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JUAN FELIPE OSORIO TOBON

EXTRAÇÃO E PRECIPITAÇÃO DE CURCUMINÓIDES DE CÚRCUMA (Curcuma longa L.) UTILIZANDO LÍQUIDOS PRESSURIZADOS E FLUIDOS SUPERCRÍTICOS

EXTRACTION AND PRECIPITATION OF CURCUMINOIDS FROM TURMERIC (*Curcuma longa* L.) USING PRESSURIZED LIQUIDS AND SUPERCRITICAL FLUIDS

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Tese de doutorado apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do titulo de Doutor em Engenharia de Alimentos

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A ata da Defesa, assinada pelos membros da Comissão examinadora, consta no processo de vida acadêmica do aluno

A Lina…

Um medo oceânico, escuro, imenso.
Um grito diabo vestido de branco,
jogo o jogo que você me deu
sintonizo com o nada
entregados ao instinto
começamos uma façanha.
Muda o áudio equalizando este momento,
areia na cidade já longe do deserto.
Entre as páginas do livro
encontro a flor dissecada,
o orgulho de um momento,
a relíquia da amada,
o momento em que dois mundos
a sorte tem sido jogada.

Um mar de amar profundo
e na superfície... todo isto...
Ulises Strumia....

Aos meus pais, Jorge Humberto e Martha Lucia,
tudo isto é graças a vocês.

Aos meus irmãos, Ricardo e Martha Inés,
pelo carinho e energia à distância.

A memoria dos meus avós.

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RESUMO

A cúrcuma (*Cúrcuma longa* L.) é uma planta cultivada em países tropicais e subtropicais que tem grande importância econômica devido à sua ampla utilização como corante natural. A cúrcuma é rica em curcuminóides, compostos que além de serem pigmentos naturais, também têm atividade para serem utilizados na prevenção de doenças devido às evidencias de suas propriedades farmacológicas. Apesar de sua utilização em grande escala, a indústria ainda utiliza processos convencionais e técnicas pouco eficientes para sua produção. A crescente exigência de qualidade e o aumento do conhecimento científico sobre o impacto dos alimentos na saúde estão promovendo o desenvolvimento de tecnologias para a produção mais eficiente e sustentável deste tipo de produtos. Entre estas tecnologias, o uso de fluidos pressurizados e fluidos supercríticos tem se mostrado como alternativas eficientes e economicamente viáveis para a extração e a formação de partículas a partir de matérias-primas vegetais, apresentando inúmeras vantagens em relação às técnicas tradicionais. Neste contexto, o objetivo deste trabalho foi desenvolver um processo de extração de curcuminóides a partir de cúrcuma desaromatizada. Etanol foi o solvente no processo de extração com liquido pressurizado (PLE = Pressurized Liquid Extraction). Na sequencia foi estudada a formação de partículas a partir dos extratos usando o processo antissolvente supercrítico (SAS = Supercritical Antisolvent). Em seguida, foi desenvolvido um método rápido de quantificação de curcuminóides via cromatografia líquida de alta eficiência (CLAE). No processo de extração de curcuminóides utilizando PLE foram avaliadas as influências da temperatura e da pressão sobre o rendimento de extração e de curcuminóides. Para a eliminação do etanol via SAS do extrato etanólico e a formação de micropartículas foram avaliados os parâmetros operacionais: tipo de injetor, temperatura, pressão e vazão de CO_2 sobre processo de precipitação. A viabilidade econômica de um processo integrado denominado SFE−PLE+SAS foi avaliada utilizando o simulador SuperPro Designer 8.5®. Inicialmente foi feita uma revisão sobre métodos de nanoencapsulação utilizando tecnologias emergentes, os quais poderiam ser utilizados na melhora da solubilidade das partículas de curcuminóides.

Palavras-chave: *Curcuminóides; Extração com líquidos pressurizados; Antissolvente supercrítico; Micropartículas; Custo de manufatura.*

ABSTRACT

Turmeric (Curcuma longa L.) is a plant widely cultivated in tropical and subtropical countries that has a large economic importance due to their use as natural dye. Turmeric is rich in curcuminoids, compounds that besides being natural pigments, also have activity to be used in the prevention of diseases due their pharmacological properties. In spite of their use in large scale, the industry still utilized traditional and inefficient processes for its production. The growing demand for quality and the increasing of the scientific knowledge about the impact of food on health are promoting the development of novel and more efficient technologies for the production of this type of natural products in a sustainable manner. Among these technologies, the use of pressurized liquids and supercritical fluids has demonstrated to be an efficient and economically viable alternative for extraction and particle formation from natural sources, presented several vantages when compared with the traditional processes. In this context, the objective of this work was the development of an extraction process of curcuminoids from deflavored turmeric. Ethanol was used as solvent in the pressurized liquid extraction (PLE) process. Afterwards the particle formation through the supercritical antisolvent process (SAS) was studied. Then, a faster method for curcuminoids quantification via high-performance liquid chromatography (HPLC) was developed. For the PLE process were evaluated the influence of the temperature and pressure on the global yield and the curcuminoid content of the ethanolic extracts. To eliminate the solvent from the extracts and produce particles through SAS process, the effects of the operational parameters: type of injector, temperature, pressure and CO2 flow rate on the precipitation process were evaluated. The economic viability of an integrated process named SFE− PLE+SAS was determined through the software SuperPro Designer 8.5®. Initially, a review about nanoencapsulation methods using emerging technologies which could be used to improve the solubility of the curcuminoids particles was done.

Keywords: *Curcuminoids; Pressurized liquid extraction; Supercritical antisolvent process, Microparticles; Cost of manufacture.*

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LISTA DE ABREVIATURAS E SIGLAS

ASE-Extração acelerada com solvente (Accelerated solvent extraction)

BDMC-Bisdemetoxicurcumina (Bisdemethoxycurcumin)

BPA-Bisfenol A (Bisphenol A)

C-Curcumina (Curcumin)

CER-Período de taxa de extração constante (Contant extraction rate)

CLAE-Cromatografia líquida de alta eficiência

COL-Custo laboral (Cost of labor)

COM-Custo de manufatura (Cost of manufacturing)

CP-Ponto critico (Critical point)

CRM-Custo da materia prima (cost of raw material)

DMC-Demetoxicurcumina (Demethoxycurcumin)

DTR-Rizomas desaromatizados (Deflavored turmeric rhizomes)

EOs-Óleos essenciais (Essential oils)

ESE-Extração melhorada com Solvente (Enhanced solvent extraction)

ESI-MS-Espectrometria de Massa (Electrospray mass spectrometry)

FCI-Custo de investimento (Cost of investment)

GAS-Processo de gás como antissolvente (Gas antisolvent process)

GC/MS - Cromatografia gasosa acoplada à espectrometria de massas (Gas chromatography–mass spectrometry)

GC–ITMS-MS - Cromatografia gasosa acoplada à espectrometria de massas de armadilha iônica (gas chromatography coupled to ion trap tandem mass spectrometry)

GC–MS-NCI - Cromatografia gasosa com detector de espectrometria de massas ionização química de íons negativos (Gas chromatography–mass spectrometry-negative chemical ionization)

GRAS-Geralmente reconhecido como seguro (Generally recognized as safe)

GT-Goma adragante (Gum tragacanth)

GYI-Isotermas de rendimento global (Global yield isotherms)

GY_{SOL}-Rendimento global de sólidos (Global yield of solids)

HD-Hidrodestilação (Hydrodistillation)

HPLC-Cromatografia líquida de alta eficiência (High-performance liquid chromatography)

HPLC-CAD - Cromatografia líquida de alta eficiência acoplada à um detector de aerossóis (High-performance liquid chromatography - charged aerosol detection)

HPLC-DAD - Cromatografia líquida de alta eficiência com detector de arranjo de diodos (High-performance liquid chromatography - diode-array detection)

HRE-Extração com refluxo de calor (Heat-reflux extraction)

HTWE-Extração com água a alta temperatura (high-temperature water extraction)

HWE-Extração com água quente (Hot water extraction)

IRR-Taxa Interna de Retorno (Internal rate of return)

LC-Cromatografia liquida (Liquid chromatography)

LC–ESI–MS-Cromatografia líquida acoplada à espectrometria de massas em tandem de alta resolução e ionização por electrospray (Liquid chromatography–electrospray ionization tandem mass spectrometry)

LC-MS - Cromatografia líquida acoplada à espectrometria de massas (Liquid chromatography–mass spectrometry)

LC–MS/MS - Cromatografia líquida acoplada à espectrometria de massas (Liquid chromatography–tandem mass spectrometry)

LOD-Limite de detecção (Limit of detection)

LOQ-Limite de quantificação (Limit of quantification)

LPSE-Extração com solvente a baixa pressão (Low pressure solvente extraction)

MAE-Extração assistida por microondas (Microwave assisted extraction)

$\mathbf{M_{CER}}$-Taxa de transferência de massa do período CER (Extraction rate for the CER period)

NPV-Valor neto presente (Net present value)

OEC-Curvas globais de extração (Overall extraction curves)

OPEO-Óleos essenciais da casca da laranja (Orange peel essential oils)

PBBs-Bifenilos polibromados (Polybrominated biphenyls)

PCBs-Bifenilos policlorados (Polychlorinated biphenyls)

PCE-Extrato rico em curcuminóides em pó (Powdered curcuminoid-rich extract)

PCP-Porcentagem de curcuminóides precipitados (Percentage of curcuminoids precipitated)

PFE-Extração com fluidos pressurizados (Pressurized fluid extraction)

PG-Goma persa (Persian gum)

PGSS - Precipitação de partículas a partir de soluções gasosas saturadas (Particles from gas saturated solutions)

PHWE-Extração com água quente pressurizada (Pressurized hot water extraction)

PLE-Extração com líquidos pressurizados (Pressurized liquid extraction)

PSE-Extração com solventes pressurizados (Pressurized solvent extraction)

$\mathbf{R_{CER}}$-Rendimento no período CER (Yield of the CER period)

RESS-Expansão rápida a partir de uma solução saturada (Rapid expansion from a saturated solution)

ROI-Taxa de retorno sobre investimento (Return of investment)

RSD-Desvio padrão relativo (Relative standard deviation)

SAS-Antissolvente supercrítico (Supercritical antisolvent)

SCF-Fluidos supercríticos (Supercritical fluids)

SFE-Extração com fluidos supercríticos (Supercritical fluid extraction)

SFEE - Extração supercrítica a partir de emulsões (Supercritical fluid extraction from an emulsion)

SSI-Impregnação de Solventes Supercríticos (Supercritical solvent impregnation)

SWE-Extração com água subcrítica (Subcritical water extraction)

Tc-Temperatura critica (Critical temperature)

$\mathbf{t_{CER}}$-Duração do período CER (Time span of the CER period)

TEO-Óleo essencial de cúrcuma (Turmeric essential oil)

$\mathbf{t_{FER}}$-Duração do período FER (End of the FER period)

UAE-Extração assistida por ultrassom (Ultra-sound assisted extraction)

ULP-Processador ultra-sônico líquido (Ultrasonic liquid processor)

WPI-Proteína isolada de soro do leite (Whey protein isolates)

$\mathbf{Y_{CER}}$ - Razão mássica de extrato na saída do leito durante o período CER (Mass ratio of extract in the supercritical phase at the bed outlet)

ZEN-Micotoxina zearalenona (Mycotoxin zearalenone)

ε-Constante dielétrica (Dielectric constant)

SÚMARIO

-*CAPÍTULO 1*-

Introdução e objetivos

1. INTRODUÇÃO

Neste trabalho foram investigados os processos de extração e formação de partículas de curcuminóides obtidos dos rizomas desaromatizados de cúrcuma (*Curcuma longa* L.) utilizando tecnologias emergentes: extração com líquidos pressurizados (PLE = Pressurized Liquid Extraction) e o processo antissolvente supercrítico (SAS = Supercritical Antisolvent). O estudo foi realizado no LASEFI (LAboratório de tecnologia Supercrítica: Extração, Fracionamento e Identificação de extratos vegetais).

Este trabalho faz parte de um processo integrado de aproveitamento da cúrcuma. Na primeira etapa deste trabalho foi feita uma revisão de literatura sobre o processo PLE. Posteriormente, na etapa de extração dos curcuminóides via PLE, foi utilizada a unidade de extração cossolvente a qual pode ser utilizada para extrações com fluidos supercríticos, fluidos supercríticos com cossolvente e líquidos pressurizados. Esta unidade foi usada inicialmente para desaromatização (retirada do óleo volátil) da matéria-prima utilizando CO_2 supercrítico (Carvalho et al., 2015) e posteriormente para extração de curcuminóides utilizando-se o etanol pressurizado. Em um trabalho anterior feito pelo nosso grupo de pesquisa Braga et al. (2007), obtiveram um extrato de curcuminóides a partir de rizomas de cúrcuma usando o processo de extração acelerada com solvente (ASE). Nesse trabalho, a baixa solubilidade dos curcuminóides em CO_2 resultou em baixos rendimentos de curcuminóides, apesar da utilização do etanol como cossolvente. Como alternativa, o processo PLE foi proveitoso devido à alta solubilidade dos curcuminóides em etanol, o que acarreta o aumento da transferência de massa para a obtenção de extrato etanólico rico em curcuminóides. Posteriormente, na segunda etapa, o solvente foi eliminado do extrato etanólico via SAS formando partículas e obtendo um extrato em pó rico em curcuminóides. Este trabalho foi realizado na unidade SAS/SFEE desenvolvida no LASEFI por Santos (2011).

A cúrcuma foi selecionada como matéria-prima pelas suas características de ingrediente natural e pelas propriedades funcionais dos seus extratos. A cúrcuma é uma planta amplamente cultivada, conhecida popularmente como açafrão da terra é utilizada comumente como conservante, condimento e corante (Nair, 2013). Os curcuminóides são um grupo formado por três compostos fenólicos, curcumina, demetoxicurcumina e bisdemetoxicurcumina, que são responsáveis pela cor amarela dos rizomas e dos extratos de

cúrcuma. Os curcuminóides apresentam efeito anti-inflamatório, anticancerígeno, antioxidante, antibacteriano e antiviral (Dao et al., 2012). Em função da funcionalidade e importância econômica desses compostos bioativos, este trabalho teve como objetivo a otimização do processo de extração de curcuminóides a partir da utilização de líquidos pressurizados, e desenvolvimento de métodos para a eliminação do solvente e formação de partículas a fim de melhorar a estabilidade dos compostos presentes e gerar extratos secos ricos em curcuminóides de alto valor econômico.

1.1. Cúrcuma (*Curcuma longa* L.)

Os rizomas da cúrcuma têm potencial de uso na indústria alimentícia e farmacêutica devido à sua funcionalidade como corante natural e atividade antioxidante. Esta planta é usada comumente como conservante e como corante no preparo de mostarda e queijo (Kotwal, 2011).

A cúrcuma contém duas classes principais de compostos: os curcuminóides responsáveis pela cor amarela da cúrcuma e os compostos aromatizantes (Bagchi e Preuss, 2004); além de possuir proteínas (6,3 %), lipídios (5,1 %), minerais (3,5 %), carboidratos (69,4 %) e umidade (13,1 %). O óleo volátil (5,8 %) é composto por uma mistura de terpenos e serquiterpenos cetônicos (53 %) (Ravindran et al., 2007) e contém mais de 40 compostos, dos quais os majoritários são ar-turmerona (21,0 – 30,3 %), α-turmerona (26,5 – 33,5 %), β-turmerona (18,9 – 21,1 %), zingibereno (25 %), felandreno (1 %), sabineno (0,6 %), cineol (1 %) e borneol (0,5 %) (Gounder e Lingamallu, 2012).

Os curcuminóides são a fração não volátil da cúrcuma, que compreende a curcumina (3 - 4 %), a curcumina I (94 %), a curcumina II (demetoxicurcumina, 5,7 %) e a curcumina III (bisdemetoxicurcumina, 0,3 %) (Chattopadhyay et al., 2004). As estruturas químicas dos compostos de interesse presentes na *Cúrcuma longa* L. são apresentadas nas Figuras 1.1 e 1.2.

Os curcuminóides não são solúveis em água, mas são solúveis em etanol, álcalis, cetonas, acido acético e clorofórmio. Sua degradação é acelerada pela luz, ar e temperatura (Suresh et al., 2007) e, em pH acima do neutro, eles sofrem degradação hidrolítica (Socaciu, 2007).

1.1.1. Produtos comerciais da cúrcuma

A cúrcuma é principalmente comercializada como rizomas frescos, os quais servem como matéria-prima para a elaboração de outros produtos. De acordo com Nair (2013), o comércio mundial de cúrcuma é de 37000 t/ano com um valor estimado de 40.160 milhões de dólares sendo a Índia o líder mundial em exportação de cúrcuma e seus derivados. Portanto, existe um mercado potencial a ser explorado para a formulação de produtos derivados da cúrcuma. Por exemplo, segundo Ravindran et al. (2007) no mercado mundial os principais derivados da cúrcuma são:

Rizomas secos: majoritariamente a cúrcuma é comercializada como rizomas inteiros, os quais são utilizados na produção de outros produtos de valor agregado, tais como açafrão da terra, óleo e oleoresina.

Cúrcuma em pó: a cúrcuma em pó é usada principalmente no mercado de varejo e pela indústria de alimentos. Os rizomas são triturados até um tamanho de partícula aproximado entre 60 e 80 *mesh*. A cúrcuma em pó é o ingrediente mais importante do curry e em outros produtos alimentícios é usado principalmente como corante e aromatizante em produtos tais como caldo de galinha, sopas, molhos, temperos e cereais.

Óleo e oleoresina: a cúrcuma contém óleo volátil, o que dá o sabor característico do tempero, no entanto, seu preço não é atraente para sua destilação comercial. A principal aplicação de óleo é em alguns produtos de confeitaria e em águas gaseificadas. A oleoresina obtida por extração com solvente, contém corantes, óleo volátil e lipídios. Os fabricantes oferecem diversas oleoresinas de cúrcuma com conteúdo de curcuminóides variando de 3,5 - 4,2 % ou mais.

1.1.2. Extração convencional dos curcuminóides

Os curcuminóides são extraídos a partir dos rizomas secos. O processo de extração convencional é descrito por Stankovic (2004) e Ravindran et al. (2007). No começo do processo, a matéria prima é moída e posteriormente a extração é realizada empregando solventes tais como acetona, dióxido de carbono, acetato de etila, diclorometano, n-butanol, metanol, etanol e hexano (Commission, 1995). Em seguida, o solvente é evaporado mediante destilação a vácuo, resultando em uma oleoresina com teor de curcuminóides entre 25 e 35 %. No entanto, para recuperar os curcuminóides o solvente é apenas parcialmente retirado, o

concentrado é resfriado e deixado em repouso para cristalizar os curcuminóides. A cristalização é reforçada por subesfriamento em água gelada. Os cristais são separados por centrifugação ou filtração a vácuo obtendo curcuminóides em bruto. Para melhorar a pureza, uma lavagem rápida com solvente é realizada. A lavagem com hexano pode remover os óleos fixos e essenciais residuários, mas não dissolve os curcuminóides. O solvente residual no produto pode ser removido através da injeção de vapor, e em seguida os cristais são secos com ar quente.

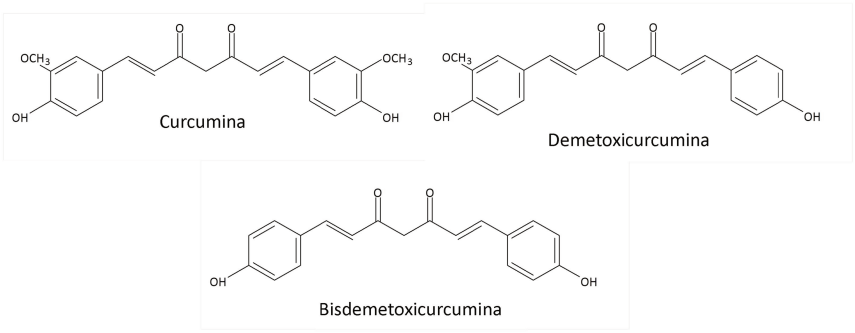

Figura 1.1. Compostos fenólicos da *Curcuma longa* L.: curcuminóides (Ravindran et al., 2007).

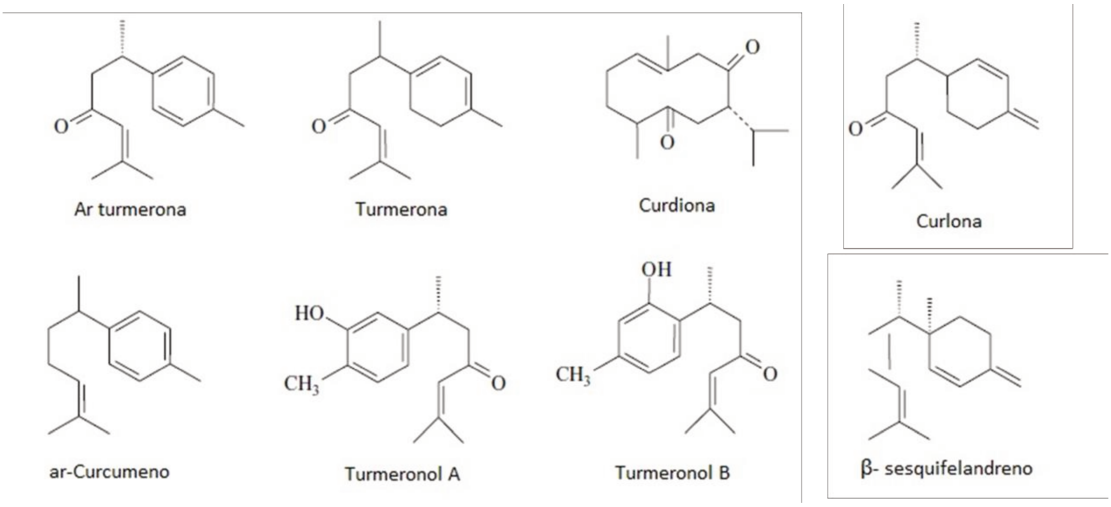

Figura 1.2. Principais compostos do óleo volátil da *Curcuma longa* L (Ravindran et al., 2007).

A funcionalidade dos curcuminóides está nos efeitos anti-inflamatório, anticancerígeno (Kuttan et al., 1985, Aggarwal et al., 2003), antioxidante (Toda et al., 1985, Al-Reza et al., 2010), cicatrizante, antimicrobiano (Wilson et al., 2005) e antiviral (Dao et al., 2012). Na Figura 1.3 são apresentadas as propriedades medicinais dos curcuminóides. Estas

propriedades podem ser exploradas a través da obtenção de extratos utilizando técnicas tais como a extração com líquidos pressurizados.

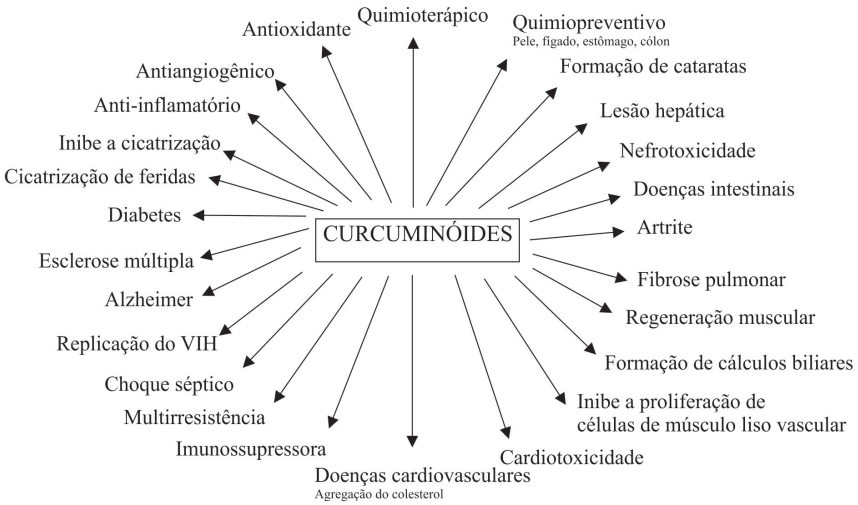

Figura 1.3. Propriedades medicinais dos curcuminóides (Ravindran et al., 2007)

1.2. Extração com Líquidos Pressurizados (PLE)

A extração com líquidos pressurizados (PLE = Pressurised Liquid Extraction) muitas vezes chamada de extração acelerada com solvente (ASE = Accelerated Solvent Extraction) utiliza temperaturas elevadas para aumentar a velocidade do processo de extração e pressões de moderadas a elevadas para manter os solventes no seu estado líquido (Xynos et al., 2012, Rostagno et al., 2009). A utilização de temperatura elevada promove a diminuição da viscosidade do solvente o que ajuda a romper as interações matriz-soluto e aumentar os coeficientes de difusão (Camel, 2001).

Esta técnica é interessante devido a eficiência de extração (5-30 min), simplicidade, facilidade de ser automatizada, ao baixo custo e utilização de menores quantidades de solventes quando comparada aos métodos tradicionais (Zaibunnisa et al., 2009). Adicionalmente, as extrações podem ser realizadas em atmosfera inerte, e na ausência de luz (Barbero et al., 2006), o que representa uma grande vantagem para a extração de compostos que podem ser sensíveis à degradação oxidativa, como os curcuminóides. Maiores detalhes sobre este processo podem ser encontrados no Capítulo 2 no qual é apresentada uma revisão sobre o processo PLE.

1.3. Formação de Partículas

De acordo com Visentin et al. (2012), a maioria dos extratos vegetais são obtidos em forma líquida, no entanto, a produção de extratos de compostos bioativos em forma seca apresenta vantagens, tais como: menor custo de estocagem, maior concentração e estabilidade dos compostos bioativos. Neste trabalho, foram produzidos extratos em forma seca mediante a precipitação do extrato. A precipitação é o processo de produção de pequenas partículas de soluto a partir de uma solução pulverizada em uma corrente de CO_2 supercrítico (Guha et al., 2011).

Usando a tecnologia supercrítica podem-se obter extratos secos com partículas de diferentes morfologias, tamanhos, distribuições, densidades aparentes e cristalinidades. Por exemplo, o controle da estrutura e da microestrutura afeta a biodisponibilidade, estabilidade (química, bioquímica e microbiológica) e a transferência de massa dos compostos bioativos. Portanto, a precipitação de compostos bioativos é uma oportunidade para melhorar a qualidade do produto (Santos et al., 2013).

Os fluidos supercríticos têm sido usados como solventes, cossolventes e antissolventes para produzir partículas com um tamanho específico. Isto se deve às condições versáteis de operação dos fluidos supercríticos e ao fato de ser possível controlar o tamanho das partículas desde a escala micrométrica até a escala nanométrica (Reverchon e De Marco, 2011).

Entre os principais processos que envolvem a utilização de fluidos supercríticos na micronização e precipitação de compostos estão: a saturação de um fluido supercrítico com um substrato sólido através do processo de expansão rápida a partir de uma solução saturada (RESS = Rapid expansion from a saturated solution), a precipitacão de compostos sólidos ou materiais hidrofóbicos que não são solúveis em fluidos supercríticos mediante o processo de gás como antissolvente (GAS = Gas antisolvent process), a utilização do fluido supercrítico como antissolvente para reduzir a solubilidade de um soluto dissolvido num solvente através do processo SAS, a combinação do processo tradicional de precipitação usando emulsões com o processo SAS, chamado processo de extração supercrítica a partir de emulsões (SFEE = Supercritical fluid extraction from an emulsion) e aquele concebido para fazer partículas de materiais que absorvem fluidos supercríticos em altas concentrações, o processo de precipitação de partículas a partir de soluções gasosas saturadas (PGSS = Particles from gas

saturated solutions) (Cocero et al., 2009).

Embora diferentes pesquisadores tenham estudado processos de formação de partículas de curcuminóides usando técnicas tradicionais tais como spray-dryer, leito fluidizado e emulsificação seguida da evaporação do solvente (Yallapu et al., 2010, Song et al., 2012, Gomez-Estaca et al., 2012, Paramera et al., 2011), até o momento nenhum deles utilizou os fluidos supercríticos na obtenção partículas de curcuminóides.

1.3.1. Antissolvente supercrítico (SAS)

No processo SAS, o CO_2 supercrítico é usado como antissolvente para reduzir a solubilidade de um soluto dissolvido num solvente. No processo SAS, o CO_2 supercrítico é bombeado em um vaso de precipitação a uma pressão e temperatura desejadas. Em seguida a solução é injetada mediante um bico difusor. O solvente é eliminado desde gotas e passa à fase supercrítica, precipitando o soluto (Kalani e Yunus, 2011). Esta técnica tem como base a possibilidade de dissolução de um grande volume de um fluido supercrítico com um solvente orgânico, a miscibilidade recíproca do CO_2 supercrítico e um solvente orgânico e a baixa afinidade de o fluido supercrítico para o soluto (Kalani e Yunus, 2011).

Este método é normalmente composto por três etapas. Inicialmente, o fluido supercrítico é introduzido no tanque de mistura antes de atingir a temperatura e a pressão desejadas. Uma vez atingidas a condições de processo, solvente puro é injetado antes que uma porção da solução seja processada. Em seguida, a solução contendo o soluto é injetada até atingir o volume de injeção desejado. Finalmente, a vazão do fluido supercrítico é mantida constante para eliminar o solvente orgânico das partículas restantes. O processo SAS é geralmente realizado sob temperaturas e pressões que variam entre 308-333 K e 8-15 MPa (Kalani e Yunus, 2011). A Figura 1.4 apresenta um esquema básico do processo.

De acordo a Kalani e Yunus (2011), as principais vantagens deste processo são: (i) as partículas são retidas no interior do sistema enquanto o CO_2 e o solvente orgânico são eliminados do sistema continuamente; (ii) uma alta supersaturação é atingida e devido a rápida mistura do CO_2 supercrítico com a solução são obtidas partículas pequenas; (iii) controlando os parâmetros operacionais é possível obter partículas uniformes; (iv) processo pode ser operado à temperatura ambiente, evitando assim a degradação térmica das partículas; (iv) este processo é adaptado para operação contínua, sendo importante para produção em larga escala; (vi) o processo ocorre num sistema fechado, o que reduz o risco de

contaminação; (vii) permite a reciclagem do solvente orgânico e do CO_2.

