PAULA SPERANZA

Production of special lipids by enzymatic interesterification of Amazonian oils and influence on the biological activity.

Produção de lipídios especiais por interesterificação enzimática de óleos da Amazônia e influência na atividade biológica

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PAULA SPERANZA

PRODUCTION OF SPECIAL LIPIDS BY ENZYMATIC INTERESTERIFICATION OF AMAZONIAN OILS AND INFLUENCE ON THE BIOLOGICAL ACTIVITY.

PRODUÇÃO DE LIPÍDIOS ESPECIAIS POR INTERESTERIFICAÇÃO ENZIMÁTICA DE ÓLEOS DA AMAZÔNIA E INFLUÊNCIA NA ATIVIDADE BIOLÓGICA

Thesis presented to the Scholl of Food Engineering of University of Campinas in partial fulfillment of the requirements for the degree of Doctor in FOOD SCIENCE

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em CIÊNCIA DE ALIMENTOS.

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ABSTRACT

This study aimed to produce, characterize and evaluate the antimicrobial properties of interesterified Amazonian oils produced by different lipases. Two blends of Amazonian oils were subjected to enzymatic interesterification: the first one was composed by buriti oil and murumuru fat and the second one was composed by patauá oil and palm stearin. The interesterification reactions were catalyzed by two microbial lipases in three different enzymatic systems: one with a commercial lipase Lipozyme-TL-IM (Novozymes); a second with a lipase from the microorganism *Rhizopus* sp.; and the third with a mixture of both lipases (commercial and *Rhizopus* sp.). In both blends, depending on the enzyme used, the lipids produced presented different characteristics. In the buriti: murumuru blend, the lipase from *Rhizopus* sp. besides being specific for the *sn*-1,3 positions of triacylglycerol was specific for the type of fatty acids (unsaturated). The commercial lipase was specific only for the type of fatty acids (unsaturated), while the use of both enzymes showed no synergistic effect in this blend; the results were intermediate to those obtained with the individual enzymes. In the patauá: palm stearin blend, the lipase from *Rhizopus* sp. is specific for the type of fatty acid (unsaturated), while commercial lipase showed no specificity to this blend. In the system with both enzymes no synergistic effect was also observed; the results obtained were similiar to those obtained using only the enzyme from *Rhizopus* sp. In both blends, with the three enzymatic systems, there was a reduction in the proportions of triacylglycerols of the types trisaturated and tri-unsaturated after the reactions, with the formation of predominantly mono -and di- unsaturated lipids. These lipids produced maintained the high concentration of tocopherols, carotenoids and phenolics, indicating that the reaction did not influence the concentration of minor compounds. In the antimicrobial evaluation, the blends before and after interesterification were emulsified, producing different responses. Emulsions produced with interesterified lipids showed lower droplet size and higher antimicrobial activity (bactericidal effect); emulsions produced with non-interesterified blends showed larger droplet size and lower antimicrobial activity (bacteriostatic effect). Therefore, lipases were able to catalyze the interesterification reactions between Amazonian oils, indicating the potential of these catalysts in these reactions. Lipids obtained showed antimicrobial activity, which encourages more detailed biological studies.

RESUMO

Este trabalho teve como objetivos produzir, caracterizar e avaliar as propriedades antimicrobianas de bases lipídicas interesterificadas produzidas com óleos e gorduras da Amazônia utilizando diferentes lipases. No trabalho foram utilizadas duas misturas de óleo e gordura da Amazônia para a produção das bases lipídicas, sendo a primeira delas composta pelo óleo de buriti e a gordura de murumuru e a segunda composta pelo óleo de patauá e a estearina de palma. As reações de interesterificação foram catalizadas por duas lipases em três sistemas enzimáticos diferentes: lipase comercial Lipozyme TL-IM (Novozymes), lipase do micro-organismo *Rhizopus* sp. e a mistura de ambas as enzimas (comercial + *Rhizopus* sp.). Em ambas as misturas, os lipídios produzidos apresentaram diferentes características dependendo da enzima utilizada. Na mistura buriti: murumuru, a lipase de *Rhizopus* sp., além de ser específica pelas posições *sn*-1,3 do triacilglicerol, foi específica para o tipo de ácido graxo (insaturado). A lipase comercial foi específica apenas pelo tipo de ácido graxo (insaturado), enquanto que a utilização de ambas as enzimas não apresentou efeito sinérgico nesta mistura, os resultados obtidos foram intermediários aos obtidos com as enzimas individualmente. Na mistura patauá: estearina de palma, a lipase de *Rhizopus* sp. foi específica para o tipo de ácido graxo (insaturados), enquanto que a lipase comercial não demonstrou específicidade para esta mistura. No sistema catalisado por ambas as enzimas também não foi observado efeito sinérgico; os resultdos obtidos foram similiares aos obtidos com a enzima de *Rhizopus* sp. Para ambas as misturas, com os três sistemas enzimáticos, houve redução nos triacilglceróis trisaturados e tri-insaturados após as reações, com a formação de bases lipídicas predominantemente mono e di-insaturados. Estes lipídios formados mantiveram a concentração elevada de tocoferóis, carotenos e fenóis, indicando que a reação não influenciou na concentração dos compostos minoritários. Na avaliação antimicrobiana, as misturas antes e após a interesterificação foram emulsificadas, produzindo diferentes respostas. Emulsões produzidas com os lipídios interesterificados apresentaram menor tamanho de partícula e maior potencial antimicrobiano, exibindo efeito bactericida; emulsões produzidas com as misturas não-interesterificadas, apresentaram maior tamanho de partícula e menor potencial antimicrobiano, exibindo efeito bacteriostático. Portanto, as lipases foram capazes de catalisar as reações de interesterificação entre os óleos da Amazônia, indicando o potencial destes catalisadores nestas reações. As frações lipídicas obtidas apresentaram atividade antimicrobiana, o que abre precedentes para que estudos biológicos mais aprofundados sejam realizados.

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"Vivendo, se aprende: mas o que se aprende, mais, é só a fazer outras maiores perguntas".

João Guimaraes Rosa - Grande Sertão Veredas.

"Perguntem, perguntem só a todos eles sem exceção: como compreendem em que consiste a felicidade? Oh, podem estar certos de que Colombo foi feliz não no momento em que descobriu a América mas quando a estava descobrindo; podem estar certos de que o momento mais elevado da felicidade foi, talvez, exatamente três dias antes do descobrimento do Novo Mundo, quando a tripulação rebelada, tomada de desespero, por pouco não mudou o curso do navio de volta para a Europa! Aí a questão não está no Novo Mundo, embora ele tenha se arruinado. Colombo morreu quase sem vê-lo e, no fundo, sem saber o que havia descoberto. A questão está na vida, apenas na vida - no seu descobrir-se, contínuo e eterno, e de maneira alguma na sua descoberta!".

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INTRODUÇÃO

A interesterificação enzimática é uma técnica de modificação de óleos e gorduras em que os ácidos graxos são redistribuidos no triacilglicerol de forma a produzir bases lipídicas com novas características físico-químicas, nutricionais e biológicas.

Embora esta técnica seja empregada há bastante tempo, o número de lipases utilizadas ainda é limitado; poucas são capazes de atuar de forma eficaz. As condições em que as reações ocorrem e as variações nos tipos de substratos exigem que estas enzimas sejam robustas e apresentem diferentes especificidades. A busca por novas lipases com estas características ainda é um dos grandes desafios.

Na interesterificação enzimática, devido à especificidade de algumas lipases e à capacidade de atuarem em condições brandas, é possível obter bases lipídicas interesterificadas em que a distribuição regioespecífica seja mais controlada e os compostos minoritários sejam preservados. Esta reação, é portanto, mais indicada quando o objetivo é produzir lipídios com composições mais específicas para aplicações funcionais e medicinais.

Os óleos e gorduras da Amazônia, neste contexto, ganham destaque; estes óleos, embora sejam popularmente conhecidos pelo potencial de utilização, são pouco explorados. A utilização destes óleos e gorduras em reações de interesterificação enzimática pode favorecer a formação de novas estruturas lipídicas, cujas características sejam mais apropriadas para aplicação em produtos cosméticos, farmacêuticos e alimentícios, com focos funcional e medicinal.

Dentre as aplicações, a busca por novos agentes antimicrobianos possui grande relevância, pois fatores como o uso indiscriminado dos antimicrobianos tradicionais resultou na seleção de diversos patógenos resistentes.

Assim sendo, a proposta deste trabalho foi produzir, caracterizar e avaliar a atividade antimicrobiana de bases lipídicas interesterificadas por lipases microbianas. Estas bases lipídicas foram produzidas utilizando-se duas misturas de óleos e gorduras da Amazônia diferentes: a primeira delas composta pelo óleo de buriti e a gordura de murumuru; e a segunda composta pelo óleo de patauá e a estearina de palma. Estas duas misturas foram interesterificadas por três diferentes sistemas enzimáticos: lipase comercial Lipozyme TL - IM (Novozymes), lipase de *Rhizopus* sp. e a mistura de ambas as lipases (comercial + *Rhizopus* sp.). Estes óleos foram selecionados devido às características físico-químicas que indicam o seu potencial biológico. O óleo de buriti é rico em ácido oleico, além de apresentar alta concentração de carotenóides e tocoferóis. A gordura de murumuru é rica em ácidos graxos de cadeia média. O óleo de patauá é rico em ácido oleico e apresenta alta concentração de tocoferóis. A estearina de palma, embora não apresente características que indiquem o potencial biológico, foi selecionada devido ao seu elevado ponto de fusão, possibilitando a obtenção de frações semi-sólidas.

Deste modo, a apresentação do trabalho foi dividida em 3 capítulos:

O capítulo 1 consiste de uma revisão bibliográfica sobre os óleos da Amazônia, com ênfase nos óleos utilizados neste trabalho. Este capítulo também faz uma revisão sobre o uso de lipases em reações de interesterificação, assim como, as características destas enzimas. Por fim, uma revisão sobre o uso de óleos como agentes antimicrobianos é apresentada.

O capítulo 2 avalia a obtenção de lipídios interesterificados à base de óleo de buriti e gordura de murumuru e de óleo de patauá e estearina de palma. Neste capítulo é abordado o modo de ação dos três sistemas de lipases utilizados, assim como a caracterização físico-química dos lipídios produzidos.

O capítulo 3 avalia o efeito da interesterificação dos óleos da Amazônia nas propriedades físicas de emulsões produzidas com estes óleos, e as correlaciona com a atividade antimicrobiana.

Em anexo é apresentado um artigo de revisão bibliográfica já publicado sobre aplicações recentes de lipases em modificação de óleos e gorduras. O artigo aborda a utilização das lipases na produção de lipídios com baixo teor de calorias, ricos em ω-3 e com a adição de compostos fenólicos.

CAPÍTULO 1
REVISÃO BIBLIOGRÁFICA

1. Lipídios

Os lipídios compõe um amplo grupo de compostos quimicamente diversos, cuja característica em comum que os define é a solubilidade em solventes orgânicos e a insolubilidade ou fraca solubilidade em água (Damodaran *et al*., 2010; Shen *et al*., 2012). Nos alimentos, os lipídios são responsáveis por características como textura e sabor; já as funções biológicas são tão diversas quanto as estruturas químicas. Estes ingredientes são portadores de vitaminas solúveis em óleo, contêm ácidos graxos essenciais, são elementos estruturais das membranas biológicas, são cofatores enzimáticos, agentes emulsificantes no trato digestivo, oferecem proteção contra agentes agressores externos, entre outros (O'Brien, 2009; Nelson e Cox, 2011; Batista *et al*., 2012; Michalski *et al*., 2013).

1.1. Óleos e gorduras vegetais

Dentre os lipídios, os óleos e gorduras vegetais são largamente utilizados como matérias-primas para as indústrias de alimentos, petroquímica, cosmética e farmacêutica (Solís-Fuentes *et al*., 2010). As maiores fontes de óleos e gorduras vegetais são obtidas de sementes de plantas cultivadas em climas relativamente temperados. Outras fontes destes óleos e gorduras são obtidas de árvores oleaginosas de climas quentes, em que o óleo é extraído em sua maioria da polpa do fruto (O'Brien, 2009).

Dentre os óleos e gorduras vegetais, alguns têm ganhado importância devido aos efeitos protetores à saúde e seu potencial de utilização pela indústria de alimentos. A

maioria deles é empregada na forma bruta, sendo portanto, ricas em compostos minoritários. Azeites como o de dendê e de oliva apresentam respostas positivas em relação às propriedades anti-inflamatórias e perfil lipídico (Cicerale *et al*., 2010; Budin *et al*., 2009). Outros óleos, como o de linhaça, devido à presença do ácido α-linolênico, está relacionado às reduções de doenças cardiovasculares e com os sintomas da menopausa, assim como, a melhora no perfil lipídico (Lemay *et al*., 2002; Rodrigues-Leyva *et al*., 2010).

Devido a estes resultados positivos, a demanda por novas fontes de óleos e gorduras vegetais com propriedades biológicas cresce continuamente. Indústrias como as de cosmético e farmacêutica buscam óleos e gorduras com melhor qualidade e diversidade. Dentre estes óleos e gorduras, os obtidos da Amazônia ganham destaque.

1.1.1. Óleos e gorduras da Amazônia

A Amazônia é a maior floresta tropical do mundo, onde o desconhecimento de sua biodiversidade ainda é uma realidade. Estima-se que a região amazônica apresente mais de 150 árvores oleaginosas, das quais mais da metade são palmeiras, que podem servir como fonte de matérias-primas (óleos e gorduras) de interesse comercial (Mambrim e Barrera-Arellano, 1997; Montúfar *et al*., 2010). Algumas destas palmeiras apresentam quantidades importantes de óleo na polpa do fruto (mesocarpo), outras na semente, e outras em ambos (Clement *et al*., 2005).

Se no passado o interesse por estes óleos e gorduras se direcionavam, prioritariamente, às poucas espécies que produziam óleos comestíveis e aromáticos, com as mudanças nos padrões alimentares e com o interesse das indústrias cosméticas e

farmacêuticas por óleos naturais, maior interesse por novas oleaginosas pode ser obsevado (Pesce *et al*., 2009).

Apesar da grande diversidade e do número de possíveis aplicações, poucas fontes de óleos e gorduras foram exploradas, tornando-se interessante o desenvolvimento de estudos mais aprofundados referentes à qualidade e composição dos mesmos.

Na Tabela 1 alguns destes óleos e gorduras de palmeiras da região amazônica são apresentados. É possível verificar que o número de trabalhos publicados com estes óleos é bastante reduzido. A baixa utilização destes óleos e gorduras em trabalhos acadêmicos pode ser atribuída à sua baixa produção, à dificuldade em se obtê-los, ao desconhecimento de suas propriedades biológicas e às características físico-químicas que dificultam a aplicação dos mesmos.

Tabela 1: Óleos e gorduras obtidos de palmeiras da região amazônica:

Nomenclatura popular	Nomenclatura botânica	Trabalhos publicados*	
		Revistas Nacionais	Revistas Internacionais
Tucumã-comum	*Astrocaryum vulgare* Mart.	4	8
Murumuru	*Astrocaryum murumuru* Mart.	2	0
Babaçu	*Attalea speciosa* Mart.	5	3
Patauá	*Oenocarpus bataua* Mart.	3	2
Pupunha	*Bactris gasipaes* Kunth	5	1
Buriti	*Mauritia flexuosa* L.f.	30	7
Açai	Euterpe oleracea Mart.	10	8

Fonte: Pesce *et al*., 2009
* Base de dados Scopus (Fevereiro/2014).

Os trabalhos disponíveis indicam que estes óleos e gorduras são ricos em compostos com propriedades biológicas comprovadas, como os carotenóides, tocóis e

esteróis (Karppi *et al*., 2012; Jaswir *et al*., 2012; Sen *et al*., 2006; Alabdulkarim *et al*., 2012). Muitos deles também são ricos em ácidos graxos com efeito biológico, como os de cadeia média, monoinsaturados e poli-insaturados.

Os carotenóides são pigmentos naturais amplamente distribuídos na natureza, conhecidos por apresentar elevada capacidade antioxidante, antitumoral, anti-inflamatória, além de inibirem a síntese de colesterol (Krinsky e Johnson, 2005; Furhrman *et al*., 1997; Khachik *et al*., 1999). Alguns carotenóides são denominados compostos pró-vitamina A por serem precursores de retinol e ácido retinóico (Benadé, 2013).

Os tocóis (tocoferóis + tocotrienóis) são considerados os antioxidantes lipossolúveis mais eficazes, pois impedem a peroxidação lipídica, agindo como sequestrantes de radicais peroxil. Além das propriedades antioxidantes, os tocóis também exibem propriedades anti-inflamatória e antialérgica em modelos *in vivo* (Mabalirajan *et al*., 2009; O`Brien, 2009; Singh *et al*., 2007; Okamoto *et al*., 2006).

Os esteróis são geralmente os principais componentes minoritários dos óleos vegetais (Choudhary *et al*., 2014). Estes compostos são conhecidos pela capacidade de diminuir a absorção de colesterol no intestino, sendo reconhecidos pelo potencial hipocolesterolêmico (Smet, 2012). Além disso, apresentam atividades anti-inflamatória e imunomodulatórias (Navarro *et al*., 2001; Yuk et al., 2007; Aherne e O'Brien, 2008; Loizou *et al*., 2010).

Os ácidos graxos de cadeia média, especialmente o ácido láurico, são conhecidos pela atividade antibacteriana e antiviral (Lieberman *et al*., 2006). Os ácidos graxos monoinsaturados, especialmente o ácido oleico, apresentam proteção contra doenças

cardiovasculares e diferentes síndromes metabólicas (Lunn, 2007). Os ácidos graxos poli-insaturados, especialmente os ácidos linoléico e linolênico, estão relacionados com a modulação de doenças autoimunes e processos inflamatórios (Galli e Calder, 2009).

Assim, os óleos e gorduras da Amazônia apresentam características importantes para a prevenção e tratamento de doenças, para a produção de alimentos com melhor qualidade nutricional e para a produção de cosméticos. Muitos destes óleos e gorduras já são utilizados, conforme tradições locais, como cicatrizante, protetor solar, no tratamento de queimaduras, prevenção do envelhecimento da pele, e como anti-inflamatório e antibiótico. Muitas vezes, são incorporados pela indústria como excipiente de cosméticos artesanais, e formulações de sabões e cremes (Hernández *et al*., 2009; Silva *et al*., 2009; Rodrigues *et al*., 2010).

Destes óleos e gorduras, o de buriti, de murumuru, de patauá e de palma e suas frações, se destacam pela composição que apresentam:

1.1.1.1. Óleo de buriti

A palmeira *Mauritia flexuosa* Mart (*Arecaceae),* conhecida como buriti, tem grande potencial para ser utilizada pela indústria cosmética e farmacêutica. O óleo extraído da polpa do fruto (Figura 1) apresenta elevada concentração de ácidos graxos monoinsaturados, podendo ser superior à do azeite de oliva (Albuquerque *et al*., 2005; França *et al*., 1999). A Tabela 2 indica a composição em ácidos graxos deste óleo.

Figura 1: Fruto do buriti (Morais e Gutjahar, 2009).

Tabela 2: Composição em ácidos graxos do óleo de buriti:

Ácido graxo		**% em massa**
C16:0	Ácido palmítico	20,8
C18:0	Ácido esteárico	1,6
C18:1 ω-9	Ácido oleico	71,6
C18:2 ω-6	Ácido linoléico	2,5
C18:3 ω-3	Ácido linolênico	1,4
Outros		2,0

Fonte: Santos *et al*., 2013.

Pode-se observar que o ácido oléico é predominante no óleo de buriti, seguido pelo ácido palmítico. Óleos ricos em ácido oleico são de grande interesse, uma vez que apresentam estabilidade oxidativa superior à dos óleos com concentração elevada de ácidos graxos poli-insaturados, e não apresentam os efeitos nocivos à saúde atribuídos aos óleos ricos em ácidos graxos saturados (Lunn, 2007).

Com relação aos compostos minoritários (Tabela 3), este óleo é rico em

carotenoides, dos quais aproximadamente 90% estão na forma de β-caroteno, o que o caracteriza como uma das maiores fontes conhecidas deste composto (Silva *et al*., 2009; Albuquerque *et al*., 2005). Além disso, este óleo é rico em tocoferóis e esterois (Santos[2] *et al*., 2013; Silva *et al*., 2009; Albuquerque *et al*., 2005). Quando, por exemplo, este óleo é comparado com o óleo de soja, conhecido por ser uma das maiores fontes de tocoferol (1170 mg kg^{-1}), observa-se que os valores para ambos os óleos são muito próximos (Matthaus e Ozcan, 2014). A mesma comparação pode ser feita com o azeite de oliva em relação aos esterois (2930 mg kg^{-1}); este óleo é rico neste composto cuja concentração é próxima à encontrada no óleo de buriti (Kamal-Eldin, 2005).

Tabela 3: Compostos minoritários presentes no óleo de buriti:

Componente	Teor (mg kg^{-1})
Carotenoides	1707
α-tocoferol	1100
β-tocoferol	466
Esterois totais	2332

Fonte: Santos[2] *et al*., 2013; Albuquerque *et al*., 2005.

Zanatta *et al*. (2010) avaliaram o efeito fotoprotetor em células (fibroblastos e queratinócitos*)* de loções tópicas formuladas com diferentes surfactantes comerciais e óleo de buriti. Os resultados indicaram que a emulsão preparada com mono-oleato de sorbitol, óleo de rícino hidrogenado e óleo de buriti foi capaz de reduzir os danos causados pelas radiações UVA e UVB após 60 minutos de exposição, quando comparada com as células não-tratadas. Os autores concluíram que a emulsão de óleo de buriti rica

em carotenóides pode ser utilizada para proteger as células contra os danos foto-oxidativos, podendo ser utilizada como adjunto de protetores solares.

Batista *et al*. (2012) avaliaram a atividade cicatrizante e antibacteriana *in vitro* do óleo de buriti em feridas realizadas em ratos. Para a avaliação da atividade cicatrizante, foram utilizados 40 ratos da linhagem Wistar, divididos em dois grupos: o grupo I, composto por 20 ratos com feridas cutâneas, tratados com aplicação tópica do creme base com 10% de óleo de buriti, e o grupo II, controle, com o mesmo número de animais, que receberam a aplicação tópica do creme base. As avaliações das feridas foram realizadas no 3º, 7º, 14º e 21º dia. Em relação à área da ferida, foi observada redução significativa da área no 14º dia do grupo tratado com o óleo de buriti em relação ao controle. No 21º dia, as feridas do grupo tratado com o óleo do buriti apresentavam completo processo de cicatrização, enquanto que as feridas do grupo controle necessitavam de mais tempo para o completo processo cicatricial. Para a avaliação antibacteriana, foram utilizados os patógenos *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumonial* e *Staphylococcus aureus* mediante o uso do método de difusão em ágar. Os resultados mostraram que houve inibição do crescimento bacteriano em quatro dos cinco patógenos testados: *E. aerogenes, B. subtilis, K. pneumoniae e S. aureus*. Com base no diâmetro dos halos de inibição, pôde-se constatar que o *B. subtilis* (15,0 mm), *K. pneumoniae* (15,0 mm) e o *S. aureus* (11,1 mm) evidenciaram maior sensibilidade ao óleo do buriti. O óleo apresentou atividade antimicrobiana tanto em cepas gram-positivas, quanto em gram-negativas.

1.1.1.2. Gordura de murumuru

O murumuru, cujo nome científico é *Astrocaryum murumuru* Mart (*Arecaceae*)

produz uma gordura na semente do fruto (Figura 2), rica nos ácidos graxos láurico e mirístico (Saraiva *et al*., 2009; Mambrim e Barrera-Arellano, 1997). Estes ácidos graxos saturados conferem à gordura de murumuru elevada estabilidade oxidativa.

Figura 2: Futo do murumuru (Komani, 2014)

Os ácidos láurico e mirístico são utilizados com frequência pela indústria de cosméticos devido às suas propriedades emulsificantes, surfactantes, perfil de fusão, além da estabilidade à oxidação (Kalustian, 1985). Na Tabela 4 apresenta a composição em ácidos graxos desta gordura.

