UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS
DEPARTAMENTO DE TECNOLOGIA DE ALIMENTOS

OBTENÇÃO DE GORDURAS ZERO *TRANS* POR INTERESTERIFICAÇÃO QUÍMICA E CARACTERIZAÇÃO PARA APLICAÇÃO EM ALIMENTOS

Ana Paula Badan Ribeiro

Profa. Dra. Lireny Aparecida Guaraldo Gonçalves
Orientadora

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BANCA EXAMINADORA

Dra. Lireny Aparecida Guaraldo Gonçalves
(Orientadora)

Dra. Denise Fabiana Silvestre Becker de Almeida
(Membro)

Dr. Luiz Antonio Gioielli
(Membro)

Dra. Thais Maria Ferreira de Souza Vieira
(Membro)

Dr. Theo Guenter Kieckbusch
(Membro)

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ÍNDICE

Resumo

A interesterificação química tem-se mostrado a principal alternativa para a obtenção de gorduras plásticas zero *trans*. Em particular, a interestesterificação de óleos líquidos com óleos totalmente hidrogenados (ou *hardfats*) consiste na alternativa de maior versatilidade para a produção de gorduras zero *trans*, produzindo bases gordurosas com propriedades diferentes para a aplicação em alimentos. Neste trabalho, misturas de óleo de soja (SO)/óleo de soja totalmente hidrogenado (FHSBO), com teores de FHSBO iguais a 10, 20, 30, 40 e 50% (m/m) e óleo de canola (CaO)/óleo de algodão totalmente hidrogenado (FHCSO), com teores de FHCSO iguais a 20, 25, 30, 35 e 40% (m/m), foram interesterificadas nas seguintes condições: 20 minutos de reação, 0,4% de catalisador metóxido de sódio, agitação de 500 rpm, a 100°C. As misturas originais e interesterificadas foram avaliadas quanto à composição triacilglicerólica, ponto de fusão, conteúdo de gordura sólida (SFC), consistência, comportamento térmico, microestrutura, cinética de cristalização e polimorfismo. A interesterificação promoveu expressivo rearranjo das espécies triacilglicerólicas em todas as misturas, com diminuição nos teores de triacilgliceróis trissaturados e aumento dos triacilgliceróis monoinsaturados-dissaturados e diinsaturados-monossaturados, o que resultou no respectivo decréscimo dos pontos de fusão. As misturas interesterificadas apresentaram diminuição do SFC em todas as temperaturas e perfis de fusão mais lineares quando comparadas às misturas originais. Os valores de *yield value* mostraram aumento da plasticidade das misturas após a reação e relação com o conteúdo de gordura sólida. Os diagramas de curvas iso-sólidas para as misturas CaO:FHCSO originais indicaram interações monotéticas, que foram atenuadas com a randomização promovida pela interesterifcação. Os termogramas de fusão e cristalização das misturas foram significativamente modificados pela randomização, que também promoveu a diminuição significativa do diâmetro dos cristais em todas as misturas, além de modificação da morfologia cristalina. A caracterização da cinética de cristalização permitiu verificar que o período de indução para formação dos

cristais e o teor máximo de gordura sólida foram alterados em função do teor de óleo totalmente hidrogenado nas misturas originais e como resultado do rearranjo ao acaso. Mudanças na constante (*k*) e expoente (*n*) do modelo de Avrami indicaram, respectivamente, que a interesterificação promoveu a queda na velocidade de cristalização das misturas e modificação na forma de cristalização das misturas originais. Análises de difração de raios-x revelaram que a interesterificação alterou o polimorfismo cristalino das misturas SO:FHSBO, mas não modificou o hábito cristalino das misturas CaO:FHCSO. As misturas interesterificadas 90:10, 80:20, 70:30 e 60:40 SO:FHSBO mostraram características adequadas à aplicação como *shortening* líquido, margarina de mesa, gordura para panificação e confeitaria, *all-purpose shortenings* e base para recheios, respectivamente; enquanto as misturas interesterificadas 80:20, 75:25, 70:30 e 65:35 CaO:FHCSO mostraram-se compatíveis ao uso em margarinas culinárias, *spreads*, gordura para panificação e *all-purpose shortenings,* coberturas do tipo *glazers*, respectivamente.

Palavras-chave: gorduras zero trans, interesterificação química, gorduras plásticas, óleos líquidos, óleos totalmente hidrogenados.

Abstract

Chemical interesterification has been used as the main alternative for obtaining zero *trans* plastic fats. In particular, interesterification of liquid oils with fully hydrogenated oils (or *hardfats*) is the most versatile way for producing zero *trans* fats, yielding fat bases with different properties for food applications. In this work, blends of soybean oil (SO)/fully hydrogenated soybean oil (FHSBO), with 10, 20, 30, 40 and 50% FHSBO (w/w) content and blends of canola oil (CaO)/fully hydrogenated cottonseed oil (FHCSO), with 20, 25, 30, 35 and 40% FHCSO (w/w) were interesterified under the following conditions: 20 minutes reaction time, 0.4% sodium methoxide, 500 rpm stirring, 100°C. The original and interesterified blends were examined for triacylglycerol composition, melting point, solid fat content (SFC), consistency, thermal behavior, microstructure, crystallization kinetics and polymorphism. Interesterification caused considerable rearrangement of triacylglycerol species, reduction of trisaturated triacylglycerol content and increase in monounsaturated-disaturated and diunsaturated-monosaturated triacylglycerols, lowering the initial melting points. The interesterified blends displayed reduced SFC at all temperatures and more linear melting profiles as compared with the original blends. *Yield values* showed a higher plasticity in the blends after the reaction, related to SFC. Iso-solid curves for original CaO:FHCSO blends indicated monotetic interactions, which were attenuated after randomization due to interesterification. Blend melting and crystallization thermograms were significantly modified by the randomization. Interesterification caused significant reductions in crystal diameter in all blends, in addition to modifying crystal morphology. Characterization of crystallization kinetics revealed that crystal formation induction period and maximum solid fat content were altered according to *hardfat* content in the original blends and as a result of the random rearrangement. Changes in Avrami model constant *(k*) and exponent (*n*) indicated, respectively, that – as compared with the original blends – interesterification decreased crystallization velocities and modified crystallization processes. X-ray diffraction analyses revealed that interesterification altered crystalline

polymorphism of SO:FHSBO blends, but did not modify CaO:FHCSO blends crystalline habit. The 90:10, 80:20, 70:30 and 60:40 interesterified SO:FHSBO blends showed characteristics suited to application, respectively, as liquid shortening, table margarine, baking/confectionery fat and all-purpose shortenings/ biscuit-filling base; while the 80:20, 75:25, 70:30 and 65:35 interesterified CaO:FHCSO blends showed characteristics suited to application as soft margarines, *spreads*, bakery fats/all-purpose shortenings and icing shortenings, respectively.

Key-words: zero trans fats, chemical interesterification, plastic fats, liquid oils, fully hydrogenated oils.

INTRODUÇÃO

A função dos óleos e gorduras na nutrição humana tem sido intensamente discutida e pesquisada nas últimas décadas. Neste contexto, destaca-se a importância do controle da ingestão de ácidos graxos *trans*, devido às suas implicações negativas em relação à saúde. Diversos estudos têm sugerido uma relação direta entre os isômeros *trans* e o aumento do risco de doenças cardiovasculares. Em resposta, muitas organizações de saúde têm recomendado a redução do consumo de alimentos contendo ácidos graxos *trans.*

Como principal fonte de ácidos graxos *trans* na alimentação temos os óleos vegetais parcialmente hidrogenados, que correspondem a aproximadamente 90% dos isômeros *trans* em alimentos. No Brasil, a hidrogenação de óleos vegetais data da década de 50, em que se iniciou a produção de gorduras técnicas (s*hortenings*), margarinas e gorduras para frituras, consolidando o emprego destas bases gordurosas em diversos alimentos, como coberturas de chocolate, biscoitos, produtos de panificação e confeitaria, sorvetes, massas e *snacks*, entre outros. Durante muitas décadas, a formação de isômeros *trans* foi considerada uma vantagem tecnológica, uma vez que a presença destes compostos está relacionada a gorduras com perfis de fusão adequados a diferentes aplicações industriais, além da manutenção do polimorfismo β', desejável para a maioria dos alimentos.

Muitos países têm mostrado preocupação no que diz respeito às informações nutricionais presentes nos rótulos das embalagens de alimentos processados ou industrializados. No Brasil, a RDC nº 360, de 23 de dezembro de 2003, determinou que a partir de 31 de julho de 2006 todos os alimentos comercializados deveriam expressar em sua rotulagem nutricional a declaração dos ácidos graxos *trans* em relação à porção harmonizada para um determinado alimento, em conjunto com as declarações para gorduras totais e saturadas. São considerados como zero *trans* os alimentos que apresentarem teor de gorduras *trans* menor ou igual a 0,2 g/porção (ANVISA, 2004).

A demanda por gorduras zero *trans* tem levado os pesquisadores a testarem diferentes matérias-primas e processos que permitam disponibilizar à indústria alimentícia gorduras para diversas finalidades. Neste sentido, a interesterificação química tem-se mostrado a principal alternativa para a obtenção de gorduras plásticas com baixos teores de isômeros *trans* ou mesmo ausência destes compostos. Em contraste à hidrogenação parcial, este processo não promove a isomerização de duplas ligações dos ácidos graxos e não afeta o grau de saturação dos mesmos. Na reação de interesterificação os ácidos graxos permanecem inalterados, mas ocorre a redistribuição dos mesmos nas moléculas triacilglicerólicas. Desta forma, a interesterificação química causa a modificação da composição triacilglicerólica de um óleo ou gordura e, conseqüentemente, de suas propriedades físicas, comportamentos de cristalização e fusão, conteúdo de gordura sólida, consistência, microestrutura e hábito polimórfico, contribuindo para uma maior disponibilidade de frações oleosas para aplicação em produtos alimentícios.

Vários tipos de óleos vegetais podem ser utilizados na produção de *shortenings*. 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. Em especial, a interesterificação de óleos líquidos com óleos totalmente hidrogenados, conhecidos como *hardfats*, consiste atualmente na alternativa de maior versatilidade para a produção de gorduras zero *trans*, pois permite o desenvolvimento de bases gordurosas com características de aplicação diferenciadas segundo a concentração e/ou tipo de óleo totalmente hidrogenado nas misturas iniciais.

Ainda que a interesterificação química seja extremamente funcional sob o ponto de vista tecnológico, a substituição de gorduras parcialmente hidrogenadas em produtos alimentícios consiste em um desafio, uma vez que apropriadas curvas de sólidos, plasticidade, propriedades de cristalização, consistência e polimorfismo são de difícil obtenção quando na ausência de ácidos graxos *trans*. Logo, o estudo e delineamento do uso de gorduras interesterificadas em alimentos deve 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.

A proposta deste trabalho foi avaliar a interesterificação química de misturas à base de óleo de soja/óleo de soja totalmente hidrogenado e óleo de canola/óleo de algodão totalmente hidrogenado, a partir de propriedades como composição triacilglicerólica, ponto de fusão, conteúdo de gordura sólida, consistência, comportamento térmico, cinética de cristalização, microestrutura e polimorfismo.

O Capítulo 1 consiste de uma revisão bibliográfica sobre as pesquisas relativas à obtenção de gorduras zero *trans* por interesterificação química, para diferentes finalidades industriais. O artigo de revisão também aborda questões como a formação dos ácidos graxos *trans*, sua ocorrência na dieta e impacto na saúde, além das progressivas modificações da legislação brasileira com respeito à presença destes compostos nos alimentos processados.

O Capítulo 2 tem como objetivo uma ampla revisão da metodologia experimental aplicada ao estudo de gorduras interesterificadas. As metodologias abordadas incluem técnicas empregadas na investigação da composição triacilglicerólica, conteúdo de gordura sólida, cinética de cristalização, comportamento térmico, polimorfismo, microestrutura e consistência. Resultados recentes verificados na literatura técnica também são discutidos.

O Capítulo 3 avalia a obtenção de gorduras interesterificadas à base de misturas de óleo de soja/ óleo de soja totalmente hidrogenado, segundo características de composição triacilglicerólica, ponto de fusão, conteúdo de gordura sólida, curvas iso-sólidas e consistência, que em conjunto fornecem subsídios para a indicação de aplicabilidade das bases gordurosas obtidas. No Capítulo 4, estas bases gordurosas são adicionalmente caracterizadas quanto às propriedades de comportamento térmico, microestrutura, cinética de cristalização e polimorfismo.

No Capítulo 5 são apresentados os resultados da interesterificação de misturas óleo de canola/ óleo de algodão totalmente hidrogenado. Modificações decorrentes da randomização são discutidas em função das propriedades de composição triacilglicerólica, ponto de fusão, conteúdo de gordura sólida, curvas iso-sólidas e consistência, permitindo avaliar potenciais usos destas bases gordurosas na indústria de alimentos. A complementação do estudo destas misturas interesterificadas é apresentada no Capítulo 6, em que as mesmas foram avaliadas segundo características de comportamento térmico, microestrutura, cinética de cristalização e polimorfismo.

O Capítulo 7 trata do estudo da influência do tempo de reação sobre a interesterificação química de mistura óleo de soja/ óleo de soja totalmente hidrogenado, realizado posteriormente à obtenção das bases interesterificadas descritas nos Capítulos anteriores, com o intuito de elucidar algumas questões acerca do comportamento diferenciado de cristalização das gorduras interesterificadas obtidas a partir destas matérias-primas.

Os resultados apresentados neste trabalho servirão como base de informação para as indústrias alimentícias, bem como para futuras pesquisas científicas que abordem a aplicação de gorduras interesterificadas em alimentos.

1.

INTERESTERIFICAÇÃO QUÍMICA: ALTERNATIVA PARA OBTENÇÃO DE GORDURAS ZERO *TRANS*

Ana Paula Badan Ribeiro, Juliana Maria Leite Nóbrega de Moura, Renato Grimaldi, Lireny Aparecida Guaraldo Gonçalves

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Chemical interesterification: alternative to production of zero trans fats

The function of lipids in the human nutrition has been intensively debated in the last decade.This context reinforces the concern about controlling the trans fat ingestion, due to its negative implications on health. The interesterification provides an important alternative to modify oils and fats consistency, without causing trans isomers formation. This article reports researches that have been done about the production of zero trans fats by chemical interesterification, for different industrial purposes. Aspects related to trans fats occurrence on diet, their impact on health and modifications in Brazilian legislation are also covered.

Keywords: trans fatty acids, interesterification, nutrition.

Lipídios e Ácidos Graxos t*rans* (AGT)

Óleos e gorduras comestíveis são nutrientes essenciais da dieta humana, apresentando papel vital mediante o fornecimento de ácidos graxos essenciais e energia. Em adição às qualidades nutricionais, os óleos e gorduras provêem consistência e características de fusão específicas aos produtos que os contém, atuam como meio de transferência de calor durante o processo de fritura e como carreadores de vitaminas lipossolúveis e aroma[1]. Além disso, os lipídios afetam a estrutura, estabilidade, sabor, aroma, qualidade de estocagem, características sensoriais e visuais dos alimentos[2].

Quimicamente, óleos e gorduras são compostos predominantemente por triacilgliceróis. Ácidos graxos saturados são menos reativos e apresentam ponto de fusão superior em relação ao ácido graxo correspondente de mesmo tamanho de cadeia com uma ou mais dupla ligações. Ácidos graxos insaturados podem existir nas configurações *cis* e *trans*, com diferentes propriedades físico – químicas. Por suas características estruturais, os ácidos graxos na forma *trans* (AGT) têm ponto de fusão mais elevado quando comparado com seu isômero *cis* correspondente, mas inferior ao ponto de fusão

do ácido graxo saturado com mesmo número de átomos de carbono. Sendo assim, os isômeros *trans* podem ser considerados como um intermediário entre um ácido graxo original insaturado e um ácido graxo completamente saturado[2]. A Figura 1 ilustra a estrutura espacial e ponto de fusão dos ácidos oléico, elaídico e esteárico. Os AGT de maior ocorrência são os monoinsaturados, mas vários isômeros diinsaturados ou mesmo triinsaturados podem ser formados a partir dos ácidos linoléico e linolênico[3].

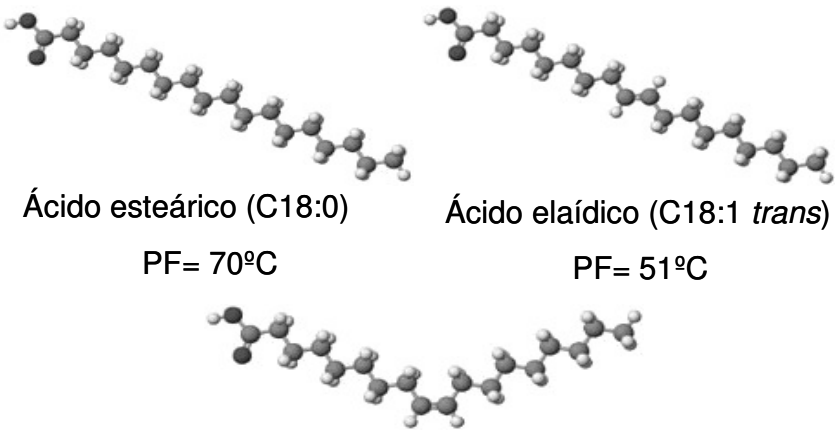

Figura 1. Estruturas e pontos de fusão[2] dos ácidos esteárico, oléico e elaídico.

Formação dos AGT

Os AGT estão presentes naturalmente em gorduras originadas de animais ruminantes, como resultado do processo de bio-hidrogenação na flora microbiana do rúmen. O teor de AGT na carne e leite varia de 1,5 a 6,5%. O isômero *trans* predominante corresponde ao C18:1 11*t*, conhecido como ácido *trans* vacênico ou ácido rumênico[4]. Estima-se que 2 a 8% dos isômeros *trans* da dieta sejam provenientes desta fonte e veiculados principalmente pelos laticínios[5].

Isômeros *trans* também podem ser formados, embora em pequenas quantidades (0,2 a 6,7%), no processo de desodorização de óleos vegetais e em operações de fritura de alimentos (0 a 35%), por mecanismo induzido termicamente[6,7].

Entretanto, os AGT são originados principalmente através da hidrogenação catalítica parcial de óleos vegetais ou marinhos[8]. Cerca de 90% dos AGT em alimentos deriva-se deste processo[9,10,11].

A hidrogenação é realizada com o intuito de modificar a composição, estrutura e consistência de um óleo. Seu resultado é a redução do grau de insaturação do óleo e aumento de seu ponto de fusão, associado ao aumento da estabilidade oxidativa e funcionalidade das frações semi-sólidas produzidas[12,13].

No Brasil, a hidrogenação comercial de óleos vegetais data da década de 50, visando à produção de gorduras técnicas (*shortenings*), margarinas e gorduras para frituras. Com o desenvolvimento de técnicas de hidrogenação seletiva, os óleos vegetais processados rapidamente substituíram as gorduras animais na dieta dos brasileiros. Estas gorduras têm sido largamente empregadas na produção de diversos alimentos, como margarinas, coberturas de chocolate, biscoitos, produtos de panificação, sorvetes, massas e batatas *chips*, entre outros [6].

A hidrogenação é realizada em tanques herméticos, onde o gás hidrogênio é intimamente misturado com o óleo na presença de 0,05 a 0,20% de catalisador níquel finamente dividido, a temperaturas superiores a 180ºC, com pressões entre 0,5 a 4 atm. No decorrer do processo, algumas das duplas ligações dos ácidos graxos são eliminadas, enquanto uma proporção significativa de duplas ligações *cis* são isomerizadas através de conversão *cis-trans* e de mudança posicional ao longo da cadeia[3].

Óleo totalmente hidrogenado é obtido quando todas as duplas ligações são saturadas no processo. Do contrário, tem-se a hidrogenação parcial, usualmente empregada[14].

As características de gorduras parcialmente hidrogenadas são controladas pelas condições de hidrogenação. Os fatores envolvidos são: temperatura, pressão, agitação,

tipo e concentração do catalisador. O aumento na temperatura e/ou decréscimo na pressão acarretam o aumento da seletividade da reação, ou seja, aumentam a conversão de linoleato sobre oleato e aumentam a velocidade de formação dos AGT[15].

No passado, a formação de isômeros *trans* foi considerada uma vantagem tecnológica, uma vez que, devido a seu maior ponto de fusão em relação aos correspondentes isômeros *cis*, favorecem a criação dos níveis de sólidos desejáveis das gorduras hidrogenadas[16,17].

Ocorrência dos AGT na dieta

Historicamente, o consumo mundial de AGT tem aumentado desde a década de 20, em paralelo com o aumento da produção comercial de margarinas e *shortenings,* constituindo-se hoje em proporção significativa da dieta nos países ocidentais. O principal isômero é representado pelo C 18:1 *trans* [18].

Em geral, os AGT são consumidos em maiores quantidades nos países industrializados, com valores médios entre 2 a 8g/dia, o que corresponde a 2,5% do total energético ou a 6 – 8% do total de energia proveniente de lipídios[5].

Estima-se que o consumo de AGT nos EUA varie de 2,6 a 12,8 g/dia. Na Europa, este valor é estimado entre 0,1 a 5,5 g/dia[19]. No Brasil não existem estimativas consensuais sobre a ingestão diária destes compostos e os teores nos alimentos são pouco conhecidos[20].

O teor de AGT nos produtos que contêm gordura parcialmente hidrogenada varia significativamente, até mesmo dentro de uma mesma categoria de produto. Essa variabilidade está associada às condições de hidrogenação da gordura utilizada e à natureza do produto. Os alimentos freqüentemente contêm misturas de diferentes tipos de gorduras parcialmente hidrogenadas e óleos não hidrogenados e o teor total de lipídios pode variar extremamente de um produto para outro[21].

Alguns estudos têm sido realizados visando obter informações acerca dos teores de AGT nos alimentos consumidos no Brasil. Block e Barrera Arellano[22] verificaram que, na década de 90, o conteúdo total de isômeros *trans* em margarinas variava de 12,3% a 38,1%; nos cremes vegetais de 15,9% a 25,1% e nas gorduras técnicas, de 30% a 40%. Basso e colaboradores[23] obtiveram em gorduras vegetais hidrogenadas resultados entre 10 a 50%. Grimaldi e colaboradores[24] avaliaram o teor de isômeros *trans* em quinze amostras de gorduras comerciais brasileiras. Os resultados encontram-se na Tabela 1 e mostram variação de 1,3 a 49,9%.

Tabela 1. Teor total de isômeros *trans* (%) em amostras de gorduras presentes em produtos alimentícios comerciais brasileiros[24].

Gorduras: Aplicação	Teor total de isômeros *trans* (%)
Sopas e caldos	32,3 – 36,4
Coberturas achocolatadas e chocolates granulados	1,3 – 49,9
Pães e bolos	19,5 – 29,9
Biscoitos recheados	21,4 – 48,3
Sorvetes, cremes e margarinas	27,0 – 36,3
Frituras	7,7 – 30,4
Doces e confeitos	3,3 - 40,3

Badolato[25] analisou 19 amostras de gorduras vegetais hidrogenadas e 14 de margarinas comercializadas no Brasil, além de 16 margarinas importadas. Foi verificada variação entre 7,3 a 40,1% de isômeros *trans* do ácido octadecenóico (C 18:1) nas gorduras hidrogenadas e de 0 a 16,1% nas margarinas comercializadas no Brasil. Dentre estas últimas, quatro apresentaram baixo teor de AGT e uma revelou ausência dos mesmos. Este resultado foi avaliado como sinalização de que algumas indústrias brasileiras estavam adotando processos tecnológicos alternativos para minimizar a

formação dos AGT. Somente duas amostras de margarinas importadas apresentaram AGT em pequenas quantidades.

Kawashima e Soares[26] constataram teores de AGT entre 12,7 a 45,1% em seis diferentes marcas de sorvetes cremosos. Sorvetes com gordura exclusivamente láctea não apresentaram AGT.

Os teores de AGT em batatas fritas, biscoitos e sorvetes consumidos no Estado do Rio de Janeiro foram determinados por Chiara e colaboradores[20]. O valor médio destes isômeros em batatas fritas provenientes de redes de *fast food* foi de 4,74g/100g. Nos sorvetes, os valores variaram de 0,041g a 1,41g/100g e em biscoitos, de 2,81g a 5,60g/100g.

Aued-Pimentel e colaboradores[27] reportaram a determinação de AGT em 26 amostras de biscoitos de 6 marcas e 4 tipos diferentes: recheados, “wafer”, salgados diversos e doces diversos. O valores médios obtidos para os teores de AGT nesta categoria de alimento foram similares (3 ± 1g/100g da amostra), independente de fabricantes ou tipo de biscoito. Martin e colaboradores[6] avaliaram o conteúdo de AGT em amostras de biscoitos tipo *cream-cracker* consumidos no Brasil. O total de isômeros *trans* variou de 12,2% a 31,2%, com média de 20,1%.

Capriles e Arêas[28] desenvolveram salgadinhos, obtidos através de extrusão, com teores reduzidos de gordura saturada e de AGT. A gordura vegetal hidrogenada, veículo convencional para aromatização de salgadinhos, foi substituída parcial ou totalmente por óleo de canola, gerando um produto com 73,8% de redução da gordura saturada em relação aos salgadinhos comercialmente disponíveis, além de apresentar ausência de AGT. Abordagens como esta podem ser utilizadas em escala industrial, gerando produtos diferenciados e contribuindo para diminuição da ingestão de ácidos graxos saturados e AGT.

AGT e suas implicações na saúde

As principais preocupações sobre os efeitos dos AGT na saúde têm aumentado, uma vez que estes isômeros são estruturalmente similares às gorduras saturadas, modificam as funções metabólicas das gorduras poliinsaturadas e competem com os ácidos graxos essenciais em vias metabólicas complexas[3].

Os isômeros *cis* são mais rapidamente metabolizados como fonte de energia que os *trans*, e são preferencialmente incorporados em fosfolipídios estruturais e funcionais. Em humanos a incorporação dos isômeros *trans* nos tecidos depende da quantidade ingerida, do tempo de consumo deste tipo de gordura, da quantidade de ácidos graxos essenciais consumida, do tipo de tecido e do tipo de isômero (configuração e posição da dupla ligação na cadeia). Os teores encontrados em tecidos adiposos refletem o consumo por longo período de tempo, apresentando normalmente correlação com histórico de ingestão por mais de um ano[29,30].

Os AGT foram, recentemente, incluídos entre os lipídios dietéticos que atuam como fatores de risco para doença arterial coronariana, modulando a síntese do colesterol e suas frações e atuando sobre os eicosanóides. Diversos estudos têm sugerido uma relação direta entre os mesmos e o aumento do risco de doenças vasculares[31,32,33,34]. No entanto, estas observações são motivos de discussões que procuram avaliar se os AGT seriam melhores ou piores do que os ácidos graxos saturados sob o aspecto nutricional.

Estudos têm mostrado que a ingestão de AGT ocasiona o aumento da lipoproteína de baixa densidade (LDL) em grau similar ao causado pelos ácidos graxos saturados. Em contraste com todos os demais ácidos graxos, os isômeros *trans* implicam na diminuição da lipoproteína de alta densidade (HDL). Logo, a razão LDL/HDL é afetada de modo desfavorável em comparação à modificação causada apenas pelos ácidos graxos saturados[31,35]. Adicionalmente, pesquisas reportam que os AGT aumentam a lipoproteína a (Lpa) e os níveis de triacilgliceróis plasmáticos, que estão independentemente associados com o aumento do risco de doenças cardiovasculares[36,37].

Além destes fatores, os AGT apresentam implicações na etiologia de várias desordens metabólicas e funcionais. Estudos indicam que os mesmos aumentam a fragilidade dos eritrócitos[38], reduzem o consumo de oxigênio e síntese de ATP pelas mitocôndrias[39] e exibem interações competitivas com os ácidos graxos essenciais ao metabolismo[40].

Entre as pesquisas voltadas para a análise da ação dos AGT sobre a saúde da criança, encontrou-se como relato comum o bloqueio e inibição da biossíntese dos ácidos graxos poliinsaturados de cadeia longa, na fase fetal e após o nascimento [41,42]. Estudo de Koletzko e Muller[43] demonstrou correlação inversamente proporcional e significativa entre o ácido linoléico e os AGT, devido possivelmente à inibição da enzima dessaturase.

Portanto, os ácidos graxos *trans* deveriam apresentar prioridade nutricional secundária quando comparados aos ácidos graxos saturados, embora o consumo destes últimos também provoque efeitos desfavoráveis à saúde e deva ser minimizado[3].

AGT e legislação

As questões controversas acerca do papel dos AGT na alimentação têm ocasionado modificações progressivas na legislação, visando a inclusão de maiores informações para os consumidores. A ingestão moderada deste tipo de gordura, com fins de promoção da saúde e redução do risco de doenças coronarianas tem sido recomendada pela Organização Mundial de Saúde desde 1995. Em 1999, a *Food and Drug Administration* (FDA) sugeriu que a quantidade de AGT fosse incluída em rótulos de produtos, recomendando, quando computada em gorduras saturadas, a demarcação por símbolo informativo da quantidade específica de AGT[44].

No Brasil, portaria da ANVISA, datada de 17 de outubro de 1997, estabelecia que a quantidade de AGT deveria estar computada como ácidos graxos saturados, contudo permanecendo desconhecidos os teores específicos de AGT[45]. Entretanto, o Código de Defesa do Consumidor brasileiro, no seu artigo 31, determina que os produtos ofertados à população devem apresentar declarações corretas e objetivas a respeito de suas

características quanto à qualidade, quantidade e composição, entre outras, além dos riscos que oferecem à saúde dos consumidores[46].

Recentemente, a Resolução RDC nº 360, de 23 de dezembro de 2003, harmonizada no Mercosul, obriga a declaração dos AGT na rotulagem nutricional dos alimentos. As empresas tiveram prazo até 31 de julho de 2006 para a adequação, devendo o teor de gorduras *trans* ser declarado em relação à porção harmonizada para um determinado alimento, em conjunto com as declarações para gorduras totais e saturadas. São considerados como zero *trans* os alimentos que apresentarem teor de gorduras *trans* menor ou igual a 0,2g/porção[47].

Além disso, vale ressaltar a necessidade de disponibilizar frações oleosas diversificadas e com isenção de AGT para adição ao chocolate, uma vez que a Resolução RDC n° 227 (28 de agosto de 2003), define como chocolate o produto contendo no mínimo 25% de sólidos totais de cacau, permitindo assim a adição de gorduras especiais a este produto, sem sua descaracterização[47].

Processo alternativo: Interesterificação Química

A interesterificação consiste em alternativa tecnológica ao processo de hidrogenação parcial, uma vez que viabiliza a produção de óleos e gorduras com funcionalidades específicas[17]. Devido à crescente preocupação sobre o impacto nutricional dos AGT na saúde, a interesterificação tem-se mostrado como o principal método para a preparação de gorduras plásticas com baixos teores de isômeros *trans* ou mesmo ausência destes compostos[48]. Em contraste à hidrogenação, este processo não promove a isomerização de duplas ligações dos ácidos graxos e não afeta o grau de saturação dos mesmos[13].

O processo de interesterificação permite a modificação no comportamento de óleos e gorduras, oferecendo contribuições importantes para o aumento e otimização do uso dos mesmos nos produtos alimentícios[49].

A interesterificação de óleos e gorduras pode ser aplicada por diversas razões: para influenciar o comportamento na fusão, fornecendo consistência desejada em temperatura 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. Na reação de interesterificação os ácidos graxos permanecem inalterados, mas ocorre a redistribuição dos mesmos nas moléculas dos triacilgliceróis, resultando na modificação da composição triacilglicerídica, cuja característica final é totalmente determinada pela composição total em ácidos graxos das matérias-primas iniciais. O processo consiste, portanto, em quebra simultânea de ligações éster existentes e formação de novas ligações nas moléculas glicerídicas[50].

Existem dois tipos de interesterificação em uso corrente: a química e enzimática. No processo enzimático, biocatalisadores, tais como lipases microbianas, são utilizados para promover a migração acila nas moléculas acilglicerídicas. Na interesterificação química, largamente utilizada, o catalisador empregado com maior freqüência é o metóxido de sódio, embora outras bases, ácidos e metais sejam disponíveis. Alquilatos de sódio são reconhecidamente os catalisadores mais ativos, inclusive a temperaturas relativamente baixas, entre 50 e 90ºC[51].

No processo químico, os 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 pré-determinado e finalizada mediante a adição de água, que promove a inativação do catalisador. Fatores que podem influenciar a reação incluem intensidade de agitação, temperatura e tamanho de partícula do catalisador[52]. Um esquema da reação de interesterificação química é ilustrado na Figura 2.

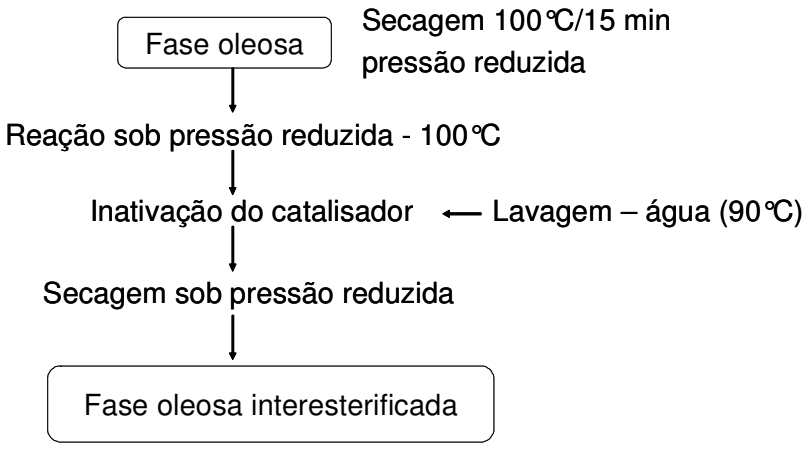

Figura 2. Esquema da reação de interesterificação química.

As mudanças nas propriedades de fusão e solidificação de óleos e gorduras interesterificados devem-se às proporções relativas dos componentes acilglicerídicos após o rearranjo dos ácidos graxos. Conseqüentemente, a estabilidade e 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* do glicerol e resultante aumento nos níveis de triacilgliceróis dissaturados e trissaturados. Logo, é possível a obtenção de produtos plásticos com consistência característica de *shortenings*[53,9].

Novas frações oleosas produzidas por interesterificação química e suas aplicações em alimentos

Interesterificação de misturas de óleos vegetais totalmente hidrogenados ou de frações mais saturadas com óleos líquidos tem sido utilizada para produção de gorduras com ausência de AGT e com propriedades funcionais específicas[17].

Zeitoun e colaboradores[54] reportaram a interesterificação química de óleo de soja totalmente hidrogenado, com nove diferentes óleos vegetais: canola, soja, girassol, algodão, milho, palma, amendoim, açafrão e coco, na proporção de 1:1. As condições de

reação corresponderam a 60 min, 95°C e 0,3% de metóxido de sódio. Os resultados indicaram que os óleos com alto conteúdo de ácido palmítico (C16:0), notadamente óleos de palma e algodão, estiveram relacionados ao perfil de sólidos e comportamento de cristalização próprios de margarinas tradicionais.

Interesterificação de óleo de soja modificado geneticamente, com teores de ácido esteárico (C18:0) entre 17,2% e 33% foi realizada por List e colaboradores [55]. Após a reação, o perfil de sólidos indicou que amostras contendo 17% de ácido esteárico seriam compatíveis para uso em margarinas para culinária. Amostras interesterificadas com teores de ácido esteárico entre 20 – 33% revelaram-se próprias para aplicação em margarinas tradicionais.

Gioielli e Baruffaldi[56] em reações de interesterificação, catalisadas por metóxido de sódio, em gorduras de babaçu, óleo de palma e mistura de ambos na proporção respectiva de 60:40, alcançaram o equilíbrio da reação em 25 minutos, sendo que o rearranjo provocou mudança significativa no perfil dos triacilgliceróis.

Petrauskaite e colaboradores[17] reportaram a interesterificação buscando obter gorduras zero-*trans* com características funcionais similares às gorduras comerciais disponíveis. Os experimentos foram realizados em escala laboratorial, mediante utilização de gorduras altamente saturadas (estearina de palma ou óleo de soja totalmente hidrogenado) com óleo de soja, em proporções variáveis entre 10:90 a 75:25 (%m/m). As condições empregadas foram 90°C durante 90 minutos e 0,2% de metóxido de sódio. As misturas de partida e interesterificadas foram comparadas a amostras comerciais, em termos de composição triacilglicerólica, teor de gordura sólida e nível de AGT. Misturas interesterificadas com 30-50% de matéria-prima saturada revelaram-se semelhantes aos *shortenings* comerciais, enquanto misturas com 40% de estearina de palma ou 25% de óleo de soja totalmente hidrogenado foram indicadas para uso em produtos de confeitaria (zero *trans*).

O efeito da interesterificação nas características físico-químicas de sistemas ternários contendo óleo de palma ou oleína de palma, com oleína de palmiste e óleo de girassol, foi avaliado por Lida e Ali[57], visando produção de coberturas sem a presença de isômeros *trans*. As matérias-primas foram misturadas em 16 diferentes proporções e sujeitas à reação sob as seguintes condições: 110°C, 30 minutos, agitação de 500 rpm e 0,2% de catalisador metóxido de sódio. A presença de óleo de palma e óleo de girassol colaborou para o aumento e a diminuição do ponto de fusão do produto interesterificado, respectivamente.

Kok e colaboradores[9] utilizaram interesterificação de óleo de soja altamente saturado (HSSBO), contendo 23,3% de ácido palmítico e 20,0% de ácido esteárico, na preparação de margarina sem AGT (70ºC, 10 min, 0,5% MeONa). Obteve-se gordura com ponto de fusão 34,5ºC, em contraste com o baixo ponto de fusão inicial (9,5ºC). Mistura de 50/50% do produto interesterificado com um óleo de soja líquido foi utilizada para a produção de margarina, que apresentou pequena diferença significativa ($p \leq 0,05$) em termos sensoriais, em relação a margarinas comerciais.

Rodriguez e colaboradores[58] estudaram a interesterificação de misturas sebo/óleo de girassol com o intuito de substituir óleos marinhos hidrogenados na formulação de *shortenings*. Para isso, foi utilizado um planejamento fatorial fracionário 2^{4-1}, em que as variáveis independentes consideradas foram: proporção de sebo (50-90%), concentração de metóxido de sódio (0,4 – 1,0% m/m), temperatura (60-120°C) e tempo de reação (15 – 60 minutos). O teor de sebo, concentração de catalisador e temperatura apresentaram efeito significativo ($p \leq 0,05$) no ponto de fusão. A interesterificação resultou em alterações nas curvas de sólidos dos referidos produtos. Para concentrações de sebo iguais a 70% e 90% obteve-se *shortenings* adequados para utilização em sorvetes e panificação, respectivamente. Rodrigues e Gioielli[59] reportaram a interesterificação química de diferentes misturas compostas por gordura de manteiga (*butterfat)* e óleo de milho, buscando bases gordurosas com as características organolépticas da manteiga,

mas com alto teor de ácidos graxos ω-6. Os resultados revelaram que o processo reduziu significativamente o conteúdo de triacilgliceróis trissaturados e triinsaturados das amostras interesterificadas.

Pontes e colaboradores[60] avaliaram as características da gordura resultante da interesterificação química de óleo de palma (OP - 75%) e estearina de palma (EP - 25%) realizada em planta-piloto. Os resultados obtidos foram comparados com os da mistura (OP + EP) antes da interesterificação. A amostra interesterificada apresentou teores de sólidos maiores que os da mistura na faixa analisada e o ponto de fusão passou de 39,1 para 42,2°C, conforme a Figura 3. Enquanto os triglicerídeos mono e dissaturados diminuíram, os triinsaturados e os trissaturados aumentaram. Concluiu-se que a gordura obtida apresentou características adequadas para ser utilizada tanto para recheio de biscoito quanto para uso geral e também para bolos e pães de forma. Porém, pelo fato de ter apresentado teor de triacilgliceróis do tipo trissaturados (SSS) da ordem de 13%, seu uso para recheios não foi recomendado, em função de poder apresentar certo grau de cerosidade.

Norizzah e colaboradores[48] estudaram a interesterificação de misturas de estearina de palma (PS) e oleína de palmiste (PKOo), nas proporções de PS:PKOo relativas a 20:80, 40:60, 60:40 e 80:20 (m/m). A reação foi conduzida a 110°C durante 60 minutos, com agitação intensa e 0,2% de MeONa. O ponto de fusão, conteúdo de gordura sólida (SFC), composição triacilglicerídica, termograma de fusão, forma polimórfica e morfologia dos cristais foram avaliados para as misturas antes e após a interesterificação. Os resultados indicaram que todas as misturas interesterificadas apresentaram menor ponto de fusão e menores teores de triacilgliceróis altamente saturados em relação às misturas de partida. As frações obtidas foram indicadas para margarinas e *shortenings* para cremes e produtos similares.

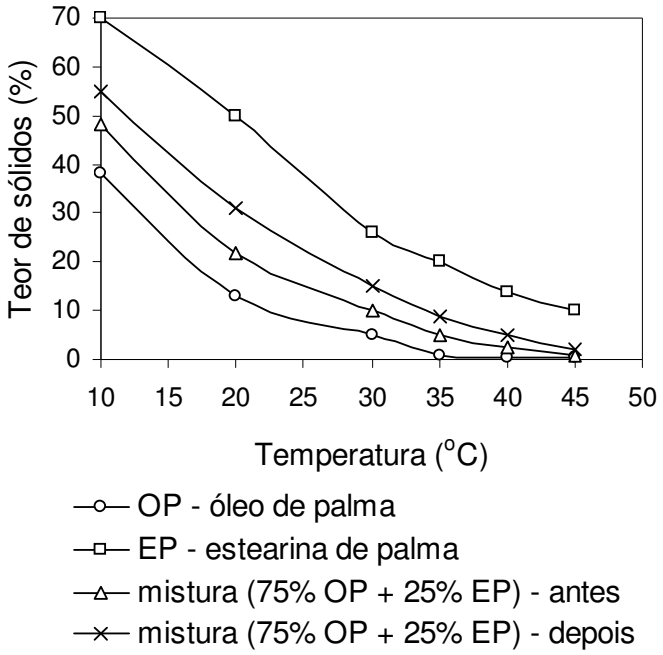

Figura 3. Curvas de sólidos das matérias-primas (OP: óleo de palma; EP: estearina de palma) e da mistura (75% OP - óleo de palma + 25% EP - estearina de palma), antes e depois da interesterificação.

Visando a obtenção de alternativas à hidrogenação parcial na produção de gorduras plásticas, Karabulut e colaboradores[61] estudaram o processo de interesterificação em escala laboratorial. Foram utilizadas misturas de estearina de palma totalmente hidrogenada ou estearina de palma com óleos de canola e de algodão, em proporções variando de 30:70 a 70:30 (%m/m). O processo resultou na diminuição do ponto de fusão e do teor de gordura sólida para todas as amostras, relacionada ao decréscimo no teor de triacilgliceróis trissaturados e monoinsaturados. As curvas de sólidos dos produtos interesterificados diferiram significativamente ($p \leq 0,05$) das amostras não interesterificadas e também entre si. As misturas interesterificadas com 30:70 canola/estearina de palma ou 30:70, 40:60 e 50:50 algodão/estearina de palma revelaram-se adequadas à produção de *shortenings* para uso geral. Em contrapartida, misturas contendo 70:30 canola/estearina de palma totalmente hidrogenada ou 70:30

algodão/estearina de palma foram recomendadas para manufatura de margarinas. Para o emprego em confeitaria, mostraram-se factíveis as misturas de 50:50 canola/estearina de palma ou 50:50 e 60:40 algodão/estearina de palma. Os autores concluíram que o emprego destas frações pode substituir seguramente as gorduras disponíveis em questão, com ausência total de AGT.

Godoy e colaboradores[62] produziram em escala piloto formulações oleosas interesterificadas a partir de misturas de óleos de palma (PO) e palmiste (PKO), visando avaliar a performance das mesmas em produtos alimentícios específicos. Além da etapa de interesterificação, o perfil de sólidos da gordura inicial foi alterado por meio de posterior adição de óleo de soja totalmente hidrogenado (HSBO), que permitiu modificação de consistência sem a necessidade de hidrogenação parcial e, conseqüentemente, sem a possibilidade de formação de isômeros *trans*. Duas formulações gordurosas interesterificadas, designadas por GE1=PO/PKO 60:40inter e GE2=PO/PKO 60:40inter + 3%HSBO, foram utilizadas no teste de aplicação, por apresentarem perfil de sólidos e pontos de fusão característicos para sorvetes e bolos, respectivamente. O estudo demonstrou a aplicação com sucesso das frações interesterificadas como ingredientes para estes produtos, sem a presença de AGT em sua composição. A Figura 4 exibe o conteúdo de gordura sólida das misturas PO/PKO 60:40 antes e após interesterificação e com adição de 3% de HSBO.

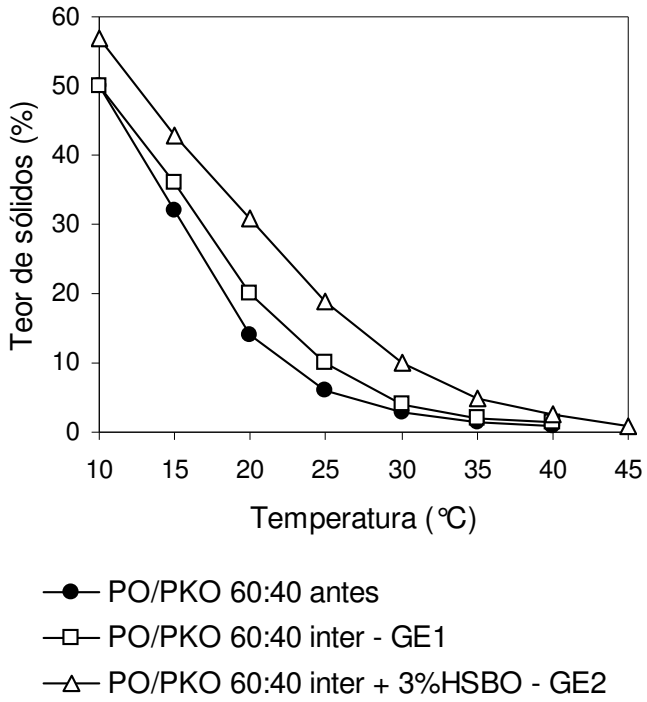

Figura 4. Curvas de sólidos das misturas PO/PKO 60:40 – antes da interesterificação, após interesterificação (GE1) e com adição de 3% de HSBO -óleo de soja totalmente hidrogenado (GE2).

Grimaldi e colaboradores[63] realizaram otimização, em escala laboratorial, da reação de interesterificação química do óleo de palma. As amostras foram submetidas a ensaio fatorial completo com 3 níveis de concentração de catalisador (metóxido de sódio comercial) e 3 níveis de tempo de reação. O processo foi desenvolvido com 100g de fase oleosa, em temperatura constante de 100°C, sob agitação magnética. Os resultados da análise estatística basearam-se na maior variação dos grupos de triacilgliceróis em relação ao controle, com preferência para a condição referente a 0,4% de MeONa e 20 min de reação. Verificou-se aumento do teor de sólidos após o processo, evidenciando a melhoria da consistência para uso em margarinas ou produtos alimentícios.

Khatoon e Reddy[64] avaliaram a obtenção de gorduras plásticas zero *trans* através de interesterificação de misturas de estearina de palma com óleos da manga ou mahua

(*Madhuca longifolia*), em diferentes proporções. Os resultados mostraram que misturas de estearina de palma/mahua (1:1 e 1:2) e estearina de palma/manga (1:1), após a reação (1h, 80°C, 0,2% metóxido de sódio) exibiram perfis de sólidos similares aos *shortenings* comerciais para panificação e ao *vanaspati*, um tipo de gordura hidrogenada tradicional na Índia e demais países do sudeste asiático.

Ramli e colaboradores[65] reportaram as alterações físico-químicas em misturas de óleo de palmiste hidrogenado e gordura proveniente do leite de cabra, após interesterificação. Os autores concluíram que o processo resultou em aumento da plasticidade das gorduras, mas não indicaram, entretanto, uma possível aplicação das mesmas em produtos alimentícios.

Conclusão

As evidências científicas relacionadas ao impacto negativo dos AGT na saúde têm fornecido subsídios para que o consumo de gordura parcialmente hidrogenada seja minimizado, fato corroborado por mudanças progressivas na legislação em diversos países.

O desafio da indústria de alimentos na substituição da gordura *trans* em diversos produtos reside no desenvolvimento de formulações que apresentem funcionalidade equivalente e viabilidade econômica, não acarretando, entretanto, aumento substancial do teor de ácidos graxos saturados nos alimentos.

A interesterificação química consiste em opção tecnológica importante para a produção de gorduras técnicas visando diversas aplicações alimentícias, mediante a facilidade do processo e baixo custo associado.

Embora inúmeros estudos tenham sido desenvolvidos na área, a exploração de novas matérias- primas e/ou suas combinações é fator preponderante para obtenção de novas frações gordurosas que possam ser empregadas na maior variedade possível de produtos alimentícios, sem restrições de ordem tecnológica ou funcional.

Referências

1. Young, F.V.K.; *J. Am. Oil Chem. Soc*. **1985**, *62*, 372.

2. O'Brien, R.D.; *Fats and Oils – Formulating and Processing for Applications*, Technomic Publishing Company: Lancaster, **1998**.

3. Valenzuela, A.; King, J.; Nieto, S.; *Grasas y Aceites*. **1995**, *46*, 369.

4. Fritsche, J.; Steinhart, H.; *Fett/Lipid*. **1998**, *6*, 190.

5. Larqué, E.; Zamora, S.; Gil, A.; *Early Human Development*. **2001**, *65*, 31.

6. Martin, C.A. ; Carapelli, R. ; Visantainer, J.V.; Matsushita, M.; Souza, N.E.; *Food Chem*. **2005**, *93*, 445.

7. Hunter, E.; *Nutr. Res*. **2005**, *25*, 499.

8. Applewhite, T.H.; *J. Am. Oil Chem. Soc*. **1981**, *58*, 261.

9. Kok, L.L.; Fehr, W.R.; Hammond, E.G.; White, P.J.; *J. Am. Oil Chem. Soc*. **1999**, *76*, 1175.

10. Mayamol, P.N. ; Samuel, T. ; Balachandran, A.; Sundaresan, A. ; Arumugham, C.; *J. Am. Oil Chem. Soc*. **2004**, *81*, 407.

11. List, G.R.; *Food Technol*. **2004**, *35*, 1015.

12. Hui, Y.H.; *Bailey's industrial oil and fat products*, 5th ed., Wiley: New York, **1996**.

13. Haumann, B.F.; *INFORM*. **1994**, *5*, 668.

14. Ghotra, B. S.; Dyal, S. D.; Narine, S. S.; *Food Res. Int.,* **2002**, *35*, 1015.

15. Woerfel, J.B.; *Grasas y Aceites*. **1995**, *46*, 357.

16. Karabulut, I.; Kayahan, M.; Yaprak, S.; *Food Chem*. **2003**, *81*, 453.

17. Petrauskaite, V.; De Greyt, W.; Kellens, M.; Huyghebaert, A.; *J. Am. Oil Chem. Soc*. **1998**, *75*, 489.

18. Jeyarani, T.; Reddy, S.Y.; *J. Am. Oil Chem. Soc*. **2003**, *80*, 1107.

19. Feldman, E.B.; Krisetherton, P.; Kritchevsky, D.; Lichtenstein, A.H.; *Am. J. Clin. Nutr*. **1996**, *63*, 663.

20. Chiara, V.L.; Sichieri, R.; Carvalho, T.S.F.; *Rev. Nutr*. **2003**, *16*, 227.

21. Padovese, R.; Mancini Filho, J.; *Entendendo a gordura: os ácidos graxos.* Editora Manole: Barueri, **2002**.

22. Block, J.M.; Barrera-Arellano, D.; *Arch. Latinoam. Nutr.* **1994**, *44*, 281.

23. Basso, R.; Almeida Gonçalves I.; Mancini Filho, J.; *Bol. Soc. Bras. Ciênc. Tecnol. Aliment.* **1999**, *33*, 57.

24. Grimaldi, R.; Gonçalves, L. A. G. ; Esteves, W.; *Braz. J. Food Technol.* **2000**, *3*, 159.

25. Badolato, E.S.G., *Dissertação de Mestrado*, Universidade de São Paulo, Brasil, **2000**.

26. Kawashima, L.M.; Soares, L.M.V.; *Ciênc. Tecnol. Aliment*. **1993**, *13*, 194.

27. Aued-Pimentel, S.; Caruso, M.S.F.; Cruz, J.M.M.; Kumagai, E.E.; Corrêa, D.U.O.*; Rev. Inst. Adolfo Lutz*. **2003**, *62*, 131.

28. Capriles, V.D.; Areas, J.A.G.; *Ciênc. Tecnol. Aliment*. **2005**, *25*, 363.

29. Garland, M.; Sacks, F.M.; Colditiz, G.A.; Rimm, E.B.; Sampson, L.A.; Willett, W.C.; *Am. J. Clin. Nutr*. **1998**, *67*, 25.

30. Chiara, V.L. ; Silva, R.; Jorge, R. ; Brasil, A.P.; *Rev. Nutr*. **2002**, *15*, 11.

31. Mensink, R.P.; Katan, M.B.; *N. Engl. J. Med*. **1990**, *323*, 439.

32. Enig, M.G.; *Cereal Foods World*. **1996**,*41*, 58.

33. Gurr, M.; *Lipid Technol*. **1990**, *2*, 105.

34. Lichtenstein, A.; *Nutr. Rev.* **1993**, *51*, 340.

35. Mensink, R.P.; Katan, M.B.; Hornstra, G.*; J. Lipid Res*. **1992**, *33*, 1493.

36. Aro, A.; Jauhiainen, M.; Partanen, R.; Salminen, I.; Mutanen, M.; *Am. J. Clin. Nutr*. **1997**, *65*, 1419.

37. Ascherio, A.; Katan, M.; Zock, P.L; Stampfer, M.J.; Willett, W.C.; *N. Engl. J. Med*. **1999**, *340*, 1994.

38. Decker, W.J.; Mertz, W.; *J. Nutr*. **1967**, *91*, 324.

39. Zevenbergen,J.L.; Houtsmuller, V.M.; Gottenbos, J.J.; *Lipids.* **1988**, *23*, 178.

40. Peacock, L.L.; Wahle, K.W.*; Biochem. Soc. Trans*. **1998**, *17*, 679.

41. Koletzko, B.; *Acta Paediatr.* **1992**, *81*, 302.

42. Carlson S.; Thomas, M.C.; Cook, H.W.; Emken, E.A.; Filer, L.; *Am. J. Clin. Nutr*. **1997**, *119*, 717.

43. Koletzko, B.; Muller, J.; *Biol. Neonate*. **1990**, *57*, 172.

44. www.cfsan.fda.gov/label.html, acessada em Dezembro **2004**.

45. Brasil. Lei n° 8078, 11 de setembro de 1990. *Diário Oficial*. Brasília, 12 set, **1990**. Seção I (supl.). p.1-12. Dispõe sobre a proteção do consumidor.

46. www.portal.saude.gov.br/portal/saude/area, acessada em Abril **2005**.

47. www.e-legis.bvs.br/leisref, acessada em Novembro **2004**.

48. Norizzah, A.R.; Chong, C.L.; Cheow, C.S. Zaliha, O.; *Food Chem*. **2004**, *86*, 229.

49. Erickson, D. R.; *Practical Handbook of Soybean Processing and Utilization*. AOCS Press, Champaign, Illinois, **1995**.

50. Rozendaal, A.; *INFORM*. **1992**, *3*, 1232.

51. Marangoni, A.G.; Rousseau, D.; *Trends Food Sci. Technol*. **1995**, *6*, 329.

52. Sreenivasan, B.; *J. Am. Oil Chem. Soc*. **1978**, *55*, 796.

53. Hustedt, H.H.; *J. Am. Oil Chem. Soc*. **1976**, *53*, 390.

54. Zeitoun, M.A.M.; Neff, W.E.; List, G.R.; Mounts, T.L.*; J. Am. Oil Chem. Soc*. **1993**, *70*, 467.

55. List, G.R.; Mounts, T.L.; Neff, W.E.; *J. Am. Oil Chem. Soc*. **1997**, *74*, 327.

56. Gioielli, L. A.; Baruffaldi, R.; *Rev. Farm. e Bioquím. Univ. S. Paulo*, **1988**, *24*, 29.

57. Lida, A.M.D.N.; Ali, A.R.; *J. Am. Oil Chem. Soc*. **1997**, *75*, 1625.

58. Rodriguez, A.; Castro, E.; Salinas, M.C.; López, R.; Miranda, M.; *J. Am. Oil Chem. Soc*. **2001**, *78*, 431.

59. Rodrigues, J. N.; Gioielli, L. A.; *Food Res.Int.,* **2003**, *36*, 149.

60. Pontes, A. E. R.; Aires, G.S.B.; Ormenese, R. C.S. C.; Grimaldi, R.; Gonçalves, L. A.G.; *Anais do Simpósio Internacional Tendências e Inovações em Tecnologia de Óleos e Gorduras*, Campinas, Brasil, **2003**.

61. Karabulut, I.; Turan, S.; Ergin, G.; *Eur. Food Res. Technol.* **2004**, *218*, 224.

62. Godoy, A.M.T.; Gonçalves, L.A.G.; Silva, M.A.A.P.; Gilabert-Escrivá, M.V.; *Anais do III Congreso Iberoamericano de Ingeniería de Alimentos, I Gongreso Español de Ingeniería de Alimentos*, Valencia, Espanha, **2001**.

63. Grimaldi, R.; Gonçalves, L.A.G.; Ando, M.Y.; *Quim. Nova,* **2005**, *28*, 633.

64. Khatoon, S.; Reddy, S.R.Y.; *Eur. J. Lipid Sci. Technol*. **2005**, *107*, 786.

65. Ramli, N.; Said, M.; Loon, N.T.; *J. Food Lipids*. **2005**, *12*, 243.

2.

INSTRUMENTAL METHODS FOR THE EVALUATION OF INTERESTERIFIED FATS

Ana Paula Badan Ribeiro, Rodrigo Corrêa Basso, Renato Grimaldi, Luiz Antonio Gioielli, Lireny Aparecida Guaraldo Gonçalves

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Instrumental methods for the evaluation of interesterified fats

Abstract

Chemical interesterification is currently the main alternative for the production of plastic fats containing no trans fatty acids. However, directing the industrial application of an interesterified fat requires a complete understanding of its physicochemical, functional and technological properties, and also of its stability during and after processing. The objective of the present article was to carry out an ample review of the experimental methodologies applied to the study of interesterified fats. The methodologies considered include techniques employed in the investigation of the triacylglycerol composition, solid fat content, crystallization kinetics, thermal behavior, polymorphism, microstructure and consistency. The methodologies considered include some official methods currently in use and new techniques recently developed and employed. Sample preparation methods, interpretation of the results and the results recently published in the literature in question are also detailed and discussed.

KEY-WORDS: Interesterification, pulsed Nuclear Magnetic Resonance, Differential Scanning Calorimetry, Cone penetrometry, X-ray Diffraction, Polarized Light Microscopy.

Introduction

The majority of natural fats and oils present limited application in their unaltered form due to their particular fatty acid and triacylglycerol compositions. Due to recent concern about the nutritional impact of *trans* fatty acids on the health, chemical interesterification has been shown to be the main method to prepare plastic fats not containing these compounds. This process allows for a modification in the behavior of the fats and oils, offering important contributions for the increase and optimization of their use in food products.[1,2]

In contrast to partial hydrogenation, interesterification does not promote isomerization of the double bonds of the fatty acids and does not affect their degree of saturation[3]. The fatty acids profile remains unaltered during the interesterification reaction, but a redistribution of these components on the triacylglycerol molecules occurs. Thus the process consists of the simultaneous breakage of existing ester bonds and the formation of new bonds on the glyceride molecules.[4] In this way, chemical interesterification causes a modification in the triacylglycerol composition of a fat or blend of fats, and consequently in its physical characteristics at all structural levels.[5,6]

A complete understanding of the functions and properties of the fats or oily bases produced by interesterification is primordial for the design of application plans for their use and to obtain food products with the desired final attributes, particularly when one considers the recent substitution of partially hydrogenated fats. The satisfactory performance of a fat depends on important elements that determine its applicability. These include, primarily, stability during the post-processing period and total compatibility of the fat base with the product for which it is destined. Apart from this, physical and functional characteristics such as plasticity, spreadability and cream formation and aeration properties should also be considered in the development of a new formulation. Thus a study of the application of interesterified fats in margarines, shortenings and other fat-rich products should be based primarily on an understanding of the relationships between parameters such as the triacylglycerol composition, melting point, solid fat content, thermal behavior and crystallization, microstructure, crystalline habit and consistency.[7] The experimental methodology associated with the study of interesterified fats includes the use of low resolution pulsed Nuclear Magnetic Resonance, Differential Scanning Calorimetry, Cone Penetrometry, X-ray Diffraction and Polarized Light Microscopy, in addition to chromatographic techniques.[5]

The objective of this article was to present a detailed description of the analytical methods most used in the study of the physicochemical properties of interesterified fats.

