	12.8±0.1	12.3±0.1	11.9±0.1	12.0±0.2	11.9±0.2	12.1±0.2
C_{50}	20.0±0.2	17.2±0.2	17.1±0.0	17.2±0.2	17.3±0.1	17.2±0.1	16.9±0.1
C_{52}	19.1±0.2	17.1±0.3	17.2±0.2	17.1±0.2	17.0±0.1	16.9±0.1	16.7±0.1
C_{54}	15.8±0.2	14.1±0.2	14.4±0.1	13.9±0.1	14.2±0.1	14.1±0.1	14.0±0.1

Values are shown as means ± SD of three replications. [a]Number of carbon derived from the triacylglycerol less glycerol.

Table 7.2b. Triacylglycerol composition (g/100g) of blend, before and after continuous enzymatic interesterification with *"Lipozyme*® RM IM" during the 1, 3, 5, and 7 days.

TAGs[a] (g/100g)	Blend	1 Day	3 Days	5 Days	7 Days
C_{26}	0.4±0.1	0.4±0.0	0.4±0.1	0.4±0.1	0.4±0.1
C_{28}	2.1±0.2	1.8±0.1	2.1±0.1	2.3±0.2	2.3±0.1
C_{30}	2.7±0.1	2.2±0.1	2.5±0.2	2.1±0.1	2.3±0.2
C_{32}	7.3±0.1	6.3±0.1	6.8±0.1	6.7±0.2	6.8±0.1
C_{34}	0.8±0.1	1.3±0.1	0.9±0.1	1.2±0.1	1.1±0.1
C_{36}	5.5±0.0	6.1±0.1	6.2±0.1	6.3±0.1	6.2±0.1
C_{38}	3.8±0.1	5.1±0.2	5.4±0.1	5.5±0.1	5.3±0.1
C_{40}	3.0±0.0	4.8±0.2	4.8±0.3	4.1±0.0	4.8±0.1
C_{42}	0.5±0.2	1.4±0.2	1.2±0.1	1.3±0.1	1.2±0.1
C_{44}	1.6±0.1	4.7±0.2	4.2±0.1	4.5±0.1	4.3±0.2
C_{46}	2.7±0.1	3.2±0.1	3.3±0.2	3.5±0.1	3.1±0.1
C_{48}	14.2±02	12.2±0.1	11.8±0.1	12.6±0.1	12.3±0.1
C_{50}	20.0±0.2	17.4±0.2	17.6±0.1	17.3±0.1	17.3±0.1
C_{52}	19.1±0.2	17.8±0.1	17.7±0.1	17.3±0.1	17.3±0.1
C_{54}	15.8±0.2	14.6±0.2	14.6±0.1	14.3±0.1	14.5±0.1

Values are shown as means ± SD of three replications. [a]Number of carbon derived from the triacylglycerol less glycerol.

More than 50% of the triacylglycerols are contributed by disaturated-monounsaturated and monosaturated-diunsaturated triacylglycerols. According to Rodrigues, Gioielli and Anton (2003), the properties of fatty foods can be related to the triacylglycerol composition of that fat which composes them. Triacylglycerols disaturated-monounsaturated, with melting points between 27 °C and 42 °C, are mainly responsible for the structure of products, whereas triacylglycerols monosaturated-diunsaturated are important with regard to their sensorial and functionality properties at ambient temperature.

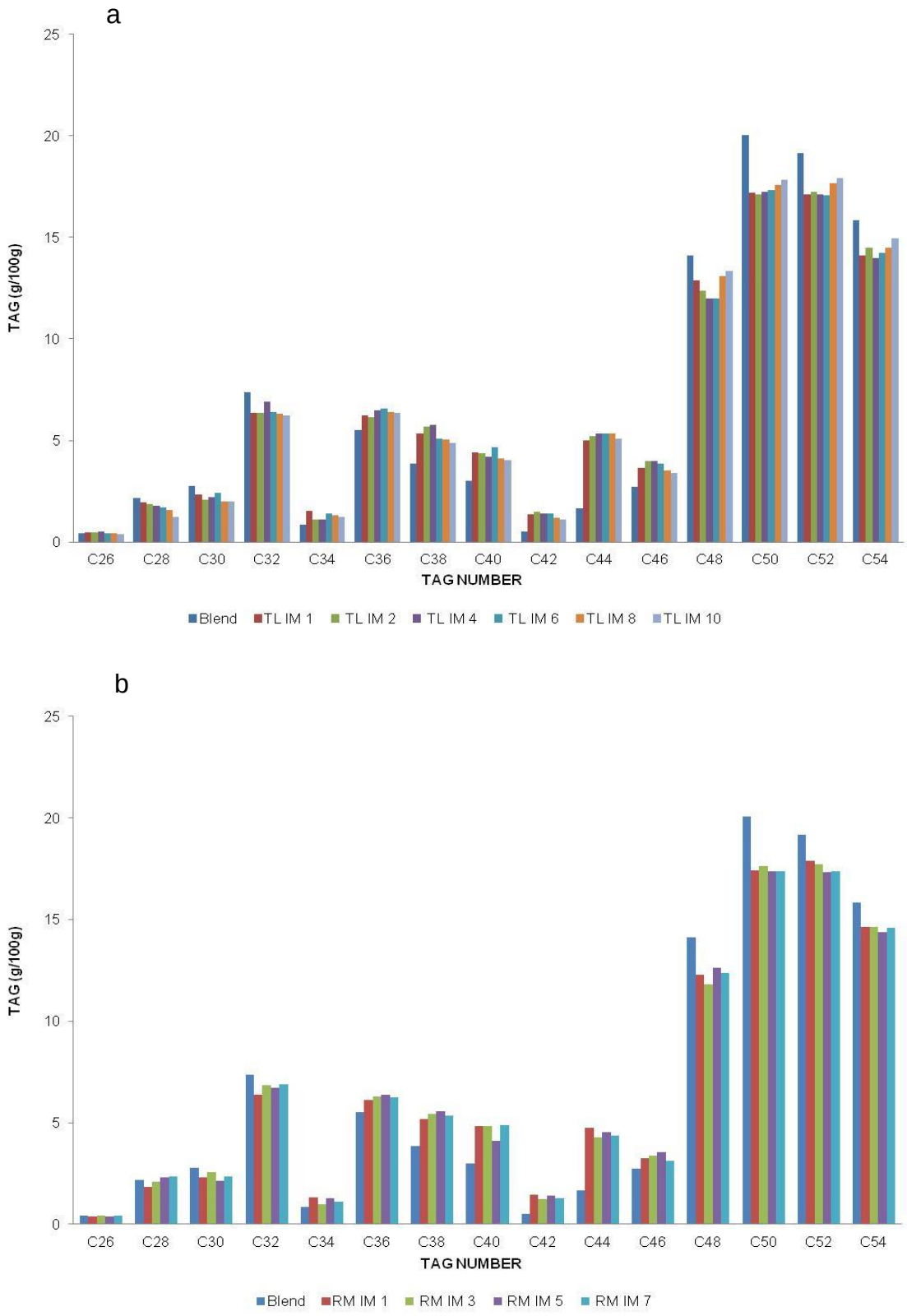


Figure 7.1. Triacylglycerol composition for the blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive oil before and after continuous enzymatic interesterification with *"Lipozyme*® TL IM" (a) and *"Lipozyme*® RM IM" (b).

In particular, the higher percentage of C_{54} triacylglycerols in the blends contributes to properties of lubricity, aeration and gloss; while the presence of C_{50} and C_{52} triacylglycerols ensures important qualities as regards structure and moisture barrier, contributing to the functionality of the bases (GHOTRA; DYAL; NARINE, 2002; RIBEIRO *et al*., 2009b).

The interesterified blend showed a decrease in C_{48} to C_{54} and an increase in C_{36} to C_{46} triacylglycerols having medium-chain fatty acids. Fats containing both medium and long-chain fatty acids, because of the fairly complex packing at the molecular level, produce smaller crystals and are more suitable as plastic fats (FLOTER; van DUIJN, 2006). The interesterified blends consisted of more diverse TAGs which enhanced crystallization in the β' form, the desirable polymorph in margarines and spreads (PANDE, AKOH, 2013).

Differences in the triacylglycerol composition of fat blends, resulting from rearrangement, can promote important alterations in the physical properties of fats, such consistency, solid fat content and melting point. Hence, the increase of the disaturated-monounsaturated and monosaturated-diunsaturated contents of palm stearin, palm kernel oil and olive oil blend, promoted by continuous enzymatic interesterification, is associated to the increase of technological functionality, the betterment of sensorial characteristics, and, therefore, to a greater potential of these interesterified bases for food application (RIBEIRO *et al.*, 2009b; WIEDERMANN,1978).

7.3.5 CONSISTENCY

Consistency is an important functional aspect of plastic fats, which are blends of fat crystals and liquid oil. The relation between the two phases and the crystalline character of the solid phase determines the consistency and firmness of samples. The rearrangement of triacylglycerols caused by enzymatic interesterification promotes changes in the physical characteristics of fat bases, especially those related to crystal lattice formation, altering the mechanical properties of initial raw materials (DEMAN, 1983; CHIU, 2006; LEE *et al*., 2008; RIBEIRO *et al*., 2009d).

Figure 7.2 a and b and Table 7.3 show the consistency profiles as a function of temperature of the blend before and after continuous enzymatic interesterification with "*"Lipozyme*® TL IM" " at 10 days and *"Lipozyme*® RM IM" at 7 days of operation in continuous packed-bed reactor.

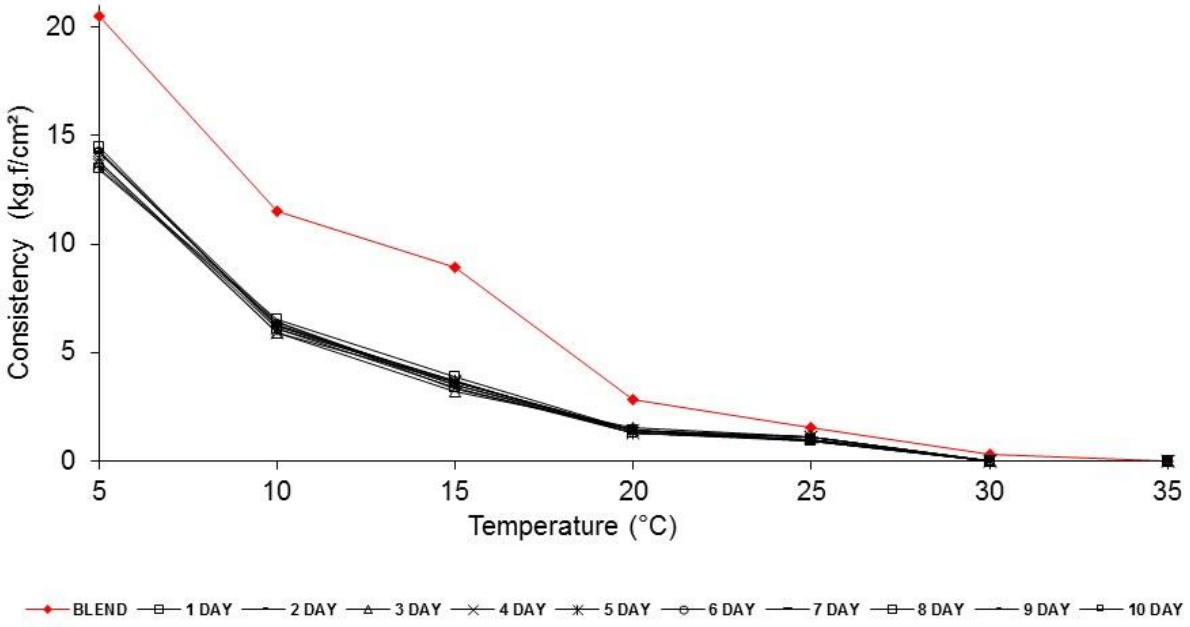
The consistency of the blend decreased as a function of temperature. This decrease may be due to the gradual melting of the crystals, leading to a structurally weaker network, which is in turn responsible for the plasticity of fats (GAMBOA; GIOIELLI, 2003 a and b; SOARES *et al*., 2009; RODRIGUES *et al*., 2007). None of the analyzed samples exhibited a measurable consistency at 35 °C. The zero value for consistency physically represents a product with very low consistency in which the equipment was unable to detect a measurable value. These products generally had the consistency of a high viscosity fluid. Crystal patterns (both polymorphism and morphology) of interesterified blends substantially differ from those of the native blend counterpart. Differences in crystal pattern and aggregation behavior could lead to an alteration in the structure of the fat crystal network in blend, resulting in altered rheological properties such as the consistency (Marangoni & Rousseau, 1998).

Table 7.3. Consistency (kgf/cm^2) of blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive oil before and after enzymatic interesterification with *"Lipozyme*® TL IM" at 10 days and with *"Lipozyme*® RM IM" at 7 days of operation in continuous packed-bed reactor.

	***"Lipozyme*® TL IM"**						
	Temperature (°C)						
Sample	**5**	**10**	**15**	**20**	**25**	**30**	**35**
Blend	20.5±3.5^{d}	12.5±0.0^{d}	8.9±0.3^{c}	2.8±0.2^{c}	1.5±0.0^{c}	0.3±0.0^{a}	0.0±0.0^{a}
1 Day	13.5±0.1^{b}	6.5±0.5^{b}	3.9±0.2^{b}	1.4±0.0^{b}	0.9±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
2 Days	13.4±0.2^{b}	6.4±0.3^{b}	3.5±0.1^{b}	1.3±0.0^{b}	0.9±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
3 Days	13.8±0.3^{b}	5.9±0.1^{b}	3.2±0.0^{b}	1.5±0.0^{b}	1.0±0.1^{b}	0.0±0.0^{a}	0.0±0.0^{a}
4 Days	13.7±0.2^{b}	5.9±0.1^{b}	3.6±0.0^{b}	1.4±0.0^{b}	1.0±0.1^{b}	0.0±0.0^{a}	0.0±0.0^{a}
5 Days	13.8±0.2^{b}	6.1±0.3^{b}	3.7±0.2^{b}	1.3±0.0^{b}	1.0±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
6 Days	14.2±0.7^{b}	6.3±0.2^{b}	3.6±0.0^{b}	1.4±0.1^{b}	0.8±0.2^{b}	0.0±0.0^{a}	0.0±0.0^{a}
7 Days	14.2±0.9^{b}	6.2±0.1^{b}	3.7±0.2^{b}	1.3±0.0^{b}	1.0±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
8 Days	14.5±0.8^{b}	6.1±0.2^{b}	3.4±0.0^{b}	1.3±0.1^{b}	1.0±0.1^{b}	0.0±0.0^{a}	0.0±0.0^{a}
9 Days	14.3±0.2^{b}	6.4±0.0^{b}	3.3±0.0^{b}	1.4±0.0^{b}	1.0±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
10 Days	14.2±0.4^{b}	6.2±0.1^{b}	3.5±0.0^{b}	1.4±0.1^{b}	0.9±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
	***"Lipozyme*® RM IM"**						
	Temperature (°C)						
Sample	**5**	**10**	**15**	**20**	**25**	**30**	**35**
Blend	19.2±2.5^{d}	10.9±1.0^{d}	8.1±1.3^{c}	3.1±0.2^{c}	1.9±0.0^{c}	0.5±0.0^{a}	0.0±0.0^{a}
1 Day	13.0±2.3^{b}	6.1±1.5^{b}	3.1±1.2^{b}	1.2±0.0^{b}	1.1±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
2 Days	13.2±1.3^{b}	6.2±1.3^{b}	3.2±1.1^{b}	1.0±0.0^{b}	1.0±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
3 Days	13.3±2.3^{b}	6.0±1.1^{b}	3.1±1.0^{b}	1.1±0.0^{b}	1.1±0.1^{b}	0.0±0.0^{a}	0.0±0.0^{a}
4 Days	13.1±2.2^{b}	6.1±1.1^{b}	3.1±1.0^{b}	1.1±0.0^{b}	1.1±0.1^{b}	0.0±0.0^{a}	0.0±0.0^{a}
5 Days	13.5±2.2^{b}	6.2±1.3^{b}	3.2±1.2^{b}	1.2±0.0^{b}	1.1±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}
6 Days	13.5±2.7^{b}	6.1±1.2^{b}	3.4±1.0^{b}	1.2±0.1^{b}	1.0±0.2^{b}	0.0±0.0^{a}	0.0±0.0^{a}
7 Days	13.4±2.9^{b}	6.0±1.1^{b}	3.5±1.2^{b}	1.4±0.0^{b}	1.0±0.0^{b}	0.0±0.0^{a}	0.0±0.0^{a}

Values are shown as means ± SD of three replications.

a



b

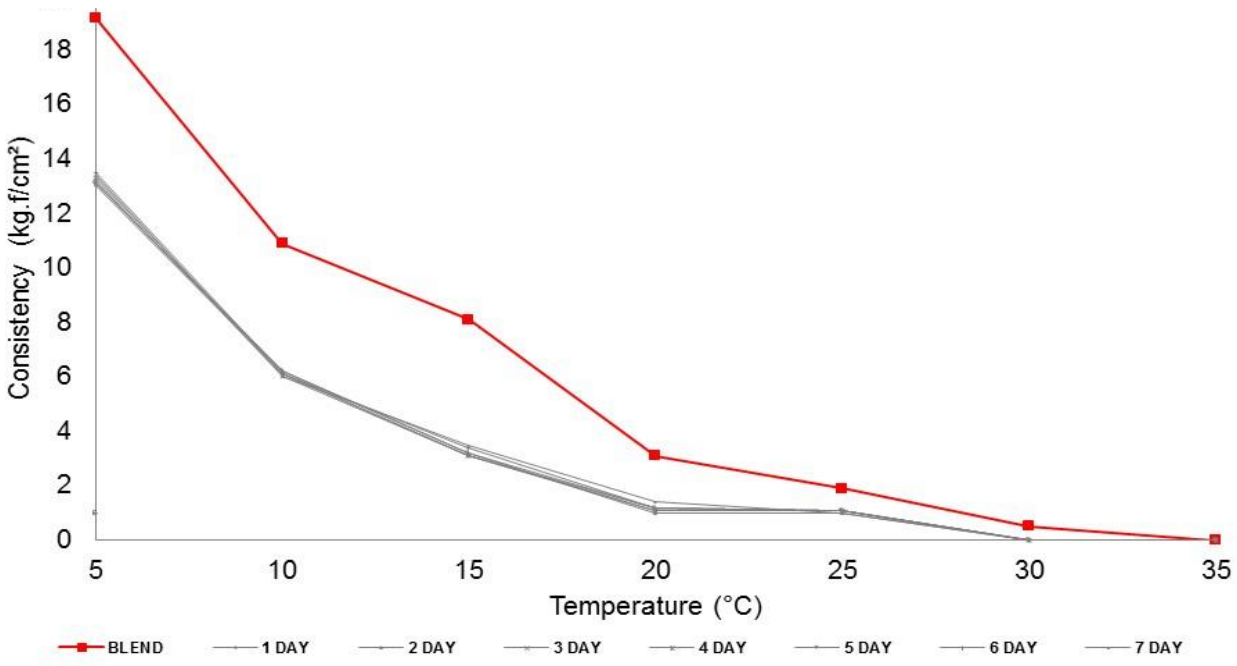


Figure 7.2. Consistency of the blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive before and after enzymatic interesterification with *"Lipozyme*® TL IM" at 10 days (a) and "*Lipozyme* RM IM" at 7 days (b) of operation in continuous packed-bed reactor.

According to Haighton (1959), a fat is plastic and spreadable at yield values ranging from 0.2 to 0.8 kgf/cm^2. The blend had no satisfactory plasticity and spreadability properties for use at refrigeration temperatures, in addition to melting point requisites for use in margarines, as mentioned earlier. However, there are other factors influencing the texture of a spread, such as the crystallization procedure including variables as cooling rate, degree of supercooling, mechanical working, tempering, solid fat content of fat used, and presence of nonfat materials (LUMOR *et al*., 2007).

Between 25 and 30°C, the interesterified blends were satisfactorily spreadable (yield values between 0.8 and 1.0 kgf/cm^2), but exhibits ideal plasticity at 35 °C, which is important for sensorial properties such as mouthfeel sensation and lack of adhesiveness. Thus, its consistency profile from 25 to 35 °C corroborates its suitability for application in bakery and confectionery products.

The blend can be classified as hard at room temperature, with a yield value between 1.6 and 2.0 kgf/cm^2 in the 25 to 30 °C interval; and is just above the spreadability limit (1.5 kgf/cm^2) at 35°C. According to Jeyarani and Reddy (2003), fats considered hard at room temperature are suitable for firmer food products, where deformations must not occur during handling or stocking.

7.3.6. SOLID FAT CONTENT

Solid fat content, the quantity of fat crystals in a fat or fat blend, has a great influence on the suitability of the fat or fat blend for a particular application. Solid fat content curves give good indications of a fat overall behavior and are useful in formulating and developing new products (RIBEIRO *et al*., 2009b). The variation of solid fat content with temperature and the sharpness of melting range, together with other factors such as the crystal morphology, determine the range within a fat can be considered plastic (RAO *et al*., 2001; SERIBURI, AKOH,1998).

The solid fat content is responsible for many product characteristics in margarines, shortenings and fat spreads, including their general appearance, ease of packing, spreadability, oil exudation and organoleptic properties (JEYARANI, KHAN, KHATOON, 2009). Excessive changes in solid fat content of the product during storage are not desirable. Ideally, the solid fat content should not change from the time of filling the product into containers throughout the storage period (GOLI *et al*., 2009). The plastic range of a fat is the temperature range over which the fat can be molded and is neither too hard nor too soft (JENNINGS, AKOH, 2010).

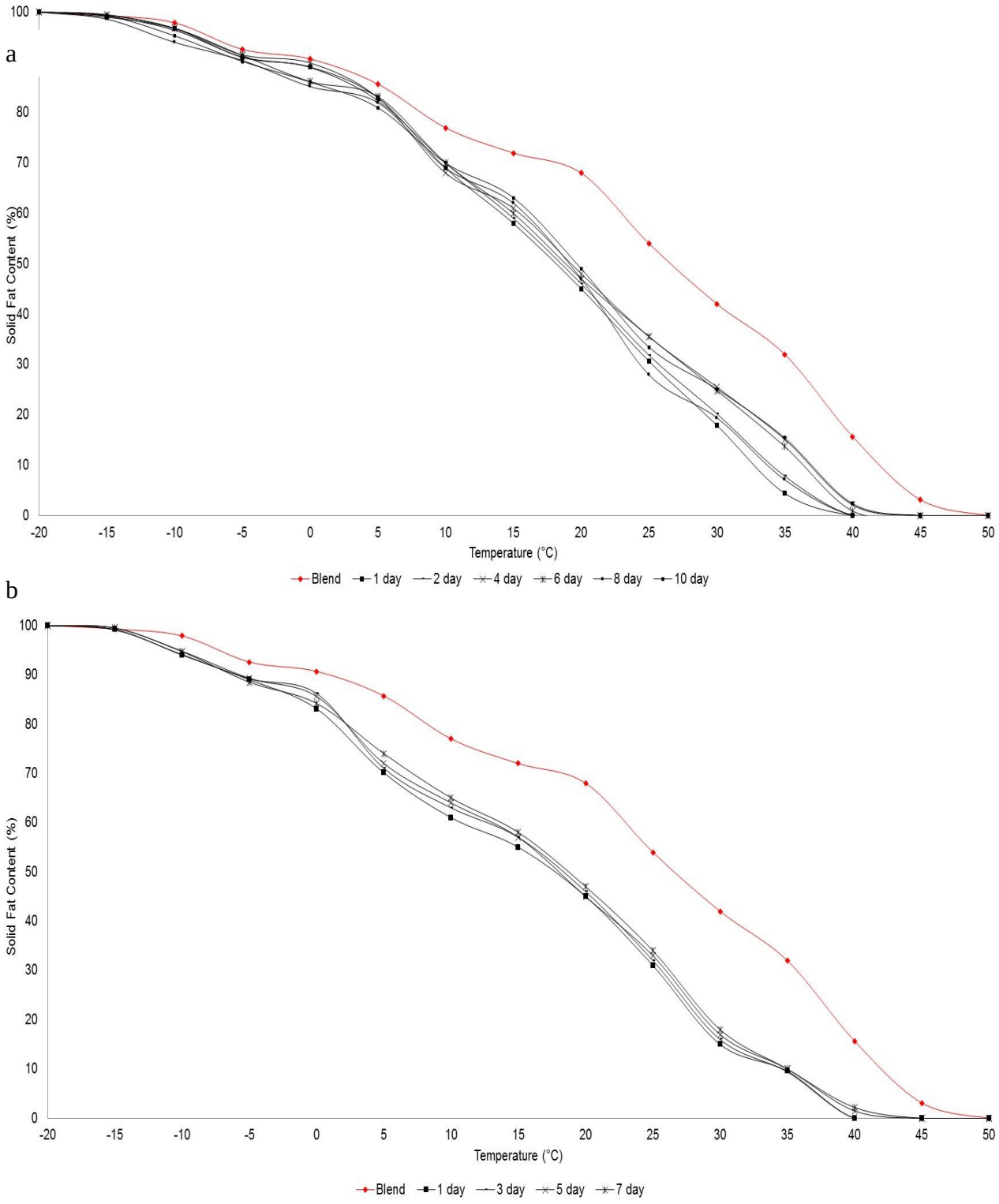


Figure 7.3. Solid fat content of the blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive before and after enzymatic interesterification with "*Lipozyme*® TL IM" at 10 days (a) and *"Lipozyme*® RM IM" at 7 days (b) of operation in continuous packed-bed reactor.

Figure 7.3 a and b shows the solid fat content as a function of temperature of the blend before and after enzymatic interesterification with "*"Lipozyme*® TL IM" " at 10 days and *"Lipozyme*® RM IM" at 7 days of operation in continuous packed-bed reactor.

The solid fat content of the interesterified blends were lower than the corresponding initial blend. Solid fat content at room temperature (25 °C) should be 15–35% for desirable spreadability as plastic fats (ADHIKARI *et al*., 2010). In the case of the interesterified blends, solid fat content at room temperature (25 °C) was within the scope of the above criteria, suggesting that blends in this study were suitable for spreadable fat or margarine stock. This effect is associated with a reduction in trisaturated triacylglycerols and a simultaneous increase in percentages of disaturated–monounsaturated and monosaturated–diunsaturated triacylglycerols, respectivel (ROUSSEAU *et al*., 1996; ROUSSEAU; MARANGONI,1998).