A gordura de murumuru, devido ao seu ponto de fusão de 32,5°C, é utilizada em misturas com óleos vegetais que se fundem a temperatura mais baixa, produzindo bases lipídicas semi-sólidas. Algumas empresas europeias e americanas, têm utilizado a gordura de murumuru em substituição à gordura de palmiste (Pesce *et al.*, 2009).

Os ácidos graxos de cadeia média presentes no murumuru são conhecidos por inibirem o crescimento de patógenos microbianos, uma vez que afetam a membrana celular e a produção de toxinas e enzimas destes micro-organismos (Bunkova *et al*., 2011; Desbois, 2012).

Tabela 4: Composição em ácidos graxos da gordura de murumuru:

Ácido graxo		% em massa
C8:0	Ácido caprílico	2,7
C10:0	Ácido cáprico	2,0
C12:0	Ácido láurico	51,6
C14:0	Ácido mirístico	25,8
C16:0	Ácido palmítico	6,0
C18:0	Ácido esteárico	2,9
C18:1 ω-9	Ácido oleico	5,7
C18:2 ω-6	Ácido linoléico	3,0
Outros		0,3

Fonte: Mambrim e Barrera-Arellano, 1997

1.1.1.3. Óleo de patauá

A palmeira *Oennocarpus bataua* Mart (Arecaceae*),* conhecida como patauá, tem grande potencial para a produção de óleos comestíveis. O óleo extraído da polpa do fruto (Figura 3) é utilizado como medicamento, cosmético e na culinária (Montúfar *et al*., 2010). Existem relatos da população local amazônica que mencionam que este óleo, por apresentar sabor semelhante ao do azeite de oliva, já foi utilizado pelos europeus como substituto deste durante a Segunda Guerra Mundial (Pesce, 2009).

Figura 3: Fruto do patauá (Rezaire, 2014)

Os resultados de caracterização do óleo indicam o seu potencial como uma nova fonte de óleo monoinsaturado, sendo comparável ao azeite de oliva e ao óleo de girassol com alto teor de ácido oléico (Montúfar *et al*., 2010; Rodrigues *et al*., 2010). Na Tabela 5 é apresentada a composição em ácidos graxos do óleo de patauá.

Tabela 5: Composição em ácidos graxos do óleo de patauá:

Ácidos graxos		**% em massa**
C16:0	Palmítico	18,1
C18:0	Esteárico	1,7
C18:1 ω-9	Oleico	72,7
C18:2 ω-6	Linoléico	1,9
C18:1 ω-7	*cis*-Vacênico	2,3
C18:3 ω-3	α-Linolênico	0,8
Outros		2,5

Fonte: Montúfar *et al*., 2010

Na fração insaponificável do óleo de patauá (Tabela 6) destaca-se a concentração de α-tocoferol e γ-tocotrienol; poucos óleos comercialmente disponíveis apresentam concentração tão elevada destes compostos (Tuberoso *et al*., 2007). Analisando-se a concentração dos tocóis no azeite de oliva (130 mg k g^{-1}), no óleo de linhaça (580 mg kg^{1}) e no óleo de girassol (670 mg kg^{-1}), constata-se que o óleo de patauá apresenta concentração destes compostos bastante superior. Apenas alguns óleos, como o de soja (1030 mg kg^{-1}), de farelo de arroz (1590 mg kg^{-1}) e de gérmen de trigo (2410 mg kg^{-1}) apresentam valores tão elevados.

Tabela 6: Compostos minoritários presentes no óleo de patauá.

Componente	Teor (mg kg^{-1})
α-tocoferol	1704
γ-tocotrienol	269
Esteróis totais	368
Fenólicos totais	51

Fonte: Hernández *et al*., 2009; Montúfar *et al*., 2010

Do teor de esteróis totais encontrado no óleo de patauá, a fração Δ^5-avenasterol (21% em relação ao total de esteróis) está presente em concentração relativamente alta quando comparada aos demais óleos vegetais (Montúfar *et al*., 2010; Phillips *et al*., 2002). Esta fração apresenta uma configuração química com alta capacidade antioxidante (Damodaran*et al*., 2010).

Segundo Schultes (1989), o óleo de patauá, devido à composição em ácido oléico e à presença de antioxidantes naturais, pode ser mantido por vários meses no ambiente

úmido e quente da região amazônica sem sofrer oxidação.

Hernández *et al.* (2009) avaliaram a capacidade antioxidante do óleo de patauá utilizando o radical DPPH (2,2-difenil-1- picril-hidrazil). Os autores observaram que a concentração de óleo utilizada para reduzir em 50% o radical foi equivalente à concentração de bifenóis e flavonóides. Este resultado é bastante promissor, uma vez que os compostos fenólicos utilizados são conhecidos pela elevada capacidade antioxidante.

1.1.1.4. Estearina de palma

O óleo de palma é um óleo vegetal original da África ocidental obtido do fruto da palma (*Elaeis guineensis* Jacq. - *Arecaceae*) (Figura 4). É largamente utilizado pela indústria e produz mais óleo por área do que qualquer outra planta, sendo a matéria-prima lipídica de maior produção mundial (Ludin *et al*., 2014, Sambanthamurthi *et al*, 2000). Os maiores produtores deste óleo são a Indonésia e a Malásia; porém outros países como o Brasil, a Colômbia, a Nigéria e a Tailândia também o produzem (Ludin *et al*., 2014). O Brasil, embora ainda seja um pequeno produtor de óleo de palma, possivelmente é o país que detém a maior área adequada para o seu cultivo, principalmente na região amazônica, que passou por uma grande expansão na última década (Villela *et al*., 2014).

Esse óleo é relativamente rico em ácidos graxos saturados, sendo o ácido palmítico (44%) o mais abundante, seguido pelos ácidos oleico (39%) e linoléico (10%). Além dos ácidos graxos, este óleo é rico em tocotrienóis, tocoferóis, carotenóides e esterois (Fattore e Fanelli, 2013).

Figura 4: Fruto da palma (Koh e Wilcove, 2007)

A estearina de palma é a fração sólida obtida do fracionamento do óleo de palma e é largamente utilizada pela indústria de alimentos como substituto dos óleos parcialmente hidrogenados ricos em ácidos graxos *trans* (Garcia-Macias *et al*., 2011). Além disso, devido ao seu elevado ponto de fusão, esta fração é utilizada em reações de interesterificação com óleos de ponto de fusão inferior, produzindo assim, bases lipídicas com maior conteúdo de gordura sólida (Silva *et al*., 2010; Adhikari[b] *et al*., 2010). Muitos trabalhos indicam a utilização da estearina de palma com diferentes óleos, como os de coco, soja e oliva em reações de interesterificação para a produção de lipídios com diferentes perfis de fusão (Khatoon *et al*., 2012; Fauzi *et al*., 2013; De Martini *et al*., 2013).

A estearina de palma é rica nos ácidos graxos palmítico e oleico, além de conter quantidade significativa dos ácidos esteárico e linoléico (Tabela 7). Alguns destes ácidos graxos, como o palmítico, apresentam atividade antimicrobiana (Ozcelik *et al*., 2005).

Tabela 7: Composição em ácidos graxos da estearina de palma (53,2°C):

Ácidos graxos		**% em massa**
C14:0	Láurico	1,1
C16:0	Palmítico	53,7
C18:0	Esteárico	5,4
C18:1 ω-9	Oleico	33,4
C18:1 ω-6	Linoléico	6,4

Fonte: Silva *et al*., 2010

A estearina de palma também apresenta uma pequena concentração de α e γ-tocoferois (36 mg kg^{-1}) e fitosterois (261 mg kg^{-1}) (Adhikari[a] *et al*., 2010).

1.1.2. Modificação de óleos e gorduras

Muitos óleos e gorduras vegetais em sua forma natural apresentam propriedades físicas, nutricionais e químicas pouco atrativas para aplicá-los diretamente em maior escala (Puligundla *et al*., 2012). Por estas razões, diferentes técnicas têm sido utilizadas para superar as limitações de uso destas matérias-primas. Em particular, tem-se procurado modificar a composição ou a distribuição dos ácidos graxos nos triacilgliceróis; sabendo que essas mudanças irão influenciar nas propriedades físicas, nutricionais e químicas destes óleos e gorduras (Gunstone, 2002).

A modificação de óleos e gorduras pode produzir lipídios com diferentes conteúdo de gordura sólida, textura, faixas de fusão e cristalização, estabilidade e interações químicas, o que pode garantir a obtenção de novas matérias-primas não encontradas na natureza.

1.1.2.1. Interesterificação

Dentre as técnicas disponíveis para a modificação da distribuição dos ácidos graxos no triacilglicerol (TAG) de óleos e gorduras, pode-se ressaltar a interesterificação. Esta reação envolve a remoção e a redistribuição dos ácidos graxos entre os TAGs, podendo ocorrer dentro da mesma molécula ou entre moléculas diferentes (De Greyt e Kellens, 2001). Na interesterificação, os ácidos graxos permanecem inalterados, ocorrendo apenas a modificação da composição triacilglicerídica (Rosenaal, 1992; Ribeiro *et al*., 2007). Esta reação geralmente ocorre entre uma gordura e um óleo, produzindo uma base lipídica com características físicas intermediárias (Tarrago-Trani *et al*., 2006).

A interesterificação pode ocorrer sem a presença de catalisadores utilizando-se altas temperaturas (300°C ou mais); entretanto, os resultados desejados não são alcançados. O equilíbrio é lentamente atingido e é acompanhado de decomposição e polimerização (Rosenaal, 1992). Geralmente, a reação é realizada na presença de um catalisador, que pode ser de natureza química ou enzimática (Akoh e Kin, 2008).

A interesterificação química, tem como principal catalisador o metóxido de sódio, embora outras bases, ácidos e metais possam ser utilizados (Marangoni e Rousseau, 1995). Nesta tipo de reação, o rearranjo dos ácidos graxos no TAG ocorre de forma aleatória (Figura 5), e o controle dos produtos da reação é obtido sob rígido controle da temperatura (Marangoni, 1995; O'Brien, 2009).

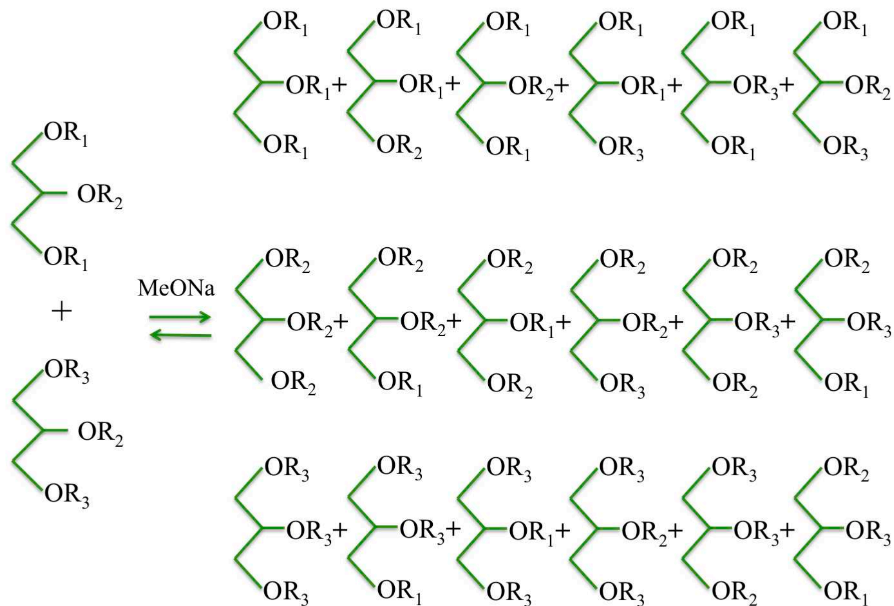

Figura 5: Triacilgliceróis produzidos por meio da reação de interesterificação química entre dois triacilgliceróis (adaptação de Willis e Marangoni, 2008).

Na interesterificação enzimática, as lipases (E.C. 3.1.1.3) são os catalisadores mais comumente utilizados. Neste tipo de reação, devido à especificidade das lipases, o rearranjo dos ácidos graxos no TAG ocorre de forma mais controlada (Figura 6), sendo possível prever mais facilmente os produtos da reação (Willis e Marangoni, 2008).

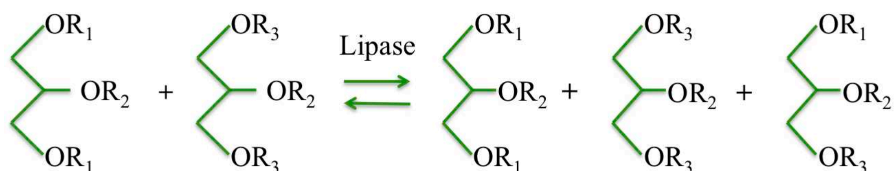

Figura 6: Triacilgliceróis produzidos por meio da reação de interesterificação enzimática entre dois triacilgliceróis (adaptação de Willis e Marangoni, 2008).

A interesterificação química é mais rápida e barata, enquanto que a interesterificação enzimática é seletiva, gera menos subprodutos indesejáveis e ocorre em

condições mais brandas de pressão e temperatura (Rodrigues e Lafuente, 2010). Desta forma, sob a perspectiva de custo e aplicação em larga escala, a interesterificação química parece ser o método mais atrativo. Contudo, sob a perspectiva de produzir lipídios com composição mais específica para aplicações funcionais e medicinais, os métodos enzimáticos são mais recomendados (Ract, 2006 e Wills *et al*., 1998).

Farmani *et al.* (2007) produziram vanaspati (tipo de *shortening*) por interesterificação enzimática e química. A interesterificação enzimática foi catalisada por 6% (m/m) da lipase comercial Lipozyme TL-IM (Novozymes) por 4 horas, enquanto que a interesterificação química foi catalisada por 0,5% (m/m) de metóxido de sódio por 30 minutos. O vanaspati produzido por interesterificação enzimática, comparado com o produzido por interesterificação química, apresentou maior estabilidade à oxidação e composição triacilglicerídica mais próxima a do vanaspati comercial. A interesterificação enzimática mostrou-se mais apropriada para a produção deste tipo específico de *shortening*.

Zarringhalami *et al*. (2012) compararam a produção de substitutos de manteiga de cacau por interesterificação química e enzimática utilizando as frações hidrogenada e sólida do óleo de semente de chá nas razões 20:80, 25:75 e 30:70. As espécies de TAGs formados após a reação de interesterificação enzimática ficaram mais semelhantes às da manteiga de cacau natural. Como a composição triacilglicerídica da manteiga de cacau define a sua qualidade, e como na interesterificação química a redistribuição dos ácidos graxos no triacilglicerol ocorre de forma randômica, o método enzimático, devido à resgioespecificidade da lipase, torna-se mais vantajoso nestas aplicações.

Em um estudo comparativo do valor nutricional do óleo de palma interesterificado por lipase e por catalisador química, verificou-se que o coeficiente de digestibilidade e teor de lipídios no fígado de ratos alimentados com estes óleos foi o mesmo. Entretanto, o nível de colesterol total dos ratos alimentados com o óleo interesterificado pela lipase apresentou redução significativa, indicando que a diferença na distribuição dos ácidos graxos dos óleos influenciou nos seus atributos nutricionais (Ray e Bhattacharyya, 1995).

1.1.2.1.1. Interesterificação enzimática

1.1.2.1.1.1. Lipases

As lipases constituem a classe de enzimas mais utilizadas em processos biotecnológicos, fatores como a capacidade de atuar em sistemas heterogêneos, resistir à condições severas de pH e temperatura e especificidade por diferentes substratos, justificam a sua elevada utilização (Kapoor e Gupta, 2012).

Estas enzimas são produzidas por várias plantas, animais e micro-organismos; no entanto, para a produção de enzimas indústriais, os micro-organismos são a fonte mais utilizada (Sharma e Kanwar, 2014). Isto porque, os micro-organismos apresentam características como crescimento rápido, elevado rendimento de conversão de substrato em produto, grande versatilidade de adaptação às condições ambientais, simplicidade na manipulação genética e condições de cultivo (Ribeiro *et al*., 2012). As lipases microbianas extra-celulares são as mais utilizadas, pois são mais fáceis de serem obtidas, são mais resistentes às condições ambientais e têm maior rendimento (Lima, 2001).

As lipases atuam na reação de interesterificação enzimática na interface óleo\água em um meio com baixo teor de umidade, suficiente apenas para manter a estrutura

proteica da enzima e a sua estrutura espacial (Marangone, 2002). A reação se processa por meio de sucessivas hidrólises e re-sínteses de TAGs, com multisubstratos, sendo os principais glicerídeos, ácidos graxos e água (Marangoni e Rousseau, 1995; Jager *et al*., 1998).

A principal vantagem das lipases que diferencia a interesterificação enzimática da interesterificação química é a especificidade (Marangoni, 2002). Devido a esta característica das lipases, lipídios para aplicação medicinais e nutricionais são mais facilmente desenvolvidos (Pande e Akoh, 2012; Devi *et al*., 2008). Existem três principais tipos de especificidades das lipases: posição, substrato e estereoespecificidade (Kapoor e Gupta, 2012):

(i) Especificidade por posição:

Algumas lipases apresentam especificidade por posição, isto é, atuam em determinadas ligações éster do TAG. A maioria destas enzimas catalisam preferencialmente a reação nas posições *sn* - 1 e *sn* - 3 do TAG (Figura 7). Devido ao impedimento estérico na posição *sn* - 2 do TAG, o sítio ativo da enzima dificilmente se liga à este ácido graxo; poucas lipases apresentam especificidade por esta posição (Marangoni, 2002).

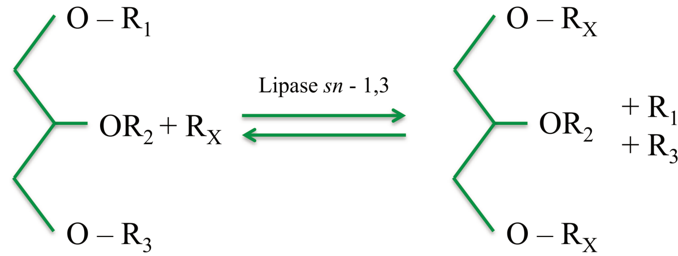

Figura 7: Reação catalisada por lipase específicapela posição do ácido graxo no triacilglicerol **(**adaptado de Weber e Mukherjee**,** 2008)

(ii) Especificidade por substrato:

Muitas lipases são específicas para um tipo particular de ácido graxo (Figura 8). Outras podem demonstrar especificidade em relação ao comprimento da cadeia, com algumas sendo específica para ácidos graxos de cadeia longa e outras para ácidos graxos de cadeia média e curta. Algumas lipases também podem ser específicas para a posição da insaturação no ácido graxo (Marangoni, 2002).

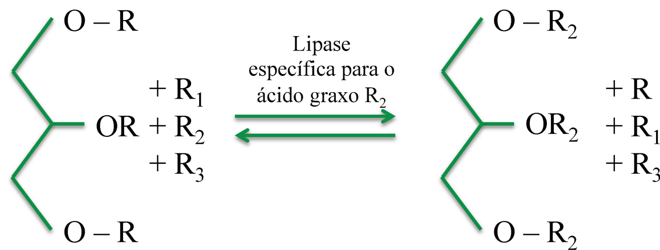

Figura 8: Reação catalisada por lipase específica por substrato **(**adaptado de Weber e Mukherjee**,** 2008)

(iii) Estereoespecificidade:

No TAG, as posições *sn* - 1 e *sn* - 3 são estericamente distintas. Algumas poucas lipases diferenciam entre os ésteres nestas posições, mas quando o fazem, as mesmas

apresentam estereoespecificidade (Figura 9). Nas reações em que a lipase é estereoespecífica, as posições *sn* - 1 e *sn* - 3 são hidrolisadas a taxas diferentes (Marangoni, 2002).

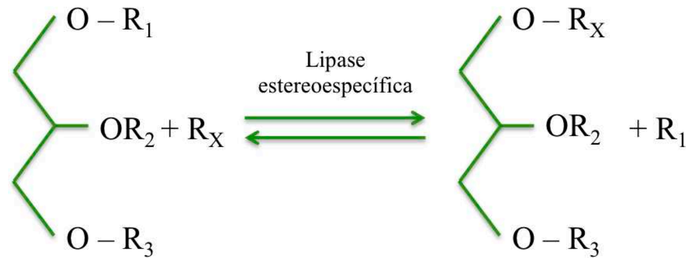

Figura 9: Reação catalisada por lipase estereoespecífica.

Há também o grupo das lipases não específicas (Figura 10). A reação com estas enzimas produz uma completa randomização dos ácidos graxos no TAG, gerando os mesmos produtos da interesterificação química (Marangoni, 2002).

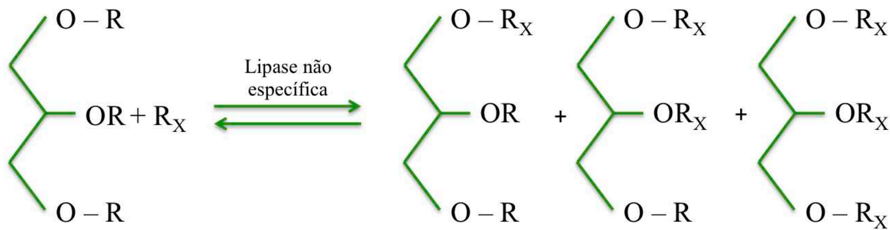

Figura 10: Reação catalisada por lipase não específica **(**adaptado de Weber e Mukherjee**,** 2008).

Entretanto, a especificidade de uma lipase individual pode mudar devido ao efeito do microambiente na reatividade dos grupos funcionais ou da molécula do substrato (Marangoni*et al*., 2002; Parida and Dordick, 1991). Fatores como quantidade de água

presente no meio, pH e solvente orgânico podem alterar a especificidade da lipase, o que possibilita a produção de lipídios com diferentes características (Akoh *et al*., 2002). Na Tabela 8, são apresentados exemplos de reação de interesterificação enzimática com diferentes substratos, em várias condições de reação. Nestas reações, os produtos obtidos apresentam diferentes propriedades e potenciais de aplicação.

Tabela 8: Reações de interesterificação catalisadas por lipases.

Lipase	Substrato	Condições da reação	Referência
Lipozyme IM-60 (*Rhizomucor miehei*)	Óleos de amendoim e linhaça	45°C por 48 horas, 200 rpm, 1% de enzima.	Sharma *et al*., 2012
Lypozyme TL-IM (*Thermomyces lanuginosa*)	Óleos de soja, colza e girassol	70°C por 4 horas, 6% de enzima.	Farmani *et al*., 2007
Lypozyme TL-IM (*Thermomyces lanuginosa*)	Óleos de farelo de arroz e coco e gordura de estearina de palma	65°C por 24 horas, 10% de enzima	Adhikari[a] *et al*., 2010
Lipozyme RM-IM (*Rhizomucor miehei*)	Óleos de soja e oliva e gordura de estearina de palma	65°C por 24 horas, 300 rpm, 10% de enzima	Lee *et al*., 2007
Novozym 435 (*Candida antarctica*)	Óleo de oliva e óleo de palma totalmente hidrogenado	75°C por 3 horas, 10% de enzima	Criado *et al*., 2008

1.1.2.1.1.2. Aplicações

A maior aplicação da interesterificação enzimática concentra-se na modificação das características físico-químicas e nutricionais de lipídios.

Criado *et al*. (2008) produziram diferentes bases lipídicas através de reação de interesterificação enzimática entre azeite de oliva extra virgem e óleo de palma totalmente hidrogenado, utilizando como catalisador da reação a lipase comercial Novozym 435 (Novozymes). Foram utilizadas diferentes proporções do óleo e da gordura, o que resultou na formação de lipídios com diferentes características físico-químicas e funcionais. Em todas as bases lipídicas houve enriquecimento de ácidos graxos insaturados na posição *sn* - 2, o que gerou temperaturas de fusão mais baixas do que as misturas físicas correspondentes. Todos os lipídios sintetizados pela ausência de ácidos graxos *trans*, pelos altos teores de ácido oleico e compostos minoritários podem apresentar diversos benefícios à saúde, com potencial de aplicação em margarinas e óleos para fritura.