The methodologies considered include techniques employed in the investigation of crystallization kinetics, thermal properties and polymorphism, texture and microstructure. With the intention of providing the reader with a complete panorama of the experimental methodologies presented, a brief theoretical introduction is included for each topic, and also some results found in the literature, so as to highlight concepts related to lipid chemistry and technology and the chemical interesterification process.

Melting point

The melting point is usually defined as the temperature at which a material changes from the solid to the liquid state. However, natural or randomized fats do not show a clearly defined melting point like pure substances, since they consist of complex mixtures of triacylglycerols that undergo gradual melting according to the individual melting points, until they become completely liquid. Thus fats present melting ranges or intervals. However, the melting point is a parameter of significant importance for the characterization and development of fats. Thus the melting point is assumed to be a value representative of the temperature within the melting range of a sample, obtained using defined experimental methods.[8]

The rearrangement of the acyl residues on the triacylglycerol molecules caused by interesterification, generally modifies the melting point of the fats.[9] The precise effect of interesterification on melting behavior depends fundamentally on the initial raw materials. Randomization can increase, decrease or even present no effect on the melting point. Fully hydrogenated fats, with high melting points, interesterified with liquid oils, show a decrease in the melting point in relation to the original material due to a decrease in the proportion of trisaturated triacylglycerols. Applying this idea to cottonseed oil, for example, which possesses a significant proportion of saturated fatty acids and minimal amounts of trisaturated triacylglycerols, the randomization process causes an increase in the melting point, associated with the formation of totally saturated triacylglycerols.[4,6]

In the industrial production of interesterified fats, the melting point is generally the most convenient way of evaluating the endpoint of the reaction. However, specific limits should be established in order to identify alterations and applicability of this analytical parameter in the control of interesterification. In some cases, as in the interesterification of fats of animal origin, there may be no change in the melting point, or the change may be so small as to be within the range of normal analytical error. Although the determination of the melting point is quick and reproducible, it is frequently shown to be inefficient as an isolated analytical method to detect the endpoint of chemical interesterification.[7,10]

The main advantage of the majority of the methods for determining the melting point of fats is their relative simplicity. A variety of procedures have been standardized by the *American Oil Chemist's Society (AOCS)*. The methods vary considerably in the way of carrying out the determination, sample tempering, level of automation, time required and degree of melting.[7,11] The two most widely used methods are described below:

Melting Point: *AOCS Method Cc 1-25 – capillary melting point.*[11] In this method, capillary tubes (internal diameter of 1mm) with the lower extremity sealed, are filled to a height of 10mm with melted fat. Tempering is carried out for 16 hours between 4 and 10ºC. The capillaries are then heated in a water bath, where the initial temperature is below (by 8 to 10ºC) the expected melting point of the sample, heating at a rate of 0.5ºC/minute until the fat is completely clear. Reproduction of the results with this method is difficult due to the subjective interpretation.

Softening point: *AOCS Method Cc 3-25 – Softening point.*[11] This method uses open capillaries and follows the same tempering procedure as *AOCS Method Cc 1-25.* The capillaries are not sealed and the determination of the endpoint corresponds to a rising of the fat column in response to hydrostatic pressure. The greater objectivity associated with this method is an advantage in obtaining better reproducibility of the results.

Kok, Fehr, Hammond & White[12] showed that the interesterification of highly saturated soybean oil (rich in stearic acid) resulted in an increase in the melting point of the original

oil from 9.5ºC to 35.4ºC. Similar results were reported by List, Emken, Kwolek, Simpson & Dutton[13] in the randomization of the same type of raw material. The interesterification of soybean oil and lard was carried out by Silva & Gioielli[14]. Initial blends containing up to 20% of disaturated-monounsaturated and trisaturated triacylglycerols produced a significant increase in the melting point after the reaction. Karabulut, Turan & Ergin[9] observed a decrease in the melting point of interesterified blends of fully hydrogenated palm stearin or palm stearin with canola and cottonseed oils, for all the proportions studied. Petrauskaite, De Greyt, Kellens & Huyghebaert[15] also determined an absolute decrease in the melting point of between 7-25ºC and 9-31ºC for the respective interesterified blends of palm oil/soybean oil and fully hydrogenated soybean oil/soybean oil in various proportions. Khatoon[16], studying the interesterification of palm oil with sunflower, rice, coconut or soybean oils, obtained an oily fraction from the palm and sunflower (4:1) oils with a melting point of 41ºC, indicated as ideal for the production of margarine bases.

Solid fat content (SFC)

The SFC is a parameter that expresses the solid:liquid ratio of a fat at various temperatures.[17] Determinations of the solid content of a fat at different temperatures up to melting can be presented in graphs, producing a curve illustrating the changes.[7] The alterations caused by interesterification in triacylglycerols of the trisaturated, disaturated-monounsaturated and monosaturated-diunsaturated types, are reflected in the curves of the solids (SFC curves) before and after the reaction.[17] The methods most used to determine the SFC curve are: SFC using the pulsed Nuclear Magnetic Resonance (NMR) technique, and the solid fat index (SFI) determined by dilatometry.[18]

Up to the early seventies, dilatometry was the standard method used. It is based on expansion, that is, the change in volume that results from complete melting of the fat at a determined temperature.[18] Thus the SFI, an empirical expression of the amount of liquid in

a fat at a determined temperature, is measured as the change in specific volume with respect to this temperature. Since the solid fat melts, the volume of the sample increases and this alteration can be measured by dilatometry.[19] Despite its importance, the SFI method is lengthy and demands intense work. In the search for a quicker, cheap and more automated analysis, in 1974, Unilever researchers were pioneers in the use of the pulsed NMR technique for the determination of the solid:liquid ratio in fats.[20] The solid:liquid ratio obtained by NMR is designated as SFC and is expressed as a percentage, where 0% corresponds to a totally liquid sample and 100% to a totally solid one.[21] The determination of the SFC by low resolution NMR is currently an internationally accepted procedure and of considerable importance in the fats and oils industry, having become the standard technique in substitution of dilatometry, since it allows for direct, quick results.[22,23]

NMR is a spectroscopic technique with a variety of applications in structural and compositional analyses. It is based on the principle that the spins of the nuclei of hydrogen atoms line up in a magnetic field. These lined up spins can be taken out of their equilibrium state (excitation) by a short electromagnetic pulse, and then return to their equilibrium position in a process called relaxation. The efficiency (and rate) of these relaxation processes is determined by the mobility of the molecules to which the nuclear spins belong. This is the basic principle of the determination of the SFC by NMR, which depends on the large difference in the time of duration of the signals from solid and liquid phases, since the signal of a solid phase fades much more quickly than that of a liquid phase.[24]

The determination of SFC does not correspond to an absolute method, that is, there is no reference fat or other material that can be used as a standard to provide a defined or known SFC value. To the contrary, it is defined in terms of the conditions of the NMR equipment and of the temperature history of the sample before the measurement.[21]

Prior to the SFC determination, the fat should be exposed to a pre-defined time/temperature sequence: complete melting of the sample aimed at destroying any traces of crystals or solid fat, cooling to complete crystallization and finally maintenance at

the temperature the reading will be taken in order to reach equilibrium. In addition, an extra stage can be used, maintaining the fat at a temperature that is not exactly the temperature of the reading, but which is required to stabilize the sample. The official methodologies used to determine SFC describe the type of heat treatment (or tempering) required according to the sample. Slight variations in the temperatures and times used during tempering can result in significant variations in the SFC values.[25]

The main methods standardized for the determination of SFC by NMR consist of the *AOCS Official Method Cd 16-81- Solid Fat Content (SFC) by NMR* (indirect method) and the *AOCS Official Method Cd 16b-93 - Solid Fat Content (SFC) by Low-Resolution NMR* (direct method).[11] The *ISO 8292* and *IUPAC 2.150* methods correspond to this latter method, and also the standard German method for the analysis of solid fat and other lipids – section *CIV – 3f*. [26,27,28]

Using any of these methods, the readings of SFC can be made in series or in parallel, the parallel mode being recommended.[11] Readings in series signify that the sample was initially measured at 20ºC, transferred to 25ºC and, after a prescribed time, measured at 25ºC; transferred to 30ºC, and so on. This measurement has the advantage of using only one sample tube to construct the SFC curve. Using parallel readings, in which various tubes are filled and each maintained at the temperature of the reading, the total time spent on obtaining the curve is substantially smaller. In addition, each determination at a given temperature is independent of the others. When compared, slight differences are found in the results obtained between these two ways of reading. In general the results obtained in parallel tend to be lower than those obtained in series.[21]

In the indirect method *AOCS Official Method Cd 16-81*, the NMR signal of the liquid phase of the sample is compared with the signal of the same sample when completely melted and the result converted into a percentage. Since this method is based on measurements of the liquid signal exclusive to the sample under analysis, a liquid oil should be analyzed concomitantly with the reference. In the direct method *AOCS Official*

Method Cd 16b-93, the signals of both the solid and liquid phases are measured and compared. In this method the SFC is defined as the ratio between the response obtained for the solid phase and the sum of the responses obtained for the solid and liquid phases of the sample, expressed as a percentage.[11] The direct method is subdivided into Methods I and II and is summarized in Table 1. Method II refers to cocoa butter and other special fats destined for use in confectionaries that demand differentiated tempering so that they can be converted into the β-polymorphic form before measuring the SFC.[29]

The data obtained from the SFC curve are used to predict the applicability of a fat. The liquid/solid contents at various temperatures provide good indications of the general behavior of the fat, information used, above all, in the formulation and development of new products.[7] The SFC is responsible for many characteristics of margarines and shortenings, including their appearance, ease in filling, spreadability, oil exudation and organoleptic properties. For example, the SFC of a margarine at 5ºC determines its ease of spreading at refrigeration temperatures; at 25ºC it is related to product stability and resistance to oil exudation at room temperature; at 35ºC the SFC the texture and properties for the liberation of flavor and aroma in the mouth.[6] According to Lida & Ali[31], the SFC of margarines at 10ºC should not be greater than 32% so that spreadability is guaranteed at refrigeration temperatures. In order to avoid producing a waxy sensation in the mouth, margarines should obligatorily present SFCs below 3.5% at 33.3ºC, in order to melt completely at body temperature.[9]

Table 1. Summary of the methods defined by the *AOCS Official Method Cd 16b-93.*[11,30]

	Method I	Method II
Use	Oils and fats that have complete crystallization within 1 hour at 0℃: margarines, conventional fats, baked goods and frying *shortenings*, interesterified fats in general.	Cocoa butter and specialty fats, with high levels of SOS triacylglycerols (cocoa butter equivalents or improvers).
Methodology	- Direct method - Parallel measurement	- Direct method - Parallel measurement
Tempering	15 minutes → 100℃ ↓ 5 minutes→ 60℃ ↓ 60 minutes→ 0℃ ↓ 30 minutes → temperature for which a measurement is needed (10, 15, 20, 25, 30, 35, 40,45℃....)	15 minutes → 100℃ ↓ 5 minutes→ 60℃ ↓ 90 minutes→ 0℃ ↓ 40 hours → 26℃ ↓ 90 minutes → 0℃ ↓ 60 minutes → temperature for which a measurement is needed (20, 25, 30, 35, 40,45, 50, 55, 60℃)

The SFC curves before and after interesterification show significant changes in the behavior of fats, providing information about:

- Plasticity: fats and oils generally show an ideal consistency for mixing and processing with SFCs between 15 and 25%. This interval is identified as the “plasticity range”. Curves which are only slightly inclined refer to plastic fats, since the solids levels remain within the

plasticity range over a larger temperature range. To the contrary there are quick melting fats, such as those destined for frying.[7]

- Melting point: this can be obtained from the SFC curve in the graphical or mathematical form. It is defined as the temperature at which the fat presents 4% of solids.[9,12]

Fig.1 shows the SFC curves obtained by Grimaldi, Gonçalves, Gioielli & Simões[32] for palm oil (PO) and palm kernel oil (PKO) before and after chemical interesterification. Randomization caused an increase in the SFC of the palm oil and a decrease in that of the palm kernel oil. It can be seen that the palm and palm kernel oils present different melting and plasticity profiles, related to their specific applications in the food industry.

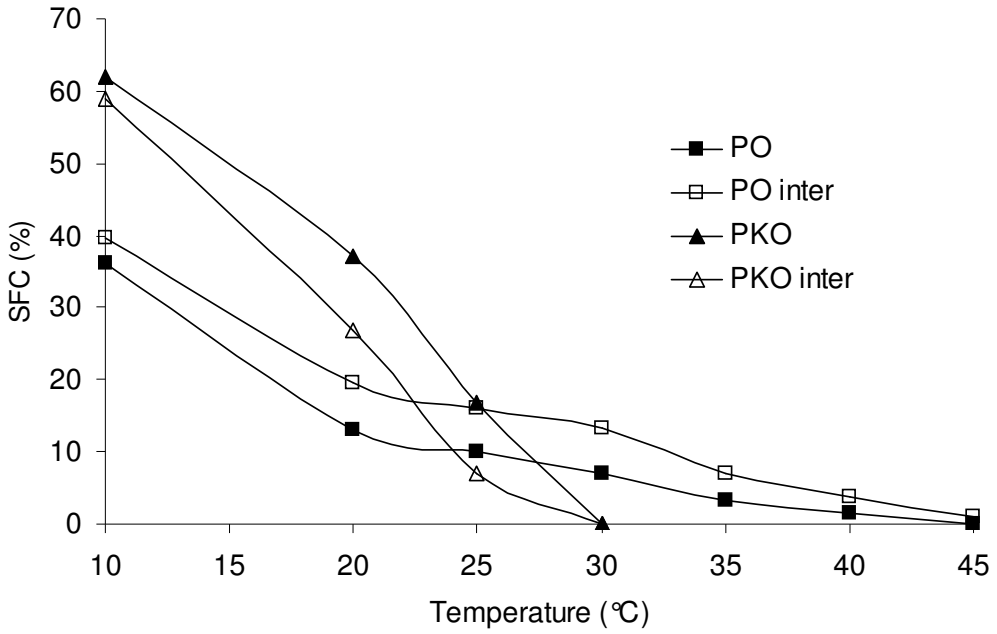

Fig.1. SFC profile of palm oil (PO) and palm kernel oil (PKO), before and after chemical interesterification.[32]

The great majority of studies on interesterification published in the literature have used the SFC curve as a tool to detect alterations caused by randomization, to delineate specific applications for interesterified fats in foods, and to optimize this chemical

modification process. Thus one can cite the studies developed by Karabulut, Turan & Ergin[9]; Kok, Fehr, Hammond & White[12]; List, Emken, Kwolek & Dutton[13]; Silva & Gioielli[14]; Petrauskaite, De Greyt, Kellens & Huyghebaert[15]; Khatoon[16]; Lida & Ali[31]; Gioielli & Baruffaldi[33]; List, Mounts, Orthoefer & Neff[34]; Marangoni & Rousseau[35,36]; Rodriguez, Castro, Salinas, López & Miranda[37]; Rodrigues, Gioielli & Anton[38]; Rodrigues & Gioielli[39]; Norizzah, Chong, Cheow & Zaliha[40]; Ramli, Said & Loon[41]; Grimaldi, Gonçalves & Ando[42]; Khatoon & Reddy[43]; & Piska, Zárubová, Louzecký, Karami & Filip[44], amongst others. One must point out, however, that despite its great use, the SFC curve should not be used alone to define the applicability of an interesterified fat. Fats showing the same solids profile can present well differentiated crystallization and texture properties.[45]

Triacylglycerol composition

The distribution of the fatty acids in natural triacylglycerols is not random. The taxonomic standard for fats and oils of vegetable origin consists of triacylglycerols that obey the *1,3-random-2-random* distribution, with saturated fatty acids located almost exclusively at positions *sn-1* and *sn-3* of the triacylglycerols and the unsaturated fatty acids preferentially at position *sn-2*. The interesterification reactions result in complete randomization of the fatty acids amongst all the triacylglycerols present, according to the laws of probability.[46] Thus the saturated fatty acids increase their participation at the central position of the triacylglycerols, whilst the unsaturated ones tend to decrease theirs until equilibrium is attained.[33]

Fig.2 presents a comparison of the triacylglycerol composition obtained by gas chromatography with respect to the number of carbons in a ternary blend between chicken fat (1/3), chicken fat stearin (1/3) and medium chain triacylglycerols (1/3), before and after chemical interesterification.[2] The reaction caused an expressive formation of triacylglycerol groups non-existent in the original sample.

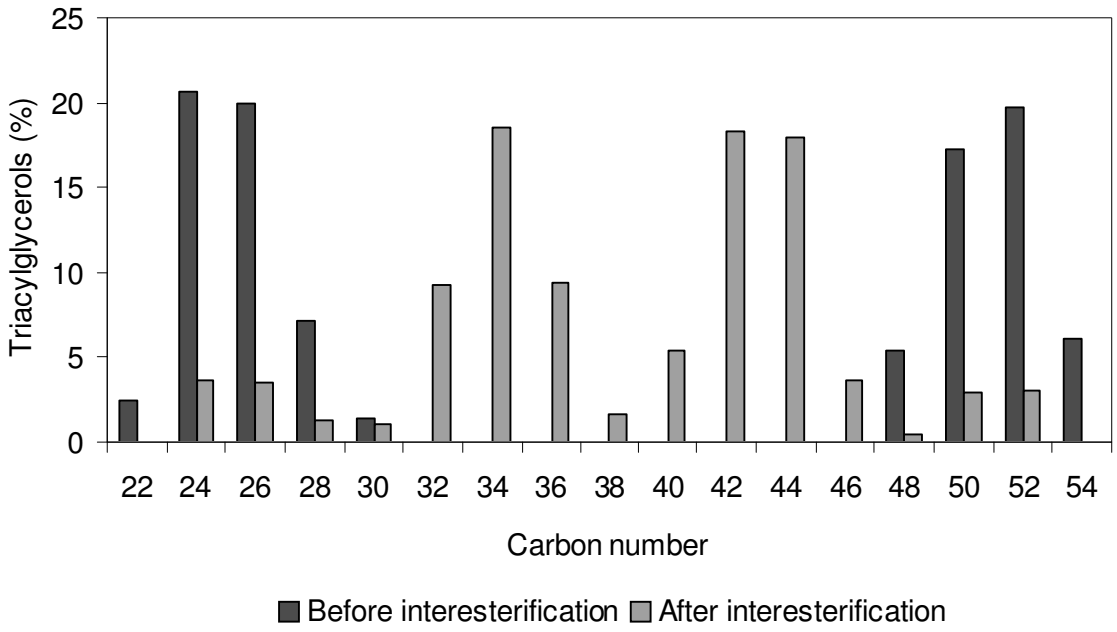

Fig.2. Triacylglycerol composition (%) by gas chromatography. Carbon number of ternary blend composed of chicken fat (1/3), chicken fat stearin (1/3) and medium-chain triacylglycerols (1/3), before and after chemical interesterification.[2]

Alterations in the physical properties provide an arbitrary measure of the structural modifications caused by interesterification, but do not provide real information about the molecular rearrangements on the triacylglycerols. Analyses of the triacylglycerolic composition represent a true indication of randomization and are extremely useful in monitoring the modifications of interesterified fats and defining specific applications for them.[7]

From the technological point of view, the molecular triacylglycerolic species profile represents the key to understanding the various physical properties of a fat or oil. In processed foods containing significant amounts of fat, the behavior of the product may depend on the triacylglycerolic composition of the fat. For example, cocoa butter, the fat predominating in chocolate, is responsible for the dispersion of all the other constituents of the chocolate and also for its physical behavior.[47] There are three main methods for

determining the triacylglycerolic composition of a fat or oil: mathematical and computer methods, gas chromatography and high performance liquid chromatography.[21,42]

Mathematical/computer methods

Fats and oils are considered to be complex samples due to the large number of triacylglycerols in their makeup. Thus the identification of the triacylglycerols is a difficult process, in which the number of possible structural forms is very large as compared to the number of fatty acids present.[48] The calculation method was widely used in the sixties and seventies, before the complete development of gas chromatography and high performance liquid chromatography methods. Although in recent decades there has been intense research on the analysis of triacylglycerols, no chromatographic method alone is capable of measuring the concentration of a specific triacylglycerol in a mixture. This incapacity can be explained by the fact that these methods can only measure properties derived from the distribution of the triacylglycerols.[49]

The theory known as *1,3-random-2-random* was developed independently by Vanderwal[50] and by Coleman.[51] Although a great variety of natural fats make up this group, the fats modified by interesterification do not obey this rule. For interesterified fats one should use the *1,2,3-random* totally at random distribution. This theory assumes that the positions *sn-1*, *sn-2* and *sn-3* are identical and equivalent to the total fatty acid composition.[33]

Antoniossi Filho, Mendes & Lanças[48] developed a program based on mathematical equations aiming at forecasting the molar percentages of the triacylglycerols present in vegetable oils as from the fatty acid composition of the samples, according to the *1,3-random-2-random* hypothesis. Chiu, Gioielli & Grimaldi[2] used the same tool to determine the triacylglycerol composition of samples prior to chemical interesterification. Gioielli & Baruffaldi[33], Rodrigues & Gioielli[39] and van Vliet & van Kempen[49] reported the use of mathematical methods and computer programs based on the *1,2,3-random* distribution to

aid in determining the triacylglycerolic composition of interesterified fats. In practice, the triacylglycerolic composition of a sample is much better defined when using a combination of statistical programs and chromatographic methods, since a greater amount of information can be obtained.[52]

Gas chromatography (GC)

The first attempts to use GC in the analysis of triacylglycerols were not successful, due to the instability of the mobile phases available and the high temperatures required to maintain the triacylglycerols in the gaseous phase (300 – 360ºC). This situation was transformed with the advent of the use of short packed columns and totally apolar mobile phases, which resulted in a reliable, reproducible and quantitative method.[19]

The standard method is the *AOCS Official Method Ce 5-86 - Triglycerides* by *Gas Chromatography* (identical to the *IUPAC 2.323* method), in which triacylglycerol groups with the same number of carbons are separated under programmed temperature conditions. A flame ionization detector is used and the carrier gas is helium or nitrogen. The measurements are based on the peaks obtained and the triacylglycerol composition, per carbon number, is expressed as a percentage relative to the total triacylglycerols in the sample.[11,27] With improvement of the method, an almost complete separation of the triacylglycerols in a sample became possible. There is no overlaping of the different triacylglycerols, and it is thus viable to separate and quantify small contents of saturated triacylglycerols. This is currently the preferred method for this analysis, with runs taking approximately 20 minutes.[21]

Fig.3 shows the chromatograms obtained by CG, of 60% soybean oil/ 40% fully hydrogenated soybean oil blend, before and after chemical interesterification. It was observed significant variation in all triacylglycerolic species as a result of the reaction, related to the randomization process. [52]

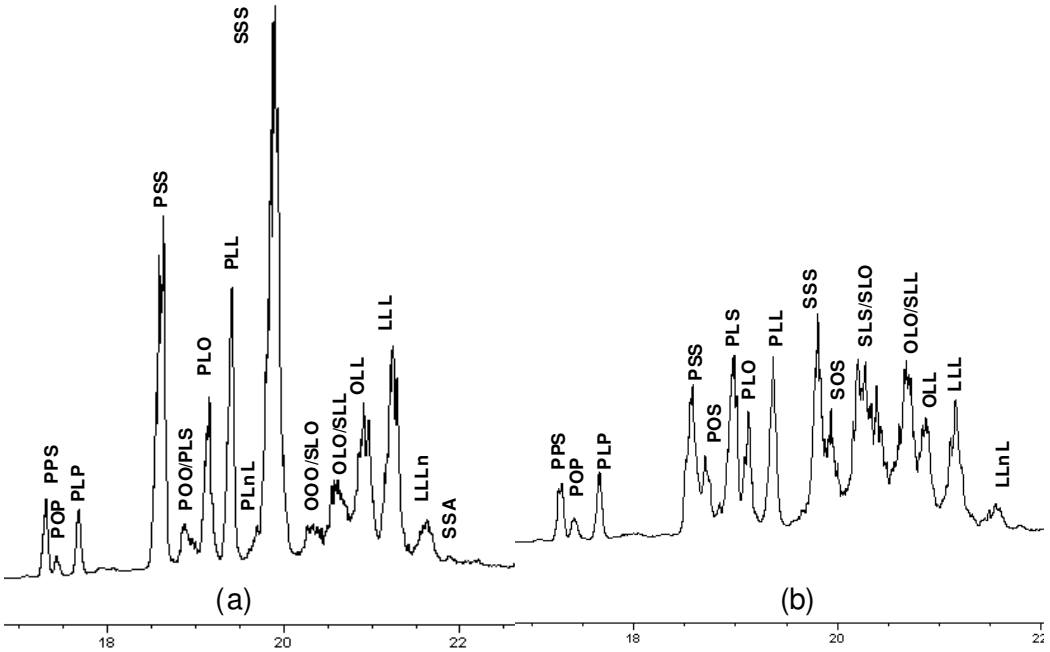

Fig. 3. Chromatograms of triacylglycerolic composition of 60% soybean oil/ 40% fully hydrogenated soybean oil blend, before (a) and after (b) chemical interesterification. P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid; A: arachidic acid. Gas chromatography; capillary column DB-17HT (Agilent Catalog 122-1811). Conditions: *split* injection*,* ratio of 1 to 100; column temperature: 250ºC, until 350ºC at 5ºC/minute; carrier gas: helium, flow rate: 1.0 mL/min; inlet temperature: 360ºC; detector temperature: 375ºC; volume injected: 1,0 µL; sample concentration: 100mg/5mL tetrahidrofurane.[52]

High performance liquid chromatography (HPLC)

The main official methods for this analysis can be found in the *AOCS Official Method Ce 5b-89* (corresponding to the *IUPAC 2.324* method) and the *AOCS Official Method Ce 5c-93* (corresponding to the *IUPAC 2.325* method).[11,27] The method *AOCS Ce 5b-89 – Triglycerides in Vegetable Oils by HPLC,* involves the separation and identification of triacylglycerols in liquid vegetable oils in terms of their molecular weights and degree of unsaturation, as a function of their "numbers of carbon equivalents – NCE", commonly referred to as the partition number. The method *AOCS Ce 5c-93 – Individual Triglycerides in Oils and Fats by HPLC,* complements the first one, aiming to separate, identify and

quantitatively determine the triacylglycerols in edible fats and oils by reference to external standards and from the fatty acid composition of the sample.[11]

In this analysis, the retention time of a determined component is related to the value of the partition number (NCE), defined as the real number of carbon atoms in the aliphatic residues (NC) less twice the number of double bonds (n) per molecule (the carbons in the glycerol chain are not counted), that is: NCE = NC – 2n. Two components with the same NCE are denominated as “critical pairs”. For example, triacylglycerols containing the following fatty acid combinations: 16:0-16:0-16:0, 16:0-16:0-18:1, 16:0-18:1-18:1, and 18:1-18:1-18:1 have the same NCE value and tend to elute together, causing a superimposition of triacylglycerol pairs, this being the main disadvantage of this technique. Even though the use of reversed phase columns and modern detectors can partially overcome this problem, the chromatograms obtained are complex and fail to show the familiar order of groups with the same number of carbons, with peaks for compounds with increasing unsaturation clearly separated. In addition, HPLC analyses are much more expensive than the use of GC and relatively slow, with runtimes of 60 to 90 minutes.[25]

The main component in the mobile phase is acetonitrile, which can be modified by the addition of solvents like chloroform, tetrahydrofurane, dichloromethane, isopropanol and acetone. The choice is usually related to the type of detector in use, of which the most common is the refractive index detector.[29]

Crystallization kinetics

Plastic fats consist of a crystalline network in a continuous oily matrix. The crystallization behavior of lipids has important implications in the industrial processing of products whose physical characteristics depend, to a large part, on fat crystals, such as chocolates, margarines and shortenings.[53]

The crystallization process is divided into two phases: nucleation and crystal growth. Nucleation involves the formation of aggregates of molecules that exceed a critical size

and are, therefore, stable. Once a crystalline nucleus has formed, it begins to grow by incorporating other molecules from the adjacent liquid layer, which is continuously filled in by supersaturated liquid from around the crystal. It is known that when the crystallization temperature increases, the rate of nucleation decreases, resulting in the formation of larger crystals.[54-56]

According to Foubert, Dewettinck, Janssen & Vanrolleghem[57], the factors influencing the crystallization process are the chemical composition of the fat and the crystallization conditions. Natural lipids are composed of a variety of triacylglycerol groups with specific requirements of activation energy for molecular diffusion and for the formation of stable crystalline nuclei. When these groups are modified by interesterification, the associated energy requirements are modified and changes occur in the speed of growth and dimensions of the crystals.[58] Thus interesterification causes important alterations in the properties of fats and oils crystallization, since it is responsible for the increase in the number of triacylglycerol species present and for changes in the inter-solubility between the triacylglycerol molecules.[44.59]

The crystallization kinetics profoundly influences the final structure of the fats and has been shown to be intrinsically related to their rheological and plastic properties. The study of the crystallization of interesterified fats has thus become of extreme importance such that their use can be adjusted to the limitations of the industrial processes and to improve the control of the processing steps involving recrystallization of the fatty fraction, guaranteeing the quality of the final product.[57,60] For example, in some specific processes, the fats should be completely crystallized by the end of the production line in order to guarantee that crystalline equilibrium is attained. If this does not occur, the standardized process times must be altered according to the characteristics of the fat used. This fact is becoming particularly important as the interesterified fats start to substitute partially hydrogenated fats in the majority of industrial applications.[61,62]

By monitoring the formation of solid crystalline material as a function of time, it is possible to determine the nature of the crystallization process. Currently, NMR is the most common and efficient analytical technique for investigating the crystallization kinetics of fats.[63-65] If the sample is maintained at a constant temperature in the NMR equipment and the SFC is determined at regular intervals, a continuous crystallization curve is obtained.[21] The graph of SFC versus time during isothermal crystallization is the simultaneous result of the nucleation and growth processes. The predominant events in the initial and final stages of the crystallization process are nucleation and growth, respectively. Nevertheless, it is difficult to measure the effects of each event separately, since they are generally superimposed on each other.[66] According to Herrera, Gatti & Hartel[67], the curves obtained can present two different forms depending on the crystallization temperature. When super-cooling is used, hyperbolic curves are obtained, whilst a low degree of super-cooling is related to sigmoid curves.

Since this technique has been relatively little explored, official methods to obtain crystallization curves by NMR are still not found. The advantages are the speed and ease of interpretation, since the values for SFC are immediately understandable in terms of the fat properties.[21] Campos[63] suggested that prior to measurement the samples should be maintained at 80ºC for 30 minutes to allow for complete melting. For fats with high melting points, 10 minutes at 120ºC would be sufficient. The crystallization temperature should be determined according to the characteristics of each sample, but 20ºC is generally defined for the majority of shortenings. The temperature of the sample compartment in the NMR equipment must be maintained at the chosen crystallization temperature. The time intervals for the measurements and the duration of the experiment depend on the type of sample and the crystallization temperature. For quick crystallizing samples or those held at low temperatures, the measurements can be taken every 30 seconds, whilst slow crystallizing samples or those held at temperatures above 25ºC can be measured at 5 minute intervals.

Characterization of the crystallization kinetics can be carried out according to the induction time (τ_{SFC}) or the nucleation period (with respect to the start of crystal formation) and the maximum solids content – SFC_{max}. The induction time is obtained graphically and reflects the time required for a stable nucleus of critical size to be formed in the liquid phase.[66,68] The τ_{SFC} generally increases with increase in isothermal crystallization temperature and with the decrease in sample melting point.[22,69] The value for SFC_{max} can decrease or increase with interesterification, depending on the raw materials used.[70]

The Avrami model, developed in 1940, is the model most used to describe the transformation kinetics of the isothermal phase, and relates the kinetics determined experimentally with the way of growth and final structure of the crystalline network.[58,65,71]

The Avrami equation gives an indication of the nature of the crystal growth process:

$$\frac{CGS(t)}{CGS(\infty)} = 1 - e^{-kt^n}$$

Where: *CGS(t)* describes the SFC (%) as a function of time, *CGS(∞)* is the limit in SFC as the time tends to infinity, *k* is the Avrami constant and *n* is the Avrami exponent.

In the study of fats this equation describes an event in which there is an initial lag period where crystallization occurs very slowly, and subsequently a rapid increase in crystalline mass. The Avrami theory considers that crystallization occurs both by nucleation and by crystal growth, and assumes that the transformation conditions are isothermal, that nucleation occurs spatially and arbitrarily, that the growth kinetics is linear, and that the growth velocity of the new phase depends only on the temperature and not on the time. The Avrami parameters provide information about the nature of the crystallization process. The constant *k* is the crystallization velocity constant. It depends mainly on the crystallization temperature and this dependence is generally expressed by the Arrhenius equation, and it takes both nucleation and the crystal growth rate into consideration. The Avrami exponent, *n*, sometimes called the crystallization index, indicates the mechanism of crystal growth. This parameter is a combined function of the time dependence of

nucleation and the number of dimensions in which growth occurred. Nucleation can be instantaneous, with the nuclei appearing all together at the start of the process, or sporadic, with the number of nuclei increasing linearly with time. Growth occurs taking the form of needles, disks or spherulites in one, two or three dimensions, respectively.[72]

Crystallization kinetics studies by way of isothermal crystallization in NMR are recent in the literature.[62,73] Campbell, Goff & Rousseau[74] studied the behavior of lard and a blend of palm stearin/canola oil at temperatures of 15; 17.5; 20; 22.5; 25 and 27.5°C. The kinetics of cocoa butter crystallization was studied by Marangoni & McGauley[75] and by Dewettinck, Foubert, Basiura & Goderis[76]. Braipson-Danthine & Deroanne[77] applied this method to the evaluation of the crystallization of binary blends of industrial shortenings at 20ºC. Martini, Herrera & Marangoni[22] verified the effect of the chemical interesterification of palm oil/canola oil and fully hydrogenated palm oil/canola oil blends at 30ºC for 100 minutes. Szydlouska-Czerniak, Karlovits, Lach & Szlyk[78] and Cerdeira, Martini, Candal & Herrera[79] studied crystallization at 10ºC for various lipid mixtures and for the saturated fraction of milk fat, respectively. Wassel & Young[60] classified interesterified fats for industrial use with respect to the time necessary to reach 25% solids at 20ºC. Silva, Escobedo & Gioielli[80] reported on the crystallization kinetics of lard/soybean oil blends before and after chemical interesterification. The curves of the initial blends presented a hyperbolic form without an initial induction period. After the reaction the samples showed a sigmoidal form, characteristic of the Avrami model, with an induction period during which crystallization occurred very slowly. Fig. 4 shows the crystallization kinetics at 25ºC of a 70% soybean oil/ 30% fully hydrogenated soybean oil, before and after chemical interesterification.[81] The values for τ_{SFC} and SFC_{max} were significantly altered by the reaction. The τ_{SFC} was altered from 4 to 8 minutes, whilst the SFC_{max} decreased by 69.4%.

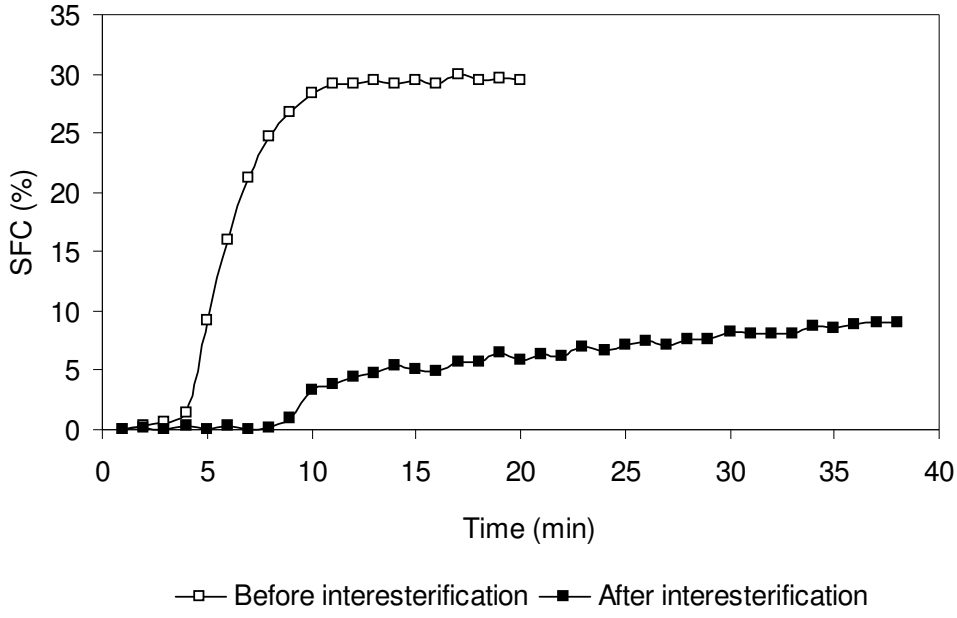

Fig.4. Crystallization kinetic of 70% soybean oil/ 30% fully hydrogenated soybean oil blend, at 25°C, before and after chemical interesterification.[81]

Polymorphism

Long chain compounds, such as fatty acids and their esters, can occur in differentiated crystalline forms. Solids with the same composition that can exist in more than one form are called polymorphs.[82] Polymorphism can be defined in terms of the ability to reveal different cell unit structures as a result of different molecular packing. The crystalline habit is defined as the form of a crystal. From a crystallographic perspective, the habit reflects the direction of growth within the crystal, whilst the morphology describes the group of faces determined by the symmetrical elements of the crystal. This distinction allows for crystals with the same morphology to present different crystalline habits.[83,84]

In a fat, the crystals are solids with atoms arranged in a periodical three dimensional pattern. A cell is the repeated unit that makes up the integral structure of a determined crystal. For its part, a sub-cell is the smallest periodic structure existing in the real unit of the cell, being defined as the transversal packing mode of the aliphatic chains on the

triacylglycerols. The polymorphic forms of a fat are identified based on the structures of the sub-cell.[54, 84,84]

Three specific types of sub-cell predominate in the lipids, referring to the α, β' and β polymorphs according to current polymorphic nomenclature. The α form is metastable with hexagonal chain packing. The β' form shows intermediary stability and orthorhombic perpendicular packing, whilst the β form shows the greatest stability and triclinic parallel packing. The melting temperature increases with increasing stability ($\alpha \rightarrow \beta' \rightarrow \beta$), as a result of the differences in density of the molecular packing.[7,85,86]

Triacylglycerols generally first crystallize in the α and β' forms, although the β form is more stable. This phenomenon is related to the fact that the β form has more free activation energy for nucleation. Polymorphic transition is an irreversible process from the less stable to the more stable form (monotrophic phase transformation), depending on the temperature and time involved. At constant temperature, the α and β' forms transform themselves into the β form as a function of time, by way of liquid-solid or solid-solid mechanisms.[85,87] The transformation velocity depends on the degree of homogeneity of the triacylglycerols. Fats with low triacylglycerol variability quickly transform themselves into the stable β form. Fats consisting of a random triacylglycerol distribution may present the β' form indefinitely. In addition, factors such as formulation, cooling velocity, heat of crystallization and level of agitation, affect the number and type of crystals formed.[53] Nevertheless, since fats are complex mixtures of triacylglycerols, it is possible that, at a determined temperature the different polymorphic forms and liquid oil can coexist simultaneously.[88]

The crystalline structure of fats is important in the formulation of shortening, margarines and fatty products in general, since each crystalline form presents unique properties with respect to plasticity, texture, solubility and aeration.[7] Fats with crystals in the β' form present greater functionality, since they are softer and allow for good aeration and

creaming properties. To the contrary, the β polymorphic form tends to produce large granular crystals, leading to sandy products with a low aeration potential, compromising the macroscopic properties of the foods.[53] Thus the β' form is the polymorph of interest for the production of fat rich foods such as margarines and confectionary and bakery products.[89]

The type of polymorph characteristic of a fat or oily is dependent on the fatty acid distribution in the triacylglycerolic molecules, the degree of randomization being particularly important. Chemical interesterification alters the triacylglycerolic composition, leading to modifications in the crystalline morphology of the fats and promoting changes in the types and/or contents of polymorphs in a natural fat.[7,63,90] In this way the chemical interesterification process represents an important means of stabilizing the β' form, since it can promote, according to the compositional characteristics of the raw materials, the formation of triacylglycerols with a greater variation in chain length. This results in a more disordered packing of the terminal methyl groups, associated with the formation of less dense crystalline structures. In addition, randomization is associated with a decrease in the content of trisaturated triacylglycerols such as SSS (triestearin) and PPP (tripalmitin), characteristics of the stabilization of the β polymorph.[46] In general, the more diverse the structure of the triacylglycerol and higher the melting point of the fat, the lower the tendency to form the β polymorph.[89] In recent years studies on the modification of the crystallization process have been carried out by chemical interesterification, resulting in various patents for the processing of fat bases for application in margarines and shortenings.[91]

X-ray diffraction is used to identify polymorphism in crystals, by determining the dimensions of the crystalline unit and sub-cells. The principle of this method consists of exciting an anti-cathode made of a mineral such as copper, for example, for the emission of X-rays that will be diffracted by the crystalline structure at a determined angle formed by

the planes of the atoms in the structure. The angle depends on the crystalline structure of the sample and the set of peaks that appear in an X-ray diffractogram function as a fingerprint of the material analyzed, that is, each crystalline substance exhibits a unique arrangement of peaks in the diffractogram.[21,92] Since fats are polycrystalline materials powder diffractometry is used, in which the technique is equivalent to the rotation of a single crystal through all the possible orientations relative to the shaft of incident X-rays. Its main application is currently in qualitative analysis.[90]

The principle of the measurement is based on Bragg's Law: $n\lambda = 2d \operatorname{sen}\theta$, where n corresponds to a whole number referring to the reflection order; λ is the wavelength of the X-rays (normally equal to 0.154 nm for copper); d is the space between the layers of atoms, to be determined; and θ is the angle of the shaft of X-rays incident on the sample. In modern crystallography, all reflections are considered to be of first order (n=1), Bragg's Law being rewritten as: $\lambda = 2d \operatorname{sen}\theta$.[21,90,93]

Due to the different geometric configurations, the polymorphs diffract the X-rays at different angles. In fats, diffractions at high angles correspond to the short spacings (SS) of the sub-cells and allow for determination of the polymorphs α, β' and β. The SS can be defined as the distances between the parallel acyl groups on the triacylglycerol, and refer to the transversal packing of the triacylglycerol chains. On the other hand, the diffractions at low angles correspond to the long spacings (LS) and provide information about the type of longitudinal packing of the triacylglycerols, which is a function of the size of the molecules in the triacylglycerols and of the angle of inclination between the axis of the triacylglycerol chain and its basal plane.[94,95] In relation to the position of the X-ray detector with respect to their direction of incidence, the LS and SS are observed, respectively, between 1º and 15º and between 16º and 25º in the 2θ system. The SS are the parameters really used to characterize the α, β' and β polymorphic forms, whereas the LS

are useful in studies of polytypism.[96] Thus in the present study, only the interpretation of the SS will be highlighted.

The polymorphs provide easily recognizable diffraction line patterns. The information reported by different authors are the interplanar distances in angstroms or nanometers, or calculated as from the angles on which these diffraction lines appear, which determine the polymorphic form.[97] The main methods can be found in the norm *AOCS Cj 2-95 – Diffraction Analysis of Fats*, applicable to all triacylglycerols that retain their format at the temperature of analysis (above 25ºC). The method includes no description of any pre-treatment of the samples. The sample is deposited on a glass or aluminum slide, which is then rotated at constant speed. The total time for the analysis is about 30 minutes under a controlled temperature.[11,21] Analyses at temperatures between 4 and 80ºC can be found in the pertinent literature, but the interval between 20 and 30ºC predominates.[34,40,74,77,82,83,92,98-107] Triestearin can be used as the reference in the analysis, since it is representative of the β form.[108]

The SS are defined as all the diffraction peaks or lines below 5Å. However in the interpretation of the results, the *d* values referring to the five SS of greatest intensity between 3.70 and 4.65Å, are calculated. Identification of the polymorphic form is only carried out as from the following peak characteristics.[11,46,64,109]

- α form: presents a single peak (or SS) at 4.15Å;
- β' form: has two main peaks at 3.8 and 4.2Å;
- β form: refers to a high intensity peak at 4.6Å, which may be accompanied by various other peaks of lower intensity.

The diffraction patterns of natural or interesterified fats present wider peaks than pure compounds, due to the presence of multiple triacylglycerols in the cell units and the concomitant presence of liquid oil.[110]

X-ray diffraction has frequently been used as a technique to evaluate chemical interesterification, aiding in the planning of the application of the fat bases produced.[91] An

important extension of the standard equipment used is the diffractometer with temperature programming, and, more recently, a development with great potential is the diffractometer with synchrotron radiation – SRXRD.[21]

Rousseau & Marangoni[46] showed the effect of chemical interesterification in milk fat/canola oil blends. The blends, both before and after the reaction, presented combinations of the β' and β polymorphs, but the proportion of β' crystals increased significantly with randomization. To the contrary, in lard/canola oil blends, interesterification resulted in an increase in the β polymorph. Woodrow & deMan[111] reported interesterification as a means of eliminating the β polymorph in milk fat. Norizzah, Chong, Cheow & Zaliha[40] only observed the polymorph β' in interesterified blends of palm stearin/palm kernel olein, due to the tremendous diversification of triacylglycerol types caused by randomization. List, Emken, Kwolek & Dutton[13] and List, Mounts, Orthoefer & Neff[34] evaluated the interesterification of various oils with *hardstocks*. The reaction favored the formation of the β' form, allowing for application of the fats produced in margarines. Lida & Ali[31] showed the same effect in ternary blends of palm oil/ sunflower oil/ palm kernel oil and palm olein/ sunflower oil/ palm kernel olein. On the other hand Ramli, Said & Loon[112] showed that interesterification did not modify the β' polymorph characteristic in blends of palm kernel oil and goat's milk fat.

Thermal behavior

Differential Scanning Calorimetry (DSC) is the thermoanalytical technique most used in the study of fats and oils. The various thermal phenomena related to these raw materials are verified by monitoring the enthalpy and phase transitions of the various triacylglycerol mixtures.[101] The DSC evaluation provides direct measurements of the energy involved in the melting and crystallization processes of fats and oils. The crystallization of oils results

in a contraction in volume associated with an exothermic effect. To the contrary the melting of fats leads to an expansion in volume, characterized by an endothermic effect.[113]

The polymorphic characteristics of the triacylglycerols make the study of the thermal and structural properties complex.[114] When a fat is heated it may exhibit multiple melting phases, and each recrystallization step represents the transition of a less stable polymorphic form to a more stable one. The peak transition temperature may be an indicator of the polymorphic form of a crystal, since the most stable crystalline form has a higher melting point.[115]

In the DSC technique, the sample (approximately 10mg) and a reference material (air) are submitted to a controlled temperature program. If a transition occurs in the sample, thermal energy is added or removed from the sample or reference compartment so as to maintain the same temperature. This energy balance is equivalent to the transition energy.[116] The register of the DSC curve, or thermogram, is expressed in terms of the heat flow versus the temperature (ºC) or time (min). The thermal phenomena suffered by the sample present themselves in the form of deviations of the baseline in the exo- or endothermic direction. Generally, the endothermic process are presented as positive (above the baseline), corresponding to an increase in heat transfer to the sample with respect to the reference.[117] In general, fats and oils can exhibit an extremely complex thermal behavior, which is highly dependent on the chemical composition and on the DSC experimental protocol. In an investigation at temperatures between -100ºC and 80ºC, various different thermal behaviors can be observed, as a reflection of the triacylglycerolic profile of the sample.[113]

DSC is considered to be a sensitive technique for the characterization of interesterified products. When the triacylglycerolic composition of a fat or oil is subject to changes by interesterification, concurrent changes in the thermal profiles can be seen. The melting and crystallization thermograms represent a tool of great use to verify the alterations caused by randomization.[118] According to O'Brien[7], DSC is the best way to determine the

final point of interesterification of lard and of lauric fats, since the crystallization curves are significantly representative of the final triacylglycerol composition desired.

The DSC analytical method is difficult to standardize, but the *AOCS Cj 1-94 - DSC Melting Properties of Fats and Oils* method, recommends the following temperature program to prepare the crystallization and melting curves in a standard evaluation of a fat or oil: 10 min (80ºC), 80ºC to -40ºC (10ºC/min); 30min at -40ºC; -40ºC to 80ºC (5ºC/min).[11,21] However the majority of studies report modified analytical conditions with respect to the temperature program.[6,31,37,40,43,112]

Various parameters can be calculated to describe the thermal behavior of a sample and evaluate the modifications that occurred as a function of interesterification.[63,119,129]

- Peak (maximum) crystallization (T_c) and melting (T_m) temperatures: correspond to the maximum evolution of the peak, that is, where the thermal effect is maximal. They are strongly influenced by the rate of cooling/heating used in the analysis and refer to the temperature at which the majority of the lipid species crystallize or melt, depending on the phase transition in question.

- *Onset* crystallization (T_{oc}) and melting (T_{om}) temperatures: temperature corresponding to the point at which the heat flow starts to deviate from the baseline, that is, the start of the transition phase. When more than one peak occurs the calculated *onset* temperature corresponds to the largest peak. T_{oc} is the temperature at which the first crystals are formed, whereas T_{om} is the temperature at which the sample starts to melt.

- Final temperature of the thermal phenomenon (T_{final}) for crystallization or melting: this is the temperature at which the curve returns to the baseline after the phenomenon has been concluded.

- Peak area, corresponding to the enthalpies of crystallization (ΔH_c) and melting (ΔH_m): this refers to the phase transition enthalpy, calculated as the area under the curve. The area under the curve is proportional to the amount of crystalline material formed during cooling or the amount of solid phase converted to liquid phase during melting. The value

for ΔH_m is strongly related to the intermolecular arrangement of the triacylglycerol species and is generally modified by randomization.

The rates of heating and cooling (scanning) show a primordial effect on the shape of the curves obtained by DSC. A correlation between the scan and the response is critical in the qualitative analysis of fats and oils.[21,113] During crystallization under high rates of cooling, the triacylglycerol molecules usually crystallize in metastable polymorphic forms, which are subsequently transformed into more stable polymorphs. On the other hand, at low rates of cooling, triacylglycerols with similar chain lengths associate together in more stable geometrical arrangements, resulting in the formation of a more stable polymorphic form. Similarly, the results of the melting thermograms are totally associated with the heating rate used. At low rates (1ºC/min or less), the heat transference allows for the occurrence of molecular rearrangements. Some thermal events, such as the melting of existing polymorphic forms, polymorphic transformations and further melting of the polymorphs formed, can be observed when a sample is slowly melted, but are not visualized when high heating rates (1ºC/min or above) are used. This results in melting of the sample before the structural alterations can establish themselves. Thus it is recommended that the majority of fats and shortenings be melted at 5ºC/min.[63] In practice it is observed that an increase in heating rate is associated with greater T_m values, whilst a decrease in the cooling rate results in increases in T_c and $T_{oc.}$[113,121]

Rossel[122] studied the effects of randomization on palm kernel oil. The formats of the melting curves were not modified by the process, occurring only a decrease in the peak temperatures as evidence of the greater variety of triacylglycerol species present. Marangoni & Rousseau[35] evaluated the interesterification of palm oil/soybean oil and of lard/canola oil blends from the parameters of T_{oc}, T_c and T_{final} for crystallization and the ΔH_c. Kok, Fehr, Hammond & White[12] observed that the interesterification of highly saturated soybean oil led to an increase in the values of T_{oc} and T_{om}. Rodríguez, Castro, Salinas, López & Miranda[37] used DSC as a tool to optimize the interesterification of lard/

sunflower oil. Khatoon & Reddy[43] reported studies on the interesterification of palm stearin/ *Mangifera indica* oil and of palm stearin/ *Madhuca latifolia* oil blends, evaluating the peak melting temperatures (T_m) and melting enthalpy (ΔH_m). The initial blends were characterized by three distinct melting endotherms indicating the heterogeneous nature of the triacylglycerols present. After randomization, the proportion of the peaks relating to high melting point triacylglycerols was reduced, whilst the peaks referring to unsaturated triacylglycerols increased significantly. Norizzah, Chong, Cheow & Zaliha[40] showed the same behavior in the interesterification of palm stearin and palm kernel olein. Chiu, Gioielli & Grimaldi[123] evaluated the properties of blends of abdominal chicken fat, chicken fat stearin and medium chain triacylglycerols by DSC, before and after chemical interesterification. In a study carried out by Ramli, Said & Loon[112], interesterified blends of fully hydrogenated palm kernel oil and goat's milk fat showed smaller and greater values for T_m and ΔH_m, respectively, as compared to the initial blends.

Grimaldi, Gonçalves, Gioielli & Simões[124] evaluated different compositions of palm (PO) and palm kernel (PKO) oils (100/0, 80/20, 60/40, 50/50, 40/60, 20/80 and 0/100), before and after chemical interesterification. The samples were characterized by melting and crystallization curves determined by DSC. Table 2 shows the values for the melting enthalpy of the natural and interesterified PO/PKO blends. The highest values for melting enthalpy of the more saturated peaks of palm oil were equal to 38.7 $J.g^{-1}$ and 48.4 $J.g^{-1}$, before and after interesterification, respectively, showing evidence of the formation of a more stable saturated group.

Table 2. Melting enthalpy - ΔH_m (J.g^{-1}) of palm oil (PO) and palm kernel oil (PKO) blends, before and after chemical interesterification.[124]

Blend PO/PKO (w/w)	Peak 1	Peak 2	Peak 3
100/0 b[1]	42.2	-	38.7
100/0 a[2]	16.2	10.5	48.4
80/20 b	22.0	56.6	-
80/20 a	7.3	80.8	-
60/40 b	4.2	80.9	-
60/40 a	1.1	90.7	-
50/50 b	2.0	84.8	-
50/50 a	2.7	79.5	-
40/60 b	3.1	98.0	-
40/60 a	2.6	68.6	-
20/80 b	1.8	98.5	-
20/80 a	1.9	82.4	-
0/100 b	-	112.5	-
0/100 a	-	96.4	-

b[1] - before interesterification; a[2]- after interesterification

Fig. 5 shows the melting curves obtained by DSC for the 100/0 PO/PKO fraction, before and after chemical interesterification. The interesterification excluded the area below the baseline in the melting curve, a fact related to the increase in crystallization velocity. Thus the process avoided the occurrence of simultaneous melting and crystallization, characterized by the portion below the baseline for the non-interesterified fraction. The DSC technique allowed for the verification that, in general, the interesterification reaction led to an increase in the velocity of crystallization of the blends and promoted better compatibility between the palm and palm kernel oils.

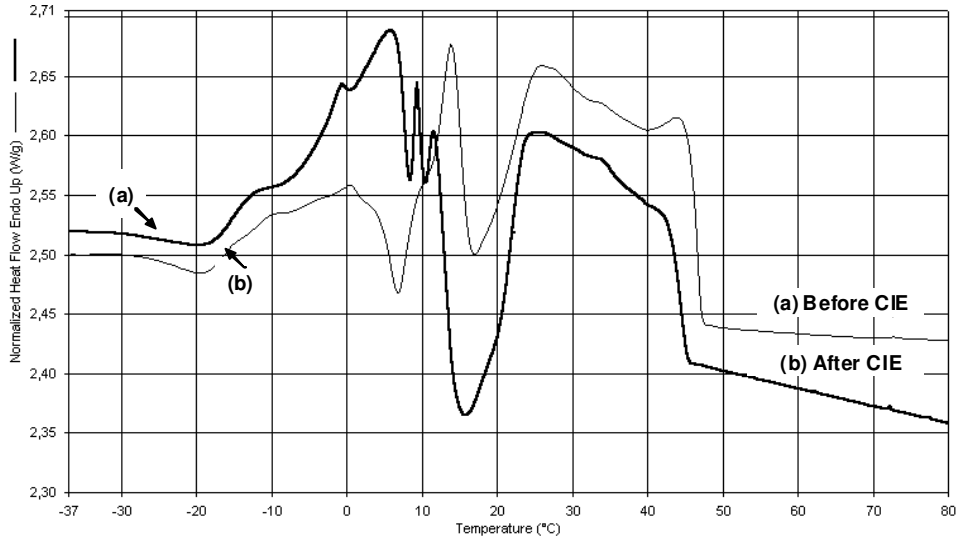

Fig.5. Melting curves of palm oil (PO), before (a) and after (b) chemical interesterification (CIE).[124]

DSC can also be used as an alternative method to determine the solid:liquid ratio in fats. The melting thermograms provide valuable information about how the products melt in the mouth during chewing. The values for the partial area identified under the melting peak (endothermic) are equivalent to the percentage of solids remaining at the temperature selected.[6] Although experiments with DSC are generally carried out at constant rates of heating or cooling, measurements under isothermal conditions can be carried out for studies of fat crystallization kinetics, according to Metin & Hartel[125] and Kawamura[126].

Microstructure

The main structural component of plastic fats is the crystalline network. The properties of crystals, such as size, morphology and polymorphic form influence this structure, which, in turn, affects the behavior of the fat in terms of texture, appearance and functionality.[110] A general relationship exists between the structure of a fat and its physical and sensory

properties. Attributes such as spreadability and the melting sensation in the mouth, depend on the mechanical strength of this crystalline network.[127]

The crystallization process is the spontaneous ordination of a system, characterized by total or partial restriction of movement as a result of chemical or physical bonds between the triacylglycerol molecules. Differences in crystalline form result from different molecular packings[26] Thus a crystal consists of molecules arranged in a fixed pattern known as a lattice. The high degree of molecular complexity allows for a determined group of triacylglycerols packing themselves into various different, relatively stable, structures. The lipid composition and the crystallization conditions influence the crystalline habit and different polymorphic and morphological forms of crystal are possible. The crystals aggregate together into larger structures forming a network, which characterizes the microstructural level of the fat.[128] The concept of microstructure conveys information about the state, quantity, form size, spatial relationship and interaction between all the components of the crystalline network and presents an enormous influence on the macroscopic properties of fats.[45]

According to Narine & Marangoni[129], the microstructural or meso-scale level of the crystalline network of a fat can be defined as the group of structures with dimensions between 0.5µm and 200 µm. It is mostly quantified by way of visualizing its geometry. The levels of a structure in a typical network are defined when the fat crystallizes from its completely melted state. Since they are nanostructural elements (0.4 – 250 µm), the triacylglycerols crystallize in particular polymorphic states. The majority of the triacylglycerols crystallizes as spherulites, implying that crystal growth occurs radially. The crystals formed grow to dimensions of 1 to 4 µm and then aggregate forming agglomerates (over 100 µm) by way of a process limited by heat and mass transfer. The process of aggregation continues until a continuous three dimensional network is formed as from the joining of these microstructures, imprisoning the liquid phase of the fat.[130,131] This structural hierarchy has been recognized by various researchers.[132,133] Nevertheless, the

arrangement of the molecules in the crystalline state also depends on factors such as the rate of cooling, crystallization temperature and velocity of agitation, if used. Static crystallization conditions induce the formation of spherical particles.[68]

Berger, Jewell & Pollitti[134] described the types of crystals that can be found in fats: spherulite A: crystal with a compact nucleus surrounded by long, fine, radially distributed needles; spherulite B: small nuclei surrounded by randomly oriented crystals; clusters: groups of small approximately spherical crystals arranged at random; bundles: crystals distributed in a parallel way, oriented at random, forming a structure similar to a net; agglomerates: aggregates of spherulite crystals and clusters.