Due to the formation of mixed triacylglycerols with intermediate melting point, the interesterified blends showed a marked decline in SFC between 10 and 25°C, and the format of the curves was significantly modified by enzymatic interesterification, which generated more linear melting profiles with more pronounced slope, similar to results obtained by Dian *et al.* (2007) in interesterification of palm stearin/sunflower oil/palm kernel olein and by Ribeiro *et al*. (2009b) in interesterification of soybean oil and fully hydrogenated soybean oil. According to Rousseau and Marangoni (1998), the linearization of SFC curves after interesterification is a consequence of the greater variability of the triacylglycerol species resulting from the reaction.

The largest alterations in SFC of the interesterified blends as compared with the original blends were observed at 25 to 35 C as a result of the high proportion of triacylglycerols that liquefy at these temperatures (Dian *et al.*, 2007; Lida *et al.*, 2002). According to Leissner *et al.* (1991), the proportional relationship between starting hardstock concentration and its effect on the temperature at which the greatest variation in SFC occurs in the interesterified blends is indicative of the increased heat resistance.

7.3.7. THERMAL PROPERTIES

Differential scanning calorimetry is the thermo-analytical technique most employed in studying oils and fats. It is considered also an important tool for characterizing interesterified products. Evaluation by differential scanning calorimetry yields direct measurements of the

energy involved in the processes of melting and crystallization of oils and fats. Crystallization of oils results in shrinking volume, associated with an exothermic effect. Conversely, when fats melt, their volume expands, characterizing an endothermic effect (RIBEIRO *et al*., 2009d; TAN, CHEN, 2002). Depending on the TAG composition and the method employed for DSC analysis, fats and oils can exhibit complicated thermal behaviour.

Figure 7.4 and 7.5 show the changes of melting (a) and crystallization (b) profiles of the blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive before and after enzymatic interesterification with "*Lipozyme*® TL IM" at 1, 2, 4, 6 and 8 days and "*Lipozyme*® RM IM" at 1, 3, 5 and 7 days.

Three peaks were observed in the melting profile of original blend at temperatures of 41.96 °C (ΔH = 110.49 J/g), 15.35 °C (ΔH = 80.78 J/g) and -10.81 °C (ΔH = 22.86 J/g) corresponding to palm stearin, palm kernel oil and olive oil, respectively. After interesterification, new peaks appeared between the peaks related to palm stearin, palm kernel oil and olive oil. These new peaks corresponded to the new TAG components formed during the reaction like disaturated–monounsaturated and monosaturated–diunsaturated triacylglycerols and their isomers. The newly formed peaks in the products were much broader than the peaks in the initial blends due to the formation of various TAG components after enzymatic interesterification. Since both SLs were not completely melted at room temperature they can be used in the production of stick margarines. Compared to blend, the SLs showed broader peaks indicating a better plastic range which may be desirable for margarine.

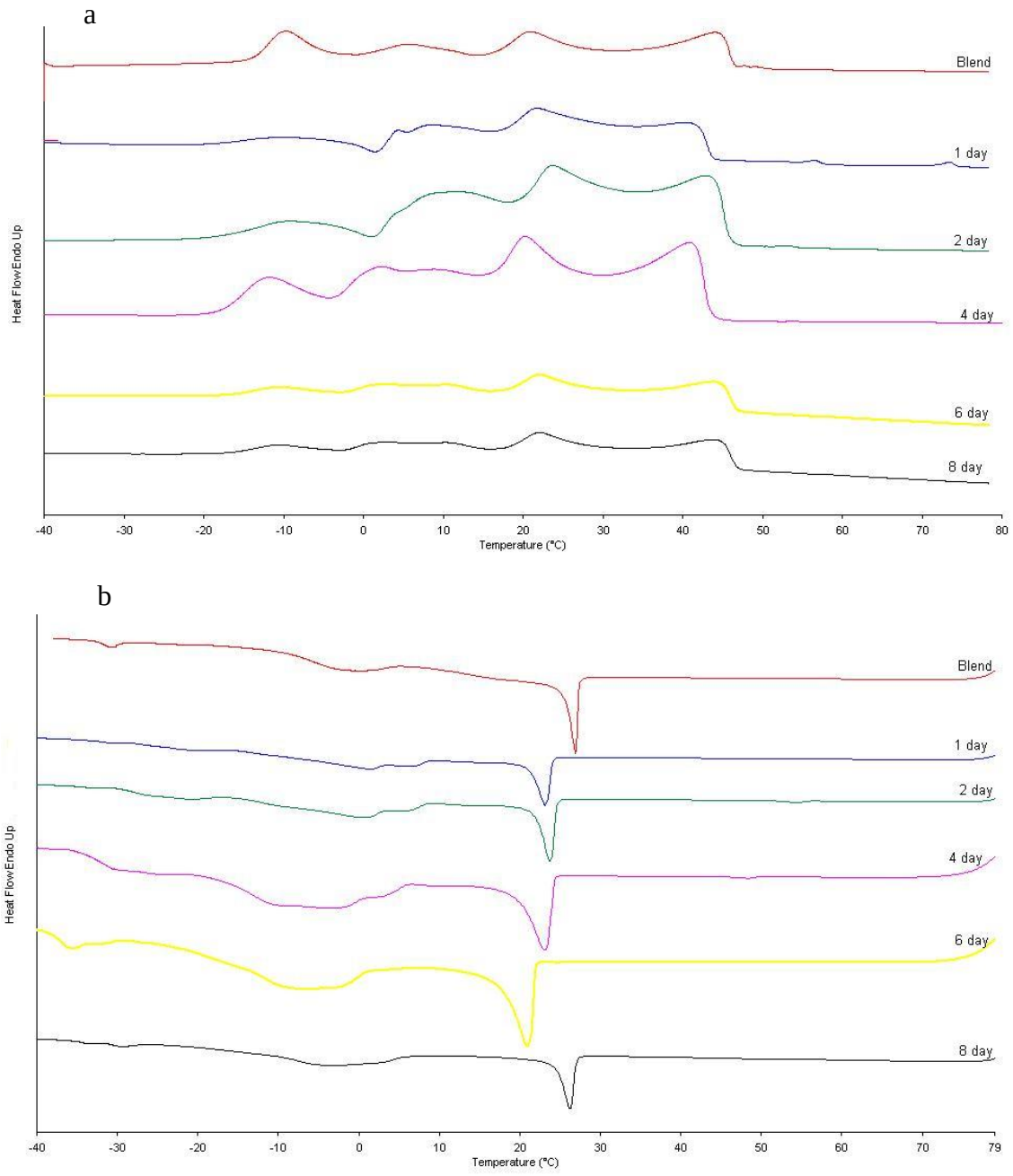


Figure 7.4. DSC melting (a) and crystallization (b) curves of the blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive before and after enzymatic interesterification with "*Lipozyme*® TL IM" at 1, 2, 4, 6 and 8 days of operation in continuous packed-bed reactor.

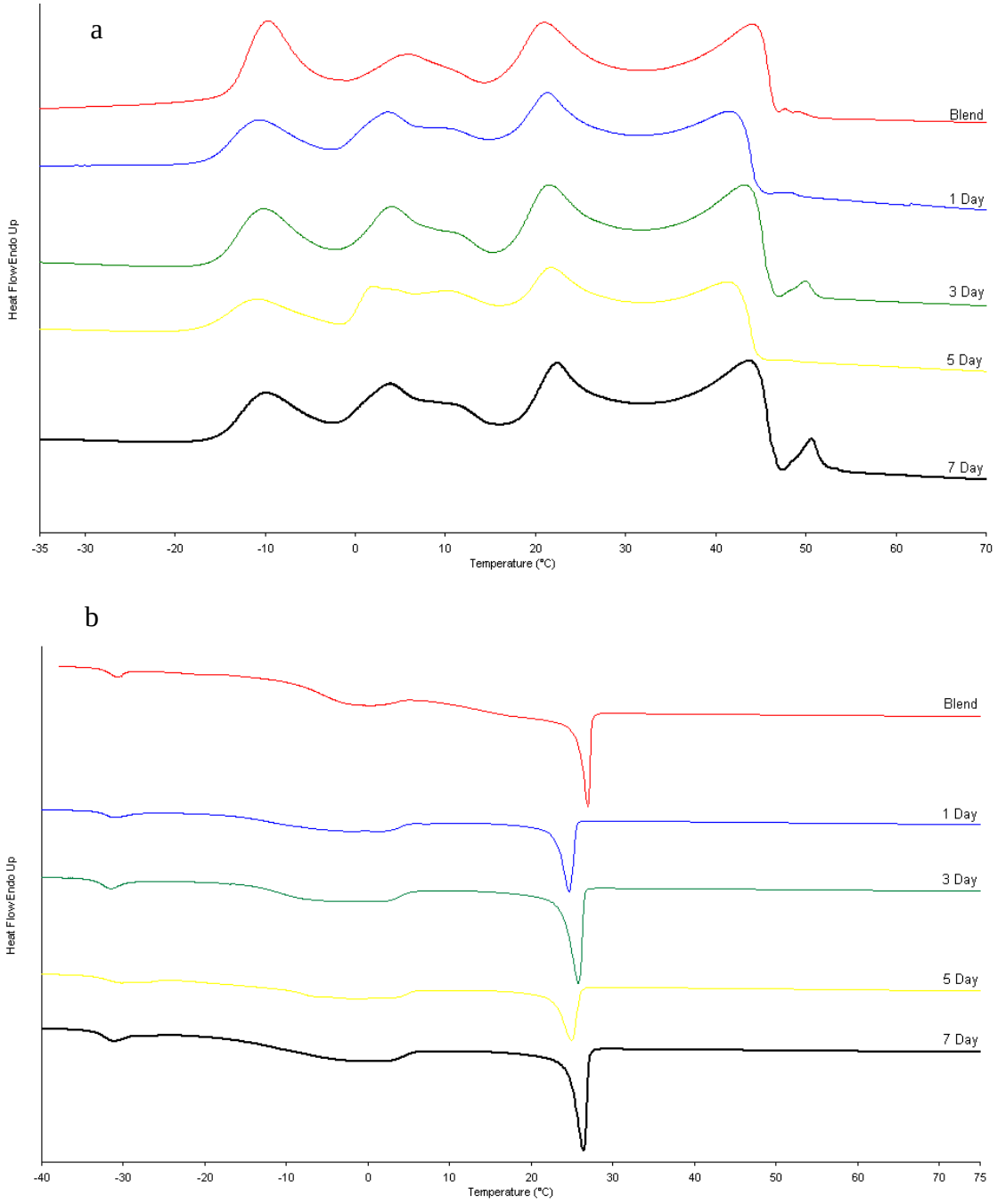


Figure 7.5. DSC melting (a) and crystallization (b) curves of the blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive before and after enzymatic interesterification with "*Lipozyme*® RM IM" at 1, 3, 5 and 7 days of operation in continuous packed-bed reactor.

The original blends showed a prominent peak in the crystallization curve, which characterizes the trisaturated fraction of palm stearin and palm kernel oil, containing high melting point triacylglycerols. These results confirmed those of Humphrey, Moquin, and Narine (2003), which showed that an increase in the number of molecules with simultaneous crystallization leads to a larger energy release by the system. Enzymatic interesterification resulted in the appearance of other peaks in the crystallization curves, which is characteristic of the increase in the middle-melting-point TAG (SSO, SOO, and POS) content due to the exchange of acyl groups between TAGs. Also, the intensity and breadth of the first peak diminished, which is typical of a mixed crystallization process starting from sporadic, instantaneous nuclei (RIBEIRO *et al.*, 2009d). This was accompanied by a reduction in crystallization onset temperatures, enabling the interesterified samples to crystallize at lower temperatures than their original blend.

7.3.8. CRYSTALLINE MICROSTRUCTURE

The concept of microstructure comprises information on the state, quantity, shape, size, spatial relations, and interaction among all the components of the crystalline network. Microstructure influences fats' macroscopic properties enormously. The microstructural level (or mesoscale) of a fat crystalline network can be defined as the structures with dimensions between approximately 0.5 and 200 μm. Polarized light microscopy is the technique most used to visualize the microstructural network of fats and has been applied with a view to explaining differences in the texture of fat blends and to show crystalline types and morphological alterations in crystal growth. Figures 7.6 and 7.7 show crystalline structures obtained by slow crystallization at 25 °C of the blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive before and after enzymatic interesterification with "*Lipozyme*® TL IM" at 1, 2, 4, 6 and 8 days and "*Lipozyme*® RM IM" at 1, 3, 5 and 7 days.

The original blends produced spherulite-shaped crystals with maximum diameters of 40 μm. According to Shi *et al.* (2001), crystalline morphology is dominated by the triacylglycerol species with the highest melting point in a blend. The presence of large spherulites or spherulite clusters gives the fat an undesirable grainy texture and is a function mainly of trisaturated TAGs in the blend.

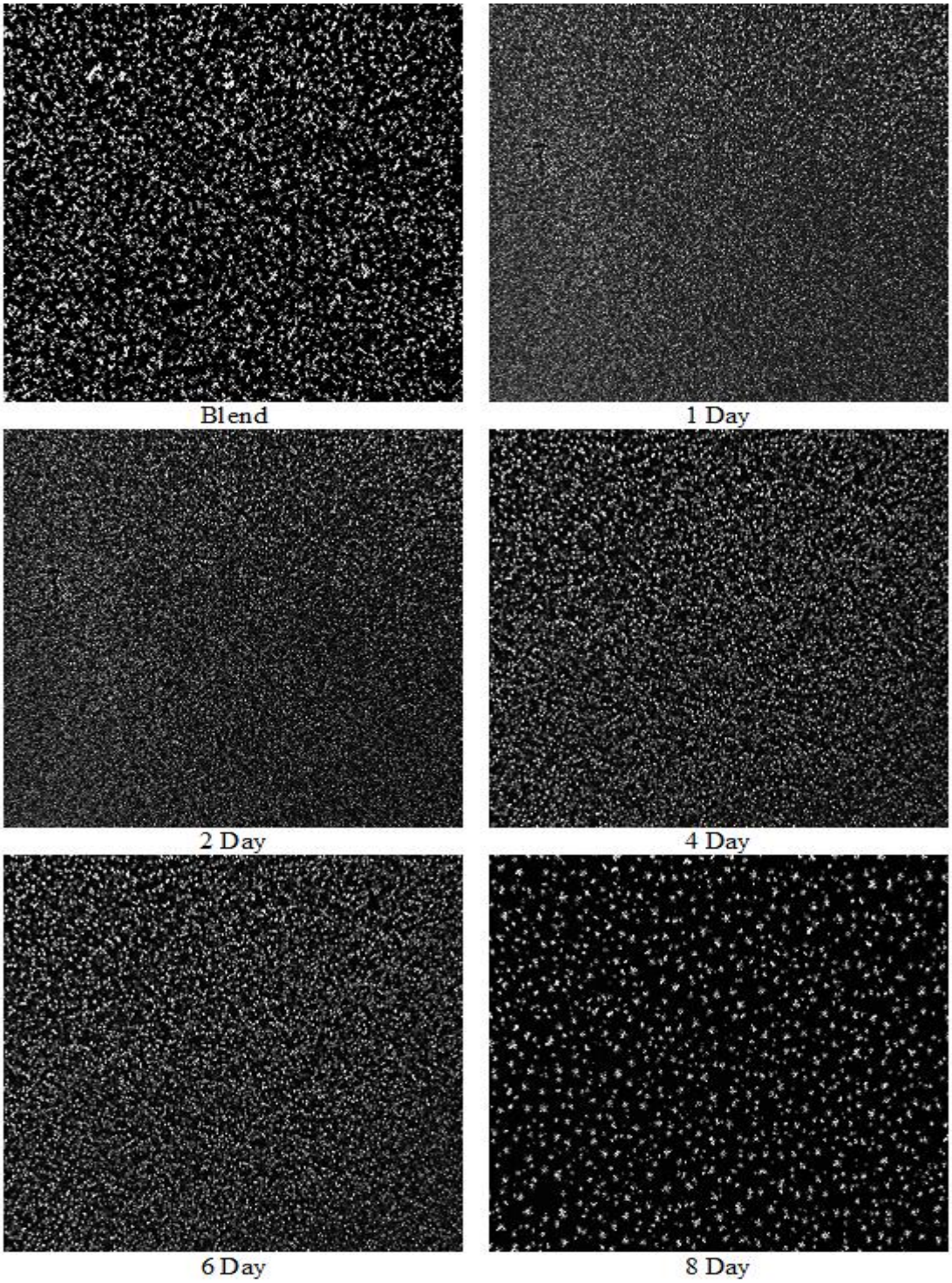


Figure 7.6. Images of the crystallization of the blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive before and after enzymatic interesterification with "Lipozyme® TL IM" at 1, 2, 4, 6 and 8 days of operation in continuous packed-bed reactor. at 25 ºC in static process.

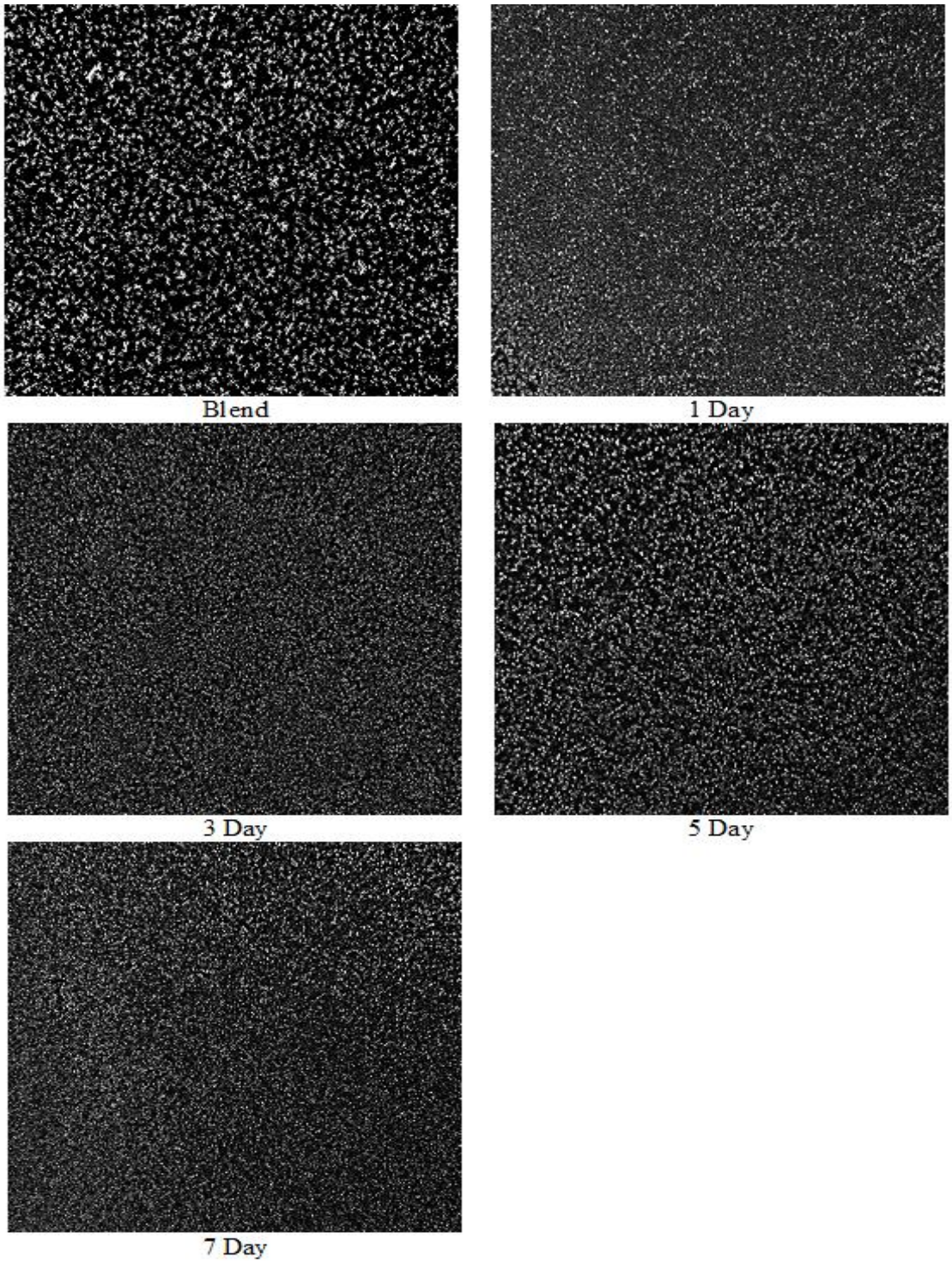


Figure 7.7. Images of the crystallization of the blend 45 % palm stearin, 30 % palm kernel oil and 25 % olive before and after enzymatic interesterification with "Lipozyme® RM IM" at 1, 3, 5 and 7 days of operation in continuous packed-bed reactor. at 25 ºC in static process.

After continuous enzymatic interesterification, the crystal morphology of the blend was completely modified: disk-shaped crystals, with similar granular crystalline structure, were observed. Interesterification produced significant reductions in crystal diameter in all samples. According to Pande and Akoh (2012) smaller crystals lead to firmer fats with smooth texture or mouthfeel adequate to margarine.

7.4. CONCLUSION

This study demonstrated that continuous enzymatic interesterification modified the triacylglycerol composition of the blend, and consequently the physicochemical properties. The enzymatic interesterification allows obtaining fats with various degrees of plasticity, increasing the possibilities for the commercial use of palm stearin, palm kernel oil, olive oil and their blends.

CONSIDERAÇÕES FINAIS

Em todas as misturas avaliadas, a interesterificação química e enzimática promoveram a diminuição nos teores de triacilgliceróis trissaturados e triinsaturados e aumento dos triacilgliceróis monossaturados-diinsaturados e dissaturados-monoinsaturados, o que resultou no respectivo decréscimo do ponto de fusão das amostras, consistência e do conteúdo de gordura sólida.

As misturas de estearina de palma, óleo de coco e óleo de canola tanto antes quanto após a interesterificação química, apresentaram interação eutética. Isto demonstra que a estearina de palma, óleo de coco e o óleo de canola apresentam certo grau de incompatibilidade no estado sólido.

Verificou-se que o alteração da composição de triacilgliceróis após a interesterificação química e enzimatica sobre as curvas de fusão e cristalização acarretou significativa alterações nos perfis das curvas. A estabilidade termica e a temperatura de oxidação foram dependentes da composição em ácidos graxos e independente da interesterificação química.

A metodologia de superfície de resposta foi aplicado com sucesso para modelar e optimizar as condições utilizadas na interesterificação de estearina de palma, óleo de palmiste e azeite de oliva, prevendo as melhores condições para aplicação da interesterificacao enzimatica continua.

Este estudo demonstrou que a mistura e interesterificação química e enzimática são um meio eficaz para modificar as propriedades de óleos e gorduras, permitindo obter gorduras com diferentes graus de plasticidade, aumentando as possibilidades de uso destas frações.

REFERÊNCIAS BIBLIOGRÁFICAS

ADHIKARI, P.; SHIN, J.A.; LEE, J.H.; HU, J.N.; AKOH, C.C.; LEE, L.T. Production of *trans*-free margarine stock by enzymatic interesterification of rice bran oil, palm stearin and coconut oil. **Journal of the Science of Food and Agriculture**, Sussex, v.90, n.4, p.703-711, 2010.

ADHIKARI, P.; HU, P. Enzymatic and chemical interesterification of rice bran oil, sheaolein, and palm stearin and comparative study of their physicochemical properties. **Journal of Food Science**. v.77, p.C1284-C1291, 2012.

AHMADI, L.; WRIGHT, A.J; MARANGONI, A.G. Chemical and enzymatic interesterification of tristearin/triolein-rich blends,chemical composition, solid fat content and thermal properties. **European Journal of Lipid Science and Technology**, Hoboken, v.110, p.1014–1024, 2008.

AHMADI, L.; WRIGHT, A.J.; MARANGONI, A.G. Structural and mechanical behavior of tristearin/triolein-rich mixtures and the modification achieved by interesterification. **Food Biophysics**, v. 4, p.64–76, 2009.

AINI, I.N.; MISKANDAR, M.S. Utilization of palm oil and palm products in shortenings and margarines. **European Journal of Lipid Science and Technology**, Hoboken, v.109, n.4, p.422-432, 2007.

AKOH, C.C. Fat replacers. **Food Technology**. v.52, n.3, p.47-53, 1998.

AKOH, C. C. Structured lipids. In C. C. AKOH, D. B. MIN (Eds.), **Food lipids chemistry, nutrition, and biology**. New York: Dekker, pp. 877–908, 2002.

ALIM, M. A., LEE, J. H., AKOH, C. C., CHOI, M. S., JEON, M. S., SHIN, J. A. Enzymatic transesterification of fractionated rice bran oil with conjugated linoleic acid: optimization by response surface methodology. **LWT Food Science and Technology**, v.41, p.764–770, 2008.

ALIM, A.M.; LEE, J.H.; LEE, K.T. Production of lipase-catalyzed solid fat from mustard oil and palm stearin with linoleic acid by response surface methodology. **Journal of Agricultural and Food Chemistry**, Washington, v.89, p.706-712, 2009.

AMERICAN OIL CHEMIST'S SOCIETY. **Official methods and recommended practices of the American Oil Chemists' Society**, Champaign: AOCS, 2009a. [Method Cd 5a-4: free fatty acid, in Official Methods and Recommended Practices of the AOCS].

AMERICAN OIL CHEMIST'S SOCIETY. **Official methods and recommended practices of the American Oil Chemists' Society**, Champaign: AOCS, 2009b. [Method Cd 8-53: peroxide value, in Official Methods and Recommended Practices of the AOCS].

AMERICAN OIL CHEMIST'S SOCIETY. **Official methods and recommended practices of the American Oil Chemists' Society**, Champaign: AOCS, 2009c. [Method Cd 1c-85: iodine value, in Official Methods and Recommended Practices of the AOCS].

AMERICAN OIL CHEMIST'S SOCIETY. **Official methods and recommended practices of the American Oil Chemists' Society**, Champaign: AOCS, 2009d. [Method Cc-3-25: softening point, in Official Methods and Recommended Practices of the AOCS].

AMERICAN OIL CHEMIST'S SOCIETY. ***Official methods and recommended practices of the American Oil Chemists' Society***, Champaign: AOCS, 2009e. [Method Cc-1-25: capillary melting point, in Official Methods and Recommended Practices of the AOCS].

AMERICAN OIL CHEMIST'S SOCIETY. **Official methods and recommended practices of the American Oil Chemists' Society**, Champaign, IL: AOCS, 2009f, (6th ed). [Method Cj 2-95: X-Ray Diffraction Analysis of Fats, in Official Methods and Recommended Practices of the AOCS].