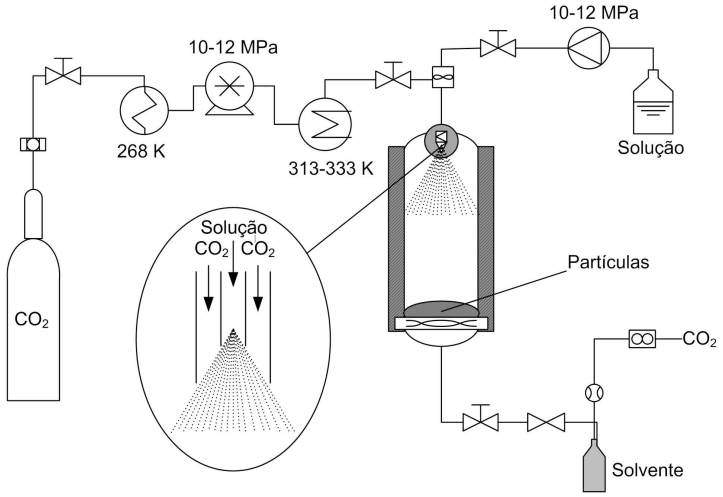

Figura1.4. Esquema básico do processo SAS (Mattea et al., 2009)

O tamanho das partículas precipitadas pelo processo SAS pode ser controlado ajustando-se os parâmetros do processo. Portanto, a otimização destes parâmetros para produzir o menor tamanho médio de partículas com uma distribuição uniforme torna-se crucial. A seguir são apresentados os efeitos dos parâmetros de operação do processo SAS sob o tamanho e morfologia:

Efeitos da pressão: Pelo fato da alta pressão incrementar o rompimento das gotas da solução em partículas menores, é possível obter partículas de tamanho menor com o aumento da pressão (Zu et al., 2012, Zhao et al., 2010). No entanto, em uma situação acima do ponto crítico, uma redução da pressão diminui a solubilidade (o que resulta em uma supersaturação mais elevada do soluto), por conseguinte, é possível também produzir partículas menores em pressões menores (Wang et al., 2013).

Efeitos da temperatura*:* O aumento da temperatura reduz a solubilidade do soluto na solução e diminui o tempo de evaporação do solvente em condições supercríticas. Portanto, aumenta a supersaturação máxima, de modo que são obtidas partículas menores quando são utilizadas temperaturas maiores (Reverchon e De Marco, 2011).

Efeitos da concentração*:* Geralmente o aumento da concentração inicial aumenta causa a formação de partículas maiores e com problemas de aglomeração (Miguel et al., 2008). Adicionalmente, em processos nos quais a supersaturação do soluto ocorre muito

lentamente, a precipitação é adiada formandos agregados, o que contribui com o crescimento das partículas (Zhao et al., 2010).

***Efeitos do bico injetor**:* Diâmetros menores do bico injetor reduzem o tamanho da partícula e partículas mais esféricas são produzidas. Quanto menor é o diâmetro do injetor, maior é a velocidade de saída e, portanto um menor tamanho da gota é obtido, gerando um menor tamanho de partícula (Guha et al., 2011).

***Efeitos das vazões de CO_2 e solvente**:* Aumentar a vazão do CO_2 acima da vazão da solução reduz o tamanho de partícula. No entanto, um menor rendimento pode ser obtido em função do pouco tempo de residência da solução dentro do vaso de precipitação (Reverchon et al., 2007). Algumas vezes não tem efeito nenhum sobre a morfologia nem o tamanho (Boonnoun et al., 2013).

***Efeitos do tipo de solvente**:* O CO_2 age como um solvente não polar e é mais solúvel em solventes menos polares. Portanto, em uma dada pressão, o nível de expansão volumétrica é maior para um solvente menos polar do que para um solvente mais polar. Geralmente, partículas menores são obtidas quando são utilizados solventes que tem grandes volumes de expansão (Kim et al., 2012). Por outro lado, se forem utilizados solventes com alta volatilidade, partículas com menor tamanho serão obtidas com o emprego de temperaturas menores (Kalani e Yunus, 2011).

Alguns processos SAS foram patenteados para a produção desde alguns quilogramas até toneladas de compostos de alto valor agregado, mas poucos alimentos ou ingredientes alimentares são produzidos industrialmente por este método. Por exemplo, para o processamento de dextrana, β-caroteno, sacarose e lecitina esta técnica é usada para produzir cerca de 200 kg de pó por hora (Weidner, 2009). Em geral este método possui vantagem industrial, além de ser uma opção para melhorar a biodisponibilidade de substâncias ativas (Majerik et al., 2007, Reverchon et al., 2007).

2. OBJETIVOS

2.1. Geral

Avaliar os processos de extração e precipitação de curcuminóides a partir de cúrcuma (*Cúrcuma Longa* L.) desaromatizada através do uso de líquidos pressurizados e fluidos supercríticos.

2.2. Específicos

- Desenvolver um método rápido de quantificação de curcuminóides por cromatografia líquida de alta eficiência (HPLC);
- Analisar o estado da arte da extração de curcuminóides a partir do uso de líquidos pressurizados;
- Determinar a condição ideal do processo PLE para maior rendimento, considerando os seguintes parâmetros: (1) temperatura (333; 343 e 353 K) e (2) pressão (10; 15, 20, 25, 30 e 35 MPa);
- Determinar a cinética de extração nas condições ótimas;
- Produzir partículas de curcuminóides empregando métodos que envolvem tecnologia supercrítica, mais especificamente a técnica SAS;
- Avaliar o efeito dos parâmetros operacionais sobre o processo de precipitação de curcuminóides;
- Analisar a eficiência de precipitação, morfologia, distribuição de tamanho de partículas e o conteúdo de curcuminóides das partículas formadas;
- Realizar a análise econômica do processo integrado utilizando o simulador comercial SuperPro Designer®.

3. ESTRUTURA DO TRABALHO

A tese está dividida em 8 capítulos, os quais apresentam o desenvolvimento da pesquisa. Estes capítulos estão compostos por artigos publicados ou que ainda serão submetidos para publicação. No **Capítulo 1 – Introdução e objetivos** é apresentada de maneira geral o tema principal deste trabalho, o objetivo geral da pesquisa e os objetivos específicos que permitirão atingir o desenvolvimento pleno do trabalho. Na Figura 1.5 são apresentadas as etapas que foram realizadas durante o doutorado.

Com o objetivo de compreender os fundamentos do processo PLE foi escrito o **Capítulo 2 – Recent applications of pressurized fluid extraction: curcuminoids extraction with pressurized liquids**. Este capítulo apresenta o estado da arte dos fundamentos do processo PLE e suas aplicações na extração de compostos bioativos, especificamente a extração de curcuminóides. Neste trabalho de revisão foram estabelecidos os principais parâmetros que influenciam o processo PLE, distinguindo entre aplicações analíticas e de processo. Esta primeira etapa teve como objetivo discutir os fundamentos da técnica que emprega líquidos pressurizados na extração de compostos bioativos, focando naqueles processos cujo objetivo era obter um extrato rico em curcuminóides. A revisão mostrou que o processo PLE, por ser altamente seletivo, usa curtos tempos de extração e baixas quantidades de solventes orgânicos, sendo excelente alternativa para substituir as técnicas de extração tradicionais, com um grande potencial na extração de compostos bioativos e na detecção de contaminantes. Quando acoplado com outra técnica analítica é altamente seletivo e utiliza tempos de extração curtos e baixas quantidades de solventes orgânicos, tornando-se uma excelente alternativa para substituir as técnicas de extração tradicionais. Porém, faltam pesquisas que permitam estabelecer a influência dos parâmetros de extração no processo de extração de curcuminóides utilizando PLE.

Paralelamente com o desenvolvimento do Capítulo 2, foi desenvolvido um método rápido e preciso de quantificação de curcuminóides com o objetivo de simplificar as análises dos extratos obtidos durante o trabalho. No **Capítulo 3 – Fast analysis of curcuminoids from turmeric (*Curcuma longa* L.) by high-performance liquid chromatography using a fused-core column,** foram determinadas as condições ótimas para levar a cabo a quantificação dos curcuminóides dos extratos obtidos nos Capítulos 4 e 5. O método desenvolvido permite a separação dos três curcuminóides principais (Curcumina, demetoxicurcumina e bisdemetoxicurcumina) em cerca de 1,3 min com um tempo total de

análise de 7 min, incluindo o período de limpeza e o reequilíbrio da coluna. O método foi validado utilizando diferentes extratos obtidos de rizomas de cúrcuma e produtos que continham cúrcuma entre os seus ingredientes (Macarrão, cúrcuma em pó e mostarda).

Dentro deste contexto, o **Capítulo 4- Extraction of curcuminoids from deflavored turmeric (*Curcuma longa* l.) using pressurized liquids: Process integration and economic evaluation** apresenta um estudo do processo de obtenção de um extrato etanólico rico em curcuminóides via PLE. Para estes experimentos, cúrcuma desaromatizada foi utilizada como matéria prima. O efeito dos parâmetros operacionais sobre o rendimento global de extração e o rendimento de curcuminóides foi avaliado. Após a análise estatística, foram estabelecidas as condições ótimas de extração. Com as condições ótimas, o processo PLE foi comparado com as técnicas de extração Soxhlet e LPSE. Finalmente, baseado nas melhores condições para o processo PLE, foi estudada a cinética de extração e feita uma análise econômica. Na simulação do processo PLE, foi avaliado o efeito do custo de matéria prima e o amento de escala.

Como alternativa aos processos convencionais de eliminação do solvente e formação de partículas, o processo supercrítico antissolvente (SAS) foi utilizado. Desta forma o **Capitulo 5- Precipitation of curcuminoids from an ethanolic turmeric extract using a supercritical antisolvent process** apresenta um estudo do efeito das condições operacionais (temperatura, pressão, vazão do CO_2 e tipo de injetor) no processo de precipitação de curcuminóides a partir do extrato etanólico obtido no Capítulo 4. Neste trabalho, foram avaliados o rendimento global de sólidos, o conteúdo de curcuminóides, o rendimento de precipitação, a morfologia e o tamanho das partículas.

O **Capítulo 6- Process integration for turmeric products extraction using supercritical fluids and pressurized liquids: process simulation and economic evaluation** foi escrito com o objetivo de propor e avaliar economicamente um processo integrado para a obtenção de óleo volátil e extrato pulverizado rico em curcuminóides a partir dos rizomas da cúrcuma. Este é um processo atualmente inexistente e utiliza simultaneamente três tecnologias emergentes distintas. Na primeira etapa do processo proposto, o óleo volátil foi extraído via SFE e no mesmo equipamento os curcuminóides foram extraídos via PLE com o solvente etanol. Finalmente, o solvente foi eliminado e os curcuminóides precipitados mediante o processo SAS. Este capítulo demonstra claramente a possibilidade da implementação de uma linha de produção destinada ao aproveitamento integral da cúrcuma e

com potencial de inserção no mercado de aditivos de origem natural.

Embora os curcuminóides sejam utilizados normalmente na indústria com a ajuda de emulsificantes, o **Capítulo 7 - Nanoencapsulation of flavors and aromas by emerging Technologies** apresenta uma revisão de técnicas utilizadas em nanoencapsulação, tais como o uso de fluidos supercríticos e a formação de emulsões assistidas por ultrassom. Estas técnicas poderiam ser exploradas em trabalho futuros com o objetivo de melhorar a solubilidade em meios aquosos do extrato em pó rico em curcuminóides obtido neste trabalho e do óleo volátil de cúrcuma obtido na etapa de desaromatização.

Finalmente, o **Capítulo 8 - Conclusões gerais** apresenta os principais resultados obtidos em cada um dos capítulos apresentados neste trabalho. Além disso, é apresentada a memória do período do doutorado com todos os trabalhos acadêmicos realizados paralelamente.

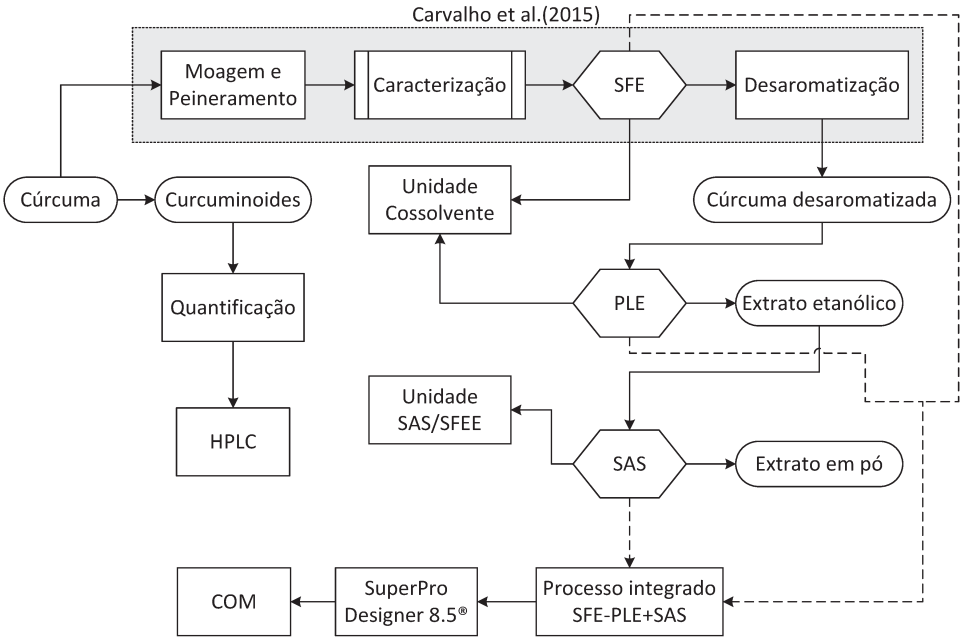

Figura 1.5. Esquema das etapas do desenvolvimento do projeto e as atividades realizadas.

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-CAPÍTULO 2-

Recent applications of pressurized fluid extraction: curcuminoids extraction with pressurized liquids

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O material suplementar deste Capítulo se encontra no Apêndice A

Recent Applications of Pressurized Fluid Extraction: Curcuminoids Extraction with Pressurized Liquids

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Abstract Pressurized liquid extraction is a versatile technique that allows the extraction of natural bioactive compounds such as curcuminoids, compounds that have medicinal properties and responsible for the yellow color of turmeric. Generally, used as an analytical tool pressurized liquid extraction uses elevated temperatures (313 – 473 K) and moderate to high (3.5 – 20 MPa) pressures to facilitate and enhance the extraction process. Various features such as the use of smaller amounts of solvents, reduced extraction time and no exposure of the compounds to oxygen and light, give this technique advantages over traditional processes of solvent extraction. This review describes the fundamentals and parameters influencing the process of pressurized liquid extraction, exploring the latest developments and trends in the extraction of bioactive compounds such as curcuminoids. It also discusses the possibility of using near room temperature (313 K) and pressures in the range of 10 – 30 MPa as opposing to the use of high temperatures, in the extraction of pigments from plant material rich in starch.

Keywords Pressurized Fluid Extraction, Extraction parameters, Bioactive compounds, Curcuminoids

1. Introduction

The increase of scientific knowledge about the impact of foods and synthetic additives on human health and customer awareness that natural compounds can replace those obtained synthetically has generated interest in the development of new processes that allow the extraction of bioactive compounds from natural matrices.

Turmeric (*Cúrcuma longa* L.) is a plant widely cultivated in countries and regions with tropical and subtropical climates, mainly in China, India and Indonesia, as well as in some Latin American countries such as Brazil and Peru, for instance[32]. Turmeric has been used popularly as a preservative, flavoring and coloring agent. The yellow color of the rhizomes is due to the presence of a group of phenolic compounds called curcuminoids. Turmeric has been investigated owing to its benefits on human health and their bioactive properties, such as: anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activity[27].

Nowadays, the curcuminoids extraction for use as potential natural food additive is made using conventional solvent extraction techniques such as Soxhlet and solid-liquid extraction. In conventional methods of extraction, factors such as light, temperature and exposure to oxygen produce curcuminoids degradation[69], limiting and restricting the application processes by which they are obtained. This aspect combined with the low yield and high cost of operation of conventional processes highlights the need to implement new processes to overcome these drawbacks and also protect the integrity of the compounds and generate environmentally friendly process.

Pressurized liquid extraction (PLE) is an attractive alternative because it allows fast extraction and reduced solvent consumption[60]. PLE has frequently been used for analytical purposes in the preparation of samples, overcoming disadvantages of the conventional methods of extraction. It is a technique characterized by being easily automated, making it a distinctive technique with low cost and favorable environmental impact because of low solvent usage[81].

PLE is performed in a wide range of conditions on the liquid compressed region, located to the left of the saturated liquid curve and below the critical temperature (Tc) line, as shown in the pressure-volume diagram for ethanol (Figure 1). In this region the liquids are highly incompressible and when the solvents are subjected to pressure changes at a constant temperature, their density and solvation power are not affected significantly[73]. However increased temperatures improve the efficiency of extraction because of enhanced rate of mass transfer and diffusion rates[60].

The aim of this article is to provide an overview of the fundamentals and parameters governing pressurized liquid extraction. As well as to explore the latest trends and applications that involves the use of PLE, focusing on the

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extraction of bioactive compounds such as curcuminoids from natural matrices.

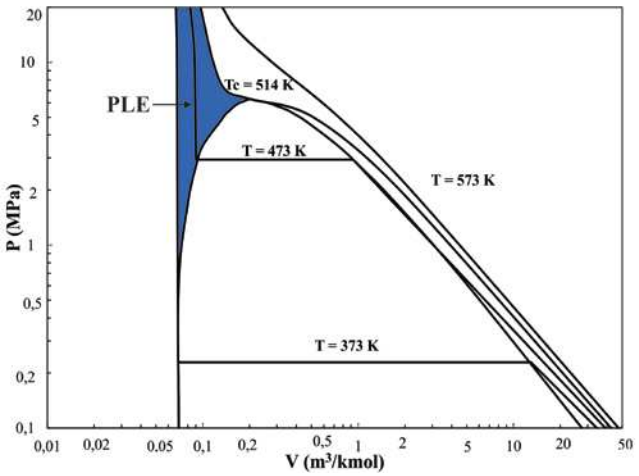

Figure 1. Pressure-volume diagram for ethanol calculated using the Peng – Robinson equation of state

2. Curcuminoids

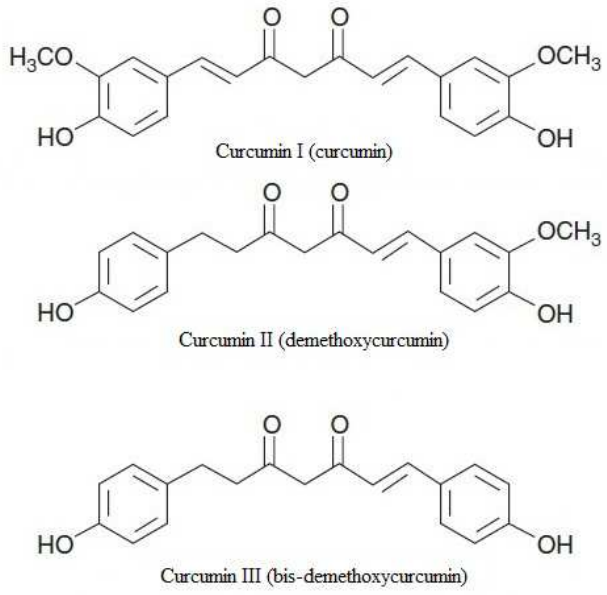

Figure 2. Chemical structures of curcuminoids

Turmeric (*Cúrcuma longa* L. and *Cúrcuma aromatica*) has been used since ancient times as a condiment (main ingredient in curry), preservative and folk medicine for treating diseases, among which are: biliary disorders, anorexia, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis[3]. Nowadays it is used mainly as a dye, due to the interest of replacing synthetic additives by natural compounds, finding application in the preparation of chutneys, pickles, mustard, butter and cheese[53]. The yellow color of turmeric is due to the presence of a group of phenolic compounds known as curcuminoids (Figure 2). Turmeric roots contain between 2 and 6.5% of curcuminoids [27]. Curcumin (I) is the main component, followed by two related compounds, demethoxycurcumin (II) and bisdemethoxycurcumin (III). Curcumin has two methoxy groups and has reddish orange color, while demethoxycurcumin has a single methoxy group and is distinguished by its orange-yellow color and the bisdemethoxycurcumin lacking methoxy groups is characterized by its yellow color[32; 53]. Curcuminoids are light sensitive in solution and solid form and undergoes hydrolytic degradation in solution at high pH[65].

Besides being chemically related and being the main constituents of turmeric, curcuminoids have innumerable functional properties. The curcuminoids are a potent antioxidant[2], whose efficiency can be compared with other antioxidants such as vitamins C and E. They have also been recognized for their biological activity associated with anticancer[1; 38], antibacterial[78] and antiviral[19] properties.

These properties can be explored in a variety of applications such as additive and raw material in the food, pharmaceutical and cosmetic industries.

2.1. Curcuminoids Extraction

Classical techniques, such as liquid–liquid extraction, sonication, Soxhlet extraction, and related methods have been used traditionally in the curcuminoids extraction[22]. The traditional process involves the preparation of the raw material (milled and dried), extraction with a suitable solvent (acetone or methanol) which selectively extracts the coloring matter, extract purification and finally remove the solvent.

This process, after distillation of the solvent, yields an oleoresin with coloring matter content of approximately 25-35 % along with volatile oils (15-20%) and other resinous extractives (20-30%). The oleoresin so obtained is subjected to further washes using selective solvents that can extract the curcumin pigment from the oleoresin. Depending on the size of the batch and of the extraction parameters, each batch takes between 6 to 24 hours. This process yields a powdered, purified food color, known as curcumin powder identified with the food code E100, with over 90 % of coloring matter content and very little volatile oil and other dry matter of natural origin[71].

The selection of solvents is done with care to meet solubilization and regulatory criteria. Curcuminoids are poorly soluble in water, but they are soluble in ethanol, alkali, ketone, acetic acid and chloroform[15]. According to the European Commission directive 45/CE[13] the following solvents are considered suitable for curcuminoids extraction: isopropanol, ethyl acetate, acetone, carbon dioxide, methanol, ethanol and hexane.

3. Pressurized Liquid Extraction

PLE is called pressurized fluid extraction (PFE), accelerated solvent extraction (ASE), pressurized liquid extraction (PLE), pressurized solvent extraction (PSE) or enhanced solvent extraction (ESE)[44; 47]. When the

solvent used is water, it is common to use other terms such as subcritical water extraction (SWE), hot water extraction (HWE), pressurized hot water extraction (PHWE), high-temperature water extraction (HTWE) superheated water extraction or hot liquid water extraction[11].

PLE uses elevated temperatures (313 – 473 K) without reaching the critical point to increase the kinetics of the extraction process while applying high pressures (3.5 – 20 MPa) to maintain the solvents in their liquid state[79]. Depending on the temperature at which the extraction is performed, PLE is a technique suitable for processes where compounds that may be sensitive to degradation through the action of the heat[5]. Furthermore, because the extractions are carried on a closed extractor, the contact of bioactive compounds with oxygen and light is avoided, thus preventing the degradation of those compounds susceptible to degradation by oxidation.

The kinetics of the extraction process with pressurized liquid is characterized by an overall extraction curve (OEC), whose kinetic parameters can be calculated by adjusting a linear spline model as used in supercritical fluid extraction (SFE)[12], which is presented in Figure 3. In the OEC can be distinguished mainly three stages (Figure 3): a first stage called constant extraction rate period (CER), followed by a falling extraction rate period (FER) and finally a diffusion controlled rate period (DC). In the PLE process the solvent is always in excess relative to the compound to be extracted. In the CER stage there is a large amount of compound available for extraction, allowing a rapid removal of compounds dissolved in the bulk solution or adsorbed at the surface of the matrix. Subsequently the extraction speed decreases at the FER period when the compounds dissolved in the solvent and/or adsorbed at the pore surface and those dissolved/adsorbed in the micro/nano pores of the matrix are extracted. Finally the extraction rate decreases almost entirely on the DC period when which begins the extraction of compounds that are chemically bonded to the matrix, the matrix-compound chemical interactions must be broken and the compound must be solvated by the solvent and subsequently released to outside the matrix in to the bulk solvent[54].

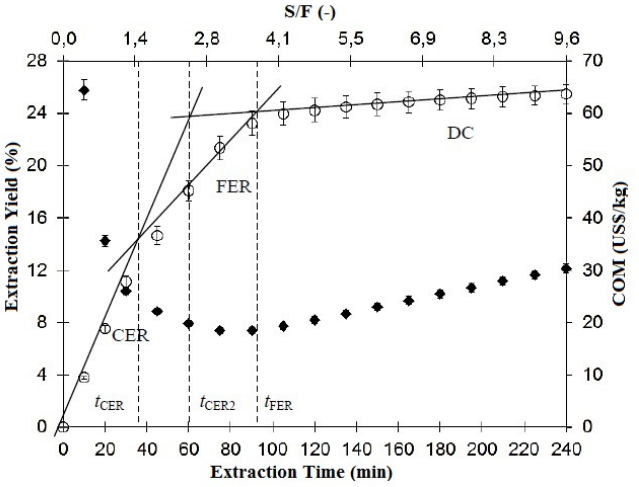

Figure 3. Overall extraction curves of Jabuticaba obtained by pressurized liquid extraction using ethanol as solvent[12]

3.1. Description of the Extraction Process

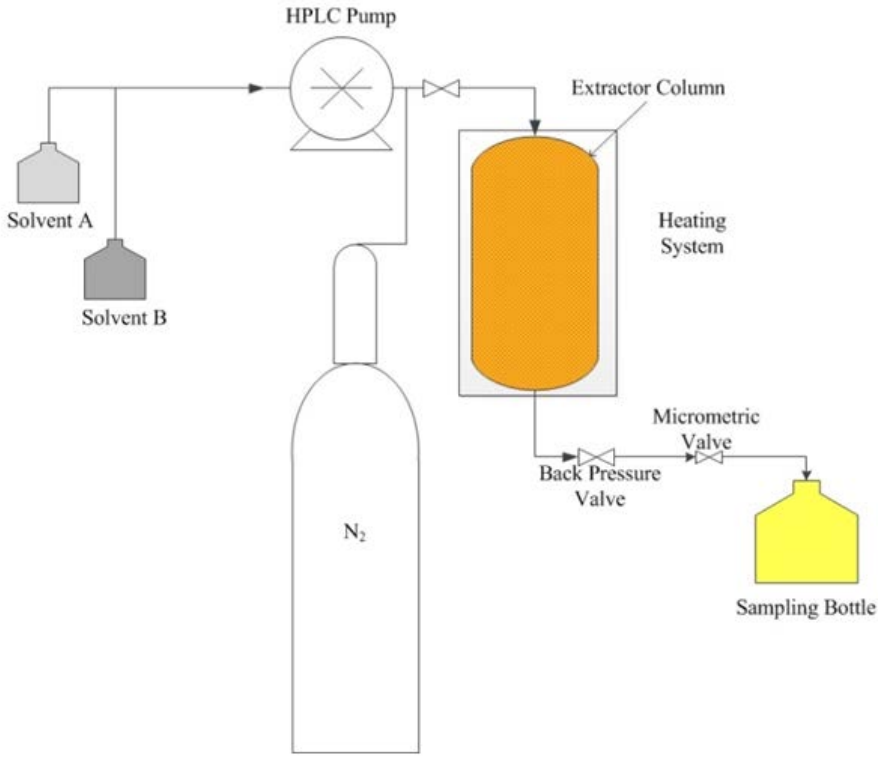

Figure 4. Basic pressurized liquid extraction set-up

PLE can be performed in either static or dynamic mode. Static extraction mode is a batch process consisting of one or more extraction cycles with addition of fresh solvent between each cycle. The extractor is pressurized through the solvent inlet while the outlet valve is kept closed. After the extraction, the valve is opened, releasing a mixture of solvent and extract to the collection collecting.

In the dynamic mode, the solvent is continuously pumped through the extractor containing the matrix, whereas the outlet valve is kept open during the extraction[11; 54]. Although one would think that the dynamic extraction may produce better results than the static extraction, avoiding the solvent saturation, by injecting continuously fresh solvent into the extractor, the static extraction is more efficient because it allows greater penetration of the solvent into the nano and micro pores of the matrix according to Nieto et al.[48].

A basic static pressurized liquid extraction setup is shown in Figure 4. The extraction process involves packing the sample into the extractor and then the solvent is pumped by a HPLC-type pump into the extraction cell, which was placed in a heating system at a desired temperature, until the required pressure was obtained. Once the temperature and pressure of the process have been reached, the extraction is performed over a preset time. If the process has more than one extraction cycle, the solvent is replaced with fresh solvent, in each extraction cycle. After the extraction cycles have finished, the back pressure valve and micrometric valves are carefully opened, keeping the pressure at an appropriate level for the desired flow. Finished the preset extraction time, the HPLC pump and the heating system are turned off. The extractor may or may not be purged using an inert gas such as nitrogen to remove the solvent remaining within the extractor[54; 10].

4. Extraction Parameters

The PLE process performance is governed mainly by the choice of solvent, temperature, extraction time and to lesser extent by the pressure. However the performance of the process depends on the matrix nature, the specific features of the target compounds and their localization inside the matrix[47]. Therefore it is necessary to know and establish the influence of these factors on the extraction process in order to obtain high yields and high purity extracts. Next, an overall review of the parameters that influence the PLE process is presented, the differences between analytical and process applications are also considered.

4.1. Analytical Applications

4.1.1. Solvent

The extraction solvent must be highly selective, with high solvation capacity of the target compound and minimize the co-extraction of other matrix components. The polarity of the solvent should be close to that of the target compound. Non-polar solvents such as n-hexane and pentane or a non-polar with medium-polarity solvents, such as pentane/dichloromethane or cyclohexane/ethyl acetate, have frequently been used in the extraction of apolar and lipophilic compounds. On the other hand, more polar solvents, such as acetonitrile, methanol, ethyl acetate or water, have been employed in the case of polar and hydrophilic compounds [11]. Other important solvent characteristic is its ability to aid in the release of compounds from matrix and helping with the breaking of the interactions matrix-compounds[57].