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 the halo/nucleus ratio of the crystal is modified, affecting the properties of texture and functionality of the interesterified fats.[40,110,135,136] The alterations in triacylglycerol composition caused by randomization modify the relative strengths of the bonds between the crystalline elements in an aggregate (intra-particle) and between aggregates (inter-particle), giving rise to the formation of differentiated structures.[45] In addition, the change in SFC inherent to the interesterification process influences the structuring of the crystalline network. When the solid fraction increases, individual crystals start to touch each other and hence crystal growth is limited. In the same way, when the SFC decreases, this leads to changes relative to the provision of a greater area and greater molecular mobility for crystal formation.[66]

The Polarized Light Microscopy (PLM) is the technique most used to visualize the microstructural network of fats and has been applied with the objective of explaining differences in texture between fat blends, showing the different types of crystal and morphological changes in crystal growth and verifying transformations in the polymorphic forms, principally with respect to interesterified fats. The microscopic image producing techniques are frequently the most appropriate means of evaluating food structure, since

they provide results in the form of images and numerical data. Reliable measurements include obtaining representative, true images of the material, a detailed analysis of the image and a numerical interpretation of the data obtained.[49,52] In recent years, the development of new software has resulted in important advances in the observation of the fat microstructure, with the recognition of structural elements and the definition of a specific nomenclature.[127,135]

PLM can distinguish between the liquid and solid phases in a fat, which refract light in different ways. The liquid phase of the crystalline network is isotropic and due to its unique refractive index, presents the same optical properties in all directions. Thus it appears to be dark under polarized light. The anisotropic solid phase presents a defined molecular orientation and optical properties that vary with the orientation of the incident light, appearing slightly brilliant under the polarizers. This anisotropic behavior is known as birefringence.[63,90,135] PLM also allows for the observation of dynamic alterations that occur during the events of nucleation and crystal growth, and can be used as a technique to obtain indicators of induction times and nucleation speeds, before and after interesterification.[63,68]

Despite the great use of PLM to visualize the microstructure of fats, there is no official methodology for this determination. In brief, the samples are first melted at a temperature that guarantees complete destruction of their crystalline history. For most samples, maintenance at 80ºC for 30 minutes is sufficient, but for palm oil, which has a higher melting point, heating at 120ºC for 10 minutes is recommended. With the aid of a pre-heated capillary, a small drop is then deposited on a pre-heated glass slide. The glass coverslip, also pre-heated, is then placed in parallel on the surface of the drop, avoiding the introduction of air bubbles into the sample and producing a uniformly thick film. Care should be taken that the glass slide, capillary and coverslip are all at the same temperature as the sample, to avoid sudden crystallization. The samples are then crystallized at the desired time and temperature, and visualization under the microscope is

generally carried out at magnifications of X 40, 100, 400 or 1000.[63,135] When the crystallization temperature is far from the melting point of the sample, a greater number of smaller crystals are formed. Near the melting point, small crystals with an indistinct shape are formed, or they may not form at all. Thus an intermediate crystallization temperature helps in the formation of a smaller number of larger crystals with distinct forms.[137]

Under certain circumstances, PLM can even differentiate the polymorphic forms of fats, based on the form and size of the crystals. The β' polymorph shows small crystals (1 – 5 μm) with the type A spherulite format, whereas the β polymorph shows relatively large crystals (20 to 100 μm) with an axial crossed format.[44,135]

Obtaining these images provides qualitative and quantitative information. The qualitative analysis can be carried out from a visual assessment of the differences between the micrographs, resulting in their classification according to their crystal morphology and size. For the quantitative analysis, descriptive numerical parameters are used, amongst which the most important are: total number of crystals, mean values for the crystal diameter and area crystallized (corresponding to the ratio, in percentage, between the sum of the areas of each crystal and the total area of the image). These results can be compared for a single sample before and after interesterification, as an additional tool to evaluate reaction performance.[63,138]

In a pioneering study, Becker (1959), cited by Rousseau & Marangoni[5], evaluated the influence of interesterification on the morphology of the crystals of binary and ternary blends of trilaurin, triolein and tristearin. Smaller crystals with different formats were found after the reaction. Hurtová, Schmidt, Zemanovic, Simon & Sekretar[139] accompanied the interesterification of sunflower oil and canola oil blends with fully hydrogenated oils. After the reaction the crystals showed the presence of small crystalline aggregates that are characteristic of the β' form. The crystallization of the original samples was very quick, resulting in aggregates with large crystals, characteristic of the β form. Rousseau, Hill & Marangoni[140] visualized blends of milk fat/canola oil before and after chemical

interesterification. Before the reaction the photomicrographs showed a dense network of spherulites with dimensions between 10 and 25 µm. After the reaction, the crystalline network was composed of small crystals measuring about 15 µm. Rousseau, Marangoni and Jeffrey[110] showed that interesterification did not alter the crystalline morphology of palm oil/soybean oil blends, but extensively modified the morphology of lard, inducing the formation of more symmetrical spherulites. Expressive modifications in the crystalline network, size and morphology of crystals as a result of randomization were also reported by Rodríguez, Castro, Salinas, López & Miranda[37] and Norizzah, Chong, Cheow & Zaliha[40] for lard/sunflower oil and palm stearin/palm kernel oil blends, respectively.

Figs. 6 and 7 show the micrographs obtained by PLM at 25ºC in a study by Grimaldi, Gonçalves, Gioielli & Simões[124] with palm oil (PO) and a 80% palm oil (PO)/20% palm kernel oil (PKO) blend, before and after chemical interesterification. Both the palm oil and the blend showed an expressive decrease in crystal diameter as a function of the chemical reaction, but the number of crystals increased significantly with the randomization process. Table 3 shows the numerical parameters relative to the images. The modification of the mean diameter of the crystals and the number of crystals after interesterification was associated with a concomitant increase in trisaturated triacylglycerols, the melting point, the consistency and the SFC of the two samples analyzed.

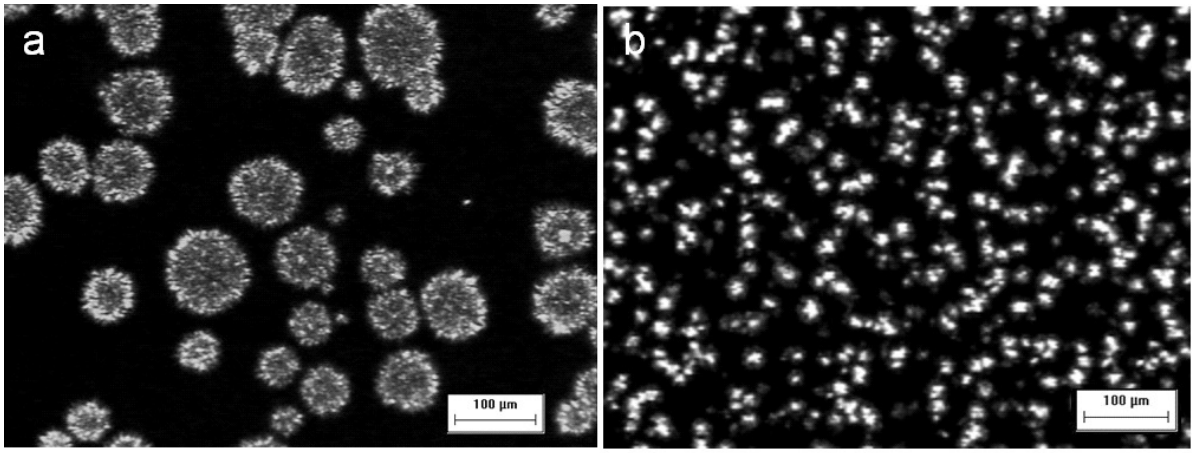

Fig.6. Photomicrography of palm oil, before (a) and after (b) chemical interesterification. Magnification: 40x. Preparation of samples: 25°C/ 20 hours.[124]

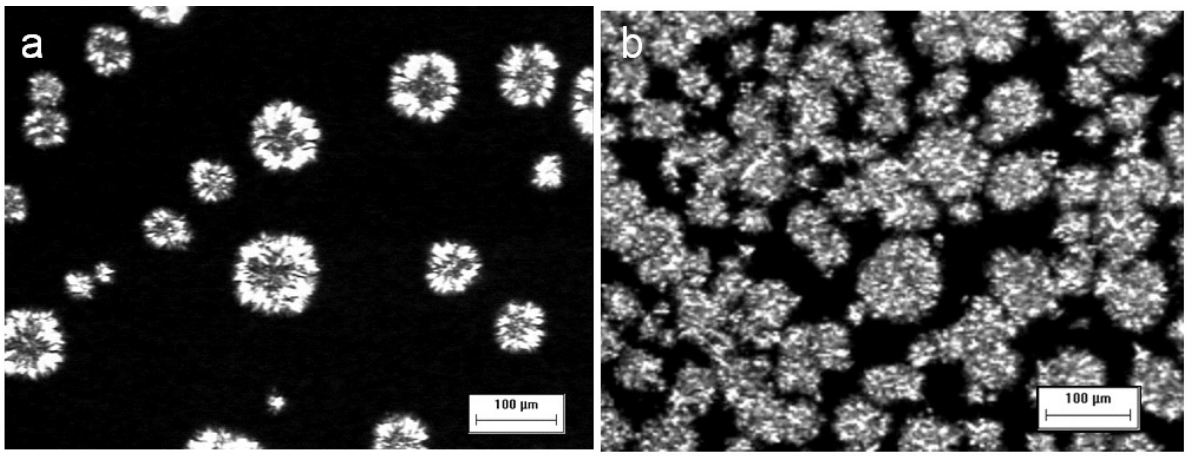

Fig.7. Photomicrography of 80% palm oil (PO)/ 20% palm kernel oil (PKO) blend, before (a) and after (b) chemical interesterification. Magnification: 40x. Preparation of samples: 25°C/ 20 hours.[124]

Table 3. Crystal parameters (25°C) by PLM of palm oil (PO) and 80% palm oil (PO)/ 20% palm kernel oil (PKO) blend, before and after chemical interesterification.[124]

	Parameter	
Sample	**Mean crystal diameter (μm)**	**Mean crystal area (μm^2)**
PO before	58.0	2700.0
PO after	14.5	119.8
80%PO/ 20%PKO before	59.6	2900.0
80%PO /20%PKO after	29.1	684.7

Consistency

Food texture is recognized as a sensory quality that manifests itself in different ways. Some of the sensory attributes identified as describing the texture of solid foods are: consistency, hardness, elasticity, brittleness and chewiness.[141] The consistency is an important functional aspect for plastic fats, which are mixtures of solid fat crystals and liquid oil. The ratio between the two phases and the crystalline character of the solid phase determine sample consistency and firmness.[142] Although consistency is related to the mechanical properties of fats, no universally accepted definition of this property exists. In general the terms consistency and hardness present similar meanings: "the resistance of the surface of a body to penetration", and are used in the same context.[143] According to O'Brien[7], consistency is a combination of effects that provide an impression of resistance, and is directly related to the proportion of material in the solid phase of a fat. The change in consistency as a function of temperature is denominated as plasticity.[10] Texture, measured as consistency or plasticity, is one of the most important characteristics of fatty products and is primarily determined from the physical properties of fats and oils.[31]

Fatty foods consist of a crystalline network of very fine particles and macromolecules, sustained by a great variety of intermolecular and colloidal forces. Its texture, stability and functionality are strongly influenced by the strength of these interactions. In addition, the

texture of a fat containing food depends on the history of structural changes occurring throughout processing. The properties of spreadability, creaminess, softness and hardness of fats are related to the rheological characteristics of their components, and also to the effects of heating on the physicochemical properties of the lipid matrixes.[144] According to Marangoni & Rousseau[36], the interactions between fat crystals and/or crystal aggregates in a network can vary from very weak van der Waals forces to solid hydrogen bridges. In this way, the lipid composition affects the crystalline arrangement and the strength of the interactions between the crystals.

Thus differences in the texture properties of fats can be attributed to the conformation of the crystalline structure (size, size distribution, crystal form and polymorphism) and to interactions between molecules in the crystalline state, and to the liquid oil in which they are dispersed, characterizing different structural levels related to the consistency of individual triacylglycerols, crystalline units and agglomerates.[45] Thus it is assumed that the consistency of a semi-solid lipid is a function of its SFC and of associated micro-structural factors.[77,145]

The rearrangement of the triacylglycerols caused by randomization causes changes in the physical characteristics of the fat bases, especially those related to the formation of the crystalline network, altering the mechanical properties of the initial raw materials. The modifications in the SFC and in the crystalline network caused by interesterification, influence sensory attributes such as mouth feel and spreadability, delineating the application of interesterified fats in margarines, chocolates, biscuits, ice creams, bakery products and confectionary products in general.[44] The interesterification of lard is a classical example of this modification. Natural lard, soft but gritty, contains large crystals and a crystalline network that is not very cohesive. On the other hand, interesterified lard contains larger numbers of smaller crystals, showing greater hardness and a homogenous texture.[7,36]

Rheological tests are used to study the mechanical properties of fats, which measure how the crystallized material responds to applied forces. Tests applying considerable deformation are generally employed under constant speed, up to the point at which the applied force exceeds the structural capacity of the sample, causing permanent deformation. The most used method involves the use of cones with uniaxial compression.[36,63]

The methodology most used to measure fat consistency involves the use of a cone penetrometer or a texture analyzer.[7] The most used method is the *AOCS Cc 16-60 - Consistency, Penetrometer Method* [11], but variations are usually applied according to the geometry of the cone or probe used. This method is applicable to plastic fats, emulsions containing solid fat like margarines, butter, shortening and similar products. It consists of an arbitrary measurement of the firmness of the plastic fats, obtained from the distance penetrated by the truncated 20º cone in a certain period of time, at a constant velocity and defined weight.[11,142] This evaluation measures the depth the cone penetrates as from the fat surface, generally for 5 seconds, as from a pre-determined height. The method *AOCS Cc 16-60* instructs the user to read the depth of penetration in mm/10 units, but fails to provide instructions about the different weights that could be used.[11]

Uniform procedures should be used in order to obtain reproducible results. To avoid heating or cooling of the samples, they should be carefully tempered at the desired temperature in the recipient specific for the measurement, usually for 24 hours. At each temperature 5 repetitions of the reading are recommended for each sample, guaranteeing that the variation in temperature between the repetitions is not more than 1ºC. The functionality of a fat can be determined as from the consistency measurements carried out in a wide range of temperatures, generally between 5 and 40ºC, at 5ºC intervals.[7] Different test conditions can be found in the literature, using cones angled between 10 and 45º. Campos[63] suggested the following conditions for the parameters used in rheological tests

for the uniaxial deformation of fats: pre-test speed: 5 mm/s; test speed: 10 mm/s; post-test speed: 5 mm/s; time: 5 seconds; force: 1000g.

With the objective of expressing the consistency of fats, methods were developed to convert the penetration depth into a more logical unit, independent of the cone format and penetration force, resulting in parameters such as *yield value* or *hardness*. The term *hardness* is defined as the ratio between the force applied and the penetration area. However the parameter *yield value* is the one most used to interpret the consistency of fats.[143] The *yield value* was described by Haighton[146] and can be analyzed independent of the equipment used. It corresponds to the tension absorbed before permanent deformation, and can be expressed in gf/cm^2. Fats behave as rigid solids until the deformation tension exceeds the *yield value*, when they start flowing as viscous liquids.[147] This relationship is expressed by Haighton:[146]

$$C = (K \times W)/(p^{1.6})$$

Where:

C = *yield value*, in gf/cm^2

K = factor that depends on the angle of the cone

W = the total force of the system in gf (for a cone penetrometer)

p = penetration depth in mm/10.

According to Haighton[146], one can classify fats at a determined application temperature as a function of the subjective property of spreadability, according to Table 4.

Table 4. Classification of margarines and shortenings according to the *yield value.*[146]

Yield value **(gf/cm^2)**	**Consistency**
< 50	Very soft, to just pourable
50-100	Very soft, not spreadable
100-200	Soft, but already spreadable
200-800	Satisfactory plastic and spreadable
800-1000	Hard, but satisfactorily spreadable
1000-1500	Too hard, limit of spreadability
> 1500	Too hard

The analysis of texture has been shown to be an extremely useful tool to outline specific applications for fats produced by interesterification. Rodríguez, Castro, Salinas, López & Miranda[37] used this technique to optimize the interesterification of fat bases produced from lard/sunflower oil. Marangoni & Rousseau[36] studied the consistency of interesterified lard/canola oil and palm oil/soybean oil blends. Interesterification caused an increase in the consistency of the lard/canola oil blends, but did not alter the consistency properties of the palm oil/soybean oil blends. The consistency of lard and its binary blends with soybean oil was significantly altered by the chemical interesterification, according to studies by Silva & Gioielli.[14] Interesterification caused an increase in consistency of the lard and of blends containing 20 to 40% soybean oil at all the temperatures analyzed. Piska, Zárubová, Louzecký, Karami & Filip[44] produced various types of shortening from interesterified fats, the values for *yield value* being proportionally dependent on the concentration of fully hydrogenated fats in the blend, before and after interesterification. De, Hakimji, Patel, Sharma, Desai & Kumar[148] developed margarines as from interesterified milk fat fractions and palm stearin, with *yield values* between 112 and 145 gf/cm^2. Kok, Fehr, Hammond & White[12] observed that margarines formulated with interesterified fats (highly saturated soybean oil) presented higher *hardness indexes* than commercial margarines. Rodrigues, Gioielli & Anton[38] showed an increase in the

consistency of blends of milk fat with corn oil for the majority of the samples studied, after chemical interesterification. This result was the opposite of that observed by Rousseau, Hill & Marangoni[86] when they interesterified blends of milk fat with canola oil.

An increase in the consistency of palm oil (PO)/ palm kernel oil (PKO) blends, especially at 25ºC, was found after interesterification, according to research by Grimaldi, Gonçalves, Gioielli & Simões[149], characterizing the plastic fats as spreadable according to Haighton.[146] Table 5 shows the values for consistency represented by the *yield value*.

Table 5. *Yield value* (gf/cm^2) of palm oil (PO)/ palm kernel oil (PKO) blends, before and after chemical interesterification.[149]

PO/PKO blends							
***Yield value* (gf/cm^2) – Before interesterification**							
T (°C)	**100/0**	**80/20**	**60/40**	**50/50**	**40/60**	**20/80**	**0/100**
10	8740.8	8522.9	10454.9	12541.1	10561.6	13780.7	10481.6
20	1027.5	449.3	489.3	1090.3	1926.1	3902.6	6743.5
25	146.8	130.5	28.2	31.1	89	269.9	1118
30	-	-	-	-	-	-	-
***Yield value* (gf/cm^2) – After interesterification**							
T (°C)	**100/0**	**80/20**	**60/40**	**50/50**	**40/60**	**20/80**	**0/100**
10	9093.7	7363.3	8634.1	9712	10204.3	8946.2	16316.2
20	1206.9	2281.9	3058.9	2769.8	3635.7	2670.4	4307.4
25	579.8	587.2	793.3	591.6	444.8	315.8	283.2
30	143.8	115.7	94.9	51.9	111.2	-	-
35	71.2	-	40	-	-	-	-

Conclusions

Chemical interesterification is currently the main means of producing low *trans* fat bases for different industrial purposes. However delineation of the application of a determined fat is a function of profound knowledge of its physicochemical properties.

Information about the triacylglycerolic composition, solid fat content, thermal behavior during crystallization and melting, crystallization velocity, microstructure, polymorphism and consistency should be evaluated as a group to determine the functionality of a fat or shortening. In some cases, the study of all these associated parameters allows one to predict the behavior of the interesterified blends during and after industrial processing. Thus a complete characterization of the fats produced by chemical interesterification can be done by the use and correct interpretation of the results obtained using analytical tools such as nuclear magnetic resonance, differential scanning calorimetry, X-ray diffraction, polarized light microscopy and a texture analysis, as well as chromatographic and computational methods.

References

1 A.P.B. Ribeiro, J.M.L.N. Moura, R. Grimaldi, and L.A.G. Gonçalves, Quim. Nova. 30, 1295 (2007).

2 M.C. Chiu, L.A. Gioielli, and R. Grimaldi, Quim. Nova. 31, 232 (2008).

3 B.F. Haumann, Inform. 5, 668 (1994).

4 A. Rozendaal, Inform. 3, 1232 (1992).

5 D. Rousseau and A.G. Marangoni, in *Food lipids: chemistry, nutrition, and biotechnology,* edited by C.C. Akoh (CRC Press, New York, 2002), p.301.

6 N.L.H.M, Dian, K. Sundram and N.A. Idris, J. Am. Oil Chem. Soc. 109, 147 (2007).

7 R.D. O'Brien, *Fats and Oils – Formulating and Processing for Applications* (CRC Press, New York, 2004).

8 D.S. Nichols and K. Sanderson, in *Chemical and functional properties of food lipids*, edited by Z.E. Sikorski and A. Kolakowska (CRC Press, New York, 2003).

9 I. Karabulut, S. Turan, and G. Ergin, Eur. Food Res. Technol. 218, 224 (2004).

10 D.R. Erickson, *Practical Handbook of Soybean Processing and Utilization* (American Oil Chemists'Society, Champaign, 1995), p.227.

11 AOCS, *Official methods and recommended practices of the American Oil Chemists' Society* (American Oil Chemists' Society, Champaign, 2004).

12 L.L. Kok, W.R. Fehr, E.G. Hammond, and P.J. White, J. Am. Oil Chem. Soc. 76, 1175 (1999).

13 G.R. List, E.A. Emken, W.F. Kwolek, and H.J. Dutton, J. Am. Oil Chem. Soc. 74, 408 (1977).

14 R.C. Silva and L.A. Gioielli, Rev. Bras. Ciênc. Farm. 42, 223 (2006).

15 V. Petrauskaite, W. De Greyt, M. Kellens, and A. Huyghebaert, J. Am. Oil Chem. Soc. 75, 489 (1998).

16 S. Khatoon, J. Food Lipids. 7, 83 (2000).

17 A.G. Marangoni and D. Rousseau, Trends Food Sci. Technol. 6, 329 (1995).

18 R.E. Timms, J. Am. Oil Chem. Soc. 62, 241 (1985).

19 F. Shahidi and P.K.J.P.D. Wanasundara, in *Food lipids: chemistry, nutrition, and biotechnology,* edited by C.C. Akoh (CRC Press, New York, 2002).

20 K. van Putte and J. van den Enden, J. Am. Oil Chem. Soc. 51, 316 (1974).

21 R.E. Timms, *Confectionery fats handbook* (The Oily Press, Dundee, 2003), p.63.

22 S. Martini, M.L. Herrera, and A.G. Marangoni, J. Am. Oil Chem. Soc. 82, 313 (2005).

23 E.M. Goh and T.H. Ker, J. Am. Oil Chem. Soc. 68, 144 (1991).

24 J. van Duynhoven, G.J. Goudappel, M.C.M. Gribnau, and V.K.S. Shukla, Inform. 10, 479 (1999).

25 A.J. Dijkstra, W.W. Christie and G. Knothe, in *The lipid handbook*, edited by F.D. Gunstone and J.L. Harwood and A.J. Dijkstra (CRC Press, New York, 2007), p.415.

26 ISO, *ISO Standard 8292: Animal and Vegetable Fats and Oils* (International Organization for Standardization, Geneva, 1991).

27 IUPAC, *Standard Methods for the Analysis of Oils, Fats and Derivatives* (International Union of Pure and Applied Chemistry, Oxford, 1987).

28 DGF, *Einheitsmethoden* (Deutsche Gesellschaft für Fettwissenschaft, Frankfurt, 2004).

29 K. Warner, in *Modifying Lipids for Use in Food*, edited by F.D. Gunstone (Woodhead Publishing, Cambridge, 2006), p.114.

30 F.Z. Vissoto, V. Luccas, N. Bragagnolo, J.M. Turatti, R. Grimaldi and M.S. Figueiredo, Braz. J. Food Technol, 2, 139 (1999).

31 A.M.D.N. Lida and A.R. Ali, J. Am. Oil Chem. Soc. 75, 1625 (1998).

32 R. Grimaldi, L.A.G. Gonçalves, L.A. Gioielli, and I.S. Simões, Grasas y Aceites. 52, 363 (2001).

33 L.A. Gioielli and R. Baruffaldi, Rev. Bras. Ciênc. Farm. 24, 29 (1988).

34 G.R. List, T.L. Mounts, F, Orthoefer and W.E. Neff, J. Am. Oil Chem. Soc. 72, 379 (1995).

35 A.G. Marangoni and D. Rousseau, J. Am. Oil Chem. Soc. 75, 1265 (1998).

36 A.G. Marangoni and D. Rousseau, J. Am. Oil Chem. Soc. 75, 1633 (1998).

37 A. Rodriguez, E. Castro, M.C. Salinas, R. López and M. Miranda, J. Am. Oil Chem. Soc. 78, 432 (2001).

38 J.N. Rodrigues, L.A. Gioielli and C. Anton, Ciênc. Tecnol. Aliment. 23, 226 (2003).

39 J.N. Rodrigues and L.A. Gioielli, Food Res. Int. 36, 149 (2003).

40 A.R. Norizzah, C.L. Chong, C.S. Cheow and O. Zaliha, Food Chem. 86, 229 (2004).

41 N. Ramli, M. Said and N.T. Loon, J. Food Lipids. 12, 243 (2005).

42 R. Grimaldi, L.A.G. Gonçalves, L.A. and M.Y. Ando, Quim. Nova. 28, 633 (2005).

43 S. Khatoon and S.R.Y. Reddy, Eur. J. Lipid Sci. Technol. 107, 786 (2005).

44 I. Piska, M. Zárubová, Louzecký, H. Karami and V. Filip, J. Food Eng. 77, 347 (2006).

45 Y. Shi, B. Liang and R.W. Hartel, J. Am. Oil Chem. Soc. 82, 399 (2005).

46 D. Rousseu and A.G. Marangoni, in *Physical Properties of Lipids,* edited by A.G. Marangoni and S.S. Narine (CRC Press, New York, 2002).

47 M. Buchgraber, F. Ulberth, H. Emons and E. Anklan, Eur. J. Lipid Sci. Technol. 106, 621 (2004).

48 N.R. Antoniossi Fillho, O.L. Mendes and F.M. Lanças, Chromatographia. 40, 557 (1995).

49 M.H. van Vliet and G.M.P. van Kempen, Eur. J. Lipid Sci. Technol. 106, 697 (2004).

50 R.J.J. Vanderwal, J. Am. Oil Chem. Soc. 37, 595 (1960).

51 M.H. Coleman, J. Am. Oil Chem. Soc. 42, 751 (1965).

52 A.P.B. Ribeiro, L.A.G. Gonçalves and R. Grimaldi in *6th Euro Fed Lipid Congress - Abstracts, Athens, 2008*, edited by European Federation for the Science and Technology of Lipids.

53 K. Sato, Chem. Eng.Sci. 56, 2255 (2001).

54 R. Boistelle, in *Crystallization and Polymorphism of Fats and Fatty Acids,* edited by N. Garti and K.Sato (Marcel Dekker, New York, 1988), p.189.

55 M.L. Herrera, C. Falabella, M. Melgarejo and M.C. Anón, J. Am. Oil Chem. Soc. 75, 1273 (1998).

56 A.G. Marangoni and D. Tang, Chem. Phys. Lett. 433, 248 (2006).

57 I. Foubert, K. Dewettinck, G. Janssen and P.A. Vanrolleghem, J. Food Eng. 75, 551 (2006).

58 S.S. Narine, K.L. Humphrey, and L. Bouzidi, J. Am. Oil Chem. Soc. 83, 913 (2006).

59 A.M.D.N Lida, K. Sundram, W.L. Siew, A. Amina and S. Mamot, J. Am. Oil Chem. Soc. 79, 1138 (2002).

60 P. Wassel and N.W.G. Young, Int. J. Food Sci. Technol. 42, 503 (2007).

61 M.L. Herrera, C. Falabella, M. Melgarejo and M.C. Anón, J. Am. Oil Chem. Soc. 75, 1 (1999).

62 A. Bell, M.H. Gordon, W. Jirasubkunakorn and K.W. Smith, Food Chem. 101, 799 (2007).

63 R. Campos, in *Fat Crystal Networks*, edited by A.G. Marangoni (Marcel Dekker, New York, 2005), p.267.

64 G.G. Rye, J.W. Litwinenko and A.G. Marangoni, in *Bailey's industrial oil and fat products,* edited by F. Shahidi (Wiley Interscience, New York, 2005).

65 I. Foubert, P.A. Vanrolleghem, B. Vanhoutte and K. Dewettinck, Food Res. Int. 35, 945 (2002).

66 C. Himavan, V.M. Starov and A.G.F. Stapley, Adv. Colloid Interface Sci. 122, 3 (2006).

67 M.L. Herrera, M.L. Gatti and R.W. Hartel, Food Res. Int. 32, 289 (1999).

68 S. Metin and R.W. Hartel, in *Bailey's industrial oil and fat products,* edited by F. Shahidi (Wiley Interscience, New York, 2005).

69 A.J. Wright and A.G. Marangoni, J. Am. Oil Chem. Soc. 79, 395 (2002).

70 S. Martini, M.L. Herrera and R.W. Hartel, J. Am. Oil Chem. Soc. 79, 1055 (2002).

71 J.F. Toro-Vazquez, E. Dibildox-Alvarado, M. Charó-Alonso, V. Charó-Alonso, and C.A. Gómez-Aldapa, J. Am. Oil Chem. Soc. 79, 855 (2002).

72 A.J. Wright, R.W. Hartel, S.S. Narine, and A.G.Marangoni, J. Am. Oil Chem. Soc. 77, 463 (2000).

73 W. Kloek, P. Walstra and T. van Vliet, J. Am. Oil Chem. Soc. 77, 48 (2000).

74 S.D. Campbell, H.D. Goff and D. Rousseau, Food Res. Int. 35, 935 (2002).

75 A.G. Marangoni and S.E. McGauley, Cryst. Growth Des. 3, 95 (2003).

76 K. Dewettinck, I. Foubert, M. Basiura and B. Goderis, Cryst. Growth Des. 4, 1295 (2004).

77 S. Braipson-Danthine and C. Deroanne, Food Res. Int. 37, 941 (2004).

78 A. Szydlouska-Czerniak, G. Karlovits, M. Lach and E. Szlyk, Food Chem. 92, 133 (2005).

79 M. Cerdeira, S. Martini, R.J. Candal and M.L. Herrera, J. Am. Oil Chem. Soc. 83, 489 (2006).

80 R.C. Silva, J.P. Escobedo and L.A. Gioielli, Quim. Nova. 31, 330 (2008).

81 A.P.B. Ribeiro, R. Grimaldi and L.A.G. Gonçalves, in *Anais do XII Congresso Latino-americano de Óleos e Gorduras, Florianópolis, 2007*, edited by Sociedade Brasileira de Óleos e Gorduras.

82 V. D'Souza, J.M. deMan, and L.M. deMan, J. Am. Oil Chem. Soc. 67, 835 (1990).

83 L.M de Man, J.M. deMan, and B. Blackman, J. Am. Oil Chem. Soc. 66, 1777 (1989).

84 P.J. Lawler and P.S. Dimick, in *Food lipids: chemistry, nutrition, and biotechnology,* edited by C.C. Akoh (CRC Press, New York, 2002).

85 S. Martini, T. Awad, and A.G. Marangoni, in *Modifying Lipids for Use in Food*, edited by F.D. Gunstone (Woodhead Publishing, Cambridge, 2006), p.142.

86 D. Rousseau, A.R. Hill, and A.G. Marangoni, J. Am. Oil Chem. Soc. 72, 983 (1996).

87 M.L. Herrera and F.J. Márquez Rocha, J. Am. Oil Chem. Soc. 73, 321 (1996).

88 C.L. Chog, Z. Kamarudin, P. Lesieur, A.G. Marangoni, C. Bourgaux, and M. Ollivon, Eur. J. Lipid Sci. Technol. 109, 410 (2007).

89 J. Oh, A.R. McCurdy, S. Clark, and B.G. Swanson, J. Am. Oil Chem. Soc. 82, 13 (2005).

90 A.G. Marangoni, in *Fat Crystal Networks*, edited by A.G. Marangoni (Marcel Dekker, New York, 2005), p.1.

91 Z. Omar, C.C. Let, C.C. Seng, and N.A. Rashid, Eur. J. Lipid Sci. Technol. 107, 634 (2005).

92 L.A. Gioielli, I.S. Simões, and J.N. Rodrigues, J. Food Eng. 57, 347 (2003).

93 D.A. Skoog, J.F. Holler, and T.A. Nieman, *Princípios de Análise Instrumental* (Bookman, Porto Alegre, 2002).

94 S.E. McGauley and A.G. Marangoni, in *Physical Properties of Lipids,* edited by A.G. Marangoni and S.S. Narine (CRC Press, New York, 2002).

95 D. Kalnin, P. Lesieus, F. Artzner, G. Keller, and M. Ollivon, Eur. J. Lipid Sci. Technol. 107, 594 (2005).

96 B.S. Ghotra, S.D. Dyal, and S.S. Narine, Food Res. Int. 35, 1015 (2002).

97 S.Martini and M.L. Herrera, J. Am. Oil Chem. Soc. 79, 315 (2002).

98 S. Sabariah, A.R.M. Ali, and C.L. Chong, J. Am. Oil Chem. Soc. 75, 905 (1998).

99 C.W. Chen, O.M. Lai, H.M. Ghazal, and C.L. Chong, J. Am. Oil Chem. Soc. 79, 403 (2002).

100 S.S. Narine and Humphrey, Food Res. Int. 37, 28 (2004).

101 Y.B. Che Man, K. Shamsi, M.S.A. Yusoff, and S. Jinap, J. Am. Oil Chem. Soc. 80, 409 (2003).

102 W. MacNaughtan, I.A. Farhat, C. Himawan, V.M. Starov and A.G.F. Stapley, J. Am. Oil Chem. Soc. 83, 1 (2006).

103 P.N. Mayamol, T. Samuel, A. Balachandran, A. Sundaresan, and C. Arumugham, J.Am. Oil Chem. Soc. 81, 407 (2004).

104 C.Loisel, G. Keller, G. Lecq, C. Bourgaux, and M. Ollivon, J.Am. Oil Chem. Soc. 75, 425 (1998).

105 T. Sonoda, Y. Takata, and K. Sato, J. Am. Oil Chem. Soc. 81, 365 (2004).

106 H. Schenk and R. Peschar, Radiat. Phys. Chem. 71, 829 (2004).

107 A. van Langevelde, R. Driessen, W. Molleman, and R. Peschar, J. Am. Oil Chem. Soc. 78, 911 (2001).

108 C.L. Chong, Z.Kamarudin, P. Lesieur, A.G. Marangoni, C. Bourgaux, and M. Ollivon, Eur. J. Lipid Sci. Technol. 109, 410 (2007).

109 P.H. Yap, J.M. deMan, and L.deMan, J. Am. Oil Chem. Soc. 66, 693 (1989).

110 D. Rousseau, A.G. Marangoni, and K.R. Jeffrey, J. Am. Oil Chem. Soc. 75, 1833 (1998).

111 I.L. Woodrow and J.M. deMan, J. Dairy Sci. 51, 996 (1961).

112 N. Ramli, M. Said, and N.T. Loon, J. Food Lipids. 12, 243 (2005).

113 C.P. Tan and Y.B. Che Man, Food Chem. 76, 89 (2002).

114 D. Kalnin, G. Garnaud, H. Amenistsch, and M. Ollivon, Food Res. Int. 35, 927 (2002).

115 M.A.M. Zeitoun, W.E. Neff, and T.L. Mounts, J. Am. Oil Chem. Soc. 70, 467 (1993).

116 Y. Pomeranz and C.E. Meloan, *Food Analysis – Theory and Practice* (Chapman & Hall, New York, 1994).

117 C.G. Biliaderis, Food Chem.10, 239 (1983).

118 J.M.N. Marikkar, H.M Ghazali, Y.B. Che Man, and O.M. Lai, J. Food Lipids. 10, 63 (2003).

119 E. Kaisersberger, Anal. Proc. 27, 64, (19990).

120 J.S. Aronhime, Thermochim. Acta. 134,1 (1988).

121 D.J. Cebula and K.W. Smith, J. Am. Oil Chem. Soc. 69, 992 (1992).

122 J.B. Rossel, J. Am. Oil Chem. Soc. 52, 505 (1975).

123 M.C. Chiu, L.A. Gioielli, and R. Grimaldi, Quím. Nova, 31, 238 (2008).

124 R. Grimaldi, L.A.G. Gonçalves, L.A. Gioielli, and I.S. Simões, Grasas y Aceites. 52, 363 (2001).

125 S. Metin and R.W. Hartel, J. Am. Oil Chem. Soc. 75, 1617 (1998).

126 K. Kawamura, J. Am. Oil Chem. Soc. 57, 48 (1979).

127 J.M. Aguilera, D.W. Stanley, and K.W. Baker, Trends Food Sci. Technol. 11, 3 (2000).

128 A.G. Marangoni and R.W. Hartel, Food Tech. 52, 46 (1998).

129 S.S. Narine and A.G. Marangoni, in *Fat Crystal Networks*, edited by A.G. Marangoni (Marcel Dekker, New York, 2005), p.179.

130 F.F.A. Hollander, D. Kaminski, D. Duret, W.J.P. van Enckevort, H. Meekes, and P. Bennema, Food Res. Int. 35, 909 (2002).

131 A.G. Marangoni and S.S Narine, Food Res. Int. 35, 957 (2002).

132 D. Tang and A.G. Marangoni, Adv. Colloid Interface Sci. 128, 257 (2006).

133 D. Tang and A.G. Marangoni, Trends Food Sci. Technol. 18, 474 (2007).

134 K.G. Berger, G.G. Jewell, and R.J.M. Pollitti, in *Food Microscopy*, edited by J.G. Vaughan (Academic Press, London, 1979), p.445.

135 S.S. Narine and Marangoni, J. Cryst. Growth. 198, 1315 (1999).

136 D. Tang and A.G. Marangoni, J. Am. Oil Chem. Soc. 83, 377 (2006).

137 O.W.D. Gamboa and L.A. Gioielli, Quím. Nova, 29, 646 (2006).

138 V.E. Sotero-Solis and L.A. Gioielli, Alimentaria, 38, 131 (2001).

139 S. Hurtová, S. Schmidt, J. Zemanovic, P. Simon and S. Sekretar, Fett/Lipid. 98, 60 (1996).

140 D. Rousseau, A.R. Hill, and A.G. Marangoni, J. Am. Oil Chem. Soc. 83, 377 (2006).

141 J. F. C. Meullenet and J. Gross, Texture Stud. 30, 167 (1999).

142 I. Simões, L.A. Gioielli and M.N. Oliveira, Ciênc. Tecnol. Aliment. 18, 325 (1998).

143 J.M. deMan, J. Am. Oil Chem. Soc. 60, 82 (1983).

144 T. Matuszek, in *Chemical and functional properties of food lipids*, edited by Z.E. Sikorski and A. Kolakowska (CRC Press, New York, 2003).

145 A.C. Juriaanse and I. Heertje, Food Microstruct. 7, 181 (1988).

146 A.J. Haighton, J. Am. Oil Chem. Soc. 36, 345 (1959).

147 M.C. Chiu and L.A. Gioielli, Rev. Bras. Ciênc. Farm. 38,95 (2002).

148 B.K. De, M. Hakimji, A. Patel, D. Sharma, H. Desai, and T. Kumar, Eur. J. Lipid Sci. Technol. 109, 32 (2007).

149 R. Grimaldi, L.A.G. Gonçalves, L.A. Gioielli, and I.S. Simões, Grasas y Aceites. 52, 349 (2001).

3.

ZERO *TRANS* FATS FROM SOYBEAN OIL AND FULLY HYDROGENATED SOYBEAN OIL: PHYSICOCHEMICAL PROPERTIES AND FOOD APPLICATIONS

Ana Paula Badan Ribeiro, Renato Grimaldi, Luiz Antonio Gioielli, Lireny Aparecida Guaraldo Gonçalves

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Zero trans fats from soybean oil and fully hydrogenated soybean oil: physicochemical properties and food applications

Abstract

Blends of soybean oil (SO) and fully hydrogenated soybean oil (FHSBO), with 10, 20, 30, 40 and 50% FHSBO (w/w) content were interesterified under the following conditions: 0.4% sodium methoxide, 500 rpm stirring, 100°C, 20 minutes. The original and interesterified blends were examined for triacylglycerol composition, melting point, solid fat content (SFC) and consistency. Interesterification caused considerable rearrangement of triacylglycerol species, reduction of trisaturated triacylglycerol content and increase in monounsaturated and diunsaturated triacylglycerols, resulting in lowering of respective melting points. The interesterified blends displayed reduced SFC at all temperatures and more linear melting profiles as compared with the original blends. Yield values showed increased plasticity in the blends after the reaction. Isosolid diagrams before and after the reaction showed no eutectic interactions. The 90:10, 80:20, 70:30 and 60:40 interesterified SO:FHSBO blends displayed characteristics suited to application, respectively, as liquid shortening, table margarine, baking/confectionery fat and all-purpose shortenings/ biscuit-filling base.

Key-words: chemical interesterification, zero trans fats, soybean oil, fully hydrogenated soybean oil, triacylglycerol composition, solid fat content, consistency.

1. Introduction

Most natural oils and fats offer limited application in their unaltered state, due to their particular fatty acid and triacylglycerol composition (Rozendall, 1992; Chiu, Gioielli, & Grimaldi, 2008). Accordingly, oils and fats are modified chemically by hydrogenation or

interesterification; or physically, by fractionation (Erickson, 1995; Fattahi-far, Sahari, & Barzegar, 2006).

Although used for a long time, partial hydrogenation also results in substantial formation of *trans* fatty acids, compounds that act as coronary artery disease risk factors by modulating the synthesis of cholesterol and its fractions and acting on the eicosanoids. A number of studies have suggested a direct relationship between *trans* isomers and increased risk of vascular disease. In response, many health organizations have recommended reducing consumption of foods containing *trans* fatty acids (Mensink & Katan, 1990; Enig, 1996; Gurr, 1990; Lichtenstein, 1993; Hunter, 2005).

In this connection, chemical interesterification has proved the main alternative for obtaining plastic fats that have low *trans* isomer content or are even *trans* isomer free. Unlike partial hydrogenation, this process does not isomerize the fatty acids' double bonds and does not affect their degree of saturation (Haummann, 1994; Norizzah, Chong, Cheow, & Zaliha, 2004; Ribeiro, Moura, Grimaldi, & Gonçalves, 2007). In the interesterification, the fatty acids are not altered, but are redistributed in the triacylglycerol molecules. The process thus consists in simultaneously breaking the existing ester bonds and forming new bonds in the glycerol molecules (Rozendall, 1992). In this way, chemical interesterification modifies the triacylglycerol composition of the oil or fat and thus its physical properties, crystallization and melting behavior, solid fat content (SFC), texture and crystal habit, contributing to greater availability of oil fractions for food product applications (Dian, Sundram, & Idris, 2007).

A number of studies have investigated the influence of chemical interesterification on the physico-chemical properties of oils and fats or their blends, including the work of Gioielli and Baruffaldi (1988); List, Emken, Kwolek, Simpson, and Dutton (1977); List, Mounts, Orthoefer, and Neff (1995); Lida and Ali (1998); Marangoni and Rousseau (1998a, 1998b); Petrauskaite, De Greyt, Kellens, and Huyghebaert (1998); Kok, Fehr, Hammond, and White (1999); Khatoon (2000); Rodríguez, Castro, Salinas, López, and

Miranda (2001); Rodrigues, Gioielli, and Anton (2003); Rodrigues and Gioielli (2003); Norizzah et al. (2004); Karabulut, Turan, and Ergin (2004); Ramli, Said, and Loon (2005); Grimaldi, Gonçalves, and Ando (2005); Khatoon and Reddy (2005); Silva and Gioielli (2006); Piska, Zárubová, Louzecký, Karami, and Filip (2006), and others. The interesterification of liquid oils with *hardfats* is the most versatile way for producing zero *trans* fats, yielding fat bases with excellent characteristics for preparation of margarines, shortenings and spreads (List et al., 1995; Karabulut et al., 2004; Khatoon & Reddy, 2005; Lo & Handel, 1983).

In world vegetable oil consumption, soybean oil (SO) stands out for its nutritional qualities, permanent supply, considerable economic value and high functionality, making it a particularly interesting raw material for producing special fats (O'Brien, 2004). Fully hydrogenated soybean oil (FHSBO), a relatively low-cost product of total hydrogenation, can be used as hardstock for producing interesterified fat bases. Zeitoun, Neff, List, and Mounts (1993) report on a chemical interesterification of blends formulated with FHSBO and nine different vegetable oils, at a ratio of 1:1 (w/w).

In addition to its economic feasibility, the advantage of using FHSBO as hardstock derives from its high (approximately 85%) content of stearic acid, which is not atherogenic and therefore has no adverse effect on cardiovascular disease risk (Hunter, 2005; O'Brien, 2004; Rao & Lokesh, 2003). Although a saturated fatty acid, consumption of stearic acid does not significantly alter total serum cholesterol and LDL cholesterol levels, unlike most – lauric, myristic and palmitic – saturated fatty acids. Numerous clinical studies have shown that the effect of stearic acid on plasma lipoprotein concentrations is similar to that of oleic acid (Tholstrup, 2005; Mensink, 2005; Sampath & Ntambi, 2005; Nielsen, 2006; Dirienzo, Lemke, Petersen, & Smith, 2008; Sanders & Berry, 2005). Therefore, FHSBO is used in producing interesterified fats with a view to the stability and functionality required of fats for food applications and does not entail adverse health effects.

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, does pose challenges, because suitable SFC curves, plasticity, crystallization properties and texture are difficult to obtain in the absence of *trans* fatty acids. Accordingly, the study of, and application guidelines for, interesterified fats must comprehend jointly their fundamental physico-chemical properties, as regards triacylglycerol composition, SFC, consistency and melting point (O'Brien, 2004; Reyes-Hernandez, Dibildox-Alvarado, Charo-Alonso, & Toro-Vazquez, 2007).

The purpose of this study was to evaluate the chemical interesterification of binary blends of SO:FHSBO, with 10, 20, 30, 40 and 50% FHSBO content, with a view to studying fat bases for application in food products. Results for triacylglycerol composition, melting point, SFC, isosolid diagram and consistency were analyzed, before and after randomization.

2. Materials and Methods

2.1 Raw materials. The materials used were refined soybean oil (SO), purchased in a local store, and fully hydrogenated soybean oil (FHSBO), kindly provided by a regional supplier. The catalyst was 99%-pure sodium methoxide powder (Sigma-Alldrich).

2.2 Blends. The blends were prepared in the proportions 90:10, 80:20, 70:30, 60:40 and 50:50 SO:FHSBO (w/w), melted at 100°C and homogenized for 10 minutes at that temperature to melt the crystals completely, prior to each interesterification reaction.

2.3 Chemical interesterification. For the reactions, a 500 mL, jacketed, borosilicate glass reactor with bottom drain port and conical ground glass joints was coupled to a thermostated circulator bath (LAUDA RE 212, -30 to +200°C, $\pm$0.02°C), stirring system (universal motor with electronic speed control up to 4000 rpm – Marconi, BR) with axial flow stirrer, vacuum pump (Vacuubrand model 30 diaphragm pump) and probe-type digital thermometer (-50 to + 300°C, ±1°C – Incoterm). The samples (200g) were dried for 20

minutes at 100°C in the reactor itself, under vacuum with stirring at 500 rpm. Catalyst content was 0.4% and the reaction was conducted under vacuum at 100°C, with stirring at 500 rpm, for 20 minutes, according to the optimization by Grimaldi et al. (2005). The reaction was terminated by adding distilled water and 5% citric acid solution. The interesterified samples were washed carefully with distilled water (80°C) to remove any soaps that had formed and were then dried under vacuum at 110°C for 30 minutes.

2.4 Fatty acid composition. Analysis of fatty acid composition was performed in a capillary gas chromatograph (CGC Agilent 6850 Series GC System) after esterification using the method of Hartman and Lago (1973). The fatty acid methyl esters were separated according to AOCS procedure 2-66 (AOCS, 2004) in a DB – 23 Agilent capillary column (50% cyanopropyl-methylpolysiloxane), dimensions 60m, ϕ int: 0.25 mm, 0.25 µm film. Oven temperature was 110°C-5min, 110°C-215°C (5°C/min), 215°C-24min; detector temperature: 280°C; injector temperature 250°C; carrier gas: helium; split ratio 1:50; injection volume: 1.0µL. Qualitative composition was determined by comparing peak retention times with the respective standards for fatty acids.

2.5 Iodine value. It was calculated from the fatty acid composition by AOCS Methods Cd 1c-85 (AOCS, 2004).

2.6 Triacylglycerol composition. Triacylglycerol composition was analyzed in a CGC Agilent 6850 Series GC System capillary gas chromatograph. A DB-17HT Agilent Catalog: 122-1811 capillary column (50%-Phenyl-methylpolysiloxane, 15 meters in length x 0.25 mm bore and containing 0.15µm film). The conditions were: split injection, ratio 1:100; column temperature: 250ºC, programmed up to 350ºC at 5ºC/min; carrier gas: helium, at 1.0 mL/min flow rate; injector temperature: 360ºC; detector temperature: 375ºC; injection volume: 1.0 µL; sample concentration: 100mg/5mL of tetrahydrofurane. Triacylglycerol groups were identified by comparing retention times, following the procedures of Antoniosi Filho, Mendes, and Lanças (1995).

2.7 SFC. It was determined using Nuclear Magnetic Resonance (NMR) spectrometry (Bruker pc120 Minispec) and TCON 2000 high precision dry baths (0-70℃) (Duratech, USA). AOCS Procedure Cd 16b- 93: direct method, taking readings from samples in series at temperatures of 10; 20; 25; 30; 35; 40, 45, 50, 55 and 60℃, with tempering for non-stabilized fats (AOCS, 2004). In addition, it was necessary to modify the standard tempering prescribed by AOCS Method Cd 16b-93 in order to ensure stabilization of the crystallization of the interesterified blends: the samples were kept at 0℃ for 2 hours and at each reading temperature for 1 hour.

2.8 Construction of isosolid diagrams. The isosolid line diagrams (i.e. showing compositions in which the SFC values of a blend are equivalent at a given temperature) were constructed from the data supplied experimentally by NMR using the modified tempering method (Braipson-Danthine & Deroanne, 2006).

2.9 Melting point. This was determined using an open capillary tube, to AOCS standards, Method Cc 3-25 (AOCS, 2004). In addition, melting point was calculated for the temperature corresponding to 4% solids content, obtained from the SFC curve given by NMR using the modified tempering described above, by way of polynomial equations adjusted with the aid of Statistica 6.0 software (Karabulut et al., 2004; Statistica, 2002). A simple linear regression model was applied to relate the data obtained by the two methods.

2.10 Consistency. Consistency was determined with a PC-controlled TA-XT2i texture analyzer (Stable Micro Systems) using an acrylic cone with an apex angle of 40° and no truncation. The samples were heated in a microwave oven to approximately 80℃ to completely melt the crystals and placed in 50mL beakers. Conditioning was performed in an incubator for 24 hours at 5℃ to crystallize the fats and then for 24 hours at the read temperatures: 5, 10, 15, 20, 25, 30, 35, 40℃ or higher, according to the sample analyzed (Rodrigues et al., 2003). The tests were conducted under the following conditions: distance = 10mm; speed = 2mm/s; time = 5s; and five readings for each sample. From

these conditions the compression force was obtained in (gf). The penetration data were converted into yield values, following Haighton (1959):

$$C = \frac{K \times W}{p^{1,6}}$$

Where: C = yield value, in gf/cm^2; K = factor dependent on the cone angle; W = compressive strength (gf); p = penetration depth (mm/10).

3. Results and Discussion

3.1 Fatty acid composition in raw materials and blends. Table 1 shows the fatty acid composition and iodine value calculated for the raw materials and blends used in the chemical interesterification. These results express the mean of the two assays and agree with the limits encountered in the literature (Erickson, 1995; O'Brien, 2004). For the SO, the predominant fatty acids were linoleic acid (54.87%), oleic acid (23.17%) and palmitic acid (11.37%). The FHSBO was high in stearic acid content (86.62%), followed by palmitic acid (11.50%). This value lies between the 83.70% and 90% reported by List et al. (1995) and Lee, Akoh, and Lee (2008) for this raw material.

Table 1. Fatty acid composition (%) and iodine value of the raw materials (SO and FHSBO) and blends.

		SO: FHSBO (%w/w)					
Fatty acid (%)	**SO**	**90:10**	**80:20**	**70:30**	**60:40**	**50:50**	**FHSBO**
C14:0 - myristic acid	0.08	0.09	0.09	0.10	0.10	0.10	0.12
C16:0 - palmitic acid	11.37	11.37	11.38	11.39	11.31	11.38	11.50
C16:1 - palmitoleic acid	0.08	0.08	0.07	0.06	-	-	-
C18:0 - stearic acid	3.46	11.80	20.39	28.82	37.54	45.79	86.62
C18:1- oleic acid	23.17	20.59	18.41	16.03	13.77	11.48	0.11
C18:2 - linoleic acid	54.87	49.59	44.08	38.55	32.69	27.33	0.18
C18:3 - linolenic acid	5.32	4.82	4.27	3.75	3.16	2.65	-
C20:0 - arachidic acid	0.38	0.40	0.44	0.48	0.54	0.56	0.74
C20:1 - gadoleic acid	0.24	0.21	0.19	0.17	0.14	0.11	-
C22:0 - behenic acid	0.51	0.49	0.50	0.48	0.54	0.53	0.53
C24:0 – lignoceric acid	0.18	0.17	0.17	0.18	0.20	0.20	0.19
∑ Saturates	15.98	24.32	32.97	41.45	50.23	58.56	99.71
∑ Unsaturates	84.02	75.68	67.03	58.55	49.77	41.44	0.29
I.V*	135	122	108	95	80	67	0.4

I.V*= Iodine value (g I_2/100g).

The percentages of unsaturated fatty acids in the SO and FHSBO were 84.02% and 0.29%, respectively and, in the blends, were between 75.68% and 41.44%. Adding FHSBO to the SO produced blends with stearic acid contents between 11.80% and 45.79%. 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 the SO and FHSBO were within the ranges of the literature and, for the blends, are proportional to the amounts of each component (Erickson, 1995; Gunstone & Harwood, 2007).

The chemical interesterification process neither affected the degree of saturation nor caused isomerization of the fatty acid double bonds. The fatty acid composition of the starting materials was thus not altered (Rozendall, 1992; Karabulut et al., 2004; Lida, Sundram, Siew, Aminah, & Mamot, 2002).

3.2 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). Table 2 shows the main individual triacylglycerols that make up the SO and FHSBO raw materials and the blends, before and after chemical interesterification. The initials represent the *sn-1*, *sn-2* and *sn-3* positions of the triacylglycerols. For the SO, 17 triacylglycerol species were found and, for the FHSBO, only 4. The LLL, OLL and PLL triacylglycerols predominated in the SO, accounting for 55.67% of total triacylglycerol composition. These results agree with those presented by Gunstone and Harwood (2007) for the triacylglycerol composition of SO. For the FHSBO, only trisaturated triacylglycerols were found, with StStSt predominating (63.35%), followed by PStSt, with 30.79% of total triacylglycerols present. Humphrey and Narine (2004) found very similar contents for this fraction, with these two preponderant triacylglycerols totaling 90%. LLL and OLL were the predominant triacylglycerols in the 90:10 SO:FHSBO blend, while LLL and StStSt were the

triacylglycerol species with highest percentages in the 80:20 and 70:30 SO:FHSBO blends. The saturated triacylglycerols PStSt and StStSt were the predominant species in the 60:40 and 50:50 SO: FHSBO blends.

Interesterification produced significant alteration in the triacylglycerol composition of the blends studied. Randomization resulted in the formation of new triacylglycerols, such as StOSt, and considerable StLSt contents (as a result of the high concentrations of oleic and stearic acids present in the starting blends), as well as substantial increases in PLSt, StLO and StLL triacylglycerol species, in all the blends evaluated. Generally, interesterification resulted in the formation and increased content of lower melting point, mixed triacylglycerols, as in the results obtained by Norizzah et al. (2004) in the interesterification of palm stearin and palm kernel olein blends.

Table 2. Triacylglycerol composition* (%) of the SO, FHSBO and blends, before and after interesterification.

		SO: FHSBO (%w/w)										
TAG (%)	**SO**	**90:10**	**90:10-I****	**80:20**	**80:20-I**	**70:30**	**70:30-I**	**60:40**	**60:40-I**	**50:50**	**50:50-I**	**FHSBO**
PPSt	-	0.48	0.60	0.87	0.81	1.23	1.47	1.86	2.03	2.25	2.63	4.17
POP	0.81	0.73	1.00	0.59	0.79	0.46	0.87	0.46	0.90	0.36	0.62	-
PLP	3.02	2.40	2.31	2.09	2.09	1.74	2.07	1.48	1.90	1.25	1.51	-
PStSt	-	3.93	-	6.84	1.39	10.20	2.42	13.07	5.37	16.00	10.40	30.79
POSt	0.22	**-**	**2.53**	**-**	**2.84**	**-**	**4.43**	**-**	**4.46**	**-**	**3.58**	**-**
POO	3.55	3.23	-	2.72	-	2.38	-	1.92	-	1.60	-	-
PLSt	0.67	**0.62**	**6.09**	**0.52**	**8.33**	**0.45**	**9.67**	**0.37**	**10.36**	**0.30**	**9.29**	**-**
PLO	10.38	8.92	8.14	7.67	6.34	6.94	4.63	5.80	3.41	4.66	3.12	-
PLL	15.62	13.19	10.56	11.74	8.78	10.07	6.75	8.07	5.02	6.83	4.52	-
PLnL	2.82	2.55	2.88	2.16	1.80	1.76	0.53	1.85	0.74	1.26	-	-
StStSt	**-**	**9.36**	**-**	**15.39**	**1.45**	**22.37**	**2.37**	**28.75**	**4.18**	**35.46**	**11.47**	63.35
StOSt	**-**	**-**	**1.41**	**-**	**2.60**	**-**	**3.47**	**-**	**7.50**	**-**	**8.54**	**-**
SOO	0.76	0.48	-	0.69	-	-	-	-	-	-	-	-
StLSt	**-**	**-**	**1.34**	**-**	**7.52**	**-**	**11.71**	**-**	**15.32**	**-**	**15.48**	**-**
OOO	3.79	**3.17**	**-**	**3.26**	**-**	**2.46**	**-**	**2.21**	**-**	**1.59**	**-**	**-**
StLO	1.68	**1.42**	**8.70**	**1.47**	**10.81**	**1.10**	**14.17**	**0.99**	**11.44**	**0.71**	**7.30**	**-**
OLO	10.25	8.53	3.35	8.05	2.07	7.07	1.79	5.77	1.37	4.46	0.98	-
StLL	1.09	**0.95**	**14.39**	**0.89**	**15.09**	**0.79**	**14.06**	**0.64**	**13.13**	**0.50**	**10.28**	**-**
OLL	18.99	17.26	15.75	14.38	11.16	12.29	7.61	11.22	5.41	9.58	4.12	-
LLL	21.06	18.43	16.30	16.68	12.92	15.02	9.67	12.27	6.39	10.69	5.17	-
LLnL	5.02	4.37	4.63	3.97	3.19	3.65	2.30	3.26	1.05	2.49	1.00	-
StStA	-	-	-	-	-	-	-	-	-	-	-	1.69
ALL	0.25	-	-	-	-	-	-	-	-	-	-	-

P= palmitic acid; St= stearic acid; O= oleic acid; L= linoleic acid; Ln= linolenic acid; A= arachidic acid.I**:interesterified blend; -: not detected; *average value between duplicate samples injection.

Fig. 1 shows the chromatographic profile of the triacylglycerol composition of the 70:30 SO:FHSBO blend before and after chemical interesterification.

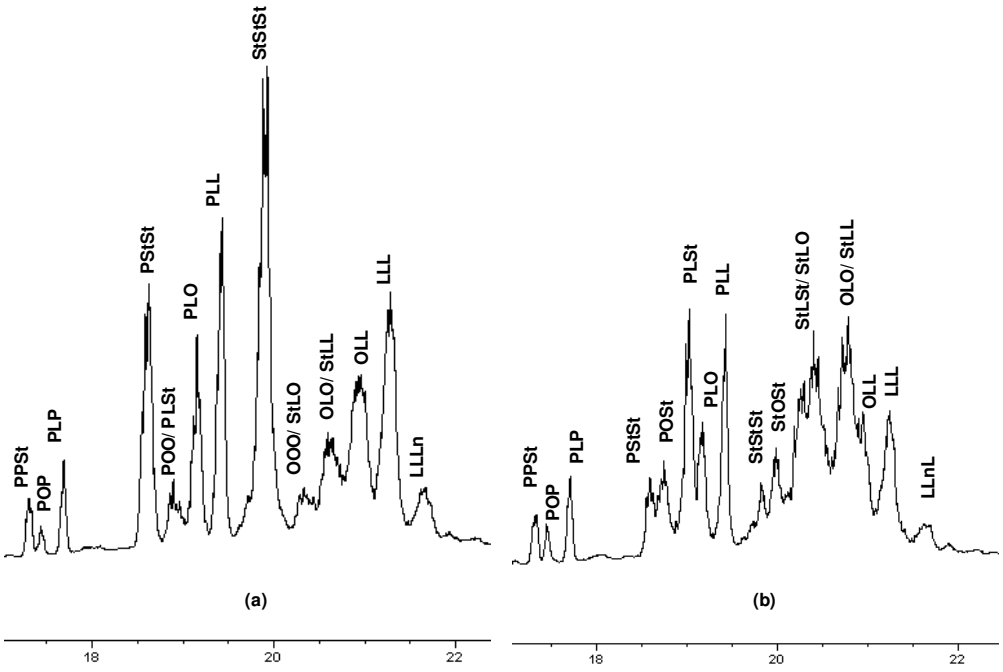

Fig.1. Chromatographic profile of the triacylglycerol composition of the 70:30 SO:FHSBO blend before (a) and after (b) interesterification. P = palmitic acid; St = stearic acid; O = oleic acid; L = linoleic acid; Ln = linolenic acid.

In particular, the percentage increasing in StLO and StLL triacylglycerols and the formation of significant amounts of StLSt and StOSt species endow the interesterified blends with properties of lubricity, aeration and gloss; while the presence of PPSt and PstSt triacylglycerols, even in small proportions, ensures important qualities as regards structure and moisture barrier, contributing to the functionality of the randomized fatty bases (O'Brien, 2004; Ghotra, Dyal, & Narine, 2002). POO and OOO triacylglycerols, on the contrary, were not detected after interesterification in any of the blends. Also, StStSt content declined substantially as a result of randomization, to percentages of 1.45%, 2.37%, 4.18% and 11.47%, respectively, in the interesterified 80:20, 70:30, 60:40 and

50:50 SO: FHSBO blends, corresponding to reductions ranging from 90.58% to 67.65%. StStSt was not detected in the interesterified 90:10 SO:FHSBO blend. Fats with low StStSt content are desirable for food applications, because this triacylglycerol is associated with a disagreeable waxy mouthfeel (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: S_3 (trisaturates), S_2U (disaturates-monounsaturates),U_2S (monosaturates-diunsaturates) and U_3 (triunsaturates), instead of by the individual triacylglycerol species (Petrauskaite et al., 1998; Neff, Byrdwell, & List, 2001). Table 3 shows the triacylglycerol composition of the blends by S_3, S_2U, U_2S and U_3 content, before and after interesterification. The higher concentration of FHSBO in the blends is associated with higher S_3 content and progressive decline in the other classes of triacylglycerols. In all the blends, interesterification produced significant decreases in S_3 triacylglycerol content and, to a lesser extent, in U_3-type triacylglycerols, with a concomitant increase in the percentages of mainly S_2U and U_2S triacylglycerols. Randomization caused the largest variations in S_3 and S_2U triacylglycerol classes for all the blends, which corresponded mainly to joint reductions in StStSt/PStSt triacylglycerols and the formation of StLSt/StOSt triacylglycerols. Khatoon and Reddy (2005) encountered a similar effect on triacylglycerol composition in the interesterification of palm stearin with fats from the species *Magnifera indica* and *Madhuca latifolia*, known as Mango fat and Mowrah butter, respectively (Lipp & Anklam, 1998). List et al. (1995) studied the interesterification of an 80:20 SO:FHSBO blend, with a view to production of margarines and shortenings. S_3, S_2U, U_2S and U_3 contents before the reaction were 23.3%, 2.5%, 27.4% and 45.3%, respectively. After randomization, the percentages of these triacylglycerol classes were, respectively, 3.3%, 20.4%, 46.9% and 28.9%, which are very close to those found in this study.

