UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

DESENVOLVIMENTO E AVALIAÇÃO DA ESTABILIDADE DE NANOCÁPSULAS POLIMÉRICAS DE LICOPENO

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PORTO ALEGRE – RS

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DESENVOLVIMENTO E AVALIAÇÃO DA ESTABILIDADE DE NANOCÁPSULAS POLIMÉRICAS DE LICOPENO

Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal do Rio Grande do Sul, como requisito para a obtenção do título de Doutor em Ciência e Tecnologia de Alimentos.

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“Eu prefiro ser essa metamorfose ambulante, do que ter aquela velha opinião formada sobre tudo...”

Raul Seixas

RESUMO

O licopeno é um composto bioativo que tem recebido atenção especial devido aos seus efeitos terapêuticos no combate e prevenção de doenças como câncer e doenças cardiovasculares. No entanto, por ser insolúvel em água, muito instável na presença de luz, oxigênio e calor, e altamente reativo, sua aplicação nos alimentos é limitada, o que prejudica o acesso do consumidor a produtos naturais como fontes de antioxidantes em substituição aos antioxidantes sintéticos. Assim, a utilização de nanocápsulas de compostos naturais, com ação antioxidante, pode ser considerada uma das tecnologias mais promissoras para disponibilizar compostos mais estáveis e disponíveis ao consumidor. Nesse contexto, nanocápsulas de licopeno extraído do tomate foram desenvolvidas e caracterizadas com o objetivo de conferir solubilidade aparente e estabilidade ao carotenoide em diferentes condições de armazenamento e processamento. O tipo de nanopartícula, a técnica de encapsulamento e o material de parede foram selecionados a partir de um levantamento na literatura, o qual originou os artigos de revisão apresentados. As nanocápsulas de licopeno (LYC-LNC) foram sintetizadas pela técnica de deposição interfacial do polímero pré-formado poli (Ɛ-caprolactona) (PCL), mantidas em meio aquoso e caracterizadas com relação aos parâmetros de diâmetro médio, potencial zeta, índice de polidispersão, eficiência de encapsulação, morfologia, pH, cor, viscosidade e concentração de licopeno. Além disso, a estabilidade das nanocápsulas foi avaliada durante armazenamento a 5 °C e 25 °C, em experimentos de fotosensibilização a 5, 15 e 25°C em condições de saturação com ar e N_2; e durante aquecimento a 60, 70 e 80 °C na ausência de luz. LYC-LNC apresentaram diâmetro médio de 193 ± 4,7 nm, índice de polidispersão de 0,069 ± 0,02, potencial zeta de -11,5 ± 0,4 mV, viscosidade de 1,09 ± 0,03 mPa.s e pH médio de 6,01 ± 0,04. Durante o armazenamento a 25°C, a suspensão LYC-LNC foi considerada estável pela ausência de alterações significativas no diâmetro, potencial zeta e apesar da redução significativa do pH, cor e concentração de licopeno, as nanocápsulas apresentaram estabilidade satisfatória com aproximadamente 50% do conteúdo total de licopeno após 14 dias de estocagem. Quando as amostras foram armazenadas a 5°C, LYC-LNC mostraram a mesma estabilidade para os parâmetros citados, exceto em relação à concentração do licopeno que teve uma melhora significativa com 40% do conteúdo total do carotenoide após 84 dias de estocagem. A degradação do licopeno durante a fotosensibilização e aquecimento seguiu uma cinética de degradação de primeira ordem e apresentaram energia de ativação de 67,0 kcal/mol e 24,9 kcal/mol respectivamente, valores superiores aos relatados pela literatura. Com base nos resultados obtidos, o presente estudo demonstrou que o nanoencapsulamento é uma técnica que pode, além de aumentar a solubilidade aparente do licopeno em meio aquoso, conferir melhoria na estabilidade em diferentes condições e por isso as LYC-LNC representam uma alternativa promissora para expandir o uso do licopeno em processos industriais, para melhorar a retenção deste composto em diferentes matrizes alimentares.

Palavras–chave: Licopeno, nanocápsulas, estabilidade, aquecimento, oxigênio singleto, nanotecnologia.

ABSTRACT

Lycopene is a bioactive compound that has received special attention due to their therapeutic effects in combating and preventing of diseases such as cancer and cardiovascular disease. However, lycopene is water insoluble, very unstable in presence of light, oxygen and heat and highly reactive, characteristics that can limit its application in food, affecting the access of consumer for natural products that can serve as sources of antioxidants, substituting synthetic antioxidants. Thus, the use of nanocapsules containing natural compounds with antioxidant action can be considered one of the most promising technologies to provide compounds more stable and available to the consumer. In this context, nanocapsules containing lycopene extracted from tomato have been characterized and developed with the objective of conferring apparent solubility and stability of this carotenoid in different storage and processing conditions. The type of nanoparticles, the encapsulation technique and the wall material were selected from a research in the literature, which resulted in review articles. The lycopene nanocapsules (LYC-LNC) were synthesized by the interfacial deposition of preformed poly(Ɛ-caprolactone) (PCL) maintained in an aqueous medium and characterized regarding to parameters such as mean diameter, zeta potential, polydispersity index, encapsulation efficiency, morphology, pH, color, viscosity and lycopene concentration. Furthermore, the stability of nanocapsules during storage was evaluated at 5 °C and 25 °C, in experiments of photosensitization at 5, 15 and 25 °C in saturated conditions with air and N_2; and during heating at 60, 70 and 80 °C in the absence of light. LYC-LNC had an average diameter of 193 ± 4.7 nm, polydispersity of 0.069 ± 0.02, zeta potential of -11.5 ± 0.4 mV, viscosity of 1.09 ± 0.03 mPa.s and pH value of 6.01 ± 0.04. During storage at 25 °C, the LYC-LNC suspension remained stable and no significative changes in diameter and zeta potential were observed, and despite of significant reductions in pH, color and lycopene concentration, nanocapsules showed satisfactory stability containing approximately 50% the total content of lycopene after 14 days of storage. However, when the samples were stored at 5 °C, LYC-LNC showed the same stability for the mentioned parameters, except for the lycopene concentration that had a significant improvement, presenting approximately 40% of the total carotenoid content after 84 days of storage. The degradation of lycopene during photosensitization and heating followed a kinetic of first order and showed activation energy of 67.0 kcal/mol and 24.9 kcal/mol respectively, activation energy higher than those reported in the literature. Based on these results, this study demonstrated that the nanoencapsulation is a technique which can, in addition to increasing apparent solubility of lycopene in aqueous medium, confer better stability under different conditions and thus, LYC-LNC represent a promising alternative to expand the use of lycopene in industrial processes by improving the retention of this compound in different food matrices.

Keywords: Lycopene, nanocapsules, stability, heating, singlet oxygen, nanotechnology.

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Sumário

1. INTRODUÇÃO

Os compostos bioativos são substâncias químicas encontradas comumente em vegetais e podem produzir efeitos biológicos terapêuticos para combater e prevenir doenças. Atualmente, existe grande interesse em compostos biologicamente ativos, oriundos de fontes naturais, que podem ser incorporados a formulações que permitam oferecer benefícios à saúde, seja na forma de alimentos funcionais ou como produtos medicinais para o tratamento de diversas doenças (ZAKYNTHINOS e VARZAKAS, 2016). No entanto, alguns compostos bioativos, como os carotenoides, são insolúveis ou fracamente solúveis em água devido sua forma cristalina, o que torna difícil a incorporação em produtos alimentares com alta atividade de água, além de apresentarem menor estabilidade a fatores externos e biodisponibilidade (RIBEIRO e CRUZ, 2005).

O licopeno é um importante carotenoide que tem recebido atenção especial devido aos efeitos benéficos à saúde, como redução do risco de câncer (BRAMLEY, 2000; NIRANJANA *et al.*, 2015), doenças cardiovasculares (SAINI *et al.*, 2015) e redução dos níveis de colesterol (RIED e FAKLER, 2011). No entanto, o licopeno é muito instável na presença de luz, oxigênio e calor devido à presença de onze ligações conjugadas e duas ligações duplas não conjugadas, o que limita a sua aplicação nos alimentos a nível industrial. Assim, uma possibilidade de superar as limitações tecnológicas, disponibilizar este composto, para aumentar sua estabilidade em condições de processamento e armazenamento, é a utilização da nanotecnologia.

A nanotecnologia rapidamente emergiu como um dos campos de pesquisa mais promissores e atraentes, com aplicações que vão do espaço aéreo para as indústrias farmacêuticas e alimentos e esta técnica oferece potencial para melhorar a biodisponibilidade e solubilidade de diferentes ingredientes funcionais, como carotenoides, ácidos graxos, entre outros (QUINTANILLA-CARVAJAL *et al.*, 2010).

A utilização da nanotecnologia tem sido promissora na área de alimentos, sendo que estudos demonstram seu potencial uso em praticamente todos os segmentos da indústria, desde a agricultura em pesticidas e fertilizantes (SUN *et al.,* 2014) até o processamento de alimentos como encapsulação de realçadores de sabor ou odor, compostos bioativos e agentes de textura, sendo utilizados para melhoria na qualidade de alimento, em embalagens e para suplementos nutricionais (EZHILARASI *et al*., 2012).

O nanoencapsulamento consiste na incorporação e dispersão de compostos em pequenos veículos com diâmetro na nanoescala tais como lipossomas, nanoemulsões ou nanocápsulas poliméricas. Os compostos incorporados podem ser protegidos contra a degradação, com aumento de sua estabilidade e solubilidade (por exemplo, a solubilização de um composto hidrofílico em matrizes hidrofóbicas e vice e versa) e, portanto, pode aumentar a biodisponibilidade (LETCHFORD e BURT, 2007). No entanto, é importante assegurar que o tipo de nanopartícula escolhida, a técnica de obtenção e o material de parede utilizado no nanoencapsulamento não comprometam a capacidade antioxidante dos compostos incorporados e aumentem a sua estabilidade, quando comparados ao composto na sua forma livre, em diferentes condições de processamento. Neste contexto, o nanoencapsulamento pode ser utilizado pela indústria como uma estratégia inovadora para expandir o uso de carotenoides em processos industriais, por exemplo, como substitutos de corantes sintéticos, uma vez que pode proteger esses compostos das diversas condições de processamento. Desta forma, este trabalho teve por objetivo desenvolver nanocápsulas poliméricas de licopeno e avaliar suas características físico-químicas, sua estabilidade térmica e fotoestabilidade.

A presente tese está estruturada em cinco capítulos conforme descritos a seguir. O capítulo 1 corresponde à fundamentação teórica que aborda aspectos relacionados aos carotenoides, suas principais fontes, em especial o licopeno, estrutura e estabilidade. Aspectos relacionados à nanotecnologia são abordados resumidamente neste capítulo, sendo a fundamentação teórica mais aprofundada no capítulo 2 que contem dois artigos de revisão que tratam exclusivamente da aplicação da nanotecnologia em compostos bioativos dando ênfase aos tipos de nanoestruturas utilizadas, as técnicas de caracterização e obtenção de nanopartícula e os tipos de material de parede utilizados na síntese de nanocápsulas.

O capítulo 3 descreve detalhadamente a metodologia adotada nesta pesquisa para a realização dos trabalhos que compõem a parte experimental da tese. O capítulo 4 corresponde aos artigos experimentais publicados em revistas de relevância internacional que justificam a importância desta pesquisa. E por fim, o capítulo 5 apresenta uma discussão geral de todos os resultados obtidos e conclusões gerais a cerca da importância da realização de estudos dessa natureza na área de Ciência e Tecnologia de Alimentos.

2. OBJETIVOS

2.1. GERAL:

O Objetivo geral do presente trabalho foi desenvolver e caracterizar nanocápsulas contendo licopeno, e avaliar a sua estabilidade em diferentes condições de armazenamento.

2.2. ESPECÍFICOS

Neste contexto, os objetivos específicos estão listados a seguir.

I. Desenvolver e caracterizar nanocápsulas de licopeno extraído de tomate, produzidas pela técnica de deposição interfacial de polímero pré-formado utilizando Poli (ε-caprolactona) (PCL) através da determinação da eficiência de encapsulamento, pH, viscosidade, análise colorimétrica, diâmetro médio da partícula, microscopia eletrônica de transmissão e potencial zeta.

II. Avaliar a estabilidade das nanocápsulas de licopeno pela determinação do diâmetro médio, potencial zeta, pH, análise colorimétrica, concentração de licopeno durante armazenamento a 5 °C e 25 °C.

III. Avaliar a estabilidade do licopeno nanoencapsulado durante fotosensibilização a 5 °C, 15 °C e 25 °C na presença de oxigênio singleto formado pela sensibilização com azul de metileno e determinar a energia de ativação no processo de degradação do licopeno.

IV. Avaliar a estabilidade do licopeno nanoencapsulado durante aquecimento a 60 °C, 70 °C e 80 °C e determinar a energia de ativação no processo de degradação do licopeno.

CAPÍTULO 1- FUNDAMENTAÇÃO TEÓRICA

Este capítulo apresenta uma revisão geral sobre carotenoides, abordando definições, fontes de obtenção, aspectos estruturais e químicos com enfoque principal no licopeno, objeto de estudo deste trabalho. O capítulo apresenta também uma breve abordagem sobre nanoencapsulamento, sendo este tema mais aprofundado no capítulo de artigos de revisão.

3. Fundamentação Teórica

3.1. Compostos bioativos

Compostos bioativos são substâncias químicas sintetizadas de forma natural pelos diferentes organismos vivos, os quais produzem vários efeitos biológicos. Atualmente, existe grande interesse em compostos biologicamente ativos, oriundos de fontes naturais, que podem ser incorporados a formulações que permitam oferecer benefícios à saúde, seja na forma de alimentos funcionais ou como produtos medicinais para o tratamento de diversas doenças (ZAKYNTHINOS e VARZAKAS, 2016).

Os compostos bioativos variam extensamente em estrutura química e, consequentemente, na função biológica, mas, apesar das diferenças, eles apresentam algumas características em comum: pertencem a alimentos do reino vegetal, são substâncias orgânicas que não são sintetizados pelo organismo humano e apresentam ação protetora na saúde humana quando presentes na dieta em quantidades significativas (LAJOLO e HORST, 2009).

As substâncias consideradas como bioativas podem ser nutrientes como a vitamina C, vitamina E, ácido fólico, cálcio e niacina ou não nutrientes, incluindo diferentes tipos de compostos químicos entre os quais se destacam aqueles que apresentam efeito preventivo contra o câncer, incluindo fibras, compostos fenólicos, terpenoides, carotenoides, isotiocianatos aromáticos, entre outros (BIESALSKI *et al.*, 2009). Além disso, alguns compostos bioativos, como os carotenoides, têm sido objeto de estudo pelo seu alto potencial antioxidativo e pela sua capacidade em conferir cor aos alimentos.

3.1.1. Carotenoides

Os carotenoides são pigmentos naturais, lipossolúveis, que proporcionam coloração viva a plantas e animais com mais de 600 compostos já identificados (DAMODARAN *et al.*, 2010); são geralmente tetraterpenoides de 40 átomos de carbono, constituídos inclusive de isômeros, todos polisoprenoides, e possuem uma cadeia poliênica que pode ter de 3 a 15 duplas ligações conjugadas. O comprimento do cromóforo determina o espectro de absorção e a cor do composto, por esta razão tais moléculas são pigmentos que variam entre o amarelo, laranja e vermelho (FRASER e BRAMLEY, 2004).

Os carotenoides (Figura 1) são classificados segundo sua estrutura química como carotenos (Figura 1 A, B, F, I, L) quando constituídos apenas por carbono e hidrogênio, ou

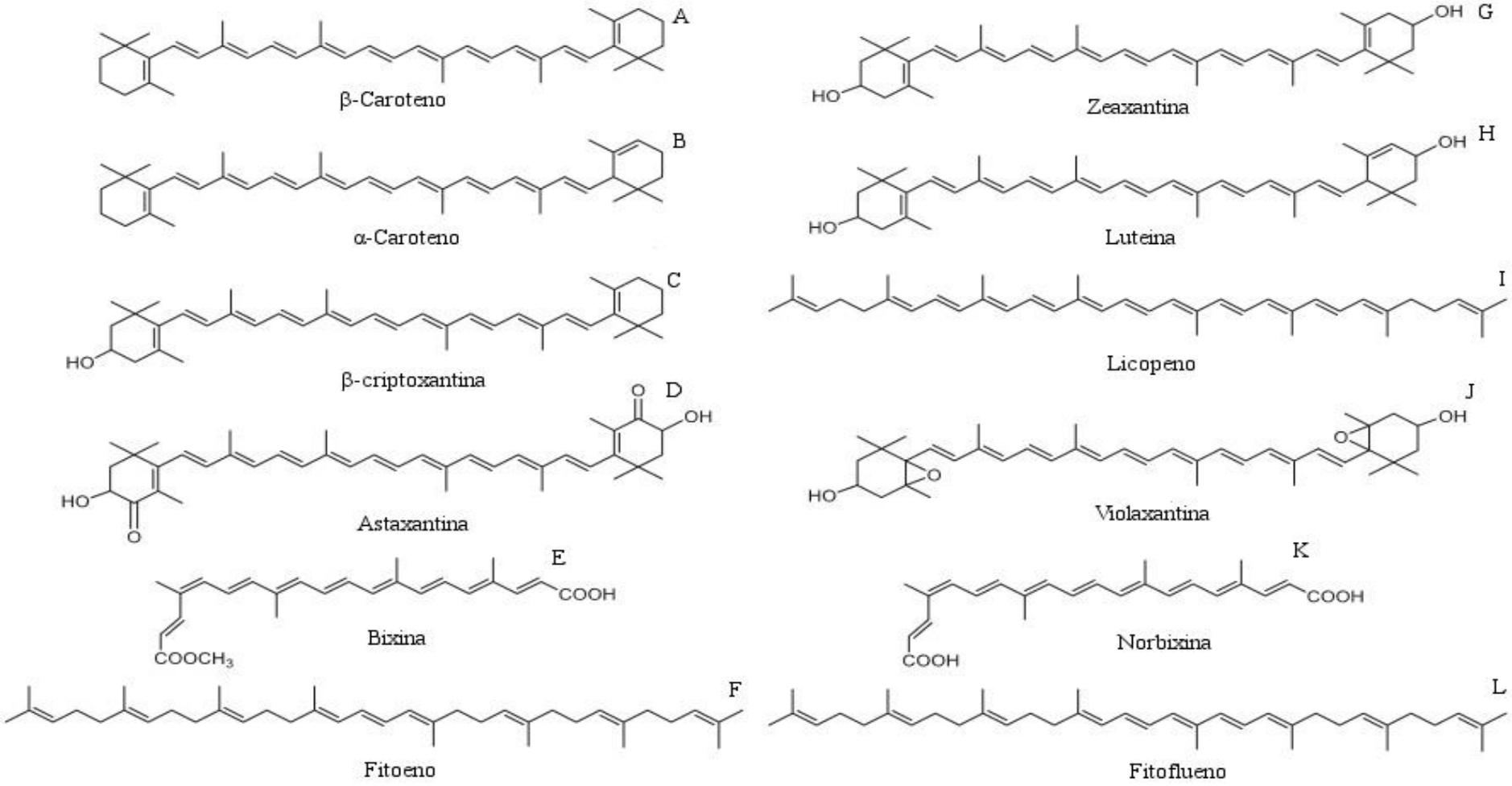
como xantofilas quando constituídos por carbono, hidrogênio e oxigênio (Figura 1 C, D, E, G, H, J, K). Estes compostos possuem sistema de ligação dupla conjugada na cadeia poliênica, podem ter ou não anel nas extremidades da cadeia e grupos funcionais (carbonila, hidroxila, entre outros), sendo que estas propriedades influenciam tanto na capacidade de absorver luz no visível, como na atividade anti-radical livre do pigmento (DAMODARAN *et al*., 2010).

Alguns carotenoides, entre eles a luteína, a violaxantina, e o β-caroteno, são largamente distribuídos na natureza, enquanto outros, como o licopeno, a capsantina e bixina, existem em grandes quantidades, mas apenas em algumas plantas (CAZZONELLI, 2011). A tabela 1 mostra as principais fontes dos carotenoides distribuídos na natureza.

Tabela 1: Carotenoides encontrados na natureza e suas principais fontes.

Carotenoide	**Fonte majoritária**	**Referência**
α-caroteno	Cenoura	MAURER *et al.* (2014)
	Abóbora	LAGO-VANZELA *et al*. (2013)
β-caroteno	Manga	MERCADANTE RODRIGUEZ-AMAYA (1998)
	Acerola	DE ROSSO e MERCADANTE (2007)
	Maracujá	SILVA e MERCADANTE (2002)
	Cenoura	MAURER *et al.* (2014)
Luteína	Couve	AZEVEDO-MELEIRO RODRIGUEZ-AMAYA (2007)
	Gema de ovo	ISLAM E SCHWEIGERT (2015)
	Rúcula	NIIZU e RODRIGUEZ-AMAYA (2005)
	Agrião	NIIZU e RODRIGUEZ-AMAYA (2005)
Criptoxantina	Tangerina	OLMEDILLA *et al.* (2003)
	Pêssego	SENTANIN e RODRIGUEZ AMAYA (2007)
Zeaxantina	Gemas de ovos	ISLAM E SCHWEIGERT (2015)
	Milho	BERARDO *et al*. (2004)
	Goji berry	WANG *et al*. (2010)
Bixina	Urucum	RODRIGUES *et al.* (2014)
Capsantina	Pimenta Vermelha	DONG *et al.* (2014)
Violaxantina	Manga	MERCADANTE RODRIGUEZ-AMAYA (1998)
Licopeno	Tomate	NIIZU e RODRIGUEZ-AMAYA (2005)
	Melancia	DIMITROVSKI *et al*. (2010)

Figura 1: Estrutura dos carotenoides.



Fonte: Adaptado de Cooperstone e Schwartz (2016)

Os carotenoides, além de pertencerem ao grupo de pigmentos naturais, também apresentam propriedades funcionais que formam a base de diversas funções e ações em organismos vivos. Alguns carotenoides apresentam importante função nutricional na dieta de humanos como precursores de vitamina A, além de outras ações benéficas como proteção contra certos tipos de câncer (KIM *et al*, 2014; ANTWI *et al.,* 2015), doenças cardiovasculares (SAINI *et al.,* 2015), degeneração macular (ROBERTS *et al*, 2009; PENG *et al*, 2016) e fortalecimento do sistema imunológico (BIESALSKI *et al.*, 2009).

A transformação dos carotenoides pró-vitamínicos em vitamina A ocorre por clivagem central (mecanismo principal), onde o composto é dividido ao meio, formando duas moléculas de retinal no caso do β-caroteno ou uma molécula no caso dos demais carotenoides pró-vitamínicos A que são, posteriormente, transformados em retinol (RAO e RAO, 2007). Existem efeitos benéficos dos carotenoides à saúde, que são independentes da atividade pró-vitamínicos A e têm sido relacionados à propriedade antioxidante, através da desativação de espécies reativas e pelo sequestro do oxigênio singleto, uma vez que a sua estrutura de base constituída por uma tetraterpene com uma série de ligações duplas conjugadas, que geram um sistema de ressonância de electrons que se deslocam ao longo de toda a cadeia de polieno (RODRIGUES *et al*., 2012).

Entre os carotenoides mais estudados encontra-se o β-caroteno (TAN e NAKAJIMA, 2005; YUAN *et al.,* 2008; GONZÁLEZ-REZA *et al.,* 2014), que é um importante membro dessa família, encontrado em muitas frutas e legumes. Este composto tem uma estrutura molecular de 8 (oito) unidades de isopreno (Figura 1.1 A). Como um precursor de retinol, com uma alta taxa de conversão, o β-caroteno proporciona uma parte substancial da vitamina A na dieta humana (LOBO *et al.,* 2012).

Por esta razão, existe um grande interesse na utilização do β-caroteno e outros carotenoides como ingredientes funcionais em alimentos. Devido a essa grande utilização, outros carotenoides vêm sendo investigados para aplicação em alimentos, entre os quais se encontram o licopeno.

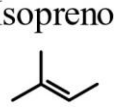
3.1.1.1. Licopeno

O licopeno é um composto lipossolúvel, constituído por onze ligações duplas conjugadas e duas ligações duplas não conjugadas, considerado o carotenoide com maior capacidade sequestrante de oxigênio singleto devido a sua estrutura química, o que lhe

oferece maior reatividade e, por isso, maior degradação oxidativa (SHI e LE MAGUER, 2000). Como um antioxidante, o licopeno tem capacidade de capturar oxigênio singleto duas vezes mais que o β-caroteno e 10 vezes mais do que o tocoferol (SILVA *et al*. 2012).

A estrutura do licopeno é considerada a estrutura fundamental dos carotenoides (Figura 2), da qual pode derivar outras estruturas por reação de hidrogenação, ciclização, aquecimento, oxidação ou combinação desses métodos (CLINTON, 1998). Diferentemente do β-caroteno, o licopeno não apresenta propriedades pró-vitamínica A, uma vez que em sua estrutura não há anéis β-iononas (Figura 1I), responsáveis pela atividade da vitamina A (DAMODARAN *et al.*, 2010).

Figura 2: Estrutura básica dos carotenoides.



Devido a sua estrutura química, o licopeno é altamente instável e as reações de degradação do licopeno podem ser influenciadas por diferentes fatores como: temperatura, estado físico e condições do ambiente, sendo os fatores mais considerados a luz, calor e a presença de oxigênio (DAMODARAN *et al.*, 2010). O elevado número de duplas ligações conjugadas torna o licopeno propenso à isomerização e oxidação durante o processamento e estocagem, resultando em desestabilização da molécula com perda da cor e atividade biológica (PELIS *et al*, 2013).

O licopeno pode ser encontrado em diversas fontes vegetais em quantidades significativas como apresentado na tabela 2, podendo ser extraído em forma de cristais com alto grau de pureza (NUNES e MERCANDANTE, 2004; DOS SANTOS *et al.*, 2015). Dentre as fontes de licopeno, o tomate é a fonte mais utilizada para obtenção deste carotenoide. O tomate possui em sua composição de 93% a 95% de água e nos 5% a 7% restantes, encontram-se compostos inorgânicos, ácidos orgânicos, açúcares, sólidos insolúveis em álcool e outros compostos como carotenoides, principalmente licopeno (FONTES e PEREIRA, 2003).

A coloração verde dos tomates imaturos é devido à presença de clorofila e à medida que ocorre a maturação, a clorofila se degrada e ocorre inicialmente a síntese de pigmentos amarelos (xantofilas e β-caroteno) e posteriormente atinge a coloração vermelha, decorrente

do acúmulo de licopeno, que compreende cerca de 80 a 90% dos pigmentos presentes nos tomates maduros, principalmente na casca (SHI e LE MAGUER, 2000).

Tabela 2: Concentração de licopeno nas frutas consideradas fontes.

Frutas/vegetais	Licopeno (µg/100 g de peso fresco)	Referência
Tomate	5900	NUNES E MERCADANTE (2004)
Melancia	3550	NIIZU e RODRIGUEZ-AMAYA (2003)
Goiaba vermelha	6150	RODRIGUEZ-AMAYA *et al.* (2008)
Pomelo	3360	XU e PAN (2013)
Mamão	1448	BARRETO *et al.* (2011)

O teor de licopeno varia com o grau de amadurecimento do fruto, aumentando o teor à medida que o grau de maturação aumenta e a biodisponibilidade também é alterada de acordo como este é consumido. O licopeno quando ingerido na sua forma natural (configuração *trans*-licopeno) é pouco absorvido, como por exemplo, suco de tomate cru. A isomerização converte isômeros all–*trans* em isômeros *cis* como resultado da adição de energia, o que torna o composto menos estável e, no entanto, mais biodisponível (CHEN *et al.*, 2009).

Stajčić *et al*. (2015) avaliaram o conteúdo de carotenoides em resíduos de tomate da indústria de sucos e encontraram altas concentrações de licopeno nesses subprodutos (13 mg/g de extrato úmido), comprovando que este resíduo pode ser utilizado como boa fonte de carotenoides para ser utilizado como suplemento alimentar. De acordo com Knoblich *et al*. (2005), os teores de carotenoides de subprodutos de tomate seco totalizaram 793,2 e 157,9 mg/g de base seca de casca e semente, respectivamente. Em outro estudo, os principais carotenoides encontrados em resíduos de processamento de tomate foram o licopeno (413,7 mg/g de peso seco) e o β-caroteno (149,8 mg/mg de peso seco) (KALOGEROPOULOS *et al*., 2012). Logo, uma solução para aproveitamento de todo esse resíduo que é produzido pela indústria produtora de tomate é a utilização deste na obtenção de cristais de licopeno para aplicação em outros alimentos, agregando maior valor ao produto final.

O licopeno tem recebido atenção especial nos últimos anos por causa de sua relação com efeitos benéficos à saúde devido à sua efetiva capacidade antioxidante no controle de doenças crônicas, atuando como antioxidante em fases lipídicas, bloqueando radicais livres que danificam as membranas proteicas (CHISTÉ *et al.*, 2014a). Numerosos estudos clínicos

apontam que a presença de licopeno na dieta tem uma relação inversa com incidência de câncer de próstata, câncer no trato digestivo, câncer pancreático e câncer em outros tecidos (KIM *et al*, 2014), além da relação com a redução de doenças cardiovasculares (SAINI *et al.*, 2015) e redução dos níveis de colesterol (RIED e FAKLER, 2011). Um estudo realizado por Jeong et al. (2009) avaliou a relação entre a concentração de micronutrientes com ação antioxidante como β-caroteno, licopeno, luteína, retinol, alfa-tocoferol e gama-tocoferol, em plasma de mulheres e o risco de câncer endometrial; foi observado que os níveis de β-caroteno e licopeno no plasma foram inversamente proporcionais ao risco do câncer.

O licopeno pode ser usado no combate ao envelhecimento cutâneo na forma de fitocosmético, devido sua capacidade de neutralizar espécies reativas de oxigênio inibindo ou diminuindo os efeitos do estresse oxidativo, provocados pelos raios UV, especialmente na epiderme. Um estudo realizado por Andreassi et al. (2004), determinou que a aplicação tópica prévia de 6% de extrato seco contendo licopeno incorporado em uma emulsão constituída de óleo de amêndoas doce, apresentando concentração final de licopeno na formulação de 0,03%, foi capaz de diminuir o edema apresentado na pele de voluntários submetidos à radiação UV, evidenciando sua provável atividade antioxidante em relação aos radicais livres provenientes da luz solar.

3.1.2. Estabilidade dos carotenoides

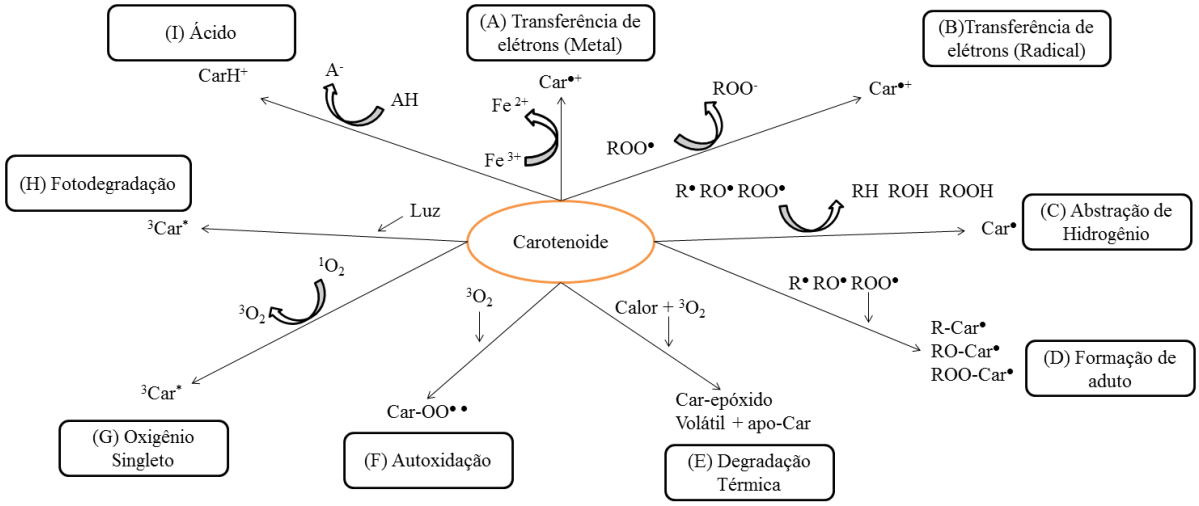
Diferentemente dos corantes sintéticos, alguns corantes naturais doam átomos de hidrogênio facilmente, potencializando assim sua ação antioxidante na mistura. Dentro deste grupo, os carotenoides são extremamente reativos e consequentemente instáveis devido a sua longa cadeia de duplas ligações conjugadas (EL-AGAMEY *et al.*, 2004).

A reduzida estabilidade dos produtos naturais se deve em parte à função que desempenham no metabolismo do organismo vegetal ou animal onde estão presentes. Desta forma, muitos compostos estão em constante transformação, em resposta a fatores externos como luz, calor oxigênio entre outros (JANNA *et al.*, 2007; SOTOMAYOR-GERDING *et al*., 2016). De acordo com o carotenoide, os grupos de extremidade terminal também podem sofrer alterações em certos ambientes e as vias de degradação são altamente influenciadas pelos agentes envolvidos no início do processo (BOON *et al.*, 2010). Os mecanismos de oxidação mais comuns são apresentados na figura 3.

A transformação mais comum que os carotenoides sofrem em alimentos é a oxidação que se processa por mecanismos de radical livre semelhante à dos lipídios insaturados

alterando a cor e até mesmo eliminando-a e este fenômeno é mais intenso em alimentos que sofrem processos de desidratação. Mesmo a liofilização sendo um método de perda de água que visa à integridade funcional e sensorial do alimento, as perdas de carotenoides são fatores preocupantes neste processo, já que a camada protetora de absorção primária de água foi reduzida e a porosidade do alimento é muito grande, aumentando a superfície de contato com O_2 (RODRIGUEZ-HUEZO *et al.*, 2004).

Figura 3: Mecanismos de oxidação de carotenoides e produtos iniciais.



Fonte: Adaptado de Boon *et al.*, 2010.

O pH também afeta a estabilidade de muitos corantes naturais, da mesma forma que a presença de determinados íons metálicos com reconhecidas características catalíticas, como ferro, alumínio, cobre ou mesmo magnésio. Estes catalisadores podem aumentar a taxa de decomposição de alguns pigmentos, o que acarreta em perda de coloração, sendo os carotenoides particularmente susceptíveis a estes efeitos (BOON *et al.*, 2010).

Além disso, os carotenoides são pouco solúveis em água (MORARU *et al.*, 2003), não sendo significativas as perdas quando o alimento é cortado ou triturado. O β-caroteno, por exemplo, é insolúvel em água e fracamente solúvel em óleo à temperatura ambiente (RIBEIRO e CRUZ, 2005). Além disso, o β-caroteno é sensível à luz, oxigênio e calor, o que limita ainda mais as suas aplicações nos alimentos industriais (RODRIGUEZ-HUEZO *et al.*, 2004).

Assim como o β-caroteno, o licopeno também é considerado instável na presença de luz, calor e oxigênio e alguns estudos avaliaram os efeitos da luz (HENRY e SCHWARTZ,

1998; LEE e CHEN, 2002) e da temperatura (LEE e CHEN, 2002; HACKETT *et al.*, 2004; SHI *et al.*, 2008; DIAS *et al.*, 2014) na sua degradação, sendo que esta pode ser conduzida também pelo estado físico, tipo de pigmento e condições ambientais (DAMODARAN *et al.*, 2010).

Henry e Schwartz (1998), em um estudo de cinética de degradação de licopeno, β-caroteno e luteína, observaram que a taxa de degradação de cristais de licopeno submetidos a diferentes temperaturas foi superior que a do β-caroteno. Resultado similar foi encontrado por Chen *et al.* (2009) que compararam a estabilidade de licopeno em um sistema modelo água e óleo, relatando que com o aumento da temperatura, ocorria um aumento da degradação dos cristais de licopeno.

Outros estudos também avaliaram a estabilidade de licopeno e β-caroteno em tomates ou em produtos derivados de tomate submetidos a diferentes temperaturas de processamento (SHI *et al.*, 2008; DEMIRAY *et al.*, 2013) e constataram a suscetibilidade desses carotenoides ao calor.

3.1.3. Ação antioxidante dos carotenoides

Um antioxidante pode ser definido como um composto ou substância química que inibe a oxidação ou, qualquer substância que, quando presente em baixa concentração comparada a do substrato diminui ou inibe significativamente a oxidação do mesmo (FUKUMOTO e MAZZA, 2000; YAN *et al.*, 2016). Os antioxidantes podem ser classificados em produtos que reagem com O_2, que atuam de forma competitiva em cadeia ou que atuam sobre os peróxidos, decompondo-os, de forma a produzirem compostos que não mais participam da reação em cadeia de radicais livres (RAMALHO e JORGE, 2006).

Espécies reativas de oxigênio (ânion superóxido e radical hidroxila), espécies reativas de oxigênio não-radical como o peróxido de hidrogênio e o oxigênio singleto, são moléculas que podem ocasionar um estresse oxidativo, sendo responsáveis pela iniciação de cadeias de oxidação que conduz a danos no DNA e ocasiona oxidação lipídica (GUTOWSKI e KOWALCZYK, 2013). A ação antioxidante e de desativação de espécies reativas têm importância para a saúde humana por, dentre outras, contribuir para a proteção de células (CHISTÉ *et al.*, 2014a).

O oxigênio singleto pode ser gerado na presença de luz, oxigênio triplete e na presença de um sensitizador que tem a capacidade de absorver luz e transferir energia para o oxigênio tripleto dando origem ao singleto (DECKER *et al.*, 2010; SKIBSTED, 2010). Carotenoides

podem atuar de duas maneiras para evitar reações causadas pelo oxigênio singleto, sendo a primeira delas a inativação do sensitizador em seu estado excitado e a segunda, desativando o próprio oxigênio singleto (Figura 3G) (CHOE e MIN, 2006).

Pesquisas têm sido realizadas sobre a extinção do oxigênio singleto por carotenoides e como esta reação protege contra oxigênio singleto mediante reações de foto-oxidação (LEE e CHEN, 2002). Os carotenoides pró-vitamínicos A (β-caroteno, α-caroteno e β-criptoxantina), assim como outros não vitamínicos (licopeno, luteína e zeaxantina) possuem capacidade de atuarem como neutralizadores de radicais livres e de outras espécies reativas de oxigênio principalmente em função de suas estruturas de duplas ligações conjugadas (CHISTÉ *et al.*, 2014b).