American Oil Chemist's Society. **Official methods and recommended practices of the American Oil Chemists' Society**, Champaign, IL: AOCS, 2009g, (6th ed). [Method Cd-16b-93: solid fat content (SFC) by magnetic resonance (direct method), in Official Methods and Recommended Practices of the AOCS].

ANDRIKOPOULOS, N.K. Triglyceride species compositions of common edible vegetable oils and methods used for their identification and quantification. **Food Reviews International**, Oxford, v.*18*, p.71–102, 2002.

ARIFIN, N.; SOO-PENG, K.; LONG, K.; TAN C.P.; YUSOFF, M.S.A.; LAI O.M. Modeling and optimization of *Lipozyme* RM IM - catalyzed esterification of medium- and long-chain triacyglycerols (MLCT) using response surface methodology. **Food Bioprocess Technology**, v.5, p. 216–225, 2012.

ARONHIME, J. S. Application of thermal analysis (DSC) in the study of polymorphic transformations. **Thermochimica Acta**, v.134, p.1–14, 1998.

ASSUNÇÃO, M.L.; FERREIRA, H.S.; SANTOS, A.F.; CABRAL JR, C.R.; FLORÊNCIO, T.M.M.T. Effects of dietary coconut oil on the biochemical and anthropometric profiles of women presenting abdominal obesity, **Lipids**, Champaign, v.44, p. 593-601, 2009.

BALBINOT, E.L.; ARENHART, M.; BATISTA, C.P; PROCHNOWS, L.R; BLASI, T.C. A interesterificação como alternativa às implicações nutricionais negativas das gorduras *trans*. **Ciências da Saúde**, Santa Maria, v.10, n.1, p.31-44, 2009.

BARROS NETTO, B.; SCARMINIO, I. S.; BRUNS, R. E. **Como fazer experimentos; pesquisa e desenvolvimento na ciência e na indústria**. Campinas: Editora da UNICAMP, **2001**.

BARROS, R.R. Consumo de alimentos industrializados e fatores associados em adultos e idosos residentes no município de São Paulo**.** 2008, 176p. [Dissertação de Mestrado – Faculdade de Saúde Pública da Universidade de São Paulo].

BASIRON, Y. Palm oil production through sustainable plantations. **European Journal of Lipid Science and Technology**, Hoboken, v.109, n.4, p.289–295, 2007.

BERGER K.G.; IDRIS N.A. Formulation of zero-trans acid shortenings and margarines and other food fats with products of the oil palm. **Journal of the American Oil Chemists' Society**, Champaign, v.82, p.775-782, 2005.

BESSLER, T.R.; ORTHOEFER, F.T. Providing lubricity in food fat systems. **Journal of the American Oil Chemists' Society**, Champaign, v.60, n.10, p.1765-1768, 1983.

BHATNAGAR, A.S; PRASANTH KUMAR, P.K; HEMAVATHY, J.; GOLAPA KRISHNA, A.G. Fatty Acid Composition, Oxidative Stability, and Radical Scavenging Activity of Vegetable Oil Blends with Coconut Oil. **Journal of the American Oil Chemists' Society**, Champaign, v.86, p.991–999, 2009.

BLOCK, J.M. Strategies for the reduction of *trans* fat consumption in Brazil: Current Status and perspectives. **INFORM**. Champaign v.20, n.1, p.53-55, 2009.

BOSKOU, D. **Olive oil chemistry and technology**. AOCS Press, Champaign, IL., 1996, 154p.

BRAIPSON-DANTHINE, S.; GIBON, V. Comparative analysis of triacylglycerol composition, melting properties and polymorphic behavior of palm oil and fractions. **European Journal of Lipid Science and Technology**, Hoboken, v.109, n.4, p.359-372, 2007.

BRASIL. Ministério da Agricultura. Divisão de Inspeção de Produtos de Origem Animal (DIPOA). Portaria n° 372, de 04 de Setembro de 1997. Regulamento técnico para fixação de identidade e qualidade de margarina. **Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, n. 172, Seção 1, p. 19702, 1997.

BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução n.360 de 23 de setembro de 2003. Aprova o regulamento técnico sobre rotulagem nutricional de alimentos embalados, tornando obrigatória à rotulagem nutricional. **Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, n. 180, Seção 1. p. 5, 2003.

BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). RDC nº 270, de 22 de setembro de 2005. Regulamento técnico para óleos vegetais, gorduras vegetais e creme vegetal. **Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, n. 184, Seção 1. p. 372, 2005.

BUCHGRABER, M.; ULBERTH, F.; EMONS, H.; ANKLAN, E. Triacylglycerol profiling by using chromatographic techniques. **European Journal of Lipid Science and Technology**, Hoboken, v.106, p.621–648, 2004.

CAMPOS, R. Experimental methodology. In A. G. MARANGONI (Ed.), **Fat crystal networks**. New York: Marcel Dekker, pp.267–349, 2005.

CANAPI, E.C.; AUGUSTIN, Y.T.V.; MORO, E.A.; PEDROSA, E.JR.; BENDAÑO, M.L., 2005. Coconut Oil. In: **Bailey's industrial oil and fat products**. 6th Edition, Volume 1 - Edible Oil and Fat Products: Chemistry, Properties, and Health Effects. Shahidi, F. (Ed). Wiley-Interscience.

CARO, Y; TURON, F; VILLENEUVE, P; PINA, M; GRAILLE, J. Enzymatic synthesis of medium-chain triacylglycerols by alcoholysis interesterification of copra oil using a crude papain lipase preparation. **European Journal of Lipid Science and Technology**, Hoboken, v. 106, n. 8, p. 503-512, 2004.

CHALEEPA, K.; SZEPES, A.; ULRICH, J. Dry fractionation of coconut oil by melt crystallization. chemical engineering research and design. **Chemistry and Physics of Lipids**, v.163, p.1217–1222, 2010.

CHE MAN, Y.B.; MARINA, A.M. Medium chain triacylglycerol. In F. Shahidi (Ed.), **Nutraceutical and specialty lipids and their coproducts**. Boca Raton: Taylor & Francis Group, p.27-56, 2006.

CHEN, C.W.; CHONG, C.L.; GHAZALI, H.M.; LAI, O.M. Interpretation of triacylglycerol profiles of palm oil, palm kernel oil and their binary blends. **Food Chemistry**, Oxford, v.100, p.178–191, 2007.

CHIARA, V.L.; SICHIERI, R.; CARVALHO, T.D.S.F.D. Teores de ácidos graxos *trans* de alguns alimentos consumidos no Rio de Janeiro. **Revista de Nutrição**, Rio de Janeiro, v.16, n.2, p.227-233, 2003.

CHIU,M.C. **Sínteses de lipídeos estruturados por interesterificação de gorduras de frango com e triacilgliceróis de cadeia média.** São Paulo, 2006. 176p. [Tese de Doutoramento – Faculdade de Ciências Farmacêuticas da Universidade de São Paulo].

CHO, F, deMAN, J., ALLEN, O. B. Physical properties and composition of low *trans* canola/palm blends modified by continuous enzymatic interesterification. **Elaies,** v.6, p. 39–49, 1994.

CHONG, C.L.; KAMARUDIN, Z.; LESIEUR, P.; MARANGONI, A.; BOURGAUX, C.; OLLIVON, M. Thermal and structural behaviour of crude palm oil: Crystallisation at very slow cooling rate. **European Journal of Lipid Science and Technology**, Hoboken, v.109, n.4, p.410-427, 2007.

CHRYSAM, M.M. Margarines and spreads, in **Bailey's Industrial Oil and Fat Products**, Vol. 3, 5th ed., HUI, Y.H., Ed., Wiley-Interscience, New York, p 67–68, 92–96, 2002.

CHU, B.S.; GHAZALI, H.M.; LAI, O.M.; MAN, Y.B.C.; YUSOF, S. Physical and chemical properties of a lipase-transesterified palm stearin/palm kernel olein blend and its isopropanol-solid and high melting triacylglycerol fractions. **Food Chemistry**, Oxford, v.76, n.2, p.155-164, 2002.

CODEX ALIMENTARIUS COMMISION. **Codex Standard for Named Vegetable Oils**., Codex Stand 210, p.24–45, 2004.

CORREIA, A.C.; FERREIRA-DIAS, S. The effect of impurities of crude olive residue oil on the operational stability of the *Candida rugosa* lipase immobilized in polyurethane foams, In: BALLESTEROS, A.; PLON, F.J.; IBORRA, J.L.; HALLING, P.J (Eds). **Stability and Stabilization of Biocatalysts**. Amsterdam:Elsevier Science, B.V., p.71-76, 1998.

COSTALE-RODRIGUES, R; GIBON, V.; VERHE, R.; DE GREYT, W. Chemical and enzymatic interesterification of a blend of palm stearin: soybean oil for low *trans*-margarine formulation. **Journal of the American Oil Chemists' Society**, Champaign, v.86, p.681–697, 2009.

CRIADO, M.; HERNÁNDEZ-MARTIN, E.; LÓPEZ-HERNÁNDEZ, A.; OTERO, C. Optimized interesterification of virgin olive oil with a fully hydrogenated fat in a batch reactor: Effect of mass transfer limitations. **European Journal of Lipid Science and Technology,** Hoboken, v.109, n.5, p.474-485, 2007a.

CRIADO, M.; HERNANDEZ-MARTIN, E.; LÓPEZ-HERNÁNDEZ, A.; OTERO. C. Enzymatic interesterification of extra virgin olive oil with a fully hydrogenated fat: Characterization of the reaction and its products. **Journal of American Oil Chemists' Society**, v.84, p.717–726, 2007b.

CRIADO, M.; HERNÁNDEZ-MARTIN, E.; LÓPEZ-HERNÁNDEZ, A.; OTERO, C. Enzymatic interesterification of olive oil with fully hydrogenated palm oil: Characterization of fats. **European Journal of Lipid Science and Technology,** Hoboken, v.110, n.8, p.714-724, 2008.

D'AGOSTINI, D. Obtenção de lipídios estruturados por interesterificação de triacilgliceróis de cadeia média e longa. São Paulo, 2001. 168p. [Tese de Doutoramento – Faculdade de Ciências Farmacêuticas da Universidade de São Paulo].

D'AGOSTINI, D.; L.A GIOIELLI, Distribuição estereoespecífica de lipídios estruturados a partir de gorduras de palma, palmiste e triacilgliceróis de cadeia média. **Revista Brasileira Ciências Farmacêuticas**, v.38, p.345-354, 2002.

DALLA-VECCHIA, R.; NASCIMENTO, M.D.; SOLDI, V. Synthetic applications of immobilized lipases in polymers. **Quimica Nova**, São Paulo, v.27, n.4, p.623-630, 2004.

de ARAUJO, G.L.B.; de FARIA, D.L.A.; ZAIM, M.H.; CARVALHO, F.M.D.; de ANDRADE, F.R.D.; MATOS, J.D.R. Thermal studies on polymorphic structures of tibolone. **Journal of Thermal Analysis and Calorimetry**, London, v.102, n.1, p.233-241, 2010.

de CASTRO, H.F.; MENDES, A.A.; dos SANTOS, J.C.; de AGUIAR, C.L. Modificação de óleos e gorduras por biotransformação. **Quimica Nova**, São Paulo, v.27, n.1, p.146-156, 2004.

DE CLERCQ, N.; DANTHINE, S.; NGUYEN, M.T.; GIBON, V.; DEWETTINCK, K. Enzymatic Interesterification of Palm Oil and Fractions: Monitoring the Degree of Interesterification using Different Methods. **Journal of the American Oil Chemists' Society**, Champaign, v.89, n.2, p.219-229, 2011.

DE GREYT, W.F.J., KELLENS, M.J. Improvement of the nutritional and physicochemical properties of milk fat. In: GUNSTONE, F.D. **Structured and modified lipids**. Marcel Dekker: New York, 2001. p. 285-312.

de LEON, S.Y; DELORES, M.I. Coconut. In: BARRET, D.M.; SOMOGYI, L.; RAMASWAMY, H. P**rocessing Fruits: Science and Technology**. 2 ed. Boca Raton: CRC Press, 2005. p.110-127.

de OLIVEIRA, A.G.; SCARPA, M.V.; CORREA, M.A.; CERA, L.F.R.; FORMARIZ, T.P. Microemulsions: Structure and application as drug delivery systems. **Quimica Nova**, São Paulo, v.27, n.1, p.131-138, 2004.

de ROOS, N.; SCHOUTEN, E.; KATAN, M. . Consumption of a solid fat rich in lauric acid results in a more favorable serum lipid profile in healthy men and women than consumption of a solid fat rich in trans -fatty acids. **The Journal of Nutrition.** Penn State, v.131, n.2, p.242-245, 2001

DEBNATH, S.; RAGHAVARAO, K.S.M.S.; LOKESH, B.R. Hydrodynamic, thermo-analytical and molecular structural investigations of enzyme interesterified oil and its thermo-oxidative stability by thermogravimetric analysis. **Journal of Food Engineering**, Ontario, v.105, n. 4, p.671-679, 2011.

DEMAN, J.M. Consistency of fats: a review. **Journal of the American Oil Chemists' Society**, Champaign, v.60, p.82-87,1983.

DEMAN, L.; DEMAN, J.M.; BLACKMAN, B. Physical and textural evaluation of some shortenings and margarines. **Journal of the American Oil Chemists' Society**, Champaign, v.66, n.1 p.128-132, 1989.

DIAN, N.L.H, SUNDRAN K., IDRIS, N.A. Effect of chemical interesterification on triacylglycerol and solid fat contents of palm stearin, sunflower oil and palm kernel olein blends. **European Journal of Lipid Science and Technology**, Hoboken, v.109, p.147–156, 2007.

DIAN, N.L.H.M.; KALYANA, S.; IDRIS, N.A. Effect of chemical interesterification on triacylglycerol and solid fat contents of palm stearin, sunflower oil and palm kernel olein blends. **European Journal of Lipid Science and Technology**, Hoboken, v.109, n.2, p.147-156, 2007.

DOURTOGLOU, V.G.; DOURTOGLOU, T.; ANTONOPOULOS, A.; STEFANOU, E.; LALAS, E.; POULOS, C. Detection of olive oil adulteration using principal component analysis applied on total and regio FA content. **Journal of the American Oil Chemists' Society**, Champaign, v.80, n.3, p.203-208, 2003.

DWECK, J.; SAMPAIO, C.M.S. Analysis of the thermal decomposition of commercial vegetable oils in air by simultaneous TG/DTA. **Journal of Thermal Analysis and Calorimetry**, London, v.75, n.2, p.385-391, 2004.

FARMANI, J.; SAFARI, M.; HAMEDI, M. Application of palm olein in the production of zero-*trans* Iranian vanaspati through enzymatic interesterification. **European Journal of Lipid Science and Technology**, Hoboken, v.108, n.8, p. 636-643, 2006.

FARMANI, J.; SAFARI, M.; HAMEDI, M. Trans-free fats through interesterification of canola oil/palm olein or fully hydrogenated soybean oil blends, **European Journal of Lipid Science and Technology,** Hoboken, v.111, p.1212–1220, 2009.

FAUR, L. Interesterification Technology In: KARLSESKIND, A.; WOLFF, J.P. (Eds.). **Oils and fats Manual**., London, Paris & New York: Association Française pour l'Étude des Corps Gras, pp.923-995, 1996.

FAUZI, S.H.M.; NORIZZAH, A.R.; ZALIHA, O. Effects of chemical interesterification on the physicochemical, microstructural and thermal properties of palm stearin, palm kernel oil and soybean oil blends. **Food Chemistry**, Oxford, v.137, p.8–17, 2013.

FLOTER, E; van DUIJIN, G. In: Trans-free Fats for Use in Food in Modifying Lipids for Food Use (edited by FG Gunstone). New York: CRC Press., 2006, pp. 429– 442.

FOMUSO, L. B., AKOH, C. C. Lipase-catalyzed acidolysis of olive oil and caprylic acid in a bench-scale packed bed bioreactor. **Food Research International**, v.35, p.15–21, 2002.

FOUBERT, I.; DEWETTINCK, K.; JANSSEN, G.; VANROLLEGHEM, P. A. Modelling two step isothermal fat crystallization. **Journal of Food Engineering**, Ontario, v.75, p.551–559. 2006.

FOUBERT, I.; DEWETTINCK, K.; VAN DE WALK, D.; DIJKSTRA, A.J.; QUINN, P.J. Physical properties, Structural and physical characteristics. In GUNSTONE, F. D., HARWOOD, J. L., DIJKSTRA, A. J. (Eds.), **The lipid handbook**. Boca Raton, CRC Press, p.471-508, 2007.

GAMBOA, O.W.D., GIOIELLI, L.A. Consistencia de lípidos estructurados a partir de aceite de pescado y grasa de palmiste. **Grasas y Aceites**, v. 54, n. 2, p. 122-129, 2003a.

GAMBOA, O.W.D., GIOIELLI, L.A. Lípidos estructurados obtenidos por interesterificación química y enzimática a partir de aceite de pescado y grasa de semilla de palma. *Grasas y Aceites*, v. 54, n. 2, p. 161-168, 2003b.

GAMBOA, O.W.D., GIOIELLI, L.A. Comportamento de cristalização de lipídios estruturados obtidos a partir de gordura de palmiste e óleo de peixe. **Química Nova**, São Paulo, v. 29, n. 4, p. 646-653, 2006.

GEE, P.T. Analytical characteristics of crude and refined palm oil and fractions. **European Journal of Lipid Science and Technology**, Hoboken, v.109, n 4, p.373-379, 2007.

GHAZALI, H. M., HAMIDAH, S., CHE MAN, Y. B. Enzymatic transesterification of palm olein with nonspecific and 1,3-specific lipases. **Journal of the American Oil Chemists' Society**, v.72, p.633–639, 1995.

GHOTRA B.S.; DYAL, S.D.; NARINE S.S. Lipid shortenings, a review. **Food Reviews International**, Oxford, v.35, p.1015–1048, 2002.

GIBON, V.; DE GREYT, W.; KELLENS, M. Palm oil refining. **European Journal of Lipid Science and Technology**, Hoboken, v.109**,** n.4, p.315–335, 2007.

GIOIELLI, L.A. Misturas de óleos e gorduras na formulação de produtos gordurosos. **Revista Óleos & Grãos**, São Caetano do Sul, n.32, p.24-28, 1996.

GIOIELLI, L.A. Interesterificação de óleos e gorduras. **Revista Engenharia de Alimentos**, São Paulo, v.4, n.21, p.22-24, 1998.

GIOIELLI, L.A. Lipídios estruturados. In: CURI, R., POMPÉIA, C., MIYASAKA, C.K. PROCOPIO, J., eds. **Entendendo a gordura: os ácidos graxos**. São Paulo: Manole, 2002. cap.33, p.457-465.

GIOIELLI, L.A., PITOMBO, R.N.M., VITOLO, M., BARUFFALDI, R., OLIVEIRA, M.N., MORENO, P.C. Enzymatic hydrolysis of oils and fats. **Rivista Italiana Sostanze Grasse**, Milano, v.72, n.3, p.115-117, 1995.

GIOIELLI, L.A.; SIMÕES, I.S.; RODRIGUES, J.N. Crystal morphology and interactions of binary and ternary mixtures of hydrogenated fats. **Journal of Food Engineering**, Ontario, v. 57, n.4, p. 347-355, 2003.

GOLD, I.L.; UKHUN, M.E.; AKOH, C.C. Characteristics of eutectic compositions of restructured palm oil olein, palm kernel oil and their mixtures. **Journal of the American Oil Chemists' Society**, Champaign, v.88, n.11, p.1659 – 1667, 2011.

GRASEFE, R. Qual a origem da margarina. **Revista Super Interessante**. v.61, 1992. Disponível em http://super.abril.com.br/alimentacao/qual-origem-margarina-440459.shtml>. Acesso em 20 de abril de 2011.

GRIMALDI, R. **Alternativa tecnológica para produção de gorduras especiais**. 1999. 162p. [Tese de Doutorado – Faculdade de Engenharia de Alimentos - UNICAMP].

GUNSTONE, F.D. Vegetable sources of lipids. In: GUNSTONE, F.D (Ed). **Modifying lipids for use in food**. Boca Raton: CRC Press, 2006. p.11-27.

GUNSTONE, F.D.; J.L. HARWOOD, J.L. Occurrence and characterization of oils and fats. In: **The lipid handbook**. Eds. F.D, GUNSTONE, J.L. HARWOOD, A.J. DIJKSTRA, Chapman and Hall, London, p.137–141, 2007.

HAALAND, P.D. **Experimental design in biotechnology. Statistics: Textbooks and monographs**, Marcel Dekker, Inc., New York (USA), 1989.

HAIGHTON, A. J. The measurement of the hardness of margarine and fats with cone penetrometers. **Journal of the American Oil Chemists' Society,** Champaign, v.36, p. 345–348, 1959.

HAMAN, F., SHAHIDI, F. Enzymatic incorporation of capric acid into a single cell oil rich in docosahexaenoic acid and docosapentaenoic acid and oxidative stability of the resultant structured lipid. **Food Chemistry**, v.91, p.583–591, 2005.

HARTMAN, L.; LAGO, R.C.A. Rapid preparation of fatty acid methyl esters from lipids. **Laboratory Practice**, v.22, p.475–476, 1973.

HASSEL, R.L. Thermal-Analysis - Alternative Method Of Measuring Oil Stability. **Journal of the American Oil Chemists' Society**, Champaign, v.53, n.5, p.179-181, 1976.

HAYATI, I.N.; AMINAH, A.; MAMOT, S.; AINI, I.N.; LIDA, H.M.N.; SABARIAH, S. Melting characteristic and solid fat content of milk fat and palm stearin blends before and after enzymatic interesterification. **Journal of Food Lipids**, Trumbull, v.7, n.3, p.175-193, 2000.

HELMAN, C. G. **Cultura, saúde e doença**. 4. ed. Porto Alegre: Artmed, 2003.

HERRERA, M. L.; FALABELLA, C.; MELGAREJO, M.; ANÓN, M. C. Isothermal crystallization of hydrogenated sunflower oil: I – Nucleation. **Journal of American Oil Chemists' Society,** Champaign, v.75, p.1273–1280, 1998.

HOLM, H.C.; COWAN, D. The evolution of enzymatic interesterification in the oils and fats industry. **European Journal of Lipid Science and Technology**, Hoboken, v.110, p.679–691, 2008.

HOUMÖLLER, L.P.; KRISTENSEN, D.; ROSAGER, H. Determination of SFC, FFA, and equivalent reaction time for enzymatically interestified oils using NIRS. **Talanta**, v.71, p.868–873, 2007.

HUI, Y.H. **Bailey's industrial oil and fat products**. 5. ed., New York: Wiley-Interscience, 1996. v.2: p.603-654; v.3: p.1-63, p.65-114.

HUMPHREY, K. L.; MOQUIN, P. H. L.; NARINE, S. S. Phase behavior of a binary lipid shortening system: From molecules to rheology. **Journal of American Oil Chemists' Society**, Champaign, v. 80, p. 1175–1182, 2003.

HUMPHREY, K. L.; NARINE, S. S. A comparison of lipid shortening functionality as a function of molecular ensemble and shear: Crystallization and melting. **Food Research International**, Amsterdam, v. 37, p. 11–27, 2004.

IDA, A.M.D.N.. SUNDRAM, K.. SIEW, W.L.; AMINAH, A.; MAMOT, S. TAG composition and solid fat content of palm oil, sunflower oil and palm kernel olein blends before and after chemical interesterification. **Journal of American Oil Chemists' Society**, Champaign, v.79, p.1138–1143, 2002.

IDRIS, N.A.; DIAN, N.L.H.M. Interesterified palm products as alternatives to hydrogenation. **Asia Pacific Journal of Clinical Nutrition**, v.14, n.4, p.396-401, 2005.

ISO 3656:2002: Animal and vegetable fats and oils – Determination of ultraviolet absorbance expressed as specific UV extinction. **International Organization for Standardization**, Genève (Switzerland), p.1–6, 2002.

ISO 3656:2002: Animal and vegetable fats and oils – Determination of ultraviolet absorbance expressed as specific UV extinction. **International Organization for Standardization**, Genève (Switzerland), p.1–6, 2002.

ISO 3960:2007: Animal and vegetable fats and oils - Determination of peroxide value - Iodometric (visual) endpoint determination. **International Organization for Standardization**, Genève (Switzerland), p.1–9, 2007.

ISO. Standard 5509 Animal and Vegetable Fats and Oils Preparation of Methyl Esters of Fatty Acids, **International Organization for Standardization**, Geneva, 2000

IWASAKI, Y.; YAMANE, T. Enzymatic synthesis of structured lipids. **Journal of Molecular Catalysis B: Enzymatic**, Amsterdam, v.10, n.1-3, p.129–140, 2000.

JAEGER, K.E.; REETZ, M.T. Microbial lipases form versatile tools for biotechnology. **Trends in Biotechnology**, Oxford, v.16, n.9, p.396-403, 1998.

JANSEN, M.; BIRCH, J. Composition and stability of olive oil following partial crystallization, **Food Research International**, v.42, p.826-831, 2009.

JAYADAS, N.H.; PRABHAKARAN NAIR, K. Coconut oil as base oil for industrial lubricants—evaluation and modification of thermal, oxidative and low temperature properties. **Tribology International**, v.39, p.873–878, 2006.

JENNINGS, B.H; AKOH, C.C. *Trans*-free plastic shortenings prepared with palm stearin and rice bran oil structured lipid. **Journal of American Oil Chemists' Society**, Champaign, v.87, p.411-417, 2010.

JENSEN, R. G. Infant formulas. In F. D. Gunstone (Ed.), **Structured and modified lipids**. New York: Dekker, p. 455–464, 2001.

JEYARANI, T.; KHAN, M.I.; KHATOON, S. Trans-free plastic shortenings from coconut stearin and palm stearin blends. **Food Chemistry**, Oxford, v.114, p.270-275, 2009.

JEYARANI, T.; YELLA REDDY, S. Preparation of plastic fats with zero *trans* FA from palm oil. **Journal of the American Oil Chemists' Society**, Champaign, v.80, p. 1107-1113, 2003.

KALLIO, H.; YLI-JOKIPII, K.; KURVINEN, J.P.; SJÖVALL, O.; TAHVONEN, R. Regioisomerism of triacylglycerols in lard, tallow, yolk, chicken skin, palm oil, palm olein, palm stearin, and a transesterified blend of palm stearin and coconut oil analyzed by tandem mass spectrometry. **Journal of Agricultural Food Chemistry**, Washington, v.49, n.7, p.336-369, 2001.