Table 3. Classes of triacylglycerols in the SO:FHSBO blends, before and after interesterification.

TAG	SO: FHSBO (%w/w)									
(%)	**90:10**	**90:10-I***	**80:20**	**80:20-I**	**70:30**	**70:30-I**	**60:40**	**60:40-I**	**50:50**	**50:50-I**
S_3	13.77	0.60	23.36	3.65	34.32	6.26	44.07	11.58	54.11	24.50
S_2U	3.74	14.68	3.20	24.17	2.65	32.63	2.31	40.45	1.91	39.02
U_2S	31.08	45.39	27.35	43.40	23.05	40.14	19.27	33.75	15.56	25.22
U_3	51.41	39.33	46.09	28.78	39.98	20.96	34.35	14.22	28.42	11.26

I*: interesterified blend.

According to Rodrigues and Gioielli (2003), the properties of fatty foods can be related to the triacylglycerol composition of the fat they contain. The S_3 triacylglycerols, with melting points from 54 to 65°C, and some S_2U triacylglycerols, with melting points from 27 to 42°C, are responsible for these products' structure. The U_2S triacylglycerols are important to their mouthfeel and their functionality at room temperature. The U_3 triacylglycerols, with melting points between -14 and 1°C, contribute to food softness (Wiedermann, 1978; Bessler & Orthoefer, 1983). Thus, the increased S_2U and U_2S content in the SO:FHSBO blends produced by chemical interesterification is associated with enhanced technological functionality, improved sensorial properties and, therefore, greater potential for applying these interesterified bases in foods.

3.3 Melting point. Melting point is a parameter of significant importance for characterizing and developing interesterified fats (Nichols & Sanderson, 2003). Timms (1985) and Karabulut et al. (2004) report that fat moves in the capillary tube when there is approximately 4-5% of solid fat, making it possible to determine the melting point at which the SFC is in this range. Fig. 2 shows the melting point values (°C), by FHSBO content of the starting and interesterified blends, determined by the open capillary tube method and calculated from the SFC curve obtained by NMR, as well as the mathematical correlations between the methods. The melting point values determined by SFC curves are lower than those given by the capillary tube method, but correlation between the methods was good, with coefficients of determination (R^2) greater than 0.99. This corroborates Nuclear Magnetic Resonance as an appropriate method for determining melting points of fats, because it differs in being quick and easy, and can be used to obtain practically instant measurements. In this study, the results will be discussed in terms of the melting points calculated from the SFC curves.

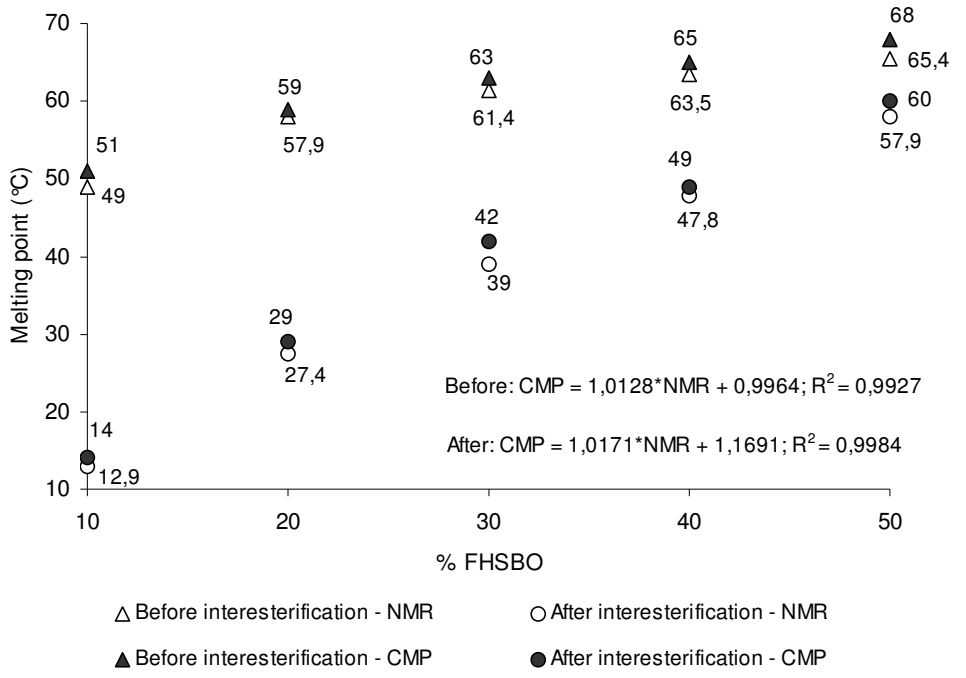

Fig.2. Melting points (°C) of the SO:FHSBO blends before and after chemical interesterification, by FHSBO concentration (%). NMR: melting point (°C) calculated from the SFC curve (Karabulut et al., 2004); CMP: melting point obtained by AOCS Method Cc 3-25 (AOCS, 2004).

Adding FHSBO to SO raised the melting point of the blends to values ranging from 49.0°C to 65.4°C. The greatest increase in melting point among the samples was seen in the 90:10 and 80:20 SO:FHSBO blends.

Interesterification lowered the melting point of all the blends evaluated, by reducing the proportion of high melting point, trisaturated triacylglycerol; and increasing the percentages of disaturated-monounsaturated and monosaturated-diunsaturated triacylglycerols, which have intermediate melting points (Norizzah et al., 2004; Wiedermann, 1978). Other researchers have reported similar results (Petrauskaite et al., 1998; Rodríguez et al., 2001; Lo & Handel, 1983). Rousseau and Marangoni (2002) found a directly proportional relationship between trisaturated triacylglycerol content and melting point. When interesterification is associated with a reduction in the percentages of triacylglycerol

species such as StStSt and PStSt, with melting points of 65°C and 61.1°C, respectively, the decrease in melting point is considerable.

The absolute variation in melting points of the interesterified blends in relation to the original blends decreased with FHSBO concentration, to between 36.0°C and 7.5°C. It was observed that the greater the ratio between the percentages of S_3 and U_3 type triacylglycerols in the blends, the smaller was the effect of interesterification on the change in melting point. This behavior was also observed by Rousseau and Marangoni (1998) in relation to milk fat concentration in blends interesterified with canola oil. According to Rozendall (1992) and Dian et al. (2007) this effect is associated with randomization of highly saturated raw materials (such as fully hydrogenated fats) with liquid oils.

The interesterified blends displayed a wide range of melting points (12.9 to 57.9°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 (Karabulut et al., 2004; Ghotra et al., 2002). Thus the interesterified 90:10 and 80:20 SO:FHSBO blends, with melting points of 12.9 and 27.4°C, fall within this group. In addition, the 90:10 SO:FHSBO blend displays the characteristics of liquid shortenings, which can be readily pumped and bottled at low temperatures and have melting points between 10°C and 15°C (O'Brien, 2004). The interesterified 70:30 SO:FHSBO blend, whose melting point is 39°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. The melting points of the interesterified blends agree with those found by Grimaldi, Gonçalves, and Esteves (2000) in characterizing Brazilian commercial fats for various different industrial purposes, where the great majority of the samples evaluated had melting points ranging from 21.9°C to 49.2°C. A recent study by Karabulut and Turan (2006) found that the melting points of 25 samples of margarines and shortenings ranged from 31.2° to 43.0°C. However, the interesterified 50:50 SO:FHSBO

blend was found to have too high a melting point (57.9°C) for food applications. It would thus seem to be restricted to use as a source of zero-*trans* hardstock (List et al., 1995; Lampert, 2000).

3.4 SFC. SFC curves give good indications of a fat's overall behavior and are useful in formulating and developing new products. The solids content is responsible for many characteristics of margarines and shortenings, including their appearance, ease of packaging, spreadability, oiling out and organoleptic properties (Lida et al., 2002).

Fig. 3 shows SFC curves for the original and interesterified blends obtained using the standard tempering of AOCS Method Cd 16b-93 (AOCS, 2004) and with the following sample tempering history: 2 hours at 0°C and 1 hour at each read temperature. The tempering prescribed by the official method proved suitable for the starting blends, but was not satisfactory for achieving stable crystallization of the interesterified blends, resulting in SFC curves with atypical behavior for the interesterified blends, as can be seen in Fig. 3b. This effect can be seen mainly in the depression in the SFC curve between 20 and 25°C. This behavior has been encountered in our laboratory in a number of SO:FHSBO blend-based interesterified samples, with FHSBO content higher than 20%, interesterified under a variety of reaction parameters. There is no information on this kind of differentiated behavior in the relevant literature. However, the interesterified blends may be regarded as requiring additional tempering time in order to complete polymorphic stabilization, mainly as a result of their triacylglycerol composition, given that the high content of StLSt, StLO and StLL type triacylglycerols, produced by randomization, seems to be associated with the samples' incomplete crystallization stabilization in the time intervals set by AOCS Method Cd 16b-93 (AOCS, 2004), as in the case of certain special fats and cacao butter, which require differential tempering because of high StOSt content (Campos, 2005).

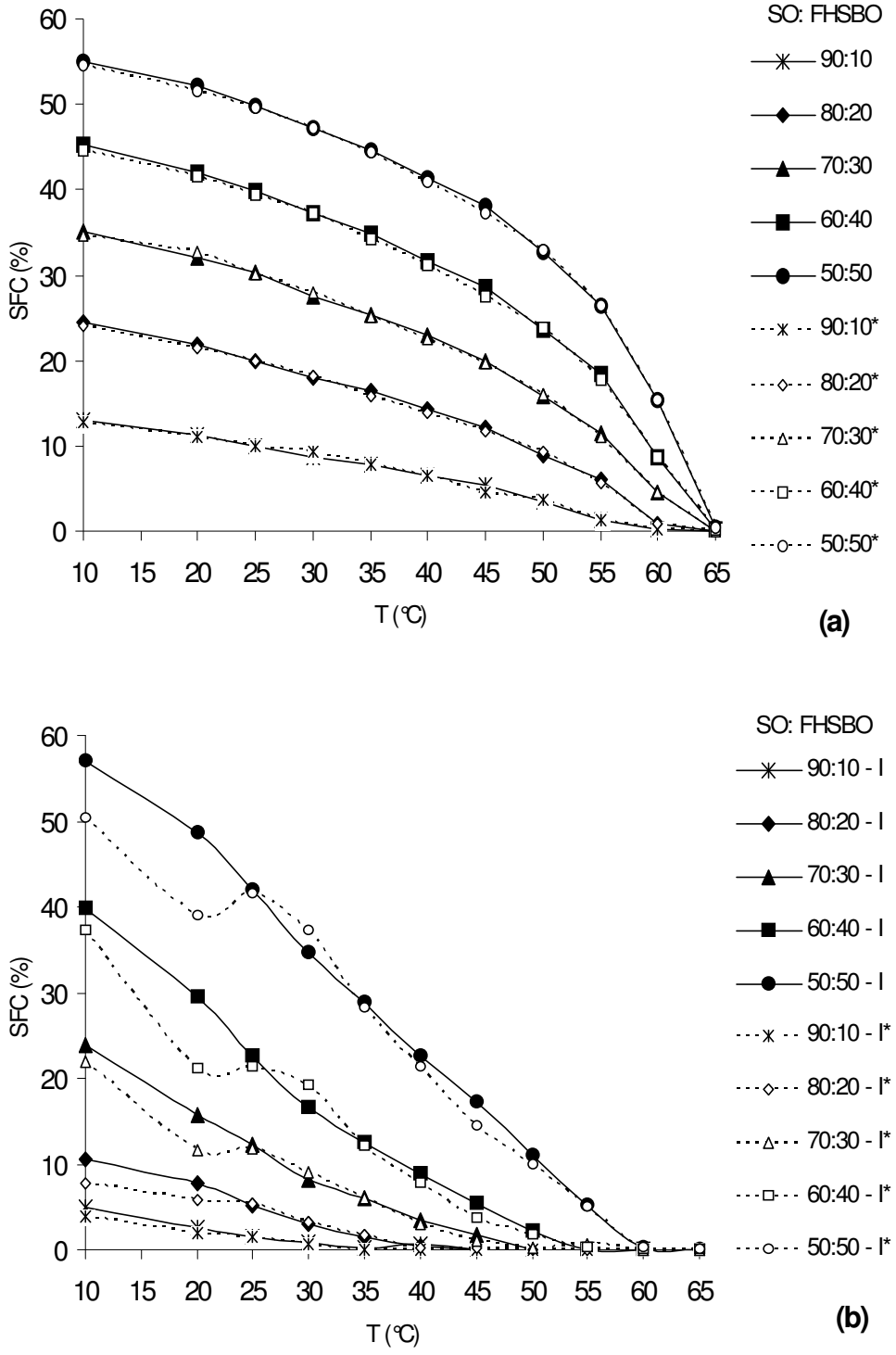

Fig.3. SFC curves of the SO:FHSBO blends, before (a) and after (b) chemical interesterification, by standard tempering* of AOCS Method Cd 16b-93 (AOCS, 2004) and modified tempering (2 hours at 0°C and 1 hour at each read temperature).

According to Dijkstra, Christie, and Knothe (2007), small variations in the temperatures and times used in tempering can result in significant variations in SFC values. Therefore, the tempering conditions were modified accordingly to ensure stable crystallization of the interesterified samples in order to construct the SFC curves. These results were used in the interpretation and discussion of results below.

The original samples gave identical SFC curves for the two tempering methods used. The results of the SFC curves show that the blends have different profiles, covering varying ranges of SFC by temperature. The SFC of the blends was directly proportional to the addition of FHSBO to SO, at all the temperatures considered. At 10°C, the blends showed SFC ranging from 12.89% to 55.09%, which decreased non-linearly until melting completely at between 60°C and 65°C. The greatest decrease in SFC occurred from 50°C onwards, due to the large proportion of trisaturated triacylglycerols that melt and solubilize at relatively high temperatures.

Interesterification resulted in decreased SFC in the blends at all temperatures. At 35°C, the SFC of the blends ranged from 7.75 to 44.70%; after randomization, the corresponding values were 0.28 to 28.72%. This effect is associated with a reduction in trisaturated triacylglycerols (PStSt and StStSt) and a simultaneous increase in percentages of disaturated-monounsaturated and monosaturated-diunsaturated triacylglycerols, represented mainly by StOSt/StLSt and StLO/StLL species, respectively (Fattahi-far et al., 2006; Rousseau & Marangoni, 1998; Rousseau, Forestiere, Hill, & Marangoni, 1996). Due to the formation of mixed triacylglycerols with intermediate melting point, the interesterified blends showed a marked decline in SFC between 25°C and 45°C, and the format of the curves was significantly modified by 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. According to Rousseau and Marangoni (1998), the linearization of SFC curves after randomization 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 orignal blends were observed at 20, 30, 40, 45 and 50°C for the 90:10, 80:20, 70:30, 60:40 and 50:50 SO:FHSBO blends, respectively, as a result of the high proportion of triacylglycerols in the randomized blends 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 produced by the increase in saturated fatty acids.

Small alterations in SFC between 15 and 25°C characterize plastic fats (Leissner et al., 1991). The plasticity of the interesterified blends diminished with increasing FHSBO concentration. The interesterified 90:10 SO:FHSBO blend showed low SFC at all the temperatures considered, endowing this sample with low plasticity. According to Petrauskaite et al. (1998), this can be explained by its low percentage of saturated fatty acids (24.32%), which should be greater than 25-30% in order to assure minimum plastic properties.

The alterations in the SFC profiles resulting from interesterification can be correlated with the melting point results. Increased FHSBO concentration produced smaller alterations in the SFC profiles of the interesterified blends as compared with the originals, in the same way that it reduced the variation among the melting points of the blends before and after the reaction.

Identification of applications for new fats is guided initially by correlating their physical properties with the properties of commercial fats for specific applications. The SFC profile, particularly, has important implications for the characteristics of margarines and shortenings at various temperatures, given that it is the main tool for specifying and selecting fats for food applications (Wassell & Young, 2007).

At low temperatures (4 a 10°C), SFC gives an indication of the fat's spreadability at refrigeration temperatures, which should not exceed 32% to 10°C (Lida & Ali, 1998; Wassell & Young, 2007). The interesterified 60:40 SO:FHSBO blend had well above 32% SFC at 10°C, indicating that it does not offer satisfactory spreadability for use in refrigerated products. SFC between 20 and 22°C is related to the product's stability and its resistance to oiling-out and should be above 10% (Lida & Ali, 1998; Wassell & Young, 2007). The interesterified 70:30 and 60:40 SO:FHSBO blends fall within this group of fats. The percentage of solid fat above 25°C represents the hardness of a specific fat, while the values between 35 and 37°C demonstrate the potential for releasing aromas during melting (Lida & Ali, 1998; Wassell & Young, 2007). Therefore, the interesterified 70:30 and 60:40 SO:FHSBO blends are regarded as suited mainly to endowing products with structure and the 80:20 and 90:10 SO:FHSBO blends, with lower SFC values at 1% to 37°C, are regarded as offering good melting characteristics at body temperature (Karabulut et al., 2004).

Petrauskaite et al. (1998), Karabulut et al. (2004), Karabulut and Turan (2006), Ghotra et al. (2002), Wassell and Young (2007), Grimaldi et al. (2000), Timms (2003a), Dijkstra (2007) and Lee et al. (2008) present SFC curves for various fats and shortenings for a variety of industrial purposes. When compared with the SFC profiles encountered in the literature, the interesterified 90:10 SO:FHSBO blend exhibits characteristicas similar to those of frying shortenings. As the frying process deposits fat on the surface of the foods, frying shortenings should be free of solids at body temperature in order to avoid the sensation of graininess in the mouth (Ghotra et al., 2002). The 80:20 SO:FHSBO blend had a melt profile close to those of fatty bases for table margarine production and meets the SFC requirements to assure spreadability under refrigeration and complete melting at body temperature for margarines of this type (Karabulut & Turan, 2006).

The interesterified 70:30 SO:FHSBO blend exhibited a SFC profile and plasticity suitable for use in bakery and confectionery shortenings. According to Wassell and Young

(2007), shortenings for these purposes should create stable emulsions, but have good creaming properties, which can be obtained when SFC ranges from 15 to 20% at 20°C, as is the case with this sample.

Meanwhile, the interesterified 60:40 SO:FHSBO blend has a SFC profile similar to most all-purpose shortenings and biscuit-filling fats, which should offer high SFC at room temperature in order to endow suitable crystalline structure and resist deformation (Ghotra et al., 2002; Woerfel, 1995). However, the SFC of this blend at 40°C is 8.91%. In data from the relevant literature, similar SFC values are encountered at this temperature for fats for use in tropical regions and lower values for most shortenings and fats for this purpose. The possibility should thus be considered that waxy mouthfeel may be associated with direct use of this interesterified blend. According to Jeyarani and Reddy (2003), List et al. (1995) and Rousseau et al. (1996), in many cases, an interesterified fat should be diluted with small proportions of liquid oil in order to adjust its physical properties. One sure option to avoid this problem would thus be to add small amounts (1 to 5%) of liquid oil, such as soybean oil, to this fraction (Dijkstra, 2007).

The interesterified 50:50 SO:FHSBO blend exhibited significantly high SFC between 35 and 40°C (28.72 to 22.55%) and at 50°C (10.91%), precluding its use at body temperature or even direct application as a fatty base for shortenings (Karabulut et al., 2004; Grimaldi et al., 2000; Dijkstra, 2007).

3.5 Isosolid diagrams. When oils and fats with differing compositions are blended, a number of behaviors may be observed: solid solutions (compatibility among the fats), monotectic behavior or eutectic behavior (Timms, 2003b). The isosolid diagrams are useful in studying compatibility among different fats. The isosolid lines represent the temperature at which the SFC of a fat or blend of fats is constant (Campos, 2005). Fig. 4 shows the isosolid diagrams of the original and interesterified blends.

The isosolid diagrams for the original blends indicate monotectic behavior, which prevails in mixed systems of fats with very different melting points (differences greater than 20°C). Monotectic behavior occurs when high melting point triacylglycerols are solubilized in the liquid triacylglycerol components (Rousseau & Marangoni, 1998; Timms, 2003b). In the original blends, FHSBO, with a melting point of 72°C, is solubilized in the SO, whose melting point is around -22°C (O'Brien, 2004). Monotectic behavior is associated with non-linear isosolid lines resulting from dilution of the solid fat by the liquid oil in a binary blend, as seen in Fig. 4a (Braipson-Danthine & Deroanne, 2006). This type of system was observed by Rousseau and Marangoni (2002) for lard/canola oil and palm oil/soybean oil blends and by Braipson-Danthine and Deroanne (2006) for canola oil/partly hydrogenated canola oil blends. When the temperature and the percentage of FHSBO in the blends increase, the isosolid lines converge to the right. The more closely spaced isosolid lines due to increased FHSBO concentration in the blends indicate that the effect of diluting is smaller when the trisaturated triacylglycerol content increases (Rousseau & Marangoni, 1998). This behavior was observed by Humphrey and Narine (2005) for a soybean oil/fully hydrogenated canola oil blend.

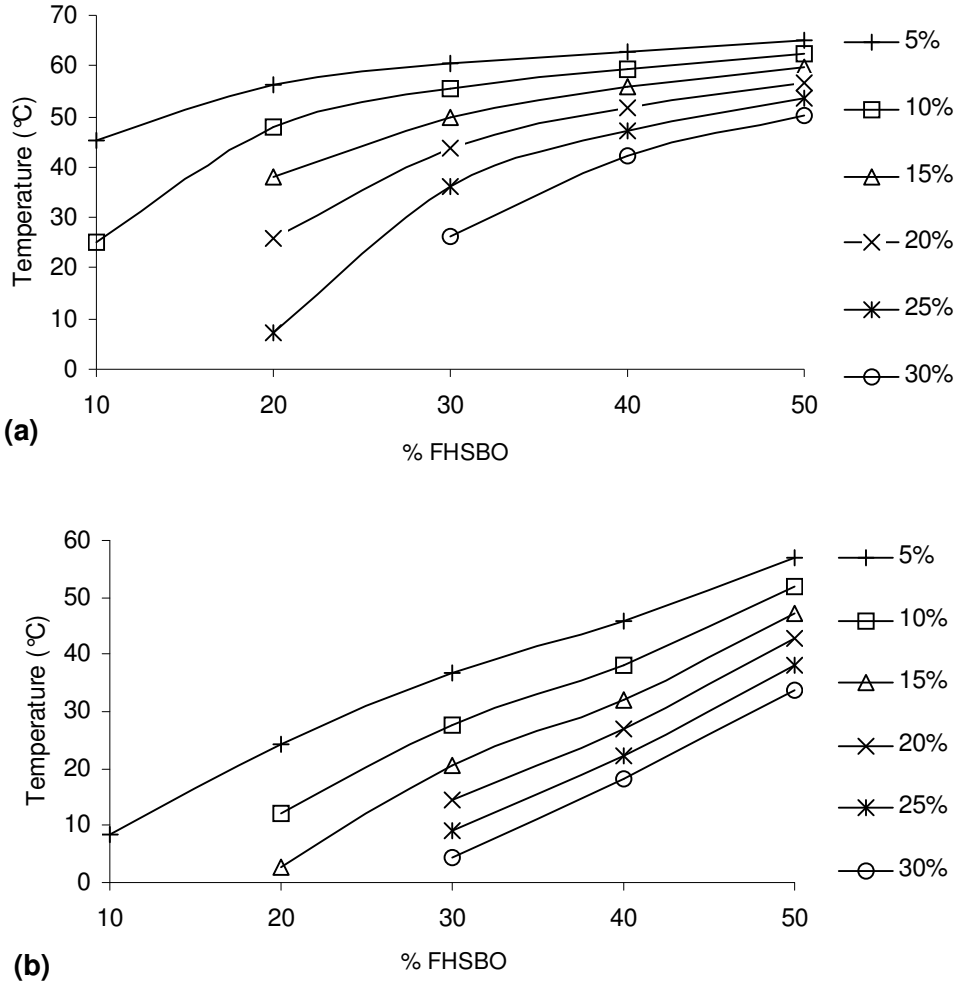

Fig. 4. Isosolid diagram of the blends before (a) and after (b) chemical interesterification.

After interesterification, the substantial decrease in temperature recorded in the diagram indicates greater intersolubility in the solid state among the triacylglycerol species present, as a result of the greater heterogeneity in the triacylglycerol composition and increased proportion of triacylglycerols with intermediate melting points (Rousseau & Marangoni, 1998). Randomization resulted in linear evolution of the isosolid lines, which characterizes totally compatible interesterified blends and the formation of continuous solid solutions, as seen in Fig. 4b (Braipson-Danthine & Deroanne, 2003a).

No depressions were observed in the isosolid lines for the original and interesterified blends. Depressions in isosolid diagrams characterize the formation of a eutectic system, which results in liquefaction of the blend in specific compositions, representing problems in fat applications where consistency and SFC are important (Humphrey & Narine, 2005).

3.6 Consistency. The consistency of a semisolid lipid system is a function of its SFC content and the microstructural properties associated with that content. Modification of SFC and crystalline network by interesterification affects the sensorial properties and technological performance of fats, thus directing their application in food products (Piska et al., 2006).

Table 4 shows the consistency of the blends with temperature, represented as yield value (gf/cm^2), before and after interesterification. It was not feasible to ascertain the consistency of the 90:10 SO:FHSBO and 90:10 SO:FHSBO-I blends, because of these samples' low melting point.

Generally, interesterification led to a reduction in yield values for all the blends at all temperatures considered, with the sole exceptions of the 70:30 and 60:40 SO:FHSBO, where consistency increased at 5°C. Consistency 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 (Deman, 1983).

Consistency proved proportionally dependent on FHSBO concentration in the blends, before and after randomization. This behavior was also observed by Piska et al. (2006) in developing shortenings from various fully hydrogenated fats. According to Chiu and Gioielli (2002) and Larsson and Quinn (1994), the increase of saturated fatty acids content of a sample strongly influences its consistency, due to their high melting point.

The greatest alterations in yield values of the interesterified blends as compared with the original blends was observed at 15, 35, 50 and 55°C for the 80:20, 70:30, 60:40 and 50:50 SO:FHSBO blends, respectively. At 25°C, the original blends gave yield values

ranging from 719.2 to 20535.7gf/cm^2; after interesterification, these values ranged from 0 to 8709.9 gf/cm^2.

According to Haighton (1959), a fat is plastic and spreadable at yield values from 200 to 800gf/cm^2. The interesterified 80:20 SO:FHSBO blend, with a yield value of 309.6 gf/cm^2 at 10°C, has satisfactory plasticity and spreadability properties for use at refrigeration temperatures, in addition to meeting SFC and melting point requisites for use in margarines, as mentioned earlier. Between 25 and 30°C, the interesterified 70:30 SO:FHSBO blend was satisfactorily spreadable (yield values between 800 and 1000 gf/cm^2), but exhibits ideal plasticity at 35°C, which is important for sensorial properties such as mouthmelt 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 60:40 SO:FHSBO blend can be classified as hard at room temperature, with a yield value between 3352.4 and 2519.6 gf/cm^2 in the 25 to 35°C interval; and is just above the spreadability limit (1500 gf/cm^2) at 40°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. With a yield value below 1500 gf/cm^2 only from 45°C up, the interesterified 50:50 SO:FHSBO blend can be considered too hard for direct application in foods (Haighton, 1959).

A number of recent studies have shown a direct relation between SFC and fat consistency (Rodrigues et al., 2003; Silva & Gioielli, 2006; Braipson-Danthine & Deroanne, 2006; Shi, Liang, & Hartel, 2005; Gioielli, Simões, & Rodrigues, 2003; Braipson-Danthine & Deroanne, 2003b, 2004). Fig. 5 shows the relation between the logarithm of yield value and the logarithm of SFC for the samples at the temperatures at which consistency was evaluated, as proposed by Braipson-Danthine and Deroanne (2003b, 2004, 2006) for studying these properties. The relationship is not shown for the interesterified 80:20 SO:FHSBO blend, because the consistency of this sample was measurable only up to 15°C, with 10°C as the only temperature in common with SFC determination.

Table 4. Yield value (gf/cm^2) of the SO: FHSO blends before and after interesterification.

Yield value (gf/cm^2)								
SO: FHSBO (%w/w)								
T (°C)	**80:20**	**80:20-I***	**70:30**	**70:30-I**	**60:40**	**60:40-I**	**50:50**	**50:50-I**
5	1891.3	1163.7	6829.5	7601.0	12647.1	17634.7	23789.8	21560.1
10	1668.9	309.6	5946.4	3120.1	11490.4	8174.5	23648.7	15552.4
15	1303.8	105.4	4791.2	1774.4	10904.2	7634.1	21527.9	14298.5
20	1170.4	-	4610.0	1263.6	10456.3	5476.3	20756.4	11737.4
25	719.2	-	4278.4	1069.1	10181.9	3352.4	20535.7	8709.9
30	601.7	-	3466.5	803.1	7473.6	2987.4	17248.8	6374.6
35	273.2	-	2234.4	273.4	5866.4	2519.6	13481.5	4402.7
40	-	-	2019.3	-	5222.1	1576.2	11735.7	2154.8
45	-	-	1606.9	-	4976.8	698.6	10709.8	1881.2
50	-	-	254.1	-	2591.4	280.4	5514.3	925.1
55	-	-	-	-	1413.3	-	2862.0	435.4
60	-	-	-	-	366.4	-	854.2	226.4

I*: interesterified blend.

A significant ($p<0.05$) linear relationship was observed between the logarithm of consistency and the logarithm of SFC of the samples, over the temperature range considered, with a correlation coefficient (R^2) greater than 0.9 in all cases. Braipson-Danthine & Deroanne (2003b, 2004, 2006) also observed this type of relationship for several binary and ternary blends of various oils and fats. They remark that significant linear relations between these magnitudes indicate that consistency is strongly dependent on SFC and can be used for predictive purposes.

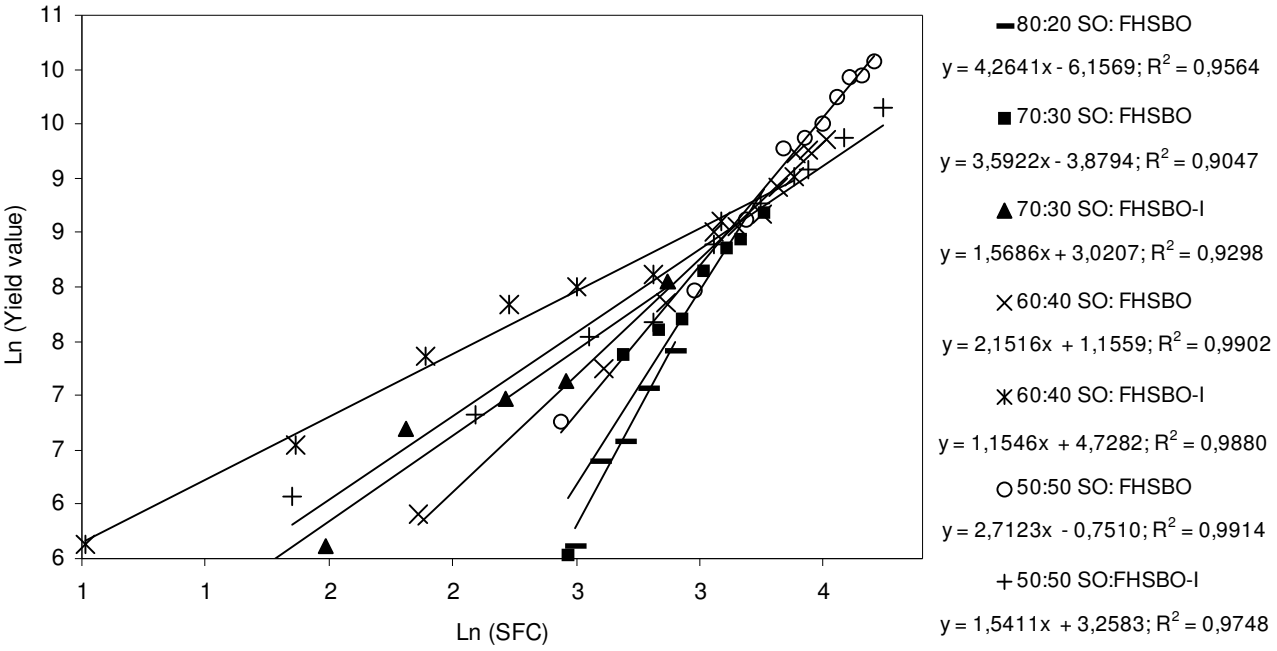

Fig.5. Linear relations between the logarithm of yield value and the logarithm of SFC measurements for the original and interesterified blends

4. Conclusion

This study demonstrated that chemical interesterification significantly modified the triacylglycerol composition, and consequently the melting point, solid fat content and consistency properties of the SO:FHSBO blends. The interesterified blends displayed characteristics suited to a variety of industrial purposes, offering alternatives to partly

hydrogenated fats. The interesterified 90:10, 80:20, 70:30 and 60:40 SO:FHSBO blends displayed characteristics suited to application as liquid shortening, table margarine, bakery/confectionery fat and all-purpose shortenings/ biscuit-filling base, respectively.

References

Antoniosi Filho, N., Mendes, O. L., & Lanças, F. M. (1995). Computer prediction of triacylglycerol composition of vegetable oils by HRGC. *Chromatographia, 40*, 557-562.

AOCS. (2004). *Official methods and recommended practices of the American Oil Chemists' Society*. Champaign: American Oil Chemists' Society.

Bessler, T. R., & Orthoefer, F. T. (1983). Providing lubricity in food fat systems. *Journal of American Oil Chemists' Society, 60*, 1765-1768.

Braipson-Danthine, S., & Deroanne, C. (2003a). Blending of hydrogenated low-erucic acid rapeseed oil, low-erucic acid rapeseed oil, and hydrogenated palm oil or palm oil in the preparation of shortenings. *Journal of American Oil Chemists' Society , 80*, 1069-1075.

Braipson-Danthine, S., & Deroanne, C. (2003b). Physical and textural characteristics of hydrogenated low-erucic acid rapeseed oil and low-erucic acid rapeseed oil blends. *Journal of American Oil Chemists' Society, 80*, 109-114.

Braipson-Danthine, S., & Deroanne, C. (2004). Influence of SFC, microstructure and polymorphism on texture (hardness) of binary blends of fats involved in the preparation of industrial shortenings. *Food Research International, 37*, 941-948.

Braipson-Danthine, S., & Deroanne, C. (2006). Determination of solid fat content (SFC) of binary fat blends and use of these data to predict SFC of selected ternary fat blends containing low-erucic rapeseed oil. *Journal of American Oil Chemists' Society, 83*, 571-581.

Campos, R. (2005). Experimental Methodology. In A. G. Marangoni (Ed.). *Fat Crystal Networks*, (pp.267-349). New York: Marcel Dekker.

Chiu, M. C., & Gioielli, L. A. (2002). Consistência da gordura abdominal de frango, de suas estearinas e de suas misturas binárias com toucinho. *Revista Brasileira de Ciências Farmacêuticas, 38*, 95-105.

Chiu, M. C., Gioielli L. A., & Grimaldi R. (2008). Lipídios estruturados obtidos a partir da mistura de gordura de frango, sua estearina e triacilgliceróis de cadeia média. I – composição em ácidos graxos e triacilgliceróis. *Química Nova, 31*, 232-237.

Deman, J. M. (1983). Consistency of fats: a review. *Journal of American Oil Chemists' Society, 60*, 82-87.

Dian, N. L. H. M., Sundram, K., & Idris, N. A. (2007). 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, 109*, 147-156.

Dijkstra, A. J. (2007). Modification processes and food uses. In F. D. Gunstone, J. L. Harwood, & A. J. Dijkstra (Eds.). *The Lipid Handbook*, (3rd ed.) (pp.263-353). New York: CRC Press.

Dijkstra, A. J., Christie, W. W., & Knothe, G. (2007). Analysis. In F. D. Gunstone, J. L. Harwood, & A. J. Dijkstra (Eds.). *The Lipid Handbook*, (3rd ed.) (pp.415-455). New York: CRC Press.

Dirienzo, M. A., Lemke, S. L., Petersen, B. J., & Smith, K. M. (2008). Effect of substitution of high stearic low linolenic acid soybean oil for hydrogenated soybean oil on fatty acid intake. *Lipids, 43*, 451-456.

Enig, M. G. (1996). *Trans* fatty acids in diets and databases. *Cereal Foods World, 41*, 58-63.

Erickson, D. R. (1995). *Practical handbook of soybean processing and utilization*. Champaign: American Oil Chemists' Society.

Fattahi-far, E., Sahari, M. A., & Barzegar, M. (2006). Interesterification of tea seed oil and its application in margarine production. *Journal of American Oil Chemists' Society, 83*, 841-845.

Ghotra, B. S., Dyal, S. D., & Narine, S. S. (2002). Lipids shortenings: a review. *Food Research International* , 35, 1015-1048.

Gioielli, L. A., & Baruffaldi, R. (1988). Acompanhamento da reação de interesterificação de gordura de babaçu e azeite de dendê. *Revista de Farmácia e Bioquímica da Universidade de São Paulo, 24*, 29-38.

Gioielli, L. A., Simões, I. S., & Rodrigues, J. N. (2003). Crystal morphology and interactions of binary and ternary mixtures of hydrogenated fats. *Journal of Food Engineering, 57*, 347-355.

Grimaldi, R., Gonçalves, L. A. G., & Ando, M. Y. (2005). Otimização da reação de interesterificação química de óleo de palma. *Química Nova, 28*, 633-636.

Grimaldi, R., Gonçalves, L. A. G., & Esteves, W. (2000). Características de gorduras comerciais brasileiras. *Brazilian Journal of Food Technology, 3*, 159-164.

Gunstone, F. D., & Harwood, J. L. (2007). Occurrence and characterisation of oils and fats. In F. D. Gunstone, J. L. Harwood, & A. J. Dijkstra (Eds.). *The Lipid Handbook*, (3rd ed.) (pp.37-141). New York: CRC Press.

Gurr, M. (1990). *Trans* fatty acids and health: an update. *Lipid Technology, 2*, 105-107.

Haighton, A. J. (1959). The measurement of the hardness of margarine and fats with cone penetrometers. *Journal of American Oil Chemists' Society, 36*, 345-348.

Hartman, L., & Lago, R. (1973). Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practice, 22*, 475-476.

Haummann, B. F. (1994). Tools: hydrogenation, interesterification. *Inform, 5*, 668-678.

Humphrey, K. L., & Narine, S. S. (2004). A comparison of lipid shortening functionality as a function of molecular ensemble and shear: Crystallization and melting. *Food Research International, 37*, 11-27.

Humphrey, K. L., & Narine, S. S. (2005). Lipid Phase Behavior. In A. G. Marangoni (Ed.). *Fat Crystal Networks*, (pp.83-115). New York: Marcel Dekker.

Hunter, J. E. (2005). Dietary levels of *trans*-fatty acids: basis for health concerns and industry efforts to limit use. *Nutrition Research, 25*, 499-513.

Jeyarani, T., & Reddy, S. Y. (2003). Preparation of plastic fats with zero *trans* FA from palm oil. *Journal of American Oil Chemists' Society, 80*, 1107-1113.

Karabulut, I., & Turan, S. (2006). Some properties of margarines and shortenings marketed in Turkey. *Journal of Food Composition and Analysis, 19*, 55-58.

Karabulut, I., Turan, S., & Ergin, G. (2004). Effects of chemical interesterification on solid fat content and slip melting point of fat/oil blends. *European Food Research & Technology, 218*, 224-229.

Khatoon, S, (2000), Semi-solid fat by interesterification of red palm oil with other vegetable oils. *Journal of Food Lipids, 7*, 83-93.

Khatoon, S., & Reddy, S.R.Y. (2005). Plastic fats with zero *trans* fatty acids by interesterification of mango, mahua and palm oils. *European Journal of Lipid Science and Technolology*, *107*, 786-791.

Kok, L. L., Fehr, W. R., Hammond, E. G., & White, P. J. (1999). *Trans*-free margarine from highly saturated soybean oil. *Journal of American Oil Chemists' Society, 76*, 1175-1181.

Lampert, D. (2000). Process and products of interesterification. In R. D. O'Bryen, W. E. Farr, & P. J. Wan (Eds.). *Introduction to fats and oils technology*, (pp.208-222). Champaign: AOCS Press.

Larsson, K., & Quinn, P. J. (1994). Physical properties: structural and physical characteristics. In F. D. Gunstone, J. L. Harwood, & F.B. Padley (Eds.). *The Lipid Handbook*, (2nd ed.) (pp.401-460). London: Chapman and Hall.

Lee, J. H., Akoh, C. C., & Lee, T. (2008). Physical properties of *trans*-free bakery shortening produced by lipase-catalyzed interesterification. *Journal of American Oil Chemists' Society, 85*, 1-11.

Leissner, R., Hogenbirk, G., Nilsson, F., Petersson, B., Alander, F., Helmbring, G., Stenmyr, C., Linghede, M., & Gunnerdal, F. (1991). *Cocoa butter alternatives*. Karlshams: Karlshams Oils and Fats Academy.

Lichtenstein, A. (1993). *Trans* fatty acids, blood lipids and cardiovascular risk: where do we stand? *Nutrition Reviews, 51*, 340-343.

Lida, A. M. D. N., & Ali, A. R. (1998). Physico-chemical characteristics of palm-based oil blends for the production of reduced fat spreads. *Journal of American Oil Chemists' Society, 75*, 1625-1631.

Lida, A. M. D. N., Sundram, K., Siew, W. L., Aminah, A., & Mamot, S. (2002). 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, 79*, 1138-1143.

Lipp, M., & Anklam, E. (1998). Review of cocoa butter and alternative fats for use in chocolate – part A. Compositional data. *Food Chemistry, 62*, 73-97.

List, G. R., Emken, E. A., Kwolek, W. F., Simpson, T. D., & Dutton, H. J. (1977). Zero *trans* margarines: preparation, structure, and properties of interesterified soybean oil-soy trisaturate blends. *Journal of American Oil Chemists' Society, 74*, 408-413.

List, G. R., Mounts, T. L., Orthoefer, F., & Neff, W. E. (1995). Margarine and shortening oils by interesterification of liquid and trisaturated triglycerides. *Journal of American Oil Chemists' Society, 72*, 379-382.

Lo, Y. C., & Handel, A. P. (1983). Physical and chemical properties of randomly interesterified blends of soybean oil and tallow for use as margarine oils. *Journal of American Oil Chemists' Society, 60*, 815-818.

Marangoni, A, G., & Rousseau, D. (1998a). The influence of chemical interesterification on physicochemical properties of complex fat systems. 3. Rheology and fractality of the crystal network. *Journal of American Oil Chemists' Society, 75*, 1633-1636.

Marangoni, A. G., & Rousseau, D. (1998b). The influence of chemical interesterification on physicochemical properties of complex fat systems. 1. Melting and crystallization. Journal *of American Oil Chemists' Society, 75*, 1265-1271.

Mensink, R. P. (2005). Effects of stearic acid on plasma lipid and lipoproteins in humans. *Lipids, 40*, 1201-1205.

Mensink, R. P., & Katan, M. B. (1990). Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *New England Journal of Medicine, 323*, 439-445.

Neff, W. E., Byrdwell, W. C., & List, G. R. (2001). Triacylglycerol structures of food fats high in saturated acids by HPLC and mass spectrometry. *Journal of Liquid Chromatography and Related Technologies, 24*, 837-854.

Nichols, D. S., & Sanderson, K, (2003). The nomenclature, structure and properties of food lipids. In Z. E. Sikorski, & A. Kolakowska (Eds.) *Chemical and functional properties of food lipids*, (pp.29-61). New York: CRC Press.

Nielsen, K. (2006). Is the quality and cost of food affected if industrially produced *trans* fatty acids are removed? *Atherosclerosis supplements, 7*, 61-62.

Norizzah, A. R., Chong, C. L., Cheow, C. S., & Zaliha, O. (2004) Effects of chemical interesterification on physicochemical properties of palm stearin and palm kernel olein blends. *Food Chemistry, 86*, 229-235.

O'Brien, R. D. (2004). *Fats and Oils – Formulating and Processing for Applications*. New York: CRC Press.

Petrauskaite, V., De Greyt, W., Kellens. M., & Huyghebaert, A. (1998). Physical and chemical properties of *trans*-free fats produced by chemical interesterification of vegetable oil blends. *Journal of American Oil Chemists' Society, 75*:489-493.

Piska, I., Zárubová, M., Louzecký, T., Karami, H., & Filip, V. (2006). Properties and crystallization of fat blends. *Journal of Food Engineering, 77*, 433-438.

Ramli, N., Said, M., & Loon, N. T. (2005). Physicochemical characteristics of binary mixtures of hydrogenated palm kernel oil and goat fat milk. *Journal of Food Lipids, 12*, 243-260.

Rao, R., & Lokesh, B. R. (2003). TG containing stearic acid, synthesized from coconut oil, exhibit lipidemic effects in rats similar to those of cocoa butter. *Lipids, 38*, 913-918.

Reyes-Hernandez, J., Dibildox-Alvarado, E., Charo-Alonso, M. A., & Toro-Vazquez, J. (2007). Physicochemical and rheological properties of crystallized blends containing *trans*-free and partially hydrogenated soybean oil. *Journal of American Oil Chemists' Society, 84*, 1081-1093.

Ribeiro, A. P. B., Moura, J. M. L. N., Grimaldi, R., & Gonçalves, L. A. G. (2007). Interesterificação química: alternativa para obtenção de gorduras zero *trans*. *Química Nova, 30*, 1295-1301.

Rodrigues, J. N., & Gioielli, L. A. (2003). Chemical interesterification of milkfat and milkfat-corn oil blends. *Food Research International, 36*, 149-159.

Rodrigues, J. N., Gioielli, L. A., & Anton, C. (2003) Propriedades físicas de lipídios estruturados obtidos de misturas de gordura do leite e óleo de milho. *Ciência e Tecnologia de Alimentos, 23*, 226-233.

Rodríguez, A., Castro, E., Salinas, M. C., López, R., & Miranda, M. (2001). Interesterification of tallow and sunflower oil. *Journal of American Oil Chemists' Society, 78*, 431-436.

Rousseau, D., & Marangoni, A. G. (1998). The effects of interesterification on the physical properties of fats. In A. G. Marangoni, & S. S. Narine (Eds.) *Physical Properties of Lipids*, (pp.479-565). Boca Raton: CRC Press.

Rousseau, D., Forestiere, K., Hill, A. R., & Marangoni, A. G. (1996). Restructuring butterfat through blending and chemical interesterification. 1. Melting behavior and triacylglycerol modifications. *Journal of American Oil Chemists' Society, 73*, 963-972.

Rousseau. D., & Marangoni, A. G. (2002). Chemical interesterification of food lipids: theory and practice. In C.C. Akoh, & D. B. Min (Eds.) *Food Lipids: Chemistry, Nutrition, and Biotechnology*, (pp.301-335). Boca Raton: CRC Press.

Rozendall, A. (1992). Interesterification of oils and fats. *Inform, 3*, 1232-1237.

Sampath, H., & Ntambi, J. M. (2005). The fate and intermediary metabolism of stearic acid. *Lipids, 40*, 1187-1191.

Sanders, T. A. B., & Berry, S. E. E. (2005). Influence of stearic acid on postprandial lipemida and hemostatic function. *Lipids, 40*, 1221-1227.

Shi, Y., Liang, B., & Hartel, R. W. (2005). Crystal morphology, microstructure, and textural properties of model lipid system. *Journal of American Oil Chemists' Society, 82*, 399-408.

Silva, R. C., & Gioielli, L. A. (2006). Propriedades físicas de lipídios estruturados obtidos a partir de banha e óleo de soja. *Revista Brasileira de Ciências Farmacêuticas, 42*, 223-235.

Statistica. (2002). *Statistica v.6.0. for Windows Vol. IV*. Tulsa: Statsoft Inc.

Tholstrup, T. (2005). Influence of stearic acid on hemostatic risk factors in humans. *Lipids, 40*, 1229-1235.

Timms, R. E. (1985). Physical properties of oils and mixtures of oils. *Journal of American Oil Chemists' Society*, *62*, 241-248.

Timms, R. E. (2003a). *Confectionery fats handbook*. Bridgwater: The Oily Press.

Timms, R. E. (2003b). Phase behavior of fats and their mixtures. *Progress in Lipid Research, 23*, 1-38.

Wassell, P., & Young, N. W. G. (2007). Food applications of *trans* fatty acids substitutes. *International Journal of Food Science and Technology, 42*, 503-517.

Wiedermann, L. H. (1978). Margarine and margarine oil, formulation and control. *Journal of American Oil Chemists' Society, 55*, 823-829.

Woerfel, J. B. (1995). Formulation of soy products. *Grasas y Aceites, 46*, 357-365.

Zeitoun, M. A. M., Neff, W. E., List, G. R., & Mounts, T. L. (1993). Physical properties of interesterified fat blends. *Journal of American Oil Chemists' Society, 70*, 467-471.

4.

THERMAL BEHAVIOR, MICROSTRUCTURE, POLYMORPHISM AND CRYSTALLIZATION PROPERTIES OF ZERO *TRANS* FATS FROM SOYBEAN OIL AND FULLY HYDROGENATED SOYBEAN OIL

Ana Paula Badan Ribeiro, Renato Grimaldi, Luiz Antonio Gioielli, Adenilson Oliveira dos Santos, Lisandro Pavie Cardoso, Lireny Aparecida Guaraldo Gonçalves

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Thermal behavior, microstructure, polymorphism and crystallization properties of zero trans fats from soybean oil and fully hydrogenated soybean oil

Abstract

Blends of soybean oil (SO) and fully hydrogenated soybean oil (FHSBO), with 10, 20, 30, 40 and 50% (w/w) FHSBO content were interesterified under the following conditions: 20 minutes reaction time, 0.4% sodium methoxide catalyst, 500 rpm stirring speed, 100°C. The original and interesterified blends were examined for triacylglycerol composition, thermal behavior, microstructure, crystallization kinetics and polymorphism. Interesterification produced substantial rearrangement of the triacylglycerol species in all the blends, reduction of trisaturated triacylglycerol content and increase in monounsaturated-disaturated and diunsaturated-monosaturated triacylglycerols. Evaluation of thermal behavior parameters showed linear relations with FHSBO content in the original blends. Blend melting and crystallization thermograms were significantly modified by the randomization. Interesterification caused significant reductions in maximum crystal diameter in all blends, in addition to modifying crystal morphology. Characterization of crystallization kinetics revealed that crystal formation induction period (τ_{SFC}) and maximum solid fat content ($SFC_{máx}$) were altered according to FHSBO content in the original blends and as a result of the random rearrangement. Changes in Avrami constant (k) and exponent (n) indicated, respectively, that – as compared with the original blends – interesterification decreased crystallization velocities and modified crystallization processes, altering crystalline morphology and nucleation mechanism. 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.

Key-words: chemical interesterification, low trans fats, thermal behavior, microstructure, crystallization kinetics, polymorphism.

Introduction

Chemical interesterification of blends of fully hydrogenated vegetable oils with liquid oils is currently the most versatile option for producing low *trans* fats for a diversity of industrial purposes.[1,2] Given its economic importance and wide availability, soybean oil (SO) is a suitable raw material for manufacturing *trans* fatty acid-free fat fractions. In order to raise the melting point of these fractions, fully hydrogenated soybean oil (FHSBO), which is also zero *trans*, has proven highly useful.[3,4] As hardstock FHSBO offers the advantage, in addition to its economic feasibility, of its high (approximately 85%) content of stearic acid, which is not atherogenic and therefore has no adverse effect on risk of cardiovascular disease.[5-7]

The main factors that influence a fat's crystallization process are its chemical composition and the conditions of crystallization. Natural lipids are composed of a variety of triacylglycerol groups with specific requirements as to activation energy for molecular diffusion and formation of stable crystalline nuclei.[8] When these groups are modified by interesterification, the related energy requirements are altered, and changes occur in crystal growth velocity and size.[9] Interesterification thus produces significant modifications in the crystallization properties of oils and fats by increasing the number of triacylglycerol species present and altering intersolubility among the triacylglycerol molecules.[10,11]

Lipid crystallization behavior has very important implications, mainly for industrial processing of products whose physical properties depend largely on fat crystals, such as chocolates, margarines and shortenings. The crystallization process is divided into nucleation and crystal growth phases. Nucleation involves the formation of molecule clusters that exceed a critical size and are therefore stable. Once a crystalline nucleus has formed, it begins to grow by incorporating other molecules. Crystal growth velocity is proportional to degree of supercooling and inversely proportional to solution viscosity. Crystallization kinetics has a profound influence on fats' final structures and is intrinsically related to their rheological and plasticity properties.[12-15]

The hierarchical organization of structural levels of the fat's crystalline network is better understood by examining the structural levels formed in the network when the fat crystallizes out of the liquid state. The microstructural level or mesoscale of a fat crystal network may be defined as structures measuring 0.5 μm to 200 μm. It is quantified mainly by visualizing its geometry. As nanostructural materials (0.4 – 250 nm), triacylglycerols crystallize into particular polymorphic forms. Most triacylglycerols crystallize as spherulites, meaning that crystal growth occurs radially. The crystals grow to a size of 1 to 4 μm and then form clusters (larger than 100 μm) in a mass and heat transfer-limited process. The clustering process continues until a continuous three-dimensional network is formed by the coupling of these microstructures, trapping the fat's liquid phase.[16-19]

Fats' tendency to crystallize is of fundamental concern to processing techniques. Triacylglycerols generally crystallize initially into the α and β' polymorphic forms, although the β form is more stable. This phenomenon relates to the fact that the β form has greater activation free energy of nucleation. The polymorphic transformation is an irreversible process from the less stable to the more stable form, and depends on the temperature and time involved. At constant temperature, the α and β' forms change, with time, into the β form by the liquid-liquid or solid-solid mechanisms.[20,21] Fats with crystals in the β' form offer greater functionality, because they are softer, support aeration better and offer creaming properties. The β' form is thus generally the polymorph of greatest interest for producing high-fat foods, such as margarines and confectionary and bakery products. However, suitable products can be obtained even using fats with a high propensity towards the β form, such as FHSBO.[22]

Also, when the triacylglycerol composition of an oil or fat is subjected to change by interesterification, a series of alterations in thermal profiles are observed. Melting and crystallization thermograms are extremely useful tools for ascertaining the changes caused by randomization, while the various thermal phenomena are examined by

monitoring changes in enthalpy and phase transitions of the various triacylglycerol blends.[23,24]

This study evaluates the effect of chemical interesterification on the thermal behavior and crystallization properties of binary SO:FHSBO blends, with a view to studying interesterified bases for food product applications. The modifications resulting from randomization were evaluated by way of triacylglycerol composition, differential scanning calorimetry, crystallization isotherms, polarized light microscopy and x-ray diffraction.

Materials and Methods

Raw materials. The materials used were refined soybean oil (SO), purchased commercially, and fully hydrogenated soybean oil (FHSBO), kindly provided by a local industry. The catalyst was 99%-pure sodium methoxide powder (Sigma-Alldrich).

Blends. The blends were prepared in the proportions 90:10, 80:20, 70:30, 60:40 and 50:50 SO: FHSBO (w/w), melted at 100°C and homogenized for 10 minutes at that temperature to melt the crystals completely, prior to each interesterification reaction.

Chemical interesterification. For the reactions, a 500 mL, jacketed, borosilicate glass reactor with bottom drain port and conical ground glass joints was coupled to a thermostated circulator bath (Lauda RE 212, -30 to +200°C, $\pm$0.02°C), stirring system (universal motor with electronic speed control up to 4000 rpm – Marconi, BR) with axial flow stirrer, vacuum pump (Vacuubrand model 30 diaphragm pump) and probe-type digital thermometer (-50 to +300°C, $\pm$1°C – Incoterm). The samples (200g) were dried for 20 minutes at 100°C in the reactor itself, under vacuum with stirring at 500 rpm. Catalyst content was 0.4% and the reaction was conducted under vacuum at 100°C, with stirring at 500 rpm, for 20 minutes, according to the optimization by Grimaldi et al.[25] The reaction was terminated by adding distilled water and 5% citric acid solution. The interesterified samples were washed carefully with distilled water (80°C) to remove any soaps that had formed and were then dried under vacuum at 110°C for 30 minutes.

Triacylglycerol composition. Triacylglycerol composition was analyzed in a CGC Agilent 6850 Series GC System capillary gas chromatograph. An Agilent DB-17HT Catalog: 122-1811 capillary column (50%-Phenyl-methylpolysiloxane, 15 meters in length x 0.25 mm bore, containing 0.15 µm film). The conditions were: split injection, ratio 1:100; column temperature 250ºC, programmed up to 350ºC at 5ºC/min; carrier gas helium, at 1.0 mL/min flow rate; injector temperature 360ºC; detector temperature 375ºC; injection volume 1.0 µL; sample concentration 100 mg/5 mL of tetrahydrofurane. Triacylglycerol groups were identified by comparing retention times, following the procedures of Antoniosi Filho et al.[26]

Thermal analysis. Thermal analysis of the samples was performed by differential scanning calorimetry according to AOCS method Cj 1-94.[27] The equipment used was a Perkin Elmer DSC 7 thermal analyzer coupled to a TAC 7/DX Thermal Analysis Controller Cooler. The data processing software used was Pyris Series Thermal Analysis System. The conditions of analysis were: sample weight ~10 mg; crystallization curves: 80ºC for 10 min, 80ºC to -40ºC (10ºC/min), and -40ºC for 30 min; and melting curves: -40ºC to 80ºC (5ºC/min). The following parameters were used in evaluating the results: crystallization and melting onset temperatures (T_{oc} and T_{om}), crystallization and melting peak temperatures (T_{pc} and T_{pm}), crystallization and melting enthalpies (ΔH_c and ΔH_m) and crystallization and melting end temperatures (T_{fc} and T_{fm}).[28]

Polarized light microscopy. The samples were melted at 70ºC in an oven and, with the aid of a capillary tube, one drop of the blend was placed on a glass slide, preheated to a controlled temperature (70ºC), and covered with a slip. Duplicate slides were prepared for each sample. The slides were kept in the oven at the analysis temperature (25ºC) for 24 hours. Crystal morphology was examined by polarized light microscope (Olympus, model BX 50) coupled to a digital video camera (Media Cybernetics). The slides were placed on the heating plate support (Mettler Toledo, FP82 Microscope Hot Stage), which was kept at the crystallization temperature. Images were captured under polarized light and 40x

magnification using Image Pro-Plus software version 4.5.1.22 (Media Cybernetics). On each slide, three fields were examined, of which only one was selected to represent the crystals observed. The evaluation parameter chosen for quantitative analysis of the images was maximum crystal diameter.[14]

Crystallization isotherm. The samples were melted (100°C/15 min) and kept in a high precision dry bath (Tcon 2000 - Duratech, USA) at 70°C for 1 hour to completely erase their prior crystalline history. Increase in the solid fat content with crystallization time was monitored by nuclear magnetic resonance spectrometer (Bruker pc120 Minispec), with the reading compartment stabilized at 25°C. Data acquisition was automatic, with measurements taken every minute, for 100 minutes. Crystallization kinetics was characterized by induction period (τ_{SFC}) – to onset of crystal formation – and maximum solid fat content ($SFC_{máx}$). Induction time was obtained graphically and reflects the time necessary for the stable, critical-size nucleus to form in the liquid phase.[29,30] The original Avrami equation, the kinetic model most used to describe isothermal phase transformation, was used to study the crystallization:[9,31]

$$\frac{CGS(t)}{CGS(\infty)} = 1 - e^{-kt^n}$$

- where *CGS(t)* describes the solid fat content (%) as a function of time, *CGS(∞)* is the solid fat content limit as time tends to infinity, *k* is the Avrami constant (min^{-n}), which considers both nucleation and crystal growth rate and *n* is the Avrami exponent, which indicates the crystal growth mechanism.[32,33] The equation was linearized and applied to the results obtained in the first 15 minutes of crystallization to determine the values of *k* and *n*.

