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INSTITUTO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E
TECNOLOGIA DE ALIMENTOS

TESE

**Microencapsulação de ômega-3 empregando emulsões
simples estabilizadas por biopolímeros.**

Juarez Vicente

2016



**UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
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DE ALIMENTOS**

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SIMPLES ESTABILIZADAS POR BIOPOLÍMEROS.**

JUAREZ VICENTE

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Tese submetida como requisito parcial para obtenção do grau de **Doutor em Ciências**, no Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, na área de concentração em ciência de alimentos.

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RESUMO

VICENTE, Juarez. **Microencapsulamento de óleo de Sacha Inchi empregando emulsões simples e estabilizadas com biopolímeros**. 2016. 163p. Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia. Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2015.

O ômega-3 (ω -3) é uma classe de ácidos graxos essenciais, de reconhecida importância para o metabolismo de diversas funcionalidades do organismo, destacam-se a prevenção de doenças cardiovasculares, imunológicas e anti-inflamatórias, câncer de cólon, favorece o desenvolvimento cerebral e da retina. Os ácidos graxos das séries ω -3 e ω -6 são precursores dos ácidos graxos poli-insaturados de cadeia longa, como araquidônico (AA), eicosapentaenoico (EPA) e docosahexaenóico (DHA), este último considerado o mais importante para o desenvolvimento neonatal e junto com o AA são os principais componentes dos ácidos graxos cerebrais. A microencapsulação empregando a formação de emulsões simples é uma estratégia que objetiva manter a estabilidade do ômega-3 e a permanência de suas atividades funcionais frente à exposição do mesmo a condições adversas como processos oxidativos. Os biopolímeros como proteínas e carboidratos (naturais) são componentes que auxiliam na manutenção da estabilidade física e morfológica destas emulsões empregadas para microencapsulação. Da mesma forma, conferem ao produto melhorias nas propriedades reológicas, nutricionais e funcionais. Com isso, objetiva-se nesta tese estudar a formação de micro-emulsões física e morfológicamente estáveis e seus comportamentos reológicos, propriedades calorimétricas e estabilidade oxidativa do ômega-3 nos sistemas poliméricos formados com Ovalbumina (surfactante natural) e suas interações com polissacarídeos naturais (Pectina e Goma Xantana), comparado a um sistema padrão com surfactante sintético (Tween 80) na microencapsulação do ômega-3 do óleo de Sacha Inchi (*Plukenetia volubilis* L.). Com esse trabalho objetiva-se também o estudo do comportamento oxidativo do óleo de Sacha Inchi e sua inserção como componente em outros alimentos na forma microencapsulada.

Palavras chave: microemulsão, goma xantana, pectina, óleo de Sacha Inchi, RMN ^1H e CG-EM.

ABSTRACT

VICENTE, Juarez. **Microencapsulation of Sacha Inchi oil employing simple emulsions stabilized with biopolymers.** 2016. 163p. Thesis (PhD in Food Science and Technology). Technology Institute. Federal Rural University of Rio de Janeiro, Seropédica, RJ.

The omega-3 (ω -3) is a class of essential fatty acids, of major importance for the metabolism of various functions of the body, highlight for the prevention of cardiovascular diseases, immunological and anti - inflammatory, colon cancer, promotes developing brain and retina. The fatty acids of the series ω -3 and ω -6 are precursors of polyunsaturated long chain fatty acids as arachidonic (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the latter considered the most important for developing neonatal and together with the AA are the main components of brain fatty acids (Silva et al., 2007). Microencapsulation employing the formation of simple emulsions is a strategy that aims to maintain the stability of omega-3 and permanence of their functional activities upon exposure to the same harsh conditions as oxidative processes. Biopolymers such as proteins and carbohydrates (natural) are components that assist in maintaining the physical and morphological stability of these emulsions employed for microencapsulation. Likewise, to give the product improvements in the rheological, nutritional and functional properties. Thus, this thesis aims to study the formation of micro-emulsions physically and morphologically stable and their behavior rheological properties of heat and oxidative stability of omega-3 in polymeric systems formed with ovalbumin (natural surfactant) and their interactions with natural polysaccharides (pectin and xanthan gum), compared to a standard system with synthetic surfactant (Tween 80) in the microencapsulation of omega-3 of Sacha Inchi (*Plukenetia volubilis* L.) oil. With this work we aim to also study the behavior of oxidative Sacha Inchi oil and its inclusion as a component in other foods in microencapsulated form.

Keywords: microemulsion, xanthan gum, pectin, Sacha Inchi oil, ^1H NMR, GC-MS.

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INTRODUÇÃO GERAL

As técnicas utilizadas para encapsulamento (como o microencapsulamento) visam assegurar, proteger e estender a vida útil de substâncias ou componentes alimentícios de importância nutricional, nutracêutica (bioativos), ou com alguma característica de interesse científico ou industrial.

O apelo de profissionais de saúde para o consumo de alimentos saudáveis requer muitas mudanças de hábitos que vão além das restrições alimentares no dia-a-dia dos consumidores. Essas mudanças certamente surtem impacto positivo na saúde, porém são muitas vezes interrompidas por falta de tempo, pelo cansaço, pela demora ou inadequado preparo dos alimentos e certamente pelo custo.

O encapsulamento de substâncias bioativas pode configurar como uma alternativa viável para o consumo ou dieta de componentes de interesse funcional sem que haja a necessidade de preparo de alimento e de mudanças na rotina diária dos consumidores para que se consiga ter uma alimentação ou suplementação saudável. Existem atualmente, diversas formas para encapsular compostos bioativos, seja para a produção de cápsulas para suplementação alimentar, como para inserir esses compostos de interesse em alimentos industrializados (fortificação de alimentos), tornando-os mais saudáveis.

As técnicas utilizadas, entretanto, apresentam vantagens e desvantagens de acordo com seu uso apropriado a cada tipo de substância a encapsular, na estabilidade química e/ou física da capsula e do componente, no tempo necessário para a operação, no gasto de energia, no impacto ambiental e em termos financeiros envolvidos. As técnicas mais utilizadas são atomização, extrusão, liofilização, coacervação e a encapsulação pela formação de emulsões, simples, que são discutidas nesta tese.

Os compostos mais utilizados para encapsulação na indústria de alimentos são pigmentos naturais como carotenoides e antocianinas, terpenos, aromatizantes, ésteres, microrganismos e compostos com interesses funcionais, como o ômega-3. A formação de emulsões para a encapsulação pode ser ainda mais estável quando associada a biopolímeros, como carboidratos e proteínas. A associação de carboidratos e proteínas na composição também melhora as suas características funcionais e tecnológicas do encapsulado.

O capítulo 1 desta tese trata da abordagem teórica acerca dos temas explorados nos capítulos seguintes. Em suma, contempla uma revisão sobre microencapsulação, emulsões, sobre a importância do ômega-3 e do uso da ressonância magnética nuclear como técnica de

avaliação da estabilidade do óleo de Sacha Inchi. No segundo capítulo, apresentamos o primeiro artigo experimental publicado desta tese, onde descrevemos uma proposta de identificação e quantificação das unidades acila de ômega-3 do óleo de Sacha Inchi usando RMN de ^1H comparados com GC-FID.

O capítulo 3 trata do estudo das propriedades de formação, estabilidade cinética e caracterização de emulsões simples (O/A) formadas por óleo de Sacha Inchi e Ovalbumina ou Tween, em várias proporções, e estabilizadas por Pectina de alta metoxilação ou Goma Xantana. De algumas emulsões estáveis obtidas, seguimos com o estudo das propriedades termogravimétricas, cristalinidade, características físicas (infravermelho, conteúdo de óleo, eficiência de encapsulamento) e estabilidade do óleo após o encapsulamento por RMN de ^1H . Estes resultados são apresentados no Capítulo 4.

No quinto capítulo, utilizamos os encapsulados para avaliar o efeito da simulação gástrica humana na composição do óleo de Sacha Inchi e seus encapsulados formados por biopolímeros com RMN de ^1H . Já no sexto e último capítulo, avaliamos por DSC e RMN de ^1H , a estabilidade térmica e oxidativa, em condições isotérmicas e sob atmosfera de oxigênio, do óleo de Sacha Inchi e seus encapsulados formados por biopolímeros.

OBJETIVO GERAL

Estudar os aspectos relacionados à caracterização, estabilidade e propriedades físico-químicas de óleo de Sacha Inchi e de seus encapsulados formados por emulsões simples (com Ovalbumina e Tween) e estabilizados com biopolímeros (pectina e goma xantana).

OBJETIVOS ESPECÍFICOS

- ✓ Identificar e quantificar os sinais das unidades acila de ômega-3 do óleo de Sacha Inchi usando RMN de ^1H comparados com GC-FID.
- ✓ Estudar as propriedades de formação, estabilidade cinética e caracterização de emulsões simples (O/A) formadas por óleo de Sacha Inchi e biopolímeros.
- ✓ Estudar as propriedades termogravimétricas, cristalinidade, características físicas (infravermelho, conteúdo de óleo, eficiência de encapsulamento) e estabilidade do óleo após o encapsulamento por RMN de ^1H de encapsulados formados por óleo de Sacha Inchi e biopolímeros.
- ✓ Avaliar o efeito da simulação gástrica humana na composição do óleo de Sacha Inchi e seus encapsulados formados por biopolímeros com RMN de ^1H .
- ✓ Avaliar por DSC e RMN de ^1H , a estabilidade térmica e oxidativa, em condições isotérmicas e sob atmosfera de oxigênio, do óleo de Sacha Inchi e seus encapsulados formados por biopolímeros.

CAPITULO I – REVISÃO DE LITERATURA

1.1 MICROENCAPSULAÇÃO

1.1.1 Histórico

As primeiras tentativas registradas da aplicação da técnica de microencapsulação foram executadas na década de 1930, embora os produtos encapsulados surgiram apenas na década de 1950 (RÉ, 2000; CLARK, 2002; SUAVE et al., 2006). Desde então, a microencapsulação vem sendo empregada em diversas áreas do conhecimento, em especial no setor farmacêutico, permitindo o desenvolvimento de fórmulas de liberação controlada de fármacos que apresentam a capacidade de liberar o agente ativo apenas no local ou órgão onde este deve agir (SUAVE et al., 2006). As pesquisas em fármacos foram inicialmente realizadas na Universidade de Wisconsin, EUA, onde as microcápsulas produzidas foram utilizadas para aumentar a estabilidade de medicamentos e para modificar ou retardar sua liberação em locais específicos de ação no organismo humano e de interesse nos experimentos (RÉ, 2000; MOREIRA, 2007).

O setor de agrotóxicos é também um ramo que vem sendo muito explorado, buscando diminuir a toxicidade e a contaminação ambiental com a criação de fórmulas de liberação controlada de defensivos agrícolas. Outras áreas como cosméticos, pigmentos, adesivos e em especial a área de alimentos também merecem destaque (SUAVE et al., 2006).

Na área de alimentos, observam-se aplicações das mais diversas, como encapsular pigmentos e/ou corantes naturais, como carotenóides e antocianinas; para estabilizar compostos bioativos (como lipídeos e peptídeos) e conferir aos alimentos propriedades nutracêuticas; óleos essenciais, ácido ascórbico, enzimas, antioxidantes, preservar propriedades de cor, aroma, *flavor*, estabilidade de vitaminas, mascarar gostos e odores indesejáveis, entre outras aplicações (BARBOSA & MERCADANTE, 2008); (CHANDRA et al., 1993; GRADINARU et al., 2003); (KIRBY et al., 1991; UDDIN et al., 2001; LEE et al., 2004; RIGHETTO & NETTO, 2006); (LEIMANN, 2008); (BARBOSA & MERCADANTE, 2008); (GONSALVES, et al., 2009); (REBELLO, 2009).

Há um consenso mundial de que os alimentos não devam mais ser vistos somente como uma fonte de nutrientes com apelo sensorial, mas que também sejam fonte de bem estar e saúde. Em função disso, há a necessidade de desenvolvimento de tecnologias de conservação de alimentos capazes de preservar ao máximo os componentes naturais presentes

no alimento e de tecnologias inovadoras para conferir características e propriedades desejáveis do ponto de vista funcional, sensorial e/ou nutricional (REBELLO, 2009).

Um ponto importante na produção de um produto microencapsulado é a escolha do material encapsulante, geralmente um polímero, que deve ser selecionado em função das propriedades físico-químicas do agente ativo, da aplicação pretendida e do método de formação das micropartículas (SUAVE et al., 2006).

O método de microencapsulação é de extrema importância para a indústria de alimentos (SUAVE et al., 2006) e pode servir como uma estratégia para a valorização, manutenção da qualidade de compostos bioativos em alimentos e de liberação em locais específicos e de interesse nutricional e/ou nutracêutico.

1.1.2 Conceitos

Microencapsulação pode ser definida como a "tecnologia de empacotamento de materiais sólidos, líquidos e gasosos em pequenas cápsulas que liberam seus conteúdos (recheios) a taxas controladas ao longo de determinados períodos de tempo" (CHAMPAGNE & FUSTIER, 2007).

Encapsular significa envolver ou capturar uma substância em outra. O núcleo é chamado de 'encapsulado', que pode ser um líquido, um sólido ou um gás. Já o material que o recobre é chamado de 'encapsulante', 'cobertura', 'parede' ou 'matriz' e é geralmente um polissacarídeo ou um peptídeo (CLARK, 2002).

O conceito de microcápsula foi idealizado, seguindo o modelo celular, em que o encapsulante possui a função semelhante à da membrana, envolvendo e protegendo o citoplasma e os demais componentes, controlando a entrada e saída de material no núcleo (encapsulado) da célula (microcápsula). Generalizando, uma microcápsula consiste em uma camada de polímero que atua como um filme protetor, isolando a substância ativa (encapsulada) e evitando os efeitos indesejáveis de sua exposição inadequada (RÉ, 2000).

A microencapsulação é definida como um processo no qual uma membrana envolve partículas (em escala de μm) de sólido, líquido ou gás, a fim de, proteger o material 'encapsulado' das condições adversas do meio, tais como luz, umidade, oxigênio, calor e interações com outros compostos, com o objetivo de estabilizar o produto, aumentando a vida útil e promovendo a liberação controlada do encapsulado em condições pré-estabelecidas (SHAHIDI & HAN, 1993).

A microencapsulação envolve um conjunto de técnicas que permitem a preparação de partículas funcionais, constituídas por um suporte (encapsulante) e um composto ativo (distribuído em seu núcleo). A microencapsulação condiciona um controle extremamente rígido de propriedades específicas do produto, que tem a sua ação controlada ou programada (IPT, 2013).

As cápsulas podem ser classificadas em dois grupos. As que são definidas como sistema do **tipo reservatório** e classificadas como ‘microcápsulas verdadeiras’. Elas são caracterizadas por seu núcleo ser nitidamente concentrado na região central, circundado por um filme definido e contínuo do material de parede. E aquelas onde o núcleo é uniformemente disperso em uma matriz, classificadas como **sistema matricial**, resulta nas camadas microsferas (KING, 1995; RÉ, 1998).

O que difere as microcápsulas das microsferas, é que nas microsferas, uma pequena fração do material “encapsulado” permanece exposta na superfície, o que é evitado pela “verdadeira” encapsulação. No entanto, o termo ‘encapsulação’ tem sido usado em seu sentido amplo, englobando tanto a formação de microcápsulas quanto de microsferas (DEPYPERE et al., 2003). As microcápsulas podem ter ainda mais de um núcleo ou várias paredes para um mesmo núcleo (CONSTANT & STRINGHETA, 2002). A Figura 1.1 ilustra os principais modelos de microcápsulas.

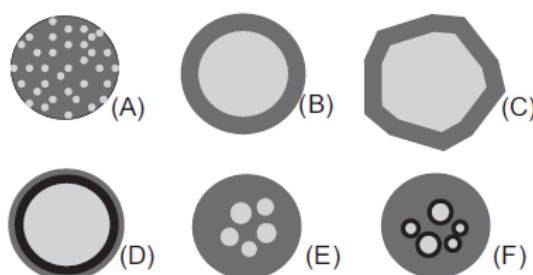


Fig. 1.1 – Modelos de microcápsulas:

(A): matriz (microsfera) – produzido por atomização; (B): microcápsula simples; (C): microcápsula simples e irregular; (D): microcápsula de duas paredes; (E): microcápsula com vários núcleos e; (F): agrupamento de microcápsulas (ARSHADY, 1993; GIBBS, 1999).

A Figura 1.2, no entanto, apresenta a distribuição do encapsulado nos sistemas possíveis, microcápsula e microsfera.



Fig. 1.2 – Modelos de microcápsula e microesfera.

Fonte: Alvin, 2012.

As microesferas funcionam como bons reservatórios para substâncias ou componentes solúveis em água que podem ser liberados, sob condições controladas, a partir das microcápsulas, após reconstituição (GARTI, 1997).

A encapsulação é realizada com os objetivos de: proteger o encapsulado das influências ambientais como luz, oxigênio, umidade, interação com outros componentes alimentares ou ingredientes, manter um encapsulado no interior do núcleo do pó formado; evitar a degradação físico-química do componente encapsulado; controlar a liberação do material presente no núcleo; retardar alterações que podem resultar em perda de aroma, alteração de cor ou perda do valor nutricional; separar componentes reativos ou incompatíveis; evitar reações prematuras de um substrato; mascarar compostos de sabor indesejáveis; promover melhor solubilidade do núcleo e melhor incorporação em sistemas secos (CLARK, 2002; RÉ, 2000; REINECCIUS, 1991; PSZCZOLA, 1998; DEPYPERE et al., 2003; MOREIRA, 2007).

Os efeitos positivos das técnicas de microencapsulação sobre a estabilidade à degradação química de compostos são citados para as antocianinas (CHANDRA et al., 1993; GRADINARU et al., 2003), ácido ascórbico (KIRBY et al., 1991; UDDIN et al., 2001; LEE et al., 2004; RIGHETTO & NETTO, 2006), óleo essencial de capim limão (LEIMANN, 2008), bixina (BARBOSA & MERCADANTE, 2008), para óleo essencial de *Citrus sinensis* (*L*)*Osbeck* (GONSALVES, et al., 2009) dentre outros compostos.

A técnica de microencapsulação tem solucionado limitações no emprego de ingredientes alimentícios, visto que pode suprimir ou atenuar sabores e odores indesejáveis,

reduzir a volatilidade e a reatividade e aumentar a estabilidade destes em condições ambientais adversas, como na presença de luz, oxigênio e pH extremos (REBELLO, 2009).

Esta técnica é aplicada em vários seguimentos, como farmacêuticos, cosméticos e as indústrias de alimentos. Quando utilizada em aplicações para a indústria de alimentos, diversos fins podem ser objetivados: i) proteger e estabilizar o produto ou material encapsulado contra possíveis danos causados por condições externas adversas, como por exemplo, a luz ou o calor durante o processamento e armazenagem de alimentos, ii) o controle da libertação do encapsulado, iii) retardar alterações que possam resultar em perda de aromas, perdas do valor nutricional ou alterações de cor; iv) garantir a homogeneidade das características sensoriais e nutricionais do material encapsulado; iv) separação de componentes reativos; v) reduzir a taxa de migração do material encapsulado para o ambiente externo; vi) evitar reações que geram compostos com sabores, odores ou qualquer propriedade indesejável; vii) melhorar a solubilidade do núcleo permitindo a inserção do encapsulado em alimentos secos ou formulados (AZEREDO, 2005; CHAMPAGNE & FUSTIER, 2007; DE VOS ET AL, 2010;. LOVEDAY & SINGH, 2008).

Além disso, enquanto a microencapsulação aumenta a capacidade de dispersão de compostos com baixa solubilidade em água, a mesma, não deve alterar as propriedades sensoriais, a cor ou o sabor dos produtos alimentares. Em relação ao encapsulamento de ingredientes alimentícios, a literatura é extremamente abundante e até mesmo em relação a compostos com propriedades funcionais, como é o caso do ômega-3 (GONNET, LETHUAUT & BOURY, 2010; LOVEDAY & SINGH, 2008).

1.1.3 Métodos para microencapsulação

Diversos são os métodos possíveis para encapsular um material ativo, a depender da aplicação, do tipo de material e do mecanismo de liberação desejado. O que difere os métodos é o tipo de “aprisionamento” do material ativo pelo agente encapsulante, podendo ser de natureza física, química ou físico-química (JACKSON & LEE, 1991; RÉ, 2000; MOREIRA, 2007; SHAHIDI & HAN, 1993; DESAI & PARK, 2005; MADENE et al., 2006; REBELLO, 2009; ALVIN, 2012).

Métodos Físicos – spray drying, spray chilling, spray cooling, leiteo fluidizado, extrusão centrífuga, extrusão estacionária, co-cristalização e liofilização;

Métodos Químicos – polimerização interfacial, indução molecular e polimerização in

situ;

Métodos Físico-químicos – coacervação simples, coacervação complexa, separação por fase orgânica, geleificação iônica, lipossomas, lipoesferas (nanopartículas) e evaporação do solvente.

A escolha do método a se utilizar depende de fatores como: tamanho da partícula desejado (micro ou nano), propriedades físicas e químicas do núcleo e da parede, aplicação da cápsula no produto final que se destina, mecanismos desejados de liberação do encapsulado, escala de produção e certamente o custo (AZEREDO, 2005; MARTIN VILLENA et al., 2009). Os métodos mais utilizados, no entanto são: atomização, extrusão, liofilização, leiteo fluidizado, inclusão de lipossomas e coacervação (REBELLO, 2009).

1.1.3.1 Atomização

A secagem por atomização (*spray drying*) é um dos métodos mais empregados para a microencapsulação, devido à grande disponibilidade de equipamentos, baixo custo do processo, possibilidade de emprego de uma ampla variedade de agentes encapsulantes, boa retenção dos compostos voláteis e estabilidade do produto final (REINECCIUS, 1989).

É uma técnica relativamente barata, a mais utilizada pela indústria de alimentos e umas das mais antigas técnicas para microencapsulação. Tem sido utilizada desde 1930 para encapsular aromatizantes (AZEREDO, 2005; DZIEZAC, 1988).

O material a ser encapsulado (geralmente hidrofóbico) é emulsificado em uma solução aquosa. Essa emulsão (O/A) é então bombeada por um atomizador a uma câmara em alta temperatura. À medida que as partículas são lançadas no meio aquoso, tomam a forma esférica, de modo que a fase oleosa seja “empacotada” no interior da fase aquosa. Quando entra em contato com o ar aquecido, a água é evaporada rapidamente, pois a superfície de contato da partícula sendo alta, permite por consequência uma alta taxa de evaporação em curto tempo de exposição, impedindo que o material a ser encapsulado permaneça por um longo tempo de contato com o aquecimento, evitando a degradação térmica, e reduzindo assim, a ocorrência de alterações indesejáveis (MUKTA & NELSON, 1988; DZIEZAC, 1988; AZEREDO, 2005).

As cápsulas produzidas por atomização geralmente são do tipo matricial e a liberação do material presente no núcleo se dá pelo mecanismo de difusão ou por ação de solventes. Uma das principais vantagens do emprego do método de atomização é de se poder trabalhar

com substâncias termolábeis; outra vantagem é a geração de partículas menores que 100µm (RÉ, 1998; AZEREDO, 2005).

A produção de microcápsulas pelo método de atomização, utilizando goma arábica e grânulos porosos de amido/gelatina como agentes encapsulantes, pode representar uma alternativa viável para proteger carotenóides presentes em pigmentos contra a oxidação. Este processo gera pós de fácil solubilidade, o que é conveniente para a indústria de alimentos (SANTOS, FÁVARO-TRINDADE & GROSSO, 2005).

1.1.3.2 Extrusão

A tecnologia de extrusão tem um papel importante na indústria de alimentos como um eficiente processo de fabricação. Essa tecnologia foi desenvolvida para melhor transportar e moldar matérias-primas processadas, como massas e pasta fluidas. A tecnologia de cozimento por extrusão é utilizada para o processamento de cereais e de proteínas em alimentos e, está intimamente relacionada com a tecnologia de *petfoods* e ao ramo de produção de rações (GUY, 2000).

Atualmente, as funções desta tecnologia incluem o transporte, a mistura, as propriedades de cisalhamento, separação, aquecimento ou arrefecimento, modelagem, co-extrusão, liberação de umidade e substâncias voláteis, a geração de sabor, encapsulamento e esterilização. São utilizados para processar em temperaturas relativamente baixas, como em massas e produtos peletizados (*pellets*), ou em altas temperaturas como em pães e *snacks* (GUY, 2000).

A extrusão se baseia na conversão de um material sólido ao estado de massa fluida, combinando fatores de umidade, calor, compressão e tensão de cisalhamento. Desta forma, ocorre a gelatinização do amido e/ou a desnaturação da proteína presente no alimento (a depender da sua composição). Têm-se dois tipos de extrusão: a quente e a frio. Na extrusão a quente, o alimento é cozido e na extrusão a frio ocorre uma mudança de conformação (UFRGS, 2013).

As condições de processo durante a extrusão, (temperatura, pressão, diâmetro de saída do orifício, velocidade e rotação do parafuso) bem como as propriedades reológicas (textura, viscosidade, composição química e estado físico) do alimento exercem grande influência sobre o produto final (UFRGS, 2013).

O objetivo da extrusão é conferir aos produtos modificações na sua textura, sabor, aroma e forma de apresentação, além de aumentar a conservação dos mesmos em função da baixa atividade de água imposta (UFRGS, 2013).

A extrusão como método de encapsulamento envolve a dispersão do composto a encapsular em uma massa fundida de carboidrato. Essa massa é forçada a passar por peças moldadas e a seguir em direção a uma solução de isopropanol (ou outro álcool desidratante) para endurecer a cobertura. Ao endurecer a mistura, em contato com o isopropanol, é removida da superfície qualquer porção oleosa. O material então extrusado é quebrado em partículas menores, separado e seco (RISCH & REINECCIUS, 1995; AZEREVO, 2005).

A extrusão como método para encapsulamento é desejada quando se objetiva a visualização de fragmentos, já que são geradas partículas com granulometria menor. Como o material presente no núcleo fica ausente da superfície na encapsulação, geram-se produtos com maior estabilidade (RISCH & REINECCIUS, 1995; AZEREVO, 2005).

Os alginatos, que são hidrocolóides aniônicos, são utilizados por este método de encapsulação pois são capazes de formar géis em contato com soluções salinas (KING, In: RISCH & REINECCIUS, 1995). A encapsulação por extrusão é utilizada para compostos termolábeis (RISCH, In: RISCH & REINECCIUS, 1995; AZEREVO, 2005).

1.1.3.3 Liofilização

A liofilização tem como base a desidratação por sublimação de um produto congelado. Consiste no congelamento preferencialmente rápido do produto e posterior sublimação do gelo sob vácuo (MAYLAND, 1968; AZEREDO, 2005).

Tanto o congelamento quanto o vácuo são, por si só, processos onerosos e, quando os liofilizadores são de sistemas de produção em batelada (*batch*) isto aumenta os custos de funcionamento, bem como diminui o rendimento. Liofilizadores contínuos apresentam melhores rendimentos, mas o custo inicial de implantação é mais elevado (GREENSRNITH, 1998).

A liofilização remove a água e outros solventes do produto congelado pelo processo de sublimação. A sublimação ocorre quando a água no estado sólido é convertida diretamente em vapor de água, sem passar pelo estado líquido, como observado na Figura 1.3. (RODRIGUES, 2008; VASCONCELOS & MELO FILHO, 2010).

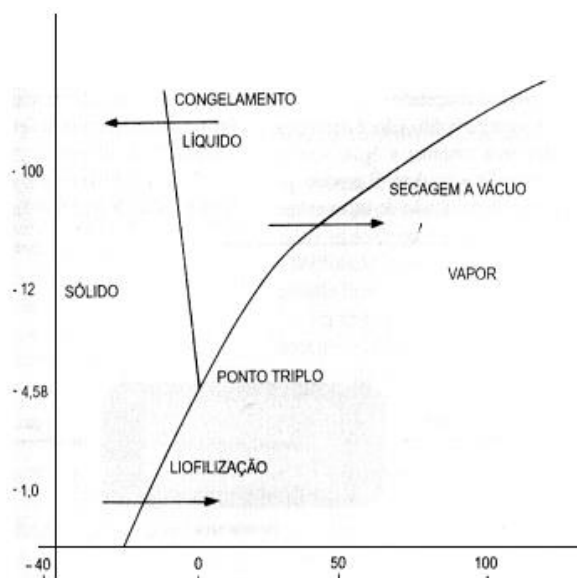


Fig. 1.3 – Ilustração da sublimação, abaixo do ponto triplo da água.

Em termos simples, a liofilização é um método de secagem de um produto no qual o conteúdo de água é convertido primeiramente para a fase sólida (gelo) e, em seguida, transformado em vapor (fase gasosa), sem passar pela fase aquosa (estado líquido). Como consequência, uma vez que o gelo tem um volume maior do que a água, o congelamento do produto faz expandi-lo, em função da pressão causada pela formação de gelo. Esta expansão não é seguida por igual contração, pois o gelo sublima para o estado de vapor sob condições de vácuo, entretanto o alongamento do sistema capilar no produto permanece estável. Isto auxilia na rápida reidratação e confere uma textura uniforme ao produto (GREENSRNITH, 1998).

Este processo tende a danificar menos o tecido vegetal que está sendo desidratado quando comparado a outros métodos da desidratação, que geralmente utilizam temperaturas mais elevadas (UFRGS, 2013b).

A liofilização ocorre quando a temperatura e a pressão parcial do vapor da água forem inferiores às do ponto triplo da água (610 Pa à temperatura de 0,01°C, para a água pura) (RODRIGUES, 2008).

Pode-se dividir o processo de liofilização nas seguintes etapas: (i) pré-congelamento (< 0°C); (ii) Secagem Primária (eliminada 90% da água – produto com 15% de umidade); (iii) Secagem Secundária (eliminada 10% da água – água ligada – produto desidratado até 2% de umidade) (RODRIGUES, 2008).

No congelamento do produto, formam-se pequenos cristais, em função da

diminuição da temperatura que permita a menor quantidade possível de água para congelar. Pode se dar por convecção forçada (para legumes, carnes e pescado), leite fluidizado (para legumes com tamanhos menores), por condução em cilindros rotativos (para alimentos líquidos) ou ainda através de criogênica (azoto líquido) (RODRIGUES, 2008).

Na secagem primária ocorre sublimação do gelo controlando a pressão de vácuo e o aumento da temperatura (calor latente de sublimação -2840 kJ/kg). Na secagem secundária ocorre eliminação da água ligada por evaporação a vácuo, aumento da temperatura (20-60°C), podendo ocorrer colapso. Este processo se estende até que seja atingido o conteúdo de água de 2%. O final do processo é controlado pela temperatura no produto ou pelo seu peso (RODRIGUES, 2008).

Como a liofilização é realizada à baixa temperatura e ausência de oxigênio atmosférico, as propriedades químicas e organolépticas dos alimentos liofilizados permanecem praticamente inalteradas (VASCONCELOS & MELO FILHO, 2010).

O acondicionamento da matéria-prima é um processo caro, exige matéria-prima de qualidade e a aplicação de tratamentos prévios adequados como: seleção, limpeza (lavagem), inativação enzimática (tratamento térmico ou químico), preparação do produto (corte em produtos sólidos ou pré-concentração em produtos líquidos) (RODRIGUES, 2008).

O armazenamento do produto desidratado dependerá do seu conteúdo residual de água, que é um fator crítico e que afeta a vida do produto em armazenado. Geram-se neste processo produtos higroscópicos, sensíveis à oxidação e devem receber uma embalagem a vácuo ou serem acondicionados em atmosfera protetora de azoto ou CO₂ (RODRIGUES, 2008; VASCONCELOS & MELO FILHO, 2010).

A liofilização é uma tecnologia largamente utilizada em países da América, é uma técnica considerada economicamente viável quando aplicada em larga escala da operação e a materiais com alto valor agregado, como carnes, aves, mariscos, temperos, condimentos, especiarias, chás e algumas especialidades não alimentares (GREENSRNITH, 1998).

Rossi & Catelli, em Parma na Itália, liofilizaram um lote de frangos com uma capacidade de produção de entrada no liofilizador de 3125Kg em uma única câmara, assumindo que o teor de sólidos totais do produto é de 20%, produziram 625Kg de produto desidratado em um dia de trabalho. Para um grande volume de produção, duas ou mais unidades poderiam ser utilizadas em bateria, permitindo mais eficiência na liofilização e, desse modo, reduzindo custos (GREENSRNITH, 1998).

Os parâmetros de temperatura, intensidade de vácuo e gradiente de aquecimento de

um liofilizador são todos programados no começo da operação, e são controlados automaticamente para a duração do ciclo, que finaliza quando o produto atinge a umidade pré-determinada (GREENSRNITH, 1998).

A liofilização é um processo de desidratação a frio que pode ser usado para preservar alimentos perecíveis, princípios ativos e bactérias. São determinantes para a manutenção da qualidade nutricional de alimentos, a baixa pressão e temperatura envolvidos no processo, pois as proteínas, assim como as vitaminas ficam protegidos das reações enzimáticas e oxidativas responsáveis pelas perdas nutricionais (UFRGS, 2013b).

A oferta de alimentos liofilizados em supermercados tem sido cada vez maior, devido a manutenção dos nutrientes, da qualidade sensorial e da facilidade de reidratação, tornando o alimento (no caso dos frutos) mais próximos de seu estado *in natura* (UFRGS, 2013b).

Entre os vegetais alimentícios em que se obtém os melhores resultados com a aplicação da liofilização são: abacaxi, maracujá, morango, banana, suco de frutas, coco, legumes diversos, cogumelo, milho, alho, cebola e extrato de café (UFRGS, 2013b).

A liofilização de uma emulsão pode ser aplicada para a encapsulamento de um componente. O método gera produtos de excelente qualidade, pois minimiza as alterações associadas a altas temperaturas (AZEREDO, 2005).

1.1.3.4 Coacervação

A coacervação refere-se a um sistema coloidal de separação de fases. Uma das fases, rica em um coloide separa-se da outra fase (solução de uma macromolécula), tendo como resultado a diminuição da solubilidade, que pode ser por alteração de algum fenômeno físico e/ou químico, por exemplo, pH, temperatura, força iônica, etc (AZEREDO, 2005; DODGE, 2013).

A técnica de microencapsulação mais antiga e uma das mais utilizadas envolve a separação de fases por coacervação (VILA JATO, 1999; SUAVE et al., 2006). O processo de coacervação foi desenvolvido pela *National Cash Register Corporation* (NCR), EUA, aplicado inicialmente no desenvolvimento de cápsulas contendo corantes que foram impregnados em papéis para substituir o papel carbono (RÉ, 2000; MOREIRA, 2007).

O termo coacervação descreve o fenômeno de agregação macromolecular formando um sistema coloidal em que existem duas fases líquidas: uma rica (coacervado) e a outra

pobre em colóides (sobrenadante) (VILA JATO, 1999). Consiste na deposição do polímero ao redor do agente ativo a ser recoberto pela alteração das características físico-químicas do meio, tais como temperatura, força iônica, pH ou polaridade (WATTS et al., 1990).

A coacervação consistiu-se de três etapas: (i) formação de um sistema trifásico, uma fase líquida (veículo), uma fase com o material a ser encapsulado e outra com o encapsulante; (ii) formação do núcleo, que é a deposição do material a ser encapsulado e; (iii) solidificação do material encapsulante (RISCH, In: RISCH & REINECCIUS, 1995; AZEREVO, 2005).

O método de coacervação geralmente produz compostos do tipo reservatório (microcápsulas) (AZEREVO, 2005);

A coacervação pode ser realizada em meio aquoso ou orgânico, dependendo das propriedades físico-químicas do polímero que será empregado e do material a ser encapsulado (SUAVE et al., 2006).

A coacervação pode ser dividida em: (i) simples (por alteração de pH, temperatura e/ou força iônica) ou (ii) complexa (quando ocorre complexação ou neutralização mútua entre dois polieletrólitos carregados com cargas opostas (KRUIF & TUINIER, 2001; KO et al., 2002; CAMILO, 2007). Para aumentar a estabilidade do complexo coacervado, forma-se uma parede enrijecida ao redor do núcleo, pela adição de um composto de reticulação, em geral glutaraldeído, o que o torna insolúvel (CAMILO, 2007).

1.1.3.5 Micro ou nanoemulsões

A formação de micro ou de nanoemulsões pode ser considerado um método de encapsulamento. Diversas técnicas podem ser aplicadas nesta metodologia com o objetivo de fornecer energia à emulsão para a diminuição do tamanho de suas partículas a que se deseja encapsular. Este método produz um material encapsulado até a escala micro (1 – 100µm) ou nano (1 – 100 nm), que pode ser através de sonicação, agitação magnética, agitação mecânica... Mais detalhes serão discutidos nesta tese no item “1.2 – Emulsões”.

1.1.3.5 Considerações na escolha do método para microencapsulação

Independentemente do método escolhido ou o mais adequado para a produção da micropartícula, a mesma pode ser realizada em três etapas principais, (i) composição do ativo

de interesse, (ii) aplicação do método mais adequado para sua produção e (iii) inserção do material de parede, como ilustrado na Figura 1.4 a seguir.

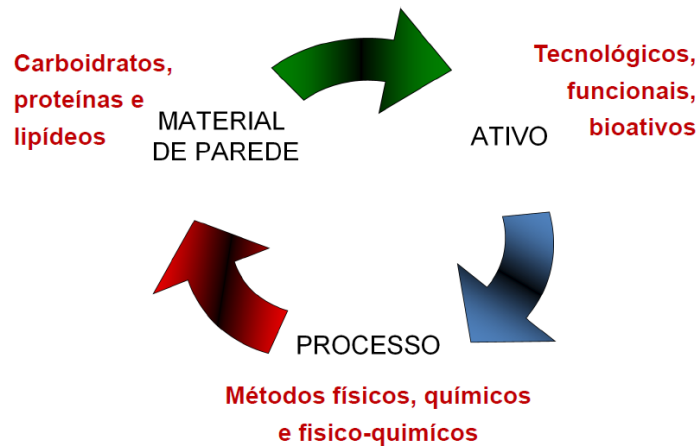


Fig. 1.4 – Sistema de produção de micropartículas.

Fonte: Alvin, 2012.

De modo geral a produção de uma microcápsula se dá em três fases, conforme ilustrado na Figura 1.5 a seguir:

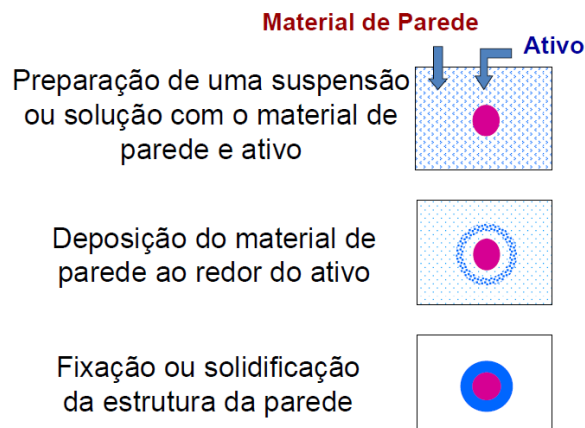


Fig. 1.5 – Etapas da produção de micropartículas.

Fonte: Alvin, 2012.