O mecanismo de ação antioxidante dos carotenoides frente a radicais livres ocorre pela transferência do elétron desemparelhado do radical livre para o pigmento (Figura 3B). Existem pelo menos quatro tipos de reação de transferência (Equações. 1, 2, 3 e 4) (BOON *et al.*, 2010):

$RO^{\bullet} + CAR \rightarrow RO^{-} + CAR^{\bullet +}$ Eq. 1

$RO^{\bullet} + CAR \rightarrow RO^{+} + CAR^{\bullet -}$ Eq. 2

$RO^{\bullet} + CAR \rightarrow ROH^{\bullet} + CAR(-H)^{\bullet}$ Eq. 3

$RO^{\bullet} + CAR \rightarrow (ROH\text{-}CAR)^{\bullet}$ Eq. 4

Onde:

$RO^{\bullet}$: Radical livre;

CAR: Carotenoide;

$CAR^{\bullet +}$ e $CAR^{\bullet}$-: Formas catiônica e aniônica de carotenoides;

CAR $(-H)^{\bullet}$: Radical formado pela transferência de H ao carotenoide;

(ROH-CAR)●: Radical aduto.

Além disso, os carotenoides têm a capacidade de estabilizar moléculas eletronicamente excitadas envolvidas na geração de oxigênio singleto como os sensitizadores e outros radicais, sendo que tal propriedade ocorre química ou fisicamente. Na estabilização química, há a união entre o carotenoide e o radical livre (Figura 3D), sendo que na física, o oxigênio singleto transfere sua energia para o carotenoide, sendo tal energia liberada na forma de calor para o ambiente, com retorno do composto ao seu estado fundamental, o qual pode

realizar novos ciclos de estabilização (CHOE e MIN, 2006). À medida que o número de duplas ligações conjugadas aumenta, a energia do estado excitado diminui e isso é refletido na dependência da taxa constante de extinção de oxigênio singleto com o comprimento da cadeia dos carotenoides (EDGE *et al.*, 1997).

Estudos em sistema-modelo têm sido muito utilizados para demonstrar a capacidade de desativação de oxigênio singleto pelos carotenoides (LOBATO *et al*, 2015; DOS SANTOS *et al.,* 2016). Dentre outras características, sabe-se que quanto maior o número de duplas ligações conjugadas, maior a capacidade de desativação (EDGE *et al.*, 1997) (Tabela 3).

Tabela 3: Constantes de reação de desativação de oxigênio singleto de alguns carotenoides em sistema-modelo.

Carotenoide	**Ligações Duplas**	***K*q (x10^{9} M^{-1}s^{-1})**
Dodecano – β – caroteno	19	23,0
Decapreno – β – caroteno	15	20,0
Tetradehidrolicopeno	15	10,7
Rodoxantina	12 (+2,C=O)	12,0
Astaxantina	12 (+2,C=O)	14,0
Cantaxantina	12 (+2,C=O)	12,0
Licopeno	11	17,0
Dihidroxilicopeno	11	5,1
todo-*trans*- β-caroteno	11	13,0
15-*cis*-β-caroteno	11	11,0
9-*cis*-β-caroteno	11	11,0
Zeaxantina	11	12,0
α-caroteno	10	12,0
β-apo-8'-carotenal	10	5,27
Luteína	10	6,64

Adaptado de: Edge e Truscott, 1997.

O licopeno, por exemplo, é conhecido por ser eficaz na desativação do oxigênio singleto em comparação a outros carotenoides (DAMODARAN *et al.*, 2010). Embora não tenha atividade pró-vitamínica A, este composto é capaz de funcionar como um antioxidante, com maior capacidade de desativar oxigênio singleto que β–caroteno, α-tocoferol

(WEISBURGER, 2002) e luteína (MÜLLER *et al.*, 2011). Esta capacidade está relacionada ao grau de insaturação da longa cadeia carbônica do licopeno que contem onze ligações duplas conjugadas e duas não conjugadas, característica química que também inibe a propagação da etapa de peroxidação lipídica (SILVA *et al.*, 2012).

3.2. Nanoencapsulamento de compostos bioativos

A tecnologia de nanopartículas, que antes era aplicada apenas em eletrônicos, em fármacos e na indústria têxtil, tem começado a influenciar as indústrias associadas aos alimentos e benefícios potenciais de tal tecnologia para os consumidores são muito promissores (BOUWMEESTER *et al.*, 2009).

Em alimentos, a nanotecnologia abrange muitos aspectos tais como segurança alimentar (VANCE *et al.*, 2015), materiais de embalagem (SOUZA e FERNANDO, 2016) tratamento de doenças (JHA *et al.*, 2016), sistemas de distribuição, biodisponibilidade de compostos (ARUNKUMAR *et al.*, 2015), novas ferramentas de biologia molecular e celular e novos materiais para detecção de patógenos (MAYNARD *et al.*, 2006).

No campo de alimentos processados, técnicas de nanoencapsulamento têm sido utilizadas para melhorar a estabilidade e proteger ingredientes alimentares como óleos essenciais (NATRAJAN *et al.*, 2015), corantes (LOBATO *et al.*, 2013) e lipídios (ILYASOGLU e EL, 2014) contra degradação, perda de voláteis e interação com outros ingredientes (GOULA e ADAMOPOULOS, 2012).

Nanopartículas são definidas como unidades que tem três dimensões de ordem menor que 1 μm e é este pequeno tamanho em combinação com a composição química e estrutura de superfície que fornece as nanopartículas características únicas e um enorme potencial de aplicação (BOUWMEESTER *et al.*, 2009). O nanoencapsulamento é definido como uma tecnologia desenvolvida para envolver substâncias gerando partículas com dimensões nanométricas pelo uso de técnicas, tais como nanoemulsificação, nanocompósitos e nanoestruturação, aumentando a estabilidade e promovendo a funcionalidade, que inclui liberação controlada do composto bioativo e solubilidade aparente destes em meio aquoso (vitaminas, antioxidantes, proteínas, lipídeos e carboidratos) (TAYLOR *et al.*, 2005; LETCHFORD e BURT, 2007; QUINTANILLA-CARVAJAL *et al.*, 2010). Além disso, o encapsulamento pode promover a melhoria da estabilidade de compostos na presença de oxigênio, luz e umidade, com aumento do tempo de vida útil e biodisponibilidade em um processo de liberação controlada (RIBEIRO *et al.*, 2008).

A aplicação de técnica de encapsulamento para carotenoides tem sido comumente utilizada para aumentar a estabilidade destes compostos na presença de oxigênio, calor e luz (CORONEL-AGUILERA e SAN MARTÍN-GONZÁLEZ, 2015; DOS SANTOS *et al.*, 2016; GOULA e ADAMOPOULOS, 2012). Barbosa *et al.* (2005) avaliaram a estabilidade de bixina encapsulada com polissacarídeos e observaram que a encapsulação reduziu a degradação da bixina causada pela luz, ar, ozônio e altas temperaturas. Em outro estudo, Rocha *et al* (2012) caracterizaram e avaliaram a estabilidade de microcápsulas de licopeno obtidas por *spray drying* e observaram que a estrutura encapsulante conferia mais estabilidade ao licopeno quando comparado ao composto livre submetido às mesmas condições de armazenamento. Goula e Adamopoulos (2012) desenvolveram uma técnica para melhorar a microencapsulamento do licopeno por *spray drying* usando ar desumidificado com maltodextrina como material de parede e constataram que o uso de ar sem umidade melhorou a estabilidade do licopeno encapsulado. Shu *et al.* (2006) também obtiveram microcápsulas de licopeno estáveis por *spray drying* utilizando gelatina e sacarose como material de parede.

Além da encapsulação, outra possibilidade de aumentar a estabilidade dos carotenoides e que vem sendo utilizada é o nanoencapsulamento, que envolve a incorporação, adsorção e dispersão de compostos em pequenos veículos com diâmetro manométrico (VENTURINI *et al.*, 2011). As nanocápsulas podem ser constituídas por um invólucro polimérico onde o composto fica adsorvido à parede ou em um núcleo oleoso como as desenvolvidas por Contri *et al.* (2013) que avaliaram diferentes óleos como núcleo para as nanocápsulas.

Lobato *et al.* (2015) sintetizaram nanocápsulas de núcleo lipídico contendo bixina e avaliaram a estabilidade dessas nanoestruturas durante aquecimento e fotosensitização em um sistema modelo etanol:água (2:8) constatando que o nanoencapsulamento promoveu estabilidade da bixina em ambas as condições. Yuan *et al.* (2008) caracterizaram nanoemulsões de β-caroteno preparadas sob homogeneização a alta pressão e avaliaram a estabilidade das mesmas por 4 semanas, constantando que o composto degradou apenas 25 % após 4 semanas de armazenamento à 25°C.

Assim, é possível notar que o uso da nanotecnologia melhora o potencial do uso de carotenoides em processos industriais devido ao aumento da sua estabilidade em diferentes condições de exposição, sendo este tema melhor discutido no capítulo seguinte que cor responde aos artigos de revisão sobre o uso de nanotecnologia em compostos bioativos.

CAPÍTULO 2- ARTIGOS DE REVISÃO

Este capítulo é constituído de dois artigos de revisão que trazem uma abordagem mais aprofundada sobre a nanotecnologia aplicada em compostos bioativos, em especial aos carotenoides focando, nos tipos de encapsulamento, nas técnicas utilizadas, nos parâmetros que devem ser avaliados durante a síntese de nanopartículas e nos principais materiais de parede utilizados.

NANOENCAPSULATION OF CAROTENOIDS: A FOCUS ON DIFFERENT DELIVERY SYSTEMS AND EVALUATION PARAMETERS

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Nanoencapsulation of carotenoids: a focus on different delivery systems and evaluation parameters

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ABSTRACT

Different types of nanoparticles have been synthesized to protect carotenoids against exposition of external factors such as light, heat and oxygen and processing conditions to increase stability and to improve the bioavailability of nanoencapsulated carotenoid. The type of nanostructure synthesized (nanoemulsions, liposomes, solid lipid nanoparticles, nanostructured lipid carrier, and polymeric nanoparticles) influences on the synthesis and nanoparticles stability, which reflect in physic-chemical characteristics such as polydispersity index, zeta potential, and encapsulation efficiency. Different nanostructures can be used to improve of stability of carotenoids; however, currently, polymeric nanocapsules are the nanostructure most utilized due to its stability during storage and high efficiency to encapsulate and to control the release of the carotenoid encapsulated and due to these considerations, they have been focus of researches for future studies regarding to application of carotenoids nanoencapsulated by food industries. The focus of this review is the presentation of different carotenoids delivery systems and the use of techniques to evaluate parameters that might limit the application of this innovative and potential technology in cosmetic, pharmaceutical, and food industry.

Keywords: Carotenoid; nanotechnology; stability; nanoparticles.

1. Introduction

Carotenoids are bioactive compounds with biological and chemical importance. Carotenoids are fat-soluble pigments that confer yellow, orange and red color to vegetables. These compounds are present in nature, such as β-carotene, α-carotene, lutein, zeaxanthin, lycopene, astaxanthin and bixin (Damodaran et al. 2010).

Carotenoids are isoprenoid compounds with polyene chains that may contain up to 15 conjugated double bonds. This chemical characteristic confers to these compounds instability to light, oxygen and heat and functionality such as provitamin A, antioxidant and anticancer activity (Tanaka et al. 2008). Therefore, carotenoids are considered human health promoters and have an important role in human nutrition and health.

Currently, carotenoids have been used to fortify food, as a supplement in pharmaceutical products and to topic use in cosmetic industry. These products usually undergo a stabilization process during its production and, for that reason; the influence of processing on carotenoid is an interesting issue, mainly for the food industry (Sáiz-Abajo et al. 2013).

Nanotechnology has improved stability, solubility and bioavailability of bioactive compounds to the application in food, cosmetic and pharmaceutical field (Okonogi and Riangjanapatee 2015). This technology has great potential in herbal products and functional foods to provide innovation on food characteristics, such as texture, taste, coloring strength and processability and aggregating improvements in human health (Ezhilarasi et al. 2012).

Nanotechnology refers to the obtention of material scales nanometer (10-1000 nm), which produces particles with the greater mass per surface area and more biologically active. Several studies have tried to encapsulate carotenoids to increase their antioxidant properties and avoid degradation (Rascón et al. 2011; Tan et al. 2014).

The industry has utilized novel applications of nanotechnologies in different sectors. It includes nanoparticles, such as micelles, nanoliposomes, nanoemulsions, biopolymeric nanoparticles and solid lipid nanoparticles, as well as the development of nanosensors, which aim to ensuring pharmaceutical and food safety (Yih and Al-Fandi 2006).

These nanostructured vehicles must be evaluated to ensure safety and stability during their application (Gaumet et al. 2008). The most important parameters in this evaluation are size, polydispersity index (PDI), zeta potential, morphology, pH, and drug loading (Couvreur et al. 2002).

Thus, the aim of this review was to collect information concerning the parameters considered important in the characterization, stability and functionality evaluation of nano-size vehicles and regarding the different types of nanostructures used as vehicles for carotenoids. Furthermore, the readers may understand how the use of nanotechnology evolved regarding carrier of bioactive compounds mainly concerning to encapsulation of carotenoids for potential application in food industry.

2. Parameters of characterization and stability evaluation of nanoencapsulated compounds.

Nanoencapsulation have been utilized to improve the stability of compounds and thus, to increase its application mainly for pharmaceutical and food industry. Different nanoencapsulation methods and types of nano-sized vehicles are utilized as delivery systems such as polymeric carriers and lipid nanostructures (Yegin and Lamprecht 2006).

After synthesis of these nanostructured vehicles, the evaluation of parameters such as size, polydispersity index (PDI), zeta potential, morphology, pH and release profile is essential to stability evaluation and safety to the biomedical application (Couvreur et al. 2002; Gaumet et al. 2008). The choice of techniques for characterization and stability evaluation is dependent on the nature of stability issues and product dosage form (Wu et al. 2011). Table 1 summarizes the techniques utilized to characterize and evaluate nanocapsules and nanoparticles.

2.1. Size and size distribution

Size (mean diameter or *z*-average) and size distribution are important parameters to nanoencapsulation evaluation due to their relation to distribution, physicochemical changes of the encapsulated compounds, viscosity, surface area and packing density of the nanoparticles (Gaumet et al. 2008). The particle size allows the selection of adequate colloidal preparations for parenteral administration and also possibly useful as sustained-release injections to target site (Reis et al. 2006). Moreover, the nanoparticle size control is a parameter that must be ensured, during storage due to the fact of physical stability is related to the periodic determination of the mean diameter (Wu et al. 2011).

Table 1: Parameters used for characterization of nanostructures

Parameter	Technique	Principle	Importance
Size and polydispersity index	Laser Diffraction (LD)	Light interaction	Ensure that particle exclusively on the nanometer scale was achieved (Lobato et al., 2013).
	Brunauer -Emmett –Teller (BET)	Adsorption	Allow the evaluation of surface area utilizing pore structure analysis (Akbari et al., 2011).
	X-ray Diffraction Peak Broadening Analysis (DPBA)	X-ray	This method is capable of yielding the crystallite size distribution (Akbari et al., 2011).
	Dynamic light scattering (DLS)	Light interaction	Allow the description of particle size distribution and destabilization phenomena (Venturini et al., 2011).
Morphology	Scanning electron microscopy (SEM)	Microcopy	Allow to obtention of information regarding to structure, wall thickness estimative and polymer porosity (Burghardt & Droleskey, 2005)
	Transmission electron microscopy (TEM)	Microscopy	
	Atomic Force Microscopy (AFM)	Microscopy	It is more appropriate for surface analysis (Gaumet et al., 2008).
Zeta potential	Zeta Potential analysis (ζ)	Electrophoresis mobility	Determine particle stability in suspension, macromolecule and material surface (Win & Feng, 2005).
Loading capacity	Ultrafiltration	Particle size	Reduce the quantity of carrier required for the administration to the target site (Lim et al., 2013)
	Tangential filtration		
	Ultracentrifugation	Density	
Release profile	Sample and Separate (SS)	Diffusion	Provide information concerning the dosage form used to assess product safety and efficacy (D'Souza, 2014).
	Continuous Flow (CF)		
	Dialysis Method (DM)	Physical separation	

The size distribution or PDI correspond the particles uniformity in suspension, in which PDI values higher than 0.5 indicate a broad distribution and between 0.1 and 0.25 show a narrow size distribution (Wu et al. 2011). The PDI is estimated considering the particle mean size, the refractive index of the solvent, the measurement angle and the variance of the distribution.

Several methods can determine the nanoparticle size and size distribution such as laser diffraction (LD); surface area analysis (BET) and X-ray diffraction peak broadening (Akbari et al. 2011). However, the most used technique to nanostructures is dynamic light scattering (DLS) (Venturini et al. 2011), which allows the description of particle size distribution and destabilization phenomena.

Dynamic light scattering (DLS), also referred to dynamic light scattering and quasi-elastic light scattering, is a rapid method for determining the mean size, the size distribution and PDI (Gaumet et al. 2008). The method consists in the particle interaction with light generally at an observation angle of 90°, and the calculation model is based on the equivalent sphere principle, in which each particle is viewed as a sphere (Gaumet et al. 2008; Yegin and Lamprecht 2006).

Studies that evaluated the size distribution of carotenoid nanoparticles used DLS or LD method to characterize and to evaluate the stability of these nanoparticles (Da Silva et al. 2016; Dos Santos et al 2015). The choose of technique depends on the size range of nanoparticles after synthesis due to that DLS and LD can determine particle sizes ranging from 0.003 to 10 μm and from 0.02 to 2000 μm, respectively (Lobato et al. 2013). The carotenoid nanoparticles presented size between 8 and 300 nm (Table 2) and due to this large range and small size of particle, DLS has been the method more used in these studies.

2.2. Zeta Potential

Zeta potential is the method most frequently used to characterize the surface charge. Zeta potential corresponds to the electrical potential of nanoparticles, which determines particle stability in suspension, macromolecule or material surface (Win and Feng 2005). Zeta potential may also be applied to investigate if a biologically active compound is linked to the core or only adsorbed into the surface and to evaluate the adsorption of plasma proteins onto the particles (Couvreur et al. 1995).

Zeta potential is measured based on the electrophoretic mobility that corresponds to the boundary of the surrounding liquid layer attached to the moving particles in the medium

(Wu et al. 2011). Values higher than 30 mV or lower than -30 mV promote high stability and prevent particles aggregation (Mohanraj and Chen 2006). Nanoparticles containing carotenoids present negative zeta potential (Table 2), between -4 and -75 mV, and this parameter is influenced by nanocapsule composition and the dispersion medium (Wu et al. 2011).

2.3. Morphology

Morphology is another important parameter for the characterization of nanoparticles where the microscopy is used to evaluate nanoparticles integrity and stability under different conditions. However, a detailed nanostructures morphology characterization is difficult due to their small size and complexity of the composition (Couvreur et al. 2002).

Many types of electron microscopy can be applied to observe nanoparticle morphology and structure; and as direct visualization techniques, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and Atomic Force Microscope (AFM) are widely used for assessment of particle morphology (Wu et al. 2011).

Currently, transmission electron microscopy (TEM) is the most used technique, mainly in carotenoid nanocapsules, which is performed after freeze–fracture of nanoparticles. TEM provides information regarding structure, wall thickness estimative and polymer porosity (Couvreur et al. 2002). The main advantage of TEM is the potential resolution provided by electron beams accelerated at high voltage that presents efficient wavelengths shorter by a factor of 105 than visible light (Burghardt and Droleskey 2005).

Despite to the morphology is an important parameter of evaluation, the applied techniques require a significant number of particles to achieve statistical size distribution and additional sample preparation that alter particle properties.

2.4. Loading capacity and release profile

Loading and release profile are important parameters that must be evaluated after the preparation of nanocarriers. The loading capacity is a parameter highly variable. This parameter is dependent upon the fabrication process and the type of carrier lipid utilized for the selected carotenoid (Gutiérrez et al. 2013).

Nanostructured systems with maximal compound loading and high entrapment efficiency can reduce the quantity of carrier required for the administration of sufficient amount of active compound to the target site (Lim et al. 2013).

The loading capacity corresponds to the percentage of a compound by weight in the final formulation. This parameter can be measured after preparation and separation of the nanocapsules from continuous phase (Couvreur et al. 1995). Carotenoids nanoparticles present loading capacity superior than 50% (Anarjan et al. 2012) and, in some cases, encapsulation efficiency of 100% (Hong et al. 2016).

Loading capacity can be measured by ultracentrifugation, size exclusion chromatography, ultrafiltration or tangential filtration. However, the last technique is the most used due to the tendency of nanoparticles clog membranes utilized in the quantification of compound loaded (Couvreur et al. 2002).

The release kinetics provides critical information concerning the dosage form used to assess product safety and efficacy. This parameter also provides details on the release mechanism and kinetics, enabling a rational and scientific approach to product development (Langer 1990). The release studies are performed at 37°C to simulate the physiological conditions. However, elevated temperatures and different pH have been explored to characterize compound release from a variety of dosage forms (D'Souza et al. 2005).

The release profile is currently evaluated using some methods such as sample and separate (SS), continuous flow (CF), dialysis membrane (DM) methods and novel techniques such as voltammetry and turbidimetry. Currently, the method most used to assess drug release from nano-sized dosage forms is the dialysis method (DM) that consists in the use of dialyzes membranes which allow for ease of sampling at periodic intervals (D'Souza 2014). In the dialyzes method, the nanostructures are added into a dialyzes bag containing release media that is subsequently sealed and placed in a vessel containing release media, agitated to minimize unstirred water layer effects (Muthu and Singh 2009).

Mathematical models to characterize compounds release have been discussed due to the possibility to elucidate release mechanisms and can be used to guide formulation development efforts; however, this field has been little explored (D'Souza 2014).

3. Types of nano carotenoids delivery systems

Carotenoids are often utilized as additives in food products despite these compounds exhibit very low water solubility or even water insolubility. Also, carotenoids present high melting point, chemical instability, and a low bioavailability, limiting the application of these compounds in pharmaceutical and food industry (Qian et al. 2012).

In this context, nanoencapsulated compounds have been developed, as presented in Table 2, to increase the stability, apparent solubility, industrial application and mainly for the design of carotenoids delivery systems, improving its bioavailability (Gutiérrez et al. 2013). Delivery system corresponds to technology in which a bioactive compound is entrapped in a carrier to control the rate of bioactive release (Fathi et al. 2012).

There are many types of nano-sized vehicles (Figure 1) and in general, some steps are involved in the encapsulation of bioactive compounds such as the formation of the wall around the material to be encapsulated and ensuring that undesired leakage does not occur (Mozafari et al. 2008).

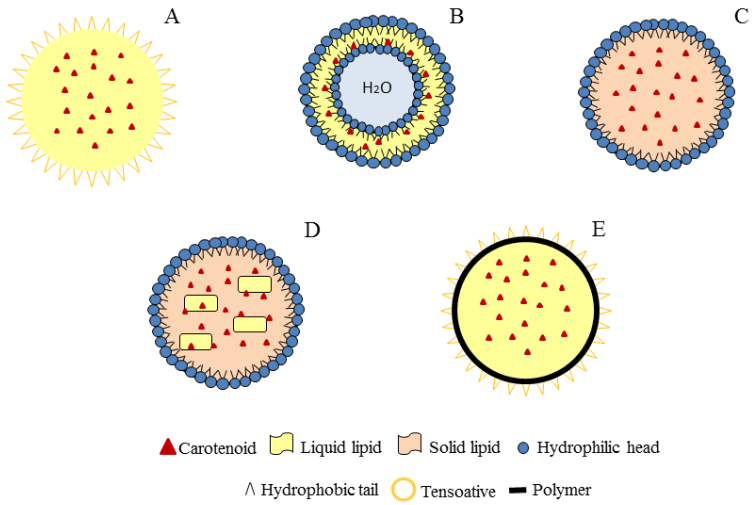

Figure 1: Types of nano delivery systems used to encapsulate carotenoids: (A) Nanoemulsion, (B) Nanoliposome, (C) Solid lipid nanoparticle (SLN), (D) nanostructure lipid carrier (NLC) and (E) Polymeric nanocapsule.

The carotenoids can be encapsulated in lipid based carriers and polymeric nanocapsules utilizing different polymers as wall material such as poly(lactic acid) (PLA) and it co-polymers, poly(lactide-co-glycolide) (PLGA); and poly(Ɛ-caprolactone) (PCL) due to their biocompatibility and biodegradability characteristics. The use of materials considered "generally recognized as safe" (GRAS) is essential to produce safe nanocapsules under the conditions of its intended purpose, along with nutritional quality and stability to food or pharmaceutical applications.

Table 2: Characteristics of carotenoids encapsulated utilizing different types of nanocarriers

Carotenoid	Carrier System	Size (nm)	Zeta Potential (mV)	Encapsulation(%)	Reference
Astaxanthin	Nanostructured lipid carriers (NLC)	85-138	-22 to -35	No reported	Tamjidi et al. (2014)
	Polymeric nanosphere	68-312	-18 to -30	98	Tachaprutinun et al. 2009
	Polymeric nanocapsule	98-157	-4 to 28	59 – 76	Anarjan et al. (2012)
Bixin	Solid lipid nanoparticle (SLN)	135.5–352.8	−17.9 to −36.5	17.96	Rao et al. (2014)
	Polymeric nanocapsule	195	-14	100	Lobato et al. (2013)
β- carotene	Liposome	1900-2500	-38 to -74	100	Toniazzo et al. (2014)
	Nanoemulsion	132-184	No reported	100	Yuan et al. (2008)
		9-177	No reported	100	Silva et al. (2011)
	Nanostructured lipid carriers (NLC)	8-15	No reported	100	Hejri et al. (2013)
	Solid lipid nanoparticle (SLN)	168	No reported	No reported	Qian et al. (2013)
	Polymeric nanocapsule	166	-18	100	da Silva et al. (2016)
	Polymeric nanosphere	32-206	-8 to -32	No reported	Yin et al. (2009)
		102-793	-24 to 9	15 – 65	Cao-Hoang et al. (2011)
Canthaxanthin	Liposome	174-425	No reported	No reported	Xia et al. (2015)
Lycopene	Nanoemulsion	100-200	-33 to -42	51-65	Ha et al. (2015)
	Nanostructured lipid carriers (NLC)	150-160	-73 to-75	100	Okonogi et al. (2015)
	Polymeric nanocapsule	193	-12	100	dos Santos et al.(2015)
Lutein	Nanoelmulsion	150	No reported	100	Vishwanathan et al. (2009)
	Nanostructured lipid carriers (NLC)	150-350	-40 to -63	No reported	Mitri et al. (2011)
	Polymeric nanocapsule	163-219	No reported	88	Jin et al. (2009)
		200	-21 to -29	72- 88	Arunkumar et al. (2015)
	Polymeric nanosphere	200	No reported	100	Hong et al. (2016)
		72 - 143	No reported	No reported	Tan et al. (2016)

3.1. Nanoemulsions

Nanoemulsion, also denominated as ultrafine emulsion or mini-emulsion, corresponds to an emulsion with disperse-phased droplets (oil) and a continuous phase (water), generally presenting diameter between 50 and 200 nm (Sanguansri and Augustin 2006). Nanoemulsions are characterized by specifying molecular constituents; quantity of these constituents and the sizes of the droplet structures (Mason et al. 2006).

Nanoemulsions (Figure 1A) can have lipid cores separated from the aqueous phase by a monomolecular layer of a surfactant material, allowing for nanoencapsulation of oil-based bioactive (Sanguansri and Augustin 2006). Nanoemulsions can be prepared using high energy (high-pressure, homogenization, microfluidization, and ultrasonication), low energy (solvent diffusion) or combined methods (high-shear stirring) (Fathi et al. 2012). During the preparation of nanoemulsion, some criteria are considered essential to stability and size of particles such as the choice of surfactant, the surfactant concentration and the flexibility of interface (Bhosale et al. 2014). The influence of different emulsifiers such Tween 20, 40, 60 and 80; decaglycerol monolaurate and sodium caseinate in the formation of nano-vehicles to carotenoids were tested by Yin et al. (2009) and Tan et al. (2016). The authors verified that emulsifiers act as a protective barrier and support in antioxidant activity of encapsulated compounds.

Encapsulation into nanoemulsions of bioactive compounds such as carotenoids (Qian et al. 2012; Yuan et al. 2008) and flavonoids (Li et al. 2012) represents the first effective approach to improve the dispersion and bioavailability. Encapsulation also protects these compounds against degradation (Donsì et al. 2011).

Nanoemulsion containing β-carotene was synthesized to evaluate the stability of carotenoid during storage at 4°C and 25°C (Yuan et al. 2008). These authors observed that refrigeration was an important factor in the stability of β-carotene nanoemulsions presenting stable range diameter of 132 to 184 nm and gradually degradation of carotenoid during storage (14–25%), with slightly greater loss occurred at 25°C than at 4°C.

Lutein nanoemulsion was prepared and compared with supplements regarding bioavailability (Vishwanathan et al. 2009). The lutein nanoemulsions presented diameter of 150 nm and were significantly more bioavailable even at 10-40% lower doses than supplements proving that nanoemulsions could improve bioavailability even at physiological doses.

Lycopene was also incorporated in nanoemulsion to protect the antioxidant activity and improve the bioaccessibility of lycopene in tomato extract, presenting sizes between 100 and 200 nm (Ha et al. 2015). In this study, lycopene nanoemulsion exhibited high antiradical efficiency, antioxidant activity, high *in vitro* bioaccessibility and high aqueous stability.

Nanoemulsions present various advantages: improving of bioavailability of compound, small-sized droplets, increase absorption rate, improvement of solubility of lipophilic compound, rapid and efficient penetration of the compound, less amount of energy requirement and proving of aqueous dosage form for water insoluble compounds (Mishra et al. 2014). Although nanoemulsions present many advantages, the use of a large surfactant and co-surfactant concentration necessary for stabilizing the nanoemulsion due to its low storage stability and limited solubilizing capacity for high-melting substances limit its application (Mishra et al. 2014).

3.2. Nano liposomes

Liposomes are structures constituted of phospholipid that presents concentric lipid bilayers alternating with aqueous compartments, encapsulating hydrophilic and lipophilic compounds with sizes in the nanometer to micrometer range (Gregoriadis 1973; Mozafari et al. 2008; Muller and Landfester 2015).

The use of liposomes as a delivery system was established for the first time by Gregoriadis (1973) to eliminate the inability of some compounds to penetrate target cells, and liposomes were employed as drug carriers to actinomycin D.

Nano-sized liposomes (Figure 1B) can be produced by different methods such as mechanical (ultrasonication, extrusion, high-pressure homogenization and membrane homogenization) and non-mechanical methods (depletion of mixed detergent-lipid micelles and reversed-phase evaporation) (Fathi et al. 2012).

Choosing the best preparation method depends on some parameters such as the physicochemical properties of encapsulated compounds and liposomal ingredients. The concentration and toxicity of encapsulated compounds; potential modifications to the features of the liposome on release and processes involved during delivery of the liposome are also parameters evaluated (Mozafari et al. 2008).

Liposomes have been used as carriers for bioactive compounds (Vanaja et al. 2013) to improve their solubility, stability, and bioavailability. The interaction between carotenoid (β –

carotene, lutein, canthaxanthin, and lycopene) and lipid bilayer and effects of carotenoid incorporation on the physical properties of liposome was evaluated by Xia et al. (2015). This study showed that the carotenoids affect morphologic characteristics of liposomes, increasing liposome size (20- 425 nm) and that carotenoid modulation on the properties of liposomes is concentration-dependent.

Another study evaluated the modulating effects of liposomes encapsulation (10-100nm) on the carotenoids bioaccessibility. The results presented that carotenoid bioaccessibility depended strongly on the incorporating ability of carotenoids into a lipid bilayer and loading content (Tan et al. 2014).

Liposomes also can be combined with biopolymers such as chitosan (chitosomes) to encapsulate carotenoids due to the capacity of chitosan coating to improve the carotenoid encapsulating efficiency of liposomes and increase the ability of liposomes to protect β-carotene and lutein (Tan et al. 2016). Gums (guar and xanthan) were utilized as stabilizing in β-carotene-loaded liposome formulations applied in yogurt. It was possible observed that liposomes protected the carotenoid incorporated and the mixture of gums was highly effective in avoiding liposome aggregation (Toniazzo et al. 2014).

The application of liposomes as carrier presents some advantages such reduction in toxicity of encapsulated compounds, increasing to stability, efficacy, and therapeutic index and improving pharmacokinetic effects (Akbarzadeh et al. 2013). However, characteristics such as low solubility, short half-life, low encapsulation efficiency, batch-to-batch irreproducibility and difficulties in controlling liposome size can be limit the manufacture and development of liposomes (Akbarzadeh et al. 2013).

3.3. Solid lipid nanoparticles (SLN)

Solid lipid nanoparticle (SLN) is the denomination for nanometric-size dispersion of lipids that should be solid at body temperatures (37°C). SLN is composed of a solid lipid core with a compound linked to lipid matrix and a surfactant and cosurfactant which stabilize the lipophilic components in an aqueous phase (Arana et al. 2015; Weiss et al. 2008). The mobility of bioactive compounds can be controlled by the physical state of the lipidic matrix and for this, crystallized lipids increase the stability of bioactive compound incorporated (Weiss et al. 2008).

The SLN was introduced in 1991 as an alternative carrier system to traditional colloidal carriers for drug delivery, such as emulsions and liposomes (Müller et al. 2000). In general, SLN (Figure 1C) can be prepared by different techniques: high-pressure homogenization, solvent emulsification-evaporation, breaking of oil-in-water microemulsion and preparation via water-in-oil-in-water double emulsion (Üner and Yener 2007).

High-pressure homogenization is the most used method to the production of SLN due to its efficiency and reliability. These nanoparticles can be obtained by hot homogenization or cold homogenization technique (Üner and Yener 2007). In both techniques, the heated lipid solubilizes the compound. For the hot homogenization method, the mixture lipid and compound are dispersed under stirring in a hot aqueous surfactant solution at the same temperature. Then, this pre-emulsion is homogenized in a controlled high pressure; the nanoemulsion is cooled down to room temperature, the lipid recrystallizes and leads in solid lipid nanoparticles (Müller et al. 2000).

Different bioactive compounds are encapsulated utilizing SLN (Arana et al. 2015; Lacatusu et al. 2013; Qian et al. 2013; Rao et al. 2014). Between these compounds, carotenoids have been the most studied. Calendula officinalis L. (Asteraceae) is a medicinal plant extract that consists to mainly carotenes and SLN containing this extract was synthesized to evaluate its toxicity and healing efficacy on a conjunctival epithelium cell. The SLN presented a diameter between 67-523 nm and zeta potential in a range of −35 to −48 mV and proved that nanoparticles were safe and improved epithelium repair on the ocular surface (Arana et al. 2015).

Bixin was loaded in solid lipid nanoparticles utilizing different lipid matrices. The nanoparticles were prepared by technique fusion-emulsification obtaining particles with size ranged from 135–352 nm, PDI of 0.185–0.572 and zeta potential of −18 to −37 mV and this SLN showed a hepatoprotective effect in rats (Rao et al. 2014).

Qian et al. (2013) evaluated the stability of β-carotene encapsulated by SLN. The authors synthesized nanoparticles (d<200nm) homogenizing lipid (cocoa butter and hydrogenated palm oil), Polysorbate 80 and water and observed that after eight days of storage, the SLN contained 50.3 ± 1.2% of the β-carotene content.

The SLN presents some advantages regarding other colloidal carriers such as incorporation of lipophilic and hydrophilic compounds, avoidance of organic solvents and the possibility to large scale production and sterilization. However, SLN has low loading

capacity, presence of others colloidal structures and stability problems due to transformations of the physical state of the lipid (Mehnert and Mäder 2001).

3.4. Nanostructure lipid carrier (NLC)

Nanostructured lipid carrier (NLC) is a new generation of lipid solids nanoparticles that consist of different lipids blended to form the lipid matrix with a particular nanostructure (Radtke and Muller 2001).

The NLC was developed by first time in 2001 by Radtke and Muller (2001) to reduce the limitations of SLN based on preparation methods described for SLN. The method of obtention of NLCs (Figure 1D) depends on the type of compound encapsulated, especially its solubility and stability, and the lipid matrix (Kaur et al. 2015).

Currently, bioactive compounds NLCs are increasingly introduced as ingredients for food applications such as carotenoids (Hejri et al. 2013; Mitri et al. 2011), and unsaturated fatty acids (Lacatusu et al. 2013).

For example, the β- carotene loaded nanostructured lipid carriers were obtained by solvent diffusion method for using in foods and oral administration. These NLCs presented small diameter (8-15 nm) and high β-carotene retention, proving that NLCs can be produced and employed as appropriate carriers for bioactive compounds in foods (Hejri et al. 2013).

Lutein was also loaded in NLCs produced by high-pressure homogenization to protect skin from photodamage (Mitri et al. 2011). In this study, NLCs showed diameter range 150 – 350 nm, zeta potential range −40 to −63 mV, and the encapsulated lutein remained in the skin, protecting the skin against UV.

In another study, astaxanthin was loaded in NLCs to the utilization of this active ingredient in food formulations, evaluating physicochemical characteristics and storage stability (Tamjidi et al. 2014). The astaxanthin-NLC presented particle size of 94 nm, zeta potential of -24 mV and stability for more than one month.

NLCs were developed to overcome the limitations of SLNs, and its main advantages are high entrapment of lipophilic and hydrophilic compounds, extended release, high stability, simple preparation and scale-up and controlled particle size (Kaur et al. 2015). Despite the potential of NLCs, some limitations were detected such as cytotoxic effects and irritative and sensitizing action of surfactants (Kaur et al. 2015).

3.5. Polymeric nanoparticles

Polymeric nanoparticles represent the most current and promise model of delivery systems in the pharmaceutical, medical and food field. These nanostructures can be classified as nanocapsules or nanospheres. Polymeric nanoparticles were synthesized for the first time by Birrenbach and Speiser (1976) to the application as adjuvants (enhancers of the immune response) in immunology.

Nanocapsules (Figure 1E) can be defined as a vesicular system in which specific compound, solubilized in an aqueous or oil core, is covered by a single polymeric membrane whereas nanospheres are a solid polymeric sphere in which particular compound is dispersed in polymer surface (Couvreur et al. 2002).

