PEDRO HENRIQUE CAMPELO FELIX

MICROENCAPSULAÇÃO DE ÓLEO ESSENCIAL DE LIMÃO (*Citrus aurantifolia)*: EMULSÕES E ESTUDO DAS PROPRIEDADES FÍSICO-QUÍMICAS

LAVRAS – MG

2017

PEDRO HENRIQUE CAMPELO FELIX

MICROENCAPSULAÇÃO DE ÓLEO ESSENCIAL DE LIMÃO (*Citrus Aurantifolia)*: EMULSÕES E ESTUDO DAS PROPRIEDADES FÍSICO-QUÍMICAS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, área de concentração em Secagem por Atomização, para a obtenção do título de Doutor.

Orientadora

Dra. Soraia Vilela Borges

LAVRAS – MG

2017

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Felix, Pedro Henrique Campelo.
Microencapsulação de óleo essencial de limão (*Citrus aurantifolia*) : Emulsões e estudo das propriedades físicos-químicas / Pedro Henrique Campelo Felix. - 2017.
17 p. : il.

Orientador(a): Soraia Vilela Borges.
Coorientador(a): Diego Alvarenga Botrel, Regiane Victória de Barros Fernandes.
Tese (doutorado) - Universidade Federal de Lavras, 2017.
Bibliografia.

1. óleo essencial de limão. 2. microencapsulação. 3. emulsões. I. Borges, Soraia Vilela. II. Botrel, Diego Alvarenga. III. Fernandes, Regiane Victória de Barros. IV. Título.

PEDRO HENRIQUE CAMPELO FELIX

MICROENCAPSULAÇÃO DE ÓLEO ESSENCIAL DE LIMÃO (*Citrus Aurantifolia)*: EMULSÕES E ESTUDO DAS PROPRIEDADES FÍSICO-QUÍMICAS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, área de concentração em Secagem por Atomização, para a obtenção do título de Doutor.

APROVADA em 19 de maio de 2017.

Dr. Diego Alvarenga Botrel	UFLA
Dr. Jefferson Luiz Gomes Correa	UFLA
Dra. Joyce Maria Gomes da Costa	UFVJM
Dra. Viviane Santos Birchal	UFMG

Dra. Soraia Vilela Borges
Orientadora

LAVRAS – MG

2017

À minha avó Maria (in memorian). Mesmo no céu, sinto sua presença e seu zelo.
“ Bença! ”

DEDICO

AGRADECIMENTOS

À minha mãe, Ligia, pelo seu amor incondicional e pelo seu maior ensinamento em minha vida: pensamento positivo e sempre lutar pelo que eu acreditar. Você e toda sua sensibilidade conseguiram extrair o melhor de mim desde pequeno.

Ao meu irmão, Matheus, pela amizade e companheirismo;

À minha companheira, Lesley (Mumu), por sempre acreditar em mim e por viver meus sonhos junto comigo. Sobreviveu à distância Lavras X BH, sempre com sorriso no rosto e emanando amor e carinho. Te amo muito, linda....

À minha orientadora, Profa. Dra. Soraia Vilela Borges. Soraia, você confiou em mim sem nem me conhecer. Acreditou no meu potencial e investiu em mim como se fosse um filho. Sempre levarei seus ensinamentos comigo. Te amo!

Aos meus coorientadores, Prof. Dr. Diego Alvarenga Botrel e Dra. Regiane Victória de Barros Fernandes, por todo apoio e ensinamentos.

À Profa. Dra. Joyce Maria Gomes da Costa, que permitiu a realização deste sonho. Você me abriu as portas para a UFLA ao me apresentar a Profa. Soraia. Minha eterna gratidão!

À Profa. Dra. Viviane Santos Birchal, que me viu crescer como pesquisador no mestrado, e hoje contribui para esta tese.

Ao Prof. Dr. Jefferson Luiz Gomes Correa, por ter aceitado o convite em participar desta banca e acrescentar com suas valiosas contribuições.

À minha amiga/braço direito/"filha", Jayne de Abreu Figueiredo. Sem sua ajuda, o curto tempo de realização deste doutorado não teria acontecido. Obrigado por sua disposição em ir ao laboratório à noite e aos finais de semana para me ajudar. Muito obrigado!

Aos amigos de departamento que contribuíram muito para este trabalho: Rhana, Hugo, Eloá, Gérson, Luciana e Viviane. A ajuda de vocês foi muito importante. Muito obrigado!

Aos amigos de DCA/UFLA (Turma da Bagaceira!). Os dias em Lavras foram mais felizes com os almoços, pausas para conversas, festas e outros eventos. Levarei vocês sempre comigo. Contem comigo, sempre. Obrigado!

Aos professores e técnicos do DCA/UFLA, principalmente aos profs. Drs. Jaime, Marali e Roney pelo apoio de sempre.

À minha família, em especial à minha madrinha (tia) Cláudia, por sempre acreditar em meus sonhos.

Aos meus amigos eternos, Javan, Flávia Borim, Igor, Dandoca, Luana e Mário. Vocês sempre elevaram meu astral nos momentos mais complicados. Obrigado pela torcida e me desculpem pelas ausências. Amo vocês!

Aos Profs. Drs. Cassiano Oliveira, Maria Irene Yoshida, Rosada Domingues Zacarias e Vany Ferraz, pela ajuda e disponibilidade com inúmeras análises deste trabalho.

Aos meus amigos de República (Júnior, Marcelo e Tiago) por cuidarem de mim durante 1 ano. Obrigado pela amizade e companheirismo de vocês!

A todas as empresas que fizeram doações importantes para o desenvolvimento deste trabalho: Metachem, Hilmar Ingredients, Alibra e Amazon Oils.

Aos amigos de carona pelas conversar e por “financiarem” minhas idas e vindas na BR 381.

Às centenas de alunos que tive na vida, tanto em aulas particulares, como em cursinho e graduação. Não há no mundo, projeto, artigo, financiamento e/ou laboratório que faça com que o crescimento de vocês (pessoal e profissional) não seja minha prioridade. Obrigado pela confiança!!

Aos professores do Departamento de Engenharia Agrícola e Solos da Universidade Federal do Amazonas, Prof. Carlos Victor Lamarão e Prof. Anderson Mathias Pereira, por otimizarem a minha vinda à Lavras para defender esta tese.

Ao Governo Federal (2003 – 2016), pelas oportunidades no ensino técnico, graduação, mestrado e doutorado. A visão e sensibilidade de construir um país melhor e igualitário através da educação gerou frutos. Obrigado e FORA TEMER!

RESUMO

O óleo essencial de limão (*Citrus aurantifolia*) é um produto muito utilizado pela indústria de alimentos, cosméticos e farmacêutica, devido as suas propriedades químicas e sensoriais marcantes. Depois de extraído, o óleo essencial de limão é muito suscetível a degradações e volatilizações. Com isso, é necessário promover processos para reduzir reações indesejáveis. O processo de microencapsulação é amplamente recomendado e utilizado para se conservar compostos bioativos por longos períodos. Sendo assim, este trabalho tem como objetivo, estudar o processo de microencapsulação do óleo essencial de limão através do processo de *spray drying*, utilizando diversos biopolímeros como material de parede. No primeiro artigo foi estudada a estabilidade de emulsões óleo essencial de limão, adicionadas de Goma Arábica (GA) e Isolado Proteico de Soro de Leite. Foi proposto delineamento meramente casualizado, variando-se a concentração de óleo essencial (5, 7,5 e 10%) e a composição do material de parede (GA, WPI e GA/WPI). As emulsões foram homogeneizadas utilizando ultrassom assistido (240W/2min). Foram avaliados pH, condutividade, potencial zeta, tamanho de gotículas, reologia (viscosidade e tensão de cisalhamento), microscopia ótica e índice de cremeação. Não se observou separação de fases durante as 4 primeiras horas de armazenamento. Em todos os tratamentos não houve variação significativa dos valores de pH e condutivadade. Maiores valores de potencial zeta foram encontrados para tratamentos com WPI e maiores concentração de óleo devido as características e grupos funcionais presentes no biopolímero e no óleo essencial de limão. Todos os tamanhos de gotículas apresentaram valores menores que 2µm. Conclui-se que as proteínas do soro de leite apresentaram emulsões mais estáveis, sendo este material de parede, a base para a produção das micropartículas de óleo essencial de limão. O segundo artigo mostra o estudo das propriedades físico-quimicas de micropartículas de óleo essencial de limão, utilizando-se proteínas de soro de leite concentradas (WPC) e maltodextrinas de valores de dextrose equivalente (DE) de 5, 10 e 20. As emulsões foram homogeneizadas através do ultrassom assistido, com potência de 240W, por 2 min. Após homogeneização, as emulsões foram submetidas a secagem por *spray drying* à 170°C, com fluxo de alimentação da emulsão de 0,7 L/h. Foram realizadas análises de reologia, microscopia ótica e tamanho de gotas, para as emulsões e; composição majoritária e atividade antioxidante do óleo cru e encapsulado, umidade, atividade de água, teor de óleo superficial, teor de óleo total, eficiência de encapsulação, retenção de óleo, tamanho de partículas, microscopia eletrônica de varredura e difração de raio-x. Emulsões com maltodextrinas de maiores valores de DE apresentaram menor viscosidade. O grau de hidrolização das cadeias de maltodextrina aumentaram a umidade e atividade de água das MP. Estrutura superficial das micropartículas

não apresentaram rachaduras. DRX mostrou a estrutura amorfa das micropartículas, ideal para processos de microencapsulação. DE 20 apresentaram melhores resultados de eficiência e atividade antioxidante. DE de maltodextrinas podem interferir nas propriedades físico-químicas de micropartículas de óleo essencial de limão. Em resumo, os biopolímeros utilizados podem ser uma alternativa para produção de emulsões e micropartículas de óleo essencial de limão.

Palavras-chaves: Óleo essencial de limão. *Spray drying*. Isolado proteico de soro de leite. Maltodextrina. Dextrose equivalente.

ABSTRACT

Lime essential oil (Citrus aurantifolia) is a product widely used by the food, cosmetics and pharmaceutical industry due of its remarkable chemical and sensory properties. After extracted, the lime essential oil is very susceptible to degradations and volatilizations. Thus, it is necessary to promote processes to reduce undesirable reactions. The microencapsulation process is widely recommended and used to preserve bioactive compounds for long periods. The objective of this work is to study the microencapsulation process of lemon essential oil through the spray drying process, using several biopolymers as a wall material. In the first article we studied the stability of lemon essential oil emulsions added of Arabic Gum (AG) and Whey Protein isolate (WPI). It was proposed a purely randomized design, varying the concentration of essential oil (5, 7,5 and 10%) and the composition of the wall material (AG, WPI and AG / WPI). The emulsions were homogenized using assisted ultrasound (240W / 2min). PH, conductivity, zeta potential, droplet size, rheology (viscosity and shear stress), optical microscopy and cream index were evaluated. No phase separation was observed during the first 4 hours of storage. In all treatments there was no significant variation of pH and conductivity values. Higher zeta potential values were found for treatments with WPI and higher oil concentration due to the characteristics and functional groups present in the biopolymer and in the lime essential oil. All droplet sizes presented values lower than 2μm. It is concluded that the whey proteins had more stable emulsions, this wall material being the basis for the production of the microparticles of lemon essential oil. The second article shows the study of the physicochemical properties of lemon essential oil microparticles using whey protein concentrated (CWP) and maltodextrins of dextrose equivalent (DE) values of 5, 10 and 20. The emulsions were homogenized by of ultrasound-assisted with power of 240W, for 2 min. After homogenization, the emulsions were spray dried at 170øC, with emulsion feed flow of 0.7 L / hr. Rheology, optical microscopy and droplet size analyzes were performed for the emulsions; and major composition and antioxidant activity of crude and encapsulated oil, moisture, water activity, surface oil content, total oil content, encapsulation efficiency, oil retention, particle size, scanning electron microscopy and x-ray diffraction. Emulsions with maltodextrins of higher DE values presented lower viscosity. The degree of hydrolyzation of the maltodextrin chains increased the moisture and water activity of microparticles. Surface structure of the microparticles showed no cracking. DRX showed the amorphous structure of the microparticles, ideal for

microencapsulation processes. DE 20 presented better results of efficiency and antioxidant activity. DE of maltodextrins may interfere with the physicochemical properties of microparticles of lemon essential oil. In summary, the biopolymers used may be an alternative for the production of emulsions and microparticles of lemon essential oil.

Keywords: lime essential oil, spray drying; whey protein isolate; maltodextrin; dextrose equivalent.

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Os produtos alimentícios são muito suscetíveis a degradações e reações indesejáveis, acarretando mudanças nas suas características químicas, físicas e sensoriais. Estas degradações podem inviabilizar o seu comércio, perdendo valor econômico e tecnológico. Os processos de conservação de alimentos tentam, através de diversas técnicas, minimizar essas perdas, disponibilizando o produto por mais tempo. Além disso, há uma necessidade de mudança no foco da indústria de alimentos: a observação das propriedades macroscópicas para aquelas que surgem em escalas micro e nanométricas, com o subsequente controle das estruturas hierárquicas no alimento e na sua funcionalidade.

Portanto, são cada vez mais imprescindíveis os estudos sobre as relações entre as estruturas micro e supramoleculares, bem como da funcionalidade nos níveis físico, nutricional e fisiológico (FAVARO-TRINDADE; PINHO, 2008).

Óleos essenciais são compostos bioativos que apresentam um grande potencial para indústria de alimentos, por sua atividade antimicrobiana e antioxidante. Suas características sensoriais também são valorizadas pela indústria de bebidas, panificados e outros; sendo um grande potencial de utilização de um aditivo natural (BIZZO et al., 2009; SANTOS et al., 2011). Devido a suscetibilidade dos óleos essenciais à temperatura, luz, oxigênio e umidade, faz-se necessário desenvolver mecanismo para reduzir reações indesejadas como volatilização, oxidação e interações com outros compostos, prolongando sua disponibilidade e tempo de vida útil (BRINGAS-LANTIGUA; VALDÉS; PINO, 2012).

O processo de microencapsulação de alimentos vem ganhando espaço nas principais pesquisas científicas da área de alimentos, como uma técnica promissora de conservação e liberação controlada de compostos bioativos. A técnica consiste no empacotamento do composto ativo em matrizes poliméricas,

muitas delas biopolímeros, com o objetivo de proteger o material do núcleo de condições estocagem não ideais de temperatura, umidade, oxigênio e luz. Dentre as diversas técnicas de microencapsulação estudadas, a secagem por atomização, mais conhecida como *spray drying* é uma das mais utilizadas, principalmente pelas formações de micropartículas mais homogêneas e íntegras.

Neste contexto, objetivou-se neste trabalho, o um estudo do processo de microencapsulação do óleo essencial de limão, utilizando diversos biopolímeros como material de parede.

Esta tese está dividida em capítulos, conforme descrição resumida seguinte. Na primeira parte, será estudada a estabilidade das emulsões utilizando o processo de homogeneização por ultrassom, variando potência e tempo de homogeneização, concentração do óleo e tipo do material de parede utilizado.

Foi avaliada a estabilidade da emulsão durante as primeiras 4 horas, estimando-se que este tempo era o suficiente desde a homogeneização até a secagem de toda emulsão, garantindo que não houvesse nenhum processo de desestabilização da emulsão. O melhor material de parede será escolhido como material de parede principal para o estudo das propriedades físico-químicas das micropartículas.

Na segunda parte, serão estudados os efeitos dos diferentes materiais de parede nas propriedades físico-químicas do produto em pó. A partir do material de parede que apresentou melhor estabilidade nas emulsões, será substituído por maltodextrinas de diferentes valores de dextrose equivalente, avaliando esta substituição nas propriedades físico-químicas das micropartículas.

2 REFERENCIAL TEÓRICO

2.1 Óleos essenciais

Óleo essencial pode ser definido como uma substância formada por diversos compostos produzidos pelo metabolismo secundário de vegetais e de composição química complexa. Estes óleos podem ser extraídos de flores, folhas, caules, sementes, cascas e outras partes do vegetal, e apresentam características sensoriais marcantes. Os óleos são encontrados em poucas plantas no mundo e, devido a isso, apresentam alto valor de mercado (ASBAHANI et al., 2015; DIMA; DIMA, 2015).

A composição dos óleos essenciais se baseia principalmente em dois grupos: os hidrocarbonetos terpênicos e os terpenóides. Os hidrocarbonetos terpênicos são compostos por monoterpenos (10 átomos de carbono) apresentando estrutura de hidrocarbonetos acíclicos e estruturas mono, bi e tricíclicas. Os terpenóides são terpenos oxigenados tais como álcoois, aldeídos, cetonas, fenóis, éteres e ésteres (ASBAHANI et al., 2015).

Os óleos essenciais apresentam diferentes propriedades biológicas como agente antimicrobiano, antifúngicos, antiviral, anti-inflamatório, combate ao câncer e atividade antioxidante. Estas características podem ser exploradas, desenvolvendo-se fármacos e alimentos (DIMA; DIMA, 2015). Diversos trabalhos relatam as atividades antimicrobianas de diferentes óleos essenciais (COTA-ARRIOLA et al., 2013; GIORDANI et al., 2006; SIMIC et al., 2004).

A aplicação dos óleos essenciais é bastante diversificada e depende da sua origem, qualidade e modo de extração. Pode ser utilizado na indústria de cosméticos (perfumes, shampoo e produtos de limpeza), alimentos (produção de bebidas, aromatizantes, conservantes e antimicrobianos) ou farmacêutica (aromaterapia e produtos dermatológicos) (PREEDY, 2016).

2.1.1 Óleo essencial de limão

O limão Tahiti (*Citrus Aurantifolia)* é um fruto da família *Rutaceae* de origem em países de clima tropical. Os frutos cítricos conferem propriedades antioxidantes que são muito importantes para saúde. Ácidos orgânicos, compostos fenólicos e açucares são os principais componentes de frutas cítricas (CRUZ-VALENZUELA et al., 2016). A Tabela 1 apresenta as características físico-químicas do limão.

Tabela 1 - Características Físico-químicas do limão tahiti.

Parâmetros	**Valores**
pH	2,56 ± 0,02
Sólidos solúveis (°Brix)	8,35 ± 0,35
Acidez Titulável (g ácido cítrico/100ml de suco)	9,80 ± 1,70
SS/AT	0,87 ± 0,12
Vitamina C (mg de ácido ascórbico/100ml de suco)	56,52 ± 3,33
Fenólicos totais (mg ácido gálico/g de	0,05 ± 0,0016
Capacidade Antioxidante	86,28 ± 3,00

Fonte: Oliveira e Diniz (2015).

Óleo essencial de limão é amplamente utilizado pela indústria alimentícia, farmacêutica e de cosméticos devido as suas características químicas e sensoriais (RAO; MCCLEMENTS, 2012). É muito utilizado em produtos como bebidas, panificados, doces, sobremesas e sorvete (GAMARRA et al., 2006). O óleo essencial de limão é uma mistura complexa de compostos químicos dividida em 3 classes: terpenos (75%), complexos oxigenados (12%) e

sesquiterpenos (3%) (Figura 1). É composto por substâncias como limoneno, γ-terpeno, citral, β-carofileno entre outros (Tabela 2) (CRUZ-VALENZUELA et al., 2016).

Tabela 2 - Características Físico-químicas do limão tahiti.

Substância	**Composição (ml/kg)**
α-thujene	1,143
α-pineno	0,868
Sabineno	0,282
β-Pineno	0,871
Mirceno	1,269
p-cimeno	1,566
d-limoneno	49,657
β-cariofileno	0,477
Trans-α-bergamoteno	0,795
β-bisaboleno	2,298

Fonte: (ASNAASHARI et al., 2010; CHISHOLM; WILSON; GASKEY, 2003; GAMARRA et al., 2006).

Autores estudaram a eficiência do uso do óleo de limão como agente antimicrobiano (JAFARI et al., 2011) e antioxidante (LUZIA; JORGE, 2010; MISHARINA; POLSHKOV, 2005).

2.2 Microencapsulação

O processo de microencapsulação pode ser definido com e empacotamento de uma substância sensível (sólido, líquido ou gás) em uma

matriz polimérica. Conceitualmente, o tamanho das partículas deve ser entre 0,1 a 10 μm. O material encapsulado pode ser definido também como núcleo, material ativo ou fase interna; podendo ser aromas, compostos ativos, óleos essenciais ou microrganismos. A matriz polimérica pode ser definida como material de parede ou agente carreador e podem ser biopolímeros ou polímeros sintéticos (VASISHT, 2014).

O principal objetivo do processo de microencapsulação é proteger e retardar reações indesejadas no material do núcleo, pois isolam parcialmente ou completamente o material encapsulado do meio externo (FERNANDES, 2013). Outras razões como redução da evaporação de compostos voláteis, redução da adsorção de água e melhoria na manipulação são relevantes para indústria alimentícia (RAY; RAYCHAUDHURI; CHAKRABORTY, 2015; SANTANA, 2013).

Outro objetivo que vem ganhando espaço nos estudos sobre a microencapsulação de alimentos é o controle da liberação do material do núcleo em uma matriz alimentícia. A liberação deste material pode promover um diferencial tecnológico aos alimentos, principalmente por promover estabilidade, aumento da vida útil e melhoria nos atributos sensoriais (PELISSARI, 2014).

Existem diferentes técnicas com potencial de aplicação para microencapsulação de alimentos, sendo que a seleção desta técnica é dependente da aplicação que será dada à micropartícula, tamanho desejado, mecanismo de liberação e propriedades físico-químicas, tanto do material do núcleo, quanto dos materiais de parede ou carreadores. De uma forma geral, a diferença está no tipo de envolvimento do material de recheio pelo agente encapsulante (ZUANON, 2012). Estas técnicas podem ser classificadas como físicas (*spray drying, freeze drying, spray chilling* e outros), químicas (polimerização interfacial e in situ) e físico-químicas (coacervação simples e complexa, evaporações de solvente e inclusão molecular) (SANTANA, 2013; TAVARES et al., 2014).