Reshma *et al*. (2008) produziram bases lipídicas por meio da reação de interesterificação enzimática entre a estearina de palma e o óleo de farelo de arroz nas proporções de 40:60, 50:50, 60:40 e 70:30, respectivamente. A reação foi catalisada pela lipase comercial Lipozyme TL-IM (Novozymes) na concentração de 3% em relação ao total de substrato por 6 horas a 60°C. As bases lipídicas produzidas apresentaram um conteúdo de ácidos graxos saturados variando de 37,6 - 52,0% e 48,0 - 62,4%, respectivamente. O conteúdo de fitoquímicos bioativos como os tocóis (839 - 1172 mg kg^{-1}), esterois (4318 - 9647 mg kg^{-1}), orizanol (3000 - 6800 mg kg^{-1}) e carotenos (121 - 180 mg kg^{-1}) foram conservadas após a reação. As bases lipídicas produzidas devido às qualidades nutricionais e a ausência de ácidos graxos *trans* podem ser utilizadas em produtos com alto valor agregado.

Feltes *et al*. (2009) por meio da reação de interesterificação enzimática utilizando 5% (m/m) da lipase comercial Lipozyme RM - IM (Novozymes), em 6 horas de reação, incorporaram ácidos graxos de cadeia média aos TAG do óleo de pescado, conservando a concentração dos ácidos eicosapentaenóico (EPA) e docosahexaenóico (DHA) próximos à do óleo original. A produção de bases lipídicas contendo ácidos graxos de cadeia média e ácidos graxos poli-insaturados na mesma molécula oferece benefícios nutricionais e terapêuticos. Estas bases podem ser aplicadas em nutrição clínica em pacientes com síndromes de má absorção.

Adhikari[b] *et al*. (2010) produziram margarinas zero-*trans* ricas em tocoferóis e fitoesteróis por meio da reação de interesterificação enzimática entre o óleo da noz de pinhão e a estearina de palma nas proporções de 40:60 e 30:70 (% m/m). As condições de reação empregada foram 65°C por 24 horas e 30% (m/m) da lipase comercial Lipozyme TL - IM (Novozymes). Os produtos obtidos, devido às condições brandas de reação, mantiveram os compostos minoritários da mistura inicial.

Borham *et al*. (2011) por meio da reação de interesterificação enzimática entre a fração média da palma, a estearina de palmiste e TAGs de cadeia média produziram bases lipídicas com conteúdo calórico reduzido para serem utilizados como substitutos da manteiga de cacau. As reações foram conduzidas sob condições controladas de temperatura, tempo e concentração da lipase comercial IM - 60 (Novo Nordisk). As bases lipídicas, devido à especificidade da lipase, apresentaram um balanço entre os ácidos graxos de cadeia média e cadeia longa. Os ácidos graxos de cadeia média, devido à alta solubilidade e a capacidade de se emulsificar, são metabolizados mais rapidamente. As

características físico-químicas das bases lipídicas foram similares às da manteiga de cacau, porém apresentaram textura levemente mais macia.

Teichert e Akoh (2011) produziram substitutos da gordura de leite materno para formulações infantis por meio de reação de interesterificação enzimática entre o óleo de soja enriquecido com ácido estearidônico nas posições *sn* - 1 e *sn* - 3 e tripalmitina. As reações foram catalisadas por duas lipases comerciais, Novozym 435 e Lipozyme TL - IM (Novozymes), em diferentes tempos, temperatura e razões molares de substrato. Como as enzimas atuaram principalmente nas posições *sn* - 1, 3 do TAG, foi possível sintetizar TAGs ricos em ácido palmítico na posição *sn* - 2 do TAG. A gordura de leite materno apresenta mais de 60% de ácido palmítico nesta posição. Durante o metabolismo dos bebês, devido à especificidade da lipase pancreática (*sn* - 1, 3), este ácido é mantido na forma de 2 - monoacilglicerol, sendo diretamente absorvido pela mucosa intestinal de forma mais rápida e eficiente. Além disso, a presença do ácido estearidônico nas posições *sn* - 1, 3 do TAG garante uma maior conversão deste ácido em EPA, importante para o crescimento e desenvolvimento.

Além da produção de lipídios com melhores características físico-químicas e nutricionais, a interesterificação enzimática também pode ampliar o potencial biológico da mistura inicial. A combinação de ácidos graxos e compostos minoritários de diferentes óleos e gorduras pode produzir um efeito sinérgico nas propriedades biológicas destas matérias-primas. Embora o número de estudos ainda seja pequeno, muitos deles têm apresentado resultados positivos, o que pode ampliar a aplicação desta técnica em áreas como a cosmética e a farmacêutica.

Reena e Lokesh (2007) produziram uma mistura e uma base lipídica interesterificadacom igual proporção de óleo de coco e óleo de farelo arroz com propriedades nutracêuticas em ratos. A reação para a produção da base lipídica foi catalisadas com 1% (m/m) da lipase comercial Lipozyme RM - IM (Novozymes), a 37°C por 72 horas. Os ratos tratados por 60 dias com a mistura dos óleos tiveram a redução do colesterol sérico, lipoproteínas de baixa densidade (LDL) - colesterol e triglicérides de 23,8%, 32,4% e 13,9%, respectivamente; enquanto que os ratos tratados com as bases lipídicas tiveram redução de 35,0%, 49,1% e 23,2%, respectivamente, quando comparados com os ratos tratados apenas com o óleo de coco. Os resultados indicaram que o potencial aterogênico dos ácidos graxos saturados contidos no óleo de coco pode ser significativamente reduzido pela mistura com os ácidos graxos insaturados do óleo de farelo de arroz, e esta redução pode ser ainda maior quando os óleos são interesterificados.

Reena e Lokesh (2012) produziram uma base lipídica por meio da reação de interesterificação enzimática entre o óleo de palma e o óleo de farelo de arroz e avaliaram as alterações lipídicas sérica e hepática em ratos alimentados com estes lipídos por 8 semanas. A reação de interesterificação dos óleos foi conduzida por 6 horas a 40°C, utilizando-se 1% (m/m) da lipase comercial Lipozyme RM - IM (Novozymes). Os ratos alimentados com a mistura dos óleos não interesterificados apresentaram redução de 51% no teor de colesterol sérico, enquanto que os ratos alimentados com a base lipídica interesterificada apresentaram redução de 56%, quando comparados com os ratos alimentados apenas com o óleo de palma. Os autores sugerem que a distribuição dos

ácidos graxos no TAG influenciam a expressão dos genes que regulam a circulação de LDL-colesterol em ratos.

Kanjilal *et al*. (2013) avaliaram o efeito hipolipidêmico de duas bases lipídicas com conteúdo calórico reduzido em ratos e coelhos. As bases foram produzidas por meio de reações de interesterificação enzimática entre etil behenato (éster metílico do ácido behênico) e os óleos de soja e de girassol, utilizando-se 4% (m/m) da lipase comercial Lipozyme TL - IM (Novozymes) por 6 horas. Os ácidos graxos de cadeia muito longa, como o ácido behênico, apresentam absorção limitada por terem ponto de fusão mais elevado do que a temperatura corporal, além de possuirem baixa capacidade de formar emulsão e de se solubilizar (Hashim *et al*, 1978). As bases lipídicas formadas apresentaram aproximadamente 29% de ácido behênico e 38% de ácido linolênico. Os animais alimentados com bases interesterificadas apresentaram redução nos níveis de LDL - colesterol e triglicérides entre 34 - 43% e 26 - 30%, respectivamente, comparados com os animais alimentados apenas com o óleo de girassol. As bases lipídicas além de apresentarem conteúdo calórico reduzido, foram capazes de diminuir os índices de lipídios sérico e hepático em ratos e coelhos.

Sanders *et al*. (2003), embora não tenham avaliado diretamente o efeito da interesterificação na resposta biológica, avaliaram a influência da estrutura do TAG na resposta da lipemia pós-prandial e ativação do Fator VII (proteína ligada à coagulação). TAGs simétricos ricos em ácido esteárico nas posições *sn* - 1, 3, parecem ser mais rapidamente absorvidos e retardam a ativação do Fator VII; enquanto que, os TAGs assimétricos, com o ácido esteárico localizado na posição *sn* - 2, têm a absorção retardada e conduzem à ativação do Fator VII.

2. Antimicrobianos

Antimicrobiano refere-se a uma subtância que impede ou inibe o crescimento de micro-organismos (Zhang *et al*., 2010). Geralmente, esse composto se liga a algum componente vital do metabolismo da bactéria, inibindo a síntese de biomoléculas funcionais ou impedindo a atividade celular normal (Walker *et al*., 1996). Apesar do grande progresso no desenvolvimento de antimicrobianos, muitas doenças infecciosas permanecem difíceis de serem tratadas. A resistência dos patógenos aos antimicrobianos, a dificuldade de transporte destas substâncias na membrana celular e a baixa atividade dos mesmos no interior das células são algumas das razões para o desenvolvimento desses novos compostos (Zhang *et al*., 2010).

Estudos com ácidos graxos indicam que a estrutura dos mesmos influencia na ação antimicrobiana. Huang *et al*. (2011) avaliaram a atividade antimicrobiana de ácidos graxos de cadeia curta, média e longa contra micro-organimos bucais. Os ácidos graxos capróico (C6:0), caprílico (C8:0) e láurico (C12:0) foram eficazes contra a levedura *Candida albicans*, enquanto que os ácidos fórmico (C1:0) acético (C2:0), propiônico (C3:0), butírico (C4:0), valérico (C5:0), cáprico (C10:0), mirístico (C14:0) e palmítico (C16:0) tiveram pouca ação contra este micro-organismo. Os ácidos fórmico, cáprico e láurico inibiram amplamente as bactérias *Streptococcus mutans*, *Streptococcus gordonii* e *Streptococcus sanguinis*. Os resultados indicaram que à atividade antimicrobiana dos ácidos graxos de cadeia curta, média e longa podem influenciar na flora microbiana da cavidade bucal.

Em um estudo com os ácidos cáprico, caprílico e láurico foi observado que a

exposição dos mesmos à bactéria *Clostridium difficile,* um patógeno comum em hospitais, apresentou diferentes respostas. Destes ácidos graxos, o láurico foi o que mais inibiu o crescimento do patógeno. Quando utilizado na concentração de 1000µM foi capaz de inibir quase completamente o crescimento do micro-organismo, e na concentração de 250 µM inibiu em 90% o crescimento (Shilling*et al*., 2013). Em um outro estudo, diferentes ácidos graxos na concentração de 50 µL mL^{-1} inibiram o crescimento do micro-organismo *Staphylococcus epidermidis*. Os ácidos graxos testados foram o burítico, capróico, enântico (C7:0), caprílico, cáprico, láurico, mirístico, palmítico e esteárico (C18:0). Dos ácidos graxos, o mirístico foi o que apresentou maior capacidade antimicrobiana, com a inibição de 80,6% do patógeno após 24 horas de incubação (Liu e Huang, 2012).

Parfene *et al*. (2013) verificaram o perfil de ácidos graxos e a atividade antimicrobiana de óleo de coco hidrolisado pelo fungo *Yarrowia lipolytica* R013 por fermentação em estado-sólido. Os resultados cromatográficos obtidos indicaram como principal ácido graxo, o ácido láurico, representando 70% do conteúdo total de ácidos graxos. Foi constatado também que este ácido foi capaz de inibir o crescimento dos micro-organimos *Salmonella enteritidis*, *Escherichia coli*, *Listeria monocytogenes* e *Bacillus cereus* após 24 horas.

Ozcelik *et al*. (2005) avaliaram à atividade antibacteriana, antifúngica e antiviral de dezesseis extratos lipofílicos de *Pistacia vera,* uma planta medicinal nativa da Ásia e largamente distribuída na região Mediterrânea e nos Estados Unidos. Os extratos foram avaliados contra os micro-organismos *Escherichia coli, Pseudomonas aeroginosa, Enterecoccus faecalis, Staphlococcus aureus, Candida albicans, Candida parapsilosis*,

vírus da herpes simples e o vírus da parainfluenza pelo método de microdiluição. Todos os extratos avaliados apresentaram baixa atividade antibacteriana nas concentrações estudadas (128 - 256 μg mL^{-1}). Porém, os mesmos apresentaram uma perceptível atividade antifúngica nestas mesmas concentrações. Dois dos extratos testados apresentaram também atividade antiviral quando comparados com antibióticos comerciais. O potencial antimicrobiano destes extratos lipofílicos foi atribuído à presença do ácido palmítico. Em um outro estudo este mesmo ácido graxo também apresentou atividade antibacteriana contra os micro-organismos *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* e *Klebsiella pneumoniae* e contra o vírus da influenza (Yff *et al*., 2002).

A utilização de óleos com potencial biológico em estudos antimicrobianos ainda é uma realidade pouco comum. Devido às dificuldades em se solubilizar estes óleos em meio aquoso, poucos estudos foram conduzidos com este objetivo.

Buthelezi *et al*. (2012) avaliaram o efeito antimicrobiano do óleo da calda do crocodilo em DMSO (dimetilsulfóxido). Este óleo é conhecido popularmente por ser eficaz no tratamento de doenças inflamatórias e microbianas. O óleo apresenta em sua composição dezesseis tipos de ácidos graxos, sendo o oleico (19,6%), o palmítico (15,4%) e o linoléico (4,0%) os principais. No estudo, o óleo foi capaz de inibir os micro-organismos *Staphylococcus aureus* (gram - positivo), *Klebsiella pneumoniae* (gram - negativo) e a levedura *Candida albicans*, indicando que as diferenças estruturais da parede celular das bactérias não modularam a eficácia do óleo. A maior atividade do óleo contra as bactérias foi obtida na concentração de 15% (m/v), com inibição de 58,4% para o *Staphylococcus aureus*e 54,0% para a *Klebsiella pneumoniae.* Com relação à levedura,

a maior atividade foi obtida na concentração de 6% (m/v), com inibição de 81,7%.

Prijck *et al*. (2008) avaliaram o efeito dos óleos de jojoba, óleo de carbol, óleo de gergelim e a mistura do óleo de jojoba com o óleo essencial de árvore do chá contra os micro-organismos patógenos *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus* e *Pseudomonas aeruginosa*. Os óleos foram misturados com as bactérias numa concentração de 10^4 UFC mL^{-1} por 24 horas. As bactérias foram extraídas dos óleos com tampão fosfato salino contendo 0,5% de Tween-20. Alíquotas das fases aquosas foram analisadas por citometria de fase sólida e contagem em placas. A contagem em placas foi igual a zero para todos os micro-organimos durante 24 horas, com todos os óleos testados. Em contrapartida, um número significativo de células viáveis ainda foi detectado pelo método de citometria de fase sólida, exceto para os micro-organismos mantidos na mistura do óleo de jojoba/óleo essencial de árvore do chá. Os resultados indicaram que esta mistura foi mais eficaz que o óleo de carbol, que é conhecido pelo potencial antimicrobiano.

3. Conclusões

Os óleos da Amazônia devido à composição em ácidos graxos e compostos minoritários, podem ser utilizados em reações de interesterificação enzimática para a produção de frações lipídicas interesterificadas com propriedades físico-químicas, nutricionais e biológicas mais atrativas para aplicações cosméticas, farmacêuticas e de alimentos. Apesar de trabalhos indicarem o potencial desta técnica para a produção de lipídios com maior potencial biológico, poucas lipases são capazes de atuar de forma eficiente nestas reações. A complexidade da reação e o grande número de substratos com

diferentes características, dificultam a ação da enzima, restringindo sua utilização a produtos de alto valor agregado. A busca por novas lipases com diferentes especificidades e maior robustez ainda é uma necessidade nesta área. Desenvolver frações lipídicas interesterificadas com óleos e gorduras da Amazônia que apresentem potencial antimicrobiano, cujo interesse é muito grande, pode ser uma oportunidade de valorização destas matérias-primas brasileiras pouco exploradas.

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CAPÍTULO 2

LIPASE-CATALYZED INTERESTERIFICATION OF AMAZONIAN BURITI OIL WITH MURUMURU FAT AND PATAUÁ OIL WITH PALM STEARIN TO PRODUCE SPECIAL-STRUCTURED LIPIDS.

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1. Introduction

Enzymatic interesterification has been shown to be a technique for improving the physico-chemical characteristics of oils and fats. The redistribution of fatty acids present in the original blend may improve its potential application (Nunes, Paula, Castro & Santos, 2011; Iwasaki & Yamane, 2000). Furthermore, enzymatic interesterification can also be used as a technique for improving the biological characteristics of oils and fats. Recent studies have revealed that the interesterified oils showed different responses for digestion, absorption and atherogenicity compared with the same oils when non-interesterified (Michalski *et al*., 2013; Fárfan, Villalón, Ortíz, Nieto & Bouchon, 2013; Berry, 2009). Lipases have a key role in these reactions. Due to the specificity of these enzymes, the lipids formed may have different characteristics and applications (Speranza & Macedo, 2012).

Thus, the use of fat blends in enzymatic interesterification reactions, which have different composition of fatty acids and minor compounds, can promote the formation of new lipids with better physicochemical, nutritional and biological characteristics. The Amazonian fats are highlighted in this context.

The Amazon is the largest tropical forest in the world, and much of its biodiversity remains to be explored. An example of the potential of this biodiversity is found in oils and fats extracted from the pulp and seed of palm trees. In the few published studies, these oils and fats have been shown to be nutritious, with features rarely found in commercially available vegetable oils (Rodrigues, Darnet & Silva, 2010; Silva, Sampaio, Taham, Rocco, Ceriani & Meirelles, 2009; Hernández, Fregapane & Moya, 2009; Albuquerque *et al*., 2005).

The oil from *Mauritia flexuosa* Mart. (*Arecaceae*) mesocarp, know as buriti, has a high concentration of monounsaturated fatty acids (MUFA), higher than that of olive oil, which is known to have high-quality nutritional oil (Albuquerque *et al*., 2005; França, Reber & Meireles, 1999). The unsaponificable fraction of buriti oil has high concentrations of tocopherol and carotenoids, which have functional importance such as antioxidant activity (Albuquerque *et al*., 2005; De Rosso & Mercadante, 2007). The fat from *Astrocaryum murumuru* Mart. (Arecaceae) seed, known as murumuru, is rich in lauric and myristic acids, important fatty acids that are used as antimicrobials (Desbois, 2012; Mambrim & Barrera-Arellano, 1997).

Oennocarpus bataua Mart (*Arecaceae*), known as patauá, is a palm that represents a great potential source of edible oils. The oil extracted from the mesocarp fruit is used for medical, cosmetic or culinary purposes (Montúfar, Laffargue, Pintaud, Hamon, Avallone, 2010). Chemical characterization of this oil indicates its potentialas a new source of monounsaturated oil (Darnet, Silva, Rodrigues & Lins, 2011; Hernandez, Fregapane, Moya, 2009). In its unsaponifiable fraction, this oil is rich inΔ^5-avenasterol, cycloartenol and α-tocopherol (Montúfar*et al*., 2010). Palm stearin is the solid fraction obtained from the oil extracted from the mesocarp of the palm fruit (*Elaeis guineensis Jacq* - *Arecaceae*) (Lee & Ofori-Boateng, 2013). Because of the high melting point of palm stearin, it is used in interesterification reactions with other oils with a lower melting point. The melting profile of the blend obtained is more suitable for commercial applications (Silva *et al*., 2010; Adhikari *et al*., 2010).

The use of buriti oil and murumuru fat and patauá oil and palm stearin blends in interesterification reactions can produce new lipids in which the individual characteristics

of these oils and these fats can be coupled, with features not found in nature. Accordingly, the aim of this study was to produce, characterize and evaluate the pattern of action of enzymes in interesterified lipids of buriti oil and murumuru fat and patauá oil and palm stearin. The lipids were produced using two lipases in three different enzyme systems: one with a commercial lipase, purified and immobilized, widely used in interesterification reactions; a second with a crude lipase from the fungus *Rhizopus* sp. produced by solid-state fermentation with an agro-industrial by product; and the third with a mixture of both lipases (commercial and *Rhizopus* sp.).

2. Materials

Crude buriti and patauá oils and murumuru fat were bought in a local market in the city of Belém, State of Pará, in the Brazilian Amazon. Palm stearin (melting point 43°C) was kindly supplied by Agropalma (Pará, Brazil). Crude lipase from *Rhizopus* sp. was produced in wheat bran in our laboratory (Macedo, Pastore & Rodrigues, 2004). Commercial, purified and immobilized lipase (Lipozyme TL-IM) was kindly supplied by Novozymes. All other reagents and solvents were of analytical grade.

3. Methods

3.1. Lipases assay

The lipase activity in both enzymes were quantified using olive oil as a substrate. One unit of lipase activity (U) is defined as 1 μmol of oleic acid released per minute (Macedo *et al*., 2004).

3.2. Fatty acid composition

Fatty acids methyl esters were prepared according to the Hartman and Lago's method (Hartman & Lago, 1973). A Perkin Elmer Clarus 600 gas chromatograph equipped with a flame ionization detector was used. A capillary chromatographic column (Perkin Elmer - 225; 30 m, 0.25 mm id and 0.25 μm film thickness) was used to analyze the fatty acid methyl esters (FAME). The split ratio was 1: 40, and the injector and detector temperature was 250°C. The operation conditions of the column were as follows: 100°C for 5 min, 100°C - 230°C (5°C/min) and 230°C for 20 min. Helium was used as the carrier gas. The qualitative composition was determined by comparison of the retention times of the peaks with those of the respective standards of fatty acids. The methyl ester profile was quantified based on relative peak areas (Basso, Almeida & Batista, 2012).

3.3. Enzymatic interesterification

The enzymatic interesterification was performed in an orbital-shaking water bath at 150 opm for 24 h at 40°C under vacuum for buriti oil and murumuru fat blend and 150 opm for 24 h at 50°C under vacuum for patauá oil and palm stearin blend. The weight ratio of oil to fat was 70: 30, with a total weight of 10 g. The reactions were performed in three different enzyme systems: commercial lipase, lipase from *Rhizopus* sp. and a mixture of both enzymes. In all systems, the final enzyme concentration ranged between 2.5 and 10% (w / w). Before the reaction, the enzymes were dried in a vacuum oven at 40°C for 30 minutes. After completion of the reaction, the interesterified blend was immediately filtered using a 0.45 μm membrane filter and frozen (Simões, Valero,

Tecelão & Ferreira-Dias, 2014; Rodrigues & Ayub, 2011). The non-interesterified blend was also subjected to the same reaction conditions. The free fatty acids and partial acylglycerols of the blends after the reaction were removed according to the methodology of Farmani, Safari & Hamedi, 2006.

3.4. Regiospecific distribution

Proton - decoupled ^{13}C NMR (Nuclear Magnetic Resonance) was used to analyze the positional distribution of classes of fatty acids on the triacylglycerol (TAG) backbone. Lipid samples were dissolved in deuterated chloroform in NMR tubes, and NMR spectra were recorded on a Burker Advance DPX spectrometer operating at 300 MHz. The determination of ^{13}C was performed at a frequency of 75.8 MHz, with a 5 mm multinuclear probe operating at 30°C (Vlahov, 1998).