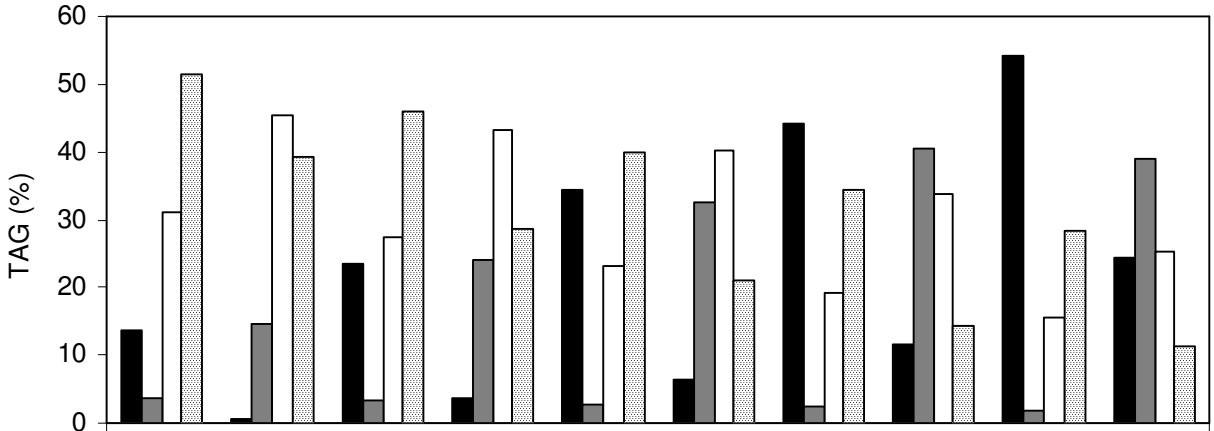
X-ray diffraction. The crystal polymorphic form of the fat sample was determined according to AOCS method Cj 2-95.[27] The analyses were performed on a Philips diffractometer (PW 1710), using Bragg-Bretano geometry (θ:2θ) with Cu-Kα radiation (λ = 1.54056 Å, at 40 KV and 30 mA). Measurements were taken at 0.02° step size in 2θ and 2

seconds acquisition time, with 5 to 40° scans (2θ scale). The samples were melted in a microwave oven at approximately 80°C and stabilized at 25°C for 24 hours in an oven. Analyses were performed in duplicate at 25°C. Polymorphic forms were identified from characteristic crystal short spacings. The α form displayed a single diffraction line at 4.15 Å. The β' form is characterized by two strong diffraction lines at 3.8 and 4.2 Å, while the β form is associated with a series of diffraction lines, one prominent at 4.6 Å and weaker lines at 3.7 and 3.8 Å.[27,34,35] β and β' type crystal contents in the samples were estimated by relative intensity of the short spacings at 4.2 and 4.6 Å.[23,27,35,36]

Results and discussion

Triacylglycerol composition. From the technological standpoint, the triacylglycerol profile is key to understanding the various physical properties of an oil or fat.[37] Figure 1 shows the triacylglycerol composition of the blends before and after interesterification, in terms of S_3 (trisaturated), S_2U (disaturated-monounsaturated), SU_2 (monosaturated-diunsaturated) and U_3 (triunsaturated) triacylglycerol content.

Interesterification caused significant alteration in the triacylglycerol composition of the blends studied. Increased FHSBO concentration in the blends is associated with higher S_3 triacylglycerol content and progressive decline in other triacylglycerol classes. In all blends, interesterification produced a significant decrease in S_3 and, to a lesser degree U_3, triacylglycerol content with a corresponding increase in the percentages of mainly SU_2 and S_2U triacylglycerols. These changes appear as a function of blend FHSBO content. With increasing FHSBO concentration in the blends, after-interesterification decreases in S_3 and U_3 triacylglycerol content ranged, respectively, from 95.64% to 54.72% and 23.50% to 60.38%. After-interesterification increases in S_2U and SU_2 triacylglycerols ranged, respectively, from 95.10% to 74.52% and 31.53% and 38.30%. Randomization thus caused the largest percentage variations in triacylglycerols of classes S_3 and S_2U.



	90:10	90:10-I	80:20	80:20-I	70:30	70:30-I	60:40	60:40-I	50:50	50:50-I
S_3	13.77	0.60	23.36	3.65	34.32	6.26	44.07	11.58	54.11	24.50
S_2U	3.74	14.68	3.20	24.17	2.65	32.63	2.31	40.45	1.91	39.02
SU_2	31.08	45.39	27.35	43.40	23.05	40.14	19.27	33.75	15.56	25.22
U_3	51.41	39.33	46.09	28.78	39.98	20.96	34.35	14.22	28.42	11.26

Fig.1. Triacylglycerol classes (%) in SO:FHSBO blends, before and after interesterification. I: interesterified blend. Triacylglycerols: S_3 (trisaturated), S_2U (disaturated-monounsaturated), SU_2 (monosaturated-diunsaturated) and U_3 (triunsaturated). Means among duplicate sample injections.

List et al.[38] evaluated the interesterification of an 80:20 SO:FHSBO blend, with a view to producing margarines and shortenings. S_3, S_2U, SU_2 and U_3 contents before the reaction were 23.3%, 2.5% 27.4% and 45.3%, respectively. After randomization, the percentages of these triacylglycerol classes were, respectively, 3.3%, 20.4%, 46.9% and 28.9%, very close to those found in this study. Interesterification of a 50:50 SO:FHSBO blend was studied by Zeitoun et al.[39] The original blend contained 50.4%, 1.5%, 17.1% and 31.0%, respectively, of S_3, S_2U, SU_2 and U_3. In the interesterified blend, the corresponding values were 12.3%, 46.4%, 32.1% and 9.2%.

Thermal behavior. Differential scanning calorimetry is the thermo-analytical technique most employed in oils and fats study. It is considered an important tool for characterizing interesterified products. Evaluation by differential scanning calorimetry yields direct

measurements of the energy involved in 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.[40] Figure 2 shows the crystallization and melting thermograms for the blends before and after chemical interesterification.

The shape of the curves was modified by the reaction, reflecting the alteration in triacylglycerol composition associated with randomization.[41] Table 1 shows the crystallization thermogram parameters for the blends before and after randomization.

Table 1. Crystallization onset temperature (T_{oc}), crystallization peak temperature (T_{pc}), crystallization enthalpy (ΔH_c) and crystallization end temperature (T_{fc}), of the original and interesterified blends. I: interesterified blend.

SO:FHSBO	T_{oc} (°C)	$T_{pc}1$ (°C)	$T_{pc}2$ (°C)	ΔH_c 1 (J/g)	ΔH_c 2 (J/g)	T_{fc} (°C)
90:10	33.82	29.47	-	9.47	-	12.52
90:10 - I	30.63	22.59	4.76	7.18	13.12	-27.15
80:20	39.31	34.63	-	30.13	-	14.82
80:20 - I	38.97	34.33	9.97	24.09	26.13	-16.90
70:30	39.48	37.33	-	57.83	-	16.38
70:30 - I	38.62	34.80	12.46	33.15	32.67	-13.28
60:40	43.97	39.33	-	75.86	-	20.02
60:40 - I	38.10	34.47	13.80	38.44	53.28	-11.63
50:50	45.34	41.30	-	85.81	-	21.03
50:50 - I	42.59	34.63	15.04	38.45	40.75	-7.59

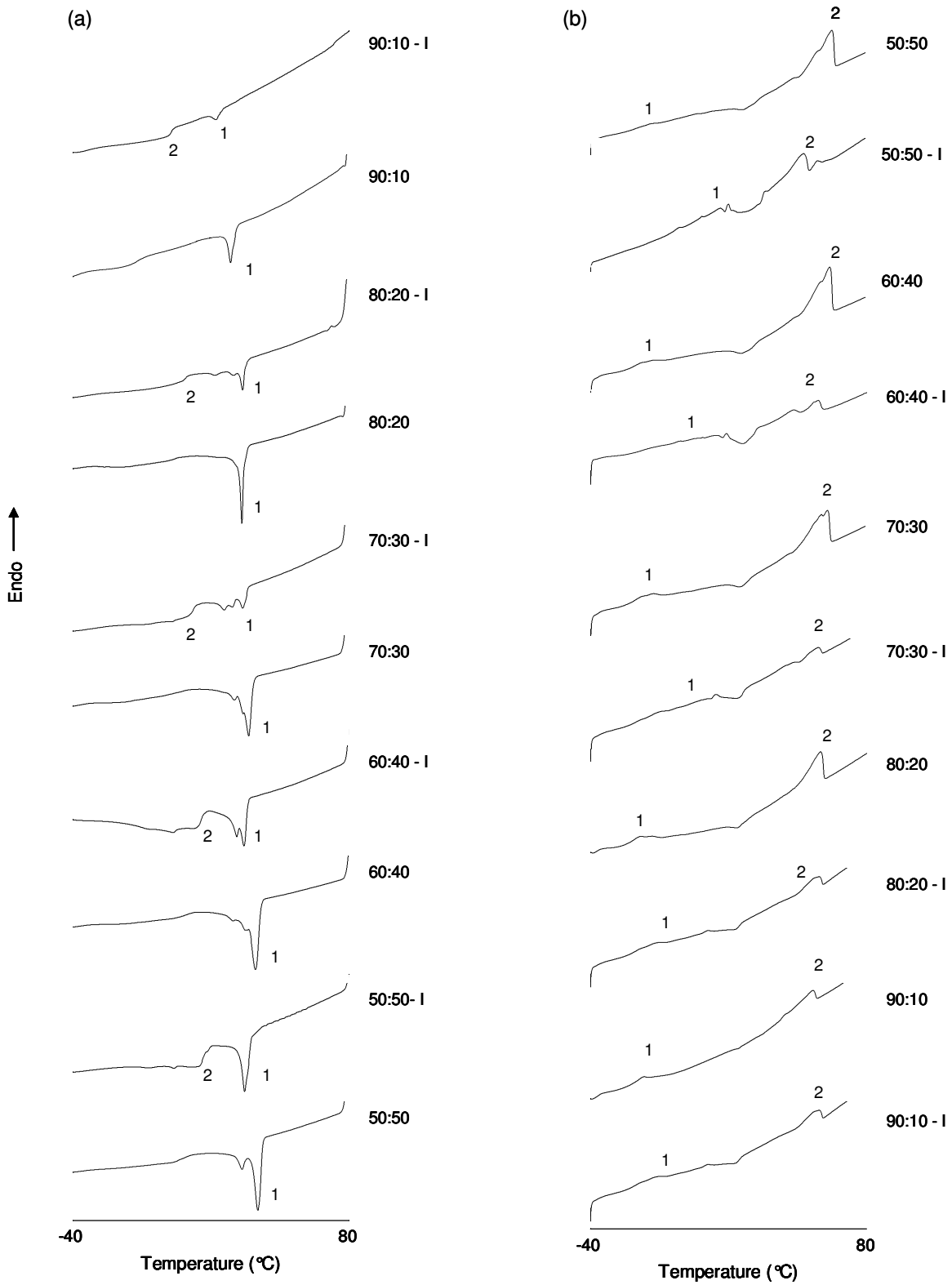


Fig. 2. Crystallization (a) and melting (b) thermograms of the SO:FHSBO blends before and after interesterification. I: interesterified blend.

The crystallization curve of an oil or fat can be subdivided into different exothermic regions reflecting different types of triacylglycerols.[40] The original blends showed a prominent peak in the crystallization thermogram (Figure 2), which characterizes the trisaturated fraction of FHSBO, containing high melting point triacylglycerols. Before the reaction, the values of T_{oc}, $T_{pc}1$ and T_{fc} increased as a function of blend FHSBO concentration, demonstrating that the crystallization process was accelerated by increased S_3 triacylglycerol content in the samples. The crystallization enthalpy values were between 9.47 and 85.81 J/g. All parameters evaluated in relation to the crystallization thermograms showed a positive linear relation to increasing FHSBO concentration in the original blends, with coefficients of determination (R^2) higher than 0.90, as in Figure 3. A similar relation among these parameters and *hardfats* concentration is observed by Humphrey et al.[42] and by Humphrey & Narine[43] in studying blends containing various liquid and fully hydrogenated oils.

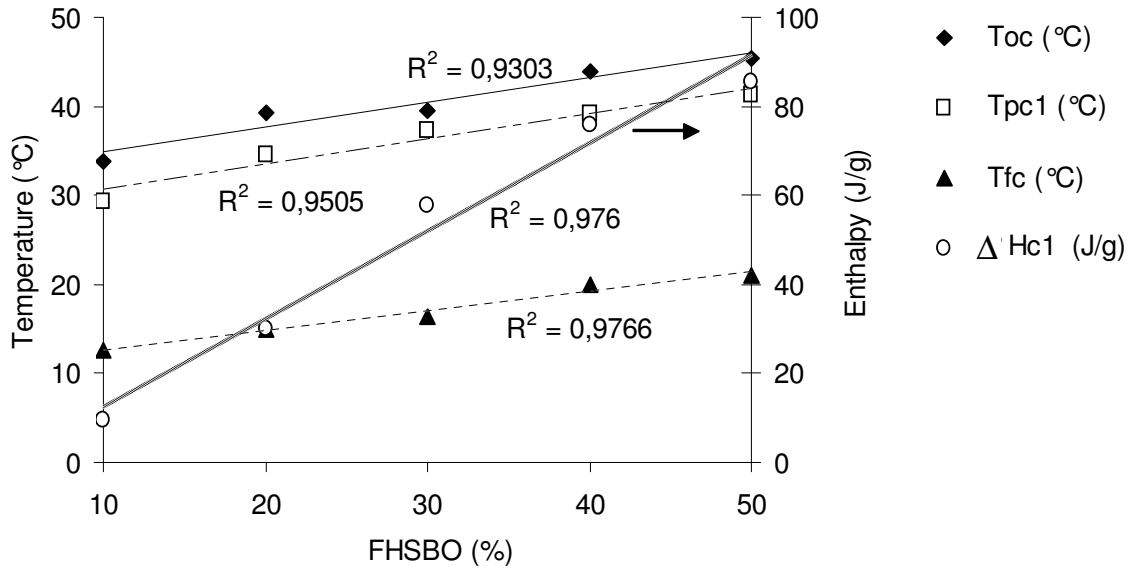


Fig. 3. Linear relations among the parameters T_{oc} (°C), $T_{pc}1$ (°C), T_{fc} (°C), ΔH_c1 (J/g) and FHSBO content in the original blends.

Interesterification caused a second peak to appear in the crystallization thermograms, characteristic of a substantial increase in the content of intermediate melting point triacylglycerols (S_2U and SU_2) as a result of the randomization process. Also, the intensity and width of the first peak diminished, which is typical of a mixed crystallization process starting from sporadic, instantaneous nuclei.[42] This was accompanied by a reduction in crystallization onset (T_{oc}) and end (T_{fc}) temperatures, enabling the interesterified samples to crystallize at lower temperatures than their original blends. $T_{pc}2$ rose as a function of the S_2U and SU_2 triacylglycerol content in the interesterified blends, indicating that the intensity of crystallization of this group of compounds is related to the proportions of these species in the samples. Values of ΔH_c1 diminished following randomization, as a result of the lower concentration of S_3 triacylglycerols in the randomized blends. Enthalpy values for the second peak observed in the thermograms (ΔH_c2) agreed with the triacylglycerol composition results, in that they displayed a relation with the sum total of the S_2U and SU_2 triacylglycerol contents in the randomized blends. Table 2 shows the melting thermogram parameters for the blends before and after randomization.

The original blends produced two characteristic peaks relating to the low melting point triacylglycerols of the unsaturated SO fraction (peak 1) and the trisaturated fraction corresponding to the FHSBO (peak 2). Peak 1 diminished, and peak 2 increased, in intensity with increasing FHSBO content in the original blends, as in Figure 2. Melting onset temperature (T_{om}) varied slightly with FHSBO content in the original blends, while the values of $T_{pm}1$, $T_{pm}2$ and T_{fm} increased with blend FHSBO content, as a result of the decrease in the percentage of triacylglycerol species from the SO and the addition of high melting point triacylglycerols characteristic of FHSBO. This effect also shows an association with the respective reduction and increase in the values of ΔH_m1 and ΔH_m2 when FHSBO is added to SO. The melting thermogram parameters evaluated displayed a linear relationship with FHSBO concentration in the original blends. However, coefficients

of determination (R^2) lower than 0.90 were encountered for the parameters T_{om} and ΔH_m1, as in Figure 4.

Table 2. Melting onset temperature (T_{om}), melting peak temperatures (T_{pm}), melting enthalpies (ΔH_m) and melting end temperature (T_{fm}) of the original and interesterified blends. I: interesterified blend.

SO:FHSBO	T_{om} (°C)	$T_{pm}1$ (°C)	T_{pm} 2 (°C)	ΔH_m1 (J/g)	ΔH_m 2 (J/g)	T_{fm} (°C)
90:10	-22.20	-17.90	56.77	7.26	20.48	60.11
90:10 - I	-21.00	-12.50	54.22	13.96	31.14	57.18
80:20	-23.31	-16.40	59.60	7.16	47.13	62.19
80:20 - I	-23.08	-11.92	57.83	14.80	42.57	61.56
70:30	-23.87	-13.07	63.43	6.11	101.98	65.94
70:30 - I	-21.04	14.60	58.68	31.58	68.54	61.81
60:40	-23.62	-12.73	64.43	5.90	105.68	66.97
60:40 - I	-20.57	18.85	58.18	59.87	84.23	61.84
50:50	-23.68	-11.96	65.35	4.09	144.92	67.83
50:50 - I	-14.06	20.18	52.60	43.75	107.02	62.67

After randomization, the first peak of the thermograms for all blends was observed to shift to the right, with respective increases in the values of T_{om} and $T_{pm}1$, relating to the significant increase in the content of intermediate melting point triacylglycerols (S_2U and SU_2). Also, ΔH_m1 values increased after the reaction, indicating increased participation by these triacylglycerol species in the interesterified blends. In parallel, interesterification caused a decline in the values of $T_{pm}2$, T_{fm} and ΔH_m2, resulting from the lower percentage of S_3 triacylglycerols in the blends. Considering only the interesterified blends, comparison of the melting thermogram parameters revealed that the tendency for T_{om}, $T_{pm}1$, $T_{pm}2$, T_{fm}, ΔH_m1 and ΔH_m 2 to increase is in complete agreement with the triacylglycerol composition results (Figure 1), with S_2U + SU_2 triacylglycerol content corresponding to 60.07%,

67.57%, 72.77%, 74.20% and 64.24%, respectively, for the 90:10-I, 80:20-I, 70:30-I, 60:40-I and 50:50-I SO:FHSBO blends.

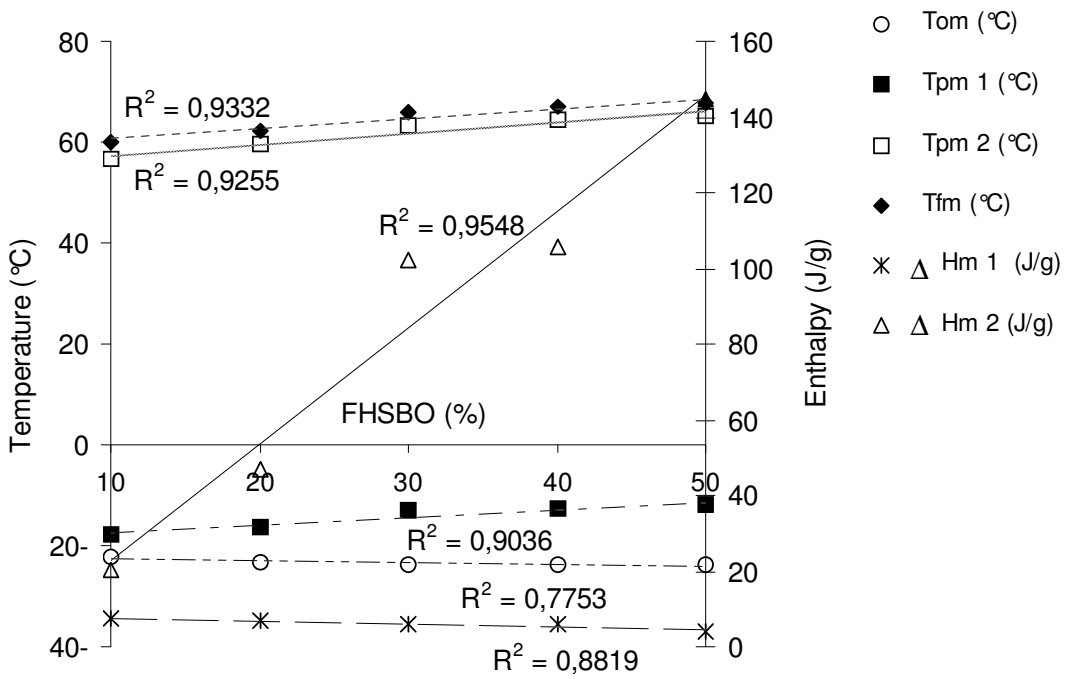


Fig. 4. Linear relations among the parameters T_{om} (°C), $T_{pm}1$ (°C), $T_{pm}2$ (°C), T_{fm} (°C), ΔH_m1 (J/g), ΔH_m2 (J/g) and original blend FHSBO content.

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.[44] 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.[17] 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.[45] Figure 5 shows the SO:FHSBO blends' before- and after-interesterification crystalline structures obtained by slow crystallization at 25°C. The maximum crystal diameter values of the SO:FHSBO

blends, before and after randomization, are shown in Table 3. The high standard deviation values for mean crystal diameter, i.e. the high coefficient of variation, are characteristic of crystallized fats when observed using polarized light microscopy.[46,47]

The original blends produced spherulite-shaped crystals with maximum diameters from 47.52 to 116.45 μm. Particularly in the 50:50 SO:FHSBO blend, the spherulites were observed to form predominantly large clusters. According to Shi et al.[44], 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 tristearin (StStSt) in the blends.[48,49] In line with these observations, crystal diameter diminished with increasing SO concentration in the blends, but crystalline morphology was not altered as a result of dilution. Rousseau et al.[49] also observed this behavior when the proportion of liquid oil was increased in palm oil/soybean oil and lard/canola oil blends.

After randomization, the crystal morphology of the 90:10, 80:20, 70:30 and 60:40 SO:FHSBO blends was completely modified: disk-shaped crystals, with similar granular crystalline structure, were observed.[50] However, the 50:50 SO:FHSBO blend maintained the tendency to crystallize predominantly into spherulites after randomization, although a smaller proportion of disk-shaped crystals was also observed. This differential behavior probably relates to the presence of considerable tristearin content in the randomized blend, given the 24.50% S_3 triacylglycerol content in this sample.

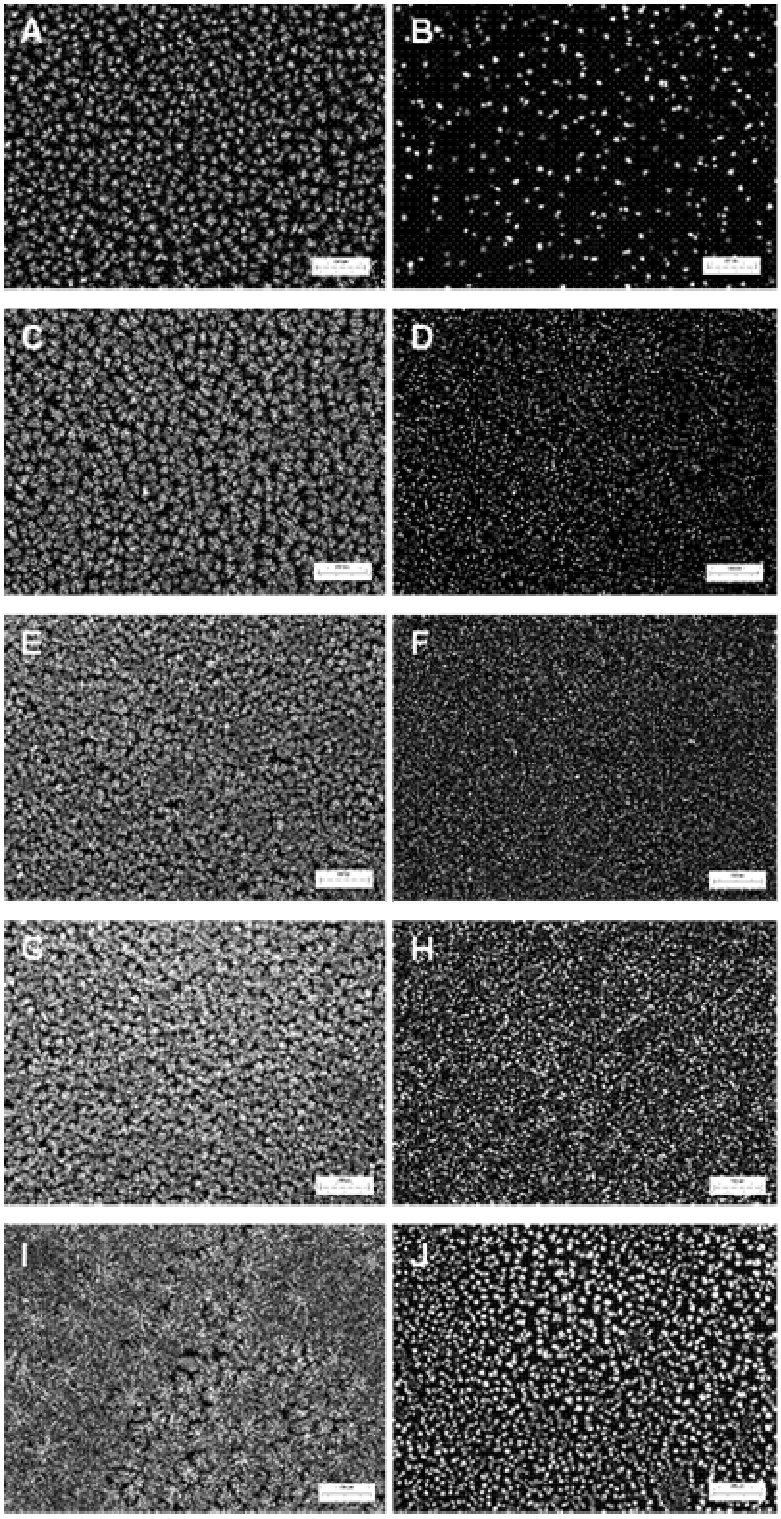


Fig.5. Images of crystallization of SO:FHSBO blends A: 90:10; B: 90:10-I; C: 80:20; D: 80:20-I; E: 70:30; F: 70:30-I; G: 60:40; H: 60:40-I; I: 50:50; J: 50:50-I. I: interesterified blend. The bar represents 200µm.

Table 3. Maximum crystal diameter of the SO:FHSBO blends, before and after interesterification.

	Maximum crystal diameter (μm)	
SO:FHSBO	Before interesterification	After interesterification
90:10	47.52 ± 5.45	18.37 ± 7.88
80:20	52.71 ± 8.13	5.97 ± 2.74
70:30	55.09 ± 7.45	6.63 ± 3.90
60:40	60.79 ± 5.22	9.08 ± 6.44
50:50	116.45 ± 16.20	25.13 ± 15.18

Interesterification produced significant reductions in crystal diameter in all blends. According to Herrera et al.[51], fats for food product applications should have crystal diameters of less than 30μm in order to prevent grainy mouthfeel. In all the blends evaluated, interesterification proved effective in yielding fat bases compatible with use in foods. Increased SO concentration in the randomized blends was also associated with decreased maximum crystal diameter, with the exception of the 90:10-I SO: FHSBO blend, which showed dimensions greater than the 80:20-I, 70:30-I and 60:40-I SO:FHSBO blends. This effect may be related to the longer time required for this sample to crystallize, due to its low melting point. Also, interesterification considerably increased the number of crystals in the 80:20-I, 70:30-I, 60:40-I and 50:50-I SO:FHSBO blends at 25°C, as can be seen from the images in Figure 4 (D, F, H, J). According to Kloek et al.[52], 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 makes for harder fat than a smaller number of large crystals, which is also associated with undesirable characteristics, such as grainy mouthfeel.[53]

According to Rousseau et al.[49], the alterations in fat microstructure caused by interesterification result from modifications to morphology and the density of the crystalline network and affect the texture and functionality of the interesterified bases. The alteration

in triacylglycerol composition caused by randomization modifies the relative strengths of the intraparticle bonds among the crystalline elements in a cluster and interparticle bonds among clusters, leading to the formation of differing structures.[44] 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.[29] Substantial changes in crystalline network density, and crystal size and morphology, due to randomization have also been reported by Rodríguez et al.[53] and Norizzah et al.[54], respectively, for lard/sunflower oil and palm stearin/palm olein blends.

Crystallization kinetics. Study of the crystallization of interesterified fat bases is extremely important in order to suit their use to industrial process constraints and to improve control of processing stages that involve recrystallization of the fat fraction, so as to ensure final product quality.[15] Figure 6 shows the crystallization isotherms obtained for the original and interesterified blends at 25°C. The 90:10-I SO:FHSBO sample did not crystallize at 25°C. interesterification changed curve format for all the blends evaluated. Before the reaction, the curves were hyperbolic. After the reaction, however, the curves showed the sigmoid form characteristic of the Avrami model, in which crystallization takes place more slowly.[55] Silva et al.[46] also observed a similar effect on isotherms in the interesterification of lard/soybean oil blends.

Table 4 shows the values of τ_{SFC}, SFC_{max}, and the Avrami parameters (and respective coefficients of determination, R^2), indicating velocity (*k*) and mechanism (*n*) of crystallization, before and after interesterification. The values of $SFC_{máx}$ and τ_{SFC}, particularly, are of significant importance for outlining applications for interesterified fats. In some specific processes, fats must be completely crystallized by the end of the production line, in order to ensure that crystallization equilibrium is reached. Otherwise, standard processing times have to be adjusted to suit the characteristics of the fat used.[55,56]

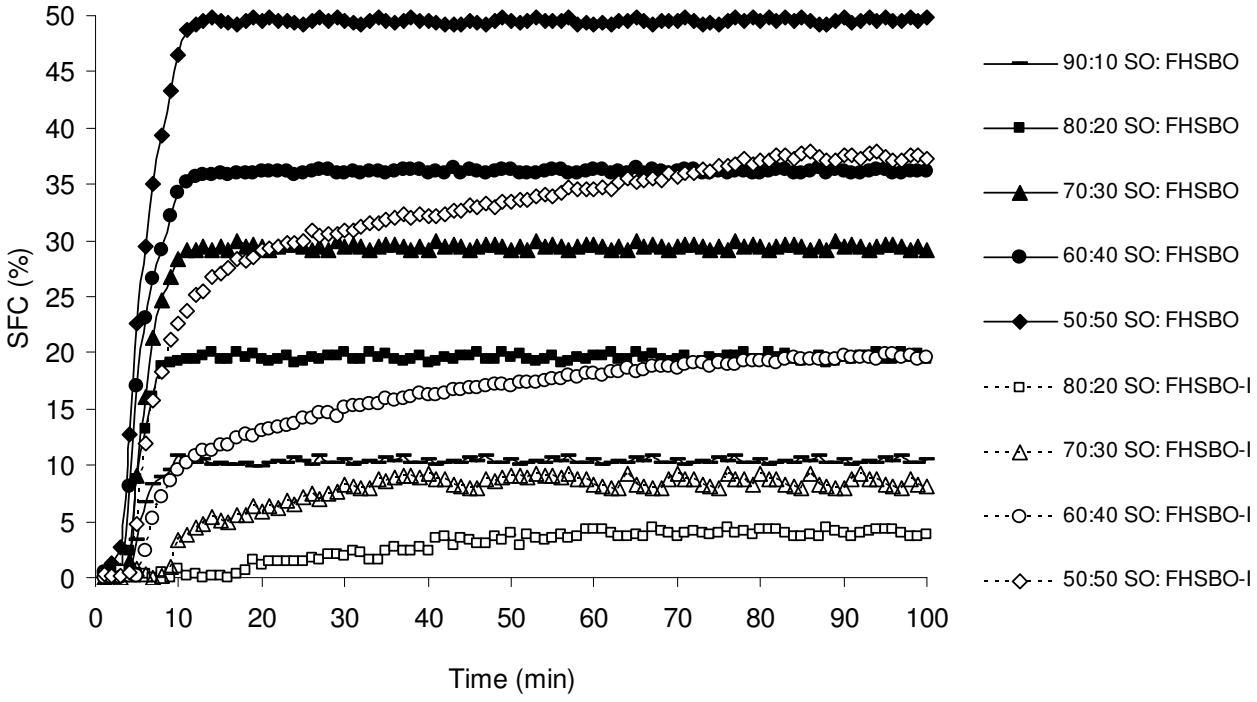


Fig. 6. Crystallization isotherms at 25°C for the original and interesterified blends (I).

Table 4. Induction period (τ_{SFC}), maximum solid fat content ($SFC_{máx}$), Avrami constant (*k*), Avrami exponent (*n*) and respective coefficients of determination (R^2) for SO:FHSBO blends before and after interesterification. I: interesterified blend.

SO:FHSBO	τ_{SFC} (min)	$SFC_{máx}$ (%)	*k* (min^{-n})	*n*	R^2
90:10	4	10.83	0.0011	3.59	0.9328
90:10 – I*	-	-	-	-	-
80:20	3	20.04	0.0014	3.38	0.8825
80:20 - I	17	4.37	0.0001	2.56	0.7314
70:30	3	30.00	0.0025	2.79	0.8943
70:30 -I	8	9.19	0.0005	2.60	0.6435
60:40	3	36.36	0.0035	2.86	0.9027
60:40 -I	5	19.82	0.0010	2.60	0.9069
50:50	1	49.77	0.0039	2.88	0.9773
50:50 -I	4	37.82	0.0020	2.40	0.8493

* The 90:10-I SO:FHSBO sample did not crystallize at 25°C.

Addition of FHSBO to soybean oil raised $SFC_{máx}$ values. Interesterification led to a decrease in $SFC_{máx}$ in all blends, an effect associated with reduced percentages of S_3 triacylglycerols and accompanying increases in S_2U and SU_2 triacylglycerols.[35,57] Randomization produced 78.19%, 69.37%, 45.49% and 24.01% reductions in $SFC_{máx}$ values for the 80:20, 70:30, 60:40 and 50:50 SO:FHSBO blends, respectively, in line with the percentage reductions in S_3 and U_3 triacylglycerols. Blend τ_{SFC} was significantly modified by chemical interesterification. Once again, the greatest alterations were observed in the 80:20 and 70:30 SO:FHSBO blends. The decline in crystallization process induction or nucleation period results primarily from changes in solid fat content inherent to the interesterification process, which influences the formation and structuring of the crystalline network.[29] In addition, the formation of a small quantity of partial acylglycerols, such as mono- and diacylglycerols, as a result of interesterification, can retard onset of nucleation.[58] At 25°C, τ_{SFC} was 1 to 4 minutes for the original blends, and 4 to 17 minutes for the interesterified blends.

The greater the difference between the sample's crystallization temperature and melting point, the greater the degree of super-cooling associated with crystallization and, consequently, the smaller the values of τ_{SFC}. When the degree of super-cooling is small, the triacylglycerol molecules are incorporated into the crystalline structure in the most appropriate configuration and the most stable polymorph is formed, because there is time sufficient for them to be properly oriented. With high degrees of super-cooling, however, triacylglycerol molecules are incorporated into the crystalline surface very quickly, and therefore imperfectly, resulting in the formation of mixed crystals and less stable polymorphs, which may persist indefinitely.[30,33] Therefore, although the percentage increase in lower melting point, mixed triacylglycerols (represented by the S_2U and SU_2 species) caused by randomization contributes to higher τ_{SFC} values, the interesterified blends are associated with greater stability and polymorphic homogeneity at 25°C, which is of significant importance for food applications.[59,60]

The Avrami constant, *k*, which is primarily a function of crystallization temperature, decreased with increased proportion of SO in the original and interesterified blends, indicating that the velocity of crystallization was lower as result of dilution of the FHSBO with liquid oil, increased U_3 triacylglycerol content and reduced S_3 triacylglycerol content. This effect was also observed by Cerdeira et al.[61] in studying the crystallization of milkfat/sunflower oil blends. Interesterification produced a drop in the crystallization velocity of the samples, related mainly to the decrease in percentage S_3 triacylglycerol content.[62]

The Avrami exponent, *n*, is sensitive to the crystallization mechanism, with respect to nucleation process and dimensions of growth. Nucleation may be sporadic or instantaneous, and crystal growth may occur in one, two or three dimensions, characterizing the formation of needle-, disk- or spherulite-shaped crystals, respectively.[33] Values of $n = 3$ correspond to spherulitic growth from instantaneous nuclei or disk-shaped growth from sporadic nuclei, while values of $n = 2$ denote needle-shaped growth from sporadic nuclei or disk-shaped growth from instantaneous nuclei.[33,62] Although *n* should be a whole number, fractional values are usually obtained. This anomaly relates primarily to the formation of crystals of similar morphology from different nucleation types (sporadic and instantaneous). Fractional values of the exponent, *n*, may also be explained by the simultaneous development of crystals with different morphologies.[62]

The value of *n* for the original blends was 3.59 to 2.88. According to Metin & Hartel[63], values of *n* increase with decreasing melting point, i.e. with decreasing degree of super-cooling of the sample, which agrees with the results of this study. Observation of the original samples' crystalline structure (Figure 5), which is made up of spherulites, corroborated the *n* values characteristic of spherulitic growth from instantaneous nuclei. The interesterified blends gave *n* values of 2.40 to 2.60. Values of *n* from 2 to 3 reflect disk-shaped growth, as observed by polarized light microscopy for the 80:20-I, 70:30-I and 60:40-I SO:FHSBO blends, denoting mixed nucleation, where crystals of the same

morphology arise from sporadic and instantaneous nuclei. However, the value of n = 2.4 for the 50:50 SO:FHSBO-I blend, where spherulites were the predominant crystal type (Figure 5), is possibly related to the concomitant presence of disk-shaped crystals, with both crystal types originating from instantaneous nucleation.[33,62] The results thus show that chemical interesterification modified the mechanism of crystallization of the original blends, altering both crystalline morphology and nucleation mechanism of the blends with from 20 to 40% FHSBO content; and with simultaneous development of crystals with different morphologies, but the same nucleation mechanism, in the 50% FHSBO blend.

Polymorphism. X-ray diffraction is often used as a technique to evaluate chemical interesterification, helping outline applications for the fat bases produced.[64] Diffraction patterns of natural or interesterified fats show broader peaks than pure compounds, due to the presence of multiple triacylglycerols in the unit cells accompanied by liquid oils.[49]

Diffractograms of the blends before and after chemical interesterification are shown in Figure 7. The 90:10 SO:FHSBO and 90:10-I SO:FHSBO samples were liquids at 25°C, and thus impossible to immobilize on the sample holder. The original blends produced peaks of strong intensity at 4.6 Å and lesser intensity at 3.7 and 3.8 Å, which is characteristic of the β form. However, the 60:40 and 50:50 SO:FHSBO blends also produced a low-intensity diffraction line at 4.2 Å, which is associated with the presence of the β' polymorph. The interesterified samples showed two peaks of varying intensity at 4.2 and 3.8 Å, which correspond to the occurrence of the β' polymorph, as well as a low-intensity peak at 4.6 Å. Due to the lower solid fat content of the 80:20-I SO:FHSBO sample at 25°C, the diffractogram at this temperature of analysis showed low-intensity peaks.

Table 5 details the polymorphic changes produced by blend and by interesterification. The original blends displayed β-type polymorphic behavior. The 80:20 and 70:30 SO:FHSBO blends showed exclusively β-type. In the 60:40 and 50:50 SO:FHSBO blends,

however, the presence of a small quantity of β' crystals was observed, although SO and FHSBO display a well known tendency to crystallize in the β form, which is associated with the low diversity of fatty acid composition and relatively homogeneous triacylglycerol composition.[59,65]

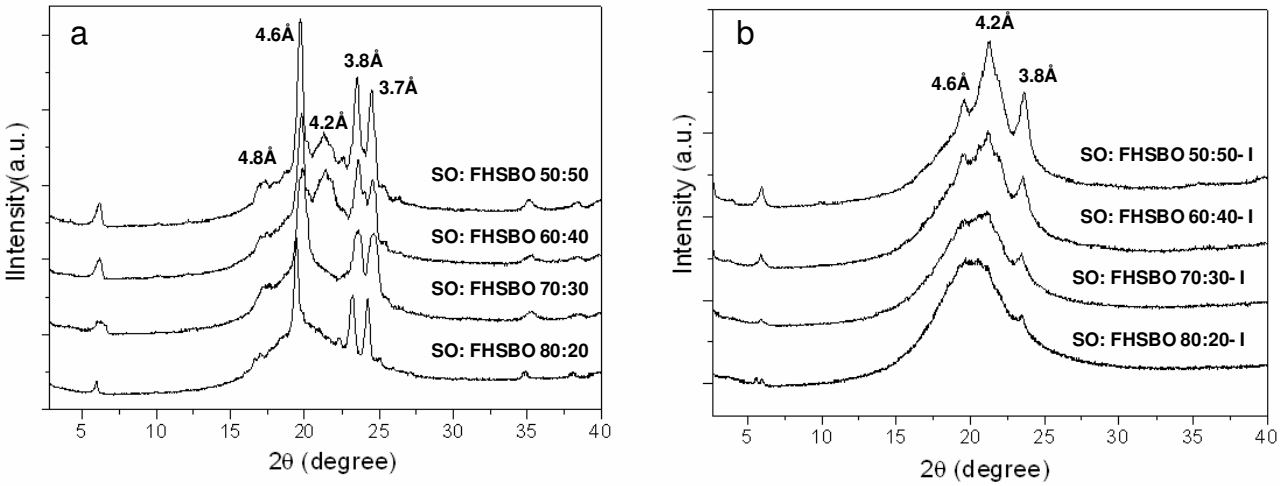


Fig.7. X-ray diffraction patterns for SO:FHSBO blends, crystallized at 25℃ for 24 hours, before (a) and after (b) interesterification.

Table 5. Polymorphic forms and short spacings of the original and interesterified SO:FHSBO blends *.

	Short spacings (Å)					
SO:FHSBO	4.8	4.6	4.2	3.8	3.7	Polymorphic form
80:20	4.83 (vw)	4.60 (s)		3.86 (m)	3.70 (m)	β
70:30	4.82 (vw)	4.59 (s)		3.85 (m)	3.69 (m)	β
60:40	4.78 (vw)	4.56 (s)	4.19 (w)	3.77 (m)	3.67 (m)	β>>β'
50:50	4.84 (vw)	4.60 (s)	4.22 (w)	3.79 (m)	3.70 (m)	β>>β'
80:20 - I		4.57 (w)	4.24 (m)	3.84 (m)		β'>>β
70:30 - I		4.63 (w)	4.23 (m)	3.84 (m)		β'>>β
60:40 - I		4.58 (w)	4.23 (s)	3.83 (m)		β'>>β
50:50 - I		4.62 (w)	4.21 (s)	3.80 (m)		β'>>β

*Intensities: v, very; w, weak; m, medium; s, strong.

Zeitoun et al.[39], studying the polymorphic behavior of a 50:50 SO:FHSBO blend, observed the simultaneous presence of β' crystals (24.7%) and β crystals (75.3%). Narine & Humphrey[48] also found the β' polymorph in a 75:25 SO:FHSBO blend. DeMan et al.[66] report the β' polymorph in fully hydrogenated canola oil, which characteristically tends to β stabilize. Humphrey et al.[42], evaluating polymorphic behavior in soybean oil/fully hydrogenated canola oil blends, found that the polymorphic forms varied with the concentration of fully hydrogenated canola oil in the samples. According to Metin & Hartel[30] and Kloek et al.[52], the persistence of β' crystals in cases where stabilization is preferentially β-type probably results from the formation of mixed crystals, which may be favored by the triacylglycerol composition of the blend and by the tempering protocol used, particularly when a high degree of super-cooling occurs. According to McGauley & Marangoni,[33] two factors relate to this phenomenon: the unfavorable configuration of triacylglycerol molecules resulting from rapid nucleation and/or rapid increase in viscosity, which limits heat and mass transfer and hinders molecular arrangement. Therefore, the formation of mixed crystals may be associated with the presence of the β' polymorph in the 60:40 and 50:50 SO:FHSBO blends.

Interesterification modified the crystalline habit of the fats evaluated towards significant predominance of the β' polymorph. The β' crystals were small and their morphology appropriate to the plasticity characteristics desirable in products such as margarines, shortenings and fats for bakery and confectionary products. Conversely, the β polymorphic form tends to produce broad granular crystals, resulting in grainy products with low aeration potential, which may impair the macroscopic properties of some foods.[13] This result agrees with the microstructure of the blends observed by polarized light microscopy, before and after randomization (Figure 5).

According to Rousseau & Marangoni,[35] stabilization of the β' polymorph caused by chemical interesterification is associated with the formation of triacylglycerols with greater

variation in chain length. This results in more disorderly packing of the terminal methyl groups, consequently forming less dense crystalline structures. Also, in all the blends evaluated, randomization caused a reduction in S_3 triacylglycerol content for the FHSBO, represented mainly by tristearin, triacylglycerol characteristic of β polymorph stabilisation.[67] List et al.[38,68] evaluated interesterification of various oils with hardstocks: the reaction also favored formation of the β' structure, enabling the bases produced to be applied in margarines.

Conclusion

A comprehensive understanding of the functions and properties of fats and oil bases produced by interesterification is essential to outlining applications for them and obtaining food products with the desired final attributes. This study allows determining that thermal behavior, microstructure, crystallization kinetics and polymorphism characteristics of SO:FHSBO blends were significantly altered by an increase FHSBO concentration and chemical interesterification process. In addition, these evaluated properties were directly related to the blends' triacylglycerol composition before and after randomization and are useful to determine the applicability of selected bases.

Acknowledgments

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References

1 D.R. Erickson, *Practical Handbook of Soybean Processing and Utilization* (American Oil Chemists'Society, Champaign, 1995), p.227.

2 A.P.B. Ribeiro, J.M.L.N. Moura, R. Grimaldi, L.A.G. Gonçalves, Quim. Nova. 30, 1295 (2007).

3 R.D. O'Brien, *Fats and Oils – Formulating and Processing for Applications* (CRC Press, New York, 2004).

4 A.G. Marangoni, D. Rousseau, Trends Food Sci. Technol. 6, 329 (1995).

5 R. Rao, B.R. Lokesh, Lipids. 38, 913 (2003).

6 J.E. Hunter, Nutr. Res. 25, 499 (2005).

7 M.A. Dirienzo, Lipids. 43, 451 (2008).

8 I. Foubert, K. Dewettinck, G. Janssen, P.A. Vanrolleghem, J. Food Eng. 75, 551 (2006).

9 S.S. Narine, K.L. Humphrey, L. Bouzidi, J. Am. Oil Chem. Soc. 83, 913 (2006).

10 I. Piska, M. Zárubová, Louzecký, H. Karami, V. Filip, J. Food Eng. 77, 347 (2006).

11 A.M.D.N Lida, K. Sundram, W.L. Siew, A. Amina, S. Mamot, J. Am. Oil Chem. Soc. 79, 1138 (2002).

12 D. Rousseau, S. Sonwai, Food Biophys. 3, 273 (2008).

13 K. Sato, Chem. Eng. Sci. 56, 2255 (2001).

14 O.W.D. Gamboa, L.A. Gioielli, Quím. Nova, 29, 646 (2006).

15 P. Wassel, N.W.G. Young, Int. J. Food Sci. Technol. 42, 503 (2007).

16 S.S. Narine, A.G. Marangoni, Food Biophys. 3, 113 (2008).

17 S.S. Narine, A.G. Marangoni, in *Fat Crystal Networks*, edited by A.G. Marangoni (Marcel Dekker, New York, 2005), p.179.

18 F.F.A. Hollander, D. Kaminski, D. Duret, W.J.P. van Enckevort, H. Meekes, P. Bennema, Food Res. Int. 35, 909 (2002).

19 A.G. Marangoni, S.S Narine, Food Res. Int. 35, 957 (2002).

20 S. Martini, T. Awad, A.G. Marangoni, in *Modifying Lipids for Use in Food*, edited by F.D. Gunstone (Woodhead Publishing, Cambridge, 2006), p.142.

21 M.L. Herrera, F.J. Marquez Rocha, J. Am. Oil Chem. Soc. 73, 321 (1996).

22 J. Oh, A.R. McCurdy, S. Clark, B.G. Swanson, J. Am. Oil Chem. Soc. 82, 13 (2005).

23 Y.B. Che Man, K. Shamsi, M.S.A. Yusoff, S. Jinap, J. Am. Oil Chem. Soc. 80, 409 (2003).

24 J.M.N. Marikkar, H.M Ghazali, Y.B. Che Man, O.M. Lai, J. Food Lipids. 10, 63 (2003).

25 R. Grimaldi, L.A.G. Gonçalves, M.Y. Ando, Quim. Nova. 28, 633 (2005).

26 N.R. Antoniossi Fillho, O.L. Mendes, F.M. Lanças, Chromatographia. 40, 557 (1995).

27 AOCS, *Official methods and recommended practices of the American Oil Chemists' Society* (American Oil Chemists' Society, Champaign, 2004).

28 C.G. Biliaderis, Food Chem. 10, 239 (1983).

29 C. Himavan, V.M. Starov, A.G.F. Stapley, Adv. Colloid Interface Sci. 122, 3 (2006).

30 S. Metin, R.W. Hartel, in *Bailey's industrial oil and fat products,* edited by F. Shahidi (Wiley Interscience, New York, 2005).

31 J.F. Toro-Vazquez, E. Dibildox-Alvarado, M. Charó-Alonso, V. Charó-Alonso, C.A. Gómez-Aldapa, J. Am. Oil Chem. Soc. 79, 855 (2002).

32 A.J. Wright, R.W. Hartel, S.S. Narine, A.G.Marangoni, J. Am. Oil Chem. Soc. 77, 463 (2000).

33 S.E. McGauley, A.G. Marangoni, in *Physical Properties of Lipids,* edited by A.G. Marangoni, S.S. Narine (CRC Press, New York, 2002).

34 P.H. Yap, J.M. deMan, L.deMan, J. Am. Oil Chem. Soc. 66, 693 (1989).

35 D. Rousseau, A.G. Marangoni, in *Food lipids: chemistry, nutrition, and biotechnology,* edited by

C.C. Akoh (CRC Press, New York, 2002), p.301.

36 A. Szydlouska-Czerniak, G. Karlovits, M. Lach, E. Szlyk, Food Chem. 92, 133 (2005).

37 M. Buchgraber, F. Ulberth, H. Emons, E. Anklan, Eur. J. Lipid Sci. Technol. 106, 621 (2004).

38 G.R. List, T.L. Mounts, F, Orthoefer, W.E. Neff, J. Am. Oil Chem. Soc. 72, 379 (1995).

39 M.A.M. Zeitoun, W.E. Neff, T.L. Mounts, J. Am. Oil Chem. Soc. 70, 467 (1993).

40 C.P. Tan, Y.B. Che Man, Food Chem. 76, 89 (2002).

41 K.A. Al-Rashood, R.R.A. Abou-Shaaban, E.M. Abdel-Moety, A. Raulf, J. Am. Oil Chem. Soc. 73, 303 (1996).

42 K.L. Humphrey, P.H.L. Moquin, S.S. Narine, J. Am. Oil Chem. Soc. 80, 1175 (2003).

43 K.L. Humphrey, S.S. Narine, Food Res. Int. 37, 11 (2004).

44 Y. Shi, B. Liang, R.W. Hartel, J. Am. Oil Chem. Soc. 82, 399 (2005).

45 L.A. Gioielli, I.S. Simões, J.N. Rodrigues, J. Food Eng. 57, 347 (2003).

46 R.C. Silva, J.P. Escobedo, L.A. Gioielli, Quim. Nova. 31, 330 (2008).

47 D. Rousseau, A.R. Hill, A.G. Marangoni, J. Am. Oil Chem. Soc. 83, 377 (2006).

48 S.S. Narine, Humphrey, Food Res. Int. 37, 28 (2004).

49 D. Rousseau, A.G. Marangoni, K.R. Jeffrey, J. Am. Oil Chem. Soc. 75, 1833 (1998).

50 R. Campos, in *Fat Crystal Networks*, edited by A.G. Marangoni (Marcel Dekker, New York, 2005), p.267.

51 M.L. Herrera, C. Falabella, M. Melgarejo, M.C. Anón, J. Am. Oil Chem. Soc. 75, 1273 (1998).

52 W. Kloek, P. Walstra, T. van Vliet, J. Am. Oil Chem. Soc. 77, 48 (2000).

53 A. Rodríguez, E. Castro, M.C. Salinas, R. López, M. Miranda, J. Am. Oil Chem. Soc. 78, 432 (2001).

54 A.R. Norizzah, C.L. Chong, C.S. Cheow, O. Zaliha, Food Chem. 86, 229 (2004).

55 M.L. Herrera, C. Falabella, M. Melgarejo, M.C. Anón, J. Am. Oil Chem. Soc. 75, 1 (1999).

56 A. Bell, M.H. Gordon, W. Jirasubkunakorn, K.W. Smith, Food Chem. 101, 799 (2007).

57 D. Rousseau, A.R. Hill, A.G. Marangoni, J. Am. Oil Chem. Soc. 72, 983 (1996).

58 M.L. Herrera, M.L. Gatti, R.W. Hartel, Food Res. Int. 32, 289 (1999).

59 L.H. Wiedermann, J. Am. Oil Chem. Soc. 55, 823 (1978).

60 S. Martini, M.L. Herrera, R.W. Hartel, J. Am. Oil Chem. Soc. 79, 1055 (2002).

61 M. Cerdeira, S. Martini, R.J. Candal, M.L. Herrera, J. Am. Oil Chem. Soc. 83, 489 (2006).

62 A.G. Marangoni, in *Fat Crystal Networks*, edited by A.G. Marangoni (Marcel Dekker, New York, 2005), p.1.

63 S. Metin, R.W. Hartel, J. Am. Oil Chem. Soc. 75, 1617 (1998).

64 Z. Omar, C.C. Let, C.C. Seng, N.A. Rashid, Eur. J. Lipid Sci. Technol. 107, 634 (2005).

65 I. Foubert, K. Dewettinck, D. Van de Walk, A.J. Dijkstra, P.J. Quinn, in *The lipid handbook*, edite by F.D. Gunstone, J.L. Harwood, A.J. Dijkstra (CRC Press, Boca Raton, 2007), p.471.

66 L. deMan, J.M. deMan, B. Blackman, J. Am. Oil Chem. Soc. 66, 1777 (1989).

67 B.S. Ghotra, S.D. Dyal, S.S. Narine, Food Res. Int. 35, 1015 (2002).

68 G.R. List, E.A. Emken, W.F. Kwolek, H.J. Dutton, J. Am. Oil Chem. Soc. 74, 408 (1977).

5.

EFFECT OF CHEMICAL INTERESTERIFICATION ON PHYSICOCHEMICAL PROPERTIES AND INDUSTRIAL APPLICATIONS OF CANOLA OIL AND FULLY HYDROGENATED COTTONSEED OIL BLENDS

Ana Paula Badan Ribeiro, Rodrigo Corrêa Basso, Renato Grimaldi, Luiz Antonio Gioielli, Lireny Aparecida Guaraldo Gonçalves

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Effect of chemical interesterification on physicochemical properties and industrial applications of canola oil and fully hydrogenated cottonseed oil blends

Abstract

Blends of canola oil (CaO) and fully hydrogenated cottonseed oil (FHCSO), with 20, 25, 30, 35 and 40% FHCSO (w/w) were interesterified under the following conditions: 0.4% sodium methoxide, 500 rpm stirring, 100°C, 20 minutes. The original and interesterified blends were examined for triacylglycerol composition, melting point, solid fat content (SFC) and consistency. Interesterification caused considerable rearrangement of triacylglycerol species, reduction of trisaturated triacylglycerol content and increase in disaturated-monounsaturated and monosaturated-diunsaturated triacylglycerols in all the blends, resulting in lowering of respective melting points. The interesterified blends showed reduced SFC at all temperatures and more linear melting profiles if compared with the original blends. Yield values significantly decreased after the reaction. Iso-solid curves indicated eutectic interactions for the original blends, which were eliminated after randomization. The 80:20, 75:25, 70:30 and 65:35 CaO:FHCSO interesterified blends showed characteristics which are appropriate for their application as soft margarines, spreads, fat for bakery/all-purpose shortenings, and ice shortenings, respectively.

PRACTICAL APPLICATIONS

Recently, a number of studies have suggested a direct relationship between *trans* isomers and increased risk of vascular disease. In response, many health organizations have recommended reducing consumption of foods containing *trans* fatty acids. In this connection, chemical interesterification has proved the main alternative for obtaining plastic fats that have low *trans* isomer content or are even *trans* isomer free. This work proposes to evaluate the chemical interesterification of binary blends of canola oil and fully

hydrogenated cottonseed oil and the specific potential application of these interesterified blends in food products.

Key-words: chemical interesterification, zero trans fats, canola oil, fully hydrogenated cottonseed oil, triacylglycerol composition, melting point, solid fat content, iso-solid diagrams, consistency.

INTRODUCTION

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, interesterification has become the main method for the preparation of plastic fat with low *trans* isomer contents or even with the absence of these compounds (Norizzah *et al*. 2004).

In the interesterification reaction, fatty acids remain unaltered, although they are redistributed in the triacylglycerol molecules. Therefore, the process consists in the simultaneous breakage of existing ester bonds and the formation of new bonds in glycerol molecules (Rozenaal 1992). In contrast with partial hydrogenation, interesterification does not promote the double-bond isomerization of fatty acids and does not affect their saturation degree (Haumann 1994). Thus, chemical interesterification causes the triacylglycerol composition of any given oil or fat to alter and, consequently, their physical properties, crystallization and fusion behaviors, solid fat content, and polymorphic texture and behavior to change as well, contributing to a greater availability of oil fractions to be used in food products (Dian *et al.* 2007).

Several studies have been investigating the influence of chemical interesterification on the physical-chemical properties of oils and fats or on their blends' (Ghotra *et al.* 2002). In particular, the interesterification of blends of fully hydrogenated vegetal oil (hardfats) with

liquid oils currently represent the most versatile option for the production of zero *trans* fats with many industrial purposes (Karabulut *et al.* 2004; Khatoon and Reddy 2005; Ribeiro *et al.* 2007). Systems that are composed of raw materials of well varied compositions provide good heterogeneity with regard to the triacylclycerol species and, consequently, can produce interesterified fats with substantially modified physical-chemical properties (Humphrey *et al*. 2003; Narine and Humphrey 2004). In this sense, canola oil (CaO) stands out among vegetal oils mainly due to its nutritional qualities, given that it is characterized by a low saturated fatty acid content and an expressive oleic acid percentage (superior to 60%), which has a neutral effect on the plasma levels of total cholesterol, consisting in raw material of particular interest for the production of special fats (O'Brien 2004; Hunter 2005). Fully hydrogenated cottonseed oil (FHCSO) presents desirable properties for the production of interesterified fat bases, for possessing the nonatherogenic stearic acid as the predominant fatty acid and, therefore, for having no adverse effect on the risk of cardiovascular diseases (Hunter 2005; Rao and Lokesh 2003). Moreover, FHCSO is characterized by its considerable palmitic acid content (20-25%), known as the promoter of polymorphic form β', which contributes for the attainment of better consistency, plasticity and aeration characteristics (Zeitoun *et al.* 1993; D'Souza *et al.* 1992).

A comprehensive understanding of the functions and properties of fats produced by interesterification is preeminent for outlining their applications and attaining food products with the final intended attributes, particularly when the replacement of partially hydrogenated fats is considered. The satisfactory performance of a given fat depends on important elements that determine its applicability. These include, firstly, 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 products that are rich in fat content

must be based mainly on the understanding of the relations among parameters such as triacylglycerol composition, melting point, solid profile, and consistency (Ghotra *et al.* 2002; O'Brien 2004).

The objective of this work was to evaluate the chemical interesterification of binary CaO:FHCSO blends, with FHCSO contents equal to 20, 25, 30, 35 and 40%, aiming at the study of fat bases to be used in food products. Triacylglycerol composition results, melting point, solid fat content, iso-solid diagram, and consistency were analyzed before and after randomization.

MATERIALS AND METHODS

Raw materials

Refined canola oil (CaO), acquired at the local market, and fully hydrogenated cottonseed oil (FHCSO), kindly provided by a regional industry, were used. The catalyst consisted of 99% sodium methoxide powder (Sigma-Alldrich).

Blends

Blends were prepared in the proportions of 80:20, 75: 25, 70:30, 65:35, and 60:40 of CaO:FHCSO (m/m), melted at 100°C and homogenized during 10 minutes at this temperature for the full melting of crystals, prior to each interesterification reaction.

Chemical interesterification

For reactions, a borosilicate-glass jacketed reactor (500 mL) was used, with bottom outlet and emery-polished conical joints, coupled to: recirculating thermostatized bath (LAUDA RE 212, -30 to +200°C, $\pm$0,02°C), agitation system (universal motor with electronic speed variator up to 4000 rpm – Marconi, BR) with axial-flow agitation shaft, vacuum pump (vacuubrand model 30, diaphragm pump), and digital skewer type thermometer (-50 to + 300°C, $\pm$1°C - Incoterm). Samples (200g) were dried in the reactor, under vacuum and agitation of 500 rpm, at 100 °C, for 20 minutes. The catalyst content used was equal to 0.4% and reaction was conducted under vacuum, at 100 °C, with

agitation of 500 rpm, during 20 minutes, according to optimization performed by Grimaldi *et al.* (2005). Reaction was finalized through the addition of distilled water and 5% citric acid solution, and interesterified samples were carefully washed with distilled water (80°C), for the removal of soaps, and subsequently dried under vacuum, at 110°C, during 30 minutes.