Para um processo de microencapsulação mais efetivo, a escolha do método de encapsulação depende de uma série de fatores tais como: tamanho de partículas requerido, propriedades físicas e químicas do núcleo e do material de parede, aplicação do produto final, mecanismos desejados de liberação, escala de produção e custo. Para aplicação na indústria de alimentos, os materiais incluem ácidos, bases, óleos, vitaminas, sais, gases, aminoácidos, óleos essenciais, corantes, enzimas e microrganismos (FAVARO-TRINDADE; PINHO, 2008).

De forma geral, o processo de microencapsulação é divido em etapas como o preparo de soluções/emulsões e a secagem. Estas etapas serão definidas a seguir.

2.3 Emulsões

Uma emulsão pode ser definida como uma mistura de dois líquidos imiscíveis, na qual gotas de um líquido estão distribuídas, fase dispersa, em outro, fase contínua. Emulsões estão presentes no cotidiano das pessoas, na forma de cosméticos, fármacos, produtos agrícolas e alimentos. Alguns exemplos de emulsões alimentícias são maionese, leite, margarina, manteiga, molho de salada, sorvete, dentre outros (ZUGE, 2012).

A propriedade física mais importante de emulsões, do ponto de vista tecnológico geral e para processos de microencapsulação, é a sua estabilidade. Pode-se definir estabilidade de uma emulsão como a sua capacidade em resistir a processos de desestabilização que podem carretar na cremeação, floculação ou até mesmo a separação de fases (MCCLEMENTS, 2005).

A estabilidade de emulsões é facilitada por agentes emulsionantes que atuam na solução, reduzindo a tensão superficial, diminuindo a energia na superfície entre as duas fases, permitindo a formação de novas superfícies

quando energia é cedida ao sistema (JUNQUEIRA, 2015).

Goma Arábica e Proteínas de Soro de Leite são bons emulsificantes, sendo amplamente utilizado em estudos de emulsões alimentícias com óleos essenciais e vegetais (KLEIN et al., 2010; KUHN; CUNHA, 2012; SILVA et al., 2016; TAVARES et al., 2014).

2.3.1 Homogeneização por ultrassom

A formação de emulsões requer a aplicação de energia, a fim de se reduzir o tamanho das gotículas de óleo, melhorando suas propriedades físico-químicas. A tecnologia ultrassônica está sendo muito utilizada para a formação de emulsões finas, com tamanho de gotículas na escala nano (MCCLEMENTS, 1995).

Ultrassom é definido como o som com frequências acima de 16 kHz. O mecanismo de formação dessas emulsões se deve à vibração da superfície da emulsão causada pela alta energia do ultrassom. A introdução de ondas sonoras em uma dispersão de água-óleo resulta em uma sucessão de depressões e compressões mecânicas, gerando bolhas de cavitação, tendendo a implodir e formar emulsões com gotículas de tamanho reduzido. O tempo e a potência das ondas do ultrassom são fatores que afetam o tamanho das gotículas formadas durante o processo de homogeneização (LEONG; MARTIN; ASHOKKUMAR, 2015; SILVA; ROSA; MEIRELES, 2015).

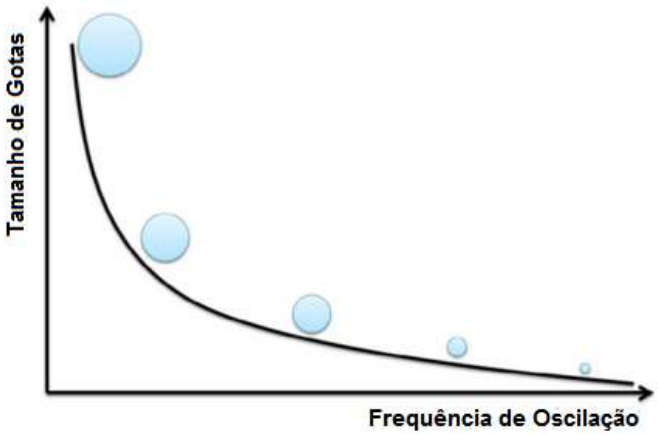

Figura 1 Representação Tamanho de Gotas x Frequência de oscilação em processos de homogeneização ultrassônica.
Fonte: Adaptado de Leong et al. (2015).

O processo de homogeneização por ultrassom apresenta várias vantagens como eficiência energética, baixo custo de produção, facilidade de manipulação e controle das variáveis de processo. Também é considerado um processo não tóxico para os alimentos e, ambientalmente seguro (SILVA; ROSA; MEIRELES, 2015).

As propriedades físicas de emulsões interferem diretamente nas propriedades de retenção em micropartículas de óleos essenciais e vegetais. Alguns estudos demostraram o potencial uso do ultrassom na homogeneização de emulsões alimentícias e sua correlação com as propriedades físico-químicas de alimentos em pó (CONSOLI et al., 2016; FERNANDES et al., 2016; SILVA; MEIRELES, 2015; SILVA; ZABOT; MEIRELES, 2015).

2.4 Secagem por atomização

A secagem por atomização (*spray drying*) é um dos processos mais utilizados em microencapsulação de alimentos (Figura 2). O processo de

secagem por atomização pode ser dividido em 3 etapas: atomização, secagem e separação. Na etapa de atomização, a solução ou emulsão é bombeada através de um dispositivo que irá reduzir este líquido a pequenas gotículas e ser injetada em uma câmara de secagem.

Na segunda etapa, o ar quente entra em contato com as gotículas, estabelecendo transferência de calor entre a fase líquida e gasosa, e na direção ar-produto como resultado da diferença das temperaturas, e a transferência de água é realizada em direção oposta produto-ar, devido à diferença das pressões de vapor. A taxa de secagem diminui rapidamente tornando-se dependente da taxa de difusão de água através da parede, e termina teoricamente quando a temperatura da partícula atinge o valor da temperatura do ar (CARMONA, 2011).

Na terceira etapa, o pó seco é submetido à separação através de um ciclone acoplado após a câmara de secagem (FILKOVÁ; HUANG; MUJUMDAR, 2014).

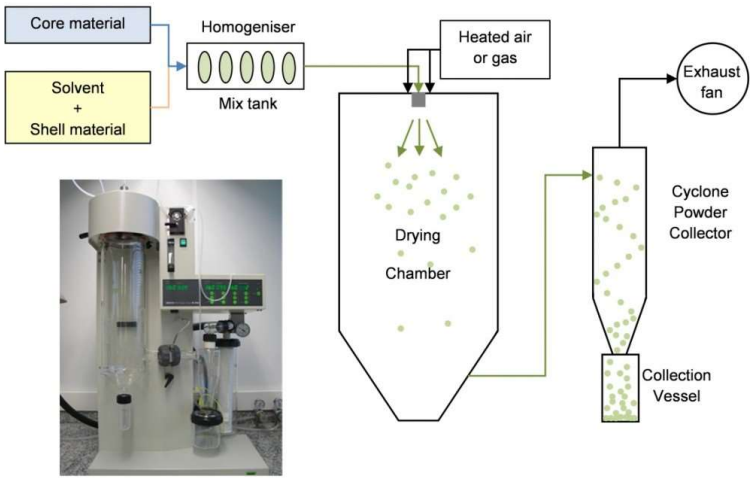

Figura 2 Desenho esquemático de um *spray dryer*.

O processo apresenta algumas vantagens, tais como a disponibilidade de equipamentos, baixo custo de processo, emprego de vários materiais encapsulantes sem necessidade de adaptações ao equipamento, boa retenção e estabilidade do material do núcleo. Mas este processo também apresenta algumas desvantagens, como produção de micropartículas não uniformes (forma e tamanho) e prejuízos ao material encapsulado já que o processo é realizado em altas temperaturas (SANTANA, 2013). A desvantagem é o gasto energético alto (sendo necessário estudo da planta para aproveitamento residual).

As primeiras informações sobre o surgimento do processo de secagem por informação datam de 1872, com o registro de patente de Samuel Percy, sobre uma técnica de atomização e desidratação simultânea de leite como o objetivo de aumentar a estabilidade do produto ao armazenamento (GAONKAR et al., 2014).

O *Spray Dryer* é o equipamento mais utilizado em processos de microencapsulação de óleos e tem diversos exemplos de estudo na literatura científica como a encapsulação de óleo de alecrim (FERNANDES et al., 2014a; FERNANDES; BORGES; BOTREL, 2013), peixe (BOTREL et al., 2014a), semente de urucum (SILVA et al., 2016), linhaça (TONON et al., 2012) e manjericão (GARCIA; TONON; HUBINGER, 2012).

2.5 Agentes encapsulantes

Uma infinidade de materiais pode ser utilizada para aprisionar, encapsular, ou revestir óleos vegetais e essenciais. Biopolímeros pode ser definido como qualquer polímero que seja produzido por um ser vivo, ou obtido a partir de fontes renováveis (PAULO, 2014). Esta classe é representada por diversas matérias como polissacarídeos, proteínas e lipídios (Tabela 3) (TELIS, 2012).

O tipo e a natureza do material encapsulante podem ser considerados um dos fatores mais importantes no processo de encapsulação, pois uma escolha errada pode acarretar numa degradação mais rápida do material do núcleo. Esta escolha deve levar em consideração uma série de fatores, como: propriedades físicas e químicas do núcleo e do material de parede, como porosidade, solubilidade, viscosidade, propriedades mecânicas, transição vítrea, capacidade de formação de filme, compatibilidade do núcleo com a parede, mecanismo de controle e fatores econômicos. O material de parede deve ser insolúvel e não reativo com o núcleo (BURNSIDE, 2014).

Para que um biopolímero possa ser adequado e utilizado em processos de microencapsulação de óleos, é necessário que ele apresente propriedades emulsificantes e estabilizante (TELIS, 2012).

Tabela 3 - Exemplos dos biopolímeros mais comuns utilizados em microencapsulação de alimentos.

Material	**Exemplos**
Polissacarídeos	Amidos e Celulose
Polissacarídeos modificados	Maltodextrinas, Ciclodextrinas e Amidos modificados
Gomas	Goma Arábica, Alginato, Goma Carragena e Pectina
Proteínas vegetais	Soja, trigo e milho
Proteínas animais	Isolado e Concentrado proteico de soro de leite, caseína e gelatina

Fonte: (GAONKAR et al., 2014).

2.5.1 Goma arábica

Goma Arábica (Figura 3), também conhecida como Goma Acácia, é uma das gomas mais antigas e mais conhecidas aplicadas em processos de alimentos. Do gênero *Acacia*, é muito encontrada em países tropicais e subtropicais sendo distribuídas mais em países do continente africado (Mauritânia, Senegal e outros), Índia, Arábia Saudita, Paquistão, Austrália e outros (TELIS, 2012).

A goma arábica é um exsudado formado por macromoléculas complexas de carboidratos e algumas proteínas. A composição química da goma pode variar dependendo da variedade da planta, do clima, estação, idade das árvores, precipitação, tempo de exsudação, e outros fatores. Em geral, a composição constitui de unidades de D-galactopiranosil ligadas entre si por ligações β (1→3). As cadeias laterais são compostas de duas a cinco unidades d-galactopiranosil, conectadas à cadeia principal, ligações 1-6. Tanto a cadeia principal quanto a lateral contêm unidades de α-L-arabinofuranosil, α-L-ramnopiranosil, β-D-glucopiranosil e 4-O-metil-β-d-glucuronopiranosil (WANDREY; BARTKOWIAK; HARDING, 2010).

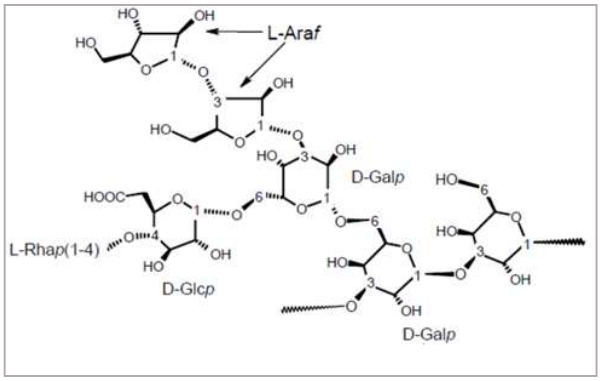

Figura 3 Estrutura da Goma Arábica.
Fonte: (FAVARO-TRINDADE; PINHO, 2008).

Algumas características físico-químicas da goma arábica são: inodoro, alta higroscopicidade, solubilidade em água e viscosidade. A goma arábica é considerada um bom material encapsulante, pois em concentrações de até 30 % em soluções, apresenta bons valores de viscosidade, não causando entupimento no bico atomizador, bem como formando gotículas de tamanhos ideais. Uma das características da goma arábica é que ela age muito bem em sinergismo com outros hidrocolóides (WANDREY; BARTKOWIAK; HARDING, 2010).

Em processos de microencapsulação de óleos, é o biopolímero mais estudado e com bons resultados de eficiência de encapsulação e retenção de óleo de café verde (SILVA; VIEIRA; HUBINGER, 2014), óleo essencial de alecrim (FERNANDES; BORGES; BOTREL, 2013, 2014), óleo de palma (FERREIRA et al., 2016) e oleoresina de cúrcuma (CANO-HIGUITA; VÉLEZ; TELIS, 2015).

2.5.2 **Isolado proteico de soro de leite:** proteínas lácteas

O soro de leite é um coproduto da indústria de laticínios composto por uma mistura de proteínas globulares, lactose, sais e gordura. O soro de leite compreende uma série de proteínas globulares encontradas em várias quantidades diferentes. β-lactoglobulina é a proteína mais abundante (50% de soro de leite bovino) e refere às propriedades de géis. α-lactalbumina é a segunda proteína mais abundante no soro de leite de bovino (12%). É o único componente capaz de realizar ligação com cálcio e ser transportado ao corpo humano. As imunoglobulinas (10%) são conhecidas por beneficiar o aumento da imunidade. Albumina de soro de bovino (5%) tem propriedades aglutinantes de gordura (TELIS, 2012).

As proteínas que compõem o soro de leite apresentam grande capacidade emulsificante e formação de géis, e devido a estas características,

vem sendo muito utilizadas pela indústria de alimentos como forma de agregar valor ao soro de leite, antes descartado e sem nenhum valor comercial. Muitos estudos já comprovaram a eficiência do uso das proteínas do soro de elite como material de parede na microencapsulação de óleos (QIU; ZHAO; MCCLEMENTS, 2015; RODEA-GONZÁLEZ et al., 2012).

O concentrado proteico de soro de leite é obtido do soro de leite, um subproduto da indústria láctea. O soro de leite representa a porção aquosa obtida no processo de produção do queijo, ou seja, é a porção restante após a retirada da coalhada. O soro de leite bruto é composto basicamente de 94 a 95% de água, 3,8 a 4,2% de lactose, 0,8 a 1,0% proteínas e 0,7 a 0,8% de minerais (NOELLO, 2016).

Em processos de microencapsulação de óleos, o isolado proteico de soro de leite vem se destacando. Óleo de peixe (BOTREL et al., 2014b), óleo de linhaça (PARTANEN et al., 2008), óleo de arroz (CHAROEN et al., 2011) e abacate (BAE; LEE, 2008) são exemplos de microencapsulação utilizando isolado proteico de soro de leite como material de parede.

2.5.3 Maltodextrinas

Maltodextrinas são amidos obtidos a partir da hidrolise parcial de amido de milho, constituído por grupos de oligossacarídeos de pelo menos três unidades de glicose. Podem ser obtidos através de hidrólise enzimática e/ou acidificação, com subsequente secagem para obtenção do maltodextrina na forma de pó.

As maltodextrinas são amplamente utilizadas na indústria de alimentos como agentes carreadores e encapsulantes, principalmente pelo baixo custo. Maltodextrinas também favorecem a redução da permeabilidade da parede das micropartículas, favorecendo a retenção de compostos bioativos (CARMONA,

2011).

Maltodextrinas não apresentam boas propriedades de retenção de compostos voláteis. Em processos de microencapsulação de óleos essenciais é sempre utilizada em combinação com outro composto emulsificante para melhorar a retenção dos compostos voláteis (FERNANDES et al., 2014b; MATIOLI; RODRIGUEZ-AMAYA, 2002).

Dextrose Equivalente (DE) é uma medida que está relacionada ao total de unidade de α-D-glicose e ao seu grau de polimerização, descrito como o número de unidade monométricas em uma molécula do polímero (DE = 100/DP). Amido não hidrolisado tem valor de DE = 0 e glicose apresenta valor de DE = 100. Para serem chamadas de maltodextrinas, o valor de DE deve ser menor que 20 (DA COSTA, 2013; TONON, 2009). Maltodextrinas de diferentes DE apresentam diferentes propriedades físico-químicas, alterando a estabilidade de micropartículas de alimentos pó, como observado em outros estudos de microencapsulação de alimentos (ABD GHANI et al., 2017; ALVES et al., 2014; MATSUURA et al., 2015; MULCAHY; MULVIHILL; O'MAHONY, 2016; TAKEITI; KIECKBUSCH; COLLARES-QUEIROZ, 2010)

REFERÊNCIAS

ABD GHANI, A. et al. Effect of different dextrose equivalents of maltodextrin on oxidation stability in encapsulated fish oil by spray drying. **Bioscience, Biotechnology, and Biochemistry**, v. 8451, p. 1-7, January, 2017.

ALVES, S. F. et al. Microencapsulation of Essential Oil from Fruits of Pterodon emarginatus Using Gum Arabic and Maltodextrin as Wall Materials: Composition and Stability. **Drying Technology**, v. 32, n. 1, p. 96–105, 2014.

ASBAHANI, A. EL et al. Essential oils: From extraction to encapsulation. **International Journal of Pharmaceutics**, v. 483, n. 1–2, p. 220–243, abr. 2015.

ASNAASHARI, S. et al. Essential Oil from Citrus aurantifolia prevents ketotifen-induced weight-gain in mice. **Phytotherapy Research**, v. 24, n. 12, p. 1893-1897, dez. 2010.

BAE, E. K.; LEE, S. J. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. **Journal of microencapsulation**, v. 25, n. 8, p. 549-560, 2008.

BIZZO, H. R. et al. Brazilian Essential Oils: General view, developments and perspectives. **Química Nova**, v. 32, n. 3, p. 588–594, 2009.

BOTREL, D. A. et al. Optimization of fish oil spray drying using a protein: inulin system. **Drying Technology**, v. 32, n. 3, p. 279–290, 2014a.

BOTREL, D. A. et al. Influence of wall matrix systems on the properties of spray-dried microparticles containing fish oil. **Food Research International**, v. 62, p. 344–352, ago. 2014b.

BRINGAS-LANTIGUA, M.; VALDÉS, D.; PINO, J. A. Influence of spray-dryer air temperatures on encapsulated lime essential oil. **International Journal of Food Science and Technology**, v. 47, n. 7, p. 1511–1517, 2012.

BURNSIDE, E. Hydrocolloids and Gum as encapsulating agents. In: GOANKAR, A. et al. (Eds.). . **Microencapsulation in the Food Industry**. First Edit ed. San Diego: Elsevier Inc., 2014. p. 241–252.

CANO-HIGUITA, D. M.; VÉLEZ, H. A. V.; TELIS, V. R. N. Microencapsulation of oleoresin in binary and ternary blends of gum Arabic , maltodextrin and modified starch. **Ciênc. Agrotec., Lavras**, v. 39, n. 2, p. 173–182, 2015.

CARMONA, P. A. O. **Secagem por atomização e microencapsulação de óleo de laranja:** estudo das propriedades da emulsão e do tipo de material de parede sobre as características do pó e a estabilidade do D-Limoneno. 2011. 154 f. Dissertação (Mestrado**)** - Universidade Estadual de Campinas, Campinas, SP, 2011.

CHAROEN, R. et al. Influence of Biopolymer Emulsifier Type on Formation and Stability of Rice Bran Oil-in-Water Emulsions: Whey Protein, Gum Arabic, and Modified Starch. **Journal of Food Science**, v. 76, n. 1, p. 165–172, 2011.

CHISHOLM, M. G.; WILSON, M. A.; GASKEY, G. M. Characterization of aroma volatiles in key lime essential oils (Citrus aurantifolia Swingle). **Flavour and Fragrance Journal**, v. 18, n. 2, p. 106–115, mar. 2003.

CONSOLI, L. et al. High solids emulsions produced by ultrasound as a function of energy density. **Ultrasonics Sonochemistry**, dez. 2016.

COTA-ARRIOLA, O. et al. Controlled release matrices and micro/nanoparticles of chitosan with antimicrobial potential: development of new strategies for microbial control in agriculture. **Journal of the Science of Food and Agriculture**, v. 93, n. 7, p. 1525–1536, maio 2013.

CRUZ-VALENZUELA, M. R. et al. Lime (Citrus aurantifolia) oils. In: PREEDY, V. R. (Ed.). **Essential oils in food preservation, flavor and safety.** First Edit ed. London: Elsevier Inc., 2016. p. 531–538.

DA COSTA, J. M. G. **Eficiência de diferentes encapsulantes e condições operacionais de Secagem por stomização na produção e aplicação de micropartículas de bioaroma de queijo suíço**. 2013. 153 f. Tese (Doutorado em Ciência dos Alimentos) - Universidade Federal de Lavras, Lavras, MG, 2013.

DIMA, C.; DIMA, S. Essential oils in foods: extraction, stabilization, and toxicity. **Current Opinion in Food Science**, v. 5, p. 29–35, 2015.