Among the most commonly used solvents in the extraction of bioactive compounds from natural sources are: ethanol[41; 46; 50; 60; 82], methanol[30; 35; 40; 68; 65] and n-hexane [16; 81; 31; 36; 55]. When water is used as solvent[9; 22; 55; 62], the combination of high temperatures, pressure and pH changes, contribute to the change of the water polarity, modifying its dielectric constant (ε). At ambient pressure and temperature, water is a polar solvent with a high dielectric constant (ε = 78) but at 573 K and 23 MPa this value decreases to 21, which is similar to the value for ethanol (ε = 24 at 298 K) or acetone (ε = 20.7 at 298 K). This means that at elevated temperatures and moderate pressures the polarity of water can be reduced considerably; thus, water can act as if ethanol or acetone were being used[11].

4.1.2. Temperature

Temperature is the main parameter responsible of the extraction process acceleration. High temperatures decrease the solvent viscosity, helping with its penetration inside the matrix and consequently, improve the extraction process. Furthermore, elevated temperature decreases the surface tension of the solvent, compounds and matrix and therefore enhances the solvent wetting of the matrix; therefore, to a higher contact between the solvent and those compounds inside the matrix[47]. The use of high temperatures increases the diffusion coefficients, increasing the mass transference rates, furthermore helps to disrupt the compounds–matrix interactions[10; 48]. Nonetheless, for some applications moderate to low temperatures maybe preferred instead in order to avoid some modifications in the solid matrix such as, for instance, the gelatinization of starch in starch-rich solid substratum.

4.1.3. Extraction Time

The duration of the static extraction time is important in the extraction efficiency since prolonged contact periods between the matrix and the solvent permits increased swelling with enhanced matrix wetting and increased penetration of solvent into the nano and micro pores with a greater solvation of compounds. Therefore, an enhanced possibility of the solvent breaking specific compounds–matrix interactions is ensured[57]. Generally, for analytical applications, extraction times between 5 and 30 minutes are enough to guarantee the extraction of the most compounds with a high yield. However, the combination of high

temperatures and long extraction times could induce the degradation of the compounds and the matrix[54].

4.1.4. Pressure

Pressure is a parameter that does not present big influence on the yield of extraction process, because liquids are not compressible fluids; therefore, even under large pressure changes the solvation power of the solvent is not significantly affected. This effect was observed by Rizvi[54] for pressures between 3 and 20 MPa. Nonetheless, when the vapor pressure of the target components is important for its solubilization in the solvent, pressure may have an important role in the PLE process. Otherwise, the use of high pressures facilitate the extraction of compounds located inside the matrix pores, due to a pressure increase which forces the solvent to penetrate into places which are normally not reached by the solvent at atmospheric pressure[33]. Depending of the structure of the matrix and the particularities of each process, the use of high pressures could be a positive or negative influence on the extraction process, for instance, at higher pressures; the matrix may be compacted, affecting the flow of the solvent[37].

4.2. Process Applications

4.2.1. Particle Size

It is important to determine the influence of particle size on the extraction process due to the fact that different size fractions are obtained by passing the milled material through a nest of sieves and extracting each fraction. According to Cheah et al.[16], the particle size exert a significant influence on the performance of PLE process, owing to the smallest particle size generate a greatest percentage of extractable solids allowing a greater recovery of bioactive compounds. Particle size reduction by milling not only increases the specific area of raw materials but also ruptures cell walls, releasing a greater amount of bioactive compounds[75].

4.2.2. Solvent

Aspects such as economy, safety and sustainability must be considered to choose the solvent. Less toxic and non-harmful solvents that are easy to remove or recover should be preferred[47]. Larger volumes of expensive and toxic organic solvents have to be used in many cases, which is not applicable for food industry; additionally, toxic solvent disposal is expensive. In recent years, continuous efforts have been made in order to reduce the amount of organic solvents required in extraction process. With continuous interests and investment in biofuel industry, ethanol has become the cheapest solvent after water. Different from industrial ethanol synthesized from petroleum, ethanol produced by fermentation is listed as safe, clean, green and sustainable[29].

4.2.3. Temperature

Temperature is an important parameter and usually the higher yields obtained with PLE in comparison with other extraction techniques are attributed to this parameter.

An increase in extraction temperature is reported to improve the efficiency of extraction because of enhanced diffusion rate; nevertheless, a maximum temperature limit should be fixed and should depend on other factors, particularly extraction time[60]. The stability of the compounds of interest and the matrix can be affected by the combination of high temperatures and pressures, therefore, the process temperature must be carefully selected so as to not cause degradation of bioactive compounds or the matrix, or the increased extraction of other undesirable compounds [29; 18].

4.2.4. Cost of Manufacturing (COM)

Although when PLE is compared with traditional extraction methods, the PLE process appears as a valuable alternative in terms of extraction, the economic viability of the process is very important and even more when considering that there are other technologies that have a lower investment cost[51]. The PLE process appears to be a technically promising and economically viable technique. Santos et al.[59] determined the cost of manufacturing (COM) for the extraction of phenolic compounds from Jabuticaba skins obtained under the optimum PLE conditions were 40-fold lower in comparison with a conventional low-pressure solvent extraction (LPSE) for a process time of 2 h.

5. PLE Applications

The increasing appearance of degenerative diseases has increased consumer concern regarding the use and ingestion of foods. In this sense, the use of vegetable extracts with functional characteristics (specific physiologic benefits due to the presence of bioactive substances), with high purity (synthetic substances, often, are suspected to cause secondary effects to health) has attracted the interest from institutions and companies involved in the formulation of food and/or products with beneficial health effects.

A literature survey was conducted regarding the use of PLE in the area of food science and technology considering analytical and process applications. A survey was conducted in Web of Science and Scopus databases considering the period between 2006 and 2013, several articles were found about the utilization of PLE in the area of food science and technology.

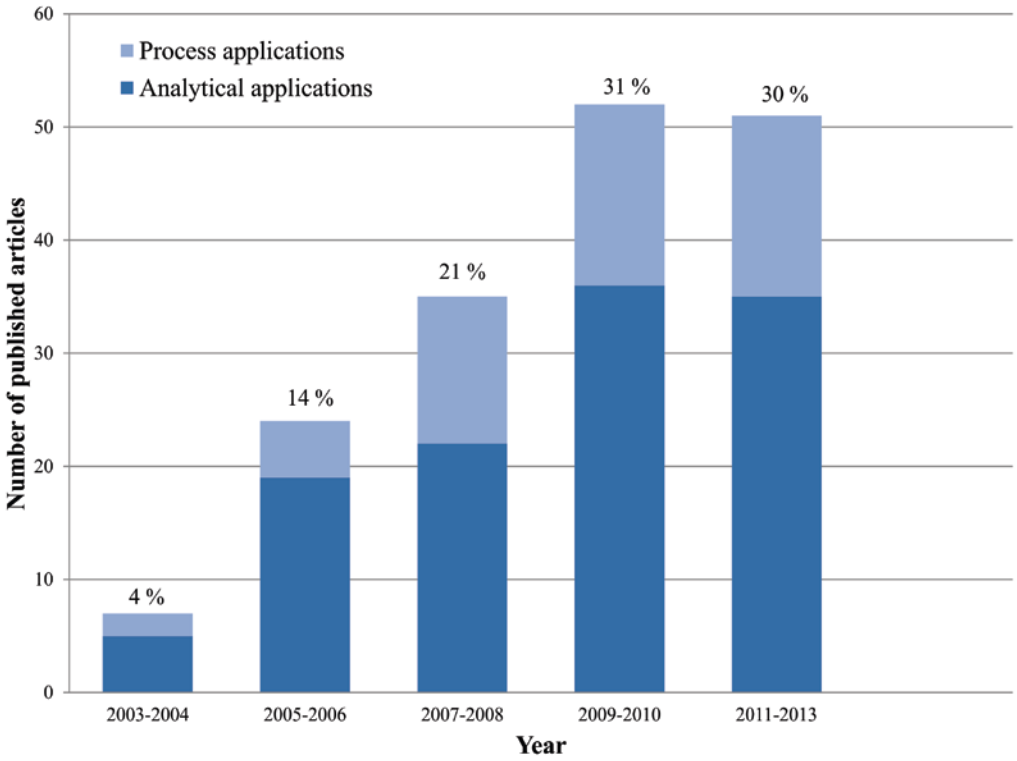

Figure 5. Distribution of the published articles using PLE in the area of food science and technology

Analyzing the literature about the evolution related to the use of pressurized fluids an increase in the number of articles published could be observed in recent years in this area particularly since 2006 to the present (Figure 5). Figure 5 shows that approximately 60% of the articles that aims application of the PLE in the area of food science and technology have been published in the past 4 years and almost 70% of all the articles are related to analytical applications.

This growing interest in the use of pressurized liquids and in particular the concentration of search in the analytical area, is mainly because there is a high level of automation in PLE equipment and its configuration allows that oxygen and light sensitive compounds to be protected. Moreover, samples require minimal pretreatment operations usually involved in conditioning, such as homogenization, drying and milling [47].

The PLE applications can be divided in two parts: 1) the study of extraction of bioactive compounds with functional characteristics extracted from natural fonts and 2) the PLE coupling with analytical techniques for the quantification and detection of contaminants and toxic substances in foods. Next, we intend to make a review of the articles published in recent years, related the extraction conditions and the effects of these on the extraction process.

5.1. Extraction of Bioactive Compounds from Natural Matrices

PLE has been utilized in extraction of several bioactive compounds such as flavonoids[70; 82], polyphenols[9] and several antioxidants compounds[77; 74] from natural matrices such as plants, fruits, vegetables and microorganisms as showed in Table 1.

Exhaustive extraction of honokiol and magnolol from *Magnolia officinalis* were studied by Cheah et al.[16]. PLE is a more economical alternative to Soxhlet in exhaustive extraction as it requires less solvent and time. The proportions of active principles in the extracts obtained by PLE were higher compared to Soxhlet (honokiol 5% vs 3%; magnolol 46% vs 30%). Only 20 minutes of PLE with 5 minutes of static time were enough to extract almost the totality of compounds extracted with Soxhlet after 8 hours of extraction[29].

PLE extraction of flavones was investigated and compared with other conventional extraction techniques such as ultrasonic-assisted extraction (UAE), heat-reflux extraction (HRE) and Soxhlet extraction. PLE and HRE. Although the efficiencies of HRE and PLE for flavones extraction were similar, PLE method was less time-consuming. The PLE method required only 20 min under 433 K to complete the extraction while Soxhlet, UAE and HRE required extraction times of 4 hours, 1 hour and 20 minutes, respectively[40].

Additionally, PLE was utilized for acetophenones extraction, 17 minutes static extraction time were necessary to overcome the extraction yield of HRE and Soxhlet, which took between 6 and 9 hours, respectively, to be completed [41]. PLE extraction of phenolics compounds from Jabuticaba (*M. cauliflora*) was compared to LPSE by Santos et al.[60], under optimized conditions both extraction techniques presented similar yield, however, PLE extracts showed in their composition higher content of anthocyanins and phenolics compounds. Likewise, the cost of

manufacturing (COM) obtained for the PLE extract was 40-fold lower than conventional low-pressure solvent extraction (LPSE).

Flavonoid extraction from onions were studied by Søltoft et al.[70] comparing PLE with Microwave-assisted extraction (MAE) and using a ultrasonic liquid processor (ULP). Although the efficiencies of MAE and ULP were comparable and at the same level as PLE, MAE and ULP needed an additional sample preparation step (e.g. centrifugation). Likewise, difficulties were observed during the filtration of extracts MAE and ULP. In contrast, PLE yielded clean extracts, which could be filtered directly after extraction.

Due to growing interesting in nutraceutical and bioactive compounds extraction from natural fonts and the parallel concerns about use of technologies that are more "green", PLE is becoming a promising extraction technology to satisfy these requirements[10].

5.2. Detection of Contaminants and Toxic Substances in Foods

PLE coupled with other analytical techniques that have been used in extraction and quantification of contaminants compounds presents in several food stuffs such as fruits, vegetables, meats and cereals (Table 2).

In articles published in recent years, it is possible to observe that PLE application in the analysis of contaminants in foods has been mainly focused on detection of organic compounds used in plastic manufacturing[23], pesticides[28] and mycotoxins[17].

Since the food matrices are complex and contaminants are at a trace level in the matrices, it requires investing a large amount of time in the conditioning and sample preparation. However, in analytical applications, PLE is a robust and time-saving technique that would enable automated sample-handling and avoid health risks caused by both the contaminants compounds and the solvents[17].

In the food analysis by PLE a dispersing agent is frequently used for improving the interactions between sample and extraction solvent, as well as ensuring the reproducibility of the extraction. In sample preparation of matrices with high lipid content, lipophilic sorbents are widely employed, for instance. To avoid this, neutral alumina, florisil, graphitized carbon (120/400 mesh) and amino propyl silica were tested[58].

Bisphenol A (BPA), is a endocrine disrupting chemical. This product is used in the manufacture of epoxy resins and polycarbonate plastics, which are, in turn, used in a wide variety of domestic products such as in dental fillings, plastic food and water containers, baby bottles, food wrap, as well as in the lining of beverage and food can[20]. Several authors have used PLE as a method for detection and quantification of these compounds[20; 23; 58]. Temperature is an important factor in bisphenols extraction, an increase in temperature has a negative effect on the recoveries of analytes, because higher amounts of matrix components that are extracted at higher temperatures affect the quantification of the analytes. For instance, Ferrer et al.[23] developed a method for quantifying the bisphenol contents in powdered milk and infant formulas. The recoveries of BPA at temperatures above 343 K were lower. A temperature of 393 K gave the worst results, because an increase of the temperature resulted in a cloudy extract with an increasing amount of material co-extracted from the matrix that hampered the quantification of the compounds. On the other hand to avoid the amount of unwanted compounds Salgueiro-González et al.[58] used an extraction temperature of 313 K for the analysis of BPA in bivalve molluscs. Besides the influence of temperature on the extraction and quantitation of BPAs, the choice of solvent is an important factor, since due to the BAPs low solubility in water is recommended to use organic solvents such as ethyl acetate and methanol, because the presence of water is responsible for low yields in the extraction[20].

Due to excessive use of agrochemical in agriculture, have been detected appreciable levels of these chemicals in soil, water and food. Those contaminant substances have been entered in food chain of customers, accumulate in the body with the consequent generation of negative effects.

Several PLE methods have been validated to pesticide detection, for example: organochlorine and organophosphorus pesticides[28; 63; 24], polychlorinated biphenyls (PCBs)[25; 39] and polybrominated biphenyls (PBBs)[42]. Temperature has a fundamental role in the extraction process and quantification of contaminant compounds, and as in the detection of other contaminants, an increase in temperature, produces a large amount of additional peaks in chromatography techniques coupled to extraction, preventing the identification of the contaminant compounds [24].

PLE detection of mycotoxins has had an important development, with successful application in detection of contaminants in different food, making it a potentially useful tool to measure with accuracy the levels of mycotoxins. The mycotoxin Zearalenone (ZEN) is produced by several fungi, including Fusarium graminearum and Fusarium culmorum in cereal flours and is associated with development of breast cancer, was analyzed by Pérez-Torrado et al.[49] using PLE coupled with liquid chromatography–mass spectrometry (LC-MS), achieving European Commission level requirements. Aflatoxins produced by Aspergillus flavus and Aspergillus parasiticus, highly toxic and carcinogenic, can be extracted by PLE, presenting higher extraction yield or recovery in comparison with the AOAC method using similar extraction solvent[67]. On the other hand, Zinedine et al.[83] and Chen et al.[17] used PLE to extract ochratoxin A in cereals. Ochratoxin A is known to cause a wide range of toxic effects due their nephrotoxic and carcinogenic. The PLE methods coupled with LC-MS demonstrated their ability to extract quickly and automatically residues of mycotoxins, allowing great accuracy over a wide range of concentrations meeting the criteria established by the European Commission.

Table 1. Summary of the works published on the extraction of bioactive compounds from natural matrices by PLE

Matrix	Compounds	Related functional activities	Extraction conditions					Analytical technique[a]	Optimized PLE parameters	Statistical analysis	Ref.
			Solvent	Temperature (K)	Pressure (MPa)	Cycles	Extraction time (min)				
Wheat flour	Folic acid	Nucleotide synthesis, cell division and growth	0.1 M phosphate buffer	313-353	10	3	3	HPLC	313 K	Comparison of means	[4]
Spicy cayenne pepper	Capsaicinoids	Color, pungency, aroma, analgesia, anticancer, anti-inflammation, antioxidant.	Water Ethanol/ Water Methanol /Water	323-473	10	1-3	5	HPLC	473 K, mehtanol 100%, 1 cycle	Comparison of means	[5]
Pomegranate	Polyphenols	Antioxidant	Water	313-363	10	1-5	4- 30	HPLC	313 K, 4 min, 2 cycles	ANOVA	[9]
Chlorella vulgaris	Carotenoids Chlorophyll	Antioxidant	Ethanol	308-160	10			HPLC ABTS$^{\bullet+}$	358 K	ANOVA	[14]
Magnolia officinalis	Magnolol Honokiol	Antioxidant, anti-inflammation	n-Hexane	343-373	10	1	5-15	HPLC	373 K, 10 min	ANOVA	[16]
American ginseng	Ginsenosides	Anti-inflammation	Agua Methanol Triton X-100 (1%)	323-393	3.45-20	1	10	HPLC	Water or methanol + Triton X-100 (1%), 323 K, 20 MPA	Comparison of means	[18]
Propolis	Phenolic compounds	Antioxidant, anti-inflammation	Ethanol/Ethylacetate /Water/HCl Methanol/HCl Ethanol/Water/HCl Methanol/Water/HCl Ethanol/Acetone/Water/ HCl Water/HCl Methanol/Water/ tert-butylhy-droquinone (tBHQ)	293-353	3.35-13. 7	1	15-120	HPLC	Methanol/Water/ tBHQ (70:30:0.1 v/v/w, 313 K, 10 Mpa, 45 min	ANOVA	[21]
Ginger	Gingerols Shogaols	Antiemetic, antitussive, analgesic, anti-inflammatory, cardiotonic, anticancer	Hexane Chloroform Ethyl acetate Methanol Bioethanol	333-403	6.89-13. 7	2	5	HPLC	Bioethanol (70%), 373 K, 10 MPa	ANOVA	[29]

Mate tea leaves	Mate tea leaves constituents	Antioxidant, diuretic, anti-inflammatory, stimulant	Hexane Methanol	323-373	10	1-3	10-30	GC/MS	Methanol, 373 K, 1 ciclo, 10 min	Factorial design	[30]
Haematococcus pluvialis microalgae	Carotenoids	Antioxidant	Hexane Ethanol	323-473	10	1	20	HPLC	Best yield: Ethanol, 473 K Best antioxidant activity: Ethanol, 323 K		[31]
Lysimachia clethroide	Flavonoids	Anti-tumor, anti-bacterial, anti-platelet aggregation	Methanol Acetonitrile	333-413	10	1	5-25	HPLC	Acetonitrile (50%), 373 K, 25 min	Response surface	[33]
Liriope platyphylla	Saponins	Anti-inflammatory, antitussive, expectorant	Ethanol	343-443	10	1	5-20	HPLC-CAD	Ethanol (86%), 403 K, 20 min	Response surface	[35]
Chlorella ellipsoidea	Zeaxanthin	Antioxidant, anticancer	Hexane Ethanol Isopropanol	313-430	10	1	0,7-23.3	HPLC	Ethanol, 387 K, 23.3 min	Response surface	[36]

Table 1. (Continued)

Matrix	Compounds	Related functional activities	Extraction conditions					Analytical technique[a]	Optimized PLE parameters	Statistical analysis	Ref.
			Solvent	Temperature (K)	Pressure (MPa)	Cycles	Extraction time (min)				
Peels of Citrus reticulata	Flavones	Anticancer, antibacterial, antioxidant, anti-leukaemia	Water Ethanol Methanol	353-453	10	1	5-30	HPLC-DAD	Methanol (70%), 433 K, 20 min	Comparison of means	[40]
Roots of *Cynanchum bungei*	Acetophenones	Anti-aging, gastroprotection, anti-tumor, immunoregulation	Ethanol	373-453	10	1	8-20	HPLC-UV	Ethanol, 393 K, 17 min	Response surface	[41]
Carrot	Carotenoids	Antioxidant	Ethanol	333-453	5	1-5	2	HPLC-DAD	333 K, 5 cycles	Response surface	[46]
Leaves of *Orthosiphon stamineus*	Volatile oil	Antioxidant	Ethanol	353-393	10	1-3	5-15	-	373 K, 10 min, 2 cycles	Response surface	[50]
Phomidium species	Bioactive Compounds	Antioxidant, antimicrobial	Hexane Ethanol Water	323-473	10	1	20	HPLC GC-MS	Ethanol, 473	-	[56]

Jabuticaba (M. *cauliflora*) skins	Anthocyanins	Colorant, antioxidant, free radical scavenger	Ethanol	313-393	5-10	1	3-5	UV–Vis	353 K, 5 MPa, 9 min	Response surface	[60]
Haematococcus pluvialis microalgae	antimicrobial agents	Antimicrobial	Hexane Ethanol	323-473	10	1	20	GC–MS	Ethanol, 373 ó 423 K	-	[61]
Eisenia bicyclis	Fucoxanthin	Antioxidant, anti-tumor	Ethanol	313-373	6.89-17.24	1	5-15	HPLC	Ethanol (90%), 383 K	Plackett–Burman design	[66]
Fruits of *Heracleum leskowii*	Coumarins	Anticoagulant, antifungal, antiviral	Dichloromethane Petroleum ether Methanol	353-383		1	10	HPLC-DAD	Methanol, 353 K	Comparison of means	[68]
Onions	Flavonoids	Anti-allergic, anti-inflammatory, anti-microbial, anti-cancer	Methanol	313-373	10	2	1	HPLC–UV	Methanol (65%), 313 K	Comparison of means	[70]
Sweet potato (*Ipomoea batatas* L.)	Anthocyanins	Colorant, antioxidant, free radical scavenger	Methanol/Water Acetic acid /Methanol/Water	293-413	10	3	5	HPLC-DAD	Acetic acid /Methanol/Water (7:75:18,v/v), 353-373 K	Response surface	[74]
Potato peel	Polyphenols	Antioxidant	Ethanol/Water	338-408	10	1	5	HPLC-DAD	Ethanol (75%), 398 K	Response surface	[77]
Flower of *Hylocereus undatus*	Flavonoids	Anti-allergic, anti-inflammatory, anti-microbial, anti-cancer	Methanol	353-413	7	1-3	5-20	HPLC	393 K, 1 cycle, 15 min	ANOVA	[80]
Houttuynia cordata Thunb	Flavonoids	Anti-allergic, anti-inflammatory, anti-microbial, anti-cancer	Ethanol Solvent rate 0.6-2.4 mL/min	313-343	8-20		Dynamic extraction (V 48 mL)	HPLC	Ethanol (50%), 343 K, 8 MPa, 1,8 mL/min	Orthogonal array design (OAD)	[82]

[a]HPLC, High-performance liquid chromatography; HPLC ABTS•+, High-performance liquid chromatography radical cation 2,2‘-azinobis-(3-ethylbenzothiazoline-6-sulfonate); GC/MS, gas chromatography–mass spectrometry; HPLC-CAD, High-performance liquid chromatography - charged aerosol detection; HPLC-DAD, High-Performance Liquid Chromatography - Diode-Array Detection

Table 2. Summary of the works published on the analysis of contaminant compounds in food by PLE

Matrix	Contaminant compound	Toxic effects	Extraction conditions					Analytical technique coupled [b]	Ref.
			Solvent	Temperature (K)	Pressure (MPa)	Cycles	Extraction time (min)		
Animal derived foods	Aflatoxins B1, B2, G1, G2, M1 and M2 Ochratoxin A	Teratogenic, mutagenic, carcinogenic, immunosuppressive	Acetonitrile /Hexane (50:50, v/v)	373	10	1	5	LC–MS/MS	[17]
Powdered milk Infant formulas	Bisphenol A Octylphenol Nonylphenol	Chronic toxicity Endocrine disruptor	Ethyl acetate	343	10	3	10	LC–MS/MS	[23]
Fish	PCBs PBDEs	Carcinogenic	n-hexane/dichloromethane (75:25, v/v)	373	10	3	5	in-cell clean-up	[25]
Fish, squid, bivalves, shells, octopus, shrimp	OCPs PCBs	Nephrotoxicity, leukemia, birth defects, peripheral neuropathies	Hexane/dichloromethane (50:50, v/v)	393	10	-	-	GC–MS-NCI	[28]
Peaches, melon, watermelon, apricot	Pesticide residues	Nephrotoxicity, leukemia, birth defects, peripheral neuropathies	Water	338	10	1	5	ESI-MS	[34]
Fish	PBBs	Carcinogenic, endocrine disruptor	n-hexane	373	-	3	5	GC–ITMS-MS	[42]
Wheat flours utilized in infant Foods	Herbicides: Chlormequat Mepiquat	Teratogen	Ethanol	393	10	1	5	LC–ESI–MS–MS	[44]
Popcorn packaging	PFCs	Carcinogenic	Methanol	373	10	1	6	UPLC–MS	[45]
Cereal flours	Zearalenone	Carcinogenic, estrogenic	Methanol/Acetonitrile (50:50, v/v)	323	10	1	5	LC–MS	[49]
Bakery foods	Chloropropanols	Carcinogenic	Ethyl acetate	403	10	1	5	GC-MS	[52]
Bivalves	Alkylphenols Bisphenol A	Endocrine disruptor	Methanol	313	10	2	5	LC–ESI-MS-MS	[58]
Spinach Eggplant	Organochlorine and organophosphorus pesticides	Nephrotoxicity, leukemia, birth defects, peripheral neuropathies	Dichloromethane/acetone (50:50, v/v)	298	10	3	5	GC-MS	[63]
Pistachio	Aflatoxins B1 and B2	Carcinogenic	Methanol/ Water (80:20, v/v)	353	5	1	5	HPLC	[67]
Fish	PCDDs PCDD/Fs dl-PCBs	Carcinogenic, hepatotoxicity, effects on reproduction, development and the immune system	Dichloromethane/Hexane (50:50, v/v)	373	10	1	5	HRGC–ECNI/MS	[72]
Breakfast and infants cereals	Ochratoxin A	Carcinogenic, immunosuppressive, teratogenic	Acetonitrile /Water(80:20, v/v)	313	10	1	5	LC	[83]

[b] LC–MS/MS, liquid chromatography–tandem mass spectrometry; GC–MS-NCI, gas chromatography–mass spectrometry-negative chemical ionization; ESI-MS, electrospray mass spectrometry; GC–ITMS-MS, gas chromatography coupled to ion trap tandem mass spectrometry; LC–ESI–MS–MS, liquid chromatography–electrospray ionization tandem mass spectrometry; GC-MS, ; HPLC, High-performance liquid chromatography; HRGC–ECNI/MS, high resolution gas chromatography coupled with electron capture negative ionization mass spectrometry; LC, liquid chromatograph

6. Curcuminoids Extraction by PLE

The need to develop more efficient processes, avoiding the massive use of organic solvents for the extraction of curcuminoids, has allowed the development of more rapid and environmentally friendly techniques.

After a literature survey in Web of Science and Scopus databases it was found several articles about curcuminoids extraction using various techniques. The extraction of curcuminoids by MAE[43; 76] and supercritical fluid extraction (SFE)[6; 7; 26; 76] was studied.

But, more specifically about curcuminoids extraction using pressurized fluids only 3 articles were found. Braga and Meireles[8] studied SFE using a high percentage of cosolvent into the extraction system, the process was denominated accelerated solvent extraction (ASE). The cosolvent (EtOH/IsoC_3, 1:1 v/v) percentages used were 10,

50 and 90% (v/v); however, the higher yield of curcuminoids was obtained when a concentration of 50% cosolvent was used at 313 K and 30 MPa for 30 minutes of static extraction.

Schieffer[64] compared PLE with ultrasonically assisted extractions. Methanol was used as solvent at 373 K, 10 MPa and 5 minutes of static extraction time. PLE demonstrated higher performance, due to the fact that ultrasonically assisted extraction left a small amount, but significant, of curcuminoids in the sample. Euterpio et al.[22] used water as solvent and due to the low solubility of the curcuminoids in water, was necessary to increase the extraction temperature to improve the extraction. However, at temperatures above 473 K, both curcumin and turmeric matrix were rapidly degraded and the extracted rhizome particles took on a deep dark brown color resulting in poor extraction yields. This situation was improved by acidifying the water (pH 1.6) and using phosphate buffer (62 g/L), which increased the solubility of curcuminoids in a dynamic extraction process with solvent rate of 0.5 mL/min at 370 K and 5 MPa.

Although curcuminoids have many potential uses in industry, until now the study by Braga and Meireles[8] has been the only study to use pressurized liquids in order to study the effect of the extraction parameters of the process of separation of the volatile oil and oleoresin using fractionated SFE with different solvents, in order to maximize the extract yield and curcuminoid content.

7. Conclusions

This review has shown that PLE is a highly selective process that uses short periods of extraction and small amounts of organic solvents. PLE is presented as an excellent alternative to replace the conventional techniques of extraction, to have a potential use in the extraction of bioactive compounds and the assessment of food contaminants when it is coupled to an analytical technique.

Likewise, PLE represents a good alternative for extraction of curcuminoids, ensuring high extractability, producing high purity extracts in conditions that avoid the degradation of curcuminoids.

However, there is still a lack of studies (only 3 articles were found in the journals indexed in the Web of Science and Scopus databases) that allow establish the influence of different parameters on the extraction process of curcuminoids using PLE and determine the best conditions for the extraction process, which in turn allows to study the viability and scale up process to implementing a production line of curcuminoids extracts using PLE.

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-CAPÍTULO 3-

Fast analysis of curcuminoids from turmeric (Curcuma longa L.) by high-performance liquid chromatography using a fused-core column

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Fast analysis of curcuminoids from turmeric (*Cúrcuma longa* L.) by high-performance liquid chromatography using a fused-core column

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Abstract

The recent development of fused-core technology in HPLC columns is enabling faster and highly efficient separations. This technology was evaluated for the development of a fast method for the analysis of main curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) present in extracts of turmeric (*Curcuma longa* L.). A step-by-step strategy was used to optimize temperature (40-55 ºC), flow rate (1.0-2.5 mL min^{-1}), mobile phase composition and equilibration time (1-5 min). A gradient method was developed using acidified water and acetonitrile combined with high column temperature (55 ºC) and flow rate (2.5 mL min^{-1}). Optimized conditions provided a method for the separation of these three curcuminoids in approximately 1.3 min with a total analysis time (sample-to-sample) of 7 min, including the clean-up and the re-equilibration of the column. Evaluation of chromatographic performance revealed excellent intraday and interday reproducibility (>99%), resolution (>2.23), selectivity (>1.12), peak symmetry (>1.24) while presenting low limits of detection (<0.40 $mg.L^{-1}$) and quantification (<1.34 $mg.L^{-1}$). The robustness of the method was calculated according to the concentration/dilution of the sample and the injection volume. Several sample solvents were evaluated and the best chromatographic results and extraction rate were obtained using 100% methanol. Finally, the developed method was validated with different extracts of turmeric rhizome and products that use turmeric in their formulation.