De acordo com Alvin (2012) caracteriza-se uma emulsão sob os seguintes aspectos:

- ✓ Eficiência da encapsulação;

- ✓ Rendimento do processo;
- ✓ Tamanho médio e distribuição de tamanho das partículas formadas;
- ✓ Perfil de liberação (do biocomposto);
- ✓ Morfologia superficial e interna;
- ✓ Atividade do encapsulado;
- ✓ Materiais de parede;

Diversos são as possibilidades para se compor um sistema de encapsulação de componentes alimentícios. Portanto, alguns critérios podem ser usados para distinguir estes diferentes sistemas, de modo que se possa seleccionar o sistema mais adequado para uma aplicação desejável. McClements et al., (2009) listam alguns dos mais importantes critérios a serem considerados na elaboração ou seleção de um sistema de encapsulação, a saber:

✓ *Capacidade de encapsulamento*: A capacidade de encapsulamento (CE) é uma medida da massa do material encapsulado (M_E) por unidade de massa de material de suporte (M_S): $CE = M_E/M_S$. Idealmente, um sistema de administração deve ter uma elevada capacidade de encapsulamento.

✓ *Eficiência de encapsulamento*: A eficiência de encapsulamento (EE) é uma medida da capacidade do sistema de fornecimento para reter o material encapsulado ao longo do tempo: $EE = 100 \times M_E(t)/M_E(0)$, onde $M_E(t)$ e $M_E(0)$ é a massa de material encapsulado no tempo t e 0, respectivamente. O ideal, é que a eficiência de encapsulamento permaneça o mais alta possível (100%) durante o armazenamento.

✓ *Eficiência de entrega*: A eficiência de entrega (DE) é a medida da capacidade do sistema de “entregar” o material encapsulado no local desejado de ação: $DE = 100 \times M_E(0)/M_E(A)$, onde $M_E(0)$ e $M_E(A)$ são as massas de material encapsulado no sistema de entrega inicial e o sistema entregue no local de ação, respectivamente. Idealmente, a eficiência de entrega deve ser o mais elevado possível (100%).

✓ *Mecanismo de entrega*: O sistema de entrega pode ser designado de modo que leve o componente funcional para um determinado local de ação e, em seguida, entregue-o. A entrega pode ser a uma taxa controlada, ou a resposta a um estímulo a uma condição imposta pelo meio (pH, força iónica, ação mecânica, atividade enzimática ou temperatura);

✓ *Proteção contra degradação química*: O sistema de entrega pode ser designado para proteger um material encapsulado contra algum tipo de degradação química (oxidação,

hidrólise). As taxas destas degradações químicas podem ser promovidas por fatores que devem ser controlados, como o calor, luz, oxigênio ou produtos químicos específicos.

✓ *Compatibilidade da matriz alimentícia*: O sistema de entrega deve ser compatível com a matriz alimentícia, ou seja, este não deve afetar as características de aparência, textura, *flavor* ou estabilidade do produto final.

✓ *Grau alimentício*: O sistema de entrega deve ser fabricado com ingredientes de grau alimentício (GRAS – “*generally recognized as safe*”), utilizando operações de processamento facilmente implementadas.

✓ *Biodisponibilidade*: O sistema de entrega deverá melhorar ou, pelo menos, não afetar adversamente a biodisponibilidade do componente encapsulado.

✓ *Produção econômica*: O sistema de entrega deve ser capaz de ser fabricado de maneira econômica, utilizando ingredientes e processos de baixo custo. Por fim, os benefícios obtidos a partir do encapsulamento do componente funcional (aumento da vida de prateleira, melhora na comercialização, funcionalidade) devem compensar os custos associados com o processo de encapsulamento.

1.2 EMULSÕES

“*Emulsões são dispersões de um líquido em outro líquido*”. As emulsões simples podem ser do tipo O/A (óleo em água) ou A/O (água em óleo) (FENEMMA, 2010; ULLMANN’S, 2013; SHAW, 1992).

1.2.1 Emulsões simples

Uma dispersão de no mínimo dois líquidos imiscíveis pode ser considerada uma emulsão. Um dos líquidos é denominado fase contínua, fase externa ou ainda fase dispersante enquanto que o outro líquido denomina-se fase interna ou fase dispersa da emulsão (SHAW, 1992; ULLMANN’S, 2013; McCLEMENTES, 2005).

As emulsões são sistemas que podem ser classificadas em função da sua distribuição de óleo na fase aquosa (O/A), como por exemplo, leite, creme, maionese, bebidas, sopas ou da fase aquosa na fase oleosa (A/O), como manteiga ou margarina (McCLEMENTES, 2005; CARNEIRO, 2011).

É muito comum encontrar exemplos de emulsões na área de alimentos, pois muitos deles os são. Os produtos lácteos, molhos e sopas são considerados emulsões O/A. A manteiga e a margarina, no entanto, possuem gotículas de água imersas em uma gordura plástica, quando a parte cristalina da gordura derrete produz uma emulsão A/O que instantaneamente separa-se em uma camada de óleo, por cima da camada aquosa. As gotículas de emulsões O/A também contém, a baixa temperatura, gordura em fase cristalina, não sendo, portanto, verdadeiras emulsões (FENEMMA et al., 2010).

As emulsões possuem diversas aplicações aos alimentos, atuam como estabilizantes em margarinas, maionese e sorvetes. Em pães e derivados evitam a inibição da retrogradação do amido. Inibem a aglomeração de gordura em chocolates e aumentam a solubilidade de alimentos em pó instantâneos (AZEVEDO-MELEIRO, 2009).

Emulsões óleo em água (O/A) são considerados fluidos muito importantes em função de suas propriedades e tem sido aplicados nas indústrias de petróleo, química, alimentos e na medicina (HUANG et al., 2001; ROUSSEAU, 2000). O contato entre moléculas de óleo e moléculas aquosas é energeticamente desfavorável, emulsões são considerados sistemas termodinamicamente instáveis (HARNSILAWAT et al., 2006). A estabilidade das emulsões é caracterizada através de vários mecanismos – cremeação ou sedimentação, floculação, coalescência ou pela separação de fases (GHARIBZAHEDI et al., 2012; GRANATO et al., 2010; GHARIBZAHEDI et al., 2013).

Nas emulsões O/A, as proteínas são os emulsificantes preferidos, pois são comestíveis, solúveis em água, são ativas na superfície e apresentam boa resistência a coalescência (FENNEMA et al., 2010).

De acordo com Carneiro (2011) a formação da emulsão tem papel crucial na obtenção de um bom desempenho no processo de microencapsulação. Na maioria dos alimentos, o diâmetro das gotas (encapsuladas) situa normalmente entre 0,1 e 100µm (DICKINSON, 1992; FRIBERG & LARRSON, 1997).

1.2.2 Emulsões duplas ou múltiplas

As emulsões múltiplas ou duplas (Figura 1.6) são designadas “*emulsões de emulsões*”. Nestes sistemas, as gotículas da fase dispersa contêm gotículas menores dispersas dentro delas (GARTI, 1997). Estas emulsões podem ser: (i) Emulsões água em óleo em água (A/O/A), onde a fase dispersa (óleo), possui gotículas de água dispersas em seu interior, ou

(ii) Emulsões óleo em água em óleo (O/A/O), onde a fase dispersa (aquosa), possui em seu interior gotículas de óleo dispersas.

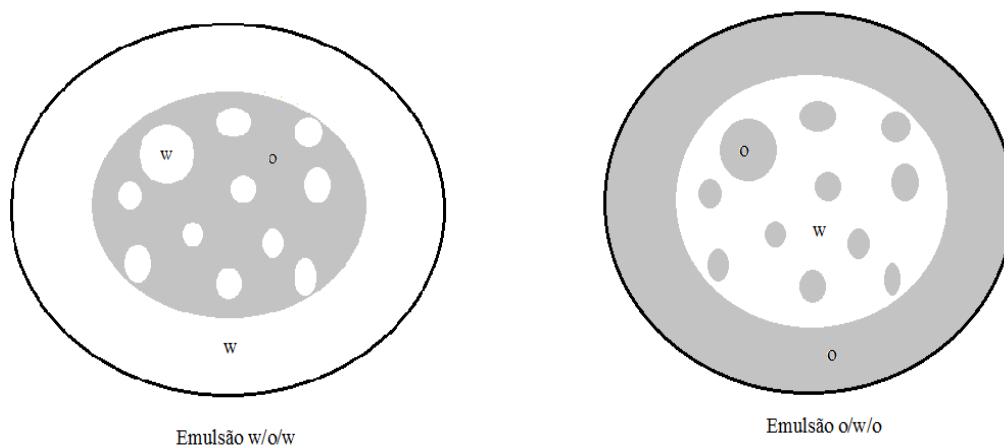


Fig. 1.6 – Emulsões duplas O/A/O e A/O/A.

As emulsões duplas são sistemas termodinamicamente instáveis, mas que podem ser estabilizadas por surfactantes lipofílicos e hidrofílicos dissolvidos na fase intermediária e na fase externa (NISISAKO et al., 2005).

Diversas indústrias do ramo farmacêutico, cosmético e de alimentos têm demonstrado interesse crescente nestes sistemas, pois apresentam um grande potencial para o controle de liberação de substâncias químicas, encapsuladas inicialmente na fase interna (NISISAKO et al., 2005).

Essas emulsões duplas, do tipo A/O/A (água em óleo em água), por possuírem mecanismos de taxa controlada de liberação de substâncias químicas, podem funcionar como um reservatório, pois há migração das substâncias da fase externa, para a interna da emulsão, tornando-se assim, útil em casos de desintoxicação ou despoluição de águas (NOGUEIRA, 2001).

O processo mais utilizado para preparação de emulsões duplas é realizado em duas etapas. Para emulsões $A_1/O/A_2$, primeiramente prepara-se uma emulsão A_1/O sob condições vigorosas de agitação ou sonicação. Esta emulsão (A_1/O) é então vertida na fase externa aquosa (A_2) sob agitação lenta ou moderada, para evitar a ruptura das cápsulas preparadas (NISISAKO et al., 2005).

O método em duas etapas apresenta problemas como a extensão na distribuição do tamanho, dificuldade de controle e reprodutibilidade do tamanho da partícula e baixo rendimento de aprisionamento. Todos são devido ao cisalhamento turbulento que ocorre no

processo. Recentemente, a técnica de membrana de emulsificação foi aplicada a formulação de emulsões duplas monodispersas, forçando emulsões individuais através de meios porosos ou pentes micro fabricados (NISISAKO et al., 2005).

Esta técnica pode produzir emulsões duplas com estreita distribuição de tamanhos e um maior rendimento de aprisionamento, mas ainda é difícil de conseguir um bom controle sobre o tamanho de gotícula interna, externa, além do número de gotas contidas em cada cápsula. Como esta técnica consiste de dois processos, isso limita a eficiência da produção (NISISAKO et al., 2005).

1.2.3 Estabilidade das emulsões

De acordo com Dickinson (2003) pode-se promover a estabilização das emulsões, adicionando emulsificante ao sistema. Os emulsificantes são moléculas classificadas como anfifílicas, pois estabilizam a emulsão formada por ação interfacial.

Outro fator que influencia muito na estabilidade das emulsões é a seleção do material de parede utilizado no processo de microencapsulação, tanto na emulsão antes da secagem como na emulsão em pó (CARNEIRO, 2011).

De acordo com Reineccius (1989) e Carneiro (2011) todas as características do material de parede “ideal” dificilmente serão reunidas em apenas um componente, desta forma faz-se o uso destes em combinação. É desejável, no entanto que o emulsificante reúna o máximo de características desejáveis, tantas quantas possíveis, como:

- ✓ Ser capaz de formar um filme na interface da microcápsula;
- ✓ Possuir baixa viscosidade em concentrações elevadas de sólidos;
- ✓ Ser pouco higroscópico;
- ✓ Ser capaz de liberar o material encapsulado quando desejável no produto final;
- ✓ Ser de baixo custo;
- ✓ Possuir alta disponibilidade e,
- ✓ Ser capaz de proteger o composto microencapsulado.

De acordo com Carneiro (2011) os processos empregados na homogeneização das emulsões, como os homogeneizadores de alta pressão, os emulsificadores ultrassônicos ou os sistemas de membrana, são processos que influenciam diretamente na adsorção da proteína à interface da cápsula, desta forma, interferem no diâmetro da gota e na estabilidade do sistema,

consequentemente produzem emulsões com propriedades físico-químicas e sensoriais diferentes.

A homogeneização por alta pressão tem o objetivo de reduzir o tamanho das gotas da “(macro)-emulsão” pré-formada (McCLEMENTS, 2005). Durante este processo, a (macro)-emulsão passa sob pressão por um orifício, chegando esse fluido a alcançar velocidade de 300m/s. A energia gerada resultante do cisalhamento, impacto e cavitação resulta na diminuição do tamanho das gotas de (macro)-emulsão à micro emulsão (ANTON et al., 2008; CARNEIRO, 2011).

Entretanto, a alta pressão utilizada pode proporcionar, durante o processo de equilíbrio dinâmico do sistema, algumas alterações físicas ou reações como a desnaturação proteica (MOZHAEV et al., 1996; CARNEIRO, 2011).

A estabilidade da emulsão é admitida quando não há mudanças perceptíveis na distribuição do tamanho das gotas em seu estado de agregação em um determinado intervalo de tempo. A instabilidade da emulsão pode ser física ou química, quando física, resulta na redistribuição espacial ou reorganização das moléculas. A estabilidade cinética deve prevenir a coalescência das gotas após a formação em um tempo razoável, conseguido quando há uma concentração alta de emulsificante durante a homogeneização. Este processo se dá pela adsorção do emulsificante (tensoativo) pela superfície das gotas, assim forma-se uma membrana protetora, que impede que as gotas que estejam muito próximas, coalesçam (McCLEMENTS, 2005).

Um dos parâmetros mais importantes e que determinam a estabilidade física da emulsão é o tamanho das gotas na emulsão. A instabilidade deste, afeta a taxa de cremação (formação de creme) e a floculação (KIM et al., 1996).

O tamanho da gota influencia na retenção do material ativo após a secagem, quanto menor o tamanho da gota, maior a retenção e menor o conteúdo de óleo na superfície da capsula após a secagem (SOOTTITANTAWAT et al., 2003)

Polissacarídeos são geralmente adicionados à fase aquosa das emulsões alimentares para melhorar a estabilidade da emulsão. Sabe-se que as propriedades reológicas das emulsões e sua estabilidade são essencialmente influenciadas pela fração de volume da fase dispersa, fatores termodinâmicos e pela composição química da fase oleosa e aquosa (SAMAVATI et al., 2012). Os polissacarídeos são compostos que aumentam a viscosidade da fase contínua das emulsões, retardando a separação gravitacional das gotículas (CHANAMAI & McCLEMENTS, 2000).

Hidrocolóides, no entanto, são conhecidos como modificadores de textura e utilizados no processamento de alimentos. A goma de xantana (GX) é um polissacarídeo extracelular de alta massa molecular produzido por bactérias do gênero *Xanthomonas campestris*. Quimicamente, a GX pode ser considerada um polieletrólito aniônico, com uma cadeia principal que consiste em β -D-glucano celulose (1 \rightarrow 4). A espinha dorsal do polímero é substituída em C- 3 em resíduos alternados de glucose com um trissacarídeo de cadeia lateral. A cadeia lateral consiste em β -*d*-manopiranosil-(1 \rightarrow 4)-(α -*d*-glucopiranosil) - (1 \rightarrow 2)- β -*d*-manopiranosídeo6-acetato (SAMAVATI et al., 2012).

1.2.3.1 Estabilidade oxidativa

A maior causa da deterioração de óleos é oxidação, tendo como consequência algumas alterações sensoriais (CARNEIRO, 2011), bem como o desenvolvimento de compostos potencialmente tóxicos (BERTON et al., 2012). Uma das principais alterações é denominada rancidez, que pode levar à rejeição do produto pelos consumidores (CARNEIRO, 2011).

De acordo com Araújo (2006), as reações oxidativas envolvem a atuação do O₂ na insaturação da cadeia carbônica de ácidos graxos. Essas reações podem ser catalisadas pela presença de luz (foto sensibilidade), de calor (termolábel) e de metais como Cobre, Zinco e Ferro.

Em geral, com o aumento da temperatura, há um aumento cinético na reação. Na oxidação da fração lipídica, os produtos desta reação são aldeídos, cetonas, álcoois e por vezes hidrocarbonetos, que são percebidos sensorialmente pela presença de odores indesejáveis nos produtos, que caracterizam a oxidação lipídica (ARAÚJO, 2006).

A fragilidade oxidativa da molécula se dá prioritariamente nas insaturações (FENEMMA et al., 2010). Por ser um ácido graxo muito insaturado e estar em uma concentração elevada no óleo de Sacha Inchi (MAURER et al., 2012); a reação oxidativa tende a ocorrer no ω -3 (ácido linolênico), prioritariamente a ω -6 e a ω -9 respectivamente (FENEMMA et al., 2010).

As emulsões O/W servem de modelo como matrizes alimentícias para o estudo do processo de oxidação lipídica. Muitos estudos têm se preocupado com o efeito do emulsificante na oxidação lipídica nessas emulsões. Tem-se mostrado que as proteínas

geralmente funcionam como uma barreira protetora da fase oleosa contra o efeito oxidativo (BERTON et al., 2012).

1.2.3.2 Coalescência

A coalescência é induzida pela quebra das camadas superficiais (filme) das gotículas que estão próximas umas das outras. As emulsões alimentícias podem sofrer coalescência no processo de congelamento, pois a formação de cristais de gelo forçará as gotículas a aproximarem-se uma das outras e no descongelamento sofrerem a coalescência (FENEMMA et al., 2010).

Durante a secagem isso também pode ocorrer, onde a coalescência é atenuada por concentrações altas de sólidos não gordurosos, pela adição ou presença de sais os quais diminuem a tensão na camada interfacial ocorrendo a coalescência (FENEMMA et al., 2010).

Os surfactantes tanto naturais (proteínas) como sintéticos (Tween) consomem diminuir a coalescência das gotículas, pois aumentam a viscosidade e a resistência interfacial e conseqüentemente a estabilidade das emulsões (FENEMMA et al., 2010).

1.2.4 Propriedades das emulsões

As propriedades das emulsões são importantes, porque refletem de forma direta na eficiência da encapsulação. A concentração de sólidos totais, viscosidade, estabilidade e tamanho da gota da emulsão, são propriedades importantes a serem consideradas (JAFARI et al., 2008; CARNEIRO, 2011).

O aumento na concentração de sólidos totais tem como consequência, o aumento da viscosidade da emulsão, alguns autores relatam em seus estudos que é ideal o uso da maior concentração de sólidos possíveis, entretanto, outros autores relatam que existe um ponto ótimo tanto de viscosidade como do teor de sólidos totais para a microencapsulação de óleos. Quando esse ponto “ideal” é ultrapassado, tem-se a diminuição na retenção do material ativo (JAFARI et al., 2008; CARNEIRO, 2011), reduzindo a mobilidade dos compostos ativos e dentro da microcápsula e reduz o tempo para formar uma camada protetora ao redor do mesmo durante a secagem (CHARVE & REINECCIUS, 2009).

A eficiência da encapsulação de óleos pode ser influenciada pela estabilidade inicial da emulsão, pois quanto mais estável a mesma for, maior será a eficiência da encapsulação (JAFARI et al., 2008; CARNEIRO, 2011).

1.2.4.1 Formação da emulsão

Para a formação de uma emulsão necessita-se de óleo, água, emulsificante e energia. A produção de gotas é relativamente fácil, mas não a sua quebra em pequenas gotículas. Como as gotas possuem resistência à deformação, que é inversamente proporcional ao seu tamanho, se faz necessário um grande aporte de energia. A energia necessária para a quebra das gotas será menor se a tensão interfacial (pressão de Laplace) for reduzida pela adição de um emulsificante. Essa energia necessária à deformação das gotas pode ser obtida por uma agitação intensa, homogeneização por alta pressão, uso do ultrassom (sonicação), entre outras formas (FENEMMA et al., 2010).

1.2.5 Considerações sobre micro e nanoemulsão

Microemulsões e nanoemulsões são cada vez mais utilizados para encapsular, proteger e liberar componentes lipofílicos de interesse nas indústrias de alimentos e farmacêutica. O tamanho das partículas nestes de sistemas são <100nm, o que significa que estas partículas apresentam vantagens para certas aplicações como maior estabilidade à longo prazo, alta transparência óptica, e aumento da biodisponibilidade (McCLEMENTS, 2012).

Atualmente, existe porém, uma confusão na literatura quanto ao uso dos termos “microemulsões” e “nanoemulsões”. Ambas são dispersões coloidais distintas: uma microemulsão é termodinamicamente estável, ao passo que uma nanoemulsão, não é. Por este motivo, é importante diferenciá-las, uma vez que isso influencia nos métodos utilizados para fabricação, nas estratégias utilizadas para estabilização das mesmas, e nas abordagens utilizadas para projetar seus atributos funcionais (McCLEMENTS, 2012).

1.2.5.1 Microemulsão

É o termo geralmente utilizado para se referir aos líquidos isotrópicos termodinamicamente estáveis formados por uma mistura de óleo, água e surfactantes. As

misturas de óleo (O), água (W), e surfactante (S) podem formar uma grande variedade de sistemas diferentes, dependendo da sua composição e às condições ambientais (particularmente a temperatura). Podem formar uma, duas, três ou mais fases separadas, que estarão em equilíbrio uma com a outra. Estas podem ser: fase contínua aquosa, fase contínua oleosa ou bi contínua (dependendo das concentrações, da natureza e arranjos das moléculas presentes) (McCLEMENTS, 2012).

As estruturas dentro destas fases podem ser esféricas (micelas ou micelas reversas), semelhantes a um cilindro (haste micelas ou micelas reversas), planares (lamelares), ou esponjosas (bi contínua). Métodos analíticos adequados são necessários para identificar com precisão as estruturas formadas dentro de um sistema S/O/W sob um conjunto de condições particulares, tais como a microscopia (luz, elétrons, ou de força atômica), métodos de dispersão (luz, raio-X, ou nêutrons) , condutividade elétrica, ressonância magnética nuclear (RMN) e reologia (McCLEMENTS, 2012).

Quando se utiliza o termo “microemulsão” deve-se definir claramente qual é o tipo de microemulsão que está sendo considerado. Daremos ênfase aos sistemas que são utilizados para encapsular componentes lipofílicos (microemulsões O/W), que consistem em pequenas partículas esféricas compostas por óleo e moléculas surfactantes dispersas em um meio aquoso. Este tipo de dispersão coloidal também é conhecido como “microemulsão de gotas” ou como “sistema de micelas inchadas” (McCLEMENTS, 2012).

A seguinte definição se propõe a descrever o tipo de microemulsão óleo-em-água como sendo uma dispersão coloidal termodinamicamente estável composta por pequenas partículas esféricas (composta de óleo, tensoativo, e possivelmente um agente co-tensioativo) disperso em um meio aquoso (McCLEMENTS, 2012).

As moléculas de surfactante em uma microemulsão óleo-em-água são organizadas de modo que as suas extremidades não polares associem-se umas com as outras formando um núcleo hidrofóbico (Fig. 1.7), uma vez que isto reduz a superfície de contato termodinamicamente desfavoráveis entre os grupos não polares e a água (McCLEMENTS, 2012).

A cabeça polar dos grupos hidrofílicos das moléculas de surfactante interage com a fase aquosa circundante. Moléculas de óleo podem ser incorporadas no interior hidrofóbico das micelas, como um núcleo separado ou entre as extremidades do surfactante (Fig. 1.8). Se as moléculas de óleo tiverem alguns grupos polares, elas podem então ser incorporadas na

micela de tal modo que estes grupos irão interagir com a cabeça polar no surfactante ou interagir com a fase aquosa (McCLEMENTS, 2012).

Embora o sistema final usado para encapsular um componente lipofílico pode ser uma microemulsão de A/O, é possível encapsular um componente lipofílico dentro de outros tipos de sistema de microemulsão (como em emulsões múltiplas) (McCLEMENTS, 2012).

1.2.5.2 Nanoemulsão

Uma nanoemulsão pode ser considerada uma emulsão convencional contendo partículas muito pequenas. Podem ser do tipo água-em-óleo (A/O) ou óleo-em-água (O/A) dependendo se o óleo é disperso como gotícula em água, ou vice-versa. As dispersões coloidais adequadas para encapsular componentes lipofílicos tratam-se das nanoemulsões óleo em água, que consistem em pequenas partículas de óleo e tensoativo dispersos em um sistema aquoso. Uma nanoemulsão de óleo em água é definida como uma dispersão coloidal termodinamicamente instável composta por dois líquidos imiscíveis com um dos líquidos a ser disperso como pequenas gotas esféricas ($r < 100\text{nm}$) em outro líquido (McCLEMENTS, 2012).

Em princípio uma nanoemulsão poderia ser formada por gotículas de óleo em água sem o uso de um tensoativo (surfactante), entretanto, na prática este sistema seria altamente instável para a coalescência da gota e necessitaria de um surfactante para facilitar a formação de uma nanoemulsão e assegurar a estabilidade cinética durante o armazenamento.

Muitas vezes, a combinação de mais de um surfactante é utilizada para estabilizar a formação das nanoemulsões. Deste modo, uma nanoemulsão é preparada muitas vezes utilizando os mesmos componentes de uma microemulsão: óleo, água e surfactante e possivelmente, com um co-surfactante (McCLEMENTS, 2012).

Outra semelhança é com relação a estrutura das partículas encontradas em nano e microemulsões, as extremidades não polares das moléculas tensoativas sobressaem para o núcleo hidrofóbico formado na fase oleosa, enquanto a cabeça polar dos grupos de moléculas dos surfactantes interagem com a fase aquosa circundante (McCLEMENTS, 2012).

A principal diferença entre uma nanoemulsão e uma microemulsão é, portanto, a sua estabilidade termodinâmica: nanoemulsões são termodinamicamente instáveis, enquanto microemulsões são termodinamicamente estáveis (McCLEMENTS, 2012).

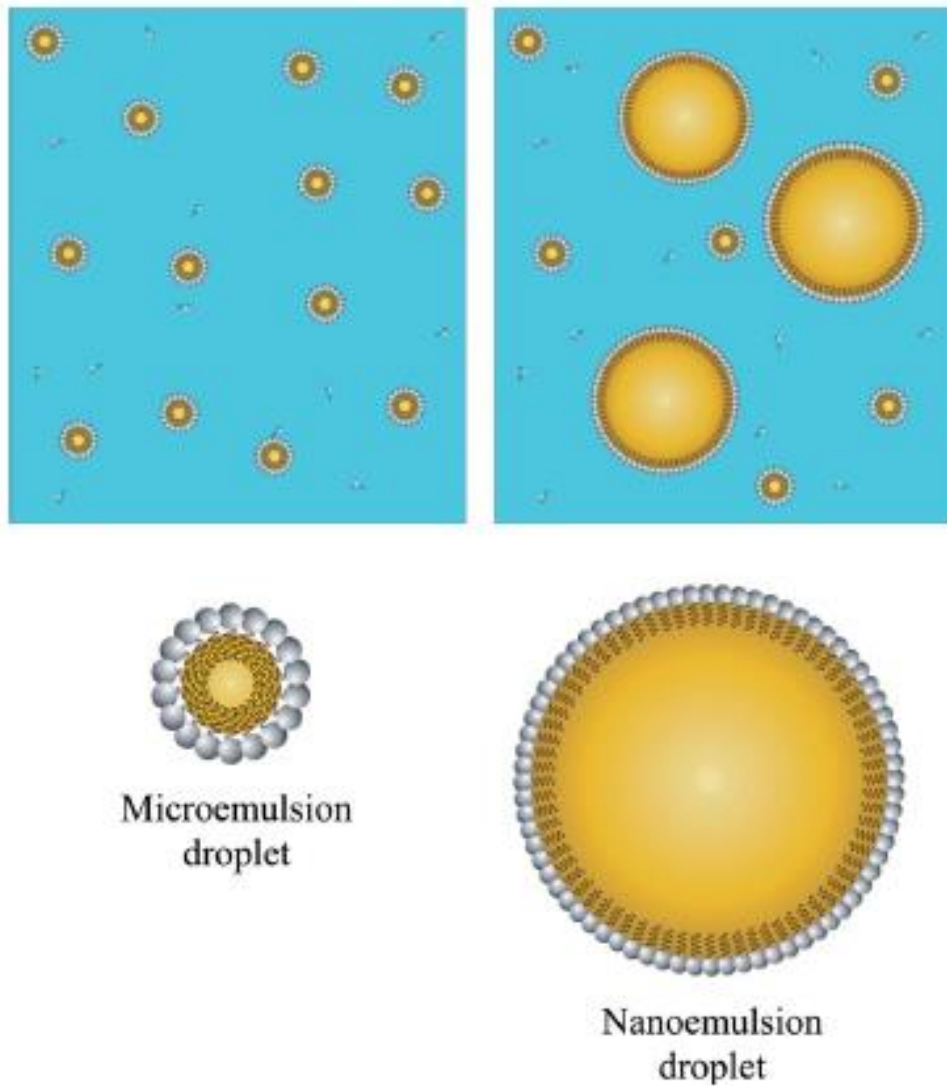


Fig. 1.7 – Diagrama de microemulsão e nanoemulsão fabricadas com óleo, água e surfactante.

Fonte: McClements, 2012.

Na Figura 1.7, a estrutura das partículas em ambos os tipos de dispersões coloidais são muito semelhantes – o núcleo hidrofóbico do óleo e da extremidade surfactante e uma camada hidrofílica da cabeça do grupo surfactante. Na Figura 1.8, as moléculas de óleo podem ser incorporadas entre a extremidade do surfactante e/ou no núcleo da micela.

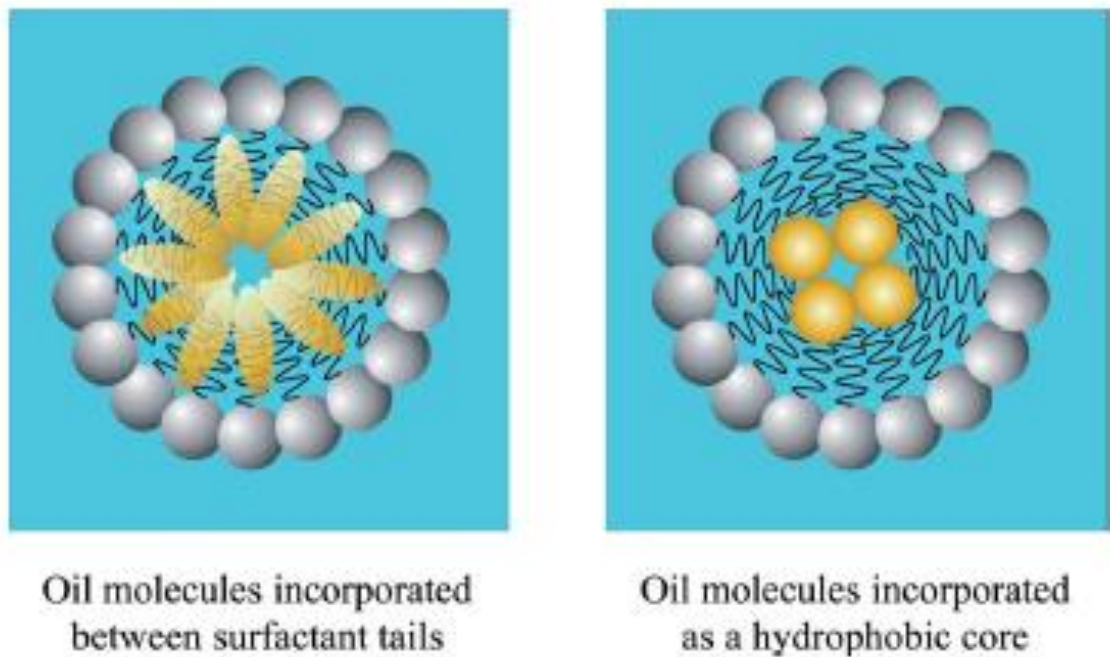


Fig. 1.8 – Microemulsões óleo-em-água consistem de uma micela de surfactante com uma molécula de óleo nela incorporada.

Fonte: McClements, 2012.

1.2.6 Estabilizantes

1.2.6.1 Pectina

Embora exista uma grande diversidade de polissacarídeos, há, entretanto algumas características que governam suas propriedades. Em geral são cadeias carbônicas rígidas e volumosas, superiores a 10 unidades de monômeros (cadeias de monossacarídeos). Essa característica permite que os polissacarídeos produzam soluções com alta viscosidade (FENEMMA et al., 2010).

As pectinas comerciais utilizadas como aditivos em alimentos são heteropolissacarídeos aniônicos contendo 65% da sua massa de unidades de ácido galacturônico. Podem estar presentes como ácido livre, metil éster ou, em pectinas amidadas, amido-ácido (Figura 1.9), obtidos por esterificação de pectina de alta metoxilação (MAY, 2000).

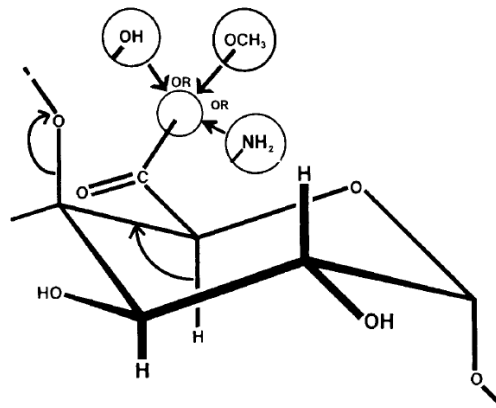


Fig. 1.9 – Ácido galacturônico (Éster) e unidades amida encontradas em pectina.

As setas indicam o potencial para degradação por β -eliminação sob a forma de éster.

Fonte: May, 2000.

O ácido galacturônico é parte da classe mais ampla de substâncias pécticas que é um dos principais polissacarídeos da parede celular das plantas terrestres e frutos. Os materiais comerciais normalmente contêm açúcar adicionado para padronizar o desempenho (MAY, 2000). Seu pKa em torno de 3,6 e seu principal monômero é glicuronato (AL-HAKKAK & AL-HAKKAK, 2010; ZIMET & LIVNEY, 2009).

1.2.6.1.1 Fontes de pectina

Atualmente a maioria das fontes de pectinas são cascas e resíduos da extração de sucos de frutos cítricos e de bagaços de maçã. Nos diversos processos comerciais de frutos cítricos, a casca de lima ou de limão são preferidas, por apresentarem pectinas de melhor qualidade, enquanto a casca de laranja é em maior quantidade e pode ser utilizada em muitas aplicações (MAY, 2000; FENEMMA et al., 2010).

A casca de frutos cítricos pode ser lavada para ser livre de acidez e cuidadosamente seca para preservar a qualidade da pectina, ou pode ser processada diretamente da matéria úmida. O processamento da casca úmida é particularmente apropriada no caso de cascas de laranja, mas requer uma grande e consistente fonte de cascas muito semelhantes para pectina de plantas (MAY, 2000).

A pectina é muito susceptível a degradação, seja por enzimas presentes na casca úmida ou pela ação do calor durante a secagem e tratamento subsequente, e sempre que possível esta perda de qualidade deve ser controlada. Os produtores de pectina devem destinar recursos para garantir tanto a disponibilidade quanto a qualidade das matérias-primas, pois a

qualidade tem um efeito muito significativo sobre os tipos de pectinas que podem ser economicamente produzidos (MAY, 2000).

1.2.6.2 Goma xantana

Goma Xantana (GX) é um polissacarídeo aniônico extracelular, de alto peso molecular, produzida por uma bactéria gram-negativa, *Xanthomonas campestris*. Esta goma é largamente utilizada na indústria de alimentos, farmacêutica e de cosmético, pois esta é reconhecidamente segura (BHATTACHARYA et al., 2012; BHATTACHARYA, et al., 2012^a; SWORN, 2000). Com base nos estudos de alimentação de curto e longo prazo, a GX foi estudada pela *US Food and Drug Administration* (FDA) em 1969 permitindo seu uso em produtos alimentícios sem qualquer limitação específica (BHATTACHARYA et al., 2013).

A GX tem sido extensivamente utilizada para produção de emulsões e outras aplicações, por apresentar excelentes propriedades de viscosidade e dispersão (fluxo altamente pseudoplástico, dispersão em água quente ou fria) (NIKIFORIDIS & KIOSSEOGLOU, 2010). A GX tem como seu principal monômero o β -D-glicose, e seu pKa é em torno de 2,8. (STEPHEN et al., 2006).

Entretanto, este polímero tende a formar estruturas em solução, mostrando baixo cisalhamento e propriedades de gel fraco que dão estabilidade às suspensões coloidais (NIKIFORIDIS & KIOSSEOGLOU, 2010). A utilização de GX, em combinação com goma arábica (GA) pode aumentar a estabilidade físico-química de emulsões de óleo de noz em água (GHARIBZAHEDI et al., 2013).

Sua viscosidade apresenta excelente estabilidade ao longo de uma ampla faixa de pH e temperatura, além de ser resistente à degradação enzimática. A GX exibe uma interação sinérgica com goma guar (GG) e goma de alfarroba (LBG) e o glucoman de *Konjac* (GK). Isto resulta no aumento da viscosidade com a GG e a baixas concentrações com LBG. Em concentrações pouco elevadas, elásticas, são formados géis termo resistentes com LBG e GK (SWORN, 2000). Uma solução de 0,1% de GX aumenta em 10 vezes a viscosidade da água (FENEMMA et al., 2010).

A estrutura da GX é mostrada na Figura 1.10, a seguir:

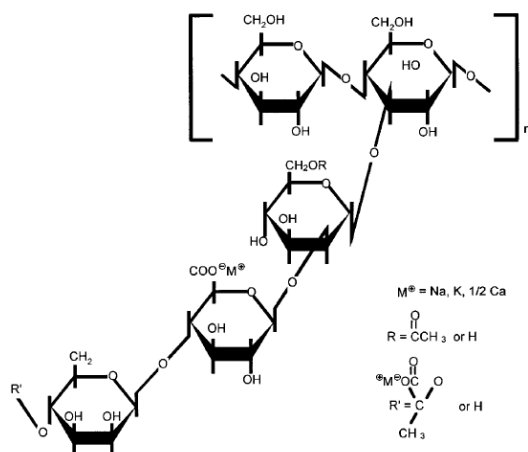


Fig. 1.10 – Estrutura primária da Goma Xantana.

Fonte: Sworn, 2000.

Soluções de GX à 1% (m/v) ou concentração maior apresentam aspecto semelhante a gel em repouso, mas essas mesmas soluções possuem reduzida resistência à mistura e ao bombeamento. Essas mesmas características são observadas em níveis normais de uso (0,1-0,3%) (SWORN, 2000).

A alta viscosidade de soluções de GX em baixas taxas de cisalhamento, impactam na sua capacidade para proporcionar uma estabilidade à longo prazo em sistemas coloidais. A redução na viscosidade em resposta ao aumento da taxa de cisalhamento é importante para as propriedades reológicas das suspensões e emulsões e para a eficácia da GX como um auxiliar de processamento (SWORN, 2000).

1.2.6.3 Ovoalbumina (Proteína)

As proteínas são moléculas anfifílicas, com uma cadeia molecular flexível, possuem a capacidade de interagir com uma variedade de diferentes substâncias de alto peso molecular (MADENE et al., 2006).

Em função das propriedades funcionais que apresentam, como solubilidade, capacidade de formação de filme interfacial, capacidade emulsificante e estabilizante, e que são desejáveis para um material de parede, as proteínas tornam-se materiais muito utilizados para a microencapsulação de alimentos e fármacos (CHARVE & REINECCIUS, 2009; JAFARI et al., 2008).

As proteínas mais utilizadas para microencapsulação na indústria de alimentos são as proteínas do leite (ou soro), da soja e gelatina. As proteínas do soro de leite apresentam boa propriedade contra a oxidação, quando comparados à goma arábica e amido modificado, por ser menos permeáveis ao oxigênio. Por ser muito utilizada e apresentar excelentes propriedades emulsificantes, a β -lactoglobulina é a proteína de soro de leite mais importante (CHARVE & REINECCIUS, 2009; JOUENNE & CROUZET, 2000).