FAVARO-TRINDADE, C. S.; PINHO, S. C. DE. Revisão : Microencapsulação de ingredientes alimentícios. **Brazilian Journal of Food Technology**, v. 11, p. 103–112, 2008.

FERNANDES, R. V. B. **Microencapsulamento de óleo essencial de alecrim (Rosmarinus officinalis L.) através de secagem por atomização.** 2013. 134 f. Dissertação (Mestrado em Ciência dos Alimentos) - Universidade Federal de Lavras, Lavras, MG, 2013.

FERNANDES, R. V. B. et al. Physical and chemical properties of encapsulated rosemary essential oil by spray drying using whey protein-inulin blends as carriers. **International Journal of Food Science & Technology**, v. 49, n. 6, p. 1522–1529, jun. 2014a.

FERNANDES, R. V. B. et al. Effect of solids content and oil load on the microencapsulation process of rosemary essential oil. **Industrial Crops and Products**, v. 58, p. 173–181, jul. 2014b.

FERNANDES, R. V. B. et al. Study of ultrasound-assisted emulsions on microencapsulation of ginger essential oil by spray drying. **Industrial Crops and Products**, v. 94, p. 413–423, 2016.

FERNANDES, R. V. B.; BORGES, S. V.; BOTREL, D. A. Influence of spray drying operating conditions on microencapsulated rosemary essential oil properties. **Ciência e Tecnologia de Alimentos**, v. 33, n. 1, p. 171–178, fev. 2013.

FERNANDES, R. V. B.; BORGES, S. V.; BOTREL, D. A. Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. **Carbohydrate Polymers**, v. 101, n. 1, p. 524–532, jan. 2014.

FERREIRA, C. D. et al. Physicochemical Characterization and Oxidative Stability of Microencapsulated Crude Palm Oil by Spray Drying. **Food and Bioprocess Technology**, v. 9, n. 1, p. 124–136, 1 jan. 2016.

FILKOVÁ, I.; HUANG, L. X.; MUJUMDAR, A. S. Industrial Srpay Drying Systems. In: MUJUMDAR, A. S. (Ed.). **Handebook of Industrial Drying**. 4th Editio ed. Boca Raton: CRC Press, 2014. p. 191–226.

GAMARRA, F. M. C. et al. Influence on the quality of essential lemon (Citrus aurantifolia) oil by distillation process. **Brazilian Journal of Chemical Engineering**, v. 23, n. 1, p. 147–151, mar. 2006.

GAONKAR, A. et al. **Microencapsulation in the Food Industry:** a pratical implemetation guide. First ed. San Diego: Elsevier Inc., 2014.

GARCIA, L. C.; TONON, R. V.; HUBINGER, M. D. Effect of Homogenization Pressure and Oil Load on the Emulsion Properties and the Oil Retention of Microencapsulated Basil Essential Oil (Ocimum basilicum L.). **Drying Technology**, v. 30, n. 13, p. 1413–1421, out. 2012.

GIORDANI, R. et al. Potentiation of antifungal activity of amphotericin B by essential oil from Cinnamomum cassia. **Phytotherapy Research**, v. 20, n. 1, p. 58–61, 2006.

JAFARI, S. M. et al. Antimicrobial Activity of Lime Essential Oil Against Food-borne Pathogens Isolated from Cream-filled Cakes and Pastries. **International Journal of Biological Chemistry**, v. 5, n. 4, p. 258–265, 1 abr. 2011.

JUNQUEIRA , L. A. Ca**racterísticas físicas e microestruturais de emulsões formadas por hidrocoloides obtidos do ora-pro-nóbis (Pereskia aculeata Miller).** 2015. 130 f. Dissertação (Mestrado em Ciência dos Alimentos)- Universidade Federal de Lavras, Lavras, MG, 2015.

KLEIN, M. et al. Interactions between whey protein isolate and gum Arabic. **Colloids and Surfaces B: Biointerfaces**, v. 79, n. 2, p. 377–383, set. 2010.

KUHN, K. R.; CUNHA, R. L. Flaxseed oil - Whey protein isolate emulsions: Effect of high pressure homogenization. **Journal of Food Engineering**, v. 111, n. 2, p. 449–457, 2012.

LEONG, T. S. H.; MARTIN, G. J. O.; ASHOKKUMAR, M. Ultrasonic encapsulation - A review. **Ultrasonics Sonochemistry**, v. 35, p. 605–614, 2015.

LUZIA, D. M. M.; JORGE, N. Potencial antioxidante de extratos de sementes de limão (Citrus limon). **Ciência e Tecnologia de Alimentos**, v. 30, n. 2, p. 489–493, jun. 2010.

MATIOLI, G.; RODRIGUEZ-AMAYA, D. B. Licopeno Encapsulado em Goma Arábica e Maltodextrina: Estudo da Estabilidade. **Brazilian Journal of Food Technology**, v. 5, p. 197–203, 2002.

MATSUURA, T. et al. Effect of dextrose equivalent of maltodextrin on the stability of emulsified coconut-oil in spray-dried powder. **Journal of Food Engineering**, v. 163, p. 54–59, 2015.

MCCLEMENTS, D. J. Advances in the application of ultrasound in food analysis and processing. **Trends in Food Science & Technology**, v. 6, n. September, p. 293–299, 1995.

MCCLEMENTS, D. J. **Food Emulsion: Principle, Practices, and Techniques**. Second Edi ed. [s.l.] CRC Press, 2005.

MISHARINA, T. A.; POLSHKOV, A. N. Antioxidant properties of essential oils: Autoxidation of essential oils from laurel and fennel and of their mixtures with essential oil from coriander. **Applied Biochemistry and Microbiology**, v. 41, n. 6, p. 610–618, 2005.

MULCAHY, E. M.; MULVIHILL, D. M.; O'MAHONY, J. A. Physicochemical properties of whey protein conjugated with starch hydrolysis products of different dextrose equivalent values. **International Dairy Journal**, v. 53, p. 20–28, 2016.

NOELLO, C. **Obtenção de emulsões multicamadas de óleo de chia (Salvia Hispânica L) estabilizadas por concentrado proteico de soro de leite/pectina microencapsuladas em spray drying**. 2016. Dissertação (Mestrado) - Universidade Estadual de Campinas, 2016.

OLIVEIRA, D. R.; DINIZ, A. B. COMPOSIÇÃO QUÍMICA DA LARANJA KINKAN E DE FRUTAS CÍTRICAS. **DEMETRA: Alimentação, Nutrição & Saúde,** v. 10, n. 4, p. 835–844, 17 dez. 2015.

PARTANEN, R. et al. Effect of relative humidity on oxidation of flaxseed oil in spray dried whey protein emulsions. **Journal of Agricultural and Food Chemistry**, v. 56, n. 14, p. 5717–5722, 2008.

PAULO, H. D. Biopolímeros: uma alternativa favorável. **Temas Atuais em Biologia**, v. 2, n. 1, 2014.

PELISSARI, J. R. et al. Elaboração de micropartículas lipídicas sólidas carregadas de licopeno: estudo da estabilidade e da morfologia. In: SLACA - SIMPÓSIO LATINOAMERICANO DE CIÊNCIA DE ALIMENTOS, 10., **Anais...** Campinas, 2013.

PREEDY, V. R. **Essential oils in food preservation, flavor and safety**. First Edit ed. London: Elsevier Inc., 2016.

QIU, C.; ZHAO, M.; MCCLEMENTS, D. J. Improving the stability of wheat protein-stabilized emulsions: Effect of pectin and xanthan gum addition. **Food Hydrocolloids**, v. 43, p. 377–387, 2015.

RAO, J.; MCCLEMENTS, D. J. Impact of lemon oil composition on formation and stability of model food and beverage emulsions. **Food Chemistry**, v. 134, n. 2, p. 749–757, 2012.

RAY, S.; RAYCHAUDHURI, U.; CHAKRABORTY, R. An overview of encapsulation of active compounds used in food products by drying technology. **Food Bioscience**, v. 13, p. 76–83, 2015.

RODEA-GONZÁLEZ, D. A. et al. Spray-dried encapsulation of chia essential oil (Salvia hispanica L.) in whey protein concentrate-polysaccharide matrices. **Journal of Food Engineering**, v. 111, n. 1, p. 102–109, jul. 2012.

SANTANA, A. A. **Obtenção da polpa de pequi e do leite de coco de babaçu microencapsulados através da secagem por asperção**. 2013. Tese (Doutorado) - Universidade Estadual de Campinas, Campinas, SP, 2013.

SANTOS, J. C. et al. Atividade antimicrobiana in vitro dos óleos essenciais de orégano, alho, cravo e limão sobre bactérias patogênicas isoladas de vôngole. **Semina: Ciências Agrárias**, v. 32, n. 4, p. 1537–1564, 19 out. 2011.

SILVA, E. K. et al. Biopolymer-prebiotic carbohydrate blends and their effects on the retention of bioactive compounds and maintenance of antioxidant activity. **Carbohydrate Polymers**, v. 144, p. 149–158, 2016.

SILVA, E. K.; MEIRELES, M. A. A. Influence of the degree of inulin polymerization on the ultrasound-assisted encapsulation of annatto seed oil. **Carbohydrate Polymers**, v. 133, p. 578–586, nov. 2015.

SILVA, E. K.; ROSA, M. T. M. G.; MEIRELES, M. A. A. Ultrasound-assisted formation of emulsions stabilized by biopolymers. **Current Opinion in Food Science**, v. 5, p. 50–59, 2015.

SILVA, E. K.; ZABOT, G. L.; MEIRELES, M. A. A. Ultrasound-assisted encapsulation of annatto seed oil: Retention and release of a bioactive compound with functional activities. **Food Research International**, v. 78, n. 2015, p. 159–168, 2015.

SILVA, V. M.; VIEIRA, G. S.; HUBINGER, M. D. Influence of different combinations of wall materials and homogenisation pressure on the microencapsulation of green coffee oil by spray drying. **Food Research International**, v. 61, p. 132–143, jul. 2014.

SIMIC, A. et al. The chemical composition of some Lauraceae essential oils and their antifungal activities. **Phytotherapy Research**, v. 18, n. 9, p. 713–717, set. 2004.

TAKEITI, C. Y.; KIECKBUSCH, T. G.; COLLARES-QUEIROZ, F. P. Morphological and Physicochemical Characterization of Commercial Maltodextrins with Different Degrees of Dextrose-Equivalent. **International Journal of Food Properties**, v. 13, n. 2, p. 411–425, 24 fev. 2010.

TAVARES, G. M. et al. Milk proteins as encapsulation devices and delivery vehicles: Applications and trends. **Trends in Food Science & Technology**, v. 37, n. 1, p. 5–20, maio 2014.

TELIS, V. R. N. **Biopolymer engineering in food processing**. First Edit ed. Boca Raton: CRC Press, 2012.

TONON, R. V. **Secagem por atomização do suco de açaí:** Influência das variáveis de processo , qualidade e estabilidade do produto. Tese (Doutorado) Campinas, Universidade Estadual de Campinas, SP, 2009.

TONON, R. V. et al. Microencapsulation of flaxseed oil by spray drying: effect of oil load and type of wall material. **Drying Technology**, v. 30, n. 13, p. 1491–1501, out. 2012.

VASISHT, N. Selection of Materials for Microencapsulation. In: GAONKAR, A. G. et al. (Eds.). **Microencapsulation in the Food Industry**. First ed. San Diego: Elsevier, 2014. p. 173–180.

WANDREY, C.; BARTKOWIAK, A.; HARDING, S. E. Materials for Encapsulation. In: **Encapsulation Technologies for Active Food Ingredients and Food Processing**. New York, NY: Springer New York, 2010. p. 31–100.

ZUANON, L. A. C. **Microencapsulação de oleoresina de cúrcuma (curcuma longa l.) em matrizes de gelatina, goma arábica e colágeno hidrolisado.** 2012. 100 f. Dissertação (Mestrado) - Universidade Estadual Paulista, Instituto de Biociências, Letras e Ciências Exatas, São Paulo, 2012.

ZUGE, L. C. B. **Estudo da inversão catastrófica e transicional de emulsões de óleo de soja com diferentes tensoativos**. Curitiba, Brasil: Universidade Federal do Paraná, 2012.

SEGUNDA PARTE - ARTIGOS

ARTIGO 1: STABILITY OF LIME ESSENTIAL OIL EMULSION PREPARED USING BIOPOLYMERS AND ULTRASOUND TREATMENT

Pedro Henrique CAMPELO[1]; Luciana Affonso JUNQUEIRA[1]; Jaime Vilela de RESENDE[1]; Rosana Domingues ZACARIAS[2], Regiane Victória de Barros FERNANDES[1]; Diego Alvarenga BOTREL[1]; Soraia Vilela BORGES[1]

[1] Departament of Food Science, Federal University of Lavras (Campus Universitário, CEP 37200-000, Lavras, Minas Gerais, Brazil).

[2] Departamento of Chemistry, Federal University of Minas Gerais (Av. Antônio Carlos 6627, Pampulha, Belo Horizonte – Minas Gerais. CEP 31270-901, Brazil)

Artigo publicado conforme normas do periódico International Journal of Food Properties.

STABILITY OF LIME ESSENTIAL OIL EMULSION PREPARED USING BIOPOLYMERS AND ULTRASOUND TREATMENT

[1] Departament of Food Science, Federal University of Lavras (Campus Universitário, CEP 37200-000, Lavras, Minas Gerais, Brazil).

[2] Departamento of Chemistry, Federal University of Minas Gerais (Av. Antônio Carlos 6627, Pampulha, Belo Horizonte – Minas Gerais. CEP 31270-901, Brazil)

ABSTRACT

The objective of this study was to investigate the kinetic stability of lime essential oil emulsion using gum arabic (GA) and whey protein isolate (WPI) biopolymers as emulsifiers and stabilizers. Different solid compositions were evaluated: GA, GA/WPI (1:1) and WPI, as well as oil concentration (5.0, 7.5 and 10% w/w). Emulsions were prepared using an ultrasonic homogenizer. Viscosity, oil droplet size, zeta potential, pH and conductivity were measured to predict the emulsion stabilityover a period of 4h (estimated time until complete drying in microencapsulation processes). Emulsion rheological behavior and microstructure were characterized. All emulsions were stable during the time period analyzed and no destabilization characteristic was observed. The pH, conductivity and viscosity showed no significant difference ($p > 0.05$) over the 4h period. Emulsions with WPI showed higher zeta potential values, confirming the biopolymer electrosteric stabilization. The combination of wall materials (GA/WPI) and the oil concentration increased the average droplet size. The results of the apparent viscosity at shear rate of 100 s^{-1} confirmed the emulsions stability. These values did not change significantly considering the fresh

emulsions and emulsions after 4 h of preparation. The emulsifying biopolymers showed to be excellent natural stabilizers for lime essential oil emulsions, highlighting the results using WPI.

Keywords: Emulsion kinetic stability; whey protein isolate; gum arabic; zeta potential; emulsion conductivity.

INTRODUCTION

Lime essential oil is widely used by the food, pharmaceutical and cosmetic industries due to its chemical and sensory characteristics (1). It is commonly used in products such as beverages, baked goods, sweets, desserts and ice cream (2). Lime essential oil is a complex mixture of chemical compounds divided into three classes: terpenes (75%), oxygenated complexes (12%) and sesquiterpenes (3%). It is composed of substances such as limonene, γ-terpene, citral, β-caryophyllene among others (2–4). This essential oil is extremely volatile and your encapsulation is an alternative to provide slow release of their bioactive compounds. The process of encapsulation begins with the preparation of stable emulsions using biopolymers and subsequent drying using spray-drier.

Emulsions can be defined as a mixture of two immiscible liquids, usually water and oil. Several products from food, pharmaceutical, chemical and other industries are characterized as emulsions (5). In foods, the emulsion behavior can be defined in three parts: 1) the oil or fat within the emulsion droplet; 2) the interfacial material between lipid and aqueous phase; and 3) the aqueous phase itself. Food systems are extremely complex and may have different behaviors in different situations. Different compositions of the interfacial material, for example, may result in very different physical and chemical characteristics. Therefore, it is necessary to understand the characteristics of each system individually and collectively (6,7). Stable emulsions are able to resist changes in their properties over a period of time. Physical and chemical processes can cause an emulsion to become unstable, causing destabilization processes such as agglomeration, creaming, coalescence, phase inversion, and others (physical instability); or oxidation and hydrolysis (chemical instability) (7–11). However, the basic mechanisms of emulsion stabilization are not accurately understood,

particularly due to biopolymer structure complexity (12,13)

Biopolymers are widely used as emulsifying and stabilizing agents, for having good gelling and emulsifying capacity (14–17). Stable emulsion production, using biopolymers such as gum arabic (GA) and whey protein isolate (WPI), is desirable and widely used in microencapsulation processes of food components (18–24).GA is a mixture of anionic carbohydrates and some proteins, while WPI is a mixture of globular proteins. GA has good emulsifying properties due to the presence of highly branched proteins that are closely linked to the polysaccharide structure (24–27). Globular proteins are quickly adsorbed onto the oil droplets surface during homogenization, facilitating the formation of small droplets (28,29).

The homogenization process is directly related to the emulsion physical properties, and is an important step in the study of microencapsulation processes (30). The process can be divided into two stages: the first in which the high shear stress leads to the drop deformation, increasing the surface area; and the second, the system stabilization by some emulsifying agent (31). In the literature, the most commonly used processes are mechanical stirring, ultrasonication and high pressure homogenization. Homogenization by ultrasound has been highlighted in food emulsion research (15,32–35) and results showed to be an efficient method for obtaining emulsions with droplet size in micrometers or nanometers. The ultrasound homogenization mechanism is based on cavitation, in which high intensity ultrasound waves impact the liquid surface, forming high velocity jets and are responsible for the droplet formation (30,36,37).

Emulsion plays an important role on efficiency and stability of the oil micro particles produced by encapsulation processes, specially spray drying. Some of

the main properties to be evaluated are stability, viscosity, droplet size and solid concentration (38). Several works (39-43) on emulsions cite and study physical and chemical parameters that predict stability. Droplet size, zeta potential, surface tension, rheology, pH, conductivity, creaming rate and microstructure are examples of the main parameters used. Ensuring emulsion stability is extremely important for processes in the food and pharmaceutical industries, such as oil microencapsulation. From emulsion homogenization to complete drying, it is necessary that all its physical and chemical properties are maintained, so the micro particles quality is not compromised. Many research works on microencapsulation give special attention to the study of the emulsions stability as an interfering in powdered products final quality (34,39–43). This work aimed to study the different physical and chemical parameters of lime essential oil emulsions using GA and WPI biopolymers, evaluating the effect of lime oil concentration and wall material composition to predict the ideal conditions for water in oil emulsion stability. All parameters were evaluated in a 4-hour interval, as this time is sufficient for emulsion use in microencapsulation processes. The emulsions, in these cases, should remain stable between the emulsion preparation and drying steps.

MATERIALS AND METHODS

Lime essential oil was used as dispersed phase (Ferquima, Vargem Grande Paulista, São Paulo). Gum Arabic (Alland & Robert, France) and Whey Protein Isolate (Alibra Ingredients Ltda, Campinas, Brazil) were used as emulsifying and stabilizing agents.

Experimental Design

The lime essential oil emulsions were obtained using gum arabic (GA) and whey protein isolate (WPI) and different concentrations of lime essential oil,in relation to the total solids as shown in Table 1. This work was carried out based on a complete factorial experimental design, where were evaluated two variable: emulsifier and lime essential oil concentration, both in three levels (WPI, GA/WPI and GA) and (5.0, 7.5 and 10.0 g oil/100 g emulsion), respectively. Solids concentration (Emulsifier + oil) was 30.0 g/100 g emulsion.

Emulsion preparation

The different formulations studied (Table 1) were added to water for 24 h to complete biopolymer hydration and saturation. The lime essential oil was added to the biopolymer solution and homogenization was carried out under ultrasound (Digital Sonifier 450, Branson Ultrasonic Corporation, USA) for 2 min using nominal power of 160 W. After homogenization, emulsions were placed in an ice bath until they reach room temperature (The temperature after homogenization was 46 ± 0.3 °C).

pH measurement

Emulsion pH measurement was carried out in triplicate using a PHS-3E pH meter (pHTek, Curitiba, Brazil). The equipment was calibrated with buffer solutions and pH values were measured by inserting the electrode directly into the sample at 25 °C (44,45).

Electrical Conductivity

The emulsion electrical conductivity measurement was determined in triplicate, using a benchtop conductivity meter (Hanna, model HI 8731, Portugal). The conductivity values were measured by inserting the electrode directly into the

sample at 25 °C (46).

Zeta Potential

The surface charge density (Zeta Potential) of the biopolymers was determined by Electrophoretic Light Scattering using ZetaSizer Nano-ZS (Malvern Instruments Ltd.,Worcestershire, UK). The emulsions were diluted in Milli-Q water (Millipore, Bedford, USA) to 2.0% (v/v), according to the equipment optimal detection range. The measurements were performed in duplicate (each measure with 10 runs or more) at 25°C (12).

Droplet size measurement

The Z-Average diameter was determined by dynamic light scattering (DLS) technology using Zetasizer Nano ZS (Malvern Instruments, UK). The emulsions were diluted in Milli-Q water (Millipore, Bedford, USA) to 2.0% (v/v), according to the equipment optimal detection range. The measurements were performed in duplicate (each measure with 5 runs or more) at 25 °C (44,47). The polydispersity index (SPAN) was determined according to Equation (1).

$$SPAN = \frac{d(90) - d(10)}{d(50)} \qquad \text{Eq. (2)}$$

where d(10), d(50) and d(90) are the diameters at 10%, 50% and 90% cumulative volume, respectively.