3.5. Triacylglycerol composition

The fatty acid composition described in item 3.2 was used to predict the groups of TAGs in the non-interesterified samples with PrÓleos software, which uses a mathematical algorithm that describes the distribution of fatty acids in TAG molecules based on the natural tendency of regiospecific distribution (Antoniosi Filho, Mendes & Lanças, 1995). For the prediction, the average values of fatty acids with more than 1% of the total composition were used, and TAGs at predicted levels below 0.5% of the total were excluded. The composition of TAGs present in interesterified lipids was analyzed according to the 1, 3 - random, 2 - random theory (non - random redistribution), and 1, 2, 3 - random theory (random redistribution), based on the analysis of regiospecific distribution described in item 3.4 (D`Agostini & Gioielli, 2002; Guedes, Ming, Ribeiro,

Silva, Gioieli & Gonçalves, 2014). The equations used are described below:

1, 3 - random, 2 - random theory:

$$\% SSS = \frac{(\%S_1)x(\%S_2)x(\%S_3)}{10^3} \quad (1)$$

$$\% SUS = \frac{(\%S_1)x(\%U_2)x(\%S_3)}{10^3} \quad (2)$$

$$\% SSU = \frac{2x(\%S_1)x(\%S_2)x(\%U_3)}{10^3} \quad (3)$$

$$\% USU = \frac{(\%U_1)x(\%S_2)x(\%U_3)}{10^3} \quad (4)$$

$$\% UUS = \frac{2x(\%U_1)x(\%U_2)x(\%S_3)}{10^3} \quad (5)$$

$$\% UUU = \frac{(\%U_1)x(\%U_2)x(\%U_3)}{10^3} \quad (6)$$

1, 2, 3 - random theory:

$$\% SSS = \frac{(\%S)x(\%S)x(\%S)}{10^3} \quad (1)$$

$$\% S_2U = \frac{3x(\%S)x(\%U)x(\%S)}{10^3} \quad (2)$$

$$\% U_2S = \frac{3x(\%U)x(\%S)x(\%U)}{10^3} \quad (3)$$

$$\% UUU = \frac{(\%U)x(\%U)x(\%U)}{10^3} \quad (4)$$

3.6. Thermal properties

Thermal analysis of the samples was performed by differential scanning calorimetry according to the AOCS method Cj 1 – 94 (AOCS, 2009). The equipment used was a TA Q2000 thermal analyzer coupled to RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle). The data processing software used was V4.7A (TA Instruments, Waters LLC, New Castle). The conditions of analysis were as follows: sample weight, ~10 mg; crystallization curves, 80°C for 10 min, 80 °C to − 40 °C (10 °C min^{-1}), and − 40 °C for 30 min; and melting curves, − 40°C to 80 °C (5 °C min^{1}). 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}) (Campos, 2005; Ribeiro, Basso, Grimaldi, Gioielli, Gonçalves, 2009).

3.7. Minor compounds

3.7.1. Tocopherols

The α, β, γ, and δ - tocopherol content were determined by High - Performance Liquid Chromatography (HPLC), according to Ce8 - 89 AOCS method (AOCS, 2009). A Perkin Elmer 250 Liquid Chromatograph coupled with Shimadzu RF - 10 AXL fluorescence detector (excitation at 290 nm; emission at 330 nm), Merck Li Chrosorb Si - 60 250 × 4 mm column was used. The mobile phase was HPLC-grade mixture of hexane: isopropanol 99: 1, at a flow rate of 1 mL min^{-1}. Peaks were identified by comparison of their retention time with authentic standards of tocopherols and where quantified based upon the peak areas relative to standard calibration plots by external standard method.

3.7.2. Total carotenoid

The total carotenoid content was obtained using the method described by Porim (1995). The solution of oil sample (0.1%) was diluted in hexane and read at 446 nm using computerized Shimadzu Spectrophotometer (Kyoto, Japan).

3.7.3. Total polyphenol

A sample of oil (0.5 g) was dissolved in 1 mL hexane, and the phenolics were extracted with 1 mL methanol/water (60: 40, v v^{-1}) for 2 minutes under nitrogen atmosphere by means of an electronic mixer. Both phases were separated by centrifugation (3500 rpm, 10 minutes), and an aliquot (40 μL) of the methanolic phase was diluted with water to a total volum of 1 mL, followed by the addition of 100 μL Folin-Ciocalteu reagent (Sigma Chemicals, Co., St. Louis - MO, USA). After 3 minutes, 200 μL sodium carbonate solution (35%, wt v^{-1}) was added to the reaction mixture, which was finally mixed and diluted with water to 1000 μL. The absorbance of the solution was measured after 2 h against a blank sample by Shimadzu Spectrophotometer (Kyoto, Japan) at a wavelenght of 725 nm. The calibration curve was constructed using standard solution of gallic acid (Sigma Chemicals Co.) within the range of 10 - 100 μg mL^{-1} (Hrncirik & Fritsche, 2004).

4. Results and Discussion

The discovery of new enzymes in interesterification reactions is a necessity in the oil industry. These enzymes should be capable of hydrolyzing ester bonds in an acylglycerol, followed by re-esterification of the new hydroxyl moiety by a free fatty acid (Criado, Hernández-Martín, López-Hernández & Otero, 2008). The lipase obtained from the fungus *Rhizopus* sp. is a crude enzyme, produced by solid-state fermentation, with lipolytic activity of 8.0 U g^{-1}. Commercial lipase is purified and immobilized in silica, with lipase activity of 12.7 U g^{-1}. These enzymes, which present different characteristics, also showed different catalytic properties, as can be seen below.

4.1. Fatty acid compositions

Few studies have evaluated the fatty acid composition of Amazonian oils. These oils have great commercial potential, and identification of the fatty acids will allow better targeting of potential applications.

4.1.1. Buriti: Murumuru

The results in Table 1 show that the buriti oil is composed mainly of oleic acid. Oils rich in oleic acid usually have a positive effect on health because of their low levels of saturated fatty acids and the potential to decrease blood triglycerides, low-density lipoprotein (LDL - type cholesterol) and total cholesterol values (O`Brien, 2009). Furthermore, lipids rich in oleic acid have a high oxidative stability, which is a useful property for cosmetic and pharmaceutical applications (Bracco, 1995). The buriti oil used in this study also presents a considerable concentration of linoleic acid, one precursor of ω-3, with numerous positive health implications (Riediger, Othamn, Suh & Moghadasian, 2009).

Murumuru fat includes large amounts of lauric and myristic fatty acids (Table 1). Medium-chain fatty acids can be used in the manufacture of topical aerosols, foams, creams, ointments and lotions (Prajapati, Dalrymple & Serajuddin, 2012). Furthermore, these fatty acids are known to exhibit antimicrobial activity (Parfene, Horincar, Tyagi, Malik & Bahrim, 2013; Huang, Alimova, Myers & Ebersole, 2011).

Table 1: Fatty acid compositions and characterization (%) of buriti oil and murumuru fat.

Fatty acids	Buriti	Murumuru	Blend*
Caprylic acid (C8:0)	-	2.2 ± 0.00	0.7
Capric acid (C10:0)	-	1.7 ± 0.00	0.5
Lauric acid (C12:0)	-	49.6 ± 0.00	14.9
Myristic acid (C14:0)	0.5 ± 0.00	26.7 ± 0.00	8.4
Palmitic acid (C16:0)	19.2 ± 0.03	6.3 ± 0.01	15.3
Stearic acid (C18:0)	1.3 ± 0.00	2.4 ± 0.00	1.6
Oleic acid (C18:1)	65.6 ± 0.0	7.5 ± 0.00	48.2
Linoleic acid (C18:2)	4.9 ± 0.05	3.7 ± 0.00	4.5
Linolenic acid (C18:3)	8.2 ± 0.01	-	5.7
∑ Saturated	**21.0**	**88.8**	**41.3**
∑ Monounsaturated	**65.6**	**7.5**	**48.2**
∑ Polyunsaturated	**13.2**	**3.7**	**10.3**
Moisture	0.19	0.04	0.13
Free Fatty Acids	5.4	3.7	3.9

* Composed of 70% buriti oil and 30% murumuru fat.

The blend of buriti oil with murumuru fat presents similar concentration of saturated and monounsaturated fatty acids; indicating the production of a blend that shows positive effects of oleic and lauric acids, in addition to greater oxidation stability.

The few previous studies that address the fatty acid composition of buriti oil confirm that oleic acid is the major fatty acid (61 to 74%), followed by palmitic acid (16 to 23%) (Pardauil, Souza, Molfetta, Zamian, Rocha Filho & Costa, 2011; Silva *et al*., 2009). The previous study characterizing the fatty acid composition of murumuru fat is consistent with findings in this study; lauric acid was the main fatty acid (52%), followed by myristic acid (26%) (Mambrim & Barrera-Arellano, 1997).

4.1.2. Patauá: Palm stearin

The results in Table 2 indicate that patauá oil is very rich in oleic acid (74.5%). From a technological standpoint, oleic acid-rich oils present greater oxidative stability, as they are low in polyunsaturated fatty acids (Séverac, Galy, Turon, Monsan & Marty, 2011). From the biological point of view, evidence from epidemiological studies suggests that oleic acid is linked to a reduced risk of coronary heart disease and reduction of inflammatory responses (Lunn, 2007; Pacheco, López, Bermúdez, Abia, Villar, Muriana, 2008). Therefore, patauá oil has great potential as a new source of monounsaturated oil.

A few other available studies also confirm the high concentration of oleic acid in the patauá oil (Montúfar*et al*., 2010; Rodrigues*et al*., 2010; Hernández*et al*., 2009). The fatty acid composition also indicates that this oil presents palmitic acid as the major saturated fatty acid (16%). Regarding polyunsaturated linoleic acid, the concentration in this oil does not exceed 6.0%, which ensures a good oxidative stability.

Regarding palm stearin, palmitic acid is its main fatty acid (47%), followed by oleic acid (28%) (Table 2). Saturated fatty acids increase the plasticity of structured lipid, in addition, providing an important moisture barrier property (O' Brien, 2009). The concentration of linoleic acid in the study sample was approximately 13%. Palm stearin is widely used to alter the physicochemical properties of oils with lower melting points (Shin, Akoh & Lee,2010). This fat has been used as a solid substrate to produce semi-solid fats via interesterification with olive and soybean oils (Silva *et al*., 2010; Costales-Rodríguez*et al*., 2009).

Table 2: Fatty acid composition and characterization (%) of patauá oil and palm stearin.

Fatty acids	Patauá	Palm stearin	Blend*
Myristic acid (C14:0)	0.6 ± 0.00	6.1 ± 0.77	2.2
Palmitic acid (C16:0)	15.7 ± 0.01	46.9 ± 0.06	25.1
Stearic acid (C18:0)	2.2 ± 0.00	5.9 ± 0.12	3.3
Oleic acid (C18:1)	74.5 ± 0.00	28.1 ± 0.01	60.6
Linoleic acid (C18:2)	5.8 ± 0.01	13.0 ± 0.04	8.0
Arachidic acid (C20:0)	1.3 ± 0.00	-	0.9
∑ Saturated	**19.8**	**58.9**	**31.5**
∑ Monounsaturated	**74.4**	**28.1**	**60.5**
∑ Polyunsaturated	**5.8**	**13.0**	**8.0**
Moisture	0.2	0.1	0.2
Free Fatty Acids	4.0	0.05	2.8

*Composed of 70% patauá oil and 30% palm stearin

Enzyme-modified patauá oil and palm stearin with a desirable fatty acid profile can be used to prepare semi-solid fats that have a health protective effect, greater structuring capacity and higher oxidative stability.

4.2. Regiospecific distribution

The regiospecific distribution of fatty acids in the TAG has implications for the both nutritional and technological qualities of oils and fats (Silva*et al*., 2012). In this study, the redistribution of fatty acids in the TAG was different depending on the enzyme used.

4.2.1. Buriti: Murumuru

The results in Figures 1 and 2 show that all three-enzyme systems used for interesterification caused changes in the distribution of fatty acids in the TAG. The redistribution of the fatty acids varied depending on the enzyme system used. Lipids produced using only lipase from *Rhizopus* sp. showed a slight change in the *sn* - 2 position of the TAG, indicating the low specificity of this enzyme for this position; whereas in the *sn* - 1,3 positions of TAG, there were several significant changes in the distribution of fatty acids. It is possible to observe an increase in the levels of unsaturated fatty acids in these positions, showing that the enzyme was specific to re-esterify unsaturated fatty acids in these positions of the TAG. Thus, the enzyme from *Rhizopus* sp. showed specificity for the position and nature of the fatty acid group on the TAG in the reaction conditions employed and considering that the oils were not refined.

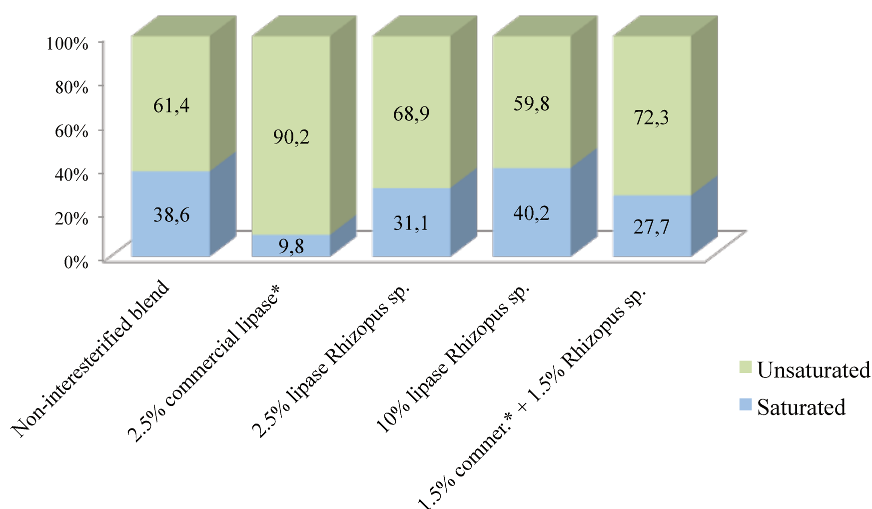

Figure 1: Regiospecific distribution of fatty acids at the *sn* - 2 position of non-interesterified and interesterified blends of buriti and murumuru catalyzed by different enzyme systems.

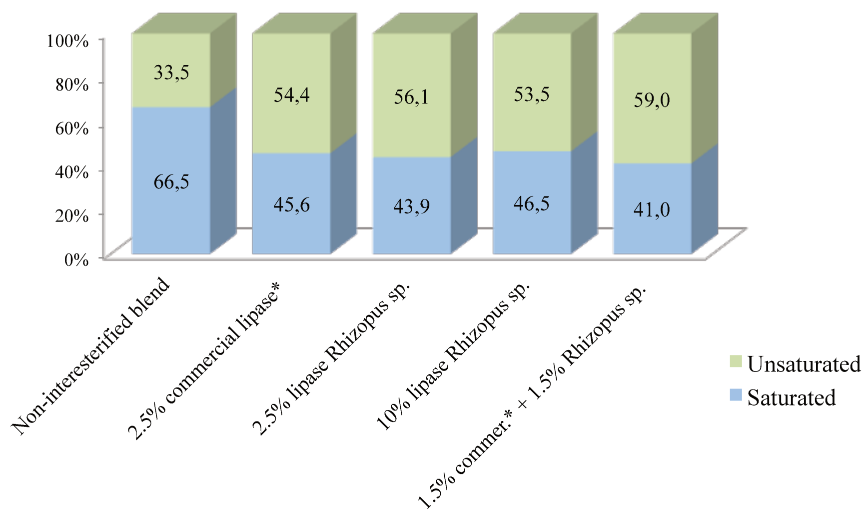

Figure 2: Regiospecific distribution of fatty acids at the *sn* - 1,3 positions of non-interesterified and interesterified blends of buriti and murumuru catalyzed by different enzyme systems.

Specific lipases make it possible to produce fats and oils with a customized TAG structure (O'Brien, 2009). Products such as substitutes for human milk-fat and cocoa butter are obtained most efficiently using specific lipases (Soumanou, Pérignon & Villeneuve, 2013; Borhan, Said & Sahri, 2011). The enzyme from *Rhizopus* sp. has been used by our group in synthesis reactions for the production of aroma compounds and waxes (Lopes, Duarte & Macedo, 2011; Melo, Pastore & Macedo, 2005; Macedo*et al.*, 2004). In these studies, this enzyme showed specificity for butyric, valeric and oleic acids, thereby confirming the specificity of this enzyme for oleic acid.

Pérignon, Lecomte, Pina, Renault, Simonneau-Deve & Villeneuve (2012) evaluated the regiospecificity of *Rhizopus oryzae* lipase in the presence of short- and medium-chain fatty acids under partial hydrolysis conditions. Their results showed that *Rhizopus oryzae*

lipase maintains its strict *sn* - 1,3 - regioselectivity even though short and medium chain fatty acids are present in the TAG structure of the substrate.

The lipid produced using the commercial enzyme showed changes in the three positions of TAG. The lipid formed with this enzyme showed high levels of unsaturated fatty acids in all three positions of the TAG. Therefore, this enzyme was specific for the type of fatty acid but was unspecific for the position of the fatty acid in TAG. The specificity of a lipase depends largely on the structure of the substrate, the interaction at the active site, and the reaction conditions (Muralidhar, Chirumamilla, Marchant, Ramachandran, Ward & Nigan, 2002; Svensson & Adlercretz, 2011). Thus, the presence of TAGs with short- and medium-chains fatty acids, the presence of free fatty acids in the medium and the reaction time may prevent the exclusivity of action of this enzyme at the *sn* - 1, 3 positions of TAG. Furthermore, the reaction conditions may have induced acyl migration, which is the migration of fatty acids from positions *sn* - 1, 3 to *sn* - 2 or from the *sn* - 2 position to the *sn* - 1, 3 (Silva *et al*., 2012).

Akanbi, Adcock & Barrow (2013) evaluated the selective hydrolysis of anchovy oil, using the commercial *Thermomyces lanuginosus* lipase (TL - 100L - Novozymes) as catalyst. After hydrolysis, there are no major changes in the distribution of the saturated and unsaturated fatty acids, indicating that *T. lanuginosus* lipase selectivity is not regioselective in this reaction system but rather fatty acid selective.

Flores, Serwalt, Janssen & Van der Padt (2000) evaluated the effect of different fatty acids with chain lengths between 4 and 18 carbons, saturated and unsaturated, on esterification reactions with butanol. Depending on the type of fatty acid, the reaction was conduced using lipases from *Candida rugosa* (L034P - Biocatalysts) or *Candida*

antarctica (Novozym 435 - Novozymes). In the esterification of either saturated or unsaturated fatty acids, higher ester mole fractions are obtained for fatty acids with higher chain lengths. For the same chain length, a higher ester mole fraction is found for the unsaturated fatty acids. Therefore, the enzymes were more specific for fatty acids that had higher chain lengths and were more unsaturated.

Karabulut, Durmaz & Hayaloglu (2009) evaluated the chain-length selectivity of immobilized lipases from *Thermomyces lanuginosus* (Lipozyme TL - IM - Novozymes), *Rhizomicor miehei* (Lipozyme RM - IM - Novozymes) and *Candida antarctica* (Novozym 435 - Novozymes) in acidolysis reaction. The fatty acids used were saturated, with chain lengths between 6 and 22 carbon atoms. Lipases preferentially incorporated fatty acids with chain lengths between 12 and 16 carbons.

Watanabe, Nagao & Shimada (2009) found that the regiospecificity of immobilized *Candida antarctica* lipase (Novozym 435 - Novozymes), generally known to be nonregiospecific, is correlated to the polarity of the reaction mixture; the higher the polarity, the stricter the regiospecificity. The highest *sn* - 1, 3 regiospecificity was obtained in the transesterification of oil with ethanol, among other alcohols investigated.

Therefore, from these examples, it can be observed that the specificity of individual lipases can change based on microenvironmental effects on the reactivity of functional groups or substrate molecules (Marangoni, 2002; Parida & Dordick, 1991).

In the lipid formed using both enzymes (commercial and from *Rhizopus* sp.), there is an intermediate behavior compared to the other lipids: an increase of unsaturated fatty acids in the three positions of the TAG, especially in positions *sn* - 1 and *sn* - 3. In this case, the enzyme system was not specific to the position on the TAG but for the type of

fatty acid. No apparently synergistic effect of the combination of the enzymes was observed.

In the literature, there are studies in which such synergistic effects can be observed. A combination of commercial and free lipases (Lipozyme TL - IM and Amano AK 20) was used in the interesterification reaction between palm stearin and coconut oil. After 4 hours of reaction, a visible enhancement of the degree of interesterification by the combination lipase system was obtained compared with either immobilized or free lipase applied alone. However, in this same study, other combinations of enzymes and analysis conditions did not have the same effect (Ibrahim, Guo & Xu, 2008). These results showed that any synergistic effect of the lipases is dependent on the lipase species used and their proportions (Fernandez - Lafuente, 2010).

4.2.2. Patauá: Palm Stearin

The results in Figures 3 and 4 show that the enzyme from *Rhizopus* sp. at both tested concentrations, 2.5% and 5.0% (w / w), displayed essentially the same behavior, indicating that lower concentrations of the enzyme were sufficient to saturate the substrate. The lipids formed with this enzyme displayed a higher concentration of saturated fatty acids in the *sn* - 2 position of the TAG and unsaturated fatty acids in the *sn* - 1,3 positions of the TAG. These results indicate that the enzyme was specific for unsaturated fatty acids, especially oleic acid, both in the hydrolysis and in the re-esterification of fatty acids in the glycerol. During re-esterification, the enzyme was also specific for the *sn* - 1, 3 position of the TAG. The formation of TAGs rich in saturated fatty acids in the *sn* - 2 position and unsaturated fatty acids in the *sn* - 1, 3 positions is

quite timely, as products such as human milk fat present this TAG profile (Simões *et al*., 2014); the action of pancreatic lipase in the human body, an enzyme that is *sn* - 1, 3 specific, makes the fatty acids located at the *sn* - 2 position of the TAG be maintained in the form of 2 - MAG, and therefore, they can be more easily freed (Quinlan & Moore, 1993).

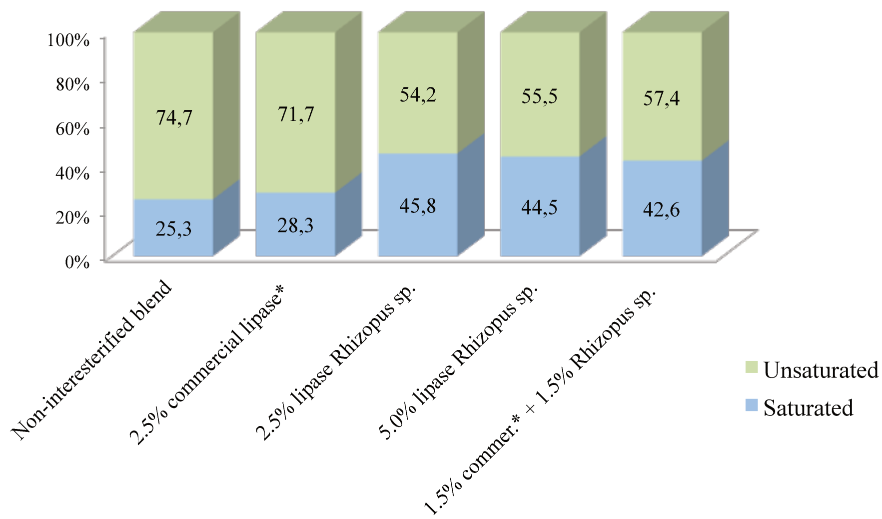

Figure 3: Regiospecific distribution of fatty acids at the *sn* - 2 position of non-interesterified and interesterified blends of patauá oil and palm stearin catalyzed by different enzyme systems.