Polymeric nanoparticles can be synthesized by different methods, classified into two broad categories: polymerization of the monomer and preformed polymer (Couvreur et al. 1995). The method most utilized currently is preformed polymer that consist of the injection of an organic phase containing the polymers, solvent accessible to remove (acetone or ethanol), oil, triglycerides and the compound of interest into in an aqueous phase containing water and a tensoative compound under magnetic stirring (Quintanar-Guerrero et al. 1998).

In addition different polymers can be utilized as wall material to obtention of polymeric nanoparticles such as poly(lactic acid) (PLA) and it co-polymers, poly(lactide-co-glycolide) (PLGA); and poly(Ɛ-caprolactone) (Fessi et al. 1989).

Polymeric nanoparticles have been used to protect and increase to stability of carotenoids such as bixin (Lobato et al. 2013), lycopene (Dos Santos et al. 2015), lutein (Jin et al. 2009) and β-carotene (Cao-Hoang et al. 2011; da Silva et al. 2016). Bixin nanocapsules were produced to improve the stability of carotenoid during storage and presented mean diameter of 195 nm and physical stability during 119 days of storage at 25°C (Lobato et al. 2013). In others study, bixin nanoencapsulated, in the same condition, was evaluated in relation to stability under photosensitization and heating (65-95°C) and the results showed that bixin encapsulated was more stable than bixin free in both experiments of photosensitization and heating (Lobato et al. 2015).

The stability of β-carotene was tested in nanocapsules containing a blend of carotenoids from carrots, and it was observed that parameters such diameter (166nm) and zeta potential (-18mV) remained stable after 100 days of storage (Da Silva et al. 2016). Nanospheres containing synthetic β-carotene and polylactic acid were also synthesized to

increase the stability of this carotenoid and verified the formation of stable structures of 102 nm of diameter and oxidation stability (Cao-Hoang et al. 2011).

The lycopene nanocapsules stability was evaluated during storage and observed that nanocapsules presented satisfactory stability compared to free lycopene, showing 50 % content after 14 days of storage at room temperature (25°C), 40% after 84 days of storage at 5°C and stability under high temperature (60, 70 and 80°C) and photosensitization (5, 15 and 25°C) (Dos Santos et al. 2015; Dos Santos et al. 2016).

Lutein was nanoencapsulated using hydroxypropylmethyl cellulose phthalate and the authors obtained nanocapsules stables ranged from 163 nm to 219 nm (Jin et al. 2009). In another study, lutein was nanoencapsulated with chitosan to improve the solubility, and the results showed that lutein nanospheres with 200 nm of diameter presented higher solubility than no-nanoencapsulated lutein (Hong et al. 2016).

The polymeric nanoparticles have attracted the attention for applications due to advantages such as high stability, high encapsulation efficiency and controlled release of encapsulated compounds (Singh et al. 2011). In addition, nanocapsules present a central cavity that avoids the direct contact of the compounds with tissues and therefore irritation after administration (Couvreur et al. 2002). Thus, this nanostructure has been the most utilized to encapsulate carotenoids conferring more stability, safety, and long-lasting use.

4. Conclusion

Nanotechnology is a promising technology used to protect and improve the carotenoids application in food, cosmetic, and pharmaceutical field. Different nanostructures can be used to encapsulate carotenoids such as nanoemulsions, liposomes, and solid lipid nanoparticle; however, currently, polymeric nanocapsules are the nanostructure most utilized due to its stability during storage and high efficiency to encapsulate and to control the release of the carotenoid encapsulated.

This review discusses regarding the importance of nanocarrier characterization and stability evaluation. Size, zeta potential, encapsulation efficiency, desired compounds release profile are parameters investigated. In addition, this review also addresses the different types of nanostructures used to encapsulated carotenoids, and its advantages and limitations.

The literature studies showed that carotenoids have greater stability when exposed to the different conditions. The nanoparticles have uniformity in particle size with a smaller

diameter and greater surface area per volume, in general, when submitted to industrial processes and storage. The nanoencapsulation reduce the degradation rate of carotenoids and it can increase the application of these fat-soluble compounds, in the different research fields.

Therefore, carotenoids nanostructures present a high potential to use for nutritional food fortification or pharmaceutical and cosmetic research. However, most studies concerning nanostructures of carotenoids are related to synthesis and characterization, with reduced attention on the biological application and on the release kinetics of carotenoid. The additional studies regarding different carotenoids nanocarrier and the behavior of the compounds nanoencapsulated *in vivo* are required.

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References

Akbari B, Tavandashti MP, Zandrahimi M (2011) Particle Size Characterization of Nanoparticles–A Practical approach. Iranian Journal of Materials Science and Engineering 8(2): 48-56.

Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K (2013) Liposome: classification, preparation, and applications. Nanoscale Res Lett 8(1): 102-102.

Anarjan N, Tan CP, Nehdi IA, Ling TC (2012) Colloidal astaxanthin: Preparation, characterisation and bioavailability evaluation. Food Chem 135(3): 1303-1309.

Arana L, Salado C, Vega S, Aizpurua-Olaizola O, Arada Idl, Suarez T, Usobiaga A, Arrondo JL, Alonso A, Goñi FM, Alkorta I (2015) Solid lipid nanoparticles for delivery of Calendula officinalis extract. Colloid Surface B 135: 18-26.

Arunkumar R, Prashanth KV, Manabe Y, Hirata T, Sugawara T, Dharmesh SM, Baskaran V (2015) Biodegradable Poly (Lactic-co-Glycolic Acid)-Polyethylene Glycol Nanocapsules: An Efficient Carrier for Improved Solubility, Bioavailability, and Anticancer Property of Lutein. J Pharm Sci 104(6): 2085-2093.

Bhosale RR, Osmani RA, Ghodake PP, Shaikh SM, Chavan, SR (2014) Nanoemulsion: A review on novel profusion in advanced drug delivery. Indian Journal of Pharmaceutical and Biological Research 2(1): 122.

Birrenbach G, Speiser PP (1976) Polymerized micelles and their use as adjuvants in immunology. J Pharma Sci 65(12): 1763-1766.

Burghardt RC, Droleskey R (2005) Transmission Electron Microscopy Current Protocols in Microbiology: John Wiley & Sons, Inc.

Cao-Hoang L, Fougère R, Waché Y (2011) Increase in stability and change in supramolecular structure of β-carotene through encapsulation into polylactic acid nanoparticles. Food Chem 124(1): 42-49.

Couvreur P, Barratt G, Fattal E, Vauthier C (2002) Nanocapsule technology: a review. Therapeutic Drug Carrier Systems 19(2): 99-134.

Couvreur P, Dubernet C, Puisieux F (1995) Controlled drug delivery with nanoparticles: Current possibilities and future trends. Eur J Pharm Biophar 41(1): 2-13.

D'Souza S (2014). A Review of In Vitro Drug Release Test Methods for Nano-Sized Dosage Forms. Advances in Pharmaceutics 1-12.

Da Silva MM, Nora L, Cantillano RFF, Paese K, Guterres SS, Pohlmann AR, Costa TMH, Rios AdO (2016) The Production, Characterization, and the Stability of Carotenoids Loaded in Lipid-Core Nanocapsules. Food Bioprocess Tech 9(7): 1148-1158.

Damodaran S, Parkin KL, Fennema OR (2010) Química de alimentos de Fennema. (4th ed.). Porto Alegre: Artmed.

Donsì F, Sessa M, Mediouni H, Mgaidi A, Ferrari G (2011) Encapsulation of bioactive compounds in nanoemulsion- based delivery systems. Procedia Food Science 1: 1666-1671.

Dos Santos PP, Paese K, Guterres SS, Pohlmann AR, Costa TH, Jablonski A, Flôres SH, Rios AdO (2015) Development of lycopene-loaded lipid-core nanocapsules: physicochemical characterization and stability study. J Nanopart Res 17(2): 1-11.

Dos Santos PP, Paese K, Guterres SS, Pohlmann AR, Jablonski A, Flôres SH, Rios AdO (2016) Stability study of lycopene-loaded lipid-core nanocapsules under temperature and photosensitization. Lebensm-Wiss Technol 71: 190-195.

D'Souza SS, Faraj JA, DeLuca PP (2005) A model-dependent approach to correlate accelerated with real-time release from biodegradable microspheres. AAPS J 6(4): E553-E564.

Ezhilarasi PN, Karthik P, Chhanwal N, Anandharamakrishnan C. (2012) Nanoencapsulation Techniques for Food Bioactive Components: A Review. Food Bioprocess Tech 6(3): 628-647.

Fathi M, Mozafari MR., Mohebbi M (2012) Nanoencapsulation of food ingredients using lipid based delivery systems. Trends Food Sci Tech 23(1): 13-27.

Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S (1989) Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 55(1): R1-R4.

Gaumet M, Vargas A, Gurny R, Delie F (2008) Nanoparticles for drug delivery: The need for precision in reporting particle size parameters. Eur J Pharm Biopharm 69(1): 1-9.

Gregoriadis G (1973) Drug entrapment in liposomes. FEBS Letters 36(3): 292-296.

Gutiérrez FJ, Albillos SM, Casas-Sanz E, Cruz Z, García-Estrada C, García-Guerra A, García-Reverter J, García-Suárez M, Gatón P, González-Ferrero C, Olabarrieta I, Olasagasti M, Rainieri S, Rivera-Patiño D, Rojo R, Romo-Hualde A, Sáiz-Abajo M-J, Mussons M-L (2013) Methods for the nanoencapsulation of β-carotene in the food sector. Trends Food Sci Tech 32(2): 73-83.

Ha TV, Kim S, Choi Y, Kwak H-S, Lee, SJ, Wen J, Oey I, Ko S (2015) Antioxidant activity and bioaccessibility of size-different nanoemulsions for lycopene-enriched tomato extract. Food Chem 178: 115-121.

Hejri A, Khosravi A, Gharanjig K, Hejazi M (2013) Optimisation of the formulation of β-carotene loaded nanostructured lipid carriers prepared by solvent diffusion method. Food Chem 141(1): 117-123.

Hong DY, Lee J-S, Lee HG (2016) Chitosan/poly-γ-glutamic acid nanoparticles improve the solubility of lutein. Int J Biol Macromol 85: 9-15.

Jin H, Xia F, Jiang C, Zhao Y, He L (2009) Nanoencapsulation of Lutein with Hydroxypropylmethyl Cellulose Phthalate by Supercritical Antisolvent. Chinese J Chem Eng 17(4): 672-677.

Kaur S, Nautyal U, Singh R, Singh S, Devi A (2015) Nanostructure Lipid Carrier (NLC): the new generation of lipid nanoparticles. Asian Pacific Journal of Health Sciences 2(2): 76-93.

Lacatusu I, Mitrea E, Badea N, Stan R, Oprea O, Meghea A (2013) Lipid nanoparticles based on omega-3 fatty acids as effective carriers for lutein delivery. Preparation and in vitro characterization studies. J Funct Foods 5(3): 1260-1269.

Langer R (1990) New methods of drug delivery. Science 249(4976): 1527-1533.

Li Y, Zheng J, Xiao H, McClements DJ (2012) Nanoemulsion-based delivery systems for poorly water-soluble bioactive compounds: Influence of formulation parameters on polymethoxyflavone crystallization. Food Hydrocolloid 27(2): 517-528.

Lim E-K, Jang E, Lee K, Haam S, Huh Y-M (2013) Delivery of Cancer Therapeutics Using Nanotechnology. Pharmaceutics 5(2): 294-317.

Lobato KBdS, Paese K, Forgearini JC, Guterres SS, Jablonski A, Rios AdO (2013) Characterisation and stability evaluation of bixin nanocapsules. Food Chem 141(4): 3906-3912.

Lobato KBdS, Paese K, Forgearini JC, Guterres SS, Jablonski A, Rios AdO (2015) Evaluation of stability of bixin in nanocapsules in model systems of photosensitization and heating. Lebensm-Wiss Technol 60(1): 8-14.

Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM (2006) Nanoemulsions: formation, structure, and physical properties. J Phys-Condens Mat 18(41): R635.

Mehnert W, Mäder K (2001) Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliver Rev 47(2–3): 165-196.

Mishra RK, Soni GC, Mishra R (2014) Nanoemulsion: A Novel Drug Delivery Tool. Int J Pharm Res Rev 3(7): 32-43.

Mitri K, Shegokar R, Gohla S, Anselmi C, Müller RH (2011) Lipid nanocarriers for dermal delivery of lutein: Preparation, characterization, stability and performance. Int J Pharm 414(1–2): 267-275.

Mohanraj VJ, Chen Y (2006) Nanoparticles – A Review. Trop J Pharm Res 5: 561-573.

Mozafari MR, Johnson C, Hatziantoniou S, Demetzos C (2008) Nanoliposomes and their applications in food nanotechnology. J Liposome Res 18(4): 309-327.

Muller LK, Landfester K (2015) Natural liposomes and synthetic polymeric structures for biomedical applications. Biochem Bioph Res Co 468(3): 411-418.

Muthu MS, Singh S (2009) Poly (D, L-lactide) nanosuspensions of risperidone for parenteral delivery: formulation and in-vitro evaluation. Current Drug Delivery 6(1): 62-68.

Müller RH, Mäder K, Gohla S (2000) Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. Eur J Pharm Biopharm 50(1): 161-177.

Okonogi S, Riangjanapatee P (2015) Physicochemical characterization of lycopene-loaded nanostructured lipid carrier formulations for topical administration. Int J Pharma 478(2): 726-735.

Qian C, Decker EA, Xiao H, McClements DJ (2012) Inhibition of β-carotene degradation in oil-in-water nanoemulsions: Influence of oil-soluble and water-soluble antioxidants. Food Chem 135(3): 1036-1043.

Qian C, Decker EA, Xiao H, McClements DJ (2013) Impact of lipid nanoparticle physical state on particle aggregation and β-carotene degradation: Potential limitations of solid lipid nanoparticles. Food Res Int 52(1): 342-349.

Quintanar-Guerrero D, Allemann E, Fessi H, Doelker E (1998) Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. Drug Dev Ind Pharm 24(12): 1113-1128.

Radtke M, Muller RH (2001) NLS^{TM} nanostructured lipid carriers: the new generation of lipid drug carriers. New Drugs 2: 48-52.

Rao MP, Manjunath K, Bhagawati ST, Thippeswamy BS (2014) Bixin loaded solid lipid nanoparticles for enhanced hepatoprotection – Preparation, characterisation and in vivo evaluation. Int J Pharm 473(1–2): 485-492.

Rascón MP, Beristain CI, García HS, Salgado MA (2011) Carotenoid retention and storage stability of spray-dried encapsulated paprika oleoresin using gum Arabic and Soy protein isolate as wall materials. Lebensm-Wiss Technol 44(2): 549-557.

Reis CP, Neufeld RJ, Ribeiro AJ, Veiga F (2006) Nanoencapsulation II. Biomedical applications and current status of peptide and protein nanoparticulate delivery systems. Nanomed-Nanotechnol 2(2): 53-65.

Sanguansri P, Augustin MA (2006) Nanoscale materials development – a food industry perspective. Trends Food Sci Tech 17(10): 547-556.

Silva HD, Cerqueira MA, Souza BWS, Ribeiro C, Avides MC, Quintas MAC, Coimbra JSR, Carneiro-da-Cunha MG, Vicente AA (2011) Nanoemulsions of β-carotene using a high-energy emulsification–evaporation technique. J Food Eng, 102(2): 130-135.

Singh S, Pandey VK, Tewari RP, Agarwal V (2011) Nanoparticle Based Drug Delivery System: Advantages and Applications. Indian Journal of Science and Technology 4(3): 177-180.

Sáiz-Abajo M-J, González-Ferrero C, Moreno-Ruiz A, Romo-Hualde A, González-Navarro CJ (2013) Thermal protection of β-carotene in re-assembled casein micelles during different processing technologies applied in food industry. Food Chem 138(2–3): 1581-1587.

Tachaprutinun A, Udomsup T, Luadthong C, Wanichwecharungruang S (2009) Preventing the thermal degradation of astaxanthin through nanoencapsulation. Int J Pharma 374(1–2): 119-124.

Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A (2014) Design and characterization of astaxanthin-loaded nanostructured lipid carriers. Innov Food Sci Emerg 26: 366-374.

Tan C, Feng B, Zhang X, Xia W, Xia S (2016) Biopolymer-coated liposomes by electrostatic adsorption of chitosan (chitosomes) as novel delivery systems for carotenoids. Food Hydrocolloid 52: 774-784.

Tan C, Zhang Y, Abbas S, Feng B, Zhang X, Xia S (2014) Modulation of the carotenoid bioaccessibility through liposomal encapsulation. Colloid Surface B 123: 692-700.

Tan TB, Yussof NS, Abas F, Mirhosseini H, Nehdi IA, Tan CP (2016) Forming a lutein nanodispersion via solvent displacement method: The effects of processing parameters and emulsifiers with different stabilizing mechanisms. Food Chem 194: 416-423.

Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. The Plant Journal 54(4): 733-749.

Toniazzo T, Berbel IF, Cho S, Fávaro-Trindade CS, Moraes ICF, Pinho SC (2014) β-carotene-loaded liposome dispersions stabilized with xanthan and guar gums: Physico-chemical stability and feasibility of application in yogurt. Lebensm-Wiss Technol 59(2, Part 2): 1265-1273.

Vanaja K, Wahl MA, Bukarica L, Heinle H (2013) Liposomes as carriers of the lipid soluble antioxidant resveratrol: Evaluation of amelioration of oxidative stress by additional antioxidant vitamin. Life Sciences 93(24): 917-923.

Venturini CG, Jäger E, Oliveira CP, Bernardi A, Battastini AMO, Guterres SS, Pohlmann AR (2011) Formulation of lipid core nanocapsules. Colloid Surface A 375(1–3): 200-208.

Vishwanathan R, Wilson TA, Nicolosi RJ (2009) Bioavailability of a Nanoemulsion of Lutein is Greater than a Lutein Supplement. Nano Biomedicine and Engineering 1(1): 38-49.

Weiss J., Decker E, McClements DJ, Kristbergsson K, Helgason T, Awad T (2008) Solid Lipid Nanoparticles as Delivery Systems for Bioactive Food Components. Food Biophysics 3(2): 146-154.

Win KY, Feng S-S (2005) Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs. Biomaterials 26(15): 2713-2722.

Wu L, Zhang J, Watanabe W (2011) Physical and chemical stability of drug nanoparticles. Adv Drug Deliver Rev 63(6): 456-469.

Xia S, Tan C, Zhang Y, Abbas S, Feng B, Zhang X, Qin F (2015) Modulating effect of lipid bilayer–carotenoid interactions on the property of liposome encapsulation. Colloid Surface B 128: 172-180.

Yegin BA, Lamprecht A (2006) Lipid nanocapsule size analysis by hydrodynamic chromatography and photon correlation spectroscopy. Int J Pharma 320(1–2): 165-170.

Yih TC, Al-Fandi M (2006) Engineered nanoparticles as precise drug delivery systems. J Cell Biochem 97(6): 1184-1190.

Yin L-J, Chu B-S, Kobayashi I, Nakajima M (2009) Performance of selected emulsifiers and their combinations in the preparation of β-carotene nanodispersions. Food Hydrocolloid 23(6): 1617-1622.

Yuan Y, Gao Y, Mao L, Zhao J (2008) Optimisation of conditions for the preparation of β-carotene nanoemulsions using response surface methodology. Food Chem 107(3): 1300-1306.

Üner M, Yener G (2007) Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. Int J Nanomed 2(3): 289-300.

BIODEGRADABLE POLYMERS AS WALL MATERIALS TO THE SYNTHESIS OF BIOACTIVE COMPOUND NANOCAPSULES

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Biodegradable polymers as wall materials to the synthesis of bioactive compound nanocapsules

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ABSTRACT

Background

Nanoparticles have been synthetized using polymers as wall materials to protect bioactive compounds against external factors (light, heat and oxygen), increasing the stability and improving the bioavailability of the nanoencapsulated compound.

Scope and Approach

The encapsulation processes and type of polymers (natural and synthetic) exert a direct impact on the synthesis of bioactive compounds nanocapsules, which reflect in parameters such as size, zeta potential, encapsulation efficiency, aqueous solubility, aqueous stability, surface permeability, desired bioactive compounds release profile and wall resistance and these characteristics might limit its use by food, pharmaceutical and cosmetic industries. This review summarizes researches on nanocapsules synthesis (advantages and limitations of different techniques) and focuses on the importance of different biodegradable polymers as wall materials to the obtention of stable and safe nanocapsules.

Key Findings and Conclusions

Different wall materials can be used to synthesize bioactive compound nanocapsules; however, biodegradable polymeric nanocapsules have proven to be one of the most stable structures during storage and showed high efficiency to control the release of encapsulated compounds and due to these characteristics, they have been focus of various studies for future applications in health and food-related areas.

Keywords: Nanotechnology; biopolymer; polymeric nanocapsule, bioactive compound.

1. Introduction

Nanotechnology has been considered one of the main technology of the 21st century and it promises a revolution in the pharmaceutical, medical and food fields. This technology involves design, synthesis, characterization and application of particles or systems with dimensions less than 1 μm (Hoyt & Mason, 2008).

The nanotechnology is currently applied in a great number of commercial products (cosmetics and sunscreens, drugs, dental fillings, food), as well as in water filtration and catalytic systems (Brayner, 2008). In drug delivery systems, the use of polymeric nanoparticles improves the stability, absorption, and therapeutic concentration of drugs within the target tissue (Kayser, Lemke, & Hernandez-Trejo, 2005). In addition, nanotechnology can also improve thermal and storage stabilities, water solubility and bioavailability of bioactive compounds for food application (Huang, Yu, & Ru, 2010), innovating the macroscale characteristics of foods, such as texture, taste, coloring strength and industrial processes (Ezhilarasi, Karthik, Chhanwal, & Anandharamakrishnan, 2012).

Bioactive compounds can be nanoencapsulated by different types of nano-sized vehicles, such as nanoemulsion, liposomes or nanocapsules. Nanoemulsion, which corresponds to preparation of oil-in-water (O/W) emulsion, improves solubility and bioavailability of bioactive compounds due to the reduction of incomplete dissolution of lipids (Yin, Chu, Kobayashi, & Nakajima, 2009), while liposomes are prepared with lipids and phospholipids, providing protection to the compound encapsulated in their core (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). However, liposomes have low encapsulation efficiency and fast leakage rate of water-soluble drugs in the blood; and nanoemulsions have very low storage stability (Sharma, Bansal, Visht, Sharma, & Kulkarni, 2010; Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). Considering this context, biodegradable polymeric nanocapsules have attracted the attention for innumerous applications due to its high stability, high encapsulation efficiency and controlled release of encapsulated compounds (Singh, Pandey, Tewari, & Agarwal, 2011).

Polymeric nanocapsules are prepared with natural or synthetic polymers as wall materials and they can be used to nanoencapsulate drugs and bioactive compounds. The use of "generally recognized as safe" (GRAS) materials is important to produce safe nanocapsules under the conditions of its intended use, along with nutritional quality and stability to food or

pharmaceutical applications. These GRAS materials generally exhibit controlled-release behavior and some examples were already referred in the literature, such as polysaccharides from plants (gum Arabic) or microbial (Xanthan gum) origin. Moreover, food proteins (whey protein), emulsifiers, such as lecithin, Tweens, Spans (Huang, Yu, & Ru, 2010) and synthetic polymers such as poly(lactic acid) (PLA) and it co-polymers, poly(lactide-co-glycolide) (PLGA); and poly(Ɛ-caprolactone) (PCL) are also frequently used due to their biocompatibility and biodegradability characteristics (Hans & Lowman, 2002).

Thus, the purpose of this review was to gather information concerning the techniques of nanocapsules synthesis and the most frequent biodegradable polymers used as wall material for polymeric nanocapsules of bioactive compounds. Furthermore, the readers may figure out how these polymers can affect the characteristics of the synthesized nanocapsules.

2. Synthesis of polymeric nanocapsules

Nanocapsules are vesicular system in which specific compounds, solubilized in an aqueous or oil core, are covered by a single polymeric membrane (wall material) (Couvreur, Barratt, Fattal, & Vauthier, 2002). After synthesis, the evaluation of the nanocapsules stability is crucial mainly in relation to important parameters, such as size, polydispersity index (PDI), zeta potential, morphology, pH and release profile.

Size and size distribution of nanocapsules are essential due to their ability to modify physicochemical and pharmaceutical behaviors of encapsulated compounds (Yegin & Lamprecht, 2006). Nanoparticles size, also denominated mean diameter or z-average, can be determined by several methods, such as laser diffraction (LD) and coulter counter; however, the most used technique is dynamic light scattering (DLS) (Venturini et al., 2011; Yegin & Lamprecht, 2006), which allows the description of particle size distribution and destabilization phenomena. The size distribution is indicated as PDI that represents the particles uniformity in suspension, in which PDI values between 0.1 and 0.25 indicate a narrow size distribution and PDI values higher than 0.5 indicate a broad distribution (Wu, Zhang, & Watanabe, 2011).

Zeta potential is a physical property that is exhibited by particles in suspension, macromolecule or material surface; it corresponds to the electrical potential of nanoparticles as influenced by the nanocapsule composition and the medium in which they are dispersed (Lobato et al, 2013; Wu, Zhang, & Watanabe, 2011). This parameter is widely used to

indicate suspension stability in colloidal dispersions, where zeta potential values higher than 30 mV and lower than -30 mV promote high stability and prevent particles aggregation (Mohanraj & Chen, 2006).

Morphology is also another important parameter for the characterization of nanocapsules and many types of electron microscopy are usually applied to observe nanoparticle morphology and structure. As an example, currently, transmission electron microscopy (TEM) is the most used technique, which is performed after freeze–fracture of nanocapsules that allow to obtain information about structure, polymer porosity and wall thickness estimative (Couvreur, Barratt, Fattal, & Vauthier, 2002).

The above-mentioned parameters should be evaluated after the preparation of nanocapsules and monitored during storage of the suspension. However, nanocapsules stability depends on the composition and chosen technique for the synthesis and, as consequence, the most efficient technique to prepare nanocapsules will depend on both the physicochemical characteristics of polymer and also the bioactive compound to be nanoencapsulated (Reis, Neufeld, Ribeiro, & Veiga, 2006). Furthermore, the choice of the organic solvent used during nanocapsules synthesis is also very important due to the possibility of providing risk to human health, limiting the application of nanocapsules.

According to the formulation, the nanocapsules can be synthesized by two different methods (Figure 1): interfacial polymerization of monomers and preformed polymer (Couvreur, Dubernet, & Puisieux, 1995).

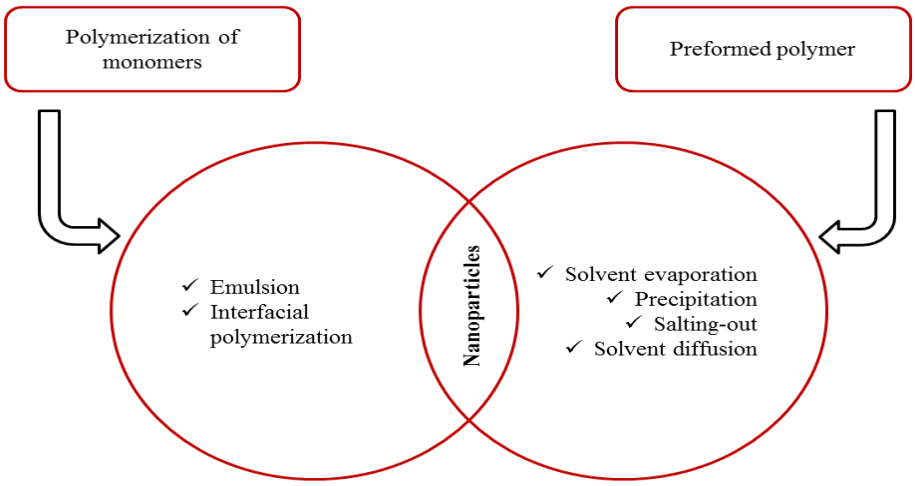

Figure 1: Scheme of techniques for polymeric nanocapsules synthesis, according to the formulation.

2.1. Nanocapsule synthesis by polymerization of monomers

In this method, monomers of cyanoacrylate are polymerized to form nanoparticles in aqueous solution. Polymerization of monomers was applied for the first time to obtain structures denominated “nanoparts” constituted of polyoxyethylene 4-lauryl ether to the application as adjuvants (enhancers of the immune response) in immunology (Birrenbach & Speiser, 1976). However, the term “nanocapsules” was introduced for this structure by Couvrier *et al.* (1977), which synthetized nanocapsules of polyacrylamide aiming to allow access of some compounds for lysosomes.

Monomer polymerization can be classified into emulsion and interfacial polymerization, where the first is one of the fastest techniques for nanoparticle obtention and the second one was reported to present high drug encapsulation efficiency (Reis, Neufeld, Ribeiro, & Veiga, 2006).

Briefly, the monomer is added in an aqueous solution containing surfactant (polymerization medium) under vigorous mechanical stirring to polymerize at room temperature (Figure 2) (Rollot, Couvreur, Roblot-Treupel, & Puisieux, 1986). During polymerization process, stabilizers and surfactants are added in the formulation and the type and concentration of these constituents are responsible for particle size and molecular mass of nanocapsules obtained; the solvents are used to disperse the oil in aqueous phase and serve as vehicle for monomers (Mohanraj & Chen, 2006; Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). The compound of interest is incorporated either by solubilization in the polymerization medium or by adsorption after completed polymerization (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). After the concentration under reduced pressure at room temperature, the suspension has to be purified to remove stabilizers and surfactants by ultracentrifugation and, then, the particles are suspended in surfactant-free medium (Mohanraj & Chen, 2006).

The monomer that is used in the synthesis of nanocapsules should present fast polymerization rate between the organic phase and aqueous phase. As an example, poly-(alkylcyanoacrylate) presents very fast polymerization rate and it is biodegradable and biocompatible (Reis, Neufeld, Ribeiro, & Veiga, 2006; Krauel, Pitaksuteepong, Davies, & Rades, 2004; Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). Furthermore, polymerization must be carried out in acidic medium and further pH increase to produce high

molecular mass, as well as stable nanocapsules (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001).

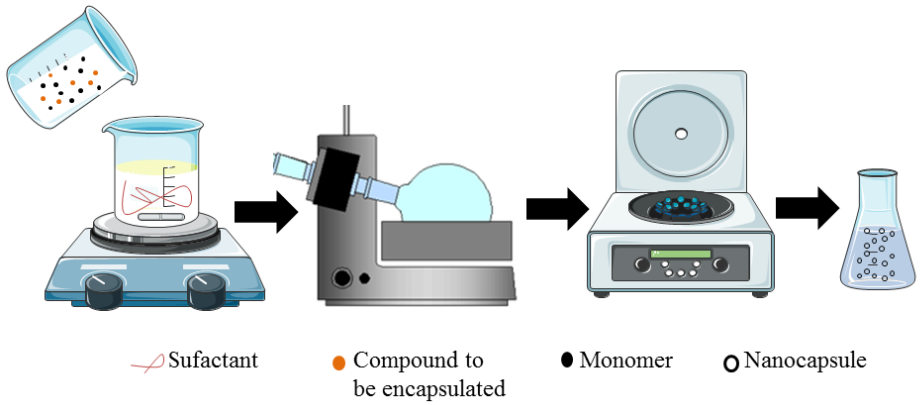

Figure 2: Schematic representation for obtention of nanocapsules by polymerization of monomers.

Nanocapsules obtained by polymerization of monomers were developed to the application with pharmaceutical compounds, such as endocytizable and lysosomotropic drugs (Couvreur, Tulkenst, Roland, Trouet, & Speiser, 1977; Birrenbach & Speiser, 1976). Moreover, researches regarding nanocapsules prepared by polymerization of monomers for the application in textile industries have been reported. As an example, rose fragrance was nanoencapsulated for aroma sustained-release in cotton textiles utilizing polybutylcyanoacrylate (Hu et al., 2011).

The main advantage of this nanoencapsulation technique is the formation of wall in contours of emulsion since the polymer is formed *in situ*; however, undesirable reactions between monomers and the compounds of interest may occur during polymerization process (Couvreur, Barratt, Fattal, & Vauthier, 2002). The possible presence of residual monomers or undesirable products formed after polymerization can be toxic and limit the potential use of these kind of nanocapsules (Fessi, Puisieux, Devissaguet, Ammoury, & Benita, 1989).

Thus, the main applications of polymerization of monomers technique in bioactive compounds are associated only with proteins and peptides (Krauel, Pitaksuteepong, Davies, & Rades, 2004) and none studies were reported concerning its application for bioactive compounds, such as carotenoids, phenolic compounds or essential oils.

2.2. Nanocapsule synthesis by preformed polymer

Due to the presence of toxic compounds in nanocapsules synthesis by polymerization of monomers, as described above, some techniques using preformed polymers, instead of monomers, have been proposed since this technique offers the possibility to control the molecular mass and PDI of the polymer (Vrignaud, Benoit, & Saulnier, 2011).

The first technique employed to prepare nanocapsules by preformed polymer was the solvent evaporation (Vanderhoff, El-Aasser, & Ugelstad, 1979) aiming to synthesize more stable and resistant nanocapsules to settling or sedimentation and it has been widely used to synthetize nanocapsules in the current literature on techniques using dispersed preformed polymers (Rao & Geckeler, 2011).

In general, in preformed polymer technique, the aqueous insoluble polymer is dispersed in an aqueous phase in the presence of stabilizers, surfactants and oil (Vanderhoff, El-Aasser, & Ugelstad, 1979). Emulsification/solvent evaporation, interfacial deposition, emulsification/solvent diffusion and salting-out are typical examples of preformed polymer techniques (Figure 3).

In the solvent evaporation method (Figure 3A), the polymer is dissolved in an organic solvent, such as chloroform, dichloromethane or ethyl acetate, and the synthesis of nanocapsules by this technique involve two steps: the emulsification of polymer solution (organic phase) in an aqueous phase and evaporation of solvent, inducing polymer precipitation and formation of nanoparticles (Reis, Neufeld, Ribeiro, & Veiga, 2006). Nanocapsules containing bacoflen (medical drug used as a skeletal muscle relaxant) (Nabi-Meibodi et al., 2013), albumin (Landry, Bazile, Spenlehauer, Veillard, & Kreuter, 1996) and bioactive compounds, such as lutein (Arunkumar et al., 2015) and flavonoids (Roussaki et al., 2014) were already obtained by this technique. The advantage of using solvent evaporation method for synthesis of nanocapsules is the utilization of aqueous phase as suspension that eliminates the need for recycling, improving the synthesis process. However, it can only be applied to fat-soluble compounds, limiting the application for other compounds (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001)

Emulsification/solvent diffusion (Figure 3B), also denominated emulsification solvent-displacement, is a technique based on organic solvents and then was adapted to the following salting-out procedure, where the polymer solvent used to prepare the emulsion needs to be partly soluble in water, such as propylene carbonate (Vauthier & Bouchemal, 2009). In this

method, the polymer is dissolved in a partially water-soluble solvent and saturated with water, promoting the diffusion to occur precipitation of the polymer and the consequent formation of nanoparticles. Finally, the solvent is eliminated by evaporation or filtration. Nanocapsules prepared by emulsification/solvent diffusion have been developed as drug carriers, as in the case of diclofenac (Mora-Huertas, Garrigues, Fessi, & Elaissari, 2012). The high encapsulation efficiency, narrow size distribution and the efficiency to encapsulate lipophilic and hydrophilic drugs are important advantages and, thus, this technique is also used in the synthesis of bioactive compounds nanocapsules for food application, such as β-carotene (González-Reza, Quintanar-Guerrero, Flores-Minutti, Gutiérrez-Cortez, & Zambrano-Zaragoza, 2015) and food grade oil (Zambrano-Zaragoza, Mercado-Silva, Gutiérrez-Cortez, Castaño-Tostado, & Quintanar-Guerrero, 2011).

Salting out is a method based on the separation of water–miscible solvent from aqueous solution by the salting-out effect that consist in the use of electrolytes for polymer desolvation (Quintanar-Guerrero, Allemann, Fessi, & Doelker, 1998). In this method (Figure 3C), both the polymer and compound of interest are dissolved in an emulsified solvent into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate). Then, a colloidal stabilizer and an aqueous solution is added in the emulsion promoting solvent diffusion, inducing the formation of nanocapsules (Figure 3C) (Quintanar-Guerrero, Allemann, Fessi, & Doelker, 1998). Acetone is the water-miscible solvent generally chosen due to its physicochemical characteristics, mainly concerning its solubilizing efficiency and good separation from aqueous solutions by salting-out with electrolytes. In addition, the selection of the salting-out agent is important to the encapsulation efficiency (Rao & Geckeler, 2011).

The most used technique to prepare nanocapsules with bioactive compounds is the interfacial deposition, also named nanoprecipitation, that is based on the interfacial deposition of a polymer after displacement of a semi-polar solvent (miscible in water) from a lipophilic solution (Rao & Geckeler, 2011). This method was first developed by Fessi *et al.* (1989) to encapsulate medical drugs, such as indomethacin, progesterone and dexamethasone, using different biodegradables polymers and stabilizers, ensuring high encapsulation efficiency of drugs and increasing the potential of use of these nanocapsules due to the complete elimination of solvents in suspension.

In deposition interfacial of preformed polymer technique (Figure 3D), the organic phase containing the polymers, an aliquot of a solvent easy to remove (acetone or ethanol),

oil, triglycerides and the compound of interest is injected in an aqueous phase containing water and a tensoative compound under magnetic stirring. After that, the polymer deposition occurs immediately on the interface between the water and the organic solvent, induced by the rapid diffusion of the solvent, leading the instantaneous formation of nanocapsules (Quintanar-Guerrero, Allemann, Fessi, & Doelker, 1998). After nanocapsules formation, the organic solvent is removed by evaporation under reduced pressure. Some factors are very important to prepare an uniform and homogeny colloidal suspension by interfacial deposition, such as polymer concentration, polymer insolubility in aqueous phase and oily phase, triglycerides concentration and diffusion of the organic solvent (Couvreur, Barratt, Fattal, & Vauthier, 2002). Venturini *et al.* (2011) evaluated the influence of these parameters in the formulation of exclusively lipid-core nanocapsules in aqueous suspensions. They concluded that the optimal proportion to obtain only lipid-core nanocapsules in suspension was 1:4.1:2.6 (w/w/w) of sorbitan monostearate, caprylic/capric triglyceride and polymer, respectively, and that the increase in the sorbitan monostearate and oil resulted in creaming.