Fatty acid composition

Analysis of fatty acid composition were performed in a capillary gas chromatograph (CGC Agilent 6850 Series GC System) after esterification using the method of Hartman and Lago (1973). The fatty acid methyl esters were separated according to AOCS procedure 2-66 (AOCS 2004) in a DB – 23 Agilent capillary column (50% cyanopropyl-methylpolysiloxane), dimensions 60m, ϕ int: 0.25 mm, 0.25 μm film. Oven temperature was 110°C-5min, 110°C-215°C (5°C/min), 215°C-24min; detector temperature: 280°C; injector temperature 250°C; carrier gas: helium; split ratio 1:50; injection volume: 1.0μL. Qualitative composition was determined by comparing peak retention times with the respective standards for fatty acids.

Triacylglycerol composition

The triacylglycerol composition analysis was performed in capillary gas chromatograph CGC Agilent 6850 Series GC System. A capillary column DB-17HT Agilent Catalog: 122-1811 (50% phenyl-methylpolysiloxane, with 15 m in length x 0.25 mm in internal diameter, and 0.15μm film) was used. Conditions were the following: split injection, ratio of 1:100; column temperature: 250ºC, programmed up to 350ºC at the ratio of 5ºC/min.; carrier gas: helium, flow of 1.0 mL/min.; injector temperature: 360ºC; detector temperature: 375ºC; volume injected: 1.0 μL; sample concentration: 100mg/5mL of tetrahydrofuran. Identification of triacylglycerol groups was performed through the comparison of retention times, according to Antoniosi Filho *et al.* (1995).

Solid fat content (SFC)

SFC was determined through the use of the Nuclear Magnetic Resonance Spectrometer (NMR) Bruker pc120 Minispec and the high-precision dry baths (0 – 70°C) Tcon 2000 (Duratech, USA), based on the method AOCS Cd 16b- 93: direct method, sample reading in series at the temperatures of 10; 20; 25; 30; 35; 40, 45, 50, 55 and 60°C, with tempering for unstabilized fats (AOCS 2004).

Construction of iso-solid curves diagrams

The diagrams of iso-solid lines, that is, the compositions in which the SFC values of a blend are equivalent, were constructed with basis on the data experimentally provided by NMR (Humphrey *et al.* 2003; Braipson-Danthine and Deroanne 2006).

Melting point

Meting point was calculated for the temperature corresponding to the solid content equal to 4%, which was attained from the NMR solid curve through adjusted polynomial equations and the aid of the Statistica 6.0 Software (Timms 1985; Karabulut *et al.* 2004; Ribeiro *et al*. 2009; Statistica 2002).

Consistency

Consistency was determined by the use of the texture analyzer equipment TA-XT2i (Stable Micro Systems), controlled by a microcomputer. Samples were heated in a microwave oven, at approximately 80°C, for complete crystal melting, and kept in 50mL beakers. Conditioning was performed in an incubator for 24 hours at 5°C, for fat crystallization, and subsequently for 24 hours at the reading temperatures: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50°C or higher, according to analyzed samples (Rodrigues *et al.* 2003). An acrylic cone with a non-truncated tip and an angle of 60° was used. Tests were operated in the following conditions: distance = 10mm; speed = 2mm/s; time = 5s. (Rodrigues *et al.* 2007). Based on these conditions, compressive strength in (gf) was attained. Penetration data was converted in yield value, according to Haighton (1959):

$$C = \frac{K \times W}{p^{1,6}}$$

Where: C = yield value, in gf/cm^2; K = factor dependent on the cone angle (equal to 2815, for a 60° cone); W = compressive strength (gf); p = penetration depth (mm/10).

Samples were analyzed in triplicates and results correspond to calculated means.

RESULTS AND DISCUSSION

Fatty acid composition in raw materials and blends

Table 1 shows the composition in fatty acids of raw materials and blends used in the chemical interesterification. The predominant fatty acids in CaO were oleic acid (61.97%), linoleic acid (20.72%), and linolenic acid (6.54%). CaO was characterized by a low saturated fatty acid content, among which palmitic acid (5.22%) and stearic acid (2.49%) stand out. These results are in accordance with studies verified in literature, in which oleic acid and linoleic acid percentages are found in the ranges of 54.60-62.41% and 19.10-26.00%, respectively (Kim and Akoh 2005; Braipson-Danthine and Deroanne 2003; List *et al.* 1995; Tan and Che Man 2000; Bell *et al.* 2007; Zambiazi *et al.* 2007; O'Brien 2004). From a nutritional point of view, the low saturated fatty acid content (7-8%) and the expressive monounsaturated fatty acid content present in CaO make it appropriate for different industrial purposes, including the interesterification process (Zambiazi *et al*. 2007; O'Brien 2004).

TABLE 1. FATTY ACID COMPOSITION* (%) OF THE RAW MATERIALS (CaO AND FHCSO) AND BLENDS.

		CaO: FHCSO (%w/w)					
Fatty acid (%)	CaO	80:20	75:25	70:30	65:35	60:40	FHCSO
C14:0	0.07	0.22	0.39	0.30	0.33	0.38	0.86
C16:0	5.22	8.39	9.46	10.41	11.48	12.42	23.39
C16:1	0.25	0.21	0.24	0.18	0.17	0.15	-
C18:0	2.49	16.61	19.89	23.85	27.77	30.99	74.25
C18:1t	0.09	0.08	0.07	0.06	0.06	0.05	0.04
C18:1	61.97	51.32	47.90	44.34	40.46	37.73	0.33
C18:2t	0.07	0.05	0.06	0.05	0.06	0.05	0.03
C18:2	20.72	15.45	14.88	14.24	13.59	12.64	0.51
C18:3t	0.21	0.16	0.14	0.14	0.14	0.13	-
C18:3	6.54	5.44	5.00	4.57	4.19	3.79	-
C20:0	0.67	0.61	0.59	0.57	0.56	0.54	0.37
C20:1	0.98	0.82	0.75	0.70	0.63	0.59	-
C22:0	0.31	0.28	0.28	0.26	0.25	0.24	0.14
C22:1	0.08	0.06	0.06	0.05	0.05	0.05	-
C24:0	0.21	0.19	0.20	0.17	0.17	0.16	0.09
C24:1	0.11	0.09	0.08	0.08	0.07	0.07	-
Saturated (%)	8.98	26.31	30.81	35.57	40.57	44.74	99.09
Monounsaturated (%)	63.49	52.59	49.10	45.42	41.45	38.65	0.37
Poliunsaturated (%)	27.54	21.10	20.08	19.01	17.98	16.61	0.54
Total (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00

-: not detected; *average value between duplicate samples injection.

FHCSO presented a high stearic acid content (74.25%), followed by palmitic acid (2.39%), totalizing 99.09% of saturated fatty acids. These values show concordance with those reported by the works of DeMan *et al.* (1989), List *et al.* (1995) and Humphrey and Narine (2004) for FHCSO, where the stearic acid and palmitic acid contents were 70.5-76.2% and 18.8-23.3%, respectively. According to Criado *et al.* (2008), small variations in the composition of a fully hydrogenated oil result mainly from the raw material origin and process conditions employed during oil modification by hydrogenation.

The percentages of unsaturated fatty acids of CaO and FHCSO correspond to 91.03 and 0.91%, respectively, and, in the blends, they ranged from 73.69 to 55.26%. The addition of FHCSO to CaO produced blends in which the sum of stearic acid and palmitic acid varied from 25.00 to 43.41%. Plasticity of fat is mainly determined by the presence of palmitic acid, which is also responsible for the crystallization of form β`, essential in most products (Jeyarani and Reddy 2003). In raw materials and, therefore, in blends as well, the presence of minimum *trans* fatty acid contents, which are associated to the refining and total hydrogenation processes, was verified (O'Brien 2004).

Triacylglycerol composition

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. In processed food which contains significant fat contents, the product's behavior may depend on the triacylglycerol composition of that fat (Buchgraber *et al.* 2004). The interesterification reaction results in complete fatty acid randomization among all triacylglycerols present, according to the laws of probability (Rousseau and Marangoni 2002). Table 2 shows the major individual triacylglycerols which compose the raw materials CaO and FHCSO, as well as the blends, before and after chemical interesterification.

15 different triacylglycerol species were verified for CaO, with the predominance of the triacylglycerols OOO, OLO, and OLnO, which totalized 73.16% of the whole triacylglycerol

composition. This significant predominance of triacylglycerols with 54 carbons is directly related to the fatty acid composition of CaO, in which oleic acid, linoleic acid, and linolenic acid accounted for 89.51% of total fatty acids, according to Table 1. Concerning to triacylglycerols with 52 carbons, the POO and PLO species showed the larger percentages. These results are in agreement with the ones presented by Antoniosi Filho (1995), Andrikopoulos (2002), Tan and Che Man (2000) and Bell *et al*. (2007), for the CaO triacylglycerol composition. FHCSO presented 98.98% of trisaturated triacylglycerols, in which PStSt (44.51%) predominated, followed by StStSt (38.07%). Triacylglycerol species with 50, 52 and 54 carbons amounted to 15.07, 44.77 and 38.82%, respectively. In a study of DeMan *et al.* (1989), these percentages corresponded to 13.60; 43.50 e 40.50%, which is very close to those ascertained in this study. OOO and OLO were the predominant triacylglycerols in the 80:20, 75:25 and 70:30 blends, whereas OOO and PStSt were the triacylglycerol species with higher percentages for the 65:35 and 60:40 blends.

Randomization promoted significant alteration in the triacylglycerol composition of evaluated samples. Reaction caused the formation of new triacylglycerols, such as POSt, StOSt, and OLL, and especially of considerable StLO contents, as a result of high concentrations of stearic, oleic, and linoleic acids which were present in the initial blends. Additionally, an expressive increase of the triacylglycerol species StLO was observed, as well as of the POP, POO, PLnO and PLnL percentages, with a concomitant decrease in the contents of triacylglycerols PPSt, PStSt, PLO, StStSt, OOO, OLO, OLnO and OLnL.

Differences in the triacylglycerol composition of blends, resulting from random rearrangement, can promote important alterations in the physical properties of fats. The presence of considerable contents of StLO and StOO, with melting points equal to 6.1 and 22.8°C, respectively, is associated to good lubricity and gloss characteristics; whereas the formation of the triacylglycerol species POSt and StOSt (respective melting points corresponding to 37.8 and 41.7°C) contributes for a better crystal lattice organization, helping to maintain fat plasticity until higher processing temperatures are reached

(Wiedermann 1978; Ghotra *et al.* 2002). Yet, the StStSt content decrease after randomization can be regarded as an advantage, for high percentages of this triacylglycerol are associated with an unpleasant waxy sensation to the mouth (O'Brien 2004).

Table 3 shows the triacylglycerol composition of blends before and after interesterification, according to the degree of unsaturation: S_3 (trisaturated), S_2U (disaturated-monounsaturated), U_2S (monosaturated-diunsaturated) and U_3 (triunsaturated).

The addition of FHCSO to CaO promoted the increase of the triacylglycerol S_3 percentages and the concomitant decrease of other triacylglycerol classes. Original blends with 20 and 25% of hardfat presented low contents of triacylglycerols S_2U, which were not detected for the other blends. For the totality of blends, randomization caused a decrease in the contents of triacylglycerols of types S_3 and U_3, with an increase in the percentages of the triacylglycerol classes S_2U and U_2S, whose sum accounted for 58.82, 60.49, 57.55, 54.65 and 46.78% of the whole triacylglycerol composition. For all blends, a significant predominance of triacylglycerols U_3 was verified, before reaction, which is associated to high contents of species OOO/OLO/OLnO, according to Table 2. After random rearrangement, triacylglycerols U_2S presented the highest concentration among the triacylglycerol classes mainly as a result of the excessive formation of triacylglycerols StLO and the StOO concentration increase. Triacylglycerols U_2S with melting points from 1 to 23℃ are important for sensorial properties and related to fat functionality at ambient temperature (Wiedermann 1978; Rodrigues and Gioielli 2003).

TABLE 2.TRIACYLGLYCEROL COMPOSITION* (%) OF THE CaO, FHCSO AND BLENDS, BEFORE AND AFTER INTERESTERIFICATION.

TAG (%)	CaO	CaO: FHCSO (%w/w)										FHCSO
		80:20	80:20-I**	75:25	75:25-I	70:30	70:30-I	65:35	65:35-I	60:40	60:40-I	
PPP	-	-	-	-	-	0.33	-	0.48	0.76	0.84	1.25	1.33
PStP	**-**	**2.90**	**0.33**	**3.27**	**0.97**	**3.61**	**1.23**	**5.52**	**2.32**	**6.64**	**3.78**	15.07
POP	0.51	0.31	1.42	0.26	1.82	-	1.29	-	1.77	-	1.03	-
PLP	0.30	0.09	0.28	-	0.37	-	0.31	-	0.38	-	0.19	-
PStSt	**-**	**9.46**	**0.60**	**11.51**	**1.96**	**12.80**	**3.62**	**15.67**	**5.96**	**18.90**	**9.95**	44.51
POSt	0.62	**-**	**4.79**	**-**	**4.93**	**-**	**5.72**	**-**	**6.91**	**-**	**7.04**	**-**
POO	6.56	5.52	9.02	5.24	7.73	5.21	7.49	5.02	6.21	4.71	5.36	-
PLSt	-	-	-	-	-	-	-	-	-	-	-	0.26
PLO	5.16	4.08	1.73	3.73	3.68	3.69	2.70	3.48	3.21	3.19	2.42	-
PLnO	2.37	1.37	5.14	1.36	4.44	1.26	4.56	1.16	4.55	1.14	3.26	-
PLnL	0.31	0.14	1.08	-	1.00	-	0.73	-	2.88	-	1.20	-
StStSt	-	**7.03**	**-**	**8.96**	**1.06**	**10.49**	**2.28**	**13.74**	**4.43**	**16.71**	**7.97**	38.07
StOSt	-	**-**	**2.62**	**-**	**4.72**	**-**	**5.32**	**-**	**8.22**	**-**	**10.48**	0.42
StOO	1.89	**1.82**	**13.42**	**1.80**	**13.78**	**1.76**	**13.98**	**1.60**	**9.67**	**1.03**	**9.81**	-
StLSt	-	-	-	-	-	-	-	-	-	-	-	0.33
StLO	-	**-**	**18.51**	**-**	**17.87**	**-**	**15.01**	**-**	**10.68**	**-**	**7.19**	-
OOO	32.96	**27.86**	**11.30**	**26.76**	**11.67**	**25.62**	**9.69**	**22.88**	**9.55**	**19.67**	**8.03**	-
OLO	23.65	19.65	20.20	19.01	14.00	17.93	15.60	15.29	10.97	13.80	11.14	-
OLL	-	**-**	**5.25**	**-**	**6.47**	**-**	**7.68**	**-**	**5.11**	**-**	**6.12**	-
OLnO	16.55	**13.35**	**2.57**	**12.49**	**2.01**	**11.98**	**1.80**	**11.37**	**2.93**	**9.86**	**1.80**	-
OLnL	6.62	4.69	0.21	4.26	0.51	4.00	0.40	3.07	2.84	2.93	1.18	-
AOO	0.99	0.90	0.81	0.77	0.15	0.66	0.44	0.48	0.17	0.39	0.80	-
OOGa	0.72	0.47	0.53	0.42	0.86	0.39	0.15	0.24	0.48	0.19	-	-
OLGa	0.80	0.37	0.19	0.16	-	0.27	-	-	-	-	-	-

P= palmitic acid; St= stearic acid; O= oleic acid; L= linoleic acid; Ln= linolenic acid; A= arachidic acid; Ga = gadoleic acid.I**:interesterified blend; -: not detected; *average value between duplicate samples injection.

TABLE 3.

CLASSES OF TRIACYLGLYCEROLS IN THE CaO:FHCSO BLENDS, BEFORE AND AFTER INTERESTERIFICATION.

TAG	CaO: FHCSO (%w/w)									
(%)	80:20	80:20-I	75:25	75:25-I	70:30	70:30-I	65:35	65:35-I	60:40	60:40-I
S_3	19.39	0.93	23.74	3.99	27.23	7.13	35.41	13.47	43.09	22.95
S_2U	0.40	9.11	0.26	11.84	-	12.64	-	17.28	-	18.74
U_2S	13.83	49.71	12.90	48.65	12.58	44.91	11.75	37.37	10.46	30.04
U_3	66.39	40.25	63.10	35.52	60.19	35.32	52.85	31.88	46.45	28.27

I: interesterified blend. -: not detected.

Melting point

Natural or randomized fats possess no definite melting point like that of a pure substance, for they consist of complex blends of triacylglycerols that suffer gradual melting according to their individual melting points, until they become completely liquid. Therefore, fats present melting ranges or intervals. Nevertheless, melting point is a parameter of significant importance for the characterization and development of fats. Thus, a representative temperature value within the melting range of a given sample is adopted as the melting point, which is attained by definite experimental methods (Nichols and Sanderson 2003). The rearrangement of the acyl residue in triacylglycerol molecules, caused by interesterification, usually modifies the melting point of fats, given that the precise interesterification effect on the melting behavior depends fundamentally on the initial raw materials (Karabulut *et al.* 2004). According to Timms (1985) and Karabulut *et al.* (2004), fat tickles down the capillary tube when there is approximately 4-5% of solid fat, which allows melting point characterization when solid content is in this range. Ribeiro *et al.* (2009a, 2009b) reported a study on the melting point of interesterified fats based on soybean oil and fully hydrogenated soybean oil. A simple linear regression model was applied, relating the melting points attained by NMR with the melting points attained by the open capillary tube method - Method AOCS Cc 3-25 (AOCS 2004). There was good correlation between methods, with coefficients of determination (R^2) superior to 0.90, which corroborates the use of the NMR technique as an appropriate method for determining the melting point of fats, given that this method stands out as easy and expeditious practice. Table 4 shows the melting point values of initial and interesterified blends, determined with basis on the solid curve attained by NMR.

The addition of FHCSO to CaO promoted the melting point increase of blends, which presented values ranging from 52.0℃ to 61.2℃. Interesterification caused the melting

point decrease for all blends evaluated, as a result of the decrease in the contents of triacylglycerols S_3, of high melting point, and a considerable increase in the percentages of triacylglycerols S_2U and U_2S, which present intermediary melting points (Schmidt *et al.* 1996; Reshma *et al*. 2008). After random rearrangement, the melting point of blends varied from 24.9 to 57.2°C. The blend with 20% of FHCSO presented the biggest relative decrease (27.1°C) in its melting point after reaction. List *et al.* (1995) attained, for this same interesterified fraction, a melting point equal to 28.4°C.

TABLE 4.

MELTING POINTS (°C) OF CaO:FHCSO BLENDS, BEFORE AND AFTER CHEMICAL INTERESTERIFICATION.

CaO: FHCSO (%w/w)	Before interesterification (°C)	After interesterification (°C)
80:20	52.0	24.9
75:25	55.5	35.2
70:30	58.0	41.4
65:35	59.0	50.4
60:40	61.2	57.2

Absolute variation of melting points of interesterified blends in relation to original blends decreased in keeping with the initial FHCSO concentration, which produced randomized blends with increasing and decreasing percentages of triacylglycerols of type S_2U and U_2S, respectively, pursuant to Table 3. According Dian *et al.* (2007), fully hydrogenated fats, interesterified with liquid oil, present a melting point decline in regard to the original blend preponderantly due to the decrease in the proportion of triacylglycerols S_3. When interesterification is associated to the reduction of the percentages of triacylglycerol species such as StStSt and PStSt (in the case of this study), with melting points of 65°C

and 61.1°C, respectively, melting point decline is significant (Rousseau and Marangoni 1998).

Fats with melting points inferior to body temperature (37°C) can be directly applied as shortenings, given that they completely melt in the mouth and produce no waxy sensation during consumption (Ghotra *et al.* 2002). Hence, 80:20 and 75:25 interesterified blends fit in this group. The 70:30 interesterified blend, with melting point equal to 41.4°C, is situated in the range of most fat bases for the production of solid and semi-solid shortenings, whose representative melting point is 42°C. Grimaldi *et al.* (2000) evaluated Brazilian commercial fats used for different industrial purposes. The authors verified that the samples studied presented melting points between 21.9 and 49.2°C; with the great majority being found in the range between 30 and 40°C. Karabulut and Turan (2006) reported that the melting points of 25 direct-use margarine and shortening samples were situated between 31.2° and 43.0°C. The 65:35 interesterified blend, with melting point equal to 50.4°C, fit into that category of stabilizing-function fats used in toppings and icings, which are characterized by having melting points ranging from 45 to 52°C (O'Brien, 2004). However, for the 60:40 interesterified blend, a considerably high melting point (57.2°C) was observed, as far as direct applicability to food is concerned. Its employment would be linked to its use as basestock for the formulation of shortenings and margarines based on the addition of liquid oils. Conversely, this becomes particularly interesting, because, from a nutritional point of view, we now have a zero *trans* basestock source in contrast with commercial basestocks that are normally produced by hydrogenation, which typically possess between 32 and 40% of *trans* fatty acids in their composition (List *et al*. 2007). Nonetheless, melting point does not amount to a real fat consistency indicator and is only to be used as complementary parameter at determining the specific use of fats in general (Jeyarani and Reddy 2005).

SFC

Solid fat content (SFC) is a parameter that expresses the solid/liquid ratio of a given fat at various temperatures (Marangoni and Rousseau 1995). SFC affects physical properties such as spreadability, consistency and stability, besides influencing important sensorial properties. Hence, solid profile is essential for plastic fat characterization (Lee *et al*. 2008a). Figure 1 shows the solid curves of original and interesterified blends. The SFC of blends was directly proportional to the addition of FHCSO to CaO at all temperatures evaluated. At 10°C, samples presented SFC values between 21.17 and 41.95%, which decreased in a nonlinear fashion until complete fusion between 60°C and 65°C.

The shape of curves was significantly modified by interesterification, which generated melting profiles that were more linear and with a more pronounced inclination, as a consequence of a greater variability of triacylglycerol species resulting from reaction (Rousseau and Marangoni 2002).

Interesterification resulted in the decrease of SFC of blends at all temperatures. At 25°C, SFC of blends was between 18.50 and 39.18%; after randomization, these values corresponded to 3.06 and 27.61%, respectively. According to Marangoni and Rousseau (1995), alterations in triacylglycerols of types S_3, S_2U and U_2S, caused by interesterification, directly reflect on the solid curves. In this sense, the decrease in percentage of species S_3 and, especially, the simultaneous increase in percentage of triacylglycerols U_2S, which presented the highest concentration among the triacylglycerol classes of randomized blends (Table 3), are associated to the SFC decrease effect in interesterified fats. Fig. 2 shows the relations between the SFC values and (S_3 + S_2U) triacylglycerols levels for the blends, before and after random rearrangement.

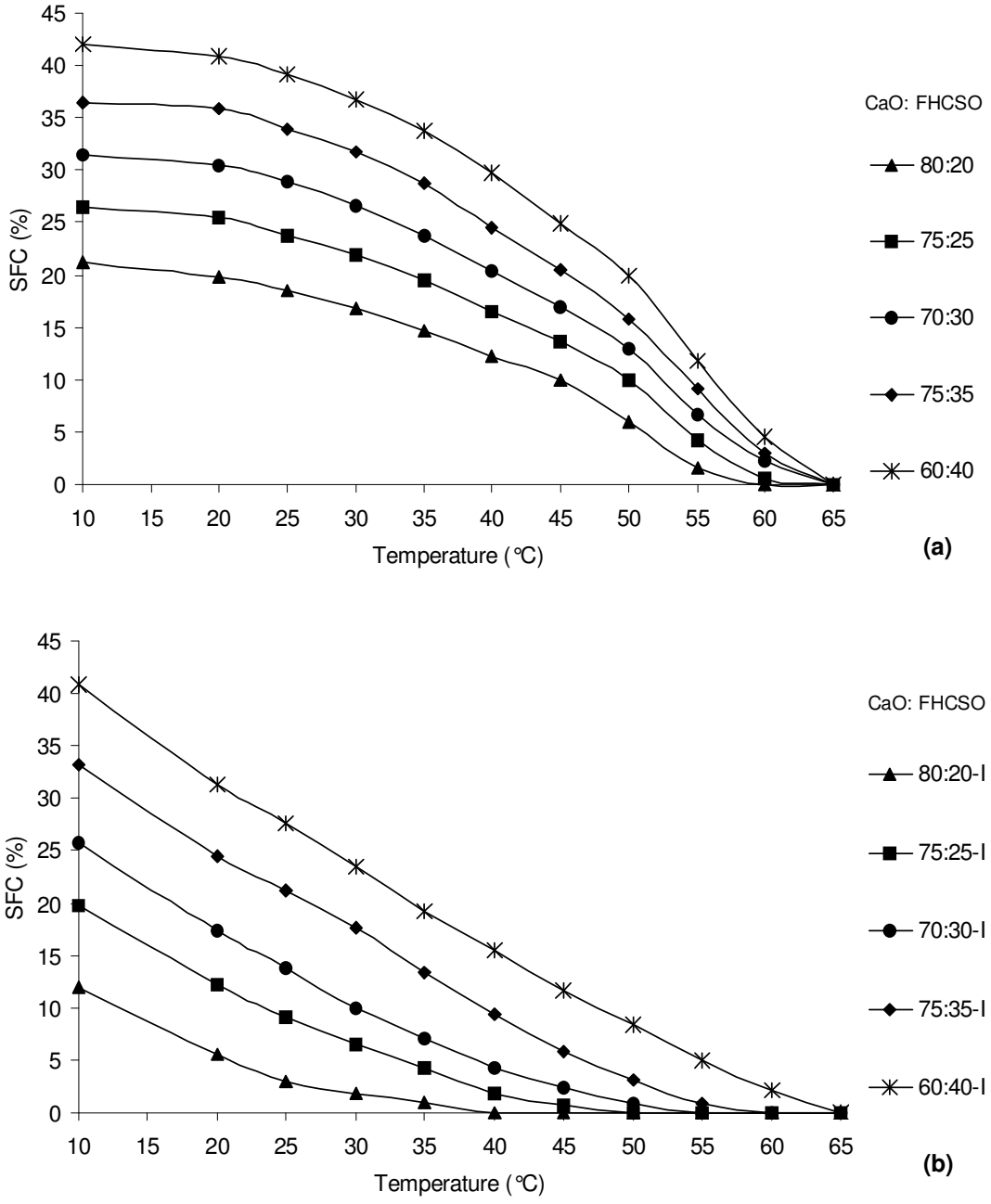


FIG.1. SFC CURVES OF CaO: FHCSO BLENDS, BEFORE (a) AND AFTER (b) INTERESTERIFICATION. I: INTERESTERIFIED BLEND.

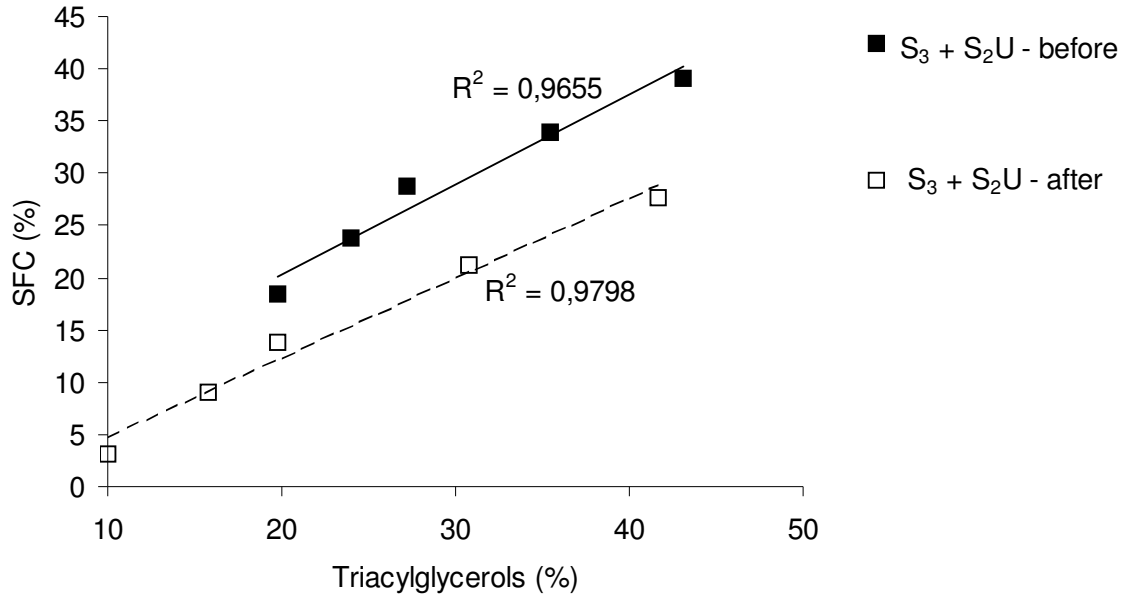


FIG.2. CORRELATION BETWEEN SFC VALUES AND (S_3 + S_2U) TRIACYLGLICEROLS CONTENT OF CaO:FHCSO BLENDS, BEFORE AND AFTER INTERESTERIFICATION.

The coefficients of determination (R^2) were greater than 0.95 in both cases. It was observed that SFC values had a positive linear variation with the (S_3 + S_2U) triacylglycerols increase. Moreover, Figure 2 shows that the FHCSO concentration increase produced smaller percentage alterations in the SFC values of interesterified blends with regard to the original blends. This behavior was also verified by Rousseau and Marangoni (2002), in regard to milk fat concentration in blends interesterified with canola oil. According to Rozenaal (1992) and Dian *et al.* (2007), this effect is associated to the randomization of raw materials that are highly saturated with liquid oils. Inclusively, these results can be considered with respect to melting points, given that such measures are directly attained from the solid profile of fats.

The application destining of new fats is initially based on the correlation of their physical properties with the properties of commercial fats for specific use. Solid profile, in particular, has important implications for margarine and shortening characteristics, at different temperatures, since it is the major fat specification and selection tool for food applications (Wassell and Young 2007). At low temperatures (4 at 10°C), SFC gives an indication of the fat's spreadability at refrigeration temperatures, which should not exceed 32% to 10°C (Lida and Ali 1998). The 80:20, 75:25, and 70:30 interesterified blends meet this requisite, which indicates that they possess compatible characteristics for their use in refrigerated products. The SFC ranging between 20 and 22°C is related to product stability and its resistance to oil exudation and must be over 10%. This is particularly important for the interesterified blend 80:20, whose SFC at 20°C is equal to 5.52%, rendering its employment in solid and semi-solid products inviable. The solid fat percentage above 25°C represents the hardness of a specific fat. Thus, the interesterified blends 70:30, 65:35 and 60:40, with SFC over 10% at 25°C, would be appropriate to provide food products with structure (Lida and Ali 1998).

Solid curves concerning several fats and shortenings for different industrial purposes were reported in the works of Lida and Ali (1998), Petrauskaite *et al.* (1998), Kok *et al.* (1999), Karabulut *et al.* (2004), Karabulut and Turan (2006), Ghotra *et al.* (2002), Khatoon and Reddy (2005), Wassel and Young (2007), Grimaldi *et al.* (2000), Timms (2003), Dijkstra (2007), Lee *et al.* (2008a, 2008b). The interesterified blend 80:20 shows a solid profile similar to that of soft margarines, or margarines for culinary use. The interesterified blend 75:25 has a solid profile close to that of fat bases used for the production of *spreads*, also meeting SFC requisites in order to ensure spreadability under refrigeration. Fats reach the melting point when SFC is reduced to 4-5% (Karabulut *et al.* 2004). At 35°C, this blend's SFC is equal to 4.23%, which ensures complete melting in the mouth.

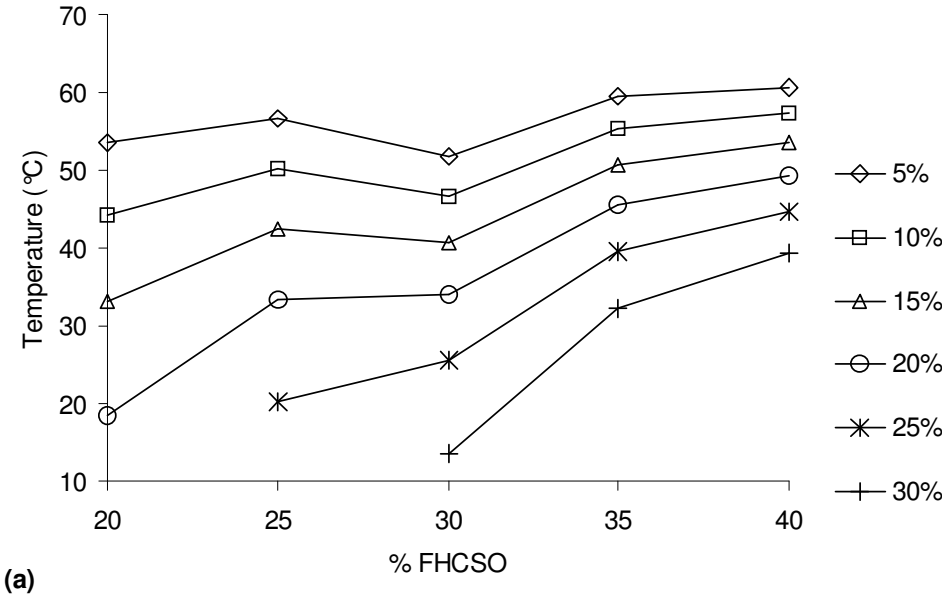
The interesterified blend 70:30 shows a solid profile appropriate for general use in bakery and as all-purpose shortening. Shortenings for bakery require sufficient SFC at ambient temperature, and higher, for dough structuring and gas retention during the initial baking stages, aiming at products bearing appropriate volume. In general, this type of fat base also serves for a different number of applications, based on the addition of specific emulsifiers (Ghotra *et al.* 2002; Karabulut and Turan 2006). According to Wassell and Young (2007), the SFC at 20°C, for all-purpose shortenings, must be between 15 and 20% in order to create stable emulsions that have good cream-formation properties; this blend's specific case, in which SFC at this temperature corresponds to 17.40%.

The interesterified blend 65:35 presents a solid profile that is very similar to that of icing shortenings. The aim of such fats is to prevent moisture loss and create a rigid matrix that can resist a wide temperature range and packaging (Timms 2003; O'Brien 2004). The interesterified blend 60:40, on its turn, presented a significantly high SFC between 35 and 40°C (19.20-15.48%) and at 50°C (8.37%), which makes its direct application in food products inviable (Dijkstra 2007). This blend has a solid profile compatible to those of soybean-oil-based basestocks, produced by hydrogenation, whose iodine values (I.V.) range between 66 and 60. However, such hydrogenated bases are regarded as nutritionally inappropriate, for they have approximately 45% of *trans* isomers in their composition (O'Brien 2004).

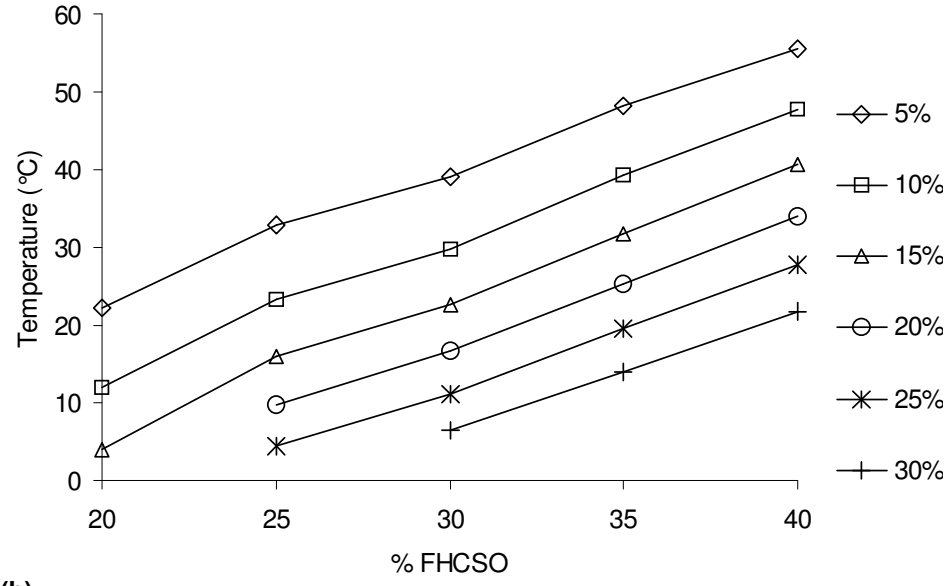
Iso-solid curves diagrams

When oils and fats with distinct compositions are blended, several behaviors can be verified: solid solutions (compatibility among fats), monotectic or eutectic behaviors. Iso-solid diagrams are useful in the compatibility study among different fats. The iso-solid curves diagrams represent the temperature in which the SFC of a given fat, or blend of

fats, is constant (Campos 2005). Figure 3 presents the iso-solid curves diagrams for the original and interesterified blends.



(a)



(b)

FIG. 3. ISOSOLID DIAGRAM OF CaO:FHCSO BLENDS BEFORE (a) AND AFTER (b) CHEMICAL INTERESTERIFICATION.

The blend of two different lipid systems can present a SFC lower than the SFC predicted through the SFC values of the individual components, characterizing incompatibility between fats (Braipson-Danthine and Deroanne 2006). The iso-solid lines show a noticeable decrease in temperature for a FHCSO concentration equal to 30%. Such declines are typical of eutectic system formation, which results in the liquefaction of blends at specific compositions, which represents a problem for the application of fats in which consistency and SFC are important (Humphrey and Narine 2005). This kind of interaction tends to occur when blend components differ in molecular volume, shape or polymorph (Lida *et al*. 2002).

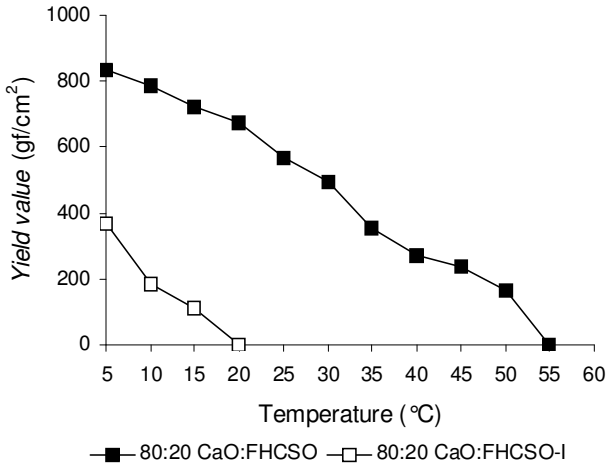
Eutectic interaction was eliminated as the result of randomization, which promoted miscibility between CaO and FHCSO. These results are consistent with those reported by Grimaldi *et al.* (2001) and Lida *et al.* (2002), which showed that interesterification can eliminate, or at least reduce, interactions in a eutectic blend. After interesterification, the expressive temperature decrease verified in the diagram indicates a better solid-state intersolubility among the triacylglycerol species present, as a result of a higher heterogeneity in the triacylglycerol composition and the increase in the contents of triacylglycerols with intermediary melting points, decreasing the monotectic effect observed in original blends (Rousseau and Marangoni 2002). Randomization promoted the linear evolution of the iso-solid lines, which characterizes interesterified blends with total compatibility and the formation of continuous solid solutions (Braipson-Danthine and Deroanne 2003).

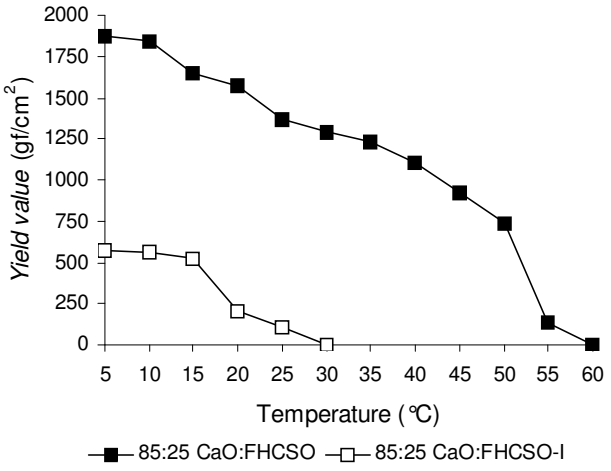
Consistency

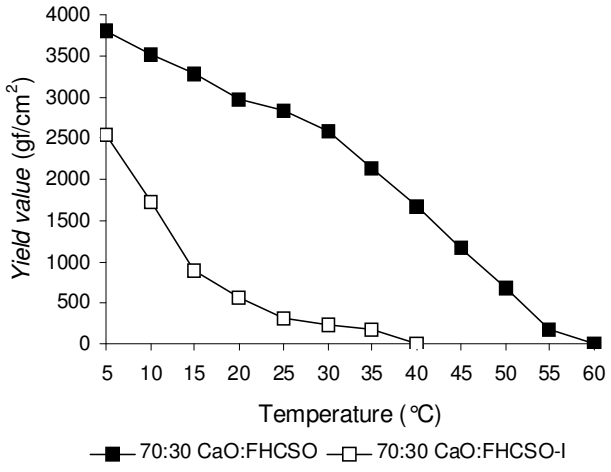
Consistency is an important functional aspect of plastic fats, which are blends of fat crystals and liquid oil. The relation between the two phases and the crystalline character of

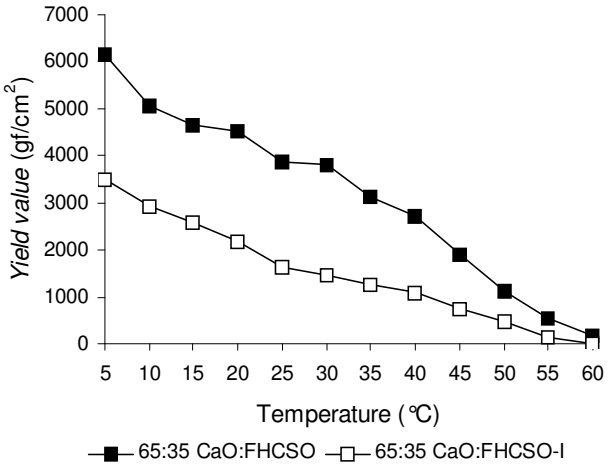
the solid phase determines the consistency and firmness of samples (Simões *et al.* 1998). The rearrangement of triacylglycerols caused by randomization promotes changes in the physical characteristics of fat bases, especially those related to crystal lattice formation, altering the mechanical properties of initial raw materials (Piska *et al*. 2006). Figure 4 shows blend consistency in keeping with temperature, represented as *yield value* (gf/cm^2), before and after interesterification.

Blend consistency decreases in keeping with temperature, as a result of gradual crystal fusion, which produces structurally weaker crystal lattices (Rousseau *et al.* 1996; Rodrigues *et al*. 2007). Interesterification promoted *yield value* decrease for all blends, at all temperatures evaluated, as in the case of Silva and Gioielli's study (2006), which verified that the consistency of lard, and its binary blends with soybean oil, was significantly altered by random rearrangement. Consistency was proportionally dependent on the FHCSO concentration of blends, before and after randomization. Such proportionality shows conformity with the results verified by melting point and SFC. The major alterations in the *yield values* of interesterified blends, in relation to original blends, were ascertained for blends 80:20, 75:25 and 70:30. At 25°C, original blends presented *yield val*ues between 569.46 and 5137.33 gf/cm^2; after interesterification, original blends ranged between 0 and 1590.98gf/cm^2.









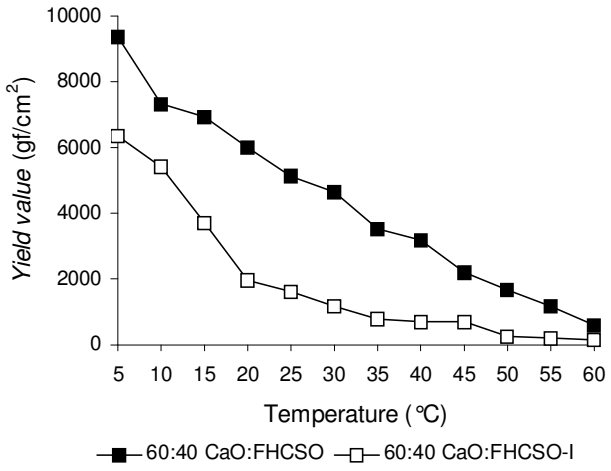


FIG. 4. *YIELD VALUE* (gf/cm^2) OF CaO:FHCSO BLENDS, BEFORE AND AFTER INTERESTERIFICATION. I: INTERESTERIFIED BLEND.

Consistency analysis has proved to be an extremely useful tool to outline specific applications of fat bases produced by interesterification. According to Haighton (1959), *yield values* between 100 and 200 gf/cm^2 characterize soft fats. The interesterified blend 80:20, with *yield value* at 10-15°, between 182.52 and 122.33 gf/cm^2, fit the classification of semi-fluid fat bases group, which corroborates its potential to be used in soft margarines (O'Brien 2004). Plastic and spreadable fats present *yield values* between 200 and 800 gf/cm^2 (Haighton 1959). In this way, the interesterified blend 75:25, with *yield values* equal to 208.06 and 561.83 gf/cm^2, at temperatures 10 and 20°C, respectively, has satisfactory plasticity and spreadability conditions to be used at refrigeration temperatures, besides having the appropriate solid fat content and melting point requisites for the application in *spreads*, as previously mentioned. Between 25 and 30°C, the interesterified blend 70:30 is satisfactorily spreadable (*yield value* between 315.25 and 239.06 gf/cm^2), but is soft at 35°C (*yield value* equal to 174.66 gf/cm^2), which is important for sensorial attributes such as melting sensation in the mouth and absence of adhesiveness. Thus, its consistency profile is also suitable to use in bakery or all-purpose shortenings (O'Brien 2004).

Yield values between 1000 and 1500 gf/cm^2 are linked to hard fats and at spreadability limit, whereas values higher than 1500 gf/cm^2 represent much harder fats of little direct applicability to food (Haighton 1959). Based on this classification, the interesterified blend 65:35 can be regarded as hard between 35 a 40°C, with *yield values* between 1472.28 and 1103.90 gf/cm^2 in this temperature interval. According to Jeyarani and Reddy (2005), fats regarded as hard are destined to firmer food products, in which there can be no deformations during handling or storage. Hence, this blend presents compatible consistency for its use in *icing* shortenings, as previously said. The interesterified blend 60:40, on its turn, would be classified as hard fat up to 30°C, because from there up its *yield values* are inferior to 1000 gf/cm^2. However, keeping high contents of solid fat at

37°C, or higher, renders its direct use in food products inappropriate, due to the undesirable waxy sensation in the mouth (Jeyarani and Reddy 2003).

Generally, it is possible to establish a correlation between consistency and SFC (Rodrigues *et al*. 2007). In this work, a power relation model between SFC values and consistency values, represented by *yield values*, was verified for all blends, before and after randomization, as shown in Figure 5. This relation is described by the equation: *yield value = a.SFC*^{b}, where "*a*" and "*b*" represent the coefficients, which are shown in Table 5, for all blends evaluated. A coefficient of correlation (R^2) superior to 0.9 was observed in all cases.

Shi *et al.* (2005) also observed this same kind of relation for different lipid systems, which is representative of different structural lattices. According to these authors, the relations between texture properties and SFC of fats can be attributed to crystal structure configuration (crystal size, size distribution, shape and polymorphism) and to interactions between molecules in crystalline state and the liquid oil in which they are dispersed, characterizing different structural levels that are related to consistency: individual triacylglycerols, crystalline units and agglomerates. Thus, it is assumed that the consistency of a semi-solid lipid system is the function of its solid fat content and of the microstructural factors associated thereto.

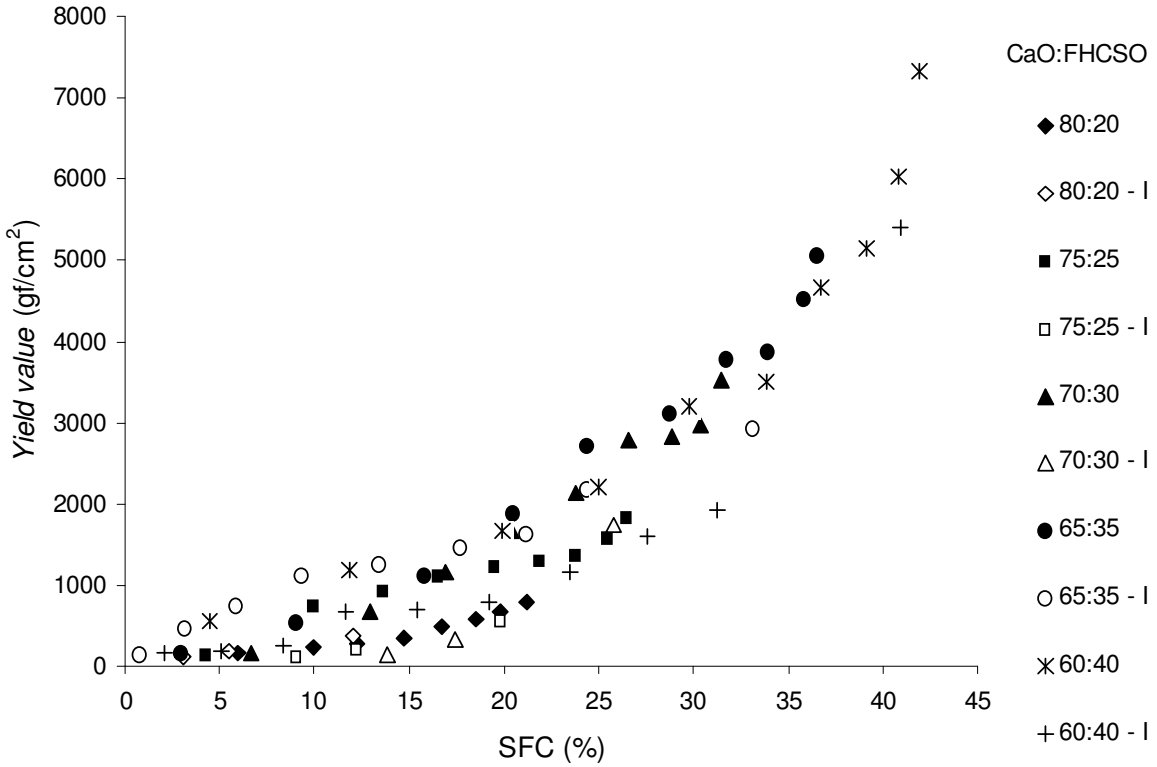


FIG. 5. RELATIONS BETWEEN Y*IELD VALUE* AND SFC VALUES FOR ORIGINAL AND INTERESTERIFIED BLENDS. I: INTERESTERIFIED BLEND.

TABLE 5.

COEFFICIENTS OF THE RELATIONS BETWEEN *YIELD VALUE* AND SFC VALUES FOR ORIGINAL AND INTERESTERIFIED BLENDS. I: INTERESTERIFIED BLEND.

CaO: FHCSO	***a***	***b***	R^2
80:20	14.9770	1.2410	0.9254
80:20 - I	41.9650	0.8711	0.9993
75:25	26.6030	1.2925	0.9428
75:25 - I	1.0437	2.1084	0.9997
70:30	4.7859	1.9181	0.9944
70:30 - I	0.0028	4.0972	0.9976
65:35	30.1760	1.3849	0.9877
65:35 - I	166.5700	0.7965	0.9864
60:40	86.2250	1.0933	0.9255
60:40 - I	38.6810	1.1272	0.9681

CONCLUSIONS

This study demonstrated that chemical interesterification significantly modified the triacylglycerol composition, and consequently the melting point, solid fat content and consistency properties of the CaO:FHCSO blends. The interesterified blends displayed characteristics suited to a variety of industrial purposes, offering alternatives to partly hydrogenated fats. All the analyses showed that the interesterified 80:20, 75:25, 70:30 and 65:35 CaO:FHCSO blends displayed characteristics suited to application as soft margarines, spreads, bakery/all-purpose shortenings and icing shortenings, respectively.

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REFERENCES

ANDRIKOPOULOS, N.K. 2002. Triglyceride species compositions of common edible vegetable oils and methods used for their identification and quantification. Food Rev. Int. *1*8, 71-102.

ANTONIOSI FILHO, N.R. 1995. Análise de óleos e gorduras vegetais utilizando métodos cromatográficos de alta resolução e métodos computacionais. University of São Paulo, São Carlos, Brazil.

ANTONIOSI FILHO, N., MENDES, O.L. and LANÇAS, F.M. 1995. Computer predition of triacylglycerol composition of vegetable oils by HRGC. Chromatographia. 40, 557-562.

AOCS. 2004. Official methods and recommended practices of the American Oil Chemists' Society. 5Ed., American Oil Chemists' Society. Champaign.

BELL, A., GORDON, M.H., JIRASUBKUNAKORN, W. and SMITH, K.W. 2007. Effects of composition on fat rheology and crystallisation. Food Chem. 101, 799-805.

BRAIPSON-DANTHINE, S and DEROANNE, C. 2003. Blending of hydrogenated low-erucic acid rapeseed oil, low-erucic acid rapeseed oil, and hydrogenated palm oil or palm oil in the preparation of shortenings. J. Am. Oil Chem. Soc. 80, 1069-1075.

BRAIPSON-DANTHINE, S. and DEROANNE, C. 2006. Determination of solid fat content (SFC) of binary fat blends and use of these data to predict SFC of selected ternary fat blends containing low-erucic rapessed oil. J. Am. Oil Chem. Soc. 83, 571-581.

BUCHGRABER, M., ULBERT, H. and ANKLAN, E. 2004.Triacilglycerol profiling by using chromatographic techniques. Eur. J. Lipid Sci. Technol. 106, 621-648.

CAMPOS, R. 2005. Experimental Methodology. In Fat Crystal Networks, (A.G. Marangoni, ed.) pp. 267-349, Marcel Dekker, New York.

CRIADO, M., HERNÁNDEZ-MARTÍN, E., LÓPEZ-HERNÁNDEZ, A. and OTERO, C. 2008. Enzymatic interesterification of olive oil with fully hydrogenated palm oil: characterization of fats. Eur. J. Lipid Sci. Technol. 110, 714-724.

DEMAN, L., DEMAN, J.M. and BLACKMAN, B. 1989. Polymorphic behavior of some fully hydrogenated oils and their mixtures with liquid oil. J. Am. Oil Chem. Soc. 66, 1777-1780.

DIAN, N. L.H.M., SUNDRAM, K. and IDRIS, N.A. 2007. Effect of chemical interesterification on triacylglicerol and solid fat contents of palm stearin, sunflower oil and palm kernel olein blends. Eur. J. Lipid Sci. Technol. 109, 147-156.

DIJKSTRA, A.J., CHRISTIE, W.W. and KNOTHE, G. 2007. Analysis. In The lipid handbook., (F.D. Gunstone, J.L. Harwood and A.J. Dijkstra, eds.) pp. 415-455, CRC Press, Boca Raton.

D'SOUZA, V., DEMAN, J.M. and DEMAN, L.M. 1992. Chemical and physical properties of the solid fats in commercial soft margarines. J. Am. Oil Chem. Soc. 69, 1198- 1205.

GHOTRA, B.S., DYAL, S.D. and NARINE, S.S. 2002. Lipids shortenings: a review. Food Res. Int. 35, 1015 -1048.

GRIMALDI, R., GONÇALVES, L.A.G., GIOIELLI, L.A. and SIMÔES, I.S. 2001.Interactions in Interesterified Palm and Palm Kernel Oils Mixtures. I – Solid fat content and consistency. Grasas y Aceites. 52, 349- 354.

GRIMALDI, R., GONÇALVES, L.A.G. and ANDO, M.Y. 2005. Otimização da reação de interesterificação química de óleo de palma. Quím. Nova. 28, 633-636.

GRIMALDI, R., GONÇALVES, L.A.G. and ESTEVES, W. 2000. Características de gorduras comerciais brasileiras. Braz. J. Food Technol. 3, 159-164.

HAIGHTON, A. J. 1959. The measurement of the hardness of margarine and fats with cone penetrometers. J. Am. Oil Chem. Soc. 36, 345-348.

HARTMAN, L. and LAGO, R. 1973. Rapid preparation of fatty acid methyl esters from lipids. Lab. Practice, 22, 475-476.

HAUMANN, B.F. 1994. Tools: hydrogenation, interesterification. Inform, 5, 668- 678.

HUMPHREY, K.L., MOQUIN, P.H.L. and NARINE, S.S. 2003. Phase behavior of a binary lipid shortening system: from molecules to rheology. J. Am. Oil Chem. Soc. 80, 1175-1182.

HUMPHREY, K.L. and NARINE, S.S. 2004. A comparison of lipid shortening functionality as a function of molecular ensemble and shear: Cristallization and melting. Food Res. Int. 37, 11-27.

HUMPHREY, K.L. and NARINE, S.S. 2005. Lipid Phase Behavior. In Fat Crystal Networks, (A.G. Marangoni, ed.) pp. 83-115, Marcel Dekker, New York.

HUNTER, J.E. 2005. Dietary levels of *trans*-fatty acids: basis for health concerns and industry efforts to limit use. Nutr. Res. 25, 499-513.

JEYARANI, T. and REDDY, S.Y. 2003. Preparation of plastic fats with zero *trans* FA from palm oil. J. Am. Oil Chem. Soc. 80, 1107-1113.

JEYARANI, T. and REDDY, Y. 2005. Physicochemical evaluation of vanaspati marketed in India. J. Food Lipids. 12, 232-242.

KARABULUT, I. and TURAN, S. 2006. Some properties of margarines and shortenings marketed in Turkey. J. Food Comp. Anal. 19, 55-58.

KARABULUT, I., TURAN, S. and ERGIN, G. 2004. Effects of chemical interesterification on solid fat content and slip melting point of fat/ oil blends. Eur. J. Lipid Sci. Technol. 218, 224-229.

KHATOON, S. and REDDY, S.R.Y. 2005. Plastic fats with zero *trans* fatty acids by interesterification of mango, mahua and palm oils. Eur. J. Lipid Sci. Technol. 107, 786-791.

KIM, B.H. and AKOH, C.C. 2005. Chemical and physical properties of butterfat-vegetable oil spread prepared with enzymatically transesterified canola oil and caprylic acid. J. Agric. Food. Chem. 53, 4954-4961.

KOK, L.L., FEHR, W.R., HAMMOND, E.G. and WHITE, P.J. 1999. *Trans*-free margarine from highly saturated soybean oil. J. Am. Oil Chem. Soc. 76, 1175-1181.

LEE, J.H., AKOH, C.C. and LEE, T. 2008a. Physical properties of *trans*-free bakery shortening produced by lipase-catalyzed interesterification. J. Am. Oil Chem. Soc. 85, 1-11.

LEE, J.H., AKOH, C.C., HIMMELSBACH, D.S. and LEE, K.T. 2008b. Preparation of interesterified plastic fats from fats and oils free of *trans* fatty acid. J. Agric. Food. Chem. 56, 4039-4046.

LIDA, A.M.D.N. and ALI, A.R. 1998. Physical-chemical characteristics of palm-based oil blends for the production of reduced fat spreads. J. Am. Oil Chem. Soc. 75, 1625-1631.

LIDA, H.M.D.N., SIEW, W.L, AMINA, A. and MAMOT, S. 2002. TAG composition and solid fat content of palm oil, sunflower oil and palm kernel olein blends before and after chemical interesterification. J. Am. Oil Chem. Soc. 79, 1138-1143.

LIST, G.R., MOUNTS, T.L., ORTHOEFER, F. and NEFF, W.E. 1995. Margarine and shortening oils by interesterification of liquid and trisaturated triglycerides. J. Am. Oil Chem. Soc. 72, 379-382.

LIST, G.R., Jackson, M., Eller, F. and Adlof, R. 2007. Low *trans* spread and shortening oils via hydrogenation of soybean oils. J. Am. Oil Chem. Soc. 84, 609-612.

MARANGONI, A.G. and ROUSSEAU, D. 1995. Engineering triacylglycerols: the role of interesterification. Trends Food Sci. Technol. 6, 329-335.

NARINE, S.S. and HUMPHREY, K.L. 2004. A comparision of lipid shortening functionality as a function of molecular ensemble and shear: microestruture, polymorphism, solid fat content and texture. Food Res. Int. 37, 28 -38.

NICHOLS, D.S. and SANDERSON, K. The nomenclature, structure and properties of food lipids. In Chemical and functional properties of food lipid, (Z.E. Sikorski and A. Kolakowska, eds.) CRC Press, New York.

NORIZZAH, A.R., CHONG, C.L., CHEOW, C.S. and ZALIHA, O. 2004. Effects of chemical interesterification on physicochemical properties of palm stearin and palm kernel olin blends. Food Chem. 86, 229-235.

O'BRIEN, R.D. 2004. Fats and Oils – Formulating and Processing for Applications. CRC Press, New York.

PETRAUSKAITE, V., DE GREYT, W., KELLENS, M. and Huyghebaert, A. 1998. Physical and chemical properties of *trans*-free fats produced by chemical interesterification of vegetable oil blends. J. Am. Oil Chem. Soc. 75, 489-493.

PISKA, I., M. ZÁRUBOVÁ, M., LOUZECKÝ, H., KARAMI, H. and FILIP, V. 2006. Properties and crystallization of fat blends. J. Food Eng. 77, 433-438.

RAO, R. and LOKESH, B.R. 2003. TG containing stearic acid, synthesized from coconut oil, exhibit lipidemic effects in rats similar to those of cocoa butter. Lipids. 38, 913-918.