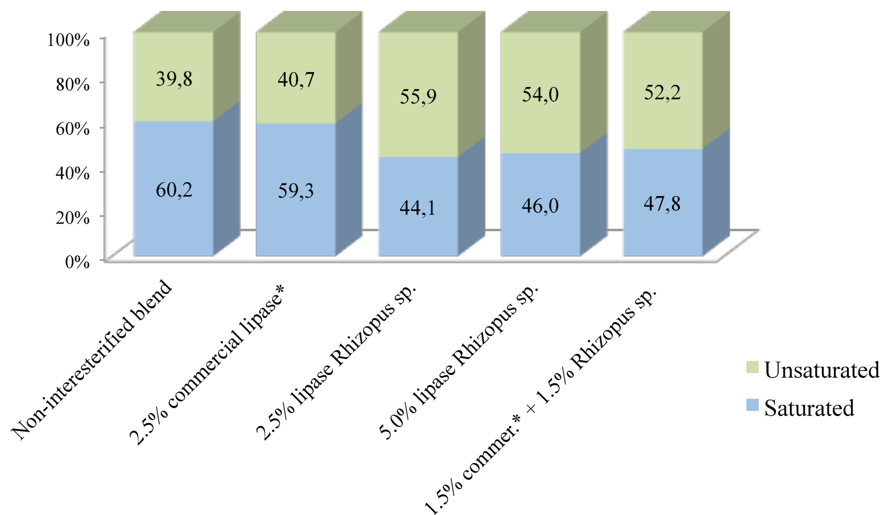

Figure 4: Regiospecific distribution of fatty acids at the *sn* - 1,3 positions of non-interesterified and interesterified blends of patauá oil and palm stearin catalyzed by different enzyme systems.

In nature, vegetable oils are generally rich in unsaturated fatty acid in the *sn*-2 position of the TAG and saturated fatty acids in the *sn* - 1,3 positions of the TAGs (Silva *et al*., 2010). Methods to produce TAGs rich in palmitic acid at *sn* - 2 position are therefore of great potential industrial interest, as these TAGs are not produced at industrial levels (Jiménez *et al*., 2010).

In another study, the enzyme from *Rhizopus oryzae* was used in the reaction between palm stearin enriched with palmitic acid and oleic acid. The enzyme was specific in the incorporation of oleic acid at the *sn* - 1,3 positions of the TAG. Because of the specificity of the enzyme, it was possible to structure TAGs of an oleic acid - palmitic acid-oleic acid type, which allow application to the synthesis of human milk fat substitutes (Esteban, Jiménez, Hita, González, Martín & Robles, 2011). In the human milk fat, palmitic acid is located mainly in the *sn* - 2 position (44% of the total fatty acids

in this position), while the oleic acid is the mainly fatty acid in the *sn* - 1, 3 positions (46.3% total fatty acids in these positions).

Regarding the reaction catalyzed by the commercial lipase, there was no change in the distribution of saturated and unsaturated fatty acids in the three positions of the TAG profile, but changes occurred in the types of TAGs (described in the next section). Most likely what occurred was the replacement of certain saturated fatty acids by other saturated fatty acids at the same position, which is the same as occurred with the unsaturated fatty acids.

Several commercial enzymes have been used in interesterification reactions using palm stearin and other oils with a lower melting point. The enzymes have different specificities depending on enzyme preparations, the reaction conditions and type of substrate (Fernandez-Lafuente, 2010; Muralidhar*et al*., 2002; Vaysse, Ly, Moulin & Dubreucq, 2002). For this reason, many results of different groups of researchers appear contradictory (Fernandez-Lafuente, 2010).

Adhikari*et al*. (2010) produced a zero-trans margarine using pine nut oil and palm stearin. The reaction was carried out for 24 h at 65°C, using 30% wt% of lipase TL - IM (Novozymes). In the *sn* - 2 position, the major fatty acids before reaction were linoleic, palmitic and oleic acids. After the reaction, it was observed that the major fatty acids in this position were palmitic and oleic acids, thus indicating the specificity of the enzyme by the type of fatty acid.

Shin*et al*. (2010) produced lipids rich in linolenic acid by an interesterification reaction between butterfat, palm stearin and flaxseed oil. The reaction was conducted at 60°C for 24 hours, using 10% by weight of commercial enzyme Novozym SP435

(Novozymes). The results revealed that the enzyme was specific for the *sn* - 1 and *sn* - 3 positions of the TAG.

In the reaction catalyzed by the mixture of both lipases (*Rhizopus* sp. + commercial), the same behavior was observed compared with using *Rhizopus* sp. alone. Apparently, the results indicate that the mixture of enzymes did not display any synergistic effect, and lipase from *Rhizopus* sp. at a concentration of 1.5% was able to catalyze the interesterification reaction alone. In other studies, the use of more than one lipase in the reactions has been used as a strategy to improve the properties of the enzymes, increasing specificity and selectivity.

Rodrigues & Ayub (2011) evaluated the effect of combining two commercial lipases (TL - IM and RM - IM - Novozymes) on the transesterification and hydrolysis of soybean oil. The results indicated that the mixture of lipases provided synergistic effects in the reaction, and the conversion rate was superior to that obtained with the individual enzymes. Complex substrates such as vegetable oils, because of the variety of fatty acids, have different specificities, and in certain situations, the use of mixtures of lipases may be required for effective transesterification and hydrolysis.

4.3. Triacylglycerol composition

TAG composition is key to understanding the various physical properties of an oil or fat (Buchgraber *et al*., 2004).

4.3.1. Buriti: Murumuru

In buriti oil, the main types of TAGs found are palmitic-oleic-oleic (POO), which represents 25% of total TAGs, and oleic-oleic-oleic (OOO), which represents 29% of total TAGs present in this oil. In murumuru fat, the most common TAGs found are lauric-lauric-myristic (LaLaM), representing 21% of the total TAGs, lauric-myristic-myristic (LaMM), representing 16% of total TAGs and lauric-lauric-lauric (LaLaLa), representing 14% of the total TAGs present in this fat (unpublished data).

Interesterification caused significant alteration in the TAG composition of the blends studied (Table 3). In the blend catalyzed by the lipase from *Rhizopus* sp. at a concentration of 2.5%, there were reductions in the proportions of TAGs of the types tri-saturated (S_3) and tri-unsaturated (U_3), by 72% and 38%, respectively. Meanwhile, the levels of disaturated-unsaturated (S_2U) and saturated-diunsaturated (SU_2) TAGs increased by 100% and 52%, respectively. Similar behavior was observed when using this same enzyme at a concentration of 10%.

Table 3: Class of triacylglycerol in buriti oil, murumuru fat and non-interesterified and interesterified blends catalyzed by different enzyme systems.

TAG	Buriti (%)	Muru muru (%)	Non-interesterified blend (%)	Interesterified blends			
				2.5%lipase *Rhizopus* sp.[1]	10% lipase *Rhizopus* sp.[1]	2.5% Comm. lipase*[2]	1.5% Comm.* + 1.5% *Rhizopus* sp. lipases[2]
SSS	0.9	69.7	21.5	6.0	8.7	2.0	4.7
S_2U	9.8	25.6	14.4	28.6	32.9	23.5	25.6
SU_2	39.3	4.3	28.8	43.7	41.2	47.7	44.6
UUU	50.0	0.5	35.3	21.7	17.1	26.7	25.1

* Lipozyme TL-IM
[1] *sn* - 1, 3 specific lipase (non-random pattern)
[2] No specific lipase(s) (random pattern)

The results obtained with the commercial enzyme at a concentration of 2.5%, as with the lipids produced with the enzyme from *Rhizopus* sp., indicate reductions in S_3 and U_3 TAGs, of 90% and 24%, respectively, and increases in S_2U and SU_2 TAGs, of 63% and 66%, respectively.

The results obtained from the lipid produced with a mixture of both enzymes had the same behavior as the other two lipids: decreases in S_3 and U_3 TAGs and increases in S_2U and SU_2 TAGs. Therefore, all lipids after the interesterification presented primarily SU_2 TAGs, followed by S_2U and U_3 TAGs. All lipids showed a greatly reduced concentration of S_3 TAGs when compared with the non-interesterified blend. The reduction in the amount of S_3 TAGs shows a great advantage from a health perspective. Certain saturated fatty acids, including palmitic and myristic acids, are related to various metabolic syndromes, especially increases in plasma triglyceride, total cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations (Lottenberg, Afonso, Lavrador, Machado, Nakandakare, 2012).

Peña-Orihuela *et al*. (2013) reported that oxidative stress in humans can be modulated by dietary fat. Their study demonstrated that monounsaturated fatty acid consumption reduces oxidative stress compared to saturated fat by inducing higher postprandial antioxidant response in adipose tissue. Thus, replacing SFA with MUFA may be an effective dietary strategy to reduce the oxidative stress in patients with metabolic syndrome and to reduce its pathophysiological consequences.

From a technological standpoint, the conversion of TAGs S_3 and U_3 to S_2U and SU_2 is also of great interest. The formation of these TAGs increases the application of the resultant lipids because they can provide structure and lubricity to the products. The TAG

S_3 only provides structure to the products, while the TAG U_3 only provides lubricity and has low stability (Gunstone, 2001; O'Brien, 2009). The S_2U - type TAGs have melting points between 27 and 42°C, while SU_2 - type TAGs have melting points between 1 and 23°C (Rodrigues & Gioielli, 2003; Hoffman, 1989). These same profiles of TAGs can be observed in interesterification reactions with different types of catalysts and oils (Silva *et al*., 2010; Ribeiro *et al*., 2009; Goli, Sahri & Kadivar, 2008).

4.3.2. Patauá: Palm stearin

In patauá oil, the main types of TAGs found are oleic - oleic - oleic (OOO), which represents 42.6% of total TAGs, and palmitic - oleic - oleic (POO), which represents 26.7% of total TAGs present in this oil. In palm stearin, the most common TAGs found are palmitic - oleic - palmitic (POP), representing 19.1% of the total TAGs, palmitic - oleic - oleic (POO), representing 13.2% of total TAGs and palmitic - palmitic - palmitic (PPP), representing 11.4% of the total TAGs present in this fat (unpublished data).

Interesterification caused significant alteration in the TAG composition of the blends studied (Table 4). In all lipids produced with the different enzymes, interesterification produced a significant increase in the percentages of S_2U TAG content, with a corresponding decrease in U_3 TAG content. The lipids produced with the lipase from *Rhizopus* sp. and the mixture of the lipases produced an increase of approximately 80% in the S_2U TAG content and an approximately 60% decrease in the concentration of U_3 TAG concentration. The lipase from *Rhizopus* sp., at both concentrations tested, produced blends with very similar TAG types, indicating again that an increase in enzyme concentration does not change the characteristics of the lipids obtained.

Table 4: Class of triacylglycerol in patauá oil, palm stearin and non-interesterified and interesterified blends catalyzed by different enzyme systems.

TAG	Patauá oil (%)	Palm stearin (%)	Non-interesterified blend (%)	Interesterified blends			
				2.5%lipase *Rhizopus* sp.[1]	5.0% lipase *Rhizopus* sp.[1]	2.5% Comm. lipase*[1]	1.5% Comm.* + 1.5% *Rhizopus* sp. lipases[1]
SSS	0.7	20.5	6.5	8.9	9.4	9.9	9.7
S_2U	8.7	41.7	18.6	33.1	33.8	38.9	34.5
SU_2	37.2	29.0	34.8	41.1	40.6	39.3	40.2
UUU	53.2	8.5	39.9	16.9	16.2	11.9	15.6

* Lipozyme TL - IM
[1] Random redistribution

The lipid produced with the commercial lipase displayed an increase of approximately 110% in the S_2U TAG content and an approximately 70% decrease in the concentration of U_3 TAGs. The concentration of SU_2 and S_3 TAGs slightly increased after the reaction in all lipids produced.

Therefore, the main TAGs of the non-interesterified blend were of the U_3 type followed SU_2, whereas the main TAGs present in the lipids after interesterification were of the SU_2 type, followed by S_2U. In these TAGs, the major unsaturated fatty acid was oleic acid, and the major saturated fatty acid was palmitic acid. The TAGs of type SU_2 and S_2U formed in the reaction by the three enzyme systems have properties of both structure and lubricity, thus making it possible to change the application of the produced lipids. Although U_3 TAGs are related to the reduction of cardiovascular diseases, these TAGs have low oxidative stability and low structuring capacity, making it difficult to implement them in semi-solid products (Lopez-Huertas, 2010; O'Brien, 2009; Rodrigues & Gioielli, 2003).

In other studies for modification of fats and oils using different lipases, the synthesis of SU_2 and S_2U has also been observed (Kadivar, De Clercq, Van de Walle, Dewettinck, 2014; Esteban*et al*., 2011; Lee, Son, Akoh, Kim & Lee, 2010).

4.4. Thermal properties

The evaluation of thermal behavior using Differential Scanning Calorimetry (DSC) measures the energy involved in melting (endothermic) and crystallization (exothermic) process of oils and fats. The melting and crystallization curves can be subdivided into different regions, reflecting the different types of TAGs present in the mixture before and after interesterification. Interesterification can change the types of TAGs initially present in the blend, thus changing their thermal properties. This analysis is considered an important tool for characterizing interesterified products (Ribeiro *et al*., 2009).

4.4.1. Buriti:Murumuru

The results of thermal melting behavior (Table 5) showed that all lipids produced using different enzyme systems showed reductions in melting onset temperature (T_{0F}) and melting end temperatures (T_{fm}) and ended with only the melting peak (peak 1) of TAGs more unsaturated. The second peak, of more saturated TAGs with higher melting points, disappeared. This effect can also be seen in the increase in the value of ΔH_1, showing the increased participation of more highly unsaturated TAGs in the lipids, and the elimination of ΔH_2, a result of the elimination of more saturated TAGs in the lipids. The tests of thermal melting behavior indicated the formation of a single endothermic region with fewer types of TAGs. This feature facilitates the use of the lipid formed, as it prevents

separation of oil and fat at a given temperatureas given crystallize at the same rate (Metin & Hartel, 2005).

The same type of modification is observed in the thermal behavior in crystallization, because the phenomena of melting and crystallization are thermodynamic reversed in the scan evaluated. However, due to the different polymorphic forms present in lipids, these phenomena do not occur at the same rate. In the crystallization of the blends (Table 6), a reduction in the enthalpy values was observed for the first peak (ΔH_{f1}), indicating a lower participation of more highly saturated TAGs in the interesterified lipids, and an increase in enthalpy values was observed for the second peak (ΔH_{f2}), indicating a higher participation of SU_2 and S_2U TAGs in the lipids. These results confirm those obtained for the composition of TAGs in the previous section.

Table 5: Melting onset temperature (T_{om}), melting peak temperature (T_{pm}), melting end temperature (T_{fm}), melting height peak (H_{pm}) and melting enthalpy (ΔH_m) of the non-interesterified and interesterified buriti:murumuru blends catalyzed by different enzyme systems.

Blends and enzymes	Peak 1					Peak 2				
	T_{0m} (°C)	T_{pm} (°C)	T_{fm} (°C)	H_{pm} (W/g)	ΔH_{m1} (J/g)	T_{om} (°C)	T_{pm} (°C)	H_{pm} (W/g)	T_{fm} (°C)	ΔH_{m2} (J/g)
Non-interesterified	**-19.28**	**-5.15**	**0.10**	**0.22**	**20.02**	**-1.30**	**7.47**	**0.24**	**27.30**	**49.01**
2.5% Commercial lipase*	-21.53	7.67	23.18	-0.20	69.06					
2.5% Lipase from *Rhizopus* sp.	-21.28	-13.26	23.30	-0.21	71.42					
10% Lipase from *Rhizopus* sp.	-22.90	-12.87	21.05	-0.23	70.85					
1.5% Comm.* + 1.5% *Rhizopus* sp.	-22.03	-12.43	21.93	-0.20	72.41					

* Lipozyme TL - IM

Table 6: Crystallization onset temperature (T_{oc}), crystallization peak temperature (T_{pc}), crystallization end temperature (Tfc), crystallization height peak (H_{pc}) and crystallization enthalpy (AH_c) of the non-interesterified and interesterified buriti: murumuru blends catalyzed by different enzyme systems.

Blends and enzymes	Peak 1					Peak 2				
	T_{0c} (°C)	T_{pc} (°C)	T_{fc} (°C)	H_{pc} (W/g)	ΔH_{c1} (J/g)	T_{oc} (°C)	T_{pc} (°C)	T_{fc} (°C)	H_{pc} (W/g)	ΔH_{c2} (J/g)
Non-interesterified blend	**5.2**	**2.9**	**-13.79**	**0.66**	**16.88**	**-13.28**	**-16.36**	**-23.65**	**0.04**	**1.438**
2.5% Commercial lipase*	4.07	1.95	-5.92	0.27	5.534	-17.32	-30.18	-37.51	0.27	22.57
2.5% Lipase from *Rhizopus* sp.	4.57	1.76	-6.67	0.33	6.167	-7.90	-32.05	-37.38	0.11	9.397
10% Lipase from *Rhizopus* sp.	4.70	1.97	-5.67	0.32	5.872	-5.74	-31.63	-37.26	0.11	11.04
1.5% Comm.* + 1.5% *Rhizopus* sp.	6.57	4.77	-6.54	0.22	5.486	-6.70	-31.07	-37.26	0.21	19.80

* Lipozyme TL-IM

The DSC results confirm the changes in the physico-chemical characteristics of the blends after interesterification, indicating that the lipids obtained exhibited characteristics that improve their utility.

4.4.2. Patauá: Palm stearin

The decrease in (T_{0C1}) of crystallization of the first peak (more saturated TAGs) in the interesterified lipids compared to non-interesterified blend may be due to an increase in the concentration of S_2U and SU_2 TAGs and the reduction of the more saturated TAGs (Table 7). This effect can also be observed when evaluating the reduction in enthalpy of the crystallization peak (AH_{c1}). In the second crystallization peak (more unsaturated TAGs), there was an increase in the T_{oc} crystallization, which indicates the reduction of TAGs more unsaturated. In this peak, it should also be noted that the lipids produced with the enzyme from *Rhizopus* sp. displayed a smaller reduction in the content of saturated TAGs when compared with the lipids produced with the commercial enzyme. Therefore, this analysis indicated a reduction in the types of TAGs, leaving predominantly SU_2 and S_2U TAGs after the reaction.

Regarding the thermal behavior in melting (Table 8), the same type of modification is observed, as the phenomena of melting and crystallization are reversed in the scan evaluated. In all lipids produced, the number of peaks was reduced, indicating a reduction in the types of TAGs presents. These results confirm those obtained in the composition of TAGs in the previous item.

The DSC results confirm the changes in the physico-chemical characteristics of the blends after interesterification, indicating that the lipids obtained exhibited characteristics that improve their applicability.

Table 7: Crystallization onset temperature (T_{oc}), crystallization peak temperature (T_{pc}), crystallization end temperature (T_{fc}), crystallization height peak (H_{pc}) and crystallization enthalpy (AH_c) of the non-interesterified and interesterified patauá: palm stearin blends catalyzed by different enzyme systems.

Blends and enzymes	Peak 1					Peak 2				
	T_{0c} (°C)	T_{pc} (°C)	T_{fc} (°C)	H_{pc} (W/g)	ΔH_{c1} (J/g)	T_{0c} (°C)	T_{pc} (°C)	T_{fc} (°C)	H_{pc} (W/g)	ΔH_{c2} (J/g)
Non-interesterified blend	**21.05**	**19.39**	**-1.17**	**0.73**	**11.39**	**-4.08**	**-7.50**	**-29.52**	**0.17**	**14.32**
2.5% Commercial lipase*	13.81	11.92	5.57	0.25	4.084	1.52	-1.39	-25.40	0.17	14.11
2.5% Lipase from *Rhizopus* sp.	15.31	12.79	3.70	0.12	4.839	0.31	-4.08	-36.51	0.19	18.24
5.0% Lipase from *Rhizopus* sp.	16.51	10.37	4.33	0.14	4.688	0.65	-3.32	-36.82	0.19	18.95
1.5% Comm.* + 1.5% *Rhizopus* sp.	15.43	12.52	1.95	0.12	3.913	1.26	-3.41	-24.02	0.15	12.01

* Lipozyme TL-IM

Table 8: Melting onset temperature (T_{om}), melting peak temperature (T_{pm}), melting end temperature (T_{fm}), melting height peak (H_{pm}) and melting enthalpy (ΔH_m) of the non-interesterified and interesterified patauá: palm stearin blends catalyzed by different enzyme systems.

Blends and enzymes	Peak 1					Peak 2					Peak 3				
	T_{0m} (°C)	T_{pm} (°C)	T_{fm} (°C)	H_{pm} (W/g)	ΔH_m (J/g)	T_{0m} (°C)	T_{pm} (°C)	T_{fm} (°C)	H_{pm} (W/g)	ΔH_m (J/g)	T_{0m} (°C)	T_{pm} (°C)	T_{fm} (°C)	H_{pm} (W/g)	ΔH_m (J/g)
Non-interesterified blend	**-21.28**	**-3.66**	**8.27**	**-0.41**	**63.92**	**7.69**	**12.57**	**41.16**	**-0.13**	**40.73**	**41.60**	**44.41**	**48.03**	**-0.08**	**3.72**
2.5% Commercial lipase*	-20.90	0.85	35.16	-0.32	79.33										
2.5% Lipase from *Rhizopus* sp.	-23.90	-0.91	34.79	-0.41	84.36										
5.0% Lipase from *Rhizopus* sp.	-23.90	-4.67	26.30	0.52	84.19										
1.5% Commer.* + 1.5% *Rhizopus* sp.	-20.78	0.88	35.91	-0.37	80.87										

* Lipozyme TL-IM

4.5. Minor compounds

As the commercial and *Rhizopus* sp. lipases in smaller concentrations (2.5%), were able to catalyze the reaction; minor compounds analysis was performed only with the lipid produced in this enzyme concentration. As the mixture of both lipases showed no synergistic effect, this enzymatic system was removed from the analysis of minor compounds.

4.5.1. Tocopherols

Tocopherols are natural antioxidants and inhibit lipid oxidation in fats and oils by modifying the radical chain autoxidation process (Azadmard-Damirchi & Dutta, 2008). They have a saturated side-chain and occur in four related forms, designated alpha (α), beta (β), gamma (γ) and delta (δ). Previous studies have shown that the tocopherol have substancial health benefits like hypocholesteremic, hypolipidemic, anticancer, anti-inflamatory and antioxidant properties and slow down the aging process (Ghaffari, Nouri, Irannejad & Rashidi, 2011; Singh & Devaraj, 2007; Hau, Adolfsson, Lee, Ordovas & Meydani, 2006).

4.5.1.1. Buriti: Murumuru

Buriti oil is naturally rich in tocopherols and as the blends are rich in these oils (70%), the non-interesterified blends are also rich in tocopherols (Table 9).

The blend buriti: murumuru maintained the concentration of α and γ-tocopherols after interesterification with both enzymes, whereas the isomers β and δ-tocopherols, already at low concentrations in the initial blend, were eliminated after the reaction. The production of lipids relatively rich in α and γ-tocopherols is quite

timely, since these isomers have different biological activities, with varying degrees of action (Devaraj & Jialal, 2005).

Table 9: Tocopherol content in buriti oil and murumuru fat; non-interesterified blend and interesterified lipids.

Blend and enzymes	**Tocopherol (mg kg^{-1})**			
	α	**β**	**γ**	**δ**
Buriti	1125.0 ± 3.91	71.3 ± 0.01	1074.0 ± 3.43	93.8 ± 0.54
Murumuru	ND	ND	ND	ND
Non-interesterified blend	746.0 ± 0.73	19.0 ± 0.16	601.0 ± 0.34	83.1 ± 0.24
2.5% Commercial lipase*	760.0 ± 4.10	3.0 ± 0.43	641.5 ± 3.95	35.2 ± 0.10
2.5% Lipase *Rhizopus* sp.	692.0 ± 2.98	ND	633.1 ± 2.75	28.0 ± 0.39

* Lipozyme TL-IM
ND - Not detected

The values obtained for total tocopherols in lipid synthesized with buriti oil could be even greater. The oil used in this study may have missed a significant portion of their tocopherols. Variation may be caused by numerous factors including: variety, region in which the buriti is grown, maturity of the fruit at harvest, extration process and condition of storage. In other published studies with this oil, the tocopherol content reaches 1500 mg kg^{-1}, the next value of soybean (1800 mg kg^{-1}) and corn oil (1618 mg kg^{-1}), known to be rich in tocopherol oil (Tuberoso, Kowalczyk, Sarritzu & Cabras, 2007).

4.5.1.2. Patauá: Palm stearin

As buriti oil, patauá oil is naturally rich in tocopherols and as the blends are rich in these oils (70%), the non-interesterified blends are also rich in tocopherols (Table 10).