Analysis of rheological behavior

Emulsion rheological behavior and viscosity were evaluated using an oscillatory

rheometer HAAKE RheoStress 6000 (Thermo Fisher Scientific) coupled to a temperature controller HAAKE UTM Controller (Thermo Fisher Scientific). The geometry used was plate-plate with a 34.997mm diameter and a 1mm GAP. The emulsions were evaluated immediately after preparation and after 4 h. Analyses were performed in triplicate at 25 °C. The rheological behavior was analyzed by the deformation rate (0-300 s^{-1}) increase, decrease and increase. After removal of time dependence (thixotropic), the third curve was obtained. The third curve data were adjusted to rheological model Power Law (Equation 3). The model adequacy was evaluated by the coefficient of determination (R^2), the residual mean square and the significance of the parameters ($p < 0.05$). Apparent viscosity of emulsions was studied at shear rate of 100 s^{-1}. The parameter was evaluated at this shear rate since it is the typical rate for food processes, such as the flow through tubes in industry and stirring and mastication processes (7).

$$\delta = \eta \times \gamma \quad (3)$$

$$\delta = k(\gamma)^n \quad (4)$$

$$\delta = \delta_0 + k(\gamma)^n \quad (5)$$

Where, δ = shear stress (Pa); = shear rate (s^{-1}); k = consistency index ($Pa.s^n$); n = flow behavior index.

Optical microscopy

Optical microscopy was performed to assess the emulsion destabilization

processes on a microstructural level. The analysis was performed after the emulsion preparation and after 4 h of storage, in an Axio Scope A1 optic microscope, Carl Zeiss (Germany), with a video camera attached. For this, an aliquot of emulsion was placed on the slide, covered with a cover slip and examined with a 100x objective lens (48,49).

Creaming index

The creaming index (CI) was determined by adding 5 mL of emulsion in plastic pots, immediately after preparation. After 4 h, the volume of the formed cream was measured and creaming percentage determined according to the Equation (5):

$$CI\ (\%) = 100 \times \left(\frac{H_C}{H_T}\right) \qquad \text{(Eq. 5)}$$

Where, H_C is the cream phase (upper phase volume) and H_T is the initial emulsion height.

Statistical Analysis

All treatments were manufactured in three repetitions. Analysis of variance (ANOVA) and rheological data adjustment to rheological models were performed using SAS software (Statistical Analysis System), version 9.1, licensed by the institution. Significant differences between the samples ($p < 0.05$) were assessed by the Scott-Knott mean comparison test.

RESULTS AND DISCUSSION

pH and electrical conductivity

The pH is a very significant parameter because a significant change in its value may suggest chemical changes of the components present in the formulation.

Thus, it can be inferred that the emulsions formed are stable systems and their components did not alter significantly over the analyzed time period. The pH and conductivity of lime essential oil emulsions are shown in Figure 1 (a) and (b), respectively. Food emulsions generally have pH in the range of 2.5 to 7.5 (7). The variation in the oil amount did not influence the emulsion pH values, except for emulsions in which GA and WPI were used as emulsifier, which presented a slight increase in its value when oil concentration was increased from 5.0 to 7.5 g oil/100 g emulsion. The pH values showed no significant difference ($p > 0.05$) after 4 h. Silva et al. (12) also found higher pH values for emulsions in which WPI was used as an emulsion stabilizer and emulsifying agent compared to the values of emulsions in which GA was used. The values found by the cited author are close to those found in this present work, around 5.0 for emulsions with GA and 6.2 for WPI emulsions, showing that pH is not influenced by the oil but by the polysaccharides used.

Electrical conductivity analysis showed reduced values with increasing oil concentrationin the emulsion, when GA was used as emulsifier. In the emulsions in which GA/WPI and WPI were used, the conductivity value decreased only at the highest oil concentration. This behavior can be explained by the poor conductive characteristic of the oil (55,56). In addition, emulsions with lower electrical conductivity values were those where the GA/WPI combination was used. Higher for GA conductivity values were observed for emulsions with WPI. It can be seen that the emulsions with pure GA have higher conductivity values compared to the other treatments. Globular proteins exhibit large structure and higher capacity links with water molecules in its chain and free water increases the emulsion conductivities (57). Klein et al. (58) studied the interaction between GA and WPI and observed that the conductivity values decrease when the two biopolymers are mixed. This observation can be explained by the cancellation of the biopolymer charges, reducing the solution conductivity. The

increase in viscosity of solutions can cause a reduction in conductivity. Also, the process of cavitation and ultrasonic homogenization can cause ion aggregations, reducing the mobile ions of these systems and consequently the electrical conductivity (59). Conductivity values also did not present statistically significant alterations ($p > 0.05$) after 4 h. Variations in conductivity values can occur when there are creaming, sedimentation or phase inversion processes and sensitive alterations in the molecular structure of the formulation components. Conductivity is a parameter frequently used to determine emulsion stability during the storage period (60).

Zeta Potentia

According to Jayme et al. (24) and Sari et al. (61), there are two types of emulsion stabilization processes: steric stability, when the emulsifier molecule adheres to the oil droplet surface, maintaining it stable; and electrostatic stability, when there is repulsion among the droplets due to the high surface charge, hindering the droplet agglomeration. Moreover, electrosteric stability can also occur, in which both types of stabilization processes are observed. The Zeta potential is the most commonly used parameter to determine the electrostatic contribution to emulsion stability (5,11,12). Surface charges control possible system droplet-droplet interactions and contribute to the emulsion stability (62).

Table 2 presents the results of Zeta Potential for the solutions (water + emulsifier) and the lime essential oil emulsion at 0 and 4 h. A significant difference in zeta potential values was observed after the homogenization process compared with the solution values, which were reduced, except for emulsions in which GA/WPI was used with oil concentrations of 5.0 and 7.5 g oil/100 g emulsion. O´Brien (37) explains that the biopolymer chains may be

broken due to the cavitations' process. This chain breakage favors the increase in biopolymersurface charge. The decrease in zeta potential values in the emulsions in which GA and WPI was used can be explained by the reduction of biopolymer charges.

Emulsions E1, E3, E5, E6 and E9, showed no significant change ($p > 0.05$) in the zeta potential values after 4 h. This behavior is the opposite to what occurred in E2, E4, E7 and E8 emulsions, which became less stable according to this parameter. The reduction in the zeta potential value, after a certain time, is especially common in emulsions, due to low oil polarity. Herculano et al. (63) and Silva et al. (39) report that emulsions are considered stable when the zeta potential values exceed ±30 mV. Zeta potential values from 0 to ±30 mV promote droplet attraction, leading to less stable emulsions (39,49).

Therefore, the most electrostatically stable emulsions, according to this parameter, are emulsions in which WPI was used as a biopolymer, since they presented zeta potential above ±30 mV and there were no statistically significant changes after 4 h of storage. Proteins are known to be easily absorbed in the water-oil interface, promoting greater emulsion kinetic stability, especially by electrostatic repulsion, due to the high surface charge concentration. Protein also hinders the water adsorption in oil due to its low wet ability and long molecular chain (22,28,54,64).

Gum arabic has less surface charge compared to whey protein isolate. Polysaccharides have the capacity to retain water causing system thickening, so even with the low charge concentration of the GA emulsion, they were stable, as confirmed by pH and conductivity data. It can be said that the gum arabic emulsifying properties are related to a steric mechanism (7,65). In fact, the emulsions in which WPI was used showed the highest zeta potential values after preparation and this value remained statistically the same after 4 h.

In emulsions with annatto oil, Silva et al. (12) observed higher Zeta Potential values for WPI compared to samples with GA, as in this work. Xu et al. (66) also observed zeta potential values lower than -30 mV in model WPI and sunflower oilemulsions. In orange oil emulsions with gum arabic at different concentrations, Mirhosseini et al. (67) observed zeta potential values varying between -22.6 and -28.7 mV. The addition of GA reduces the charges around the emulsion droplets, becoming less electrostatically stable than emulsions with WPI. Sukhotu et al. (68) observed the same behavior in peanut oil emulsions.

Polidispersion index (PDI), oil droplet size and emulsions optical microscop

PDI and oil droplet size values for the emulsions are shown in Table 3. The PDI values are related to the distribution homogeneity of oil droplet size and the closer to zero, the more uniform is the distribution (46, 66). For all biopolymers used, there is an increase in the PDI with the increase in the oil concentration used. With the increase in the oil concentration, the droplets were not perfectly mono-disperse and the PDI values or deviation from the average size increased (Figure 2). Increased PDI values may be associated with destabilization phenomena, such as emulsion oil droplet coalescence. However, the phenomenon was not enough to promote creaming or phase separation.

The oil droplet size is an impact factor for predicting food emulsion stability (7). The average droplet size (Table 3) is in the range of 0.282 to 1.306 μm for fresh emulsions; and of 0.256 to 1.968 μm for emulsions after 4 h of storage. The biopolymer influenced significantly ($p < 10^{-10}$) the average oil droplet size and WPI emulsions showed smallest average droplet size in both time intervals (0 and 4 h). When comparing the influence of oil concentration on the average droplet size, only the GA/WPI blend presented significant difference ($p < 10^{-8}$) compared with the other biopolymers. In the study of WPI emulsions by Kim and Morr (68) and Bae and Lee (48), they also observed an increase in the oil

droplet size by increasing the amount of oil in the emulsions. Taneja et al. (22) observed a reduction of average soybean oil droplet size when the WPI concentration was increased in the emulsion. Dluzewska et al. (23) observed an increase in droplet size with increasing oil concentration in gum arabic emulsions. The same author also did not observe significant increase in droplet size during storage.

When comparing the droplet size over time (0 to 4 h), we observed that there was a significant increase ($p < 0.05$) in the dropletsize in the GA/WPI blend. When studying the stability of essential oil and WPI emulsions, Dluzewska et al. (23) found no significant increase in droplet size over time. Microscopic images (Figure 3) show the increase in droplet size with increasing oil amount for the GA/WPI treatment and also confirm the increase in droplet size after 4 h. There is a tendency for the oil droplet size to increase with increased amount of oil in the emulsions, since the amount of oil is higher for a smaller amount of emulsifier available to bind to the oil surface (40,48,70). Garcia et al. (52) observed a tendency of increasing average droplet size with increasing oil concentration in emulsions of Bassil essential oil with gum arabic.

Rheology

The Power Law rheological model was that best fit to all the experimental data due to the mean square low value (E(%) < 4.49). In addition, this model presented significant ($p < 0.05$) parameters (k and n) for all treatments. This model has been extensively used to describe emulsion rheological behavior (12,17,65,71–73). Table 5 shows the Power Law model parameters. Except for the E8 and E9 emulsions, all emulsions presented a flow behavior index (n) value very close to 1, which shows a tendency of such systems toward a Newtonian behavior, in which the relation between shear stress and shear rate

is linear and viscosity is constant, which can be evidenced by the rheograms shown in Figure 4. E8 and E9 emulsions have a higher oil concentration and were produced using WPI as emulsifier (GA/WPI and WPI, respectively). The *n* values found in these two cases were lower, showing that in these emulsions the rheological behavior deviates from the Newtonian behavior and shear-thinning behavior being more evident. This behavior is shown in Figure 4 in which there is reduction in the apparent viscosity values with increasing shear rate.

The consistency index increased with the increasing oil concentrations within each emulsifier, presenting the highest value in the emulsions in which GA/WPI was used. The consistency index is a parameter that also informs about fluid viscosity. Therefore, according to İbanoglu (74) the consistency index increases with the solids content and with the increase in oil concentration. The parameters behavior remained the same after 4 h, which is the time necessary for microencapsulation

Figure 4 shows the relationship between the apparent viscosity and shear rate. It can be noted in the Figure 4 that, except for E8 and E9 emulsions, all emulsions exhibit nearly constant viscosity values with the increase in shear rate that is behavior of Newtonian fluids. In the E8 and E9 emulsions there is reduction in apparent viscosity that is typical behavior of pseudoplastic fluids. İbanoglu (74), when studying the rheological behavior of emulsions with milk protein isolate and different gum arabic concentrations, also found the pseudoplastic behavior present in these emulsions. Moschakis et al. (51)verifies that emulsions stabilized with low WPI concentrations, present a behavior very close to Newtonian, while in the presence of high polysaccharide concentrations, such as gum arabic, he found that these systems exhibit a pseudoplastic behavior. According to the author, this tendency toward pseudoplastic behavior,

as occurred in the E8 and E9 emulsions, is due to weak associative interactions and suggests the formation of a weak network around the droplets. Silva et al. (65) studied emulsions stabilized by different polysaccharides for green coffee oil microencapsulation. The author also found that the behavior of these systems can vary between Newtonian and pseudoplastic. Jafari et al. (17) found that at concentrations above 4%, emulsions which had Newtonian behavior start exhibiting a pseudoplastic behavior.

The apparent viscosity (Table 5) of the emulsions at shear rate of 100 s^{-1} did not change significantly ($p < 0.05$) between the fresh emulsions and emulsions after 4 h of preparation, showing kinetic stability of these systems during the studied period. According to Silva et al. (12), rheological changes which may occur in the emulsions after preparation period are mainly associated to destabilization processes, since in these processes the oil is released into the continuous phase, altering the system rheological properties. Since oils have higher viscosity than water, the properties change. Viscosity values ranged from 0.791 to 0.7987 Pa s, for the lowest and highest emulsifier agent concentrations, respectively.

Creaming index

Creaming can be defined as a destabilization process due to the upward movement of the oil droplets since its density is lower than the continuous phase (water). Generally, this process occurs due to gravitational or centrifugal forces, which cause droplets to agglomerate, increase in size and accumulate at the top of the system (7,50). In this study during the 4 h of storage, none of the samples exhibited a visible process of destabilization (creaming, phase separation or agglomeration). Even with the increase in oil concentration (Figure 5), there was no emulsion creaming process in the 4 h interval. In the microencapsulation process, the emulsion must remain stable throughout the process, so that there is

no interference in the powder properties, particularly as to the encapsulation efficiency reduction.

Moschakis et al. (51) observed that sunflower oil emulsions with GA and WPI are stable and did not exhibit a creaming process over a week. Garcia et al. (52) studied Bassil essential oil emulsions with GA and found no system creaming process in 24 h. Tonon et al. (53) observed that, even at high oil concentrations (30%), there was no oil phase separation in emulsions with flaxseed oil. In studies with flaxseed oil emulsions with WPI, Kuhn and Cunha (54) did not observed creaming in 9 days of storage.

In order to have emulsion stability, there can be no creaming, flocculation or coalescence (26). The creaming index is a parameter which provides indirect information about the emulsion droplet agglomeration, and the larger the drops, the faster the creaming process (12,50). Thus, it can be inferred that the emulsions of all lime oil concentrations and different biopolymers have small oil droplets which did not undergo agglomeration, which can lead to the creaming process, in the 4-hour period. That is, over the 4-hour period, the emulsion was visibly stable.

CONCLUSION

In this work, the evaluation of physicochemical properties indicated that these are related with the charges that were originated after application of ultrasound treatment. The treatments with WPI proved to have more surface charges than GA. These charges resulted in increases in pH values, increases in the zeta potential values, decreases the PDI values, Newtonian behavior changes (n) and increases of the consistency index (k) values. The ultrasonic homogenization process favors breaking of the biopolymer chain and the increase in surface loads. These charges are concentrated around the droplets

forming stable emulsions. The presence of oil and second biopolymer (GA) influences the electrostatic stability and the effects on the physicochemical parameters are mitigated, as noted. Considering the emulsion stability between the time after preparation and feeding in the spay-dryer to produce microcapsules, the use of biopolymers with good emulsifying capacity was confirmed, highlighting the importance of this feature in essential oil encapsulation. The results show that for all biopolymer formulations, no phase separation during the first 4 hours of storage was observed. In all treatments there was no significant change in pH and conductivity. Higher zeta potential values were found for WPI and higher oil concentration treatments due to the characteristics and functional groups present in the biopolymer and lime essential oil. The viscosity values do not change with time, demonstrating kinetic stability. All oil droplets were less than 2μm in size. GA e WPI showed high emulsifying and stabilizing capacity in emulsions prepared with lime essential oil and can be used as wall material for microencapsulation systems. The results were satisfactory for all essential oil and biopolymer combinations, being more relevant in emulsions in which WPI was used as biopolymer

ACKNOWLEDGEMENTS

The authors thank FAPEMIG (Minas Gerais State Foundation for Research Development, Brazil) (CAG-PPM-00318-11) and CNPq (National Council for Scientific and Technological Development, Brazil) (Grant number: 448530/2014-7) for the financial support

REFERENCES

1. Rao, J., McClements, D.J. Impact of lemon oil composition on formation and stability of model food and beverage emulsions. Food Chemistry, 2012, 134, 749– PubMed ;572.

2. Gamarra, F.M.C., Sakanaka, L.S., Tambourgi, E.B., Cabral, F.A. Influence on the quality of essential lemon (Citrus aurantifolia) oil by distillation process. Brazilian Journal of Chemical Engineering, 23,147–51.

3. Santos, J,C, dos. Avaliação do efeito antimicrobiano de óleos essenciais sobre micro-organismos patogênicos em vôngole (Anomalocardia brasiliana). Universidade Federal da Bahia; 2010.

4. Cruz-Valenzuela, M.R., Tapia-Rodriguez, M.R., Vazquez-Armenta, F.J,, Silva-Espinoza, B.A., Ayala-Zavala JF. Lime (Citrusaurantifolia) oils. In: Preedy V.R. Essential oils in food preservation, flavor and safety. First Edit. London: Elsevier Inc.; 2016. p. 531–8.

5. Achouri, A., Zamani, Y., Boye, J.I. Stability and Physical Properties of Emulsions Prepared with and without Soy Proteins. Journal of Food Research, 2012,1, 254–267.

6. Dalgleish, D.G. Food emulsions—their structures and structure-forming properties. Food Hydrocolloids, 20, 415–22.

7. McClements, D.J. Food Emulsion: Principle, Practices, and Techniques. Second Edi.CRC Press; 2005. 628p.

8. Dickinson E.,Bergenstahl, B. Food Colloids - Proteins, Lipids and Polysaccharides. 2004. 417 p.

9. Charoen, R., Jangchud, A., Jangchud, K., Harnsilawat, T., Decker, E.A., McClements, D.J. Influence of interfacial composition on oxidative stability of oil-in-water emulsions stabilized by biopolymer emulsifiers. Food Chemistry, 2012, 131,1340– PubMed ;1346.

10. Charoen, R., Jangchud, A., Jangchud, K., Harnsilawat, T., Naivikul, O., McClements, D.J. Influence of Biopolymer Emulsifier Type on Formation and Stability of Rice Bran Oil-in-Water Emulsions: Whey Protein, Gum Arabic, and

Modified Starch. Journal of Food Science. 2011, 76(1), 165 PubMed –72.

11. Bouyer, E., Mekhloufi, G., Rosilio, V., Grossiord, J., Agnely, F. Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: Alternatives to synthetic surfactants in the pharmaceutical field? International Journal of Pharmaceutics, 2012, 436(1–2), 359–78.

12. Silva, E.K., Gomes, M.T.M.S., Hubinger, M.D., Cunha, R.L,, Meireles, M.A.A. Ultrasound-assisted formation of annatto seed oil emulsions stabilized by biopolymers. Food Hydrocolloids, 2015, 47, 1– PubMed ;13.

13. Amine, C., Dreher, J., Helgason, T., Tadros, T. Investigation of emulsifying properties and emulsion stability of plant and milk proteins using interfacial tension and interfacial elasticity. Food Hydrocolloids, 2014, 39, 180– PubMed ;6.

14. Matsumura, Y., Satake, C., Egami, M., Mori, T. Interaction of gum arabic, maltodextrin and pullulan with lipids in emulsions. Bioscience, biotechnology, and biochemistry. 2000, 64, 1827–1835.

15. Gharibzahedi, S.M.T., Razavi, S.H., Mousavi, S.M. Ultrasound-assisted formation of the canthaxanthin emulsions stabilized by arabic and xanthan gums. Carbohydrate Polymers. 2013, 96(1), 21–30.

16. Ozturk, B., McClements, D.J. Progress in natural emulsifiers for utilization in food emulsions.Current Opinion in Food Science, 2015, 7, 1–6.

17. Jafari, S.M., Beheshti, P., Assadpoor, E. Rheological behavior and stability of d-limonene emulsions made by a novel hydrocolloid (Angum gum) compared with Arabic gum. Journal of Food Engineering, 2012, 109(1), 1–8.

18. Rosenberg, M., Sheu T-Y. Microencapsulation of volatiles by spray-drying in whey protein-based wall systems. International Dairy Journal. 1996, 6(3), 273–84.

19. Frascareli, E.C., Silva, V.M., Tonon, R.V., Hubinger, M.D. Effect of process conditions on the microencapsulation of coffee oil by spray drying. Food and Bioproducts Processing, 2012, 90(3), 413–24.

20. Wang, B, Wang, L.J., Li, D., Adhikari, B., Shi, J. Effect of gum Arabic on stability of oil-in-water emulsion stabilized by flaxseed and soybean protein. Carbohydrate Polymers, 2011, 86(1), 343–51.

21. Turchiuli, C., Munguia, M.T.J., Sanchez, M.H., Ferre, H.C., Dumoulin, E. Use of different supports for oil encapsulation in powder by spray drying. Powder Technology, 2014, 255, 103–108.

22. Taneja, A., Ye, A., Jones, J.R., Archer, R., Singh, H. Behaviour of oil droplets during spray drying of milk-protein-stabilised oil-in-water emulsions. International Dairy Journal, 2013, 28(1), 15–23.