RIBEIRO, A.P.B., MOURA, J.M.L.N., GRIMALDI, R. and GONÇALVES, L.A.G. 2007. Interesterificação química: alternativa para obtenção de gorduras zero *trans*. Quím. Nova. 30, 1295-1301.

RIBEIRO, A.P.B., MASUCHI, M.H., GRIMALDI, R. and GONÇALVES, L.A.G. 2009. Chemical interesterification of soybean oil and fully hydrogenated soybean oil: Influence of the reaction time. Quím. Nova. Food Res. Int. 42, 401-410.

RODRIGUES, J.N., GIOIELLI, L.A. and ANTON, C. 2003. Propriedades físicas de lipídios estruturados obtidos de misturas de gordura do leite e óleo de milho. Ciênc. Tecnol. Aliment. 23, 226-233.

RODRIGUES, J.N., TORRES, R.P., MANCINI-FILHO, J. and GIOIELLI, L.A. 2007. Physical and chemical properties of milkfat and phytosterol esters blends. Food Res. Int. 40, 748-755.

ROUSSEAU, D., HILL, A.R. and MARANGONI, A.G. 1996. Restructuring butterfat through blending and chemical interesterification. 2. Microestructure and polymorphism. J. Am. Oil Chem. Soc. 73, 973- 981.

ROUSSEAU, D. and MARANGONI, A.G. 1998. The influence of chemical interesterification on physicochemical properties of complex fat systems. 1. Melting and crystallization. J. Am. Oil Chem. Soc. 75, 1265-1271.

ROUSSEAU, D. and MARANGONI, A.G. 2002. The effects of interesterification on the physical properties of fats. In Physical Properties of Lipids, (A.G. Marangoni and S.S. Narine, eds.) pp.479-565, CRC Press, Boca Raton.

ROZENAAL, A. 1992. Interesterification of oils and fats. Inform. 3, 1232- 1237.

SHI, Y., LIANG, B. and HARTEL, R.W. 2005. Crystal morphology, microstructure, and textural properties of model lipid system. J. Am. Oil Chem. Soc. 82, 399-408.

SILVA, R.C. and GIOIELLI, L.A. 2006. Propriedades físicas de lipídios estruturados obtidos a partir de banha e óleo de soja. Rev. Bras. Ciênc. Farm. 42, 223-235.

SIMÕES, I., GIOIELLI, L.A. and OLIVEIRA, M.N. 1998. Consistência de mistura de gorduras hidrogenadas e óleo de soja. Ciênc. Tecnol. Aliment. 18, 325-330.

STATISTICA, 2002. Statistica, v.6.0. for Windows Vol. IV. Statsoft Inc, Tulsa.

TAN, C.P. and CHE MAN, Y.B. 2000. Differential scanning calorimetric analysis of edible oils: comparision of thermal properties and chemical composition. J. Am. Oil Chem. Soc. 77, 143-155.

TIMMS, R.E. 1985. Physical properties of oils and mixtures of oils. J. Am. Oil Chem. Soc. 62, 241-248.

TIMMS, R.E. 2003. Confectionery fats handbook. The Oily Press, England.

ZAMBIAZI, R.C., PRZYBYLSKI, R., ZAMBIAZI, M.W. and MENDONÇA, C.B. 2007. Fatty acid composition of vegetable oils and fats. B. CEPPA, 25, 111-120.

ZEITOUN, M.A.M., NEFF, W.E. and MOUNTS, T.L. 1993. Physical properties of interesterified fat blends. J. Am. Oil Chem. Soc. 70, 467-471.

WASSEL, P. and YOUNG, N.W.G. 2007. Food applications of *trans* fatty acids substitutes. Int. J. Food Sci. Technol. 42, 503-517.

WIEDERMANN, L.H. 1978. Margarine and margarine oil, formulation and control. J. Am. Oil Chem. Soc. 55, 823-829.

6.

INFLUENCE OF CHEMICAL INTERESTERIFICATION ON THERMAL BEHAVIOR, MICROSTRUCTURE, POLYMORPHISM AND CRYSTALLIZATION PROPERTIES OF CANOLA OIL AND FULLY HYDROGENATED COTTONSEED OIL BLENDS

Ana Paula Badan Ribeiro, Rodrigo Corrêa Basso, Renato Grimaldi, Luiz Antonio Gioielli, Adenilson Oliveira dos Santos, Lisandro Pavie Cardoso, Lireny Aparecida Guaraldo Gonçalves

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Influence of chemical interesterification on thermal behavior, microstructure, polymorphism and crystallization properties of canola oil and fully hydrogenated cottonseed oil blends

Abstract

This work evaluated chemical interesterification of canola oil (CaO) and fully hydrogenated cottonseed oil (FHCSO) blends, with 20, 25, 30, 35 and 40% (w/w) FHCSO content. Interesterification produced reduction of trisaturated and increase in monounsaturated-disaturated and diunsaturated-monosaturated15 triacylglycerols contents, which caused important changes in temperatures and enthalpies associated with the crystallization and melting thermograms. It was verified reduction in medium crystal diameter in all blends, in addition crystal morphology modification. Crystallization kinetics revealed that crystal formation induction period and maximum solid fat content were altered according to FHCSO content in original blends and as a result of random rearrangement. Changes in Avrami constant (k) and exponent (n) indicated, respectively, that interesterification decreased crystallization rates and altered crystalline morphology. However, X-ray diffraction analyses showed randomization did not change the original crystalline polymorphism. The original and interesterified blends had significant predominance of β' polymorph, which is interesting for several food applications.

Key-words: chemical interesterification, canola oil, fully hydrogenated cottonseed oil, thermal behavior, microstructure, crystallization kinetics, and polymorphism.

1. Introduction

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, interesterification has become the main method for the preparation of plastic fats with low *trans* isomer contents, or even with the absence of these compounds, given that it allows the modification of oil and fat behaviors, rendering important contributions for the increase and optimization of their use in food products (Haumann, 1994).

Particularly , the interesterification of fully hydrogenated vegetal oil (*hardfats*) and liquid oil blends currently represents the most versatile option for the production of low *trans* fats for several industrial purposes (Ribeiro, Moura, Grimaldi, & Gonçalves, 2007). Systems that are composed of raw materials with well varied compositions provide good heterogeneity with regard to the triacylclycerol species and, consequently, can produce interesterified fats with substantially modified physical-chemical properties (Humphrey, Moquin, & Narine, 2003). In view of its nutritional qualities, canola oil (CaO) stands as interesting raw material for the making of fat fractions that are exempt of *trans* fatty acids. Fully hydrogenated cottonseed oil (FHCSO) shows desirable properties for the production of interesterified fat bases, for it is characterized by its considerable palmitic acid content (20-25%), known as the promoter of polymorphic form β', which contributes for the attainment of better consistency, plasticity and aeration characteristics (D'Souza, Deman, & Deman, 1992; Zeitoun, Neff, List, & Mounts, 1993).

The major structural component of plastic fats is their crystal network. Crystal properties such as size, morphology, and polymorphic form influence this structure, which, on its turn, affects texture behavior, appearance, and functionality of fats (Rousseau, Marangoni, & Jeffrey, 1998). Crystallization process is the spontaneous system ordering, which is

characterized by total or partial movement restriction caused by the chemical or physical bonds among triacylglycerol molecules. Differences in crystal shapes result in different molecular packing (Kawamura, 1979). A crystal, therefore, consist of molecules arranged in a fixed pattern known as reticulate. Its high degree of molecular complexity permits that the same triacylglycerol group packs into many different and relatively stable structures. Lipid composition and crystallization conditions influence the crystal habit – different polymorphic forms and crystal morphologies are possible. Crystals aggregate into larger structures, creating a network that characterizes the microstructural level of a given fat (Marangoni & Hartel, 1998). 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 form β', 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 ration take place, affecting the texture

and functionality properties of interesterified bases (Rousseau et al. 1998; Tang & Marangoni, 2006).

By monitoring the formation of crystalline solid material in keeping with time, it is possible to verify the nature of crystallization. Crystallization kinetics profoundly influences the final structure of fats and is intrinsically related to their rheologic and plasticity properties. The study of crystallization kinetics of interesterified fat bases becomes, therefore, of significant importance so that their use may be adjusted to industrial process limitations and the control over processing steps which include fat fraction recrystallization is improved, ensuring final product quality (Foubert, Dewettinck, Janssen, & Vanrolleghem, 2006; Wassel & Young, 2007). Additionally, when the triacylglycerol composition of a given oil or fat is subject to change by interesterification, conjoined alterations in the thermal profiles are visualized. The fusion and crystallization thermograms are very useful tools to verify the alterations caused by randomization, being the various thermal phenomena verified by the monitoring of changes in enthalpy and phase transitions of the various triacylglycerol blends (Che Man, Shamsi, Yusoff, & Jinap, 2003; Marikkar, Ghazali, Che Man, & Lai, 2003).

In recent years, several studies on crystallization process modification as a result of interesterification have been carried out, originating many processing patents of oily bases to be used in margarines and shortenings (Omar, Let, Seng, & Rashid, 2005).

The objective of this work was to evaluate the effect of chemical interesterification on the thermal behavior and crystallization properties of binary CaO:FHCSO blends, aiming at the study of interesterified bases for food product application. The modifications resulting from randomization were evaluated by triacylglycerol composition, differential scanning calorimetry, crystallization isotherms, polarized light microscopy, and x-ray diffraction.

2. Material and Methods

2.1 Raw materials. Refined canola oil (CaO), acquired at the local market, and fully hydrogenated cottonseed oil (FHCSO), kindly provided by a regional industry, were used. The catalyst consisted of 99% sodium methoxide powder (Sigma-Alldrich).

2.2 Blends. Blends were prepared in the proportions of 80:20, 75:25, 70:30, 65:35, and 60:40 of CaO:FHCSO (w/w), melted at 100°C and homogenized during 10 minutes at this temperature for the complete melting of crystals, prior to each interesterification reaction.

2.3 Chemical interesterification. For reactions, a borosilicate-glass jacketed reactor (500 mL) was used, with bottom outlet and emery-polished conical joints, coupled to: recirculating thermostatized bath (Lauda RE 212, -30 to +200°C, $\pm$0.02°C), agitation system (universal motor with electronic speed variator up to 400 rpm – Marconi, BR) with axial-flow agitation shaft, vacuum pump (vacuubrand model 30, diaphragm pump), and digital skewer type thermometer (-50 to + 300°C, ±1°C - Incoterm). Samples (200g) were dried in the reactor, under vacuum and agitation of 500 rpm, at 100 °C, for 20 minutes. The catalyst content used was equal to 0.4% and reaction was conducted under vacuum, at 100 °C, with agitation of 500 rpm, during 20 minutes, according to optimization performed by Grimaldi, Gonçalves, and Ando (2005). Reaction was finalized through the addition of distilled water and 5% citric acid solution, and interesterified samples were carefully washed with distilled water (80°C), for the removal of soaps, and subsequently dried under vacuum, at 110°C, during 30 minutes.

2.4 Triacylclycerol composition. The triacylglycerol composition analysis was performed in capillary gas chromatograph CGC Agilent 6850 Series GC System. A capillary column DB-17HT Agilent Catalog: 122-1811 (50%-Phenyl-methylpolysiloxane, with 15 m in length x 0.25 mm in internal diameter, and 0.15µm film) was used. Conditions were the following: split injection, ratio of 1:100; column temperature: 250ºC, programmed up to 350ºC at the

ratio of 5ºC/ min.; carrier gas: helium, flow of 1.0 mL/min.; injector temperature: 360ºC; detector temperature: 375ºC; volume injected: 1.0 μL; sample concentration: 100mg/5mL of tetrahydrofuran. The analysis were carried out in duplicate and the identification of triacylglycerol groups was performed through the comparison of retention times, according to Antoniosi Filho, Mendes, and Lanças (1995)'s procedures.

2.5 Thermal analysis. Thermal analysis of samples was performed by differential scanning calorimetry (DSC), according to the method AOCS Cj 1-94 (AOCS, 2004). The equipment used was a thermal analyzer DSC 7 (Perkin Elmer) coupled to a Thermal Analysis Controller Cooler TAC 7/DX. The data processing system used was the Pyris Series Thermal Analysis System software. The analysis conditions were: sample weight ~ 10 mg; crystallization curves: 80ºC for 10 min, 80ºC to -40ºC (10ºC/ min), -40ºC for 30 min; melting curves: -40ºC to 80°C (5°C/min). The following parameters for result assessment were used: onset crystallization and melting temperatures (T_{oc} and T_{om}), peak crystallization and melting temperatures (T_{pc} and T_{pm}), crystallization and melting enthalpies (ΔH_c and ΔH_m), and final crystallization and melting temperatures (T_{fc} and T_{fm}) (Biliaderis, 1983). The thermal analysis was carried out only once for each sample.

2.6 Polarized light microscopy. Samples were melted at the temperature of 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 hours. Crystal morphology was evaluated by means of the polarized light microscope (Olympus, model BX 50) coupled to the digital video camera (Media Cybernetics). Glass sliders were placed on a hot plate (Mettler Toledo, FP82 Microscope Hot Stage), 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 40 times. For each glass slider, 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). To obtain these parameters, 180 crystals were measured for the original blends. For interesterified blends, the totality of crystals was considered in calculations.

2.7 Crystallization isotherms. Samples were melted (100°C/15min) and kept in high precision dry bath (TCON 2000 - Duratech, USA) at 70°C for complete destruction of its crystal history (Campos, 2005). The solid fat content increase in keeping with crystallization time was monitored by the Nuclear Magnetic Resonance Spectrometer (NMR) Bruker pc120 Minispec. Each sample tube was placed in the sample holder in the NMR equipment, with reading compartment stabilized at 15 and 25°C. The analyses were performed in duplicated for each temperature. Data acquisition was automatic, with measures being taken at every minute, during 60 minutes. Characterization of crystallization kinetics was performed in accordance with the induction period (τ_{SFC}) – referent to the beginning of crystal formation – and the maximum solid content ($SFC_{máx}$). Induction time is graphically attained and reflects the time needed for a stable nucleus of critical size to be formed in liquid phase (Himawan, Starov, & Stapley, 2006; Metin & Hartel, 2005). The original Avrami equation, which consists in the most used model for the isothermal phase transformation kinetics description, was used for crystallization study at 25°C (Narine, Humphrey, & Bouzidi, 2006; Toro-Vazquez, Dibildox-Alvarado, Charó-Alonso, Charó-Alonso, & Gómez-Aldapa, 2002):

$$\frac{CGS(t)}{CGS(\infty)} = 1 - e^{-kt^n}$$

Where: *CGS(t)* describes solid fat content (%) as time function, *CGS(∞)* is the solid fat content limit when time tends to infinite, *k* is the Avrami constant (min^{-n}), which takes into

account both crystal nucleation and growth rate, and *n* is the Avrami exponent, which indicates crystal growth mechanism (McGauley & Marangoni, 2002; Wright, Hartel, Narine, & Marangoni, 2000). The equation was linearized and applied to the results attained during the first 20 minutes of crystallization in order to determine the *k* and *n* values.

2.8 X-ray diffraction. The samples fat crystals' polymorphic form was determined according to the method AOCS Cj 2-95 (AOCS, 2004). Analyses were carried out in a diffractometer Philips (PW 1710), using Bragg-Bretano (θ:2θ) geometry with radiation of Cu-Kα (λ = 1.54056Å, tension of 40 KV and 30 mA). Measures were attained with 0.02° in 2θ steps and acquisition time of 2 seconds, with scans of 5 to 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 4.15Å. Form β' is characterized by two strong diffraction lines at 3.8 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 and 3.8 Å (AOCS, 2004; Rousseau & Marangoni, 2002). The crystal contents of types β and β', in the samples, were calculated from normalized areas by sum of peak areas. The areas were calculated by removing the background. The β form was calculated from the area of the short spacings at 4.6 Å and the β' was calculated from the areas of the short spacings at 3.8 and 4.2 Å, using the equation:

$$\%\beta = (A_{\beta} \times 100)/(A_{\beta} + A_{\beta'}); \qquad \%\beta' = (A_{\beta'} \times 100)/(A_{\beta} + A_{\beta'});$$

Where A_{β} e $A_{\beta'}$ were the peak areas of β and β' polymorphs, respectively (Yap, Deman, & Deman, 1989; Reshma, Saritha, Balachandran, & Arumughan, 2008).

3. Results and Discussion

3.1 Triacylclycerol composition. From a technological point of view, the triacylglycerol profile is the key for the understanding of several physical properties of a given oil or fat (Buchgraber, Ulberth, Emons, & Anklan, 2004). The chemical interesterification reactions result in complete fatty acid randomization among all triacylglycerols present, according to the laws of probability (Rousseau & Marangoni, 2002). Fig. 1 shows the triacylglycerol compositions of blends before and after interesterification, according to the triacylglycerol contents of types S_3 (trisaturated), S_2U (disaturated-monounsaturated), SU_2 (monosaturated-diunsaturated), and U_3 (triunsaturated).

The addition of FHCSO to CaO promoted the increase of triacylglycerol S_3 percentages and the concomitant decrease of other triacylglycerol classes. Original blends with 20 and 25% of *hardfat* presented low contents of triacylglycerols S_2U, which were not detected for the other blends. For the totality of blends, randomization caused a decrease in the contents of triacylglycerols of types S_3 and U_3, with an increase in the percentages of the triacylglycerol classes S_2U and U_2S, whose sum accounted for 58.82, 60.49, 57.55, 54.65 and 46.78% of the whole triacylglycerol composition for the 80:20, 75:25, 70:30, 65:35 and 60:40 blends, respectively. For all blends, a significant predominance of triacylglycerols U_3 was verified, before reaction. After random rearrangement, triacylglycerols U_2S presented the highest concentration among the triacylglycerol classes. List, Mounts, Orthoefer, and Neff (1995) evaluated the interesterification of 80:20 CaO:FHCSO blend, with a view to producing margarines and shortenings. S_3, S_2U, SU_2 and U_3 contents before the reaction were 20.8, 1.4, 19.9 and 57.6%, respectively. After randomization, the percentages of these triacylglycerol classes were, respectively, 4.1, 11.9, 46.0 and 36.2%, very close to those found in this study.

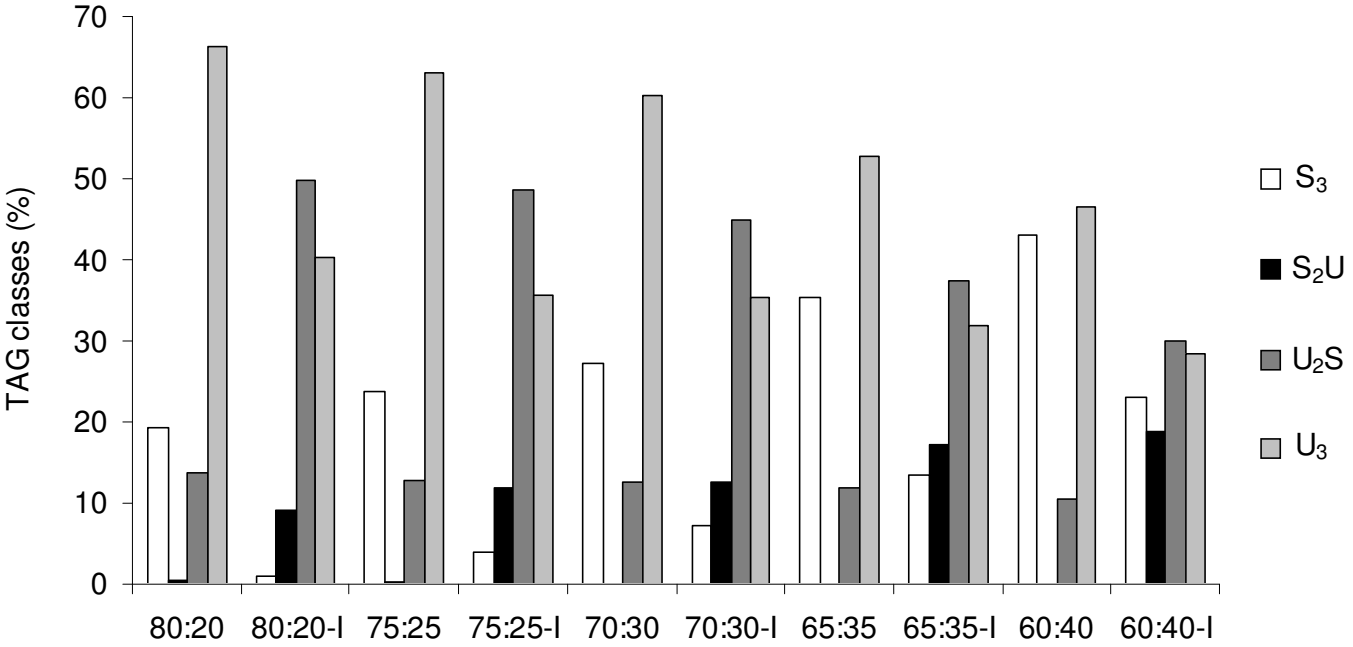

Fig.1. Triacylglycerol classes (%) in CaO:FHCSO blends, before and after interesterification. I: interesterified blend. Triacylglycerols: S_3 (trisaturated), S_2U (disaturated-monounsaturated), SU_2 (monosaturated-diunsaturated) and U_3 (triunsaturated). Average values among duplicate sample injections.

According to Rodrigues and Gioielli (2003), the properties of fatty foods can be related to the triacylglycerol composition of that fat which composes them. Triacylglycerols S_2U, with melting points between 27 and 42°C, are mainly responsible for the structure of products, whereas triacylglycerols U_2S are important with regard to their sensorial and functionality properties at ambient temperature. Hence, the increase of the S_2U and U_2S contents of CaO:FHCSO 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 (Wiedermann, 1978).

3.2 Thermal behavior. Differential Scanning Calorimetry (DSC) is the most used thermoanalytical 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 triacylglycerol blends (Che Man et al., 2003). Evaluation by DSC provides direct measures on the energy involved in the melting and crystallization processes of oils and fats. 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 (Tan & Che Man, 2002). Table 1 shows the parameters regarding the crystallization thermograms for the blends, before and after randomization.

Table 1. Crystallization onset temperature (T_{oc}), crystallization peak temperature (T_{pc}), crystallization enthalpy (ΔH_c) and crystallization end temperature (T_{fc}), of the original and interesterified blends. I: interesterified blend.

CaO: FHCSO	T_{oc} (°C)	T_{pc} 1 (°C)	T_{pc} 2 (°C)	ΔH_c 1 (J/g)	ΔH_c 2 (J/g)	T_{fc} (°C)
80:20	36.75	31.04	-	33.59	-	13.64
80:20 - I	32.97	28.80	0.15	13.30	6.23	-9.93
75:25	37.59	32.47	-	42.17	-	14.55
75:25 - I	33.62	31.27	3.30	18.30	6.83	-5.86
70:30	38.45	33.97	-	52.94	-	15.52
70:30 - I	34.28	31.77	4.97	20.27	7.49	-3.97
65:35	40.34	35.80	-	68.01	-	16.55
65:35 - I	37.41	33.97	6.63	22.45	8.20	-2.69
60:40	42.21	37.13	-	78.32	-	18.45
60:40 - I	40.82	36.09	10.63	24.87	13.90	-1.82

The crystallization curve subdivision of a given oil or fat, in different exothermic regions, corresponds to different types of triacylglycerols (Tan & Che Man, 2002). Original blends presented a single peak in the crystallization thermogram related to the FHCSO trisaturated fraction, with high-melting-point triacylglycerols, mainly represented by the species PStP, PStSt, and StStSt (Deman, Deman, & Blackman, 1989). The T_{oc}, $T_{pc}1$ and T_{fc} values are functions of the FHCSO concentration increase in the blends, proving that the crystallization process was accelerated by the increase in the triacylglycerols S_3 content of samples. The onset crystallization temperature (T_{oc}) represents the beginning of the transition phase, that is, the temperature at which the first crystals are formed (Aronhime, 1988). The addition of *hardfat* to liquid oil produced blends with crystallization onset between 36.75 and 42.21°C.

Peak crystallization temperature, on the other hand, refers to the temperature at which the biggest proportion of lipid species crystallizes with maximum thermal effect (Campos, 2005). Original blends presented $T_{pc}1$ values between 31.04 and 37.13°C. At the beginning of a blend's crystallization process, 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 will be. Consequently, the increase in the number of molecules with simultaneous crystallization leads to a larger energy release by the system (Campos, 2005; Humphrey and Narine, 2004). The crystallization enthalpy values of original blends were between 33.59 and 78.32J/g.

Interesterification caused the appearance of a second peak in the crystallization thermograms, which is characteristic of the expressive increase in the middle-melting-point triacylglycerols (S_2U and SU_2) contents, as a result of the randomization process. Interesterified blends showed increasing T_{oc}, $T_{pc}1$, $T_{pc}2$, and T_{fc} values according to the

gradual increase in the triacylglycerol S_3 content (Fig. 1). There was onset (T_{oc}) and final (T_{fc}) crystallization temperature decrease, allowing interesterified blends to crystallize at temperatures lower than those of their original blends (between 32.97 and 40.82°C). $T_{pc}2$ presented values between 0.15 and 10.63°C, especially increasing as the function of the triacylglycerol S_2U content in interesterified blends, which indicates that the crystallization intensity of this group of compounds directly relates to its proportion in the blends.

According to Campos (2005), crystallization enthalpy value is strongly related to the intermolecular arrangement of triacylglycerol species and, therefore, usually modified by randomization. ΔH_c1 values decreased as a consequence of randomization, resulting from the lower triacylglycerol S_3 content in the blends which suffered random rearrangement. The enthalpy decrease values regarding the second peak verified in the thermograms (ΔH_c2) presented concordance with the triacylglycerol composition results, given that they showed a relation with the triacylglycerols S_2U contents, a predominant class in the randomized blends.

Fig. 2 shows the differences between the T_{oc} and $T_{pc}1$, (ΔT_{o-p}), values for the original and interesterified blends. This parameter is proportional to crystal growth rate (Marangoni & Rousseau, 1998). The ΔT_{o-p} values of original blends are higher than those of interesterified blends. According to Rousseau and Marangoni (2002), this effect may be due to the randomization of saturated fatty acids among all triacylglycerol species present, potentially leading to concomitant increase in nucleation and decrease in crystalline growth rates. In terms of crystal packing, the random rearrangement of fatty acids in the triacylglycerols would destroy any structural complementarity among triacylglycerol molecules, disfavoring crystal growth. Hence, the interesterification of CaO:FHCSO blends results in a less kinetically and/or thermodynamically favorable crystal growth (Rousseau & Marangoni, 2002).

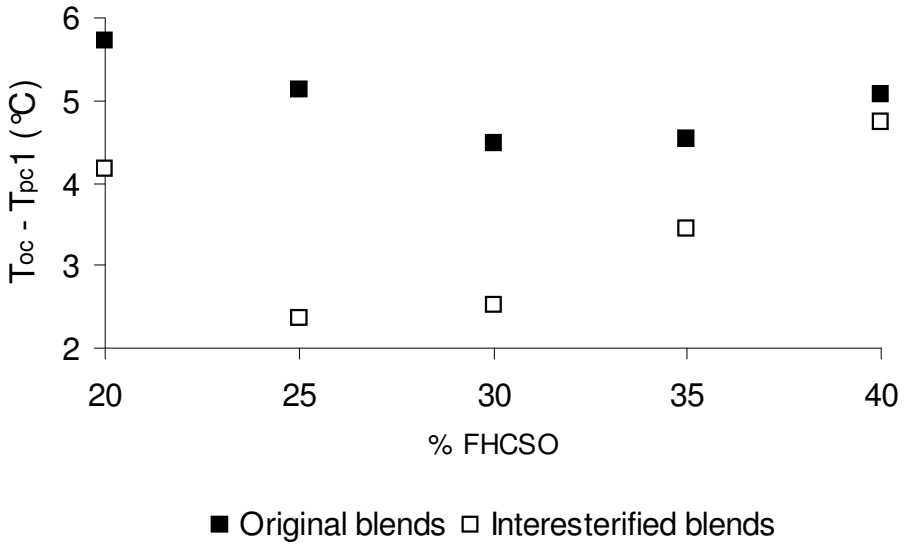

Fig.2. Differences between onset (T_{oc}) and peak crystallization temperatures ($T_{pc}1$) of original and interesterified blends.

Table 2 shows the parameters regarding the melting thermograms for the blends, before and after randomization. The melting thermograms of original blends were characterized by two peaks pertaining to the unsaturared fraction of CaO ($T_{pm}1$), with low-melting-point triacylglycerols, and the trisaturated fraction corresponding to FHCSO ($T_{pm}2$), which were situated in the intervals from -15.07 to -14.98°C and from 54.27 to 58.22°C, respectively. Onset melting temperature (T_{om}) presented small variation in keeping with the FHCSO content of original blends, whereas the $T_{pm}1$, $T_{pm}2$, and T_{fm} values increased according to FHCSO addition in the blends, as a result of the percentage decrease of triacylglycerol species coming from CaO and the increment of high-melting-point triacylglycerols that are characteristic of FHCSO. This effect is also associated to the decrease and increase, respectively, of ΔH_m1 and ΔH_m2, upon the addition of FHCSO to CaO. According to Humphrey and Narine (2004), when *hardfat* content of a given sample increases, high-melting-point components proportionally increase.

Table 2. Melting onset temperature (T_{om}), melting peak temperatures (T_{pm}), melting enthalpies (ΔH_m) and melting end temperature (T_{fm}) of the original and interesterified blends. I: interesterified blend.

CaO: FHCSO	T_{om} (°C)	T_{pm} 1 (°C)	T_{pm} 2 (°C)	ΔH_m 1 (J/g)	ΔH_m 2 (J/g)	T_{fm} (°C)
80:20	-19.00	-15.07	54.27	17.13	33.79	57.87
80:20 - I	-18.72	-14.48	45.77	28.11	23.13	50.94
75:25	-19.03	-14.82	55.52	15.70	44.54	58.73
75:25 - I	-17.33	-12.40	47.76	29.80	39.50	53.09
70:30	-19.09	-14.90	56.35	14.66	55.75	59.60
70:30 - I	-16.21	-11.48	51.45	31.79	45.99	54.70
65:35	-18.32	-14.65	57.76	9.22	65.07	61.14
65:35 - I	-15.56	-12.32	51.35	33.91	53.83	53.89
60:40	-18.57	-14.98	58.22	4.24	82.00	61.31
60:40 - I	-14.97	-10.90	53.12	36.17	65.22	56.36

After randomization, an increase in the values of T_{om} and $T_{pm}1$ was verified, which is related to the significant increase in the contents of middle-melting-point triacylglycerols (S_2U and SU_2). Yet, the ΔH_m1 values significantly increased after reaction, indicating an increase in the participation of these triacylglycerol species in the interesterified blends. In parallel, interesterification promoted the reduction of $T_{pm}2$, T_{fm}, and ΔH_m2 values as a result of the triacylglycerol S_3 percentage decrease in the blends.

However, among original and interesterified blends, it was observed that the variations in peak melting temperatures were low and not directly proportional to the triacylglycerol S_3 increase. For the original blends, variation was between 0.08 and 1.4°C, whereas for the interesterified blends a variation between 0.1 and 3.7°C was verified. Similar effect was verified by Humphrey and Narine (2004), who evaluated the fusion thermograms of 24 shortenings containing different fully hydrogenated oils, diluted in soybean oil, in 5% increments. For the same *hardfat* type, the maximum peak temperature variation was

small, not exceeding 5℃. According to the authors, the *hardfat* choice, and not its concentration, is the determining factor for the attainment of blends with differentiated fusion properties.

3.3 Microstructure. According to Narine and Marangoni (2005), the microstructural level, or mesoscale, of a fat's crystal network can be defined as the group of structures with dimensions ranging from 0.5 to 200μm. 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. Polarized light microscopy (PLM) is the most used technique for the visualization of fat microstructural network and it has been applied with the intent of explaining the texture differences of fat blends and showing morphological alterations in crystal growth (Gioielli, Simões, & Rodrigues, 2003).

Fig. 3 shows the crystal structure of CaO:FHCSO blends, before and after interesterification by slow crystallization at 25℃. Crystal mean diameter values of blends and the range of crystalline distribution, before and after randomization, are shown in Table 3. 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, Escobedo, & Gioielli, 2008; Rousseau, Hill, & Marangoni, 1996).

Original blends presented crystals with mean diameters between 79.95 and 92.77 μm, in the form of spherulites. Especially in the 60:40 blend, spherulites were preponderantly observed in the form of agglomerates. Narine and Humphrey (2004) reported that the mean diameter of the spherulites observed for the CaO:FHCSO 75:25 blend, statically crystallized at 20℃, was approximately 100μm, that is, close to that verified in this study. According to Shi et al. (2005), the highest-melting-point triacylglycerol species of a blend dominates crystal morphology. FHCSO presents PStSt (~ 43%) and StStSt (~ 40%) as the

predominant triacylglycerols, with melting points equal to 61 and 65°C, respectively. The presence of large spherulites can be attributed, therefore, to the significant triestearine content in the blends, which gives that undesirable sandy texture to fat (Deman et al., 1989; Rousseau et al., 1998). Concordantly with these observations, crystal diameter decreased as CaO concentration increased in the blends, although crystal morphology was not altered by the dilution effect. Rousseau et al. (1998) also observed this behavior upon the liquid-oil proportion increase in palm oil/soybean oil and lard/canola oil blends. For original blends with FHCSO contents between 20 and 35%, the minimum and maximum mean diameters of crystals were very similar. The minimum mean diameters showed values from 61 to 68 μm, whereas the maximum mean diameters presented values between 96 and 109 μm. The 60:40 original blend exhibits crystalline distribution characterized by larger crystals, which mean diameters were from 73 to 121 μm.

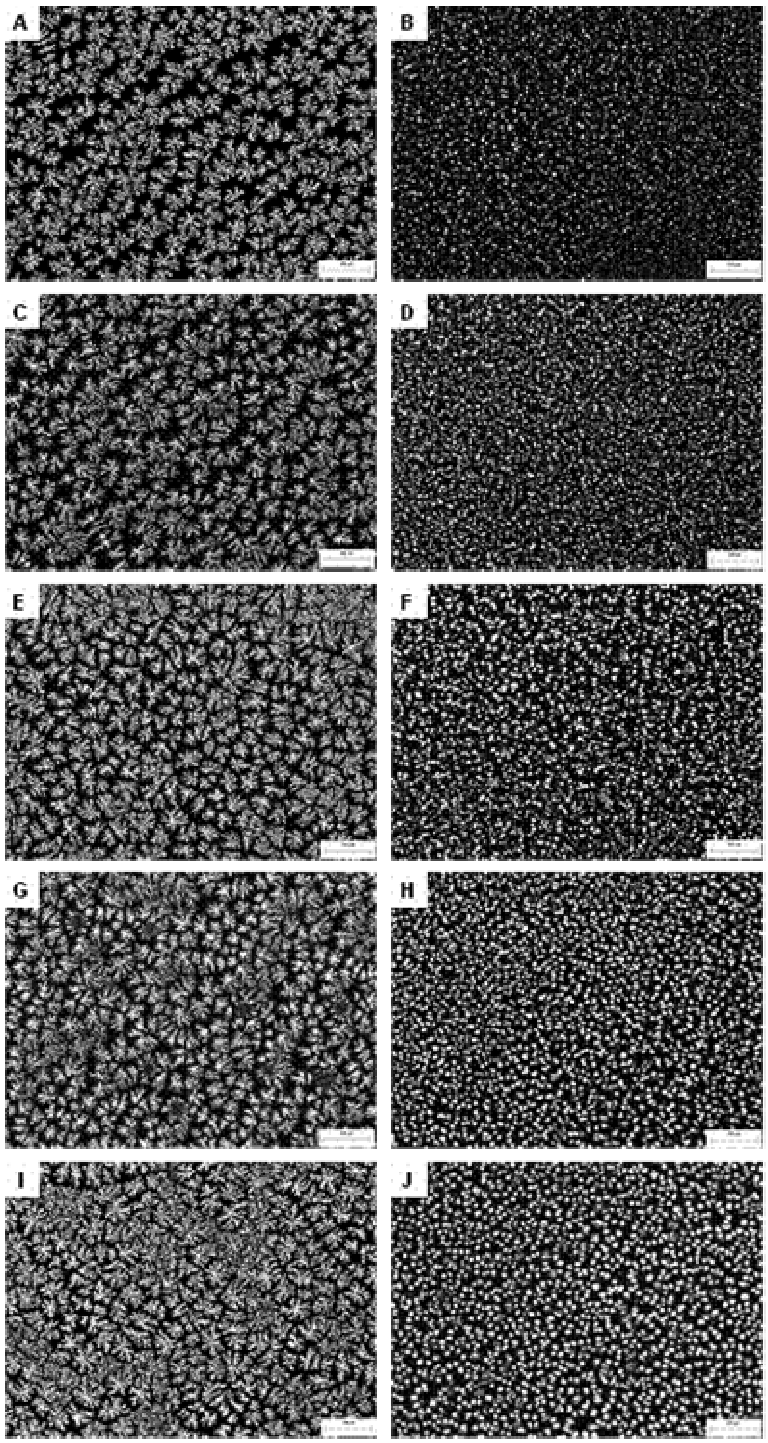

Fig.3. Images of crystallization of CaO:FHCSO blends, before and after chemical interesterification. A: 80:20; B: 80:20-I; C: 75:25; D: 75:25-I; E: 70:30; F: 70:30-I; G: 65:35; H: 65:35-I; I: 60:40; J: 60:40-I. I: interesterified blend. The bar represents 200μm.

Table 3. Mean crystal diameter and minimum/ maximum mean crystal diameter of CaO:FHCSO blends, before and after interesterification.

	Before interesterification		**After interesterification**	
CaO: FHCSO	Medium crystal diameter (μm)	Range - medium crystal diameter variations (μm)	Medium crystal diameter (μm)	Range - medium crystal diameter variations (μm)
80:20	79.95 ± 7.84	61.13 - 103.33	6.13 ± 2.81	2.21 - 13.46
75:25	79.93 ± 7.36	62.28 - 100.01	7.92 ± 4.10	2.04 - 13.71
70:30	80.85 ± 8.94	68.11 - 96.43	10.72 ± 5.86	2.52 - 30.20
65:35	80.58 ± 9.63	65.63 - 109.24	11.80 ± 5.59	2.09 - 39.06
60:40	92.77 ± 10.76	73.19 - 121.63	15.60 ± 7.04	2.19 - 41.19

After randomization, complete modification of crystal morphology took place for the totality of blends. Disc-shaped crystals, with granular distribution, were observed (Campos, 2005). Additionally, random rearrangement promoted significant crystal diameter decrease for all blends. Notwithstanding, the mean diameter of crystals increased in keeping with the triacylglycerol S_3 content in randomized blends (as shown in Fig. 1), presenting dimensions between 6.13 and 15.60 μm. Interesterified blends with 20 and 25% FHCSO contents showed similar crystalline distributions, with crystals in range of 2 -14 μm. For the 70:30, 65:35 and 60:40 interesterified blends, the minimum mean diameter of crystals was also close to 2 μm, but the maximum mean diameter of crystals was considerably high, presenting values between 30 and 41 μm.

According to Herrera, Falabella, Melgarejo, & Anón (1998), the application of fat in food products demands that the mean diameter of crystals be inferior to 30μm in order to prevent that sandy sensation in the mouth. For all blends evaluated, interesterification was effective at attaining fat bases which are compatible with food application. Fig. 3 also shows that randomization promoted an expressive increase in the number of crystals in the blends as well. The crystal dispersion of fats with a large number of small crystals can provide desirable properties such as good spreadability. 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 (Kloek, Walstra, & van Vliet, 2000; Lee, Akoh, Himmesbach, & Lee, 2008).

According to Rousseau et al. (1998), alterations in the microstructure of fats, caused by interesterification, result from modifications in the morphology and density of the crystalline network and affect the texture and functionality properties of interesterified bases. The triacylglycerol composition alteration caused by randomization modifies the relative strength of bonds among crystal elements in an aggregate (intraparticle) and among

aggregates (interparticle), generating the formation of differentiated structures (Shi et al., 2005). Moreover, the change in solid fat content inherent to the interesterification process influences crystal network structuring. When solid content decreases, as verified in this study, changes regarding the larger area and a greater molecular mobility for crystal formation take place (Himavan et al., 2006).

3.4 Crystallization kinetics. Crystallization kinetics deeply influences the final structure of fats and is intrinsically related to their rheologic and plasticity properties. The crystallization kinetics study of interesterified fats is of significant importance so that fat can be adjusted to industrial process limitations and the control over processing steps which include fat fraction recrystallization is improved, ensuring final product quality (Wassel & Young, 2007). Fig. 4 shows crystallization isotherms attained at 15 and 25°C for original and interesterified blends. Crystallization of the 80:20 interesterified blend, at 25°C, did not occur, as a result of its low solid fat content. Table 4 presents the induction period (τ_{SFC}) and maximum solid fat content (SFC_{max}) values for the original and interesterified blends. These parameters are to be considered when outlining the application of interesterified fats. In some specific industrial processes, fats must be completely crystallized until the end of the production line in order to ensure that crystal equilibrium is reached. Otherwise, the standardized processing times must be altered in accordance with the characteristics of the fat in use. This fact became particularly important as interesterified fats started to replace partially hydrogenated fats in most industrial applications (Herrera, Gatti, & Hartel, 1999).

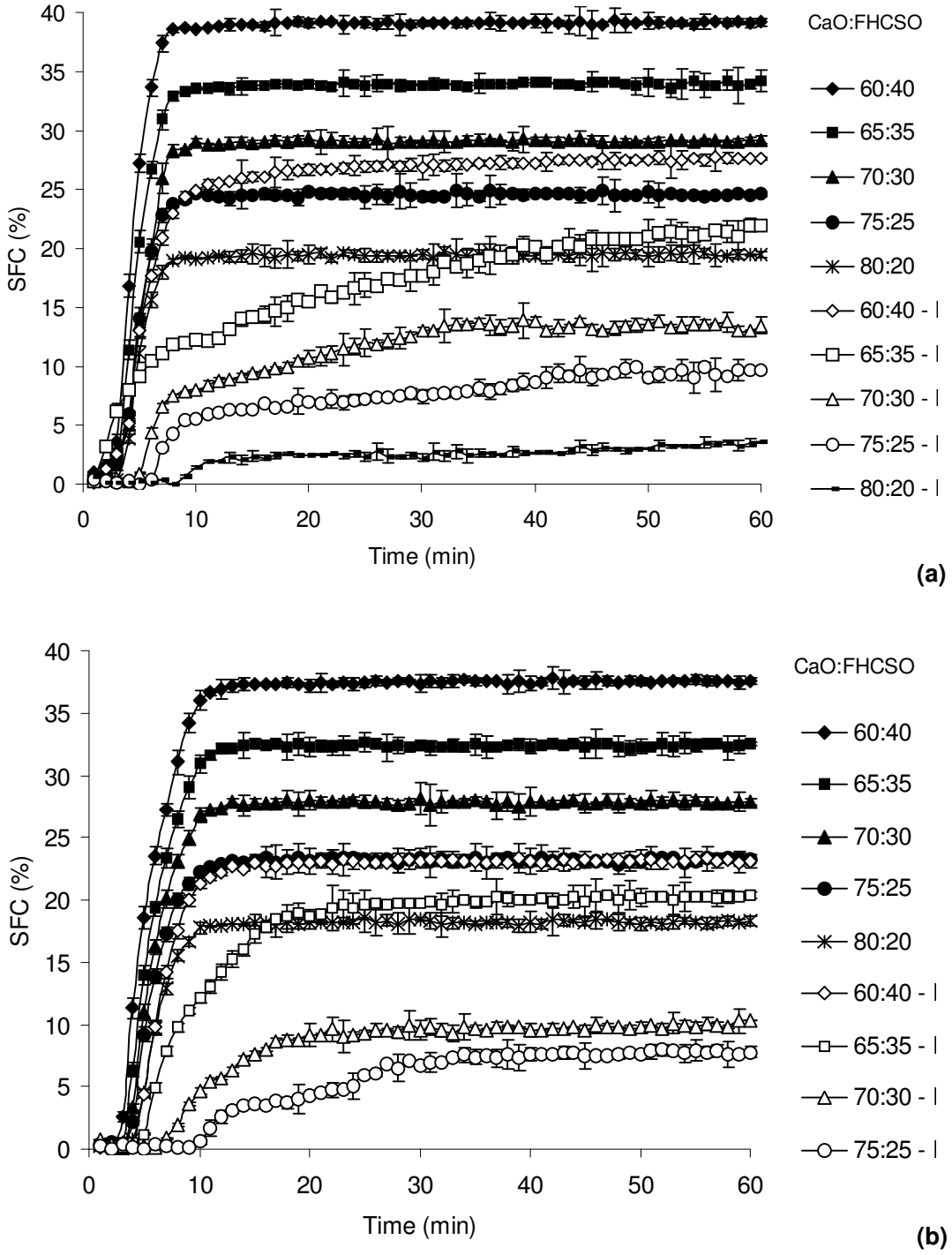

Fig. 4. Crystallization isotherms at 15℃ (a) and 25℃ (b) for original and interesterified blends (I).

Table 4. Induction period (τ_{SFC}), maximum solid fat content ($SFC_{máx}$) for the CaO:FHCSO blends, at 15 and 25℃, before and after interesterification. I: interesterified blend.

	15℃		25℃	
CaO: FHCSO	**τ_{SFC} (min)**	**$SFC_{máx}$ (%)**	**τ_{SFC} (min)**	**$SFC_{máx}$ (%)**
80:20	3	19.77 ± 0.78	4	18.48 ± 2.56
80:20 – I	9	3.58 ± 0.76	-	-
75:25	2	24.87 ± 2.39	3	23.42 ± 1.34
75:25 - I	6	9.97 ± 0.66	9	7.94 ± 0.85
70:30	2	29.29 ± 1.35	3	28.12 ± 2.50
70:30 -I	5	13.88 ± 2.91	6	10.27 ± 1.96
65:35	2	34.22 ± 1.66	3	32.63 ± 1.53
65:35 -I	2	21.98 ± 1.17	4	20.40 ± 0.61
60:40	2	39.31 ± 2.07	2	37.81 ± 1.93
60:40 -I	2	27.77 ± 2.44	4	23.35 ± 2.32

The addition of FHCSO to CaO promoted proportional increases in the $SFC_{máx}$ value. Original blends presented maximum $SFC_{máx}$ between 19.77 and 39.31% at 15℃ and between 18.48 and 37.81% at 25℃. For all evaluated blends, the $SFC_{máx}$ values showed small decline with the increase of crystallization temperature. According to Rousseau et al. (1998), the increase of crystallization temperature promotes an effect related to formation of weaker fat crystal networks, which reflect directly in the solid fat content. The increase of the temperature of isothermal crystallization was also associated to decrease in τ_{SFC} for most original and interesterified blends. The 60:40 original blend, however, kept its induction period characteristics. Additionally, the examination of Fig.4 makes perceptible that at 25℃ all evaluated blends displayed longer times to attain complete crystallization equilibrium when compared to blends crystallized at 15℃.

Interesterification caused a $SFC_{máx}$ decrease of all blends, an effect which is associated to the decrease of triacylglycerols S_3 and simultaneous increase in the percentages of triacylglycerols S_2U and SU_2 (Rousseau & Marangoni, 2002). The reduction of $SFC_{máx}$ caused by randomization corresponded to 66.10, 63.48, 37.48 and

38.24% for the 75:25, 70:30, 65:35 and 60:40 blends at 25°C, respectively. The τ_{SFC} of original blends was also modified by random rearrangement, especially in relation to the blends with smaller percentages of FHCSO. Nevertheless, for blends with 35 and 40% of *hardfat*, this alteration was little significant. At 25°C, original blends presented τ_{SFC} between 2 and 4 minutes, whereas for the interesterified blends, values between 4 and 9 minutes were observed.

The alteration in the induction or nucleation period of crystallization primely results from the change in solid fat content that is inherent to the interesterification process, which influences crystal network formation and structuring (Himavan et al., 2006). Moreover, the bigger the difference between crystallization temperature and melting point of sample, the higher the supercooling degree imposed on the system and, consequently, the smaller the τ_{SFC} values will be. When supercooling degree is small, the incorporation of triacylglycerol molecules in the crystal structure occurs in the most suitable configuration and the stablest polymorph is formed, for there is enough time for the molecules to orient themselves perfectly. At a high supercooling degree, however, the incorporation of triacylglycerol molecules on the crystal surface is very fast and, therefore, imperfect, which can result in the formation of mixed crystals and polymorphs that can persist indefinitely (McGauley & Marangoni, 2002; Metin & Hartel, 2005). Hence, although the percentage increase of middle-melting-point triacylglycerols (represented by species S_2U and SU_2, as shown in Fig. 1), caused by randomization, concurs for bigger τ_{SFC} values, interesterified blends with lower melting points are associated to greater polymorphic stability and homogeneity at the temperature of 25°C, which is of significant importance for food application (Martini, Herrera, & Hartel, 2002; Wiedermann, 1978).

In the study of fats, the Avrami model describes an event in which there is an initial *lag* period, where crystallization occurs slowly, and a subsequently rapid crystal mass increase. The Avrami theory considers that crystallization occurs both by nucleation and crystal growth, and assumes that transformation conditions are isothermic, that nucleation

occurs spatially and arbitrarily, and that growth kinetics is linear, in which the new phase's growth velocity depends solely on temperature, and not on time. The Avrami parameters provide information on the nature of the crystallization process. Constant *k* is the crystallization velocity constant and is mainly dependent on crystallization temperature. The Avrami exponent, *n*, is the combined function of time dependency, on the part of nucleation, and the number of dimensions in which growth takes place. Nucleation can be instantaneous, with nuclei appearing all at once at the beginning of the process, or sporadic, with the number of nuclei linearly increasing with time. Growth takes place in the form of needles, discs or spherulites, in one, two or three dimensions, respectively (McGauley & Marangoni, 2002; Wright et al., 2000). Fig. 5 shows the Avrami parameters (for crystallization at 25°C) which indicate velocity (*k*) and crystallization form (*n*), before and after interesterification.

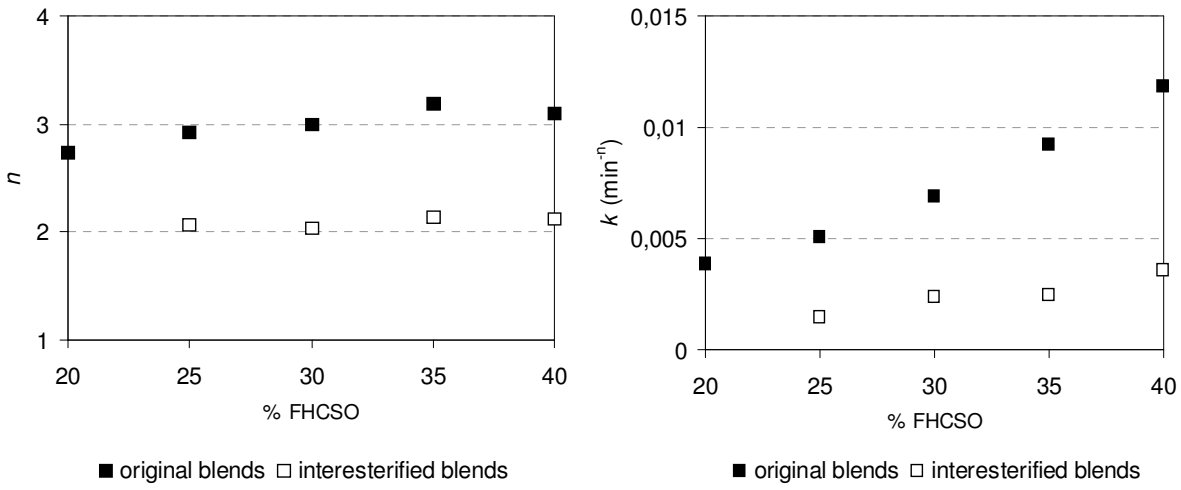

Fig.5. Avrami constant (*k*) and Avrami exponent (*n*) of CaO:FHCSO blends, before and after interesterification, crystallized at 25°C.

Avrami constant (*k)* gradually decreased with the increase in the proportion of CaO for the original and interesterified blends, indicating that crystallization velocity was lower in keeping with the dilution effect of FHCSO with liquid oil as well as the triacylglycerol U_3 content increase and triacylglycerol S_3 content decrease. Interesterification promoted the crystallization rate drop of blends, which is related to the decrease in the percentages of triacylglycerols S_3, characterized by instantaneous crystallization (Marangoni, 2005). These results corroborate those previously discussed, with relation to parameter $\Delta T_{o\text{-}p}$ (Fig. 2), which evidence that randomized blends show crystal growth thermodynamically less favorable than that of original blends.

n values equal to 3 correspond to spherulitic growth from instantaneous nuclei or disc-shaped growth from sporadic nuclei, whereas *n* values equal to 2 denote needle-shaped growth from sporadic nuclei or disc-shaped growth from instantaneous nuclei. Although *n* should correspond to an integer, fractional values are usually attained (McGauley & Marangoni, 2002; Marangoni, 2005).

As shown in Fig. 5, original blends showed *n* values very close to 3. In parallel, the observation of the crystal structure of these blends (Fig. 3) attest that crystals are shown as spherulites. Thus, it is concluded that the crystallization of the original blends is characterized by spherulitic growth from instantaneous nuclei. Interesterified blends showed *n* values very close to 2. Additionally, they were properly characterized by disc-shaped crystals, according to Fig. 3. Therefore, the conjoint result analysis leads to the conclusion that randomized blends also present crystal growth from instantaneous nuclei. In summary, results show that chemical interesterification only modified the crystal morphology of CaO:FHCSO blends, but caused no alterations as to the nucleation mechanism.

3.5 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. Generally, triacylglycerols first crystallize in polymorphic forms α and β', although form β is the most stable. Transformation velocity is dependent on the homogeneity degree of triacylglycerols. Fats with low variability of triacylglycerols rapidly transform into the stable form β. Fats that consist in random distribution of triacylglycerols can present the form β' indefinitely (Sato, 2001). However, since fats are complex triacylglycerol blends, different polymorphic forms and liquid oil can simultaneously coexist at certain temperature (Chong, Kamarudin, Lesieur, Marangoni, Bourgaux, & Ollivon, 2007).

Diffractograms of blends, before and after chemical interesterification, attained at 20°C, are shown in Fig. 6. Diffraction patterns of natural or interesterified fats presented larger peaks than those of pure compounds, due to the presence of multiple triacylglycerols in the cellular units and the concomitant presence of liquid oil (Rousseau et al., 1998). For original and interesterified blends, peaks at 4.6 Å, and at 4.2 and 3.8 Å were verified,

which is characteristic of polymorphic forms β and β', respectively. Due to the lower solid fat content of the 80:20 interesterified blend at 20°C, the diffractogram at this analysis temperature presents broad peaks, which is typical of amorphous materials.

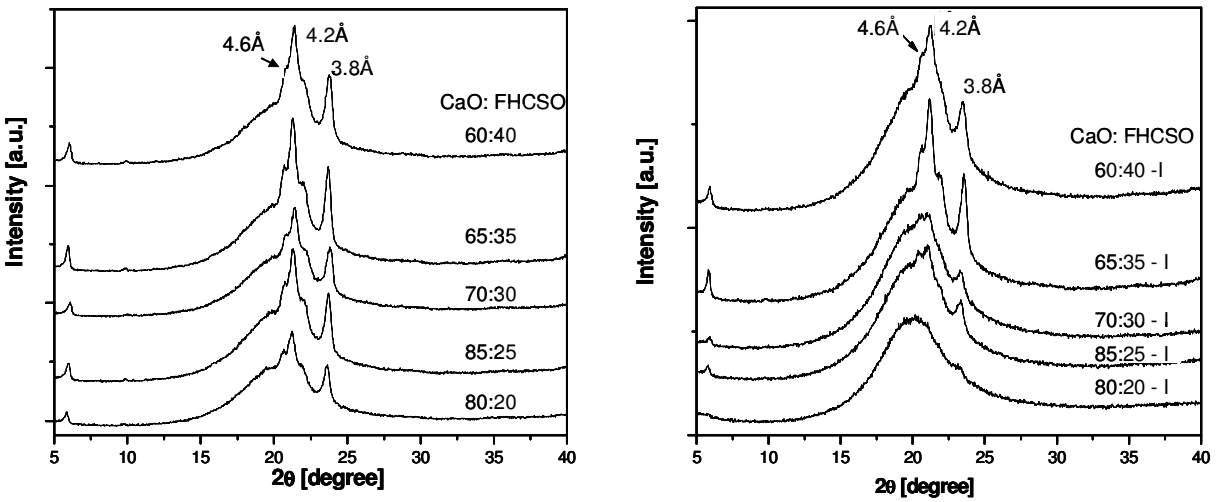

Fig. 6. X-ray diffraction patterns for original and interesterified (I) CaO:FHCSO blends, at 20°C.

Table 5 details the polymorphic forms of original and interesterified blends. Original blends presented predominance of polymorphic form β' (79.21 to 84.26%), although form β was verified in smaller proportions (15.74 to 20.79%), in all blends. As far as physical blends between CaO and FHCSO are concerned, which are known for possessing polymorphism β and β', respectively, the results presented herein are consistent with those verified in pertinent literature (Deman et al., 1989; Foubert, Dewettinck, Van de Walk, Dijkstra, & Quinn, 2007; Ghotra, Dyal, & Narine, 2002; Lawler & Dimick, 2002; Narine & Humphrey, 2004; Rousseau, Hodge, Nickerson, & Paulson, 2005). CaO presents polymorphism β associated to the low diversity in fatty acid composition, which provides relative homogeneity in its triacylglycerol composition (Foubert et al., 2007; Wiedermann, 1978). According to Ghotra et al. (2002), palmitic acid contents higher than 20% are determinant to promote stability of polymorphic form β' in oils and fats. FHCSO with

palmitic acid percentages between 20 and 24% fit, therefore, in the form β' promoter category. Moreover, FHCSO presents considerable contents of triacylglycerols PStP (~ 15%), which are responsible for the exceptional stability of polymorph β' (Ghotra et al., 2002; Lawler & Dimick, 2002). Additionally, it was verified that, in the original blends, the percentage increase of FHCSO promoted the increase in the intensity of characteristic peaks of form β', with consequent decrease in the intensity of the peaks regarding form β, which is inherent to CaO. Deman et al. (1989) also observed similar effect on CaO:FHCSO blends.

Table 5. Polymorphic forms (%) of CaO:FHCSO blends, before and after interesterification.

	Polymorphic forms (%)			
	Before interesterification		**After interesterification**	
CaO: FHCSO	**β'**	**β**	**β'**	**β**
80:20	84.26	15.74	78.88	21.12
75:25	80.82	19.18	liquid	liquid
70:30	79.21	20.79	74.05	25.95
65:35	82.03	17.97	80.84	19.16
60:40	80.83	19.17	78.01	21.99

The crystal habit of blends was not significantly altered by the random rearrangement process. Randomized blends kept crystallization tendency in form β', which is probably associated to that 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). Ramli, Said and Loon (2005) also verified that interesterification did not modified polymorph β', which is characteristic of palmiste oil and goat milk fat blends.

Crystals β' are small and present morphology that is suitable for the plasticity characteristics which are desirable in products such as margarines, shortenings, and fat for bakery 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. Conclusion

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 showed that triacylglycerol composition, thermal behavior, microstructure and crystallization kinetics properties of CaO:FHCSO blends were significantly altered by both increasing *hardfat* concentration and chemical interesterification process. The randomization, however, did not change the original crystalline polymorphism.

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References

Antoniosi Filho, N., Mendes, O. L., & Lanças, F. M. (1995). Computer prediction of triacylglycerol composition of vegetable oils by HRGC. *Chromatographia, 40*, 557-562.

AOCS. (2004). *Official methods and recommended practices of the American Oil Chemists' Society*. Champaign: American Oil Chemists' Society.

Aronhime, J.S. (1998). Application of thermal analysis (DSC) in the study of polymorphic transformations. *Thermochimica Acta*, *134*, 1-14.

Biliaderis, C.G. (1983). Differential scanning calorimetry in food research – a review. *Food Chemistry*, *10*, 239-265.

Buchgraber, M., Ulberth, F., Emons, H., & Anklan, E. (2004). Triacylglycerol profiling by using chromatographic techniques. *European Journal of Lipid Science Technology*, *106*, 621-648.

Campos, R. (2005). Experimental Methodology. In A. G. Marangoni (Ed.). *Fat Crystal Networks*, (pp.267-349). New York: Marcel Dekker.

Che Man, Y.B., Shamsi, K., Yusoff, M.S.A., & Jinap, S. (2003). A study on the crystal structure of palm oil-based whipping cream. *Journal of American Oil Chemists' Society*, *80*, 409-415.

Chong, C.L., Kamarudin, Z., Lesieur, P., Marangoni, A.G., Bourgaux, C., & Ollivon, M. (2007). Thermal and structural behavior of crude palm oil: crystallization at very slow cooling rate. *European Journal of Lipid Science and Technology*, *109*, 410-421.

Deman, L, Deman, J.M., & Blackman, B. (1989). Polymorphic behavior of some fully hydrogenated oils and their mixtures with liquid oil. *Journal of American Oil Chemists' Society*, 66, 1777-1780.

D'Souza, V., Deman, J.M., & Deman, L.M. (1992). Chemical and physical properties of the solid fats in commercial soft margarines. *Journal of American Oil Chemists' Society, 69*, 1198- 1205.