The blend patauá oil and palm stearin after interesterification with both enzymes, kept the concentration of α and γ-tocopherol practically constant, whereas the isomers β and δ-tocopherols were not detected in this blend (Table 10). The sythetized lipids showed a relatively high concentration of α-tocopherol, even after interesterification with palm stearin, poor in this compound.

Table 10: Tocopherol content in patauá oil and palm stearin; non-interesterified blend and interesterified lipids.

Blends and enzymes	Tocopherol (mg kg^{-1})			
	α	**β**	**γ**	**δ**
Patauá	1504.7 ± 1.71	ND	258.6 ± 1.23	ND
Palm stearin	33.0 ± 1.91	ND	5.0 ± 0.01	ND
Non-interesterified blend	992.0 ± 2.82	ND	161.1 ±1.21	ND
2.5% Commercial lipase*	1001.3 ± 0.92	ND	152.0 ±0.33	ND
2.5% Lipase from *Rhizopus* sp.	1019.0 ± 1.73	ND	158.2 ±1.01	ND

* Lipozyme TL - IM
ND - Not detected

In another study, Sundram, Khor, Ong & Pathmanathan (1989) added tocopherol from palm oil in corn oil (500 or 1000 mg kg^{-1}), and the results indicated lower incidence of tumors and occurrence compared to rats fed corn oil alone.

Results similar to those obtained in this study were obtained by Reshma, Saritha, Balachandran & Arumughan (2008). In this study, lipids were produced using interesterified palm stearin and rice bran oil rich in tocopherol and tocotrienol (tocols). The conditions employed for the reaction, showed only a marginal decrease (4 - 8%) in the tocols content, with no preferential reduction of any particular isomer. Due to the use of lipase, which allows the reaction occurs under mild conditions, and the use of rice bran oil which is rich in tocols, the synthesized lipids showed high concentrations of these compounds.

The use of two different lipases, commercial and non-commercial, had little influence on tocopherol concentration of the synthesized lipid. Although the enzymes have different characteristics (crude and purified) and have catalyzed the production of lipids with different types and concentrations of TAGs, these feature did not influence the ultimate concentration of tocopherols.

4.5.2. Total Carotenes

Carotenoids were shown to influence diverse molecular and cellular process, which can provide the basis for the effects of carotenoids on human health and disease prevention (Benadé, 2013).

4.5.2.1. Buriti: Murumuru

Buriti oil, one of the largest known sources of carotenoids, in the few studies available, showed photoprotection against UV radiation, in addition to antibacterial and healing capabilities (Batista *et al*., 2012; Zanatta, Ugartondo, Mitjans, Rocha-Filho & Vinardell, 2010; Tuberoso *et al*., 2007). In Table 11 it is possible to evaluate the effect of interesterification of buriti oil with murumuru fat regarding the content of carotenoids. It is observed that the interesterification of this blend does not significantly alter the final concentration of carotenoids, that is, the content of carotenes from the buriti in the initial blend, still present after interesterification.

The process of interesterification with the enzyme from *Rhizopus* sp. increased carotenoid content, this effect may have been due to the fact that this enzyme is crude and compounds present therein have influenced the analysis of carotenoids.

Table 11: Carotene content in buriti oil and murumuru fat; non-interesterified blend and interesterified lipids.

Blends and enzyme	Concentration (mg kg^{-1})
Buriti	510. 4 ± 0.68
Murumuru	5.8 ± 0.05
Non-interesterified blend	406.0 ± 1.35
2.5% Commercial lipase*	364.8 ± 2.71
2.5% Lipase from *Rhizopus* sp.	429.4 ± 11.5

*Lipozyme TL- IM

As with the concentration of tocopherols, carotenes concentration of buriti oil sample used in this study was lower than other studies published with this oil. In other published studies with buriti oil, the content of carotenes showed near 1700 mg kg^{-1}, indicating that in the case of blend buriti: murumuru, the carotene content of the synthesized lipids could get even bigger (Albuquerque *et al*., 2005).

4.5.2.2. Patauá: Palm stearin

Palm stearin contained 301.2 mg kg^{-1} of carotene and it was absent in patauá (Table 12). On blending palm stearin with patauá, the carotene content ranged between 83.8 and 89.4 mg kg^{-1}. After interesterification with both lipases, the concentration of carotenoids in the synthetized lipids remained practically constant.

In clinical studies carotenoids presents photoprotective capacity responsible for the prevention of premature skin aging, provide high degree of antioxidant protection and photoprotective against UV radiation (Stahl & Sies, 2002). Thus, such lipids can be synthesized for use as nutraceuticals and may be applied in various cosmetic and pharmaceutical products. In addition, these lipids synthesized presented after the interesterification reaction, triacylglycerols more homogeneous (less types of TAGs)

and only one phase, which improves the characteristics of these lipids to be used in emulsions (unpublished results).

Table 12: Carotene content in patauá oil and palm stearin; non-interesterified blend and interesterified lipids.

Blends and enzyme	Concentration (mg kg^{-1})
Patauá	ND
Palm stearin	301.2 ± 0.68
Non-interesterified blend	85.3± 0.68
2.5% Commercial lipase*	83.8± 2.71
2.5% Lipase from *Rhizopus* sp.	89.4 ± 4.71

*Lipozyme TL-IM
ND - Not detected

Reshma, Saritha, Balachandran & Arumughan (2008) produced lipids (shortenings) where carotenes concentration was maintained after the enzymatic interesterification. In the reaction, rice bran oil and palm stearin were used, the latter being the source of carotenoids. Generally shortenings are devoid of carotenes, so the mixture of palm stearin, which has carotene, with other oils poor in this compound may contribute to the production of shortenings rich in this beneficial nutrient.

In another study, Khatoon, Khan & Jeyarani (2012) produced different interesterified lipids using palm stearin and coconut oil as substrates. In blends with higher concentration of palm stearin, source of carotenes, there was a greater loss of this minority (up 40%). The authors atribute part of the loss due to further processing after enzymatic interesterification reaction.

4.5.3. Total phenol content

The phenolic compounds present significant biological potential, especially in preventing oxidative stress, inflammation and bacterial infection, where all of them have a promoter role in the development of many severe chronic diseases (Lesjak *et al*., 2014). Among the potential benefits, the use of these compounds in cosmetic and nutraceutical formulations, have offered protection against the damage caused to DNA by solar radiation (Matsui *et al*., 2009).

In addition to its effects on health, such phenolic compounds due to the antiradical activity can protect the tocopherols present in the oils during storage and handling. Also, as the phenolic compounds act as free radical scavengers, may protect against autooxidation of unsaturated fatty acids, thereby increasing the shelf life of the oil (Valavanidis *et al*., 2004).

4.5.3.1. Buriti: Murumuru

The results in Table 13 show that the buriti oil is a source of phenolic compounds. Although the concentration of this compound in these oil is not so high as olive oil (170 - 210 mg gallic acid equivalents $kg-^{1}$) it is superior to many other vegetable oils (Tuberoso *et al*., 2007).

The interesterified and non-interesterified blends of buriti: murumuru have practically the same concentration of phenolic compounds, indicating that the interesterification itself had no effect on the concentration of phenol. However, due to the reaction conditions, these blends with and without the presence of enzymes, have suffered a loss of approximately 30% of phenolic compounds. Even after the loss, the phenols content in the synthesized lipids showed values close to the soybean oil (60 -

80 mg kg^{-1}) and higher than sunflower oil (3 - 4 mg kg^{-1}) and maize oil (1 mg kg^{-1}) (Valavanidis *et al*., 2004).

Table 13: Phenol content in buriti oil and murumuru fat; non-interesterified blend and interesterified lipids.

Blends and enzymes	Gallic acid (mg kg^{-1})
Buriti	107.0 ± 1.25
Murumuru	16.2 ± 0.63
Non-interesterified blend	52.4 ± 0.64
2.5% Commercial lipase*	45.3 ± 1.58
2.5% Lipase from *Rhizopus* sp.	50.3 ± 0.24

* Lipozyme TL-IM
ND - Not detected

4.5.3.2. Patauá: Palm stearin

The results in Table 14 indicate that the patauá oil is a source of phenolic compounds. Regarding patauá: palm stearin blend the same losses that occurred with the buriti: murumuru blend can be observed. However, in this blend, losses of phenolic compounds reaches 40%, which can be explained by the higher temperature (50°C) used in the reaction.

Table 14: Phenol content in patauá and palm stearin; non-interesterified blend and interesterified lipids.

Blends and enzymes	Gallic acid (mg kg^{-1})
Patauá	50.0 ± 0.72
Palm stearin	N.D.
Non-interesterified blend	20.3 ± 0.48
2.5% Commercial lipase*	22.7 ± 5.05
2.5% Lipase from *Rhizopus* sp.	20.3 ± 1.34

* Lipozyme TL-IM. ND - Not detected

Ochoa-Herrera, Huertas, Quites & Matix (2001) compared the effect of virgin olive oil and high oleic sunflower oil on antioxidant activity in rabbits. The study indicated that in addition to the fatty acid composition of the dietary lipids, the minor constituents such as polyphenols present in olive oil can greatly influence antioxidant status in animal (Ochoa-Herrera *et al.*, 2001; Nagaraju & Belur, 2008).

No other studies in the literature evaluating the effect of interesterification of oils in phenolic compounds was found.

5. Conclusions

The process development presented here is an ecofriendly approach to the utilization of two blends of Amazonian oils. Due to the mild conditions of the reaction, enzymatic interesterification proved to be a suitable strategy for producing alternative new oils with improved distributions of fatty acids. The interesterification of the Amazonian oils, using different enzyme systems, produced lipids with more homogeneous triacylglycerols, especially the mono and di-unsaturated triacylglycerols. The lipases used in this study showed different specificities in these blends. For the buriti oil and murumuru fat blend, the commercial lipase was specific for unsaturated fatty acids, whereas the *Rhizopus* sp. lipase, even as a crude enzyme, was specific for both unsaturated fatty acids and the positions *sn*-1 and *sn*-3 of the fatty acid on the triacylglycerol. For the patauá oil and palm stearin blend, the commercial lipase was not specific, whereas the *Rhizopus* sp. lipase was specific for unsaturated fatty acids. The mixture of both lipases, for both blends, showed no synergistic effect: the results were intermediate between the two enzymes applied alone. The use of different lipases in the interesterification reaction, although it produced lipids with different compositions of triacylglycerols, did not influence the concentration of minor compounds. The lipids obtained, because of the characteristics

of the oils, may be applied to the formulation of cosmetics, pharmaceuticals and foods.

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CAPÍTULO 3

INFLUENCE OF EMULSION DROPLET SIZE ON ANTIMICROBIAL ACTIVITY OF INTERESTERIFIED AMAZONIAN OILS.

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Abstract

This study evaluated the antimicrobial potential of emulsions formulated with interesterified Amazonian oils. The results indicated that the antimicrobial activity of the emulsions is directly related to the properties of the emulsion, and the interesterification process of these oils influences these characteristics. When the antimicrobial emulsion was produced with interesterified Amazonian oils (2 mL 100 mL^{-1}), the oil droplet presented smaller size, and resulted in the complete inhibition of pathogens growth, *Bacillus cereus* and *Escherichia coli,* after 24 h (bactericidal effect). When the antimicrobial emulsion was produced with non-interesterified oils (only physical mixing), the oil droplets were larger and the emulsions did not completely inhibit the growth of the pathogens after 24 h (bacteriostatic effect). The results suggest that the interesterification of these oils may be responsible for changes in the physicochemical characteristics of the emulsions, producing droplets with smaller size and greater antimicrobial activity.

1. Introduction

The enzymatic interesterification is one of the techniques available to improve the properties of oils and fats. The changes in the original triacyglycerol (TAG) composition can modify the physical, nutritional and biological properties of the lipid produced, increasing its potential application (Iwasaki & Yamane, 2000 and Nunes *et al*., 2011). Furthermore, these reactions can reduce the types of TAGs initially present in the blend, forming a lipid with a narrower range of crystallization, with ease of packaging and greater capacity for interaction with other components of the medium (Meten & Hartel, 2005).

Thus, this technique may be an alternative for the production of lipids with a higher commercial value. In this sense, Amazonian oils stand out. These oils are little explored, and using them in these reactions can enlarge the interest in these materials due to the new characteristics of the lipids produced.

Among the oils and fats of the Amazon, some due to the composition of fatty acids and minor compounds, are highlighted: buriti (*Mauritia flexuosa* L.f. - *Arecaceae*) and patauá (*Oenocarpus bataua* Mart. - Arecaceae) oils have higher concentration of monounsaturated fatty acids, and some minor compounds such as tocols, carotenoids and phenolics (Zanatta *et al*., 2010 and Montúfar *et al.,* 2010); murumuru (*Astrocaryummurumuru* Mart.- *Arecaceae*) fat is rich in lauric and myristic fatty acids (Mambrim & Barrera-Arellano, 1997) and palm stearin (*Elaeis guineensis* Jacq. - *Arecaceae*) is rich in palmitic and oleic acids, and contains significant amounts of stearic and linoleic acids (Adhikari *et al*., 2010). Several of these compounds have antimicrobial activity (Batista *et al*., 2012; Desbois, 2012 and García-Ruiz *et al*., 2013). Therefore the production of blends with these oils and fats and their use in

interesterification reactions can result in lipids with modified characteristics, and increased antimicrobial potential.

Some pathogenic bacteria cause food spoilage and are responsible for many human illnesses. Among them the gram-positive *Bacillus* genus form stable spores and are resistant to harsh conditions and extreme temperatures. *B.cereus* is a common pathogen involved in foodborne diseases, local sepsis and wound and systemic infections (Hamouda *et al*., 1999). Another genus of bacteria that cause damage is the gram-negative *Escherichia,* which is resistant to many antibiotics. *E.coli* is a common pathogen responsible for various extra intestinal or intestinal infections (Croxen *et al*., 2013; Rather *et al*., 2013).

Thus, this study aimed to evaluate the antimicrobial effect of emulsions formulated with interesterified Amazonian oils produced by different enzymes. The pathogens tested were the gram-positive bacteria *Bacillus cereus* and gram-negative *Escherichia coli*. In addition, this study evaluated whether the physical characteristics of the emulsion influence their antimicrobial potential. The interesterified lipids tested were composed of buriti oil and murumuru fat (first blend) and patauá oil and palm stearin fat (second blend). The lipids were produced using two lipases in three different enzyme systems: first, a commercial lipase; second, a crude lipase from the fungus *Rhizopus* sp.; and third, a mixture of both lipases (commercial and *Rhizopus* sp.).

2. Materials

2.1. Chemicals

Buriti, patauá oils and murumuru fat were bought in a local market in the city of Belém, State of Pará, in the Brazilian Amazon. Palm stearin was kindly supplied by

Agropalma (Pará, Brazil). Commercial purified and immobilized lipase (Lipozyzme TL-IM) was kindly supplied by Novozymes. Crude lipase from *Rhizopus* sp. was produced in a solid medium in our laboratory (Macedo *et al*.,2004). All other reagents and solvents were of analytical grade.

2.2. Microorganism

The bacterial strains *Bacillus cereus* (*B.cereus*) and *Escherichia coli* (*E.coli*) were provided by Pluridisciplinary Center for Chemical, Biological and Agricultural Research (Cpqba - Unicamp, Brazil).

3. Methods

3.1. Lipases assay

Lipase activities in both enzymes were quantified using olive oil as substrate. One unit of lipase activity (U) is defined as 1 µmol of oleic acid released per minute (Macedo *et al*., 2004).

3.2. Enzymatic interesterification

The enzymatic interesterification was performed in an orbital-shaking water bath at 150 opm for 24 h at 40°C under vacuum for buriti oil and murumuru fat blend and 150 opm for 24 h at 50°C under vacuum for patauá oil and palm stearin blend. The weight ratio of oil to fat was 70:30, with a total weight of 10 g. The reactions were performed in three different enzyme systems: commercial lipase, lipase from *Rhizopus* sp. and a mixture of both enzymes. In all systems, the final enzyme concentration ranged between 2.5g to 10g 100^{-1}g . Before the reaction, the enzymes were dried in a vacuum oven at 40°C for 30 minutes. After completion of the

reaction, the interesterified blend was immediately filtered using a 0.45 μm membrane filter and frozen (Rodrigues & Ayub, 2011 and Simões*et al*., 2014). The non-interesterified blend was also subjected to the same reaction conditions.

3.3.Emulsions preparation and inoculation

The emulsions were prepared in tubes with screw using a fixed concentration of the culture medium (Nutrient broth - Merck Millipore), different concentrations of water and interesterified and non-interesterified oils, followed by Triton X-100 emulsifier (Buthelezi *et al*., 2012). The mixtures were shaken (vortex) for 70 seconds. Finally the culture medium containing the microorganism with the optical density of 0.4 - 0.6 at 490 nm were added. The concentrations of these components are described in Table 1. The final volume of the emulsions was kept constant at 1500 μL. After inoculation of the microorganism, the emulsion was kept under stirring at 150 rpm for 24 hours at 37°C. This preparation was performed under aseptic conditions.

Table 1: Concentration of the components used for the production of emulsions.

Culture medium (mL 100 mL $^{-1}$)	Water (mL 100 mL $^{-1}$)	Oil (mL 100 mL $^{-1}$)	Emulsifier (mL 100 mL $^{-1}$)	Culture medium with microorganism (mL 100 mL $^{-1}$)
33.5	31	0	2	33.5
33.5	29	2	2	33.5
33.5	25	6	2	33.5
33.5	21	10	2	33.5
33.5	17	14	2	33.5
33.5	13	18	2	33.5
33.5	13	22	2	33.5

3.4. Antimicrobial assay

After the incubation period, aliquots of 150 μL of the emulsions were transferred to 96-well sterile microplates. Adding 20μL of the dye of *p*-iodonitrotetrazolium violet, and after 15 minutes, the microbial growth was read by colorimetry at 490 nm in a microplate reader (BMG Labtech - Fluostar Optima) (Buthelezi *et al.*, 2012). The growth of microorganisms was compared to plates prepared without the presence of oil (control).

3.5. Emulsion droplet size distribution

Emulsions droplet size distribution (PSD) was measured using a laser light scattering analyzer after 1 hour of preparation (Malvern Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). Aliquots of emulsions were added to distilled water under stirring (1750 rpm) until an obscuration rate of 10% was gained. The average droplet size was calculated as the Sauter Mean Diameter (D_{32}), which is defined as the diameter of the sphere having the same volume / surface area of the particle of interest (Equation 1). The Specific Surface Area (SSA) was calculated and this parameter is defined as the total area of the droplets divided by their total weight. It was calculated based on the assumption that the particles are both spherical and nonporous (Equation 2).

$$D_{32} = \frac{\sum \mathbf{n_i} \cdot \mathbf{d_i}^3}{\sum \mathbf{n_i} \cdot \mathbf{d_i}^2} \qquad \text{(Equation 1)}$$

Where n_i is the number of particles with diameter d_i

$$SSA = \frac{6 \sum \frac{V_i}{d_i}}{\rho \sum V_i} = \frac{6}{\rho \, D[3,2]} \qquad \text{(Equation 2)}$$

Where V_i is the relative volume of particles with diameter d_i and ρ is the particle density.

4. Results and Discussion

4.1. Lipases

In this study, two different lipases were used in interesterification reactions. The commercial lipase, purified and immobilized, presented lipolytic activity of 12.7 U g^{1}. The semi-purified and non-immobilized lipase from *Rhizopus* sp. presented lipolytic activity of 8.0 U g^{-1}. These enzymes were able to catalyze the reaction of interesterification of oils and fats from the Amazon, and effects on lipids formed can be observed below.

4.2. Antimicrobial activity of emulsified Amazonian interesterified oils

In this study, the Amazonian interesterified oils produced using different lipases were able to inhibit the growth of microorganism's *B. cereus* and *E.coli* when emulsified with Triton X-100. Bacteria were tested with different concentrations of non-interesterified and interesterified oils in the emulsion (between 2 and 22 mL 100 mL^{-1}), in order to observe the effect on bacterial growth. All oils were evaluated at a concentration of 2 mL 100 mL^{-1} in the emulsion. The other concentrations (between 6 and 22 mL 100 mL^{-1}), varied according to the oil, this is because, due to the large number of samples, it was not possible to evaluate all concentrations for each microorganism.

A different effect was observed for each type of oil used, although in all of them the lowest concentration of oil in the emulsion (2 mL 100 mL^{-1}) had greater antimicrobial effect, regardless of whether the oil was interesterified or not (Figure 1). However, it was observed that the emulsions formulated with oils interesterified at this concentration (2 mL 100 mL^{-1}) did not completely inhibit the growth of the pathogens after 24 hours (bacteriostatic effect), while the emulsions formulated with

non-interesterified oils complete inhibition of pathogens grown (bacteriostatic effects). Furthermore, emulsions prepared with interesterified oils had higher antimicrobial activity at most of the concentrations tested.

Thus, it can be concluded that the composition of the oils was not primarily responsible for antimicrobial activity, since the best results were observed at lower concentrations of oil, that means it does not present a dose-response effect. However, the emulsions prepared with interesterified oils showed higher antimicrobial activity, suggesting that the interesterification of these oils may be responsible for changes in the characteristics of the emulsion, and this factor influenced the results of antimicrobial activity.

4.3. Characteristics of the emulsions

Mean diameter (D_{32}) and specific surface area of the droplets in the emulsion were evaluated in order to verify whether the interesterification of oils changed the characteristics of the emulsions (Tables 2 and 3).

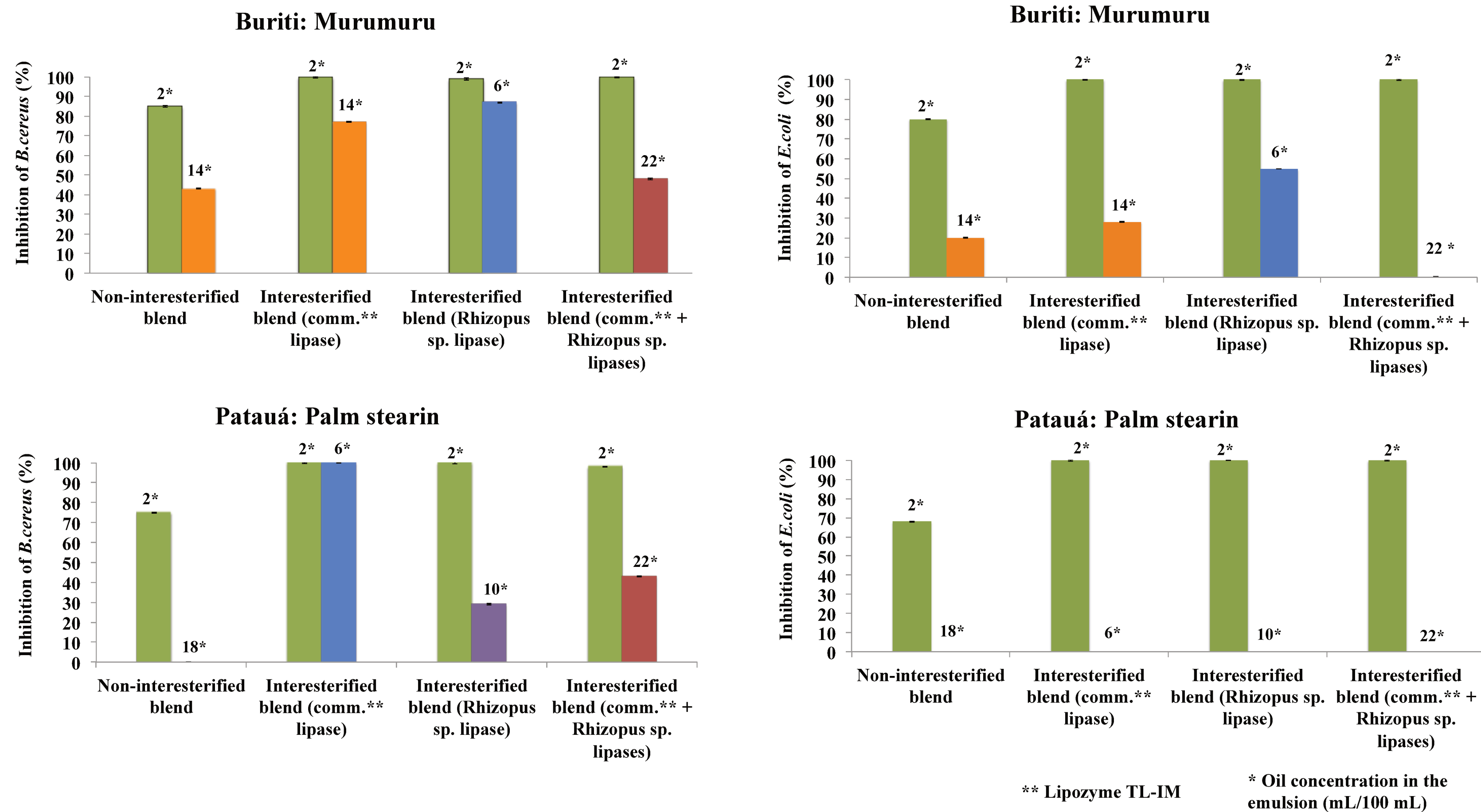

Figure 1: Effect of non-interesterified blends and interesterified lipids at different concentrations (2, 6, 10, 14, 18 or 22 mL 100 mL^{-1}) on the antimicrobial activity of *B.cereus* and *E.coli*.