23. Dluzewska, E., Sobiecka, A., Maszewska, M. Effect of oil phase concentration on rheological properties and stability of beverage emulsions. Acta Scientiarum Polonorum Technologia Alimentaria, 2006, 5(2), 147–156.

24. Jayme, M., Dunstan, D., Gee, M. Zeta potentials of gum arabicstabilised oil in water emulsions. Food Hydrocolloids, 1999, 13(6), 459 PubMed –465.

25. Shao, P., Shao, J., Jiang, Y., Sun, P. Influences of Ulva fasciata polysaccharide on the rheology and stabilization of cinnamaldehyde emulsions. Carbohydrate Polymers, 2016, 135, 27–34.

26. Thevenet, F. Acaciagum (gumarabic). In: Imeson A, editor. Food stabilisers, thinckeners and gelling agents.Blackwell Publishing; 2006. p. 11–30.

27. Patel, S., Goyal, A. Applications of Natural Polymer Gum Arabic: A Review. International Journal of Food Properties, 2015, 18(5), 986–98.

28. McClements, D.J. Protein-stabilized emulsions. Current Current Opinion in

Colloid and Interface Science, 2004, 9(5), 305–13.

29. Akhtar, M., Dickinson, E. Whey protein-maltodextrin conjugates as emulsifying agents: An alternative to gum arabic. Food Hydrocolloids, 2007, 21(4), 607 PubMed –616.

30. Jafari, S.M., He, Y., Bhandari, B. Nano-Emulsion Production by Sonication and Microfluidization - A Comparison. International Journal of Food Properties. 2006, 9(3), 475–85.

31. Perrier-Cornet, J.M., Marie, P., Gervais, P. Comparison of emulsification efficiency of protein-stabilized oil-in-water emulsions using jet, high pressure and colloid mill homogenization. Journal of Food Engineering, 2005, 66(2), 211–217.

32. Le, H. D., Le, V.V.M. Application of ultrasound to microencapsulation of coconut milk fat by spray drying method. Journal of Food Science and Technology. 2015, 52(4), 2474–2478.

33. Shanmugam, A., Ashokkumar, M. Ultrasonic preparation of stable flax seed oil emulsions in dairy systems – Physicochemical characterization. Food Hydrocolloids, 2014, 39, 151–162.

34. Silva, E.K., Azevedo, V.M., Cunha, R.L., Hubinger, M.D., Meireles, M.A.A. Ultrasound-assisted encapsulation of annatto seed oil - Whey protein isolate versus modified starch.Food Hydrocolloids, 2016, 56, 71– PubMed ;83.

35. Ramisetty, K.A., Pandit, A.B., Gogate, P.R. Ultrasound assisted preparation of emulsion of coconut oil in water: understanding the effect of operating parameters and comparison of reactor designs. Chemical Engineering and Processing: Process Intensification. 2015, 88, 70–77.

36. Cucheval, A, Chow, R.C.Y. A study on the emulsification of oil by power ultrasound. Ultrasonics Sonochemistry, 2008, 15(5), 916–920.

37. O'Brien, W.D. Ultrasound-biophysics mechanisms. Progress in Biophysics & Molecular Biology. 2007, 93(1– PubMed ;3), 212–255.

38. Jafari, S. M, Assadpoor, E., He, Y., Bhandari, B. Encapsulation Efficiency of Food Flavours and Oils during Spray Drying. Drying Technology, 2008, 26, 816– PubMed ;835.

39. Silva, H.D., Cerqueira, M.Â., Vicente, A.A. Nanoemulsions for Food Applications: Development and Characterization. Food and Bioprocess Technology, 2012, 21, 5(3), 854–867.

40. Tonon, R. V., Pedro, R.B., Grosso, C.R.F., Hubinger, M.D. Microencapsulation of Flaxseed Oil by Spray Drying: Effect of Oil Load and Type of Wall Material. Drying Technology, 2012, 30(13),1491–1501.

41. Botrel, D.A., Borges, S.V., Fernandes, R.V. de B., Carmo, E.L. Optimization of Fish Oil Spray Drying Using a Protein : Inulin System. Drying Technology, 2014, 32(3), 279 PubMed –290.

42. Carneiro, H.C.F., Tonon, R.V., Grosso, C.R.F., Hubinger, M.D. Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. Journal of Food Engineering, 2013, 115(4), 443–451.

43. Beristain, C.I., García, H.S., Vernon-Carter, E.J. Spray-dried Encapsulation of Cardamom (Elettariacardamomum) Essential Oil with Mesquite (Prosopisjuliflora) Gum. LWT - Food Science and Technology, 2001, 34(6), 398–401.

44. Adjonu, R., Doran, G., Torley, P., Agboola, S. Formation of whey protein isolate hydrolysate stabilised nanoemulsion. Food Hydrocolloids, 2014, 41, 169– PubMed ;177.

45. Mirhosseini, H., Tan, C.P., Hamid, N.S.A., Yusof, S. Effect of Arabic gum,

xanthan gum and orange oil contents on ζ-potential, conductivity, stability, size index and pH of orange beverage emulsion. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2008, 315(1–3), 47–56.

46. Mirhosseini, H., Tan, C.P., Hamid, N.S.A., Yusof, S. Optimization of the contents of Arabic gum, xanthan gum and orange oil affecting turbidity, average particle size, polydispersity index and density in orange beverage emulsion. Food Hydrocolloids, 2008, 22(7), 1212–1223.

47. Wang, B., Li, D., Wang, L-J, Özkan, N. Effect of concentrated flaxseed protein on the stability and rheological properties of soybean oil-in-water emulsions. Journal of Food Engineering, 2010, 96(4), 555–561. 48. Bae, E.K., Lee, S.J. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. Journal of Microencapsulation, 2008, 25(8), 549–60.

49. Rosa, M.T.M.G., Silva, E.K., Santos, D.T., Petenate, A.J., Meireles, M.A.A. Obtaining annatto seed oil miniemulsions by ultrasonication using aqueous extract from Brazilian ginseng roots as a biosurfactant. Journa of Food Engineering, 2016, 168, 68–78.

50. Tadros, T.F. Rheology of Dispersions: Principles and Applications. First Edit. Rheology of Dispersions: Principles and Applications. Wiley-VCH; 2010.218 p.

51. Moschakis, T., Murray, B.S., Biliaderis, C.G. Modifications in stability and structure of whey protein-coated o/w emulsions by interacting chitosan and gum arabic mixed dispersions. Food Hydrocolloids, 2010, 24(1), 8 PubMed –17.

52. Garcia, L.C., Tonon, R.V., Hubinger, M.D. Effect of Homogenization Pressure and Oil Load on the Emulsion Properties and the Oil Retention of Microencapsulated Basil Essential Oil (Ocimumbasilicum L.). Drying Technology, 2012, 30(13), 1413 PubMed –1421.

53. Tonon, R. V., Pedro, R.B., Grosso, C.R.F., Hubinger, M.D. Microencapsulation of Flaxseed Oil by Spray Drying: Effect of Oil Load and Type of Wall Material. Drying Technology, 2012, 30(13),1491–1501.

54. Kuhn, K.R., Cunha, R.L. Flaxseed oil - Whey protein isolate emulsions: Effect of high pressure homogenization. Journal of Food Engineering, 2012, 111(2), 449–457.

55. Al-Malah, K.I., Azzam, M.O.J., Omari, R.M. Emulsifying properties of BSA in different vegetable oil emulsions using conductivity technique. Food Hydrocolloids, 2000, 14(5), 485 PubMed –490.

56. Mirhosseini, H., Tan, C.P., Taherian, A.R. Effect of glycerol and vegetable oil on physicochemical properties of Arabic gum-based beverage emulsion. European Food Research and Technology, 2008, 228(1), 19–28.

57. Masson, D.S., Morais, G.G., de Morais, J.M., de Andrade, F.F., Dos Santos, O.D.H., de Oliveira, W.P., et al. Polyhydroxy Alcohols and Peach Oil Addition Influence on Liquid Crystal Formation and Rheological Behavior of O/W Emulsions. Journal of Dispersion Science and Technology, 2005, 26(4), 463–468.

58. Klein, M., Aserin, A., Ishai, P Ben, Garti, N. Interactions between whey protein isolate and gum Arabic. Colloids and Surfaces B: Biointerfaces, 2010, 79(2), 377 PubMed –83.

59. Jambrak, A.R., Mason, T.J., Lelas, V., Herceg, Z., Herceg, I.L. Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. Journal of Food Engineering, 2008, 86, 281–7.

60. Casteli, V.C., Mendonça, C.C., de Campos, M.A.L., Ferrari, M., Machado, S.R.P. Desenvolvimento e estudos de estabilidade preliminares de emulsões O/A contendo Cetoconazol 2.0%. Acta Scientiarum. Health Sciences, 2008, 30(2),

121 PubMed –128.

61. Sari, T.P., Mann, B., Kumar, R., Singh, R.R.B., Sharma, R., Bhardwaj, M., et al. Preparation and characterization of nanoemulsion encapsulating curcumin. Food Hydrocolloids, 2015, 43, 540–546.

62. Mohammadzadeh, H., Koocheki, A., Kadkhodaee, R., Razavi, S.M.A. Physical and flow properties of d-limonene-in-water emulsions stabilized with whey protein concentrate and wild sage (Salvia macrosiphon) seed gum. Food Research International, 2013, 53(1), 312–318.

63. Herculano, E.D., de Paula, H.C.B., de Figueiredo, E.A.T., Dias, F.G.B., Pereira, V.D.A. Physicochemical and antimicrobial properties of nanoencapsulated Eucalyptus staigeriana essential oil. LWT - Food Science ande Technology, 2015, 61(2), 484–491.

64. Jayasundera, M., Adhikari, B., Aldred, P., Ghandi, A. Surface modification of spray dried food and emulsion powders with surface-active proteins: A review. Journal of Food Engineering, 2009, 93(3), 266–277.

65. Silva, V.M., Vieira, G.S., Hubinger, M.D. Influence of different combinations of wall materials and homogenisation pressure on the microencapsulation of green coffee oil by spray drying. Food Research International, 2014, 61, 132–143.

66. Xu, D., Yuan, F., Wang, X., Li, X., Hou, Z., Gao, Y. The effect of whey protein isolate-dextran conjugates on the freeze-thaw stability of oil-in-water emulsion. Journal of Dispersion Science and Technology 2010, 32(1), 77–83.

67. Mirhosseini, H., Tan, C.P., Hamid, N.S.A, Yusof, S., Chern, B.H. Characterization of the influence of main emulsion components on the physicochemical properties of orange beverage emulsion using response surface methodology. Food Hydrocolloids, 2009, 23(2), 271–280.

68. Sukhotu, R., Guo, S., Xing, J., Hu, Q., Wang, R., Shi, X., et al. Changes in physiochemical properties and stability of peanut oil body emulsions by applying gum arabic. LWT - Food Science and Technology, 2016, 68, 432–438.

69. Kim, Y.D., Morr, C. V. Microencapsulation Properties of Gum Arabic and Several Food Proteins: Spray-Dried Orange Oil Emulsion Particles. Journal of Agriculture and Food Chemistry, 1996, 44(5), 1314–1320.

70. Garti, N. Hydrocolloids as emulsifying agents for oil-in-water emulsions. Journal of Dispersion Science and Technology, 1999, 20(1–2), 327–355.

71. Camino, N.A., Pilosof, A.M.R. Hydroxypropylmethylcellulose at the oil-water interface. Part II. Submicron-emulsions as affected by pH. Food Hydrocolloids, 2011, 25(5), 1051 PubMed –1062.

72. Kuhn, K.R., Silva, F.G.D., Netto, F.M,, Cunha, R.L. Assessing the potential of flaxseed protein as an emulsifier combined with whey protein isolate. Food Research International, 2014, 58, 89–97.

73. Conceição,M. C., Junqueira, L. A., Silva, K. C. G., Prado, M. E. T., Resende J V. Thermal and microstructural stability of a powdered gum derived from Pereskiaaculeata Miller leaves. Food Hydrocolloids, 2014, 40, 104– PubMed ;114.

74. İbanoğlu, E. Rheological behaviour of whey protein stabilized emulsions in the presence of gum arabic. Journal of Food Engineering, 2002, 52(3), 273–7.

FIGURE CAPTIONS

Figure 1 - (a) pH and (b) Conductivity of Lime emulsions oils.

Figure 2–Size distributions of emulsions. (a) GA; (b) GA/WPI; (c) WPI; (1) 0 h and (2) after 4 h.

Figure 3 – Microscopy images of emulsions prepared with GA and different essential oil concentration: (a) 5.0 g oil/100 g emulsion; (b) 7.5 g oil/100 g emulsion; (c) 10.0 g oil/100 g emulsion; (1) fresh and (2) after 4 h.

Figure 4– Apparent viscosity as function of shear rate for lime essential oil with GA (A), GA/WPI (B) e WPI (C)

Figure 5- Creaming index to lime essential oil emulsions after 4 h.

Figure 1

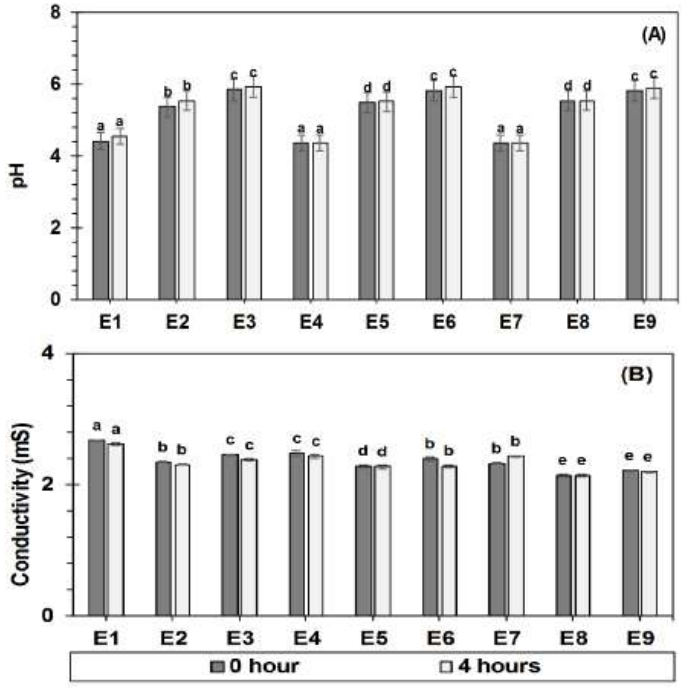
(A)
pH
8
6
4
2
0
E1
E2
E3
E4
E5
E6
E7
E8
E9
(B)
Conductivity (mS)
4
2
0
0 hour
4 hours

Figure 2

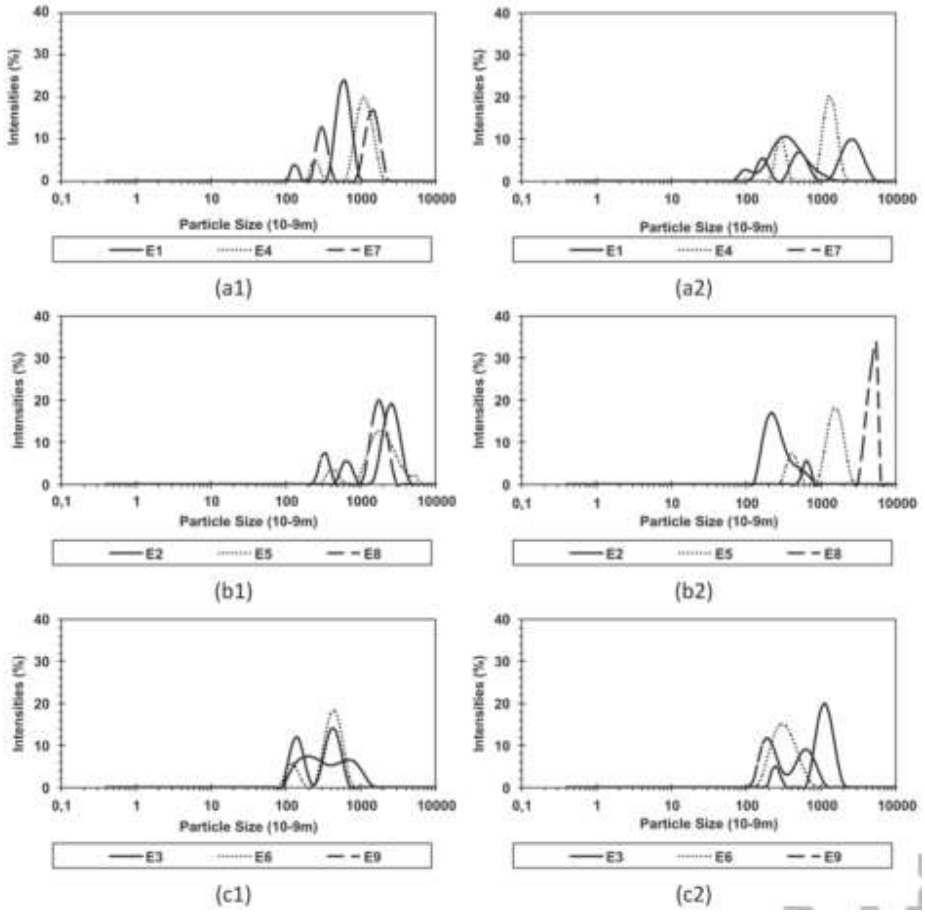
Intensities (%)
Particle Size (10-9m)
E1
E4
E7
(a1)
(a2)
E2
E5
E8
(b1)
(b2)
E3
E6
E9
(c1)
(c2)

Figure 3

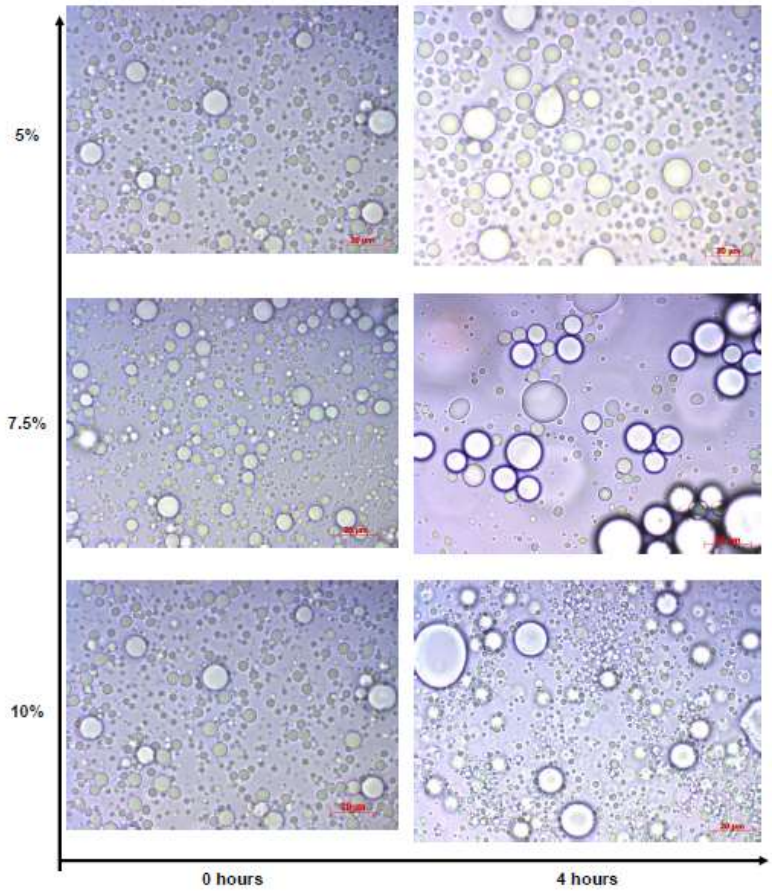

Figure 4

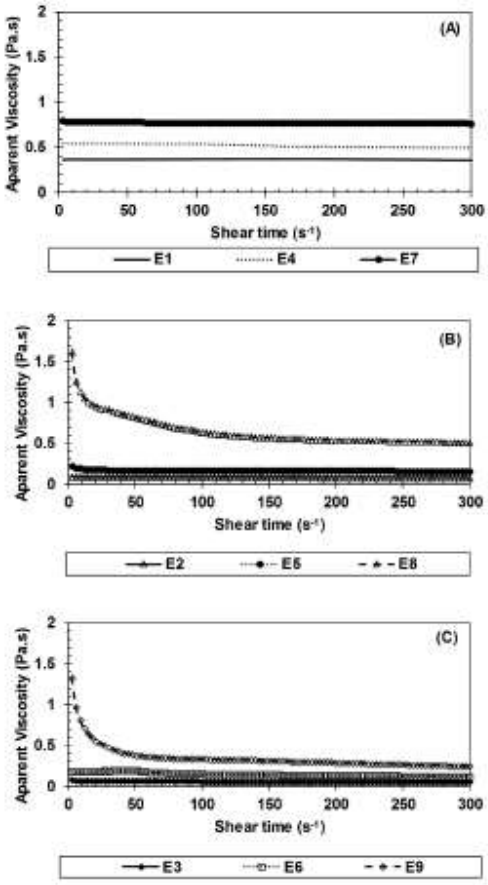
(A)
Aparent Viscosity (Pa.s)
Shear time (s⁻¹)
E1
E4
E7
(B)
Aparent Viscosity (Pa.s)
Shear time (s⁻¹)
E2
E5
E8
(C)
Aparent Viscosity (Pa.s)
Shear time (s⁻¹)
E3
E6
E9

Figure 5

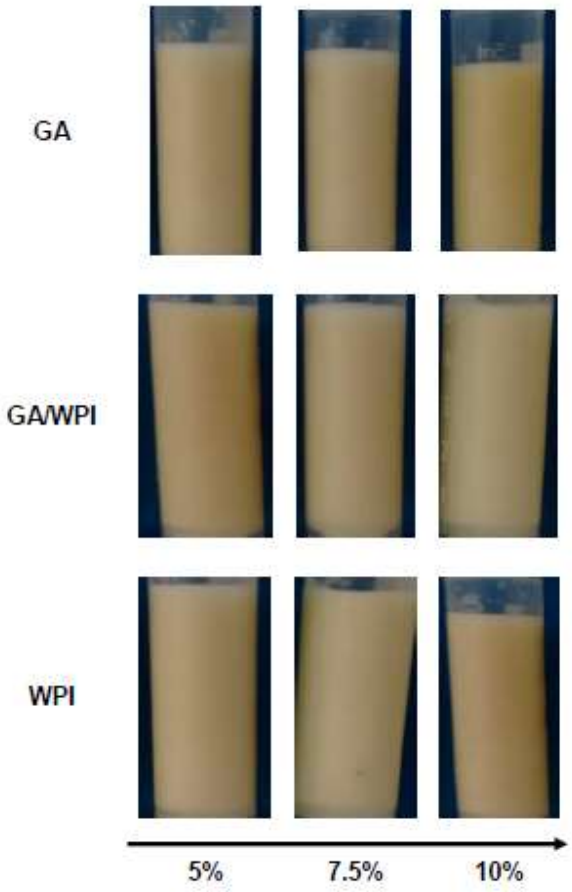

Table 1

Table 1 - Experimental Design to lime essential oil emulsions.