Nanocapsules containing polylactic acid (PLA), poly-d,l-lactide-co-glycolide (PGLA) and poly-ε-caprolactone (PCL) as wall materials were already obtained by interfacial deposition of preformed polymer technique (Coradini et al., 2014; Fessi, Puisieux, Devissaguet, Ammoury, & Benita, 1989). Some medical drugs, such as indomethacin (Bernardi et al., 2009) and clobetasol propionate (Fontana, Coradini, Guterres, Pohlmann, & Beck, 2009) and several bioactive compounds, such as carotenoids (dos Santos et al., 2015; Lobato et al, 2013), flavonoids (Coradini et al., 2014) and food grade oils (Contri, Ribeiro, Fiel, Pohlmann, & Guterres, 2013) have also been nanoencapsulated by this technique for food application. The main advantage of this technique is the possibility of complete removal of organic solvents in its composition (Fessi, Puisieux, Devissaguet, Ammoury, & Benita, 1989) that allows the encapsulation of bioactive compounds for food and pharmaceutical applications (Coradini et al., 2014; dos Santos et al., 2015; Lobato et al., 2013). Moreover, this technique allowed the use of natural and synthetic polymers, such as the ones described in details in the following section.

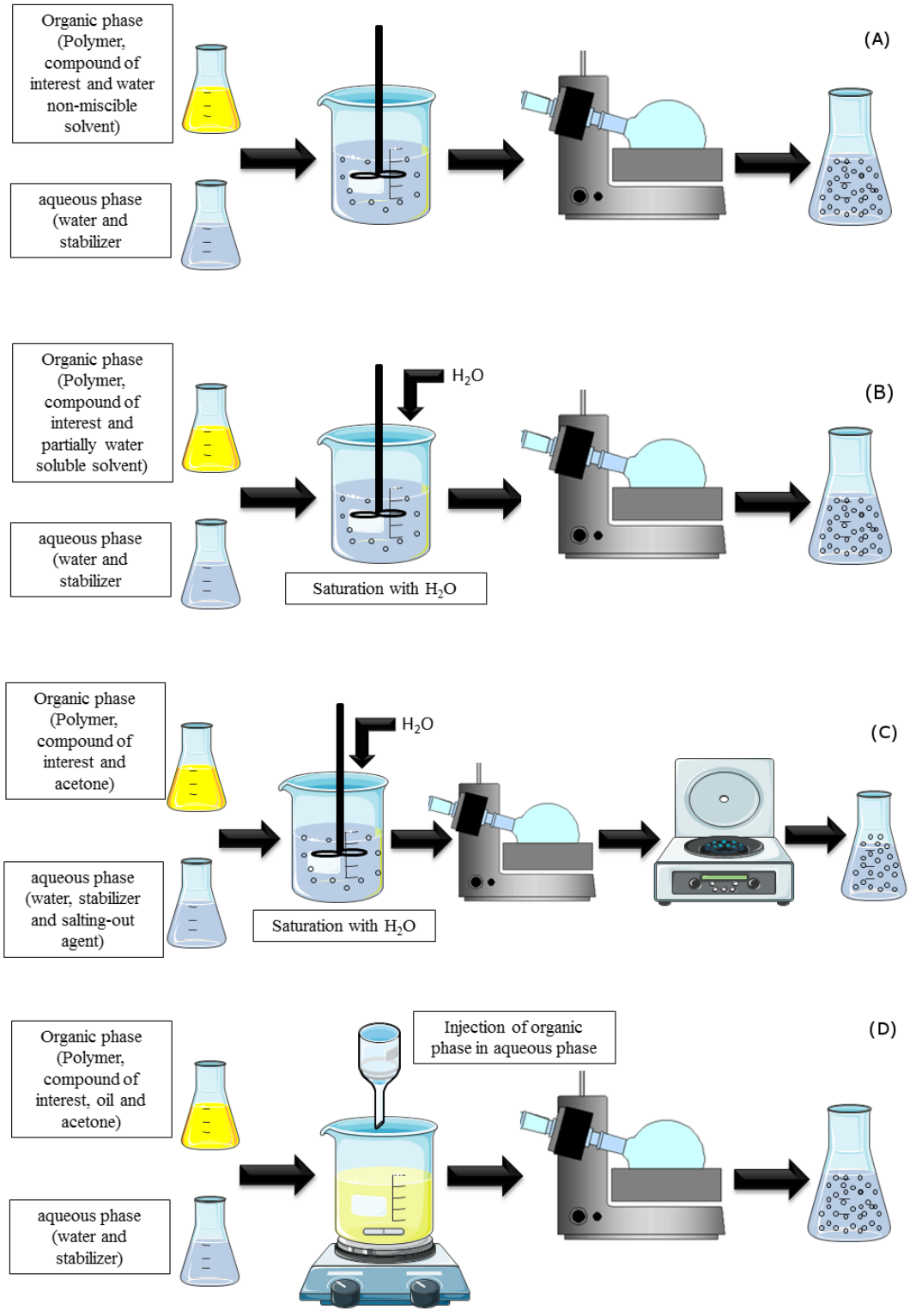

Figure 3: Schematic representation of nanocapsules synthesis by preformed polymer: (A) Solvent evaporation method, (B) Emulsification/solvent diffusion method, (C) Salting-out method and (D) Interfacial deposition method.

3. Biodegradable polymers: characteristics and effects on nanocapsules formulations

Polymeric nanocapsules have been synthetized using different polymers (Figure 4) according to the application and type of compound to be nanoencapsulated to improve therapeutic benefits and minimize side effects (Nair & Laurencin, 2007). The polymer choice depends on the size of required nanoparticles, aqueous solubility, aqueous stability, surface permeability and desired drug release profile (Mohanraj & Chen, 2006). Moreover, polymeric nanocapsules are used to facilitate drug administration and drug delivery system (Reis, Neufeld, Ribeiro, & Veiga, 2006) and recently, nanocapsules have also been used to increase application and stability of bioactive compounds, as summarized in Table 1.

In this sense, nanoparticles can be prepared from a variety of compounds as wall materials, such as proteins, polysaccharides and synthetic polymers and their physicochemical characteristics can be influenced by the combination of different polymers, as described below.

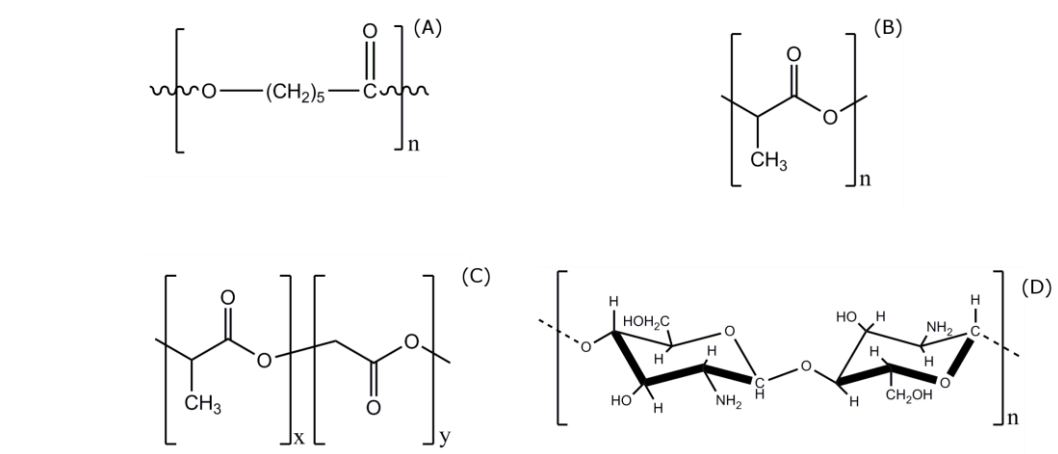

Figure 4: Chemical structures of some biodegradable polymers used for nanocapsules synthesis: (A) Poly- (Ɛ-caprolactone); (B) Polylactic acid; (C) Poly-D, L-lactide-co-glycolide and (D) chitosan.

Table 1: Characteristics of bioactive compound nanocapsules synthesized with biodegradable polymers as wall materials.

Polymer	Encapsulated compound	Diameter (nm)	PDI	zeta Potential(mV)	Encapsulation Efficiency (%)	Release (%) in neutral pH	Reference
PCL	α- tocopherol	172	0.28	-19	97	Not reported	Khayata et al. (2012)
		184	0.11	−30.6 to −51.1	99	Not reported	Noronha et al. (2013)
	Bixin	195	0.09	-14	100	Not reported	Lobato et al. (2013)
	β-carotene	191	0.16	-34	Not reported	Not reported	González-Reza et al. (2015)
	Curcumin	200	0.12	-8 to -10	100	35% after 72 h [water/Tween 80®/ethanol (80:2:20, v/v/v)]	Coradini et al. (2014)
	Lycopene	193	0.07	-12	98	Not reported	dos Santos, et al. (2015)
	Quercetin	216 – 253	0.09-0.19	-12	66 – 69	66% after 48 h [phosphate buffered saline/methanol (80:20,v/v)]	Kumar et al. (2015)
	Tocopherols/tocotrienols	200	0.09	-9	Not reported	Not reported	Rigo et al. (2015)
PLA	Auresidine	231 – 376	0.2-0.3	-2 to -20	98	Not reported	Roussaki et al., 2014
	Quercetin	130	Not reported	Not reported	97	100% after 72 h (phosphate buffered; Saline 0.01M/0.1% NaN_3 (v/v))	Kumari et al. (2010)
		250	Not reported	Not reported	40	100% after 14 days (phosphate buffered	Kumari et al. (2011)

						Saline 0.01M/0.1% NaN_3 (w/v))	
PLGA	Lutein	200	0.20	-21 - -29	72 – 88	66% after 72h (phosphate buffered Saline 0.01M)	Arunkumar, et al. (2015)
	Campomanesia xanthocarpa O. Berg extract	153-155	0.28-0.21	Not reported	84-98	92% after 1 h/ 77% after 6 h (0.1 M sodium dodecyl sulfate and 0.1 M NaCl)	Pereira et al. (2015)
	Tocopherols/tocotrienols	124 – 131	0.11	-6	4	Not reported	Alqahtani et al. (2015)
Chitosan	Cinnamon essential oil	112 – 527	0.35-0.62	Not reported	12-20	not reported	Hu et al. (2015)
	Curcumin	300-500	Not reported	Not reported	19-58	87% after 50 h (phosphate buffered saline)	Liu et al. (2015)
		159	0.14	-17	100	Not reported	Abbas, et al. (2015)
	Lemongrass oil	59-226	Not reported	-11 – 36	87	42% after 48 h (phosphate buffered saline)	Natrajan et al. (2015)
	Lutein	200	0.074	Not reported	100	not reported	Hong et al. (2016)
		80 – 600	Not reported	Not reported	85	54% after 8 h (Plasma)	Arunkumar et al. (2013)
	Turmeric oil	92 – 256	Not reported	27-37	71	90% after 48 h (phosphate buffered saline)	Natrajan et al. (2015)
	Vitamin B_2	104	0.32	-29	56	< 1% after 24 h (phosphate buffered saline)	Azevedo et al. (2014)

Abbreviations: PCL: Poly-Ɛ-caprolactone; PLA: Polylactic acid; PLGA: Poly-D, L-lactide-co-glycolide; PDI: Polydispersity index

3.1. Poly-Ɛ- caprolactone (PCL)

Poly-Ɛ-caprolactone $(C_6H_{10}O_2)_n$ (Figure 4A) is a polymer obtained by ring-opening polymerization of the cyclic monomer Ɛ-caprolactone utilizing anionic, cationic, coordination or radical catalysts mechanism (Langer & Chasin, 1990).

PCL is a semi-crystalline polymer and its crystallinity is directly related to its molecular weight. This polymer is only soluble in organic solvents, presents low melting point (59–64 ◦C) and good blend-compatibility that allows change in chemical characteristics, such as solubility, porosity, degradation and drug release (Dash & Konkimalla, 2012; Nair & Laurencin, 2007). Furthermore, PCL has flexible mechanical characteristics, such as elasticity and tensile strength that are important to pharmaceutical and paramedical application (Woodruff & Hutmacher, 2010). Additionally, due to its characteristics to be a biodegradable and biocompatible polymer, PCL is used in several formulations for tissue engineering and controlled drug delivery due to its long time degradation, reducing the drug release up to several months (Dash & Konkimalla, 2012).

Due to its safety and cytocompatibility, it has been also used in the synthesis of bioactive compounds nanocapsules (Kumar, Verma, & Singh, 2015; Khayata, Abdelwahed, Chehna, Charcosset, & Fessi, 2012; Noronha, de Carvalho, Lino, & Barreto, 2014). Large-scale production of α-tocopherol nanocapsules by the nanoprecipitation technique was optimized using PCL as wall material and the nanocapsules presented 172 nm of mean diameter, PDI of 0.28, zeta potential of -19 mV and 97% of encapsulation efficiency (Khayata, Abdelwahed, Chehna, Charcosset, & Fessi, 2012).

In another study, α-tocopherol nanocapsules prepared with high encapsulation efficiency (99%), 184.6 nm of diameter and PDI of 0.112 were applied as antioxidant in films to active packaging (Noronha, de Carvalho, Lino, & Barreto, 2014). These authors observed that α-tocopherol nanocapsules conferred antioxidant potential to the films due to the fast α-tocopherol release of nanocapsules with a burst effect after 1 h and sustained release over 10 days.

Rice bran oil was reported to contain considerable levels of bioactive compounds, such tocopherols, tocotrienols and γ-oryzanol (Chen & Bergman, 2005). Nanocapsules of rice bran oil were synthetized using PCL as wall material to evaluate its protective effect against UVB radiation-induced skin injury in mice and the authors concluded that rice bran

nanocapsules (200 nm, potential zeta of -9 mV and low PDI of <0.2) inhibited 60% of edema induced by UVB-irradiation (Rigo et al., 2015).

The use of PCL in nanocapsules formulations also allows the production of carotenoids nanocapsules in order to improve solubility, stability and increasing industrial applications for these compounds, such as bixin, lycopene and β-carotene (Coradini et al., 2014; dos Santos et al., 2015; González-Reza, Quintanar-Guerrero, Flores-Minutti, Gutiérrez-Cortez, & Zambrano-Zaragoza, 2015; Lobato et al., 2013). Nanocapsules prepared with these kind of compounds presented similar behaviors in relation to the parameters of diameter (195-209 nm), PDI (0.008- 0.304, zeta potential (-12 to -9 mV) and stability; and these characteristics can be associated to the synthesis technique and the wall material (Dash & Konkimalla, 2012).

PCL presents innumerous advantages over other polymers, such as low cost, ease of shaping and manufacture enabling appropriate pore sizes conducive to tissue and the controlled delivery of drugs (Dash & Konkimalla, 2012). However, its long-term degradation and intracellular resorption pathways can limit the application of this polymer (Woodruff & Hutmacher, 2010).

3.2. Polylactic acid (PLA)

Polylactic acid [$(C_3H_4O_2)n$] (Figure 4B) can be synthetized by ring-opening polymerization of lactide with metal catalyst in solution, in the melt, or by direct condensation of lactic acid monomers (Södergård & Stolt, 2010).

PLA has received especial attention due to its physicochemical characteristics, such as high melting point (175 °C), high degree of transparency and simple chemical synthesis (Tokiwa & Calabia, 2006). Moreover, this type of polymer can be amorphous glassy with a glass transition of 60 °C or semi crystalline/highly crystalline with crystalline melting points ranging from 130 to 180°C (Lunt, 1998).

PLA is biocompatible, biodegradable and renewable; it has attractive mechanical properties and, therefore, it can be utilized for packaging, textile fibers and medical applications (Raquez, Habibi, Murariu, & Dubois, 2013). PLA is also used for the encapsulation of many therapeutic agents due to its physicochemical properties, such as hydrophobicity, biodegradability, no toxicity, biocompatibility and slow drug release (Lee, Zhang, & Feng, 2007).

Regarding the application of PLA for bioactive compounds, it was already used to encapsulate flavonoids by the solvent evaporation (Kumari et al., 2011; Kumari, Yadav, Pakade, Singh, & Yadav, 2010; Roussaki et al., 2014) and by the nanoprecipitation technique (Pandey et al., 2015). Retinyl palmitate nanocapsules was prepared by preformed polymer interfacial deposition method utilizing PLA in its composition and nanocapsules with a mean diameter of 220 nm and sustained release was obtained (Teixeira et al., 2012).

Quercetin nanocapsules, synthetized by the solvent evaporation technique presented diameters between the range of 130–230 nm and 97% of nanoencapsulation efficiency with complete release after 72 h phosphate buffered saline 0.01M contained 0.1% NaN_3 (v/v) (Kumari, Yadav, Pakade, Singh, & Yadav, 2010). These same authors also reported that the antioxidant activity of quercetin was retained even after nanoencapsulation, while quercetin nanocapsules obtained by the nanoprecipitation technique (32-152 nm) was lower than those previously described.

Although PLA presents modulus and tensile strength comparable to petroleum-based polymers, it has lower thermal and impact resistance properties; in addition, the cost of PLA is higher than the other polymers, limiting its application (Lim, Auras, & Rubino, 2008). Nevertheless, PLA is biodegradable and derived from sustainable sources (Lim, Auras, & Rubino, 2008) and, therefore, it may be considered a good choice as wall material in the development of nanoparticles with bioactive compounds.

3.3. Poly-D,L-lactide-co-glycolide (PLGA)

Poly-D, L-lactide-co-glycolide ($[C_3H_4O_2]x[C_2H_2O_2]y$) (Figure 4C) is a polymer which is obtained by random melt co-polymerization of lactide (PLA) and glycolide (PGA) under high vacuum in the presence of a catalyst, for example, stannous octoate (Kapoor et al., 2015).

The physicochemical properties of PLGA, such as viscosity, density, transition temperature can be affected by changes in the concentration of lactic acid (Kapoor et al., 2015). PLGA is soluble in organic solvents (acetone, dichloromethane and chloroform) and its physical properties (molecular weight, polydispersity index, glass transition and degree of crystallinity) can affect the biodegradation rate and mechanical strength (Kapoor et al., 2015).

PLGA is an important non-toxic and biocompatible synthetic co-polymer that has been used for the development of nanocapsules to controlled and targeted drug delivery system

(Sharma, Parmar, Kori, & Sandhir, 2016). In formulations of nanocapsules, it has been used to bioactive compounds due to its adequate biodegradability, biocompatibility and controlled delivery properties (Arunkumar, et al., 2015; Pereira et al., 2015). Bioactive compounds, such as quercetin, can be encapsulated with hydrophilic drugs using PLGA as wall material to provide synergic therapeutic effects to these compounds and improving their effectiveness in free administration (Ghosh et al., 2011).

The use of PLGA to encapsulate lutein, a lipophilic compound, increased the solubility of carotenoids in aqueous medium, which may improve its bioavailability and anticancer properties (Arunkumar et al., 2015). The lutein nanocapsule obtained in the referred study was prepared by the emulsion sonication-solvent evaporation technique, presented 200 nm (mean diameter) and a controlled sustainable release of 66% up to 72 h. Therefore, the coating polymer may improve not only the biological availability of such compound, but also its controlled and sustained release properties (Sharma, Parmar, Kori, & Sandhir, 2016).

Moreover, PLGA can be utilized in blend with other polymers in order to modify its chemical characteristics, such as internalization and encapsulation efficiency (Patil, Swaminathan, Sadhukha, Ma, & Panyam, 2010). As an example, nanocapsules of α-tocopherol and γ-tocotrienol covered with chitosan and PLGA (1:1, w/w) was developed to evaluate the effect of nanoparticles entrapment on cellular uptake, antioxidant, and antiproliferative activity. These authors observed that nanocapsules containing chitosan in its composition were more effective in cellular uptake than those containing only PLGA, and this fact could be attributed to the positive zeta potential that chitosan confers to nanoparticles inducing high internalization in cells (Alqahtani et al., 2015).

The main advantages for the application of PGLA in nanocapsules are the fact that the hydrolysis of PGLA in physiologic system forms metabolites (monomers of lactic acid and glycolic acid) that are easily metabolized by the human body via the Krebs cycle (Di Toro, Betti, & Spampinato, 2004).

3.4. Chitosan

Chitosan (Figure 4D) can be defined as a natural carbohydrate polymer composed of β-(1-4)-linked d-glucosamine (deacetylated unit) and N-acetyl-d-glucosamine (acetylate unit) obtained from crustacean chitin by partial N-deacetylation, the second most abundant natural biopolymer next to cellulose (Azevedo, Bourbon, Vicente & Cerqueira, 2014).

Chitosan is a semi crystalline, nontoxic, biocompatible and biodegradable cationic polymer with capacity to form gels, soluble in acid solution and insoluble at alkaline and neutral pH, which solubility is direct linked to the degree of deacetylation and it is susceptible to chemical modification (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Hejazi & Amiji, 2003).

Due to the chemical characteristics of conferring adherence to negatively charged surfaces and due to its biological properties, such as mucoadhesive properties and antimicrobial activity, chitosan has been used to coat nanoparticles conferring more stability and allowing biologic interactions improving the capacity of delivery systems (de la Fuente et al., 2010; Oyarzun-Ampuero, Garcia-Fuentes, Torres, & Alonso, 2010).

Despite chitosan has been widely used to encapsulate drugs, it is still not much explored as isolated wall material for bioactive compounds. However, there are some studies about its application to cover functional compounds, generally combined with other polymers (Hong, Lee, & Lee, 2016; Natrajan, Srinivasan, Sundar, & Ravindran, 2015).

As examples, chitosan nanoparticles containing cinnamon essential oil were obtained with diameter ranging from 112 to 527 nm using different chitosan concentrations and presented a significant decrease of microbial growth, pH and peroxide value when applied in meat (Hu, Wang, Xiao, & Bi, 2015). In this sense, chitosan nanoparticles with turmeric oil or lemongrass oil presented similar behaviors and kept its antiproliferative activities (Natrajan, Srinivasan, Sundar, & Ravindran, 2015).

Chitosan can be used to involve nanocapsules that present have solid core, such as the research developed to Azevedo, Bourbon, Vicente & Cerqueira (2014) that synthetized Vitamin B_2 nanocapsules of chitosan and alginate as the main materials. The average size of these nanoparticles was 104 nm, PDI of 0.45 with nanoencapsulation efficiency of 56% and stability of 5 months after incubation at 4°C.

Curcumin and lutein nanoparticles have been developed to improve the solubility of these natural compounds and increase its technologic applications. The lutein nanoparticles encapsulated with chitosan/poly-γ-glutamic acid showed 200 nm of mean diameter and presented higher solubility than free lutein (Hong, Lee, & Lee, 2016). Regarding curcumin, chitosan nanoparticles with diameter between 300-500 nm presented good incorporation of curcumin (64%) and 87% of curcumin was released after 50 h in phosphate buffered saline (Liu, Yang, Ao, & Zhou, 2015). In another study, curcumin nanoemulsion was covered with chitosan and carboximetylcellulose and the prepared core–shell structures exhibited size of

159 nm, PDI of 0.140 and negative zeta potential (−17.2 mV) without aggregation after storage for 4 weeks ate 4 °C (Abbas et al., 2015).

The advantages of using chitosan for nanocapsules synthesis include the ability to control the release profile, use of safe organic solvents, possibilities for structural modifications due to presence of free amine group, easing its application combined to other polymers (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Pillai, Paul, & Sharma, 2009).

3.5. Polysaccharide gums

Natural gums are polysaccharides of considerable molecular weight (ranging from ~ 2,000 to >2 million) produced by the protection mechanism of plants and they are constituted of multiple linked sugar units that generate smaller structure after hydrolysis, such as galactose, mannose and others (Rana et al., 2011). The polysaccharide gums are one of the most abundant raw material and have been received special attention due to their biodegradable, sustainable, low cost, biosafety characteristics and availability (Rana et al., 2011).

The most known physicochemical characteristics of polysaccharide gums is the ability to form gel (Fiszman & Varela, 2013), that depends on ionic strength, pH and temperature. Moreover, the polysaccharides gums are also known for their swelling properties and such properties are due to the entrapment of large amounts of water between their chains and branches (Rana et al., 2011). Polysaccharide gums can exhibit neutral charge, negative charge, or positive charge according to the presence of various chemical groups attached to individual monosaccharide units (Nieto, 2009).

Gums have been studied for several applications in industrial and pharmaceutical fields, such as film coating, viscous formulations and, recently, drug delivery systems due to the capacity of intestinal microflora metabolize and degrade gums in individual sugar components, ensuring the release of nanoencapsulated compounds (Prajapati, Jani, Moradiya, & Randeria, 2013; Rana et al., 2011).

Ghayempour *et al.* (2015) developed antimicrobial nanocapsules containing plant extract using *Tragacanth gum* as biocompatible and biodegradable wall material and observed that in the optimal formulation, the nanocapsules presented the average size of 22 nm, parameter that according to this study, is directly associated to the concentration of wall

material. Furthermore, antimicrobial activity was confirmed, showing 100% microbial reduction after 12 h stirring and this suggest the release of plant extract from nanocapsules.

In another study, Ghayempour & Mortazavi (2015) used alginate to encapsulate peppermint oil with different methods of stirring and obtained nanocapsules with 56 nm of size by ultrasonic stirrer. Moreover, stirring, quantity of emulsifier and polymer concentration can affect the homogeneity and size of nanocapsules.

Cashew gum is a heteropolysaccharide from the exudate of *Anacardium occidentale* that has also been used as wall material for the obtention of nanocapsules. In a recent study (Herculano et al., 2015), nanocapsules with essential oil of *Eucalyptus staigeriana* were synthesized using cashew gum and the nanocapsules size varied from 27 nm to 432 nm, with negative zeta potential. These authors observed that the encapsulation efficiency varied between 25% and 27% and that the increase in gum concentration favored rapid oil release (90% after 7 h in distilled water).

Gums can be combined with others gums or other materials, such as gelatin and synthetic polymers to change physicochemical characteristics and improve stability, protection and increase wall resistance (Lv, Yang, Li, Zhang, & Abbas, 2014; Zambrano-Zaragoza et al., 2014). Gum Arabic and gelatin was combined (1:1, w/w) to develop nanocapsules of essential oil of jasmine flowers in different pH values and the nanocapsules obtained in alkaline conditions showed high stability after 7 h of incubation at 80 °C with 112 nm of diameter, PDI of 0.25 and zeta potential of -8 mV (Lv, Yang, Li, Zhang, & Abbas, 2014). In another study, gum Arabic was also combined with sodium caseinate to synthesize nanocapsules containing fish oil to use in the enrichment of fruit juice and these nanocapsules presented particle size of 232 nm with encapsulation efficiency of 79% and sedimentation stability that depends mainly on biopolymer ratio (Ilyasoglu & El, 2014).

Although gums are natural polymers and they are cheaper than the synthetic polymers, improving the wall resistance of nanocapsules and protection of encapsulated compound, they vary in purity and often require crosslinking that could denature the embedded drug (Hans & Lowman, 2002), limiting its application.

3.6. Other materials

The nanoencapsulation of bioactive compounds has been a scientific field extensively explored concerning the utilization of different wall materials in its composition. In addition

to the previously cited wall materials, casein micelles, whey protein, pectin, cellulose, albumin and others are also used as wall materials in nanocapsules synthesis (Ghasemi & Abbasi, 2014; Jin, Xia, Jiang, Zhao, & He, 2009).

Hydroxypropylmethyl cellulose is an enteric coating material used to protect encapsulated compounds from degradation by gastric acid or to prevent them from causing side effects in stomach (Chung et al., 2014). Due to its gastric juice resistance, lutein was nanoencapsulated with hydroxypropylmethyl cellulose phthalate to be used as a functional food ingredient (Jin, Xia, Jiang, Zhao, & He, 2009). These nanocapsules presented the mean diameter ranging from 163 nm to 219 nm and encapsulation efficiency of 88%, increasing the application of lutein in food industries.

Casein micelles are natural vehicles, proline-rich, open-structured rheomorphic proteins, which have different hydrophobic and hydrophilic domains (Livney, 2010). Fish and vegetable oils were already nanoencapsulated using casein micelles as wall material to evaluate the protective properties of casein micelles (Ghasemi & Abbasi, 2014). These authors observed that the decrease of pH reduced the size of nanoparticle and this behavior is due to the strong electrostatic repulsion. In another study, curcumin was encapsulated in casein with 83% of efficiency with 169 nm of diameter, PDI of 0.24 and zeta potential of -31 mV (Pan, Zhong, & Baek, 2013).

There are some advantages in the use of casein micelles to nanoencapsulation of hydrophobic compounds. Among them, the high nutritional value of the raw material can be mentioned, as well as good sensory properties, the increase of bioavailability of the active compounds consumed as casein nanoparticles, the higher stability as compared to emulsions or liposomes and cost reduction and environmental protection (Gutiérrez et al., 2013).

Whey protein can be also used to nanoencapsulate bioactive compounds due to its functional properties, such as surface activity, gelation, shielding and some protective properties, such as biocompatibility and biodegradability (Livney, 2010). Madadlou *et al.* (2014) obtained nanocapsules with whey protein via heat gelation of enzymatically reinforced, using caffeine as drug model and confirmed that this method was responsible for obtention of stable nanocapsules with 45 nm of diameter.

4. Conclusion

Nanotechnology proved to be a promising and viable strategy to protect and improve the application of bioactive compounds in both the food and pharmaceutical industries. Different materials can be used to nanoencapsulate bioactive compounds; however, polymeric nanocapsules are the most stable ones during storage and showed high efficiency to control the release of the encapsulated compound and due to these characteristics, it has been focus of various studies for future use in humans and food.

This review contributes with the discussion of different techniques to the obtention of polymeric nanocapsules and its advantages and limitations. Polymeric nanocapsules of bioactive compounds are widely synthetized by preformed polymer method due to the ease removal of residual substances, such as organic solvents.

Not only the chosen technique, but the choice of the wall material is an important factor to the synthesis of nanocapsules in relation to the particular parameters, such as size, zeta potential and encapsulation efficiency and others physicochemical characteristics, such as aqueous solubility, aqueous stability, surface permeability and desired bioactive compounds release profile and wall resistance. However, these parameters are not determinant since stirring, temperature, pH and presence or not of enzymes can also interfere in the nanoparticles characteristics.

Most studies concerning encapsulated bioactive compounds are directly related to the development, synthesis and characterization of nanocapsules, with reduced focus on the implementation and on release kinetics of bioactive compounds. Future trends in synthesis of polymeric nanocapsules should comprise focus on researches regarding the intrinsic properties and interactions of these nanostructures in food, pharmaceutical and cosmetic applications, as well as the use of new and safe wall materials followed by *in vivo* studies.

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References:

Abbas, S., Karangwa, E., Bashari, M., Hayat, K., Hong, X., Sharif, H. R., & Zhang, X. (2015). Fabrication of polymeric nanocapsules from curcumin-loaded nanoemulsion templates by self-assembly. *Ultrasonics Sonochemistry, 23*, 81-92.

Agnihotri, S. A., Mallikarjuna, N. N., & Aminabhavi, T. M. (2004). Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *Journal of Controlled Release, 100*, 5-28.

Alqahtani, S., Simon, L., Astete, C. E., Alayoubi, A., Sylvester, P. W., Nazzal, S., Shen, Y., Xu, Z., Kaddoumi, A., & Sabliov, C. M. (2015). Cellular uptake, antioxidant and antiproliferative activity of entrapped α-tocopherol and γ-tocotrienol in poly (lactic-co-glycolic) acid (PLGA) and chitosan covered PLGA nanoparticles (PLGA-Chi). *Journal of Colloid and Interface Science, 445*, 243-251.

Arunkumar, R., Prashanth, K. V. H., & Baskaran, V. (2013). Promising interaction between nanoencapsulated lutein with low molecular weight chitosan: Characterization and bioavailability of lutein in vitro and in vivo. *Food Chemistry, 141*, 327-337.

Arunkumar, R., Prashanth, K. V. H., Manabe, Y., Hirata, T., Sugawara, T., Dharmesh, S. M., & Baskaran, V. (2015). Biodegradable Poly (Lactic-co-Glycolic Acid)-Polyethylene Glycol Nanocapsules: An Efficient Carrier for Improved Solubility, Bioavailability, and Anticancer Property of Lutein. *Journal Of Pharmaceutical Sciences, 104*, 2085-2093.

Azevedo, M. A., Bourbon, A. I., Vicente, A. A., & Cerqueira, M. A. (2014). Alginate/chitosan nanoparticles for encapsulation and controlled release of vitamin B2. *International Journal of Biological Macromolecules, 71*, 141-146.

Bernardi, A., Braganhol, E., Jäger, E., Figueiró, F., Edelweiss, M. I., Pohlmann, A. R., Guterres, S. S., & Battastini, A. M. O. (2009). Indomethacin-loaded nanocapsules treatment reduces in vivo glioblastoma growth in a rat glioma model. *Cancer Letters, 281*, 53-63.

Birrenbach, G., & Speiser, P. P. (1976). Polymerized micelles and their use as adjuvants in immunology. *Journal of Pharmaceutical Sciences, 65*, 1763-1766.

Brayner, R. (2008). The toxicological impact of nanoparticles. *Nano Today, 3*, 48-55.

Chen, M. H., & Bergman, C. J. (2005). A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ-oryzanol contents. *Journal of Food Composition and Analysis, 18*, 319-331.

Chung, J.-H., Lee, S.-J., Chung, J.-O., Oh, Y.-J., Hwang, J.-A., Kim, Y.-K., Ko, S., & Shim, S.-M. (2014). Effect of hydroxypropyl methyl cellulose phthalate coating on digestive stability and intestinal transport of green tea catechins. *Integrative Medicine Research, 3*, 34-37.

Contri, R. V., Ribeiro, K. L. F., Fiel, L. A., Pohlmann, A. R., & Guterres, S. S. (2013). Vegetable oils as core of cationic polymeric nanocapsules: influence on the physicochemical properties. *Journal of Experimental Nanoscience, 8*, 913-924.

Coradini, K., Lima, F. O., Oliveira, C. M., Chaves, P. S., Athayde, M. L., Carvalho, L. M., & Beck, R. C. R. (2014). Co-encapsulation of resveratrol and curcumin in lipid-core nanocapsules improves their in vitro antioxidant effects. *European Journal of Pharmaceutics and Biopharmaceutics, 88*, 178-185.

Couvreur, P., Barratt, G., Fattal, E., & Vauthier, C. (2002). Nanocapsule Technology: A Review.*Therapeutical Drug Carrier Systems, 19*, 99-134.

Couvreur, P., Dubernet, C., & Puisieux, F. (1995). Controlled drug delivery with nanoparticles: Current possibilities and future trends. *European Journal of Pharmaceutics and Biopharmaceutics, 41*, 2-13.

Couvreur, P., Tulkenst, P., Roland, M., Trouet, A., & Speiser, P. (1977). Nanocapsules: A new type of lysosomotropic carrier. *FEBS Letters, 84*, 323-326.

Dash, T. K., & Konkimalla, V. B. (2012). Poly-ϵ-caprolactone based formulations for drug delivery and tissue engineering: A review. *Journal of Controlled Release, 158*, 15-33.

de la Fuente, M., Raviña, M., Paolicelli, P., Sanchez, A., Seijo, B., & Alonso, M. J. (2010). Chitosan-based nanostructures: A delivery platform for ocular therapeutics. *Advanced Drug Delivery Reviews, 62*, 100-117.

Di Toro, R., Betti, V., & Spampinato, S. (2004). Biocompatibility and integrin-mediated adhesion of human osteoblasts to poly(DL-lactide-co-glycolide) copolymers. *European Journal of Pharmaceutical Sciences, 21*, 161-169.

dos Santos, P. P., Paese, K., Guterres, S. S., Pohlmann, A. R., Costa, T. H., Jablonski, A., Flôres, S. H., & Rios, A. d. O. (2015). Development of lycopene-loaded lipid-core nanocapsules: physicochemical characterization and stability study. *Journal of Nanoparticle Research, 17*, 1-11.

Ezhilarasi, P. N., Karthik, P., Chhanwal, N., & Anandharamakrishnan, C. (2012). Nanoencapsulation Techniques for Food Bioactive Components: A Review. *Food and Bioprocess Technology, 6*, 628-647.

Fessi, H., Puisieux, F., Devissaguet, J. P., Ammoury, N., & Benita, S. (1989). Nanocapsule formation by interfacial polymer deposition following solvent displacement. *International Journal of Pharmaceutics, 55*, R1-R4.

Fiszman, S., & Varela, P. (2013). The role of gums in satiety/satiation. A review. *Food Hydrocolloids, 32*, 147-154.

Fontana, M. C., Coradini, K., Guterres, S. S., Pohlmann, A. R., & Beck, R. C. R. (2009). Nanoencapsulation as a Way to Control the Release and to Increase the Photostability of Clobetasol Propionate: Influence of the Nanostructured System. *Journal of Biomedical Nanotechnology, 5*, 254-263.

Ghasemi, S., & Abbasi, S. (2014). Formation of natural casein micelle nanocapsule by means of pH changes and ultrasound. *Food Hydrocolloids, 42*, 42-47.

Ghayempour, S., Montazer, M., & Rad, M. M. (2015). Tragacanth gum as a natural polymeric wall for producing antimicrobial nanocapsules loaded with plant extract. *International Journal of Biological Macromolecules, 81*, 514-520.

Ghayempour, S., & Mortazavi, S. M. (2015). Preparation and investigation of sodium alginate nanocapsules by different microemulsification devices. *Journal of Applied Polymer Science, 132*, 1-8.

Ghosh, S., Dungdung, S. R., Chowdhury, S. T., Mandal, A. K., Sarkar, S., Ghosh, D., & Das, N. (2011). Encapsulation of the flavonoid quercetin with an arsenic chelator into nanocapsules enables the simultaneous delivery of hydrophobic and hydrophilic drugs with a synergistic effect against chronic arsenic accumulation and oxidative stress. *Free Radical Biology and Medicine, 51*, 1893-1902.

González-Reza, R. M., Quintanar-Guerrero, D., Flores-Minutti, J. J., Gutiérrez-Cortez, E., & Zambrano-Zaragoza, M. L. (2015). Nanocapsules of β-carotene: Thermal degradation kinetics in a scraped surface heat exchanger (SSHE). *LWT - Food Science and Technology, 60*, 124-130.