Table 2: Mean diameter and specific surface area of emulsions droplets produced with non-interesterified and interesterified buriti oil and murumuru fat blends.

Buriti:Murumuru Enzyme system	Oil in the emulsion (mL 100 mL^{-1})	$D_{3,2}$ (μm)	Specific surface area ($m^2 g^{-1}$)
Non-interesterified blend	2	2.8 ± 0.01	2.1 ± 0.01
	14	5.0 ± 0.01	1.2 ± 0.02
Interesterified lipid (commercial lipase*)	2	2.3 ± 0.01	2.6 ± 0.00
	14	3.0 ± 0.01	2.0 ± 0.00
Interesterified lipid (lipase from *Rhizopus* sp.)	2	2.0 ± 0.02	3.1 ± 0.01
	6	5.0 ± 0.01	1.2 ± 0.01
Interesterified lipid (commercial* + *Rhizopus* sp. lipases)	2	1.8 ± 0.01	3.3 ± 0.01
	22	9.8 ± 0.02	0.6 ± 0.00

* Lipozyme TL - IM

Table 3: Mean diameter and specific surface area of emulsions droplets produced with non-interesterified and interesterified patauá oil and palm stearin fat blends.

Patauá:Palm stearin Enzyme system	Oil in the emulsion (ml 100 mL^{-1})	$D_{3,2}$ (μm)	Specific surface area ($m^2 g^{-1}$)
Non-interesterified blend	2	3.5 ± 0.00	1.7 ± 0.02
	18	4.6 ± 0.01	1.3 ± 0.01
Interesterified lipid (commercial lipase*)	2	2.1 ± 0.00	2.8 ± 0.02
	6	2.2 ± 0.01	2.7 ± 0.01
Interesterified lipid (lipase from *Rhizopus* sp.)	2	1.6 ± 0.01	3.7 ± 0.00
	10	3.2 ± 0.02	1.8 ± 0.01
Interesterified lipid (commercial* + *Rhizopus* sp. lipases)	2	2.2 ± 0.00	2.7 ± 0.02
	22	3.6 ± 0.00	1.7 ± 0.01

* Lipozyme TL-IM

The results showed that there are differences in these parameters when using the same concentration of the tested oils in the emulsions (2 mL 100 mL^{-1}). The

emulsions prepared with the interesterified blends showed a smaller droplet diameter and higher specific surface area. These characteristics can be observed in both blends, with all three enzymatic systems used for interesterification.

Thus, the results indicate that indeed chemical modification caused by enzymatic interesterification was responsible for the change in the characteristics of the emulsions.

4.4. Emulsion, Droplet size and Antimicrobial activity

In order to verify whether the characteristics of the emulsion were related to antimicrobial activity, a correlation table of these parameters is shown below (Tables 4 and 5). The results confirm that the antimicrobial activity of the emulsions is more closely related to the droplet size than to the chemical characteristics of the oils. In emulsions in which the specific surface area was larger, the antimicrobial feature was more effective to both microorganisms tested.

Table 4: Concentration of the non-interesterified and interesterified buriti oil and murumuru fat blends in the emulsion, specific surface area of the droplets in the emulsions, microbial inhibition of *B.cereus* and *E.coli*.

Buriti: Murumuru Enzyme system	Oil in the emulsion (mL 100mL^{-1})	Specific surface area (m^{2}g^{-1})	Inhibition of *B.cereus* (%)	Inhibition of *E.coli* (%)
Non-interesterified blend	2	$2.1^A \pm 0.01$	$85.1^A \pm 0.11$	$80.0^A \pm 0.13$
	14	$1.2^B \pm 0.02$	$43.0^B \pm 0.09$	$20.0^B \pm 0.06$
Interesterified blend (commercial lipase*)	2	$2.6^A \pm 0.00$	$100.0^A \pm 0.00$	$100.0^A \pm 0.00$
	14	$2.0^B \pm 0.00$	$77.5^B \pm 0.05$	$28.0^B \pm 0.04$

Means with the same letter in the column for each type of oil are not significantly different at the 5% significance level.
* Lipozyme TL - IM

Table 5: Concentration of the non-interesterified and interesterified patauá oil and palm stearinfat blends in the emulsion, specific surface area of the droplets in the emulsions, microbial inhibition of *B.cereus* and *E.coli*.

Patauá: Palm stearin Enzyme system	Oil in the emulsion (mL $100mL^{-1}$)	Specific surface area (m^2g^{-1})	Inhibition of *B.cereus* (%)	Inhibition of *E.coli* (%)
Non-interesterified blend	2	$1.7^A \pm 0.02$	$79.0^A \pm 0.09$	$68.0^A \pm 0.10$
	18	$1.3^B \pm 0.01$	$0.0^B \pm 0.00$	$0.0^B \pm 0.00$
Interesterified blend (commercial lipase*)	2	$2.8^A \pm 0.02$	$100.0^B \pm 0.00$	$100.0^A \pm 0.00$
	6	$2.7^B \pm 0.01$	$100.0^A \pm 0.00$	$0.0^B \pm 0.00$

Means with the same letter in the column for each type of oil are not significantly different at the 5% significance level.
* Lipozyme TL - IM

Interesterified oils showed greater antimicrobial activity probably because it allows the formation of an emulsion with reduced droplets size. The non-interesterified blend due to the different properties of their components, such as viscosity and melting range, separates into two phases, producing emulsions with less ability to stabilize, forming larger droplets. The interesterification process produces lipids with a single phase and do not exhibit phase separation yielding more stable emulsions (Grimaldi *et al*., 2001; Meten & Hartel, 2005).

This paper only shows results of the interesterified oils by commercial enzyme, nonetheless the same behavior has been observed for the other interesterified blends (data not shown).

Enzymatic interesterification induces exchange of fatty acids in the structure of glycerol, and this change is related to the specificity of the enzyme (Kapoor & Gupta, 2012). The interesterified blends with different enzymes show different behavior in emulsion, thereby modifying the drop size, hydrophobicity and their effectiveness as an antimicrobial agent. Interesterified blends used in this study showed a significant change in the relationship between saturated and unsaturated fatty acids. There was a

significant increase of unsaturated fatty acids, in the *sn* - 1 and *sn* - 3 positions of the triacylglycerol, and the predominance of mono and di-unsaturated triacylglycerols. The interesterification decreased the types of TAGs present in the initial blend, forming lipids with similar properties, such as melting and crystallization rates (unpublished results). This properties obtained may have influenced the physicochemical properties of the emulsions formed, as discussed above.

The characteristic of the interesterified Amazonian oils used in this study is of great interest since recent studies have indicated that the interesterification may influence the biological activity (Berry, 2009; Speranza & Macedo, 2012; Farfán *et al*., 2013; Michalski *et al*., 2013). The intramolecular triacylglycerol structure can influence the digestion and absorption of lipids, affecting some metabolic responses (Michalski *et al*., 2013). Thus, the use of these interesterified oils for the production of emulsions for biological applications appears to be more advantageous than the use of simple blending of these oils and fats. By presenting droplets with higher specific surface area, the performance of this emulsified oil becomes more effective in biological systems.

Wang *et al*. (2008) evaluated the effects of curcumin (polyphenol) encapsulated in oil in water emulsion regarding anti-inflammatory activity in rats. The authors observed increased inhibition of edema in the ears of mice with decreasing diameter of the emulsion droplets. This effect is due to the increase in the surface area-to-volume ratio as the droplet size decreases. As the interfacial area increases, the number of dispersed droplets becomes higher, and the number of interactions is also higher (Nunez *et al.,* 2000).

In order to facilitate visualization, Table 6 shows only the results of the emulsions that produced higher microbial inhibition (formulated with a 2 ml 100^{-1} mL

oil concentration). It is clearly observed that the emulsions produced with interesterified blends show larger specific surface area and higher inhibition of pathogens.

Table 6: Specific surface area of the droplets in the emulsions produced with 2 mL 100 mL^{-1} of the non-interesterified and interesterified blends and microbial inhibition of *B.cereus* and *E.coli.*

Oil Blends Enzyme system	**Specific surface area (m^2 g^{-1})**	**Inhibition of *B.cereus* (%)**	**Inhibition of *E.coli* (%)**
Buriti: Murumuru			
Non-interesterified blend	2.1 ± 0.01	85.1 ± 0.09	80.2 ± 0.07
Interesterified blend (comm. lipase*)	2.6 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
Interesterified blend (lipase from *Rhizopus* sp.)	3.1 ± 0.01	98.6 ± 0.07	100.0 ± 0.00
Interesterified blend (comm.* + *Rhizopus* sp. lipases)	3.3 ± 0.01	100.0 ± 0.00	100.0 ± 0.00
Patauá: Palm stearin			
Non-interesterified blend	1.7 ± 0.02	79.0 ± 0.07	68.0 ± 0.05
Interesterified blend (comm. lipase*)	2.8 ± 0.02	100.0 ± 0.00	100.0 ± 0.00
Interesterified blend (lipase from *Rhizopus* sp.)	3.7 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
Interesterified blend (comm.* + *Rhizopus* sp. lipases)	2.7 ± 0.02	98.9± 0.09	100.0 ± 0.00

* Lipozyme TL-IM

A recent study showed that there is a close relation between the physicochemical properties of emulsions and their potential effects as antimicrobial agents (Al-Adham *et al.,* 2012). The results suggested that the high levels of antimicrobial activity are due to the unique characteristic of the structure of oil-in-water emulsion system, rather than the chemical activity of their individual

components. Our results confirmed that the physicochemical characteristics of the emulsion are related to the antimicrobial activity. In the emulsions in which the droplet size was smaller, the antimicrobial effect was more relevant. This correlation was observed for all oils, interesterified or not. Droplet with smaller sizes has significantly larger specific surface area, ensuring that the components of the emulsion have greater contact with the bacterial membrane. The interaction between the droplets and the bacterial membrane decreases the hydrophobicity of the bacterial cell, causing rapid loss of cell viability (Zhang *et al*., 2009).

Two recently published studies have evaluated the antimicrobial activity of emulsions and related it to the droplet size (Buranasuksombat *et al*., 2011 and Terjung *et al*., 2012). In these two studies, the authors find no correlation between droplet size and antimicrobial activity. However, many other studies correlate biological activity with droplet size (Salvia-Trujillo *et al*., 2013; McClements and Xiao 2012; Acosta, 2009; Wang *et al*., 2008). These latter studies show that the reduction in droplet size produces larger absorption of active ingredients and increased intake of these droplets in cells and tissues.

Therefore, the results obtained in this study are in accordance with those that relate the increase in biological activity with a reduction in droplets size. The interesterification of these oils produces emulsions with smaller droplet size, and this feature enhances the biological potential of the emulsion. New applications for interesterified oils can be investigated from this finding.

5. Conclusions

This study verified that the antimicrobial activity of emulsified Amazonian oils is influenced by the oil droplet sizes in the emulsions, and these sizes are determined

by the properties of the oils used. The emulsions produced with the non-interesterified blends of Amazonian oils showed a higher oil droplet size and presented a bacteriostatic effect against *B. cereus* and *E. coli* pathogens, while the emulsions produced with interesterified blends showed smaller oil droplets size and presented bactericidal effect. The use of these interesterified oils for biological applications seems to be more advantageous than the use of simple blending of these oils.

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CONCLUSÕES GERAIS

Os resultados obtidos neste trabalho permitiram estabelecer as seguintes conclusões:

- As lipases Lipozyme TL - IM (Novozymes) e a produzida pelo fungo *Rhizopus* sp. foram capazes de catalisar a reação de interesterificação dos óleos da Amazônia;

- A lipase de *Rhizopus* sp., quando utilizada na mistura buriti: murumuru, foi específica para o tipo de ácido graxo (insaturado) e também pela posição do tipo de ácido graxo no triacilglicerol (*sn* - 1, 3). Esta lipase, quando utilizada na mistura patauá: estearina de palma, foi específica apenas para o tipo de ácido graxo (insaturado);

- A lipase comercial Lipozyme TL - IM (Novozymes), quando utilizada na mistura buriti: murumuru, foi específica para o tipo de ácido graxo (insaturado). Esta lipase, quando utilizada na mistura patauá: estearina de palma, não apresentou específicidade;

- A mistura de ambas as enzimas não melhorou a performance catalítica das enzimas isoladamente, isto é, não foi observado efeito sinérgico em ambas as misturas avaliadas;

- Na maioria das misturas avaliadas, a interesterificação enzimática, com os três sistemas de enzimas utilizados, promoveu diminuição nos teores de triacilgliceróis trisaturados e tri - insaturados e aumento dos triacilgliceróis monoinsaturados-

disaturados e di-insaturados - monosaturados, o que resultou na formação de lipídios com número menor de tipos de triacilgliceróis;

- Os lipídios formados apresentaram mudanças nas características físico - químicas, quando comparados com as misturas físicas não - interesterificadas. Os perfis de fusão e cristalização, de uma maneira geral, apresentaram redução no número de picos, confirmando a redução no número de tipos de triacilgliceróis;

- A interesterificação destes óleos e gorduras da Amazônia não reduziu de forma significativa o conteúdo de compostos minoritários. O teor de tocóis, carotenos e fenólicos foram preservados após a reação;

- Os óleos interesterificados, quando aplicados em emulsões, influenciaram no tamanho da partícula. Quando a emulsão era produzida com o óleo interesterificado, a gota apresentava tamanho menor do que a produzida com a mistura não-interesterificada. Por esta razão, as emulsões produzidas com óleos interesterificados apresentaram maior atividade antimicrobiana;

- Os óleos interesterificados apresentaram efeito bactericida contra os patógenos gram - positivo *Bacillus cereus* e gram - negativo *Escherichia coli*; efeito não observado com as misturas não - interesterificadas.

Portanto, a interesterificação dos óleos da Amazônia, com as lipases utilizadas neste estudo, produziu bases lipídicas com novas características físico-químicas e biológicas para possíveis aplicações nas áreas de alimentos, cosmética e farmacêutica.

O trabalho mostrou que a modificação biotecnológica destes óleos brasileiros, pode ser uma alternativa para a valorização destas matérias - primas, garantindo maior interesse por sua exploração.

SUGESTÕES PARA TRABALHOS FUTUROS

- Otimização das condições de reação de interesterificação enzimática: tempo de reação e concentração de enzima;

- Avaliar novos efeitos biológicas dos lipídios produzidos;

- Aprofundar os estudos do efeito da interesterificação dos óleos em sistemas emulsificados.

ANEXO

LIPASE-MEDIATED PRODUCTION OF SPECIFIC LIPIDS WITH IMPROVED BIOLOGICAL AND PHYSICOCHEMICAL PROPERTIES.

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Abstract

The use of lipases in the modification of lipids has grown significantly in recent years. This increased interest is mainly due to the ability of these enzymes to catalyze the production of lipids with specific distributions of fatty acids that better fit the current needs of consumers, who are looking for healthier foods that are manufactured with the highest quality. The successful use of lipases to obtain modified lipids with low caloric content, high concentrations of n-3 fatty acids or high amounts of phenolic compounds demonstrate the great potential of these enzymes. The lipase - catalyzed production of lipids with reduced caloric content is made possible by the addition of a medium or a very long chain fatty acid to the triacylglyceride. Diacylglicerols with low caloric content can also be produced using lipases. Due to the deficiency of n-3 fatty acids in the current diet, strategies for the lipase-mediated incorporation of these acids in the TAG have shown promising results. Finally, studies have successfully used lipases for the incorporation ofphenolic compounds in the lipid structure, which produce compounds with improved oxidative stability and more beneficial health effects.

Abbreviations:

PUFA: polyunsaturated fatty acid
MAG: monoacylglycerides
TAG: triacylglycerol
MCFA: medium-chain fatty acid
LCFA: long-chain fatty acid
MCT: medium chain triacylglycerol
LCT: long-chain fatty acid
FA: fatty acid
MUFA: monounsaturated fatty acid
DAG: diacylglycerol

VLCFA: very long-chain saturated fatty acid
EA: erucic acid
HEA: high-erucic-acid
EPA: eicosapentaenoic acid
DHA: docosahexaenoic acid
ALA: α-linolenic acid
SDA: stearidonic acid

1. Lipases in lipid modification

Lipases, esterases, and phospholipases are different classes of biocatalysts that can be used for the modification of lipids. Of these biocatalysts, lipases are the most versatile catalysts in the field of lipid biotechnology and have a variety of industrial applications [1]. The use of lipases for the production of specific lipids is a technique that has been used for many years with promising results [2, 3, 4, 5]. The production of these specific lipids allows the industry to meet the changing dietary requirement of consumers. The use of lipases for the incorporation of desired FAs at specific positions of the glycerol moiety or the selective removal of FAs from specific positions has the potential to generate a concentrated amount of a particular type of fatty acid. It is well - established that most lipases, particularly those that are microbial, display certain FA selectivity, catalyze reactions under unnatural conditions, have broad substrate specificity, possess regioselective properties and show enantioselective catalytic behavior [6]. In particular, FA selectivity and regioselectivity are critical properties for the synthesis of specific lipids [1, 7, 8]. The use of oils and fats could therefore be upgraded to the use of nutritional synthetic lipids, such as cocoa butter substitutes, human milk fat analogues, low calorie TAGs and PUFA-enriched oils [9, 10, 11, 12]. It is also possible to change the physical properties of natural oils and convert them into margarines or hard butter with higher

melting points or into special low calorie spreads with short MCFAs [8]. Lipases can be used to carry out not only hydrolytic reactions but also synthetic reactions, such as esterification, acidolysis, alcoholysis and interesterification [6]. The hydrolysis of oils is the natural function of lipases. It is therefore possible to utilize lipases to produce special glycerides or free fatty acids that have many different applications, such as the production of docosahexanoic and erucic acids-enrichments [13, 14, 15]. In some cases, glyceride alcoholysis or transesterification, which involves the replacement of water by an alcohol to form the corresponding ester, can occur. This reaction is mainly used to produce MAGs, which are the most widely used emulsifiers in the food and pharmaceutical industries and have a number of beneficial effects and nutritional properties, such as their antimicrobial activity [16, 17]. Esterification can also be used for the production of mono -, di - and triacylglycerides of interest or to reduce the acidity of acid oil [18, 19, 12]. In addition, acidolysis and interesterification are strategies to improve the physical properties of oils and fats, such as lowering their melting point and changing their solid fat content [20]. Furthermore, it is possible, through the use of these reactions, to produce healthier fats, such as those that are rich in PUFAS or have a low number of calories [21, 22]. In addition to their specificity and selectivity, which ensures the production of the desired compounds, lipases can operate under mild temperature and pressure conditions, which would minimize the formation of side products and thus facilitate the subsequent separation and processing of the products [8].

This review will describe recent studies that have utilized lipases in the modification of fats and oils for the production of compounds of interest. The studies have successfully synthesized low calorie lipids that are rich in n-3 and exhibit high antioxidant capacity; these compounds meet the current needs of consumers and thus

the food industry.

2. Lipid modification

2.1. Low calorie lipids

Currently, more than 1 billion adults are overweight, and at least 300 million are clinically obese, which is a serious problem that continues to increase in industrialized countries and also in the urban areas of developing countries [23]. In addition, obesity and increased weight are major risk factors for a number of chronic diseases, including type - 2 diabetes, cardiovascular disease, hypertension, stroke, and certain forms of cancer [24]. Conventional TAG-based fats and oils represent, with an approximately 9 kcal g^{-1} content, the richest source of dietary calories; carbohydrates and proteins, however, have an approximately 4 kcal g^{-1} energy content [25]. Therefore, fats are of critical importance in the totaldaily calorie intake. Consumers concerned about excess weightand its health consequences have driven the food industry to reduce the fat and caloric content of foods [26]. Changing the composition of FAs in the TAGs through biotechnology is a promising option for the reduction of the caloric value of fats and oils. These new compounds would exhibit (a) the reduced energy content of short-chain organic acids, (b) the lowered energy content of MCFAs, and (c) the reduced gastric absorption of saturated VLCFAs and partially hydrolyzed TAGs [26]. Recent work indicates that the combination of these structural feature shave produced reduced - calorie lipids with potential industrial applications.

2.1.1. Medium-chain fatty acids

There is increasing interest in the synthesis of TAGs that contain MCFAs. These FAs, which belong to the family of FAs that contain between 6 and 12 carbons atoms, have become attractive because of their low caloric content and the fact that their fat content is not stored in body tissues. The caloric values of MCTs and TAG-containing LCTs are 8.3 kcal and 9.0 kcal, respectively. During digestion, the MCFAs, which are released from the TAGs by the action of the *sn*-1,3-specific pancreatic lipase, are preferentially transported via the portal vein to the liver because these FAs are more soluble than LCFAs and are metabolized as rapidly as glucose. Because the MCFAs are not readily re - esterified into new TAGs, they have little tendency to accumulate in the body as stored fat, which proves beneficial for weight control [27]. Weight maintenance studies that investigated the effect of diets with 20% and 30% lipid concentrations found that MCTs may be useful in the control of obesity [28]. In addition, because these TAGs are thought to provide less metabolizable energy per gram than traditional fats and oils, they are an efficient food source for patients with pancreatic insufficiency and other forms of malabsorption [29].

As shown in Table 1, studies on the production of TAGs with MCFAs have been recently published. In these studies, 1, 3 - specific lipases are capable of promoting the addition of MCFAs to the *sn* - 1 and *sn* - 3 positions of the TAGs (MLM). These synthesized TAGs have a different FA composition than the original TAGs, which accounts for their different characteristics. In addition, TAGs containing MUFA at the sn - 2 position can be hydrolyzed by pancreatic lipase into 2 - MAG and FAs, which are efficiently absorbed by the intestinal mucosa cells in normal adults [30]. The replacement of LCFA by MCFA in the *sn* - 1, 3 positions can also modify

the physicochemical properties of the TAG formed, which extends its range of applications. Recent publications indicate that the enrichment of palm olein with MCFA proves advantageous for healthy food applications due to the reduction of palmitic acid and no reported turbidity or solid separation at lower (refrigerated) temperatures [31]. Thus, the incorporation of MCFA in the production of margarine forms a product with desirable physical properties and a reduced caloric content [32].

Table 1: Lipase-mediated production of TAGs with MCFAs.