Keywords: *Analysis; Curcuminoids; Fused-core columns; HPLC; Curcuma longa L.*

1. Introduction

Turmeric (*Cúrcuma longa* L.) has been used since ancient times as a seasoning, preservative, flavoring and coloring agent as well as folk medicine for treating several types of diseases. Turmeric has been largely investigated due to its potential benefits on human health and bioactive properties. They may act as potent antioxidants by inducing endogenous cellular antioxidant defense mechanisms (Al-Reza, Rahman, Sattar, Rahman, & Fida, 2010) and also have been recognized for their biological activity associated with anticancer (Aggarwal, Kumar, & Bharti, 2003; Kuttan, Bhanumathy, Nirmala, & George, 1985), antibacterial (Wilson, Abraham, Manju, Mathew, Vimala, Sundaresan, et al., 2005), antiviral (Dao, Nguyen, Won, Kim, Park, Won, et al., 2012), chemopreventive and chemotherapeutic activities (Hatcher, Planalp, Cho, Tortia, & Torti, 2008).

Nowadays, turmeric application as a dye is increasing greatly due to the interest of replacing synthetic additives by natural compounds (Ravindran, Babu, & Sivaraman, 2007). The yellow color of the rhizomes is due to the presence of a group of phenolic compounds named curcuminoids (Figure 1). In turmeric rhizomes there are three main curcuminoids (Figure 1): curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC).

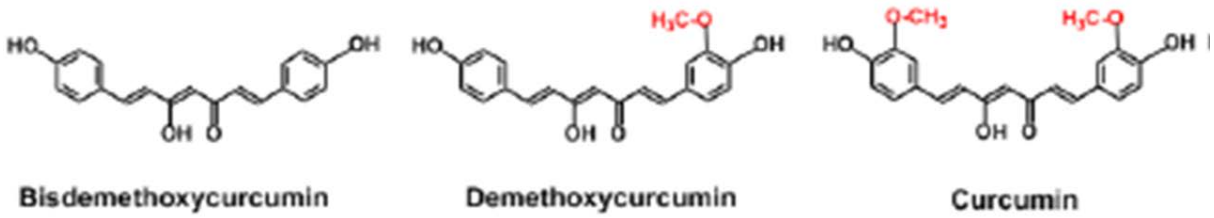

Figure 1. Structure of the tree main curcuminoids found in turmeric rhizomes.

A variety of HPLC methods for the quantification of the curcuminoids have been reported. Jayaprakasha, Rao, and Sakariah (2002) reported a suitable HPLC method for the routine analysis of a large number of commercial samples of *C. longa* with a runtime of 20 minutes. Another method was developed by Li, Xiang, Ye, Li, Zhang, and Guo (2011) for the analysis of curcuminoids in herbal medicines derived from Curcuma species with a runtime of 60 minutes. Wichitnithad, Jongaroonngamsang, Pummangura, and Rojsitthisak (2009) developed an isocratic method for the determination of individual curcuminoids for the quality control of turmeric extracts achieving the separation in 16 minutes. Currently, the interest in the separation of curcuminoids by using HPLC is increasing, but all methods reported to date require between 8 and 18 minutes to reach an acceptable separation of the

main curcuminoids present (Ali, Haque, & Saleem, 2014; Kim, Lee, & Shin, 2013; Koop, de Freitas, de Souza, Martinez, & Silveira, 2013; Long, Zhang, Wang, & Chen, 2014). Furthermore, since this analysis time range does not include the time necessary to clean the column for elution of other compounds present in the sample, to return to initial conditions and to re-equilibrate the column/detector before the next run, the actual sample-to-sample interval is much greater.

In contrast, it is possible to greatly reduce analysis time by employing ultra-performance liquid chromatography (UHPLC) systems. Illustratively, an UHPLC method was developed by Cheng, Weijun, Yun, Jiabo, Haitao, Qingmiao, et al. (2010) for the rapid quantification of curcuminoids in *Curcuma longa* Linn. (C. longa) with a runtime of 2 min. The performance of UHPLC methods in terms of resolution and time is largely due to the use of short columns packed with small diameter particles. However, the main disadvantage of sub-2-μm particles is the increased column backpressure generated which makes it impossible to use them in conventional HPLC systems. To overcome this problem a new type of superficially porous silica particles, termed "fused-core particles" or "core-shell", was developed. The characteristic of those particles is a solid core wrapped with a thin, porous outer layer with an overall diameter less than 3 μm.

Columns packed with superficiality porous particles match the efficiency of columns with sub-2-μm totally porous particles, but with only about one-half the operating pressure (Manchón, D'Arrigo, García-Lafuente, Guillamón, Villares, Martínez, et al., 2011). The high efficiency may be a result of narrower particle size distribution that makes the packing more homogeneous reducing eddy diffusion and improving mass transfer (Borges, Rostagno, & Meireles, 2014; González-Ruiz, Olives, & Martín, 2015; M. A. Rostagno, Debien, Vardanega, Nogueira, Barbero, & Meireles, 2014).

Therefore, fused core technology has a great prospect for analysis of natural products with complex matrixes like turmeric. In fact, there are several applications of this new technology for the analysis of a number of different classes of compounds present in the most diverse types of samples, such as phenolics in tea, coffee, mate and wine, isoflavones in soybeans, flavonoids, among others. (Aznar, Checa, Oliver, Hernández-Cassou, & Saurina, 2011; Borges, Rostagno, & Meireles, 2014; Manchón, et al., 2011; Olszewska, 2012; M. A. Rostagno, Debien, Vardanega, Nogueira, Barbero, & Meireles, 2014; Verardo, Riciputi, Garrido-Frenich, & Caboni, 2015).

The characteristics of the methods available in the recent literature have some disadvantages including the use of complex solvent mixtures, poor resolution and relatively

long analysis runs, combined with availability of new column technology suggest that separation of curcuminoids has a great potential for overcome these drawbacks. Since there are no applications of fused-core columns for the analysis of curcuminoids in foods in the literature, the aim of the present work was to evaluate the suitability of this new column technology for the development of a fast HPLC method for the analysis of these compounds.

2. Materials and Methods

2.1. Chemicals and solvents

HPLC grade acetonitrile and methanol were obtained from Scharlau (Barcelona, Spain), acetic acid was obtained from Ecibra (Sao Paulo, Brazil) and ethanol was obtained from Dinâmica (Sao Paulo, Brazil). Standards (> 95%) of curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) were obtained from Sigma-Aldrich. Ultra-pure water was supplied by a Milli-Q Advantage 8 Water Purifier System from Millipore (Bedford, MA, USA).

2.2. Samples

Noodles, mustard sauce, curry powder, instant pumpkin soup and instant noodle seasoning powder were obtained from a local supermarket in Campinas - Brazil. Turmeric rhizome was obtained from Oficina Das Ervas (Ribeirão Petro, Brazil). Upon receiving the samples, they were stored on a domestic freezer (-20^{o}C) before being processed for the extraction. Before the extraction, samples were milled on a knife mill (Marconi, model MA340, Piracicaba, Brazil) and passed through a set of sieves (24-48 mesh) and the larger and smaller particles were eliminated from the sample. Methanolic stock solutions of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were prepared separately at a concentration of 150 mg/L and diluted in methanol for the preparation of the standard curve. Stock solutions were stored at -32 ºC until chromatographic analysis.

2.3. Ultrasound-assisted extraction

To evaluate the influence of the extraction solvent on the chromatographic performance of the analysis method the samples were extracted with ethanol (100%, 75%, 50% and 25%) and methanol (100%, 75%, 50% and 25%) on an ultrasound bath (40 kHz and 135 W, model Max Clean 1400, Unique, Indaiatuba, Brazil). The extraction time was fixed in 20 minutes and the solvent to sample ratio was 10:1 (i.e. 10 mL of solvent for 1.0 g of sample), except for noodles that employed a 2:1 ratio (10 mL of solvent for 5.0 g of sample), due its lower turmeric content regarding the other samples. After the extraction, all the

extracts were filtered through filter paper and the volume measured. Afterwards, the extract was diluted with methanol to half the concentration and an aliquot of the extract was filtered through a 0.45 μm nylon seringe filter (Sinergia Cientifica, Campinas, Brazil) directly into 1.5 mL HPLC vials. The vials were then transferred to the HPLC system for analysis. All the extractions were performed in triplicate.

2.4. High performance liquid chromatography (HPLC)

HPLC analysis was performed on an Alliance 2695/2695D Separations Module (Waters, Milford, USA) equipped with a chromatographic oven and a 2998 photodiode array detector (Waters, Milford, USA). The software for control and data collection was Empower 3 (Waters, Milford, MA, USA). Separation of curcuminoids was carried out on a fused-core C_{18} column (Kinetex, 100 × 4.6 mm i.d.; 2.6 μm; Phenomenex, Torrance, CA, USA) using a gradient of acidified water (0.1% (v/v) acetic acid, solvent A) and another acidified solvent (acetonitrile or methanol) with 0.1% (v/v) acetic acid (solvent B). Using an ethanolic extract of turmeric rhizomes obtained by ultrasound assisted extraction as sample, different mobile phase compositions (mixtures of acidified water (0.1% v/v acetic acid) and acidified methanol (0.1% v/v acetic acid) or acidified acetonitrile (0.1% v/v acetic acid)), temperatures (35-55 ºC), flow rates (1.0-2.5 mL min^{-1}) and equilibration times (1-5 min) were tested. UV absorbance was monitored between 240-600 nm and the peaks of the curcuminoids were integrated at 425 nm. Identification of the curcuminoids present in the samples was achieved by the comparison of retention times and UV spectra of separated compounds with the authentic standard. Column efficiency was evaluated on basis of retention time, peak width, *K* prime, selectivity, symmetry factors, width at baseline and resolution of the three peaks of curcuminoids: curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). All performance parameters were calculated using the US Pharmacopeia (USP) choice using the Empower 3 software. The standard curve of each curcuminoid was prepared by plotting the concentration (0.1; 0.25; 0.5; 1; 2.5; 5; 10; 25; 50; 75 and 100 $mg.L^{-1}$) against area of the peak. Regression equations, correlation coefficient (r^2) and a lack-of-fit test were calculated using Minitab® v. 16 software. The lack-of-fit test was performed according the metodolgy described by (Araujo, 2009) through a *F*-test. Detection and quantitation limits (LOD and LOQ, respectively) were determined by considering a value 10 times the deviation of the background noise obtained from blank samples (n = 10) divided by the slope of the calibration curve line and a value 10 times the deviation of the background noise obtained for blank samples (n = 10) divided by the slope of the calibration curve line, respectively.

3. Results and discussion

3.1 Development of the HPLC method

For the initial separation a series of runs using a linear gradient of solvent A to solvent B (0% to 100%) were tested by modifying the initial concentration of solvent B in solvent A while maintaining the analysis time fixed in 10 minutes and a flow-rate of 1 $mL.min^{-1}$. The initial tests were performed using a mobile phase composed by water with 0.1% of acetic acid, as solvent A, and methanol or acetonitrile (solvent B) with 0.1% of acetic acid, as solvent B. Using pure acetonitrile, the system back pressure was lower than with pure methanol. A similar difference in pressure was also obtained by mixing these solvents with water during the gradient, although at different proportions. It was observed that the highest system pressure using mixtures of methanol and acetonitrile with water was obtained with 60% and 80% of water, respectively. Additionally, it was observed a much lower pressure of the mixture with water (approximately 60%) was produced by acetonitrile when compared to methanol. On the other hand, an overall better partial separation and peak shape was obtained with acetonitrile when compared to methanol as well as a faster separation of the peaks. In terms of initial concentration of acetonitrile, the best resolution was obtained starting the gradient with 40-60% of this solvent. Due to the lower backpressure generated associated with an increased chromatographic performance, acetonitrile, was selected as mobile phase B to continue the development of the method.

Using a gradient of acidified water to acidified acetonitrile (0-100%) in 10 minutes the influence of column temperature (35-55 ^{o}C) was evaluated. The use of higher temperatures is a useful tool to reduce analysis time since mobile phase viscosity is significantly reduced which in turn decreases the pressure drop across the column allowing higher linear velocities of the mobile phase. In addition, as known by the Strokes-Einstein relationship, the diffusion coefficient is directly proportional to the absolute temperature and inversely proportional to the viscosity. The lower viscosity and higher diffusivity of a mobile phase at high temperatures produce much lower mass transfer resistance, thereby decreasing the peak width and leading to flatter van Deemter curves. Therefore, on increasing column temperature it can be expected an improvement of analyte resolution through an increased diffusion coefficient of the mobile phase and a lower mass transfer resistance (Borges, Rostagno, & Meireles, 2014; Manchón, et al., 2011; Nováková & Vlčková, 2009; M. A. Rostagno, Debien, Vardanega, Nogueira, Barbero, & Meireles, 2014).

Based on these principles, the column temperature was gradually increased from 35 to 55 ºC, in 5 ºC intervals. Increasing the column temperature to 45, 50 and 55 ºC from 35 ºC resulted in a significant reduction of retention time of the three curcuminoids. It was also observed a clear trend with the increase of the temperature of the column, with narrower peaks, increased peak heights and better resolution in the separation of the three curcuminoids present in the sample. Another positive effect of increasing column temperature was the significant reduction of the column backpressure generated. However, it is also important not to exceed the column maximum operating temperature (60 ºC) since it may damage the column. Therefore 55 ºC was selected as the maximum working temperature and used for further development of the method.

Once the optimum temperature was selected, the reduced column back pressure allowed exploring the flow-rate to shorten analysis time. Consequently, the flow rate was increased step-by-step from 1.0 to 2.5 mL min^{-1}. The maximum flow rate was determined by the system pressure's limitation, which was set to 5000 psi. As the flow-rate was increased, a proportional reduction of the gradient was applied in order to maintain the separation of the three peaks. For example, if the flow rate was doubled, the gradient time was reduced to half while maintaining the same percentage of solvents of the mobile phase. It was observed that by increasing the flow rate the analysis time was shortened proportionally and that peak width is reduced maintaining an optimum separation of the three chromatographic peaks.

This performance is only possible due to the characteristic van Deemter curve of fused-core columns, allowing the use of higher linear velocities without affecting column efficiency. In fact, the combination of a fused-core column with a mobile phase with low viscosity and high temperature greatly reduce the system pressure to a point that it is not only possible to increase flow-rate but it is necessary, otherwise the low mobile phase velocity will reduce column efficiency. It was clear that combination of the operational conditions allowed only partially exploring the potential of the fused-core column, since flow-rate was limited by the system´s pressure limitation and not by the column pressure limitation (~8700 psi).

However, separation of all compounds using higher temperatures (55 ºC) and flow-rates (2.5 mL min^{-1}) was not satisfactory and different initial and final concentration of solvent B and duration of the linear gradient were tested by using small steps to change the inclination of the gradient curve towards solvent B. After several trials and errors, the best separation was achieved a linear gradient starting from 45% of solvent B to 65% in 1.5 minutes. Separation of all curcuminoids was achieved in approximately 1.3 minutes which is an extremely short time for the separation of three structurally similar compounds. It is

important to notice that the gradient continues and include a clean-up step. The gradient uses 1.5 min at 90% of mobile phase B for column clean-up (2.5-4.0 min) and 1 min to return to initial conditions (4.0-5.0 min). This is an important aspect which is often disregarded when developing gradient methods. The clean-up of the column is extremely necessary when dealing with natural products since these are complex samples and may present several different classes of compounds that may remain in the column after the analytes of interest left the column. In this case, a very short time was necessary to clean the column and return to initial conditions due to the high flow-rate used.

However, total analysis time is the amount of time from injection to injection and includes the run time, column clean-up and re-equilibration time. Re-equilibration time is necessary in gradient HPLC in order to ensure that the column has returned to initial conditions after clean-up and that detector signal is stable. These conditions are particularly important when using gradient elution since the difference between initial and final organic composition of the mobile phase is significant. The importance of equilibration time is even greater since the failure to optimize re-equilibration time can lead to unnecessary overextension of analysis time, with the increased cost and reduced sample output associated.

Usually, equilibration time is recommended by manufacturers on basis of the column volume and flow rate. Standard recommendations are approximately 10 times the column volume, although it depends on the applications and more importantly the mobile phases and gradient used. In this study, all previous sets of experiments were carried out using 4 min between runs, which is equivalent to approximately 45% of the total method duration (including elution, clean-up and re-equilibration times) and equivalent to 15.4 volumes of the column. Therefore, in order to keep this equilibration time as low as possible, and consequently reduce the total method duration, shorter re-equilibration times (1-3 min) were evaluated.

Equilibration time was implemented as a delay after the mobile phase composition returned to initial conditions (5.0 min), after which a new sample was injected into the column. Using 4 min to re-equilibrate the column between runs provided a mean (n=18; interday) area and retention time variability lower than 0.3 and 0.2% respectively. By reducing the equilibration time to 3, 2 and 1 min resulted in mean area variability values lower than 0.3, 0.4 and 0.8%, and mean retention time variability values lower than 0.3, 0.3 and 0.5%, respectively. Although with the use of very short re-equilibration times variability was within the normal range, a slight higher reproducibility for the analysis of the three curcuminoids was observed by using equilibration times higher than 2 min, and therefore 2

minutes was considered as the most appropriate re-equilibration time in order to achieve the highest possible reproducibility while not over extending total run time. This equilibration time is equivalent to 7.7 times the column´s volume and slightly lower than that recommended. This is in agreement with previous studies showing that for other compounds present in natural products very short equilibration times can be used with fused-core columns. (Manchón, D'Arrigo, García-Lafuente, Guillamón, Villares, Ramos, et al., 2010; M. Rostagno, Manchón, D'Arrigo, Guillamón, Villares, García-Lafuente, et al., 2011; M. A. Rostagno, Debien, Vardanega, Nogueira, Barbero, & Meireles, 2014; Zabot, Moraes, Rostagno, & Meireles, 2014).

3.2 Characteristics of the HPLC method

Briefly, the optimized conditions use a simple gradient (0 min, 45% of solvent B; Step 1: 1.5 min, 65% solvent B; Step 2: 2.5 min, 90% solvent B; Step 3: 4.0 min, 90% solvent B; Step 4: 5.0 min, 45% solvent B) with a fuse-core column maintained at 55 ºC, working with a flow rate of 2.5 mL/min and employing 2 minutes of re-equilibration time between runs. These conditions provide the best balance between analysis time and separation of the three curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin). The chromatographic properties of the developed method are reported in Table 1.

Table 1. Chromatographic characteristics of the developed method.

	RT (min)	Width	K prime	Selectivity	Resolution	Symmetry Factor	Width at baseline
BDMC	1.07	6.65	1.68		2.34	1.42	0.11
DMC	1.17	5.47	1.92	1.14	2.23	1.30	0.09
C	1.26	8.44	2.15	1.12	2.31	1.24	0.17

With the developed method, retention time of BDMC, DMC and C were 1.07, 1.17 and 1.26 minutes respectively. Total run time is 5 minutes and total analysis time (sample-to-sample) is 7 minutes, including the column clean-up and re-equilibration. Resolutions of BDMC, DMC and C peaks were calculated as 2.34, 2.23 and 2.31, respectively. As can be seen in Figure 2, the developed method achieved an excellent separation between the three peaks of curcuminoids. The width of peaks, *K* prime, selectivity, symmetry factors and width at baseline were also calculated by Empower 3 software as are shown in Table 1. These results also indicate an excellent chromatographic performance of the fused-core column for the separation of curcuminoids.

Several parameters of the method, including linearity, repeatability (intraday and interday), limit of quantification (LOQ) and limit of detection (LOD), were also evaluated.

The linearity was verified with an analytical curve consisting of eleven points (0.1-100 $mg.L^{-1}$; in triplicate) for each of the three curcuminoids. A Lack-of-fit test was applied to check statistical significance of the regression equations at significance level of 0.05. According to the results, for all analytes the model fits the data well in a linear range of 100 – 0.5 $mg.L^{-1}$, 75 – 0.1 $mg.L^{-1}$ and 75 - 0.25 $mg.L^{-1}$ for BDMC (p_{value} = 0.071), DMC (p_{value} = 0.137) and C (p_{value} = 0.059), respectively. The LOQ and LOD were estimated as 3 and 10 times the signal-to-noise ratio, respectively. The repeatability and reproducibility of the developed method was evaluated in terms of peak area, peak resolution and retention time of the three curcuminoids. A total of 30 HPLC analyses were performed on three successive days (10 analyses per day) using the same sample, a methanolic extract of turmeric rhizomes obtained by ultrasound assisted extraction. The intraday and interday relative standard deviation of retention time, peak area and peak resolution are shown in Table 2.

In terms of retention time, the resulting RSD values were lower than 0.67% for repeatability and less than 0.78% for reproducibility. Regarding peak area, the resulting RSD values were lower than 0.80% for repeatability and lower than 0.88% for reproducibility. In terms of resolution, RSD values were lower than 0.96% for repeatability and less than 1.02% for reproducibility.

Table 2. Validation parameters for the developed method.

	Calibration curve	*n*	R^2	F-test	LOD (ppm)	LOQ (ppm)	Retention time		Peak area		Peak resolution	
							Intraday*	Interday**	Intraday*	Interday**	Intraday*	Interday**
BDMC	y = 52661x - 46072	9	0.9999	17233.74	0.19	0.64	0.67	0.78	0.80	0.88	0.96	1.02
DMC	y = 52952x - 15770	10	0.9999	55587.37	0.20	0.67	0.57	0.70	0.45	0.59	0.70	0.79
C	y = 66075x - 1610	9	0.9999	86017.46	0.40	1.34	0.48	0.65	0.50	0.60	0.51	0.61

n, number of concentration levels (data points) used for construction of the regression equation; F-test, value of the statistical Fisher variance ratio for the experimental data (the critical values at α = 0.05 are 2.373 for n = 9 and 2.266 for n = 10 calibration data points)
* Intraday Relative Standard Deviation (%) (n = 10).
* Interday Relative Standard Deviation (%) (n = 30).

3.3 Sample concentration/dilution of the sample

Most samples are expected to contain some amount of organic solvents, as they were obtained by extraction methods, which employ organic solvents, mainly methanol or ethanol. Furthermore, in several methods, a final concentration step is included to increase the analytical signal in the detection systems, thus changing the initial solvent concentration. As a result, the robustness of the chromatographic method related to the sample dilution should be

checked. Different dilutions factors ([Xo]/2, [Xo]/3, [Xo]/4 and [Xo]/5) with methanol of the initial sample were studied. The results for resolution, concentration, width of the peak, *K* prime, selectivity, symmetry factor and also peak retention time for BDMC, DMC and C is presented in Table 3.

Table 3. Effect of sample concentration on the chromatographic performance of the developed method.

Dilution	Compound	RT (min)	Concentration (mg L^{-1})*	Width (s)	*K* prime	Selectivity	Resolution	Symmetry factor
	BDMC	1.07	9.33	6.65	1.68			1.42
[Xo]/1	DMC	1.17	12.48	5.47	1.92	1.14	2.23	1.30
	C	1.26	30.36	8.44	2.15	1.12	2.31	1.24
	BDMC	1.07	4.68	6.62	1.68			1.46
[Xo]/2	DMC	1.16	5.68	5.64	1.91	1.14	2.22	1.34
	C	1.26	15.18	8.50	2.15	1.12	2.30	1.28
	BDMC	1.07	3.07	6.71	1.67			1.53
[Xo]/3	DMC	1.16	3.8	5.63	1.90	1.14	2.18	1.38
	C	1.26	10.09	8.45	2.14	1.12	2.27	1.31
	BDMC	1.07	2.27	6.61	1.68			1.58
[Xo]/4	DMC	1.16	2.83	5.48	1.91	1.14	2.15	1.41
	C	1.26	7.47	8.41	2.15	1.12	2.25	1.33
	BDMC	1.07	1.63	6.60	1.68			1.65
[Xo]/5	DMC	1.17	2.29	5.56	1.91	1.14	2.13	1.43
	C	1.26	5.92	8.42	2.15	1.12	2.23	1.35

* Concentration of curcuminoids in the extract (10 mL of extract).

Regarding peak resolution, the developed method showed that a dilution of the sample did not affect the separation between the three curcuminoids. It was further verified that a lower concentration of the sample did not affect the reproducibility of the method. It was also confirmed that the sample dilution within the studied range did not significantly affect the retention time, width of the peaks, K prime, selectivity and symmetry factor of the tree peaks studied. Finally it should be noticed that ideally the sample should be with a curcuminoid concentration below 75 mg L^{-1}. Furthermore, between 15 and 75 mg L^{-1} there are no noteworthy differences in the chromatographic performance.

3.4 Injection volume

There are several types of samples where the target analytes may be present in low concentration in the extract obtained by the extraction method. In these cases, analysts sometimes choose to overcome this problem by injecting a larger volume of the sample to add

enough amounts of analytes to quantify them. However, injection of a greater volume of sample may dramatically affect the resolution and peak shape. This effect can be important depending on the solvent in which the sample is dissolved and the solvent of the mobile phase. As a result, the robustness of the chromatographic method related to the injection volume should be checked. In this context, different injection volumes (10, 20, 30, 40 and 50 µL) and different dilutions while maintaining constant the amount of curcuminoids injected (1X, 2X, 3X, 4X and 5X respectively) were combined to simulate the analysis of a diluted sample by using a higher injection volume. The results in term of resolution, concentration, peak width, *K* prime, selectivity, symmetry factor and peak retention time for BDMC, DMC and C are presented in Table 4.

Table 4. Effect of injection volume on the chromatographic performance of the developed method.

Injection volume (µL)	Compound	RT (min)	Concentration (mg L^{-1})*	Width (s)	*K* prime	Selectivity	Resolution	Symmetry factor
	BDMC	1.07	9.33	6.65	1.68			1.42
10	DMC	1.17	12.48	5.47	1.92	1.14	2.23	1.30
	C	1.26	30.36	8.44	2.15	1.12	2.31	1.24
	BDMC	1.07	9.74	7.33	1.68			-
20	DMC	1.16	11.70	5.66	1.91	1.14	1.47	-
	C	1.26	31.77	12.15	2.15	1.12	1.50	1.13
	BDMC	1.08	9.07	7,59	1.69			-
30	DMC	1.17	11.23	5.37	1.93	1.14	1.03	-
	C	1.26	31.05	14.82	2.16	1.12	1.07	-
	BDMC	1.09	13.06	10.95	1.72			-
40	DMC	1.18	14.96	5.55	1.95	1.13	0.76	-
	C	1.27	20.79	10.53	2.18	1.12	0.77	-
	BDMC	1.09	19.47	15.95	1.73			-
50	DMC	1.18	11.53	5.24	1.94	1.12	-	-
	C	1.27	17.80	10.25	2.17	1.12	-	-

* Concentration of curcuminoids in the extract.

Regarding concentration, peak area, resolution, width, and symmetry factor of the peaks it can be observed that separation of these three curcuminoids is highly dependent of the injection volume. With an injection volume of 20 µl there is significant a loss of resolution in the chromatographic separation of the three curcuminoids, although they retain their shape. Injection of sample volumes higher than 30 µl causes a negative effect on peak shape and symmetry causing double peaks and leading to serious overlapping. It is important to notice that the optimum injection volume for the developed method is 10 µL (or lower) but

higher injection volumes can be used to some extent to increase the amount of curcuminoids that reach the detector allowing the analysis of samples with small amounts of curcuminoids. The drawback is that there is an associated loss of performance and peak shape which will increase as the injection volume increase. This effect is caused by an increased diffusion of the sample in the solvent leading to differences in the retention of the curcuminoids molecules and to poor peak shape.

3.5 Sample solvent

In order to study the effect of the sample (i.e. extraction) solvent on chromatographic performance of the methods, several extractions of a turmeric rhizome (*Cúrcuma longa* L.) were performed with different solvents, as detailed in Section 2.3. The solvents used in the extractions were methanol (25, 50, 75 and 100%) and ethanol (25, 50, 75 and 100%), both solvents diluted with water. The extracts obtained with each solvent were analyzed using the chromatographic method developed to test whether the extraction solvent affects the chromatographic separation of the peaks studied. The studied parameters were the retention time, the amount of curcuminoids extracted, width of the peaks, *K* prime, selectivity, resolution and symmetry factor for the three chromatographic peaks of curcuminoids. Injection volume was set at 10 µL and every extraction was done in triplicate. The obtained results for each solvent are shown in Table 5.

It can be observed in Table 5 that higher the percentage of methanol or ethanol in the extraction solvent leads to higher amount of curcuminoids in the extracts. It is also noteworthy that 100% of methanol extracted greater amount of curcuminoids than 100% ethanol. When 25% of ethanol was used solvent, the curcuminoids are not extracted. On the other hand, percentages of methanol higher than 50% are required in order to extract the curcuminoids. This is due to the differences in polarity of methanol in comparison to the ethanol. These results indicate that solubility of curcuminoids is greatly affected by the solvent and that methanol and ethanol with small amounts of water still are efficient for their solubilization.

It is important to notice that the purpose of the test was to evaluate the influence of the extraction solvent on the chromatographic performance of the HPLC method. In this context, it can be observed ethanol provided wider peaks than methanol and that adding small amounts of water to the extraction solvent improved peak characteristics. The best chromatographic resolution was obtained when 75% of methanol was used. This is an

important information since it is quite common the need of diluting the sample to avoid saturating the detector during the analysis.

Table 5. Effect of the sample solvent on the chromatographic performance of the developed method.