Assays	Biopolymer (%)		Oil content (%)
	GA	WPI	
E1	25	0	5
E2	12.5	12.5	5
E3	0	25	5
E4	22.5	0	7.5
E5	11.25	11.25	7.5
E6	0	22.5	7.5
E7	20	0	10
E8	10	10	10
E9	0	20	10

Table 2 - Zeta Potential by biopolymers solutions and emulsions at 0 and 4 hours.

Wall Material	Samples	Zeta Potential (mV)		
		Solution (Water + Wall Material)	0 hours	4 hours
GA	E1	-19.42 ± 0.60 ^{g,B}	-20.13 ± 0.01 ^{e,A}	-19.75 ± 0.29 ^{e,A}
	E4	-18.28 ± 1.88 ^{c,C}	-22.08 ± 0.12 ^{d,A}	-19.9 ± 0.55 ^{e,B}
	E7	-19.94 ± 0.90 ^{b,B}	-30.44 ± 0.30 ^{c,A}	-20.11 ± 0.09 ^{c,C}
GA/WPI	E2	-23.74 ± 0.54 ^{e,A}	-23.19 ± 0.10 ^{d,A}	-18.49 ± 0.08 ^{e,B}
	E5	-20.30 ± 0.64 ^{d,A}	-20.13 ± 0.24 ^{e,A}	-20.39 ± 0.13 ^{e,A}
	E8	-16.68 ± 1.33 ^{a,C}	-32.25 ± 0.08 ^{b,A}	-25.16 ± 0.52 ^{d,B}
WPI	E3	-26.50 ± 0.90 ^{d,B}	-31.92 ± 0.16 ^{b,A}	-29.55 ± 0.10 ^{c,A}
	E6	-18.54 ± 0.68 ^{e,B}	-32.52 ± 0.23 ^{b,A}	-31.52 ± 0.13 ^{b,A}
	E9	-17.32 ± 1.27 ^{f,B}	-33.93 ± 0.30 ^{a,A}	-33.99 ± 0.06 ^{a,A}

Same letter in same column (^{a,b,c,d,e,f}) or same line (^{A,B}) represent not significant difference (p > 0.05%). Results are presented as the mean ± standard deviation.

Table 3

Table 3 - Polidispersion Index (PDI) and droplet size by emulsions at 0 and 4 hours.

Wall Material	Samples	PDI		Droplet size (µm)	
		0 hours	4 hours	0 hours	4 hours
GA	E1	$0,65 \pm 0,05^{a,A}$	$1,31 \pm 0,07^{a,B}$	$0.694 \pm 0.024^{a,A}$	$0.709 \pm 0.029^{a,A}$
	E4	$1,27 \pm 0,03^{b,A}$	$1,77 \pm 0,1^{b,B}$	$0.812 \pm 0.032^{a,A}$	$0.792 \pm 0.022^{a,A}$
	E7	$2,11 \pm 0,05^{c,A}$	$2,48 \pm 0,08^{c,B}$	$0.806 \pm 0.098^{a,A}$	$1.037 \pm 0.141^{a,A}$
GA/WPI	E2	$1,22 \pm 0,05^{b,A}$	$1,45 \pm 0,06^{a,A}$	$0.97 \pm 0.016^{b,A}$	$1.622 \pm 0.027^{b,B}$
	E5	$1,28 \pm 0,02^{b,A}$	$1,71 \pm 0,08^{b,B}$	$1.305 \pm 0.075^{c,A}$	$1.968 \pm 0.088^{c,B}$
	E8	$1,71 \pm 0,04^{d,A}$	$2,2 \pm 0,09^{d,B}$	$1.306 \pm 0.017^{c,A}$	$1.634 \pm 0.028^{c,B}$
WPI	E3	$0,94 \pm 0,04^{e,A}$	$1,11 \pm 0,05^{a,A}$	$0.282 \pm 0.025^{d,A}$	$0.256 \pm 0.01^{d,A}$
	E6	$1,34 \pm 0,05^{b,A}$	$1,63 \pm 0,09^{b,A}$	$0.288 \pm 0.007^{d,A}$	$0.289 \pm 0.001^{d,A}$
	E9	$1,54 \pm 0,05^{f,B}$	$2,16 \pm 0,13^{d,B}$	$0.294 \pm 0.002^{d,A}$	$0.294 \pm 0.002^{d,A}$

Same letter in same column ($^{a.b.c.d.e.f}$) or same line ($^{A.B}$) represent not significant difference ($p > 0.05\%$). Results are presented as the mean ± standard deviation.

Table 4

Table 4 - Correlation coefficient, residual mean square and Power-Law model parameters for emulsions prepared with different biopolymers and lime essential oil.

Wall Material	Assay	R^2		E (%)		Consistency Index, k (Pa.s^n)		Flow Behavior Index, n	
		Fresh emulsion	4h	Fresh emulsion	4h	Fresh emulsion	4h	Fresh emulsion	4h
GA	E1	0.994	0.996	2.319	1.437	0.391 ± 0.007^a	0.434 ± 0.016^a	0.984 ± 0.006^b	0.9923±0.0065^b
	E4	0.994	0.995	3.361	3.303	0.699 ± 0.272^b	0.790 ± 0.230^b	0.946 ± 0.060^b	0.9320±0.0403^b
	E7	0.999	0.997	0.828	3.632	0.800 ± 0.012^b	0.967 ± 0.293^b	0.992 ± 0.007^b	0.965 ± 0.048^b
GA/WPI	E2	0.994	0.979	0.509	1.122	0.089 ± 0.013^a	0.109 ± 0.008^a	0.972 ± 0.012^b	0.964 ± 0.005^b
	E5	0.984	0.999	1.724	0.248	0.203 ± 0.021^a	0.217 ± 0.025^a	0.950 ± 0.000^b	0.939 ± 0.020^b
	E8	0.989	0.983	42.293	4.491	1.848 ± 0.136^d	1.621 ± 0.577^b	0.770 ± 0.001^a	0.766 ± 0.048^a
WPI	E3	0.995	0.981	0.296	0.618	0.070 ± 0.004^a	0.077 ± 0.012^a	0.939 ± 0.002^b	0.933 ± 0.010^b
	E6	0.998	0.999	0.252	0.090	0.139 ± 0.007^a	0.130 ± 0.003^a	0.908 ± 0.001^b	0.909 ± 0.000^b
	E9	0.996	0.994	1.291	1.613	1.173 ± 0.083^c	1.179 ± 0.634^b	0.725 ± 0.015^a	0.749 ± 0.104^a

Table 5

Table 5 – Apparent viscosity value at 100 s^{-1}

Wall Material	Assay	Apparent viscosity at 100s^{-1} (Pa.s)	
		Fresh emulsion	4 hours
GA	E1	0,3622 ± 0,01*ª	0,4177 ± 0,02 *
	E4	0,5347 ± 0,07 *	0,5574 ± 0,05 *
	E7	0,7698 ± 0,01 *	0,7987 ± 0,05 *
GA/WPI	E2	0,0791 ± 0,007 *	0,0929 ± 0,009 *
	E5	0,1612 ± 0,01 *	0,1622 ± 0,002 *
	E8	0,6405 ± 0,04 *	0,6278 ± 0,08 *
WPI	E3	0,0522 ± 0,002 *	0,0573 ± 0,006 *
	E6	0,0918 ± 0,007 *	0,0856 ± 0,002 *
	E9	0,3221 ± 0,006 *	0,3437 ± 0,013 *

**Same letters in the same line represent no significant difference (p <0.05)*

ARTIGO 2: EFFECT OF DEXTROSE EQUIVALENT ON PHYSICAL AND CHEMICAL PROPERTIES OF LIME ESSENTIAL OIL MICROPARTICLES

Pedro Henrique CAMPELO[1]; Eloá Lourenço do CARMO[1]; Rosana Domingues ZACARIAS[2], Maria Irene YOSHIDA[2]; Vany Perpétua FERRAZ[2;] Regiane Victória de Barros FERNANDES[1]; Diego Alvarenga BOTREL[1]; Soraia Vilela BORGES[1]

1 Departament of Food Science, Federal University of Lavras (Campus Universitário, CEP 37200-000, Lavras, Minas Gerais, Brazil).

2 Departamento of Chemistry, Federal University of Minas Gerais (Av. Antônio Carlos 6627, Pampulha, Belo Horizonte – Minas Gerais. CEP 31270-901, Brazil)

Artigo publicado conforme normas do periódico Industrial Crops.

EFFECT OF DEXTROSE EQUIVALENT ON PHYSICAL AND CHEMICAL PROPERTIES OF LIME ESSENTIAL OIL MICROPARTICLES

Pedro Henrique CAMPELOa,b,*; Eloá Lourenço do CARMOa; Rosana Domingues ZACARIASc; Maria Irene YOSHIDAc; Vany Perpétua FERRAZc; Regiane Victória de Barros FERNANDES a; Diego Alvarenga BOTRELa; Soraia Vilela BORGES a.

[a]Departament of Food Science, Federal University of Lavras, Lavras, Minas Gerais, Brazil

[b]Departament of Agricultural Engineering and Soil, Federal University of Amazonas, Manaus, Amazonas, Brazil

[c]Departament of Chemistry, Federal University of Minas Gerais, Minas Gerais, Brazil

*Correspondent author: pcampelo.felix@gmail.com

ABSTRACT

This study seeks to evaluate the influence of different dextrose equivalent values on the characteristics of emulsions assisted by ultrasound and lime essential oil microparticles. Four treatments were evaluated: whey protein concentrate (WPC), and blends of maltodextrin with dextrose equivalents of 5 (WM5), 10 (WM10) and 20 (WM20). The physicochemical properties of the emulsions (viscosity and droplet size) and dry powders, by spray drying (moisture content, water activity, particle size, surface oil, encapsulation efficiency, oil and

limonene retention, x-ray diffraction, thermal and antioxidant activity analysis), were studied to measure the quality of the encapsulated materials. Maltodextrins with high DE values (10 and 20) had lower viscosities and consequently smaller droplet and particle sizes. The treatments showed significantly different and good retention for the majority compounds of the lime essential oil and maintained high antioxidant activity. Maltodextrin of DE 20 presented better significant oil retention values (77.6%) and encapsulation efficiency (83.3%). In conclusion, the degree of maltodextrin hydrolyzation can interfere with the physicochemical parameters of lime essential oil microparticles, observing the best results at higher DE values.

Keywords: Dextrose equivalent; lime essential oil; ultrasound homogenization; spray drying; whet protein isolate; maltodextrin.

1 INTRODUCTION

Lime essential oil is one of the most aromatic oils used by the food, pharmaceutical and cosmetic industries, and is widely used in beverages, soft drinks, bakery products, perfumes and others. Lime essential oil is a complex mixture of chemical compounds such as limonene, γ-terpinene, citral, linalool and β-caryophyllene, among others, which may be represented by three main classes: terpenes, the oxygenated compounds and sesquiterpenes (Cruz-Valenzuela et al., 2016; Gamarra et al., 2006). In addition, limonene, myrcene, octanal, and γ-terpinene, among others, contribute to the organoleptic characteristics of the oil (Cruz-Valenzuela et al., 2016; Gamarra et al., 2006).

Microencapsulation can provide numerous benefits to the encapsulated material, as it can protect it from oxidation, degradation and volatilization reactions

(Carmo et al., 2015). Spray drying is the most popular technique for microencapsulation of food, particularly in the ease of operation and for allowing continuous production of dry material. The technique is described as the atomization of an emulsion, with high solids concentration, into small droplets in a drying chamber, being subjected to contact with air at high temperatures (Asbahani et al., 2015; Botrel et al., 2014a).

The wall material is one of the most important parameters in the food microencapsulation processes. Its chemical composition and structure can affect the quality of the powdered product and criteria, such as good barrier formation, high glass transition temperature and consequent stability during storage must be observed and studied to produce good, low cost emulsifiers (Gharsallaoui et al., 2007; Zuidam and Shimoni, 2010). Whey protein, a by-product of the dairy industry, is one of the most commonly studied emulsifiers for encapsulation of oils (Bakry et al., 2016) and essential oils. Whey protein concentrate (WPC), the main source of globular proteins made up of β-lactoglobulin, α-lactalbumin, immunoglobulin and albumin, is widely used as an emulsifier (Fernandes et al., 2014; Tavares et al., 2014; Telis, 2012). Its emulsifying capacity is ideal for microencapsulation process of oils widely used in some studies (Takeungwongtrakul et al., 2015; Tontul and Topuz, 2014; Carneiro et al., 2013). Carbohydrates have good ability to absorb volatiles and retain them in its structure, and are widely used in the microencapsulation processes (Liu et al., 2001). Maltodextrins are widely used as a secondary wall material since they have low emulsifying capacity. These carbohydrates have characteristics such as low cost, high solubility and high soluble solids, providing additional protection for the encapsulated material. Moreover, maltodextrins of different dextrose equivalents (DE) are commonly used by the wall materials because if their high water solubility, low viscosity, low sugar content and their colorless solutions (Akhavan Mahdavi et al., 2016). The DE of maltodextrin can be defined as the

level of starch hydrolysis, which can interfere with the retention and protection of microencapsulated essential oils. The DE values of maltodextrins range from 3-20 (Dziedzic and Kearsley, 1995).

As such, the objective of this study is to evaluate the effect of substitution of WPC by maltodextrins of different DE on the physicochemical properties of lime essential oil microparticles. We evaluated the emulsion parameters (viscosity, droplet size and creaming index) and physicochemical characteristics (equilibrium moisture content, water activity, particle size and morphology, the total oil, surface oil, encapsulation efficiency, thermogravimetric analysis, diffraction x-ray and antioxidant activity).

2 MATERIALS AND METHODS

2.1 Materials

Lime (Citrus aurantifolia) essential oil (Ferquima Ind. e Com. Ltda, Vargem Grande Paulista, Brazil) was used as the core material. The biopolymers used with wall material were Whey protein concentrate (WPC), was donated from Alibra (Campinas, Brazil) and Maltodextrin in different dextrose equivalent (GLOBE® 1905, 1910 and 1920) was donated from Ingredion (São Paulo, Brazil).

2.2 Experimental Design

The experiments were conducted in a completely randomized design with three replications, as shown in Table 1 to evaluate the effects of the four encapsulation formulations on the characteristics of powders of lime essential oil microencapsulated.

2.3 Preparation of emulsion

The wall materials were dissolved in distilled water and the solutions were prepared 24 hours before being emulsified at room temperature to ensure full

saturation of the polymer molecules. Then, lime essential oil was added to the wall material solution and submitted homogenization (Model 450 - Branson Ultrasonic, USA). Aliquots of 60 mL were subjected to ultrasonication (2 min/240 W) to completely emulsify the lime essential oil. The emulsion was used as the feed liquid for the spray-drying process (Fernandes et al., 2014; Silva and Meireles, 2015). Characterization of emulsions properties

Freshly prepared emulsions were diluted to a droplet concentration of approximately 1:1000 with MilliQ water and placed into the measurement chamber of a microelectrophoresis instrument (Nano ZS Zetasizer, Malvern Instruments Ltd., Worcestershire UK). The measurements were performed at least three replicates, at 25 °C.

Emulsion droplet size were measured by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, U.K.), using 1.5 mL emulsion samples diluted 1000x with MilliQ water to avoid multiple light scattering effects. The particle size data are reported as Z-average mean diameter and polydispersity index (PDI).

Rheological measurements were analysed according (Botrel et al., 2014b) using a oscillatory rheometer HAAKE RheoStress 6000 (Thermo Fisher Scientific, USA) coupled to a temperature controller HAAKE UTM Controller (Thermo Fisher Scientific, USA). T The parameters of Power Law (Eq. 1) were estimated by correlating the mathematical models to the experimental data via a quasi-Newton nonlinear regression, with a 5% significance level. The model considered most suitable was that with lower relative mean error (E), defined as follows in Eq. 2.

$$\sigma = K\gamma^n \qquad \text{Eq. 1}$$

$$E = \frac{100}{N} \sum_{i=1}^{N} \frac{(m_i - m_{pi})}{m_i} \quad \text{Eq. 2}$$

2.4 Spray-drying process

The emulsions were dried using a spray-dryer (model MSD 1.0; Labmaq do Brasil, Ribeirão Preto, Brazil) with a double fluid nozzle system of diameter 3 mm in a drying chamber of di- mensions 670 × 200 mm. Feeding was performed using a peristaltic pump with a airflow of 40 L/min and air pressure of 400 kPa. The outlet air temperatures ranged from 90 to 97 °C, depending on the feed concentration and inlet temperature, and the feed flow rate was maintained at 0.70 L/h. The microparticles obtained were stored under refrigeration (4 to 7 °C) in sealed aluminum packing, protected from light penetration and gas permeation until further analysis (da Costa et al., 2015; Fernandes et al., 2013). All the drying were done in triplicate.

2.5 Gas Chromatography

To determine the major components of the lime essential oil in the microparticles approximately 30 mg were mixed with 500 uL of chloroform ultrasonic bath for 15 min. Then it was added 500 uL of water and subjected to vortexing for 10 s and 10 min in ultrasonic bath. After centrifugation at 6500 min-1, a rate of 2 µL the organic layer was injected into the chromatograph for analysis. The analysis was performed on a gas chromatograph HP7820A (Agilent, USA) equipped with ionization detector flame. A HP5 column 30 m x 0.32 mm x 0.25 µm (Agilent, USA) was used. The column was heated to 50-150 °C at a rate of 3 °C / min with injector (splitless) at 200 °C and detector 220 °C. Hydrogen was used as carrier gas at a rate of 3 mL / min and injection volume

1µl. Data were acquired by EZChrom Compact Elite software (Agilent, USA).

2.6 Moisture and Water Activity

The moisture content of the powders was determined by the AOAC method. The powder weight loss percentage (%) after oven-drying at 105 °C until a constant weight was obtained, and moisture content (%) was calculated. Water activity measurements were using an AquaLab 4TE as described by the manufacturer (Decagon Devices Inc., USA) were performed at 25 °C.

2.7 Surface Oil, Total Oil, Encapsulation Efficiency and Oil Retention

Surface oil (SO) was determined by method described by (Carneiro et al., 2013) with some modifications. Twenty milliliters of petroleum ether (30 – 10 °C boiling point) were added to approximately 2 grams of microparticles and subjected to vortexing for 1 min for the extraction of non-encapsulated oil. The mixture solution was filtered through Whatman filter paper No. 1. Three rinses of microparticles with petroleum ether was conducted. The collected solvent was left to evaporate at room temperature and after at 60°C until constant weight (balance accuracy = ± 0.001 g). The surface oil was determined by mass difference between the initial and final sample.

To determine total oil (TO) of the lime essential oil microparticles were used the values of the amount of oil extracted from the microparticles as described procedure of Section 2.5.

Encapsulation efficiency (EE) is defined as the ratio between the oil inside the microparticles and total oil present in the microparticles (Tonon et al., 2012). EE was calculated using Eq. 3:

$$EE\ (\%) = 100 \times \left(\frac{TO - SO}{TO}\right) \quad \text{Eq. 3}$$

The retention oil (or entrapment efficiency) (Eq.4) was defined as the ratio between the total mass of oil present in the microparticles and the mass of oil added at the beginning of the process (Silva and Meireles, 2015):

$$RO\ (\%) = 100\times\left(\frac{Total\ oil\ (\%)}{Initial\ oil\ load\ (\%)}\right) \quad \text{Eq. 4}$$

2.8 Particle size

Particle size of lime essential oil microencapsulated were measured by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, U.K.), using 1.5 grams of microparticles samples diluted 1000x with isopropanol to avoid multiple light scattering effects. The particle size data are reported as Z-average mean diameter and polydispersity index (PDI).

2.9 Scanning Electronic Microscopy (SEM)

The morphologies of the lime essential oil microcapsules were examined by scanning electron microscopy (SEM). Microcapsules samples were distributed over a pieceof double-sided carbon tape adhered to a metallic support (stub). Then, the samples were analyzed with scanning electron microscopy JSM-6360 (JEOL; Tokyo, Japan), with an accelerating voltage of 20 kV.