Gutiérrez, F. J., Albillos, S. M., Casas-Sanz, E., Cruz, Z., García-Estrada, C., García-Guerra, A., García-Reverter, J., García-Suárez, M., Gatón, P., González-Ferrero, C., Olabarrieta, I., Olasagasti, M., Rainieri, S., Rivera-Patiño, D., Rojo, R., Romo-Hualde,

A., Sáiz-Abajo, M.-J., & Mussons, M.-L. (2013). Methods for the nanoencapsulation of β-carotene in the food sector. *Trends in Food Science & Technology, 32*, 73-83.

Hans, M. L., & Lowman, A. M. (2002). Biodegradable nanoparticles for drug delivery and targeting. *Current Opinion in Solid State and Materials Science, 6*, 319-327.

Hejazi, R., & Amiji, M. (2003). Chitosan-based gastrointestinal delivery systems. *Journal of Controlled Release, 89*, 151-165.

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

Hong, D. Y., Lee, J.-S., & Lee, H. G. (2016). Chitosan/poly-γ-glutamic acid nanoparticles improve the solubility of lutein. *International Journal of Biological Macromolecules, 85*, 9-15.

Hoyt, V. W., & Mason, E. (2008). Nanotechnology: Emerging health issues. *Journal of Chemical Health and Safety, 15*, 10-15.

Hu, J., Wang, X., Xiao, Z., & Bi, W. (2015). Effect of chitosan nanoparticles loaded with cinnamon essential oil on the quality of chilled pork. *LWT - Food Science and Technology, 63*, 519-526.

Hu, J., Xiao, Z., Zhou, R., Ma, S., Wang, M., & Li, Z. (2011). Properties of Aroma Sustained-release Cotton Fabric with Rose Fragrance Nanocapsule. *Chinese Journal of Chemical Engineering, 19*, 523-528.

Huang, Q., Yu, H., & Ru, Q. (2010). Bioavailability and delivery of nutraceuticals using nanotechnology. *J Food Sci, 75*, R50-57.

Ilyasoglu, H., & El, S. N. (2014). Nanoencapsulation of EPA/DHA with sodium caseinate–gum arabic complex and its usage in the enrichment of fruit juice. *LWT - Food Science and Technology, 56*, 461-468.

Jin, H., Xia, F., Jiang, C., Zhao, Y., & He, L. (2009). Nanoencapsulation of Lutein with Hydroxypropylmethyl Cellulose Phthalate by Supercritical Antisolvent. *Chinese Journal of Chemical Engineering, 17*, 672-677.

Kapoor, D. N., Bhatia, A., Kaur, R., Sharma, R., Kaur, G., & Dhawan, S. (2015). PLGA: a unique polymer for drug delivery. *Ther Deliv, 6*, 41-58.

Kayser, O., Lemke, A., & Hernandez-Trejo, N. (2005). The Impact of Nanobiotechnology on the Development of New Drug Delivery Systems. *Current Pharmaceutical Biotechnology, 6*, 3-5.

Khayata, N., Abdelwahed, W., Chehna, M. F., Charcosset, C., & Fessi, H. (2012). Preparation of vitamin E loaded nanocapsules by the nanoprecipitation method: From laboratory scale to large scale using a membrane contactor. *International Journal of Pharmaceutics, 423*, 419-427.

Krauel, K., Pitaksuteepong, T., Davies, N., & Rades, T. (2004). Entrapment of bioactive molecules in poly (alkylcyanoacrylate) nanoparticles. *American Journal of Drug Delivery, 2*, 251-259.

Kumar, V. D., Verma, P. R. P., & Singh, S. K. (2015). Development and evaluation of biodegradable polymeric nanoparticles for the effective delivery of quercetin using a quality by design approach. *LWT - Food Science and Technology, 61*, 330-338.

Kumari, A., Yadav, S. K., Pakade, Y. B., Singh, B., & Yadav, S. C. (2010). Development of biodegradable nanoparticles for delivery of quercetin. *Colloids and Surfaces B: Biointerfaces, 80*, 184-192.

Kumari, A., Yadav, S. K., Pakade, Y. B., Kumar, V., Singh, B., Chaudhary, A., & Yadav, S. C. (2011). Nanoencapsulation and characterization of Albizia chinensis isolated antioxidant quercitrin on PLA nanoparticles. *Colloids and Surfaces B: Biointerfaces, 82*, 224-232.

Landry, F. B., Bazile, D. V., Spenlehauer, G., Veillard, M., & Kreuter, J. (1996). Degradation of poly(d,l-lactic acid) nanoparticles coated with albumin in model digestive fluids (USP XXII). *Biomaterials, 17*, 715-723.

Langer, R., & Chasin, M. (1990). Biodegradable polymers as drug delivery systems. In): Marcel Dekker, New York.

Lee, S. H., Zhang, Z., & Feng, S. S. (2007). Nanoparticles of poly(lactide)-tocopheryl polyethylene glycol succinate (PLA-TPGS) copolymers for protein drug delivery. *Biomaterials, 28*, 2041-2050.

Lim, L. T., Auras, R., & Rubino, M. (2008). Processing technologies for poly(lactic acid). *Progress in Polymer Science, 33*, 820-852.

Liu, M., Yang, J., Ao, P., & Zhou, C. (2015). Preparation and characterization of chitosan hollow nanospheres for anticancer drug curcumin delivery. *Materials Letters, 150*, 114-117.

Livney, Y. D. (2010). Milk proteins as vehicles for bioactives. *Current Opinion in Colloid and Interface Science, 15*, 73-83.

Lobato, K. B. d. S., Paese, K., Forgearini, J. C., Guterres, S. S., Jablonski, A., & Rios, A. d. O. (2013). Characterisation and stability evaluation of bixin nanocapsules. *Food Chemistry, 141*, 3906-3912.

Lunt, J. (1998). Large-scale production, properties and commercial applications of polylactic acid polymers. *Polymer Degradation and Stability, 59*, 145-152.

Lv, Y., Yang, F., Li, X., Zhang, X., & Abbas, S. (2014). Formation of heat-resistant nanocapsules of jasmine essential oil via gelatin/gum arabic based complex coacervation. *Food Hydrocolloids, 35*, 305-314.

Madadlou, A., Jaberipour, S., & Eskandari, M. H. (2014). Nanoparticulation of enzymatically cross-linked whey proteins to encapsulate caffeine via microemulsification/heat gelation procedure. *LWT - Food Science and Technology, 57*, 725-730.

Mohanraj, V. J., & Chen, Y. (2006). Nanoparticles – A Review. *Tropical Journal of Pharmaceutical Research, 5*, 561-573.

Mora-Huertas, C. E., Garrigues, O., Fessi, H., & Elaissari, A. (2012). Nanocapsules prepared via nanoprecipitation and emulsification–diffusion methods: Comparative study. *European Journal of Pharmaceutics and Biopharmaceutics, 80*, 235-239.

Nabi-Meibodi, M., Navidi, B., Navidi, N., Vatanara, A., Rouini, M. R., & Ramezani, V. (2013). Optimized double emulsion-solvent evaporation process for production of solid lipid nanoparticles containing baclofene as a lipid insoluble drug. *Journal of Drug Delivery Science and Technology, 23*, 225-230.

Nair, L. S., & Laurencin, C. T. (2007). Biodegradable polymers as biomaterials. *Progress in Polymer Science, 32*, 762-798.

Natrajan, D., Srinivasan, S., Sundar, K., & Ravindran, A. (2015). Formulation of essential oil-loaded chitosan–alginate nanocapsules. *Journal of Food and Drug Analysis, 23*, 560-568.

Nieto, M. (2009). Structure and Function of Polysaccharide Gum-Based Edible Films and Coatings. In K. C. Huber & M. E. Embuscado (Eds.), *Edible Films and Coatings for Food Applications*, (pp. 57-112): Springer New York.

Noronha, C. M., de Carvalho, S. M., Lino, R. C., & Barreto, P. L. (2014). Characterization of antioxidant methylcellulose film incorporated with alpha-tocopherol nanocapsules. *Food Chemistry, 159*, 529-535.

Noronha, C. M., Granada, A. F., de Carvalho, S. M., Lino, R. C., Maciel, M. V. d. O. B., & Barreto, P. M. L. (2013). Optimization of α-tocopherol loaded nanocapsules by the nanoprecipitation method. *Industrial Crops and Products, 50*, 896-903.

Oyarzun-Ampuero, F. A., Garcia-Fuentes, M., Torres, D., & Alonso, M. J. (2010). Chitosan-coated lipid nanocarriers for therapeutic applications. *Journal of Drug Delivery Science and Technology, 20*, 259-265.

Pan, K., Zhong, Q., & Baek, S. J. (2013). Enhanced dispersibility and bioactivity of curcumin by encapsulation in casein nanocapsules. *Journal of Agricultural and Food Chemistry, 61*, 6036-6043.

Pandey, S. K., Patel, D. K., Thakur, R., Mishra, D. P., Maiti, P., & Haldar, C. (2015). Anti-cancer evaluation of quercetin embedded PLA nanoparticles synthesized by emulsified nanoprecipitation. *International Journal of Biological Macromolecules, 75*, 521-529.

Patil, Y. B., Swaminathan, S. K., Sadhukha, T., Ma, L., & Panyam, J. (2010). The use of nanoparticle-mediated targeted gene silencing and drug delivery to overcome tumor drug resistance. *Biomaterials, 31*, 358-365.

Pereira, M. C., Hill, L. E., Zambiazi, R. C., Mertens-Talcott, S., Talcott, S., & Gomes, C. L. (2015). Nanoencapsulation of hydrophobic phytochemicals using poly (dl-lactide-co-glycolide) (PLGA) for antioxidant and antimicrobial delivery applications: Guabiroba fruit (Campomanesia xanthocarpa O. Berg) study. *LWT - Food Science and Technology, 63*, 100-107.

Pillai, C. K. S., Paul, W., & Sharma, C. P. (2009). Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Progress in Polymer Science, 34*, 641-678.

Prajapati, V. D., Jani, G. K., Moradiya, N. G., & Randeria, N. P. (2013). Pharmaceutical applications of various natural gums, mucilages and their modified forms. *Carbohydrate Polymers, 92*, 1685-1699.

Quintanar-Guerrero, D., Allemann, E., Fessi, H., & Doelker, E. (1998). Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. *Drug Development and Industrial Pharmacy, 24*, 1113-1128.

Rana, V., Rai, P., Tiwary, A. K., Singh, R. S., Kennedy, J. F., & Knill, C. J. (2011). Modified gums: Approaches and applications in drug delivery. *Carbohydrate Polymers, 83*, 1031-1047.

Rao, J. P., & Geckeler, K. E. (2011). Polymer nanoparticles: Preparation techniques and size-control parameters. *Progress in Polymer Science, 36*, 887-913.

Raquez, J.-M., Habibi, Y., Murariu, M., & Dubois, P. (2013). Polylactide (PLA)-based nanocomposites. *Progress in Polymer Science, 38*, 1504-1542.

Reis, C. P., Neufeld, R. J., Ribeiro, A. J., & Veiga, F. (2006). Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine, 2*, 8-21.

Rigo, L. A., da Silva, C. R., de Oliveira, S. M., Cabreira, T. N., da Silva, C. d. B., Ferreira, J., & Beck, R. C. R. (2015). Nanoencapsulation of rice bran oil increases its protective effects against UVB radiation-induced skin injury in mice. *European Journal of Pharmaceutics and Biopharmaceutics, 93*, 11-17.

Rollot, J. M., Couvreur, P., Roblot-Treupel, L., & Puisieux, F. (1986). Physicochemical and morphological characterization of polyisobutyl cyanoacrylate nanocapsules. *Journal of Pharmaceutical Sciences, 75*, 361-364.

Roussaki, M., Gaitanarou, A., Diamanti, P. C., Vouyiouka, S., Papaspyrides, C., Kefalas, P., & Detsi, A. (2014). Encapsulation of the natural antioxidant aureusidin in biodegradable PLA nanoparticles. *Polymer Degradation and Stability, 108*, 182-187.

Sharma, N., Bansal, M., Visht, S., Sharma, P. K., & Kulkarni, G. T. (2010). Nanoemulsion: A new concept of delivery system. *Chronicles of Young Scientists, 1*, 2-6.

Sharma, S., Parmar, A., Kori, S., & Sandhir, R. (2016). PLGA-based nanoparticles: A new paradigm in biomedical applications. *Trends in Analytical Chemistry*, 80, 30-40.

Singh, S., Pandey, V. K., Tewari, R. P., & Agarwal, V. (2011). Nanoparticle Based Drug Delivery System: Advantages and Applications. *Indian Journal of Science and Technology; 4*, 177-180.

Soppimath, K. S., Aminabhavi, T. M., Kulkarni, A. R., & Rudzinski, W. E. (2001). Biodegradable polymeric nanoparticles as drug delivery devices. *Journal of Controlled Release, 70*, 1-20.

Södergård, A., & Stolt, M. (2010). Industrial Production of High Molecular Weight Poly(Lactic Acid). In *Poly(Lactic Acid)*, (pp. 27-41): John Wiley & Sons, Inc.

Teixeira, Z., Dreiss, C. A., Lawrence, M. J., Heenan, R. K., Machado, D., Justo, G. Z., Guterres, S. S., & Durán, N. (2012). Retinyl palmitate polymeric nanocapsules as carriers of bioactives. *Journal of Colloid and Interface Science*, 382, 36-47.

Tokiwa, Y., & Calabia, B. P. (2006). Biodegradability and biodegradation of poly(lactide). *Applied Microbiology Biotechnology*, *72*, 244-251.

Vanderhoff, J. W., El-Aasser, M. S., & Ugelstad, J. (1979). Polymer emulsification process. In): Google Patents.

Vauthier, C., & Bouchemal, K. (2009). Methods for the preparation and manufacture of polymeric nanoparticles. *Pharmaceutical Research, 26*, 1025-1058.

Venturini, C. G., Jäger, E., Oliveira, C. P., Bernardi, A., Battastini, A. M. O., Guterres, S. S., & Pohlmann, A. R. (2011). Formulation of lipid core nanocapsules. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 375*, 200-208.

Vrignaud, S., Benoit, J.-P., & Saulnier, P. (2011). Strategies for the nanoencapsulation of hydrophilic molecules in polymer-based nanoparticles. *Biomaterials, 32*, 8593-8604.

Woodruff, M. A., & Hutmacher, D. W. (2010). The return of a forgotten polymer—Polycaprolactone in the 21st century. *Progress in Polymer Science, 35*, 1217-1256.

Wu, L., Zhang, J., & Watanabe, W. (2011). Physical and chemical stability of drug nanoparticles. *Advanced Drug Delivery Reviews, 63*, 456-469.

Yegin, B. A., & Lamprecht, A. (2006). Lipid nanocapsule size analysis by hydrodynamic chromatography and photon correlation spectroscopy. *International Journal of Pharmaceutics, 320*, 165-170.

Yin, L.-J., Chu, B.-S., Kobayashi, I., & Nakajima, M. (2009). Performance of selected emulsifiers and their combinations in the preparation of β-carotene nanodispersions. *Food Hydrocolloids, 23*, 1617-1622.

Zambrano-Zaragoza, M. L., Mercado-Silva, E., Del Real L, A., Gutiérrez-Cortez, E., Cornejo-Villegas, M. A., & Quintanar-Guerrero, D. (2014). The effect of nano-coatings with α-tocopherol and xanthan gum on shelf-life and browning index of fresh-cut "Red Delicious" apples. *Innovative Food Science & Emerging Technologies, 22*, 188-196.

Zambrano-Zaragoza, M. L., Mercado-Silva, E., Gutiérrez-Cortez, E., Castaño-Tostado, E., & Quintanar-Guerrero, D. (2011). Optimization of nanocapsules preparation by the emulsion–diffusion method for food applications. *LWT - Food Science and Technology, 44*, 1362-1368.

CAPÍTULO 3- MATERIAL E MÉTODOS

Esta seção descreve detalhadamente a metodologia empregada na presente tese relacionada aos artigos científicos apresentados no capítulo seguinte.

4. MATERIAL E MÉTODOS.

Os experimentos de bancada foram realizados no Laboratório de Compostos Bioativos, do Instituto de Ciência e Tecnologia de Alimentos (ICTA) da Universidade Federal do Rio Grande do Sul (UFRGS). As análises de diâmetro, potencial zeta e viscosidade das nanocápsulas de licopeno foram realizadas na Faculdade de Farmácia (UFRGS), e as análises de microscopia eletrônica foram realizadas no Centro de Microscopia Eletrônica (CME) – UFRGS.

4.1. Materiais

Os tomates utilizados para obtenção dos cristais de licopeno foram obtidos de um mercado local, em Porto Alegre, Brasil. O polímero poli (Ɛ-caprolactona) (Mw = 80.000) (PCL), azul de metileno (conteúdo seco 82%) e monoestearato de sorbitano foram obtidos a partir da Sigma (St. Louis, MO, EUA). O triglicerídeo Cáprico/caprílico (TCC) e polissorbato 80 foram obtidos da Delaware (Porto Alegre, Brasil). Todos os outros produtos químicos e solventes utilizados como acetona e etanol são de grau analítico.

4.2. Isolamento do licopeno e obtenção de cristais

Para isolamento do licopeno e obtenção de cristais foi utilizado método adaptado de Nunes e Mercadante (2004) que consiste na cristalização do composto com diferentes solventes. Para tal, foram utilizados tomates em uma etapa prévia de retirada de água constituída por um processo mecânico de compressão da massa do tomate por um espremedor, e posteriormente foram realizadas duas extrações com solvente acetato de etila, onde o tomate e o solvente, na relação massa/volume de 1:0,7, foi submetido à agitação em agitador elétrico por 120 minutos.

Para a cristalização, o extrato obtido foi seco em um balão de fundo redondo em um evaporador rotatório (40°C) para remoção completa do acetato de etila e redissolvido em diclorometano/etanol (1:4) à temperatura de 50-60ºC. A solução foi submetida a um banho de gelo para abaixamento gradual da temperatura e em seguida levado ao freezer por uma noite para a formação dos cristais. Os cristais de licopeno foram filtrados em papel Whatman Chr 3

mm (Figura 1), lavados com etanol gelado, secos à temperatura ambiente, armazenados em frascos âmbar e congelados para posterior avaliação da pureza.

Figura 1- Filtração dos cristais de licopeno.

4.3. Avaliação da pureza dos cristais de licopeno

A pureza dos cristais de licopeno foi avaliada por Cromatografia Líquida de Alta Eficiência (CLAE) em um cromatógrafo (Agilent series 1100) (Figura 2), equipado com um desgaseificador *online*, bomba quaternária, e injetor automático com coluna C 30 YCM (250 x 4,6mm d.i. x 3µm tamanho da partícula) acoplada e ambientada a 33°C.

Figura 2- CLAE com detector UV e coluna C30.

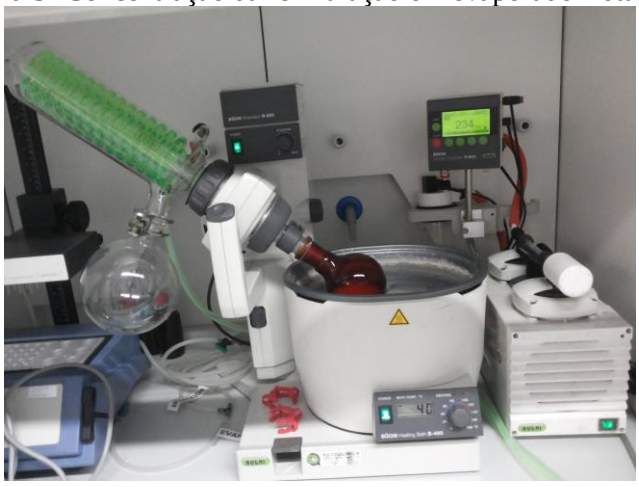
O gradiente de eluição utilizado na análise teve um fluxo de 1 mL/min constituído de água/ éter metil-terc-butílico/ metanol, inicialmente numa proporção de 5: 90: 5 v/v e

atingindo 0: 95: 5 v/v em 12 min, 0:89:11 v/v em 25 min, 0:75:25 v/v em 40 min e finalmente 00:50:50 v/v em 80 min. Os cromatogramas foram processados no comprimento de onda máximo de absorção do licopeno (470 nm).

4.4. Obtenção das nanocápsulas de licopeno

Formulações de nanocápsulas de núcleo lipídico contendo licopeno (LYC-LNC) foram preparadas pela técnica de deposição interfacial do polímero pré-formado utilizando concentrações dos constituintes, exceto o licopeno, já estabelecidas por Venturini *et al.* (2011). A fase orgânica foi preparada dissolvendo o polímero PCL (100 mg), o triglicerídeo de cadeia média (TCC) (160 µL), monoestearato de sorbitano (38 mg) e licopeno (850 µg) em uma mistura de acetona (24 mL) e etanol (3 mL) sob agitação magnética a 40 °C; e a fase aquosa foi preparada dissolvendo o polissorbato 80 (77 mg) em água (53 mL). A fase orgânica, então, foi injetada na fase aquosa, sob agitação durante 10 minutos e, por fim, a formulação foi concentrada sob pressão reduzida até um volume final de 10 mL (Figura 3). As nanocápsulas foram acondicionadas em frasco âmbar e submetidas a diferentes condições de armazenamento. Em testes preliminares, foram testadas várias concentrações de licopeno até saturação da fase orgânica.

Figura 3- Concentração da formulação em evaporador rotatório.



4.5. Caracterização de LYC-LNC

As nanocápsulas obtidas (Figura 4) foram analisadas imediatamente após a preparação em relação aos parâmetros de diâmetro, potencial zeta, viscosidade, pH, morfologia, análise

colorimétrica, teor e eficiência de encapsulação, cuja metodologia empregada será descrita a seguir.

Figura 4- Nanocápsulas de licopeno (LYC-LNC)

4.5.1. *Determinação de diâmetro*.

As análises preliminares de medição de diâmetro foram realizadas por Difração de Laser (LD) (Mastersizer 2000®), onde a amostra foi dispersa em água e as leituras realizadas em triplicata. Para determinação inicial de diâmetro das partículas, os dados foram analisados por Mastersizer 2000 5.54 e os valores de Span foram determinados pelo software e calculados dividindo-se a diferença entre o $D_{0,1}$ e $D_{0,9}$ por $D_{0,5}$.

Subsequentemente, o diâmetro de partícula e o índice de polidispersão foram medidos a 25°C por espalhamento de luz dinâmico (DLS) (Zetasizer® nano-ZS ZEN mod. 3600) (Figura 5). As amostras foram diluídas 1000 vezes (V/V) em agua ultrapura filtrada através de membrana 0,45 µm e os resultados avaliados através da média de três repetições usando software de tecnologia de dispersão (versão 7.4, 2013, Malvern Instruments Ltd).

Figura 5- Equipamento Zetasizer Nano ZS utilizado para medir diâmetro e potencial zeta.

4.5.2. Potencial zeta

O potencial zeta das suspensões das nanocápsulas foi obtido por mobilidade eletroforética (Zetasizer® nano-ZS ZEN mod. 3600) (Figura 5). As amostras foram diluídas 1000 vezes em solução de Nacl 10 mM previamente filtrada por membranas de 0,45 µm e os resultados avaliados através da média de três repetições.

4.5.3. pH

O pH das amostras foi medido diretamente nas suspensões, sem diluições prévias, a 25°C por meio de um potenciômetro (Digimed®, Brasil) calibrado com tampão pH 4,0 e 7,0. Os resultados foram avaliados através da média de três repetições.

4.5.4. Viscosidade

A viscosidade de cada formulação foi medida imediatamente após a preparação, usando um viscosímetro rotacional Brookfield (Figura 6) a 25°C e os dados foram analisados utilizando a Brookfield Software Rheocalc 32.

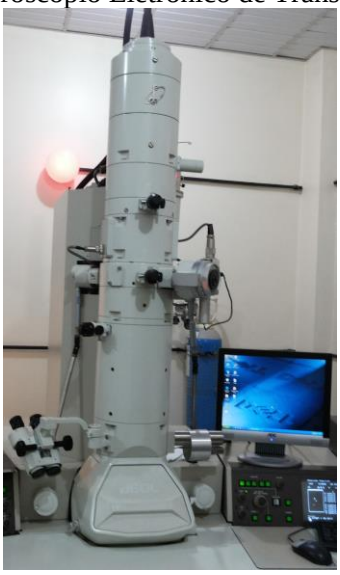
Figura 6- Viscosímetro rotacional Brookfield

4.5.5. Avaliação da morfologia

A morfologia de LYC-LNC foi avaliada através de microscopia eletrônica de transmissão (MET) no Centro de Microscopia Eletrônica da UFRGS (Figura 7). Neste

experimento, a suspensão foi diluída em água ultrapura na proporção 1:10 (v/v) e depositadas em *grids* de cobre (revestimento de formar/carbono 400 *mesh*), usando como contraste negativo acetato de uranila em solução aquosa (2%). As amostras foram colocadas em dessecador até o momento da análise e posteriormente em microscópio operando a 80kV.

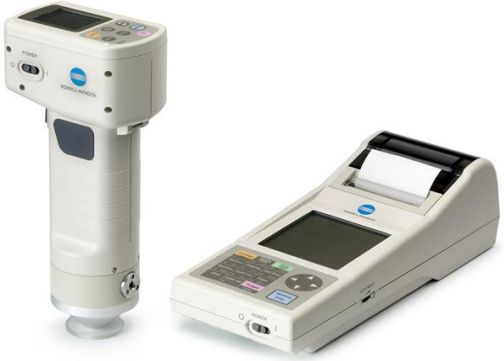
Figura 7- Microscópio Eletrônico de Transmissão (MET)



4.5.6. Análise colorimétrica

As medições de cor foram realizadas utilizando um colorímetro portátil (Konica Minolta modelo CR 400) (Figura 8). Os parâmetros colorimétricos foram obtidos de acordo com a Comission Internationale de l'Eclairage (Sistema CIELAB) e foram determinados os valores de L* (luminosidade), e das coordenadas a* (componente vermelho-verde) e b* (componente amarelo-azul).

Figura 8- Colorímetro (Modelo Konica Minolta CR 400)

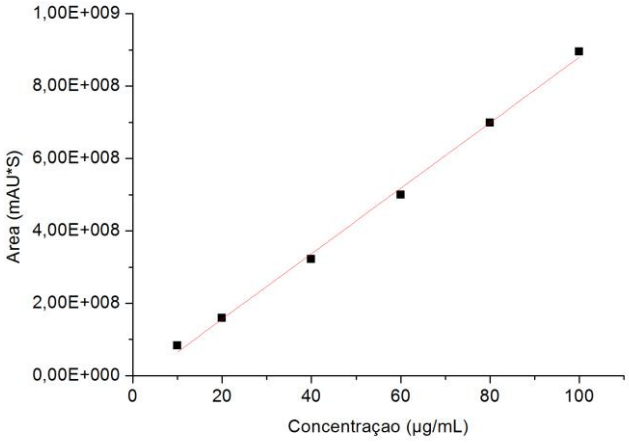


4.5.7. Determinação do teor de licopeno na nanocápsula e eficiência de encapsulação.

O teor de licopeno nas nanocápsulas foi determinado por cromatografia líquida de alta eficiência em um cromatógrafo (Agilent series 1100), equipado com um desgaseificador online, bomba quaternária, e injetor automático, com uma coluna C 30 acoplada.

Antes das injeções, foi construída uma curva padrão de licopeno diluído em éter metil-terc-butílico. A curva padrão (Figura 9) foi obtida através da representação gráfica das áreas dos picos de cinco diferentes concentrações previamente quantificadas por espectrofotometria (UV-Visível).

Figura 9- Curva padrão para obtenção da equação da reta.



Os cromatogramas foram processados no comprimento de onda máximo de absorção do licopeno (470 nm). O licopeno foi quantificado de acordo com a equação da reta obtida (R^2=0,99) através da curva:

$$y = 9E^6x - 2E^7 \qquad (1)$$

Onde:

y corresponde à área do pico no cromatogramas.

x corresponde à concentração de licopeno na solução.

O licopeno total presente nas nanocápsulas foi extraído de uma alíquota de nanocápsulas de licopeno (500µL) com acetonitrila (2,5 mL) por sonicação (30 min) e em

seguida centrifugado (30 min a 3500 x g). Todas as amostras foram então filtradas antes da injeção em filtros 0,45 µm (Millex com modificada membrana PTFE para solventes aquosos e orgânicos, Millipore, Barueri, SP, Brasil), secas com nitrogênio, diluídas em MTBE (1 mL) e, em seguida, injetadas no CLAE. O teor de licopeno na fase aquosa da dispersão foi determinado após ultrafiltração-centrifugação, sendo o filtrado injetado diretamente no cromatógrafo.

A eficiência de encapsulação foi determinada, de acordo com Venturini *et al.*(2011), dividindo-se a diferença entre a concentração total do pigmento e a sua concentração em fase aquosa, pela concentração total, multiplicada por 100.

Todos os solventes utilizados são de grau cromatográfico e previamente filtrados através do sistema de filtração a vácuo Millipore, utilizando uma membrana de 0,22 µm para solventes orgânicos (Millipore). As injeções foram realizadas em duplicata.

4.6. **Avaliação da Estabilidade de LYC-LNC**

LYC-LNC foram avaliadas com relação à sua estabilidade ao armazenamento em diferentes temperaturas, em altas temperaturas e na presença do oxigênio singleto. Segue a descrição das metodologias utilizadas:

4.6.1. Estabilidade das nanocápsulas de licopeno durante armazenamento.

LYC-LNC foram armazenadas à temperatura de refrigeração (4°C) e a temperatura ambiente (25°C) por 12 semanas e 4 semanas, respectivamente. As amostras foram acondicionadas em frascos âmbar, alíquotas retiradas semanalmente e as análises da estabilidade das nanocápsulas em relação aos parâmetros diâmetro, índice de polidispersão, potencial zeta, pH, análises colorimétricas e teor total de licopeno ao longo do período de armazenamento, foram realizadas de acordo com as metodologias já descritas anteriormente.

4.6.2. Estabilidade das nanocápsulas de licopeno ao aquecimento.

Para os experimentos de aquecimento, 500 µL da suspensão de LYC-LNC foi aquecida em *Eppendorf*s selados com para-filme, protegidos da luz e aquecidos em banho-maria (DeLeo® B450) a 60, 70 e 80°C e em seguida resfriados em um banho de gelo. A

concentração de licopeno foi periodicamente avaliada por CLAE em diferentes tempos (0, 5, 10, 15, 20, 30, 50, 70, 100, 130 e 180 min).

Os resultados obtidos foram utilizados para determinar os parâmetros cinéticos que correspondem ao valor de *K* (constante cinética) através de uma cinética de primeira ordem:

$$[A] = [Ao]e^{-kt} \quad (2)$$

Onde:

[A] é a concentração final do licopeno

[Ao] é a concentração inicial do licopeno

K é a constante de velocidade da reação

t é o tempo que ocorre a reação.

A Energia de ativação (*Ea*), por sua vez, foi calculada através da equação de Ahrrenius:

$$K = Ae^{\frac{-Ea}{RT}} \quad (3)$$

Onde:

A é a constante pré-exponencial;

K é a constante de velocidade da reação;

Ea é a energia de ativação;

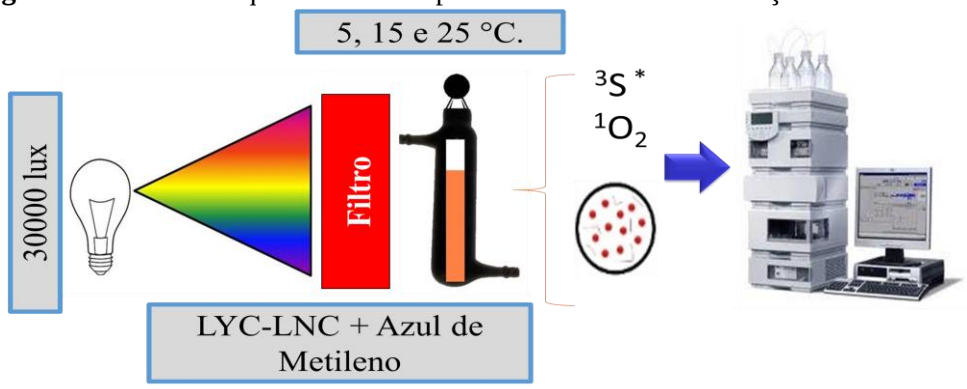
R é a constante dos gases;

T é a temperatura.

4.6.3. Estabilidade das nanocápsulas de licopeno à fotosensibilização

Para o estudo de fotosensibilização, foi preparada uma solução contendo LYC-LNC e 5 mg/(mL de LYC-LNC) de azul de metileno. Os experimentos foram realizados a 5, 15 e 25 °C, temperatura controlada por um sistema de água do banho de refrigeração. A iluminação foi fornecida por uma lâmpada de 150W e a intensidade da luz foi medida por um luxímetro (30000 lux). Um filtro vermelho de acrílico foi acoplado à lâmpada para excitar exclusivamente a banda do sensibilizador azul de metileno que ocorre acima de 625 nm. A figura 10 apresenta um esquema ilustrativo do aparato montado para a realização deste experimento.

Figura 10- Resumo esquemático do experimento de fotosensibilização.



Neste experimento, foram testadas diferentes condições. O primeiro estudo foi realizado na ausência de oxigênio através da injeção de N_2 (99,99% de pureza) para a célula de fotosensibilização à 25 °C e na presença do azul de metileno; o segundo estudo foi realizado em um sistema saturado com ar à 5, 15 e 25 °C na presença de azul de metileno; e o terceiro realizado num estado saturado com ar à 25 °C, na ausência do azul de metileno.

No experimento de fotosensibilização, a luz filtrada excita o azul de metileno, e a energia é transferida para o oxigênio (oxigênio atmosférico), gerando assim o oxigênio singleto. Na presença de oxigênio (condições de saturação com ar), tanto o sensibilizador quanto o oxigênio singleto podem participar simultaneamente na perda de licopeno, que, na ausência de oxigênio (condições de N_2 saturada), apenas o sensibilizador pode atuar. Todos os experimentos ocorreram durante 400 min e a concentração de licopeno foi periodicamente avaliada por CLAE em diferentes tempos (0; 0,5; 1; 1,5; 2; 3; 4; 5 e 6h). Os dados obtidos foram então utilizados para determinar os parâmetros cinéticos que correspondem ao valor de *K* (constante cinética) e a Energia de ativação (*Ea*).

4.7. Análise estatística dos resultados

Os valores de *K* e *Ea* foram calculados a partir da curva obtida com os dados experimentais e das equações citadas anteriormente. Os demais resultados foram avaliados pela análise de Variância (ANOVA) e os valores médios analisados pelo teste de Tukey ao nível de 5% de significância, utilizando o software STATISTICA ® 8.0.

CAPÍTULO 4 – ARTIGOS CIENTIFICOS

Neste capitulo serão apresentados os trabalhos desenvolvidos: foram escritos dois artigos científicos que já estão publicados em revistas internacionais.

DEVELOPMENT OF LYCOPENE-LOADED LIPID-CORE NANOCAPSULES: PHYSICOCHEMICAL CHARACTERIZATION AND STABILITY STUDY

Artigo publicado na revista 'Journal of Nanoparticle Research'
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Development of Lycopene-loaded lipid-core nanocapsules: physicochemical characterization and stability study

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ABSTRACT

The objective of this study was to develop lycopene-loaded lipid-core nanocapsules (Lyc-LNC) by the interfacial deposition of preformed poly(ε-caprolactone) (PCL). Lycopene extract (93.9%) was obtained from tomatoes, and the organic phase was prepared with polymer (PCL), caprylic/capric triglycerides (CCT), sorbitan monostearate and lycopene in a mixture of acetone and ethanol under magnetic stirring at 40°C. The organic phase was injected into an aqueous phase containing polysorbate 80, and the suspension was concentrated under reduced pressure. The formulation with a lycopene concentration of 85 µg/mL was characterized in terms of size distribution, zeta potential, encapsulation efficiency, pH, viscosity and color. The Lyc-LNC formulation presented stable values for the *z*-average (193 ± 4.7 nm) and zeta potential (-11.5 ± 0.40 mV). Despite the lower pH, lycopene content and color change of the suspension, the nanocapsules showed satisfactory stability, presenting around 50% lycopene content after 14 days of storage at room temperature (25°C).

Keywords*:* Lycopene; lipid-core nanocapsules; nanoencapsulation; stability.

1. Introduction

Tomatoes are sources of carotenoids, particularly lycopene and β–carotene. The green color of unripe tomatoes is due to the presence of chlorophyll. As maturation occurs, the chlorophyll degrades and the synthesis of yellow pigments (carotenes and xanthophylls) occurs. Eventually, a red color is achieved due to the accumulation of lycopene, which represents 80 to 90% of the pigment in ripe tomatoes, mainly in the peel (Shi and Le Maguer 2000).

In addition to its color, lycopene is important because of its recognized health benefits. Epidemiological studies have shown that serum levels of lycopene and dietary intake of lycopene are inversely related to the risk of certain types of cancer (Bramley 2000; Levy and others 1995; Southon 2000).

Lycopene is a fat-soluble compound consisting of eleven conjugated bonds and two non-conjugated double bonds. Among carotenoids, this compound has the highest scavenging ability for singlet oxygen due to its chemical structure, which offers high reactivity. Unlike β-carotene, lycopene has no pro-vitamin properties because its structure lacks β-ionone rings, which are responsible for vitamin A activity (Damodaran 2008).

Carotenoids are often used as additives or colorants in food products. However, most of the fat-soluble compounds exhibit very low water solubility or even water insolubility (Moraru and others 2003). Carotenoids may also serve as antioxidants and free-radical quenching agents. They interact with free radicals and oxygen, inhibiting the propagation step of lipid peroxidation (Silva and others 2012).

Lycopene is considered to be unstable in the presence of oxygen, heat and light. As an antioxidant, lycopene has a singlet-oxygen quenching ability that is twice that of β-carotene and 10 times higher than that of α-tocopherol (Silva and others 2012). However, some studies have showed that encapsulation techniques increase the stability of lycopene in the presence of oxygen, heat and light (Chiu and others 2007; Goula and Adamopoulos 2012; Montenegro and others 2007; Patricia Blanch and others 2007).