KARABULUT, I.; TURAN, S.; ERGIN, G. Effects of chemical interesterification on solid fat content and slip melting point of fat/oil blends. **European Journal of Lipid Science and Technology**, Hoboken, v.218, p.224–229, 2004.

KARABULUT, I.; TURAN, S.; VURAL, H.; KAYAHAN, M. Human milk fat substitute produced by enzymatic interesterification of vegetable oil blend. **Food Technology and Biotechnology**, Zagred, v.45, p.434-438, 2007.

KARABULUT, I.;TURAN, S. Some properties of margarines and shortenings marketed in Turkey. **Journal of Food Comp. Analytic**, v.19, p.55–58, 2006.

KARUPAIAH, T., SUNDRAM, K. Effects of stereospecific positioning of fatty acids in triacylglycerol structures in native and randomized fats: a review of their nutritional implications. **Nutrition & Metabolism**, Londres, v.4, n.16, p.1-17, 2007.

KAWANARI, M. Butter's characteristics: Effect of processing. **INFORM**, Champaign, v.7, p.1104-1110, 1996.

KELLENS, M.; GIBON, V.; HENDRIX, M.; DE GRYT, W. Palm oil fractionation. **European Journal of Lipid Science and Technology**, Hoboken, v.109, n.4, p.336–349, 2007.

KHATOON, S.; REDDY, S.R.Y. Plastic fats with zero trans fatty acids by interesterification of mango, mahua and palm oils. **European Journal of Lipid Science and Technology**, Hoboken, v. 107, n. 11 p. 786-791, 2005.

KIM, B.H.; AKOK, C.C. Chemical and Physical Properties of Butterfat-Vegetable Oil Blend Spread Prepared with Enzymatically Transesterified Canola Oil and Caprylic Acid, **Journal of Agricultural and Food Chemistry**, Washington, v.53, p.4954-4961, 2005.

KIM, B.H.; LUMOR, S.E; AKOH, C.C. *trans*-Free margarines prepared with canola oil/palm stearin/palm kernel oil-based structured lipids. **Journal of Agricultural and Food Chemistry**, Washington, v.56, p.8195–8205, 2008.

KIM, J. S.; MAENG, I. K.; KIM, C. K.; KWON, Y. J.; LEE, B. O.; KIM, Y. J. Lipase catalyzed acidolysis of butterfat with R-linolenic acid from perilla oil. **Food Science Biotechnology**, v.*11*, p.66-70, 2002.

KLOEK, W.; WALSTRA, P.; VAN VLIET, T. Crystallization kinetics of fully hydrogenated palm oil in sunflower oil mixtures. **Journal of American Oil Chemists' Society,** Champaign, v.77, p.389–398, 2000.

LARQUÉ, E. ZAMORA, S.; GIL, A. Dietary *trans* fatty acids in early life: a review. **Early Human Development**, Clare, v.65, n.2, p.31s-41s, 2001.

LAWLER, P.;DIMICK, P. Crystallization and polymorphism of fats. In AKOH, C., MIN, D. B., (Edis), **Food lipids: Chemistry, nutrition and biotechnology**, Boca Raton: CRC Press, 2002.

LEE, J.H.; AKOH, C.C.; LEE, K.T. Physical properties of *trans*-free bakery shortening produced by lipase-catalyzed interesterification. **Journal of the American Oil Chemists' Society**, Champaign, v.85, n.1, p.1-11, 2008.

LEE, J.H; AKOH, C.C; HIMMELSBACH, D.S; LEE, K.T. Preparation of interesterified plastic fats from fats and oils free of trans fatty acid. **Journal of Agricultural and Food Chemistry**, v. 56, n. 11, p.4039–4046, 2008.

LIDA, H.M.D.N; SUNDRAM, K.; SIEW, W.L.; AMINAH, A.; MAMOT, S. TAG composition and solid fat content of palm oil, sunflower oil, and palm kernel olein blends before and after chemical interesterification. **Journal of the American Oil Chemists Society**, Champaign, v.79, n.11, p.1137-1144, 2002.

LIN, S.W. Palm Oil. In: F.D.GUSNTONE. **Vegetable oils in food technology: Composition, Properties and Uses**, 2002, 59-97.

LIST, G.R.; MOUNTS, T.L.; ORTHOEFER, F.; NEFF, W.E. Margarine and shortening oils by interesterification of liquid and trisaturated triglycerides. **Journal of American Oil Chemists' Society**, Champaign, v.72, p.379–382, 1995.

LITWINENKO, J.W.; ROJAS, A. M.; GERSCHENSON, L. N.; MARANGONI, A.G. Relationship between crystallization behavior, microstructure, and mechanical properties in a palm oil-based shortening. **Journal American Oil Chemist' Society**, Champaign, v.79, n.7, p.647–654, 2002.

LIU, H. R.; WHITE, P. J. Oxidative stability of soybean oils with altered fatty acid compositions. **Journal of the American Oil Chemists' Society**, Champaign, v.69, p.528–532, 1992.

LIU, Y.F; MENG, Z.; ZHANG, F.Q.; SHAN, L.; WANG, X.G. Influence of lipid composition, crystallization behavior and microstructure on hardness of palm oil-based margarines. **European Food Research and Technology**, New York, v.230, n.5, p.759-767, 2010.

LONG, K.; ZUBIR, I.; HUSSIN, A.B.; IDRIS, N.; GHAZALI, H.M.; LAI, O.M. Effect of enzymatic transesterification with flaxseed oil on the high-melting glycerides of palm stearin and palm olein. **Journal of the American Oil Chemists' Society**, Champaign, v.80, n.2, p.133-137, 2003.

LUMOR, S.E.; JONES, K.C.; ASHBY, R.; STRAHAN, G.D.; KIM, B.H.; LEE, G.C.; SHAW, J.F.; KAYS, S.E.; CHANG, S.W.; FOGLIA, T.A.; AKOH, C.C. Synthesis and characterization of canola oil-stearic acid-

based *trans*-free structured lipids for possible margarine application. **Journal of Agricultural and Food Chemistry**, Washington v.55, n.26, p.10692–10702, 2007.

MARANGONI, A. G. Crystallization kinetics. In A. G. MARANGONI (Ed.), **Fat crystal networks**. New York: Marcel Dekker, p.21-83, 2005.

MARANGONI, A.G. LENCKI, R.W. Ternary Phase Behavior of Milk Fat Fractions**. Journal of Agricultural and Food Chemistry**, Champaign, v.46, p.3879-3884, 1998.

MARANGONI, A.G., ROUSSEAU, D. Engineering triacylglycerols: the role of interesterification. **Trends Food Science Technology**, v.6, n.10, p.329-335, 1995.

MARIKKAR, J.M.N.; LAI, O.M.; GHAZALI, H.M.; MAN, Y.B.C. Detection of lard and randomized lard as adulterants in refined-bleached-deodorized palm oil by differential scanning calorimetry. **Journal of the American Oil Chemists' Society**, Champaign, v.78, n.11, p.1113-1119, 2001.

MARTIN, C.A.; MATSHUSHITA, M.; SOUZA, N.E. Ácidos graxos *trans*: implicações nutricionais e fontes da dieta. **Revista da Nutrição**, Campinas, v.17, n.3, p.361-368, 2004.

MASSIELLO, F.J. Changing trends in consumer margarines. **Journal of the American Oil Chemists' Society**, Champaign, v. 55, n.2, p.262-265, 1978.

MATOS, J.R. ; MERCURI, L.P. ; ARAÚJO, G.L.B. . Análise Térmica Aplicada a Fármacos e Medicamentos. In: STORPIRTIS, S.; GONÇALVES, J.E.; CHIANN, C.; GAI, M.N. (Org.). **Ciências Farmacêuticas: Biofarmacotécnica.** 1ªed.Rio de Janeiro: Guanabara Koogan S.A., p. 32-65, 2009.

MATOS, J.R.; MACHADO, L.D.B. Análise térmica – termogravimetria. In: CANEVAROLO JUNIOR., S.V.,(Ed.). **Técnicas de caracterização de polímeros**. São Paulo: Artliber, p.209-228, 2004.

MENARD, K.F.; SICHINA, W.J. Prediction of solid fat index (SFI) values of food fats using DSC. **Perkin Elmer Application Note**, 1-3, 2000.

MENSINK, R.P.; KATAN, M.B. Effect of dietary *trans* fatty acids on high-density and low density lipoprotein cholesterol levels I healthy subjects. The New England *Journal* of *Medicine*, Massachusetts, v.323, n. 7, p.439-450, 1990.

MING, C.C; GIOIELLI, L.A; SOLIS, V.E.S. Fracionamiento de la grasa abdominal de pollo. **Grasas y Aceites**, v. 53, n. 3, p. 298-303, 2002.

MING, L.O.; GHAZALI, H.M.; LET, C.C. Use of enzymatic transesterified palm stearin-sunflower oil blends in the preparation of table margarine formulation. **Food Chemistry**, v.64, p.83–88, 1999.

MISKANDAR, M.S.; MAN, Y.B.C.; YUSOFF, M.S.A.; RAHMAN, R.A. Effect of emulsion temperature on physical properties of palm oil-based margarine. **Journal of the American Oil Chemists' Society**, Champaign, v.79, n.12 p.1163-1168, 2002.

MONTGOMERY, D.C. **Design and analysis of experiments**, John Wiley and Sons, New York (USA), 1991.

MOUNTS, T. L.; WARNER, K.; LIST, G. R.; KLEIMAN, R.; FEHR, W. R.; HAMMOND, E. G. Effect of altered fatty acid composition on soybean oil stability. **Journal of the American Oil Chemists' Society**, Champaign, v.65, p.624–628, 1998.

MOUSTAFA, A. Consumer and industrial margarines. In: **Practical handbook of soybean processing and utilization**. Ed. D.R. ERICKSON, AOCS Press and United Soybean Board, Champaign, IL (USA), p. 339–362, 1995.

MU, H., KALO, P., XU, X., HOY, C.E. Chromatographic methods in the monitoring of lipase-catalyzed interesterification. **European Journal of Lipid Science and Technology**, Hoboken, v.102, n.3, p.202-11, 2000.

MU, H.; XU, X.; HOY, C.E. Production of specific-structured triacylglycerols by lipase-catalyzed interesterification in a laboratory-scale continuous reactor. **Journal of American Oil Chemists' Society**, v.75, p.1187–1193, 1998.

NARINE, S. S.; HUMPHREY, K. L.; BOUZIDI, L. Modification of the Avrami model for application to the kinetics of the melt crystallization of lipids. **Journal of American Oil Chemists' Society**, Champaign, v.83, p.913–921, 2006.

NARINE, S.S., MARANGONI, A. G. Relating structure of fat crystal networks to mechanical properties: a review. **Food Research International**, Amsterdam, v. 32, n. 4, p. 227-248, 1999a.

NARINE, S.S.; MARANGONI, A. G. Microscopic and rheological studies of fat crystal networks. **Journal of Crystal Growth**, North Holland, v.198/199, n. 2, p;1315- 1319, 1999b.

NARINE, S.S.; MARANGONI, A.G. In A. G. MARANGONI (Ed.), **Fat crystal networks**. New York: Marcel Dekker, p.179, 2005.

NASCIMENTO, A.C., TECELÃO, C.S.R., GUSMÃO, J.H., da FONSECA, M.M.R., FERREIRA DIAS, S. Modelling lipase-catalysed transesterification of fats containing *n*-3 fatty acids monitored by their Solid Fat Content, **European Journal of Lipid Science and Technology**, Hoboken, v.106, n.9, p.599-612, 2004.

NASIRULLAH, R.S., UMESHA, S.S.; REDDY, S.Y.. Development of chemically interesterified healthy coconut oil blends. **International Journal of Food Science and Technology**, v.45, p.1395–1402, 2010.

NASRI, N., KHALDI, A., FADY, B., TRIKI, S., Fatty acids from seeds of Pinus pinea L.: composition and population profiling. **Phytochemistry**, London, v.66, p.1729–1735, 2005.

NASSU, R. T.; GONÇALVES, L.A.G. Solid fat content determination: comparision between pNMR and DSC techniques. **Grasas y Aceites**, v.46, p. 337-343, 1995.

NEFF, W. E.; BYRDWELL, W. C.; LIST, G. R. Triacylglycerol structures of food fats high in saturated acids by HPLC and mass spectrometry. **Journal of Liquid Chromatography and Related Technologies**, Florida, v.24, p.837–854, 2001.

NEHDIA, I.; OMRIB, S.; KHALIL, M.I.; AL-RESAYESA, S.I. Characteristics and chemical composition of date palm (Phoenix canariensis) seeds and seed oil. **Industrial Crops and Products**, London, v.32, p.360–365, 2010.

NIELSEN, N. S., YANG, T., XU, X., JACOBSEN, C. Production and oxidativestability of a human milk fat substitute produced from lard by enzyme technology in a pilot packed-bed reactor. **Food Chemistry**, v.94, p.53–60, 2006.

NOOR LIDA, H. M. D.; SUNDRAM, K.; IDRIS, N. A. Effect of chemical interesterification on triacylglycerol and solid fat contents of palm stearin, sunflower oil and palm kernel olein blends. **European Journal of Lipid Science and Technology**, v.109, p.147–156, 2007.

NORIZZAH, A.R.; CHONG,C.L.; CHEOW, C.S.; ZALIHA, O. Effects of chemical interesterification on physicochemical properties of palm stearin and palm kernel olein blends. ***Food Chemistry***, Oxford, v.86, n.2, p.229-235, 2004.

O'BRIEN, R.D. **Fats and oils: formulating and processing for applications.** Boca Raton: CRC Press LLC, 387p., 2004.

OIL WORD. Statistic world production of 7 oilseeds, production of vegetable oils. Disponível em:<www.oilworld.de>. Acesso em 20 de abril de 2012.

OLIVER, W.R.; MAcGILL, D.C. Butter and margarine: Their chemistry, their conflict. Journal of Chemistry Education. Washington, v.64, n.7, p.596-598, 1987.

OMAR, Z.; LET, C. C.; SENG, C. C.; RASHID, N. A. Crystallization and rheological properties of hydrogenated palm oil and palm oil blends in relation to crystal network. **European Journal of Lipid Science and Technology**, Hoboken, v.107, p. 634–640, 2005.

OSÓRIO, N.M. GUSMÃO, J.H.; da FONSECA, M.M.; FERREIRA-DIAS, S. Lipase-catalysed interesterification of palm stearin with soybean oil in a continuous fluidized-bed reactor. **European Journal of Lipid Science and Technology**, Hoboken, v.107, n.7-8, p.455-463, 2005.

OSÓRIO, N.M.; da FONSECA, M.M.; FERREIRA-DIAS, S. Operational stability of *Thermomyces lanuginosa* lipase during fats interesterification in continuous- packed-bed reactors. **European Journal of Lipid Science and Technology**, Hoboken, v.108, n.7, p.545-553, 2006.

OSÓRIO, N.M.; DUBREUCQ, E.; da FONSECA, M.M.R.; FERREIRA-DIAS, S. Lipase/acyltransferase-catalysed interesterification of fat blends containing *n*-3 polyunsaturated fatty acids. **European Journal of Lipid Science and Technology**, v.111, p.120–134, 2009.

OSÓRIO, N.M.; FERREIRA-DIAS, S.; GUSMÃO, J.H.; da FONSECA, M.M.R. Response surface modelling of the production of ω-3 polyunsaturated fatty acids-enriched fats by a commercial immobilized lipase. **Journal of Molecular Catalysis B-Enzymatic**, v.11, p.677–686, 2001.

OSÓRIO, N.M.F.R.M. **Estudos de interesterificação de gorduras enriquecidas em ácidos gordos ómega-3, catalisadas por lipases imobilizadas.** Lisboa, 2008,173p. [Tese de doutoramento – Instituto Superior de Agronomia da Universidade Técnica de Lisboa].

OTERO, C.; LOPEZ-HERNÁNDEZ, A.; GARCÍA, H.S.; HERNANDEZ-MARTIN, E.; HILL, C.G. Continuous enzymatic transesterification of sesame oil and a fully hydrogenated fat: effects of reaction conditions on product characteristics. **Biotechnology and Bioengineering**, v.94, p.877–887, 2006.

PADOVESE, R.; MANCINI FILHO, J. Ácidos graxos *trans*. In: CURI, R., POMPÉIA, C., MIYASAKA, C.K. PROCOPIO, J. (Eds.). **Entendendo a gordura: os ácidos graxos**. São Paulo: Manole, 2002. cap. 36, p. 509-521.

PANDE, G.; AKOH, C.C. Enzymatic synthesis of trans-free structured margarine fat analogs with high stearate soybean oil and palm stearin and their characterization. **LWT-Food Science Technology**, v.50, p. 232-239, 2013.

PANDE, G.; AKOH, C.C.; SHEWFELT, R.L. Utilization of enzymatically interesterified cottonseed oil and palm stearin-based structured lipid in the production of trans-free margarine. **Biocatalysis and Agricultural Biotechnology**. v.2, p.76-84, 2013.

PANTZARIS, T.P.; Y. BASIRON, Y. The lauric oil (coconut oil and palm kernel oil). In: F.D.Gusntone. VEGETABLE OILS IN FOOD TECHNOLOGY: **Composition, Properties and Uses**, 2002, 157-202.

PAULA, A.V. **Reestruturação da gordura de leite por interesterificação enzimática empregando lipase imobilizada: otimização das condições reacionais e operacionais**. 2011, 212. [Tese de Doutoramento – Escola de Engenharia de Lorena da Universidade de São Paulo].

PAVAN, R. Avaliação dos teores de ácidos graxos *trans* em margarinas e cremes vegetais apos a resolução RDC 360 (ANVISA). São Paulo, 2008. 83p. Dissertação (Mestrado em Bromatologia) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2008.

PETRAUSKAITE, V.; DE GREYT, W.; KELLENS, M.; HUYGHEBAERT, A. Physical and chemical properties of *trans*-free fats produced by chemical interesterification of vegetable oil blends. **Journal of the American Oil Chemists' Society**, Champaign, v.75, p.489–495, 1998.

PISKA, M.; ZÁRUBOVÁ, T.; LOUZECKÝ, H.; KARAMI, FILIP, V. Properties and crystallization of fat blends. **Journal of Food Engineering**, Ontario, v.77, p. 379-471, 2006.

PORTE, A. Interesterificação enzimática na obtenção de Substitutos da manteiga de cacau. **Boletim CEPPA**, Curitiba, v. 17, n. 2, p. 115-126, 1999.

PROENÇA, R.P. da C.; HISSANAGA, V. M. O controle de gorduras *trans* no processo produtivo de refeições. **Revista do Conselho Regional de Nutricionistas – 2ª Região, RS e SC (CRN 2),** Porto Alegre, v. 2, n. 16, p. 13, 2008.

QUINLAN, P.T., LOCKTON, S., IRWIN, J., LUCAS, A.L. The relationship between stool hardness and stool composition in breast and formula-fed infants. **Journal of Pediatric Gastroenterology Nutition**. v.20, p.81–90. 1995.

RANJITH, H.M.P. Water continuous emulsions. In: RAJAH, K.K. (Ed). **Fats in food technology**. Sheffield Academic Press: CRC Press. 2002, p. 69-122.

RAO R.; SANKAR, K.U.; SAMBAIAH, K.; LOKESH, B. Differential scanning calorimetric studies on structured lipids from coconut oil triglycerides containing stearic acid. **European Food Res Techology**., v. 212, p.334–343, 2001.

RASERA, K.; OSÓRIO, N. M.; MITCHELL, D. A.; KRIEGER, N.; FERREIRA-DIAS S. Interesterification of fat blends using a fermented solid with lipolytic activity. **Journal of Molecular Catalysis B-Enzymatic**, v.76, p.75–81, 2012.

REENA, M.B.; LOKESH, B.R. Hypolipidemic effect of oils with balanced amounts of fatty acids obtained by blending and interesterification of coconut oil with rice bran oil or sesame oil. **Journal of Agricultural and Food Chemistry**, Washington, v.55, n.25, p. 10461-10469, 2007.

REENA, M.B.; REDDY, S. R. Y.; LOKESH, B.R. Changes in triacylglycerol molecular species and thermal properties of blended and interesterified mixtures of coconut oil or palm oil with rice bran oil or sesame oil. **European Journal of Lipid Science and Technology**, Hoboken, v.111, p.346–357, 2009.

RESHMA, M.V.; SARITHA, S.S.; BALACHANDRAN, C.; ARUMUGHAN, C. Lipase catalyzed interesterification of palm stearin and rice bran oil blends for preparation of zero *trans* shortening with bioactive phytochemicals. **Bioresource Technology**, v.99, p.5011–5019, 2008.

REYES-HERNÁNDEZ, J.; DIBILDOX-ALVARADO, E.; CHARÓ-ALONSO, M.A; TORO-VAZQUEZ, J.F. Physicochemical and Rheological Properties of Crystallized Blends Containing trans-free and Partially Hydrogenated Soybean Oil. **Journal of the American Oil Chemists Society**, Champaign, v.84, p. 1081–1093, 2007.

RIBEIRO, A,P,B.; MOURA, J.M.L.N.; GRIMALDI, R.; GONÇALVES, L.A.G. Interesterificação química: alternativa para obtenção de gordura *trans*. **Química Nova**, São Paulo, v. 30, n. 5, p. 1295-1300, 2007.

RIBEIRO, A.P.B.; GRIMALDI, R.; GIOIELLI, L.A.; GONÇALVES, L.A.G. Zero *trans* fats from soybean oil and fully hydrogenated soybean oil, Physico-chemical properties and food applications, **Food Research International**, Amsterdam, v.42, p.401-410, 2009a.

RIBEIRO, A.P.B.; BASSO, R.C.; GRIMALDI, R.; GIOIELLI, L.A.; GONÇALVES, L.A.G. Effect of chemical interesterification on physicochemical properties and industrial applications of canola oil and fully hydrogenated cottonseed oil blends. **Lipids**, Champaign, v.16, p.362-384, 2009c.

RIBEIRO, A. P. B.; BASSO, R. C.; GRIMALDI, R.; GIOIELLI, L. A.; GONÇALVES, L. A. G. Instrumental methods for the evaluation of interesterified fats. **Food Analytical Methods**, v.2, p.282-302, 2009d.

RIBEIRO, A.P.B.; BASSO, R.C.; GRIMALDI, R.; GIOIELLI, L.A.; GONÇALVES, L.A.G. Effect of chemical interesterification on physicochemical properties and industrial applications of canola oil and fully hydrogenated cottonseed oil blends. **Journal of Food Lipids**, Trumbull, v.16, p.362-384, 2009e.

RIBEIRO, A.P.B.; BASSO, R.C.; GRIMALDI, R.; GIOIELLI, L.A.; SANTOS, A.O.; CARDOSO; L.P.; GONÇALVES L.A.G. Influence of chemical interesterification on thermal behavior, microstructure, polymorphism and crystallization properties of canola oil and fully hydrogenated cottonseed oil blends. **Food Research International**, Amsterdam, v.42, p. 1153-1162, 2009f.

RIBEIRO, A.P.B., GRIMALDI, R.C.,GIOIELLI, L.A., SANTOS,A.O., CARDOSO, L.P, GONÇALVES, L.A.G. Thermal behavior, microstructure, polymorphism, and crystallization properties of zero trans fats from soybean oil and fully hydrogenated soybean oil. **Food Biophysics**, New York, v.4, p.106-118, 2009g.

RIBEIRO, A.P.B.; MASUCHI, M.H.; GRIMALDI, R.; GONCALVES, L.A.G. Interesterificação química de óleo de soja e óleo de soja totalmente hidrogenado, influência do tempo de reação. **Química Nova**, São Paulo, v.32, p.939-945, 2009b.

RITTNER, H. **Óleo de palma: processamento e utilização**. São Paulo, 1 ed, 1996. 302p.

RODRIGUES, J. N.; GIOIELLI, L. A. Chemical interesterification of milkfat and milkfat–corn oil blends. **Food Research International**, Amsterdan, v.36, p.149–159, 2003.

RODRIGUES, J.N.; GIOIELLI, L.A.; ANTON, C. Propriedades físicas de lipídios estruturados obtidos de misturas de gordura do leite e óleo de milho. **Ciência e Tecnologia Alimento**, Campinas, v.23, p.226-233, 2003.

RODRIGUES, J. N.; TORRES, R.; MANCINI-FILHO, J.; GIOIELLI, L.A. Physical and chemical properties of milkfat and phytosterol esters blends. **Food Research International**, Amsterdam, v.40, p.748–755, 2007.

RØNNE, T.H.; YANG, T.; MU, H.; JACOBSEN, C.; XU, X. Enzymatic interesterification of butterfat with rapeseed oil in a continuous packed bed reactor. **Journal of Agricultural and Food Chemistry**, v.53, p.5617-5624, 2005.

ROSSELL, J.B; KING B.; DOWNES, M.L. Composition of Oil. **Journal of the American Oil Chemists' Society**, Champaign, v. 62, p. 221-230, 1985.

ROUSSEAU, D., HILL, A. R., MARANGONI, A. G. Restructuring butterfat through blending and chemical interesterification. 2. Microstructure and polymorphism. **Journal of the American Oil Chemists' Society**, Champaign, v.73, n.8, p.973-981, 1996a.

ROUSSEAU, D., HILL, A. R., MARANGONI, A. G. Restructuring butterfat through blending and chemical interesterification. 3. Rheology. **Journal of the American Oil Chemists' Society**, Champaign, v.73, n.8, p.983-989, 1996b.

ROUSSEAU, D.; MARANGONI, A. G.; JEFFREY, K. R. The influence of chemical interesterification on the physicochemical properties of complex fat systems. 2. Morphology and polymorphism. **Journal of American Oil Chemists' Society**, Champaign, v.75, p.1833–1839, 1998.

ROUSSEAU, D., MARANGONI, A.G. The effects of interesterification on physical and sensory attributes of butterfat and butterfat-canola oil spreads. **Food Research International**, Amsterdam, v.31, n.5, p.381-388, 1999.

ROUSSEAU, D.; MARANGONI, A. G. Chemical interesterification of food lipids: Theory and practice. In C. C. Akoh & D. B. Min (Eds.), **Food lipids: Chemistry**, **nutrition, and biotechnology**. Boca Raton: CRC Press, 301-335, 2002.

ROZENAAL, A. Interesterification of oils and fats. **INFORM**, Champaign, v.3, n.11,p.1232-1237, 1992.

SAADI, S.; ARIFFIN, A.A.; GHAZALI, H.M.; MISKANDAR, M.S.; BOO, H.C.; ABDULKARIM, S.M. Application of differential scanning calorimetry (DSC), HPLC and Pnmr for interpretation primary crystallisation caused by combined low and high melting TAGs. **Food Chemistry**, Oxford, v.132, p.603–612, 2012.