Foubert, I., Dewettinck, K., Janssen, G., & Vanrolleghem, P.A. (2006). Modelling two-step isothermal fat crystallization. *Journal of Food Engineering*, *75*, 551-559.

Foubert, I., Dewettinck, K., Van de Walk, D., Dijkstra, A.J., & Quinn, P.J. (2007). Physical properties: structural and physical characteristics. In F.D. Gunstone, J.L. Harwood, & A.J. Dijkstra (Eds.) *The lipid handbook,* (pp.471-508). Boca Raton: CRC Press.

Gamboa, O.W.D., & Gioielli, L.A. (2006). Comportamento de cristalização de lipídios estruturados a partir de gorduras de palmiste e óleo de peixe. *Química Nova*. *29*, 646-653.

Ghotra, B. S., Dyal, S. D., & Narine, S. S. (2002). Lipids shortenings: a review. *Food Research International* , *35*, 1015-1048.

Gioielli, L. A., Simões, I. S., & Rodrigues, J. N. (2003). Crystal morphology and interactions of binary and ternary mixtures of hydrogenated fats. *Journal of Food Engineering*, *57*, 347-355.

Grimaldi, R., Gonçalves, L. A. G., & Ando, M. Y. (2005). Otimização da reação de interesterificação química de óleo de palma. *Química Nova*, *28*, 633-636.

Haummann, B. F. (1994). Tools: hydrogenation, interesterification. *Inform*, *5*, 668-678

Herrera, M.L., Falabella, C., Melgarejo, M., & Anón, M.C. (1998). Isothermal crystallization of hydrogenated sunflower oil: I – nucleation. *Journal of American Oil Chemists' Society*, *75*, 1273-1280.

Herrera, M.L., Gatti, M.L., & Hartel, R.W. (1999). A kinetic analysis of crystallization of a milk fat model system. *Food Research International*, *32*, 289 -298.

Himavan, C., Starov, V.M., & Stapley, A.G.F. (2006). Thermodynamic and kinetic aspects of fat crystallization. *Advances in Colloid and Interface Science*, *122*, 3-33.

Humphrey, K.L., Moquin, P.H.L., & Narine, S.S. (2003). Phase behavior of a binary lipid shortening system: from molecules to rheology. *Journal of American Oil Chemists' Society*, *80*, 1175-1182.

Humphrey, K. L., & Narine, S. S. (2004). A comparison of lipid shortening functionality as a function of molecular ensemble and shear: Crystallization and melting. Food Research International, 37, 11-27.

Kawamura, K. (1979). The DSC thermal analysis of crystallization behavior in palm oil. *Journal of American Oil Chemists' Society*, 57, 48-52.

Kloek, W., Walstra, P., & van Vliet, T. (2000). Crystallization kinetics of fully hydrogenated palm oil in sunflower oil mixtures. *Journal of American Oil Chemists' Society*, *77*, 389-398.

Lawler, P.J., & Dimick, P.S. (2002). Crystallization and polymorphism of fats. In In C.C. Akoh, & D. B. Min (Eds.) *Food Lipids: Chemistry, Nutrition, and Biotechnology*, (pp.275-300). Boca Raton: CRC Press.

Lee, J.H., Akoh, C.C., Himmesbach, D.S., & Lee, K.T. (2008). Preparation of interesterified plastic fats from fats and oils free of *trans* fatty acid. *Journal of Agricultural and Food Chemistry*, *56*, 4039-4046.

List, G. R., Mounts, T. L., Orthoefer, F., & Neff, W. E. (1995). Margarine and shortening oils by interesterification of liquid and trisaturated triglycerides. *Journal of American Oil Chemists' Society*, *72*, 379-382.

Marangoni, A.G. Crystallization kinetics. In A. G. Marangoni (Ed.). *Fat Crystal Networks*, (pp.21-83). New York: Marcel Dekker.

Marangoni, A.G., & Hartel, R.W. (1998). Visualization and structural analysis of fat crystal networks. *Food Technology*, 52,46 -51.

Marangoni, A. G., & Rousseau, D. (1998). The influence of chemical interesterification on physicochemical properties of complex fat systems. 1. Melting and crystallization. Journal *of American Oil Chemists' Society, 75*, 1265-1271.

Marikkar, J,M.N., Ghazali, H.M., Che Man, Y.B., & Lai, O.M. (2003). Differential scanning calorimetric analysis for determination of some animal fats as adulterants in palm olein. *Journal of Food Lipids*, *10*, 63-79.

Martini, S., Herrera, M.L, & Hartel, R.W. (2002). Effect of cooling rate on crystallization behavior of milk fat fraction/sunflower oil blends. *Journal of American Oil Chemists' Society*, *79*, 1055-1062.

McGauley, S.E., & Marangoni, A.G. (2002). Static crystallization behavior of cocoa butter and its relationship to network microstructure. In A. G. Marangoni, & S. S. Narine (Eds.) *Physical Properties of Lipids*, (pp.85-123). Boca Raton: CRC Press.

Metin, S., & Hartel, R.W. (2005). Crystallization of fats and oils. In F. Shahidi (Ed.) *Bailey´s Industrial Oil and Fat Products*, (pp.45-76). New York: Wiley Interscience.

Narine, S.S., Humphrey, K.L., & Bouzidi, L. (2006). Modification of the Avrami model for application to the kinetics of the melt crystallization of lipids. *Journal of American Oil Chemists' Society*, *83*, 913-921.

Narine, S.S., & Marangoni, A.G. Microstructure. In A. G. Marangoni (Ed.). *Fat Crystal Networks*, (pp.179-255). New York: Marcel Dekker.

Omar, Z., Let, C.C., Seng, C.C. & Rashid, N.A. (2005). Crystallization and rheological properties of hydrogenated palm oil and palm oil blends in relation to crystal network. *European Journal of Lipid Science and Technology*, *107*, 634-640.

Ramli, N., Said, M., & Loon, N. T. (2005). Physicochemical characteristics of binary mixtures of hydrogenated palm kernel oil and goat fat milk. *Journal of Food Lipids*, 12, 243-260.

Reshma, M.V., Saritha, S.S., Balachandran, C., & Arumughan, C. (2008). Lipase catalysed interesterification of palm stearin and rice bran oil blends for preparation of zero *trans* shortening with bioactive phytochemicals. *Bioresource Technology*, 99, 5011-5019.

Ribeiro, A. P. B., Moura, J. M. L. N., Grimaldi, R., & Gonçalves, L. A. G. (2007). Interesterificação química: alternativa para obtenção de gorduras zero trans. *Química Nova*, 30, 1295-1301.

Rodrigues, J. N., Gioielli, L. A., & Anton, C. (2003) Propriedades físicas de lipídios estruturados obtidos de misturas de gordura do leite e óleo de milho. *Ciência e Tecnologia de Alimentos*, 23, 226-233.

Rousseau, D., Hodge, S.M., Nickerson, M.T., & Paulson, A.T. (2005). Regulating the$\beta' \rightarrow \beta$ polymorphic transition in food fats. *Journal of American Oil Chemists' Society*, *82*, 7-12.

Rousseau. D., & Marangoni, A. G. (2002). Chemical interesterification of food lipids: theory and practice. In C.C. Akoh, & D. B. Min (Eds.) *Food Lipids: Chemistry, Nutrition, and Biotechnology*, (pp.301-335). Boca Raton: CRC Press.

Rousseau, D., Hill, A.R., & Marangoni, A.G. (1996). Restructuring butterfat through blending and chemical interesterification. 2. Microestructure and polymorphism. *Journal of American Oil Chemists' Society*, *73*, 973- 981.

Rousseau, D., Marangoni, A.G., & Jeffrey, K.R. (1998). The influence of chemical interesterification on the physicochemical properties of complex fat systems. 2. Morphology and polymorphism. *Journal of American Oil Chemists' Society*, *75*, 1833-1839.

Sato, K. (2001). Crystallization behavior of fats and lipids: a review. *Chemical Engineering Science*, *56*, 2255-2265.

Shi, Y., Liang, B., & Hartel, R.W. (2005). Crystal morphology, microstructure, and textural properties of model lipid system. *Journal of American Oil Chemists' Society*, *82*, 399-408.

Silva, R.C., Escobedo, J.P., & Gioielli, L.A. (2008). Comportamento de cristalização de lipídios estruturados por interesterificação química de banha e óleo de soja. *Química Nova*, *31*, 330-335.

Tan, C.P., & Che Man, Y.B. (2002). Differential scanning calorimetric analysis of palm, palm oil based products and coconut oil: effects of scanning rate variation. *Food Chemistry*, *76*, 89-102.

Tang, D., & Marangoni, A.G. (2006). Microstructure and fractal analysis of fat crystal networks. *Journal of American Oil Chemists' Society*, *83*, 377-388.

Toro-Vazquez, J.F., Dibildox-Alvarado, E., Charó-Alonso, M., Charó-Alonso, V., & Gómez-Aldapa, C.A. (2002). The Avrami index and the fractal dimension in vegetable oil crystallization. *Journal of American Oil Chemists' Society*, *79*, 855-866.

Zeitoun, M. A. M., Neff, W. E., List, G. R., & Mounts, T. L. (1993). Physical properties of interesterified fat blends. *Journal of American Oil Chemists' Society*, *70*, 467-471.

Yap, P.H., Deman, J.M., Deman, L. (1989). Polymorphism of palm oil and palm oil products. *Journal of American Oil Chemists' Society*, *66*, 693-697.

Wassel, P., & Young, N.W.G. (2007). Food applications of *trans* fatty acids substitutes. *International Journal of Food Science and Technology*, *42*, 503-517.

Wiedermann, L.H. (1978). Margarine and margarine oil, formulation and control. *Journal of American Oil Chemists' Society*, *55*, 823-829.

Wright, A.J., Hartel, R.W., Narine, S.S., & Marangoni, A.G. (2000). The effect of minor components on milk fat crystallization. *Journal of American Oil Chemists' Society*, *77*, 463-475.

7.

INTERESTERIFICAÇÃO QUÍMICA DE ÓLEO DE SOJA E ÓLEO DE SOJA TOTALMENTE HIDROGENADO: INFLUÊNCIA DO TEMPO DE REAÇÃO

Ana Paula Badan Ribeiro, Monise Helen Masuchi, Renato Grimaldi, Lireny Aparecida Guaraldo Gonçalves

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Chemical interesterification of soybean oil and fully hydrogenated soybean oil: Influence of the reaction time

Chemical interesterification is an important alternative to produce zero trans fats. In practice, however, excessive reaction times are used to ensure complete randomization. This work evaluated the influence of the reaction time on the interesterification of soybean oil / fully hydrogenated soybean oil blend, carried out in the following conditions: 100°C, 500 rpm stirring speed, 0.4% (w/w) sodium methoxide catalyst. The triacylglycerol composition, solid fat content and melting point analysis showed that the reaction was very fast, reaching the equilibrium within 5 minutes. This result suggests the interesterification can be performed in substantially lower times, with reduction in process costs.

Keywords: chemical interesterification, reaction time, low trans fat.

INTRODUÇÃO

A maioria dos óleos e gorduras naturais apresenta aplicação limitada em suas formas inalteradas, impostas pela sua composição particular em ácidos graxos e triacilgliceróis (TAG).[1] Devido à crescente preocupação sobre o impacto nutricional dos ácidos graxos *trans* na saúde, a interesterificação química tem-se mostrado o principal método para preparação de gorduras plásticas com ausência destes compostos.[2] Este processo permite a modificação de óleos e gorduras, oferecendo contribuições importantes para o aumento e otimização do uso dos mesmos nos produtos alimentícios.[3]

Em particular, neste processo, óleos e gorduras, isentos de umidade, são aquecidos a 80-100°C e o catalisador, geralmente metóxido de sódio, é adicionado em proporções apropriadas (0,1-0,5%). A reação é conduzida em um intervalo de tempo pré-determinado e finalizada com adição de água ou solução ácida, desativando o catalisador. Na reação

de interesterificação, os grupamentos ésteres, derivados de ácidos graxos, presentes nos triacilgliceróis iniciais não são quimicamente modificados, porém ocorre uma troca desses fragmentos, gerando novos triacilgliceróis com composição geral diferenciada da inicial. As propriedades finais da nova composição triacilglicerólica são totalmente determinadas pela composição total em ácidos graxos das matérias-primas iniciais. A reação é freqüentemente monitorada através de alteração do ponto de fusão e perfil de sólidos.[4]

A reação de interesterificação química consiste de três estágios: ativação do catalisador (indução), clivagem das ligações éster e intercâmbio de ácidos graxos. As diferenças de energia entre as várias combinações de triacilgliceróis são insignificantes e não conduzem, portanto, à seletividade de ácidos graxos. Logo, a reação é randômica e entropicamente dirigida até que o equilíbrio termodinâmico seja alcançado.[5,6]

Embora existam diversos estudos sobre o mecanismo da interesterificação química e produtos interesterificados, a cinética da reação ainda é pouco explorada na literatura pertinente.[7] Neste sentido, apenas a temperatura de reação revela-se como parâmetro praticamente consolidado. Konishi *et al*.[8] mostraram que a faixa de temperatura ideal para ativação do metóxido de sódio encontra-se entre 100 a 110°C. Segundo revisão de Dijkstra,[9] a temperatura de 100°C é empregada na maioria dos trabalhos sobre o tema.

Entretanto, verifica-se tipicamente que a interesterificação química tem sido conduzida em intervalos relativamente longos, de 20 a 40 minutos, com o objetivo de garantir completa randomização. Contudo, com a diminuição desse tempo de reação, haveria conseqüentemente uma diminuição de custo do processo e melhor aproveitamento da capacidade dos reatores, além de diminuir a probabilidade de formação de sabões, principal subproduto da reação.[5,10,11] Ainda, a importância de um estudo minucioso do tempo de reação reside no fato de que a intensidade de formação de novas espécies triacilglicerólicas pode afetar consideravelmente as propriedades físicas das bases oleosas.[12] Gorduras parcialmente interesterificadas apresentam teor de sólidos, composição triacliglicerólica e comportamento de cristalização que diferem

substancialmente da mistura inicial e do produto completamente randomizado. Estes produtos consistem em frações oleosas com propriedades únicas, que podem ser aplicados diretamente como ingredientes plásticos.[10,11,13]

Gioielli e Baruffaldi[14] estudaram a interesterificação de misturas de óleos de babaçu/dendê, com coleta de frações a cada 5 minutos, durante 45 minutos. O equilíbrio da reação foi alcançado entre 20 e 25 minutos após a introdução do catalisador metóxido de sódio. Marangoni e Rousseau[10] acompanharam a interesterificação de misturas óleo de palma/ óleo de soja e banha/óleo de canola, durante 12 e 24 horas, respectivamente, com uso de 0,5% de metóxido de sódio, a 80°C. A maior variação nas proporções relativas dos triacilgliceróis ocorreu em 30 minutos para a mistura óleo de palma/ óleo de soja e em 1 hora para a mistura banha/óleo de canola. A otimização da interesterificação do óleo de palma foi realizada por Grimaldi *et al*.[15] A variabilidade nos teores de triacilgliceróis em relação ao controle, em conjunto com o menor tempo de reação, foi o fator determinante na escolha da melhor condição, que consistiu em 0,4% de metóxido de sódio e 20 minutos de reação. Dijkstra *et al*.[13], estudando a interesterificação de misturas 1:1 trilaurina/trioleína, a 90°C e com 0,2% de metóxido de sódio, verificaram que o equilíbrio ocorreu em 10 minutos de reação.

A interesterificação de misturas de óleos vegetais totalmente hidrogenados com óleos líquidos representa atualmente a melhor opção para produção de gorduras *low trans* com diversas finalidades industriais. Face à sua importância econômica e grande disponibilidade, o óleo de soja (OS) apresenta-se como matéria-prima interessante para a elaboração de frações gordurosas isentas de ácidos graxos *trans*. Para que haja aumento do ponto de fusão destas frações, o uso do óleo de soja totalmente hidrogenado (OSTH), também zero *trans*, mostra-se altamente favorável.[16,17]

A interesterificação de misturas OS/OSTH tem sido comumente relatada para teores de OSTH de 10 a 50%, com 0,2 a 0,5% de MeONa e temperaturas entre 60°C a 110°C, com duração de 15 a 95 minutos.[18-21]

Este trabalho teve como objetivo realizar o monitoramento da interesterificação química de mistura 60%OS/40%OSTH, a fim de verificar as alterações químicas e físicas do processo como função do tempo de reação. Esta fração foi escolhida devido a seu alto ponto de fusão e elevado teor de triacilgliceróis trissaturados, de forma a viabilizar o acompanhamento destas propriedades durante a interesterificação química.

PARTE EXPERIMENTAL

Material

Matérias-primas. Foram utilizados óleo de soja refinado, adquirido em comércio local, e óleo de soja totalmente hidrogenado, gentilmente cedido por indústria regional. O catalisador empregado para as reações de interesterificação foi o metóxido de sódio (em pó 99%, Sigma- Alldrich).

Equipamentos. As reações foram realizadas em reator de vidro em borossilicato (500 mL), encamisado, com saída de fundo e juntas cônicas esmerilhadas, acoplado a: banho termostatizado com recirculação (Lauda RE 212, -30 a +200°C, $\pm$0,02°C), sistema de agitação (motor universal com variador eletrônico de velocidade até 4000 rpm – Marconi, BR) com haste de agitação para escoamento axial, bomba de vácuo (Vacuubrand modelo 30, bomba tipo diafragma) e termômetro digital tipo espeto (-50 a + 300°C, $\pm$1°C - Incoterm).

Interesterificação química

Para a reação utilizou-se 200g de mistura 60%OS/40%OSTH (m/m), previamente fundida e homogeneizada durante 10 minutos a 100°C até completa fusão dos cristais. Procedeu-se à secagem da mesma no reator, sob vácuo e agitação de 500 rpm, a 100 °C, por 20 minutos. O teor de catalisador utilizado foi igual a 0,4% (m/m), conforme otimização realizada por Grimaldi *et al.*[15] A reação foi conduzida sob vácuo, a 100 °C,

com agitação de 500 rpm, durante 40 minutos. Foram coletadas amostras (20g) em intervalos regulares de 5 minutos, garantindo-se ausência de ar no sistema devido ao forte vácuo utilizado. As amostras foram imediatamente agitadas com adição de água destilada e solução de ácido cítrico 5% para interrupção da reação. As alíquotas foram cuidadosamente lavadas com água destilada (80°C) para retirada dos sabões formados; e em seguida secas a 110ºC, por 30 min.

Metodologias Analíticas

Composição em ácidos graxos. A análise da composição em ácidos graxos foi realizada em cromatógrafo em fase gasosa com coluna capilar – CGC Agilent 6850 Series GC System, após esterificação utilizando método de Hartman e Lago.[22] Os ésteres metílicos de ácidos graxos foram separados de acordo com o método AOCS[23] Ce 2-66 em coluna capilar DB – 23 Agilent (50% cianopropilmetilpolisiloxano), dimensões 60m, ϕ int: 0,25 mm, 0,25 μm filme. Temperatura do forno de 110°C-5min, 110°C-215°C (5°C/min), 215°C-24min; temperatura do detector: 280°C; temperatura do injetor 250°C; gás de arraste: hélio; razão split 1:50; volume injetado: 1,0μL. A composição qualitativa foi determinada por comparação dos tempos de retenção dos picos com os dos respectivos padrões de ácidos graxos.

Composição triacilglicerólica. A análise da composição em triacilgliceróis foi realizada em cromatógrafo gasoso capilar CGC Agilent 6850 Series GC System. Foi utilizada coluna capilar DB-17HT Agilent Catalog: 122-1811 (50% fenilmetilpolisiloxano), com 15 metros de comprimento x 0,25 mm de diâmetro interno e contendo 0,15μm de filme) As condições foram: injeção *split,* razão de 1:100; temperatura da coluna: 250ºC, programada até 350ºC à razão de 5ºC por minuto; gás de arraste: hélio, em vazão de 1,0 mL por minuto; temperatura do injetor: 360ºC; temperatura do detector: 375ºC; volume injetado: 1,0 μL; concentração da amostra: 100mg/5mL de tetrahidrofurano. A identificação dos grupos de triacilgliceróis foi realizada através da comparação dos tempos de retenção,

segundo os procedimentos de Grimaldi[24] e Antoniossi Filho.[25,26] A composição em triacilgliceróis das matérias-primas foi também obtida teoricamente através do software *1,2,3 Óleos*, baseada na hipótese de distribuição *1,3-random-2-random*, que prevê a porcentagem molar dos triacilgliceróis presentes em óleos vegetais, a partir da composição em ácido graxos destas amostras.

Conteúdo de gordura sólida. Determinada utilizando Espectrômetro de Ressonância Magnética Nuclear (RMN) Bruker pc120 Minispec e banhos secos de alta precisão (0 – 70°C) Tcon 2000 (Duratech, EUA). Método AOCS[23] Cd 16b- 93: método direto, leitura das amostras em série nas temperaturas de 10; 20; 25; 30; 35; 40, 45, 50, 55 e 60°C, com temperagem para gorduras não estabilizadas.

Ponto de fusão. Determinado utilizando tubo capilar aberto, segundo as normas da AOCS[23], método Cc 3-25. Adicionalmente, o ponto de fusão foi calculado para a temperatura correspondente ao teor de sólidos igual a 4% obtido por Ressonância Magnética Nuclear, segundo metodologia de Karabulut *et al.*[27], através de equações polinomiais ajustadas com auxílio do Software Statistica 6.0.[28] Foi aplicado um modelo de regressão linear simples relacionando os resultados obtidos pelos dois métodos.

Índice de iodo e Índice de saponificação. Calculados a partir da composição em ácidos graxos segundo os métodos AOCS[23] Cd 1c-85 e Cd 31-94, respectivamente.

Ácidos Graxos Livres: Método AOCS[23] Ca 5a-40.

Índice de Peróxido. Método AOCS[23] Cd 8b-90.

Sabões. Método AOCS[23] Cc 17-79.

RESULTADOS E DISCUSSÃO

A qualidade do óleo utilizado na reação de interesterificação química é fundamental. "Venenos" catalíticos, como ácidos graxos livres, peróxidos, umidade e sabões podem provocar queda na atividade do MeONa.[29] As características de qualidade das matérias-primas utilizadas constam da Tabela 1 e atendem os parâmetros de qualidade descritos

por Erickson.[30] A Tabela 2 apresenta a composição em ácidos graxos e os valores de índice de iodo e saponificação do OS, OSTH e da mistura 60%OS/40%OSTH. Estes resultados exprimem a média de duas determinações e estão de acordo com os limites encontrados na literatura.[16,30]

Tabela 1. Características de qualidade das matérias-primas.

Determinações	OS	OSTH
AGL (%)	0,03	0,04
Índice de peróxido (meq O_2/kg amostra)	1,04	0
Sabões (mg/kg)	0	0

Tabela 2. Composição em ácidos graxos (%) e índices de iodo e saponificação das matérias-primas e mistura 60%OS/40%OSTH.

Ácidos graxos (%)	OS	60%OS/40%OSTH	OSTH
C14:0 mirístico	0,08	0,10	0,12
C16:0 palmítico	11,37	11,31	11,50
C16:1 palmitoléico	0,08	-	-
C18:0 esteárico	3,46	37,54	86,62
C18:1 oléico	23,17	13,77	0,11
C18:2 linoléico	54,87	32,69	0,18
C18:3 linolênico	5,32	3,16	-
C20:0 araquídico	0,38	0,54	0,74
C20:1 gadoléico	0,24	0,14	-
C22:0 behênico	0,51	0,54	0,53
C24:0 lignocérico	0,18	0,20	0,19
∑ Saturados	15,98	50,23	99,71
∑ Insaturados	84,02	49,77	0,29
I.V*	135	80	0,4
I.S*	193	192	189

I.V*= Índice de iodo (g I_2/100g); I.S*= Índice de saponificação (mg KOH/g)

Óleos e gorduras são considerados amostras complexas, devido ao grande número de diferentes triacilgliceróis que os compõe. Desta maneira, a identificação de triacilgliceróis torna-se um difícil processo, no qual o número de possíveis formas estruturais é muito grande comparado ao número de ácidos graxos presentes.[31] A Tabela 3 mostra os principais triacilgliceróis individuais que compõem os diversos grupos classificados segundo o número de carbonos, para as matérias-primas, conforme análise experimental e resultados teóricos.

Comparando-se os resultados experimental e teórico, verifica-se que os mesmos apresentam boa concordância. Para o OS, os triacilgliceróis em maior quantidade obtidos teoricamente foram os mesmos obtidos através do método cromatográfico: PLO, PLL, OLO, OLL e LLL. As espécies PLSt, OOO e StLL não foram detectadas pelo método teórico. A Tabela 4 apresenta a composição das matérias-primas segundo teores de triacilgliceróis dos tipos trissaturados (SSS), monoinsaturados (SSU), diinsaturados (SUU) e triinsaturados (UUU), conforme as duas metodologias empregadas. O teor total de triinsaturados do OS correspondeu a 59,12% e a 63,33% segundo os métodos experimental e teórico, respectivamente. O OSTH apresentou totalidade de triacilgliceróis do tipo SSS. No que se refere à distribuição experimental quanto ao número de carbono, o OS e o OSTH perfazem, respectivamente: C50 – 3,83% e 4,17%; C52 – 33,27% e 30,79%; C54 – 62,65% e 63,35%; C56 – 0,25% e 1,69%.

As análises da composição triacilglicerólica representam uma indicação verdadeira de como ocorre o processo de randomização, sendo extremamente úteis para monitorar a modificação de gorduras interesterificadas e delinear aplicações específicas para as mesmas.[16] Na Tabela 5 são mostrados os resultados de composição triacilglicerólica da mistura 60%OS/40%OSTH segundo o tempo de reação.

Tabela 3. Composição em triacilgliceróis individuais e quanto ao número de carbono (NC) do óleo de soja (OS) e óleo de soja totalmente hidrogenado (OSTH), obtida teoricamente (T*) e por cromatografia em fase gasosa.

NC	TAG (%)	OS	OS (T*)	OSTH	OSTH (T*)
C50	PPSt	-	-	4,17	3,82
	POP	0,81	0,95	-	-
	PLP	3,02	2,27	-	-
C52	PStSt	-	-	30,79	26,70
	POSt	0,22	0,59	-	-
	POO	3,55	3,29	-	-
	PLSt	0,67	-	-	-
	PLO	**10,38**	9,27	-	-
	PLL	**15,62**	12,32	-	-
	PLnL	2,82	2,18	-	-
C54	StStSt	-	-	63,35	67,43
	StOO	0,76	0,99	-	-
	OOO	3,79	-	-	-
	StLO	1,68	4,22	-	-
	OLO	**10,25**	12,86	-	-
	StLL	1,09	-	-	-
	OLL	**18,99**	23,54	-	-
	LLL	**21,06**	21,67	-	-
	LLnL	5,02	5,26	-	-
C56	StStA	-	-	1,69	2,05
	ALL	0,25	0,59	-	-
	SLA	-	-	-	-

T* - Resultado Teórico, obtido através do Software *1,2,3 Óleos*. P= ácido palmítico; St= ácido esteárico; O= ácido oléico; L= ácido linoléico; Ln= ácido linolênico; A= ácido araquídico.

-: não detectado

Tabela 4. Teores de SSS, SSU, SUU e UUU do óleo de soja (OS) e óleo de soja totalmente hidrogenado (OSTH), obtidos teoricamente (T*) e por cromatografia em fase gasosa.

TAG (%)	OS	OS (T*)	OSTH	OSTH (T*)
SSS	-	-	100,00	100,00
SSU	4,72	3,81	-	-
SUU	36,16	32,86	-	-
UUU	59,12	63,33	-	-

T* - Resultado Teórico, obtido através do Software *1,2,3 Óleos*. -: não detectado. S = saturados; U = insaturados.

Verifica-se igual número de triacilgliceróis distintos antes e durante a reação de interesterificação. Entretanto, três novas espécies triacilglicerólicas foram formadas: POSt, StOSt e SLSt, esta última em proporções relativamente altas em relação à composição total, enquanto os triacilgliceróis POO, PLnL e OOO, que se apresentavam em pequenas quantidades na mistura de partida, desapareceram com a reação. Ainda, os teores de PLSt, StLO e StLL aumentaram expressivamente com a interesterificação. Ocorreu variação de todos os tipos de triacilgliceróis com a reação, comprovando-se o rearranjo dos ácidos graxos, conforme a Figura 1.

Tabela 5. Composição triacilglicerólica da mistura 60%OS/40%OSTH segundo o tempo de reação de interesterificação química, obtida através de cromatografia em fase gasosa.

	Tempo de reação (min)								
TAG (%)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
PPSt	1,86	1,94	1,71	1,89	1,92	1,39	1,97	2,32	1,86
POP	0,46	0,72	0,54	0,73	0,77	0,44	1,00	1,21	0,75
PLP	1,48	1,66	1,84	1,79	1,82	1,67	2,02	2,48	1,84
PStSt	13,07	7,49	7,36	5,87	7,41	6,36	7,29	7,96	7,12
POSt	**-**	**3,46**	**2,77**	**4,87**	**2,73**	**2,57**	**2,84**	**3,16**	**3,33**
POO	1,92	-	-	-	-	-	-	-	-
PLSt	**0,37**	**8,14**	**8,12**	**8,37**	**9,54**	**8,33**	**7,89**	**8,80**	**8,61**
PLO	5,80	4,89	4,28	3,60	4,21	4,78	4,36	4,52	4,31
PLL	8,07	6,57	5,98	5,88	6,62	7,17	5,89	6,14	6,02
PLnL	1,85	-	-	-	-	-	-	-	-
StStSt	**28,75**	**10,18**	**7,70**	**6,20**	**8,10**	**10,02**	**11,55**	**10,98**	**10,43**
StOSt	**-**	**6,50**	**8,35**	**6,71**	**6,03**	**4,25**	**4,92**	**5,25**	**4,86**
StLSt	**-**	**11,90**	**11,76**	**13,61**	**12,97**	**11,49**	**11,58**	**11,85**	**11,73**
OOO	2,21	-	-	-	-	-	-	-	-
StLO	**0,99**	**8,88**	**8,78**	**9,97**	**9,48**	**8,67**	**8,74**	**8,55**	**9,43**
OLO	5,77	1,10	1,22	1,30	1,11	1,28	1,26	1,07	1,23
StLL	**0,64**	**11,10**	**12,34**	**13,18**	**11,28**	**12,95**	**12,71**	**10,83**	**12,45**
OLL	11,22	6,90	6,89	6,73	6,01	7,41	5,47	5,93	6,06
LLL	11,88	7,60	8,31	7,84	8,42	9,28	8,64	7,65	8,18
LLnL	3,26	0,98	2,04	1,46	1,55	1,97	1,85	1,26	1,78

P= ácido palmítico; St= ácido esteárico; O= ácido oléico; L= ácido linoléico; Ln= ácido linolênico. -:não detectado

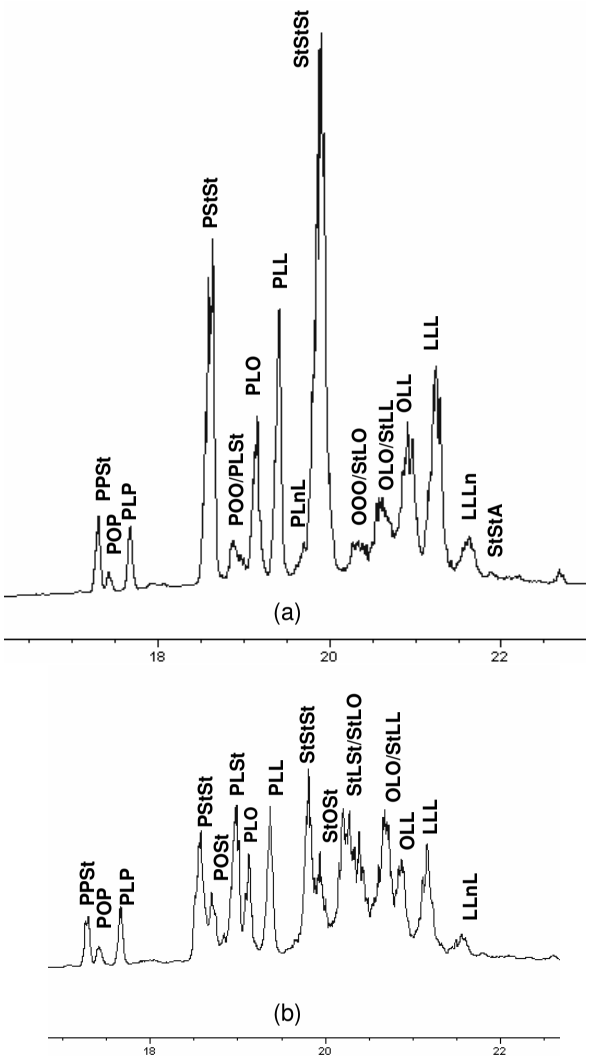

Figura 1. Cromatogramas de composição triacilglicerólica (a) da mistura inicial; (b) da mistura interesterificada aos 5 minutos de reação. P= ácido palmítico; St= ácido esteárico; O= ácido oléico; L= ácido linoléico; Ln= ácido linolênico; A= ácido araquídico.

Os triacligliceróis predominantes na mistura interesterificada, independentemente do tempo de reação, consistiram do StLSt e StLL, com teores médios em torno de 12%. Os demais ácidos graxos presentes na mistura avaliada e que não constituem as espécies triacilglicerólicas verificadas na Tabela 5, provavelmente contribuíram para a formação de triacilgliceróis em quantidades mínimas, de difícil separação ou detecção.

É possível observar que a maior variação nos teores de triacilgliceróis ocorreu entre os

tempos 0 e 5 minutos, ou seja, praticamente no início da reação. A partir dos 5 minutos de reação, as alterações relativas nas porcentagens de cada espécie triacilglicerólica mostram-se pouco significativas em função do tempo de reação. Este fato pode ser facilmente verificado mediante a queda expressiva no teor de StStSt durante os 5 minutos iniciais de reação, em que esta porcentagem passa de 28,75% a 10,18%, o que representa uma variação de 64%. Adicionalmente, observa-se que a formação dos triacilgliceróis StOSt e StLSt e o expressivo acréscimo de 89% e 94%, respectivamente, nos teores de StLO e StLL, ocorreu nos 5 minutos iniciais da interesterificação. A partir dos 5 minutos de reação, os resultados sugerem pequenas oscilações segundo o tempo de reação, que podem estar associadas a uma reação em estado de equilíbrio, conforme estudos de Konishi *et al*.[8] A Figura 2 mostra o acompanhamento da reação de interesterificação em função dos teores de triacilgliceróis trissaturados, monoinsaturados, di- e triinsaturados.

A interesterificação resultou na diminuição simultânea dos triacilgliceróis de maior e menor pontos de fusão, com formação expressiva de espécies intermediárias (SSU e SUU). Segundo O'Brien,[16] as propriedades de alimentos gordurosos podem ser relacionadas à composição triacilglicerólica da gordura que os compõe. Os triacilgliceróis SSU, com pontos de fusão entre 27 a 42°C, são responsáveis pela estrutura destes produtos. Os triacilgliceróis SUU são importantes no que se refere às propriedades de fusão à temperatura corporal e plasticidade dos mesmos à temperatura ambiente. Logo, o acréscimo nos teores de SSU e SUU promovido pela interesterificação química, está associado ao aumento da funcionalidade tecnológica, melhoria das características sensoriais e, portanto, maior potencial de aplicação desta gordura interesterificada em alimentos.

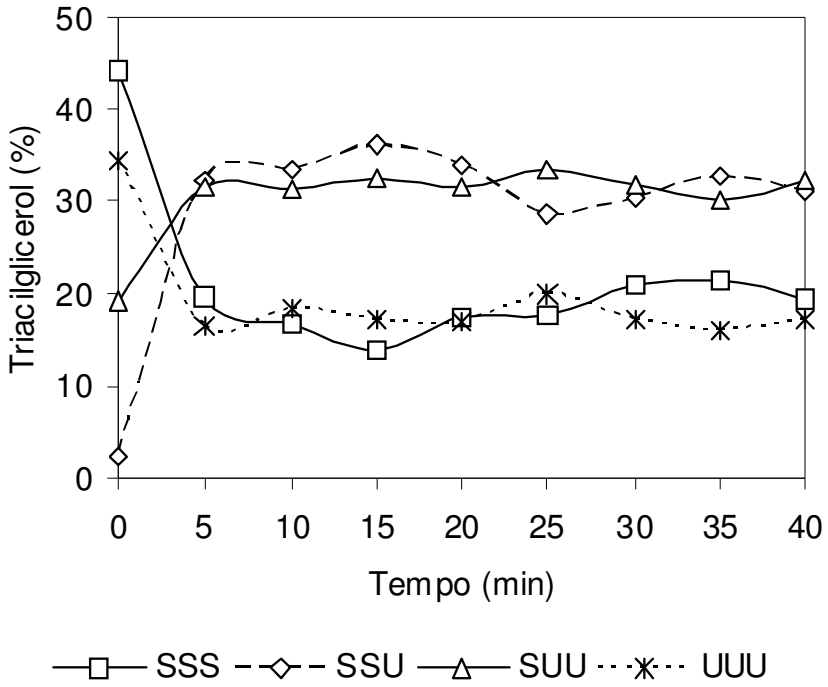

Figura 2. Teores de SSS, SSU, SUU e UUU em função do tempo de reação de interesterificação química de mistura 60%OS/40%OSTH.

Verificou-se que nos 5 minutos iniciais da reação os teores de SSS e UUU diminuíram consideravelmente (55,5% e 51,7%, respectivamente), enquanto ocorreu o rápido acréscimo nos teores de triacillgliceróis dos tipos SSU e SUU, nas proporções de 92,9% e 38,7%. Aos 15 minutos de reação, os teores de SSS e SSU alcançaram o teor mínimo (13,9%) e máximo (36,1%), respectivamente. Porém, aos 30 minutos de reação a porcentagem de SSS foi igual a 20,8%, similar ao teor verificado aos 5 minutos de reação (19,6%), o mesmo ocorrendo para o teor de SSU. Assim, é possível observar que a partir dos 5 minutos iniciais, os teores de SSS, SSU, SUU e UUU oscilaram conjuntamente, com variações percentuais negativas e positivas ao longo do tempo de interesterificação. Estas observações corroboram com os estudos realizados por Grimaldi *et al.*[15] e Wada.[32] Estes autores ressaltam que o processo de rearranjo dos ácidos graxos ocorre durante um determinado período, mas em longos tempos de reação pode conduzir ao rearranjo termodinamicamente mais favorável. Observação semelhante foi verificada por

Meneghetti *et al*.[33] em reações de transesterificação. Em particular, a etanólise de óleo de mamona, realizada a 80°C com catalisador metóxido de sódio (etanol/óleo/catalisador em razão molar de 6:10:2) e monitorada durante 10 horas, apresentou máxima taxa de conversão em ésteres etílicos em 6 horas de reação. Após este período, verificou-se reversibilidade da reação.

A análise da Figura 2 permite a observação deste fenômeno. Contudo, embora ocorra reversão do rearranjo das espécies triacliglicerólicas, este acontece apenas após um estado de relativo equilíbrio alcançado nos minutos iniciais de reação. Comportamento similar foi reportado por Musavi *et al*.,[7] a partir do estudo da cinética de interesterificação do óleo de soja. A proporção inicial de ácido palmítico na posição *sn-2* dos triacilgliceróis era igual a 1,28% e aumentou para 12,5% quando a reação alcançou o equilíbrio, em menos de 5 minutos de reação, a 80°C, 0,4% de MeONa e agitação de 600 rpm. O teor de ácido palmítico na posição *sn-2* atingiu 4% imediatamente após adição do catalisador, indicando uma reação extremamente rápida.

A alteração da composição triacilglicerólica refletiu-se em modificação do conteúdo de gordura sólida da mistura interesterificada. A partir de metodologia descrita por Coenen[34] e adaptada por Rousseau e Marangoni,[6] foi construído o gráfico do conteúdo de gordura sólida em função do tempo de reação, para cada temperatura de leitura, que consta da Figura 3.

A 30°C, a porcentagem de gordura sólida da amostra inicial correspondeu a 37,2%. Considerando a média de todas as amostras, após a interesterificação o conteúdo de gordura sólida a esta temperatura foi igual a 22,4%. Estes resultados são coerentes com os obtidos por Petrauskaite *et al*.,[20] para mistura 60%OS/40%OSTH interesterificada, em que o teor de sólidos a 30°C consistiu de 20,3%.

A análise da Figura 3 permite observar que o conteúdo de gordura sólida apresentou decréscimo significativo nos minutos iniciais de reação, mantendo-se praticamente inalterado após 5 minutos de processo. De acordo com Coenen,[34] este comportamento

sugere que a reação é bastante rápida, e que, uma vez que haja concentração suficiente de catalisador, a reação requer somente alguns minutos, a menos que sejam utilizadas baixas temperaturas.

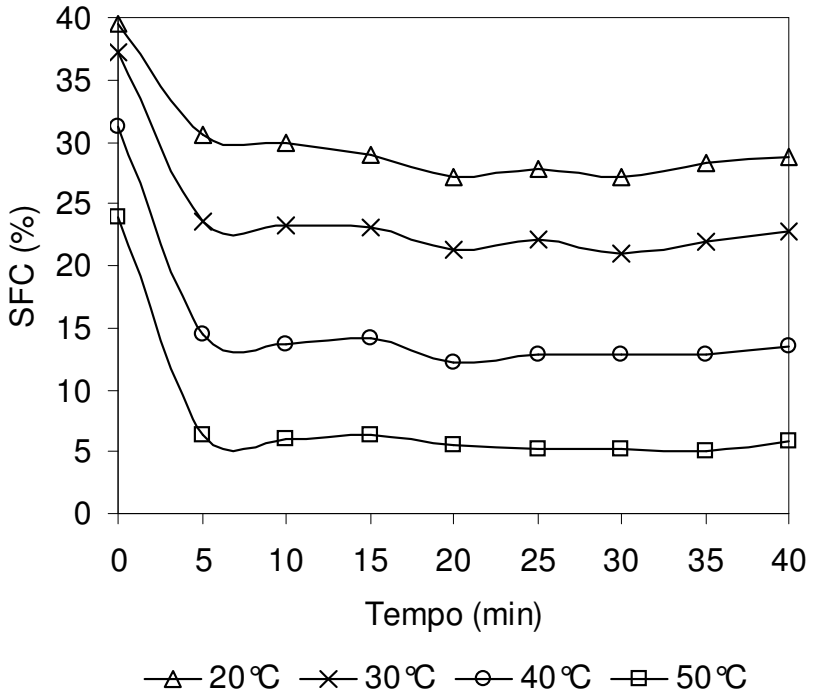

Figura 3. Conteúdo de gordura sólida a 20, 30, 40 e 50℃, da mistura 60%OS/40%OSTH, em função do tempo de reação de interesterificação química.

As oscilações nos teores dos diversos grupos de triacilgliceróis, a partir dos 5 minutos de reação, verificadas na Figura 2, não se refletiram em oscilações no conteúdo de gordura sólida com o tempo de interesterificação. Este fato pode estar relacionado a uma dinâmica de compensação que não afeta a curva de sólidos.

A Figura 4 mostra os valores dos pontos de fusão (obtidos através do método matemático descrito por Karabulut *et al.*[27]) e o teor de triacligliceróis trissaturados em função do tempo de reação. É possível observar que o declínio do ponto de fusão é expressivo nos primeiros 5 minutos de reação e oscila conforme as variações nos teores de SSS. A mistura inicial apresentava ponto de fusão igual a 63℃. Após a reação, as

temperaturas de fusão variaram entre 50,9 e 53,7°C. O menor valor ocorreu a 15 minutos de reação, podendo ser explicado pelo concomitante decréscimo nas proporções de triacilgliceróis do tipo SSS, em concordância com os resultados de composição triacilglicerólica obtidos. Ainda, ocorre um acréscimo do ponto de fusão a 30 minutos de reação (53°C), associado provavelmente ao aumento de 34,3% no teor de SSS em relação ao verificado a 15 minutos de interesterificação. Portanto, o acompanhamento do ponto de fusão da mistura interesterificada também evidenciou certo grau de reversão da interesterificação química, embora a níveis delimitados após expressiva modificação da matéria-prima.

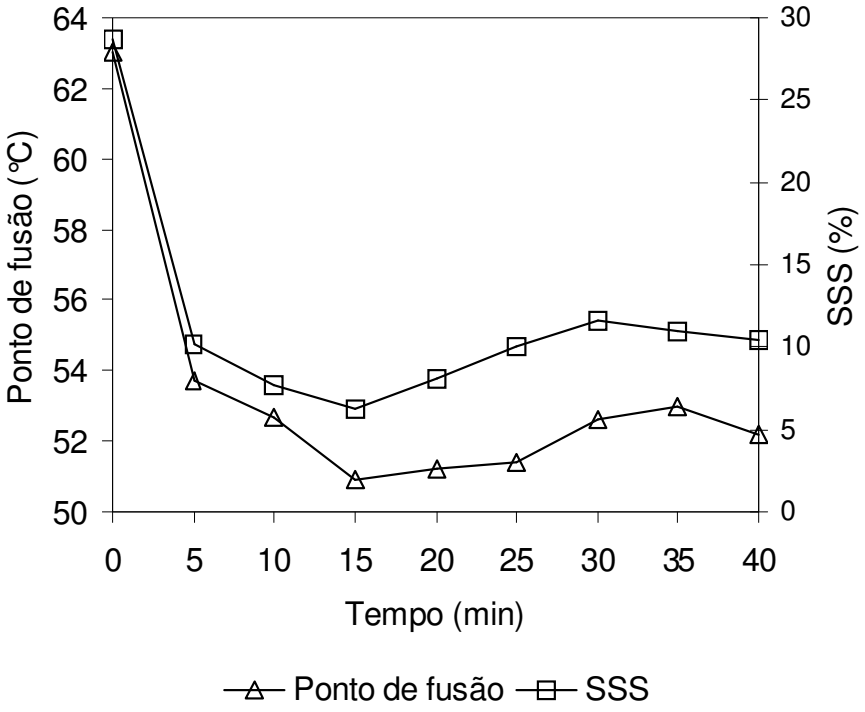

Figura 4. Ponto de fusão e teor de triacilgliceróis trissaturados em função do tempo de reação de interesterificação química de mistura 60%OS/40%OSTH.

Em resumo, uma análise conjunta de todos os resultados permitiu verificar que a reação de interesterificação química apresentou um período inicial associado à intensa

randomização, no qual as modificações de composição triacilglicerólica, conteúdo de gordura sólida e ponto de fusão foram representativas da mistura interesterificada. Após este período, a reação foi caracterizada por um estado de relativo equilíbrio, no qual, devido ao tempo relativamente longo de reação, na presença de catalisador, ocorreu pequena reversão da randomização previamente estabelecida aos 5 minutos de reação. Poderia-se assumir, portanto, que é possível a utilização de tempos de reação significativamente menores, escolhidos em função de objetivar-se interesterificação parcial ou total.

Um modelo de regressão linear simples foi aplicado relacionando os pontos de fusão obtidos por RMN com os pontos de fusão obtidos pelo método do tubo capilar aberto (Figura 5), para mistura inicial e frações, cuja equação é descrita a seguir:

P.F. (°C) capilar = 0,8725*[P.F. (°C) RMN] + 4,12; com coeficiente de correlação (r) = 0,905.

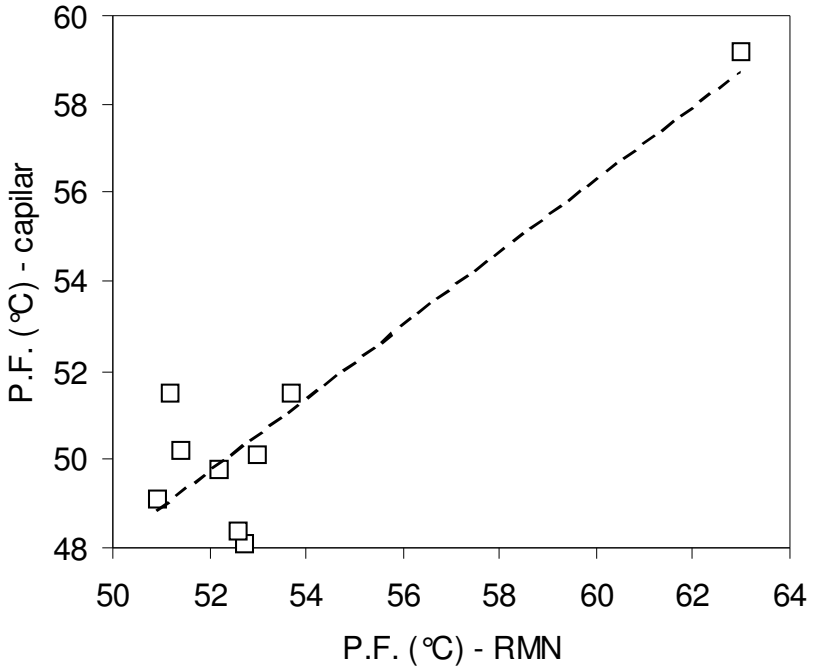

Figura 5. Correlação entre os métodos de ressonância magnética nuclear e tubo capilar aberto para obtenção de pontos de fusão.

Houve boa correlação entre os métodos. Este fato corrobora a utilização da técnica de RMN como meio seguro para obtenção do ponto de fusão de gorduras, uma vez que o mesmo se diferencia como uma prática fácil, rápida e não subjetiva, podendo ser utilizado para medidas praticamente instantâneas.

CONCLUSÃO

A interesterificação química consiste em uma alternativa fundamental para o desenvolvimento de bases gordurosas com ausência de ácidos graxos *trans*. Apesar dos vários estudos já realizados, tempos relativamente longos de reação ainda são empregados. Neste trabalho, a interesterificação total de mistura 40%OS/60%OSTH foi alcançada aos 5 minutos de reação, sob condições de 100°C, 0,4% de catalisador metóxido de sódio e 500 rpm de agitação. Este resultado, verificado a partir de técnicas distintas como a cromatografia em fase gasosa e a ressonância magnética nuclear, indica que é possível a obtenção de gorduras total ou parcialmente interesterificadas em tempos substancialmente menores, com redução dos custos associados ao processo.

Referências

1. Chiu, M.C.; Gioielli, L.A.; Grimaldi, R.; *Quim. Nova.* **2008**, *31*, 238.

2. Norizzah, A.R.; Chong, C.L.; Cheow, C.S. Zaliha, O.; *Food Chem*. **2004**, *86*, 229.

3. Ribeiro, A.P.B.; Moura, J.M.L.N.; Grimaldi, R.; Gonçalves, L.A.G.; *Quim. Nova.* **2007**, *30*, 1295.

4. Rozendaal, A.; *Inform.* **1992**, *3*, 1232.

5. Liu, L.; *J. Am. Oil Chem. Soc*. **2004**, *81*, 331.

6. Rousseau, D.; Marangoni, A.G.; Em *Food Lipids: Chemistry, Nutrition, and Biotechnology;* Akoh, C.C.; Min, D.B., eds.; CRC Press: Boca Raton, **2008**, cap. 10.

7. Musavi, A.; Tekin, A.; Kaya,M.; Sanal, I.; *J. Food Lipids*. **2003**, *10*, 277.

8. Konishi, H.; Neff, W.E.; Mounts, T.L.; *J. Am. Oil Chem. Soc*. **1993**, *70*, 411.

9. Dijkstra, A.J. Em *The Lipid Handbook*; Gunstone, F.D., ed.; CRC Press: Boca Raton, 2007, cap. 4.

10. Marangoni, A.G.; Rousseau, D.; *J. Am. Oil Chem. Soc*. **1998**, *75*, 1265.

11. Liu, L.; Lampert, D.; *J. Am. Oil Chem. Soc*. **1999**, *76*, 783.

12.Ramamurthi, S.; McCurdy, A.R.; Em Development *and Processing of Vegetable Oils for Human Nutrition;* Przybylski, R.; McDonald, B.E., eds.; AOCS Press: Champaign, **2005**, cap. 6.

13. Dijkstra, A.J.; Toke, E.R.; Kolonits, P.; Recseg, K.; Kovári,K.; Poppe, L.; *Eur. J. Lipid Sci. Technol*. **2005**, *107*, 912.

14. Gioielli, L. A.; Baruffaldi, R.; *Rev. Farm. e Bioquím. Univ. S. Paulo*. **1988**, *24*, 29.

15. Grimaldi, R.; Gonçalves, L.A.G.; Ando, M.Y.; *Quim. Nova.* **2005**, *28*, 633.

16. O'Brien, R.D.; *Fats and Oils – Formulating and Processing for Applications*, 2nd ed., CRC Press: New York, **2004**.

17. Marangoni, A.G.; Rousseau, D.; *Trends Food Sci. Technol*. **1995**, *6*, 329.

18. Zeitoun, M.A.M.; Neff, W.E.; List, G.R.; Mounts, T.L.*; J. Am. Oil Chem. Soc*. **1993**, *70*, 467.

19. List, G.R.; Mounts, T.L.; Orthoefer, F.; Neff, W.E.; *J. Am. Oil Chem. Soc*. **1995**, *72*, 379.

20. Petrauskaite, V.; De Greyt, W.; Kellens, M.; Huyghebaert, A.; *J. Am. Oil Chem. Soc*. **1998**, *75*, 489.

21. Zomorrodi, S.; Shokrani, R.; Shahedi, M.; Dokhani, S.; *Iranian J. Agric. Sci.* **2003**, *34*, 587.

22. Hartman, L.; Lago, R.C.A.; *Lab. Pract.* **1973**, *22*, 475.

23. AOCS; *Official methods and recommended practices of the American Oil Chemists' Society.* AOCS Press: Champaign, **2004**.

24.Grimaldi, R.; *Tese de Doutorado*, Universidade Estadual de Campinas, Brasil, **1999**.

25. Antoniossi Filho, N.R.; *Tese de Doutorado*, Universidade de São Paulo, Brasil, **1995**.

26. Antoniossi Filho, N.R.; Mendes, O.L., Lanças, F.M.; *Chromatographia.* **1995**, *40*, 557.

27. Karabulut, I.; Turan, S.; Ergin, G.; *Eur. Food Res. Technol.* **2004**, *218*, 224.

28. Statsoft Inc.; *Statistica v.6.0. for windows Vol. IV;* Tulsa, Estados Unidos, **2002**.

29. Sreenivasan, B.; *J. Am. Oil Chem. Soc*. **1978**, *55*, 796.

30. Erickson, D. R.; *Practical Handbook of Soybean Processing and Utilization*. AOCS Press: Champaign, **1995**.

31.http://biodiesel.gov.br, acessada em Fevereiro **2008**.

32. Wada, J.K.A.; *Dissertação de Mestrado*, Universidade Estadual de Campinas, Brasil, **2007**.

33. Meneghetti, S.M.P.; Meneghetti, M.R.; Wolf, C.R.; Silva, E.C.; Gilvan, E.S.L.; Coimbra, M. A.; Soletti, J.I.; Carvalho, S.H.V.; *J. Am. Oil Chem. Soc*. **2006**, *83*, 819.

34. Coenen, J.W.E.; *Rev.Fr. Corps Gras*. **1974**, *21*, 403.

CONCLUSÕES GERAIS

Os resultados obtidos neste trabalho permitiram estabelecer as seguintes conclusões:

- Em todas as misturas avaliadas, a interesterificação química promoveu diminuição nos teores de triacilgliceróis trissaturados e aumento dos triacilgliceróis monoinsaturados-dissaturados e diinsaturados-monossaturados, o que resultou no respectivo decréscimo do ponto de fusão das amostras. Conseqüentemente, as misturas interesterificadas apresentaram diminuição do conteúdo de gordura sólida em todas as temperaturas e perfis de fusão mais lineares quando comparadas às misturas originais, bem como maior plasticidade após o rearranjo ao acaso;

-Verificou-se que o efeito da randomização sobre os termogramas de fusão e cristalização foi semelhante para os dois tipos de misturas binárias avaliadas, em todas as proporções. A interesterificação acarretou significativa diminuição nas dimensões dos cristais em todas as misturas, além de total modificação da microestrutura cristalina. A caracterização da cinética de cristalização permitiu verificar que o período de indução para formação dos cristais e o teor máximo de gordura sólida foram alterados em função do teor de óleo totalmente hidrogenado nas misturas originais e como resultado do rearranjo ao acaso;

- A interesterificação química alterou o polimorfismo cristalino das misturas à base de óleo de soja/ óleo de soja totalmente hidrogenado, com promoção significativa do polimorfo β', de interesse para a grande maioria das aplicações em alimentos. Para as misturas à base de óleo de canola/ óleo de algodão totalmente hidrogenado, a tendência original de cristalização no hábito polimórfico β' foi mantida após a randomização;

- As misturas interesterificadas à base de óleo de soja/ óleo de soja totalmente hidrogenado, com teores de óleo totalmente hidrogenado iguais a 10, 20, 30 e 40% apresentaram pontos de fusão, perfis de sólidos, consistência, dimensões e hábito cristalino apropriados ao uso como *shortening* líquido, margarina de mesa, gordura para panificação/confeitaria e *all-purpose shortenings*/ base para recheios de biscoitos, respectivamente. Entretanto, para a mistura com 40% de óleo totalmente hidrogenado, o conteúdo de gordura sólida residual a 37°C pode estar associado à possível sensação de residual gorduroso na boca, limitando a sua aplicação;

- Para as misturas à base de óleo de soja/ óleo de soja totalmente hidrogenado, verificou-se que concentrações de óleo totalmente hidrogenado entre 20 e 40% forneceram as melhores propriedades para aplicação quanto à plasticidade, consistência, microestrutura e comportamento polimórfico. Os extremos avaliados (10 e 50% de óleo totalmente hidrogenado) produziram bases interesterificadas com características de conteúdo de gordura sólida, consistência e propriedades de cristalização incompatíveis com a maioria das aplicações em alimentos. Portanto, considera-se que teores de óleo de soja totalmente hidrogenado abaixo de 20% sejam evitados quando da interesterificação desta matéria-prima com óleos líquidos;

- As misturas interesterificadas à base de óleo de canola/ óleo de algodão totalmente hidrogenado, com teores de óleo totalmente hidrogenado iguais a 20, 25, 30 e 35% apresentaram pontos de fusão, perfis de sólidos, consistência, dimensões dos cristais e hábito cristalino compatíveis com sua aplicação em margarinas culinárias, *spreads*, gordura para panificação*/all-purpose shortenings* e gorduras para coberturas do tipo *glazers*, respectivamente. Considerando a interesterificação com óleos líquidos, teores de óleo de algodão totalmente hidrogenado entre 20 e 30% seriam os ideais para a produção de bases gordurosas com as características da maioria das gorduras comerciais

brasileiras. Percentuais deste óleo totalmente hidrogenado superiores a 30% devem ser criteriosamente avaliados nas formulações, pois produzem gorduras de alto ponto de fusão e alto conteúdo de gordura sólida a temperaturas relativamente altas;

- Para a totalidade das misturas avaliadas, a interesterificação produziu cristais com diâmetro inferior a 30μm, essencial para o uso em alimentos; e estabilização do polimorfo β' como forma cristalina predominante;

- Os óleos totalmente hidrogenados utilizados neste estudo mostraram comportamentos muito distintos entre si. O percentual de óleo de soja totalmente hidrogenado igual a 50% produziu mistura interesterificada com ponto de fusão igual a 57°C e conteúdo de gordura sólida de 10% a 50°C. Mistura interesterificada com as mesmas características foi obtida para uma concentração inferior de óleo de algodão totalmente hidrogenado, igual a 40%. Nesta comparação, exclui-se o óleo de canola como promotor deste efeito, uma vez que seu grau de insaturação é superior ao do óleo de soja. Portanto, conclui-se que maiores percentuais de ácido palmítico, bem como interações entre os triacilgliceróis dos tipos PStSt e StStSt (predominantes no óleo de algodão totalmente hidrogenado) podem estar relacionados a este comportamento diferencial;

- Na determinação do conteúdo de gordura sólida, as misturas interesterificadas à base de óleo de soja/ óleo de soja totalmente hidrogenado não apresentaram estabilização da cristalização segundo temperagem padrão prescrita pelo Método AOCS Cd 16b-93. Fez-se necessária a modificação do processo de temperagem, para garantir a completa estabilização da cristalização das misturas interesterificadas: as amostras foram mantidas por 2 horas a 0°C e por 1 hora a cada temperatura de leitura;

- No estudo da cristalização das misturas antes e após randomização, os resultados de microestrutura obtidos a partir da microscopia sob luz polarizada apresentaram concordância com os resultados calculados para o expoente de Avrami (*n*), corroborando a significância da caracterização da cinética de cristalização por este modelo;

- O estudo da influência do tempo de reação sobre a interesterificação química de mistura óleo de soja/ óleo de soja totalmente hidrogenado permitiu verificar que o equilíbrio da reação foi alcançado após 5 minutos da introdução do catalisador, indicando que é possível a obtenção de gorduras interesterificadas, à base de óleos líquidos e óleos totalmente hidrogenados, em tempos substancialmente menores, com redução dos custos associados ao processo;

- As correlações matemáticas verificadas entre os resultados de ponto de fusão obtidos a partir do teor de 4% de sólidos por RMN e os obtidos a partir do método do tubo capilar aberto (método AOCS Cc 3-25) corroboram a utilização da técnica de RMN como meio seguro para a obtenção do ponto de fusão de gorduras, uma vez que o mesmo se diferencia como uma prática fácil e rápida.