Solvent	Compound	RT (min)	Concentration (mg L^{-1})*	Width (s)	*K* prime	Selectivity	Resolution	Symmetry factor
25% EtOH	BDMC	1.08	0.003	3.40	1.70			1.23
	DMC	1.18	0.006	3.70	1.95	1.14	2.00	1.19
	C	1.27	0.004	3.60	2.17	1.12	1.77	1.08
50% EtOH	BDMC	1.07	2.127	6.25	1.69			1.44
	DMC	1.17	6.055	5.65	1.92	1.14	2.33	1.39
	C	1.26	7.158	11.30	2.16	1.12	2.39	1.33
75% EtOH	BDMC	1.07	5.284	6.75	1.67			1.49
	DMC	1.16	12.477	5.55	1.90	1.14	2.10	1.40
	C	1.26	14.454	10.85	2.14	1.13	2.12	1.34
100% EtOH	BDMC	1.06	9.846	7.10	1.66			-
	DMC	1.15	24.844	5.55	1.89	1.14	1.53	-
	C	1.25	30.354	11.85	2.13	1.13	1.51	1.15
25% MeOH	BDMC	1.08	0.003	3.60	1.70			1.21
	DMC	1.18	0.006	4.10	1.94	1.14	2.26	1.29
	C	1.27	0.004	4.45	2.18	1.12	2.11	1.39
50% MeOH	BDMC	1.08	0.003	3.75	1.70			1.53
	DMC	1.17	0.009	3.65	1.93	1.14	2.19	1.48
	C	1.27	0.014	4.85	2.17	1.12	2.18	1.86
75% MeOH	BDMC	1.07	5.538	6.95	1.68			1.38
	DMC	1.17	12.876	5.50	1.92	1.14	2.39	1.33
	C	1.26	14.827	10.85	2.16	1.13	2.44	1.27
100% MeOH	BDMC	1.07	10.423	6.60	1.67			1.39
	DMC	1.16	25.607	5.50	1.91	1.14	2.24	1.32
	C	1.26	30.766	9.25	2.15	1.13	2.29	1.26

* Concentration of curcuminoids in the extract (10 mL of extract).

Our results indicate that the best solvent to dilute the sample is water since it improved the chromatographic performance. Although the solubility of curcuminoids is reduced by adding a small amount of water to the extract, it is still possible to solubilize curcuminoids and analyze the sample with improved performance.

A recovery test was performed in order to evaluate the accuracy of this method. The procedure used to evaluate the accuracy was similar to the described by Li, Xiang, Ye, Li, Zhang, and Guo (2011) with some modifications. Approximately 0.15 g of the turmeric rhizomes sample 2 were extracted with 30 mL of methanol using the procedures discussed in Section 2.3. A 700 µL aliquot was transferred in a vial for HPLC analysis. Three different concentrations (high, middle, low) of three curcuminoids were added from stock solutions of 100 $mg.L^{-1}$ into the vials. In terms of accuracy, the method had a satisfactory accuracy with the overall recovery of three analytes ranging for 97.0% to 103.3% (Table 6).

3.6. Application to real samples

A recovery test was performed in order to evaluate the accuracy of this method. The procedure used to evaluate the accuracy was similar to the described by Li, Xiang, Ye, Li, Zhang, and Guo (2011) with some modifications. Approximately 0.15 g of a turmeric rhizome (sample 2) were extracted with 30 mL of methanol using the procedures discussed in Section 2.3. A 700 µL aliquot was transferred in a vial for HPLC analysis. Three different concentrations (high, middle, low) of three curcuminoids were added from stock solutions of 100 $mg.L^{-1}$ into the vials. In terms of accuracy, the method had a satisfactory accuracy with the overall recovery of three analytes ranging for 97.0% to 103.3% (Table 6).

Table 6. Recoveries of three curcuminoids (n = 3).

Analytes	Original amount (µg)	Spiked (µg)	Found (µg)	Recovery (%)	RDS (%)
BDMC	15.663	5	20.519	99.1	3.22
	15.640	3.5	18.677	97.0	
	15.310	2	17.820	103.3	
DMC	17.826	5	22.283	97.0	2.45
	17.809	3.5	21.453	100.8	
	17.478	2	19.739	101.5	
C	26.056	5	31.094	100.1	1.28
	26.007	3.5	28.953	97.9	
	25.679	2	27.670	100.0	

The developed method was also used for the analysis of other samples of turmeric rhizome and different products where turmeric extract is used, such as curry, mustard sauce, instant pumpkin soup, noodles and instant noodle seasoning powder in order to determine the curcuminoids content (Table 7).

Table 7. Concentration of curcuminoids (mg g-1 FW ± RSD) in turmeric rhizome and different turmeric byproducts.

	BDMC*	DMC*	C*	Total Curcuminoids*
Turmeric rhizome sample 1	2.139 ± 0.019	5.863 ± 0.035	10.237 ± 0.061	18.239 ± 0.115
Turmeric rhizome sample 2	6.122 ± 0.025	6.976 ± 0.019	10.211 ± 0.017	23.310 ± 0.045
Curry sample 1	0.217 ± 0.002	0.628 ± 0.004	1.421 ± 0.009	2.266 ± 0.014
Curry sample 2	0.152 ± 0.002	0.103 ± 0.003	0.201 ± 0.002	0.457 ± 0.007
Curry sample 3	0.149 ± 0.003	0.139 ± 0.003	0.303 ± 0.013	0.591 ± 0.012
Mustard	0.020 ± 0.000	0.041 ± 0.000	0.064 ± 0.000	0.125 ± 0.001
Instant pumpkin soup	0.032 ± 0.000	0.020 ± 0.000	0.029 ± 0.000	0.081 ± 0.001
Noodles	$2.6*10^{-4}$ ± 0.000	$7.9*10^{-4}$ ± 0.000	$1.34*10^{-4}$ ± 0.000	$2.4*10^{-4}$ ± 0.000
Instant noodle seasoning powder	0.011 ± 0.000	0.006 ± 0.000	0.007 ± 0.000	0.024 ± 0.001

* (mg g^{-1} FW ± RSD).

A representative chromatogram obtained with each sample is presented in Figure 2. It can be observed that turmeric rhizome (Figure 2A) samples had a total amount of curcuminoids of 18.2 and 23.3 mg of curcuminoids per gram of rhizome for samples 1 and 2, respectively. The turmeric byproduct with a higher amount of curcuminoids is curry (Figure 2B) which has 2.3 mg of curcuminoids per gram of fresh product for sample 1. Mustard (Figure 2C), instant pumpkin soup (Figure 2E) and instant noodle seasoning powder (Figure 2F) are other products made with turmeric, but they only have a small amount of curcuminoids (0.13 mg of curcuminoids per gram of mustard, 0.081 mg of curcuminoids per gram of instant soup and 0.024 mg of curcuminoids per gram of seasoning powder). The product with lower concentration of curcuminoids was the noodles (Figure 2D), which had only 0.002 mg of curcuminoids per gram of product. The low amounts of curcuminoids present in this sample required to adjust the solvent: sample ratio from 10:1 to 2:1 in order to increase the amount of curcuminoids to concentrations above the LOD and LOQ. In all the samples, curcumin was the major curcuminoid, then demethoxycurcumin and the minority one was bisdemethoxycurcumin. These results indicate that the developed method is efficient and reliable for the analysis of curcuminoids in turmeric rhizome and in processed products which turmeric is used as a colorant. It also indicates that it may be necessary to adjust extraction conditions depending of the sample and the amount of curcuminoids present.

However, these are only a few examples of applications of the developed method for the analysis of curcuminoids and other types of samples, such as other foods and even to biological fluids and tissues, may be potentially analyzed with it. The suitability of the

method to other types of samples will depend of several factors, especially the concentration of curcuminoids and the presence of other compounds/metabolites that may co-elute with them.

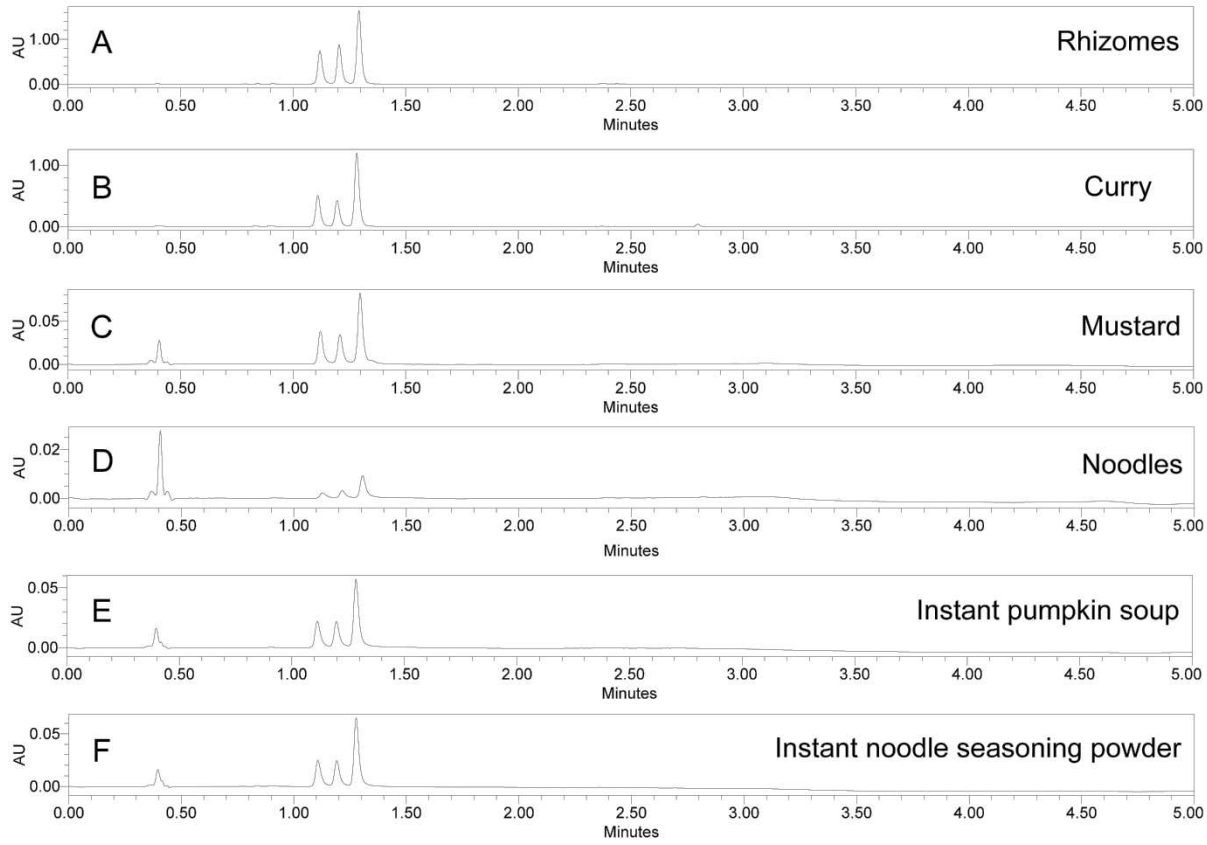

Figure 2. Representative chromatograms of different types of samples obtained with the developed method**.**

In the case of samples with low concentration of curcuminoids other sample preparation procedures, such as solid-phase extraction, may be required to allow proper quantitation. Alternatively a larger injection volumes may be explored to overcome these limitations as discussed previously. In this case, it is important to note that chromatographic performance may be seriously affected with injections volume above 20 µL. Lower injection volumes may also be used to reduce the amount of curcuminoids that reach the detector when analyzing highly concentrated samples and avoid detector saturation problems and will only be limited by the sampler precision.

On the other hand, the presence of other compounds or metabolites in the sample may be required that conditions are adjusted in order to properly separate them from curcuminoids. Although, the success of the application of the developed method to other sample may require adjustment of the separation conditions it may prove useful as starting reference.

4. Conclusions

In the present study, a step-by-step optimization strategy of chromatographic parameters (flow rate, mobile phase composition, temperature of the column, gradient and re-equilibration time) was used to develop a fast and reproducible analysis method for the determination of curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) in turmeric (*Cúrcuma longa* L.) extracts. Separation of these three curcuminoids was achieved in approximately 1.3 min and the total analysis time, including column clean-up, and re-equilibration time, was 7 min. The optimized method showed an excellent chromatographic performance in term of resolution, peak symmetry, repeatability, reproducibility, selectivity, quantification and detection levels and was successfully used for the analysis of different real samples with similar performance. The developed method has presented an excellent robustness according to the concentration/dilution of the samples. The robustness according the injection volume is not good. It has also been observed that the best solvent for the extraction process and the dilution of the samples is 100% methanol. The combination of state-of-the art column technology and optimized conditions significantly increased sample throughput in standard chromatographic systems when compared to conventional methods. Based on the results gathered during the method development, it is clear that fused-core column technology has a great potential to deliver faster and more sensitive methods for the analysis of curcuminoids and other natural products.

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-CAPÍTULO 4-

Extraction of curcuminoids from deflavored turmeric (Curcuma longa l.) using pressurized liquids: Process integration and economic evaluation

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Extraction of curcuminoids from deflavored turmeric (*Curcuma longa* L.) using pressurized liquids: Process integration and economic evaluation

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ABSTRACT

Pressurized liquid extraction (PLE) of curcuminoids from deflavored turmeric rhizomes was optimized. The rhizomes were initially deflavored by extraction with supercritical CO_2. Immediately after SFE, PLE process was performed using ethanol as the solvent and a static extraction time of 20 min, and the independent variables were the temperature (333–353 K) and pressure (10–35 MPa). The results indicate that the optimum extraction temperature and pressure were 333 K and 10 MPa, respectively. PLE required three and six times less extraction time than low-pressure solvent extraction and Soxhlet extraction, respectively, to produce similar extraction yields. The cost of manufacturing (COM) decreased from US$ 94.92 kg^{-1} to US$ 88.26 kg^{-1} when the capacity of the two-extractor system increased from 0.05 m^3 to 0.5 m^3 and from US$ 94.92 kg^{-1} to US$ 17.86 kg^{-1} when the cost of the raw materials decreased from US$ 7.91 kg^{-1} to US$ 0.85 kg^{-1} for a two 0.05 m^3 extractor system.

1. Introduction

Turmeric (*Curcuma longa* L.) is an important commercial crop grown for its aromatic rhizomes. Turmeric has been used as a condiment, preservative and traditional medicine for treating certain diseases since 4000 B.C. India is the largest turmeric producer, importer and exporter. However, many other Asian, Latin American and Caribbean countries now produce turmeric. Global turmeric production is approximately 1100,000 t/year [1]. Today, it is used primarily as a natural coloring agent to replace synthetic dyes in chutneys, pickles, mustard, butter and cheese among other products [2]. Most importantly, turmeric is employed in the preparation of curry.

The color of turmeric is attributed mainly to a group of phenolic compounds classified as curcuminoids. Curcuminoids are also potent antioxidants and exhibit anticancer, antibacterial and antiviral activities. Curcumin (I) is the main curcuminoid compound, and two related compounds, demethoxycurcumin (II) and bisdemethoxycurcumin (III), are also important [3].

Conventional solid–liquid extraction, sonication, Soxhlet extraction and related techniques have traditionally been used to extract curcuminoids [4]. However, exposure to light, high temperatures and oxygen during these traditional extractions triggers the degradation of curcuminoids [5], limiting the efficiency of these processes and the application of the extracted curcuminoid compounds in food products.

Pressurized liquid extraction (PLE) is an attractive alternative to traditional extraction processes because it usually requires smaller amounts of solvent and is more efficient [6]. PLE can be used over a wide range of temperatures (313–473 K) and moderate to high pressures (3.5–35 MPa) to accelerate the extraction and keep the solvent in the compressed liquid region [7].

Only a few studies on the extraction of curcuminoids using pressurized fluids have appeared in the literature [8]. Braga and Meireles [9] studied supercritical fluid extraction (SFE) at a pressure of 30 MPa, temperature of 303 K and cosolvent percentage of 50% v/v. Schieffer [10] and Euterpio et al. [11] studied PLE using methanol (373 K and 10 MPa) and water (370 K and 5 MPa) as the solvent. In these studies, PLE was shown to be more efficient than traditional extraction processes.

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However, in today's market, new processes must be not only efficient but also relatively cheap to be competitive. Factors such as performance (obtain as much product as possible), productivity (require the least amount of processing time) and selectivity (obtain a product rich in the substance of interest) should be considered when determining the economic viability of a process [12].

On the other hand, process integration is an attractive approach for the production of valuable products and higher quality at lower costs [13]. This work corresponds to the second step of an integrated extraction process. In the first step of the integrated process, the rhizomes were deflavored using supercritical CO_2 according to work done by Carvalho et al. [14], obtaining volatile oil rich in ar-turmerone. Subsequently, in the second step (this work), curcuminoids were extracted by PLE.

Therefore, the aim of this study was to evaluate PLE for obtaining curcuminoids from turmeric rhizomes deflavored by SFE. To optimize the process temperature and pressure, the global yield isotherms (GYIs) were determined. Based on the GYI results, a kinetic experiment was performed to determine the required data to evaluate the process economics. Ethanol was used as the solvent because curcuminoids are highly soluble in this solvent and it is a generally recognized as safe (GRAS) solvent.

2. Materials and methods

2.1. Raw materials

Turmeric rhizomes (*Curcuma longa* L.) were provided by the "Oficina das Ervas" (Ribeirão Preto, Brazil). The turmeric rhizomes were kept at 275 K before use as the raw material in the extractions. The rhizomes were ground in a knife mill (Marconi, model MA340, Piracicaba, São Paulo). The particle size distribution was determined using a set of 24 to 48 mesh sieves (WS Tyler, Wheeling, USA). The apparent bed density was calculated by dividing the sample mass loaded into the extraction cell by its volume in the cell.

2.2. Extraction procedures

2.2.1. SFE and PLE

SFE and PLE processes were performed sequentially in a home-built equipment described by Farias-Campomanes and Meireles [15]. This equipment can be used to perform both SFE (with or without a cosolvent) and PLE. To obtain a more uniform temperature profile, temperature controllers were installed at the input and output of the extraction cell (Fig. 1).

In order to integrate the SFE and PLE processes, rhizomes were initially subjected to SFE obtaining Deflavored Turmeric Rhizomes (DTRs) [14]. Immediately after the SFE process, without removing the raw material from the extraction vessel, the curcuminoids were extracted by PLE using ethanol as solvent. Table 1 shows the experimental conditions used in the SFE and PLE processes.

The procedure used to deflavor the rhizomes was similar to that described by Veggi [16]. Turmeric (47 g) was placed inside the extraction cell (415 cm^3). The sample represented approximately 13% of the total volume of the extraction cell. To fill the extraction vessel completely, 8–10 mesh glass beads and a Teflon column were added to the extraction cell vessel. The solvent was carbon dioxide (99.9% CO_2, Gama Gases Especiais Ltd., São Bernardo do Campo, Brazil). First, the CO_2 solvent was cooled to 263 K in a thermostatic bath (Marconi, model MA—184, Piracicaba, SP) and then pumped into the extractor. The extraction vessel was heated by a heating jacket through which soybean oil was circulated from a heating bath (Marconi, model MA—184, Piracicaba, SP). The extraction cell assembly containing the raw material was heated, filled with CO_2 and pressurized. After reaching the desired pressure, the extraction cell was maintained at the desired temperature for a static period of 20 min. Then the block valves were opened, and

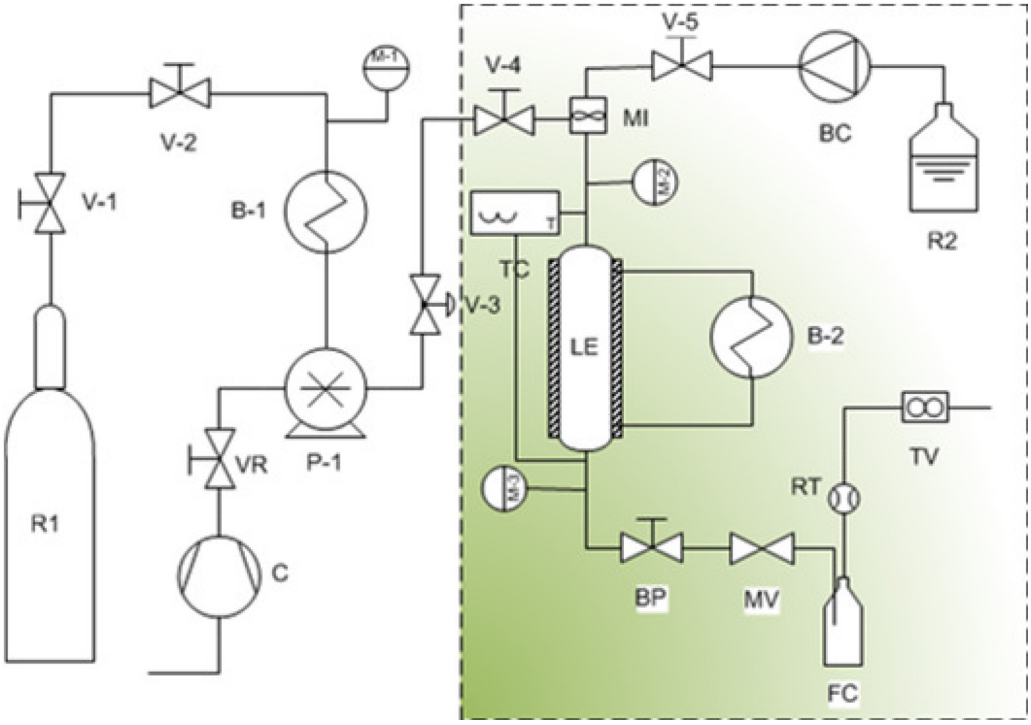

Fig. 1. Schematic diagram of the home-built equipment designed to perform SFE (with or without a cosolvent) and PLE to deflavor and recover curcuminoids from turmeric. R1—CO_2 reservoir; R2—extracting solvent reservoir; P-1—CO_2 pump; C—compressor; BC—HPLC pump; B-1—thermostatic bath; B-2—heating bath; LE—extraction cell; TC—temperature controllers; FC—collector flask; M—manometers; RT—glass float rotameter; TV—flow totalizer; V—blocking valves; MV—micrometric valve with a heating system; MI-fluid mixer; BP—back pressure regulator.

Table 1
List of experiments performed on turmeric rhizomes under different operating conditions by SFE and PLE.

Experiment	SFE		PLE		Experiment	SFE		PLE	
	T(K)	P(MPa)	T(K)	P(MPa)		T(K)	P(MPa)	T(K)	P(MPa)
1	313	15	343	35	19	323	25	343	15
2	313	10	353	35	20	333	25	343	15
3	333	30	343	20	21	323	10	353	15
4	313	25	343	25	22	313	15	353	15
5	313	35	333	20	23	313	30	333	15
6	323	35	333	35	24	323	30	343	30
7	323	20	333	30	25	313	25	353	30
8	333	10	343	30	26	323	25	343	25
9	333	25	333	10	27	333	35	353	25
10	313	30	333	25	28	313	20	353	20
11	323	35	333	30	29	313	20	333	35
12	333	20	343	35	30	333	20	343	10
13	323	10	333	20	31	333	30	333	15
14	323	15	333	10	32	333	10	353	25
15	323	30	333	25	33	333	35	353	20
16	323	20	353	10	34	333	15	353	10
17	313	10	343	10	35	333	15	343	20
18	323	15	353	30	36	313	35	353	35

the micrometering valve was carefully adjusted to maintain the system pressure, a constant CO_2 flow of 8.6×10^{-3} kg/min and a solvent-to-feed ratio (S/F ratio) of 12.1. After the SFE process was completed, PLE of the deflavored turmeric rhizomes was performed in the extraction cell. Three temperatures (333, 343 and 353 K) and six pressures (10, 15, 20, 25, 30 and 35 MPa) were evaluated. Extraction yield and curcuminoid content were based on the amount of dry raw material.

During the PLE process, the solvent was first pumped into the extractor using a HPLC pump (Thermo Separation Products, model ConstaMetric 3200 P/F, Fremont, USA). The extraction vessel was heated as previously described. After reaching the desired pressure, the extraction cell was maintained at the desired temperature for a static period of 20 min. Then the block valves were opened, and the micrometering valve was carefully adjusted to maintain the system pressure and a constant solvent flow of 1.11×10^{-4} kg/s. The dynamic extraction time was 60 min, and the solvent-to-feed ratio (S/F ratio) was 9.5. After the PLE process, the extract was rapidly cooled and protected from light to prevent the degradation of the curcuminoids. The cell was then purged with carbon dioxide (99.9% CO_2, Gama Gases Especiais Ltd., São Bernardo do Campo, Brazil) at a flow rate of 8.6×10^{-3} kg/min for 10 min. The extracting solvent was 99.5% ethanol (Dinâmica, Sao Paulo, Brazil). After extraction, an aliquot of the extract (1.5 cm^3) was removed to determine the curcuminoid content. The solvent was evaporated from the remaining extract using a vacuum-controlled rotary evaporator (Laborota, model 4001, Vertrieb, Germany). The thermostatic bath was maintained at 313 K. All vials and extracts were refrigerated and protected from light before analysis.

2.2.2. Soxhlet extraction

DTRs (17 g) were placed in a Soxhlet apparatus and extracted with ethanol. The S/F ratio was 9.5, and the extraction was performed for 6 h at a temperature of 351 K and atmospheric pressure. Then the extract was filtered, and an aliquot of the extract (1.5 cm^3) was placed in a vial to determine the curcuminoid content by HPLC. The solvent was evaporated under the conditions described previously to determine the yield. The extractions were performed in triplicate.

2.2.3. Low-pressure solvent extraction (LPSE)

DTRs (5 g) were placed in a 125 cm^3 Erlenmeyer flask containing ethanol. The S/F ratio was 9.5, and the extraction was performed on a shaker (Marconi, model MA420, Piracicaba, Brazil) at a temperature of 313 K and atmospheric pressure. The extraction was performed under stirring for 3 h. Then the extract was filtered, and an aliquot of the extract (1.5 cm^3) was placed in a vial to determine the curcuminoid content by HPLC. The solvent was evaporated in an evaporator under the same conditions described previously to determine the yield. The extractions were performed in triplicate.

2.3. High-performance liquid chromatography

The total curcuminoid content of the extracts was determined by HPLC on a Waters Alliance separation module (model 2695D, Milford, USA) equipped with a diode array detector (2998). The individual compounds in the extracts were separated on a Kinetex C_{18} column (150 × 4.6 mm id, 2.6 μm, Phenomenex, Torrance, USA) maintained at 328 K using a flow rate of 2.5 cm^3/min [17].

The mobile phase consisted of 0.1% water (v/v) in acetic acid (solvent A) and 0.1% acetonitrile (v/v) in acetic acid (solvent B). The curcuminoids were separated by increasing the percent of solvent B from 45% to 65% at a constant rate in 1.5 min. The solvent B concentration was then increased from 65% to 90% after 2.5 min and decreased to its initial value (45% solvent B) in 1 min. The compounds were detected at 425 nm and quantified using Empower 2 software (Waters, Milford, MA, USA). They were identified by comparing their retention times and UV–vis spectra to those of reference standards (Sigma Aldrich, St. Louis, USA).

The relative curcuminoids content was calculated based on the yield of curcuminoids. The highest yield of curcuminoids obtained at 333 K and 10 MPa was considered as 100% of the relative content of curcuminoids. The other curcuminoids yields were expressed as percentage of this value to obtain the relative yields.

2.4. Statistical analysis

Minitab® v. 16 software was used to determine the effects of the extraction conditions (temperature and pressure) on the overall performance and curcuminoid content. A completely randomized factorial experiment with a replica was used to determine the ideal conditions for the extraction process.

2.5. Overall extraction curves and process modeling

The overall extraction curve (OEC) was determined at the GYI-optimized temperature and pressure. The experimental OEC was fitted to a spline of three straight lines using the SAS® v. 9

software according to the procedure described by Meireles [18]. The fitted lines were attributed to three different mass transfer mechanisms based on classic descriptions of the periods: the constant extraction rate (CER), falling extraction rate (FER) and diffusion-controlled (DC) periods. The three straight lines fitted to the OEC were described by the following equations [19]:

For $t \leq t_{CER}$:

$$m_{Ext}(t) = b_0 + a_1 t \quad (1)$$

For $t_{CER} \leq t \leq t_{FER}$, that is, for the second straight line or the FER period:

$$m_{Ext}(t) = (b_0 - t_{CER} a_2) + (a_1 + a_2)\, t \quad (2)$$

For $t \geq t_{FER}$, that is, for the third straight line or the DC period:

$$m_{Ext}(t) = (b_0 - t_{CER} a_2 - t_{FER} a_3) + (a_1 + a_2 + a_3)\, t \quad (3)$$

here, m_{ext} is the extract mass; b_0 is the linear coefficient (zero-order term) of the CER line (g); a_1,a_2 and a_3 are the slopes (first-order terms) of the CER, FER and DC lines, respectively (g/min); t_{CER} is the CER time span (min) and t_{FER} is the end of the FER period (min) [20]. The extraction and curcuminoid yields were used to estimate the PLE kinetic parameters.

2.6. Process simulation: Technical and economic evaluation

Simulations of the PLE, Soxhlet and LPSE processes were performed using the SuperPro Designer® software (v. 8.5). The PLE process flow diagram was similar to that described by Santos et al. [6] and consisted of a solvent storage tank, a pump to pressurize the system, two extractors allowing for semi-continuous operation (while one extractor was in operation, the other one was cleaned and prepared for the next extraction cycle), a flow controller and a distiller. The process flow diagrams used to simulate the Soxhlet extraction and LPSE extraction were similar to those described by Veggi et al. [21]. For the LPSE process, the flow diagram consisted of two extractors (while one of the vessels was in operation, the other one was cleaned and recharged), an extract-solution tank, a pump, a distiller, a condenser and a recycled solvent tank. For the Soxhlet extraction, the LPSE flow diagram was modified to include a condenser, which was connected to the extractor to simulate the condensation step of the solvent and reflux of the condensed fluid to the extractor.