A ovoalbumina (ou ovalbumina) é a principal glicoproteína presente na clara de ovos, representa aproximadamente 54% do total, seguida da ovotransferrina (13%) e lisozima (3,5%). A ovoalbumina assume um papel importante na alimentação e é responsável pela gelificação dos ovos (VACHIER et al., 1995).

Ovoalbumina possui propriedades funcionais que interessam muito a indústria de alimentos e que a torna muito utilizada, tais como formação de gel e espuma, para a fabricação de biscoitos, tortas, produtos cárneos, produtos de panificação (ZAYAS, 1997; (ORDÓÑEZ et al., 2005).

Muitas das propriedades funcionais que as proteínas desempenham, como solubilidade, por exemplo, podem ser atribuídas às interações proteína-proteína ou proteína-solvente em função das diversas maneiras e condições em que estas interações ocorrem (KAKALIS & REGENSTEIN, 1986; ZAYAS, 1997).

1.3 ÁCIDOS GRAXOS – ÔMEGA-3

Os ácidos graxos são as unidades fundamentais dos lipídeos. Podem ser saturados (contendo apenas ligações simples entre carbono-carbono) ou insaturados (contendo de 1 a 6 ligações duplas carbono-carbono). As insaturações variam na posição e na conformação das duplas ligações (VISENTAINER & FRANCO, 2006).

Denominam-se os ácidos graxos todos os ácidos monocarboxílicos alifáticos. Salvo algumas exceções, todos os ácidos encontrados na natureza são de alta massa molecular, lineares, saturados e insaturados, podendo apresentar substituintes em sua cadeia, como grupos metílicos, hidroxílicos, carbonílicos. Os principais ácidos graxos saturados são o láurico, palmítico e o esteárico, e os insaturados o oleico, linoleico e linolênico (BOBBIO & BOBBIO, 2003).

Lipídeos de animais e de vegetais possuem ácidos graxos com cadeias de dezesseis a dezoito carbonos (predominando este último). Em gorduras de animais marinhos é comum

cadeias com vinte ou mais átomos de carbono. A maioria dos ácidos graxos encontrados de forma natural nas gorduras (animais e vegetais) possuem números pares de carbonos, e quando insaturados, predominam a conformação *cis* (BOBBIO & BOBBIO, 2003).

O ômega-3 (ω -3) é uma classe de ácidos graxos poli-insaturados essenciais, como o ácido α -linolênico (ALA – C18:3), ácido estearidônico (EDA – C18:4), ácido eicosapentaenoico (EPA C20:5), ácido docosapentaenóico (DPA – C22:5) e ácido docosahexaenóico (DHA – C22:6). A classe de ácidos graxos ω -3 apresenta diversos benefícios à saúde humana, pois exerce um papel importante na prevenção de doenças cardiovasculares, câncer de cólon e doenças imunológicas, além de serem vitais para o desenvolvimento do cérebro e a retina (CASTRO-GONZALEZ, 2002).

1.3.1 Benefícios do consumo de ω -3

Os ácidos graxos da série ω -3 compõem a estrutura da retina e do cérebro, sendo portanto, extremamente importantes que sejam consumidos pela mãe durante a gravidez para que o feto tenha um pleno desenvolvimento. Estima-se que aproximadamente 600mg de ácidos graxos essenciais são transferidos da mãe saudável para o feto durante o prazo de gestação. A dieta que a gestante adota é de extrema importância, uma vez que a alimentação da mãe determina o tipo de gorduras que se acumulam nos tecidos fetais (CASTRO-GONZALEZ, 2002; CONNOR, 1996).

A placenta transporta o Ácido Araquidônico (AA) e o DHA da mãe para o feto, o que é vital no desenvolvimento do sistema nervoso, que ocorre próximo ao terceiro trimestre da gravidez (CASTRO-GONZALEZ, 2002; CONNOR, 1996). Estudos sugerem que o consumo prolongado de ω -3 durante a gravidez pode reduzir a incidência de parto prematuro e aumentar o peso do bebê ao nascer (CONNOR et al., 1996).

Connor (1996) observou que em crianças alimentadas com leite materno ou com formulas que contenham DHA, houve melhora na acuidade visual e maior capacidade de responder à luz, que está associado com a melhor habilidade cognitiva para integrar informações e um melhor coeficiente intelectual.

Sabe-se que os ácidos graxos ω -3 são essenciais para o crescimento e desenvolvimento normal, e desempenham um importante papel na prevenção e tratamento de diversas enfermidades, como o sistema cardiovascular, sistema imunológico, sistema nervoso, diabetes do tipo 2, câncer de cólon, úlcera, obstrução pulmonar, enfermidades renais,

psoríase, artrite reumatoide e artrose (CASTRO-GONZALEZ, 2002).

1.3.2 Fontes de ômega-3.

As principais fontes de alimentos que se encontram uma maior quantidade de ácidos graxos ω -3 (EPA e DHA) são os peixes, moluscos, crustáceos e algas (CASTRO-GONZALEZ, 2002). Entretanto fontes vegetais como o óleo de Linhaça e o de Sacha Inchi, têm sido estudadas e apresentam-se como fontes em concentrações tão altas quanto as de origem animal (MAURER et al., 2012; FANALI et al., 2001; GUILLÉN et al., 2003).

1.3.2.1 Sacha Inchi

Sacha Inchi (*Plukenetia volubilis* L.) é uma planta oleaginosa, da família *Euphorbiaceae*, nativa da região amazônica florestal Peruana, conhecida pelos nativos há milhares de anos. Seus frutos (Figura 1.11) têm um formato de estrela e produzem as sementes comestíveis (amêndoas) de cor marrom-escura (FANALI et al., 2001).

O sachá inchi também é conhecida por diversos nomes, tais como “Oro Inka”, “Inca Peanut”, “Wild Peanut” dentre outros. O óleo de Sacha Inchi é extraído de suas sementes (amêndoa) por prensagem. Suas sementes são compostas de aproximadamente 48-50% de óleo e 27-28% de proteínas. Da fração oleosa aproximadamente 90% referem-se a ácidos graxos insaturados, sendo 42-48% de ácido linolênico (ω -3), 32-37% de ácido linoléico (ω -6) e 9-12% de ácido oléico (ω -9) (MAURER et al., 2012; FANALI et al., 2001; GUILLÉN et al., 2003).

A composição em ácidos graxos do óleo de Sacha Inchi permite classificá-lo como alimento funcional e seu consumo pode trazer benefício à saúde. Em função da sua composição altamente insaturada (<90%), o óleo de Sacha Inchi é muito suscetível à oxidação lipídica. Dessa forma, a microencapsulação torna-se uma estratégia importante na conservação desses compostos, em especial o ômega-3 (MAURER et al., 2012).

O óleo de Sacha inchi apresenta uma relação ω -3/ ω -6 = 1,33, muito superior à da maioria óleos vegetais (oliva = 0,07; canola = 0,33; algodão = 0,01; milho = 0,02; girassol = 0,01), ficando atrás apenas do óleo de linhaça, em que a relação ω -3/ ω -6 = 3,44 (MAURER et al., 2012). Quanto maior essa relação, maior será a redução dos níveis de colesterol e melhora do perfil lipídico do plasma (RIEDIGER et al, 2008; CARNEIRO, 2011).



Fig. 1.11 – Frutos de Sacha Inchi (*Plukenetia volubilis* L.).

1.4 RESSONANCIA MAGNÉTICA NUCLEAR

A ressonância magnética nuclear (RMN) tem sido extensivamente utilizada desde os anos 40 (GUTOWSKY et al, 1949) na elucidação de estruturas químicas e bioquímicas. Trata-se de uma das mais importantes técnicas utilizadas não só na identificação de substâncias como para acompanhamento do progresso de reações. O uso constante da espectroscopia de ressonância magnética nuclear (RMN) em análise de lipídeos alimentares (incluindo animais e vegetais) inclui não somente a determinação de conteúdo de lipídeos totais bem como a identificação da composição em ácidos graxos (MARCONE et al, , 2013). De acordo com Barison et al., (2010) o método de RMN para análise do perfil lipídico pode ser realizado diretamente na fração oleosa. Particularmente não necessita de padrões de ácidos graxos, não envolve a hidrólise de triacilglicerol e tem baixo consumo de reagentes químicos comparado ao método oficial da AOCS (American Oil Chemists' Society) que é feito por cromatografia gasosa (CG). Trata-se de uma técnica que pode ser utilizada como uma ferramenta útil para detecção de fraudes e processos oxidativos em óleos (GUILLÉN & RUIZ, 2005) e pode ser utilizada também para determinar o conteúdo de gordura sólida em alimentos (COUPLAND, 2002; McCLEMENTS & DECKER, 2010). Na literatura encontram-se varias aplicações para a técnica de RMN para a área de alimentos, tais como, a determinação do conteúdo lipídico em leite (HU et al, 2007), determinação do conteúdo de ômega-3 em óleo não refinado de atum e salmão (IGARASHI, 2000) e em carne de kebabs (FORATO et al, 2009).

Em óleos vegetais, pode ser utilizada para a determinação do perfil de ácidos graxos,

como feito por Barison et al, (2010) e por Guillén et al, (2003), para avaliação da termo-oxidação (GUILLÉN & RUIZ, 2005) e frequentemente utilizada para verificar adulteração em azeite de oliva (FANG et al, 2013; PARKER et al, 2014). Para avaliação do perfil em ácidos graxos, a técnica de RMN mostra uma boa correlação com os dados obtidos por CG, assim constitui-se uma técnica eficiente e uma poderosa ferramenta para determinação da composição lipídica em alimentos (FANG et al, 2013).

De acordo com Guillén et al (2003) o uso de RMN de ^1H é vantajoso em relação a outros métodos químicos de avaliação de estabilidade oxidativa em função da praticidade, rapidez e sensibilidade. Segundo Colzato et al (2008) a nuvem eletrônica blindam fracamente os núcleos de uma molécula cuja densidade varia com o ambiente químico, assim esta variação origina os diferentes deslocamentos químicos. A comparação dos deslocamentos químicos se dá utilizando tetrametilsilano (TMS) como padrão de referencia, o qual se considera com deslocamento químico de δ_{H} 0.00 ppm. Os lipídeos no entanto, possuem sempre valores positivos em relação ao TMS.

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CAPITULO II – Fatty acids profile of Sacha Inchi oil and blends by ^1H NMR and GC-FID

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ABSTRACT

This study aimed at the characterization of blends of Sacha Inchi oil (SIO) with different ratios of SO (soybean oil) and CO (corn oil) by nuclear magnetic resonance (^1H NMR), compared with the data obtained by gas chromatography with a flame ionization detector (GC-FID). The ^1H NMR and GC-FID data from different ratios of SIO were adjusted by a second order polynomial equation. The two techniques were highly correlated (R^2 values ranged from 0.995 to 0.999), revealing that ^1H NMR is an efficient methodology for the quantification of omega-3 fatty acids in oils rich in omega-6 fatty acids or vice versa such as SO and CO and, on the other hand, can be used to quantify ω -6 in oils rich in ω -3, such as SIO.

Keywords: Sacha inchi oil, ^1H nuclear magnetic resonance, gas chromatography-flame ionization detection, blends, ω -3 and ω -6.

2.1 INTRODUCTION

Sacha Inchi (*Plukenetia volubilis* L.) is a plant native from the Peruvian Amazon region and is known by various names, such as “Oro Inka”, “Inca peanut”, and “wild peanut”. Sacha Inchi oil (SIO) is extracted from the seeds by pressing. The seeds are composed of about 48-50% oil and 27-28% protein. Approximately 90% of the oil fraction is comprised of unsaturated fatty acids, including 42-48% linolenic acid (ω -3), 32-37% linoleic acid (ω -6) and 9-12% oleic acid (ω -9) (Guillén, Ruiz, Cabo, Chirinos, & Pascual, 2003; Follegatti-Romero, Piantino, Grimaldi & Cabral, 2009).

SIO has levels of ω -3 fatty acids ranging from 44 to 51.5% in crude oil and 53.3% in the refined oil, in addition to 33.5-36.2% ω -6 and 8.9-10.7% ω -9 fatty acids (Maurer, Hatta-Sakoda, Pascual-Chagman & Rodriguez-Saona, 2012; Fanali et al., 2011; Guillén, Ruiz, Cabo, Chirinos, & Pascual, 2003; Gutiérrez, Rosada, & Jiménez, 2011).

Proton nuclear magnetic resonance (^1H NMR) has been increasingly used in different applications in the food industry. Nowadays, it is one of the most important techniques used for molecular structure identification, due to the practicality of the use of this method for the identification of fatty acids present in samples (Guillén & Ruiz, 2004; Marcone, Wang, Albabish, Nie, Somnarain & Hill, 2013). Routinely, the evaluation of fatty acid composition

is carried out using gas chromatography with a flame ionization detector (GC-FID) (ISO 5508, 1990). However, this analysis requires prior conversion of triacylglycerol into methyl esters, and the identification of components necessarily depends on a previously established internal or external standard (Fang, Goh, Tay, Lau & Li, 2013; Barison et al., 2010). However, when using ^1H NMR for lipid profile analysis, there is no need for methyl ester standards, since identification is based on the chemical shifts of protons; ^{13}C NMR analysis can be also used (Barison et al., 2010). With the ^1H NMR technique, lipid profile analysis can be performed either using methyl esters (or ethyl esters) obtained by transesterification, as well as directly from the oil (triacylglycerol form). Thus, chemical consumption is also lower (Guillén, Ruiz, Cabo, Chirinos & Pascual, 2003; Guillén & Ruiz, 2004; Barison et al., 2010; Fang, Goh, Tay, Lau, & Li, 2013).

Igarashi (2000) observed a good correlation ($r = 0.994$) between ^1H NMR (500 MHz) and GC methods to determine the ω -3 content in unrefined bonito, tuna and salmon oils. The data obtained by both gas chromatography/mass spectrometry (GC/MS) and ^1H NMR also showed an excellent ability to build predictive models for the classification and discrimination of oils and fats (Fang, Goh, Tay, Lau, & Li, 2013), thus showing that the NMR technique appears to be a promising tool for the determination of the fatty acid composition in lipid fractions of food.

In this study, the aims were to identify and to quantify the omega-3 fatty acids and omega-6 fatty acids contents and to characterize the profile of SIO (rich in ω -3 fatty acids) blended with different ratios (100:0, 75:25, 50:50, 25:75 and 0:100) of soybean oil (SO) and corn oil (CO), both rich in ω -6 fatty acids, using ^1H NMR spectral analysis and to compare these results with the data obtained by GC-FID. In addition, physicochemical characterization was performed.

2.2. MATERIAL AND METHODS

2.2.1. Chemical and materials

Sacha Inchi (*P. volubilis* L.) oil was purchased from a local market in Lima, Peru. Sodium methoxide (NaOCH_3), methanol (CH_3OH) and n-hexane (C_6H_{14}) were purchased from Sigma-Aldrich[®] (St. Louis, MO, USA). CDCl_3 (99%) was used as the solvent in NMR analysis. Soybean oil and corn oil were purchased at the local market (Volta Redonda, RJ,

Brazil).

2.2.2. Physicochemical characterization of Sacha Inchi oil

The physicochemical analyses to qualify the used SIO were: acid value (Cd 3d-63), peroxide value (Cd 8b-90), saponification value (Cd 3-25) and iodine value (Cd 1c-85) according to the AOCS methods (2009).

The refractive index (Cc 7-25) and density ($\text{g}\cdot\text{cm}^{-3}$) were assessed using a digital density meter - Anton Paar DMA 4500 M (Graz, St, Austria). The average molecular weight (AMW) of SIO was calculated according to the Kay rule (Moraes, 2010).

2.2.3. Study design

The study was performed using SIO and blends with SO and CO in different ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 (w/w). These proportions/ratios (w/w) of SIO with SO and CO were used to obtain methyl esters (ME) to be analyzed by ^1H NMR and GC-FID.

2.2.4. Methyl ester preparation

The methyl esters (ME) were prepared according to the method proposed by Bannon et al. (1982), with a few adaptations. Approximately 75 mg of oil were weighed and added to 500 μL of NaOCH_3 in a methanolic solution (30%, w/v) and 500 μL of hexane. Then, the mixture was subjected to sonication with a nominal frequency of approximately 40 kHz, for 30 min. After filtration, the obtained ME were dried under N_2 .

2.2.5. Methyl ester analysis with ^1H NMR

NMR spectra were recorded on a Bruker Advance III spectrometer (Billerica, MA, USA) operating at 500 MHz for ^1H . The acquisition parameters of 1D NMR experiments employed were: TD (time domain data) = 65536, AQ (acquisition time) = 3.17 s, NS (number of scans) = 16, SW (spectral width) = 7500 Hz, D1 (relaxation delay between successive scans/transients) = 1.0 s, LB (exponential line broadening prior to Fourier transformation) = 0.3 Hz.

The ME of Sacha Inchi oil and mixtures with soybean oil and corn oil were assessed. The samples were solubilized in CDCl_3 with tetramethylsilane as the reference. The mixture was introduced into a tube with a diameter of 5 mm and the experiments were conducted at 25°C. CDCl_3 was the solvent that showed the best signs of separation of the allylic methylene protons of ω -3 and ω -6 fatty acids, compared to benzene and acetone, also considered in the analysis. The spectra were processed using the ACD/NMR Processor Academic Edition Program (Version 12.0).

2.2.6. Fatty acid profile with GC-FID

The analysis of the fatty acid methyl ester profiles of SIO and mixtures with SO and CO were performed using a Hewlett Packard HP 5890 – Series II, (Palo Alto, CA, USA) gas chromatograph, equipped with a flame ionization detector (GC-FID) and a BPX70-ms (Thermo Scientific, Waltham, MA, USA) capillary column (30 m x 0.25 mm x 0.25 μm) composed of 70% cyanopropylpolysilphenylene-siloxane. The initial temperature was 150°C/min, which increased by 5°C/min up to 240°C and was held constant for 30 min. The injector temperature was 240°C and the detector temperature was 280°C, with a split ratio of 1:30. The carrier gas used was He at 1 mL/min, at a pressure of 11 psi.

2.2.7. Statistical analysis

All analyses were performed in triplicate and the results are expressed as average \pm confidence interval (CI) (1). Correlation coefficients were calculated from the results obtained by ^1H NMR and GC-FID by the Pearson correlation coefficient-equation (2) (Moore, 2007).

$$CI = \frac{\sigma}{\sqrt{n}} \cdot t \quad (1)$$

where CI is the confidence interval, σ is the standard deviation, n is the number of repetitions and t is the Student's t -test inverse function (significance level of 5%).

$$r = \frac{1}{n-1} \sum \left(\frac{x_i - \bar{X}}{s_x} \right) \left(\frac{y_i - \bar{Y}}{s_y} \right) \quad (2)$$

where r is the Pearson correlation coefficient, n is the number of repetitions, $s_{x,y}$ is the standard deviation of variables x, y ; x_i is the observation value I of variable x ; y_i is the observation value I of variable y ; \bar{X} is the average value of variable x and \bar{Y} is the average value of variable y .

2.3. RESULTS AND DISCUSSION

2.3.1. Physicochemical characterization of Sacha Inchi oil

The physicochemical parameters are important for the characterization of vegetable oils, both from the view point of quality and the identity of SIO. This verification is particularly important for the detection of possible adulterations during the processing of SIO. The results of the physicochemical characterization of SIO are shown in Table 2.1.

Table 2.1. Physicochemical characterization of Sacha Inchi oil.

Properties	Unit	Values	CI
Saponification value	(mg KOH/g)	190.5 ±	1.1
Iodine value	(g I ₂ /100g)	192.5 ±	3.1
Refractive index	(20 °C)	1.47514 ±	0.00008
Density	(g.cm ⁻³) à 20 °C	0.92073 ±	0.00005
Average molecular weight	(g.mol ⁻¹)	863.5 ±	22
ω-3/ω-6 ratio	Dimensionless	1.88 ±	0.05
Acid value	(mg KOH/g)	2.40 ±	0.11
Acids (%)	(linolenic acid)	1.19 ±	0.06
Peroxide value	(meq/Kg)	7.36 ±	0.20

*CI – confidence interval at 95% probability.

The acid value and peroxide value are parameters that indicate the quality or conservation state of oils and fats, and are not used as identification parameters. The lower the value the lower the extent of oxidation and hydrolysis. On the other hand, slightly higher values (as obtained) indicate that the SIO probably had not undergone processing, and it may

be considered an unrefined or extra virgin oil (McClements & Decker, 2010; Jorge, Soares, Lunardi & Malacrida, 2005).

The refractive index is a measure to identify the oil, as it indicates the degree of unsaturation of bonds, the presence of oxidation compounds and whether thermal treatment of the oil has been performed. The value of the refractive index and the iodine value decrease with increasing oil saturation (Allen 1978; Paul, Mittal & Chinnan, 1997). The refractive index should be evaluated with other parameters together to provide more accurate conclusions, because their values differ slightly in vegetable oils. Pereira, Bridges, Neto, Santos, Galvão and Araújo (2008) reported refractive index data for different vegetable oils: 1.467 (± 0.001) for olive oil, 1.473 (± 0.003) for soybean oil, 1.473 (± 0.001) for sunflower oil, 1.473 (± 0.002) for corn oil and 1.468 (± 0.005) for cotton oil. The refractive index of 1.47514 (± 0.00008) and density value of 0.92073 (± 0.00005) g.cm⁻³ found in this study are similar to data found for SIO by Gutiérrez, Rosada and Jiménez (2011).

The iodine value is a measure of the degree of unsaturation of an oil or a fat. The higher its value, the greater the amount of triglycerides with double or triple bonds that will be present in the oil or fat. The value of 192.5 (± 3.1) g I₂/100g for SIO found in the present study is higher than the reported values for soybean oil at 131.5 (± 11.5), corn oil at 115.5 (± 12.5), sunflower oil at 126.5 (± 16.5), cotton oil at 109 (± 10) and flax seed oil at 177 (± 10); however, it resembles that of canola oil at 190.5 (± 2.5) g I₂/100 g (Knothe, 2002).

The higher saponification value indicates a shorter chain length, because a greater mass of KOH will be required to neutralize the lipid fraction. The obtained value of 190.5 (± 1.1) mg KOH/g indicates that the greatest portion of the fatty acids that make up SIO are intermediate chains (Chira, Todașcă, Nicolescu, Păunescu & Roșca, 2009).

The calculation of the average molecular weight (AMW) of oil considers the percentage areas, of each fatty acid obtained in the lipid profile multiplied by the molecular weight of the corresponding triacylglycerol. Thus, one obtains the weight contribution of each fatty acid to the total molecular weight. The value of 863.5 (± 22) g.mol⁻¹ obtained in the present study indicates a considerable contribution of linolenic acid (ω -3) in the composition of SIO, since the molecular weight of omega-3 fatty acid is 872.0 g.mol⁻¹ (Moraes, 2010; Chaves, 2008).

The ω -3: ω -6 ratio obtained in this study was 1.88 (± 0.05), greater than 1.33 found to SIO by Maurer, Hatta-Sakoda, Pascual-Chagman and Rodriguez-Saona (2012). This ratio is one of the highest in the case of vegetable oils (olive = 0.07, canola = 0.33, cotton = 0.01,

corn = 0.02 and sunflower = 0.01), just behind that of flax seed oil, in which the ω -3: ω -6 ratio is 3.44 (Maurer, Hatta-Sakoda, Pascual-Chagman, & Rodriguez-Saona, 2012). A higher ω -3: ω -6 ratio has been shown to reduce cholesterol levels and improve the plasma lipid profile (Riediger, Othman, Fitz, Pierce, Suh & Moghadasian, 2008).

The parameters of identification found in this study indicate that SIO had not undergone adulteration during processing. The experimental data are close to the data in the literature for the saponification value, iodine value, refractive index and density. The SIO used in this study is therefore authentic.

2.3.2. Methyl ester analysis with ^1H NMR

The ^1H NMR spectra of the methyl esters of the studied vegetable oils (Sacha Inchi, soybean oil and corn oil) (Fig. 2.1) were very similar; however, the differences in the range of the signals between 2.68 and 2.77 ppm may be observed by the intensities of the signals. These signals correspond to the doubly allylic hydrogens (between two double bonds), and the ratio between them can be used to identify adulteration by measuring the percentage ratio of soybean oil or corn oil in Sacha Inchi oil (or any other oil rich in ω -3). Fig. 1 illustrates the ^1H NMR spectra of methyl esters obtained from the Sacha Inchi oil in different proportions with SO (Fig. 2.1a) and CO (Fig. 2.1b).

In both cases, the signals of the proton located at the α carbonyl position were observed with chemical shifts of δ_{H} 2.22-2.25 ppm. In the β position, protons appeared with chemical shift between δ_{H} 1.52 and 1.61 ppm. The signals from δ_{H} 1.19 to 1.27 ppm represent methylene protons (γ); the intensity of this signal can be related to the CH_2 intermediate. The δ is the signal of allyl protons (neighboring to double bonds) is shown, represented by the δ_{H} 1.95-2.03 ppm signals. The signals between 5.25 and 5.34 ppm (ϵ) represent the protons of the double bond, confirming the presence of unsaturation. The singlet at 3.60 ppm represents the methoxyl group (OCH_3) of esters (ME). There were no signals from glycerol protons (δ_{H} 4.1 - 4.4 ppm) in the ^1H NMR spectrum, which are characteristic of oils in the triacylglycerol form, as observed by Guillén, Ruiz, Cabo, Chirinos, and Pascual (2003) and Barison et al. (2010).

Fig. 2.1c and 2.1e shows the expansion of the δ_{H} 2.62-2.78 ppm region for doubly allylic protons (*double double bonds*, ddb), characteristic of the presence of ω -6 (2.70 ppm) and ω -3 (2.74 ppm) fatty acids (ζ signal). These observations are in agreement with the results

of Guillén, Ruiz, Cabo, Chirinos and Pascual (2003). Fig. 2.2 shows that with an increase in the SIO ratio in relation to SO (Fig. 2.1c) or CO (Fig. 2.1e), the signal intensity at δ_H 2.74 ppm increased and the signal δ_H 2.70 ppm decreased. The reverse is also true.

Fig. 2.1d and 2.1f shows the expansion of the region between δ_H 0.70 and 0.94 ppm for SIO:SO (Fig. 2.1d) and SIO:CO (Fig. 2.1f). In these expansions, it can be observed that with an increased SIO ratio, the signal at δ_H 0.91 ppm increased, whereas the signal decreased proportionally at δ_H 0.81 ppm. In addition, with an increase in the proportion of SO or CO, the signal at δ_H 0.81 ppm (ω -6) became more intense than the signal at δ_H 0.91 ppm (ω -3). The signal at δ_H 0.81 ppm also indicates the presence of saturated and monounsaturated compounds.

The identification of the signals at δ_H 0.81 and 0.91 ppm are in accordance with the results observed by Barison et al. (2010) with various vegetable oils (soybean, corn, rice, canola, sunflower and olive) as triacylglycerols. Similarly, this can be seen in Fig. 2.1d to SIO:SO and 2.1f to SIO:CO.

It was observed that with a decrease in the amount of SIO and increase in SO or CO in the oil blends, the areas of the representatives signals of doubly allylic hydrogens varied proportionally. In Fig. 2.1 (c, e), the first triplet is represented by peaks at δ_H 2.69 ppm, 2.70 ppm and 2.72 ppm, and the second triplet by peaks at δ_H 2.73, 2.74 and 2.75 ppm, both with frequencies centralized, respectively, on δ_H 2.70 ppm and 2.74 ppm. These signals represent, respectively, the doubly allylic hydrogens of ω -6 and ω -3 fatty acids.

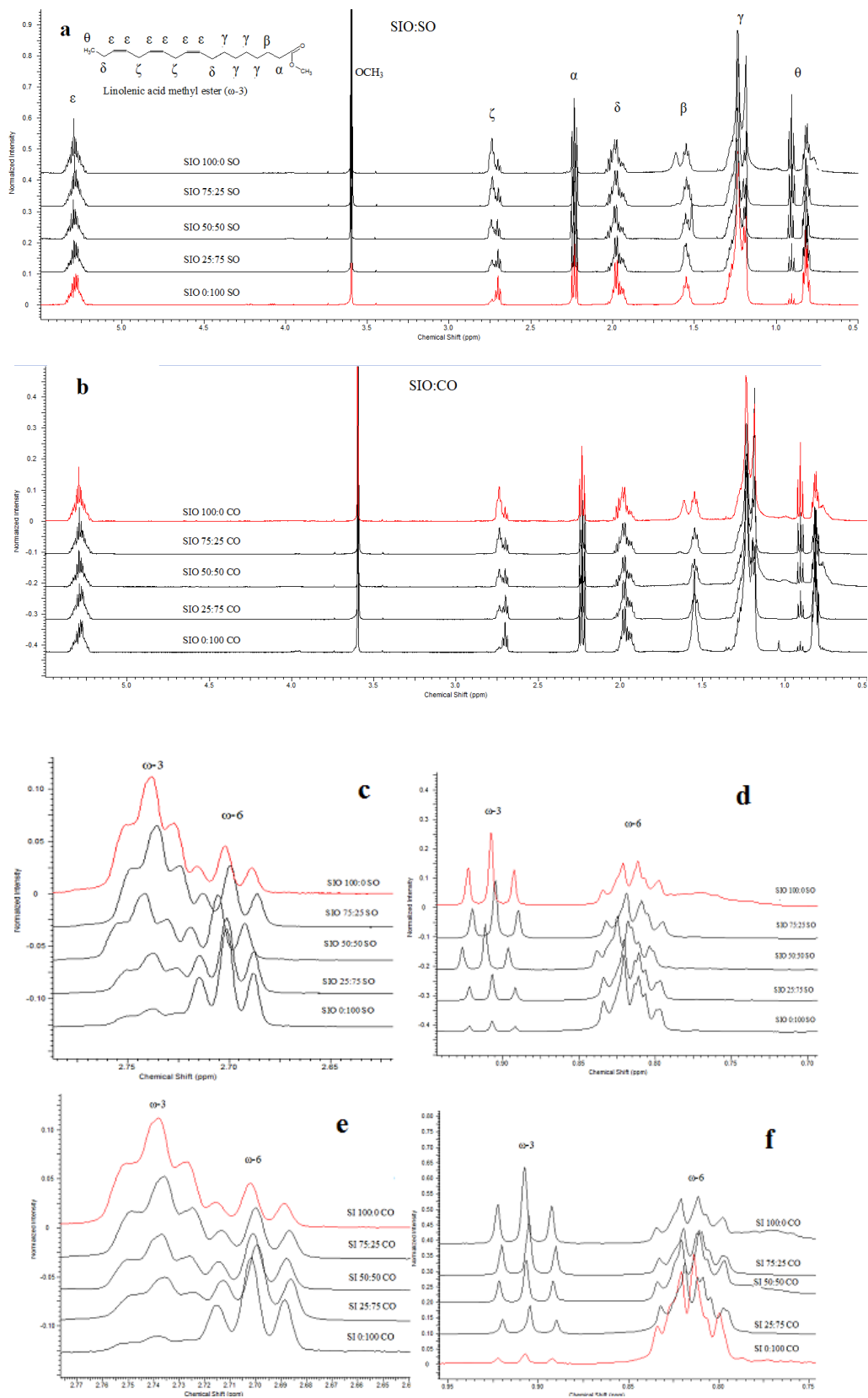


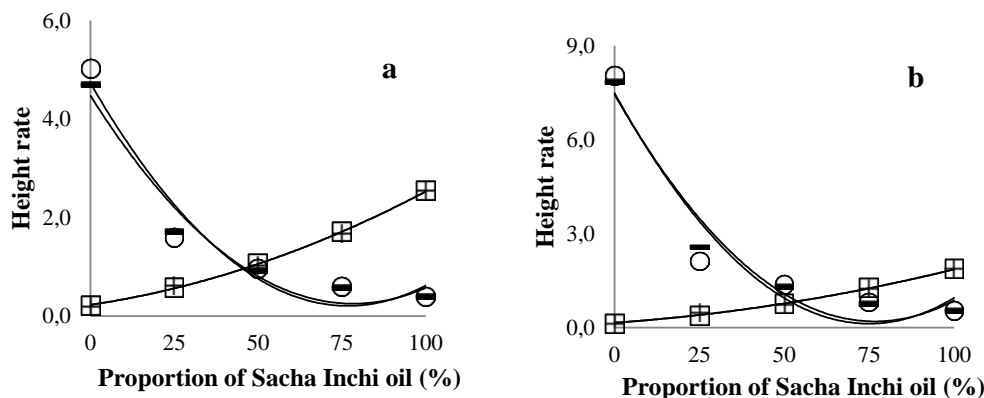
Fig. 2.1. ¹H NMR spectra of SIO methyl esters in ratios different, (a) with SO; and (b) with SIO:CO. Expansions of the regions of the doubly allylic hydrogens between 2.62 and 2.78 ppm to SIO:SO (c), SIO:CO (e) and between 0.70 and 0.95 ppm to SIO:SO (d) and to SIO:CO (f).

The areas ratio of the signals δ_{H} 2.74 and 2.70 ppm, omega-3/omega-6, respectively, to SIO:SO (100:0) is 2.549 and the ratio decreases until 0.213 to SIO:SO (0:100). This behavior can be estimated by a second order polynomial equation, which when the value of the ratio (omega-3/omega-6) is less than 2.549, indicate adulteration of SIO. Similar parameters may be considered for adulteration with CO, with an SIO:CO (0:100) was 0.199. If done calculations with inverse ratio, omega-6/omega-3, the value is 0.392 for SIO (100%), which also follows a second order polynomial equation, reaching values of 4.695 and 5.025, respectively, SO (100%) and CO (100%). Likewise ratios of greater than 0.392 (omega-6/omega-3) also indicate adulteration of SIO. The data of the areas ratio are presented in Table 2, and can be calculated as illustrated in Fig. 2.2a.

Table 2.2. Percentage quantifications of areas ratio of the signals at 2.70 to 2.74 ppm of SO or CO in SIO by ^1H NMR, and areas ratio between ω -6: ω -3 and vice versa to oils studied by GC-FID.

^1H NMR	2.70/2.74	2.74/2.70	2.70/2.74	2.74/2.70
Blends	(SIO:SO)	(SIO:SO)	(SIO:CO)	(SIO:CO)
100% SIO (0% SO/CO)	0.392	2.549	0.392	2.549
75% SIO (25% SO/CO)	0.580	1.724	0.595	1.681
50% SIO (50% SO/CO)	0.930	1.075	0.969	1.032
25% SIO (75% SO/CO)	1.720	0.581	1.591	0.628
0% SIO (100% SO/CO)	4.695	0.213	5.025	0.199
GC-FID	ω -6/ ω -3	ω -3/ ω -6	ω -6/ ω -3	ω -3/ ω -6
Blends	(SIO:SO)	(SIO:SO)	(SIO:CO)	(SIO:CO)
100% SIO (0% SO/CO)	0.533	1.875	0.533	1.875
75% SIO (25% SO/CO)	0.779	1.283	0.812	1.232
50% SIO (50% SO/CO)	1.319	0.758	1.367	0.731
25% SIO (75% SO/CO)	2.573	0.389	2.128	0.470
0% SIO (100% SO/CO)	7.857	0.127	8.047	0.124

Fig. 2.2a shows the polynomial equations of the curves with the data obtained by ^1H NMR. This Table shows that the second order polynomial equations between the area ratios for the signals at δ_{H} 2.74/2.70 ppm, with an accuracy ranging from 99.8 to 99.9% reliability. Values that were higher than those found when the calculation was performed with the ratio of the δ_{H} areas between 2.70 and 2.74 ppm had reliability ranging from 94.6 to 96.5%.



δ_{H} $^1\text{H NMR}$	Polynomial equation	R^2	fatty acids GC-FID	Polynomial equation	R^2
2.70/2.74 (SIO:SO)	$y = 0.0007x^2 - 0.1077x + 4.4717$	0.965	ω -6/ ω -3 (SIO:SO)	$y = 0.7708x^2 - 2.9808x + 3.0756$	0.959
2.74/2.70 (SIO:SO)	$y = 0.0001x^2 + 0.011x + 0.2181$	0.999	ω -3/ ω -6 (SIO:SO)	$y = 0.0584x^2 - 0.7893x + 2.6122$	0.999
2.70/2.74 (SIO:CO)	$y = 0.0008x^2 - 0.1177x + 4.7254$	0.946	ω -6/ ω -3 (SIO:CO)	$y = 0.8204x^2 - 3.2881x + 3.4173$	0.929
2.74/2.70 (SIO:CO)	$y = 0.0001x^2 + 0.0102x + 0.2276$	0.998	ω -3/ ω -6 (SIO:CO)	$y = 0.0596x^2 - 0.7842x + 2.5832$	0.996

Fig. 2.2. Polynomial relations between the areas ratio of: (a) the triplet signals: $-2.70/2.74$ (SIO:SO), $\square 2.74/2.70$ (SIO:SO), $\circ 2.70/2.74$ (SIO:CO) and $+ 2.74/2.70$ (SIO:CO) obtained by ^1H NMR and of the: (b) peaks: $- \omega$ -6/ ω -3 (SIO:SO), $\square \omega$ -3/ ω -6 (SIO:SO), $\circ \omega$ -6/ ω -3 (SIO:CO) and $+ \omega$ -3/ ω -6 (SIO:CO) obtained by GC-FID.

This is a useful technique to quantify the percentage of adulteration of oils rich in ω -6 (such as SO and CO) in oils rich in ω -3 (such as SIO or flax seed oil).

It is worth mentioning that Guillén and Ruiz (2005), when working with olive, hazelnut and peanut oil, observed signals next to those characterized by the chemical shifts of doubly allylic hydrogens after 48 days of oil treatment at 70°C . The signals were between

2:50 and 2.70 ppm, with another close to 2.90 ppm. These signals were highlighted because they indicate thermal degradation.

The signals between δ_H 0.80 and 0.92 ppm observed by Barison et al (2010) for ω -3 identification can also be quantified as the signals obtained in this study in the range of δ_H 2.67-2.75 ppm.

2.3.3. Fatty acid profile analysis with GC-FID

Fig. 2.3 shows the chromatograms of the studied oil blends, with the identification of ω -3 and ω -6 fatty acids. The chromatograms shown in Fig. 2.3 (A-I) illustrate the fatty acid profile of SIO (Fig. 2.3A), with increasing proportions of SO (Fig. 2.3B, 2.3C, 2.3D and 2.3E) and CO (Fig. 2.3F, 2.3G, 2.3H and 2.3I). With an increase in SO or CO in the SIO blends, the peak area of ω -6 increased and therefore the peak area of ω -3 decreased proportionally. This figure illustrates the data obtained from GC chromatograms of methyl esters of the oil blends.

Similar patterns to the data obtained by 1H NMR were observed in the data obtained by GC-FID. Table 2.2 shows the ratio of peak areas between ω -3 and ω -6 and between ω -6 and ω -3 in the studied oils. The ratios between of the ω -3 and ω -6 areas obtained by GC-FID and shown in Table 2.2 were adjusted by a second order polynomial equation, as shown in Fig. 2.2b.

Second order polynomial behavior was observed in Fig. 2.2b, similar to what occurred with the data obtained by 1H NMR to analyze the ω -3 and ω -6 contents. In this Figure are shows the polynomial equations of the curves with the data obtained by GC-FID. Also shows that the second order polynomial equations between the area ratios of ω -3 and ω -6 describe with high accuracy (99.6 to 99.9%) the behavior of the lipid profiles of SIO and its blends with SO and CO. This was even better than when the calculation was performed with the ratio of the areas of ω -6 and ω -3 where the polynomial fit varied from 92.9 to 95.9% reliability.