Another possibility for increasing the availability of lycopene is the use of nanotechnology to increase its stability under processing and storage conditions. Recently, nanotechnology has emerged as one of the most promising and attractive fields of research, with applications ranging from the aerospace to the healthcare and food industries. This technique offers the potential to improve the stability and solubility of various bioactive

compounds (Yuan and others 2008). Sari and collaborates (2015) evaluated Curcumin encapsulated in medium chain triglyceride oil droplets of nanoemulsion prepared by ultrasonification using whey protein concentrate-70 and Tween-80 as emulsifiers and observed that the nanoemulsion was stable to pasteurization and to pH ranging from 3.0 to 7.0.

Nanoencapsulation involves the incorporation, adsorption and dispersion of compounds in small vehicles with diameters on the nanometer scale. The incorporated bioactive compounds can be protected against degradation, increasing their stability and solubility (e.g., the solubilization of a hydrophobic compound and hydrophilic matrices) (Letchford and Burt 2007; Sari and others 2015, Taylor and others 2005). Fontana, Coradini, Guterres, Pohlmann and Beck (2009) evaluated the photostability of clobetasol propionate (CP)-loaded nanoparticles under UVA radiation and found that the photostability of CP was improved by its incorporation into nanoparticles.

In the food-processing field, techniques for improving stability have been widely used to protect food ingredients, such as flavors, essential oils, lipids, oleoresins and colorants, against deterioration, volatile loss and interaction with other ingredients (Goula and Adamopoulos 2012, Onwulata 2013). Lycopene encapsulation and complexation techniques have been studied by Blanch et al. (2007), Goula and Adamopoulos (2012) and Chiu et al. (2007). Blanch et al. (2007) evaluated the stability and isomerization of lycopene complexed in cyclodextrin and concluded that lycopene with β-CD remained stable for six months. Goula and Adamopoulos (2012) developed a new technique for improved lycopene microencapsulation via a new spray-drying system using dehumidified air with maltodextrin as the wall material; in which the use of dehumidified air was proven to be an effective way to increase the encapsulation efficiency of lycopene. Chiu et al. (2007) encapsulated lycopene extract from tomato pulp waste and processed it into a powder with both gelatin and poly(γ-glutamic acid) (γ-PGA) as carriers. After one month, 50% of the lycopene content was lost. However, to date, no studies on the production and stability evaluation of lycopene nanocapsules have been published.

Therefore, considering the importance of lycopene for human health and the increased lycopene stability and solubility that can be achieved using nanotechnology, the objective of this study was to develop and characterize lycopene lipid-core nanocapsules and evaluate their stability during storage.

2. Materials and Methods

2.1. Materials

Tomatoes were obtained from a local market in Porto Alegre, Brazil. The polymer poly(ε-caprolactone) (PCL) (Mw=80,000) and sorbitan monostearate were obtained from Sigma (St. Louis, MO, USA). Capric/caprylic triglycerides (CCT) and polysorbate 80 were obtained from Delaware (Porto Alegre, Brazil). All other chemicals and solvents were of analytical or pharmaceutical grade.

2.2. Lycopene extract

Lycopene extract was prepared using an adaptation of the method of Nunes and Mercadante (2004), who described the production of lycopene extract from tomatoes. Tomatoes (500 g) were submitted to water removal followed by mechanical compression, and the lycopene was extracted with ethyl acetate (1000 mL). Each extraction was carried out under magnetic stirring over 120 min, and the extract was filtered and concentrated under reduced pressure in a rotary evaporator (Fisatom model 801/802, São Paulo, SP, Brazil). After concentration, the vessel containing the extract was placed in a cold bath, and dichloromethane (5 mL) was added slowly to this extract. After the addition of dichloromethane, ethanol (99.7%) was added slowly (20 mL). This solution was held at -18°C for 12 h for crystallization, and the crystals formed in the bottom of the vessel were filtered, washed with 50 mL of ethanol (99.7%) and dried under reduced pressure (T < 30°C). The purity of the extract was evaluated by high-performance liquid chromatography (HPLC).

2.3. Preparation of lipid-core nanocapsules

Lycopene-loaded lipid-core nanocapsules were prepared by the interfacial deposition of preformed polymers according to the method of Venturini et al. (2011). The organic phase was prepared from a polymer (PCL) (200 mg), triglycerides (CCT) (320 µL), sorbitan monostearate (76 mg) and lycopene (93.9%) in a mixture of acetone (48 mL) and ethanol (6 mL) under magnetic stirring at 40°C. After the solubilization of PCL, CCT and sorbitan monostearate, the lycopene extract (93.9%) was added, and the solution was subjected to magnetic stirring for 10 min (40°C). The organic phase was injected into an aqueous phase

(106 mL) containing polysorbate 80 (154 mg), and stirring was maintained for 10 min. The suspension was concentrated under reduced pressure until it reached a final volume of 20 mL. This formulation was called Lyc-LNC.

Considering the solubility of lycopene in the organic phase, an optimal formulation (85 µg/mL) was prepared in triplicate and characterized in terms of particle diameter, zeta potential, color, pH, viscosity, lycopene content and stability over four weeks at 25°C.

2.4. Determination of diameter and zeta potential

The mean diameter ($D_{4.3}$) of Lyc-LNC was measured by laser diffraction (LD) (Mastersizer 2000® 5.54, Malvern Instruments, UK) using water as a dispersant. The refractive indexes used for the polymer and water were 1.590 and 1.330, respectively. The data were analyzed using the Mastersizer 2000® 5.54 software program. The span values were determined by dividing the difference between $d_{(0.1)}$ and $d_{(0.9)}$ by $d_{(0.5)}$, in the software program.

The *z*-average and polydispersity index (PDI) were measured at 25°C by dynamic light scattering (DLS), and the zeta potential was measured based on the electrophoretic mobility (Zetasizer Nano ZS®, Malvern, UK). The samples were diluted with a pre-filtered aliquot (0.45 µm) of 10 mM NaCl aqueous solution or MilliQ® water to determine the zeta potential and *z*-average, respectively. Data analysis was performed using Dispersion Technology Software (version 7.4, 2013, Malvern Instruments Ltd). DLS was used to evaluate changes during storage because the Zetasizer Nano ZS® instrument is able to determine particle sizes ranging from 0.003 to 10 µm. The Lyc-LNC *z*-average and zeta potential were measured over a four-week period.

2.5. Morphological analysis

Morphological analysis of the Lyc-LNC suspension was performed by transmission electron microscopy (TEM) (Jeol, JEM 1200 Exll, Electron Microscopy Center, UFRGS, Brazil). The Lyc-LNC suspension was diluted in pre-filtered ultrapure water (1:10 v/v) and dispersed on a grid (Formvar-Carbon support films mesh 400). The TEM was operated at 80kV and uranyl acetate solution (2% w/v) was used as a negatively stained standard.

2.6. Colorimetric analysis

The Lyc-LNC suspension (10 mL) was analyzed using a portable colorimeter (Konica Minolta model CR 400, Singapore), and the suspension was prepared in triplicate with a lycopene concentration of 85 µg/mL. The colorimetric parameters were obtained according to the Commission Internationale de l'Eclairage (CIELAB system); the coordinates included *L* (lightness) and the color coordinates *a** (red green component) and *b** (yellow-blue component), which were measured using the illuminant D_{65} and a viewing angle of 0°. The color was measured over a four-week period.

2.7. pH

The pH of the Lyc-LNC suspension was measured at 25°C using a DM-22 potentiometer (Digimed, Brazil). The pH was evaluated over four weeks.

2.8. Viscosity

The viscosity of the suspension was measured immediately after preparation using a Brookfield rotational viscometer (model DV-II + Pro, spindle LV2, Brookfield Engineering, USA) at 25°C. The data were analyzed using Brookfield Rheocalc 32 software.

2.9. High-performance liquid chromatography (HPLC)

The stability of the nanocapsules was determined by high-performance liquid chromatography (HPLC) (Agilent series 1100, Santa Clara, CA, USA) using an instrument equipped with an online degasser, a quaternary pump and an automatic injector coupled to a C30 polymeric column YCM (250 x 4.6 mm i.d.; 3-µm particle size) at 33°C. Data acquisition and processing were performed using the ChemStation® software program. The gradient elution at a flow rate of 1 mL/min consisted of water/methanol/methyl tert-butyl ether (MTBE) starting from 5:95:5 v/v and reaching 0:95:5 v/v at 12 min, 0:89:11 v/v at 25 min, 0:75:25 v/v at 40 min and finally 0:50:50 v/v at 80 min. The chromatograms were processed at the maximum absorption wavelength of lycopene (470 nm).

Before injection, the lycopene extract was diluted in acetonitrile, and the lycopene in the nanocapsule (500 µL) was extracted with acetonitrile (10 mL), homogenized by ultrasonication (30 min) and centrifuged (30 min at 3,500 x g). All samples were filtered before injection (0.45 µm, Millex with modified PTFE membrane for aqueous and organic solvents, Millipore, Barueri, SP, Brazil).

For the quantification of lycopene, a calibration curve with a determination coefficient (R^2) greater than 0.99 was used. This calibration curve was obtained by plotting the peak areas (from HPLC) of five solutions containing different concentrations of lycopene (from 10 µg/mL to 100 µg/mL) quantified previously using a spectrophotometer (Agilent 8453 UV-visible spectrometer, Santa Clara, CA, USA) at 470 nm with an absorptivity coefficient of 3,450 in petroleum ether. The limits of detection (LOD) and quantification (LOQ) were 0.007 and 0.033 µg/mL, respectively, and were determined according to the method described by Valente Soares (2001).

All of the solvents used in the HPLC separation were of chromatographic grade and were previously filtered through a Millipore vacuum filtration system using a 0.22-µm membrane for organic solvents (Millipore, Barueri, SP, Brazil). The injections were performed in triplicate.

2.10. Determination of lycopene content and encapsulation efficiency

To determine the total lycopene content, the method consisted of lycopene extraction from a 500-µL aliquot of the formulation with acetonitrile (10 mL). This extract was ultrasonicated (30 min) and centrifuged (30 min at 3500 x g). The supernatant was filtered before injection into the HPLC (0.45 µm, Millex with modified PTFE membrane for aqueous and organic solvents, Millipore, Barueri, São Paulo, Brazil).

The encapsulation efficiency was determined by the ultrafiltration/centrifugation technique (Ultrafree Microcon 10,000 MW, Merck Millipore, Darmstadt, Germany). The free lycopene content in the aqueous phase of the Lyc-LNC suspension was determined over the HPLC injection of the ultrafiltrate obtained after the ultrafiltration/centrifugation (30 min at 3500 x g) of an aliquot of lycopene nanocapsule suspension (500 µL). The value was obtained by dividing the difference between the total concentration of lycopene and the concentration of lycopene in the aqueous phase by the total concentration and multiplying the result by 100.

2.11. Statistical analysis

The results were evaluated by one-way analysis of variance (ANOVA), and the mean values were analyzed by Tukey's test using STATISTICA® 8.0 software and the graphic program used was ORIGIN® 8.0.

3. Results and discussion

3.1. Lycopene extract

The lycopene extract obtained was 93.9 ± 0.33% pure. The purities of the lycopene extracts reported by Matioli and Rodriguez–Amaya (2003), Nunes and Mercadante (2004) and Liu et al. (2010) were 97%, 96% and 99%, respectively. These values correspond to differences in the type of extraction, the number of extractions, the solvents used and the number of crystallization steps, affecting the purity of the lycopene extract. In this study, the lycopene extract was submitted to a unique crystallization step which reduced the degree of purity obtained when compared with studies previously mentioned. However, the purity obtained was sufficient to produce lycopene nanocapsules.

3.2. Preparation and physicochemical characterization of lipid-core nanocapsules

The proposed encapsulation method was chosen for lycopene because of the approval of its reagents by the FDA (Food and Drugs Administration). Furthermore, the isolated formulation utilized in this study was optimized by Venturini et al. (2011) and applied in pharmaceutical experiments (Jager and others 2009) and pigment experiments (Lobato and others 2013).

The preliminary formulation was obtained by dissolving the lycopene in the organic phase. The total concentration of soluble lycopene was 85 µg/mL. The formulation was analyzed by LD and DLS immediately after being produced. Over a four-week period, the formulation was analyzed by DLS.

In the aqueous phase of Lyc-LNC, the free lycopene concentration was 4.15 µg/mL. The encapsulation efficiency was determined by the following equation: Encapsulation efficiency = [(85 – 4.15)/85] x 100. Thus, the encapsulation efficiency was determined to be 95.12 ± 0.42%. The high encapsulation efficiency indicates that the majority of the lycopene

in the suspension was present in the nanocapsule structures. Montenegro et al. (2007) obtained lycopene microcapsules in gum arabic with 95% encapsulation efficiency, and Silva et al. (2012) obtained lycopene microcapsules in a gelatin-pectin complex with 93.2% encapsulation efficiency. However, the encapsulant chosen, such as gelatin, gum arabic, gellan gum and maltodextrin, can affect the encapsulation efficiency (Rocha and others 2012; Rodriguez-Huezo and others 2004; Shu and others 2006). Tan and Nakajima (2005) attribute this phenomenon to the formation of free radicals in CCT during the solubilization of the compound in the organic phase.

Immediately after being produced, this formulation presented a unimodal distribution (volume and number analysis), with an average diameter of less than 1 μm for each method (Fig. 1). The volume-weighted mean diameter ($D_{4,3}$) observed was 153 nm, with a span value of 1.319. Similar values (132 and 184 nm) were observed for a β-carotene nanoemulsion, with a span of approximately 0.181 to 0.360 (Yuan and others 2008). Ribeiro et al. (2008) obtained nanoparticles incorporated with β-carotene ranging from 74 to 77 nm in diameter using different compounds in the formulation, such as poly(D,L-lactic acid) (PLA) and poly(D,L-lactic-co-glycolic acid) (PLGA). Tan and Nakajima (2005) produced β-carotene nanodispersions by the solvent displacement method, with diameters $D_{4,3}$ varying from 60 to 135 nm and a span value varying from 0.4 to 0.7.

Low span values indicate a homogeneous particle size distribution. Thus, the above-described results indicate that the formulation was homogeneous because the span values are lower than those reported by Lobato et al. (2013) and Venturini et al. (2011), who reported spans of 1.397 and 1.28, respectively.

The determination of the particle size distribution in terms of volume allowed for the analysis of the effect of particles with diameters greater than 1 μm, whereas the distribution in terms of number did not allow for this analysis because these particles (diameter>1 μm) were present in small amounts.

The *z*-average observed for Lyc-LNC was 193 ± 4.70 nm, with a PDI of 0.069 ± 0.02. Similar values were observed by Lobato et al. (2013), who studied bixin nanocapsules and found a *z*-average of 190 ± 9 nm and a PDI of 0.098 ± 0.03 and Gonzaléz-Reza et al. (2014) observed diameter of 191nm in β-carotene nanocapsules utilizing PCL polymer. Contri, Ribeiro, Fiel, Pohlmann, & Guterres (2013), who evaluated the effect of oils on the physicochemical properties of nanocapsules, observed that nanocapsules with capric/caprylic triglycerides (CCT-NC) presented a *z*-average of 145 ± 16 nm.

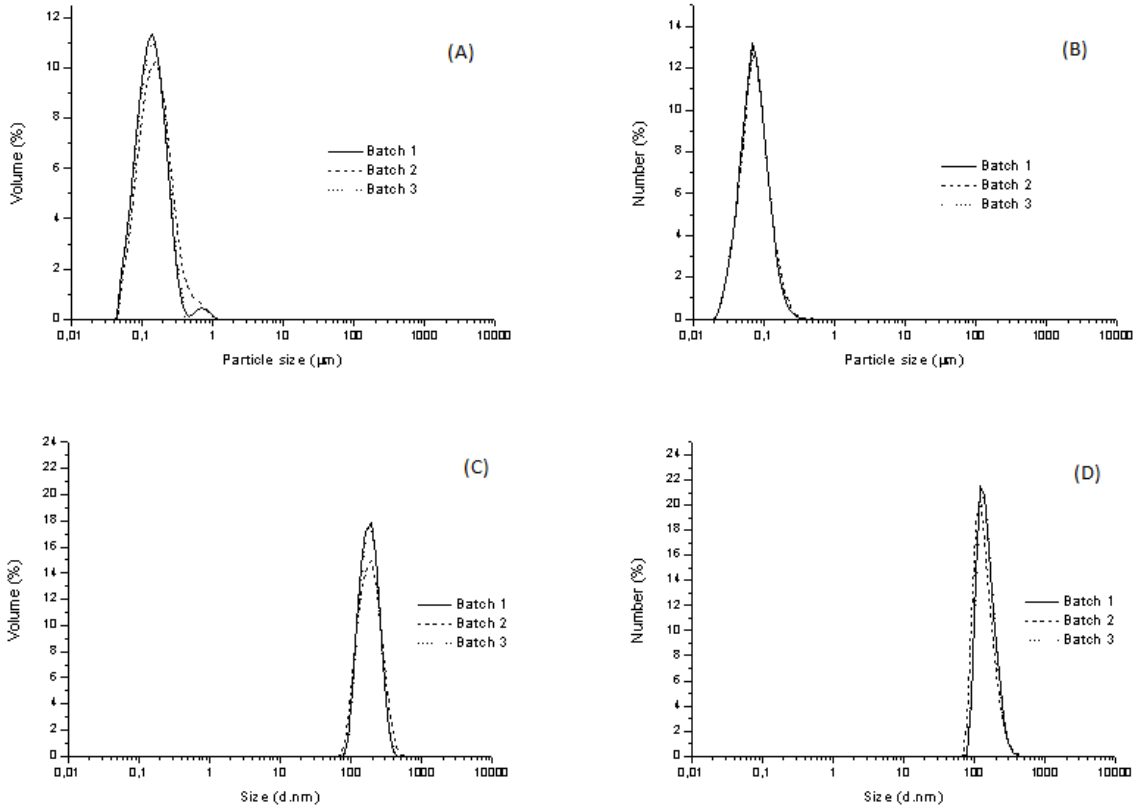

Fig 1: (A) Size distribution (volume) and (B) size distribution (number) obtained by LD after preparation. (C) Size distribution (volume) and (D) size distribution (number) obtained by DLS after preparation.

Besides LD and DLS, TEM also are used to measure size distribution and particle size (Wu, Zhang, & Watanabe, 2011). As presented by DLS and LD, in the TEM analysis, the Lyc-LNC showed small particle size and the homogeneity of the nanocapsule suspension (Fig. 2).

The charge acquired by a particle or molecule in a given medium is its zeta potential, and it arises from the surface charge and the concentration and types of ions in solution (Dickinson 2006). The Lyc-LNC suspension presented a mean potential zeta of -11.5 ± 0.40 mV immediately after preparation. Nanostructured lipid carriers utilizing glyceryl behenate, glycerol 1-monostearate and Miglyol 812N (C_8 – C_{12} triglycerides) presented zeta potential between -13.4 ± 2.0 mV and -24.7 ± 1.4 mV (Zhuang and others 2010). A benzophenone-3 loaded lipid-core nanocapsule suspension prepared with PCL and polysorbate 80 exhibited zeta potentials of -9.5 ± 1.0 mV. The negative zeta potential is a consequence of the negative charge density of the carboxylate groups in the PCL backbone (Paese and others 2009).

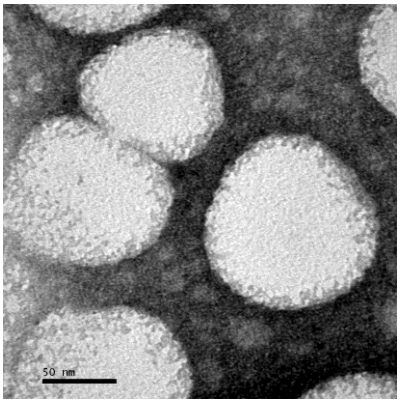

Fig 2: TEM images of lycopene nanocapsules directly after preparation [bar = 100 nm (500,000)].

The viscosity can be defined as the internal resistance of a liquid to flow, property considered important to evaluate some process steps, such as mixing, pumping, filling and quality control. The behavior of Lyc-LNC was typical for a Newtonian fluid (Fig. 3) in that the viscosity is not changed in relation shear stress/shear rate.

The Lyc-LNC suspension exhibited a viscosity of 1.09 ± 0.03 mPa.s. Similar results were observed by Contri et al. (2013), who studied the use of different oil-core nanocapsules and reported values of 1.3 ± 0.1 mPa.s for lipid-core nanocapsules of capric/caprylic triglycerides. The formulation of Lyc-LNC was expected to present Newtonian behavior due to its very low viscosity, which is similar to that of water, as well as its small particle size and the homogeneity of the nanocapsule suspension.

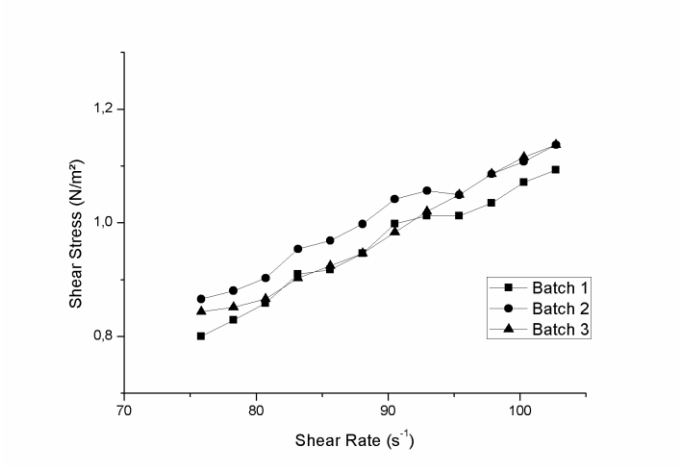

Fig 3: Rheological behavior of Lyc-LNC, in triplicate (Batch 1, Batch 2 and Batch 3), which corresponds to that of a Newtonian fluid.

The Lyc-LNC suspension showed an initial pH of 6.01 ± 0.04. Contri et al. (2013) and Paese et al. (2009) reported similar values (pH 5.5 ± 0.3 and 6.56 ± 0.09, respectively) utilizing the same technique for synthesizing nanocapsules.

Concerning the color parameter, the Lyc-LNC suspension initially exhibited an orange color, with CIELAB coordinates of L = 54.22 ± 2.33, a^* = 14.16 ± 0.28 and b^* = 41.06 ± 1.93. Compared to the same concentration of lycopene in tomatoes analyzed by Nunes and Mercadante (2004), with parameters L = 39.0 ± 0.30, a^* = 20.7 ± 0.60 and b^* = 17.7 ± 0.6, the lycopene nanocapsules exhibited a higher luminosity and yellow color and a slightly reduced red color, likely due to the presence of polymer, which conferred turbidity to the suspension.

3.3. Stability evaluation of Lyc-LNC

The lycopene nanocapsule suspension was prepared in triplicate with a mean lycopene concentration of 85 ± 0.12 µg/mL. Nanocapsules with different concentrations of other compounds were produced in previous studies, such as that of Lobato et al. (2013), who produced bixin-loaded nanocapsules with a mean concentration of 16.92 µg/mL, and that of Venturini et al. (2011), who utilized a high concentration of indomethacin ethyl ester (1 mg/mL); both groups used the same formulation. These differences are related to the type of compound encapsulated, which affects the amount of compound incorporated into the formulation. Table 1 presents the results of the four-week stability evaluation of the Lyc-LNC suspension.

Table 1: Stability of Lyc-LNC over four weeks

	7 days	**14 days**	**21 days**	**28 days**
z-average (nm)	179 ± 0.00 ^{a}	189 ± 1.50 ^{a}	184 ± 9.19 ^{a}	187 ± 9.89 ^{a}
PDI	0.056 ± 0.02 ^{a}	0.075 ± 0.01 ^{a}	0.075 ± 0.01 ^{a}	0.079 ± 0.01 ^{a}
Zeta potential (mV)	-12.50 ± 6.70 ^{a}	-15.3 ± 5.60 ^{a}	-10.95 ± 2.60 ^{a}	-12.7 ± 2.82 ^{a}
pH	5.79±0.03^{a}	5.69±0.04^{b}	5.62±0.03^{bc}	5.60±0.02^{c}
Lycopene content (µg/mL)	67±0.05 ^{a}	40±0.00 ^{b}	6±0.00^{c}	4±0.00^{c}
L^*	53.78 ± 1.32^{b}	61.42 ± 0.63^{a}	63.97 ± 2,61^{a}	65.69 ± 1.12^{a}
a^*	8.22 ± 0.44 ^{a}	-1.13 ± 0.52^{b}	-0.85 ± 1.42^{b}	-6.26 ± 0.08^{c}
b^*	28.38 ± 1.19^{a}	23.84 ± 1.46^{b}	21.18 ± 0.82^{b}	17.30 ± 0.57^{c}

Mean ± standard deviation values in the same line followed by the same superscripts are not significantly different (n = 9; p > 0.05)

The stability of the particle diameter size is very important because particle agglomeration in drug products designed for intravenous use can cause blood capillary blockage and obstruct blood flow (Wu and others 2011). In this study, the diameter of the lycopene nanocapsules was stable, and no evidence of flocculation or coalescence was observed during the evaluation of the diameter and z-average over 28 days of storage at 25°C (Fig. 4). This stability is attributed to the surfactant polysorbate 80, which has been shown to be responsible for the stability of this type of nanocapsule formulation (Jager and others 2009; Sessa and others 2013, Venturini and others 2011).

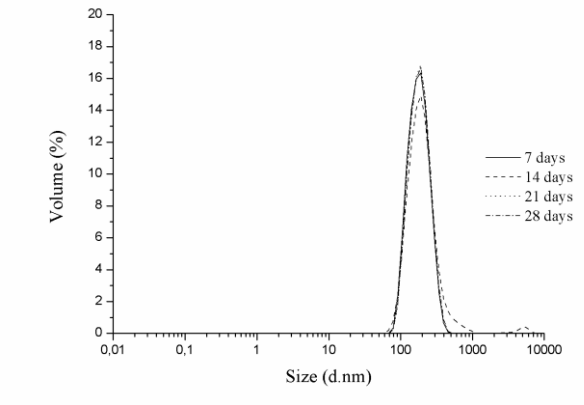

Fig 4: Size distribution (volume) obtained by DLS as a function of time.

The zeta potential indicates the charge of a particle. Because particles of similar charge repel each other, those with high charges will resist flocculation and aggregation for longer periods, making such samples more stable. Therefore, the stability can be modified by altering the pH, ionic concentration and type of ions used and by using additives, such as surfactants and polyelectrolytes (Dickinson 2006). During storage, no significant difference in the zeta potential was observed, allowing the suspension to remain stable. Contri et al. (2013) reported no significant difference in the zeta potential after 90 days of storage.

After 28 days, the pH presented a value of 5.60 ± 0.02, which was significantly lower than the initial value. This decrease may be related to the partial degradation of the polymer content in the formulation and the consequent liberation of the polyester monomer during poly(ε-caprolactone) hydrolysis (Kasperczyk and others 2008; Kishore and others 2011). Schaffazick et al. (2003) observed that a reduction in pH in the nanocapsules suspension was attributed to the exposure of a greater number of terminal carboxylic acid groups as a function of time, promoted by the relaxation of the polymer chains.

Concerning the stability of the CIELAB color parameters during storage, the parameters changed significantly after 14 days (Table 01), and the Lyc-LNC suspension presented increased luminosity and reductions in the parameters a^* and b^* due to the degradation of lycopene, leading to a loss of color and an increase in the white color component.

During storage, the Lyc-LNC suspension exhibited a decrease in lycopene content. This decrease may have occurred as a result of the presence of oxygen in the amber bottle used to hold the nanocapsules during storage. The Lyc-LNC suspension retained approximately 50% of the lycopene content after 14 days of storage (T 1), even without refrigeration (25°C). Generally, these compounds are stored at very low temperatures, as demonstrated by Dias, Camões and Oliveira (2014), who evaluated free lycopene stored at -20 and -70°C and observed the degradation of this compound in six weeks. The location in nanocapsule and the release rate of bioactive compound influences the stability of the nanocarrier formulation (Fathi and others 2012), which explains the slow degradation of lycopene that migrates slowly of the lipid core to polymeric wall of the nanocapsule during storage.

Yuan et al. (2008) reported the characterization of a β-carotene oil-in-water nanoemulsion prepared by high-pressure homogenization and evaluated its stability over a period of four weeks. Unlike lycopene, the β-carotene was gradually degraded, and by the end of the four weeks of storage at 25°C in an amber bottle, approximately 25% of the β-carotene was lost. This loss can be explained by the chemical structure of β-carotene. Whereas lycopene features conjugated double bonds in the same chain and the absence of a β-ionone ring, β-carotene features double bonds in β-ionone rings, causing it to become less reactive and more susceptible to isomerization and oxidation during storage than lycopene (Matioli and Rodriguez-Amaya 2003).

One method for reducing the degradation of lycopene during storage is the use of antioxidant agents in the nanocapsule composition. The addition of α-tocopherol is an approach utilized to prevent the degradation of β–carotene in nanoemulsions (Ribeiro and others 2008).

4. Conclusions

Lycopene is a fat-soluble compound with the highest scavenging ability of singlet oxygen among all carotenoids due to its chemical structure, which offers greater reactivity,

similarly to a powerful antioxidant. However, the application of lycopene is limited because of its reactivity and instability; even when stored at freezing temperatures, free lycopene can be degraded within a few weeks. Thus, the nanoencapsulation of lycopene produced stable particles, with a satisfactory z-average of 193 ± 4.7 nm and a zeta potential of -11.5 ± 0.40 mV. Even at room temperature (25°C), the nanocapsules retained a content of 50% lycopene after 14 days of storage, which would be unviable conditions for free lycopene. The use of nanotechnology offers the potential to expand the use of lycopene in industrial processes by improving the stability and solubility of lycopene in different foods.

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References

Blanch GP, Luisa Ruiz Del Castillo M, Del Mar Caja M, Perez-Mendez M, Sanchez-Cortes S (2007) Stabilization of all-trans-lycopene from tomato by encapsulation using cyclodextrins. *Food Chemistry 105:1335-1341*.

Bramley PM (2000) Is lycopene beneficial to human health? *Phytochemistry 54:233-236.*

Chiu YT, Chiu CP, Chien JT, Ho GH, Yang J, Chen BH (2007) Encapsulation of lycopene extract from tomato pulp waste with gelatin and poly(gamma-glutamic acid) as carrier. *Journal of Agricultural and Food Chemistry 55:5123-5130.*

Contri RV, Ribeiro KLF, Fiel LA, Pohlmann AR, Guterres SS (2013) Vegetable oils as core of cationic polymeric nanocapsules: influence on the physicochemical properties. *Journal of Experimental Nanoscience 8:913-924.*

Damodaran SPKLFOR (2008) *Food chemistry*. London: CRC Press.

Dias MG, Camões MFGFC, Oliveira L (2014) Carotenoid stability in fruits, vegetables and working standards – Effect of storage temperature and time. *Food Chemistry 156:37-41*.

Dickinson E (2006) Food Emulsions: Principles, Practices, and Techniques, 2nd edition, Julian McClements. CRC Press, Boca Raton. *Food Hydrocolloids 20:137.*

Fathi M, Mozafari MR, Mohebbi M (2012) Nanoencapsulation of food ingredients using lipid based delivery systems. *Trends in Food Science & Technology 23:13-27.*

Fontana MC, Coradini K, Guterres SS, Pohlmann AR, Beck RCR (2009) Nanoencapsulation as a Way to Control the Release and to Increase the Photostability of Clobetasol Propionate: Influence of the Nanostructured System. *Journal of Biomedical Nanotechnology 5:254-263.*

Goula AM, Adamopoulos KG (2012) A New Technique for Spray-Dried Encapsulation of Lycopene. *Drying Technology 30:641-652.*

González-Reza RM, Quintanar-Guerrero D, Flores-Minutti JJ, Gutiérrez-Cortez E, Zambrano-Zaragoza ML (2014) Nanocapsules of β-carotene: Thermal degradation kinetics in a scraped surface heat exchanger (SSHE). *LWT - Food Science and Technology 60:124–130.*

Jager E, Venturini CG, Poletto FS, Colome LM, Pohlmann JPU, Bernardi A, Battastini AMO, Guterres SS, Pohlmann AR (2009) Sustained Release from Lipid-Core Nanocapsules by Varying the Core Viscosity and the Particle Surface Area. *Journal of Biomedical Nanotechnology 5:130-140.*

Kasperczyk J, Hu Y, Jaworskam J, Dobrzynski P, Wei J, Li S (2008) Comparative study of the hydrolytic degradation of glycolide/L-lactide/epsilon-caprolactone terpolymers initiated by zirconium(IV) acetylacetonate or stannous octoate. *Journal of Applied Polymer Science 107:3258-3266.*

Kishore RSK, Kiese S, Fischer S, Pappenberger A, Grauschopf U, Mahler H-C (2011) The Degradation of Polysorbates 20 and 80 and its Potential Impact on the Stability of Biotherapeutics. *Pharmaceutical Research 28:1194-1210.*

Letchford K, Burt H (2007) A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *European Journal of Pharmaceutics and Biopharmaceutics 65:259-269.*

Levy J, Bosin E, Feldman B, Giat Y, Miinster A, Danilenko M, Sharoni Y (1995) Lycopene is a more potent inhibitor of human cancer cell-proliferation than either alpha-carotene or beta-carotene. *Nutrition and Cancer-an International Journal 24:257-266.*

Liu Y, Liu J, Chen X, Liu Y, Di D (2010) Preparative separation and purification of lycopene from tomato skins extracts by macroporous adsorption resins. *Food Chemistry 123:1027-1034.*

Lobato KBdS, Paese K, Forgearini JC, Guterres SS, Jablonski A, Rios AdO (2013) Characterisation and stability evaluation of bixin nanocapsules. *Food Chemistry 141:3906-3912.*

Matioli G, Rodriguez-Amaya DB (2003) Microencapsulação do licopeno com ciclodextrinas. *Ciência e Tecnologia de Alimentos 23:102-105.*

Montenegro MA, Nunes IL, Mercadante AZ, Borsarelli CD (2007) Photoprotection of vitamins in skimmed milk by an aqueous soluble lycopene-gum arabic microcapsule. *Journal of Agricultural and Food Chemistry 55:323-329.*

Moraru CI, Panchapakesan CP, Huang QR, Takhistov P, Liu S, Kokini JL (2003) Nanotechnology: A new frontier in food science. *Food Technology 57:24-29.*

Nunes IL, Mercadante AZ (2004) Obtenção de cristais de licopeno a partir de descarte de tomate. *Food Science and Technology (Campinas) 24:440-447.*

Onwulata CI (2013) Microencapsulation and functional bioactive foods. *Journal of Food Processing and Preservation 37:510-532.*

Paese K, Jäger A, Poletto FS, Pinto EF, Rossi-Bergmann B, Pohlmann AR, Guterres SS (2009) Semisolid formulation containing a nanoencapsulated sunscreen: Effectiveness, in vitro photostability and immune response. *Journal of Biomedical Nanotechnology 5:240-246.*

Ribeiro HS, Chu B-S, Ichikawa S, Nakajima M (2008) Preparation of nanodispersions containing beta-carotene by solvent displacement method. *Food Hydrocolloids 22:12-17.*

Rocha GA, Favaro-Trindade CS, Ferreira Grosso CR (2012) Microencapsulation of lycopene by spray drying: Characterization, stability and application of microcapsules. *Food and Bioproducts Processing 90:37-42.*

Rodriguez-Huezo ME, Pedroza-Islas R, Prado-Barragan LA, Beristain CI, Vernon-Carter EJ (2004) Microencapsulation by spray drying of multiple emulsions containing carotenoids. *Journal of Food Science 69:E351-E359.*

Sari TP, Mann B, Kumar R, Singh RRB, Sharma R, Bhardwaj M, Athira S (2015) Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloids 43:540-546.*

Schaffazick SR, Guterres SSU, Freitas LD, Pohlmann AR (2003) Physicochemical characterization and stability of the polymeric nanoparticle systems for drug administration. *Quimica Nova 26:726-737.*

Sessa M, Casazza A, Perego P, Tsao R, Ferrari G, Donsì F (2013) Exploitation of Polyphenolic Extracts from Grape Marc as Natural Antioxidants by Encapsulation in Lipid-Based Nanodelivery Systems. *Food and Bioprocess Technology 6:2609-2620.*

Shi J, Le Maguer M (2000) Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Reviews in Biotechnology 20:293-334.*

Shu B, Yu WL, Zhao YP, Liu XY (2006) Study on microencapsulation of lycopene by spray-drying. *Journal of Food Engineering 76:664-669.*

Silva DF, Favaro-Trindade CS, Rocha GA, Thomazini M (2012) microencapsulation of lycopene by gelatin-pectin complex coacervation. *Journal of Food Processing and Preservation 36:185-190.*

Southon S (2000) Increased fruit and vegetable consumption within the EU: potential health benefits. *Food Research International 33:211-217.*

Tan CP, Nakajima M (2005) beta-Carotene nanodispersions: preparation, characterization and stability evaluation. *Food Chemistry 92:661-671.*

Taylor TM, Davidson PM, Bruce BD, Weiss J (2005) Liposomal nanocapsules in food science and agriculture. *Critical Reviews in Food Science and Nutrition 45:587-605.*

Valente Soares LM (2001) Como obter resultados confiáveis em cromatografia / How to obtain reliable results in chromatography. Rev. Inst. Adolfo Lutz, 79-84.

Venturini CG, Jäger E, Oliveira CP, Bernardi A, Battastini AMO, Guterres SS, Pohlmann AR (2011) Formulation of lipid core nanocapsules. *Colloids and Surfaces A: Physicochemical and Engineering Aspects 375:200-208.*

Wu L, Zhang J, Watanabe W (2011) Physical and chemical stability of drug nanoparticles. *Advanced Drug Delivery Reviews 63:456-469.*

Yuan Y, Gao Y, Zhao J, Mao L (2008) Characterization and stability evaluation of beta-carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. *Food Research International 41:61-68.*

Zhuang C-Y, Li N, Wang M, Zhang X-N, Pan W-S, Peng J-J, Pan Y-S, Tang X (2010) Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. *International Journal of Pharmaceutics 394:179-185.*

STABILITY STUDY OF LYCOPENE-LOADED LIPID-CORE NANOCAPSULES UNDER TEMPERATURE AND PHOTOSENSITIZATION

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Stability study of Lycopene-loaded lipid-core nanocapsules under temperature and photosensitization

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ABSTRACT

The objective of this study was to evaluate the stability of nanoencapsulated lycopene (LYC-LNC) prepared by interfacial deposition of preformed poly(ε-caprolactone) (PCL) during photosensitization (5°C – 25°C), heating (60°C – 80°C) and refrigeration (5°C). LYC-LNC presented a concentration of 80.71 μg/mL and was also analyzed in terms of mean diameter, zeta potential and morphology, as well as the position of the lycopene in the particle. During photosensitization and heating in air-saturated conditions, LYC-LNC showed activation energy of 67 kcal/mol and 24.9 kcal/mol, respectively. Those values were superior to that of free lycopene as found in the literature for both experiments. During refrigeration at 5°C, the mean diameter and zeta potential of LYC-LNC remained stable for 84 days and presented approximately 40% of lycopene content. All experiments suggest that nanoencapsulation improves the stability of lycopene under different processing conditions.