2.6.1. Economic evaluation and process scale-up

The cost of manufacturing (COM) of the curcuminoid-rich extract via the PLE, Soxhlet and LPSE processes was calculated. COM of the PLE extract was calculated using the kinetic assay data. For the Soxhlet and PLSE processes, COM was estimated using the information described in the extraction procedures section. COM can be determined by the sum of three main components: direct costs, fixed costs and general expenses. COM was estimated according to the methodology proposed by Turton et al. [22], where the three principal components of the COM can be estimated in terms of five major costs: raw materials, operating costs, utilities, waste treatment, and initial investment by the equation:

$$\mathrm{COM} = 0.304\ \ \mathrm{FCI} + 2.73\ \ \mathrm{COL} + 1.23 \times (\mathrm{CUT} + \mathrm{CWT} + \mathrm{CRM}) \quad (4)$$

where FCI is the fixed capital of investment, COL is the cost of operational labor, CUT is the cost of utilities, CWT is the waste treatment cost and CRM is the cost of raw material). FCI involves expenses related to the implementation of the production line (extraction units and other equipment). COL is related to the operators of the extraction units (1, 2 and 3 operators to the 0.05, 0.3 and 0.5 m^3 extraction units, respectively). CUT considers the energy used in the solvent cycle for steam generation, water refrigeration and electricity requirements. CRM consists of the raw material cost and the cost of the solvent. Finally, CWT was considered to be zero because the waste generated by the process can be considered harmless and clean and may be reused in other applications or simply disposed of as ordinary vegetable waste. More details can be found in Rosa and Meireles [23] and Albuquerque and Meireles [24].

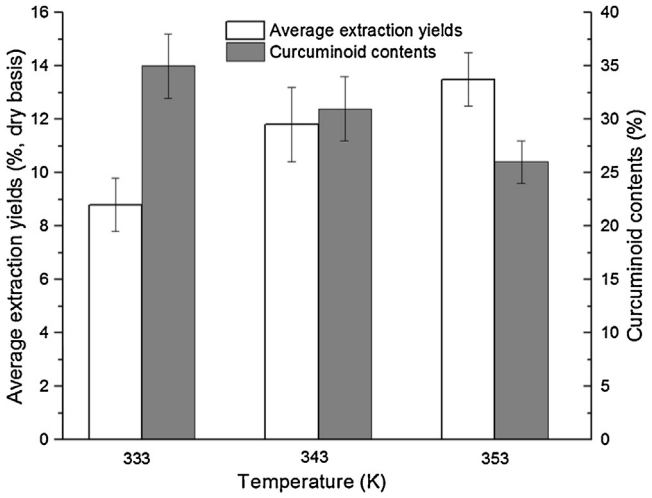

Fig. 2. Average extraction yields (open bars) and curcuminoid contents (filled bars) in the extracts. The values are the averages of all extract yield data at each temperature evaluated.

For the process scale-up, a procedure similar to that described by Santos et al. [6] was used: it was assumed that the yield and extract composition obtained at the laboratory scale would also be obtained at the industrial scale when the same processing conditions were used (temperature, pressure, S/F ratio, bed porosity, etc.). The process was designed to operate for 7920 h per year, which corresponds to 3 daily shifts for 330 days per year. Industrial-scale extractor volumes of 0.05, 0.3 and 0.5 m^3 were considered. The amount of material (DTRs) to be extracted in each stage was calculated based on the extractor size. The solvent loss during the process was assumed to be 2%, which was the loss from a distiller calculated by the simulator. The apparent density of the material was 838 kg/m^3. The cost of the raw material (DTRs) was assumed to be US$ 7.91 kg^{-1} (Oficina das Ervas, Ribeirão Preto, Brazil). The input economic parameters, which were similar to those described by Cavalcanti [25] and Veggi [26], are presented in Table 2. To evaluate the influence of the cost of the raw material on COM, the costs of the raw material from two additional suppliers were evaluated (US$ 4.5 kg^{-1}, Ceasa, Goias, Brazil and US$ 0.85 kg^{-1}, Commodity Online, India, www.commodityonline.com, last accessed on 17/01/2014). Additionally, because the raw material can be considered as the residue of the first stage of the integrated process, i.e., obtaining of the volatile oil rich ar-turmerone, in another possible scenario, the cost of the raw material was also assumed equal to zero.

3. Results and discussion

3.1. Effects of the process variables on the extraction yield and curcuminoid content in the extract

Table 3 lists the experimental conditions and the extraction, curcuminoid and relative curcuminoid yields obtained by PLE, Soxhlet extraction and LPSE. Over the temperature (333–353 K) and pressure (10–35 MPa) ranges studied, only the temperature had a significant impact on the extraction yield (p_{value} = 0.000). The average extraction yield and curcuminoid content in the extract obtained at various temperatures are presented in Fig. 2. The results show that the average extraction yield increased as the

Table 2
Input economic parameters used in the SuperPro Designer 8.5® software.

	Industrial units*		
	$2 \times 0.05\ m^3$	$2 \times 0.3\ m^3$	$2 \times 0.5\ m^3$
Fixed capital investment (FCI)			
LPSE unit extraction	US$ 60,000	–	–
Soxhlet unit extraction	US$ 115,000	–	–
PLE unit extraction	US$ 117,000	US$ 317,000	US$ 417,000
Distiller	US$ 20,000	US$ 53,000	US$ 76,000
Depreciation rate	10%/year	10%/year	10%/year
Operational labor (COL)	US$ 6.9 h^{-1}	US$ 6.9 h^{-1}	US$ 6.9 h^{-1}
Number of workers	1	2	3
Cost of the raw material (CRM)			
Deflavored turmeric	US$ 7.91 kg^{-1}	US$ 7.91 kg^{-1}	US$ 7.91 kg^{-1}
	US$ 4.40 kg^{-1}	US$ 4.40 kg^{-1}	US$ 4.40 kg^{-1}
	US$ 0.85 kg^{-1}	US$ 0.85 kg^{-1}	US$ 0.85 kg^{-1}
	US$ 0.00 kg^{-1}	US$ 0.00 kg^{-1}	US$ 0.00 kg^{-1}
Ethanol	US$ 0.85 kg^{-1}	US$ 0.85 kg^{-1}	US$ 0.85 kg^{-1}
Water	US$ 0.04 kg^{-1}	US$ 0.04 kg^{-1}	US$ 0.04 kg^{-1}
Utilities (CUT)			
Electricity	US$ 0.092 $(k Wh)^{-1}$	US$ 0.092 $(kW h)^{-1}$	US$ 0.092 $(kW h)^{-1}$
Water steam	US$ 4.2 t^{-1}	US$ 4.2 t^{-1}	US$ 4.2 t^{-1}

* The first value indicates the number of extractor vessels, and the second value is their capacity in cubic meters.

extraction temperature increased as expected. Higher extraction temperatures lead to higher extraction efficiencies because the mass transfer rate and the solubilization of components within the matrix increase [6,27]. However, the effects of the temperature on the extraction of the target compounds and other components in the raw material might be different. If the extraction yield of the other components increases more than that of the target compounds, the content of the target compounds in the extract is reduced. This effect can be clearly observed in Fig. 2. As the temperature increased, the curcuminoid content in the extract decreased, although the total amount of curcuminoids increased because the extract yield increased at a higher rate than the amount of curcuminoids extracted. Consequently, the selectivity of the extraction suffered, and the curcuminoid content in the extract was lower.

The curcuminoid yield is given as a function of temperature and pressure in Table 3. The yield varied from 2.2% d.b. (recovery of 50%) to 4.4% d.b. (recovery of 100%). These values agree with those reported for curcuminoids content in turmeric [28]. Clearly, the PLE process is an attractive alternative to traditional extraction processes due to its ability to remove large amounts of naturally occurring curcuminoids from turmeric. It should also be noted that over the temperature and pressure ranges studied (333–353 K and 10–35 MPa, respectively), the curcuminoid yield was significantly affected by the combination of pressure and temperature effects ($p_{value} = 0.000$). Although the pressure generally did not impact the PLE process considerably, the combination of temperature and pressure effects was not always negligible. At lower temperatures (between 333 and 343 K), higher yields were obtained when the extraction was performed at 343 K because of the higher mass transfer rate and curcuminoid solubility in the extraction solvent. At higher temperatures (between 343 K and 353 K), the influence of the temperature was dependent of the pressure. These results indicate that although the temperature is considered to be the most important parameter for ensuring good performance of the PLE process, the extraction yield might also be affected by the combination of temperature and pressure effects depending on the extraction conditions. In this work, when the process was performed at 333 and 343 K, increasing the pressure negatively affected the curcuminoid extraction. This behavior can be explained by the changes in the raw material matrix induced by the pressure increase; the active surface was reduced, and compounds leached from the matrix [27]. Furthermore, because the raw material bed was compacted as the pressure increased, channels might have formed, preventing proper contact between the solvent and the compounds of interest and thus reducing the extraction efficiency [29]. In contrast, when the process was performed at 353 K, the combination of the extraction temperature and pressure effects was complex and variable. Under certain temperature and pressure conditions, the negative effects of compacting the raw material bed could not be overcome by the higher mass transfer rate at the higher temperature.

Although a few studies on the extraction of curcuminoids from turmeric using PLE have appeared in the literature, the yields obtained in this study are much higher than those previously

Table 3
Summary of the extraction process conditions and extraction, curcuminoid and relative curcuminoid yields obtained by PLE. The results for Soxhlet extraction and LPSE of DTRs are also given.

Process	Pressure (MPa)	Extraction yield (%)			Curcuminoid yield (%)			Relative curcuminoid yield (%)		
Soxhlet	0.1	12 ± 1			4.2 ± 0.1			93 ± 1		
LPSE	0.1	12 ± 1			3.7 ± 0.1			80 ± 1		
Temperature (K)[1]										
		333	343	353	333	343	353	333	343	353
PLE	10	11 ± 1	13 ± 2	15 ± 2	4.3 ± 0.2	4.4 ± 0.3	3.5 ± 0.3	99 ± 5	100.0 ± 7	81 ± 8
	15	9 ± 1	12 ± 1	12 ± 5	3.7 ± 0.3	4.3 ± 0.1	2.2 ± 0.2	85 ± 8	98 ± 1	50 ± 6
	20	11 ± 3	13 ± 3	14 ± 4	3.1 ± 0.1	3.8 ± 0.3	3.6 ± 0.1	71 ± 3	88 ± 7	83 ± 3
	25	8 ± 2	11 ± 1	14 ± 5	2.7 ± 0.3	3.4 ± 0.2	3.3 ± 0.1	62 ± 7	77 ± 5	76 ± 3
	30	6 ± 1	10 ± 5	14 ± 1	2.2 ± 0.1	2.8 ± 0.3	4.0 ± 0.1	51 ± 3	64 ± 7	93 ± 1
	35	7 ± 1	11 ± 1	13 ± 2	2.2 ± 0.1	2.7 ± 0.3	3.2 ± 0.3	50 ± 1	62 ± 6	73 ± 8

Data yield based on dry mass. The relative curcuminoid yields are expressed as percentages of the highest curcuminoid yield obtained at 333 K and 10 MPa.
[1] The temperatures used in the Soxhlet and LPSE processes were 351 and 313 K, respectively.

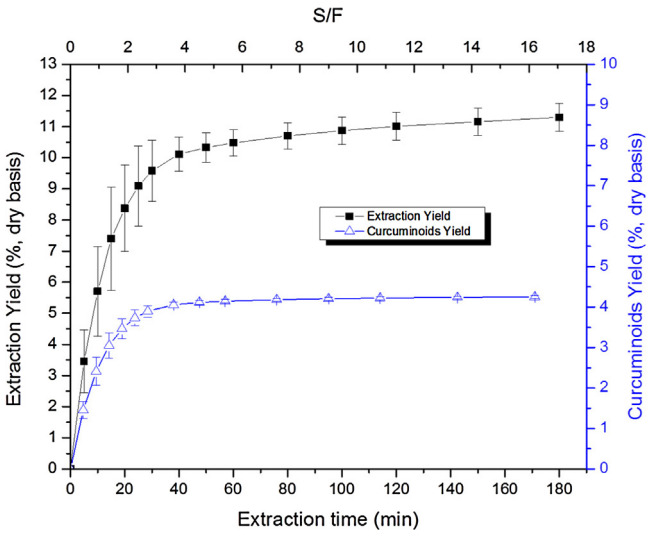

Fig. 3. Extraction yield curve for PLE performed at 333 K and 10 MPa.

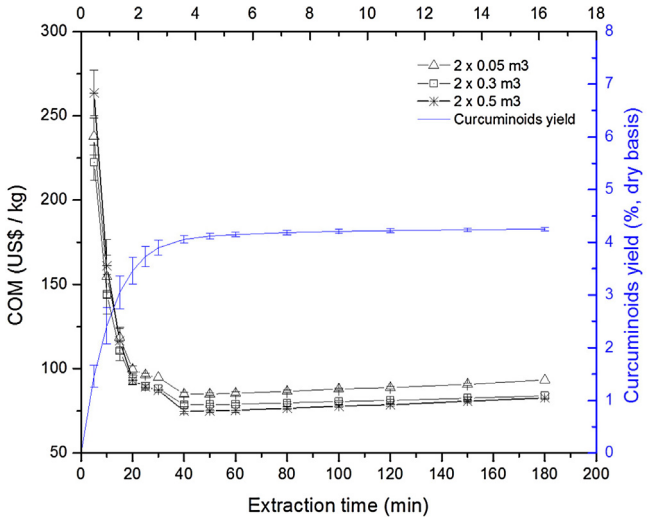

Fig. 4. COM of curcuminoid-rich extracts as a function of processing time for different extractor vessel capacities. The raw material was purchased at US$ 7.91 kg^{-1}.

reported in the literature (between 0.12% and 1.6%) [9–11]. The observed differences might be due to different extraction conditions (temperature, pressure, solvent, S/F, etc.), natural variations in the raw material, pretreatment effects and the equipment configuration.

3.2. Extraction process optimization

A statistical analysis of the results led to the identification of three sets of conditions, namely 333 K and 10 MPa, 343 K and 10 MPa and 343 K and 15 MPa, under which the extraction process performed similarly ($p_{value} = 0.002$). Based on the process yields, the optimal operating parameters were determined to be 333 K and 10 MPa. Under these conditions, the process yield was 99%, and the curcuminoid content in the extract was 39%. These extraction conditions are also less severe, requiring less energy than the other conditions tested. In addition, increases in the temperature and pressure could compromise the stability of the curcuminoids, triggering the extraction of undesired compounds, and lead to a compacted sample, preventing the extraction process from working properly.

3.3. Comparison of the efficiencies of PLE and other extraction techniques

Table 3 presents the extraction yields obtained by PLE and the other extraction techniques evaluated in this study. Although the PLE extraction yield was slightly lower than the Soxhlet extraction and LPSE extraction yields, the curcuminoid yield in the PLE extracts was similar to that in the Soxhlet extracts and higher than that in the LPSE extracts. However, the PLE process required 78% less time to achieve a performance similar to that of the Soxhlet process (80 min vs. 6 h). Compared to LPSE, the PLE process used 56% less time and resulted in a 16% higher curcuminoid yield. Furthermore, PLE resulted in a higher relative curcuminoid yield than both techniques. The high curcuminoid extraction yield and short extraction time of PLE clearly indicate that PLE is more efficient than Soxhlet extraction and LPSE.

3.4. OEC modeling

The OEC was determined using the optimized temperature and pressure (333 K and 10 MPa, respectively). Fig. 3 shows the overall extraction curve and overall curcuminoid yield curve. Both curves were quantitatively described by the spline model. The t_{CER} and t_{FER} kinetic parameters obtained from the overall extraction curve were 14 and 34 min, respectively. The M_{CER} value was 6.0×10^{-6} kg/s, Y_{CER} was 5.4×10^{-2} kg/kg_{EtOH} and R_{CER} was 7.6% d.b. The following kinetic parameters were obtained from the overall curcuminoid yield curve: t_{CER} of 16 min and t_{FER} of 37 min. The M_{CER} value was 2.5×10^{-6} kg/s, Y_{CER} was 2.2×10^{-2} kg/kg_{EtOH} and R_{CER} was 3.4% d.b.

According to Pereira and Meireles [30], 50–90% (w/w) of the total amount of extract can be obtained at the end of the CER period, which is consistent with the results obtained in this work. The R_{CER} values obtained from the extraction and curcuminoid curves were 67% and 87%, respectively, of the total extraction yields (4.2 and 11.3%, respectively).

Although the t_{CER} and t_{FER} kinetic parameters were similar, the DC period, which occurred after the FER period, was more pronounced for the curcuminoid yield than for the extraction yield as demonstrated by the yield curves in Fig. 3. For instance, the a_3 parameter obtained from the curcuminoid yield curve was 7.6 times lower than that obtained from the extraction yield curve.

According to Palma et al. [31], it is possible that after the CER and FER periods, the target compounds are no longer easily extractable, and the solvent starts to interact with other matrix compounds. The intermolecular interactions between these molecules and the matrix are disrupted, allowing the molecules to be released. Therefore, as shown in Fig. 3, the extraction yield curve did not exhibit a DC period as the extraction continued, in contrast to the curcuminoid content curve.

3.5. Economic evaluation of the extraction process

To determine the COM of the PLE extracts, the process was simulated using the SuperPro Designer 8.5® software. The raw material price was US$ 7.91 kg^{-1}, and three different production scales (0.05, 0.3 and 0.5 m^3) were considered as shown in Fig. 4. At the beginning of the extraction, COM was higher due to low raw material usage. If the extraction time was too short, the raw material would still retain an appreciable amount of curcuminoids that could be easily removed [32]. COM decreased as the process was scaled-up. Very similar COMs were also obtained when the process was simulated using 0.3 and 0.5 m^3 units. For example, assuming a raw material cost of US$ 7.91 kg^{-1} and a minimum extraction time of 30 min, the COMs of the extracts were estimated to be US$ 94.92 kg^{-1}, US$

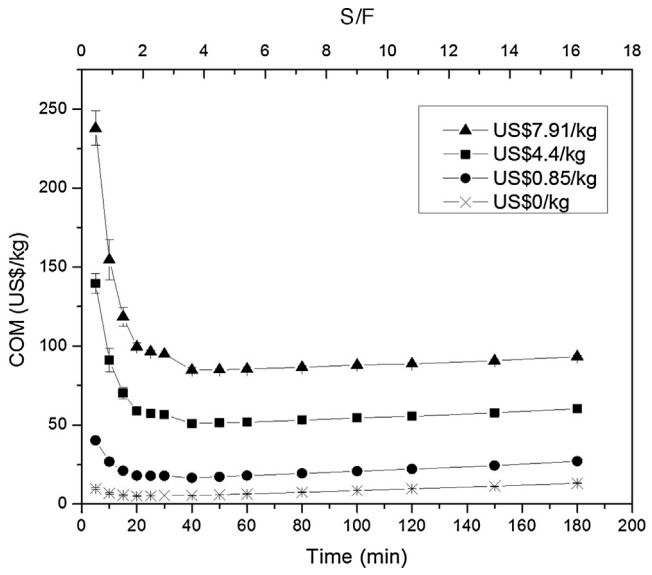

Fig. 5. Influence of the cost of the raw material on COM for the industrial unit in Fig. 1. The extractor capacity was 0.05 m^3.

88.44 kg^{-1} and US$ 88.26 kg^{-1} for two 0.05 m^3, 0.3 m^3 and 0.5 m^3 extraction units, respectively.

According to the economic analysis at the three different scales, the lowest production cost was achieved after a processing time of 40 min, which is close to t_{FER} (37 min), indicating that t_{FER} is a good parameter for making an initial COM estimate [32]. After 40 min of processing time, the extraction yield no longer increased, and the production cost remained relatively constant, increasing slightly as the extraction time increased. These results were due to the exhaustion of the raw materials (leading to lower yields) and the increasing solvent and energy costs associated with extended extraction times.

Another important factor influencing COM is the raw material costs. As the raw material cost decreased, the relative percentage of the other COM components increased because the costs associated with the raw material accounted for a large portion of COM [33]. It is noteworthy that using more expensive raw materials resulted in a higher FCI and COL. For example, when CRW decreased from US$ 7.91 kg^{-1} to US$ 0.85 kg^{-1} and were processed in two 0.05 m^3 extraction units, the FCI and COL contribution increased from 6.7% to 35.5% and from 1.28% to 6.8%, respectively, after a processing time of 30 min because the FCI is directly related to the raw material costs (US$/year) and amount of extract obtained (kg/year) [6].

The influence of the CRM on the COM of curcuminoids in the two 0.05 m^3 vessel system is presented in Fig. 5. COM decreased as the CRM decreased. The effect of decreasing the CRM was more significant for the smaller system (2×0.05 m^3) than for the larger capacity systems (2×0.3 and 2×0.5 m^3). For example, when the CRM decreased from US$ 7.91 kg^{-1} to US$ 4.4 kg^{-1}, US$ 0.85 kg^{-1} and US$ 0.00 kg^{-1} and the two 0.05 m^3 unit system was used for 30 min, COM decreased from US$ 94.92 kg^{-1} to US$ 56.6 kg/kg, US$ 17.86 kg^{-1} and US$ 5.39 kg^{-1}, respectively; which are 1.7, 5.3 and 17.6 times smaller than the COM obtained using the most expensive raw material. In this case, as the extraction time increased, fewer batches were necessary, and thus, a smaller amount of raw material was used, reducing the percentage of the cost due to the raw material. The percentages of the other COM components increased accordingly as the extraction time increased.

A comparison of PLE, Soxhlet extraction and LPSE shows that the PLE process is advantageous mainly due to the relatively short duration of this extraction method. As expected, the COM associated with using the PLE process for 30 min was lower than those associated with using conventional LPSE for 3 h and Soxhlet extraction for 6 h. For example, using two 0.05 m^3 extraction units and raw materials costing US$ 7.91 kg^{-1} resulted in COMs of US$ 94.92 kg^{-1}, US$ 245.38 kg^{-1} and US$ 193.55 kg^{-1} for PLE, LPSE and Soxhlet extraction, respectively. The LPSE and Soxhlet extraction COMs correspond to increases of 2 and 2.5 times, respectively, the PLE COM. Although the extraction yield obtained by PLE was similar to those obtained by Soxhlet extraction and LPSE, the time-consuming LPSE and Soxhlet extraction processes use more energy and are more expensive.

4. Conclusions

The results of this study show that curcuminoid-rich extracts can be obtained from deflavored turmeric rhizomes using an environmentally friendly process. The results indicate that the curcuminoid extraction was affected primarily by the extraction temperature and pressure. Specifically, increasing the temperature improved the extraction efficiency, while increasing the pressure negatively affected the extraction process.

The optimum conditions for the curcuminoid extraction were a temperature of 333 K and pressure of 10 MPa; these parameters were used to simulate the process and determine the COM. Similar curcuminoid yields were obtained by PLE, Soxhlet extraction and LPSE, but the PLE extraction time was 3 and 6 times less than the Soxhlet extraction and LPSE extraction times, respectively. Likewise, the PLE COM was 2 and 2.5 times lower that the LPSE and Soxhlet extraction COMs, respectively.

The CRM plays a very important role in COM. When the CRM was US$ 0.85 kg^{-1} and a two 0.05 m^3 vessel system was operated for 30 min, COM was US$ 17.86 kg^{-1} of extract, which was approximately 1.7 and 5.3 times lower than the COMs obtained when the CRM was US$ 7.91 kg^{-1} and US$ 4.4 kg^{-1}, respectively. Thus, PLE appears to be an attractive and economically feasible technique for obtaining curcuminoid-rich extracts from deflavored turmeric rhizomes.

Conflict of interest statement

The authors confirm that there are no conflicts of interest regarding this paper.

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-CAPÍTULO 5-

Precipitation of curcuminoids from an ethanolic turmeric extract using a supercritical antisolvent process

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ABSTRACT

This study examined the precipitation of curcuminoids from an ethanolic extract using a supercritical antisolvent process (SAS). The ethanolic extract was obtained from deflavored turmeric using pressurized liquid extraction (PLE). A Split-Plot experimental design was used to evaluate the effects of process parameters, such as nozzle type (T-mixer and coaxial), temperature (313 and 333 K), pressure (10 and 12 MPa) and CO_2 flow (500 and 800 g/h), on the curcuminoids precipitation process. The results indicate that the T-mixer nozzle obtained a higher yield and a lower particle size than the coaxial nozzle. Particles of curcuminoids were precipitated with a global yield of solids of 69% and a curcuminoid content of 554 mg/g. This corresponds to a precipitation efficiency of 97%. The particles precipitated via SAS contained a curcuminoid content 2 and 31 times higher than the extracts obtained by rotary evaporation and the ethanolic extract, respectively, obtained by PLE. Depending on experimental conditions, the particles were characterized as polydispersed and agglomerated into larger structures (by up to 100 μm) with different morphologies.

1. Introduction

Turmeric (*Curcuma longa* L.) is a plant widely cultivated in countries and regions with tropical and subtropical climates. Turmeric is known for its aromatic rhizomes and is commonly used as a condiment, preservative, flavoring and coloring agent, or in folk medicine [1]. Turmeric has been investigated for its biological activity associated with the presence of phenolic compounds classified as curcuminoids. Curcuminoids are responsible for the yellow coloration of the rhizomes and exhibit anticancer [2], antibacterial [3], chemopreventive and chemotherapeutic [4] activities. Curcuminoids are mainly used as colorants due to their coloring ability and the interest in replacing synthetic additives by natural compounds. The color tone of curcuminoids is comparable with tartrazine, and it is possible obtain a bright yellow color using low doses (5–20 ppm) [5]. According to Gomez-Estaca et al. [6] certain properties of curcuminoids could limit their use in foods: low water solubility, which may limit their dispersion in food matrices; low bioavailability, which negatively affects biological efficacy; and rapid degradation under neutral or alkaline pH conditions or when exposed to light. However, curcuminoids are commonly used in the preparation of several products, such as chutneys, pickles, mustard, butter and cheese [7].

Generally, natural extracts are marketed in the form of liquid, viscous preparations or as powders resulting from the drying of the liquid extract. Nevertheless, dried extracts have some advantages over liquid extracts, including lower storage costs and a higher concentration and stability of active substances [8]. Diverse techniques, such as spray drying, spray chilling, spray cooling, lyophilization, crystallization solvent or dry/milling processes, have been studied and employed to obtain powdered extracts and produce particles. However, these methods have several disadvantages, such as the degradation of the product, contamination with organic solvents, and the production of large sized particles [9].

As an alternative to conventional processes, precipitation and particle formation through a supercritical antisolvent process (SAS) is proposed. In the SAS process, a liquid solution of a solvent and a solute is injected into a supercritical fluid, which acts as an antisolvent. This leads to supersaturation of the solute, which is compensated by nucleation and particle growth [10]. In the SAS process, the properties of the particles produced can be strongly

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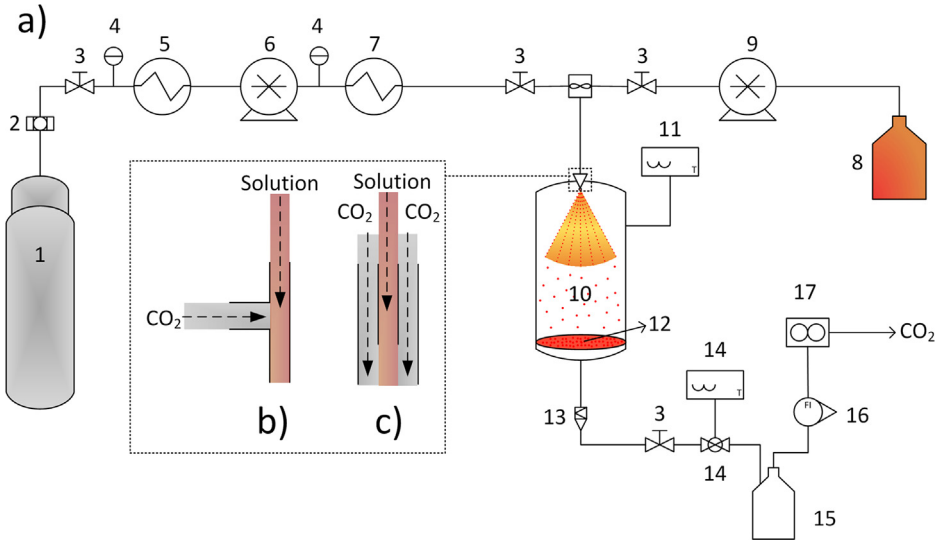

Fig. 1. (a) Schematic diagram of the SAS apparatus and types of nozzles. (1) CO_2 cylinder; (2) CO_2 filter; (3) blocking valves; (4) manometers; (5) thermostatic bath; (6) CO_2 pump; (7) heating bath; (8) solution (solute/solvent) reservoir; (9) HPLC pump; (10) precipitation vessel; (11) temperature controllers; (12) filter; (13) line filter; (14) micrometric valve with a heating system; (15) glass flask; (16) glass float rotameter; (17) flow totalizar. (b) T-mixer and (b) coaxial nozzle.

influenced by varying process parameters such as pressure, temperature, solvent type, solute concentration, and the flow-rate ratio of the solution and the antisolvent [11]. In SAS process, carbon dioxide (CO_2) is the supercritical fluid most widely used because it is considered nontoxic and nonflammable. Moreover, due to its relatively low critical point (304.2 K and 7.38 MPa), CO_2 allows for operation at a moderate temperature, providing conditions suitable for maintaining the integrity of the bioactive compounds [12]. These features, combined with very low solubility of curcuminoids in CO_2 and acceptable solubility of ethanol in CO_2, makes CO_2 a suitable antisolvent for the precipitation process. Although much research has been published on the precipitation and encapsulation of curcuminoids using different techniques [13–17], further research is necessary in order to better understand the precipitation of curcuminoids from an ethanol extract using supercritical fluids.

This work corresponds to the third step of an integrated process. In the first step, the rhizomes were deflavored using supercritical CO_2 according to work done by Carvalho et al. [19], obtaining volatile oil rich in ar-turmerone. In the second step, curcuminoids were extracted by pressurized liquid extraction (PLE), obtaining ethanolic curcuminoid-rich extracts from deflavored turmeric rhizome [18]. Subsequently, in the third step (this work), curcuminoids were precipitated by SAS. Therefore, the objective of this work was to study the precipitation of curcuminoids from an ethanolic extract obtained by PLE using supercritical fluids. The effects of temperature, pressure, type of nozzle and CO_2 flow rate on the SAS process were evaluated.