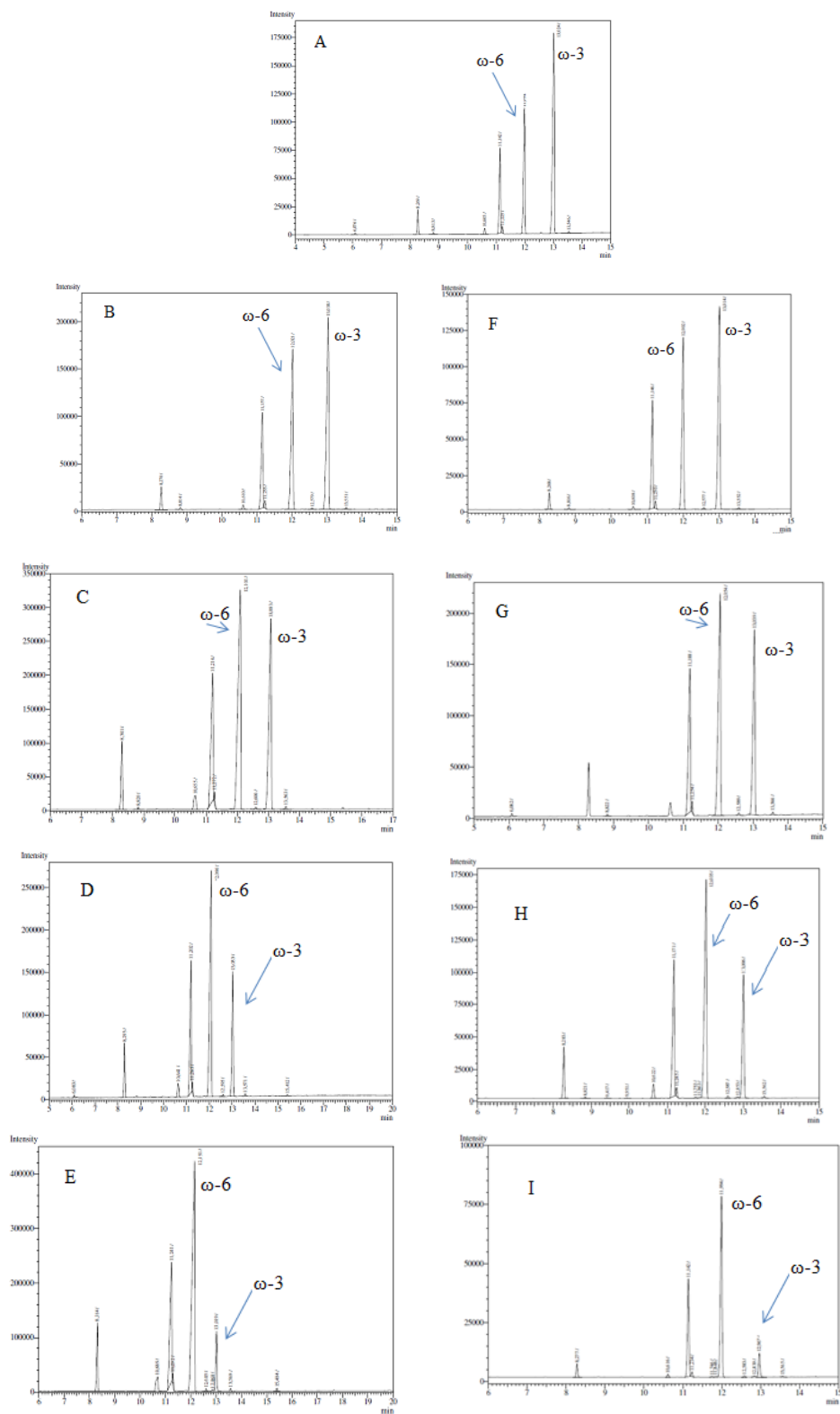


Fig. 2.3. Chromatograms of the oils blends studied, obtained by GC-FID. Where (A) SIO 100:0 SO; (B) SIO 75:25 SO; (C) SIO 50:50 SO; (D) SIO 25:75 SO; (E) SIO 0:100 SO; (F) SIO 75:25 CO; (G) SIO 50:50 CO; (H) SIO 25:75 CO e (I) = SIO 0:100 CO.

2.3.4. Analysis of the Pearson correlation coefficient

The calculation of the correlation coefficient was done by applying equation (2) to the ratio data between the compounds obtained by ^1H NMR and GC-FID, as shown in Table 2.3.

Table 2.3. Correlation coefficient between the ratios obtained by ^1H NMR and GC-FID.

^1H NMR	2.70/2.74	2.74/2.70	2.70/2.74	2.74/2.70
GC-FID	(SIO:SO)	(SIO:SO)	(SIO:CO)	(SIO:CO)
ω -6/ ω -3 (SIO:SO)	0.999	-0.775	0.999	-0.776
ω -3/ ω -6 (SIO:SO)	-0.789	0.999	-0.767	0.999
ω -6/ ω -3 (SIO:CO)	0.995	-0.746	0.999	-0.749
ω -3/ ω -6 (SIO:CO)	-0.795	0.998	-0.776	0.999

The correlation coefficient between the sum of the areas of the triplets at δ_{H} 2.70 ppm and those at δ_{H} 2.74 ppm obtained by ^1H NMR and the ratio of the areas between ω -6 and ω -3, ranging from 0.995 to 0.999, indicated a strong correlation between the two methods. Likewise, there was a strong correlation between the ratios of the triplets 2.74 and 2.70 ppm and the area ratio between ω -3 and ω -6, respectively, for both SO and CO.

The correlation between the two methods indicates that the use of ^1H NMR for the quantification of ω -6 and ω -3, identified by the signals at 2.70 and 2.74 ppm, respectively, can replace, without loss of information, the GC-FID technique to identify and quantify the fatty acid profile of vegetable oils such as Sacha Inchi oil.

It was observed that the correlation coefficients shown in Table 2.3 between the intensities of the signals at δ_{H} 2.70 ppm and the area ratio of ω -3, as well as the intensity of the signals at δ_{H} 2.74 ppm and the area ratio of ω -6, were inversely proportional (negative correlation coefficient) and far from the absolute value of 1. Thus, we can infer that the signal at δ_{H} 2.70 ppm refers to linoleic acid (ω -6) and that at δ_{H} 2.74 ppm refers to linolenic acid (ω -3).

2.4. CONCLUSIONS

The ratio of oils rich in ω -6 fatty acids such as CO and SO in oils rich in ω -3 fatty acids such as SIO can be quantified by second order polynomial equations.

Both techniques were highly correlated (r varied from 0.995 to 0.999), revealing that ^1H NMR is an efficient methodology for the quantification of ω -3 fatty acids in oils rich in ω -6 fatty acids such as SO and CO, and for the quantification of ω -6 fatty acids in oils rich in ω -3 fatty acids such as SIO. Thus, fatty acid profile analysis in oil with ^1H NMR can replace analysis with GC-FID, for both ME and for triacylglycerols.

The proton signals used to for the identification of ω -3 fatty acids and ω -6 fatty acids can be seen in the shifts of 0.81 ppm and 2.70 ppm for ω -6 fatty acids and 0.91 ppm and 2.74 ppm for ω -3 fatty acids.

^1H NMR can be used to identify and quantify both fraudulent and different proportions of oils rich in ω -6 fatty acids (CO and SO) in oils rich in ω -3 fatty acids (SIO and linseed oil) as well as oils rich in ω -9 fatty acids (olive oil).

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CAPITULO III – Simple emulsions formed from Sacha Inchi oil and ovalbumin or Tween 80 and stabilized with pectin and xanthan gum

ABSTRACT

The oil-in-water emulsions have been used on a large scale in the food industry and they have many applications, such as stabilizing lipophilic components in aqueous systems. Consequently, they can add value to a product, maintaining the quality of sensitive compounds and increasing the use of those components in several areas of food industry. The research goal was to evaluate the formation, stability and characterization of simple emulsions (oil-in-water), formed by Sacha Inchi oil (SIO) 8 % (w/w), and Ovalbumin (Ova) or Tween 80 (Tw 80) (as emulsifiers) at 0.5-2.0 % (w/w) and stabilized with pectin (Pec) at 1.0-3.0 % (w/w) or xanthan gum (XG) at 0.25-1.0 % (w/w) (as stabilizing). The emulsions were evaluated during 0, 1, 7 and 14 days after preparation and kept at temperature of $25 \pm 1^\circ\text{C}$ for the ζ -potential, particle size distribution, polydispersion (PdI), emulsion stability index. The characterization was evaluated for optical microscopy, viscosity and rheological behavior. The results showed that it is possible to form simple emulsions with SIO-Ova and Pec stable at 25°C for at least 14 days, with PdI value between 0.2 - 0.5. However the emulsions with SIO-Ova and XG are stable at certain concentrations of XG, even though it generates more viscous emulsions, leading to form aggregates. In addition, the presence of the biopolymer is essential for the kinetic stability in emulsions containing Ova as emulsifier. The same happens for the emulsions containing Tw80, this conclusion applies only to emulsions with XG in concentrations around 0.5-1.0 %, although the stability mechanism is distinct.

Keywords: emulsion stability, particle size distribution, PdI, rheological behavior, omega-3.

3.1 INTRODUCTION

Emulsions are used in large scale in the food industry and have many applications, such as stabilizing lipophilic components in aqueous systems, as oil-in-water (o/w) emulsion or hydrophilic components in lipid systems, as water-in-oil (w/o) emulsion. Both ways, can be added-value to a product, maintaining the quality and sensorial properties stay longer and expand the use of these in several areas of the food industry (DICKINSON, 2008; CHIVERO et al, 2015). Studies involving emulsions have grown in part due to the application of functional ingredients for food, such as omega-3 from Sacha Inchi oil (SIO). The SIO has a high concentration of polyunsaturated fatty acids (around 90 %), which almost 50 % of these

compounds refers to the α -linolenic acid, omega-3, responsible for several features and benefits to consumers health (STANLEY et al., 2007; SIEGEL & ERMILOV, 2012).

The emulsions requires emulsifiers or surfactants to be formed, which can be natural, such as ovalbumin (Ova) or synthetic such as polysorbate (Tween) In food emulsions, the interfacial region, where is the contact region between the dispersed lipids and the aqueous phase, it is the critical area to ensure the emulsion stability (DICKINSON, 2009) and also represents a critical area for oxidation development. Surface-active compounds (emulsifiers, polar lipid oxidation products, amphiphilic antioxidants) that adsorb in the oil/water interface has an evident influence on the lipid oxidation (BERTON-CARABIN et al, 2013).

The ovalbumin is a globular protein which has 3 nm diameter, molecular weight around 45 kDa, isoelectric point (pI) that ranges between 4.6 to 5.2 (HU & DU, 2000; WEIJERS, VISSCHERS & NICOLAI, 2004) Ova is widely used in the food industry due to it is emulsifying and foaming capabilities and it is ability to form gels under heating (DING, CAI & GUO, 2015; DAMODARAN, 2010), whereas, Tween is a nonionic synthetic surfactant, formed by polyethoxylated sorbitan and oleic acid. Tween 80 symbolizes the lipophilic group percentage, in this case the oleic acid (WILLIAMS et al., 1997; GAJDOVÁ, JAKUBOVSKY & VÁLKY, 1993).

Biopolymers (especially polysaccharides) have been used as stabilizers in emulsions (BOUYER et al., 2012). It has the ability to reduce the electrophoretic mobility in the system and, thus maintain stable emulsified components last longer. Xanthan gum (XG) is an anionic polysaccharide of high molecular weight produced by a gram-negative bacterium (*Xanthomonas campestris*). This gum is widely used in the food, pharmaceutical and cosmetic industries, due to safety concerns (BHATTACHARYA et al, 2012; SWORN, 2000). Based on short and long term feeding studies, the XG was evaluated by the US Food and Drug Administration (FDA) in 1969 allowing its use in food products without any specific limitation (BHATTACHARYA et al., 2013). The XG has been extensively used to produce emulsions and has also other applications, due to its excellent properties of dispersion and viscosity (highly pseudoplastic flow and dispersion in hot or cold water) (NIKIFORIDIS & KIOSSEOGLOU, 2010). The XG has as its main monomer the β -D-glucose, and pKa around 2.8 (STEPHEN, PHILLIPS & WILLIAMS., 2006). Pectins are anionic heteropolysaccharide containing 65% of weight of galacturonic acid units. It can be present as free acid (methyl ester) or in amidated pectin (starch acid), obtained by esterification of high methoxyl pectin (MAY, 2000). The galacturonic acid is one of the pectic substances class, which is the major

polysaccharide present in the cell's wall of terrestrial plants and fruits. Commercial pectins often containing sugar added to standardize performance (MAY, 2000). Its pKa is about 3.6 and its main monomer is the glucuronate (AL-HAKKAK & AL-HAKKAK, 2010; ZIMET & LIVNEY, 2009). Therefore, the microscopic and rheological properties as well the formation and stability of the systems formed are essential to the study flow. Due to the capacity to offer better measurement and use in an industrial scale for these emulsions (McCLEMENTS, 2004a; McCLEMENTS, 2015).

The aim of this study was to study the formation, stability and characterization of simple emulsions (oil-in-water), formed by Sacha Inchi oil (SIO) and ovalbumin (Ova) or Tween80 (Tw80), stabilized with pectin (Pec) or xanthan gum (XG).

3.2 MATERIAL AND METHODS

3.2.1. Material

The ovalbumin (Ova) (from chicken egg white, A5253) containing 62 to 68 % of protein, the Tween 80 (Tw 80), the xanthan gum (XG) and the pectin (Pec) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). The Sacha Inchi oil (SIO) was obtained from the manufacturer El Olivar (Lima, Peru). All the chemical reagents used to evaluate the study was analytical grade class and deionized water.

3.2.2. Preparation of emulsions

The emulsions were prepared and divided in four systems (as described at systems 1 to 4, in Table 3.1). The emulsions were prepared with Ova or Tw 80 in the 0.5, 1.0, 1.5 and 2.0 % (w/w) concentrations, with 8 % (w/w) of SIO and XG (0.25, 0.5 and 1.0 %, w/w) or Pec (1.0, 2.0 and 3.0 %, w/w), depending on the system. Also, it was prepared a control sample (without xanthan gum or pectin) as reference. In all emulsions was added 0.01% of sodium azide (NaN_3) to inhibit the growth of micro-organisms.

The emulsions were homogenized in T25 digital Ultra-Turrax[®] (IKA-Labortechnik, Germany) at 18000 rpm by 1 min. After, the emulsions were submitted to sonication in ultrasound bath (Ultrasonic Processor, Hilscher, Germany) for 12 min at 100 % of amplitude at 30 kHz of frequency and 0.5 cycles per minute to form emulsions. The pH of ultrapure

water utilized to prepare of emulsions was adjusted to 7.0 in buffer phosphate solution (10 mM).

Table 3.1. Composition of emulsions, % (w/w) of the systems formed by SIO and biopolymers.

	Code	SIO (%)	Ova (%)	XG (%)	Pec (%)
System 1	S1-control	8.0	0.5	0.0	0.0
	S1.1	8.0	0.5	0.25	0.0
	S1.2	8.0	0.5	0.5	0.0
	S1.3	8.0	0.5	1.0	0.0
	S1.4	8.0	0.5	0.0	1.0
	S1.5	8.0	0.5	0.0	2.0
	S1.6	8.0	0.5	0.0	3.0
System 2	S2-control	8.0	1.0	0.0	0.0
	S2.1	8.0	1.0	0.25	0.0
	S2.2	8.0	1.0	0.5	0.0
	S2.3	8.0	1.0	1.0	0.0
	S2.4	8.0	1.0	0.0	1.0
	S2.5	8.0	1.0	0.0	2.0
	S2.6	8.0	1.0	0.0	3.0
System 3	S3-control	8.0	1.5	0.0	0.0
	S3.1	8.0	1.5	0.25	0.0
	S3.2	8.0	1.5	0.5	0.0
	S3.3	8.0	1.5	1.0	0.0
	S3.4	8.0	1.5	0.0	1.0
	S3.5	8.0	1.5	0.0	2.0
	S3.6	8.0	1.5	0.0	3.0
System 4	S4-control	8.0	2.0	0.0	0.0
	S4.1	8.0	2.0	0.25	0.0
	S4.2	8.0	2.0	0.5	0.0
	S4.3	8.0	2.0	1.0	0.0
	S4.4	8.0	2.0	0.0	1.0
	S4.5	8.0	2.0	0.0	2.0
	S4.6	8.0	2.0	0.0	3.0
	Code	SIO (%)	Tw80 (%)	XG (%)	Pec (%)
System 5	S5-Control	8.0	0.5	0.0	0.0
	S5.1	8.0	0.5	0.25	0.0
	S5.2	8.0	0.5	0.5	0.0
	S5.3	8.0	0.5	1.0	0.0
	S5.4	8.0	0.5	0.0	1.0
	S5.5	8.0	0.5	0.0	2.0
	S5.6	8.0	0.5	0.0	3.0

System 6	S6-control	8.0	1.0	0.0	0.0
	S6.1	8.0	1.0	0.25	0.0
	S6.2	8.0	1.0	0.5	0.0
	S6.3	8.0	1.0	1.0	0.0
	S6.4	8.0	1.0	0.0	1.0
	S6.5	8.0	1.0	0.0	2.0
	S6.6	8.0	1.0	0.0	3.0
System 7	S7-control	8.0	1.5	0.0	0.0
	S7.1	8.0	1.5	0.25	0.0
	S7.2	8.0	1.5	0.5	0.0
	S7.3	8.0	1.5	1.0	0.0
	S7.4	8.0	1.5	0.0	1.0
	S7.5	8.0	1.5	0.0	2.0
	S7.6	8.0	1.5	0.0	3.0
System 8	S8-control	8.0	2.0	0.0	0.0
	S8.1	8.0	2.0	0.25	0.0
	S8.2	8.0	2.0	0.5	0.0
	S8.3	8.0	2.0	1.0	0.0
	S8.4	8.0	2.0	0.0	1.0
	S8.5	8.0	2.0	0.0	2.0
	S8.6	8.0	2.0	0.0	3.0

3.2.3. Emulsions stability evaluation

The emulsions stability was done by measurements on emulsion stability (%), on the zero, 1st, 7th and 14th days after preparing. The emulsions that there was no phase separation during 14 days at 25 °C was considered stable.

Aliquots of each emulsion were poured into a cylindrical graduated glass tube, sealed and stored at 25 °C for 14 days. The emulsion stability was measured by the volume of the upper phase over the storage period. The emulsion stability (ES) measurements were performed immediately after emulsions preparation (0 day), and at 1st, 7nd and 14nd as well. The ES was given by equation (1). (BOUYER et al, 2012)

$$ES (\%) = \frac{H_R}{H_T} \cdot 100 \quad (1)$$

where H_R is the upper phase volume (remaining phase) and H_T represents the total (initial) phase of the emulsion.

3.2.3.1 ζ -Potential, particle size distribution and Pdl analysis

For the ζ -potential, particle size distribution and polydispersity index (Pdl) analysis, the emulsions were diluted in ultrapure water in the 1:200 (v/v) proportions. The measurements were performed in the Zetasizer Nano (Nano-ZS 90, Malvern Instruments, Worcestershire, UK). The samples were equilibrated in the instrument during 10 seconds. The data were collected through at least 100 measurements and processed using the Smoluchowski model.

3.2.4. Emulsions characterization

3.2.4.1 Optical microscopy

For optical microscopy analysis the emulsions were performed 7 days after their preparation. The samples were poured onto microscope slides, covered with glass cover slips and observed using an Axioplan optical microscope (Zeiss, Gottingen, Germany) equipped with AxioCam MRc (Zeiss, Gottingen, Germany) using $\times 100$ objective lenses and immersion oil.

3.2.4.2 Viscosity and rheological characterization

For rheological characterization the emulsions were measured using a rotational rheometer (HaakeTM Mars III, Thermo Scientific Inc., Germany) at 25 °C, attached with cone and plate, C60/1° Ti L (60 mm, angle: 1°) and gap of 0.025 mm between the elements. The storage (G') and dissipation (G'') modulus were measured while the frequency varied from 0.01 to 10 Hz. The rheological measurement data were analyzed using the Rheowin Data Manager software Version 4.30 (Thermo Haake Co. Ltd., Karlsruhe, Germany). To classify the fluid behavior, flow and viscosity curves were generated.

3.2.5 Statistical analysis

All analyses were performed in duplicate and the results expressed as average \pm confidence interval (CI) Eq. (2) (MOORE, 2007).

$$CI = \frac{\sigma}{\sqrt{n}} \cdot t \quad (2)$$

where CI is the confidence interval, σ is the standard deviation, n is the number of repetitions and t is the Student's t -test inverse function (significance level of 5 %).

The ANOVA (at 95% probability) was applied to check the significant statistical differences between several compositions of the emulsions at different days of storage studied for emulsion stability, zeta potential and particle size distribution analysis.

3.3. RESULTS AND DISCUSSION

3.3.1 Emulsion formation

Regarding the emulsions formation presented in Table 3.1, the systems 1-4 are composed of Ova (natural emulsifier) and systems 5-8 are formed with Tween 80 (artificial emulsifier). In both cases, according to McClements (2015), the emulsification took place by interaction of hydrophobic groups between and SIO and Ova, and between SIO and Tween 80 (polysorbate), respectively. The polysaccharides (Pec and XG) used, are known for changing the texture and also to increase the viscosity of the aqueous phase of the emulsion to stop movement (and hence coalescence) between the dispersed oil droplets (DICKINSON, 2009; McCLEMENTS 2004).

In this context, most emulsions (in different compositions S1-S8) evaluated in this paper were formed. Obviously that the proportions between SIO, Ova or Tween and Pec or XG influenced in the greater interaction or emulsion kinetic stabilization (which are discussed in section 3.3.2). In case of emulsions containing XG, Dickinson (2009) states that it is possible to have gel formation due to the fact of an intermolecular network between XG molecules (discussed in 3.3.3.2).

3.3.2 Emulsion stability

The emulsion stability is a measurement that represents the portion of emulsion that remains stable, where 100 % represent stable emulsion, and any other value means unstable emulsion (BOUYER et al, 2012). The data are shown in the Table 3.2. The emulsions-control

(SIO and Ova) with 0.5 and 1.0% Ova (S1 and S2) were stable for 1 day, whereas the emulsions-control with 1.5 and 2.0% Ova (S3 and S4) were stable until the 7th day after prepared. However, it showed instability on the 14th day.

The use of Tw80 provided stability in the emulsions-control and in the emulsions containing 1.0 - 2.0 % (S6, S7 and S8) for 14 days. The only exception was the emulsion containing 0.5 % Tw 80 (S5). This shows that increased concentrations of Ova as well as with increased concentration of Tw 80 used in the control-emulsions had a positive effect on the stability of emulsions. Since higher emulsifier concentration (Ova or Tw 80) increases the amount of hydrophobic groups and thus a greater amount of oil in the emulsion can be stabilized (LAM & NICKERSON, 2013; McCLEMENTS, 2012; McCLEMENTS, 2004; DICKINSON, 2009).

From the first day after preparing, all emulsions containing 0.25 % XG showed instability, independently of the Ova or Tw 80 concentration (0.5, 1.0, 1.5 or 2.0 %, w/w) utilized. This XG concentration was not enough to stabilize the emulsion by decreasing the collisions numbers between oil droplets of the emulsion (CHIVERO et al, 2015). Although the presence of 0.25 % XG had not been enough to stabilize the emulsion, in general, the presence of the biopolymer, such as Pec or XG in the emulsion containing SIO and Ova, increased the stability of the emulsion system, when compared with the emulsion-control. A fact that should be consider according to Dickinson (2009) and Lam & Nickerson (2013) is that at low concentrations, the added non-adsorbing hydrocolloid (xanthan gum) has a destabilizing effect on the emulsion causing enhanced its serum separation. But at higher added hydrocolloid concentrations when the depletion interactions are stronger, creaming is inhibited due to the viscoelastic character of the interconnected regions of emulsion droplets which became flocculated into a gel-like network (CHANAMAI & McCLEMENTS, 2006).

On the other hand, all emulsion containing Ova and Pec, independently of the Pec concentration (1.0, 2.0 or 3.0 %, w/w), showed stability until the 14th day after emulsion preparation, when kept at 25 °C. These biopolymers (proteins and polysaccharides) prevents flocculation and coalescence by combined mechanisms (BOUYER et al., 2012). However, the opposite was shown for emulsions contend Tw 80 and Pec where in all Pec concentrations, the emulsions were destabilized on the first day after preparation. The emulsions formed with Ova were more stable than Tw80, probably, due to its better adsorption at the oil-water interface. The Ova is a globular protein and it has amphiphilic properties, hence, it migrate to the oil droplet surface, creating a resistant interfacial layer around the oil droplet, maintaining

the lower droplet to stabilize it. Moreover it is allow that the aqueous phase, with XG or Pec increase the viscosity and thus prevent the coalescence (TAHERIAN et al, 2011; FIORAMONTI et al, 2015).

All emulsions formed with Tw 80 and stabilized with Pec, independently of the concentration, were unstable almost immediately (in the first day). This effect may be related to fact that Tw80 has nonionic properties and has high concentrations of hydrophobic groups, which can lead to a solubilization in the oil phase (WILLIAMS et al., 1997; GAJDOVÁ, JAKUBOVSKY & VÁLKY, 1993). Due to it is solubility, is not only Tw 80 but the interface SIO and the droplets formed by Tw 80+SIO also becomes too large, thus, the pectin loses its ability to stabilize via the network formation between the droplets and end up separating from the aqueous phase (containing pectin) by difference in polarity. The same occurred with the use of XG, in contrast the systems S5, S6, S7 and S8, with the emulsion containing 1% XG. This concentration of XG allowed the emulsions to stabilize by increasing the viscosity, hindering the shock between the oil droplets and hence preventing the phase separation (CHIVERO et al, 2015). This may have happened, considering that xanthan gum is a non-adsorbing polymer and has higher molecular mass. It could be observed that the emulsion stability is a consequence of increasing of viscosity and a network formation that decreases droplets' movement and the number of collisions (KRSTONOŠIĆ et al, 2015; LAM & NICKERSON, 2013).

Table 3.2. Emulsion stability index (%) of the emulsions formed with Ova and Tw 80.

Ova 0.5%	Day 0	Day 01	Day 07	Day 14	Ova 1.0%	Day 0	Day 01	Day 07	Day 14
Control	100.0 ± 0.0	100.0 ± 0.0	45.2 ± 1.2	22.3 ± 1.4	Control	100.0 ± 0.0	100.0 ± 0.0	60.8 ± 0.6	35.6 ± 0.5
S1.1	100.0 ± 0.0	98.6 ± 0.9	64.8 ± 1.1	49.2 ± 1.3	S2.1	100.0 ± 0.0	98.6 ± 1.0	88.2 ± 0.8	76.8 ± 0.8
S1.2	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S2.2	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S1.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S2.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S1.4	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S2.4	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S1.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S1.6	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S2.6	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Ova 1.5%	Day 0	Day 01	Day 07	Day 14	Ova 2.0%	Day 0	Day 01	Day 07	Day 14
Control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	31.6 ± 0.3	Control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	40.6 ± 0.5
S3.1	100.0 ± 0.0	76.5 ± 0.8	63.2 ± 0.6	59.6 ± 0.6	S4.1	100.0 ± 0.0	100.0 ± 0.0	89.4 ± 0.4	84.5 ± 0.6
S3.2	100.0 ± 0.0	100.0 ± 0.0	95.5 ± 0.8	93.9 ± 0.7	S4.2	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S3.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S4.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S3.4	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S4.4	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S3.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S4.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S3.6	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S4.6	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Tw80 0.5%	Day 0	Day 01	Day 07	Day 14	Tw80 1.0%	Day 0	Day 01	Day 07	Day 14
Control	100.0 ± 0.0	100.0 ± 0.0	11.0 ± 0.4	11.0 ± 0.5	Control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S5.1	100.0 ± 0.0	38.2 ± 0.3	15.4 ± 0.4	14.4 ± 0.3	S6.1	72.4 ± 0.9	20.0 ± 0.7	15.1 ± 0.6	11.5 ± 0.5
S5.2	100.0 ± 0.0	100.0 ± 0.0	89.6 ± 0.6	80.5 ± 0.6	S6.2	100.0 ± 0.0	100.0 ± 0.0	82.4 ± 0.3	62.1 ± 0.4
S5.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S6.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S5.4	66.4 ± 1.0	25.8 ± 0.7	18.1 ± 0.6	17.0 ± 0.4	S6.4	33.2 ± 1.1	23.5 ± 0.8	15.1 ± 0.8	13.2 ± 0.6
S5.5	100.0 ± 0.0	50.6 ± 0.6	28.2 ± 0.8	25.0 ± 0.4	S6.5	100.0 ± 0.0	44.0 ± 1.0	26.5 ± 0.7	20.5 ± 0.6
S5.6	100.0 ± 0.0	79.0 ± 0.9	47.0 ± 0.6	44.9 ± 0.5	S6.6	100.0 ± 0.0	63.5 ± 0.7	42.2 ± 0.7	35.9 ± 0.8
Tw80 1.5%	Day 0	Day 01	Day 07	Day 14	Tw80 2.0%	Day 0	Day 01	Day 07	Day 14
Control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	Control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S7.1	83.3 ± 1.2	32.1 ± 0.9	17.4 ± 0.7	13.5 ± 0.4	S8.1	100.0 ± 0.0	32.1 ± 0.8	20.3 ± 0.3	14.4 ± 0.3
S7.2	100.0 ± 0.0	100.0 ± 0.0	93.6 ± 0.8	91.7 ± 0.6	S8.2	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S7.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	S8.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
S7.4	36.0 ± 1.0	27.9 ± 0.8	19.3 ± 0.7	14.8 ± 0.5	S8.4	34.8 ± 0.6	20.8 ± 0.4	13.5 ± 0.4	12.4 ± 0.3
S7.5	100.0 ± 0.0	44.3 ± 0.6	27.9 ± 0.4	23.9 ± 0.3	S8.5	100.0 ± 0.0	24.5 ± 0.6	19.5 ± 0.5	17.9 ± 0.3
S7.6	100.0 ± 0.0	72.1 ± 0.9	42.4 ± 0.5	36.3 ± 0.2	S8.6	100.0 ± 0.0	83.2 ± 0.9	50.6 ± 0.6	45.7 ± 0.4

3.3.2.1 ζ -potential

The ζ -potential represents the charge of electric double layer, which means that the further away from neutrality, the greater the electrostatic repulsion between the components. Therefore smaller is the interaction at the interface, as more stable this system becomes (WIACEK, & CHIBOWSKI, 1999; JAYME, DUNSTAN, & GEE, 1999)

As shown in Tab. 3.3, except for emulsions with 0.5 % Ova (S1), in all other emulsions-control (S2, S3 and S4), independently of the Ova concentration, the ζ -potential value were always far from zero value. The lowest values were observed in emulsion containing XG, it is because XG is known to be negatively charged hydrocolloid (KHOURYIEH et al., 2015; CHIVERO et al, 2015), and, therefore, showed negatives values for ζ -potential in the all ranges studied. The negative values in the evaluated emulsions could occurred for two reasons. Firstly is that both Pec and XG are anionic hydrocolloids, and secondly is that Ova in pH = 7.00 are over the pI (4.6-5.2) which leaves the negatively charged. When ζ -potential has is (\pm) 25 mV, in absolute values it is indicative of flocculated (less than) and deflocculated (greater than) (LEIBERMAN, RIEGER & BANKER, 1989; MIRHOSSEINI et al, 2008).

In the emulsion with 0.5 % Ova, the ζ -potential values varied in the unstable range, from -20.0 mV to almost 4.0 mV. Therefore, except for the emulsion-control and with 0.25% XG, all S1 system was stable until 14 days. We observe that over the days, the ζ -potential diverged more from zero charge, it is because the system tends to reach its thermodynamic balance, the oil droplets migrates to the emulsion interface, which decreases the interaction between the phases, thus neutralizing the charge of the system (DICKINSON, 2009; MIRHOSSEINI et al, 2008).

3.3.2.2 Particle size distribution and PDI

The average particle size distribution (PSD), as well as, PDI value (polydispersity index) are shown in the Tab. 3.3. In the Fig. 3.2 are shown the particle size distribution curves. The Figures shows the variation of the particle size distribution of the emulsions containing Ova (represented by S3) and emulsions containing Tw 80 (represented by S7). In the control-emulsions, it was observed that it had a comparatively low PDI value (approximately 0.2 to 0.4), however, by increasing concentration of the emulsion XG or Pec,

the PDI value also increases. Emulsions containing XG presented the greatest polydispersion, with values above 0.6. The greatest PDI value (0.941) was achieved in the emulsion containing 0.5 % Ova and 1.0 % Tw 80 (S5.3) in the 7st day after its preparation. In emulsions containing Ova and XG (S4) the PDI values reached the range of 0.8. PDI values as higher as the suggest aggregation of the particles (MALVERN, 2015).

It was observed that with increasing concentration of Ova in the emulsions, it also increased the average PSD, although the values observed for samples between 1.0 and 1.5 % Ova did not differ significantly in the emulsion control (no polysaccharide). It was observed an increase of the PSD over days, probably due to aggregation of the particles involved in the emulsion, thus favoring coalescence of the droplets and subsequent destabilization (FIORAMONTI et al., 2015).

Emulsions containing Pec, even though it was shown PDI values greater than 0.2, showed greater homogeneity of its particle size distributions, which can be seen clearly in Fig. 3.2. It was also observed that the averages particle size distributions were smaller compared to emulsions containing XG. The averages particle size distributions increased with the increase of Pec concentration, even better in the emulsions containing Ova (S1-S4) than in the emulsions containing Tw 80 (S5-S8), but in the last case, all emulsions had become with respect to analysis time (14 days). The emulsions with 1% XG showed the highest average particle size distribution, and, also PDI values, indicating that the occurrences of population's were very dispersed in the same emulsion. We observed that increasing the Pec or XG concentration, in relation the emulsions-control lead an increase in the particle size distribution average and PDI value. Another factor that enabled the increase of average particle size distribution and PDI value was the time.

Silva et al., (2011) suggest that when $PDI > 0.2$, the particles do not show good homogeneity. On the other hand, McClements (2005) cites that real food emulsions always contain a distribution of droplet sizes and PDI higher than 0.2 (polydisperse systems), and its droplet size is more complex than monodisperse systems forms. For values over 0.50 refers to the systems with very broad distribution (ZEEB, WEISS & McCLEMENTS, 2015). In that way, it is possible to observe that all the emulsions formed were characterized as polydispersion emulsions ($PDI > 0.2$).

It is known that only the average particle size is not sufficient to describe and represent the diameter of particles of the dispersed phase of an emulsion. According to Malvern (2015), the PDI value gives us an idea of the data dispersion in relation to the average

value and therefore becomes extremely important. The PDI is dimensionless and scaled such values smaller than 0.05 (rarely seen) and the maximum value is arbitrarily limited to 1.0. A PDI value of 1 indicates that the sample has a very broad size distribution and may contain large particles or aggregates that could be slowly sedimented.

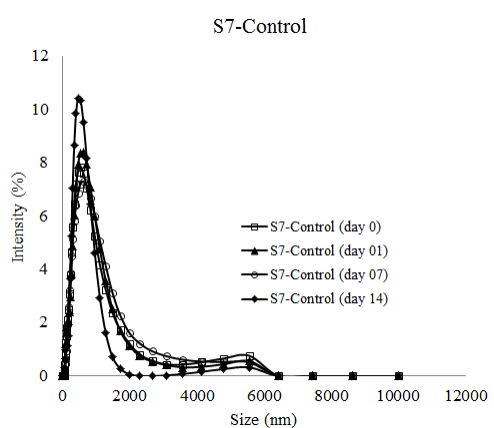
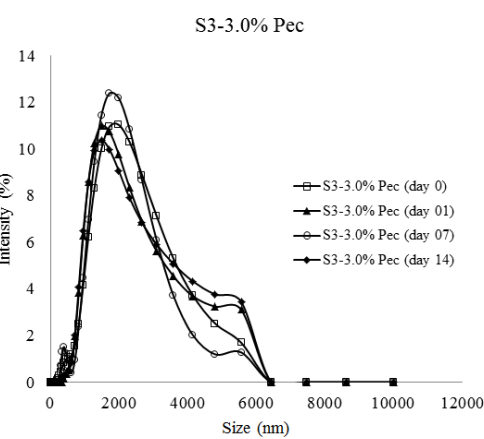
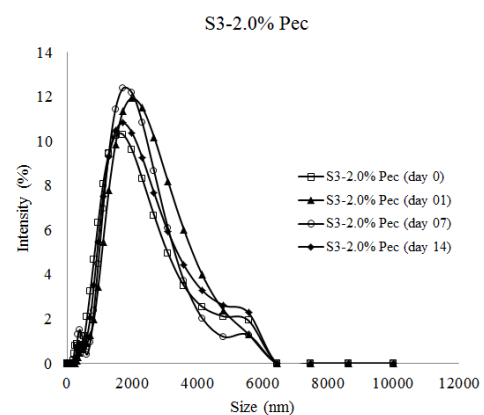
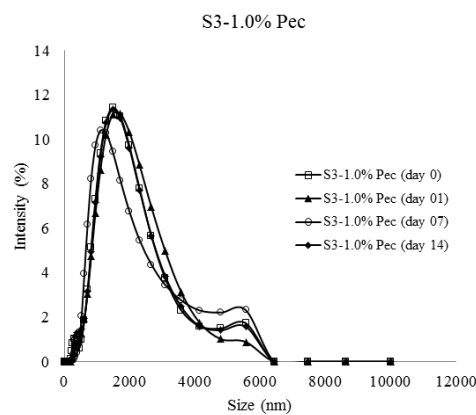
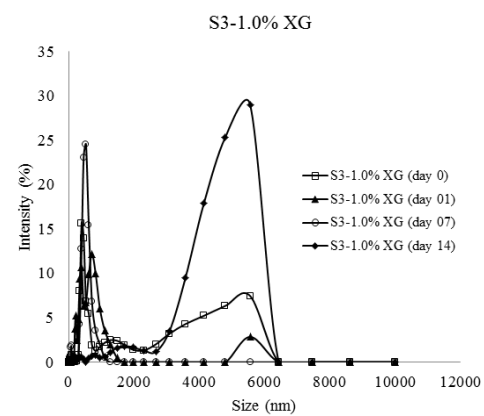
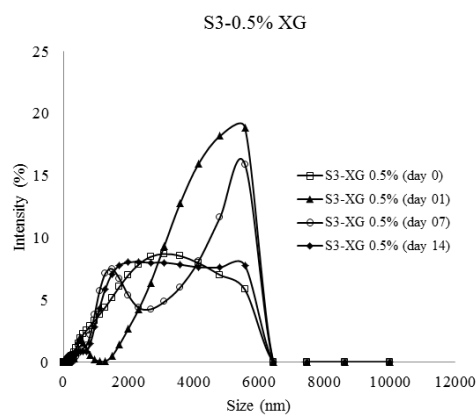
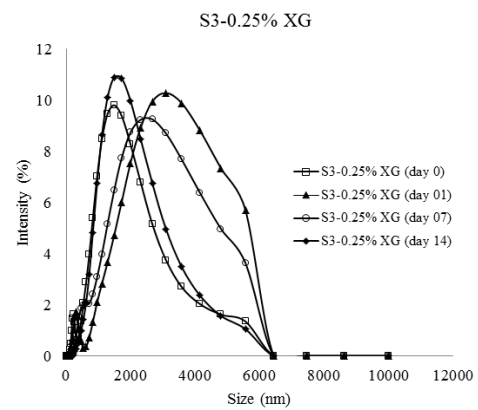
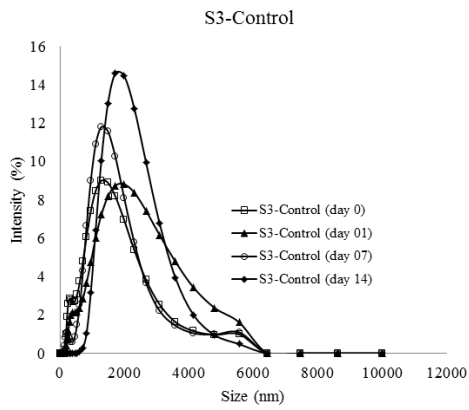
The emulsions which obtained kinetic stability (until the 14th day) and the smaller average particle size distribution, as well as, the PDI value was achieved in emulsions containing Ova and Pec. In these cases the averages ranged from 0.96 to 2.7 μm . The emulsions that had higher average particle size distribution and PDI value were the emulsions which had the smaller droplet sizes, as can be seen in Fig. 3.1, thus showing that particle size distribution does not refer to the droplets emulsion but the emulsion particles overall.