Keywords: heating; refrigeration, oxygen singlet; sensitizer; nanotechnology.

1. Introduction

Lycopene is an important carotenoid that has received special attention in recent years because of its beneficial effects to health. These benefits arise from antioxidant properties that are effective in the control of several pathologies such as reduction of the risk of certain types of cancer (Bramley, 2000; Levy et al., 1995; Southon, 2000), cardiovascular disease and hepatic fibrogenesis (Kitade, Watanabe, Masaki, Nishioka, & Nishino, 2002). Lycopene also regulates the immune system and decreases cholesterol level (Ried & Fakler, 2011). However, this compound is very unstable due to the presence of eleven conjugated bonds and two non-conjugated double bonds. This chemical structure is also responsible for the high scavenging ability for singlet oxygen (Damodaran, Parkin, & Fennema, 2010) and the ability to twine β-carotene, which is 10 times higher than α-tocopherol (Weisburger, 2002).

The singlet oxygen can be generated in the presence of light, triplet oxygen and a sensitizer that absorbs light and transfers energy to the triplet oxygen to form the singlet oxygen (Decker, Chen, Panya, & Elias, 2010; Skibsted, 2010). Carotenoids can act in two ways to avoid subsequent reactions induced by the singlet oxygen: by deactivating the sensitizer in its excited state or by quenching the singlet oxygen (physical and chemical quenching) (Choe & Min, 2006).

Lycopene is a free-radical quenching agent due to chemical characteristics that allow for a high scavenging ability for singlet oxygen. This compound interacts with free radicals and oxygen, inhibiting the propagation step of lipid peroxidation (Silva, Favaro-Trindade, Rocha, & Thomazini, 2012). On the other hand, light decreases the stability of food components by both photolytic autoxidation and photosensitized oxidation.

In addition, some studies regarding lycopene stability evaluated the effects of illumination (Henry, Catignani, & Schwartz, 1998; Lee & Chen, 2002) and temperature (Hackett, Lee, Francis, & Schwartz, 2004; Lee & Chen, 2002; Shi, Dai, Kakuda, Mittal, & Xue, 2008) on the degradation. Degradation reactions are influenced by many factors, such as temperature, physical state, type of pigments and environmental conditions (Pesek & Warthesen, 1987). Henry and Schwartz (1998) found that the degradation rate of lycopene was higher than that of β-carotene when safflower oil was heated at different temperatures. Chen, Shi, Xue & Ma (2009) compared the stability of lycopene in water- and oil-based food model systems and observed an increase in temperature or irradiation intensity promoted by the increased degradation of lycopene. In that study, the rate of degradation of total lycopene

contents was smaller in oil-based model systems than in water-based samples under different conditions.

Techniques to improve the stability of lycopene are being studied. One technique already used for carotenoids is the encapsulation (Yuan, Gao, Zhao, & Mao, 2008). In general, encapsulation improves the stability and apparent solubility of the encapsulated compound and promotes its controlled release (Ribeiro, Chu, Ichikawa, & Nakajima, 2008; Tan & Nakajima, 2005). Barbosa et al. (2005) evaluated the light stability of bixin encapsulated with different edible polysaccharide preparations and observed that the microencapsulation decreases the degradation rates of bixin caused by light, ozone, air, oxygen and high temperature. In relation to lycopene, Rocha et al. (2012) characterized and evaluated the stability of microencapsulation of lycopene by spray drying, but did not evaluate its stability under heating or oxidation conditions.

Another technique to promote stability is nanoencapsulation, a process by which one compound is covered by another; it involves the incorporation, adsorption and dispersion of those compounds in small vehicles or matrix systems of nanometric diameters (Letchford & Burt, 2007). Lobato et al. (2014) evaluated the stability of bixin in lipid-core nanocapsules (BIX-LNC) during photosensitization and heating in model systems of ethanol:water (2:8) and concluded that the nanoencapsulation increased the stability of bixin in both experiments of photosensitization and heating. However, no studies about the thermal or oxidative stability of encapsulated or nanoencapsulated lycopene have been published.

Therefore, considering that nanoencapsulation can promote lycopene stability, the aim of this study was to evaluate the stability of nanoencapsulated lycopene prepared by interfacial deposition of preformed poly(ε-caprolactone) during photosensitization, heating and refrigeration.

2. Materials and Methods

2.1. Materials

Tomatoes were acquired from a market in Porto Alegre, Brazil. The polymer poly(ε-caprolactone) (PCL) (Mw=80,000), Methylene Blue (dye content ≥82%) and sorbitan monostearate were acquired from Sigma (St. Louis, MO, USA). The polysorbate 80 and

capric/caprylic triglycerides (CCT) were obtained from Delaware (Porto Alegre, Brazil). All other solvents were of analytical or pharmaceutical grade.

2.2. Lycopene extract

The lycopene extract was obtained using an adaptation of Nunes and Mercadante's method (2004). Tomatoes (500 g) were submitted to water removal by mechanical compression, and the lycopene was extracted using ethyl acetate (1000 mL). Each extraction occurred under magnetic stirring during 120 min, and the extract was filtered and concentrated in a rotary evaporator (Fisatom model 801/802, São Paulo, SP, Brazil) until complete removal of solvent. After concentration, the vessel containing the extract was placed in a cold bath, and dichloromethane (7 mL) followed by ethanol (99.7%, 28 mL) was slowly added to this extract. This solution was maintained at –18°C for 12 h for crystallization, and the crystals formed in the vessel were filtered, washed with 50 mL of ethanol (99.7%) and dried (T < 30°C).

2.3. Preparation of lipid-core nanocapsules

Interfacial deposition of the preformed polymers was carried out to obtain lycopene-loaded lipid-core nanocapsules according to the method of Venturini et al. (2011). The organic phase was prepared from a polymer (PCL) (100 mg), triglycerides (CCT) (160 µL), sorbitan monostearate (38 mg) and lycopene (0.85 mg) in a mixture of acetone (24 mL) and ethanol (3 mL) under magnetic stirring at 40°C. After the solubilization of PCL and sorbitan monostearate, the lycopene extract (93.9%) was added, and the solution was submitted to magnetic stirring for 10 min (40°C). The organic phase was injected into an aqueous phase (53 mL of water) containing polysorbate 80 (78 mg), and stirring was maintained for 10 min. The suspension was concentrated under reduced pressure until it reached a final volume of 10 mL. This formulation was called LYC-LNC.

2.4. Characterization of LYC-LNC

The granulometric profile and the $D_{4.3}$ of LYC-LNC was measured by laser diffraction (LD) (Mastersizer 2000® 5.54, Malvern Instruments, UK) using water as a dispersant, and the data were analyzed using the Mastersizer 2000® 5.54 software program.

The *z*-average and polydispersity index (PDI) were measured at 25°C by dynamic light scattering (DLS) at measurement angles of 13° + 173°, and the zeta potential was measured by electrophoretic mobility (Zetasizer Nano ZS®, Malvern, UK). The samples were diluted (1:500) with a pre-filtered aliquot (0.45 µm) of 10 mM NaCl aqueous solution or MilliQ® water to determine the zeta potential and *z*-average, respectively. The Zetasizer nano ZS® is able to determine particle sizes ranging from 0.003 to 10 µm and zeta potential of +/-500mV and data analysis was performed using the Dispersion Technology Software (version 7.4, 2013, Malvern Instruments Ltd). The pH of the LYC-LNC was measured at 25°C using a DM-22 potentiometer (Digimed, Brazil) without prior sample dilution, and the viscosity of the suspension was measured using a Brookfield rotational viscometer (model DV-II + Pro, spindle LV2, Brookfield Engineering, USA) at 25°C. The torque used was above 10%, and data were analyzed using the Brookfield Rheocalc 32 software.

The total concentration of lycopene was determined through the extraction of lycopene from the LYC-LNC suspension. This method consisted in the extraction of 500 µL of LYC-LNC suspension from an aliquot using acetonitrile (2.5 mL). This extract was sonicated in ultrasound (30 min), dried in nitrogen, diluted in MTBE (1 mL) and was then injected in the HPLC.

The encapsulation efficiency was obtained by dividing the difference between the total concentration of lycopene and the concentration of lycopene in the aqueous phase by the total concentration and multiplying the result by 100. The free lycopene content in the aqueous phase of the LYC-LNC was determined by HPLC on a sample of the ultrafiltrate obtained after ultrafiltration of an aliquot of the LYC-LNC suspension (500 µL).

The lipophilicity of the lycopene was estimated by calculating the logarithm of the distribution coefficient (log D), which is defined as the ratio of the equilibrium concentration of a species of a molecule (unionized) in octanol to that of the same species in the water phase (ionized or unionized) (Oliveira et al., 2013). The ACD Log D 6.0 software (Advanced Chemistry Development, Inc., Toronto, Canada) was used to determine the log D as a function of pH.

2.5. Photosensitized oxidation of LYC- LNC in the model system

For the photosensitization study, a solution containing LYC-LNC and 5 µg/ (mL LYC-LNC) of methylene blue (MB) was prepared. The photosensitization experiments were

performed at 5, 15 and 25°C, controlled by a water refrigerator system. The illumination was provided by a 150 W filament lamp (30,000 lux) coupled to a red cut-off filter to exclusively excite the band of the sensitizer which occurs above 625 nm.

In this experiment, different conditions were tested. The first study was performed in the absence of oxygen by injecting N_2 (99.99% purity) to the photosensitization cell (25°C) in the presence of MB; the second study was carried out in an air-saturated condition at 5°C, 15°C and 25°C in the presence of MB; and the third was carried out in an air-saturated condition at 25°C in the absence of MB. In the photosensitization experiment, the filtered light excites the methylene blue, and energy is transferred to oxygen (atmospheric oxygen), thereby generating singlet oxygen. In the presence of oxygen (air-saturated condition), both the sensitizer (MB) and the singlet oxygen simultaneously participate in the loss of lycopene, whereas in the absence of oxygen (N_2-saturated condition), only the sensitizer is excited. All experiments were carried out for 400 min.

The concentration data were used to determinate the kinetic parameters using the Origin Pro 8.0 software (Origin lab Co., MA, USA).

2.6. Heating of model system and refrigeration stability

For the heating experiments, LYC-LNC was heated in a sealed Eppendorf tube in a heating bath at 60, 70 and 80°C, then cooled in an ice bath. Every step was carried out in the dark. The lycopene content was periodically evaluated by HPLC at different times (0, 5, 10, 15, 20, 30, 50, 70, 100, 130 and 180 min.), and the data were used to determine the kinetic parameters using the Origin Pro 8.0 software (Origin lab Co., MA, USA).

For the refrigeration experiments, the LYC-LNC was stored at 5°C, and the zeta potential, *z*-average and lycopene content were evaluated over a period of 12 weeks. The results were evaluated by one-way analysis of variance (ANOVA), and the mean values were analyzed by Tukey's test utizing the STATISTICA® 8.0 software. These analyses were performed in triplicate.

2.7. High performance liquid chromatography (HPLC) analysis

The total concentration of lycopene in all experiments was determined by high-performance liquid chromatography (HPLC) (Agilent series 1100, Santa Clara, CA, USA).

The analysis used an instrument equipped with an online degasser, a quaternary pump and an automatic injector coupled to a C30 polymeric column YCM (250 x 4.6 mm i.d.; 3-µm particle size) at 33°C. Data acquisition and processing were performed using the ChemStation® software package. The isocratic elution at a flow rate of 1 mL/min consisted of methanol/methyl tert-butyl ether (MTBE) of 20:80 v/v for 10 min. The chromatograms were processed at the maximum absorption wavelength of lycopene (470 nm).

Before injection, the lycopene extract was diluted in acetonitrile, and the lycopene in the nanocapsule (500 µL) was extracted with acetonitrile (2.5 mL), homogenized by ultrasonication (30 min), dried in nitrogen and diluted in MTBE (1 mL). All samples were filtered before injection (0.45 µm, Millex with modified PTFE membrane for aqueous and organic solvents, Millipore, Barueri, SP, Brazil).

The limits of detection (LOD) and quantification (LOQ) were 0.007 and 0.033 µg/mL, respectively, and were determined according to the method described by Valente Soares (2001).

All of the solvents used in the HPLC separation were of chromatographic grade and were filtered through a Millipore vacuum filtration system using a 0.22-µm membrane (Millipore, Barueri, SP, Brazil). The injections were performed in triplicate.

3. Results and discussion

3.1. Characterization of LYC-LNC

The LYC-LNC were fabricated with a lycopene concentration of 80.71 ± 2.22 µg/mL with a purity of 93.9 ± 0.33% and presented a monomodal distribution with an average diameter of less than 1 µm for both methods. The observed volume-weighted mean diameter ($D_{4,3}$) was 153 nm, and the *z*-average was 179 ± 4.70 nm, with a PDI of 0.054 ± 0.02. The LYC-LNC presented a mean zeta potential of –9.7 ± 0.50 mV immediately after preparation and exhibited a viscosity of 1.09 ± 0.03 mPa.s. Moreover, the LYC-LNC showed an initial pH of 6.01 ± 0.04 and encapsulation efficiency of 95.12 ± 0.42% (dos Santos et al., 2015).

Other authors using this same formulation for the production of nanocapsules found similar results (Contri, Ribeiro, Fiel, Pohlmann, & Guterres, 2013; Jager et al., 2009; Lobato et al., 2013; Paese et al., 2009). Paese et al. (2009) developed benzophenone-3-loaded lipid-core nanocapsules with a mean diameter of 247 ± 4 nm, a pH of 6.56 ± 0.09 and a zeta

potential of –9.5 ± 1.0 mV. In another study, nanostructured lipid carriers using glyceryl behenate, glycerol 1-monostearate and Miglyol 812N (C_8 – C_{12} triglycerides) presented a zeta potential in the range of –13.4 ± 2.0 mV to –24.7 ± 1.4 mV (Zhuang et al., 2010). Lobato et al. (2013) developed bixin nanocapsules, and found a mean diameter of 195 ± 27 nm, a pH of 5.9 ± 0.70 and a zeta potential of –14.45 ± 0.92 mV. Contri et al. (2013) studied the use of different oil-core nanocapsules and reported viscosities of 1.3 ± 0.1 mPa.s for nanocapsules of capric/caprylic triglycerides.

In relation to the lipophilicity of lycopene, the log D was of 15.46 and this value remains the same in the pH range of 0 to 14. The log D serves as a quantitative descriptor of lipophilicity (Chiang & Hu, 2009), and this high value indicated that 100% of lycopene content is found in the nanoparticle core. Oliveira et al. (2013) affirmed that log values higher than 2 presented low solubility in water, and the interaction between the compound and PCL or core is related to the chemical nature of the functional group. Because lycopene does not have functional groups forming hydrogen bonds that can interact with PCL, this compound tends to remain in the lipid core. This suggests that the LYC-LNC is more protected from external factors such as light, heat and oxygen than free lycopene.

3.2. Photosensitized oxidation of LYC- LNC in the model system

When comparing the different conditions used in this study, the rate of LYC-LNC loss increased with the increase in temperature (5°C, 15°C and 25°C) in the presence of MB and air (Figure 1b). It is also observed that oxygen is the most important factor in the degradation of LYC-LNC at 25°C. Indeed, at the same temperature and under identical illumination conditions, the LYC-LNC degradation was slow under N_2-saturated conditions and with oxygen but without MB, the greatest loss occurring with oxygen (Figure 1a). According to Chen et al. (2009), lycopene is an acyclic open-chain unsaturated carotenoid, and its open-chain unsaturated structure renders it more reactive towards oxygen, resulting in oxidative degradation. On the other hand, the degradation rates of LYC-LNC in both N_2-saturated solutions of MB and in aerated solutions in the absence of MB were similar.

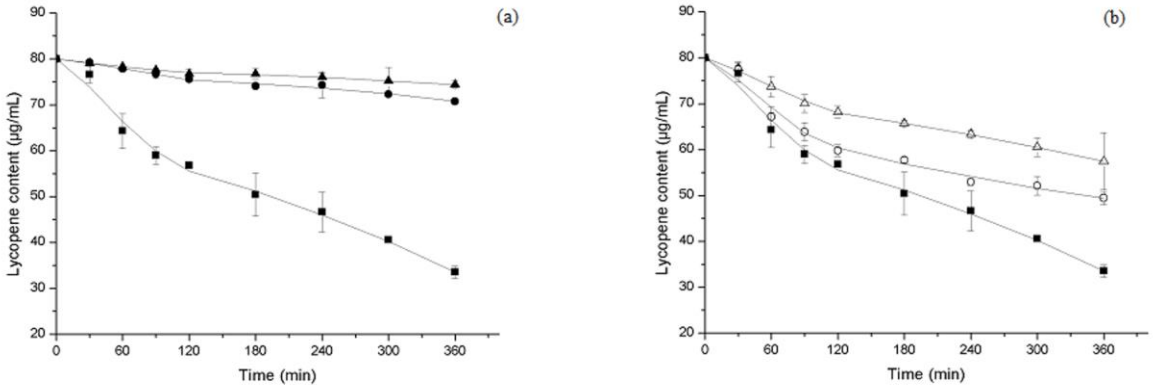

Figure 1: Lycopene concentration during photosensitization of LYC-LNC in same temperature and in different conditions (a) and in different temperatures in air-saturated (b), respectively, being 5°C (Δ), 15°C (○) and 25°C (■) in model system in saturated air with MB; 25°C (●) in saturated air without MB and 25°C (▲) with N_2 and MB.

In all conditions of LYC-LNC degradation, first-order reaction kinetics were observed, and a rate constant K_{obs} (Table 1) was obtained by exponential fitting of the experimental data (coefficient of correlation $R^2 > 0.98$).

Table 1: Rate constants (k) and activation energy for the degradation of encapsulated lycopene (LYC-LNC) during photosensitization.

Methylene blue concentration (MB) (µg/mL)	Temperature (°C)	Experimental conditions	k_{obs} x 10^{-4} (min^{-1})	Activation Energy (kcal/mol)
5	5	Air	0.88	
5	15	Air	1.30	67.0
5	25	Air	2.26	
0	25	Air	0.32	-
5	25	N_2	0.18	

The values of K_{obs} found in this study for LYC-LNC loss due to photosensitization might be related to the ability of the nanocapsule to protect the free lycopene. Lee et al. (2002) evaluated the stability of standard lycopene during illumination of a model system, where the lycopene was dissolved in hexane and evaporated to dryness with nitrogen in a vial. The vial was then placed in a closed incubator and illuminated at 25°C. The authors observed that K_{obs} was 2.9 x 10^{-4} min^{-1} while for LYC-LNC at 25°C, K_{obs} was 2.26 x 10^{-4} min^{-1} (Table 1). This result can be explained by the ability of the lipid-core nanocapsule to retain more of the core compound (Jager et al., 2009). Similar results were reported by Lobato et al. (2014) for bixin nanocapsules prepared by interfacial deposition of the preformed polymer, which is

the same technique utilized in this study. They observed a lower rate constant for BIX-LNC than for free bixin because of the permeability of singlet oxygen in the nanocapsule structure in model system of ethanol: water (2:8).

The effect of temperature on the K value was evaluated by activation energy of 67 kcal/mol in systems with saturated air. Lobato et al. (2014) found the activation energy for bixin nanocapsule photosensitization under the same conditions at 5°C, 15°C and 25°C to be of 12 kcal/mol. This high value may indicate that the nanoencapsulation improves the stability of lycopene during photosensitization.

3.3. Heating in model system and refrigeration stability

The concentration change of LYC-LNC during heating is shown in Figure 2. It can be observed that no significant change in lycopene content occurred within the first 10 min ($p > 0.05$) at either temperature (60°C, 70°C and 80°C). However, the content began to decline thereafter, following first-order kinetics. The rate constant K_{obs} (Table 2) was obtained by exponential fitting of the experimental data (correlation coefficient $R^2 > 0.94$).

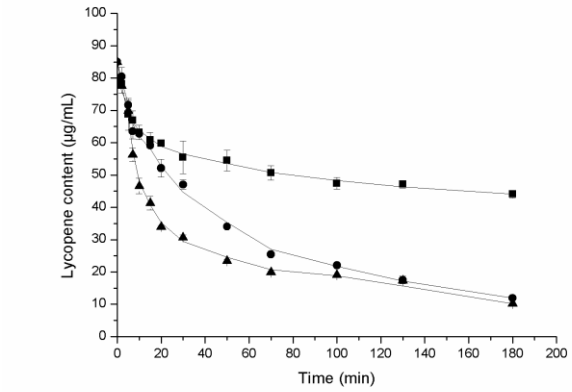

Figure 2: Lycopene concentration during heating LYC-LNC at 60°C (■), 70°C (●) and 80°C (▲).

Similar values were reported by Demiray, Tulek and Yilmaz (2013) upon evaluating the degradation kinetics of lycopene, β-carotene and ascorbic acid in tomato quarters of the *Rio Grande* variety during hot air drying. They observed that the K_{obs} at 60°C, 70°C and 80°C were 1.3 x 10^{-3} min^{-1}, 2.3 x 10^{-3} min^{-1} and 5.4 x 10^{-3} min^{-1}, respectively, and the activation energy was 10.9 Kcal/mol. Chen et al. (2009) verified that in the water-based food model system under thermal treatment, the rate of degradation kinetics of lycopene in tomato pulp were 8.1 x 10^{-4} min^{-1}, 1.79 x 10^{-3} min^{-1}, 5.13 x 10^{-3} min^{-1}, 6.8 x 10^{-3} min^{-1} at 80°C, 100°C,

120° and 140°C, respectively. When compared with the values in Table 2, it is observed that the wall of the nanocapsule reduced the thermal degradation of lycopene. According to Shi et al. (2008) who studied the effect of a heating treatment on the stability of lycopene in tomato purée, if the tomato cell walls were not completely disrupted during the tomato purée preparation, temperatures lower than 80°C could release the free lycopene but would not be could enough to disrupt the cell walls.

Table 2: Rate constants (k) and activation energy for lycopene loss during heating of encapsulated lycopene (LYC – LNC).

Temperature (°C)	**Experimental conditions**	k_{obs} **x** 10^{-4} **(**min^{-1}**)**	**Activation Energy (kcal/mol)**
60	Air	2.95	
70	Air	7.82	24.9
80	Air	10.48	

Lee et al. (2002) evaluated the stability of standard lycopene during heating of a model system where lycopene was dissolved in hexane and the mixture was evaporated to dryness with nitrogen. A thin film was formed on the bottom and the degradation rate constant was found to be 7.5 x 10^{-3} min^{-1}, 1.24 x 10^{-2} min^{-1} and 1.65 10^{-1} min^{-1} at 50°C, 100°C and 150°C, respectively. The activation energy was of 14.5 kcal/mol and that obtained in this study was 24.9 kcal/mol for LYC-LNC. These results confirmed the protecting effect that nanoencapsulation can provide to free lycopene, and it can be suggested that the biodegradable and biocompatible polymer (poly- ε -caprolactone) used in the formation of the nanocapsule confers thermal resistance and stability to lycopene.

Other studies involving encapsulation showed that this technique could improve the thermal stability of other bioactive compounds. González-Reza, Quintanar-Guerrero, Flores-Minutti, Gutiérrez-Cortez and Zambrano-Zaragoza (2015) analyzed the influence of a heat treatment on the degradation of β-carotene when nanoencapsulated and supported in model fluid in a scraped surface heat exchanger (SSHE). They observed a 6.93% loss of β-carotene, hinting at a more stable compound. Barbosa, Borsarelli and Mercadante (2005) encapsulated bixin by spray-drying using maltodextrin or arabic gum and evaluated its stability in aqueous solution both under illumination and in the dark at 21°C. They observed that encapsulated bixin presented a stability 10 times greater than the non-encapsulated system in the absence of light.

During refrigeration at 5°C, LYC-LNC presented a decrease in lycopene content, and approximately 40% of the lycopene concentration in the nanocapsule was stable ($p > 0.05$) after 42 days of storage (Table 3). Dias, Camões and Oliveira (2014) evaluated the stability of carotenoids stored at very low temperatures and observed that free lycopene degraded in 42 days when stored at –20°C and –70°C. Tan and Nakajima (2005) prepared nanodispersed β-carotene and observed that after 72 days of storage at 4°C, 25 to 44% of β-carotene was detected in the nanodispersed samples.

Table 3: Stability of LYC-LNC over 12 weeks under refrigeration at 5°C.

	14 days	**28 days**	**42 days**	**84 days**
***z*-average (nm)**	176.4 ± 4.94 [a]	178.6 ± 4.87 [a]	178.2 ± 5.29 [a]	179.3 ± 6.42 [a]
Polydispersity index	0.095 ± 0.02 [a]	0.082 ± 0.02 [a]	0.083 ± 0.01 [a]	0.077 ± 0.02 [a]
Zeta Potential (mV)	-9.43 ± 2.54 [a]	-8.89 ± 1.05 [a]	-8.64 ± 0.67 [a]	-9.44 ± 1.30 [a]
Lycopene content (µg/mL)	72.26 ±3.60 [a]	50.53±1.29 [b]	40.15±0.02[c]	38.24±1.14[c]

Mean ± standard deviation values in the same line followed by the same superscripts are not significantly different ($n = 9$; $p > 0.05$)

The *z*-average and zeta potential of LYC-LNC remained stable during 84 days of storage at 5°C ($p > 0.05$) (Table 3), contributing to the stability of lycopene. This study showed better results than Tan and Nakajima (2005) who observed that the mean particle diameter of nanodispersed β-carotene had a significant influence on the compound stability: the degradation of β-carotene increased with decreasing diameter. The zeta potential, having negative values, remained stable after 84 days of storage. This parameter is widely utilized to predict the stability of a suspension, where the higher the zeta potential, the more stable the suspension (Wu, Zhang, & Watanabe, 2011). Contri et al. (2013) reported no significant difference in the zeta potential of nanocapsules with different oil cores after 90 days of storage.

The stability of the *z*-average and zeta potential can also be explained by steric stabilization, which consists in using non-ionic amphiphilic stabilizers such as polysorbate 80. They are absorbed onto the particles through an anchor segment that strongly interacts with the dispersed particles, while the other well-solvated tail segment extends into the solution (Wu et al., 2011).

Considering the industrial use of antioxidants as a strategy to improve the stability of carotenes such as lycopene, this study showed that encapsulation increases the stability of lycopene under processing conditions such as heating, light and oxygen, mitigating the use of oxidants. Thus, nanoencapsulation can allow an increased use of lycopene in processed foods that need hard conditions of processing.

4. Conclusions

The present study showed that nanoencapsulation provides an additional protecting effect that impedes the quick degradation of lycopene under light, oxygen and temperature. It is possible to produce LYC-LNC that remains stable under photosensitization and heating because nanoencapsulated lycopene exhibited higher activation energies than free lycopene under all conditions. This study also indicated that nanoencapsulation, in addition to protecting the lycopene, can prolong its ability to quench excited singlet oxygen, due to a slow release. Additionally, this study showed that refrigeration increases the stability of the nanocapsule, indicating that the use of nanotechnology can improve the retention of lycopene in different industrial processes.

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References

Barbosa, M. I. M. J., Borsarelli, C. D., & Mercadante, A. Z. (2005). Light stability of spray-dried bixin encapsulated with different edible polysaccharide preparations. *Food Research International*, *38*(8–9), 989-994.

Bramley, P. M. (2000). Is lycopene beneficial to human health? *Phytochemistry*, *54*(3), 233-236.

Chen, J., Shi, J., Xue, S. J., & Ma, Y. (2009). Comparison of lycopene stability in water- and oil-based food model systems under thermal- and light-irradiation treatments. *Lwt-Food Science and Technology, 42*(3), 740-747.

Chiang, P.-C., & Hu, Y. (2009). Simultaneous Determination of LogD, LogP, and pK(a) of Drugs by Using a Reverse Phase HPLC Coupled with a 96-Well Plate Auto Injector. *Combinatorial Chemistry & High Throughput Screening, 12*(3), 250-257.

Choe, E., & Min, D. B. (2006). Chemistry and reactions of reactive oxygen species in foods. *Critical Reviews in Food Science and Nutrition, 46*(1), 1-22.

Contri, R. V., Ribeiro, K. L. F., Fiel, L. A., Pohlmann, A. R., & Guterres, S. S. (2013). Vegetable oils as core of cationic polymeric nanocapsules: influence on the physicochemical properties. *Journal of Experimental Nanoscience, 8*(7-8), 913-924.

Damodaran, S., Parkin, K. L., & Fennema, O. R. (2010). Química de alimentos de Fennema.

Decker, E. A., Chen, B., Panya, A., & Elias, R. J. (2010). 10 - Understanding antioxidant mechanisms in preventing oxidation in foods. In E. A. Decker (Ed.), *Oxidation in Foods and Beverages and Antioxidant Applications* (pp. 225-248): Woodhead Publishing.

Demiray, E., Tulek, Y., & Yilmaz, Y. (2013). Degradation kinetics of lycopene, β-carotene and ascorbic acid in tomatoes during hot air drying. *LWT - Food Science and Technology, 50*(1), 172-176.

Dias, M. G., Camões, M. F. G. F. C., & Oliveira, L. (2014). Carotenoid stability in fruits, vegetables and working standards – Effect of storage temperature and time. *Food Chemistry, 156*(0), 37-41.

Dos Santos, P. P., Paese, K., Guterres, S. S., Pohlmann, A. R., Costa, T. H., Jablonski, A., . . . Rios, A. d. O. (2015). Development of lycopene-loaded lipid-core nanocapsules: physicochemical characterization and stability study. *Journal of Nanoparticle Research, 17*(2), 1-11.

González-Reza, R. M., Quintanar-Guerrero, D., Flores-Minutti, J. J., Gutiérrez-Cortez, E., & Zambrano-Zaragoza, M. L. (2015). Nanocapsules of β-carotene: Thermal degradation kinetics in a scraped surface heat exchanger (SSHE). *LWT - Food Science and Technology, 60*(1), 124-130.

Hackett, M. M., Lee, J. H., Francis, D., & Schwartz, S. J. (2004). Thermal stability and isomerization of lycopene in tomato oleoresins from different varieties. *Journal of Food Science, 69*(7), C536-C541.

Henry, L. K., Catignani, G. L., & Schwartz, S. J. (1998). Oxidative degradation kinetics of lycopene, lutein, and 9-cis and all-trans β-carotene. *Journal of the American Oil Chemists' Society, 75*(7), 823-829.

Jager, E., Venturini, C. G., Poletto, F. S., Colome, L. M., Pohlmann, J. P. U., Bernardi, A., . . . Pohlmann, A. R. (2009). Sustained Release from Lipid-Core Nanocapsules by Varying the Core Viscosity and the Particle Surface Area. *Journal of Biomedical Nanotechnology, 5*(1), 130-140.

Kitade, Y., Watanabe, S., Masaki, T., Nishioka, M., & Nishino, H. (2002). Inhibition of liver fibrosis in LEC rats by a carotenoid, lycopene, or a herbal medicine, Sho-saiko-to. *Hepatology Research, 22*(3), 196-205.

Lee, M. T., & Chen, B. H. (2002). Stability of lycopene during heating and illumination in a model system. *Food Chemistry, 78*(4), 425-432.

Letchford, K., & Burt, H. (2007). A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *European Journal of Pharmaceutics and Biopharmaceutics, 65*(3), 259-269.

Levy, J., Bosin, E., Feldman, B., Giat, Y., Miinster, A., Danilenko, M., & Sharoni, Y. (1995). Lycopene is a more potent inhibitor of human cancer cell-proliferation than either alpha-carotene or beta-carotene. *Nutrition and Cancer-an International Journal, 24*(3), 257-266.

Lobato, K. B. d. S., Paese, K., Forgearini, J. C., Guterres, S. S., Jablonski, A., & Rios, A. d. O. (2013). Characterisation and stability evaluation of bixin nanocapsules. *Food Chemistry, 141*(4), 3906-3912.

Lobato, K. B. d. S., Paese, K., Forgearini, J. C., Guterres, S. S., Jablonski, A., & Rios, A. d. O. (2015). Evaluation of stability of bixin in nanocapsules in model systems of photosensitization and heating. *LWT - Food Science and Technology, 60*,8-14

Nunes, I. L., & Mercadante, A. Z. (2004). Obtenção de cristais de licopeno a partir de descarte de tomate. *Food Science and Technology (Campinas), 24*, 440-447.

Oliveira, C. P., Venturini, C. G., Donida, B., Poletto, F. S., Guterres, I. S., & Pohlmann, A. R. (2013). An algorithm to determine the mechanism of drug distribution in lipid-core nanocapsule formulations. *Soft Matter, 9*(4), 1141-1150.

Paese, K., Jäger, A., Poletto, F. S., Pinto, E. F., Rossi-Bergmann, B., Pohlmann, A. R., & Guterres, S. S. (2009). Semisolid formulation containing a nanoencapsulated

sunscreen: Effectiveness, in vitro photostability and immune response. *Journal of Biomedical Nanotechnology, 5*(3), 240-246.

Pesek, C. A., & Warthesen, J. J. (1987). Photodegradation of Carotenoids in a Vegetable Juice System. *Journal of Food Science, 52*(3), 744-746.

Ribeiro, H. S., Chu, B.-S., Ichikawa, S., & Nakajima, M. (2008). Preparation of nanodispersions containing beta-carotene by solvent displacement method. *Food Hydrocolloids, 22*(1), 12-17.

Ried, K., & Fakler, P. (2011). Protective effect of lycopene on serum cholesterol and blood pressure: Meta-analyses of intervention trials. *Maturitas, 68*(4), 299-310.

Rocha, G. A., Favaro-Trindade, C. S., & Ferreira Grosso, C. R. (2012). Microencapsulation of lycopene by spray drying: Characterization, stability and application of microcapsules. *Food and Bioproducts Processing, 90*(C1), 37-42.

Shi, J., Dai, Y., Kakuda, Y., Mittal, G., & Xue, S. J. (2008). Effect of heating and exposure to light on the stability of lycopene in tomato puree. *Food Control, 19*(5), 514-520.

Silva, D. F., Favaro-Trindade, C. S., Rocha, G. A., & Thomazini, M. (2012). Microencapsulation of lycopene by gelatin-pectin complex coacervation. *Journal of Food Processing and Preservation, 36*(2), 185-190.

Skibsted, L. H. (2010). 1 - Understanding oxidation processes in foods. In E. A. Decker (Ed.), *Oxidation in Foods and Beverages and Antioxidant Applications* (pp. 3-35): Woodhead Publishing.

Southon, S. (2000). Increased fruit and vegetable consumption within the EU: potential health benefits. *Food Research International, 33*(3-4), 211-217.

Tan, C. P., & Nakajima, M. (2005). beta-Carotene nanodispersions: preparation, characterization and stability evaluation. *Food Chemistry, 92*(4), 661-671.

Valente Soares, L. M. (2001). Como obter resultados confiáveis em cromatografia / How to obtain reliable results in chromatography (Vol. 60, pp. 79-84): Rev. Inst. Adolfo Lutz.

Venturini, C. G., Jäger, E., Oliveira, C. P., Bernardi, A., Battastini, A. M. O., Guterres, S. S., & Pohlmann, A. R. (2011). Formulation of lipid core nanocapsules. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 375*(1–3), 200-208.

Weisburger, J. H. (2002). Lycopene and tomato products in health promotion. *Experimental Biology and Medicine, 227*(10), 924-927.

Wu, L., Zhang, J., & Watanabe, W. (2011). Physical and chemical stability of drug nanoparticles. *Advanced Drug Delivery Reviews, 63*(6), 456-469.

Yuan, Y., Gao, Y., Zhao, J., & Mao, L. (2008). Characterization and stability evaluation of beta-carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. *Food Research International*, *41*(1), 61-68.

Zhuang, C.-Y., Li, N., Wang, M., Zhang, X.-N., Pan, W.-S., Peng, J.-J., Tang, X. (2010). Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. *International Journal of Pharmaceutics*, *394*(1-2), 179-185.

CAPÍTULO 5 – DISCUSSÃO GERAL E CONCLUSÕES

Este capítulo apresenta a discussão geral e a correlação de todos os resultados obtidos ao longo deste trabalho e traz conclusões gerais a cerca da importância da realização de estudos dessa natureza.

5. DISCUSSÃO GERAL

Compostos bioativos são substâncias que têm despertado interesse devido aos seus efeitos terapêuticos, principalmente no que se refere ao combate e prevenção de doenças. Estes compostos podem ser incorporados em formulações que permitam oferecer benefícios à saúde seja na forma de alimentos funcionais ou como produtos medicinais. No entanto, alguns compostos bioativos, como o licopeno, têm características físico-químicas que limitam sua aplicação como, por exemplo, insolubilidade em água devido a sua forma cristalina, além de instabilidade a fatores externos como luz, calor e oxigênio, o que dificulta a aplicação em alimentos que passam por processos industriais térmicos e mecânicos. Com base nisso, o presente trabalho desenvolveu uma possibilidade de disponibilizar o licopeno, com aumento de sua estabilidade em condições de processamento e armazenamento através da utilização do nanoencapsulamento.

A partir da pesquisa experimental combinada com a pesquisa teórica, foram produzidos dois artigos de revisão e dois práticos, respectivamente: “Nanoencapsulation of carotenoids: a focus on different delivery systems and evaluation parameters”, “Biodegradable polymers as wall materials to the synthesis of bioactive compound nanocapsules”, “Development of Lycopene-loaded lipid-core nanocapsules: physicochemical characterization and stability study” e “Stability study of Lycopene-loaded lipid-core nanocapsules under temperature and photosensitization”.