2. Materials and methods

2.1. Preparation of curcuminoid extract

The extract was obtained using pressurized liquid extraction (PLE) according to Osorio-Tobón et al. [18]. Approximately 332 g of turmeric were placed inside the extraction cell (415 cm^3) to fill the entire volume. According to the work of Carvalho et al. [19], the turmeric rhizomes were initially subjected to supercritical fluid extraction (SFE) pretreatment to remove volatile oils. This pretreatment was performed at 333 K and 25 MPa. The static period was 20 min, and the CO_2 (99.9% CO_2, White Martins Praxair, Campinas, Brazil) flow was 8.6×10^{-3} kg/min, with a solvent (S) to feed (F) mass ratio of 2.5. Without removing the sample from the equipment, the ethanolic extract, which was rich in curcuminoids, was then obtained by PLE. Ethanol (99.5%, Dinâmica, Sao Paulo, Brazil) was used as an extraction solvent at 333 K and 10 MPa. The static period was 20 min, and solvent flow was 1.22×10^{-4} kg/s, with a solvent (S) to feed (F) mass ratio of 2.4. After PLE, the curcuminoid extract was vacuum filtered (MF-Millipore^{TM}, pore size 0.45 μm) at 8×10^{-2} MPa. The liquid phase was recovered and stored at 275 K before use.

2.2. SAS: Precipitation experiments

A schematic diagram of the homemade SAS equipment used in this work is shown in Fig. 1a. Further details and a description can be found in previous work by Santos and Meireles [20]. The procedure was similar to that performed by Santos et al. [21]. The process began with the cooling of CO_2 (99.9% purity, White Martins Praxair, Campinas, Brasil) to 263 K in a thermostatic bath (Marconi, MA-184, Piracicaba, Brazil) to ensure the liquefaction of the gas. Next, CO_2 was pumped by an air-driven liquid pump (Maximator, M111 CO2, Germany) and heated using a second thermostatic bath (Marconi, MA-126, Piracicaba, Brazil) before entering the precipitation vessel (volume of 500 cm^3; 6.8-cm inner diameter). The precipitation vessel was fitted with an electric heating jacket (Autic, Campinas, Brazil). The desired conditions of pressure, temperature and CO_2 flow rate were achieved and stabilized by holding them constant for 10 min. Then, the ethanolic extract was injected into the precipitation vessel by a high-performance liquid chromatography (HPLC) pump (Jasco, PU-2080, Japan) at a flow rate of 0.5 cm^3/min. Once the extract was injected to 40 cm^3, 4 cm^3 of pure ethanol was injected to wash the tubes to avoid obstruction of the nozzles. The HPLC pump was then stopped and a minimum of 625 g of CO_2 was fed at the same operating conditions to ensure drying of the particles and complete removal of the ethanol from the precipitation vessel. The particles were collected using a stainless steel porous filter (AISE, 316, screen size of 2 μm) fixed at the bottom of the precipitation vessel. Two types of nozzles were used to mix the ethanolic extract and the CO_2. The T-mixer nozzle (Fig. 1b) is a 1/8 in. tube (inner diameter (i.d.): 317 mm) through which the ethanolic extract and the CO_2 entered simultaneously. The coaxial nozzle is a 1/16 in.

tube (inner diameter (i.d.):177.8 mm), placed inside the 1/8 in. tube when circulating the CO_2. The ethanol was recovered in a glass flask (100 cm^3) connected to a micrometric valve. This valve was maintained at 393 K to avoid freezing and subsequent blocking of the outlet caused by the Joule–Thompson effect of expanding CO_2. The CO_2 flow rate was measured using a glass float rotameter (ABB, 16/286A/2, Warminster, USA) coupled with a flow totalizer (LAO, G 0.6, Osasco, Brazil). Finally, the precipitation vessel was depressurized, and the particles were recovered by a porous filter fixed at the bottom of the vessel and by a secondary porous filter at the outlet of the first. The particles were carefully collected with a soft brush and were stored at 275 K in a glass desiccator away from light until subsequent analysis.

2.3. Analysis and characterization of the particles

The global yield of solids was determined by weighing the total amount of particles collected in the precipitator vessel and comparing it to the total amount of solids in the ethanolic extract. The precipitation efficiency was determined by weighing the total amount of particles collected in the precipitator vessel compared to the total amount of curcuminoids injected. The total amount of solids in the ethanolic extract was determined by weighing the amount of solids after drying a sample (25 cm^3) in a rotary evaporator (Laborota, model 4001, Vertrieb, Germany) at 313 K compared to the initial weight of the sample.

2.3.1. Determination of morphology and size distribution

The morphology of the curcuminoid particles was examined by scanning electron microscopy (SEM; LEO Electron Microscopy/Oxford, Leo 440i, Cambridge, England) with an energy dispersive X-ray analyzer (LEO Electron Microscopy/Oxford, 6070, Cambridge, England). Samples were coated with a thin layer of gold in a Polaron sputter coater (VG Microtech, SC7620, Uckfield, England) and were examined using an SEM at 20 kV accelerating voltage and 100 pA beam current. The mean particle size distribution was measured by laser light scattering with a Malvern Mastersizer-2000 (Malvern Instruments, Malvern, UK). The curcuminoid particles were suspended in a 0.1% (v/v) tween® 80 solution. The particle size distribution was monitored for each measurement until the readings were constant.

2.3.2. Curcuminoid content determination

The curcuminoid content of the particles was determined by HPLC on a Waters Alliance separation module (model 2695D, Milford, USA) using a Kinetex C18 column (150 × 4.6 mm id, 2.6 μm, Phenomenex, Torrance, CA, USA). This method is described by Osorio-Tobón et al. [18]. The mobile phase consisted of 0.1% water (v/v), acetic acid (solvent A), and 0.1% acetonitrile (v/v) acetic acid (solvent B). Compounds were detected at 425 nm and quantified using the Empower 2 software (Waters, Milford, MA, USA). The injected samples were prepared by diluting 10 mg of particles in 25 cm^3 of ethanol.

2.4. Statistical analysis

The SAS® v. 9.2 software was used to determine the effects of process conditions on the global yield of solids, curcuminoid content and size of the particles. The factors evaluated on the sub-plot were temperature (313 and 333 K), pressure (10 and 12 MPa), and CO_2 flow rate (500 and 800 g/h), totaling 32 experimental units (Table 1).

A Split-Plot [21] experimental design with a statistical significance of 0.05 was used in which the nozzle type was the constant parameter applied in to whole plots with one replication. Split-plot designs were originally developed for experimentation in

Table 1
Summary of the process parameters and results of the precipitation process of curcuminoids by SAS.

Run	Nozzle	Temperature (K)	Pressure (MPa)	CO_2 flow rate (g/h)	GY_{SOL} (%)	C_{CC} (mg/g)	PE (%)	$d_{[3,4]}$ (μm)	PDI
1	T-mixer	313	12	500	48.2	415	73	206	2.9
2	T-mixer	313	10	500	53.1	524	91	111	2.2
3	T-mixer	333	12	800	47.4	397	69	134	3.2
4	T-mixer	333	12	500	43.2	446	78	236	2.2
5	T-mixer	313	10	800	69.6	485	85	287	11.1
6	T-mixer	333	10	500	–	–	–	–	–
7	T-mixer	313	12	800	66.8	344	60	850	1.4
8	T-mixer	333	10	800	–	–	–	–	–
9	Coaxial	333	10	800	55.9	558	97	331	2.4
10	Coaxial	333	12	800	–	–	–	–	–
11	Coaxial	333	12	500	34.4	316	55	245	3.4
12	Coaxial	333	10	500	34.1	474	83	238	2.6
13	Coaxial	313	10	800	40.3	392	69	432	5.7
14	Coaxial	313	10	500	41.7	445	78	522	3.4
15	Coaxial	313	12	800	47.1	443	77	212	4.9
16	Coaxial	313	12	500	38.5	528	92	356	4.3
17	Coaxial	333	12	800	–	–	–	–	–
18	Coaxial	313	12	500	38.0	487	85	244	3.9
19	Coaxial	313	10	500	40.9	426	74	512	2.5
20	Coaxial	333	12	500	35.2	303	53	142	4.5
21	Coaxial	313	12	800	48.9	469	82	229	5.3
22	Coaxial	333	10	800	53.6	551	96	315	2.6
23	Coaxial	333	10	500	36.1	470	82	322	2.6
24	Coaxial	313	10	800	45.0	420	73	297	3.5
25	T-mixer	333	10	800	–	–	–	–	–
26	T-mixer	333	10	500	–	–	–	–	–
27	T-mixer	313	10	800	68.0	490	86	300	1.4
28	T-mixer	333	12	500	41.6	486	85	364	3.7
29	T-mixer	313	12	800	66.9	337	59	829	1.4
30	T-mixer	313	10	500	53.9	547	96	111	2.6
31	T-mixer	333	12	800	53.3	395	69	222	2.9
32	T-mixer	313	12	500	48.5	427	75	126	2.3

GY_{SOL}: global yield of solids; C_{CC}: curcuminoid content; PE: precipitation efficiency; $d_{[3,4]}$: particle size; PDI: polydispersity index.

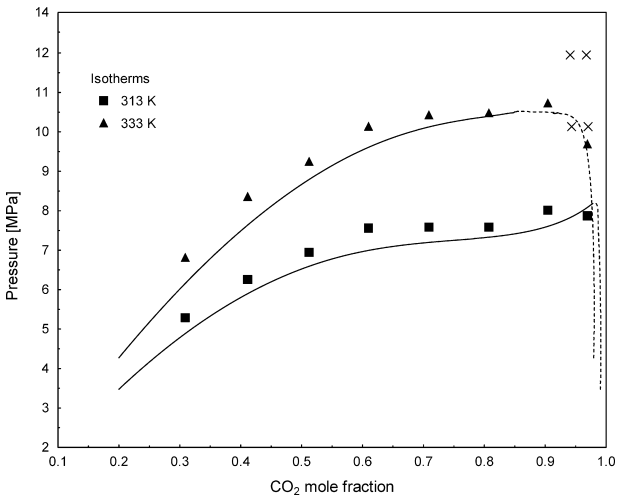

Fig. 2. *P–x–y* VLE diagram for the system CO_2 + ethanol + curcumin at 313 and 333 K reproduced from Giufrida et al. [22]. Symbols (×) indicate the experimental conditions used.

agriculture and have important application in planned experimentation. They are the choice when the levels of some treatment factors are more difficult to change than others when running the experiment. In this work, as the levels of the nozzle factor was more difficult to change compared to the others factors, the type of nozzle was randomly allocated to the "whole plots" and the combinations of the levels of the factors temperature, pressure and CO_2 rates were randomly allocated to the "split-plots". With this design it was possible to evaluate the effects of all factors and interactions. With this experimental design the SAS apparatus configuration was changed 3 times instead of the possible up to 16 times using a completely randomized factorial design.

3. Results and discussion

In this work the ethanolic extract flow was fixed at 0.5 cm^3/min. Preliminary tests showed that when a higher flow rate was used (0.75 and 1 cm^3/min), there was no possible way to form particles. The solvent was not removed quickly enough, and because it was presented as an accumulation of the ethanolic extract within the precipitation vessel, the ethanolic solvent was dragged to the glass flask where the solvent was collected. The process remained incomplete when a lower ethanolic extract flow (0.25 cm^3/min) was used due to nozzle obstructions.

Fig. 2 presents the *P–x–y* VLE diagram for the system CO_2 + ethanol + curcumin at 313 and 333 K [22]. It is evident that almost all of the experimental points executed in the supercritical fluid phase were fully developed, with the exception of the experiments conducted at 333 K and 10 MPa. However, in this study, because of the presence of soluble solids other than curcuminoids in the ethanolic solution, the phase diagram of the system could have changed. For example, in this work, the curcuminoids represented approximately 32% of the total soluble solids present in the ethanolic extract. Depending on the system temperature and pressure and due to the large differences in size, shape, and polarity of the molecules forming the ethanolic extract, three or more phases may have been produced [23]. For example, at 333 K and 10 MPa, the operating point was located in subcritical conditions or in the two phase region. This phase formation, combined with the effect of each type of nozzle and equipment settings, could cause a lack of particle formation. In this work, regardless of the CO_2 flow rate used (500 or 800 g/h of CO_2), no particles were formed when the experiments were performed with the T-mixer nozzle at 333 K and 10 MPa. Moreover, when the coaxial nozzle was used at 333 K, at 12 MPa and with 800 g h of CO_2, particles were not formed.

3.1. Effect of the process parameters on the global yield of solids

The effect of the process parameters, temperature, pressure, CO_2 flow rate, and type of nozzle on the characteristics of the particles formed by SAS was examined in this study. The ethanolic extract rich in curcuminoids used for the experiments had a solid content of 5.5 ± 0.1 wt.% and a curcuminoid concentration of 17.8 ± 0.6 mg/g. The results in terms of global yield of solids (GY_{SOL} (%)), curcuminoid content (C_{CC} (mg/g)), precipitation efficiency (PE (%)) and particle size ($d_{[3,4]}$) are presented in Table 1.

The respective global yield of solids between 35 ± 1% and 69 ± 1% were obtained using the following process conditions: coaxial nozzle, 313 K, 12 MPa and CO_2 flow rate of 500 g/h, and T-mixer nozzle, 313 K, 10 MPa and CO_2 flow rate of 800 g/h. According to the literature, the SAS process has resulted in a global yield of solids between 6.8% and 62% [8,24,25]. The results obtained in this work are similar and even slightly higher.

In the range of temperature (313 and 343 K), pressure (10 and 12 MPa), CO_2 flow rate (500 and 800 g/h), and type of nozzle (T-mixer and coaxial), the interaction among temperature × pressure ($p_{value} < 0.0143$) and nozzle type × temperature × CO_2 ($p_{value} < 0.0001$), flow rate had a significant effect on the global yield of solids. Fig. 3 shows the interaction plot of the process parameters on the global yield of solids. Generally, a higher global yield of solids was obtained when the process was performed at 313 K. In contrast, an increase in the process pressure caused a slight decrease in the global yield of solids at both temperatures but was still more pronounced for the 333 K temperature case (Fig. 3a). An increase in the pressure and temperature contributed to an increase the solubility of the solids in the CO_2 + ethanol phase, causing the entrainment of solids and, therefore, a decrease in the global yield of solids. For both types of nozzles, (Fig. 3b and c) an increase in the CO_2 flow rate resulted in an increase in the global yield of solids. For the T-mixer nozzle, higher yields were obtained when a temperature of 313 K was used. In contrast, for the coaxial nozzle, when the temperature was 333 K and the CO_2 flow rate was increased, the global yield of solids was greater. These results can be explained by the fact that when the CO_2 flow rate increased, the ethanol fraction in the CO_2 + ethanol phase decreased and, therefore, the solubility of the solutes in the CO_2 + ethanol phase reduced and the solutes were recovered in a greater proportion. Additionally, the high speed of CO_2 allowed for a quicker mixture between the ethanolic extract and the CO_2, reaching supersaturation more quickly, and thus, a higher global yields of solids.

3.2. Effect of the process parameters on curcuminoid content of the particles

Curcuminoid contents between 310 ± 9 mg/g and 554 ± 5 mg/g were obtained using the following process conditions: coaxial nozzle, 333 K, 12 MPa and CO_2 flow rate of 500 g/h and coaxial nozzle, 333 K, 10 MPa and CO_2 flow rate of 800 g/h. The large amount of curcuminoids recovered during the precipitation should be highlighted. Depending on the process conditions, between 54% and 97% of the total curcuminoids present in the ethanolic extract were precipitated. The values of precipitation efficiency (PE) are presented in Table 1. Additionally, although the extract obtained after solvent elimination by rotary evaporation was an oleoresin, its curcuminoid content (297 ± 21 mg/g) was almost 2 times lower than the curcuminoid content of the powdered extract obtained by SAS. The SAS process, besides being excellent in the precipitation of curcuminoids, allows fractionation of the solution. It was therefore possible

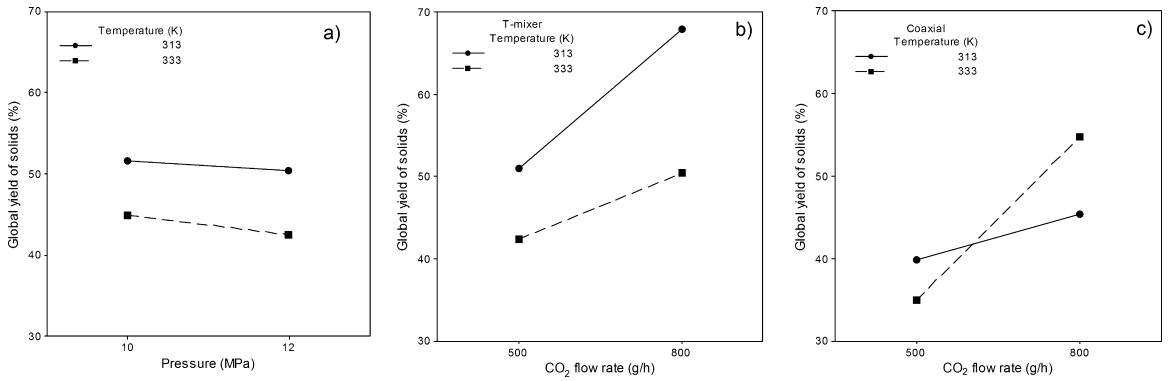

Fig. 3. The effect of the process parameters on global yield of solids. (a) temperature × pressure, (b) and (c) nozzle × temperature × CO_2 flow rate.

to obtain higher yields of curcuminoid than in the traditional process.

The powdered extract obtained by SAS has curcuminoid content 31 times higher than that of the injected extract. Thus, the precipitation of curcuminoids using the SAS process represents a more efficient alternative for the curcuminoids purification from a liquid extract than the conventional solvent elimination technique by rotary evaporation.

For the curcuminoid content, the interactions among the following process parameters were significant: nozzle type × pressure ($p_{value} < 0.0001$), pressure × CO_2 flow rate ($p_{value} = 0.0013$) and nozzle type × temperature × CO_2 flow rate ($p_{value} = 0.0049$). Generally, as with the global yield of solids, higher curcuminoid contents were obtained using the T-mixer. Again, the pressure exerted had a negative effect on the curcuminoid content (Fig. 4a). This decrease can be explained by the increase in the solubility of the CO_2 + ethanol mixture at higher pressures [24]. A cosolvent effect was produced, causing a higher dissolution of the curcuminoids in the mixture. Furthermore, combined with the increased flow rate of CO_2, a higher fraction of the curcuminoids was dragged to the solvent flask, causing an overall reduction in the curcuminoids content (Fig. 4b–d).

The results regarding the curcuminoid content as presented in Fig. 4 show that the process efficiency was higher using a CO_2 flow rate of 500 g/h, whereas a better global yield of solids was obtained using a CO_2 flow rate of 800 g/h as showed in Fig. 4. These results can be explained due the solubility difference between the curcuminoids and the remaining solids in the CO_2 + ethanol phase at process conditions. With a greater pressure and CO_2 flow rate, the solubility of the curcuminoids in the CO_2 + ethanol phase increased and the curcuminoids were dragged out of the system. For example, at a lower CO_2 flow rate, the solubility of curcuminoids in CO_2 + ethanol

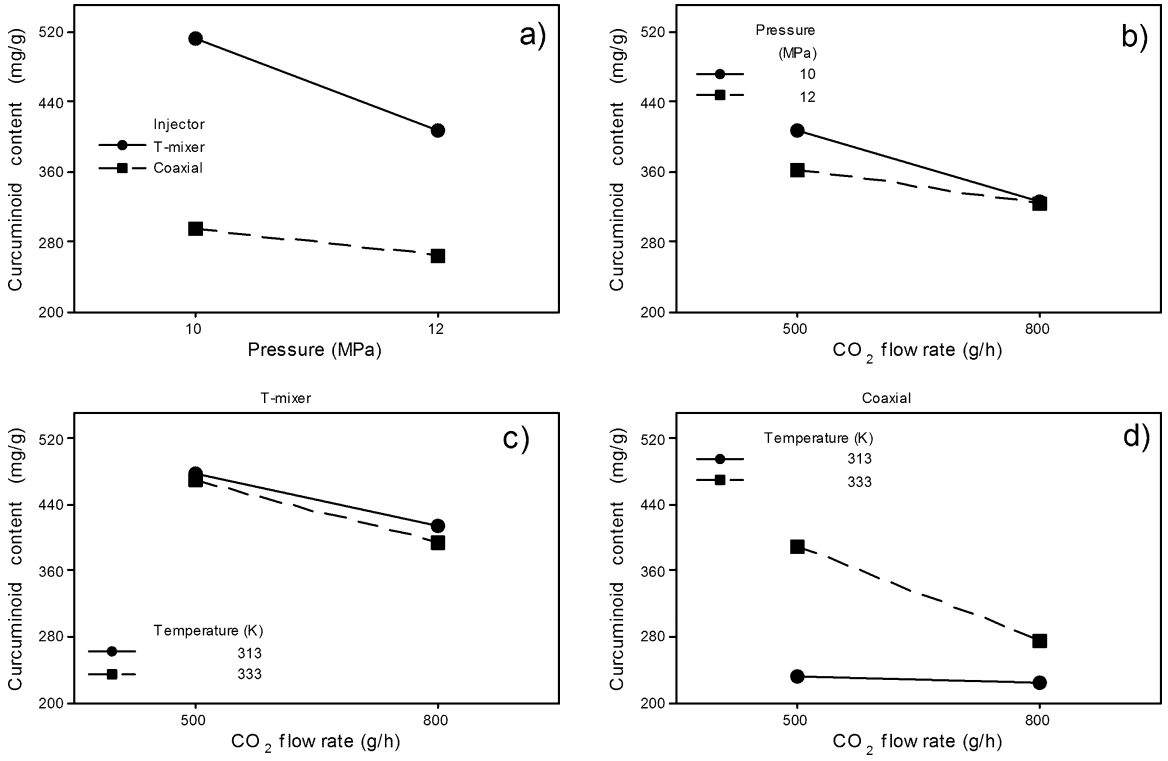

Fig. 4. The effect of the process parameters on curcuminoid content. (a) nozzle × pressure, (b) temperature × CO_2 flow rate, (c) and (d) nozzle × temperature × CO_2 flow rate.

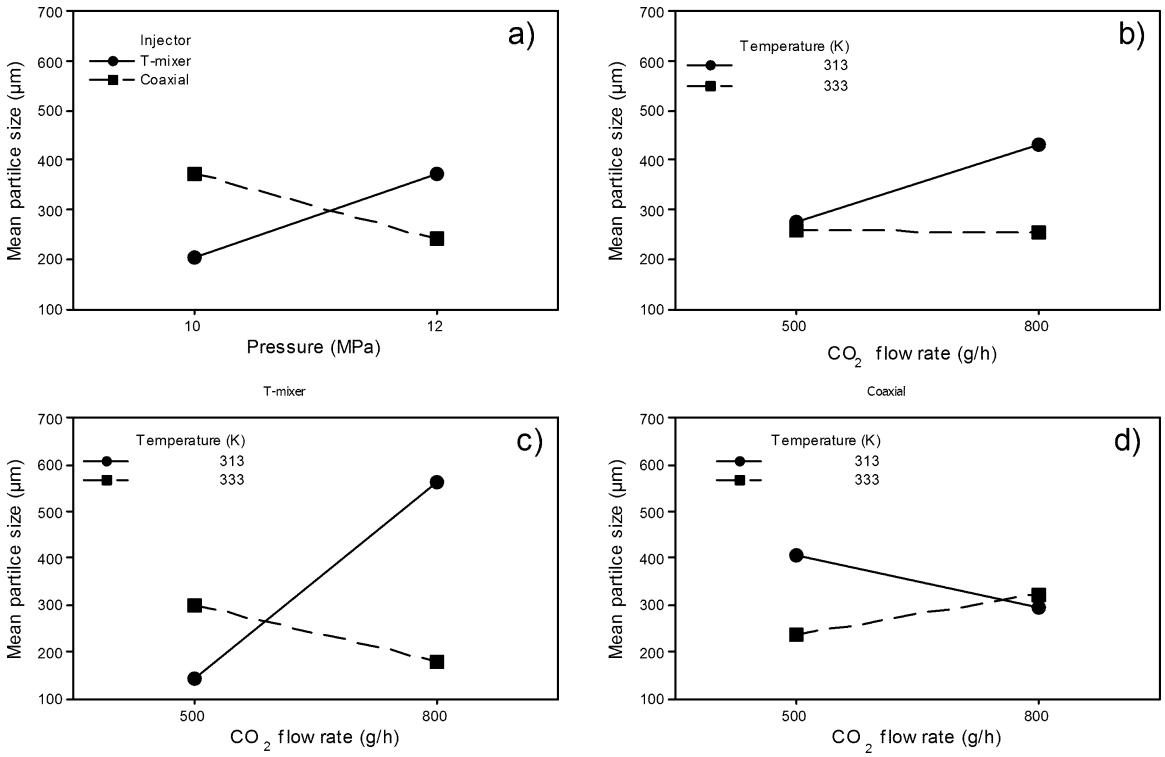

Fig. 5. The effect of the process parameters on mean particle size. (a) nozzle × pressure, (b) temperature × CO_2 flow rate, (c) and (d) nozzle × temperature × CO_2 flow rate.

phase decreased in relation to the remaining solids, resulting in a lower global yield of solids and a higher content of curcuminoids in the particles.

Moreover, a higher curcuminoid content was reached using the coaxial nozzle at 333 K, 10 MPa and 800 g/h. This can be explained due the combination of the experimental conditions under which the process was performed. The coaxial nozzle produced a smaller droplet size, which, combined with the high speed of the CO_2, promoted the breaking of the droplets. This allowed a more rapid rate of mixing and thus supersaturation was more rapidly reached. Furthermore, the higher temperature contributed to reach faster supersaturation, and the low pressure reduced the cosolvent effect.

3.3. *Effect of the process parameters on the mean particle size*

Particles with sizes between 111 ± 1 μm and 840 ± 15 μm were obtained, corresponding to the following process conditions: T-mixer nozzle, 313 K, 10 MPa and CO_2 flow rate of 500 g/h and T-mixer nozzle, 313 K, 12 MPa and CO_2 flow rate of 800 g/h, respectively. These results are comparable to the following results: the precipitation of rosemary antioxidants by SAS from an ethanolic extract (particle size > 200 μm) [8], milk powder produced by pneumatic conveying (20–200 μm), spray drying (300–2000 μm), and integrated fluid beds (100–400 μm) [26]. However, compared to prior work, the mean particle size obtained in this work was larger. The complex composition of the ethanolic extract, process conditions, and limitations of the equipment [20] prevented the formation of smaller particles.

The particle size was significantly affected by the following parameters: nozzle type × pressure ($p_{value} < 0.0001$), temperature × CO_2 flow rate ($p_{value} = 0.0155$), and nozzle type × temperature × CO_2 flow rate ($p_{value} < 0.0001$). The effect of the process parameters on the particle size was different for both nozzle types. These differences may be explained by process conditions with respect to high pressure phase equilibrium, as well as the influence of the type of nozzle in the atomization of ethanol extract in CO_2. For example, the initial droplet size may play an important role in the time required for the complete evaporation of the solvent, which may influence the characteristics of the particles formed [27]. In this work, the droplet size produced by the coaxial nozzle was lower than that produced by the T-mixer nozzle.

For the coaxial nozzle, as the pressure increased, the particle size decreased. In contrast, when the T-mixer nozzle was used, an increase in pressure increased the particle size (Fig. 5a). Due the smaller inner diameter of the coaxial nozzle and high pressure, turbulence was increased and faster supersaturation was achieved, leading to early nucleation and therefore to the formation of smaller particles [28]. However, in the T-mixer nozzle, an increase in pressure resulted in an increase in particle size. At higher pressure, the solubility of the compounds in the CO_2 + ethanol phase is higher, resulting in a lower supersaturation [29]. This fact, combined with the larger drop size, retards nucleation and therefore particle formation. At low temperatures, an increase in the CO_2 flow rate causes an increase in particle size (Fig. 5b). This may be caused by delayed supersaturation which occurs because the flying time of droplets is too short for drying before reaching the end of the precipitation vessel [27].

Fig. 5c and d shows the temperature and CO_2 flow rate effects for both nozzles. The particle size increased as the CO_2 flow rate increased, when the process was performed using the T-mixer nozzle at 313 K (Fig. 5c). Similar to the results of Rantakylä et al. [30], it is possible that the drying droplets collided with other drying particles on the bottom of the precipitation vessel. This delayed the supersaturation process, decreased the particle formation speed and, thus, formed larger particles. On the other hand, smaller particles were obtained as a product of faster supersaturation, due to the shorter time of solvent evaporation when the process was performed at 333 K and CO_2 flow rate of 800 g/h.

For the coaxial nozzle (Fig. 5d), the effects of temperature and CO_2 flow rate interaction had a contrary effect compared with that of the T-mixer nozzle. In the T-mixer nozzle, at a low temperature,

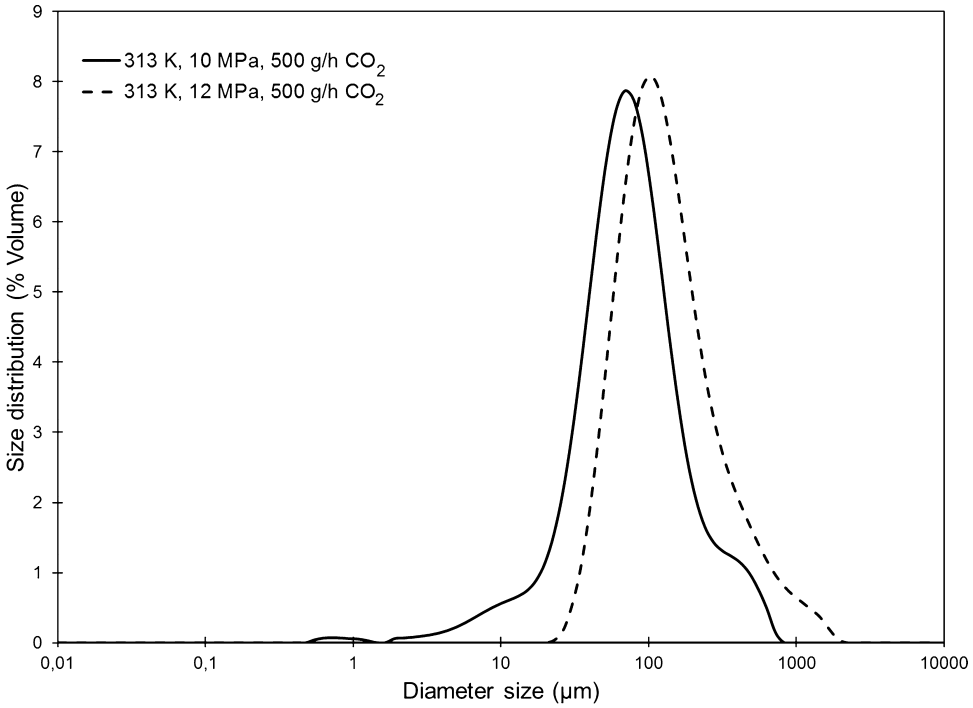

Fig. 6. Particle size distributions calculated using the T-mixer nozzle at 313 K, CO_2 flow rate of 500 g/h, 10 and 12 MPa.