Table 3.3. Particle size distribution, Pdl and ζ -potential of the emulsions formed by Ova and Tween.

	S1-Control			S1-XG 0.25%			S1-XG 0.50%			S1-XG 1.0%			S1-Pec 1.0%			S1-Pec 2.0%			S1-Pec 3.0%		
	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot
Day 0	806	0,336	-20,6	1121	0,337	-6,6	1321	0,451	4,2	3124	0,228	-0,4	816	0,397	2,5	756	0,36	0,1	744	0,321	2,5
Day 01	694	0,218	-8,8	1794	0,336	-11,3	2247	0,283	4,3	2698	0,317	-0,9	757	0,384	4,1	775	0,358	4,4	811	0,413	3,5
Day 07	876	0,303	-0,6	2267	0,269	0,7	2378	0,245	4,0	3561	0,324	2,2	945	0,397	5,7	836	0,387	3,0	859	0,356	3,0
Day 14	494	0,299	-53	2129	0,359	-5,7	2533	0,193	2,5	2349	0,248	-7,7	825	0,400	-9,6	791	0,375	5,1	743	0,397	6,0
	718	0,289	-20,7	1828	0,325	-5,7	2120	0,293	3,8	2933	0,279	-1,7	836	0,394	0,7	790	0,37	3,2	789	0,372	3,7
	S2-Control			S2-XG 0.25%			S2-XG 0.50%			S2-XG 1.0%			S2-Pec 1.0%			S2-Pec 2.0%			S2-Pec 3.0%		
	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot
Day 0	966	0,233	-33,3	1148	0,412	-50,6	2371	0,455	-38,5	4631	0,733	-46,1	1197	0,285	-42,5	1215	0,32	-42,3	1143	0,318	-39,9
Day 01	1534	0,324	-18	1895	0,516	-49,9	3712	0,461	-40,1	5341	0,62	-47,1	1554	0,256	-43,8	1572	0,247	-41,5	1601	0,31	-39,4
Day 07	1279	0,327	-21	2418	0,497	-35,9	3739	0,461	-38,1	4306	0,689	-39,4	1249	0,261	-38,9	1617	0,278	-39,9	1497	0,273	-38,6
Day 14	1387	0,174	-19,4	4558	0,365	-60,8	4329	0,333	-42,8	5632	0,651	-40	1507	0,308	-40,7	1606	0,304	-40,6	1694	0,264	-39,3
	1292	0,264	-22,9	2505	0,447	-49,3	3538	0,427	-39,9	4977	0,673	-43,1	1377	0,278	-41,5	1503	0,287	-41	1484	0,291	-39,3
	S3-Control			S3-XG 0.25%			S3-XG 0.50%			S3-XG 1.0%			S3-Pec 1.0%			S3-Pec 2.0%			S3-Pec 3.0%		
	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot
Day 0	869	0,391	-29,2	1002	0,439	-33,9	1699	0,435	-36	4115	0,618	-43,5	1285	0,304	-41,5	1365	0,289	-41,2	1541	0,297	-39
Day 01	1158	0,426	-29,1	1714	0,523	-31,6	3376	0,378	-35,3	4882	0,768	-44,4	1342	0,278	-42,9	1642	0,254	-40,8	1663	0,267	-39,2
Day 07	1079	0,337	-22,5	1493	0,442	-21,7	2588	0,532	-35	4749	0,761	-50,9	1254	0,291	-40,8	1534	0,282	-38,9	1634	0,236	-39
Day 14	1377	0,253	-20,7	2039	0,45	-31,1	4222	0,269	-35,4	5580	0,773	-39,7	1285	0,302	-41,7	1553	0,293	-40,6	1657	0,295	-39,2
	1121	0,352	-25,4	1562	0,464	-29,6	2971	0,404	-35,4	4831	0,73	-44,6	1292	0,294	-41,7	1523	0,279	-40,4	1624	0,274	-39,1
	S4-Control			S4-XG 0.25%			S4-XG 0.50%			S4-XG 1.0%			S4-Pec 1.0%			S4-Pec 2.0%			S4-Pec 3.0%		
	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot
Day 0	936	0,367	-30	933	0,425	-35,1	1168	0,48	-35,9	4414	0,821	-41,3	1210	0,297	-40,7	1383	0,285	-39,5	2074	0,413	-39,8
Day 01	1947	0,308	-36	2294	0,492	-35,6	3621	0,439	-40,6	5235	0,769	-42,4	1307	0,275	-39,3	1730	0,226	-37,5	1679	0,306	-37
Day 07	2031	0,187	-29,7	2049	0,469	-35,6	4773	0,582	-79,3	4565	0,748	-47,2	1041	0,295	-42,8	1507	0,257	-44,8	1544	0,272	-38,9
Day 14	1872	0,29	-29,9	2649	0,537	-34,6	5484	0,409	-43,5	3163	0,703	-35,1	960	0,267	-39	1557	0,253	-38,6	1372	0,276	-38,1
	1696	0,288	-31,4	1981	0,481	-35,2	3761	0,478	-49,8	4344	0,76	-41,5	1130	0,283	-40,4	1544	0,255	-40,1	1667	0,317	-38,4
	S5-Control			S5-XG 0.25%			S5-XG 0.50%			S5-XG 1.0%			S5-Pec 1.0%			S5-Pec 2.0%			S5-Pec 3.0%		
	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot
Day 0	553	0,301	-25,5	619	0,46	-57	990	0,515	-63,8	5182	0,791	-72,5	549	0,441	-51,7	559	0,373	-37,7	442	0,296	-68,5
Day 01	473	0,369	-40,3	748	0,396	-51,5	1508	0,66	-59,4	2678	0,758	-62	562	0,366	-48,8	575	0,425	-50,4	494	0,352	-45,5
Day 07	417	0,337	-40	793	0,325	-41,6	1408	0,593	-57,2	1280	0,941	-64,9	599	0,352	-50,1	566	0,431	-46,8	521	0,357	-43,9
Day 14	1288	0,194	-38	767	0,309	-40,2	1117	0,582	-56,9	1692	0,673	-57,9	606	0,344	-43,1	603	0,434	-42,4	501	0,36	-47
	682	0,3	-36	732	0,372	-47,6	1256	0,587	-59,3	2708	0,791	-64,3	579	0,376	-48,4	576	0,416	-44,3	489	0,341	-51,2
	S6-Control			S6-XG 0.25%			S6-XG 0.50%			S6-XG 1.0%			S6-Pec 1.0%			S6-Pec 2.0%			S6-Pec 3.0%		
	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot	AS (nm)	PdI	ζ -Pot
Day 0	421	0,412	-49,5	500	0,42	-48,1	835	0,541	-51	3047	0,619	-49,9	401	0,447	-37,8	535	0,485	-25	557	0,454	-30,9

Day 01	432	0,423	-44,3	623	0,337	-41,4	825	0,557	-46,7	1573	0,609	-39,8	467	0,462	-40,9	524	0,463	-24,6	506	0,427	-33,3
Day 07	530	0,435	-39,6	607	0,394	-36,6	767	0,554	-42,8	2416	0,635	-41,4	516	0,478	-34,1	526	0,455	-26,6	498	0,433	-24,9
Day 14	373	0,303	-45,5	602	0,397	-43,6	704	0,489	-55,5	2305	0,637	-47,4	527	0,505	-35,7	588	0,478	-38,1	507	0,444	-31,9
	439	0,393	-44,7	583	0,387	-42,4	783	0,535	-49	2335	0,625	-44,6	478	0,473	-37,1	543	0,47	-28,6	517	0,439	-30,2
S7-Control	S7-XG 0.25%			S7-XG 0.5%			S7-XG 1.0%			S7-Pec 1.0%			S7-Pec 2.0%			S7-Pec 3.0%					
	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot
Day 0	391	0,402	-39,8	453	0,449	-45,8	1553	0,62	-53,5	3068	0,632	-54,2	372	0,427	-29,7	424	0,413	-19,9	472	0,417	-28,2
Day 01	406	0,415	-39,3	478	0,44	-44,7	1209	0,59	-46,3	1855	0,572	-40	435	0,404	-36,6	446	0,419	-27,5	455	0,428	-31,5
Day 07	423	0,412	-32,6	579	0,401	-33,9	962	0,567	-32,7	3528	0,586	-46,2	566	0,468	-36,6	476	0,405	-36,9	454	0,406	-29,9
Day 14	375	0,302	-38,9	561	0,386	-46,6	1278	0,61	-52,3	3561	0,626	-41,4	637	0,496	-34,2	501	0,438	-34,9	435	0,379	-32,2
	399	0,383	-37,7	517	0,419	-42,8	1251	0,597	-46,2	3003	0,604	-45,5	502	0,449	-34,3	462	0,418	-29,8	454	0,408	-30,5
S8-Control	S8-XG 0.25%			S8-XG 0.5%			S8-XG 1.0%			S8-Pec 1.0%			S8-Pec 2.0%			S8-Pec 3.0%					
	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot	AS (nm)	PdI	ζ-Pot
Day 0	384	0,425	-40,6	434	0,455	-40,7	2006	0,532	-46,5	4489	0,65	-45,3	580	0,47	-26,5	423	0,455	-24,3	896	0,508	-12,7
Day 01	406	0,418	-44,7	384	0,456	-39,5	1830	0,585	-44,9	2680	0,706	-41,8	419	0,552	-27,8	413	0,413	-22,5	393	0,358	-18,4
Day 07	438	0,438	-30,4	592	0,396	-30,4	1646	0,576	-37	4344	0,578	-39,7	513	0,481	-27	452	0,45	-17,8	475	0,405	-12,3
Day 14	341	0,309	-35,1	553	0,437	-38	1828	0,663	-41,6	4340	0,614	-40	437	0,499	-28,1	499	0,486	-24,3	410	0,337	-20,8
	392	0,397	-37,7	491	0,436	-37,1	1827	0,589	-42,5	3963	0,637	-41,7	487	0,501	-27,4	447	0,451	-22,2	543	0,402	-16

where, AS = Average particle size distribution (nm), ζ-Pot = ζ-Potential (mV) and PdI = Polydispersity index



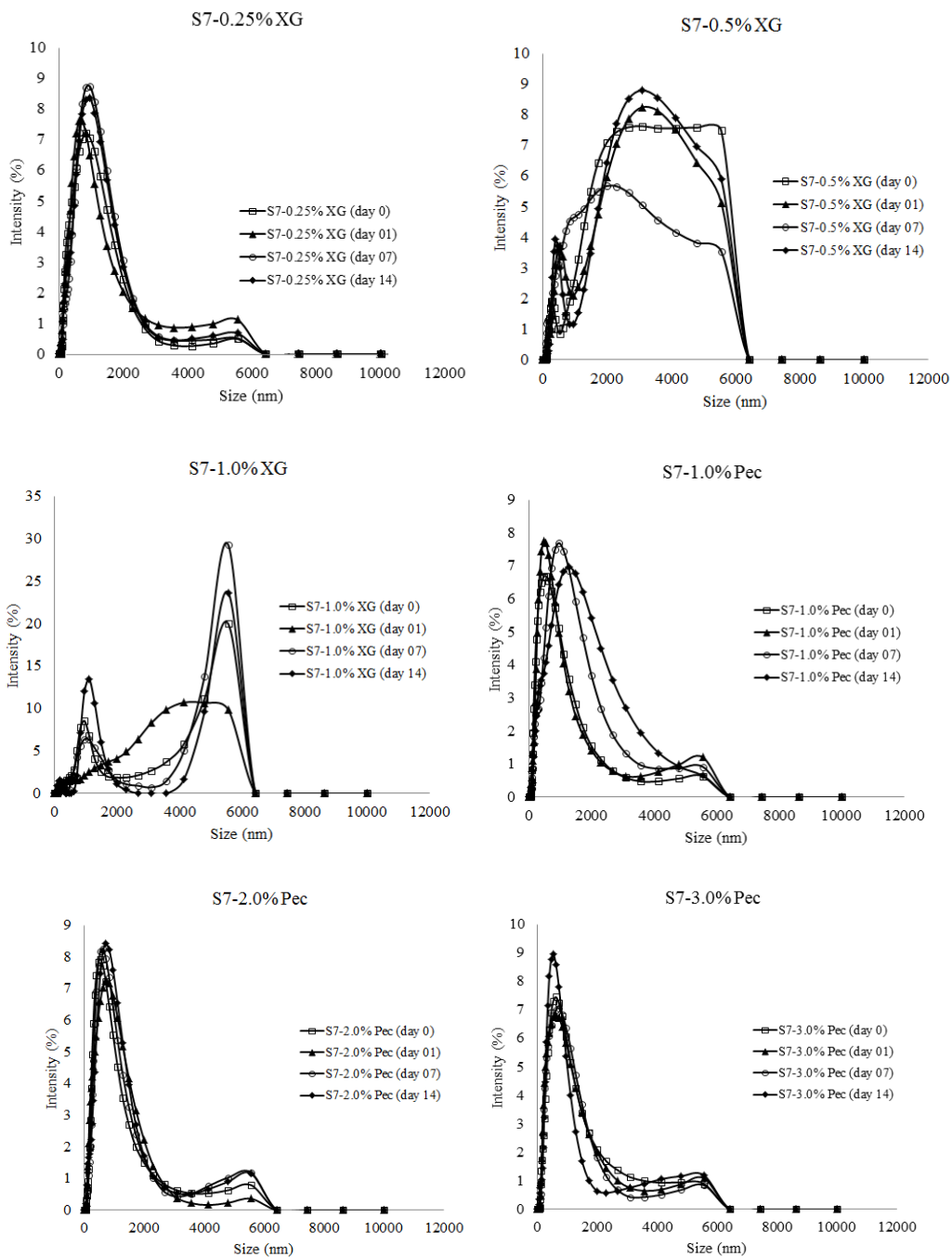


Fig. 3.1. Particle size distribution of S3 (Ova) and S7 (Tw80) systems.

3.3.3 Emulsion characterization

3.3.3.1 Optical microscopy

Fig. 3.2 shows the optical microscopy of the emulsion formed. In Fig. 3.2(a) it is possible to observe that in the SIO-Ova, the particle size increase visually, favoring the

creaming process and consequently reducing the emulsion stability. In the other hand, in the samples containing pectin (Fig. 3.2b) and xanthan gum (Fig 3.2c), respectively, the droplets are visually smaller. This shows the effectiveness of both biopolymers to reduce the electrophoretic mobility and consequently increasing the emulsion stability.

In Fig. 3.2(d), emulsion formed with SIO-Tw80-Control, we can observe that particle size is bigger than Fig 3.2(e), even the emulsion contained SIO-Tw80-1% XG. There is a possibility that for the same reason caused the emulsions stability formed with Ova, due to the fact that the XG has the power to increase the viscosity of the emulsion reducing electrophoretic mobility, and hence reduces droplet coalescence (DICKINSON, 2009; CHIVERO et al, 2015).

In both emulsions (Fig. 3.2) containing Ova, even the one with Tw80. The XG formed a more viscous emulsion, and decreased the possibility of droplets movement, resulting in a less droplet diameter compared with emulsions stabilized with Pec, which are smaller than the control emulsions (without polysaccharide). Thus, the use of pectin or XG favored increasing the kinetic stability of emulsions studied.

According to Dickinson (2009) it is important that emulsion droplets to be smaller as possible to minimize gravity creaming effect. In our work we found that the XG was the best given this effect, however only combined with Ova, because with Tw80 the emulsions were unstable. The stabilizing effect of the biopolymers was observed by Chivero et al, (2015) when they introduced xanthan and guar gums to stabilize oil-in-water emulsions containing Perilla seed oil.

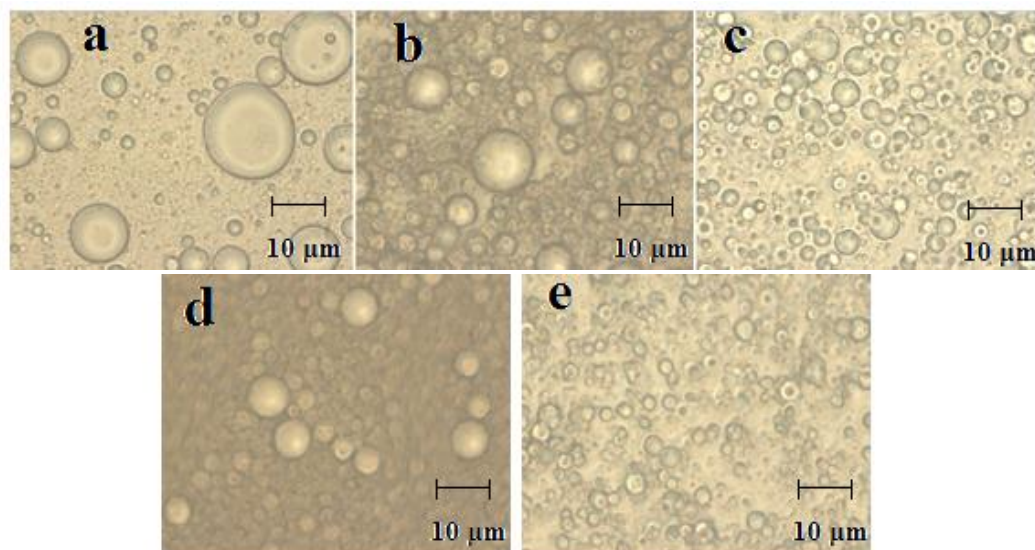


Fig. 3.2. Optical microscopy of (a) S2-Control (b) S3.4 (SIO-Ova-1%Pec), (c) S3.3 (SIO-Ova-1%XG) (d) S7-Control and (e) S7.3 (SIO-Tw80-1%XG) systems.

3.3.3.2 Viscosity and rheological behavior

Fig. 3.3 shows the flow curves (3.3a) Viscosity (η) at 25°C and (3.3b) storage (G'), dissipation (G'') moduli of emulsions studied. The data are shown in the Tab. 3.4, and presents the models that had better fit, in all emulsions, were the Power Law and Herschel-Bulkley models. However, in the Power Law model there are not initial shear stress (τ_0), in both emulsions-control (S4, with Ova and S8, with Tw80), also for the emulsions containing Pec. In all emulsion studied it is shows that these are non-Newtonian systems, as it was expected (LAM & NICKERSON, 2013; McCLEMENTS, 2004a; DAMODARAN, 2010).

The emulsions-control, although has less viscous, showed similarly with the emulsion contend Ova and Pec, as shown in Fig. 3.3. Thus, it shows that the Pec, although it had an increase in the viscosity emulsion, it did not changed the model used to adjust it and the fluid behavior was kept. Therefore, both the emulsions-control such as emulsions containing Pec and Ova, can be well adjusted to Power Law model.

The emulsions with Tw80 and XG (S8-1% XG) obtained the lowest adjust ($R^2 = 0.9378$) to Power Law model. In this case, the hydrocolloid is commonly applied to slow down or even prevent creaming by modifying the rheology of the continuous phase (DICKINSON, 2009). According to BeMiller & Huber (2010), the presence of XG leads to a formation of a pseudoplastic fluid, increasing substantially the fluid viscosity. In case of emulsions (O/W), this is an interesting behavior, because in this way it is possible to trap the oil droplets, hindering the collision between it and thereby preventing the coalescence. XG is especially effective in this type of stabilizing role. This simple rheological control mechanism is most effective at low oil volume fractions, where individual droplets are separately immobilized in an entangled biopolymer network, and the small buoyancy force acting on each droplet is hardly sufficient to overcome the effective yield stress of the surrounding weak gel-like biopolymer matrix (DICKINSON, 2009).

The rheological behavior was studied in emulsions containing Ova-XG and Tw80-XG. The results observed in Fig. 3.3, demonstrate elastic behavior, where $G' > G''$. However, in emulsions containing Ova-XG indicates the formation of a weak gel ($G' > G''$ but almost parallel). Unlike emulsions containing Tw80-XG, wherein the gel formed was stronger. As mentioned by Ding, Cai & Guo (2015) and Damodaran (2010) it is due the XG has the gel-forming capacity in aqueous systems.

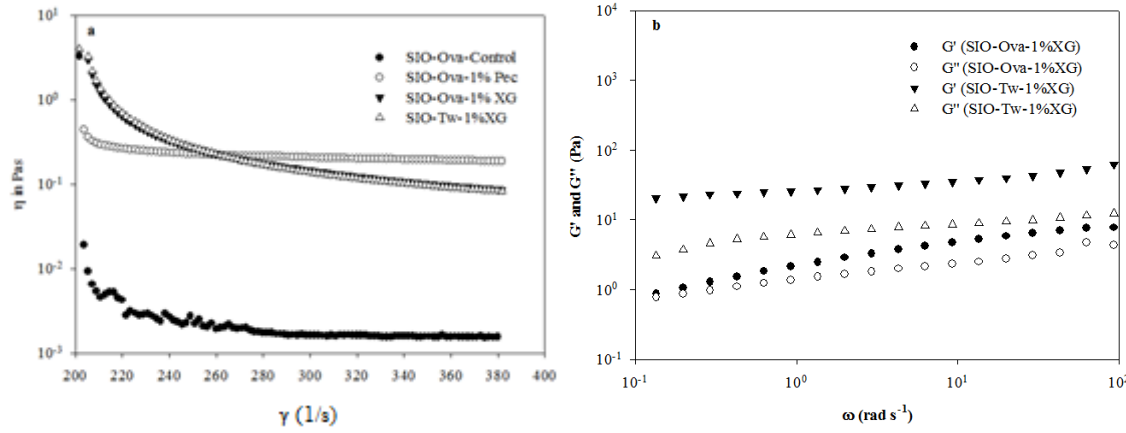


Fig. 3.3. (a) Viscosity (η) at 25°C; (b) storage (G'), dissipation (G'') moduli of emulsions formed by SIO and biopolymers.

Table 3.4. Parameters of the rheological models of emulsions formed by SIO and biopolymers.

System	Newton ^a		Power Law ^b			Bingham ^c			Casson ^d				Herschel-Bulkley ^e			
	η	R^2	K	n	R^2	τ_0	η_B	R^2	τ_0	η	K_C	R^2	τ_0	K	n	R^2
S4-Control	0.01513	0.9921	0.0021	0.9444	0.9928	0.02164	0.00145	0.9934	0.001545	0.5	0.00135	0.9931	0.03101	0.00112	1.041	0.9935
S4-1% Pec	0.02580	0.9991	0.0357	0.9449	0.9999	0.2093	0.02517	0.9995	0.01542	0.5	0.0237	0.9997	-0.1248	0.04069	0.925	0.9999
S4-0.5% XG	0.03410	-1.021	2.475	0.2656	0.9958	6.402	0.01498	0.9390	4.645	0.5	0.00467	0.9753	0.5064	2.147	0.2824	0.996
S8-Control	0.00121	0.9893	0.00143	0.971	0.9895	0.01267	0.00117	0.9900	-	-	-	-	0.02678	0.00071	1.079	0.9903
S8-1% XG	0.11550	-3.608	23.38	0.0886	0.9378	32.17	0.01943	0.6402	28.7	0.5	0.002365	0.7217	-735.3	758	0.00367	0.9818

τ = shear stress (Pa), η = viscosity (Pa.s), $\dot{\gamma}$ = shear rate (s^{-1}), τ_0 = initial shear stress (Pa), K = consistency index (Pa.sn), K_C = Casson plastic viscosity ($Pa.s^{0.5}$), n = flow behavior index.

$$^a \tau = \eta (\dot{\gamma})$$

$$^b \tau = K (\dot{\gamma})^n$$

$$^c \tau = \tau_0 + \eta_B (\dot{\gamma})$$

$$^d \tau^{0.5} = \tau_0^{0.5} + K_C (\dot{\gamma})^{0.5}$$

$$^e \tau = \tau_0 + K (\dot{\gamma})^n$$

3.4. CONCLUSIONS

It is possible to form simple emulsions composed of 8% SIO-Ova (1.0-2.0%) and Pec (1-3%), stable at 25°C for at least 14 days, which has PdI values between 0.2 - 0.5 and may be used to form hydrophilic foods rich in lipophilic active compounds (as omega-3). Moreover the emulsions formed with Tw80 instead of Ova are not stable in any Pec concentrations in this present study. XG is stable only at concentration of 1%, which can generate aggregates because of the higher amount of PdI founded on those systems. The presence of biopolymer in appropriate quantities is essential for the kinetic stability of emulsions containing Ova as emulsifier. The same is applicable for the emulsions containing Tw80, however this conclusion applies only for emulsions with XG in concentrations of between 0.5-1.0%, although the stability mechanism is distinct.

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**CAPITULO IV – Omega-3 encapsulation by emulsion-based systems
formed by biopolymers.**

ABSTRACT

In this study, omega-3 fatty acids from Sacha Inchi oil (SIO) was encapsulated by simple emulsion formation using Ovalbumin (Ova), pectin (Pec) and xanthan gum (XG) followed by freeze drying. The encapsulation was confirmed using Fourier transform infrared (FTIR), X-ray diffraction (XDR), thermogravimetric analysis (TGA) and scanning electronic microscopy (SEM). The encapsulation efficiency (EE) was measured in all composition studied. The SEM revealed a distinct and characteristic structure between the control microcapsules, just SIO-Ova (no Pec or XG) and between containing Pec or XG. The thermograms showed an thermal resistance increased by using biopolymers, which indicates that this may be a way to protect the omega-3 contained in the Sacha Inchi oil. Another conclusion was that the encapsulation also conferred increased crystallinity degree, indicating a higher degree in the structures organization. Finally the analysis by ^1H NMR allowed concluding that this encapsulation method did not affect oxidative state of Sacha Inchi oil.

Keywords: encapsulated by emulsion-based, Sacha Inchi oil, TGA, FTIR, ^1H NMR, omega-3.

4.1 INTRODUCTION

Microencapsulation is a technique that aims to ensuring the quality and physical-chemical stability of heat or photosensitive components which offers interest for the food industry, such as omega-3 (GAONKAR et al, 2014; McCLEMENTS, 2004). The source of omega-3 used in this study was Sacha Inchi oil (SIO), which has high concentration of polyunsaturated fatty acids (around 90%), almost 50% of these refers to α -linolenic acid (omega-3). The SIO is a rare vegetable source (along with flaxseed oil) which has in its composition greater than or equal to 50% concentration of omega-3 (MAURER et al., 2012; FANALI et al., 2011). That can explain why it is known as “Oro Inca” (FOLLEGATTI-ROMERO et al., 2009; GUILLEN et al., 2003). The polyunsaturated fatty acids (such as omega-3) are responsible for several features and benefits to consumer in health such as prevent fatal cardiovascular disease (ARAB-TEHRANY et al., 2012).

One of the most inexpensive and practical ways to produce encapsulated is by simples emulsion formation following by freeze drying. It is a useful tool to encapsulate

lipophilic components that naturally have low water solubility, and thus make it possible the food fortification and/or hydrophilic systems with hydrophobic components, which increases the diversity of application thereof in foods that were thermodynamically unfavorable (McCLEMENTS, 2004). There is growing interest in the formulation of added-value products containing healthy ingredients (i.e., Omega-3) for the functional food market (ACHOURI, ZAMANI & BOYE, 2012). The emulsions are used on a large scale in the food industry and have many applications such as microencapsulation of lipophilic components, adding value and keeping quality of sensitive compounds. The use of polysaccharides such as pectin and/or xanthan gum aims to reduce electrophoretic mobility of formed emulsion, preventing coalescence and consequently, providing greater stability to the system (DICKINSON, 2009). The biopolymers (protein and polysaccharides) are widely used to encapsulate bioactive compounds, as omega-3, carotenoids, vitamins, and others lipophilic compound with nutraceutical purpose. Carneiro et al (2013) used gum Arabic, maltodextrin, whey protein concentrate and two types of modified starch whereas Fioramonti et al (2015) used whey protein isolate and sodium alginate to microencapsulate flaxseed oil, which is rich in omega-3, as the SIO.

The aim of this paper was to study the encapsulated of SIO formed by ovalbumin, pectin and xanthan gum for several processes, such as, thermal characteristics, crystallinity degree, FT-IR and NMR spectra analysis, in the loaded oil content, efficiency, omega-3 stability when compared with microcapsule-control (no polysaccharide) freeze-dried to find a better way for the encapsulation.

4.2 MATERIAL AND METHODS

4.2.1 Material

The ovalbumin (Ova) (from chicken egg white, A5253) containing 62 to 68% of ovalbumin, the xanthan gum (XG) and the pectin (Pec) were purchased from Sigma-Aldrich (St. Louis, USA). The Sacha Inchi oil (SIO) was obtained from the manufacturer El Olivar (Peru). In this experiment were used analytical grade reagents and ultrapure water (GEHAKA, BRAZIL) of conductivity $0.05 \pm 0.01 \mu\text{/cm}$.

4.2.2 Methods

4.2.2.1 Preparation of microencapsulated

Firstly, the emulsions were prepared and divided in four systems (according described at systems 1 to 4, in Table 1). The emulsions were prepared with Ova in the 0.5, 1.0, 1.5 and 2.0 % (w/w) concentrations, with 8 % (w/w) of SIO and XG (0.25, 0.5 and 1.0 %) or Pec (1.0, 2.0 and 3.0 %), depending of the system. The pH of ultrapure water utilized to prepare the emulsions was adjusted to 7.0 in buffer phosphate solution (10 mM).

The emulsions were homogenized in UltraTurrax-T25 (IKA-Labortechnik, Germany) at 18000rpm by 1 min. Subsequently, they were submitted to sonication in ultrasound probe (Ultrasound Processor, Hilscher, Germany) for 12 min at 30 kHz frequency (100% of amplitude and 0.5 cycles per minute). Then, the samples were freeze dried to form the microencapsulated. The oil content (expected) after freeze dried are shown in the Tab. 4.1.

4.2.2.2 Microcapsules characterization

The microencapsulated freeze dried was characterized by thermogravimetric analysis (TGA), scanning electronic microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy and X-ray diffraction.

4.2.2.2.1 Thermogravimetric analysis

The thermograms of microencapsulated as well as XG, Pec, SIO and Ova, were performed by TA Instruments SDT-Q600 (USA). Each sample (10mg) was heated on a platinum pan (as reference) between 25 and 600 °C at a rate of 10 °C/min, under N₂ flow of 100 mL.min⁻¹.

4.2.2.2.2 Spectrometric analysis

The FTIR spectra of microencapsulated samples and all used components (XG, Pec, SIO and Ova) were obtained on a Bruker FTIR spectrometer (Vertex 70, Germany), using the KBr pellets and the range of 4000 to 500 cm⁻¹. The NMR spectra were acquired on a 400

MHz Bruker Advance II spectrometer 400 MHz, dissolving the sample in CDCl₃ in a 5 mm tube.

4.2.2.2.3 X-ray diffraction (XRD)

The crystallinity assays (or XRD patterns) were recorded over a 2θ range 1-60° using X-ray diffractometer (Rigaku, MiniFlex II, Japan) operating at 30 kV and 15 A, with a step angle of 0.02°/min and acquisition time of 2s.

4.2.2.2.4 Scanning electron microscopy (SEM)

The microencapsulated freeze dried was deposited in flesh mica and they were metalized with gold-palladium using a sputter coater (Emitech, K550X, United Kingdom). The morphology of the samples and all components (XG, Pec, SIO and Ova) used to be produced was observed by SEM (ZEISS, EVO MA 10, Germany) at acceleration of 5 to 10 kV.

4.2.2.2.5 Loaded oil content and encapsulation efficiency.

According to Karaca, Nickerson & Low (2013), approximately 20-30 mg of microcapsule were weighed in a falcon tube (15 mL), added 5 mL of isopropanol, 2 mL of ultrapure water and 2 mL of hexane. The tube was homogenized on Vortex (Pheonix, AP 56, Brazil) and centrifuged (Orto Alresa, Digicen 21 R, Spain) at 4000 rpm for 30 min. Then, the supernatant phase was removed (washed twice with hexane) and oil mass was measured, the oil content is given by Eq. 1.

$$OC (\%) = \frac{w_{oil}}{w_c} \cdot 100 \quad (\text{Eq. 1})$$

where w_{oil} is the oil mass after centrifugation, and w_c is the capsule mass utilized in this analysis. The EE (encapsulation efficiency) is the % loaded oil content divided by % oil content (theoretical), given by Eq. 2.

$$EE (\%) = \frac{\% \text{ Loaded oil content}}{\% \text{ oil content (theoretical)}} \cdot 100 \quad (\text{Eq. 2})$$

4.2.2.2.6 Omega-3 stability

After the microcapsule formation, the omega-3 stability was evaluated in the microcapsule control (SIO-Ova), with XG (SIO-Ova-XG) and Pec (SIO-Ova-Pec). Approximately 100 mg of samples were measured and solubilized in deuterated chloroform (CDCl_3) to separate oil phase. The oil phase was analyzed in ^1H NMR to quantify the omega-3 content as done by Vicente, Carvalho and Garcia-Rojas (2015).

4.3. RESULTS AND DISCUSSION

For structural characterizations, it was chosen the samples with higher efficiency of encapsulation in each composition (Tab. 4.1), therefore the samples were S.2.2 control (SIO-Ova), S.3.2 (SIO-Ova-1% XG) and S.3.3 (SIO-Ova-1% Pec).

4.3.1 Thermogravimetric analysis

The thermograms of the microencapsules as well as XG, Pec, SIO and Ova are shown in the Fig. 4.1 (a) TGA and (b) DTG (derivate of TGA curve). The first step of weight loss from 40 to 120°C refers to loss of moisture (adsorbed and bound water). The second step from 200 to 340°C (to Ova, Pec and XG, lines 2, 3 and 4, respectively, in Fig. 4.1a) is attributed to dehydration, saccharide rings, depolymerization and decomposition of the acetylated and deacetylated units of the polymers. For SIO, SIO-Ova, SIO-Ova-Pec and SIO-Ova-XG, this second step occurs at a temperature ranging from 350 to 480°C (HOSSEINI et al, 2013; KAKKAR et al, 2014).

The DTG is shown in Fig. 4.1b; in this graph, it is clearly the maximum temperature of degradation (T_d) of each component alone and the ones with formed microcapsules. In this analysis it was observed that SIO was incorporated and interacts harmoniously with the biopolymer to form the microcapsules.

The formation of microcapsules lead to an increased in T_d (from 95 to 175°C, approximately) when compared to the T_d in the biopolymers alone, getting close to T_d of SIO (411.56°C). The difference among T_d of microcapsules-control (401.43°C), with Pec (397.17°C) and with XG (407.29°C) did not differ much from each other, but they do differ

compared to the initially biopolymers, Ova (302.86°C), Pec (232.53°C) and XG (276.76°C). The displacement of T_d value of the microcapsules closest to of T_d of SIO can be explained by two mutually reasons. First it has to interaction between SIO and Ova (in forming the microcapsule by emulsion-based) and second due to the high concentration of oil (about 73-80%) which causes prevail the SIO characteristics (McCLEMENTS, 2004; DICKINSON, 2009).

The thermograms showed characteristic peaks of change state for each component, and between other analyzed microcapsules, as well, the FT-IR data. Because the diffraction patterns showed low crystallinity for the formed microcapsules, the data was prevailing only the characteristic peak of the SIO presence.

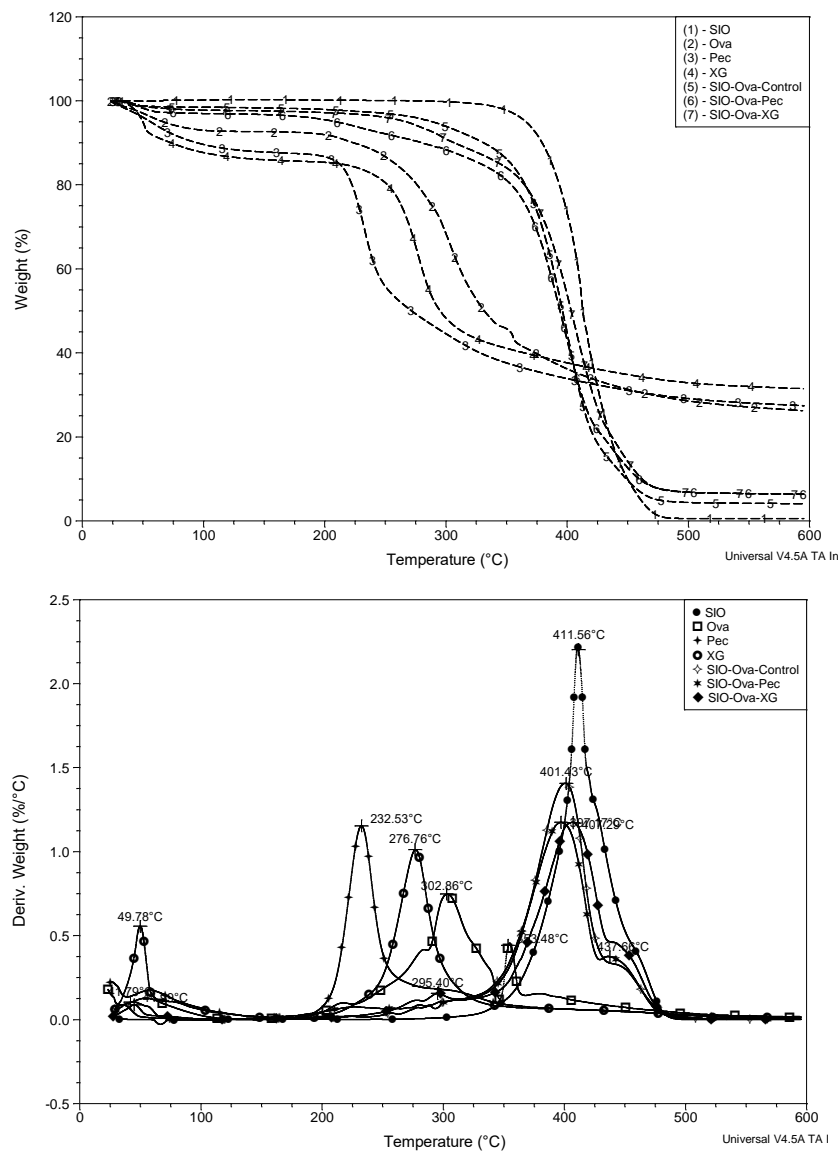


Fig. 4.1(a). TGA and **(b)** DTG (derivate of TGA curve) of SIO, biopolymers and encapsulated formed by SIO and biopolymers.

4.3.2 FT-IR spectra

The FTIR spectra of encapsulated and all used components (XG, Pec, SIO and Ova) to be produced are shown in Fig. 4.2a and 4.2b, respectively. For the composition of all biopolymers, the principals observed signals of oil were: ν_{\max} (cm^{-1}): next to de 3010 (*w*, =CH), at 2962-2853 and 1463 (C-H of CH_2 and CH_3), 1746 (C=O of ester), 1648 (*w*, C=C). The additional signals in the spectra are the strong absorptions of the biopolymers (Ova, Pec and XG) used to form the encapsulated, even for that in minor concentrations (HOSSEINI et al., 2013; ROSA et al, 2013). The *w* represents a weak signal.