SAFRA, N.M.; VILLAMIL, A.P. Production of structured lipids by enzymatic transesterification of soybean oil and palm kernel oil in a packed-bed reactor. **Grasas Y Aceites**, Sevilla, v.59, n.4, p.337-345, 2008.

SAMBANTHAMURTHI, R; SUNDRAM, K; TAN, Y.A. Chemistry and biochemistry of palm oil. **Progress in Lipid Research**, Oxford, v.39, n.6, p. 507-558, 2000.

SANTOS, A. M. dos. O excesso de peso da família com obesidade infantil. **Revista Virtual Textos & Contextos**, Porto Alegre, n.2**,** p.1-10, 2003.

SCHMIDELL, W.; LIMA, A. U.; AQUARONE, E.;BORZANI, W. **Biotecnologia Industrial**. São Paulo: E.Blücher, 2001. v. 2, 254 p.

SCHORKEN, U.; KEMPERS, P. Lipid biotechnology: Industrially relevant production processes. **European Journal of Lipid Science and Technology**, v.111, p.627–645, 2009.

SEMMA, M. *Trans* fatty acids: properties, benefits and risks. **Journal Health Science**, Tokyo,v.48, n.1, p.7-13, 2002.

SERIBURI, V.; AKOH, C.C. Enzymatic transesterification of triolein and stearic acid and solid fat content of their products. **Journal of American Oil Chemists' Society**, v.75, p.511–516, 1998.

SHAHIDI, F ed. **Bailey´s industrial oil and fat products.** 6ª. ed., New York: Wiley, v.2: p.603-654, v.3: p.1-63, 2005.

SHI, Y.; LIANG, B.; HARTEL, R. W. Crystal morphology, microstructure, and textural properties of model lipid system. **Journal of the American Oil Chemists Society**, Champaign, v.82, p.399–408, 2005.

SHIN, J.A.; AKOH, C.C.; LEE, K.T. Enzymatic interesterification of anhydrous butterfat with flaxseed oil and palm stearin to produce low-*trans* spreadable fat. **Food Chemistry**, Oxford, v.120, n.1, p.1-9, 2010a.

SHIN, J.A.; AKOH, C.C.; LEE, K.T. Physicochemical properties of lipase-catalyzed interesterified fat containing alpha-linolenic acid. **Journal of the American Oil Chemists' Society**, Champaign, v.87, n. 6, p. 647-657, 2010b.

SIEW, W.L.; CHEAH, K.Y.; TANG, W.L. Physical properties of lipase-catalyzed interesterification of palm stearin with canola oil blends. **European Journal of Lipid Science and Technology**, Hoboken, v.109, n.2, p. 97-106, 2007.

SIEW, W.L.; NG, W.L. Influence of diglycerides on crystallization of palm oil. **Journal of the Science of Food and Agriculture**, v.79, p.722–726, 1999.

SIL ROY, S.; BHATTACHATYYA, D.K. Distinction between enzymically and chemically catalyzed interesterification. **Journal of the American Oil Chemists' Society**, Champaign, v.70, n.12 p.1293-1294, 1993.

SILVA, E.C.; de PAOLA, M.V.R.V.; MATOS, J.R.M. Análise térmica aplicada à cosmetologia. **Brazilian Journal of Pharmaceutical Sciences**. Sao Paulo, v.43, n.3, jul./set., 2007.

SILVA, R.C; ESCOBEDO, J.P; GIOIELLI, L.A. Comportamento de cristalização de lipídios estruturados por interesterificação química de banha e óleo de soja. **Química Nova**, São Paulo, v.31, n.2, p.330-335, 2008.

SILVA, R.C.; COTTING, L.N.; POLTRONIERI, T.P;. BALCÃO, V.M; ALMEIDA, D.B.; GONÇALVES, L.A.G.; GRIMALDI, R.; GIOIELLI, L.A. Enzymatic interesterification of lard with soybean oil for production of human milk fat analog. **LWT Food Science and Technology**, Amsterdam, v.42, n.7, p.1275-1282, 2009.

SILVA, R.C.; GIOIELLI, L.A. Lipídios estruturados: alternativa para a produção de sucedâneos da gordura do leite humano. **Quimica Nova**, São Paulo, v. 32, n. 5, p. 1253-1261, 2009.

SILVA, R.C.; SOARES, F.A.S.D.; FERNANDES, T.G.; CASTELLS, A.L.D.; DA SILVA, K.C.G.; GONCALVES, M.I.A; MING, C.C.; GONCALVES, L.A.G.; GIOIELLI, L.A. Interesterification of lard and soybean oil blends catalyzed by immobilized lipase in a continuous packed bed reactor. **Journal of the American Oil Chemists' Society**, Champaign, v. 88, n.12 p.1925-1933, 2011.

SILVA, R.C; SOARES, F.A.S.D.M; HAZZAN, M.; CAPACLA, I.R; GONÇALVES, M.I.A.; GIOIELLI, L.A. Continuous enzymatic interesterification of lard and soybean oil blend: Effects of different flow rates on physical properties and acyl migration. **Journal of Molecular Catalysis B: Enzymatic**, Amsterdam, v. 76, p. 23-28, 2012.

SILVA, R.C., RIBEIRO, A.P.B., SOARES, F.A.S.D.M., HAZZAN, M., CAPACLA, I.R., SANTOS, A.O., CARDOSO, L.P, GIOIELLI, L.A. Microstructure and Thermal Profile of Structured Lipids Produced by Continuous Enzymatic Interesterification. **Journal of the American Oil Chemists Society**, Champaign, v.90, p.631-639, 2013.

SIMOES, I.S.; GIOIELLI, L.A.; OLIVEIRA, M.N. Consistência de mistura de gorduras hidrogenadas e óleo de soja. **Ciência Tecnologia Alimentos**. Campinas, v.18, n.3, pp.325-330, 1998.

SIMON, P.; KOLMAN, L. DSC study of oxidation induction periods. **Journal of Thermal Analysis and Calorimetry**, London, v.64, n.2, p. 813-820, 2001.

SLABAS, A. R.; SIMON, J. W.; ELBOROUGH, K. M. Information needed to create new oil crops. **INFORM**, Champaign, v.6, p.159–166, 1995.

SMALLWOOD, N.J. Using computers for oil blending. **Journal of the American Oil Chemists' Society**, Champaign, v.66, n.5, p.644-648, 1989**.**

SOARES, F.A.S.D.M; da SILVA, R.C.; da SILVA, K.C.G; LOURENÇO, M.B; SOARES, D.F.; GIOIELLI, L.A. Effects of chemical interesterification on physicochemical properties of blends of palm stearin and palm olein. **Food Research International**, Amsterdam, v. 42, n. 9, p. 1287-1294, 2009.

SOARES, F.A.S.M.; SILVA, R.C.; HAZZAN, M.; CAPACLA, R.; VICCOLA, E.R.; MARAYAMA, J.M.; GIOIELLI, L.A. Chemical Interesterification of Blends of Palm Stearin, Coconut Oil and Canola Oil, Physicochemical Properties. **Journal of Agricultural and Food Chemistry,** Washington, v.60, p.146-1469, 2012.

SONNTAG, N. O. V. Fat splitting, esterification, and interesterification. In: SWERN, D., ed. - **Bailey's industrial oil and fat products**. 4. ed. New York: Wiley-Interscience, 1982. v.2, p.97-173.

SOUZA, C.H.B.; GIOIELLI, L.A, SAAD, S.M.I. Desafios na tecnologia de fabricação de emulsões alimentícias funcionais. In: SAAD, S.M.I; CRUZ, A.G.; FARIA, J.A.F. **Probioticos e Prebioticos em Alimentos Fundamentos e Aplicações tecnológicas**, São Paulo:Varela, cap 20, p.471-502, 2011.

SREENIVASAN, B. Interesterification of fats. **Journal of the American Oil Chemists' Society**, Champaign, v. 55, n.11, p.796–805, 1978.

STATISTICA, 11.0 for Windows, Vol. IV, **Statsoft Inc**., Tulsa, OK, 2013.

STRUIJVENBERG, J.H. **La Margarine: Histoire et Évolution**. 1869-1969. Dunod-Paris, pp.90-96.

SUNDRAM, K.; ISMAIL, A.; HAYES, K.C.; JEYAMALAR R.; PATHMANATHAN, R. Trans celaidial fatty acids adversely affect the lipoprotein prof,ile relative to specific saturated fatty acids in humans. **Journal of Nutrition** , v.127, p.5143-5203, 1997.

TAN, C. P.; CHE MAN, Y. B. Differential scanning calorimetry analysis of edible oils: comparison of thermal properties and chemical composition. **Journal of the American Oil Chemists' Society**, Champaign, v.77, p.143–155, 2000.

TAN, C. P.; CHE MAN, Y.B. Differential scanning calorimetric analysis of palm, palm oil based products and coconut oil, Effects of scanning rate variation. **Food Chemistry**, Oxford, v.76, p.89–102, 2002.

TANG, D.; MARANGONI, A. G. Microstructure and fractal analysis of fat crystal networks. **Journal of American Oil Chemists' Society**, Champaign, v.83, p.377–388, 2006.

TARRAGO-TRANI, M.T.; PHILLIPS, K.M.; LEMAR, L.E.; HOLDEN, J.M. New and existing oils and fats used in products with reduced *trans*-fatty acid content. **Journal of the American Dietetic Association**, Chicago, v.106, n.6, p.867-880, 2006.

TEXEIRA, J.A.; da FONSECA, M.M.; VICENTE, A.A. Geometrias e modos de operação. In da FONSECA, M.M., TEXEIRA, J.A. (Eds.). **Reatores biológicos – Fundamentos e aplicações**. Lisboa:Lidel Edições Lda., pp.27-68, 2007.

TIMMS, R. E. Phase behaviour of fats and their mixtures**. Progress in Lipid Research,** Oxford, v.23, n.1, p.1-38, 1984.

ULBRICHT, T. L. V.; SOUTHGATE, D.A.T. Coronary heart disease: Seven dietary factors. **Lancet**, v.338, p.985−992, 1991.

UPRITCHARD, J.E.; ZEELENBERG, M.J.; HUIZINGA, H.; VERSCHUREN, P.M.; TRAUTWEIN, E.A. Modern fat technology: what is the potential for heart health? **Proceedings of the Nutrition Society**. Wallingford, v.64, n.3, p.379-386.

VAN der HOCK. Effets of crystallization, tempering and composition on the quality and structural stability of margarine. **American soybean**, p.74-78, 1983.

VISSERS, M.N.; ZOCK, PL; POODENBURG, A.J.C.; LEENEN, R.; KATAN, M.B. Olive oil phenols are absorbed in humans. **Journal of Nutrition**, Bethesda, v.132, n.3, p.409-417, 2002.

VLAHOV, G. ^{13}C nuclear magnetic resonance spectroscopy to check 1,3-random, 2-random pattern of fatty acid distribution in olive oil triacylglycerols. **Spectroscopy**, v.19, p.109-117, 2005.

WASSELL, P.; YUONG, N.W.G. Food applications of *trans* fatty acid substitutes. **International Journal of Food Science and Technology**, Malden, v.42, n.5, p.503-517, 2007.

WIEDERMANN, L. H. Margarine and margarine oil, formulation and control. **Journal of the American Oil Chemists Society**, Champaign, v.55, n.11, p.823-829, 1978.

WILLIS, W.M.; LENCKI, R.W.; MARANGONI, A.G. Lipid modification strategies in the production of nutritionally functional fats and oils. **Critical Reviews in Food Science Nutrition**, Philadelphia, v.38, n.8, p.639-674, 1998.

WILIIS, W.M.; MARANGONI, A.G. Assessment of lipase – and chemically catalized lipid modification strategies for the production of structured lipids. **Journal of the American Oil Chemists' Society**, Champaign, v.76, n.4, p.443-450, 1999.

WILLIS, W.M. Enzymatic interesterification. In: AKOH, C.C., MIN, D.B (Eds). **Food lipids: chemistry, nutrition, and biotechnology**. New York: Marcel Dekker, 2002. p.839-875.

WILLIS, W.M.; MARANGONI, A.G. Enzymes Interesterification. In AKOH, C.C.; MIND, D.B. **Food lipids: chemistry, nutrition and biotechnology**. Florida: CRC Press, cap.30, p.807, 2008.

WILSON, R. F. Practical biotechnological strategies for improving the quality and value of soybeans. **INFORM**, Champaign, v.6, p.193–200, 1993.

XIE, W.; QI, C. Interesterification of Soybean Oil and Lard Blends Catalyzed by SBA-15-pr-NR3OH as a Heterogeneous Base Catalyst. **Journal of Agricultural and Food Chemistry**, Washington, v.61, p.3373-3381, 2013.

XU, X., Production of specific-structured triacylglycerols by lipase-catalyzed reactions: a review. **European Journal of Lipid Science and Technology**, Hoboken, v.102, n.4, p.287–303, 2000.

XU, X.; BALCHEN, S.; HOY, C.-E.; ADLER-NISSEN, J. Pilot batch production of specific-structured lipids by lipase-catalized interesterification: preliminary study on incorporation and acyl migration. **Journal of the American Oil Chemists' Society**, Champaign, v.75, n.2, p.301-308, 1998a.

XU, X.; FOMUSO, L.B.; AKOH, C. C. Synthesis of structured triacylglycerols by lipase-catalyzed acidolysis in a packed bed bioreactor. **Journal of Agricultural and Food Chemistry,** Washington, v.*48*, p.3-10, 2000.

XU, X.; PORSGAARD, T.; ZANG, H.; ADLER-NISSEN, J.; HØY, C.E. Production of structured lipids in a packed-bed reactor with *Thermomyces lanuginosa* lipase. **Journal of American Oil Chemists' Society**, v.79, p.561-565, 2002.

XU, X.; SKANDS, A.R.H.; HOY, C.-E.; MU, H.; BALCHEN, S., ADLER-NISSEN, J. Production of specific-structured lipids by enzymatic interesterification: Elucidation of acyl migration by response surface design. **Journal of the American Oil Chemists' Society**, Champaign, v.75, n.9, p.1179-1186, 1998b.

XU, X.B. Engineering of enzymatic reactions and reactors for lipid modification and synthesis. **European Journal of Lipid Science and Technology**, Hoboken, v.105, n.6, p.289-304, 2003.

Yamane, T. Enzyme technology of lipid industry: an engineering overview. **Journal of the American Oil Chemists' Society**, v.64, p.1657–1662, 1987.

YOUNG, F.V.K. Interchangeability of fats and oils. **Journal of the American Oil Chemists' Society**, Champaign, v.62, n.2 p.372-376, 1985 .

ZAINAL, Z., YUSOFF, M. S. A., Enzymatic interesterification of palm stearin and palm kernel olein. **Journal of American Oil Chemists' Society**, v.76, p.1003–1008, 1999.

ZALIHA, O.; CHONG, C.L.; CHEOW, C.S., Crystallization properties of palm oil by fry fractionation. **Food Chemistry**, Oxford, v.30, p.30–36, 2004.

ZEITOUN, M. A. M.; NEFF, W. E.; LIST, G. R.; MOUNTS, T. L. Physical properties of interesterified fat blends. **Journal of American Oil Chemists' Society**, Champaign, v.70, p.467–471, 1993.

ZHANG, H.; XU, X.; NILSSON, J.; MU, H.; ADLER-NISSEN, J.; HOY, C.E. Production of margarine fats by enzymatic interesterification with silica-granulated *Thermomyces lanuginosa* lipase in a large-scale study. **Journal of American Oil Chemists' Society** v.78, p. 57–64, 2001.

ZHANG, H.; SMITH, P.; ADLER-NISSEN, J. Effects of degree of enzymatic interesterification on the physical properties of margarine fats: Solid fat content, crystallization behavior, crystal morphology, and crystal network. **Journal of Agricultural and Food Chemistry**, Washington, v.52, n.14, p.4423-4431, 2004.

ZHAO, G.; ETHERTON, T.D.; MARTIN, K.R.; GILLIES, P.J.; WEST, S.G.; KRIS-ETHERTON, P.M. Dietary α-linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. **American Journal of Clinical Nutrition**, v.85, p.385–389, 2007.

Anexos

Figura 10.1. Esquema de reator para interesterificação química em escala de laboratório.

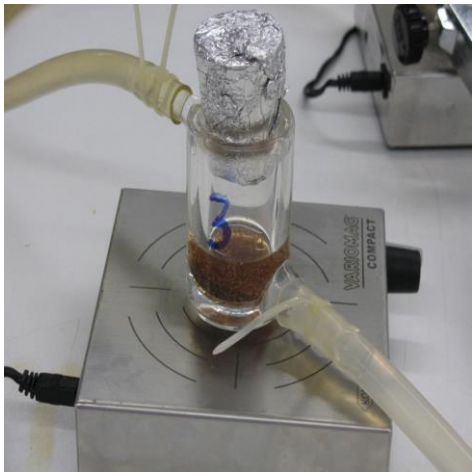


Figure 10.2. Cylindrical glass reactors (25 mL) under magnetic stirring for batch interesterification.

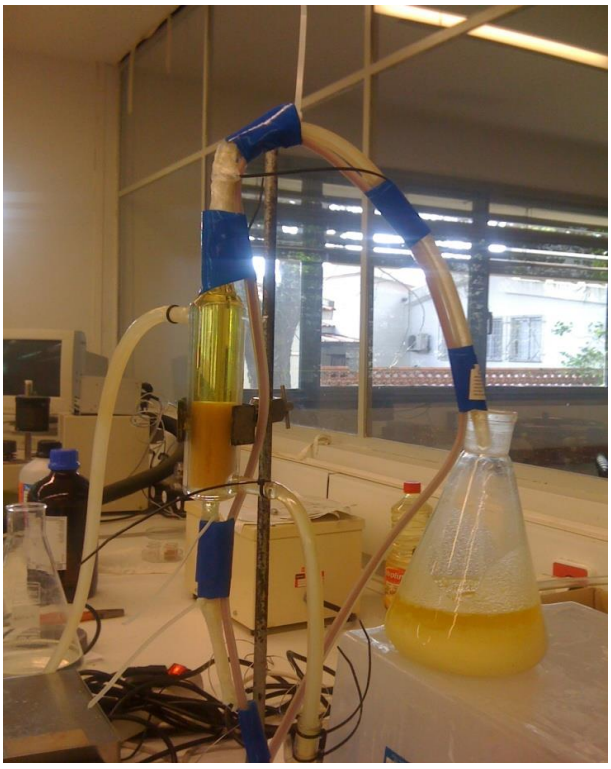


Figure 10.3. Continuous packed-bed reactor for interesterification.

CRYSTALLIZATION AND E MELTING CURVES PARAMETERS FOR THE BLENDS TO PALM STEARIN, COCONUT OIL AND CANOLA OIL BEFORE AND AFTER CHEMICAL INTERESTERIFICATION

Table 11.1. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil before chemical interesterification.

Blends	C_1				C_2				C_4			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
NIE1	30.0±0.2	25.7±0.2	29.1±0.2	-58.8±2.8	7.6±0.1	-7.6±0.7	3.4±0.0	-51.6±4.9	------	------	------	------
NIE4	24.5±0.0	20.1±0.0	23.3±0.0	-31.7±0.0	------	------	------	------	7.5±0.2	1.4±0.2	4.4±0.0	-2.9±0.2
NIE5	24.7±0.2	21.4±0.5	23.4±0.3	-14.1±1.4	-0.8±0.1	-16.9±0.6	-4.7±0.4	-10.3±0.8	------	------	------	------
NIE7	21.3±0.0	16.4±0.1	19.7±0.1	-15.5±0.2	-15.1±0.2	-24.9±0.4	-20.0±0.0	-9.7±0.8	5.3±0.1	-1.9±0.3	1.4±0.1	-1.6±0.1
NIE8	26.7±0.6	24.4±1.0	26.6±0.8	-32.5±1.6	------	------	------	------	------	------	------	------
NIE9	28.0±0.0	25.3±0.0	27.2±0.1	-3.7±0.1	10.5±0.1	-9.7±0.4	-0.7±0.5	-14.7±4.4	17.7±0.1	13.2±0.1	15.9±0.1	-5.9±0.3
NIE10	17.3±0.4	11.5±0.3	15.8±1.7	-7.6±0.5	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.1. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil before chemical interesterification.

Blends	C_5				C_2+C_5				C_2+C_4				ΔHcT (J/g)
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	
NIE1	------	------	------	------	------	------	------	------	------	------	------	------	-110.3±4.8^{f}
NIE4	------	------	------	------	0.4±0.1	-20.7±0.1	-13.1±0.1	-40.3±1.1	------	------	------	------	-74.8±0.8^{e}
NIE5	------	------	------	------	------	------	------	------	------	------	------	------	-43.5±0.1^{c}
NIE7	-30.1±0.2	-38.2±1.5	-34.2±0.2	-7.6±0.4	------	------	------	------	------	------	------	------	-26.8±1.0^{b}
NIE8	-26.8±2.0	-38.2±2.8	-33.8±2.0	-9.9±1.0	------	------	------	------	3.5±0.3	-11.1±2.0	-0.1±0.7	-22.7±2.5	-65.1±3.9^{d}
NIE9	-11.2±0.6	-18.6±0.5	-15.0±0.1	-8.1±5.5	------	------	------	------	------	------	------	------	-24.3±4.7^{a}
NIE10	------	------	------	------	-7.3±0.2	-22.4±0.3	-13.2±0.1	-7.4±0.1	------	------	------	------	-59.0±1.0^{d}

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.2. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil before chemical interesterification.

Blends	H_1				H_2				H_3			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
NIE1	-20.4±0.1	-13.4±0.2	-16.6±0.3	0.8±0.1	-2.5±0.2	4.5±0.1	2.1±0.0	3.0±0.4	5.2±0.2	11.0±0.1	9.6±0.0	8.3±1.0
NIE4	------	------	------	------	------	------	------	------	------	------	------	------
NIE5	------	------	------	------	------	------	------	------	------	------	------	------
NIE7	------	------	------	------	------	------	------	------	------	------	------	------
NIE8	------	------	------	------	------	------	------	------	------	------	------	------
NIE9	------	------	------	------	------	------	------	------	------	------	------	------
NIE10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.2. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil before chemical interesterification.

Blends	H_4				H_5				H_6			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
NIE1	25.2±0.3	33.8±0.3	27.1±0.2	4.9±0.8	39.4±0.3	48.1±0.1	46.0±0.7	18.7±0.4	48.9±0.1	55.3±0.0	53.0±0.0^{e}	28.0±0.8
NIE4	------	------	------	------	------	------	------	------	47.4±0.0	50.8±0.0	49.3±0.0^{c}	2.6±0.1
NIE5	13.6±0.5	20.9±0.3	15.7±0.2	1.7±0.1	33.8±0.2	43.3±0.3	40.7±0.2	6.5±1.5	44.0±0.1	51.2±0.1	48.5±0.2^{b}	9.2±1.7
NIE7	------	------	------	------	32.6±0.4	43.8±0.1	41.1±0.0	13.0±0.7	44.7±1.7	50.4±0.1	48.6±0.1^{b}	1.7±0.1
NIE8	------	------	------	------	------	------	------	------	47.9±0.0	53.9±0.0	51.2±0.0^{d}	12.1±0.5
NIE9	------	------	------	------	40.0±0.5	47.3±0.1	45.1±0.1	1.8±0.1	48.8±0.2	53.4±0.0	51.5±0.0^{d}	0.8±0.0
NIE10	------	------	------	------	23.9±0.1	40.7±0.2	36.9±0.1	7.0±0.5	41.7±0.9	47.3±0.7	45.5±0.1^{a}	0.2±0.0

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.2. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil before chemical interesterification.

Blends	$H_7+H_3+H_2$				H_5+H_4				H_1+H_8			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
NIE1	------	------	------	------	------	------	------	------	------	------	------	------
NIE4	3.0±0.0	28.7±0.0	23.1±0.1	84.1±0.2	34.3±0.1	44.9±0.0	42.5±0.1	29.1±0.8	------	------	------	------
NIE5	------	------	------	------	------	------	------	------	-23.6±0.3	-11.6±0.2	-17.5±0.0	4.5±1.2
NIE7	------	------	------	------	------	------	------	------	-23.4±0.4	-9.0±0.1	-16.0±0.2	10.4±1.0
NIE8	------	------	------	------	------	------	------	------	-21.4±0.3	1.6±0.2	-1.3±0.1	11.2±1.2
NIE9	11.0±0.3	28.4±0.1	23.2±0.1	72.1±1.1	------	------	------	------				
NIE10	------	------	------	------	------	------	------	------	-21.7±0.2	-6.3±0.1	-15.6±0.1	19.8±0.5

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.2. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil before chemical interesterification.

Blends	H_2+H_3				H_7+H_4				$H_7+H_4+H_5$			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
NIE1	------	------	------	------	------	------	------	------	------	------	------	------
NIE4	------	------	------	------	------	------	------	------	------	------	------	------
NIE5	-10.5±0.0	8.3±0.1	-6.0±0.1	18.2±2.4	------	------	------	------	------	------	------	------
NIE7	------	------	------	------	15.3±0.3	25.2±0.2	19.5±0.1	8.3±0.9	------	------	------	------
NIE8	2.7±0.3	11.2±0.5	4.4±0.1	14.8±0.5	------	------	------	------	15.3±0.1	47.3±0.1	44.6±0.2	104.9±2.6
NIE9	------	------	------	------	------	------	------	------	------	------	------	------
NIE10	------	------	------	------	7.8±0.1	19.0±0.1	13.4±0.2	4.4±0.1	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.2. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil before chemical interesterification.