2.10 X-Ray Diffration

X-ray diffraction (XRD) analysis of the microparticles and wall material was performed on Shimadzu XRD-6000 (Shimadzu, Tokyo, Japan) using a graphite

crystal as the monochromator with a filter radiation of Cu-Kα1 at 30kV and 30mA. Samples were analyzed at angles from 4° to 40° in 2θ with a 0.02° (1°/min) step.

2.11 Antioxidant activity

Antioxidant activity (AA%) was evaluated by DPPH radical (1,1-diphenyl-2-picrylhydrazyl) according to the method described by Mensor et al. (2001) with modifications. For extracting oil from the microparticles, 0.1 g of microcapsules was added in 50 mL of ethanol solution (1:3 v/v). The mixture was homogenized by an ultrasonic homogenizer (Branson, USA) in a 1min/250W.

In tubes were added 2.4 mL of absolute ethanol, 1 mL of DPPH solution (2 mg / 50 mL) and 0.1 mL of sample after extraction. For the correction of a possible contribution of staining of the samples was carried out in parallel, consisting of a blank test sample volume (0.1 mL) and 3.4 mL of absolute ethanol. The control was prepared by mixing 1.0 mL of DPPH solution with 2.5 mL of absolute ethanol. After 1-hour incubation in the dark at room temperature, the absorbances were recorded at 517 nm. Tests were performed in triplicates and the inhibition of free radical DPPH was calculated by Eq. 7:

$$AA\ (\%) = 100 - \left[\left(100 \times \frac{A_A - A_B}{A_C}\right)\right] \qquad \text{Eq. 7}$$

Where AA is sample absorbance, AB is blank absorbance and AC is control absorbance.

2.12 Statistical analysis

Analyses of variance were also carried out using R package software to verify the effects of the wall materials on the characteristics of the lime essential oil microparticles. The results of the Rheology, Gas Chromatograph and XRD were analyzed descriptively. Differences in the mean values obtained were examined

by Scott-Knott´s test of means at a significance level of 5%.

3 RESULTS

3.1 Rheology

Rheological parameters are widely used to predict the size behavior of dry particle in microencapsulation processes because it is related to the size of the droplet that will be atomized and dried. Moreover, the emulsion viscosity plays an important role in determining encapsulation efficiency and oil retention (Bakry et al., 2016). Figure 1 presents the apparent viscosity profile in relation to the deformation rate for lime essential oil emulsions. Higher apparent viscosity values were found for WPC and WM5 samples. Due to their high molecular weight, proteins increase the viscosity of the medium (Botrel et al., 2014b). It can be seen that the increase in the maltodextrins reduced viscosities compared to emulsions with only WPC. Similar behavior was observed by Castro et al. (2016) when studying maltodextrin solutions with different DE values. This behavior is related to the size of the maltodextrin chains, since the larger the chains are, the more the water molecules will bind to its structure, favoring a emulsion viscosity increase. Moreover, the abundance presence of high molecular-weight molecules tends to increase the aqueous phase resistance to flow, leading to an increase in the apparent viscosity of the emulsion (Bakry et al., 2016; Klongdee et al., 2012). Short chain maltodextrins interact with the oil better, reducing the size of the drops and reducing the viscosity of the medium (Bae and Lee, 2008; Dokic-Baucal et al., 2004).

The rheological data were adjusted by the Power Law (Table 2). The consistency index (K) is related to emulsion viscosity values. It can be seen that the K values were obtained for the WPC and WM5 emulsions. The emulsion viscosity may interfere with encapsulation efficiency because the increase in

viscosity to an optimum value can reduce the movement of these droplets, reducing phenomena such as flocculation and agglomeration. In contrast, an excessive increase in viscosity, by hindering the oil retention, increases the droplet size and exposure during drying due to the large surface area (Jafari et al., 2008).

3.2 Droplet Size, PDI and Droplet Size Distribution

The droplet size and PDI values are shown in Table 3. The droplet size presented significant difference (P=0.001) for the different wall materials. The increasing maltodextrin DE promoted a reduction in the average size of lime essential oil droplets. In many oil microencapsulation studies the average oil droplet size in emulsions is generally over 2 μm (Carneiro et al., 2013; Rodea-González et al., 2012; Silva et al., 2014), whereas in our study the values found were between 0.28 – 0.31 μm. This observation can be explained by the homogenization process used (ultrasonic), which generates much smaller droplets when compared with that used in other studies (mechanical) (Tontul and Topuz, 2014).

The results corroborate the apparent viscosity profile in Figure 1. This phenomenon can be explained because at high viscosities, the ultrasonic waves can not promote high agitation and shear stress in emulsion oils, generating larger droplets. When studying different biopolymers as stabilizer for annatto oil emulsions Silva et al. (2015) confirmed this hypothesis on the relationship between viscosity and droplet size after the homogenization process. Bae and Lee (2008) observed a size increase of avocado oil droplets from 2.27 μm to 2.29, 3.10 and 4.10 μm when using maltodextrin DE 5 in partial replacement of the proteinaceous biopolymer. The same authors explain that the low emulsifying capacity of maltodextrin, compared to WPI, may have resulted from the droplet size increase. Figure 2 shows the light microscopy images and the

lime essential oil emulsion droplet size distribution profile. Visual assessment demonstrates larger droplet size for WPC and WM5 treatments compared to WM10 and WM20 treatments, corroborating the average size results and those of the droplet size distribution. A higher viscosity of proteins (Figure 1), and larger-chain maltodextrins of low emulsifying capacity demonstrate the formation of larger droplets. Two peak can be seen in the particle size distribution graph with the maltodextrin DE increase. A more heterogeneous distribution may be associated with the emulsion viscosity, which hinders the formation of smaller droplets, favoring the agglomeration and increase of these droplets.

3.3 Gas Chromatography

In volatile compound microencapsulation processes, it is important to evaluate the changes the core material goes through during drying, since its chemical composition is useful for the application of these ingredients (Fernandes et al., 2014). Figure 3 presents the concentration profile of the majority compounds present in the pure lime essential oil and microencapsulated oil. The components noted in higher concentrations in the pure oil were α-pinene (2.1%), β-pinene (11.6%), myrcene (1.6%), limonene (55.3%), γ-terpinene (11.8%) and other compounds (17.4%). Gamarra et al. (2006) observed a limonene concentration of 49.66%.

For the different wall materials used, we observed that a slight reduction of the majority compound amounts present in the lime essential oil. Based on limonene (the most abundant compound) we note that there was a slight reduction in the concentration for the WPC and WM5 treatments. This observation can be explained by the high molecular weight proteins and low maltodextrin DE which may hinder the maintenance physical and chemical properties of the encapsulated oil. The surface oil content can also be an

explanation for the reduction of some lime oil compounds, since this oil is less protected and is susceptible to volatilization and degradation (Baranauskienė et al., 2006).

3.4 Moisture and Water Activity

Moisture and water activity are important parameters for preservation of food products because a large amount of retained water can accelerate degradation reactions and microorganism growth. Depending on the microcapsule storage temperature, the glass transition will occur at critical moisture content and activity values, which is an important factor for the stability of the powder (Escalona-García et al., 2016). It is necessary that its equilibrium moisture content is less than 5 g water/100 g of dry solid and its water activity is in the range from 0.1 to 0.4, ensuring greater stability for dry food (Tontul and Topuz, 2014).

The results of moisture and water activity for lime essential oil microparticles are shown in Table 2. Although the drying parameters are constant, there was a variation of the moisture content of microparticles after drying. In this case, the different equilibrium moisture values are related to the different types of biopolymers used (Díaz et al., 2015). For equilibrium moisture values, the WPC and WM5 treatments differ significantly ($p < 0.01$) from the WM10 and WM20 treatments. The equilibrium moisture values ranged from 3.83 to 5.35 g.100 g water-1. Higher moisture values were observed for samples with higher dextrose equivalent maltodextrin Fazeli et al. (2012), Goula and Adamapoulos (2005) and Matusuura et al. (2015) observed increased relative moisture by increasing the maltodextrin dextrose equivalent for blackberry, tomato and coconut oil microparticle, respectively. On encapsulating jussara pulp (Euterpe edulis Martius), (Tonon et al., 2009) also observed an increase in the equilibrium moisture content of the microparticles with the maltodextrin DE increase. High

equilibrium moisture values (> 5.00 g. 100 g water-1) may cause degradation reactions in powdered food (Masters, 1991). The moisture content observed for WM10 and WM20 treatments may be associated with the rapid formation of the drop around the bioactive component, preventing water diffusion during drying (Goula and Adamopoulos, 2010).

There was a significant difference (p = 10-5) for water activity among the different wall materials. Samples with maltodextrin (0.297, 0.267 and 0.342) presented higher water activity values compared to samples only with WPC (0.124). The increase in the the degree of maltodextrin hydrolysis increased the water activity of the microparticles. These results are related to the different biopolymers used. Carbohydrates that are more hydrolyzed have shorter chains and become more hygroscopic, thus retaining more water on their surface. When evaluating maltodextrins of different hydrolysis degrees in the microencapsulation of açai, Tonon et al. (2009) obtained water activity results for samples with DE 10 (0.229) and DE 20 (0.245), corroborating the data observed in this present study. Carvalho et al. (2014) observed increased activity values (0.245 to 0.314) with the DE increase (10 to 30) of maltodextrins used as a wall material in jussara (Martius Euterpe edulis) encapsulation.

3.5 Particle Size and Distribution Profile

The size of microencapsulated oil particles is an important factor, as it is associated with the stability and retention of the oils in the particles (Tontul and Topuz, 2014). Table 2 shows the particle size values and the polydispersion index for the treatments. Only the WM5 treatment differs significantly (p = 0.02) from the other treatments, presenting the highest average particle size (3.97 μm.) One reason for this observation may be the larger structure of the maltodextrin DE 5 that does not allow the formation of small droplets in the homogenization process and consequently the formation of large dry particles.

Particle size distribution profiles shown in Figure 4 corroborate the PDI values shown in Table 2 in which PDI values closer to zero indicate better particle size uniformity. Choi et al. (2010) also observed a size increase of conjugated linoleic acid microparticles encapsulated with WPC/MD compared to the use of only WPC. Generally, the size distribution of powdered food particles is more important than the average diameter measurement, as it may influence processing, handling and shelf-life (Tonon et al., 2009).

The morphology of the particles is an important quality parameter in microencapsulation processes because cracked or damaged structures can compromise the stability of the encapsulated material (Botrel et al., 2014b). All treatments showed microparticles with a rough surface. The addition of maltodextrins of different DE slightly improved particle roughness and occurrence of agglomeration. Smoother particle surfaces have been observed in other works when using maltodextrin as wall material (Ersus and Yurdagel, 2007; Osorio et al., 2011; Silva et al., 2014). Incorporating carbohydrates as wall materials can result in improvements in core material retention due to the formation of a complete crust without ruptures (Simon-Brown et al., 2016). The occurrence of roughness on the surface of the microparticles may be associated with shrinkage of the wall material during initial stages of the drying process. Smoother surfaces can occur when drying rates are favorable and the wall material has viscoelastic properties (Botrel et al., 2014b), as in the case of WPC.

3.6 Surfacel oil, Total oil and Encapsulation efficiency

Figure 5 shows surface oil values (%), encapsulation efficiency (%) and oil retained (%), for the lime essential oil microparticles. The amount of unencapsulated oil is a crucial parameter in the microencapsulation of oils because the exposed material is more susceptible to oxidation reactions, reducing the product shelf life (Karaca et al., 2013). The surface oil content

ranged between 2.15 to 3.28%, the treatments being significantly different from each other (p =10-7). The unencapsulated oil content was influenced by the dextrose equivalent of the maltodextrins used. We observed that the hydrolysis increase of the maltodextrin reduced the microparticles surface oil values. Botrel et al. (2014b), Turasan et al. (2015) and Young et al. (1993) found lower amounts of surface oil in blends of maltodextrins and whey protein, compared to treatments with only proteins in the microencapsulation of fish oil, rosemary essential oil and anhydrous milk fat.

For EE values (%), there is also a significant difference (p=10-5) among the different treatments. The increase of DE increases EE (%) and therefore increases the RO (%) values. The increase in DE values contributed to higher anthocyanin retention in kokum fruit (Nayak and Rastogi, 2010). The authors explained that the increase in DE values favored the barrier formation for the microparticles. High DE values improved protection of encapsulated materials since the higher the amount of carbohydrate units, the lower the permeability to oxygen (Jafari et al., 2008). Carbohydrates (mono- and disaccharides) favor crust formation during emulsions drying, reducing oil diffusion through the wall material layer, increasing the oil retention (R. V. D. B. Fernandes et al., 2014). Hogan et al. (2003) observed encapsulation efficiency values above 93.8% for fish oil microparticles encapsulated with maltodextrins with DE>14. Coalescence of droplets during drying, formation of air vacuoles during emulsion homogenization and biopolymer molecules structural properties are factors associated with oil retention in the microencapsulation process (Drusch et al., 2006; Silva and Meireles, 2015).

3.7 X-ray Diffraction

X-ray Diffraction techniques are widely utilized in bioactive compound microencapsulation studies because it demonstrates the degree of crystallinity of

the encapsulating material. The diffractograms for the wall materials and the lime essential oil microparticles are shown in Figure 6. For all results there are broadband regions at 2θ = 20°, which can indicate the interaction between wall material and lime essential oil. Broad bands are characteristic of amorphous material structure since in this kind of structure the molecules are in a more disordered state, increasing the x-ray diffraction band (Caparino et al., 2012). Amorphous solids have higher solubility and hygroscopicity compared to crystalline materials (Botrel et al., 2014b; Silva and Meireles, 2015). Matsuura et al. (2015) observed a very similar x-ray pattern for different maltodextrins used to microencapsulate coconut oil.

Furthermore, x-ray patterns for the microparticles are similar to the wall material patterns. It can be concluded that drying by spray-drying did not change the crystal structure of the materials. Amorphous biopolymer structures are more likely to be formed in drying processes by spray-drying (Botrel et al., 2014b; Langrish and Wang, 2009). In microencapsulation processes of food compounds, it is desirable that the polymeric structure of the encapsulant material has a vitreous matrix because it favors the core material retention (Drusch et al., 2006).

3.8 Antioxidant Activity

Figure 7 shows the antioxidant activity values obtained by the DPPH method for the lime essential oil microparticles. The addition of maltodextrin presented a significant difference ($p < 4.10$) for the DPPH values compared to the WPC-only treatment. The antioxidant activity was lower (72.72%) compared to treatments with the addition of different maltodextrins (90.65, 89.08, 94.71%). In acai microparticles (Tonon et al., 2009) observed higher antioxidant activity values for samples containing maltodextrin DE 10 (1165.84 TE micromol/g juice dried matter) in comparison to tapioca starch (1010.87 μmol TE/g juice dried matter).

Nayak e Rastogi (2010) observed an increase of antioxidant activity from 53.84 to 69.9% for dextrose equivalent values between 5 - 33. The authors explained that the increase in DE values favored the microparticle barrier formation, protecting the encapsulated bioactive compounds. Maltodextrins can offer good protection for encapsulated lipophilic materials, as less permeable barriers are related to high dextrose equivalent values (Jafari et al., 2008).

4 CONCLUSION

The emulsion physicochemical property results confirmed that maltodextrins with higher DE values have lower viscosities and consequently smaller droplet size. The degree of hydrolysis of the maltodextrin chains influenced particles with higher moisture and water activity. The surfaces of the particles had smoother surfaces for WM10 and WM20 treatments and visible fractures were not observed, which may protect the encapsulated material further. It was found the formation of particles with amorphous structures, typical of spray drying processes. Treatment with maltodextrin DE 20, showed better results for encapsulation efficiency and antioxidant activity of lime essential oil. In conclusion, the hydrolyzation degree of maltodextrins may interfere with the physicochemical parameters of lime essential oil microparticles; the best results for oil retention and protection were observed at higher DE values.

ACKNOWLEDGEMENT

The authors thank FAPEMIG and CNPq for the financial support.

REFERENCES

Akhavan Mahdavi, S., Jafari, S.M., Assadpoor, E., Dehnad, D., 2016. Microencapsulation optimization of natural anthocyanins with maltodextrin, gum Arabic and gelatin. Int. J. Biol. Macromol. 85, 379–385. doi:10.1016/j.ijbiomac.2016.01.011

Asbahani, A. El, Miladi, K., Badri, W., Sala, M., Addi, E.H.A., Casabianca, H., Mousadik, A. El, Hartmann, D., Jilale, A., Renaud, F.N.R., Elaissari, A., 2015. Essential oils: From extraction to encapsulation. Int. J. Pharm. 483, 220–243. doi:10.1016/j.ijpharm.2014.12.069

Bae, E.K., Lee, S.J., 2008. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. J. Microencapsul. 25, 549–560. doi:10.1080/02652040802075682

Bakry, A.M., Fang, Z., Ni, Y., Cheng, H., Chen, Y.Q., Liang, L., 2016. Stability of tuna oil and tuna oil/peppermint oil blend microencapsulated using whey protein isolate in combination with carboxymethyl cellulose or pullulan. Food Hydrocoll. 60, 559–571. doi:10.1016/j.foodhyd.2016.04.026

Baranauskienė, R., Venskutonis, P.R., Dewettinck, K., Verhé, R., 2006. Properties of oregano (Origanum vulgare L.), citronella (Cymbopogon nardus G.) and marjoram (Majorana hortensis L.) flavors encapsulated into milk protein-based matrices. Food Res. Int. 39, 413–425. doi:10.1016/j.foodres.2005.09.005

Botrel, D.A., Borges, S.V., Fernandes, R.V. de B., Carmo, E.L. do, 2014a. Optimization of fish oil spray drying using a protein: inulin system. Dry. Technol. 32, 279–290. doi:10.1080/07373937.2013.823621

Botrel, D.A., de Barros Fernandes, R.V., Borges, S.V., Yoshida, M.I., 2014b. Influence of wall matrix systems on the properties of spray-dried microparticles containing fish oil. Food Res. Int. 62, 344–352. doi:10.1016/j.foodres.2014.02.003

Caparino, O.A., Tang, J., Nindo, C.I., Sablani, S.S., Powers, J.R., Fellman, J.K., 2012. Effect of drying methods on the physical properties and microstructures of mango (Philippine “Carabao” var.) powder. J. Food Eng. 111, 135–148.

doi:10.1016/j.jfoodeng.2012.01.010

Carmo, E.L. do, Fernandes, R.V. de B., Borges, S.V., 2015. Microencapsulation by spray drying, new biopolymers and applications in food technology. J. Chem. Eng. Chem. 1, 30–44. doi:10.18540/2446941601022015030

Carneiro, H.C.F., Tonon, R. V., Grosso, C.R.F., Hubinger, M.D., 2013. Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. J. Food Eng. 115, 443–451. doi:10.1016/j.jfoodeng.2012.03.033

Carneiro, H.C.F., Tonon, R. V, Grosso, C.R.F., Hubinger, M.D., 2013. Effect of different combination of wall materials on the encapsulation efficiency of flaxseed oil microencapsulated by spray drying 115, 443–451.

Carvalho, A.G.S., Silva, V.M., Hubinger, M.D., 2014. Microencapsulation by spray drying of emulsified green coffee oil with two-layered membranes. Food Res. Int. 61, 236–245. doi:10.1016/j.foodres.2013.08.012

Castro, N., Durrieu, V., Raynaud, C., Rouilly, A., 2016. Influence of DE-value on the physicochemical properties of maltodextrin for melt extrusion processes. Carbohydr. Polym. 144, 464–473. doi:10.1016/j.carbpol.2016.03.004

Choi, K., Ryu, J., Kwak, H., Ko, S., 2010. Spray-dried conjugated linoleic acid encapsulated with Maillard reaction products of whey proteins and maltodextrin. Food Sci. Biotechnol. 19, 957–965. doi:10.1007/s10068-010-0134-7

Cruz-Valenzuela, M.R., Tapia-Rodriguez, M.R., Vazquez-Armenta, F.J., Silva-Espinoza, B.A., Ayala-Zavala, J.F., 2016. Lime (Citrus aurantifolia) oils, in: Preedy, V.R. (Ed.), Essential Oils in Food Preservation, Flavor and Safety. Elsevier Inc., London, pp. 531–538.

da Costa, J.M.G., Silva, E.K., Toledo Hijo, A.A.C., Azevedo, V.M., Malta, M.R., Ferreira Alves, J.G.L., Borges, S. V, 2015. Microencapsulation of Swiss

cheese bioaroma by spray-drying: Process optimization and characterization of particles. Powder Technol. 274, 296–304. doi:10.1016/j.powtec.2015.01.037

Díaz, D.I., Beristain, C.I., Azuara, E., Luna, G., Jimenez, M., 2015. Effect of wall material on the antioxidant activity and physicochemical properties of Rubus fruticosus juice microcapsules. J. Microencapsul. 32, 247–254. doi:10.3109/02652048.2015.1010458

Dokic-Baucal, L., Dokic, P., Jakovljevic, J., 2004. Influence of different maltodextrins on properties of O/W emulsions. Food Hydrocoll. 18, 233–239. doi:10.1016/S0268-005X(03)00068-7

Drusch, S., Serfert, Y., Van Den Heuvel, A., Schwarz, K., 2006. Physicochemical characterization and oxidative stability of fish oil encapsulated in an amorphous matrix containing trehalose. Food Res. Int. 39, 807–815. doi:10.1016/j.foodres.2006.03.003

Dziedzic, S.Z., Kearsley, M.W., 1995. Handbook of starch hydrolysis products and their derivates, First. ed. Blackie Academic & Professional, London.