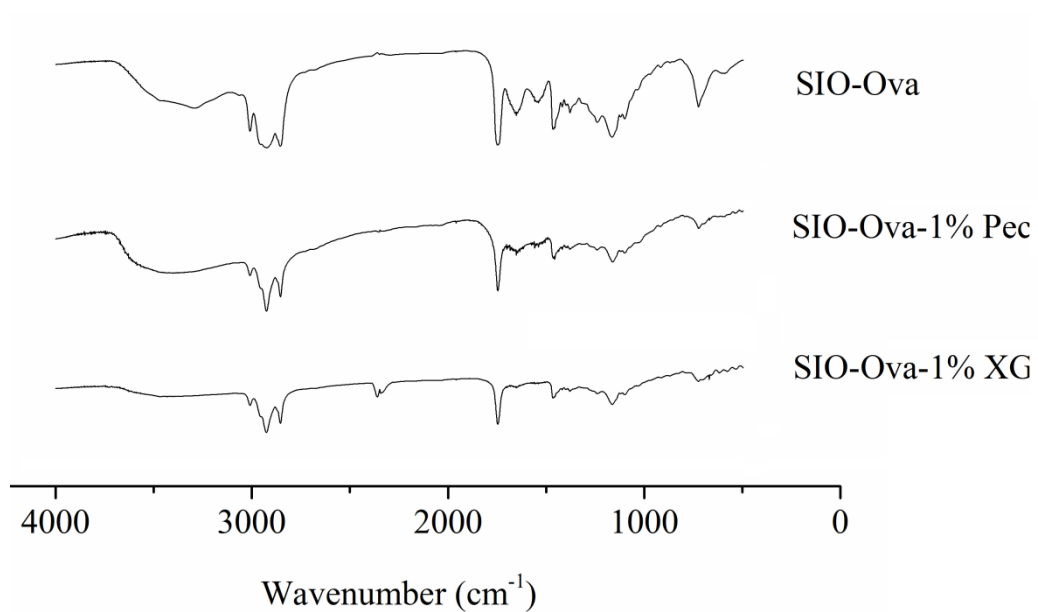


Fig. 4.2a. FTIR spectra of encapsulated formed by SIO and biopolymers.

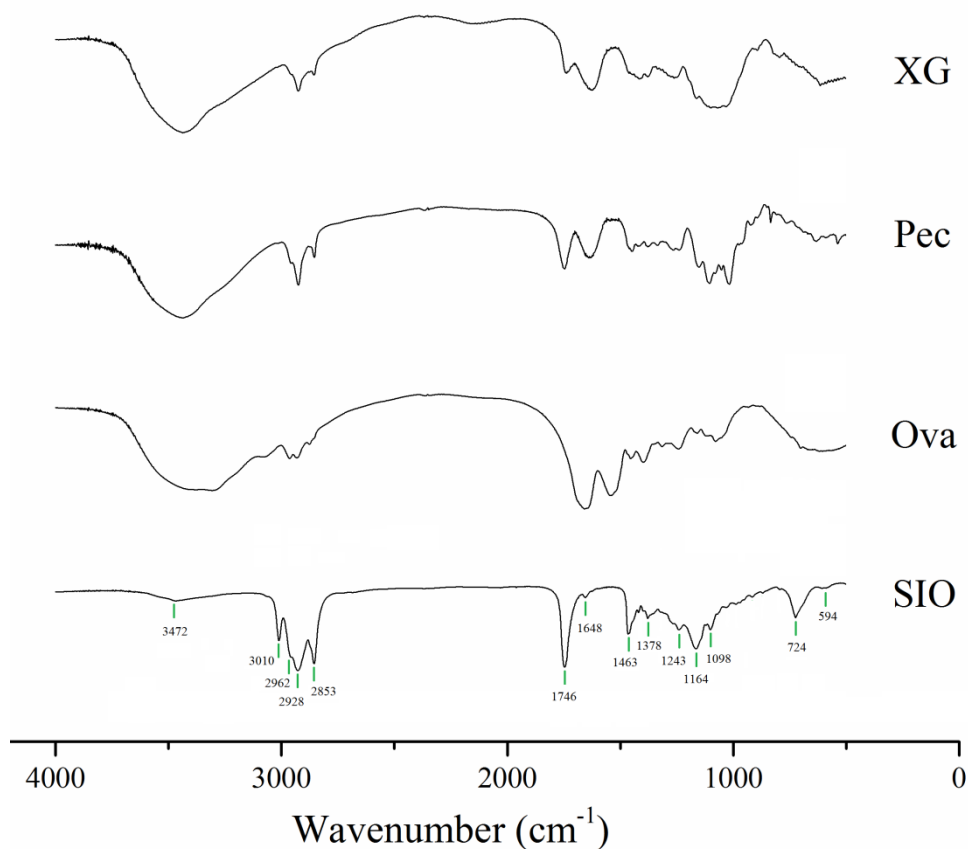


Fig. 4.2b. FTIR spectra of SIO, Ova, Pec and XG used to produce of encapsulated.

4.3.3 X-ray diffraction (XRD)

The XRD patterns are showed in the Fig. 4.3. The structure of SIO, Ova, Pec and XG were evaluated separately. It was observed that all three polymers showed similar values to each other at their characteristic 2θ of 18.9° (XG) 19.6° (Ova) and 21.2° (Pec) indicates a low crystallinity degree.

It can perceive that all of utilized components (XG, Pec, SIO and Ova) in this study have an amorphous structure. However, the microcapsules formed among SIO-Ova-Pec, SIO-Ova-XG, as well as the microcapsule-control (SIO-Ova) showed higher degree of crystallinity, and consequently a better defined structure than the compounds alone. The signals are observed between $5 - 14^\circ$. Thus, the formation of microcapsule can provide a more organized structure of the biopolymers, compared to the initial structure. The amorphous characteristics are linked directly to the hygroscopicity and hydrophilicity capacity of the biopolymers. It is known however that the peak width is related to the crystallinity degree of components (PICKUP et al, 2014; SOUZA & GARCIA-ROJAS, 2015). In the Fig. 4.3, cps

(counts per sec) is the intensity of the peak and theta θ is the inclination degree.

It is important to point it out that the improved structural organization that was observed with the increase in the degree of the crystallinity may be possibly related to the low OH stretching frequency (around 3470 cm^{-1}) of FTIR spectra (Fig. 4.3). It is observed that in the SIO and encapsulated the frequency is lower than that seen in Ova, Pec and XG. Based on this, it is suggested that when the frequency is smaller it indicates that the hydrogen bonding between the components is bigger, confirming in this case, a greater degree of structural organization.

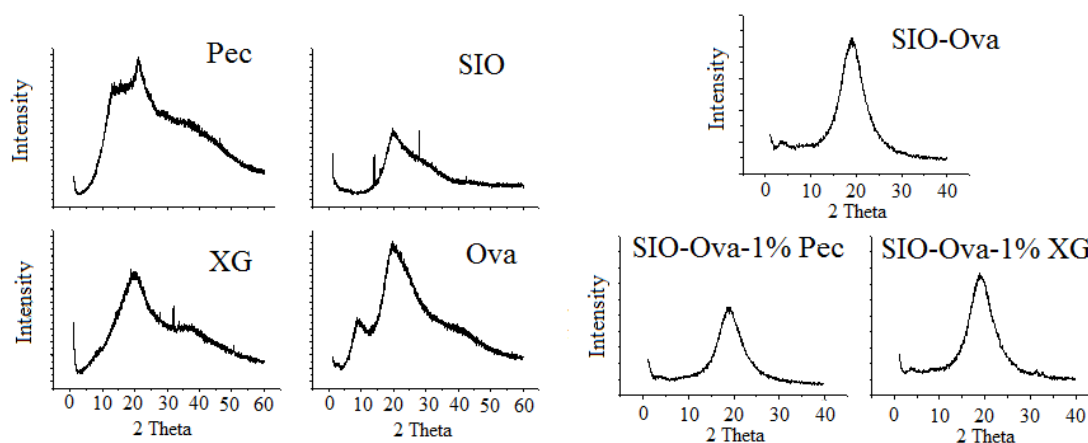


Fig. 4.3. XDR patterns of each component (SIO, Ova, Pec and XG) and microcapsules formed (SIO-Ova-Pec and SIO-Ova-XG).

4.3.4 Scanning electron microscopy (SEM)

The SEM images of the XG, Pec, Ova and samples with SIO-Ova, SIO-Ova-1% Pec and SIO-Ova-1% XG are shown in Fig. 4.4. The images revealed a distinct and characteristic structure between the control microcapsules. Just SIO and Ova (SIO-Ova-Control) when they no Pec and no XG) and between containing Pec or XG. This microcapsule shows a droplet with an undefined structure as is shown in the microcapsules containing 1% Pec or 1% XG (SIO-Ova-1% Pec and SIO-Ova- 1% XG, respectively).

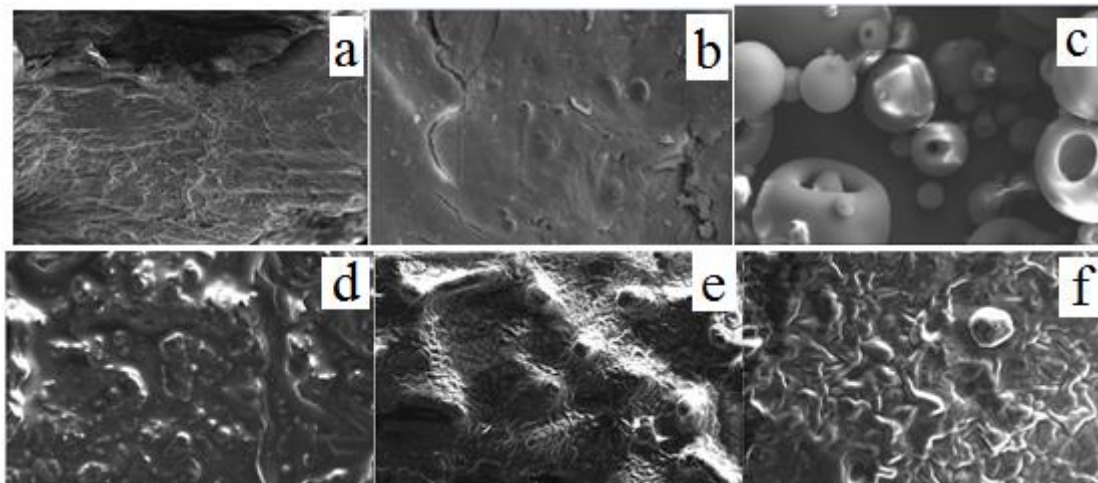


Fig. 4.4. SEM images of (a) XG, (b) Pec, (c) Ova, (d) SIO-Ova, (e) SIO-Ova-1% Pec and (f) SIO-Ova-1% XG

4.3.5. ^1H NMR spectra

The ^1H NMR spectra analysis (Fig. 4.5) were used to evaluate the oxidative stability of Sacha Inchi oil encapsulated. The signals (θ) with a range of chemical shift at δ_{H} 0.80 - 0.97 related to CH_3 , γ (δ_{H} 1.23 - 1.37) refers to the $(\text{CH}_2)_n$; δ_{H} 1.49-1.68 ($\text{CH}_2\text{-CH}_2\text{-CO}$); δ_{H} 2.25 - 2.32 ($\text{CH}_2\text{-C=O}$); δ (δ_{H} 1.96-2.09) to allylic protons; (ϵ) δ_{H} 5.27 - 5.40 (HC=CH); (ζ) δ_{H} 2.73 - 2.83 of doubly allylic protons ($=\text{C-CH}_2\text{-C=}$) of omega-3 (δ_{H} 2.78 ppm) and the omega-6 (δ_{H} 2.74 ppm) fatty acids; the signals at δ_{H} 4.10 - 4.29 and 5.22 - 5.26 represents the methylene and methynic protons of glycerol unity, respectively. There were no additional signs (Figure 4.5) to justify the oxidation of the encapsulated oil, such as the formation of epoxides and peroxides (FANG et al, 2013; BARISON et al, 2010). Thus, the encapsulation of the emulsion formation followed by freeze drying is an effective way to keep the omega-3 contained in Sacha Inchi oil oxidatively stable.

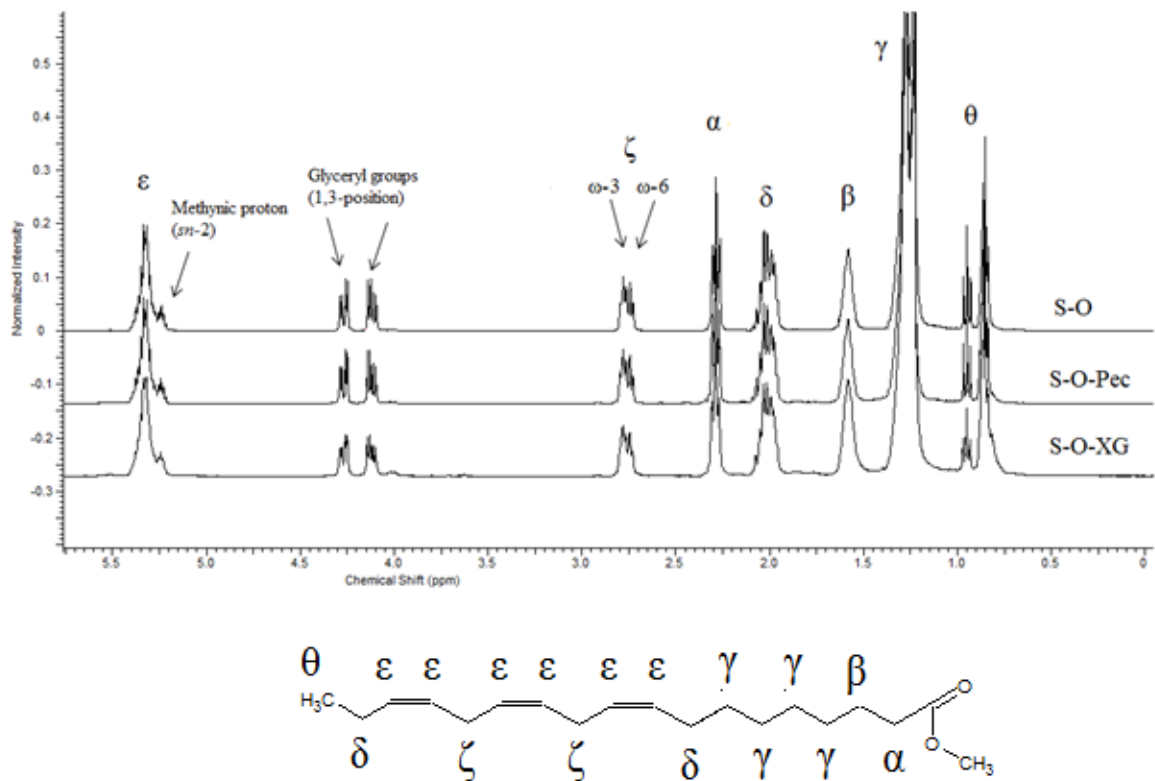


Fig. 4.5. ^1H NMR spectra of encapsulated formed by SIO and biopolymers.

4.3.6. Loaded oil content and encapsulation efficiency.

The data are shown in Table. 4.1. The encapsulation efficiency (EE) ranged from $86.6 \pm 0.7\%$ to $96.6 \pm 0.2\%$ in the range of Ova (0.5 - 2.0%) studied, indicating the high capacity of biopolymers used to form encapsulated. However, in comparison to the influence of the Pec and XG in the same Ova concentration, it is observed that the encapsulated containing Pec had greater efficiency to hold the SIO than those using XG, although the range of XG concentration utilized has been smaller. The presence of Pec or XG had lower EE compared to the encapsulated-control (SIO-Ova) in the same Ova concentration.

Table 4.1. Composition, oil content (%), loaded oil content and efficiency of microencapsulated studied.

	Code	SIO (%)	Ova (%)	XG (%)	Pec (%)	Oil content (%)*	Loaded oil content (%)**			Efficiency (%)***		
System 1	Control	8.0	0.5	0.0	0.0	94.1	89.8	±	0.5	95.4	±	0.5
	S1.1	8.0	0.5	0.25	0.0	91.4	84.5	±	0.9	92.4	±	0.9
	S1.2	8.0	0.5	0.5	0.0	88.9	79.0	±	0.4	88.9	±	0.5
	S1.3	8.0	0.5	1.0	0.0	84.2	75.0	±	0.3	89.1	±	0.3
	S1.4	8.0	0.5	0.0	1.0	84.2	78.9	±	0.3	93.7	±	0.3
	S1.5	8.0	0.5	0.0	2.0	76.2	69.3	±	0.2	90.9	±	0.2
	S1.6	8.0	0.5	0.0	3.0	69.6	62.1	±	0.4	89.2	±	0.5
System 2	S2-control	8.0	1.0	0.0	0.0	88.9	85.8	±	0.2	96.6	±	0.2
	S2.1	8.0	1.0	0.25	0.0	86.5	78.9	±	0.5	91.2	±	0.5
	S2.2	8.0	1.0	0.5	0.0	84.2	74.2	±	0.3	88.2	±	0.3
	S2.3	8.0	1.0	1.0	0.0	80.0	69.4	±	0.5	86.8	±	0.7
	S2.4	8.0	1.0	0.0	1.0	80.0	74.8	±	0.3	93.5	±	0.3
	S2.5	8.0	1.0	0.0	2.0	72.7	65.7	±	0.3	90.4	±	0.4
	S2.6	8.0	1.0	0.0	3.0	66.7	59.2	±	0.5	88.8	±	0.7
System 3	S3-control	8.0	1.5	0.0	0.0	84.2	79.4	±	0.3	94.2	±	0.4
	S3.1	8.0	1.5	0.25	0.0	82.1	73.9	±	0.6	90.1	±	0.7
	S3.2	8.0	1.5	0.5	0.0	80.0	75.0	±	0.6	93.7	±	0.7
	S3.3	8.0	1.5	1.0	0.0	76.2	70.3	±	0.9	92.3	±	1.2
	S3.4	8.0	1.5	0.0	1.0	76.2	69.8	±	0.2	91.7	±	0.2
	S3.5	8.0	1.5	0.0	2.0	69.6	61.6	±	0.5	88.6	±	0.7
	S3.6	8.0	1.5	0.0	3.0	64.0	56.9	±	0.3	89.0	±	0.5
System 4	S4-control	8.0	2.0	0.0	0.0	80.0	76.4	±	0.3	95.5	±	0.4
	S4.1	8.0	2.0	0.25	0.0	78.0	72.1	±	0.2	92.3	±	0.3
	S4.2	8.0	2.0	0.5	0.0	76.2	68.9	±	0.5	90.4	±	0.6
	S4.3	8.0	2.0	1.0	0.0	72.7	64.0	±	0.4	88.0	±	0.5
	S4.4	8.0	2.0	0.0	1.0	72.7	63.8	±	0.4	87.8	±	0.6
	S4.5	8.0	2.0	0.0	2.0	66.7	61.8	±	0.5	92.7	±	0.8
	S4.6	8.0	2.0	0.0	3.0	61.5	57.0	±	0.6	92.6	±	0.9

* Oil content (is the theoretical value) after freeze drying; ** Loaded oil content (%) is the oil content (experimental); *** Efficiency is the percentage of oil loaded by oil content (theoretical).

4.4. CONCLUSIONS

The encapsulation by emulsion formation was successfully carried out, confirmed by FTIR, TGA and XDR. This method provides increases in the thermal resistance in the biopolymers used for their formation, which, indicates that this may be a way to protect the

omega-3 contained in the Sacha Inchi oil.

The encapsulation also conferred increased of crystallinity degree when compared to the biopolymers alone, this indicates a higher degree of organization of their structures. The emulsion formation and freeze dried as an encapsulation method did not affect the oxidative state of Sacha Inchi oil.

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CAPITULO V – Effect of human digestion simulation in the omega-3 content of Sacha Inchi oil and microcapsules formed by biopolymers.

ABSTRACT

This study aimed to evaluate the stability of omega-3 content of Sacha Inchi oil (SIO) and microencapsulated with S-O, S-O-Pec and S-O-XG, when it is submitted to the human gastric simulation (HGS) process by proton nuclear magnetic resonance (^1H NMR). The decrease rates of omega-3 content in SIO and S-O and the omega-3 release rates in S-O-Pec and S-O-XG microencapsulated were calculated. It was also adjusted mathematically and correlated to the decrease and/or increase with proton signals observed by ^1H NMR during the HGS procedure. In SIO, the release of omega-3 acyl units was correlated with the decrease of the methinic proton (*sn*, 2 position) of glyceryl of ester (TAG), in contrast, the increase of 1,3-diglycerides was negatively correlated with the decrease of glyceryl groups (*sn* 1,3). It was shown that the HGS conditions did not significantly alter the omega-3 content of SIO over 180 min and also did not promote the development of oxidized compound such as epoxides and hydroperoxides. The S-O had a similar behavior to the SIO, and Ova presence was not enough to prevent the decrease of omega-3 content over 180 min. The S-O-Pec and S-O-XG microcapsules shown to be effective for the omega-3 protection and can be used to transport nutraceutical compounds because these can be resistant to the human gastric conditions.

Keywords: Sacha inchi oil, ^1H nuclear magnetic resonance, omega-3 stability, human gastric simulation, microencapsulated.

5.1 INTRODUCTION

Sacha Inchi (*Plukenetia volubilis* L.) oil has a high concentration of unsaturated fatty acids (close to 90%), where 48-50% represents to acyl chain from linolenic acid (omega-3), 32-37% acyl chain from linoleic acid (omega-6) and 9-12% from oleic acid (omega-9) (GUILLÉN et al, 2003; FOLLEGATTI-ROMERO et al, 2009, VICENTE, CARVALHO & GARCIA-ROJAS, 2015). The main reasons to encapsulate bioactive compounds are (i) for protection reasons, to avoid exposure to factors such as oxygen, light, heat, metals and water, to ensure their properties, (ii) to mask flavors, colors or odors, (iii) to facilitate manipulation (iv) to prevent undesirable reactions between the bioactive compound and food ingredients and (v) to ensure the release controlled of the same in the human gastrointestinal tract (HGT) (GAONKAR et al, 2014).

The bioactive compounds, such as omega-3, commonly referred as nutraceuticals has constituents that typically occur in small amounts in food and also have the ability to modulate one or more metabolic processes (AJILA, JAGANMOHAN RAO, & PRASADA RAO, 2010). Many bioactives compounds are lipophilic, which hinders its addition in aqueous foods. The low solubility in water also results in less absorption in the gastrointestinal tract and therefore limits the bioavailability (DONSÌ et al, 2011). The fortification of foods with polyunsaturated fatty acids, such as omega-3, it is of great interest not only because of the increasing awareness of nutrition over the world, but mainly in the prevention of malnutrition in developing countries. However the main difficulty for the food fortification with functional lipids is to maintain the physicochemical stability due to their high susceptibility to undergo oxidation (McCLEMENTS, DECKER & WEISS, 2007; HUR, LIM, DECKER, & McCLEMENTS, 2011). Emulsion-based as a way to encapsulate can be a strategy to prevent the changes in the oil compositions (omega-3, specially) when submitted to human gastric conditions. According to McClements (2015), the most commonly used encapsulation technology for this type of lipid is oil-in-water emulsions.

In the human gastrointestinal tract (HGT), foods are digested by a combination of physical and chemical processes. Chemical digestive processes are catalyzed by digestive enzymes that are secreted in the stomach, and have the function to reduce the foods to a molecular scale. Physical processes are mainly induced by peristalsis, which plays an important role by promoting food digestion at HGT (KOZU et al, 2013). Several research groups have reported that the stomach fluids movements promote emulsification of the oil components and the release of these presents compounds (SCHWIZER et al, 2006; ABRAHAMSSON et al., 2005).

In view of the foregoing, it is clear the importance to know the behavior of the Sacha Inchi oil stability and microencapsulated in human gastric simulation (HGS) conditions. Moreover, the use of ^1H NMR can help to understand how changes occur in the oil in the free and microencapsulated structure when subjected to gastric conditions which aim to evaluate the chemical stability and effectiveness of omega-3 release in system.

5.2 MATERIAL AND METHODS

5.2.1. Chemical and materials

Sacha Inchi (*Plukenetia volubilis* L.) oil was purchased from a local market in Lima, Peru. The pepsin 1/1000 (proteolytic activity) was purchased from Proquimios (Rio de Janeiro, RJ, Brazil). CDCl_3 (99%) was used as a solvent in NMR analysis. The HCl was purchased from Vetec (Rio de Janeiro, RJ, Brazil). The ovalbumin (Ova) (from chicken egg white, A5253) containing 62 to 68% of ovalbumin, the xanthan gum (XG) and the pectin (Pec) were purchased from Sigma-Aldrich (St. Louis, USA).

5.2.2. Microencapsulated formation

The study was performed using four different samples: (i) Sacha Inchi oil (SIO), and three microencapsulated content (ii) SIO and Ova (S-O), (iii) SIO, Ova and Pec (S-O-Pec) and (iv) SIO, Ova and XG (S-O-XG).

The microencapsulated were produced: first of all, by the formation of a simple emulsion (O/W) followed by freeze drying. The S-O emulsion was prepared with Ova 2.0 % (w/w) and SIO 8 % (w/w). The S-O-Pec and S-O-XG were prepared with Ova 2.0 % (w/w) and SIO 8 % (w/w) and with Pec 1% (w/w) or XG 1% (w/w), respectively. The pH of ultrapure water utilized to prepare the emulsions was adjusted to 7.0 in buffer phosphate solution (10 mM).

The emulsions were homogenized in the UltraTurrax-T25 at 18000 rpm by 1 min. After, they were submitted to sonication in the ultrasound probe (Ultrasonic Processor, Hilscher, Germany) for 12 min at frequency of 30 kHz (100% of amplitude and 0.5 cycles per minute). Then, the samples were frozen dried (Serie Enterprise, Terroni, Brazil) to form the microencapsulated. After being frozen dried, the microencapsulated now have the concentration (theoretical) of 80 % SIO and 20% Ova (S-O), and 73% SIO, 18% Ova and 9% Pec or XG to S-O-Pec or S-O-XG, respectively.

5.2.3 Preparation of gastric juice

The gastric juice was prepared following the conditions suggested by Kozu et al (2014), where NaCl (8.775 g.L^{-1}) and pepsin (1.0 g.L^{-1}) were dissolved in HCl 0.5 mol.L^{-1} and the pH was kept in the range of 1 to 3.

5.2.4. Human digestion simulator

The human gastric simulation (HGS) was done in a distillation flask with four rings (Fig. 5.1) with volumetric capacity of 500mL. It was added 200 mL of gastric juice (corresponding to the stomach gastric juice volume) to the distillation flask on a Hotplate Stirrer (AccuPlate, Labnet International Inc, USA) at 400rpm. The temperature was controlled by an alcohol thermometer (-10 to 110°C), keeping on range of 36.0 to 37.0°C and the pH (Analyser[®] pHmeter 300M, Brasil) in the range of 1 – 3, as done by Kozu et al (2014).

Approximately 1000 mg of SIO (~ 500 mg of omega-3) and equivalent amounts of 1000mg (oil mass fraction) of microcapsules were inserted in HGS. Aliquots of gastric juice containing 10 mL samples were taken at 10, 20, 30, 40, 50, 60, 90, 120 and 180 min. After withdrawal of each aliquot, 10 mL of gastric juice was added to continue the digestion. The oil phase was separated to aqueous phase by hexane addition (AUGUSTIN et al, 2014). The oil with hexane was dried under N₂. The omega-3 contents as well as the compounds formed after digestion (and their possible correlation) were evaluated by ¹H NMR for each sample (SIO, S-O, S-O-Pec and S-O-XG) separately.

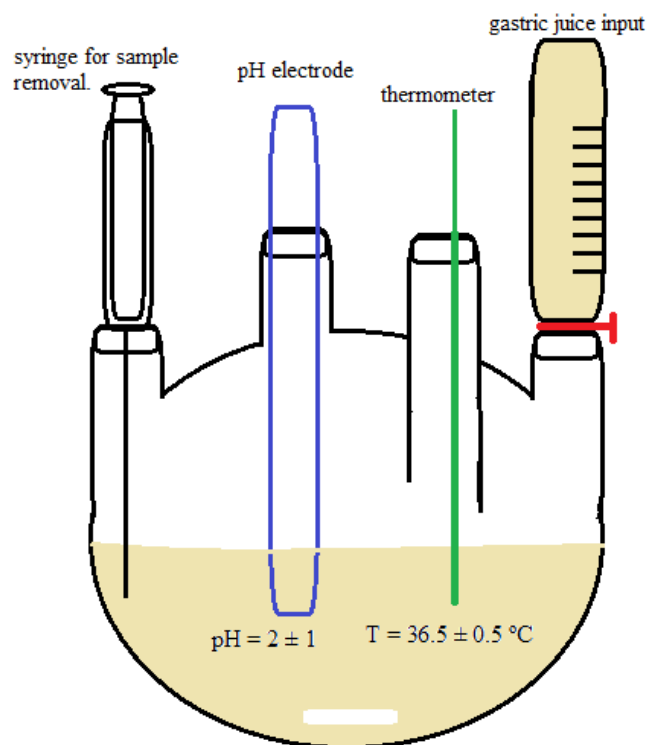


Fig. 5.1. HGS used to experiments.

5.2.5. Triacylglycerol analysis by ¹H NMR

NMR spectra were recorded on a Bruker Advance II spectrometer (Billerica, MA, USA) operating at 400 MHz for ¹H. The acquisition parameters of 1D NMR experiments employed were: TD (time domain data) = 65536, AQ (acquisition time) = 3.98 s, NS (number of scans) = 16, SW (spectral width) = 8224 Hz, D1 (relaxation delay between successive scans/transients) = 1.0 s, LB (exponential line broadening prior to Fourier transformation) = 0.3 Hz.

The aliquot of samples from Sacha Inchi oil (SIO), S-O, S-O-Pec and S-O-XG were collected for each time (from 10 to 180 min) set in human gastric simulation (HGS) and solubilized in CDCl₃ with tetramethylsilane as reference. The mixture was introduced into a tube with a diameter of 5 mm and the experiments were conducted at 25°C. The spectra were processed using the ACD/NMR Processor Academic Edition Program (Version 12.0). The omega-3 content after HGS was performed according with Vicente, Carvalho, and Garcia-Rojas (2015) by ¹H NMR.

5.2.6. Statistical analysis

All analyses were performed in triplicate and the results were expressed as average ± confidence interval (CI) (1). Correlation coefficients were calculated from the different signal obtained by ¹H NMR by the Pearson correlation coefficient equation (2) (MOORE, 2007).

$$CI = \frac{\sigma}{\sqrt{n}} \cdot t \quad (1)$$

where *CI* is the confidence interval, σ is the standard deviation, *n* is the number of repetitions and *t* is the Student's *t*-test inverse function (significance level of 5%).

$$r = \frac{1}{n-1} \sum \left(\frac{x_i - \bar{X}}{s_x} \right) \left(\frac{y_i - \bar{Y}}{s_y} \right) \quad (2)$$

where *r* is the Pearson correlation coefficient, *n* is the number of repetitions, *s_{x,y}* is the standard deviation of variables *x*, *y*; *x_i* is the observation value *I* of variable *x*; *y_i* is the observation value *I* of variable *y*; \bar{X} is the average value of variable *x* and \bar{Y} is the average

value of variable y .

5.3. RESULTS AND DISCUSSION

5.3.1. Omega-3 content of Sacha Inchi oil and microencapsulated

As it shown in the Fig. 5.2, that the HGS procedure did not alter significantly the omega-3 intensity (acyl units) and did not cause the epoxides formation and hydroperoxides in SIO over 180 min. The omega-3 acyl units decreased 2% (from 50.7% to 48.7%); this shows the stability of the SIO front of HGS tested conditions. A very similar behavior was observed to S-O, which the omega-3 acyl units ranged from 50.3% to 48.6%. In this case, the presence of Ova did not prevent the release of omega-3 acyl units.

In Fig. 5.2 it shows that the microcapsules formed with S-O-Pec and S-O-XG, the release of omega-3 acyl units did not occur instantaneously as occurred with the microcapsule without polysaccharide (S-O). The omega-3 acyl units of the S-O-Pec and S-O-XG showed a similar release profile. In both cases, there was a slow and growing way, which can be estimated by Eq. 3 (S-O-Pec) and 4 (S-O-XG).

$$\text{S-O-Pec}_{(10-180\text{min})}: y = 0.0338 (\pm 0.0011)x + 8.99 (\pm 0.09) \quad (3)$$

$$R^2 = 0.9925$$

$$\text{S-O-XG}_{(10-180\text{min})}: y = 0.046 (\pm 0.004)x + 10.9 (\pm 0.3) \quad (4)$$

$$R^2 = 0.9477$$

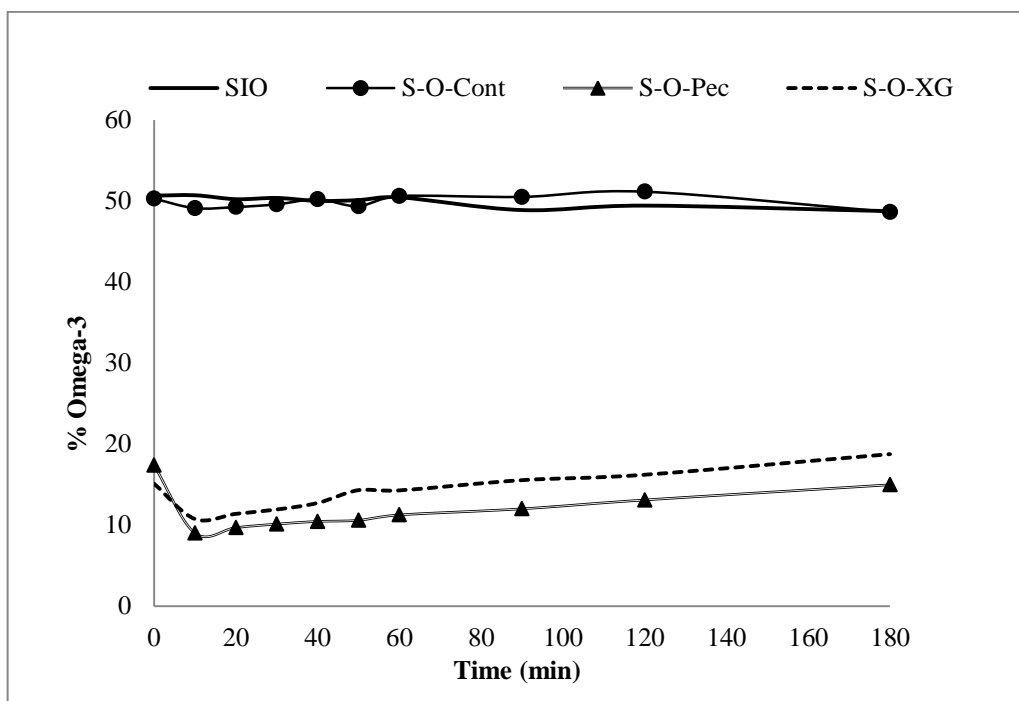


Fig. 5.2. Release of omega-3 acyl units (from 0 to 180 min of HGS).

In Fig. 5.3 the omega-3 acyl units released rates are shown for a given time (t_x) in relation to its previous time. The change in this rate was measured from 10 to 180 min, because the time 0 min was measured before introducing the samples in HGS. For S-O-Pec and S-O-XG, it can be observed that there are greater variations of these release rates, but after 120 min of HGS, they show an increasing trend. However, for SIO and S-O, the rate showed a decrease in omega-3 acyl units over time. The global rate of decrement of omega-3 acyl units after contact with HGS was $-0.20 \pm 0.01 \%. \text{min}^{-1}$ for SIO and of $-2.64 \pm 0.16 \%. \text{min}^{-1}$ for S-O. The negative sign indicates decrease in the omega-3 acyl groups. The global release rates of omega-3 from 10 to 180 minutes were $1.19 \pm 0.08 \%. \text{min}^{-1}$ for S-O-Pec and $1.91 \pm 0.29 \%. \text{min}^{-1}$ for S-O-XG. It was observed that although the omega-3 release rates were similar, the microcapsule formed with S-O-Pec retained almost 4% more omega-3 acyl units than the formed with S-O-XG and the end of HGS. Table 5.1 show the release rate of omega-3 acyl units during the HGS.

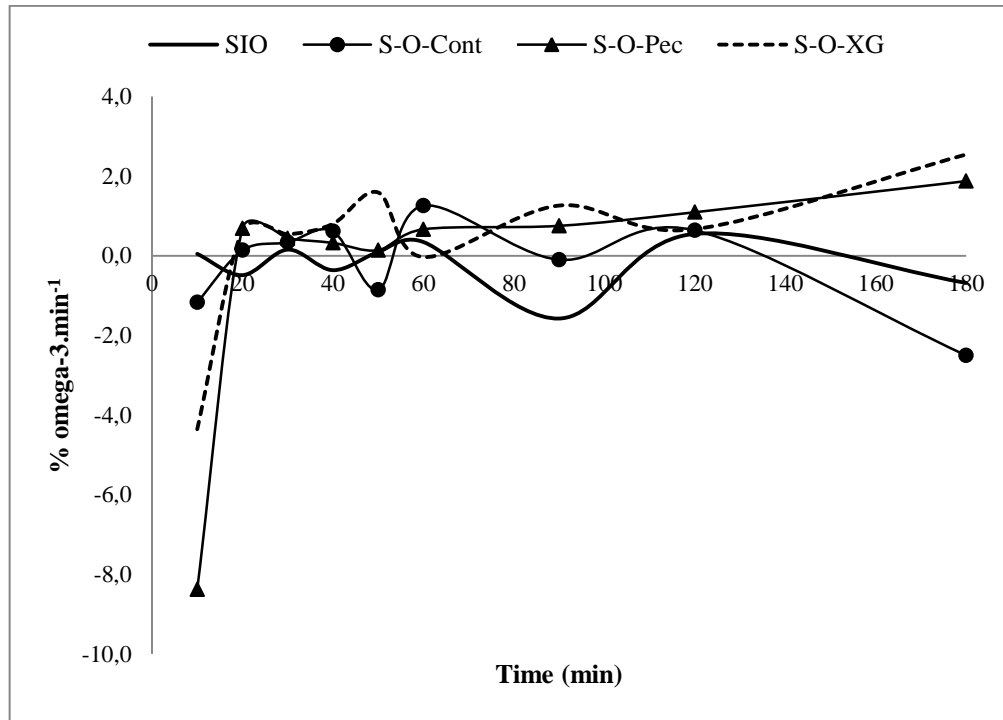


Fig. 5.3. Release rate of omega-3 content.

Table 5.1. Omega-3 contents and omega-3 release rate over 180 min over HGS.