Lipase	Substrate	References
Thermomyces lanuginosus (Lipozyme TL IM), *Rhizomucor miehei* (Lipozyme RM IM) and *Candida antarctica* (Novozym 435) (Novozymes)	Virgin olive oil with caprylic or capric acids	[30]
Lipozyme RM IM (Novozymes)	Palm olein with caprylic and/or capric acids	[31]
Candida antarctica (CAL B) (Novozymes)	Rice bran oil, ground nut oil, or mustard oil with capric acids	[33]
Lipozyme RM IM (Novozymes)	Glycerol with oleic or capric acids	[32]
Rhizopus delemar (Tanabe Seiyaku), *Rhizopus oryzae* and *Pseudomonas fluorescens* (Amano)	Tuna oil with caprylic acid	[34]
Novozym 435, Lipozyme RM IM (Novozyme), *Rhizopus oryzae* (Amano) and *Alcaligenes* sp. (Meito Sangyo)	Fish oils with ethanol (to produce 2- MAG with caprylic acid)	[35]

2.1.2. Diacylglycerol

DAGs, which are esters of the trihydric alcohol glycerol in which two of the hydroxyl groups are esterified with FAs, can be found in two different isomeric forms: 1,2 (2, 3) DAG and 1, 3 DAG. These forms are natural components of the glycerides that are found in various fats and oils at levels up to 10% (w/w) [36]. The consumption of DAG is shown to reduce body weight and the accumulation of visceral abdominal fat more effectively than consumption of TAG [37, 38]. It is suggested that the beneficial health effects of DAG compared to TAG is due to the differences in their digestion and absorption [39]. During digestion, TAGs are normally hydrolyzed by pancreatic lipase to form 2-MAGs and FAs, which are subsequently incorporated into the intestinal mucosa. In epithelial cells, TAGs are re-synthesized from 2 - MAGs and FAs, and a portion of these re-synthesized TAGs is accumulated in adipose tissue. Meanwhile, the re-synthesis of DAGs is difficult after the incorporation of the digested components (mainly 1 - MAGs and free FA) into the intestinal mucosa, which results in the reduction of body fat [40]. Through the biotechnological use of lipases, DAGs can be produced through the esterification of FA and alcohol, the glycerolysis of TAG and glycerol, the partial hydrolysis of TAG, or a combination of these methods (Figure 1). Table 2 presents studies that produced DAGs using these different methods.

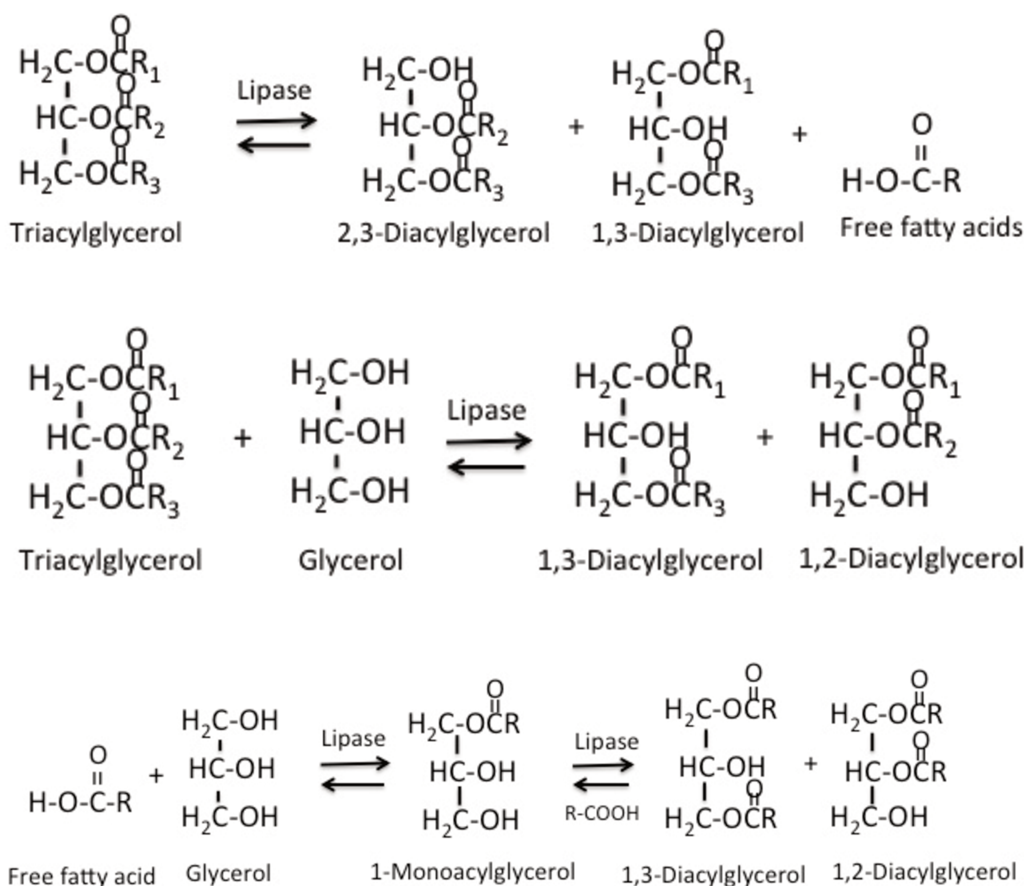

Figure 1: Possible products of lipase-catalyzed reactions of esterification, glycerolysis and hydrolysis, respectively.

Wang *et al*. [41] evaluated the effect of utilizing commercially immobilized lipases for the conversion of TAG into DAG using the glycerolysis of soybean oil as the model system. The product mixture was purified through molecular distillation to remove all of the produced MAGs. The authors observed that the removal of MAGs in the reaction ensured a higher production of DAG. The use of molecular or short-path distillation for the processing of lipids, particularly functional lipids, is increasing. This separation method is a continuous separation process that works under vacuum conditions in which the low evaporation temperature and short residence time allows the distillation of thermosensitive products with minimal

thermal stress [42].

Gonçalves *et al*. [43] produced DAGs using ultrasound irradiation with a commercial lipase as the biocatalyst. These researchers investigated the effect of ultrasound irradiation on the rate of DAG production by the enzymatic hydrolysis of the TAG contents in palm oil. With the use of ultrasound irradiation, the authors were able to produce DAGs under milder temperature and mechanical stirring conditions and lower enzyme concentrations. The use of ultrasound in bioprocesses has an extensive history with many positive results [44, 45, 46]. Consequently, ultrasound irradiation has the potential to influence the activities of enzymatic processes greatly, provided that the energy required is not so high that it would disrupt the function of the enzyme under study [46].

There is great interest in the synthesis of the *sn*-1, 3 - isoform of DAG, due to its rare manifestation in natural form and its beneficial health effects [47, 48, 49]. The 1(3) - MAG is poorly re-esterified via the 2 - MAG pathway and thus has an insignificant contribution toward the re-synthesis of TAG. Duan *et al*. [50] proposed that the enzymatic esterification of FA and glycerol was the ideal approach for the production of 1, 3 - DAG in high yield. According to the authors, the addition of t-butanol to the reaction allows the solubilization of glycerol, which eliminates its detrimental effect on the lipase. As an alternative for conventional volatile organic solvents, ionic liquids are a promising new type of reaction medium for biocatalytic processes because of their low volatility, tunable solvent properties and their ability to preserve enzymatic activity [51, 52]. It has also been observed that the addition of an organic solvent to an ionic liquid solvent could reduce its high viscosity, which has limited their use, without altering the levels of cations or anions in the system [53, 54, 55].

Table 2: Lipase-catalyzed production of DAGs.

Lipase	Substrate	References
Lipozyme RM IM, Lipozyme TL IM and Novozym 435 (Novozymes)	Soybean oil with glycerol	[41]
Burkholderia cepacia (PS IM) (Amano) and Lipozyme TL IM (Novozymes)	Palm oil	[43]
Novozym 435 (Novozymes)	Oleic acid with glycerol	[50]
Novozym 435 (Novozymes)	Triolein with glycerol	[55]
Lipozyme RM IM (Novozymes)	Oleic and linoleic acids with glycerol	[56]
Lipozyme RM IM and Novozym 435 (Novozymes)	Tuna oil with glycerol	[57]

2.1.3. Very long-chain saturated fatty acids

The TAGs that contain high amounts of VLCFA are poorly absorbed, partly because these acids have a melting point that is higher than the body temperature. In addition, these TAGs exhibit poor emulsion formation and micellar solubilization [58]. The decreased absorption capacity of saturated VLCFA makes these TAGs potential substrates for low calorie structured lipids with industrial applications. An example of VLCFA is behenic acid, a saturated FA with 22 carbons (22:0). Studies in rats indicate that this FA is safe for use in food [59, 60]. Behenic acid can be obtained by the hydrogenation of EA, a monounsaturated n-9 fatty acid (22:1) that has poor

absorption such as behenic acid, however there are doubts regarding the safety of EA for human consumption [61, 62, 63].

The major source of EA is the HEA seed oils of the *Crucifereae* family, which includes rapeseed, mustard, crambe, and wallflower, all of which contain approximately 45% to 60% EA [64]. In these seed oils, EA is primarily foundin the *sn*-1 and *sn*-3 positions of the TAGs. Therefore, the distribution of EA in the TAGs is ideal for the action of *sn*-1,3-specific lipases, which allows the efficient isolation of the acid efficiently and thus reduces the costs of down-stream processing to produce behenic acid. An alternative source for the production of behenic acid is crambe oil, which contains up to 60% EA that is localized exclusively on the *sn*-1 and *sn*-3 positions of the TAG [65, 66, 67].

The studies that investigated the lipase-catalyzed production of TAGs with VLCFAs are presented in Table 3. Kojima *et al.* [68] examined the effects of structured TAGs that contain behenic acid on the visceral fat depositions in rats. The authors observed that TAGs with behenic acid at the *sn*-1,3 positions (BOB) is resistant to hydrolysis by pancreatic lipase, which results in a reduction of the visceral fat depositions and reduces the levels of plasma and liver TAGs. When 10% of the dietary fat is replaced with BOB, the postprandial serum TAG levels suppress the intestinal absorption of dietary fat [69].

Tynek and Ledóchowska [60] prepared low calorie structured lipids containing behenic acid. The products had different compositions of TAGs and FAs and therefore exhibited different melting points and solid fat contents. These differences enabled the use of these products in low calorie formulations. Kanjilal *et al.* [59] synthesized structured lipids that contained 30 - 35% behenic acid from natural vegetable oils with semi solid characteristics and favorable melting behavior. The

enzymatically synthesized product had a reduced calorie value of 5.36 kcal g^{-1}, which is 59.5% of the calories found in natural oil. Therefore, due to its physical and chemical characteristics, this structured lipid product can be used as a reduced calorie fat.

Stearic acid (C18:0) is another FA that is used to produce low or reduced energy lipids. The absorption of LCFA by the human body is determined by its stereoposition on the TAG [70]. If the stearic acid is located at the *sn*-2 position on the TAG, the 2-MAG that is produced by the pancreatic lipase-catalyzed hydrolysis is well-absorbed [71]. This finding explains why saturated FAs in human milk, which contain saturated acids at the *sn*-2 position of the TAGs, are well-absorbed by infants compared to vegetable oils with the same FA composition; the saturated FAs in vegetable oils are mainly situated at the *sn*-1,3-positions [72]. In the case of lipids with low caloric content, the LCFAs must be primarily located at the *sn*-1,3 positions of the TAGs [73].

Table 3: Lipase-mediated production of TAGs with VLCFAs.

Lipase	Substrate	References
Rhizopus niveus	Sunflower oil with behenic acid	[68]
Lipozyme RM IM (Novozymes)	Olive oil with high erucic rapeseed oil; Olive oil with behenic acid	[60]
Candida cylindracea (Sigma Chemical) and *Mucor miehei* (Novo Nordisk)	Mustard oil (erucic acid source) with sunflower oil	[59]
Lipozyme RM IM (Novozymes)	Tributyrin with methyl stearate	[73]

Several commercially available modified fats include Caprenin (Procter & Gamble Co., Cincinnati, Ohio) and Benefat/Salatrim (Cultor Food Science, New York, NY). Caprenin is composed of C8:0, C10:0 and C22:0 FAs that are esterified to the glycerol moiety. Caprenin is produced by the chemical transesterification of coconut, palm kernel and rapeseed oils. The presence of behenic acid contributes to its low caloric value. Benefat/salatrim contains C2:0-C4:0 and C18:0 FAs that are esterified to the glycerol moiety [74]. This product is produced through the base-catalyzed interesterification of highly hydrogenated vegetable oils with TAGs that contain acetic, propionic and butyric acids [75]. Benefat/salatrim, which contains stearic acid, is a reduced calorie product, particularly if this fatty acid is esterified to the *sn*-1 and *sn*-3 positions of glycerol [74].

2.2. Lipids rich in n-3 polyunsaturated fatty acids

The beneficial effects of n-3 PUFAs on human health have been widely recognized. An enormous number of epidemiological and clinical studies have focused on the health effects of n-3 PUFAs, particularly EPA (20:5) and DHA (22:6), which are the two most important n-3 PUFAs. These studies indicated that these acids play an important role in the prevention and treatment of cardiovascular diseases, hypertension, diabetes, cancer, arthritis and other inflammatory and autoimmune disorders and are essential for normal growth and development, especially in the brain and retina [76]. Several oleaginous microorganisms have the ability to synthesize PUFA [77, 78]. Marine protists, such as species of the *Thraustochytrium*, *Schizochytrium* and *Crypthecodinium* genera, are rich sources of DHA, whereas microalgae, such as *Phaeodactylum* and *Monodus,* are good sources of EPA. The

major challenge in the production of PUFAs using microbial biotechnology is the improvement of the lipid yield [79]. Marine fish oil can be another source of EPA and DHA [80] because fish do not synthesize these long chain n-3 FAs but accumulate EPA and DHA by consuming plankton and algae as part of the marine food chain [81]. However, the concentrations of these FAs in fish oil are often low. Moreover, fish oil is not readily available in certain regions; these two drawbacks, in addition to the problems of odor, flavor, stability and purification, do not make fish oil a desirable candidate for the synthesis of PUFAs. A major source of n-3 in vegetable oils is ALA, which, by the action of certain enzymes, is converted to EPA and DHA. However, in the human body, ALA is converted to EPA and DHA at an efficiency of only 5 - 10% and 1 - 5%, respectively [82]. Strategies for the isolation of EPA and DHA and their subsequent introduction into TAG shave been used as alternatives to increasing the concentrations of these acids. However, to make this method economically feasible, it is necessary to produce these FAs at lower costs and with higher purity. The use of lipase has been considered promising because these enzymes are environmentally friendly, require mild reaction conditions and form more easily isolated products than chemical processes [83].

Some examples of lipase transformations of PUFAs are shown in Table 4. Kahveci and Xu [84] produced concentrated n-3 PUFA from the glyceride fraction of salmon oil that was obtained from salmon processing waste. The concentration of unsaturated FAs in the waste oil was increased by more than 300% after repeated hydrolysis. This use of inexpensive waste for the production of n-3 PUFA is a low cost alternative and has shown satisfactory results [85, 86]. High-acid crude fish oil is another low-cost product that can be used for the production of n-3 PUFA because, similar to the salmon waste, this product would otherwise be discarded. The

enzymatic production of glycerides with high concentrations of n-3 PUFA (EPA and DHA) from high-acid crude fish oils was reported [87]. The authors noted that the use of lipase for the deacidification of the crude fish oil instead of the conventional alkali neutralization reduces the loss that occurs during the refining process. The resulting PUFA-enriched glycerides are more beneficial for human dietary intake and have better oxidative stability.

A novel and sustainable source of n-3 FAs is the metabolic reengineering of large-scale oil seed crops, such as soy and canola [88]. The SDA - enriched soybean oil is in fact approaching commercialization [89]. SDA is an intermediate metabolite of the n-3 pathway through which ALA is converted to EPA and DHA. SDA, unlike ALA, does not need the action of the Δ - 6 desaturase enzyme, which is limited in humans; therefore, the use of SDA ensures a more efficient *in vivo* production of EPA and DHA [90].

In addition, premature infants have limited ability to make EPA and DHA from ALA; therefore, SDA-enriched soybean oil has been used as a substitute for human milk [91]. In human milk fat, the PUFAs are mainly found at the *sn*-1,3 positions of the TAGs, whereas a large amount of palmitic acid is found at the *sn*-2 position. The large amount of palmitic acid in this position provides readily absorbed energy for the development and growth of the infant and improves fat and calcium absorption [92, 93]. The great difficulty in synthesizing these human milk substitutes arises from the fact that vegetable oils have major unsaturated FAs at the *sn*-2 position. The use of lipases has been shown to represent an alternative to the production of human milk substitutes that are rich in n-3 with the specific distribution of FAs that is found in human milk. Teichert and Akoh [90] synthesized human milk fat analogues by the enzymatic interesterification of SDA - enriched soybean oil and tripalmitin. The

resulting human milk fat analogues contained over 60% palmitic acid at the *sn*-2 position and over 6% total SDA. The authors concluded that the modified lipids have the potential to exhibit absorption characteristics and FA contents that are similar to human milk fat with the added health benefits that are associated with n-3 FAs.

There are only a small number of SDA sources in nature, although there are still a large number of untested species. To date, SDA has been detected in variable amounts in several species of algae, fungi and animals tissues, although the seeds of certain plant families seem to be better sources of SDA, especially the *Echium (Boraginaceae*) species [94]. The enzymatic production of human milk fat analogues containing SDA from *Echium plantagineum* oil was recently published [95].

Table 4: Lipase-mediated production of lipids rich in n-3 PUFAs.

Enzyme process	Lipase	Substrate	References
Hydrolysis	*Candida rugosa* (Meito Sangyo)	Salmon oil	[84]
Esterification and Transesterification	Novozym 435 (Novozymes)	High-acid crude fish oil; de-acidified fish oil with ethanol	[87]
Interesterification	Novozym 435 and Lipozyme TL IM (Novozymes)	SDA-enriched soybean oil with tripalmitin	[90]
Esterification and Acidolysis	Lipozyme RM IM and Novozym 435 (Novozymes)	Glycerol with PUFAs; sn-1,3 diolein with PUFA-ethyl ester	[96]
Acidolysis	Lipozyme RM IM (Novozymes)	Groundnut oil with FAs obtained from linseed oil	[97]

2.3. Lipids rich in phenolic compounds

Phenolic compounds include flavonoids, phenolic acids, tannins, lignans and lignins among others [98, 99]. In addition to their vital biological properties, such as their antimicrobial, anticarcinogenic and antimutagenic activities, most phenolic compounds are known to be potent antioxidants [100, 101, 102]. Dietary oils, which are recommended in healthful nutrition, often contain high amounts of PUFAs, which are particularly sensitive to oxidation. Therefore, the oxidation potential of fats and oils rich in PUFAs, such as vegetable oils, should be considered in the processing of these products. The natural content of polyphenol plays an important role in extra-virgin olive oil because phenols protect the TAG from oxidation and contribute to their flavor and aroma [103]. Studies also indicate that the high content of polyphenols in extra-virgin olive oil is responsible for its positive health effects [104, 105]. Almost all vegetable oils contain phenolic substances that possess antioxidant activity; however, in most of these oils, the amount of these substances is insufficient to prevent the oxidation of the TAGs that are also present in the oil. Therefore, the inclusion of additional antioxidants is desirable in these cases. Although synthetic antioxidants could be used, natural antioxidants are preferred in dietary oils and other healthful lipids products [106]. In addition, the incorporation of phenolic acids into TAGs could result in the production of novel modified phenolic lipids that could impart both the physiological and the biological health benefits of PUFAs and phenolic acids [107]. Products that naturally contain phenols as esters of the PUFAs have been reported to have significantly improved anti-inflammatory activities, as well as antiviral and anticancer activities, which were not present in the original phenolic molecule. This finding suggests that PUFA moieties contribute to the bioactivities of the ester derivatives [108]. However, the use of phenols as antioxidants in fat and oils is limited by their hydrophilic nature [109, 110].

Therefore, it is necessary to alter the chemical structure of these compounds, possibly to improve their solubility and expand their use in emulsion systems [111]. The use of lipases in modifying these phenolic compounds has shown encouraging results.

The lipase-catalyzed esterification of phenolic acids with aliphatic alcohols can be used to alter the phenolic solubility in oil-based formulas and emulsions. Dihydrocaffeic acid, a metabolite of caffeic acid, can be found in many fruits and vegetables. Caffeic acid, similar to other phenolic acids, has several proven biological activities [112, 113]. The synthesis of a phenolic ester from dihydrocaffeic acid and octanol was recently published [114]. The novel modified lipids that were obtained are likely to confer ultraviolet radiation protection in addition to their improved emulsifying capability. The introduction of aromatic groups, which is the case in the octyl dihydrocaffeate compound, into a structured lipid is most likely to increase the protection against UV rays [115]. The authors conclude that because the structures of the new lipids have both hydrophobic (linear carbon chain) and hydrophilic (phenolic) groups, a greater activity in the oil-water interface is reached with these compounds. Viskupicova *et al*. [116] synthesized lipophilic derivates of flavonoids in lipophilic food matrices via the enzymatic esterification of rutin, a type of flavonoid, with different saturated and unsaturated FAs. Flavonoids play an important role in the prevention and/or treatment of several diseases that involve uncontrolled lipid peroxidation [117]. The authors observed that selective modification of the rutin molecule maintains the antioxidant capacity of the initial flavonoid and more effectively inhibits the lipid peroxidation in the β-carotene linoleate system. Esters with longer fatty acid chain lengths were more effective in inhibiting lipid peroxidation. The elongation of the fatty acid chain length resulted in longer induction times (i.e., the time in which the oil was completely oxidized) and therefore improved

oxidative stability. The results indicate that the synthesized rutin derivates might be useful agents for the protection of oil/fat-based foods against oxidation during their storage or processing.

Ferulic acid, a phenolic compound, has been widely studied for its health-benefiting properties and its capacity to inhibit oxidation [118]. The preparation of lipophilic derivates of ferulic acid through enzymatic transesterification was recently evaluated [119]. The results suggest that the novel feruloylated lipids exhibited a strong effect against oxidation in lipophilic systems, which makes them promising antioxidants. Table 5 shows examples of phenolic lipids produced using lipases.

Table 5: Lipase-mediated production of phenolic lipids.

Enzyme process	Lipase	Substrate	References
Esterification and Interesterification	Novozym 435 (Novozymes)	Dihydrocaffeic acid with octanol; Produced ester and tricaprylin	[114]
Esterification	Novozym 435 (Novozymes)	Caffeic acid with octanol	[120]
Esterification	CAL B (Novozymes)	Rutin with saturated and/or unsaturated fatty acids	[116]
Acidolysis	Novozym 435 (Novozymes)	Ferulic acid with tributyrin	[119]
Acidolysis	Lipozyme IM-20 (*Rhizomucor miehei*)and Novozym 435 (Novozymes)	Dihydroxyphenylacetic acid with fish liver oil	[121]
Esterification	Novozym 435 (Novozymes)	Rutin and naringin with MUFA and PUFA	[122]

The studies presented show the potential of lipases for the production of special lipids with potential industrial applications. In addition to the studies mentioned in this review that employ commercial immobilized enzymes, many other research studies using different enzymes in the modification of oils and fats are being carried out with promising results [123, 124, 125]. Consequently, studies on the isolation, identification and characterization of novel lipases are of great importance. A few of these new enzymes, which can be immobilized on different supports, have been successfully used in the modification of lipids; this finding illustrates the great capacity of lipases on the synthesis of novel lipids. Our laboratory has been working on finding new lipases and esterases that exhibit great potential for the catalysis of hydrolysis and synthesis reactions [126, 127, 128, 129, 130, 131].

3. Conclusions

The studies described in this review report the satisfactory results that were obtained by the use of lipases in the modificationof oils and fats. However, there is still much more research that can be performed.The search for new sources of lipases with greater activity and specificity and the exploration of new sources of vegetable oils and fats are several examples of the challenges in this area of biotechnology. In addition, the screening of new oilseed crops can lead to the identification of new and unusual fatty acids, such as very long chain fatty acids. The use of 1,3-positional specific lipases produced high-value TAGs and FAs more quickly and less expensively than did other methods. Based on the demonstrated potential of lipases, the oil industry has shown considerable interest in using these lipases to catalyze the modification of fats and oils.

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