Blends	$H_7+H_4+H_2+H_3$				ΔHfmT (J/g)
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	
NIE1	------	------	------	------	63.6±1.7[b]
NIE4	------	------	------	------	115.8±1.0[d]
NIE5	------	------	------	------	42.5±1.6[a]
NIE7	11.5±0.2	26.3±0.0	19.4±0.2	39.0±0.7	64.2±2.0[b]
NIE8	------	------	------	------	142.9±3.8[e]
NIE9	10.4±0.2	29.3±0.6	23.5±0.0	92.6±4.0	81.5±3.6[c]
NIE10	------	------	------	------	31.3±0.3[a]

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.3. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

	C_1				C_2				C_{2A}			
Blends	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**
CIE 1	27.6±0.2	21.6±0.1	25.5±0.1	-43.4±0.5	7.4±0.1	-4.8±0.1	4.0±0.1	-23.4±0.3	10.3±0.1	8.3±0.2	9.3±0.1	-0.4±0.0
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.3. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Blends	C_{2B}				C_7				C_8			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 1	-8.7±0.1	-12.8±0.1	-10.4±0.0	-0.6±0.1	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	17.6±0.0	15.3±0.1	16.9±0.1	-8.9±0.2	1.3±0.1	-4.7±0.2	-1.4±0.1	-10.7±0.4
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Table 11.3. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	C_9				C_{10}				C_{11}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	-8.8±0.2	-17.3±0.2	-12.5±0.2	-11.2±0.5	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	13.9±0.0	9.1±0.7	12.1±0.4	-8.4±0.1	1.2±0.1	-6.9±0.3	-2.3±0.1	-9.0±0.9
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.3. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	C_{12}				C_{13}				C_{17}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	-10.4±0.3	-20.1±0.3	-14.6±0.4	-12.4±0.7	-38.5±0.6	-54.6±2.0	-45.6±1.4	-22.5±2.4	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	9.3±0.1	4.7±0.1	7.6±0.0	-4.1±0.1
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.3. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Blends	C_{18}				C_{19}				C_{20}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	0.5±0.1	-2.1±0.4	-1.4±0.3	-0.2±0.0	-3.4±0.1	-23.6±0.5	-14.4±0.3	-8.8±0.5	-31.3±1.3	-43.1±0.2	-37.4±0.4	-13.1±0.6
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Table 11.3. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	C_{21}				C_{22}				C_{23}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	20.1±0.3	17.2±0.5	19.0±0.1	-21.1±0.8	8.5±0.4	4.4±0.1	7.2±0.5	-4.8±0.4	3.9±0.3	-0.5±0.2	0.6±0.1	-27.5±0.4
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.3. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	C_{24}				C_{25}				C_{26}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	-21.5±1.3	-44.3±1.1	-38.7±1.4	-10.6±0.6	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	7.7±±0.2	4.1±0.2	6.5±0.0	-15.1±0.0	0.2±0.0	-12.6±0.3	-5.2±0.0	-68.3±0.3
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.3. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

	C27				C28				C29			
Blends	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	-30.9±0.1	-30.9±0.1	-44.0±0.1	-3.3±0.2	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	-0.3±0.0	-3.0±0.2	-1.6±0.2	-1.3±0.1	-8.6±0.1	-19.6±0.0	-13.4±0.1	-18.8±0.1

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.3. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	C_{30} TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	ΔHcT J/g
CIE 1	------	------	------	------	-67.5±1.0
CIE 4	------	------	------	------	-30.9±0.2
CIE 5	------	------	------	------	-52.4±3.2
CIE 7	------	------	------	------	-13.1±0.6
CIE 8	------	------	------	------	-64.0±0.3
CIE 9	------	------	------	------	-86.3±0.2
CIE 10	-40.0±0.2	-52.7±0.1	-45.9±0.0	-20.8±0.9	-40.8±0.6

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.4. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	H_1				H_2				H_3			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 1	-21.2±0.3	-14.1±0.6	-17.7±0.1	0.7±0.1	-9.5±0.1	-7.6±0.1	7.61±0.1	0.5±0.0	-5.6±0.2	4.9±0.1	2.4±0.0	14.8±0.7
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.4. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	H_4				H_5				H_6			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 1	17.7±0.1	30.9±0.7	24.6±0.1	14.8±1.3	33.7±0.2	45.7±0.1	43.7±0.0	38.5±1.6	47.0±0.0	49.8±0.0	48.5±0.0	0.7±0.0
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.4. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	H_9				H_{10}				H_{11}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	-5.0±0.1	19.5±0.0	16.5±0.1	15.8±0.1	21.8±0.1	34.3±0.0	31.5±0.1	13.5±0.2	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	-24.5±0.6	-6.4±0.1	-16.3±0.2	8.1±0.4
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.4. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	H_{12}				H_{13}				H_{14}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	-4.1±0.3	4.6±0.1	0.2±0.2	18.6±0.4	-24.5±0.6	-6.4±0.1	-16.3±0.2	8.1±0.4	7.2±0.6	19.7±0.1	12.8±0.3	14.1±1.8
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.4. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	H_{15}				H_{17}				H_{18}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	20.0±0.3	24.5±0.0	31.8±0.1	5.5±0.4	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	-32.5±0.1	-21.5±0.0	-26.4±0.1	1.2±0.0	-19.5±0.1	-12.8±0.6	-16.4±0.1	0.3±0.0
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	-21.7±0.2	-6.3±0.1	-15.6±0.1	19.8±0.5	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.4. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	H_{19}				H_{20}				H_{21}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 1	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	-8.0±0.2	29.5±0.1	11.1±0.3	52.2±1.0	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	0.7±0.2	38.6±0.0	18.0±0.3	188.5±0.7	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	-0.7±0.1	26.5±0.3	19.2±0.6	119.1±0.6
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.4. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of palm stearin and its blends with coconut oil and canola oil after chemical interesterification.

Blends	H_{22}				H_{23}				ΔHmT (J/g)
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	
CIE 1	------	------	------	------	------	------	------	------	70.1±0.6
CIE 4	------	------	------	------	------	------	------	------	31.9±0.5
CIE 5	------	------	------	------	------	------	------	------	43.0±0.6
CIE 7	------	------	------	------	------	------	------	------	53.6±1.0
CIE 8	------	------	------	------	------	------	------	------	188.0±0.7
CIE 9	------	------	------	------	------	------	------	------	119.1±0.6
CIE 10	-32.0±0.1	-24.0±0.1	-27.6±0.0	2.7±0.0	-15.7±0.1	19.7±0.0	3.9±0.2	59.1±1.0	61.8±1.0

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.5. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil before chemical interesterification.

Blends	C_3				C_4				C_5			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
NIE2	30.8±0.1	27.7±0.0	29.5±0.1	-1.7±0.1	8.4±0.1	3.9±0.1	6.9±0.1	-42.8±3.1	-4.7±0.1	-13.9±0.0	-9.3±0.0	-45.8±1.5
NIE4	------	------	------	------	7.5±0.2	1.4±0.2	4.4±0.0	-2.9±0.2	------	------	------	------
NIE6	------	------	------	------	1.3±0.2	-2.6±0.3	0.2±0.3	-26.8±0.9	-12.4±4.3	-25.5±3.5	-20.5±5.4	-31.3±4.1
NIE7	------	------	------	------	5.3±0.1	-1.9±0.3	1.4±0.1	-1.6±0.1	-30.1±0.2	-38.2±1.5	-34.2±0.2	-7.6±0.4
NIE8	------	------	------	------	------	------	------	------	-26.8±2.0	-38.2±2.8	-33.8±2.0	-9.9±1.0
NIE9	------	------	------	------	17.7±0.1	13.2±0.1	15.9±0.1	-5.9±0.3	-11.2±0.6	-18.6±0.5	-15.0±0.1	-8.1±5.5
NIE10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.5. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil before chemical interesterification.

Blends	C_2+C_5				C_2+C_4				ΔHcT (J/g)
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	
NIE2	------	------	------	------	------	------	------	------	-90.4±4.4^{d}
NIE4	0.4±0.1	-20.7±0.1	-13.1±0.1	-40.3±1.1	------	------	------	------	-74.8±0.8^{c}
NIE6	------	------	------	------	------	------	------	------	-58.1±3.2^{b}
NIE7	------	------	------	------	------	------	------	------	-26.8±1.0^{a}
NIE8	------	------	------	------	3.5±0.3	-11.1±2.0	-0.1±0.7	-22.7±2.5	-65.1±3.9^{b}
NIE9	------	------	------	------	------	------	------	------	-24.3±4.7^{a}
NIE10	-7.3±0.2	-22.4±0.3	-13.2±0.1	-7.4±0.1	------	------	------	------	-59.0±1.0^{b}

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.6. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of coconut oil and its blends with palm stearin and canola oil before chemical interesterification.

Blends	H_7				$H_7+H_3+H_2$				H_7+H_4			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
NIE2	13.6±0.2	27.6±0.0	24.2±0.1	164.4±0.8	------	------	------	------	------	------	------	------
NIE4					3.0±0.0	28.7±0.0	23.1±0.1	84.1±0.2	------	------	------	------
NIE6	3.4±0.5	23.7±0.0	18.5±0.0	124.1±3.2	------	------	------	------	------	------	------	------
NIE7	------	------	------	------	------	------	------	------	15.3±0.3	25.2±0.2	19.5±0.1	8.3±0.9
NIE8	------	------	------	------	------	------	------	------	------	------	------	------
NIE9	------	------	------	------	11.0±0.3	28.4±0.1	23.2±0.1	72.1±1.1	------	------	------	------
NIE10	------	------	------	------	------	------	------	------	7.8±0.1	19.0±0.1	13.4±0.2	4.4±0.1

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.6. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of coconut oil and its blends with palm stearin and canola oil before chemical interesterification.

Blends	$H_7+H_4+H_5$				$H_7+H_4+H_2+H_3$				ΔHmT (J/g)
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	
NIE2	------	------	------	------	------	------	------	------	164.4±0.8^{f}
NIE4	------	------	------	------	------	------	------	------	115.8±1.0^{d}
NIE6	------	------	------	------	------	------	------	------	157.0±1.1^{f}
NIE7	------	------	------	------	11.5±0.2	26.3±0.0	19.4±0.2	39.0±0.7	64.2±2.0^{b}
NIE8	15.3±0.1	47.3±0.1	44.6±0.2	104.9±2.6	------	------	------	------	142.9±3.8^{e}
NIE9	------	------	------	------	10.4±0.2	29.3±0.6	23.5±0.0	92.6±4.0	81.5±3.6^{c}
NIE10	------	------	------	------	------	------	------	------	31.3±0.3^{a}

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.7. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	C_3				C_4				C_5			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 2	------	------	------	------	1.3±0.1	-4.7±0.2	-1.4±0.1	-10.7±0.4	1.2±1.0	-7.4±0.2	-2.1±0.2	-13.8±0.5
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.7. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	C_7				C_8				C_9			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 2	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	17.6±0.0	15.3±0.1	16.9±0.1	-8.9±0.2	1.3±0.1	-4.7±0.2	-1.4±0.1	-10.7±0.4	-8.8±0.2	-17.3±0.2	-12.5±0.2	11.2±0.5
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.7. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	C_{14}				C_{15}				C_{16}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 2	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	9.5±0.3	3.2±0.6	5.8±0.5	-0.4±0.1	-0.5±0.2	-4.0±0.1	-1.4±0.1	-1.5±0.2	-7.7±0.1	-19.1±0.1	-12.3±0.0	-18.7±1.5
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.7. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	C_{17}				C_{18}				C_{19}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 2	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	9.3±0.1	4.7±0.1	7.6±0.0	-4.1±0.1	0.5±0.1	-2.1±0.4	-1.4±0.3	-0.2±0.0	-3.4±0.1	-23.6±0.5	-14.4±0.3	-8.8±0.5
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.7. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

	C_{20}				C_{21}				C_{22}			
Blends	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**
CIE 2	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	-31.3±1.3	-43.1±0.2	-37.4±0.4	-13.1±0.6	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	20.1±0.3	17.2±0.5	19.0±0.1	-21.1±0.8	8.5±0.4	4.4±0.1	7.2±0.5	-4.8±0.4
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.7. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	C_{23}				C_{24}				C_{25}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 2	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	3.9±0.3	-0.5±0.2	0.6±0.1	-27.5±0.4	-21.5±1.3	-44.3±1.1	-38.7±1.4	-10.6±0.6	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	7.7±±0.2	4.1±0.2	6.5±0.0	-15.1±0.0
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.7. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

	C_{26}				C_{27}				C_{28}			
Blends	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**
CIE 2	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	0.2±0.0	-12.6±0.3	-5.2±0.0	-68.3±0.3	-30.9±0.1	-30.9±0.1	-44.0±0.1	-3.3±0.2	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	-0.3±0.0	-3.0±0.2	-1.6±0.2	-1.3±0.1

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.7. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	C_{29}				C_{30}				ΔHcT (J/g)
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	
CIE 2	------	------	------	------	------	------	------	------	-20.4±1.0
CIE 4	------	------	------	------	------	------	------	------	-30.9±0.2
CIE 6	------	------	------	------	------	------	------	------	-20.6±1.6
CIE 7	------	------	------	------	------	------	------	------	-13.1±0.6
CIE 8	------	------	------	------	------	------	------	------	-64.0±0.3
CIE 9	------	------	------	------	------	------	------	------	-86.3±0.2
CIE 10	-8.6±0.1	-19.6±0.0	-13.4±0.1	-18.8±0.1	-40.0±0.2	-52.7±0.1	-45.9±0.0	-20.8±0.9	-40.8±0.6

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.8. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	H_7				H_8				H_9			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 2	9.4±0.3	26.3±0.0	23.0±0.3	118.6±0.1	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	-5.0±0.1	19.5±0.0	16.5±0.1	15.8±0.1
CIE 6	------	------	------	------	-31.6±0.3	-12.9±0.1	-20.1±0.2	52.1±0.7	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.8. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	H_{10}				H_{16}				H_{17}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 2	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	21.8±0.1	34.3±0.0	31.5±0.1	13.5±0.2	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	-6.3±0.4	20.0±0.2	10.7±0.0	50.1±0.8	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	-32.5±0.1	-21.5±0.0	-26.4±0.1	1.2±0.0
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE10	------	------	------	------	------	------	------	------	-21.7±0.2	-6.3±0.1	-15.6±0.1	19.8±0.5

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.8. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	H_{18}				H_{19}				H_{20}			
	TOnm (°C)	**TEnm (°C)**	**TPm (°C)**	**ΔHm J/g**	**TOnm (°C)**	**TEnm (°C)**	**TPm (°C)**	**ΔHm J/g**	**TOnm (°C)**	**TEnm (°C)**	**TPm (°C)**	**ΔHm J/g**
CIE 2	------	------	------	------	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	-19.5±0.1	-12.8±0.6	-16.4±0.1	0.3±0.0	-8.0±0.2	29.5±0.1	11.1±0.3	52.2±1.0	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	0.7±0.2	38.6±0.0	18.0±0.3	188.5±0.7
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.8. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	H_{21}				H_{22}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 2	------	------	------	------	------	------	------	------
CIE 4	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------
CIE 9	-0.7±0.1	26.5±0.3	19.2±0.6	119.1±0.6	------	------	------	------
CIE 10	------	------	------	------	-32.0±0.1	-24.0±0.1	-27.6±0.0	2.7±0.0

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.8. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of coconut oil and its blends with palm stearin and canola oil after chemical interesterification.

Blends	H_{23}				Δ HmT (J/g)
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	
CIE 2	------	------	------	------	118.6±0.1
CIE 4	------	------	------	------	31.9±0.5
CIE 6	------	------	------	------	50.1±0.8
CIE 7	------	------	------	------	53.6±1.0
CIE 8	------	------	------	------	188±0.7
CIE 9	------	------	------	------	119.1±0.6
CIE 10	-15.7±0.1	19.7±0.0	3.9±0.2	59.1±1.0	61.8±1.0

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.9. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of canola oil and its blends with palm stearin and coconut oil before chemical interesterification,

Blends	C_6				Δ HcT (J/g)
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	
NIE3	-47.4±1.9	-52.6±2.5	-50.0±2.3	-99.8±2.5	-99.8±2.5^{d}
NIE5	-35.3±0.2	-53.1±0.4	-48.2±0.2	-19.1±0.6	-43.5±0.1^{b}
NIE6	------	------	------	------	-58.1±3.2^{c}
NIE7	------	------	------	------	-26.8±1.0^{a}
NIE8	------	------	------	------	-65.1±3.9^{c}
NIE9	------	------	------	------	-24.3±4.7^{a}
NIE10	-29.7±0.2	-39.5±0.4	-34.4±0.3	-43.9±0.7	-59.0±1.0^{c}

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.10. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of the canola oil and its blends with palm stearin and coconut oil before chemical interesterification,

Blends	H_8				H_1+H_8				ΔHmT (J/g)
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	
NIE3	-24.6±0.3	-6.7±0.2	-16.0±0.7	140.9±2.0	------	------	------	------	140.9±2.0^{d}
NIE5	------	------	------	------	-23.6±0.3	-11.6±0.2	-17.5±0.0	4.5±1.2	42.5±1.6^{a}
NIE6	-25.2±0.3	-13.5±0.1	-17.7±0.1	32.9±1.2					157.0±1.1^{e}
NIE7	------	------	------	------	-23.4±0.4	-9.0±0.1	-16.0±0.2	10.4±1.0	64.2±2.0^{b}
NIE8	------	------	------	------	-21.4±0.3	1.6±0.2	-1.3±0.1	11.2±1.2	142.9±3.8^{de}
NIE9	-25.5±0.4	-11.9±0.5	-18.8±0.1	4.5±0.3					81.5±3.6^{c}
NIE10	------	------	------	------	-21.7±0.2	-6.3±0.1	-15.6±0.1	19.8±0.5	31.3±0,3^{a}

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.11. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of canola oil and its blends with palm stearin and coconut oil after interesterification after chemical interesterification,

Blends	C_6				C_{6B}				C_{6A}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 3	-40.5±0.3	-54.4±1.3	50.1±1.1	66.7±2.1	-0.8±0.1	-8.9±0.0	-4.7±0.0	2.6±0.2	-7.2±0.1	-3.3±0.3	-5.9±0.1	2.3±0.1
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.11. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of canola oil and its blends with palm stearin and coconut oil after interesterification after chemical interesterification,

	C_{10}				C_{11}				C_{12}			
Blends	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**
CIE 3	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	13.9±0.0	9.1±0.7	12.1±0.4	-8.4±0.1	1.2±0.1	-6.9±0.3	-2.3±0.1	-9.0±0.9	-10.4±0.3	-20.1±0.3	-14.6±0.4	-12.4±0.7
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.11. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of canola oil and its blends with palm stearin and coconut oil after interesterification after chemical interesterification,

Blends	C_{13}				C_{14}				C_{15}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 3	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	-38.5±0.6	-54.6±2.0	-45.6±1.4	-22.5±2.4	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	9.5±0.3	3.2±0.6	5.8±0.5	-0.4±0.1	-0.5±0.2	-4.0±0.1	-1.4±0.1	-1.5±0.2
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.11. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of canola oil and its blends with palm stearin and coconut oil after interesterification after chemical interesterification,

Blends	C_{19}				C_{20}				C_{21}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 3	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	-3.4±0.1	-23.6±0,5	-14,4±0,3	-8,8±0,5	-31.3±1.3	-43.1±0.2	-37.4±0.4	-13.1±0.6	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	20.1±0.3	17.2±0.5	19.0±0.1	-21.1±0.8
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.11. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of canola oil and its blends with palm stearin and coconut oil after interesterification after chemical interesterification,

Blends	C_{22}				C_{23}				C_{24}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 3	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	8.5±0.4	4.4±0.1	7.2±0.5	-4.8±0.4	3.9±0.3	-0.5±0.2	0.6±0.1	-27.5±0.4	-21.5±1.3	-44.3±1.1	-38.7±1.4	-10.6±0.6
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table 11.11. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of canola oil and its blends with palm stearin and coconut oil after interesterification after chemical interesterification,

	C_{25}				C_{26}				C_{27}			
Blends	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**	**TOnc (°C)**	**TEnc (°C)**	**TPc (°C)**	**ΔHc J/g**
CIE 3	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	7.7±±0.2	4.1±0.2	6.5±0.0	-15.1±0.0	0.2±0.0	-12.6±0.3	-5.2±0.0	-68.3±0.3	-30.9±0.1	-30.9±0.1	-44.0±0.1	-3.3±0.2
CIE10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.11. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of canola oil and its blends with palm stearin and coconut oil after interesterification after chemical interesterification,

Blends	C_{28}				C_{29}			
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g
CIE 3	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------
CIE 10	-0.3±0.0	-3.0±0.2	-1.6±0.2	-1.3±0.1	-8.6±0.1	-19.6±0.0	-13.4±0.1	-18.8±0.1

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05).

Table 11.11. Onset crystallization temperature (TOnc), final crystallization temperature (TEnc), peak crystallization temperature (TPc), crystallization enthalpy (ΔHc), and total crystallization enthalpy (ΔHcT) of canola oil and its blends with palm stearin and coconut oil after interesterification after chemical interesterification,

Blends	C_{30}				ΔHcT (J/g)
	TOnc (°C)	TEnc (°C)	TPc (°C)	ΔHc J/g	
CIE 3	------	------	------	------	-71.5±2.2
CIE 5	------	------	------	------	-52.4±3.2
CIE 6	------	------	------	------	-20.6±1.6
CIE 7	------	------	------	------	-13.1±0.6
CIE 8	------	------	------	------	-64.0±0.3
CIE 9	------	------	------	------	-86.3±0.2
CIE 10	-40.0±0.2	-52.7±0.1	-45.9±0.0	-20.8±0.9	-40.8±0.6

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$).

Table .11.12. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of canola oil and its blends with palm stearin and coconut oil after chemical interesterification after chemical interesterification,

Blends	H_8				H_{8a}				H_{11}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 3	-31.6±0.3	-12.9±0.1	-20.1±0.2	52.1±0.7	12.1±0.2	24.8±0.1	20.6±0.1	11.6±0.3	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	-24.5±0.6	-6.4±0.1	-16.3±0.2	8.1±0.4
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$),

Table 11.12. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of canola oil and its blends with palm stearin and coconut oil after chemical interesterification after chemical interesterification,

Blends	H_{12}				H_{13}				H_{14}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 3	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	-4.1±0.3	4.6±0.1	0.2±0.2	18.6±0.4	-24.5±0.6	-6.4±0.1	-16.3±0.2	8.1±0.4	7.2±0.6	19.7±0.1	12.8±0.3	14.1±1.8
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$),

Table 11.12. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of canola oil and its blends with palm stearin and coconut oil after chemical interesterification after chemical interesterification,

Blends	H_{15}				H_{16}				H_{17}			
	TOnm (°C)	**TEnm (°C)**	**TPm (°C)**	**ΔHm J/g**	**TOnm (°C)**	**TEnm (°C)**	**TPm (°C)**	**ΔHm J/g**	**TOnm (°C)**	**TEnm (°C)**	TPm (°C)	ΔHm J/g
CIE 3	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	20.0±0.3	24.5±0.0	31.8±0.1	5.5±0.4	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	-6.3±0.4	20.0±0.2	10.7±0.0	50.1±0.8	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------	-32.5±0.1	-21.5±0.0	-26.4±0.1	1.2±0.0
CIE 8	------	------	------	------	------	------	------	------	------	------	------	------
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	-21.7±0.2	-6.3±0.1	-15.6±0.1	19.8±0.5

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$),

Table 11.12. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of canola oil and its blends with palm stearin and coconut oil after chemical interesterification after chemical interesterification,

Blends	H_{18}				H_{19}				H_{20}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 3	------	------	------	------	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------	------	------	------	------
CIE 7	-19.5±0.1	-12.8±0.6	-16.4±0.1	0.3±0.0	-8.0±0.2	29.5±0.1	11.1±0.3	52.2±1.0	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------	0.7±0.2	38.6±0.0	18.0±0.3	188.5±0.7
CIE 9	------	------	------	------	------	------	------	------	------	------	------	------
CIE 10	------	------	------	------	------	------	------	------	------	------	------	------

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$),

Table 11.12. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of canola oil and its blends with palm stearin and coconut oil after chemical interesterification after chemical interesterification,

Blends	H_{21}				H_{22}			
	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g	TOnm (°C)	TEnm (°C)	TPm (°C)	ΔHm J/g
CIE 3	------	------	------	------	------	------	------	------
CIE 5	------	------	------	------	------	------	------	------
CIE 6	------	------	------	------	------	------	------	------
CIE 7	------	------	------	------	------	------	------	------
CIE 8	------	------	------	------	------	------	------	------
CIE 9	-0.7±0.1	26.5±0.3	19.2±0.6	119.1±0.6	------	------	------	------
CIE 10	------	------	------	------	-32.0±0.1	-24.0±0.1	-27.6±0.0	2.7±0.0

Means (n = 3) values with different superscript letters in the same column are significantly different ($P<0.05$),

Table 11.12. Melting onset temperature (TOnm), final melting temperature (TEnm), melting peak temperature (TPm), melting enthalpie (ΔHm), and total melting enthalpie (ΔHmT) of canola oil and its blends with palm stearin and coconut oil after chemical interesterification after chemical interesterification,

		H_{23}			ΔHmT (J/g)
Blends	**TOnm (°C)**	**TEnm (°C)**	**TPm (°C)**	**ΔHm J/g**	
CIE 3	------	------	------	------	63.8±1.0
CIE 5	------	------	------	------	43.0±0.6
CIE 6	------	------	------	------	50.1±0.8
CIE 7	------	------	------	------	53.6±1.0
CIE 8	------	------	------	------	188.5±0.7
CIE 9	------	------	------	------	119.1±0.6
CIE 10	-15.7±0.1	19.7±0.0	3.9±0.2	59.1±1.0	61.8±1.0

Means (n = 3) values with different superscript letters in the same column are significantly different (P<0.05),

Declaramos para os devidos fins que o presente trabalho dispensou análise do Comitê de Ética em Pesquisa e/ou Comitê de Ética em Experimentação Animal.

São Paulo, 10 de março de 2014.