Omega-3 content					Omega-3 release rate (% .min ⁻¹)					
Time (min)	SIO	S-O-Cont	S-O-Pec	S-O-XG	Time	SIO	S-O-Cont	S-O-Pec	S-O-XG	
0	50,7 ± 2,6	50,3 ± 3,1	17,4 ± 3,1	15,1 ± 2,5	0 min					
10	50,7 ± 2,6	49,1 ± 2,9	9,0 ± 1,9	10,7 ± 2,0	10 (10-0) min	0,05 ± 0,00	-1,17 ± -0,19	-8,37 ± -1,22	-4,35 ± -0,53	
20	50,2 ± 2,6	49,2 ± 3,0	9,7 ± 2,2	11,4 ± 2,1	20 (20-10) min	-0,49 ± -0,02	0,14 ± 0,01	0,68 ± 0,25	0,63 ± 0,13	
30	50,4 ± 2,6	49,6 ± 3,0	10,1 ± 1,6	11,9 ± 2,6	30 (30-20) min	0,15 ± 0,01	0,34 ± 0,02	0,44 ± -0,54	0,54 ± 0,47	
40	50,0 ± 2,6	50,2 ± 3,0	10,4 ± 1,7	12,7 ± 1,8	40 (40-30) min	-0,36 ± -0,02	0,61 ± 0,04	0,32 ± 0,12	0,80 ± -0,80	
50	50,1 ± 2,6	49,3 ± 3,0	10,6 ± 1,8	14,3 ± 2,4	50 (50-40) min	0,09 ± 0,00	-0,85 ± -0,05	0,14 ± 0,08	1,59 ± 0,60	
60	50,5 ± 2,6	50,6 ± 3,0	11,3 ± 1,9	14,3 ± 1,9	60 (60-50) min	0,35 ± 0,02	1,26 ± 0,08	0,66 ± 0,06	-0,03 ± -0,49	
90	48,9 ± 2,5	50,5 ± 3,0	12,0 ± 2,0	15,5 ± 2,4	90 (90-60) min	-1,58 ± -0,08	-0,10 ± -0,01	0,75 ± 0,14	1,26 ± 0,48	
120	49,4 ± 2,5	51,1 ± 3,1	13,1 ± 2,3	16,2 ± 2,7	120 (120-90) min	0,55 ± 0,03	0,64 ± 0,04	1,09 ± 0,33	0,67 ± 0,32	
180	48,7 ± 2,5	48,6 ± 2,9	15,0 ± 2,7	18,8 ± 3,1	180 (180-120) min	-0,68 ± -0,03	-2,50 ± -0,15	1,87 ± 0,34	2,54 ± 0,42	
Δ (% omega-3)	-1,9 ± -0,1	-1,6 ± -0,2	-2,4 ± -0,4	3,7 ± 0,6	0 - 180min	average rate (% .min ⁻¹)	-0,21 ± -0,01	-0,18 ± -0,02	-0,27 ± -0,05	0,41 ± 0,07
Δ time (min)	180 ± 180	180 ± 180	180 ± 180	180 ± 180		global rate (% .min ⁻¹)	-0,73 ± -0,04	-1,33 ± 0,04	10,25 ± 1,55	6,90 ± 0,96
Δ (% omega-3)	-2,0 ± -0,1	-0,5 ± 0,0	6,0 ± 0,8	8,0 ± 1,1	10 - 180min	average rate (% .min ⁻¹)	-0,25 ± -0,01	-0,06 ± 0,00	0,75 ± 0,10	1,00 ± 0,14
Δ time (min)	170 ± 170	170 ± 170	170 ± 170	170 ± 170		global rate (% .min ⁻¹)	-0,20 ± -0,01	-2,64 ± -0,16	1,19 ± 0,08	1,91 ± 0,29

5.3.2. ^1H NMR spectra of SIO and microencapsulated

Based on the omega-3 structure (α linolenic acid) (Fig. 5.5), it was identified the signals present in Fig. 5.4 (a-d). The signals (θ) with shift δ_{H} 0.80 to 0.90 ppm and δ_{H} 0.92 to 0.98 ppm refers to terminal methyl groups, CH_3 aliphatic and CH_3 bonded to CH_2 allylic, respectively. The signals from δ_{H} 1.15 to 1.35 ppm represent methylene protons (γ); this signal can be related to the CH_2 intermediate.

In the β position, protons appeared with chemical shift between δ_{H} 1.50 and 1.70 ppm. In Fig 4a and 4b, it can be see that occurs anisotropy effect, it is decreased is the measure that increases the contact time with HGS. However, this effect is not visible in S-O-Pec (Fig. 5.4c) and S-O-XG (Fig. 5.4d), because the microcapsules better protect the omega-3 structure compared to SIO and S-O. As the process of hydrolysis lead to generation of "free" acid and not as ester (as triacylglycerol, TAG), it can see in β -position a lower scattering of this signal (KARUPAIAH & SUNDRAM, 2007).

The δ is the signal of allyl protons (neighboring to double bonds) is shown, represented by the δ_{H} 1.95-2.10 ppm signals. The signals of the proton located at the α carbonyl position were observed with chemical shifts of δ_{H} 2.25 to 2.35 ppm. At ζ signals of doubly allylic protons (*double double bonds*, ddb) refer to omega-6 (2.68 to 2.75 ppm) and omega-3 (2.77 to 2.83 ppm) acyl units. The signals between 4.10 to 4.29 ppm refer to methylenic protons bonded in the 1 and 3 positions of the glyceryl and the signal at δ_{H} 5.20 to 5.25 ppm refers to methynic proton (*sn*, 2 position) of glyceryl of ester (TAG). Based on the data it is possible to observe that the signal at δ_{H} 4.00 to 4.07 ppm (*sn*-1,3-diglycerides) increases over time while the glyceryl protons (at δ_{H} 4.10 to 4.29 ppm) decreases proportionally in the same period. The signal (ϵ) at δ_{H} 5.30 to 5.40 ppm represents the protons of the double bonds (unsaturated). The protons at δ_{H} 4.10 – 4.29 ppm (shown in Fig. 5.4) refers to glyceryl groups in 1 and 3 positions attached to TAG.

5.3.3. Analysis of the Pearson correlation coefficient

The Pearson correlation coefficient (r) was determined by applying Eq. (2) between signals of Fig. 5.4 on each one of (a) SIO, (b) S-O, (c) S-O-Pec and (d) S-O-XG. The r data are shown in Tab. 5.2. Although the HGS has not promoted formation of oxidized compounds (epoxides and hydroperoxides) in the SIO and the S-O, the slight decrease in omega-3 acyl

units observed in Fig. 5.2 was evaluated by of the Pearson correlation coefficient (r) to the increase of other signals seen in Fig. 5.4.

For SIO, it is observed in Table 5.2, which over time increased in intensity of 1,3-diglycerides signals (at δ_H 4.00 – 4.07 ppm) was directly correlated ($r = -1.000$) to the reduction of the protons attached to the glyceryl in 1 and 3 positions (at δ_H 4.10 – 4.29 ppm). The same occurred, however at a lower intensity ($r = -0.635$) with the signals of omega-3 acyl units (δ_H 2.77 to 2.83 ppm) and 1,3-diglycerides signals. These, however, cannot be related directly because the omega-3 signals of TAG as well as “free” acids are identified with the same chemical shift (δ_H 2.77 to 2.83 ppm). Thus, it can only assign that omega-3 release occur of the *sn*-1,3 positions of TAG.

The methynic protons (*sn*, 2-positions) of the glyceryl of ester (δ_H 5.20 to 5.25 ppm) decrease while decreases the omega-3 acyl units of doubly allylic protons at 2.77 to 2.83 ppm ($r = 0.741$). In this case, it can be attributed to the steric hindrance of the *sn*-2 position compared with other (*sn*-1 and *sn*-3), which have greater ease of being hydrolyzed (KARUPAIAH & SUNDRAM, 2007). Thus, it is easier for the protons of the *sn*-2 position to remain attached to the omega-3 acyl units, as soon as, when a signal decreases, the other also decreases. Although there was anisotropic effect on acyl groups (δ_H 1.51 to 1.65 ppm), it is observed in Table 5.2 that they have no correlation with the omega-3 acyl units ($r = -0.030$).

For S-O, we can observe in Table 5.2 that the intensity of omega-3 acyl units (δ_H 2.77 to 2.83 ppm) were negatively correlated with 1,3-diglycerides signals ($r = -0.873$). For the same reason that occurred with SIO, the correlation cannot be assigned in this case, since both the “free” acid and the TAG form occur at the same chemical shift, which block this interpretation. The anisotropic effect (on acyl groups at δ_H 1.51 to 1.65 ppm) was lower than the observed in SIO due to the Ova presence. Other signal that was strongly positively correlated with omega-3 acyl groups was the methynic protons (*sn*, 2-positions) of the glyceryl of ester ($r = 0.915$), it means that the decrease in omega-3 acyl units was almost proportional to the decrease in the signal intensity of δ_H 5.20 to 5.25 ppm, which indicates that omega-3 is in the TAG form (attached at *sn*, 2-position).

For S-O-Pec can be observed that there is a strong positive correlation between the decrease in the omega-3 acyl units and increasing signals of the methynic protons (*sn*, 2-positions) ($r = 0.799$) and another positive correlation ($r = 0.526$) but weak with increased of methylenic protons (1,3-diglycerides). In S-O-XG the correlation was almost zero ($r = -0.041$) between the formation of methylenic protons and decrease the omega-3 acyl units. However

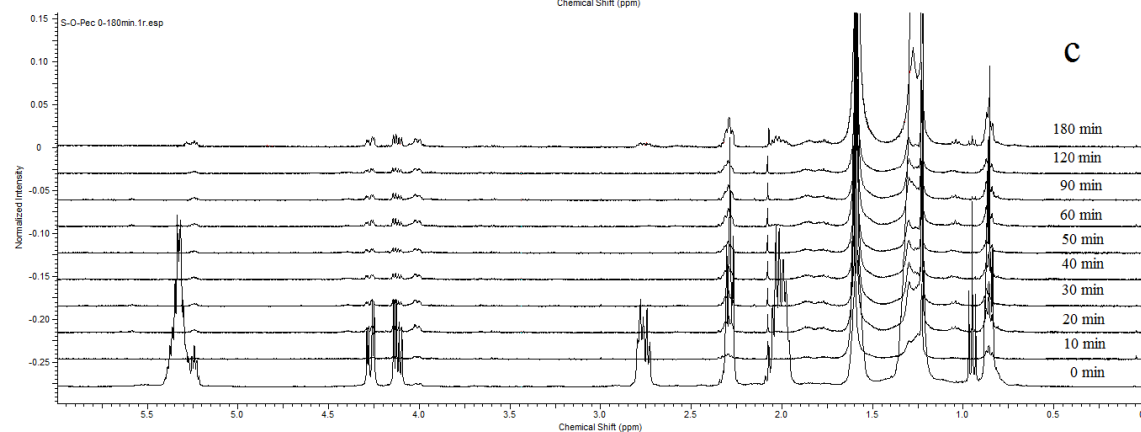
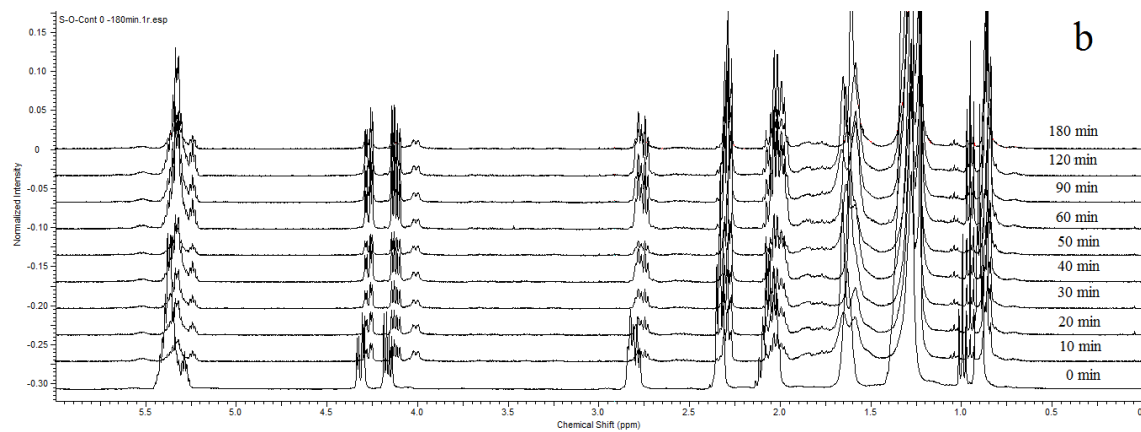
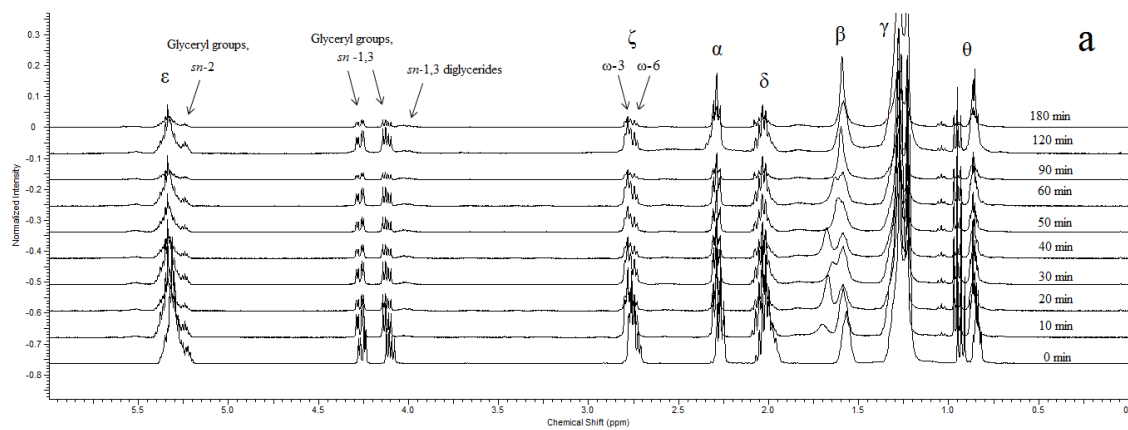
there was a weak positive correlation ($r = 0.554$) between the decrease in omega-3 acyl units and the increase of the methynic protons (*sn*, 2-positions).

The positive correlation between the decrease of omega-3 acyl units and the increase in signal related to the methynic protons (*sn*, 2-positions) showed a downward trend in microcapsules S-O-C>S-O-Pec>S-O-XG, wherein $r = 0.915$, 0.799 and 0.554 respectively. In this case, the presence of Ova, Ova with Pec and Ova with XG decreased proportionally the signs involving the methynic groups (*sn*, 2-position); thus, it can be inferred that the release of omega-3 acyl groups of the 2-position is affected by the presence of biopolymers (described sequentially).

Table 5.2. Pearson coefficient correlation over time.

SIO				
δ_H ppm (10-180min)	4.00-4.07 (1,3-diglycerides)	4.10-4.29 (Glyceryl groups, <i>sn</i> -1,3)	2.77-2.83 (Omega-3 acyl units)	1.51-1.65 (Acyl group)
4.00-4.07 (1,3-diglycerides)				
4.10-4.29 (Glyceryl groups, <i>sn</i> -1,3)	-1.000			
2.77-2.83 (Omega-3 acyl units)	-0.635	0.635		
1.51-1.65 (Acyl group)	0.515	-0.515	-0.030	
5.20-5.25 (Glyceryl groups, <i>sn</i> -2)	-0.730	0.730	0.741	-0.209
S-O				
δ_H ppm (10-180min)	4.00-4.07 (1,3-diglycerides)	4.10-4.29 (Glyceryl groups, <i>sn</i> -1,3)	2.77-2.83 (Omega-3 acyl units)	1.51-1.65 (Acyl group)
4.00-4.07 (1,3-diglycerides)				
4.10-4.29 (Glyceryl groups, <i>sn</i> -1,3)	-1.000			
2.77-2.83 (Omega-3 acyl units)	-0.873	0.873		
1.51-1.65 (Acyl group)	-0.327	0.327	0.395	
5.20-5.25 (Glyceryl groups, <i>sn</i> -2)	-0.900	0.900	0.915	0.585
S-O-Pec				
δ_H ppm (10-180min)	4.00-4.07 (1,3-diglycerides)	4.10-4.29 (Glyceryl groups, <i>sn</i> -1,3)	2.77-2.83 (Omega-3 acyl units)	1.51-1.65 (Acyl group)
4.00-4.07 (1,3-diglycerides)				
4.10-4.29 (Glyceryl groups, <i>sn</i> -1,3)	-1.000			
2.77-2.83 (Omega-3 acyl units)	0.526	-0.526		
1.51-1.65 (Acyl group)	0.264	-0.264	0.140	
5.20-5.25 (Glyceryl groups, <i>sn</i> -2)	0.100	-0.100	0.799	0.042
S-O-XG				
δ_H ppm	4.00-4.07	4.10-4.29	2.77-2.83	1.51-1.65

(10-180min)	(1,3-diglycerides)	(Glyceryl groups, <i>sn</i> -1,3)	(Omega-3 acyl units)	(Acyl group)
4.00-4.07 (1,3-diglycerides)				
4.10-4.29 (Glyceryl groups, <i>sn</i> -1,3)	-1.000			
2.77-2.83 (Omega-3 acyl units)	-0,041	0.041		
1.51-1.65 (Acyl group)	0.713	-0.713	-0.040	
5.20-5.25 (Glyceryl groups, <i>sn</i> -2)	-0.487	0.487	0.554	-0.660



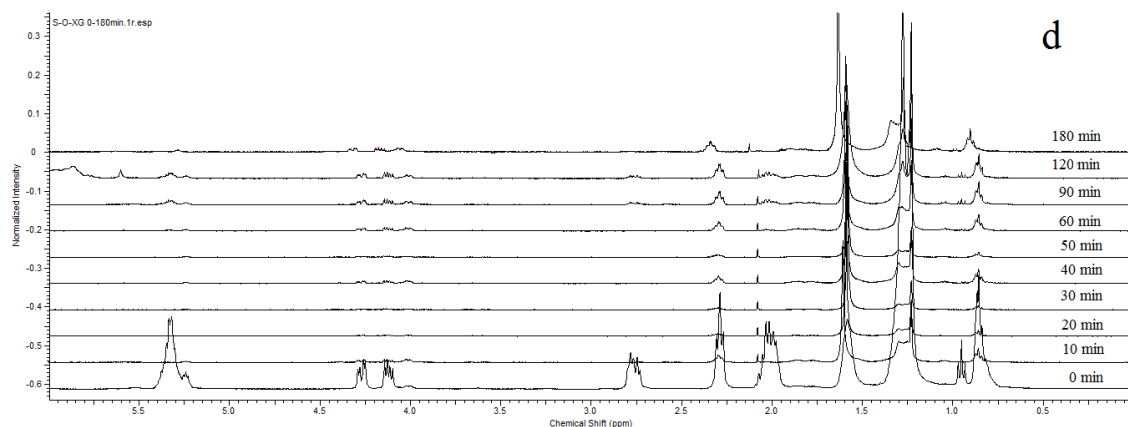


Fig. 5.4. ^1H NMR spectra of (a) SIO, (b) S-O, (c) S-O-Pec and (d) S-O-XG over 180 min.

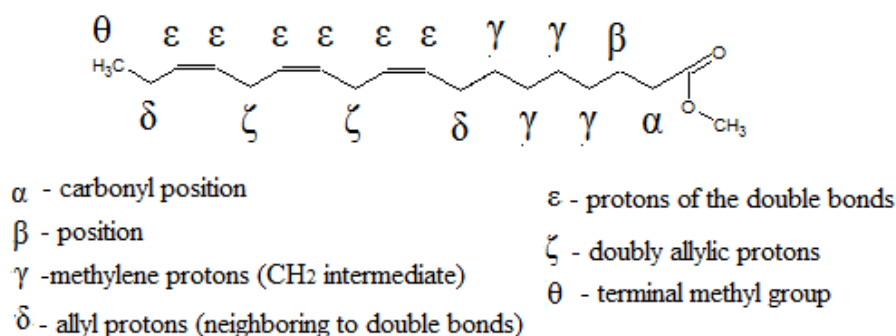


Fig. 5.5. Linolenic acid (omega-3) structure.

The lipids digestion occurs in the small intestine (as with most components) in particular because of the presence of pancreatic lipase. However, although the gastric conditions are appropriate for digestion of proteins, McClements (2015) reports that the absorption of bioactive components can occur even in smaller proportions, for gastrointestinal tract, ie, mouth, esophagus or stomach, before reaching the small intestine.

The analysis of ^1H NMR spectra for different reaction times indicates that there was not significant consumption of triglyceride. However, it can perceive the appearance of signals at δ_{H} 4.00 to 4.07 ppm (CH-OH) which can be attributed to the diglycerides (*sn*-1,3) formation, justified by the conversion (hydrolysis) of the triglycerides (*sn*-1,2,3) to (*sn*-1,3). Thus, we can infer that the hydrolysis occurred (preferably) at the *sn*-2 position of the ester derived from glycerol, Figure 5.6. The spatial orientation of the methynic carbon, shown in Figure 5.6, can justify the hydrolysis in *sn*-2. Another relevant consideration is the absence of signals which justify of the oxidation products formation, revealing the continuing source of omega-3 for metabolism future.

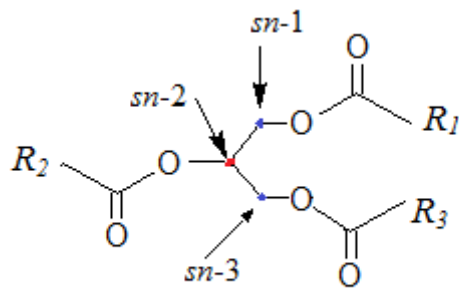


Fig. 5.6. Spatial positions of the protons in the triglyceride structure.

The red sign indicates the *sn*-2 position of the proton, it can be seen, therefore most free to be hydrolyzed in relation to other positions (*sn*-1 and 3) shown in blue.

This interpretation is according with Zeeb, Weiss & McClements (2015) which observe that, although the rate is slower. It may be a conversion (even that in less proportion) of triglycerides to diglycerides and may even reach monoglycerides, in the emulsions formed with biopolymers. The coatings biopolymers, however, improve the lipids stability because there is a decrease in the surface area of the lipid droplets exposed to gastric conditions, thus reducing hydrolysis and consequently the fatty acids release.

Zhang et al (2015) observed (as expected) that the digestion of triacylglycerol containing PUFAs (polyunsaturated fatty acids, as SIO) is slower than monounsaturated fatty acids (MUFAs) and the rate and extent of lipid digestion was also lower for the emulsions (containing oil) than for triacylglycerol oils. There is a reasonable reason for this, due the structure of the colloidal particles formed in the emulsion may have been resistant to digestion (VERRIJSEN et al, 2015; ZHANG et al, 2015). The hydrolysis of triacylglycerol generates sequentially, diacylglycerol and monoacylglycerol (GUILLÉN & URIARTE, 2012) before being absorbed (McCLEMENTS, 2015). In oil-in-water emulsions of olive oil enriched with b-carotene and containing L- α -phosphatidylcholine (as emulsifier) was observed by Verrijssen et al (2015) that the lipid digestion is not dependent on the presence of pectin.

5.4. CONCLUSIONS

The HGS conditions did not significantly alter the intensity of omega-3 acyl units of SIO over 180 min as well as did not promote the development of oxidized compound such as epoxides and hydroperoxides. In the SIO, the increase of 1,3-diglycerides is directly

correlated ($r = -1.000$) with the decrease of glyceryl groups (*sn*-1,3 positions). In the other hand, the proportional reduction of the methynic protons (*sn*, 2-position) of the glyceryl positively correlated ($r = 0.741$) with is the decrease of omega-3 acyl groups. The S-O had a similar behavior to the SIO, and Ova presence was not enough to prevent the decrease of omega-3 acyl units over 180 min.

The S-O-Pec and S-O-XG microcapsules were shown to be effective for the omega-3 protection and can be used to transport nutraceutical compounds due to their resistance the human gastric conditions.

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CAPITULO VI – Thermal and oxidative stability of Sacha Inchi oil and encapsulated formed with biopolymers by DSC and ¹H NMR.

ABSTRACT

This study aimed at evaluating the omega-3 concentration and kinetic parameters of Sacha Inchi oil (SIO) and encapsulated formed with biopolymers by differential scanning calorimetry (DSC) and proton nuclear magnetic resonance (^1H NMR). The SIO and encapsulated formed by emulsion-based were submitted by DSC under oxygen atmosphere to determine the oxidative induction time (OIT) value in isothermal conditions at 100, 120 and 150 °C. It was possible to obtain the kinetic parameters (activation energy, pre-exponential factor and z value) of SIO and then to predict the stability time by extrapolation at different temperatures. The ^1H NMR spectra allowed assess the identification and percentage relative before and after isothermal DSC oxidation of omega-3 concentration and the oxidized compounds formed in the samples. The encapsulated formed by emulsion-based were not efficient in omega-3 content protection against the SIO, but the Isothermal DSC is a useful and practical technique to determine the OIT (min) and kinetic parameters that become possible predict the stability oxidation in different edible oils.

Keywords: Sacha inchi oil, ^1H NMR, omega-3 stability, DSC, oxizide compounds.

6.1 INTRODUCTION

Oxidation is the main undesirable reactions and that affect the oil and fat stability in the food industry, changing their nutritional properties, decreasing benefits, altering the taste, flavor, texture (MICIĆ et al, 2015) and can become potentially toxic due to its decomposition and formation (mainly) of hydroperoxides and aldehydes (McCLEMENTS & DECKER, 2010; ARONSON et al., 2001). Highly oxidized oils may also produce polyaromatic hydrocarbons, which have carcinogenic effect (FAO/WHO, 1988; DEBNATH et al, 2012).

The microencapsulation is a technique that aims at preserving the stability of bioactive compounds (McCLEMENTS, 2015; GAONKAR et al, 2014) and has been used as a strategy to oxidation prevent of lipid fractions and therefore increase the stability of bioactive lipid components, such as omega-3. Several studies have reported the use of microencapsulation to prevent bioactive compounds, for example, eugenol (CORTÉS-ROJAS, SOUZA & OLIVEIRA, 2014), flavonoids (AKHTAR et al, 2014) and flaxseed oil (CARNEIRO et al, 2013). The Sacha Inchi (*Plukenetia volubilis* L.) oil is a rare vegetal

source (together with flax oil) with a high concentration of omega-3 fatty acids (48-50%) (GUILLÉN et al, 2003; FOLLEGATTI-ROMERO et al, 2009; FANALI et al, 2011; MAURER et al, 2012; VICENTE, CARVALHO & GARCIA-ROJAS, 2015).

Among the several ways available to measure the lipid oxidation, one of the most direct and effective is differential scanning calorimetry (DSC), by the oxidative induction time (OIT) test. The OIT test is defined by Gabbott (2008) as an isothermal test which is used frequently in oil, polymers and foods to evaluate their decomposition measurement. The oxidation induction time (OIT) is measured keeping the sample under isothermal conditions under an air or oxygen atmosphere (Bevis, 2008). In practice measures the time required for the oil (or sample) begin the oxidative decomposition process (GABBOTT, 2008; BEVIS, 2008). In addition, the proton nuclear magnetic resonance (^1H NMR) is another technique very useful and able to monitoring the oxidation state of edible oils and fats. It possible observes the presence of compounds from oxidative decomposition (mainly hydroperoxides and aldehydes) (GUILLÉN et al. 2003; BARISON et al, 2010; ALMOSELHY et al, 2014; ALONSO-SALCES, HOLLAND, & GUILLOU, 2011; CORDELLA et al, 2012; DUGO et al, 2015; GUILLÉN & URIARTE, 2012; SICILIANO et al, , 2013).

This way, become useful evaluate the stability of SIO and it encapsulated, formed by emulsion-based, through DSC (under O_2 flow) and then, complementing, the ^1H NMR can help us better understand how changes occur in the SIO structure and encapsulated, by identification and proportional relation of the compounds formed after isothermal DSC oxidation.

6.2 MATERIAL E METHODS

6.2.1. Chemical and materials

Sacha Inchi (*Plukenetia volubilis* L.) oil was purchased from a local market in Lima, Peru. The pepsin 1/1000 (proteolytic activity) was purchased from Proquimios (Rio de Janeiro, RJ, Brazil). CDCl_3 (99%) was used as the solvent in NMR analysis. HCl was purchased from Vetec (Rio de Janeiro, RJ, Brazil). The ovalbumin (Ova) (from chicken egg white, A5253) containing 62 to 68% of protein, the xanthan gum (XG) and the pectin (Pec) were purchased from Sigma-Aldrich (St. Louis, USA).

6.2.2. Microencapsulated formation

The microencapsulated were produced by of the formation of an emulsion (O/W) followed by freeze drying. The S-O (SIO + Ova) emulsion was prepared with Ova 2.0 % (w/w) and SIO 8 % (w/w). The S-O-Pec (SIO + Ova + Pec) and S-O-XG (SIO + Ova + XG) were prepared with Ova 2.0 % (w/w) and SIO 8 % (w/w) and with Pec 1% (w/w) or XG 1% (w/w), respectively. The pH of ultrapure water utilized to prepare of emulsions was adjusted to 7.0 in buffer phosphate solution (10 mM).

The emulsions were homogenized in UltraTurrax-T25 at 18000 rpm by 1 min. After, they were submitted at sonication in ultrasound probe (Ultrasonic Processor, Hilscher, Germany) for 12 min at frequency of 30 kHz (100% of amplitude and 0.5 cycles per minute). Then, the samples were freeze dried to form the microencapsulated. After being freeze dried, the microencapsulated now have the concentration (theoretical) of 80 % SIO and 20% Ova (S-O), and 73% SIO, 18% Ova and 9% Pec or XG to S-O-Pec or S-O-XG, respectively.

6.2.3 Isothermal DSC oxidation

The evaluation of thermal and oxidative stability of Sacha Inchi oil and encapsulated was performed in a DSC (Perkin Elmer Instruments, Pyris Diamond, USA). Approximately 3.0 mg of sample were measured in (aluminum) pans and the isothermal experiments at 100°C, 120°C and 150°C were conducted under oxygen (purity 99.995%) flow at 50 mL.min⁻¹, as done by (Micić, Ostojić, Simonović, Krstić, Pezo, & Simonović, 2015). The software TAC 7/DX Thermal Analysis Controller was utilized to evaluate the curves generated. The OIT (min) were determined by intersection of extrapolated baseline and the tangent line (leading edge) of exothermal peak. The integral isoconversional method was used for the calculation of activation energy (E_a), as done by Micić et al., (2015).

$$\ln t_{\alpha,i} = \ln \left(\frac{g(\alpha)}{A_\alpha} \right) + \left(\frac{E_{a,\alpha}}{RT_i} \right) \quad (1)$$

where $t_{\alpha,i}$ (min) is the time to reach a given extent of conversion (α) at different temperatures T_i (K), $g(\alpha)$ is integral form of reaction model ($g(\alpha) = \text{const}$ for $\alpha = \text{const}$), $E_{a,\alpha}$ and A_α are activation energy and pre-exponential factor at a given extent of conversion.

For OIT, Eq (1) can be rewritten as:

$$\ln k_{\text{OIT},i} = \ln A'_{\text{OIT}} - \frac{E_{a,\text{OIT}}}{RT_i} \quad (2)$$

where $k_{\text{OIT}} = 1/\text{OIT}$, and A'_{OIT} is proportional to A_{OIT} . Activation energy and frequency factor were determined from the slope and intercepts, respectively, of the curves generated by regressing $\ln k_{\text{OIT},i}$ vs $1/T_i$ using least squares linear regression, with implemented Levenberg-Marquardt algorithm. In this case, the slope and intercept were $-E_{a,\text{OIT}}/R$ and $\ln A'_{\text{OIT}}$, respectively.

6.2.3.1 D and z kinetics parameters

The D_T and z values are kinetic parameters important to assess the thermal stability of microorganisms and enzymes, where D_T , means the decimal reduction time value, being defined as the time required to inactivate 90% of the original enzyme activity at a constant temperature, and it is obtained from plots of \log (enzyme activity) as a function of time. The z value is the temperature needed to reduce the D_T value by one log-unit, and it is obtained from the plot of $\log D_T$ against temperature ($^{\circ}\text{C}$) (ANESE & SOVRANO, 2006). However, in this study, these values were used to assess oxidative stability of SIO, as z value as the temperature difference (ΔT) required for a \log (10X) increase in the time required for reach the OIT value.

6.2.4. ^1H NMR analysis

After the oxidative process in the DSC, the residue was evaluated by ^1H NMR. The NMR spectra were recorded on a Bruker Advance II spectrometer (Billerica, MA, USA) operating at 400 MHz for ^1H . The acquisition parameters of 1D NMR experiments employed were: TD (time domain data) = 65536, AQ (acquisition time) = 3.98 s, NS (number of scans) = 16, SW (spectral width) = 8224 Hz, D1 (relaxation delay between successive scans/transients) = 1.0 s, LB (exponential line broadening prior to Fourier transformation) = 0.3 Hz.

The residue obtained from oxidative process of DSC was solubilized in CDCl_3 with tetramethylsilane as the reference. The mixture was introduced into a tube with a diameter of

5 mm and the experiments were conducted at 25°C. The spectra were processed using the ACD/NMR Processor Academic Edition Program (Version 12.0). The omega-3 content and other compounds formed after DSC oxidation process was performed to according with Vicente, Carvalho, and Garcia-Rojas (2015) by ¹H NMR.

6.2.5. Statistical analysis

All analyses were performed in triplicate and the results are expressed as average ± confidence interval (CI) (1). Correlation coefficients were calculated from the different signal obtained by ¹H NMR through the Pearson correlation coefficient equation (2) (Moore, 2007).

$$CI = \frac{\sigma}{\sqrt{n}} \cdot t \quad (3)$$

where *CI* is the confidence interval, σ is the standard deviation, *n* is the number of repetitions and *t* is the Student's *t*-test inverse function (significance level of 5%).

$$r = \frac{1}{n-1} \sum \left(\frac{x_i - \bar{X}}{s_x} \right) \left(\frac{y_i - \bar{Y}}{s_y} \right) \quad (4)$$

where *r* is the Pearson correlation coefficient, *n* is the number of repetitions, $s_{x,y}$ is the standard deviation of variables *x*, *y*; x_i is the observation value *I* of variable *x*; y_i is the observation value *I* of variable *y*; \bar{X} is the average value of variable *x* and \bar{Y} is the average value of variable *y*.

6.3. RESULTS AND DISCUSSION

6.3.1. Isothermal DSC oxidation

Isothermal DSC oxidation curves obtained for SIO, S-O, S-O-Pec and S-O-XG at three different temperatures (100, 120 and 150°C) and the way of determination of OIT are shown in the Fig. 6.1.

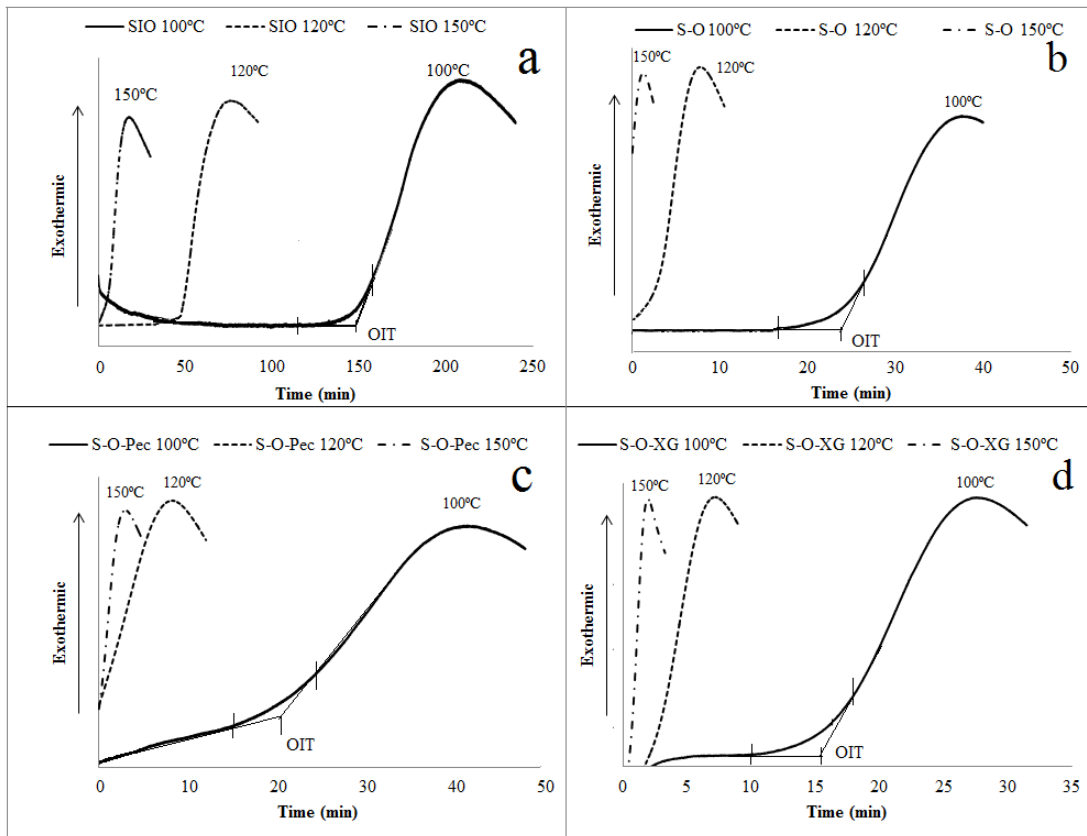


Fig. 6.1. DSC curves (a) SIO, (b) S-O, (c) S-O-Pec and (d) S-O-XG.

The OIT value represents the time that the oxidative decomposition begins for each sample, and the maximum height (plateau) of the curve indicates the state maximum value of oxidative decomposition of sample (LITWINIENKO & KASPRZYCKA-GUTTMAN, 1998; GABBOTT, 2008). In all cases, we can see in the Fig. 1 that the temperature had strong influence on the OIT value, such as demonstrate in studies done by Pardaul et al, 2011, Cibulková, Čertík, & Dubaj, 2014 and Micić et al, 2015. As can be seen in Tab. 6.1, the OIT to SIO found in this study was 148.2 ± 2.7 min at 100°C , 48.4 ± 0.7 min at 120°C and 3.6 ± 1.1 min at 150°C . However, Rodriguez et al (2015) studied the oxidative stability of SIO and found $\text{OIT} = 1.59 \pm 0.06$ h (95.4 ± 3.6 min) at 100°C and 0.49 ± 0.01 h (29.4 ± 0.06 min) at 110°C but working Rancimat method (air flow of 15L/h), we can attribute to the shorter time of SIO stability in our study due to the presence of oxygen in the isothermal DSC oxidation. Micić et al, (2015) reports OIT (in min) by DSC under O_2 to Blackberry seed oil as 284.9 ± 10.7 (at 110°C), 138.3 ± 2.2 (at 120°C) and 21.6 ± 1.8 (at 145°C), the same authors working with Raspberry seed oil found OIT of 142.7 ± 2.9 (at 110°C), 72.2 ± 2.8 (at 120°C) and 12.7 ± 1.1 (at 145°C), the latter, close to SIO. The SIO (50.7 ± 2.6 %) and the Raspberry seed oil have greater omega-

3 concentration (27.8 ± 0.21 %) than Blackberry seed oil (14.62 ± 0.32 %), that justify the lower OIT, due the unsaturated fatty acids presence. Cibulková, Čertík, & Dubaj, (2014) studied poopy seed and obtained the OIT of 19.8 min (at 130 °C) and 13.8 min (at 140 °C) by DSC (under O₂ flow). Pardaul et al, (2011) determined the OIT (under oxygen atmosphere, by T_0) in Passion fruit (116.28 min) Rubber seeds (106.25 min) and Buriti (778.86 min) at 100 °C and of 23.82 min, 25.04 min and 165.10 min (at 120 °C), respectively. In this study, the authors attribute the increase in OIT value by reducing the unsaturation degree of oils.