Para a escolha do melhor sistema de encapsulamento, foi feito levantamento das principais estruturas nanométricas já empregadas na proteção de carotenoides, entre as quais se encontram nanoemulsões, lipossomas, nanopartículas sólidas lipídicas, transportador lipídico nanoestruturado e nanopartículas poliméricas. Cada nanoestrutura têm vantagens e desvantagens que limitam a sua aplicação, mas, apesar disso, todos esses sistemas são capazes de melhorar a estabilidade de carotenoides.

Atualmente, nanocápsulas poliméricas têm sido as partículas mais utilizadas para encapsular compostos bioativos devido à sua estabilidade durante armazenamento e alta eficiência de encapsulação quando comparada aos demais sistemas. Além disso, nanocápsulas poliméricas são capazes de controlar a liberação do carotenoide encapsulado e devido a estas considerações, que foi escolhida como estrutura para o encapsulamento de licopeno e possível aplicação em alimentos.

Além do tipo de nanopartícula, o método de síntese e o material de parede utilizado na obtenção das nanocápsulas poliméricas precisaram ser estudados e avaliados para a escolha da melhor combinação. Nanocápsulas poliméricas podem ser sintetizadas por dois métodos: síntese por polimerização de monômeros ou por polímero pré-formado. No entanto, devido à presença de compostos tóxicos em nanocápsulas sintetizadas pela polimerização de monômeros, as técnicas usando o método de polímeros pré-formados têm sido consideradas mais seguras além de proporcionar o controle de parâmetros considerados importantes na síntese de nanopartículas como tamanho de partícula e índice de polidispersão.

Dentre as técnicas de síntese de nanocápsulas por polímero pré-formado está a de deposição interfacial, técnica utilizada neste trabalho, que se baseia na deposição interfacial de um polímero através do deslocamento de um solvente semi-polar a partir de uma solução lipofílica. A técnica foi escolhida para o desenvolvimento das nanocápsulas de licopeno devido à possibilidade da remoção completa dos solventes orgânicos utilizados durante a síntese, permitindo o nanoencapsulamento de carotenoides para aplicações alimentares e farmacêuticas.

Com relação à escolha do material de parede, foi possível observar, a partir de levantamento bibliográfico, que nanocápsulas têm sido sintetizadas utilizando diferentes polímeros sintéticos e naturais, sendo a escolha do polímero diretamente relacionada com a aplicação e o tipo de composto a ser encapsulado. Poli (Ɛ-caprolactona) (PCL) foi o polímero escolhido neste trabalho como material de parede devido à sua segurança e citocompatibilidade e, por isso, tem sido utilizado na síntese de compostos bioativos como bixina e β-caroteno (LOBATO *et al*., 2013; DA SILVA *et al*., 2016). Além disso, PCL apresenta baixo custo, facilidade de formação das nanopartículas e porosidade propícia para a liberação controlada do composto encapsulado, características que também foram consideradas na escolha para síntese das nanocápsulas de licopeno.

Definido, a partir da pesquisa teórica, o tipo de nanopartícula, a técnica e o material de parede, diferentes concentrações de licopeno foram testadas para a obtenção de nanocápsulas de licopeno (LYC-LNC) e a melhor formulação com concentração de 85 µg/mL foi sintetizada e avaliada quanto a suas características: diâmetro médio, índice de polidispersão, potencial zeta, morfologia, viscosidade, análise de cor, pH e conteúdo total de licopeno, além da estabilidade das nanocápsulas ao longo do período de estocagem em temperatura ambiente (25°C).

A suspensão de LYC-LNC apresentou aparente solubilidade no meio aquoso, alta eficiência de encapsulação (95%), o que corresponde a uma concentração final de 80 µg/mL, distribuição monomodal e parâmetros de tamanho, índice de polidispersão, potencial zeta, pH e viscosidade satisfatórios. A análise por microscopia eletrônica de transmissão permitiu visualizar o formato esférico das nanopartículas e confirmar o tamanho nanométrico e a uniformidade destas na suspensão.

Após 28 dias de estocagem à temperatura ambiente (25°C), as nanocápsulas permaneceram estáveis com relação ao diâmetro, índice de polidispersão e potencial zeta, sendo a estabilidade desses parâmetros atribuída à presença do surfactante Polissorbato 80 que tem demonstrado ser responsável pela estabilidade deste tipo de nanocápsulas já que é depositado sobre as partículas por meio de um segmento de ancoragem que interage fortemente com as partículas dispersas, enquanto o outro segmento de cauda estende-se na solução. No entanto, os valores de pH da suspensão diminuíram significativamente ao longo dos 28 dias provavelmente devido a degradação parcial do polímero na formulação e a consequente libertação do monômero de poliéster durante a hidrólise do PCL. A cor das nanocápsulas também foi afetada durante o armazenamento e os parâmetros de cor mudaram significativamente após 14 dias de estocagem devido à redução do conteúdo de licopeno na suspensão, ocorrendo assim um aumento de luminosidade e redução nos parâmetros de cor a* e b*.

Além destes parâmetros, a melhoria da estabilidade do licopeno nanoencapsulado ao longo do período de armazenamento à temperatura ambiente também foi avaliada e foi possível observar que a técnica de nanoencapsulamento protegeu o licopeno da degradação por fatores externos, mantendo 50% do conteúdo total do carotenoide após 14 dias de armazenamento, o que indica a capacidade de proteção do PCL que foi utilizado como material de parede.

Devido a esses resultados promissores a cerca do nanoencapsulamento do licopeno, se constatou a necessidade de avaliar a estabilidade das LYC-LNC também em condições que mimetizam processamento e em diferentes condições de armazenamento, já que reações de degradação são influenciadas por muitos fatores como temperatura, iluminação e presença de oxigênio. O efeito da refrigeração (5°C) na estabilidade de LYC-LNC foi avaliado e após 84 dias de armazenamento foi observado que todas as características físico-químicas permaneceram estáveis e o teor de licopeno nas nanocápsulas apresentou redução de apenas

40% do conteúdo total após 42 dias, indicando que a refrigeração associada à nanotecnologia melhora a estabilidade de compostos suscetíveis à degradação.

Com relação à estabilidade do licopeno encapsulado ao aquecimento (60, 70 e 80 °C) foi verificado que nenhuma mudança significativa ocorreu no conteúdo de licopeno, nos 10 primeiros minutos de aquecimento. No entanto, após esse tempo, o licopeno apresentou uma cinética de degradação de primeira ordem com coeficiente de correlação maior que 0,99. Apesar da redução do conteúdo do carotenoide após ter sido submetido ao aquecimento, quando comparado aos estudos da literatura sobre degradação de licopeno, LYC-LNC apresentou maior estabilidade ao calor, com energia de ativação de 24,9 kcal/mol, superior ao reportado por Demiray *et al.* (2013) que avaliaram a influência da temperatura (60, 70 e 80 °C) nos tomates e encontrou energia de ativação de 10,9 kcal/mol. Esta alta energia de ativação indica que o licopeno está fortemente ligado ao núcleo devido à sua lipofilicidade e protegido por uma parede polimérica na nanocápsula e, por isso, é necessária uma quantidade maior de energia para degradar o licopeno.

Quanto à avaliação da estabilidade das LYC-LNC a fotosensibilização, constatou-se que a estrutura das nanocápsulas não impediu que o licopeno desativasse os radicais formados pelo sensitizador azul de metileno e o oxigênio singleto, seguindo uma cinética de degradação de primeira ordem com um coeficiente médio de correlação acima de 0,99. A energia de ativação das LYC-LNC na presença de oxigênio singleto foi de 67,0 kcal/mol, aproximadamente 3 vezes maior que a energia de ativação do licopeno livre reportado na literatura o que pode indicar que o nanoencapsulamento também melhora a estabilidade do licopeno durante fotosensibilização.

As nanocápsulas desenvolvidas neste trabalho mostraram que o encapsulamento conferiu solubilidade aparente ao licopeno e aumentou a sua estabilidade em diferentes condições de armazenamento e processamento, tais como aquecimento, luz e oxigênio e por isso, o nanoencapsulamento pode permitir um aumento da utilização do licopeno nos alimentos processados que precisam ser submetidos a condições drásticas de processamento.

6. CONCLUSÃO

O presente trabalho demonstrou que após uma pesquisa aprofundada da literatura científica disponível sobre nanoencapsulamento, foi possível produzir e caracterizar nanocápsulas poliméricas capazes de melhorar a estabilidade do licopeno, que é um carotenoide altamente reativo, demonstrando que a técnica de nanoencapsulamento proporciona tanto a solubilidade aparente do carotenoide em meio aquoso quanto efeito protetor que impede a rápida degradação do licopeno na presença de luz, oxigênio e temperatura.

A caracterização das nanopartículas demonstrou alta eficiência de encapsulamento, conformação esférica e o tamanho nanométrico. As nanocápsulas quando armazenadas a temperaturas de 5°C e 25°C, apresentaram estabilidade em relação aos parâmetros de diâmetro, índice de polidispersão e potencial zeta, além de melhor estabilidade do licopeno encapsulado quando comparado aos dados reportados na literatura para licopeno livre.

De modo geral, as nanocápsulas também melhoraram a estabilidade do licopeno durante aquecimento e fotosensibilização em diferentes condições e temperaturas sem afetar a capacidade do licopeno de desativar oxigênio singleto. A partir desses experimentos, foi possível a determinação da energia de ativação do processo de degradação do licopeno.

Com base nos resultados obtidos, o presente estudo evidenciou que a nanotecnologia é uma estratégia potencial e viável para proteger e melhorar a aplicação de carotenoides. As nanocápsulas de licopeno desenvolvidas são uma alternativa promissora para expandir o uso de licopeno em processos industriais, melhorando a retenção destes compostos em diferentes alimentos submetidos ao processamento.

REFERÊNCIAS

ANDREASSI, M. et al. Antioxidant activity of topically applied lycopene. **Journal European Academy Dermatology Venereology**, v. 18, n. 1, p. 52-5, 1// 2004. ISSN 0926-9959 (Print).

ANTWI, S. O. et al. Plasma carotenoids and tocopherols in relation to prostate-specific antigen (PSA) levels among men with biochemical recurrence of prostate cancer. **Cancer Epidemiology**, v. 39, n. 5, p. 752-762, 10// 2015. ISSN 1877-7821. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1877782115001368 >.

ARUNKUMAR, R. et al. Biodegradable Poly (Lactic-co-Glycolic Acid)-Polyethylene Glycol Nanocapsules: An Efficient Carrier for Improved Solubility, Bioavailability, and Anticancer Property of Lutein. **Journal Pharmaceutical Science**, v. 104, n. 6, p. 2085-93, 6// 2015. ISSN 0022-3549. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25824524 >.

AZEVEDO-MELEIRO, C. H.; RODRIGUEZ-AMAYA, D. B. Qualitative and quantitative differences in carotenoid composition among Cucurbita moschata, Cucurbita maxima, and Cucurbita pepo. **Journal Agricultural and Food Chemistry**, v. 55, n. 10, p. 4027-33, 5// 2007. ISSN 0021-8561 (Print).

BARBOSA, M. I. M. J.; BORSARELLI, C. D.; MERCADANTE, A. Z. Light stability of spray-dried bixin encapsulated with different edible polysaccharide preparations. **Food Research International**, v. 38, n. 8–9, p. 989-994, 10// 2005. ISSN 0963-9969. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0963996905001079 >.

BARRETO, G. P. M. et al. Influence of ethylene on carotenoid biosynthesis during papaya postharvesting ripening. **Journal of Food Composition and Analysis**, v. 24, n. 4–5, p. 620-624, 6// 2011. ISSN 0889-1575. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0889157511000767 >.

BERARDO, N. et al. Carotenoids concentration among maize genotypes measured by near infrared reflectance spectroscopy (NIRS). **Innovative Food Science & Emerging Technologies,** v. 5, n. 3, p. 393-398, 9// 2004. ISSN 1466-8564. Disponível em: < http://www.sciencedirect.com/science/article/pii/S146685640400030X >.

BIESALSKI, H.-K. et al. Bioactive compounds: Definition and assessment of activity. **Nutrition**, v. 25, n. 11–12, p. 1202-1205, 11// 2009. ISSN 0899-9007. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0899900709002251 >.

BOON, C. S. et al. Factors influencing the chemical stability of carotenoids in foods. **Critical Reviews in Food Science and Nutrition**, v. 50, n. 6, p. 515-532, 5// 2010. Disponível em: < https://www.scopus.com/inward/record.uri?eid=2-s2.0-77953194780&partnerID=40&md5=3f9de18dc26dfd790f4cd1540bee881c >.

BOUWMEESTER, H. et al. Review of health safety aspects of nanotechnologies in food production. **Regulatory Toxicology and Pharmacology**, v. 53, n. 1, p. 52-62, 2// 2009. ISSN

0273-2300. Disponível em: <http://www.sciencedirect.com/science /article/pii/S0273230008002468 >.

BRAMLEY, P. M. Is lycopene beneficial to human health? **Phytochemistry**, v. 54, n. 3, p. 233-236, 6// 2000. ISSN 0031-9422. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10870177 >.

CAZZONELLI, C. I. Carotenoids in nature: insights from plants and beyond. **Functional Plant Biology**, v. 38, n. 11, p. 833-847, 10// 2011. ISSN 1445-4416.

CHEN, J. et al. Comparison of lycopene stability in water- and oil-based food model systems under thermal- and light-irradiation treatments. **Lwt-Food Science and Technology**, v. 42, n. 3, p. 740-747, 4// 2009. ISSN 0023-6438. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0023643808002521 >.

CHISTÉ, R. C. et al. Carotenoids are effective inhibitors of in vitro hemolysis of human erythrocytes, as determined by a practical and optimized cellular antioxidant assay. **Journal of Food Science**, v. 79, n. 1750-3841 (Electronic), p. H1841-H1847, 9// 2014a. Disponível em: < < http://www.ncbi.nlm.nih.gov/pubmed/25141990> >.

_____. Carotenoids inhibit lipid peroxidation and hemoglobin oxidation, but not the depletion of glutathione induced by ROS in human erythrocytes. **Life Sciences**, v. 99, n. 1–2, p. 52-60, 3// 2014b. ISSN 0024-3205. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0024320514001611 >.

CHOE, E.; MIN, D. B. Chemistry and reactions of reactive oxygen species in foods. **Critical Reviews in Food Science and Nutrition**, v. 46, n. 1, p. 1-22, 2006. ISSN 1040-8398. Disponível em: < http://www.tandfonline.com/doi/abs/10.1080/10408 390500455474 >.

CLINTON, S. K. Lycopene: Chemistry, Biology, and Implications for Human Health and Disease. **Nutrition Reviews**, v. 56, n. 2, p. 35-51, 1998. Disponível em: < http://nutritionreviews.oxfordjournals.org/content/56/2/35.abstract >.

CONTRI, R. V. et al. Vegetable oils as core of cationic polymeric nanocapsules: influence on the physicochemical properties. **Journal of Experimental Nanoscience**, v. 8, n. 7-8, p. 913-924, 10// 2013. ISSN 1745-8080; 1745-8099. Disponível em: < http://www.tandfonline.com/doi/abs/10.1080/17458080.2011.620019 >.

COOPERSTONE, J.; SCHWARTZ, S. **Recent Insights Into Health Benefits of Carotenoids.** 2016.

CORONEL-AGUILERA, C. P.; SAN MARTÍN-GONZÁLEZ, M. F. Encapsulation of spray dried β-carotene emulsion by fluidized bed coating technology. **LWT - Food Science and Technology**, v. 62, n. 1, p. 187-193, 2015. Disponível em: < https://www.scopus.com/inward/record.uri?eid=2-s2.0-4923168436&partnerID=40&md5=d3700b20423f940d74f402e5c5e7bcc6 >.

DA SILVA, M. M. et al. The Production, Characterization, and the Stability of Carotenoids Loaded in Lipid-Core Nanocapsules. **Food and Bioprocess Technology**, p. 1-11, 2016. ISSN 1935-5149. Disponível em: < http://dx.doi.org/10.1007/s11947-016-1704-3 >.

DAMODARAN, S.; PARKIN, K. L.; FENNEMA, O. R. **Química de alimentos de Fennema**. 2010.

DE ROSSO, V. V.; MERCADANTE, A. Z. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. **Journal Agricultural and Food Chemistry**, v. 55, n. 13, p. 5062-72, 6// 2007. ISSN 0021-8561 (Print) 0021-8561.

DECKER, E. A. et al. 10 - **Understanding antioxidant mechanisms in preventing oxidation in foods.** In: DECKER, E. A. (Ed.). Oxidation in Foods and Beverages and Antioxidant Applications: Woodhead Publishing, 2010. p.225-248. ISBN 978-1-84569-648-1.

DEMIRAY, E.; TULEK, Y.; YILMAZ, Y. Degradation kinetics of lycopene, β-carotene and ascorbic acid in tomatoes during hot air drying. **LWT - Food Science and Technology**, v. 50, n. 1, p. 172-176, 1// 2013. ISSN 0023-6438. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0023643812002460 >.

DIAS, M. G.; CAMÕES, M. F. G. F. C.; OLIVEIRA, L. Carotenoid stability in fruits, vegetables and working standards – Effect of storage temperature and time. **Food Chemistry**, v. 156, n. 0, p. 37-41, 8// 2014. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0308814614000715 >.

DIMITROVSKI, D. et al. The concentration of trans-lycopene in postharvest watermelon: An evaluation of analytical data obtained by direct methods. **Postharvest Biology and Technology**, v. 58, n. 1, p. 21-28, 10// 2010. ISSN 0925-5214. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0925521410001043 >.

DONG, X. et al. Stage extraction of capsaicinoids and red pigments from fresh red pepper (Capsicum) fruits with ethanol as solvent. **LWT - Food Science and Technology**, v. 59, n. 1, p. 396-402, 11// 2014. ISSN 0023-6438. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0023643814002564 >.

DOS SANTOS, P. P. et al. Development of lycopene-loaded lipid-core nanocapsules: physicochemical characterization and stability study. **Journal of Nanoparticle Research**, v. 17, n. 2, p. 1-11, 02// 2015. ISSN 1388-0764. Disponível em: < http://dx.doi.org/10.1007/s11051-015-2917-5 >.

_____. Stability study of lycopene-loaded lipid-core nanocapsules under temperature and photosensitization. **LWT - Food Science and Technology**, v. 71, p. 190-195, 9// 2016. ISSN 0023-6438. Disponível em: < http://www.sciencedirect.com/science/article /pii/S0023643816301724 >.

EDGE, R.; MCGARVEY, D. J.; TRUSCOTT, T. G. The carotenoids as anti-oxidants — a review. **Journal of Photochemistry and Photobiology** B: Biology, v. 41, n. 3, p. 189-200, 12// 1997. ISSN 1011-1344. Disponível em: < http://www.sciencedirect.com /science/article/pii/S1011134497000924 >.

EL-AGAMEY, A. et al. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. **Archives of Biochemistry and Biophysics**, v. 430, n. 1, p. 37-48, 2004. ISSN 0003-9861.

EZHILARASI, P. N. et al. Nanoencapsulation Techniques for Food Bioactive Components: A Review. **Food and Bioprocess Technology**, v. 6, n. 3, p. 628-647, 2012. ISSN 1935-5149. Disponível em: < http://dx.doi.org/10.1007/s11947-012-0944-0 >.

FONTES, P. C. R.; PEREIRA, P. R. G. Nutrição mineral do tomate para **mesa Informe Agropecuário Belo Horizonte,** MG. 24: p. 27-34. 2003.

FRASER, P. D.; BRAMLEY, P. M. The biosynthesis and nutritional uses of carotenoids. **Progress in Lipid Research**, v. 43, n. 3, p. 228-265, 5// 2004. ISSN 0163-7827. Disponível em: < http://www.sciencedirect.com/science/article/pii/S01637827 0300064X >.

FUKUMOTO, L. R.; MAZZA, G. Assessing Antioxidant and Prooxidant Activities of Phenolic Compounds. **Journal of Agricultural and Food Chemistry**, v. 48, n. 8, p. 3597-3604, 8// 2000. ISSN 0021-8561. Disponível em: < http://dx.doi.org/10.1021 /jf000220w >.

GONZÁLEZ-REZA, R. M. et al. Nanocapsules of β-carotene: Thermal degradation kinetics in a scraped surface heat exchanger (SSHE). **LWT - Food Science and Technology,** 2014. ISSN 0023-6438. Disponível em: < http://www.sciencedirect.com /science/article/pii/S0023643814005751 >.

GOULA, A. M.; ADAMOPOULOS, K. G. A New Technique for Spray-Dried Encapsulation of Lycopene. **Drying Technology**, v. 30, n. 6, p. 641-652, 2012. ISSN 0737-3937. Disponível em: < http://dx.doi.org/10.1080/07373937.2012.655871>.

GUTOWSKI, M.; KOWALCZYK, S. A study of free radical chemistry: their role and pathophysiological significance. **Acta Biochimica** Pol, v. 60, n. 1, p. 1-16, 2013. ISSN 0001-527x.

HACKETT, M. M. et al. Thermal stability and isomerization of lycopene in tomato oleoresins from different varieties. **Journal of Food Science**, v. 69, n. 7, p. C536-C541, 9// 2004. ISSN 0022-1147. Disponível em: < http://onlinelibrary.wiley.com/doi/10.1111 /j.1365-2621.2004.tb13647.x/abstract >.

HENRY, L. K.; CATIGNANI, G. L.; SCHWARTZ, S. J. Oxidative degradation kinetics of lycopene, lutein, and 9-cis and all-trans β-carotene. **Journal of the American Oil Chemists' Society**, v. 75, n. 7, p. 823-829, 7// 1998. ISSN 0003-021X. Disponível em: < http://dx.doi.org/10.1007/s11746-998-0232-3 >.

ILYASOGLU, H.; EL, S. N. Nanoencapsulation of EPA/DHA with sodium caseinate–gum arabic complex and its usage in the enrichment of fruit juice. **LWT - Food Science and Technology**, v. 56, n. 2, p. 461-468, 5// 2014. ISSN 0023-6438. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0023643813004738 >.

ISLAM, K. M. S.; SCHWEIGERT, F. J. Comparison of three spectrophotometric methods for analysis of egg yolk carotenoids. **Food Chemistry**, v. 172, p. 233-237, 4// 2015. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article /pii/S0308814614014125 >.

JANNA, O. A.; KHAIRUL, A. K.; MAZIAH, M. Anthocyanin stability studies in Tibouchina semidecandra L. **Food Chemistry**, v. 101, n. 4, p. 1640-1646, 2007. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article/pii/S03 08814606003402 >.

JEONG, N. H. et al. Preoperative levels of plasma micronutrients are related to endometrial cancer risk. **Acta Obstetricia et Gynecologica Scandinavica**, v. 88, n. 4, p. 434-9, 2009. ISSN 0001-6349.

JHA, R. et al. Probing suitable therapeutic nanoparticles for controlled drug delivery and diagnostic reproductive health biomarker development. **Materials Science and Engineering**: C, v. 61, p. 235-245, 4// 2016. ISSN 0928-4931. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0928493115306536 >.

KALOGEROPOULOS, N. et al. Bioactive phytochemicals in industrial tomatoes and their processing byproducts. **LWT - Food Science and Technology**, v. 49, n. 2, p. 213-216, 12// 2012. ISSN 0023-6438. Disponível em: < http://www.sciencedirect.com /science/article/pii/S0023643812000473 >.

KIM, Y.-S. et al. β-Carotene inhibits neuroblastoma cell invasion and metastasis in vitro and in vivo by decreasing level of hypoxia-inducible factor-1α. **The Journal of Nutritional Biochemistry**, v. 25, n. 6, p. 655-664, 6// 2014. ISSN 0955-2863. Disponível em: < http://www.sciencedirect.com/science/article/pii/S09552863140004 5X >.

KNOBLICH, M.; ANDERSON, B.; LATSHAW, D. Analyses of tomato peel and seed byproducts and their use as a source of carotenoids. **Journal of the Science of Food and Agriculture**, v. 85, n. 7, p. 1166-1170, 2005. ISSN 7.

LAGO-VANZELA, E. S. et al. Edible coatings from native and modified starches retain carotenoids in pumpkin during drying. **LWT - Food Science and Technology**, v. 50, n. 2, p. 420-425, 3// 2013. ISSN 0023-6438. Disponível em: < http://www.science direct.com/science/article/pii/S0023643812003702 >.

LAJOLO, F. M.; HORST, M. A. Biodisponibilidade de compostos bioativos em alimentos. In), S. M. F. C. O. (Ed.). **Biodisponibilidade de nutrientes**. 3°. São Paulo: Manole, v.1, p.772-807. 2009.

LEE, M. T.; CHEN, B. H. Stability of lycopene during heating and illumination in a model system. **Food Chemistry**, v. 78, n. 4, p. 425-432, 2002. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0308814602001462 >.

LETCHFORD, K.; BURT, H. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 65, n. 3, p. 259-269, 3// 2007. ISSN 0939-6411. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17196803 >.

LOBATO, K. B. D. S. et al. Characterisation and stability evaluation of bixin nanocapsules. **Food Chemistry**, v. 141, n. 4, p. 3906-3912, 12// 2013. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article/pii/S030881461 3008182 >.

_____. Evaluation of stability of bixin in nanocapsules in model systems of photosensitization and heating. **LWT - Food Science and Technology**, v. 60, n. 1, p. 8-14, 2015. ISSN 0023-6438. Disponível em: < http://www.sciencedirect.com/science/ article/pii/S0023643814006008 >.

LOBO, G. P. et al. Mammalian Carotenoid-oxygenases: Key players for carotenoid function and homeostasis. **Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids**, v. 1821, n. 1, p. 78-87, 1// 2012. ISSN 1388-1981. Disponível em: < http://www.sciencedirect.com/science/article/pii/S138819811100062X >.

MAURER, M. M. et al. An improved UHPLC-UV method for separation and quantification of carotenoids in vegetable crops. **Food Chemistry**, v. 165, p. 475-482, 12// 2014. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science /article/pii/S0308814614007420 >.

MAYNARD, A. D. et al. Safe handling of nanotechnology. **Nature**, v. 444, n. 7117, p. 267-269, 11// 2006. ISSN 0028-0836. Disponível em: < http://dx.doi.org/10.10 38/444267a >.

MERCADANTE, A. Z.; RODRIGUEZ-AMAYA, D. B. Effects of Ripening, Cultivar Differences, and Processing on the Carotenoid Composition of Mango. **Journal Agricultural and Food Chemistry**, v. 46, n. 1, p. 128-130, 1// 1998. ISSN 0021-8561. Disponível em: < http://pubs.acs.org/doi/abs/10.1021/jf9702860 >.

MORARU, C. I. et al. Nanotechnology: A new frontier in food science. **Food Technology**, v. 57, n. 12, p. 24-29, 12// 2003. ISSN 0015-6639.

MÜLLER, L.; FRÖHLICH, K.; BÖHM, V. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. **Food Chemistry**, v. 129, n. 1, p. 139-148, 11// 2011. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0308814611006108 >.

NATRAJAN, D. et al. Formulation of essential oil-loaded chitosan–alginate nanocapsules. **Journal of Food and Drug Analysis**, v. 23, n. 3, p. 560-568, 9// 2015. ISSN 1021-9498. Disponível em: < http://www.sciencedirect.com/science/article/pii/ S1021949815000228 >.

NIIZU, P. Y.; RODRIGUEZ-AMAYA, D. B. A melancia como fonte de licopeno. **Revista Instituto Adolfo Lutz**, v. 62, n. 3, p. 195-199, 2003. ISSN 0073-9855.

_____. New data on the carotenoid composition of raw salad vegetables. **Journal of Food Composition and Analysis**, v. 18, n. 8, p. 739-749, 12// 2005. ISSN 0889-1575. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0889157504001450 >.

NIRANJANA, R. et al. Carotenoids modulate the hallmarks of cancer cells. **Journal of Functional Foods**, v. 18, Part B, p. 968-985, 10// 2015. ISSN 1756-4646. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1756464614003235 >.

NUNES, I. L.; MERCADANTE, A. Z. Obtenção de cristais de licopeno a partir de descarte de tomate. **Food Science and Technology** (Campinas), v. 24, n. 3, p. 440-447, 2004-09 2004. ISSN 1678-457X. Disponível em: < www.scielo.br/ pdf/cta/v24n3/21940.pdf >.

OLMEDILLA, B. et al. Lutein, but not α-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. **Nutrition**, v. 19, n. 1, p. 21-24, 1// 2003. ISSN 0899-9007. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0899900702008614 >.

PELIS, F. M.; RONA, M. S. S.; MATIOLI, G. O Licopeno e suas contribuições na prevenção de doenças. **Arquivos do Museu Dinâmico Interdisciplinar**, v. 11, n. 3, p. 5-11, 2013. ISSN 1980-959X.

PENG, M.-L. et al. Influence/impact of lutein complex (marigold flower and wolfberry) on visual function with early age-related macular degeneration subjects: A randomized clinical trial. **Journal of Functional Foods**, v. 24, p. 122-130, 6// 2016. ISSN 1756-4646. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1 756464616300603 >.

QUINTANILLA-CARVAJAL, M. X. et al. Nanoencapsulation: A New Trend in Food Engineering Processing. **Food Engineering Reviews**, v. 2, n. 1, p. 39-50, 3// 2010. ISSN 1866-7910. Disponível em: < http://link.springer.com/article/10.1007/s12393-009-9012-6 >.

RAMALHO, V. C.; JORGE, N. Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos. **Química Nova**, v. 29, p. 755-760, 2006. ISSN 0100-4042. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-40422006000400023&nrm=iso >.

RAO, A. V.; RAO, L. G. Carotenoids and human health. **Pharmacological Research**, v. 55, n. 3, p. 207-216, 3// 2007. ISSN 1043-6618. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1043661807000357 >.

RIBEIRO, H. S. et al. Preparation of nanodispersions containing beta-carotene by solvent displacement method. **Food Hydrocolloids**, v. 22, n. 1, p. 12-17, 2008. ISSN 0268-005X. Disponível em: < http://agris.fao.org/agris-search/search.do? recordID=US201300795221 >.

RIBEIRO, H. S.; CRUZ, R. C. D. Biliquid foams containing carotenoids. **Engineering in Life Sciences,** v. 5, n. 1, p. 84-88, 2// 2005. ISSN 1618-0240.

RIED, K.; FAKLER, P. Protective effect of lycopene on serum cholesterol and blood pressure: Meta-analyses of intervention trials. **Maturitas**, v. 68, n. 4, p. 299-310, 4// 2011. ISSN 0378-5122. Disponível em: < http://www.sciencedirect.com/science/ article/pii/S0378512210004469 >.

ROBERTS, R. L.; GREEN, J.; LEWIS, B. Lutein and zeaxanthin in eye and skin health. **Clinics in Dermatology**, v. 27, n. 2, p. 195-201, 3// 2009. ISSN 0738-081X. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0738081X08000126 >.

ROCHA, G. A.; FAVARO-TRINDADE, C. S.; FERREIRA GROSSO, C. R. Microencapsulation of lycopene by spray drying: Characterization, stability and application of microcapsules. **Food and Bioproducts Processing**, v. 90, n. C1, p. 37-42, 1// 2012. ISSN 0960-3085; 1744-3571. Disponível em: < http://www.sciencedirect .com/science /article/pii/S0960308511000034 >.

RODRIGUES, E. et al. Development of a novel micro-assay for evaluation of peroxyl radical scavenger capacity: Application to carotenoids and structure–activity relationship. **Food Chemistry**, v. 135, n. 3, p. 2103-2111, 12// 2012. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0308814612010473 >.

RODRIGUES, L. M. et al. Bixin extraction from defatted annatto seeds. **Comptes Rendus Chimie**, v. 17, n. 3, p. 268-283, 3// 2014. ISSN 1631-0748. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1631074813003482 >.

RODRIGUEZ-AMAYA, D. B. et al. Updated Brazilian database on food carotenoids: Factors affecting carotenoid composition. **Journal of Food Composition and Analysis**, v. 21, n. 6, p. 445-463, 9// 2008. ISSN 0889-1575. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0889157508000458 >.

RODRIGUEZ-HUEZO, M. E. et al. Microencapsulation by spray drying of multiple emulsions containing carotenoids. **Journal of Food Science**, v. 69, n. 7, p. E351-E359, 9// 2004. ISSN 0022-1147. Disponível em: < http://onlinelibrary.wiley.com/doi /10.1111/j.1365-2621.2004.tb13641.x/abstract >.

SAINI, R. K.; NILE, S. H.; PARK, S. W. Carotenoids from fruits and vegetables: Chemistry, analysis, occurrence, bioavailability and biological activities. **Food Research International**, v. 76, Part 3, p. 735-750, 10// 2015. ISSN 0963-9969. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0963996915301356 >.

SENTANIN, M. A.; RODRIGUEZ AMAYA, D. B. Teores de carotenóides em mamão e pêssego determinados por cromatografia líquida de alta eficiência. **Food Science and Technology** (Campinas), v. 27, p. 13-19, 2007. ISSN 0101-2061.

SHI, J. et al. Effect of heating and exposure to light on the stability of lycopene in tomato puree. **Food Control**, v. 19, n. 5, p. 514-520, 2008. ISSN 0956-7135. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0956713507001223 >.

SHI, J.; LE MAGUER, M. Lycopene in tomatoes: Chemical and physical properties affected by food processing. **Critical Reviews in Biotechnology**, v. 20, n. 4, p. 293-334, 2000. ISSN 0738-8551. Disponível em: < http://www.ncbi.nlm.nih. gov/pubmed/11192026 >.

SHU, B. et al. Study on microencapsulation of lycopene by spray-drying. **Journal of Food Engineering**, v. 76, n. 4, p. 664-669, 10// 2006. ISSN 0260-8774. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0260877405004036 >.

SILVA, D. F. et al. Microencapsulation of lycopene by gelatin-pectin complex coacervation. **Journal of Food Processing and Preservation**, v. 36, n. 2, p. 185-190, 4// 2012. ISSN 0145-

8892. Disponível em: < http://onlinelibrary.wiley.com/doi /10.1111/j.1745-4549.2011.00575.x/abstract >.

SILVA, S. R. D.; MERCADANTE, A. Z. Composição de carotenóides de maracujá-amarelo (Passiflora edulis flavicarpa) in natura. **Food Science and Technology** (Campinas), v. 22, p. 254-258, 2002. ISSN 0101-2061.

SKIBSTED, L. H. 1 - Understanding oxidation processes in foods. In: DECKER, E. A. (Ed.). **Oxidation in Foods and Beverages and Antioxidant Applications**: Woodhead Publishing, 2010. p.3-35. ISBN 978-1-84569-648-1.

SOMMERBURG, O. et al. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. **The British Journal of Ophthalmology**, v. 82, n. 8, p. 907-910, 1998. ISSN 0007-11611468-2079. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1722697/ >.

SOTOMAYOR-GERDING, D. et al. High carotenoid bioaccessibility through linseed oil nanoemulsions with enhanced physical and oxidative stability. **Food Chemistry**, v. 199, p. 463-470, 5//2016. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article/pii/S030881461530282X >.

SOUZA, V. G. L.; FERNANDO, A. L. Nanoparticles in food packaging: Biodegradability and potential migration to food—A review. **Food Packaging and Shelf Life**, v. 8, p. 63-70, 6// 2016. ISSN 2214-2894. Disponível em: < http://www.sciencedirect.com/science/article/pii/S221428941630028X >.

STAJČIĆ, S. et al. Tomato waste: Carotenoids content, antioxidant and cell growth activities. **Food Chemistry**, v. 172, n. 0, p. 225-232, 4// 2015. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0308814614014368 >.

SUN, C. et al. Encapsulation and controlled release of hydrophilic pesticide in shell cross-linked nanocapsules containing aqueous core. **International Journal of Pharmaceutics**, v. 463, n. 1, p. 108-114, 3// 2014. ISSN 0378-5173. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0378517313011265 >.

TAN, C. P.; NAKAJIMA, M. beta-Carotene nanodispersions: preparation, characterization and stability evaluation. **Food Chemistry**, v. 92, n. 4, p. 661-671, 10// 2005. ISSN 0308-8146. Disponível em: < http://agris.fao.org/agris-search /search.do?recordID=US201301000581 >.

TAYLOR, T. M. et al. Liposomal nanocapsules in food science and agriculture. **Critical Reviews in Food Science and Nutrition**, v. 45, n. 7-8, p. 587-605, 2005. ISSN 1040-8398; 1549-7852. Disponível em: < http://www.ncbi.nlm.nih.gov /pubmed/16371329 >.

VANCE, M. E. et al. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. **Beilstein Journal of Nanotechnology**, v. 6, n. 1, p. 1769-1780, 2015. Disponível em: < https://www.scopus.com/inward/record.uri?eid=2-s2.0-84945955942&partnerID=40&md5=28f9d12e772203856ba6afb9876d935a >.

VENTURINI, C. G. et al. Formulation of lipid core nanocapsules. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 375, n. 1–3, p. 200-208, 2// 2011. ISSN 0927-7757. Disponível em: < http://www.sciencedirect.com/science /article/pii/S0927775710007429 >.

WANG, C. C. et al. Isolation of carotenoids, flavonoids and polysaccharides from Lycium barbarum L. and evaluation of antioxidant activity. **Food Chemistry**, v. 120, n. 1, p. 184-192, 5// 2010. ISSN 0308-8146. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0308814609011832 >.

WEISBURGER, J. H. Lycopene and tomato products in health promotion. **Experimental Biology and Medicine**, v. 227, n. 10, p. 924-927, 11// 2002. ISSN 1535-3702. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12424336 >.

XU, Y.; PAN, S. Effects of various factors of ultrasonic treatment on the extraction yield of all-trans-lycopene from red grapefruit (Citrus paradise Macf.). **Ultrasonics Sonochemistry**, v. 20, n. 4, p. 1026-1032, 7// 2013. ISSN 1350-4177. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1350417713000333 >.

YAN, F. et al. Antioxidant capacity variation in the oregano (Origanum vulgare L.) collection of the German National Genebank. **Industrial Crops and Products**, v. 92, p. 19-25, 12// 2016. ISSN 0926-6690. Disponível em: < http://www.sciencedirect.com/ science/article/pii/S0926669016304873 >.

YUAN, Y. et al. Characterization and stability evaluation of beta-carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. **Food Research International**, v. 41, n. 1, p. 61-68, 2008. ISSN 0963-9969. Disponível em: < http://link.springer.com/article/10.1007/s10068-014-0014-7 >.

ZAKYNTHINOS, G.; VARZAKAS, T. Carotenoids: From Plants to Food Industry. **Current Research in Nutrition and Food Science**, v. 4, n. Special Issue Carotenoid, 2016.