Fabiana Andreia Schäfer De Martini Soares

Prof. Dr. Luiz Antonio Gioielli

Orientador

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas
FICHA DO ALUNO

9133 - 5720677/2 - Fabiana Andréia Schäfer De Martini Soares

Email: fabimarti@usp.br
Data de Nascimento: 09/05/1975
Cédula de Identidade: RG - 2.971.498 - SC
Local de Nascimento: Estado de Santa Catarina
Nacionalidade: Brasileira

Graduação: Farmacêutico - Universidade Federal de Santa Catarina - Santa Catarina - Brasil - 1998

Mestrado: Mestre em Ciências - Área: Tecnologia de Alimentos - Faculdade de Ciências Farmacêuticas - Universidade de São Paulo - São Paulo - Brasil - 2010

Curso: Doutorado
Programa: Tecnologia Bioquímico-Farmacêutica
Área: Tecnologia de Alimentos
Data de Matrícula: 08/03/2010
Início da Contagem de Prazo: 08/03/2010
Data Limite para o Depósito: 10/03/2014

Orientador: Prof(a). Dr(a). Luiz Antonio Gioielli - 08/03/2010 até o presente. E.Mail: lagio@usp.br

Proficiência em Línguas: Inglês, Aprovado em 21/10/2010

Data de Aprovação no Exame de Qualificação: Aprovado em 24/02/2012

9133 - 5720677/2 - Fabiana Andréia Schäfer De Martini Soares

Sigla	Nome da Disciplina	Início	Término	Carga Horária	Cred.	Freq.	Conc.	Exc.	Situação
FBT5729-6/2	Liofilização e suas Aplicações a Alimentos e à Medicamentos	02/04/2010	03/06/2010	90	6	100	A	N	Concluída
FBT5781-3/3	Culturas Probióticas: Aplicações Tecnológicas	04/05/2010	15/06/2010	60	4	100	A	N	Concluída
QFL5714-5/1	Introdução à Análise Térmica (Instituto de Química - Universidade de São Paulo)	17/08/2010	27/09/2010	90	6	100	A	N	Concluída
QFL5715-6/1	Projetos em Análise Térmica (Instituto de Química - Universidade de São Paulo)	14/03/2011	23/05/2011	90	6	100	A	N	Concluída
FBT5773-6/2	Tópicos Especiais em Tecnologia Bioquímico-Farmacêutica	07/04/2011	16/06/2011	30	2	80	A	N	Concluída
Atividade do Programa	Publicação do trabalho "Chemical Interesterification of Blends of Palm Stearin, Conconut Oil, and Canola Oil: Physicochemical Properties", na Journal of Agricultural and Food Chemistry, v. 1, p. 1461-1469, Estados Unidos, 2012 (1)	09/01/2012	09/01/2012	-	3	0	-	-	-

	Créditos mínimos exigidos	Créditos obtidos

	Para exame de qualificação	Para depósito de tese	
Disciplinas:	0	25	27
Estágios:			
Total:	0	25	27

Créditos Atribuídos à Tese: 167

Histórico de Ocorrências: Ingressou no Doutorado em 08/03/2010
Matrícula de Acompanhamento em 03/02/2014

Observações:

1) Créditos atribuídos de acordo com o disposto na Portaria GR 3588, de 11.05.2005 e aprovados pela Comissão de Pós-Graduação, em Sessão de 18/04/2012.

Conceito a partir de 02/01/1997:

A - Excelente, com direito a crédito; B - Bom, com direito a crédito; C - Regular, com direito a crédito; R - Reprovado; T - Transferência.

Um(1) crédito equivale a 15 horas de atividade programada.

Anexo 4

Fabiana Andreia Schäfer De Martini Soares
Curriculum Vitae

Formação acadêmica/titulação

2010 Doutorado em Ciências Farmacêuticas.
Universidade de São Paulo, USP, São Paulo, Brasil
Com período sanduíche em Instituto Superior de Agronomia (Orientador: Prof. Dra. Suzana Ferreira Dias)
Título: Interesterificação química e enzimática contínua de misturas de estearina e oleína de palma, óleo de coco e óleo de canola para formulação de margarinas com baixas concentrações de isômeros trans
Orientador: Dr Luiz Antônio Gioielli
Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo

2012 - 2012 Doutorado em Ciências Farmacêuticas.
Instituto Superior de Agronomia, ISA, Portugal
Com período sanduíche em Instituto Superior de Agronomia (Orientador: Suzana Ferreira-Dias)
Título: Interesterificação química e enzimática continua de estearina de palma, óleo de coco e óleo de canola, Ano de obtenção: 2013
Orientador: Luiz Antônio Gioielli
Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo

2008 - 2010 Mestrado em Tecnologia Bioquímico-Farmacêutica.
Universidade de São Paulo, USP, São Paulo, Brasil
Título: Efeito da interesterificação química sob as propriedades físico-químicas de misturas de estearina e oleína de palma, Ano de obtenção: 2010
Orientador: Dr Luiz Antônio Gioielli
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2003 - 2004 Especialização em Biologia Aplicada a Biotecnologia e Saúde.
Universidade de Rio Verde, FESURV, Rio Verde, Brasil
Título: Controle microbiológico de carcaças de frango
Orientador: Msc Débora Cabral Machado

1998 - 2000 Graduação em Farmácia Bioquímica Tecnologia de Alimentos.
Universidade Federal de Santa Catarina, UFSC, Florianópolis, Brasil
Título: Obtenção e caracterização de concentrado e hidrolisado proteico de soro doce
Orientador: Dr Marilde Bordignon Luiz e Dr Maria Teresa Bertoldo Pacheco

1995 - 1998 Graduação em Farmácia.
Universidade Federal de Santa Catarina, UFSC, Florianópolis, Brasil
Título: Acompanhamento hospitalar de paciente com leucemia
Orientador: Dr Ricardo Marques da Rocha Pereira

1990 - 1993 Ensino Profissional de nível técnico em Técnico de Alimentos.
Universidade do Oeste de Santa Catarina, UNOESC, Videira, Brasil

Formação complementar

2012 - 2012 Curso de curta duração em Palestra Prof. John Mittchel.
Instituto Superior de Agronomia, ISA, Portugal

2011 - 2011 Curso de curta duração em Treinamento de Estatística Básica.
StatSoft South América Comercio de Software Ltda., STATSOFT, Brasil
Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo

2011 - 2011 Extensão universitária em Workshop de Capacitação para Pesquisadores da USP.
Universidade de São Paulo, USP, São Paulo, Brasil

2011 - 2011 Curso de curta duração em Modification of fats: New Technologies for Change.
AOCS Latin American, AOCS, Argentina

2010 - 2010 Curso de curta duração em Tecnologia de Chocolates.
Universidade de São Paulo, USP, São Paulo, Brasil

2009 - 2009 Curso de curta duração em Introdução a análise térmica.
Perkin Elmer, PH, Brasil

2009 - 2009 Curso de curta duração em Treinamento Operacional em DSC + Software Pyris.
Perkin Elmer, PH, Brasil

2009 - 2009 Curso de curta duração em Treinamento Operacional em GC 430 + Software Galax.
Varian Industria e Comércio Ltda, VARIAN, Brasil

2008 - 2008 Extensão universitária em Introdução à análise Exploratória de Dados e Método.
Instituto de Matemática e Estatística, IME USP, São Paulo, Brasil

2007 - 2007 Extensão universitária em Tópicos em Tratamento de Dados para Ciência e Tecn.
Universidade de São Paulo, USP, São Paulo, Brasil

2007 - 2007 Extensão universitária em Compostos Bioativos em Alimentos e sua Relação com.
Universidade de São Paulo, USP, São Paulo, Brasil

2007 - 2007 Extensão universitária em Processos Deteriorantes de Alimentos pelo Desenvolvimento de Microrganismo.
Universidade de São Paulo, USP, São Paulo, Brasil

2007 - 2007 Extensão universitária em Microrganismos Patogênicos em Alimentos I.
Universidade de São Paulo, USP, São Paulo, Brasil

2007 - 2007 Extensão universitária em Bacteriocinas e suas Aplicações em Alimentos.
Universidade de São Paulo, USP, São Paulo, Brasil

Atuação profissional

1. Universidade de São Paulo - USP

Vínculo institucional

2010 - Atual Vínculo: Bolsista, Enquadramento funcional: Doutorado, Carga horária: 44, Regime: Dedicação exclusiva

2008 - 2010 Vínculo: Livre Enquadramento funcional: Mestrado Carga horária: 44, Regime: Dedicação exclusiva

Atividades

08/2010 - 12/2010 Estágio, Faculdade de Ciências Farmacêuticas
Estágio:
Estagio de Aperfeiçoamento Didático na Disciplina de Física Industrial

03/2009 - 07/2009 Estágio, Faculdade de Ciências Farmacêuticas
Estágio:
Programa de Aperfeiçoamento de Ensino na disciplina 0900100 Informação Científica para o curso de Farmácia e Bioquímica

06/2008 - 12/2008 Estágio, Faculdade de Ciências Farmacêuticas
Estágio:
Programa de Aperfeiçoamento de Ensino na disciplina FBT 0201 Tecnologia de Alimentos para o curso de Nutrição Aplicada a Humanos

02/2008 - 02/2010 Pesquisa e Desenvolvimento, Faculdade de Ciências Farmacêuticas
Linhas de pesquisa:
Interesterificação química de estearina e oleína de palma

2. Perdigão - Filial Rio Verde - PERDIGÃO

Vínculo institucional

2000 - 2006 Vínculo: Colaborador, Enquadramento funcional: Bioquímica Junior, Carga horária: 44, Regime: Dedicação exclusiva

Atividades

09/2000 - 04/2006 Serviço Técnico Especializado, Laboratório da Garantia da Qualidade
Especificação:
Preparo e Padronização de Reagentes e Soluções Químicas.

09/2000 - 04/2006 Direção e Administração, Laboratório da Garantia da Qualidade
Cargos ocupados:
Responsabilidade pela coordenação dos trabalhos referentes ao Laboratório de Análise de Alimentos, Rações quanto a operação, controle e elaboração de documentação e suporte às Missões Técnicas e/ ou Comerciais, nacionais e/ ou internacionais;

09/2000 - 04/2006 Pesquisa e Desenvolvimento, Laboratório da Garantia da Qualidade
Linhas de pesquisa:
Implantações de novos métodos de análises físico-químicos e microbiológicos, Pesquisa de microrganismos patogênicos nas linhas de produção

09/2000 - 04/2006 Treinamento, Laboratório da Garantia da Qualidade
Especificação:
Ministrante de Treinamento de BPF, HACCP e PPHO, Responsabilidade por treinar usuários de equipamentos, quanto aos métodos de utilização, verificação e técnicos em análises microbiológicas, físico-químicas e sensoriais;

09/2000 - 04/2006 Serviço Técnico Especializado, Laboratório da Garantia da Qualidade
Especificação:
Calibração de Equipamentos e Vidrarias de Laboratório.

09/2000 - 04/2006 Outra atividade técnico-científica, Laboratório da Garantia da Qualidade
Especificação:
Levantamento estatístico em relação as análises físico-químicas, microbiológicas e sensoriais das matérias-primas e produtos finais da indústria de processamento de carne e rações e derivados

09/2000 - 04/2006 Outra atividade técnico-científica, Laboratório da Garantia da Qualidade
Especificação:
Análises Químicas, Físico-Químicas, Microbiológicas, Bromatológica, Padronização e Controle de Qualidade de matérias primas de origem animal, vegetal e mineral e dos produtos finais da indústria de processamento de carne, rações e derivados

09/2000 - 04/2006 Direção e Administração, Laboratório da Garantia da Qualidade
Cargos ocupados:
- Avaliação de desempenho operacional via People Soft;

09/2000 - 04/2006 Direção e Administração, Laboratório da Garantia da Qualidade
Cargos ocupados:
- Elaboração e implementação de plano anual de investimentos;

09/2000 - 04/2006 Serviço Técnico Especializado, Laboratório da Garantia da Qualidade
Especificação:
-Elaboração de Manual de Qualidade de laboratório segundo a. ISO 17025

09/2000 - 04/2006 Direção e Administração, Laboratório da Garantia da Qualidade
Cargos ocupados:
- Negociação com fornecedores quanto à compra de equipamentos, desenvolvimento de produtos e equipamentos;

09/2000 - 04/2006 Serviço Técnico Especializado, Laboratório da Garantia da Qualidade
Especificação:
Elaboração e Implantação de novas Técnicas de Análises Físico-Químicas, Microbiológicas e de Análise Sensorial

3. Universidade de Rio Verde - FESURV

Vínculo institucional

2005 - 2006 Vínculo: Professor visitante, Enquadramento funcional: Professor substituto, Carga horária: 20, Regime: Parcial

Atividades

08/2005 - 04/2006 Graduação, Farmácia e Bioquímica
Disciplinas ministradas:
Bromatologia

4. Universidade Federal de Santa Catarina - UFSC

Vínculo institucional

2000 - 2000 Vínculo: Livre, Enquadramento funcional: Analista de Laboratório Superior, Carga horária: 40, Regime: Dedicação exclusiva
2000 - 2000 Vínculo: Colaborador, Enquadramento funcional: Técnico de laboratório superior, Carga horária: 44, Regime: Dedicação exclusiva
1999 - 1999 Vínculo: Monitoria, Enquadramento funcional: Bolsista, Carga horária: 20, Regime: Parcial
1996 - 1997 Vínculo: Outro, Enquadramento funcional: Estagiário, Carga horária: 12, Regime: Dedicação exclusiva

Atividades

02/2000 - 06/2001 Outra atividade técnico-científica, Centro de Ciências Agrárias, Departamento de Ciência e Tecnologia dos Alimentos
Especificação:
Análise físico-química de produtos produzidos por agricultores do programa de Agricultura familiar do FUNCITEC. Bolsa de Desenvolvimento Tecnológico CNPQ

01/2000 - 08/2000 Pesquisa e Desenvolvimento, Centro de Ciências Agrárias, Departamento de Ciência e Tecnologia dos Alimentos
Linhas de pesquisa:
Análise físico-químicas de alimentos de origem animal e vegetal

08/1996 - 07/1997 Estágio, Departamento de Química
Estágio:
Iniciação Científica Bolsista CNPQ

5. Instituto de Tecnologia de Alimentos - ITAL

Vínculo institucional

1999 - 1999 Vínculo: Colaborador, Enquadramento funcional: Estagiário, Carga horária: 44, Regime: Dedicação exclusiva

Atividades

08/1999 - 11/1999 Estágio, Centro de Química e Nutrição Aplicada, Bioquímica
Estágio:
Produção de um hidrolisado ácido de proteína do soro de leite

08/1999 - 11/1999 Estágio, Centro de Química e Nutrição Aplicada, Bioquímica
Estágio:
Determinação da composição centesimal do hidrolisado ácido de proteína de soro de leite

08/1999 - 11/1999 Estágio, Centro de Química e Nutrição Aplicada, Bioquímica
Estágio:
Acompanhamento da suplementação de camundongo com o hidrolisado ácido de proteína de soro, caseinato de sódio e proteína de soja comerciais

08/1999 - 11/1999 Estágio, Centro de Química e Nutrição Aplicada, Bioquímica

Estágio:
Acompanhamento de análise de fibra alimentar

08/1999 - 11/1999 Estágio, Centro de Química e Nutrição Aplicada, Bioquímica
Estágio:
Padronização do método de determinação de glutationa em fígado de camundongo

---

Revisor de periódico

1. Journal of the American Oil Chemists´ Society

Vínculo

2012 - Atual Regime: Parcial

Prêmios e títulos

2013 Melhor Pôster de Iniciação Científica 18ª Semana Farmacêutica de Ciências e Tecnologia, Faculdade de Ciências Farmacêuticas Universidade de São Paulo

2013 PREMIO BUNGE SBOG, BUNGE ALIMENTOS

2012 Melhor apresentação de Pôster no Simpósio Biocatalysis in Lipid Modification, Euro Fed Lipid, Greifswald, Alemanha

2011 AOCS Health and Nutrition Division 2nd Place- Student Poster Award, American Oil Chemistry Society AOCS Health and Nutrition Division

2010 Menção Honrosa em Pôster em Iniciação Cientifica da XLV Semana Universitária Paulista de Farmácia e Bioquímica, Universidade de São Paulo/ Faculdade de Ciências Farmacêuticas

2009 Melhor Pôster de Iniciação Científica 17ª Semana Farmacêutica de Ciências e Tecnologia, Universidade de São Paulo

2008 Menção Honrosa Apresentação Oral 16ª Reunião de Iniciação Científica, Faculdade de Ciências Farmacêuticas

2008 Menção Honrosa Pôster 16ª Reunião de Iniciação Científica, Faculdade de Ciências Farmacêuticas

Produção

Produção bibliográfica
Artigos completos publicados em periódicos

1. **De Martini Soares, Fabiana Andreia Schäfer**, OSÓRIO, NATÁLIA M., DA SILVA, ROBERTA CLARO, GIOIELLI, LUIZ ANTONIO, FERREIRA-DIAS, SUZANA
Batch and continuous lipase-catalyzed interesterification of blends containing olive oil for -free margarines. European Journal of Lipid Science and Technology (Print). , v.115, p.413 - , 2013.

2. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, MARUYAMA, J. M, DAGOSTINHO, N. R., CALLIGARIS, G., RIBEIRO, A. P. B., CARDOSO, L.P., GIOIELLI, LUIZ ANTONIO
Effect of diacylglycerol addition on crystallization properties of pure triacylglycerols. Food Research International. v.55, p.436 - 444, 2013.

3. LEITE, P. B., LANNES, S. C. S., Rodrigues, A.M., SOARES, S. E., Bispo, E.S, **De Martini Soares, Fabiana Andreia Schäfer**
Estudo reológico de chocolates elaborados com diferentes cultivares de cacau (Theobroma cacao L.). Brazilian Journal of Food Technology (Online). , v.16, p.192 - 197, 2013.

4. SILVA, Roberta Claro da, RIBEIRO, A. P. B., **Soares, Fabiana Andreia Schäfer De Martini**, CAPACLA, I. R., HAZZAN, M., SANTOS, A.O., CARDOSO, L.P., Gioielli, L.A.
Microstructure and thermal profile of structured lipids produced by continuous enzymatic interesterification. Journal of the American Oil Chemists' Society. v.90, p.631 - 639, 2013.

5. BOGSAN, C. S. B., Florence, Ana Carolina Rodrigues, PERINA, N., HIROTA, C., Fabiana, SILVA, Roberta Claro da, SILVA, Roberta Claro da, OLIVEIRA, M. N.
Survival of Bifidobacterium Lactis hn019 and Release of Biogenic. J Prob Health. , v.1, p.1 - 7, 2013.

6. SANTOS O. V., CORRÊA, N. C. F., **SOARES, F. A. S. M.**, Gioielli, L.A., COSTA, C. E. F., LANNES, S. C. S.
Chemical evaluation and thermal behavior of Brazil nut oil obtained by different extraction processes. Food Research International. , v.47, p.253 - 258, 2012.

7. **Soares, Fabiana Andreia Schäfer De Martini**, HAZZAN, M., CAPACLA, I. R., VICCOLA, E.R., MARUYAMA, J. M, Gioielli, L.A.
Chemical Interesterification of Blends of Palm Stearin, Coconut Oil, and Canola Oil: Physicochemical Properties. Journal of Agricultural and Food Chemistry. , v.60, p.1461 - 1469, 2012.

8. SILVA, Roberta Claro da, **Soares, Fabiana Andreia Schaffer De Martini**, HAZZAN, M., CAPACLA, I. R., GONCALVES, M. I. A., Gioielli, L.A.
Continuous enzymatic interesterification of lard and soybean oil blend: Effects of different flow rates on physical properties and acyl migration. Journal of Molecular Catalysis. B, Enzymatic (Print). v.76, p.23 - 28, 2012.

9. SANTO, A. P. E., CARTOLANO, N.S., Silva, Thaiane F., **Soares, Fabiana A.S.M.**, Gioielli, L.A., PEREGO, P., CONVERTI, A., OLIVEIRA, M. N
Fibers from fruit by-products enhance probiotic viability and fatty acid profile and increase CLA content in yoghurts. International Journal of Food Microbiology. v.154, p.135 - 144, 2012.

10. FLORENCE, A. C. R, OLIVEIRA, R.P.S., SILVA, Roberta Claro da, **Soares, Fabiana Andreia Schäfer De Martini**, Gioielli, L.A., OLIVEIRA, M. N
Organic milk improves Bifidobacterium lactis counts and bioactive fatty acids contents in fermented milk. Lebensmittel-Wissenschaft Technologie / Food Science + Technology. , v.49, p.89 - 95, 2012.

11. PRESTES, P. S., **SOARES, F. A. S. M.**, OLIVEIRA, A. M., AREAS, E.P.G., Gioielli, L.A., KANEKO, T.M., GUIMARÃES, K.L., ZANIN, M.H.A., VELASCO, M.V.R., BABY, A.R.
Rheological Measurements and Thermal Characterization of Lamellar Gel Phase Emulsions Developed with Cetearyl Alcohol/Nonionic Ethoxylated Surfactants. Journal of Dispersion Science and Technology. , v.33, p.1621 - 1628, 2012.

12. SILVA, Roberta Claro da, **Soares, Fabiana Andreia Schaffer De Martini**, FERNANDES, T.G., SILVA, K. C. G., GONCALVES, M. I. A., CHIU, M.C., GONCALVES, L.A.G., Gioielli, L.A.
Interesterification of Lard and Soybean Oil Blends Catalyzed by Immobilized Lipase in a Continuous Packed Bed Reactor. Journal of the American Oil Chemists' Society. , v.88, p.1925 - 1933, 2011.

13. SEGURA, N., SILVA, Roberta Claro da, **M. Soares, Fabiana A. Schäfer**, Gioielli, L.A., JACHMANIAN, I.
Valorization of Beef Tallow by Lipase-Catalyzed Interesterification with High Oleic Sunflower Oil. Journal of the American Oil Chemists' Society. v.88, p.1945 - 1954, 2011.

14. SANTO, A. P. E., SILVA, Roberta Claro da, Fabiana, ANJOS, D., Gioielli, L.A., OLIVEIRA, M. N, SILVA, Roberta Claro da, SILVA, Roberta Claro da
Açai pulp addition improves fatty acid profile and probiotic viability in yoghurt. International Dairy Journal. v.20, p.415 - 422, 2010.

15. SILVA, Roberta Claro da, SOARES, D.F., LOURENCO, M. B., **SOARES, F. A. S. M.**, SILVA, K. C. G., GONCALVES, M. I. A., Gioielli, L.A.
Structured lipids obtained by chemical interesterification of olive oil and palm stearin. Lebensmittel-Wissenschaft + Technologie / Food Science + Technology. , v.43, p.752 - 756, 2010.

16. **Soares, Fabiana Andreia Schäfer De Martini**, SILVA, Roberta Claro da, SOARES, D.F.
Effects of chemical interesterification on physicochemical properties of blends of palm stearin and palm olein. Food Research International. v.42, p.1287 - 1294, 2009.

Artigos aceitos para publicação

1. MARUYAMA, JESSICA MAYUMI, **SOARES, FABIANA ANDREIA SCHAFER DE MARTINI**, DAGOSTINHO, NATÁLIA ROQUE, GONÇALVES, MARIA INES ALMEIDA, GIOIELLI, LUIZ ANTONIO, DA SILVA, ROBERTA CLARO
Effects of emulsifier addition on the crystallization and melting behavior of palm olein and coconut oil. Journal of Agricultural and Food Chemistry. 2014.

2. BOGSAN, C. S. B, FLORENCE, A. C. R, PERINI, N. P., HIROTA, C. Y., **Soares, Fabiana A.S.M.**, SILVA, Roberta Claro da, OLIVEIRA, M. N
Survival of Bifidobacterium lactis HN019 and release of biogenic compounds in unfermented and fermented milk is affected by

chilled storage at 4 C. Journal of Probiotic and Health. , 2014.

Trabalhos publicados em anais de eventos (resumo)

1. **SOARES, F. A. S. M.**, SILVA, K. C. G., DAGOSTINHO, N. R., MARUYAMA, J. M, OSORIO, N. M., FERREIRA-DIAS, S., Gioielli, L.A.
Continuous lipase-catalyzed interesterification of blends containing olive oil for trans-free margarines In: XVIII Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2013, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2013. v.49. p.26 - 26

2. DAGOSTINHO, N. R., SILVA, Roberta Claro da, MARUYAMA, J. M, **SOARES, F. A. S. M.**, SILVA, Y. A., GIOIELLI, LUIZ ANTONIO
Crystallization and polymorphism behavior of pure triacyglycerol's added with monoacylglycerols In: XVIII Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2013, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2013. v.49. p.27 - 27

3. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, MARUYAMA, J. M, DAGOSTINHO, N. R., CALLIGARIS, G., RIBEIRO, A. P. B., CARDOSO, L.P., GIOIELLI, LUIZ ANTONIO
Effect of diglyceride addiction on crystallization properties of pure triacylglycerols In: SBOG 20 ANOS Novos Horizontes para Ciência e Tecnologia de Óleos e Gordura, 2013, Florianópolis.
SBOG 20 ANOS Novos Horizontes para Ciência e Tecnologia de Óleos e Gordura. Florianópolis: 2013. v.1. p.95 - 95

4. MARUYAMA, J. M, MARTINI, S., DAGOSTINHO, N. R., **SOARES, F. A. S. M.**, GIOIELLI, LUIZ ANTONIO, SILVA, Roberta Claro da
Effect of high intensity ultrasound in criystalline properties of palm olein and coconut oil added emulsifiers In: XVIII Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2013, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2013. v.49. p.45 - 45

5. SILVA, Y. A., SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, MARUYAMA, J. M, DAGOSTINHO, N. R., GIOIELLI, LUIZ ANTONIO
Effects of Diacylglycerides Addiction (3%) on Crystallization Properties of Pure Triacylglycerol In: XVIII Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2013, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2013. v.49. p.66 - 66

6. **Soares, Fabiana A.S.M.**, DA SILVA, ROBERTA CLARO, OSÓRIO, NATÁLIA M., MARUYAMA, J. M, GONCALVES, M. I. A., FERREIRA-DIAS, SUZANA, Gioielli, L.A.
Effects of enzymatic interesterification on physicochemical properties of blends of palm stearin, palm kernel oil and olive oil to produce trans-free margarine analags. In: 104rd AOCS Annual Meeting & Expo, 2013, Montreal.
104rd AOCS Annual Meeting & Expo. , 2013.

7. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, MARUYAMA, J. M, DAGOSTINHO, N. R., OSORIO, N. M., FERREIRA-DIAS, S., Gioielli, L.A.
Influence of continuous enzymatic interesterification on thermal behavior, microstructure, polymorphism and crystallization properties of palm stearin, palm kernel oil and olive oil blends. In: SBOG 20 ANOS Novos Horizontes para Ciência e Tecnologia de Óleos e Gordura, 2013, Florianópolis.
SBOG 20 ANOS Novos Horizontes para Ciência e Tecnologia de Óleos e Gordura. Florianópolis: 2013. v.1. p.90 - 90

8. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, OSORIO, N. M., MARUYAMA, J. M, DAGOSTINHO, N. R., FERREIRA-DIAS, S., Gioielli, L.A.
Influence of continuous enzymatic interesterification on thermal behavior, microstructure, polymorphism and crystallization properties of palm stearin, palm kernel oil and olive oil blends In: 11th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health, 2013, Antalya.
11th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health. , 2013. v.1.

10. MARUYAMA, J. M, SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, HARES JUNIOR, S. J., Gioielli, L.A.
Crystallization and melting behavior of commercial emulsifiers In: XVII Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2012, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2012. v.48. p.80 - 80

11. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, MARUYAMA, J. M, Gioielli, L.A.