In addition, the OIT value of encapsulated had great influence by Ova and polysaccharides (Pec and XG) presence. According to Goetz & Koehler, (2005) the thermal denaturation (T_d) of ovalbumin is 84.5 °C, but it is known that this temperature can be altered due the salts or sugar concentrations, pH, aggregation with other biopolymers and also by process temperature. Thus, the temperatures (100, 120 and 150°C) of the isotherms performed in the tests (possibly) caused denaturation of biopolymers used, especially of Ova and the same drags the SIO is emulsified, causing oxidation of the oily fraction, because as the encapsulation was done by emulsion-based, in this case, there are interactions between SIO and Ova by emulsion formation, bonded by hydrophobic groups, thus, the temperatures used in DSC can have provoked the protein denaturation and hence the decrease of OIT value (LAM & NICKERSON, (2013). Another possible reason which may explain the degradation is the occurrence of Maillard reaction which occurs between an amino group and an aldehyde by covalent bond involving therefore a protein and a carbohydrate at high temperatures (OLIVER, MELTON & STANLEY, 2006; McCLEMENTS, 2015), as undesirable products are generated polar high molecular weight compounds resulting from the oxygen addition (DAMODARAN, 2010).

The mass value (%) presented in the Table 6.1, indicate gain (positive signal) or mass loss (negative signal) after isothermal DSC oxidation. It can be observed that the mass increase of SIO is associated at “O₂ incorporation” in the oil and hence hydroperoxide formation. At lower temperature there bigger mass increase because at higher temperature also occurs, with more facilities, volatilization of oxidative decomposition compounds, that slightly decrease of final mass (GUILLEN & URIARTE, 2012; CORDELLA et al, 2012). In the case of encapsulated, there was predominance of mass loss, which is associated with the denaturation of protein and polysaccharides due to the high temperatures used in the process. In addition, the mass loss was also temperature dependent, although, at lower temperatures had lower weight loss when compared with higher temperatures. In both S-O and S-O-XG at

100 °C was observed mass increase. In the S-O-Pec at 120°C and 150°C and S-O-XG at 150°C, it was not possible to obtain the OIT value, because the high temperature, degrade the encapsulated (quickly), as can be seen in Fig. 6.1c and 6.1d, respectively.

Table 6.1. The OIT value and loss (or gain) mass (%)

	OIT (min)			<i>k</i>	mass (%)
SIO 100°C	148.2 ^a	±	2.7	0.00675	7.10
SIO 120°C	48.4 ^b	±	0.7	0.02066	5.41
SIO 150°C	3.6 ^f	±	1.1	0.27778	4.26
S-O 100°C	22.5 ^c	±	2.6	0.04444	2.83
S-O 120°C	2.35 ^g	±	1.17	0.42553	-0.60
S-O 150°C	0.000 ⁱ	±	0.000	-	-5.84
S-O-Pec 100°C	19.1 ^d	±	3.8	0.05236	-0.90
S-O-Pec 120°C	0.000 ⁱ	±	0.000	-	-2.67
S-O-Pec 150°C	0.000 ⁱ	±	0.000	-	-5.49
S-O-XG 100°C	14.5 ^e	±	1.7	0.06897	0.25
S-O-XG 120°C	1.9 ^h	±	0.7	0.52632	-0.84
S-O-XG 150°C	0.000 ⁱ	±	0.000	-	-2.42

6.3.1.1 Kinetic parameters

Table 6.2 shows the kinetic parameters (activation energy, pre-exponential factor and z value) to SIO at isothermal conditions.

Table 6.2. Kinetic parameters obtained by isothermal conditions.

Estimated Arrhenius parameters for SIO oxidation on Isothermal conditions ($\ln k_{OIT}$ vs $1/T_i$)		
Parameters		Unit
a (interception)	27 ± 4	-
b (inclination)	-11871 ± 1743	-
R ²	0.98	
E_{aOIT}	99 ± 14	kJ/mol
A'_{OIT}	$3.73 \times 10^{11} \pm 83$	min ⁻¹
z	30.7	°C

Where $y = \ln k_{OIT}$; $x = 1/T_i$ (K); $a = \ln A'$ and $b = -E_a/R$

The E_a (activation energy) obtained in this study was 99 ± 14 kJ/mol, lower than found by Rodríguez et al (2015) of 137.90 kJ/mol for SIO using the Rancimat method. However E_a is statistically equal that found by Micić et al, (2015) for Blackberry seed oil (96 ± 3 kJ/mol) and Raspberry seed oil (92 ± 4 kJ/mol), common to the SIO, in both Blackberry as Raspberry has a high concentration of unsaturated fatty acids ($< 90\%$), according to Adhvaryu et al, (2000) and Pereira et al (2008), the greater the unsaturation degree of the oil, the lower its E_a and thus the higher the reactivity of oil and greater their oxidative instability.

The z value indicates that the decrease of $30.7\text{ }^\circ\text{C}$ increases the stability time (OIT) in a log (10 fold). On the other hand, the increase of storage temperature in $30.7\text{ }^\circ\text{C}$ result in the decrease of a log (10 fold less) on the OIT value. Thus we can estimate the stability of the SIO stored at different temperatures under flowing oxygen, based on the z value. Table 6.3 extrapolates the OIT of SIO for different temperatures.

Table 6.3. Shelf-life extrapolation based on the z value.

time	20 °C	25 °C	30 °C	50 °C	70 °C
min	1055888	535041	277268	24510	2875
h	17598	8917	4621	408	48
days	733.26	371.56	192.55	17.02	2.00
year	2.01	1.02	0.53	0.05	0.01

The values presented in Table 6.3 can be compared with extrapolation done by Rodríguez et al, (2015) to SIO. According to these authors, the useful life (in years) of SIO is 0.79 ($30\text{ }^\circ\text{C}$), 1.79 ($25\text{ }^\circ\text{C}$) and 3.29 ($20\text{ }^\circ\text{C}$). The Table 3 show that the data obtained in our study are slightly more than half that found by Rodríguez et al (2015), however our study was done under oxygen flow, which may explain this difference.

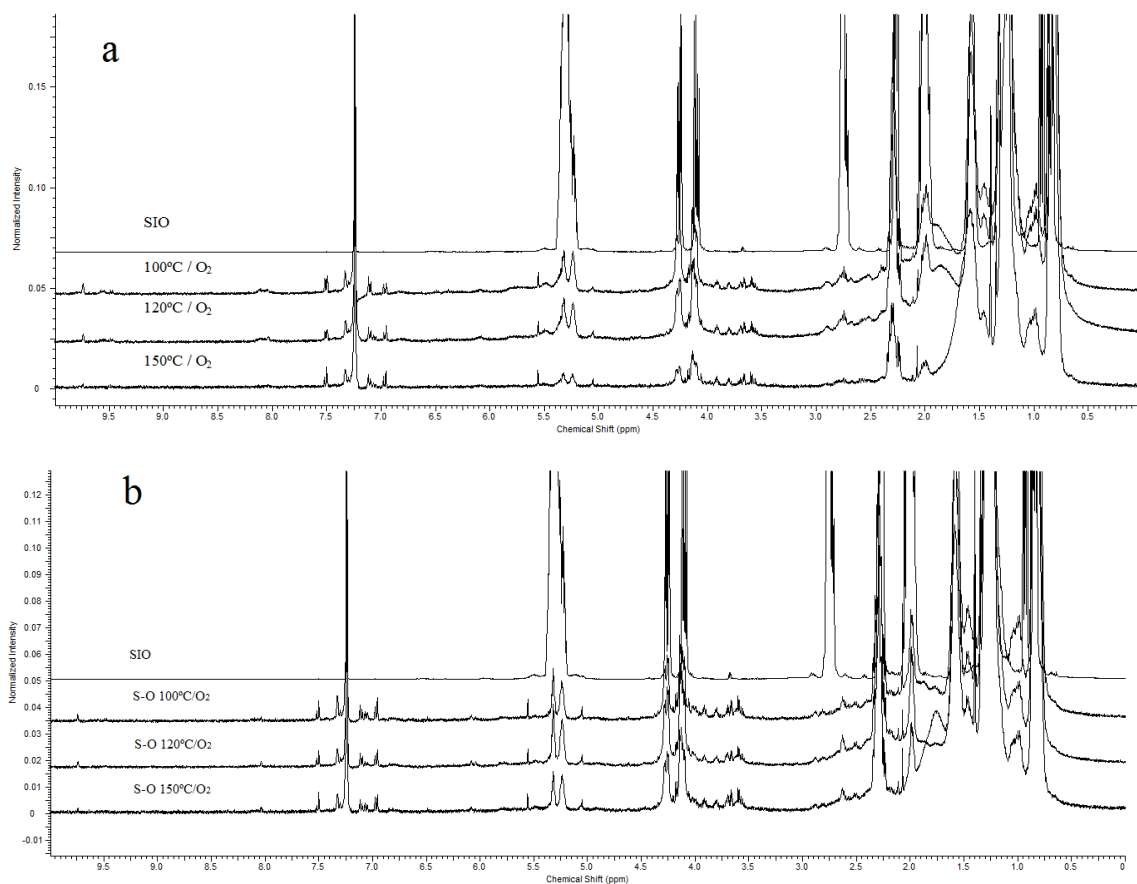
6.3.2 ^1H NMR spectra (after isothermal DSC oxidation).

Fig 6.2 shows the ^1H NMR spectra to SIO and encapsulated evaluated after isothermal DSC oxidation. It is possible to observe clearly that the isothermal DSC oxidation under O_2 flow modified considerably the ^1H NMR spectra of SIO. There was a decrease in the ω -3 concentration (Tab. 6.4) and all signals associated at unsaturated compounds and as result the increase of derived products from the oxidative degradation as hydroperoxides and aldehydes. It can be perceived that in the encapsulated the decrease in the ω -3

concentration was bigger in the temperatures at 100 e 120 °C when compared with SIO at the same temperatures, this can be linked with the interaction between SIO and Ova discussed above. The ¹H NMR spectra analysis allowed us to identify which oxidative compound were formed after isothermal DSC oxidation and are presented in Tab 6.5.

Table 6.4. Omega-3 concentration after isothermal DSC oxidation.

Isothermal condition/sample	Omega-3 (%)	
	50.7 ±	2.6
SIO (no heated)	50.7 ±	2.6
SIO 100°C	1.50 ±	0.05
SIO 120°C	1.76 ±	0.06
SIO 150°C	0.001 ±	0.000
S-O 100°C	0.344 ±	0.011
S-O 120°C	0.283 ±	0.009
S-O 150°C	0.256 ±	0.008
S-O-Pec 100°C	0.165 ±	0.005
S-O-Pec 120°C	0.105 ±	0.003
S-O-Pec 150°C	0.206 ±	0.006
S-O-XG 100°C	0.188 ±	0.006
S-O-XG 120°C	0.283 ±	0.009
S-O-XG 150°C	0.648 ±	0.020



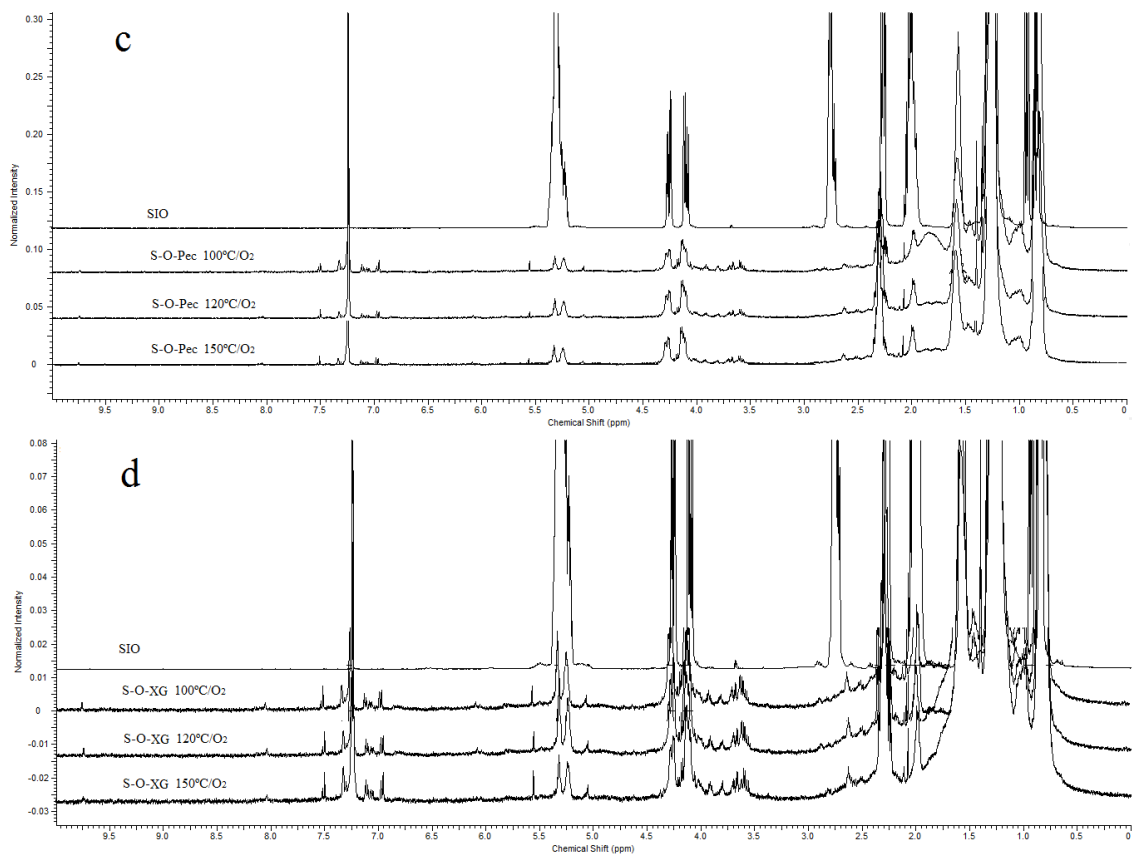


Fig. 6.2. ^1H NMR spectra of SIO and encapsulated after isothermal DSC oxidation.

The identified compounds in the ^1H NMR spectra are shown in the Table 6.5. The identification was based and compared with the information presented by Almoselhy et al, (2014); Guillén & Goicoechea, (2009); Guillén & Uriarte, (2012); Alonso-Salces et al, (2011), Cordella et al, (2012) and Barison et al, (2010).

Table 6.5. Average height of signals obtained after isothermal DSC oxidation compared to SIO (no heated).

Chemical shift (ppm)		SIO (no heated)	SIO 100°C	SIO 120°C	SIO 150°C	S-O 100°C	S-O 120°C	S-O 150°C	S-O-Pec 100°C	S-O-Pec 120°C	S-O-Pec 150°C	S-O-XG 100°C	S-O-XG 120°C	S-O-XG 150°C
	n-3 (%)	50,69	1,50	1,76	0,00	0,34	0,28	0,26	0,17	0,11	0,21	0,19	0,28	0,65
0.81 - 0.93	CH ₃ (acyl groups)	1,6638	1,4483	1,0282	0,8820	1,3099	1,3004	1,0327	1,4368	1,1658	0,9754	1,8977	1,4281	1,2930
1.13 - 1.31	methylene protons	2,1450	2,3637	2,2145	1,1405	2,0273	2,0805	1,8212	1,9723	1,7638	1,5567	2,3224	2,0595	1,6573
1.40 - 1.46	(E-E)-2,4-Alkadienals	0,0000	0,1503	0,1791	0,1326	0,1518	0,1310	0,1312	0,1601	0,1054	0,0865	0,0877	0,1291	0,1615
1.58	β-position (Acyl groups)	0,1693	0,1484	0,1447	0,0823	0,1065	0,1224	0,1087	0,1001	0,1033	0,1008	0,1230	0,1182	0,1067
1.97 - 2.17	δ (allyl protons) - neighboring to double bonds	1,1717	0,2861	0,2551	0,0817	0,1559	0,1385	0,1326	0,1209	0,1251	0,1248	0,3176	0,1619	0,0885
2.23 - 2.34	α-position	0,9984	0,3570	0,3207	0,1857	0,3875	0,4549	0,3642	0,3376	0,3733	0,3890	0,5421	0,4357	0,3485
2.37 - 2.40	Alkanal	0,0000	0,0442	0,0142	0,0208	0,0085	0,0087	0,0078	0,0779	0,0086	0,0092	0,0067	0,0072	0,0108
2.50	Oleyl acyl chains	0,0000	0,0096	0,0132	0,0186	0,0144	0,0276	0,0153	0,0065	0,0159	0,0099	0,0324	0,0259	0,0077
2.68	4-Oxo-alkanals	0,0000	0,0091	0,0106	0,0043	0,0000	0,0000	0,0000	0,0083	0,0103	0,0147	0,0030	0,0035	0,0057
2.88	(Z)-9,10-Epoxystearate	0,0000	0,0072	0,0083	0,0031	0,0043	0,0038	0,0037	0,0038	0,0021	0,0016	0,0024	0,0040	0,0056
3.56 - 3.66	1 Monoglycerides	0,0000	0,0397	0,0438	0,0333	0,0419	0,0313	0,0382	0,0414	0,0342	0,0356	0,0663	0,0506	0,0492
3.67 - 3.70	1,2-Diglycerides	0,0014	0,0229	0,0264	0,0178	0,0200	0,0120	0,0223	0,0222	0,0183	0,0096	0,0211	0,0219	0,0231

3.80	2 Monoglycerides	0,0000	0,0067	0,0075	0,0054	0,0093	0,0045	0,0053	0,0060	0,0045	0,0041	0,0096	0,0089	0,0066
3.91	Saturated alcohol	0,0000	0,0081	0,0087	0,0064	0,0112	0,0041	0,0092	0,0125	0,0157	0,0180	0,0134	0,0114	0,0062
4.03 - 4.06	<i>sn</i> 1,3-diglycerides	0,0000	0,0151	0,0117	0,0115	0,0148	0,0146	0,0144	0,0150	0,0132	0,0109	0,0102	0,0148	0,0162
4.10 - 4.28	Glyceryl groups	0,7898	0,2667	0,2559	0,1003	0,2197	0,2749	0,2165	0,1752	0,1952	0,2314	0,3076	0,2850	0,2103
4.34 - 4.38	Hydroperoxide (methine proton)	0,0000	0,0131	0,0055	0,0072	0,0064	0,0041	0,0041	0,0081	0,0063	0,0034	0,0019	0,0020	0,0038
5.07	1,2-diglycerides	0,0000	0,0039	0,0060	0,0053	0,0056	0,0048	0,0051	0,0061	0,0040	0,0031	0,0035	0,0047	0,0054
5.24	Glyceryl groups	0,1542	0,0223	0,0199	0,0075	0,0153	0,0182	0,0144	0,0133	0,0153	0,0162	0,0189	0,0177	0,0122
5.32 - 5.36	ϵ diene conjugated (protons of the double bonds)	1,7867	0,0666	0,0356	0,0134	0,0201	0,0241	0,0159	0,0146	0,0213	0,0278	0,0376	0,0295	0,0141
5.56	Hydroperoxy-(Z,E)-conjugated-dienic systems	0,0000	0,0116	0,0107	0,0091	0,0087	0,0070	0,0075	0,0099	0,0059	0,0033	0,0057	0,0076	0,0096
6.08	(E,E)-2,4-Alkadienals	0,0000	0,0030	0,0032	0,0022	0,0027	0,0027	0,0025	0,0025	0,0027	0,0031	0,0035	0,0029	0,0029
6.49	Hydroxy-(Z,E)-conjugated-dienic system	0,0000	0,0031	0,0015	0,0015	0,0019	0,0008	0,0020	0,0021	0,0000	0,0000	0,0000	0,0000	0,0000

6.83	(E)-2-Alkenals	0,0000	0,0027	0,0000	0,0000	0,0018	0,0015	0,0022	0,0016	0,0016	0,0015	0,0012	0,0017	0,0018
6.95 - 6.98	Phenolic compounds	0,0000	0,0123	0,0136	0,0154	0,0149	0,0114	0,0132	0,0162	0,0119	0,0103	0,0113	0,0121	0,0156
7.10 - 7.12	(E-E)-2,4-Alkadienals (Aldehydic groups)	0,0000	0,0171	0,0134	0,0121	0,0114	0,0099	0,0089	0,0122	0,0075	0,0060	0,0078	0,0092	0,0121
7.30 - 7.33	Olefins	0,0000	0,0199	0,0175	0,0158	0,0245	0,0108	0,0118	0,0163	0,0059	0,0062	0,0098	0,0119	0,0170
7.49 - 7.51	Unidentified signal	0,0000	0,0274	0,0119	0,0167	0,0122	0,0101	0,0110	0,0127	0,0082	0,0067	0,0092	0,0107	0,0133
8.11	Hydroperoxide group	0,0000	0,0036	0,0023	0,0015	0,0009	0,0011	0,0012	0,0012	0,0009	0,0008	0,0006	0,0011	0,0009
9.47	(E)-2-Alkenals	0,0000	0,0020	0,0014	0,0011	0,0012	0,0009	0,0010	0,0010	0,0013	0,0001	0,0008	0,0009	0,0011
9.55	4,5-epoxy-trans-2-alkenals	0,0000	0,0029	0,0018	0,0014	0,0009	0,0007	0,0014	0,0009	0,0007	0,0004	0,0006	0,0010	0,0008
9.74	Alkanal	0,0000	0,0064	0,0044	0,0019	0,0010	0,0023	0,0019	0,0023	0,0025	0,0021	0,0002	0,0025	0,0010

After signal identification, shown in Tab. 6.5, we split into two groups to facilitate the assessment and interpretation. Table 6.6 lists the signals that were detected with (only) SIO (no heated), and Table 6.7 the signals that were formed after isothermal DSC oxidation.

In the Tab. 6.6, it can observe the increase (+ signal) or decrease (- signal) relative of compounds that were identified in the ^1H NMR spectra of SIO unheated. The omega-3 concentration decreased about 97% at 100°C and 120°C and all their content at 150°C to SIO. A similar decrease was observed in the encapsulated, that the decreases of the omega-3 content ranged from 98.7 to 99.8%. Thus, it can be assumed that the encapsulated were unable to prevent thermal and oxidative decomposition of omega-3 contained in the SIO and their encapsulated. We can observe similar drop in the signal of conjugated diene percentage (protons linked to double bonds) observed at δ_{H} 5.32 to 5.36 ppm. The intensities decreases of the signals of CH_3 (acyl groups, at δ_{H} 0.81-0.93ppm), methylene protons (δ_{H} 1.13 to 1.31 ppm), protons bonded on the α (δ_{H} 2.23-2.34 ppm) and β -positions (δ_{H} 1.58 ppm), allyl protons (δ_{H} 1.97 – 2.17 ppm), glyceryl groups of 1,3 positions (δ_{H} 4.10 – 4.28 ppm), glyceryl groups of 2-position (δ_{H} 5.24 ppm) and the diene conjugated (δ_{H} 5.32-5.36 ppm) were greater the higher the temperature of the isotherm and were slightly lower in the SO and SO-Pec encapsulated, but not with respect to S-O-XG compared to SIO (at all temperatures). It therefore refers to either related to proton of double bonds or omega-3 in the triacylglycerol (TAG) form, which underwent oxidation. Importantly that the increase in diglycerides (δ_{H} 3.67-3.70 ppm) is due to decrease in the TAG fraction and release of R' forming di- and then monoglycerides (GUILLÉN & URIARTE, 2012).

Table 6.6. Increase (+) or decrease (-) percentage of signals intensity observed on ^1H NMR compared to SIO (no heated).

Chemical shift (ppm)		SIO 100°C	SIO 120°C	SIO 150°C	S-O 100°C	S-O 120°C	S-O 150°C	S-O-Pec 100°C	S-O-Pec 120°C	S-O-Pec 150°C	S-O-XG 100°C	S-O-XG 120°C	S-O-XG 150°C
	n-3 (%)	-97,0	-96,5	-100,0	-99,3	-99,4	-99,5	-99,7	-99,8	-99,6	-99,6	-99,4	-98,7
0.81 - 0.93	CH ₃ (acyl groups)	-13,0	-38,2	-47,0	-21,3	-21,8	-37,9	-13,6	-29,9	-41,4	14,1	-14,2	-22,3
1.13 - 1.31	methylene protons	10,2	3,2	-46,8	-5,5	-3,0	-15,1	-8,1	-17,8	-27,4	8,3	-4,0	-22,7
1.58	β -position (Acyl groups)	-12,3	-14,5	-51,4	-37,1	-27,7	-35,8	-40,9	-39,0	-40,5	-27,3	-30,2	-37,0
1.97 - 2.17	δ (allyl protons) - neighboring to double bonds	-75,6	-78,2	-93,0	-86,7	-88,2	-88,7	-89,7	-89,3	-89,3	-72,9	-86,2	-92,4
2.23 - 2.34	α -position	-64,2	-67,9	-81,4	-61,2	-54,4	-63,5	-66,2	-62,6	-61,0	-45,7	-56,4	-65,1
3.67 - 3.70	1,2-Diglycerides	1536	1786	1171	1329	757	1493	1486	1207	586	1407	1464	1550
4.10 - 4.28	Glyceryl groups	-66,2	-67,6	-87,3	-72,2	-65,2	-72,6	-77,8	-75,3	-70,7	-61,1	-63,9	-73,4
5.24	Glyceryl groups	-85,5	-87,1	-95,1	-90,1	-88,2	-90,7	-91,4	-90,1	-89,5	-87,7	-88,5	-92,1
5.32 - 5.36	ϵ diene conjugated (protons of the double bonds)	-96,3	-98,0	-99,3	-98,9	-98,7	-99,1	-99,2	-98,8	-98,4	-97,9	-98,3	-99,2

The Table 6.7 presents compounds which were formed only after the isothermal DSC oxidation and that therefore were not present in SIO (no heated). As result of the omega-3 degradation, the relative percentages of oxidized compounds for each sample are shown in the Table 6.7.

It can be seen that most of the oxidized compounds is related to the formation of a multiplet (E-E)-2,4-Alkadienals, their relative percentage varied from 30.5 to 45.9% of height total (chemical shift at δ_H 1.40 – 1.46 ppm) in all samples. It is the main aldehyde found in our study, it can also be observed at δ_H 6.8 ppm in low concentrations, ranging from 0.6 to 1.3% (as doublet) and at δ_H 7.10 - 7.12 ppm, again as multiplet in the concentration of 2.7 to 4.1%. Together, the relative percentage varied from 34.4 (S-O-XG at 100°C) until 50.1 % (SIO at 120°C). The average (E-E)-2,4-Alkadienals in all samples was 44.3 ± 3.0 % of the oxidized compounds. The (E-E)-2,4-Alkadienals was found by Guillén & Uriarte (2012) in extra virgin olive oil submitted to frying temperature. The Alkanal signals was observed in the chemical shift at δ_H 2.37 – 2.40 ppm and at δ_H 9.74 ppm, it is also aldehyde and is obtained by oxidation of primary alcohols. In our study the Alkanal was more intense in S-O-Pec 100°C, followed by the SIO 100 °C, but its intensity decreased with increasing temperature in both cases. In other samples, the relative concentration ranged from 2.4 (S-O-XG 100°C) to 6.9 % (SIO 150°C). The Oleyl acyl chain (δ_H 2.50 ppm) was more intense in S-O-XG 100°C and at 120°C. This signal was observed by Almoselhy et al, (2014) in Moringa oil, Sunflower oil and Apricol kernel oils unheated. In the SIO we believed that have formed due the TAG hydrolysis, but is not a toxic product.

Other important products observed were the hydroperoxide groups. Even that at low percentage relative, mainly because of the decomposition them to form aldehyde groups (McCLEMENTS & DECKER, 2010). The relative percentages (summed) ranged from 2.8 to 7.5 %. The hydroperoxides groups can be seen at δ_H 4.34 to 4.38 ppm, at δ_H 5.56 and 6.08 ppm (hydroperoxy-(Z,E)-conjugated diene systems and at δ_H 8.11 ppm. Erkan, Ayranci & Ayranci (2009) also found conjugated dienes hydroperoxides, in the study of oxidation kinetic in sunflower oil under microwave heating. The presence of monoglycerides groups (at δ_H 3.56 – 3.66 ppm and δ_H 3.80 ppm) with a reasonable intensity (ranged from 11.1 to 26.4 %) and the diglycerides groups (δ_H 4.03 to 4.06 ppm and at δ_H 5.07 ppm) summed reach an average of 5.6 % refers to hydrolysis of TAG and hence increase of these signals. The same behavior was observed by Guillén & Uriarte (2012), Guillén & Goicoechea (2009) and Alonso-Salces et al, (2011).

Table 6.7. Signal intensity percentage of oxidized compounds (formed after isothermal DSC oxidation).

Chemical shift (ppm)		SIO 100°C	SIO 120°C	SIO 150°C	S-O 100°C	S-O 120°C	S-O 150°C	S-O-Pec 100°C	S-O-Pec 120°C	S-O-Pec 150°C	S-O-XG 100°C	S-O-XG 120°C	S-O-XG 150°C
1.40 - 1.46	(E-E)-2,4- Alkadienals	35,9	45,9	40,5	43,3	44,6	43,9	37,7	39,1	36,4	30,5	39,9	45,5
2.37 - 2.40	Alkanal	10,5	3,6	6,4	2,4	3,0	2,6	18,3	3,2	3,9	2,3	2,2	3,0
2.50	Oleyl acyl chains	2,3	3,4	5,7	4,1	9,4	5,1	1,5	5,9	4,2	11,3	8,0	2,2
2.68	4-Oxo-alkanals	2,2	2,7	1,3	0,0	0,0	0,0	2,0	3,8	6,2	1,0	1,1	1,6
2.88	(Z)-9,10- Epoxy stearate	1,7	2,1	0,9	1,2	1,3	1,2	0,9	0,8	0,7	0,8	1,2	1,6
3.56 - 3.66	1 Monoglycerides	9,5	11,2	10,2	12,0	10,7	12,8	9,8	12,7	15,0	23,0	15,6	13,9
3.80	2 Monoglycerides	1,6	1,9	1,7	2,7	1,5	1,8	1,4	1,7	1,7	3,3	2,7	1,9
3.91	Saturated alcohol	1,9	2,2	2,0	3,2	1,4	3,1	2,9	5,8	7,6	4,7	3,5	1,7
4.03 - 4.06	<i>sn</i> 1,3- diglycerides	3,6	3,0	3,5	4,2	5,0	4,8	3,5	4,9	4,6	3,5	4,6	4,6
4.34 - 4.38	Hydroperoxide (methine proton)	3,1	1,4	2,2	1,8	1,4	1,4	1,9	2,3	1,4	0,7	0,6	1,1
5.07	1,2-diglycerides	0,9	1,5	1,6	1,6	1,6	1,7	1,4	1,5	1,3	1,2	1,5	1,5
5.56	Hydroperoxy- (Z,E)-conjugated- dienic systems	2,8	2,7	2,8	2,5	2,4	2,5	2,3	2,2	1,4	2,0	2,3	2,7
6.08	(E,E)-2,4- Alkadienals	0,7	0,8	0,7	0,8	0,9	0,8	0,6	1,0	1,3	1,2	0,9	0,8
6.49	Hydroxy-(Z,E)- conjugated-dienic system	0,7	0,4	0,5	0,5	0,3	0,7	0,5	0,0	0,0	0,0	0,0	0,0
6.83	(E)-2-Alkenals	0,6	0,0	0,0	0,5	0,5	0,7	0,4	0,6	0,6	0,4	0,5	0,5
6.95 - 6.98	Phenolic compounds	2,9	3,5	4,7	4,3	3,9	4,4	3,8	4,4	4,3	3,9	3,7	4,4

7.10 - 7.12	(E-E)-2,4-Alkadienals (Aldehydic groups)	4,1	3,4	3,7	3,3	3,4	3,0	2,9	2,8	2,5	2,7	2,8	3,4
7.30 - 7.33	Olefins	4,7	4,5	4,8	7,0	3,7	3,9	3,8	2,2	2,6	3,4	3,7	4,8
7.49 - 7.51	Unidentified signal	6,5	3,0	5,1	3,5	3,4	3,7	3,0	3,0	2,8	3,2	3,3	3,7
8.11	Hydroperoxide group	0,9	0,6	0,5	0,3	0,4	0,4	0,3	0,3	0,3	0,2	0,3	0,3
9.47	(E)-2-Alkenals	0,5	0,4	0,3	0,3	0,3	0,3	0,2	0,5	0,1	0,3	0,3	0,3
9.55	4,5-epoxy-trans-2-alkenals	0,7	0,5	0,4	0,3	0,2	0,5	0,2	0,3	0,2	0,2	0,3	0,2
9.74	Alkanal	1,5	1,1	0,6	0,3	0,8	0,6	0,5	0,9	0,9	0,1	0,8	0,3

6.3.2.1 Analysis of the Pearson correlation coefficient

The Pearson correlation coefficient (r) was done by applying Eq. (4) between the omega-3 concentrations and all signals observed in the ^1H NMR spectra (Tab. 6.5). The data are shown in the Tab. 6.8. The r value can vary from -1 to 1. The $r = -1$ (negative correlation) is when a variable increases while the other decreases linearly. The $r = 1$ (positive correlation) is when a variable increases while the other also increase linearly, or both decreases. And $r = 0$ (when there is no linear correlation), i.e., the variable value is independent of each other.

Thus, we can see in Table 6.8 that as the omega-3 concentration decreased brutally after the isothermal DSC oxidation (Table 6.2), all positive r values (Table 6.6) also decreased, the example of diene conjugated groups (at δ_{H} 5.32 to 5.36 ppm) and allyl protons (at δ_{H} 1.97 to 2.17 ppm) were proportional linearly to the concentration decrease ($r \sim 1$) in all samples. Fact expected, since in both signals are representing positions related to the double bonds.

On the other hand, all negatives r values represents that the decrease of omega-3 concentration is proportional to an increase of their concentrations. When the r value is > 0.90 mean that the correlation is strong. The intensity increase of the (E-E)-2,4-Alkadienals (that is the main oxidized compound formed) is strongly correlated with the decrease of omega-3 concentration. The same behavior is observed to most of the oxidized compounds.

Table 6.8. Pearson coefficient correlation of omega-3 in relation to other compounds.

Chemical shift (ppm)		SIO	S-O	S-O-Pec	S-O-XG
	n-3 (%)	1,000	1,000	1,000	1,000
0.81 - 0.93	CH ₃ (acyl groups)	0,762	0,869	0,779	0,227
1.13 - 1.31	methylene protons	0,244	0,603	0,746	0,227
1.40 - 1.46	(E-E)-2,4-Alkadienals	-0,963	-0,990	-0,883	-0,899
1.58	β -position (Acyl groups)	0,613	0,971	0,999	0,967
1.97 - 2.17	δ (allyl protons) - neighboring to double bonds	0,988	1,000	1,000	0,980
2.23 - 2.34	α -position	0,985	0,992	0,998	0,960
2.37 - 2.40	Alkanal	-0,712	-0,996	-0,440	-0,911
2.50	Oleyl acyl chains	-0,894	-0,846	-0,811	-0,730
2.68	4-Oxo-alkanals	-0,812	-	-0,901	-0,862
2.88	(Z)-9,10-Epoxystearate	-0,792	-0,991	-0,799	-0,833
3.56 - 3.66	1 Monoglycerides	-0,969	-0,973	-0,986	-0,964
3.67 - 3.70	1,2-Diglycerides	-0,938	-0,884	-0,824	-0,996
3.80	2 Monoglycerides	-0,958	-0,834	-0,948	-0,958
3.91	Saturated alcohol	-0,962	-0,806	-0,960	-0,866
4.03 - 4.06	<i>sn</i> 1,3-diglycerides	-0,964	-1,000	-0,969	-0,935
4.10 - 4.28	Glyceryl groups	0,975	0,995	0,997	0,986
4.34 - 4.38	Hydroperoxide (methine proton)	-0,794	-0,913	-0,838	-0,822
5.07	1,2-diglycerides	-0,946	-0,992	-0,868	-0,943
5.24	Glyceryl groups	0,998	1,000	1,000	0,999
5.32 - 5.36	ϵ diene conjugated (protons of the double bonds)	1,000	1,000	1,000	1,000
5.56	Hydroperoxy-(Z,E)-conjugated- dienic systems	-0,975	-0,983	-0,761	-0,920
6.08	(E,E)-2,4-Alkadienals	-0,946	-0,997	-0,984	-0,985
6.49	Hydroxy-(Z,E)-conjugated- dienic system	-0,796	-0,821	-0,333	-
6.83	(E)-2-Alkenals	-0,322	-0,955	-0,998	-0,946
6.95 - 6.98	Phenolic compounds	-0,988	-0,977	-0,932	-0,959
7.10 - 7.12	(E-E)-2,4-Alkadienals (Aldehydic groups)	-0,952	-0,980	-0,851	-0,935
7.30 - 7.33	Olefins	-0,978	-0,782	-0,700	-0,902
7.49 - 7.51	Unidentified signal	-0,820	-0,988	-0,875	-0,954
8.11	Hydroperoxide group	-0,806	-0,974	-0,943	-0,902
9.47	(E)-2-Alkenals	-0,885	-0,972	-0,628	-0,964
9.55	4,5-epoxy-trans-2-alkenals	-0,838	-0,862	-0,852	-0,925
9.74	Alkanal	-0,737	-0,848	-0,990	-0,538

The (-) signal indicate the absence of compound in the sample, and then become impossible calculate the “r” value.

6.4. CONCLUSIONS

The Isothermal DSC is a useful and practical technique to determine the OIT (min) and kinetic parameters that become possible predict the stability oxidation in different edible oils. However, when complemented by ^1H NMR, one can enrich more the understanding of what happened about the process (oxidation), because it becomes possible to identify compounds which were formed. Finally the encapsulated formed by emulsion-based were not efficient in omega-3 content protection against the SIO.

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CONCLUSÃO GERAL

Diante do trabalho realizado podemos concluir que o uso de emulsões simples (óleo-em-água) utilizando Ovalbumina como emulsificante, pode servir como estratégias para inserção de compostos lipossolúveis como o óleo de Sacha Inchi em alimentos ou sistemas aquosos. O uso de biopolímeros (como Pectina e Goma Xantana) confere a essas emulsões estabilidade termodinâmica e cinética por ao menos 14 dias à temperatura de 25 °C, por aumentarem a viscosidade da fase aquosa. Quando estabilizadas com Goma Xantana, apresentam características reológicas típicas de gel.

Estas emulsões depois de liofilizadas podem produzir encapsulados sólidos capazes de reterem em torno de 80% de óleo (m/m) em sua composição. As técnicas de cristalinidade (XDR) e de infravermelho (FTIR) confirmam que estes encapsulados apresentam uma melhor organização estrutural quando comparados aos biopolímeros isoladamente utilizados. Estes encapsulados produzidos com Pectina ou Goma Xantana, mantem as gotículas mais bem definidas quando comparadas com as produzidas apenas com Ovalbumina, mostrando deste modo que os polissacarídeos contribuem para a estabilidade da mesma.

Os encapsulados produzidos podem ainda servir como forma de transportar compostos nutracêuticos como ômega-3 contido no óleo de Sacha Inchi por resistirem bem as condições gástricas humanas (*in vitro*) e apresentarem um perfil de liberação de ômega-3 bastante lento, o que impede a perda antes de chegarem ao intestino.

Embora as microencapsulações não resistam às condições térmicas e oxidativas testadas neste estudo, foi possível identificar e quantificar os principais produtos da oxidação do óleo de Sacha Inchi e observar sua relação com a diminuição do conteúdo de ômega-3. Além disso, foi possível conhecer melhor o comportamento do óleo de Sacha Inchi em relação aos parâmetros cinéticos (E_a , A e z) e seu valor de tempo de indução oxidativa a diferentes temperaturas.

Por fim, a utilização da técnica de RMN de ^1H foi de extrema utilidade neste estudo e configura-se como uma ferramenta prática, eficaz e de altíssima sensibilidade para detecção de fraudes e para avaliação da estabilidade térmica e oxidativa não só para óleo de Sacha Inchi, mas para diversos óleos comestíveis.