Ersus, S., Yurdagel, U., 2007. Microencapsulation of anthocyanin pigments of black carrot (Daucus carota L.) by spray drier. J. Food Eng. 80, 805–812. doi:10.1016/j.jfoodeng.2006.07.009

Escalona-García, L.A., Pedroza-Islas, R., Natividad, R., Rodríguez-Huezo, M.E., Carrillo-Navas, H., Pérez-Alonso, C., 2016. Oxidation kinetics and thermodynamic analysis of chia oil microencapsulated in a whey protein concentrate-polysaccharide matrix. J. Food Eng. 175, 93–103. doi:10.1016/j.jfoodeng.2015.12.009

Fazaeli, M., Emam-Djomeh, Z., Kalbasi Ashtari, A., Omid, M., 2012. Effect of spray drying conditions and feed composition on the physical properties of black mulberry juice powder. Food Bioprod. Process. 90, 667–675.

doi:10.1016/j.fbp.2012.04.006

Fernandes, R.V. de B., Borges, S.V., Botrel, D.A., Silva, E.K., Costa, J.M.G. da, Queiroz, F., 2013. Microencapsulation of rosemary essential oil: characterization of particles. Dry. Technol. 31, 1245–1254. doi:10.1080/07373937.2013.785432

Fernandes, R.V.B., Marques, G.R., Borges, S. V., Botrel, D.A., 2014. Effect of solids content and oil load on the microencapsulation process of rosemary essential oil. Ind. Crops Prod. 58, 173–181. doi:10.1016/j.indcrop.2014.04.025

Fernandes, R.V.D.B., Borges, S.V., Botrel, D.A., 2014. Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. Carbohydr. Polym. 101, 524–532. doi:10.1016/j.carbpol.2013.09.083

Gamarra, F.M.C., Sakanaka, L.S., Tambourgi, E.B., Cabral, F.A., 2006. Influence on the quality of essential lemon (Citrus aurantifolia) oil by distillation process. Brazilian J. Chem. Eng. 23, 147–151. doi:10.1590/S0104-66322006000100016

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R., 2007. Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Res. Int. 40, 1107–1121. doi:10.1016/j.foodres.2007.07.004

Goula, A.M., Adamopoulos, K.G., 2010. A new technique for spray drying orange juice concentrate. Innov. Food Sci. Emerg. Technol. 11, 342–351. doi:10.1016/j.ifset.2009.12.001

Goula, A.M., Adamopoulos, K.G., 2005. Spray drying of tomato pulp in dehumidified air: II. The effect on powder properties. J. Food Eng. 66, 35–42. doi:10.1016/j.jfoodeng.2004.02.031

Hogan, S.A., O'Riordan, E.D., O'Sullivan, M., 2003. Microencapsulation and oxidative stability of spray-dried fish oil emulsions. J. Microencapsul. 20, 675–

688. doi:10.3109/02652040309178355

Jafari, S.M., Assadpoor, E., He, Y., Bhandari, B., 2008. Encapsulation efficiency of food flavours and oils during spray drying. Dry. Technol. 26, 816–835. doi:10.1080/07373930802135972

Karaca, A.C., Nickerson, M., Low, N.H., 2013. Microcapsule production employing chickpea or lentil protein isolates and maltodextrin: Physicochemical properties and oxidative protection of encapsulated flaxseed oil. Food Chem. 139, 448–457. doi:10.1016/j.foodchem.2013.01.040

Klongdee, S., Thongngam, M., Klinkesorn, U., 2012. Rheology and microstructure of lecithin-stabilized tuna oil emulsions containing chitosan of varying concentration and molecular size. Food Biophys. 7, 155–162. doi:10.1007/s11483-012-9253-4

Langrish, T.A.G., Wang, S., 2009. Crystallization rates for amorphous sucrose and lactose powders from spray drying: A comparison. Dry. Technol. 27, 606–614. doi:10.1080/07373930802716391

Liu, X.-D., Atarashi, T., Furuta, T., Yoshii, H., Aishima, S., Ohkawara, M., Linko, P., 2001. Microencapsulation of emulsified hydrophobic flavors by spray drying. Dry. Technol. 19, 1361–1374. doi:10.1081/DRT-100105293

Masters, K., 1991. Spray Drying: an introduction to principles, operation practice and application, Fifith. ed. Longman Scientific & Technical, London. doi:10.1002/cite.330451311

Matsuura, T., Ogawa, A., Tomabechi, M., Matsushita, R., Gohtani, S., Neoh, T.L., Yoshii, H., 2015. Effect of dextrose equivalent of maltodextrin on the stability of emulsified coconut-oil in spray-dried powder. J. Food Eng. 163, 54–59. doi:10.1016/j.jfoodeng.2015.04.018

Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C. dos, Coube,

C.S., Leitão, S.G., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phyther. Res. 15, 127–130. doi:10.1002/ptr.687

Nayak, C.A., Rastogi, N.K., 2010. Effect of selected additives on microencapsulation of anthocyanin by spray drying. Dry. Technol. 28, 1396–1404. doi:10.1080/07373937.2010.482705

Osorio, C., Forero, D.P., Carriazo, J.G., 2011. Characterisation and performance assessment of guava (Psidium guajava L.) microencapsulates obtained by spray-drying. Food Res. Int. 44, 1174–1181. doi:10.1016/j.foodres.2010.09.007

Rodea-González, D.A., Cruz-Olivares, J., Román-Guerrero, A., Rodríguez-Huezo, M.E., Vernon-Carter, E.J., Pérez-Alonso, C., 2012. Spray-dried encapsulation of chia essential oil (Salvia hispanica L.) in whey protein concentrate-polysaccharide matrices. J. Food Eng. 111, 102–109. doi:10.1016/j.jfoodeng.2012.01.020

Silva, E.K., Gomes, M.T.M.S., Hubinger, M.D., Cunha, R.L., Meireles, M.A.A., 2015. Ultrasound-assisted formation of annatto seed oil emulsions stabilized by biopolymers. Food Hydrocoll. 47, 1–13. doi:10.1016/j.foodhyd.2015.01.001

Silva, E.K., Meireles, M.A.A., 2015. Influence of the degree of inulin polymerization on the ultrasound-assisted encapsulation of annatto seed oil. Carbohydr. Polym. 133, 578–586. doi:10.1016/j.carbpol.2015.07.025

Silva, V.M., Vieira, G.S., Hubinger, M.D., 2014. Influence of different combinations of wall materials and homogenisation pressure on the microencapsulation of green coffee oil by spray drying. Food Res. Int. 61, 132–143. doi:10.1016/j.foodres.2014.01.052

Simon-Brown, K., Solval, K.M., Chotiko, A., Alfaro, L., Reyes, V., Liu, C., Dzandu, B., Kyereh, E., Goldson Barnaby, A., Thompson, I., Xu, Z., Sathivel,

S., 2016. Microencapsulation of ginger (Zingiber officinale) extract by spray drying technology. LWT - Food Sci. Technol. 70, 119–125. doi:10.1016/j.lwt.2016.02.030

Takeungwongtrakul, S., Benjakul, S., H-kittikun, A., 2015. Wall materials and the presence of antioxidants influence encapsulation efficiency and oxidative stability of micro-encapsulated shrimp oil. Eur. J. Lipid Sci. Technol. 117, 450–459. doi:10.1002/ejlt.201400235

Tavares, G.M., Croguennec, T., Carvalho, A.F., Bouhallab, S., 2014. Milk proteins as encapsulation devices and delivery vehicles: Applications and trends. Trends Food Sci. Technol. 37, 5–20. doi:10.1016/j.tifs.2014.02.008

Telis, V.R.N., 2012. Biopolymer engineering in food processing, First Edit. ed. CRC Press, Boca Raton.

Tonon, R. V., Pedro, R.B., Grosso, C.R.F., Hubinger, M.D., 2012. Microencapsulation of flaxseed oil by spray drying: effect of oil load and type of wall material. Dry. Technol. 30, 1491–1501. doi:10.1080/07373937.2012.696227

Tonon, R. V, Brabet, C., Pallet, D., Brat, P., Hubinger, M.D., 2009. Physicochemical and morphological characterisation of açai (Euterpe oleraceae Mart.) powder produced with different carrier agents. Int. J. Food Sci. Technol. 44, 1950–1958. doi:10.1111/j.1365-2621.2009.02012.x

Tontul, I., Topuz, A., 2014. Influence of emulsion composition and ultrasonication time on flaxseed oil powder properties. Powder Technol. 264, 54–60. doi:10.1016/j.powtec.2014.05.002

Turasan, H., Sahin, S., Sumnu, G., 2015. Encapsulation of rosemary essential oil. LWT - Food Sci. Technol. 64, 112–119. doi:10.1016/j.lwt.2015.05.036

Young, S.L., Sarda, X., Rosenberg, M., 1993. Microencapsulating properties of

whey proteins. 2. Combination of whey proteins with carbohydrates. J. Dairy Sci. 76, 2878–2885. doi:10.3168/jds.S0022-0302(93)77626-2

Zuidam, N.J., Shimoni, E., 2010. Overview of microencapsulates for use in food products or processes and methods to make them, in: Encapsulation Technologies for Active Food Ingredients and Food Processing. Springer New York, New York, NY, pp. 3–29. doi:10.1007/978-1-4419-1008-0_2

FIGURE CAPTIONS

Figure 1 - Apparent viscosidade for emulsions of lime essential oil.

Figure 2 - Optical micrographs and droplet size distribution of Lime essential oil.

Figure 3 – Chemical composition of lime essential oil crude and microencapsulated.

Figure 4 - Particle Size distributions and MEV of Lime essential oil microparticles

Figure 5 -Surface oil, Total oil, Encapsulation Efficiency and Retention oil of Lime essential oil. Same letters in the same column do not differ significantly ($p > 0.05$).

Figure 6 - X-ray diffraction patterns of wall materials and lime essential oil microparticles.

Figure 7 - Antioxidant activity (%) to crude oil and microparticles of lime essential oil. Same letters in the same column do not differ significantly ($p > 0.05$).

Figure 1

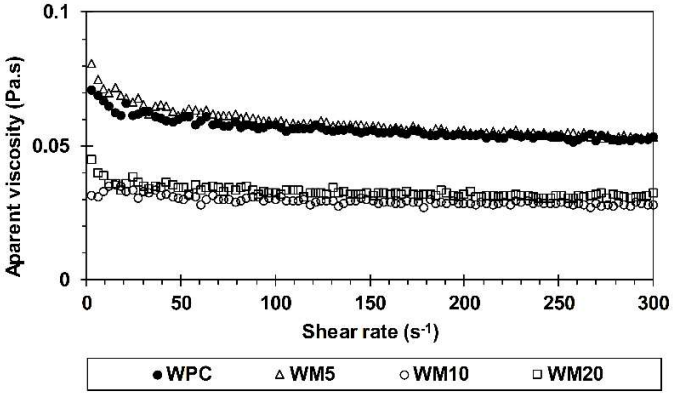
0.1
0.05
0
Aparent viscosity (Pa.s)
0
50
100
150
200
250
300
Shear rate (s-1)
WPC
WM5
WM10
WM20

Figure 2

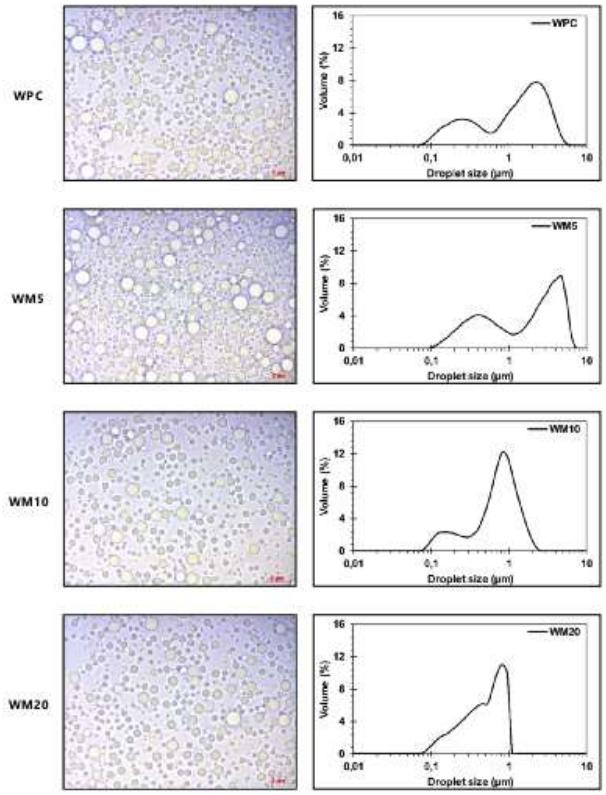

Figure 3

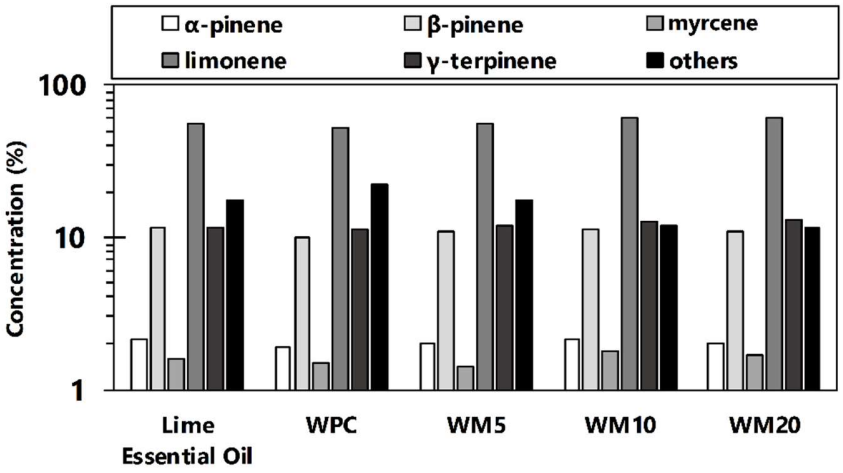
α-pinene
β-pinene
myrcene
limonene
γ-terpinene
others
100
10
1
Concentration (%)
Lime Essential Oil
WPC
WM5
WM10
WM20

Figure 4

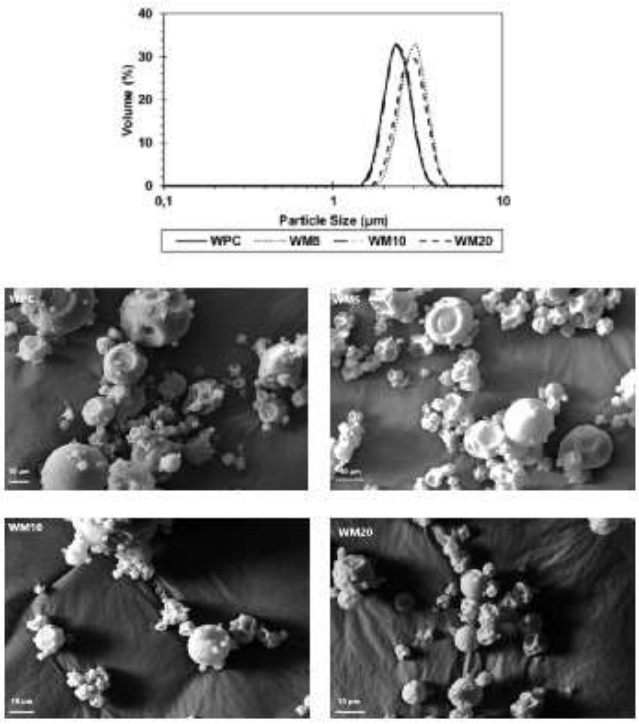
40
30
20
10
0
Volume (%)
0,1
1
10
Particle Size (µm)
WPC
WM5
WM10
WM20
WPC
WM5
WM10
WM20

Figure 5

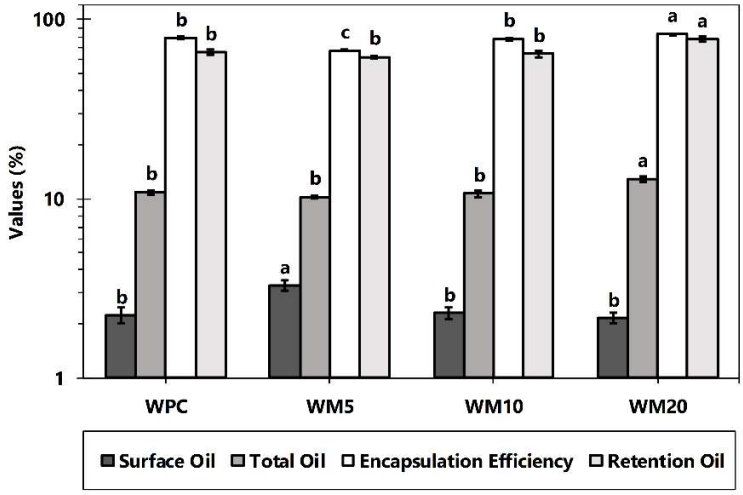
100
10
1
Values (%)
b
b
b
b
a
b
c
b
b
b
b
b
b
a
a
a
WPC
WM5
WM10
WM20
Surface Oil
Total Oil
Encapsulation Efficiency
Retention Oil

Figure 6

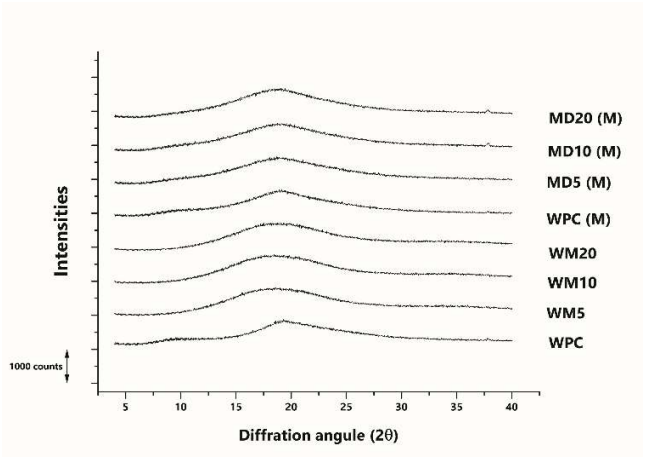
MD20 (M)
MD10 (M)
MD5 (M)
WPC (M)
WM20
WM10
WM5
WPC
Intensities
1000 counts
5
10
15
20
25
30
35
40
Diffration angule (2θ)

Figure 7

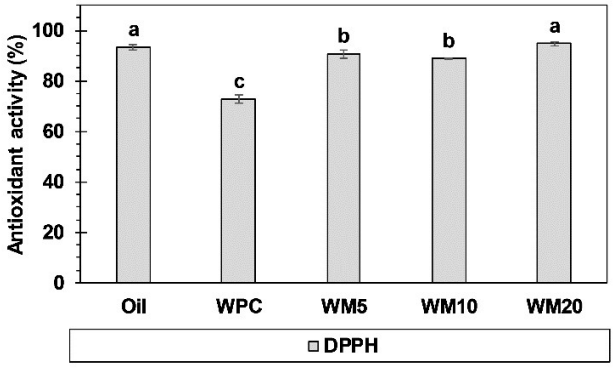
Antioxidant activity (%)
100
80
60
40
20
0
a
c
b
b
a
Oil
WPC
WM5
WM10
WM20
DPPH

Table 1 - Composition of the wall materials for each treatment used as a feed solution for the spray-drying process

Assay	Wall material (g.100g^{-1} of solution)				Core material (g.100g^{-1} of solution)
	WPC	**MD5**	**MD10**	**MD20**	**Lime essential Oil**
WPC	25				5
WM5	15	10			5
WM10	15		10		5
WM20	15			10	5

Table 2 - Rheological parameter to power law of lime essential oil emulsions.

Assay	*Rheological Parameters*		*E(%)*
	Consistency coefficient, K (mPa.sn)	***Flow Behaviour index, n***	
WPC	8.07	0.92	1.26
WM5	9.38	0.9	1.16
WM10	3.84	0.94	2.24
WM20	4.26	0.94	1.95

Table 3 - Lime essential oil emulsion and particles characteristics

Assay	Droplet size (µm)	PDI (Droplet)	Moisture Content (g water.100g dry solid^{-1})	Water Activity	Particle size (µm)	PDI (Particle)
WPC	0.30 ± 0.01^{a}	0.367 ± 0.02^{a}	3.83 ± 0.34^{b}	0.124 ± 0.02^{c}	2.74 ± 0.31^{b}	0.364 ± 0.05^{a}
WM5	0.31 ± 0.02^{a}	0.425 ± 0.02^{a}	3.91 ± 0.59^{b}	0.279 ± 0.03^{b}	3.97 ± 0.35^{a}	0.239 ± 0.01^{c}
WM10	0.28 ± 0.01^{b}	0.434 ± 0.01^{a}	5.35 ± 0.04^{a}	0.297 ± 0.03^{b}	2.67 ± 0.09^{b}	0.275 ± 0.06^{b}
WM20	0.28 ± 0.02^{b}	0.288 ± 0.02^{b}	5.31 ± 0.35^{a}	0.342 ± 0.05^{a}	2.7 ± 0.02^{b}	0.371 ± 0.02^{a}

Same letters in the same column do not differ significantly (p> 0.05).

CONCLUSÃO GERAL

A avaliação da utilização de biopolímero como estabilizante em emulsões de óleo essencial de limão se mostrou viável, visto que se obteve emulsões estáveis durante as primeiras 4 horas após o processo de homogeneização.

A adição de maltodextrina otimizou as propriedades físico-químicas das micropartículas, protegendo mais o óleo essencial de limão principalmente por estes carboidratos reduzirem a permeabilidade da parede das micropartículas.

`Portanto, a utilização de biopolímeros se mostrou uma alternativa interessante no processo de microencapsulação de óleo essencial de limão, obtendo emulsões estáveis e micropartículas com alta eficiência e retenção dos compostos voláteis.