Crystallization and polymorphism behavior of lipid systems containing triacylglycerols added monoglycerides and diglicerydes. In: 103rd AOCS Annual Meeting & Expo, 2012, Long Beach.
Abstract 103rd Annual Meeting. USA: AOCS PRESS, 2012. v.1. p.3 - 3

12. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, MARUYAMA, J. M, Gioielli, L.A.
Crystallization and polymorphism behavior of lipid systems containing triacylglycerols with added diacylglycerols In: XVII Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2012, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2012. v.48. p.3 -

13. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, MARUYAMA, J. M, RIBEIRO, A. P. B., SANTOS, A.O., CARDOSO, L.P., MATOS, J. R., Gioielli, L.A.
Crystallization behavior of structured lipids by chemical interesterification of palm stearin, coconut oil and canola oil In: XVII Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2012, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2012. v.48. p.67 -

14. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, MARUYAMA, J. M, RIBEIRO, A. P. B., SANTOS, A.O., CARDOSO, L.P., MATOS, J. R., Gioielli, L.A.
Crystallization Propriety of Structured Lipids of Palm Stearin, Coconut Oil and Canola Oil in: 10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health, 2012, Cracovia.
10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health. , 2012. v.1. p.121 - 121

15. **SOARES, F. A. S. M.**, RODRIGUES, H.G., BORTOLON, J.R., MURATA, G.M., GONCALVES, M. I. A., HATANAKA, E., CURI, R., Gioielli, L.A.
Effects of Topical Application of Vegetable Oil Blends and Structured Lipids on Wound Healing In: 103rd AOCS Annual Meeting & Expo, 2012, Long Beach.
Abstract 103rd Annual Meeting. USA: AOCS PRESS, 2012. v.1. p.6 - 6

16. RODRIGUES-RACT, J. N., **SOARES, F. A. S. M.**, RODRIGUES, H.G., BORTOLON, J.R., MURATA, G.M., GONCALVES, M. I. A., HATANAKA, E., CURI, R., Gioielli, L.A.
Effects of topical application of vegetable oil blends and structured lipids on wound healing In: XVII Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2012, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2012. v.48. p.59 - 59

17. HARES JUNIOR, S. J., **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, VICCOLA, E.R., MARUYAMA, J. M, Gioielli, L.A.
Functionality of Fats in the Formulation of Peanut Butter in: 103rd AOCS Annual Meeting & Expo, 2012, Long Beach.
Abstract 103rd Annual Meeting. USA: AOCS PRESS, 2012. v.1. p.5 - 5

18. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, MARUYAMA, J. M, Gioielli, L.A.
Microscopic approach of the polymorphism of tripalmitin and tristearin In: 10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health, 2012, Cracovia.
10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health. , 2012. v.1. p.122 -

19. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, MARUYAMA, J. M, OSORIO, N. M., GONCALVES, M. I. A., Gioielli, L.A., FERREIRA-DIAS, S.
Modelling lipase-catalysed interesterification of fats containing palm stearin, palm kernel and olive oil monitored by their solid fat content. In: XVII Semana Farmacêutica de Ciências e Tecnologia, 2012, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2012. v.48. p.66 - 66

20. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, MARUYAMA, J. M, OSORIO, N. M., Gioielli, L.A., FERREIRA-DIAS, S.
Operational stability of *Thermomyces lanuginosa* and *Rhizomucor miehei* lipase during interesterification of a blend of palm stearin, palm kernel and olive oil in continuous packed-bed reactors In: 10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health, 2012, Cracovia.
10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health. , 2012. v.1. p.120 -

21. PERINI, N. P., HIROTA, C. Y., **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, OLIVEIRA, M. N
Replacement of milk fat by a vegetable oil emulsion in symbiotic yogurts improves fatty acids profile whilst do not affects fermentation performance neither sensorial properties In: 10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health, 2012, Cracovia.
10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health. , 2012. v.1. p.123 -

22. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, HAZZAN, M., CAPACLA, I. R., Gioielli, L.A.
Crystallization and melting behavior of strutured lipids produced with lard and soybean oil by enzymatic interesterification in a

continuous packed bed reactor In: 102nd AOCS Annual Meeting & Expo, 2011, Cincinnati, Ohio, USA.
Abstracts 102nd AOCS Annual Meeting & Expo. USA: AOCS PRESS, 2011. v.1. p.33 - 34

23. SILVA, Roberta Claro da, RIBEIRO, A. P. B., **SOARES, F. A. S. M.**, CAPACLA, I. R., HAZZAN, M., CARDOSO, L.P., SANTOS, A.O., Gioielli, L.A.
Crystallization and polymorphism of human milk fat substitute produced by continuous enzymatic interesterification In: XVI Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2011, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2011. v.47. p.77 - 77

24. MARUYAMA, J. M, VICCOLA, E.R., **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, Gioielli, L.A.
Crystallization behavior of human milk fat and betapol In: XVI Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2011, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2011. v.47. p.84 - 84

25. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, VICCOLA, E.R., MARUYAMA, J. M, GONCALVES, M. I. A., Gioielli, L.A.
Effects of chemical interesterification on physicochemical properties of blends of palm stearin, coconut oil, and canola oil In: XVI Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2011, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2011. v.47. p.47 - 47

26. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, HAZZAN, M., CAPACLA, I. R., VICCOLA, E.R., GONCALVES, M. I. A., Gioielli, L.A.
Effects of chemical interesterification on physicolchemical properties of blends of palm stearin, coconut oil and canola oil In: 102nd AOCS Annual Meeting & Expo, 2011, Cincinnati, OHIO, USA.
Abstract 102nd AOCS Annual Meeting & Expo. AOCS PRESS, 2011. v.1. p.44 - 44

27. RODRIGUES-RACT, J. N., **SOARES, F. A. S. M.**, RODRIGUES, H.G., BORTOLON, J.R., MURATA, G.M., CURI, R., Gioielli, L.A.
Macroscopic analysis of wounds treated with vegetable oil blends and structured lipids In: XVI Semana Farmacêutica de Ciências e Tecnologia, 2011, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2011. v.47. p.45 - 45

28. RODRIGUES-RACT, J. N., **SOARES, F. A. S. M.**, RODRIGUES, H.G., BORTOLON, J.R., HATANAKA, E., CURI, R., Gioielli, L.A.
Physical and chemical properties of structured lipids for topical application in wounds In: 102nd AOCS Annual Meeting & Expo, 2011, Cincinnati, OHIO, USA.
Abstracts 102nd AOCS Annual & Expo. USA: AOCS PRESS, 2011. v.1. p.65 -

29. POLAK, R., **SOARES, F. A. S. M.**, MATOS, J. R., Gioielli, L.A., PITOMBO, R. N. M
Thermoanalytical characterization of lipids and their interactions to obtain echogenic liposomes via freeze-drying In: XVI Semana Farmacêutica de Ciências e Tecnologia da FCF/USP, 2011, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Bibliotecas e documentação do Conjunto das Químicas da USP, 2011. v.47. p.9 - 9

30. PRESTES, P. S., SPEGIORIN, C. S., BALOGH, T.S., PINTO, C.S.D., GIMENES, P., AREAS, E.P.G., MATOS, J. R., OLIVEIRA, A. M., GUIMARÃES, K. L., ZANIN, M. H. A., CONSIGLIERI, V. O., **SOARES, F. A. S. M.**, Gioielli, L.A., KAMEKO, T.M., VELASCO, M.V.R., BABY, A.R.
Caracterização física de sistemas emulsionados contendo tensoativos etoxilados In: 24° Congresso Brasileiro de Cosmetologia. 2010, São Paulo.
Livro de Resumo 24° Congresso Brasileiro de Cosmetologia. 2010.

31. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, HAZZAN, M., CAPACLA, I. R., GONCALVES, M. I. A., Gioielli, L.A.
Characterization of physico-chemical properties of ternary blends of palm stearin, coconut oil and canola oil In: XV SEMANA FARMACÊUTICA DE CIÊNCIA E TECNOLOGIA da FCF/USP, 2010, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2010. v.46. p.14 - 14

32. SANTOS O. V., CORRÊA, N. C. F., **SOARES, F. A. S. M.**, Gioielli, L.A., LANNES, S. C. S.
Chemical evaluation of Brazil nut fats obtained from different extraction systems In: 15th World Congress of Food Science and Technology, 2010, Cape Town.
Abstracts of the 15th IUFOST. , 2010.

33. HAZZAN, M., **SOARES, F. A. S. M.**, Gioielli, L.A.
Cinética de cristalização isotérmica de lipídios estruturados produzidos a partir de banha e óleo de soja por interesterificação enzimática contínua In: 18° Simpósio Internacional de Iniciação Científica, 2010, Ribeirão Preto.
Livro de Resumos 18° Simpósio Internacional de Iniciação Científica. , 2010.

34. CAPACLA, I. R., **SOARES, F. A. S. M.**, Gioielli, L.A.
Comparação entre os métodos de DSC e RNM para determinação de conteúdo de gordura sólida de misturas de óleo de soja e banha antes e após a interesterificação enzimática contínua In: 18° Simpósio Internacional de Iniciação Científica, 2010, Ribeirão Preto.
Livro de Resumos 18° Simpósio Internacional de Iniciação Científica. , 2010.

35. CAPACLA, I. R., SILVA, Roberta Claro da, HAZZAN, M., **SOARES, F. A. S. M.**, Gioielli, L.A.
Comparison of DSC and NMR methods for determining solid fat content of binary mixtures of lard and soybean oil In: XV SEMANA FARMACÊUTICA DE CIÊNCIA E TECNOLOGIA, 2010, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2010. v.46. p.33 - 33

36. HAZZAN, M., SILVA, Roberta Claro da, CAPACLA, I. R., **SOARES, F. A. S. M.**, Gioielli, L.A.
Crystallization and melting behavior of structured lipids produced with lard and soybean oil In: XV SEMANA FARMACÊUTICA E DE CIÊNCIA E TECNOLOGIA da FCF/USP, 2010, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2010. v.46. p.24 - 24

37. PRESTES, P. S., BALOGH, T.S., PEDFRALI, C.A., SILVA, D.G., GIMENES, P., AREAS, E.P.G., **SOARES, F. A. S. M.**, Gioielli, L.A., PINTO, C.S.D., KAMEKO, T.M., VELASCO, M.V.R., BABY, A.R.
Influence of *Punica granatum L*. extract on physical properties of sunscreens developed with noionic ethoxylated surfactants. In: 70th FIP World Congress of Pharmacy & Pharmaceutical Sciences, 2010, Lisboa.
70th International Congress of FIP. , 2010.

38. HAZZAN, M., SILVA, Roberta Claro da, CAPACLA, I. R., **SOARES, F. A. S. M.**, Gioielli, L.A.
Isothermal crystallization kinetics of structured lipids produced with lard and soybean oil In: XV SEMANA FARMACÊUTICA DE CIÊNCIA E TECNOLOGIA da FCF/USP, 2010, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2010. v.46. p.25 - 25

39. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, CAPACLA, I. R., HAZZAN, M., GONCALVES, M. I. A., Gioielli, L.A.
Regiospecific analysis of crude palm oil and their fractions using quantitative 13C In: VX SEMANA FARMACÊUTICA DE CIÊNCIA E TECNOLOGIA da FCF/USP, 2010, São Paulo.
Brazilian Journal of Pharmaceutical Sciences (Impresso). São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2010. v.46. p.91 -

40. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, CHIU, M.C., GRIMALDI, R., SILVA, K. C. G., FERNANDES, T.G., CASTELLS, A. L., GONCALVES, L.A.G., GONCALVES, M. I. A., Gioielli, L.A.
Composition and stereospecific distribution of triacylglycerols of lipids obtained by chemical interesterication of palm stearin and palm olein In: 8° Simpósio Latino America de Ciências de Alimentos, 2009, Campinas.
8° SLACA Simpósio Latino Americano de Ciência de Alimentos. Campinas: Faculdade de Engenharia de Alimentos, 2009. v.1.

41. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, RODRIGUES-RACT, J. N., SILVA, K. C. G., FERNANDES, T.G., CASTELLS, A. L., Gioielli, L.A.
Crystallization behavior of structured lipids obtained by chemical interesterification of palm stearin and palm olein In: XIV Semana Farmacêutica de Ciência eTecnologia da FCF/USP, 2009, São Paulo.
Brazilian Journal of Pharmaceutical Sciences. São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2009. v.44. p.28 -

42. SILVA, Roberta Claro da, FERNANDES, T.G., CASTELLS, A. L., SILVA, K. C. G., **SOARES, F. A. S. M.**, Gioielli, L.A.
Crystallization behavior of structured lipids produced with lard and soybean oil by enzymatic interesterification in a continuos packed-bed reactor In: 8° Simpósio Latino America de Ciências de Alimentos, 2009, Campinas.
8° Simpósio Latino Americano de Ciências de Alimentos. , 2009.

43. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, RODRIGUES-RACT, J. N., SILVA, K. C. G., FERNANDES, T.G., CASTELLS, A. L., Gioielli, L.A.
Crystallization behavior of strutured lipids by chemical interesterification of palm stearin and palm olein In: 2009 World Congress on Oils and fats & 28 th ISF Congress, 2009, Sydney.
2009 World Congress on Oils and fats & 28 th ISF Congress. , 2009. p.459 -

44. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, RODRIGUES-RACT, J. N., SILVA, K. C. G., CASTELLS, A. L., FERNANDES, T.G., LOURENCO, M. B., SOARES, D.F., Gioielli, L.A.

Effects of chemical interesterification on physicochemical properties of blends of palm stearin and palm olein In: V Simpósio Ácidos Graxos e Saúde, 2009, São Paulo.
Livro de Resumo. , 2009.

45. SILVA, Roberta Claro da, FERNANDES, T.G., CASTELLS, A. L., SILVA, K. C. G., **SOARES, F. A. S. M.**, Gioielli, L.A.
Phisico-chemical properties of structured lipids produced with lard and soybean oil by enzymatic interesterification in a continuous packed-bed reactor In: World Congress on Oils and Fats & 28th ISF Congress, 2009, Sydney.
World Congress on Oils and Fats & 28th ISF Congress. , 2009.

46. SILVA, K. C. G., **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, RODRIGUES-RACT, J. N., FERNANDES, T.G., CASTELLS, A. L., Gioielli, L.A.
Physicochemical properties of mixtures and structured lipids obtained from palm stearin, palm olein and olive oil by chemical interesterification In: XIV Semana Farmacêutica de Ciência eTecnologia da FCF/USP, 2009, São Paulo.
Brazilian Journal of Pharmaceutical Sciences. São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2009. v.44. p.27 - 27

47. FERNANDES, T.G., SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, SILVA, K. C. G., CASTELLS, A. L., Gioielli, L.A.
Solid fat content and consistency of binary mixtures between lard and soybean oil after enzymatic interesterification In: XIV Semana Farmacêutica de Ciência e Tecnologia da FCF/USP, 2009, São Paulo.
Brazilian Journal of Pharmaceutical Sciences. São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2009. v.44. p.44 - 44

48. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, GONCALVES, M. I. A., FERNANDES, T.G., CASTELLS, A. L., Gioielli, L.A.
Structured lipids produced with lard and soybean oil by enzimatic interesterification in a continuous packed-bed reactor In: XIV Semana Farmacêutica de Ciência e Tecnologia da FCF/USP, 2009, São Paulo.
Brazilian Journal of Pharmaceutical Sciences. São Paulo: Divisão de Biblioteca e Documentos do Conjunto das Químicas da USP, 2009. v.45. p.33 -

49. SOARES, D.F., SILVA, Roberta Claro da, LOURENCO, M. B., **SOARES, F. A. S. M.**, Gioielli, L.A.
Caracterização das propriedades físico-químicas de misturas binárias entre banha e óleo de soja In: XIII Semana Farmacêutica de Ciência e Tecnologia da FCF/USP, 2008, São Paulo.
RBCF. Revista Brasileira de Ciências Farmacêuticas (Cessou em 2008. Cont. ISSN 1984-8250 Brazilian Journal of Pharmaceutical Sciences). São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2008. v.44. p.60 -

50. Fabiana, SILVA, Roberta Claro da, SILVA, K. C. G., LOURENCO, M. B., SOARES, D.F., Gioielli, L.A.
Efeito da interesterificação química nas propriedades físico-químicas de misturas de estearina e oleína de palma In: XII Semana Farmacêutica de Ciência e Tecnologia da FCF/USP, 2008, São Paulo.
RBCF. Revista Brasileira de Ciências Farmacêuticas (Cessou em 2008. Cont. ISSN 1984-8250 Brazilian Journal of Pharmaceutical Sciences). São Paulo: Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP, 2008. v.44. p.27 -

51. LOURENCO, M. B., SILVA, Roberta Claro da, SOARES, D.F., **SOARES, F. A. S. M.**, SILVA, K. C. G., Gioielli, L.A.
Lipídios estruturados obtidos por interesterificação química a partir de estearina de palma e azeite de oliva In: XIII Semana Farmacêutica de Ciência e Tecnologia da FCF/USP, 2008, São Paulo.
RBCF. Revista Brasileira de Ciências Farmacêuticas (Cessou em 2008. Cont. ISSN 1984-8250 Brazilian Journal of Pharmaceutical Sciences). São Paulo: Divisão de Bibliotecas e Documentos do Conjuntos das Químicas, 2008. v.44. p.27 -

Trabalhos publicados em anais de eventos (resumo expandido)

1. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, HAZZAN, M., CAPACLA, I. R., GONCALVES, M. I. A., Gioielli, L.A.
Characterization of physico-chemical properties of ternary blends of palm stearin, coconut oil and canola oil In: V Simpósio International Tendências e Inovações de Óleo e Gorduras, 2010
Cd-Rom V Simpósio Internacional Tendências e Inovações de Óleo e Gorduras. , 2010. p.63 - 63

2. HAZZAN, M., SILVA, Roberta Claro da, CAPACLA, I. R., **SOARES, F. A. S. M.**, Gioielli, L.A.
Cinética de cristalização isotérmica de lipídios estruturados produzidos a partir de banha e óleo de soja por interesterificação enzimática contínua In: V Simpósio Internacional Tendências e Inovações de Óleo e Gorduras, 2010, Campinas.
CD-ROM V Simpósio Internacional Tendências e Inovações de Óleo e Gorduras. 2010. p.65 - 65

3. SILVA, Roberta Claro da, HAZZAN, M., CAPACLA, I. R., **SOARES, F. A. S. M.**, Gioielli, L.A.
Comportamentos de cristalização e fusão de lipídios estruturados produzidos a partir de banha e óleo de soja In: V Simpósio Internacional Tendências e Inovações de Óleo e Gorduras, 2010, Campinas.
CD-ROM V Simpósio Internacional Tendências e Inovações de Óleo e Gorduras. 2010. p.66 - 66

4. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, SILVA, K. C. G., LOURENCO, M. B., SOARES, D.F., Gioielli, L.A.
Efeito da interesterificação química nas propriedades físico-químicas de misturas de estearina e oleína de palma In: XXI Congresso

Brasileiro de Ciência e Tecnologia de Alimentos e XV Seminário Latino Americano e do Caribe de Ciência e Tecnologia de Alimentos, 2008, Belo Horizonte.
XXI Congresso Brasileiro de Ciência e Tecnologia de Alimentos. , 2008.

Artigos em revistas (Magazine)

1. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, HAZZAN, M., CAPACLA, I. R., VICCOLA, E.R., RODRIGUES-RACT, J. N., GONCALVES, M. I. A., Gioielli, L.A.
Distribuição regioespecífica de ácidos graxos em triacilgliceróis de óleos derivados do óleo de palma. Revista O&G Alimentos e Tecnologia. São Caetano do Sul, p.32 - 41, 2011.

Apresentação de trabalho e palestra

1. SILVA, Roberta Claro da, **Soares, Fabiana A.S.M.**, MARUYAMA, J. M, Gioielli, L.A.
Microscopic Approach of the Polymorphism of Tripalmitin and Triestearin, 2013. (Outra, Apresentação de Trabalho)

2. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, MARUYAMA, J. M, MATOS, J. R., Gioielli, L.A.
Crystallization Behavior of Structured Lipids by Chemical Interesterification of Palm Stearin, Coconut Oil and Canola Oil, 2012. (Congresso, Apresentação de Trabalho)

3. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, MARUYAMA, J. M, OSORIO, N. M., GONCALVES, M. I. A., Gioielli, L.A., FERREIRA-DIAS, S.
Modelling lipase-catalysed interesterification of fats containing palm stearin, palm kernel and olive oil monitored by their solid fat content. 2012. (Simpósio, Apresentação de Trabalho)

5. SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, MARUYAMA, J. M, Gioielli, L.A.
Crystallization and polymorphism behavior of lipid systems containing triacylglycerols added monoglycerides and diglicerydes: preliminary studies. 2011. (Seminário, Apresentação de Trabalho)

6. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, RIBEIRO, A. P. B., CAPACLA, I. R., HAZZAN, M., CARDOSO, L.P., SANTOS, A.O., Gioielli, L.A.
Crystallization and polymorphism of human milk fat substitute produced by continuous enzymatic interesterification, 2011. (Congresso, Apresentação de Trabalho)

7. JACHMANIAN, I., SEGURA, N., SUESCUN, L, SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, Gioielli, L.A.
Grasas con bajo contenido de ácidos grasos trans a partir de mezclas sebo vacuno/aceites vegetales interesterificadas mediante catálisis enzimática, 2011. (Congresso, Apresentação de Trabalho)

8. RODRIGUES-RACT, J. N., **SOARES, F. A. S. M.**, BORTOLON, J.R., MURATA, G.M., RODRIGUES, H.G., HATANAKA, E., CURI, R., Gioielli, L.A.
Macroscopic analysis of wounds treated with vegetable oil blends and structured lipids, 2011. (Congresso, Apresentação de Trabalho)

9. SANTOS O. V., **SOARES, F. A. S. M.**, CORRÊA, N. C. F., Gioielli, L.A., COSTA, C. E. F., LANNES, S. C. S.
Nutritional evaluation and thermal analysis of Brazil nut oil derived by different extraction processes, 2011. (Congresso, Apresentação de Trabalho)

10. **SOARES, F. A. S. M.**, SILVA, Roberta Claro da, VICCOLA, E.R., MARUYAMA, J. M, GONCALVES, M. I. A., Gioielli, L.A.
Physical and chemical properties of strutured lipids of palm stearin, coconut oil and canola oil, 2011. (Congresso, Apresentação de Trabalho)

11. SANTO, A. P. E., SILVA, Roberta Claro da, **SOARES, F. A. S. M.**, ANJOS, D., Gioielli, L.A., OLIVEIRA, M. N
Acidification and fatty acids profile of yogurt containing açaí pulp and different probiotic cultures, 2009. (Congresso, Apresentação de Trabalho)

Educação e Popularização de C&T
Participação em eventos, congressos, exposições, feiras e olimpíadas

1. Apresentação de Poster / Painel no(a) **Biocatalysis in Lipid Modification**, 2012. (Simpósio)
Modelling lipase-catalysed interesterification of fats containing palm stearin, palm kernel and olive oil monitored by their solid fat content...

2. Apresentação de Poster / Painel no(a) **10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health**, 2012. (Congress)
Operational Stability of Thermomyces lanuginosa and Rhizomucor miehei Lipase during Interesterification of a Blend of Palm

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Eventos

Eventos
Participação em eventos

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Effects of enzymatic interesterification on physicochemical properties of blends of palm stearin, palm kernel oil and olive oil to produce trans-free margarine analags...

2. Apresentação de Pôster / Painel no(a) **104rd AOCS Annual Meeting & Expo**, 2013. (Congresso)
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3. Apresentação de Pôster / Painel no(a) **SBOG 20 ANOS Novos Horizontes para Ciência e Tecnologia de óleos e Gorduras**, 2013. (Congresso)
Influence of continuous enzymatic interesterification on thermal behavior, microstructure, polymorphism and crystallization properties of palm stearin, palm kernel oil and olive oil blends...

4. Apresentação Oral no (a) **11th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health**, 2013. (Congresso)
Influence of continuous enzymatic interesterification on thermal behavior, microstructure, polymorphism and crystallization properties of palm stearin, palm kernel oil and olive oil blends.

5. Apresentação Oral no(a) **103rd AOCS Annual Meeting & Expo**, 2012. (Congresso)
Crystallization Behavior of Structured Lipids by Chemical Interesterification of Palm Stearin, Coconut Oil and Canola Oil.

6. Apresentação de Poster / Painel no(a) **Biocatalysis in Lipid Modification**, 2012. (Simpósio)
Modelling lipase-catalysed interesterification of fats containing palm stearin, palm kernel and olive oil monitored by their solid fat content..

7. Apresentação de Poster / Painel no(a) **10th Euro Fed Lipid Congress Fats, Oils and Lipids: from Science and Technology to Health**, 2012. (Congresso)
Operational Stability of Thermomyces lanuginosa and Rhizomucor miehei Lipase during Interesterification of a Blend of Palm Stearin, Palm Kernel and Olive Oil in Continuous Packed-bed Reactors.

8. Apresentação de Pôster / Painel no(a) **Avanços Tecnológicos na Qualidade e Estabilidade de Chocolates.**, 2011. (Seminário)
CRYSTALLIZATION AND POLYMORPHISM BEHAVIOR OF LIPID SYSTEMS CONTAINING TRIACYLGLYCEROLS ADDED MONOGLYCERIDES AND DIGLICERYDES: PRELIMINARY STUDIES...

9. Apresentação de Pôster / Painel no(a) **102nd AOCS Annual Meeting & Expo**, 2011. (Congresso)
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10. Apresentação de Poster / Painel no(a) **14th AOCS Latin American Congress and Exhibition on ats and Oils**, 2011. (Congresso)
Physical and chemical properties of strutured lipids of palm stearin, coconut oil and canola oil.

11. Apresentação de Pôster / Painel no(a) **V Simpósio Internacional Tendências e Inovações de Óleo e Gorduras**, 2010. (Simpósio)
of physico-chemical properties of ternary blends of palm stearin, coconut oil and canola oil.

12. Apresentação de Poster / Painel no(a) **2009 World Congress on Oils and fats & 28 th ISF Congress**, 2009. (Congresso)
Crystallization bahavior of structured lipids by chemical interesterification of palm stearin and palm olein.

13. Apresentação de Pôster / Painel no(a) **8° Simpósio Latino America de Ciências de Alimentos**, 2009. (Simpósio)
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14. Apresentação de Pôster / Painel no(a) **V Simpósio Ácidos Graxos e Saúde**, 2009. (Simpósio)
Effects of chemical interesterification on physicochemical properties of blends of palm stearin and palm olein.

15. Apresentação de Pôster / Painel no(a) **XXI Congresso Brasileiro de Ciência e Tecnologia de Alimentos e XV Seminário Latino Americano e do Caribe de Ciência e Tecnologia de Alimentos**, 2008. (Congresso)
Efeito da interesterificação química nas propriedades físico-químicas de misturas de estearina e oleína de palma.

16. **II Forum de Ética em Pesquisa da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo**, 2008. (Outra)

.

Citações

Web of Science Total de citações : 13;Total de trabalhos : 10;Data : 22/06/2012; Fator H: 2;
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