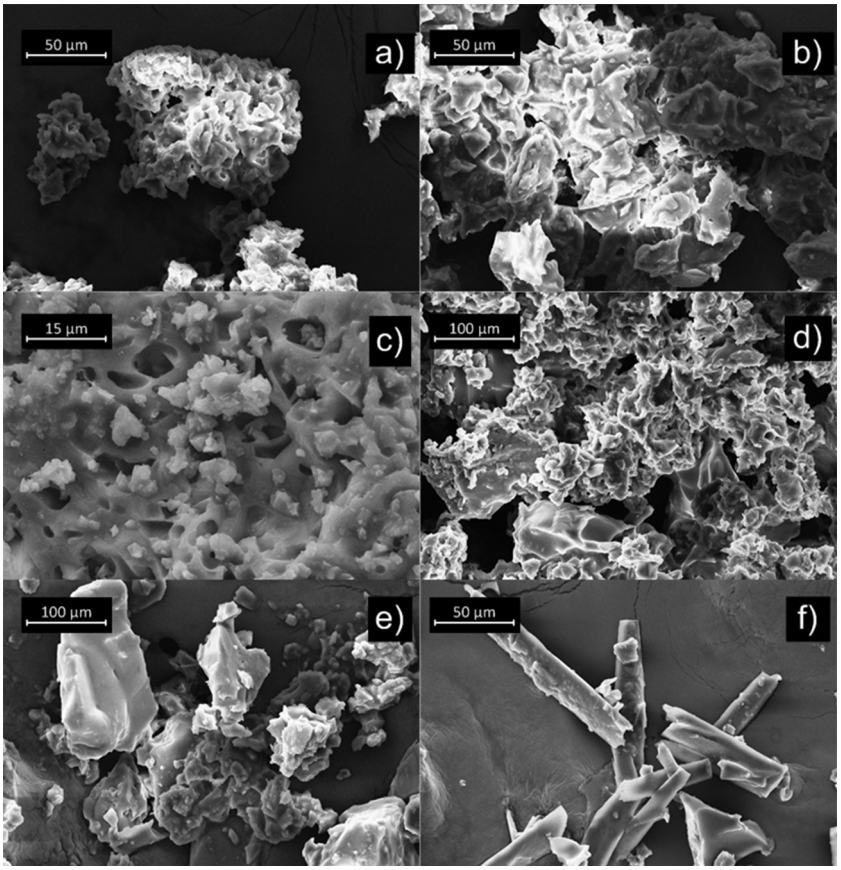

Fig. 7. SEM images of curcuminoid precipitated by SAS. (a) and (b) T-mixer nozzle, 313 K, 500 g/h of CO_2, 12 MPa; (c) T-mixer nozzle, 333 K, 800 g/h of CO_2, 12 MPa; (d) T-mixer nozzle, 313 K, 800 g/h of CO_2, 10 MPa; (e) T-mixer nozzle, 313 K, 500 g/h of CO_2, 10 MPa; (f) coaxial nozzle, 333 K, 500 g/h of CO_2, 10 MPa; (f) coaxial nozzle, 313 K, 800 g/h of CO_2, 12 MPa and (f) coaxial nozzle, 333 K, 500 g/h of CO_2, 12 MPa.

as the CO_2 flow rate increased, the droplet size decreased, thereby increasing the diffusion of the CO_2 into the drop. The use of a high CO_2 flow rate caused a pronounced turbulence inside the precipitation vessel, leading to an increase in the kinetic energy of the atomizing CO_2. This caused the mass transfer rates between CO_2 and the organic solvent to increase, with CO_2 diffusing more rapidly into the droplet. Thus, this phenomenon could have accelerated the supersaturation and nucleation, resulting in the formation of smaller particles [31]. On the other hand, at a temperature of 333 K as shown in Fig. 5d, an increase in the CO_2 flow rate caused a slight increase in the particle size. At these process conditions the supersaturation could have been delayed and thus, the particle size was increased.

The polydispersity index was used to express the uniformity of the process in relation to particle size distribution (Table 1). According to Gottlieb and Schwartzbach [32], polydispersity index values below 2 indicate a narrow and uniform distribution. Although, in general, the polydispersity index values were high, in the conditions where smaller particles, were obtained, they had a polydispersity index value close to 2. In Fig. 6, the particle size distributions calculated using the T-mixer nozzle at 313 K, CO_2 flow rate of 500 g/h, 10 and 12 MPa are presented.

Summarizing, when the process is performed using the T-mixer nozzle at 313 K, 10 MPa and a CO_2 flow rate of 500 g/h is possible to obtain the smallest particle size with a narrow size distribution. As can be observed in Table 1, at these process conditions, approximately 93.5% of the curcuminoids wee precipitated obtaining a powdered extract with higher purity (535.5 mg/g). Indeed, according to our experience with the equipment, when the experiments were performed under these conditions the system presented better stability regarding to maintaining of the pressure and CO_2 flow rate. On the other hand, considering the integrated process it would be better working with less severe process conditions because requires less energy.

3.4. *Effect of the process parameters on the particle morphology*

The effect of process parameters on the particle morphology was examined in this work. Fig. 7 shows the SEM micrographs of the particles obtained for some of the experimental conditions. Depending on the process conditions, irregular aggregates greater than 100 μm with different morphologies were obtained. For both types of nozzle, individual polydisperse particles agglomerated into larger structures were produced. Owing to the presence of various compounds with a complex molecular structure in the injected ethanolic extract, the particle growth process was altered [33]. For example, it is possible that during the precipitation process, new chemical bonds between neighboring molecules were created, forming more complex structures of a larger size [34].

Aggregates with a more irregular morphology and porous regions formed by the agglomeration of smaller particles were observed when the T-mixer nozzle was used (Fig. 7a–d). Moreover, aggregates showed a tendency to form more lengthened rod-like structures without porosities when the coaxial nozzle was used (Fig. 7e and f). In particular, as is shown in Fig. 7e and f, when the T-mixer nozzle was used at low temperatures with a low CO_2 flow rate and high pressure, slightly oval aggregates were obtained. A continuous surface with a porous morphology was observed when the T-mixer nozzle was used at a low temperature and pressure and with a high CO_2 flow rate (Fig. 7c). On the other hand, when the process temperature or pressure was increased and the CO_2 flow rate was low, the continuous surface disappeared, and caves were formed by smaller aggregates. For the coaxial nozzle, the combination of low temperature, high pressure and high CO_2 flow rate allowed the formation of particles with a more defined rod-shape (Fig. 7f). When the temperature was increased in combination with a low CO_2 flow rate, regardless of process pressure, larger non-porous structures were formed (Fig. 7e).

4. Conclusions

The precipitation of curcuminoids using the SAS process is an attractive option to eliminate the solvent of an ethanolic extract and produce a powdered extract rich in curcuminoids. Due the functional properties of the curcuminoids, the particles obtained in this work could have applications in food and pharmaceutical industries. The precipitation temperature, pressure, CO_2 flow rate, and nozzle type had a significant effect. The use of T-mixer nozzle combined with a temperature of 313 K, a pressure of 10 MPa and a flow rate of 500 g/h represent the best conditions for the global yield of solids, curcuminoid content, and small particle size. These conditions present a good alternative to having to carry out the precipitation process of curcuminoids. These conditions also result in producing a powdered extract with greater purity and a smaller particle size. Owing to the complexity of the ethanolic extract and the characteristics of the process, individual polydisperse particles agglomerated in structures larger than 100 μm were produced. The results obtained by SAS are comparable to those of other traditional techniques; however, further research into phase equilibrium and the behavior of the ethanol extract within the system is suggested. The SAS process is useful for both the micronization and the fractionation of the extract and makes it possible to obtain a preferential precipitation of curcuminoids. In the future, it will be study the technical and economic viability of the integrated process for production of volatile oil and powdered curcuminoid-rich from turmeric.

Conflict of interest statement

The authors confirm that there are no conflicts of interest regarding this paper.

Acknowledgements

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.supflu.2015.09.012.

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-CAPÍTULO 6-

Process integration for turmeric products extraction using supercritical fluids and pressurized liquids: economic evaluation

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Process integration for turmeric products extraction using supercritical fluids and pressurized liquids: economic evaluation

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Abstract

An economic evaluation of an integrated process to produce derived from turmeric was performed. Process integration of supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and antisolvent process (SAS) process have been investigated in order to obtain turmeric essential oil (TEO) and powdered curcuminoid-rich extract (PCE). Scale-up caused a decrease in the cost of manufacture (COM) for both products. Volatile oil and powdered curcuminoid-rich extract COMs decreased from US$ 112.70 kg^{-1} to US$ 85.58 kg^{-1} and from US$ 174.80 kg^{-1} to US$ 141.63 kg^{-1}, respectively, when was used a raw material cost of US$ 7.27 kg^{-1} and the capacity of the system increased from 2×50 L to 2×500 L. The raw material cost has a significantly influence in the process expenses. When the capacity of the system is 2×50 L and the raw material cost decreased from US$ 7.27 kg^{-1} to US$ 1.59 kg^{-1}, volatile oil and powdered curcuminoid-rich extract COMs decreased from US$ 112.70 kg^{-1} to US$ 64.97 kg^{-1} and from US$ 174.80 kg^{-1} to US$ 140.96 kg^{-1}, respectively. The economic evaluation results of this work clearly show that the integrated process is a feasible alternative and an attractive inversion to produce derivatives from turmeric.

Keywords: *Turmeric; Volatile oil; Curcuminoids; Process integration; Cost of manufacture; Supercritical fluids; Pressurized liquids*

1. Introduction

Turmeric (*Curcuma longa* L.) is a plant is a plant widely cultivated in countries and regions with tropical and subtropical climates, mainly in India, China and Indonesia, as well as in some Latin American countries such as Brazil and Peru, for instance [1]. Turmeric annual production is estimated in 1100,000 t/year, and worldwide, India is the largest producer, exporter and consumer of turmeric, being responsible by 82% of the world production and 45% of the overall exports [2]. Turmeric is known for the characteristics of its rhizomes, which have been used since ancient times as condiment, preservative, flavoring agent and in folk medicine. Nowadays, turmeric and its derivatives have aroused great interest due to their pharmacological properties [3]. Additionally, due to its properties and the interest in replacing synthetic additives by natural compounds, turmeric and derivative products such as turmeric powder, extracts and oleoresins, have a great potential of insertion in food industry and in market of natural additives. According to Sloan and Adams [4], in 2012, whole-food supplement sales topped US$1.2 billion and turmeric and its derivative products are projected to be among the top 10 best-selling supplements through 2016 by Nutrition Business Journal (NBJ), representing a market about US$235 million. Functional and pharmacological properties of turmeric derivative products are due to mainly the presence in the rhizomes of two secondary metabolites, volatile oil and curcuminoids [5]. Turmeric owes its characteristic aroma to an essential oil present in the rhizome. Due to its properties, such as antioxidant, antimutagenic, anti-carcinogenic, anti-fungal, anti-bacterial and insect repellent activities, turmeric volatile oil is widely used in pharmaceutical applications [6]. In food industry, volatile oil is mainly used in the production of some confectionary products and soft drinks [7]. Curcuminoids are a group of phenolic compounds responsible by the yellow color of the rhizomes. These compounds have been recognized by its biological activity associated with anti-inflammatory, anti-bacterial, anti-carcinogenic and antioxidant activities [8]. Additionally, due to its properties and colorant power, curcuminoids have great potential use in food and pharmaceutical industries. Today, curcuminoids are used primarily as a natural coloring agent to replace synthetic dyes in chutneys, pickles, mustard, butter and cheese among other products [9].

In the conventional process to obtain volatile oil and curcuminoids (Figure 1a), turmeric comminuted to predetermined particle size and loaded in the extractors. It should be dried to optimum moisture level since excessive moisture affects the percolation rate and

product quality. Then, Solvent, admitted from the top, is sprayed on to the charge to exhaust the raw material. Solvents such as methanol, ethanol and acetone are commonly used in industrial production, due to the high solubility of the curcuminoids in these solvents [10]. Afterwards, volatile oil is be recovered from the mother liquor left over after solvent extraction. The above extract is re-extracted with hexane to recover a composite of fixed and volatile oils, and this composite could be subjected to fractional distillation to get the volatile fraction. However, steam distillation, which only removes the volatile oil, is a tedious and expensive operation [11]. After removing the volatile oil and the solvent, the concentrated extract is dissolved in alkali, filtered, and acidified with acid to precipitate the pigments [7].

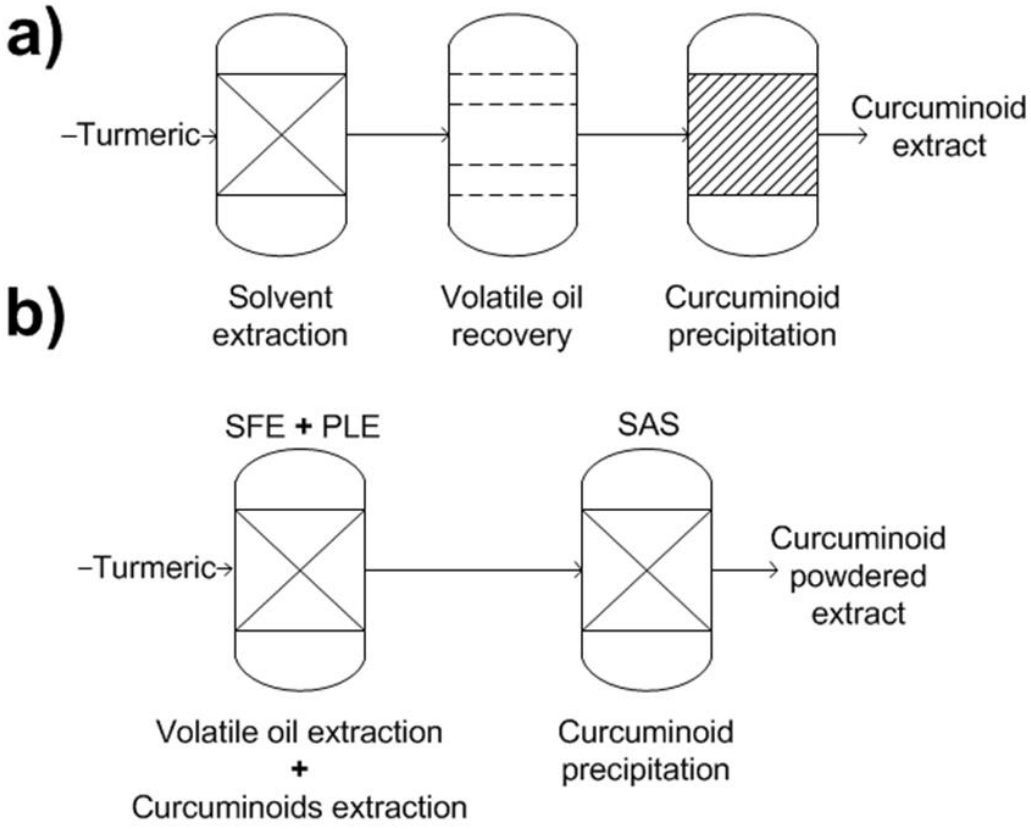

Figure 1. a) Process flow diagram for traditional volatile oil recover and curcuminoids extraction production; b) Process flow diagram for the SFE+PLE−SAS process.

Factors such as contact with light, oxygen, high temperatures and use of toxic solvents, highlight the need of develop novel processes that allows overcome these drawbacks. Alternatives techniques that involve the use of supercritical fluids and pressurized liquids have great potential to overcome the conventional processes limitations in environmentally friendly process that protects the integrity of the compounds. Meanwhile,

extracting volatile compounds using supercritical carbon dioxide ($scCO_2$) is one of the most interesting applications of supercritical technology because of the high solubility of these substances in CO_2 and the easy removal of the solvent through a pressure reduction [12].

For example, according to Carvalho et al. [13], the supercritical fluid extraction (SFE) of turmeric volatile oil allows obtain a high extraction yield in a faster extraction with a relatively low solvent consumption. On the other hand, pressurized liquid extraction (PLE) involves extraction using liquids solvents at temperatures above their atmospheric boiling point, enhancing solubility and mass transfer properties [14]. For instance, Osorio-Tobón et al. [15], demonstrated the feasibility of use PLE to obtain curcuminoid-rich extracts using ethanol as extraction solvent. Additionally, $scCO_2$ is the supercritical fluid that is most widely used due to its favorable characteristics, such as its low toxicity, low cost, easy removal and non-flammability [16]. Supercritical antisolvent (SAS) process takes advantage of these characteristics and combines with the slight solubility of curcuminoids in CO_2 and the acceptable solubility of the ethanol in CO_2 to eliminate the solvent and precipitate the curcuminoids, obtaining a powdered extract.

Process integration and intensification is an attractive approach to produce valuable products of higher quality, in processes with reduced energy consumption and therefore, with reduced costs [17]. According to Moraes et al. [18], integrating processes is different from intensifying processes. In the first case, the best process for each product is searched while in the second case the same equipment is used for different, nonetheless similar, unit operations. Process integration have been successfully applied using supercritical fluids, as in SFE integrated with low-pressure solvent extraction for bixin production from annatto seeds [18]. Fujii [19] developed a process integration of SFE and acid treatment for astaxanthin extraction from a vegetative microalga. On the other hand, process intensification using supercritical fluids also have been successfully applied, as in biodiesel production [20] as well in the production of pharmacological compounds as levodopa [21].

In this context, an integrated process named SFE+PLE−SAS is proposed in order to produce turmeric volatile oil (TEO) and powdered curcuminoid-rich extract (PCE). As shown in Figure 1b, the first stage is characterized by intensification of the SFE and PLE processes. Initially, volatile oil is obtained using $scCO_2$ through SFE. Immediately in the same equipment and after the SFE process, without removing the raw material from the extraction vessel, the curcuminoids are extracted by PLE using ethanol as solvent. Finally in a second

stage, the solvent is eliminated and curcuminoids are precipitated using $scCO_2$ through SAS process. Is important to highlight that contrary the conventional process, the integrated process proposed in this work uses solvents recognized as safe (GRAS) and process conditions that contribute to preserve the characteristics of the bioactive compounds. Additionally due to low number of unit operations and used processes, this integrated process represents a more efficient, economic and environmentally friendly alternative to obtain TEO and PCE.

The objective of the work presented here was provide an economical evaluation of an integrated process for production of volatile oil and powdered curcuminoid-rich extract using emerging technologies such as the use of supercritical fluids and pressurized liquids.

2. MATERIALS AND METHODS

2.1. Process simulation model

SFE+PLE−SAS process simulations were performed using the SuperPro Designer 8.5® software (Intelligen Inc., Scotch Plains, NJ, USA). Figure 2 shows the flowsheet of the SFE+PLE−SAS process developed for the simulation. The process is divided in two sections, one responsible by volatile oil production (SFE+PLE) and another responsible by the production of a powdered curcuminoid-rich extract (SAS). The input parameters and process conditions for the simulations of the SFE+PLE section were obtained from previous works done by Carvalho et al. [22] and Osorio-Tobón et al. [15]. Input parameters and process conditions for the second section (SAS) were obtained from a previous work done by Osorio-Tobón et al. [23]. Yield data and operation conditions, such as temperature, pressure, apparent density, solvent (S) to dry feed (F) mass ratio, bed porosity were used as input data of the model (Table 1).

2.1.1. SFE+PLE−SAS Process

As it was previously stated, curcuminoids are sparingly soluble in $scCO_2$, whereas TEO is quite soluble. Therefore, SFE+PLE section will not affect the extraction process of the coloring material, and instead, it will contribute to obtain a powdered extract with higher purity. Initially, ground turmeric is loaded in the extractor (P-5). Afterwards, CO_2 (stream 101) is cooled (278 K) using P2/HX-101 and pressurized (25 MPa) through P-3/PM-101. CO_2 reaches its supercritical point after be heated (333 K) by P-4/HX-103 and enters to P-5. Once

reached the temperature and pressure conditions into P-5, 20 min of static time are counted. Posteriorly, supercritical extraction is performed and the CO_2 + TEO mixture is recovered using the stream S-105. Finally, the TEO is recovered through decreasing pressure and temperature in P-6/V-104 (303 K and 4 MPa). CO_2 is recovered from P-6/V-104 and is reused through stream S-107.

Table 1. Data used to simulate the SFE−PLE−SAS process.

Parameter	Value
Volatile oil extraction [a]	
Extraction Yield	6.4%
Extraction time	95 min
Temperature	333 K
Pressure	250 MPa
S/F	2.4
Curcuminoid extraction [b]	
Extraction Yield	7.6%
Extraction time	110 min
Temperature	333 K
Pressure	10 MPa
S/F	2.4
Curcuminoid precipitation [c]	
Global precipitation yield	69.6%
Precipitation time	80 min
Temperature	313 K
Pressure	10 MPa

Values were obtained from [a] Carvalho et al. [22], [b] Osorio-Tobón et al. [15] and [c] Osorio-Tobón et al. [23].

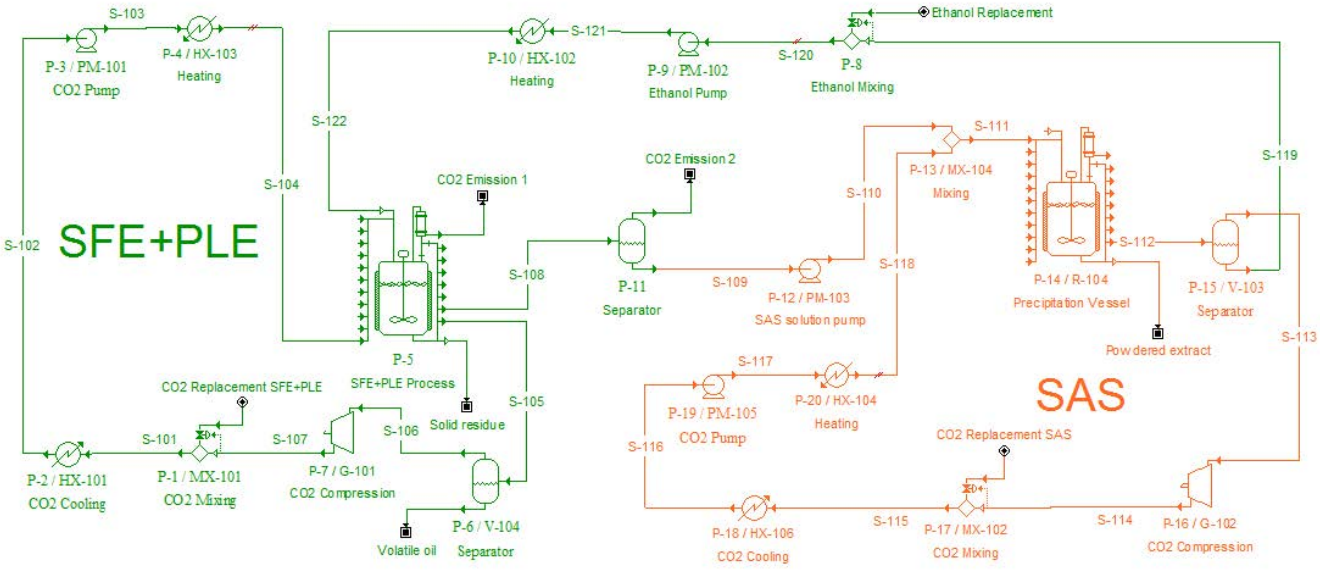

Figure 2. Flowsheet of the SFE+PLE−SAS process, designed by the SuperPro Designer 8.5® software.

Once finished SFE process and without remove the raw material from P-5, PLE process is begun. Ethanol is pressurized (10 MPa) by P-8/PM-102 and heated (333 K) using

P-9/HX-102. Again, once reached the temperature and pressure conditions into P-5, 20 min of static time are counted. The extraction begins and the ethanolic curcuminoid-rich extract is flows across stream S-108 to separator P-11 and the solvent elimination and curcuminoid precipitation starts in SAS section. An important feature of the process is that whereas SAS process is being performed and when SFE+PLE process is finished, the exhausted raw material is removed from P-5, and cleaning and reload processes begin to start a new SFE+PLE process with fresh turmeric (Figure 3). In SAS process, initially CO_2 from P-17/MX-102 is cooled (268 K) using P18/HX-106 and pressurized (10 MPa) through P-19/PM-105. CO_2 reaches its supercritical state after being heated (313 K) by P-20/HX-104 and enters into the precipitation vessel P-13/R-104. After a system stabilization time of 10 min with $scCO_2$, the ethanolic extract is pressurized (10 MPa) using P-12/PM-103 and injected into P-14/R-104 through a T-mixer injector. Once injected the desired quantity of ethanolic extract, a quantity pre-established of CO_2 is injected to finish the drying process. PCE remains into P-14/R-104 whereas the etanol-CO_2 mixture leaves the precipitation vessel through stream S-112. Afterwards, CO_2 and ethanol are separated using a flash tank (P-15/V-103). CO_2 is reused using a compressor (P-15/G-101) and flows back to P-17/MX-101 through stream S-114. On the other hand, ethanol for reuse is conducted using the gravity force by stream S-119. PCE is recovered through stream "powdered extract" and the cleaning and maintenance of the precipitation system (precipitation vessel, filters and injectors) are performed whereas the SFE+PLE process is performed.

2.2. Economic evaluation

2.2.1. Economic evaluation parameters

Generally there is a difficulty to obtain equipment quotations and detailed specifications in the first stages of a project. However, values based in past vendor quotations and literature are a good alternative [24]. On the other hand, although current vendor quotations are the most accurate to obtain the values of the equipment, generally these costs are for equipment costs for capacities other than what is required. To scale the equipment cost to the required capacity is possible through equation (1), where C_1 is the equipment cost with capacity Q_1; C_2 is the known base cost for equipment with capacity Q_2 and n is a constant depending on equipment type. Values of n were collected from literature [24-27]. In Table 2 are presented the base costs used is this work.

$$C_1 = C_2 \left(\frac{Q_2}{Q_1}\right)^n \quad (1)$$

The cost of manufacturing (COM) can be determined by the sum of three main components: direct costs, fixed costs and general expenses. COM was estimated according to the methodology proposed by por Turton et al. [27], where the three principal components of the COM for each process section were estimated in terms of five major costs: fixed capital of investment (FCI), cost of operational labor (COL), cost of utilities (CUT), waste treatment cost (CWT) and cost of raw material (CRM). FCI involves expenses related to the implementation of the production line (extraction and precipitation units and other equipment). COL is related to the operators of the extraction units. CUT considers the energy used in the solvent cycle for steam generation, water refrigeration and electricity requirements. CRM consists of the raw material cost and the cost of the solvent. Finally, CWT was considered to be zero because the waste generated by the process can be considered harmless and clean and may be reused in other applications or simply disposed of as ordinary vegetable waste. More details can be found in Rosa and Meireles [28] and Peters et al. [29]. Considering these aspects, Table 3 provides important information about data used for COM simulation.

2.2.2. Scale-up process

For the process scale-up, a procedure similar to that described by Santos et al. [30] was used: it was assumed that the yield and extract composition obtained at the laboratory scale would also be obtained at the industrial scale when the same processing conditions were used (temperature, pressure, S/F ratio, bed porosity, etc.). The process was designed to operate for 7920 h per year, which corresponds to 3 daily shifts for 330 days per year. Industrial scale extractor volumes of 2×5 L, 2×50 L and 2×500 L were considered. The number of operators required to operate the industrial units were similar those defined by Veggi et al. [31]. 1, 2 and 3 operators were defined for industrial units of 2×5 L, 2×50 L and 2×500 L, respectively. The amount of turmeric to be processed in each stage was calculated based on the extractor size and apparent density of raw material (838 kg/m^3). The CO_2 and ethanol losses during the recirculation process were assumed to be 2%, which was the loss from a distiller calculated by the simulator. A raw material pre-treatment cost of US$ 40 t^{-1} was used [31]. The cost of the raw material (DTRs) was assumed to be US$ 7.27 kg^{-1} (Oficina das Ervas, Ribeirão Preto, Brazil). To evaluate the influence of the turmeric

purchasing cost on COMs, other additional turmeric supplier was evaluated (US$ 1.59 kg^{-1}, Commodity Online, India, www.commodityonline.com, last accessed on 21/04/2015). The other input economic parameters presented in Table 3 were similar to those described by Veggi et al. [31] and Cavalcanti et al. [32].

Table 2. Base cost for equipment composing the extraction plant.

Equipment	n [a]	Unit base cost (US$) [b]
SFE+PLE		
Jacketed extraction vessel [c]	0.82	6,270.00
Air-driven CO_2 pump	0.55	2,470.00
Electric liquid pump	0.55	3,920.00
Cooler	0.59	2,080.00
Heater	0.59	820.00
Separation vessel	0.49	1,460.00
Manometer	0	410.00
Block valve	0.60	220.00
Back-pressure valve	0.60	1,780.00
Micrometering valve	0.60	1,090.00
Flowmeter	0.60	700.00
Safety Valve	0.60	310.00
Temperature controller	0.60	310.00
CO_2 compressor	0.46	2,200.00
Piping. connectors. crossheads. mixers and splitters [d]	0.60	3,660.00
Structural material for supporting the equipment [d]	0.60	4,060.00
SAS		
Jacketed precipitation vessel [c]	0.82	6,270.00
Air-driven CO_2 pump	0.55	2,470.00
Electric liquid pump	0.55	3,920.00
Cooler	0.59	2,080.00
Heater	0.59	820.00
Separation vessel	0.49	1,460.00
Manometer	0	410.00
Block valve	0.60	220.00
Micrometering valve	0.60	1,090.00
Flowmeter	0.60	700.00
Safety Valve	0.60	310.00
Temperature controller	0.60	310.00
CO_2 compressor	0.46	2,200.00
Piping, connectors, crossheads, mixers, splitters and filters [d]	0.60	3,660.00
Structural material for supporting the equipment	0.60	4,060.00
Total SFE+PLE−SAS process	−	**61,010.00**

Table 3. Input economic parameters used in the SuperPro Designer 8.5® software.

	Industrial Units		
	2×5 L	2×50 L	2×500 L
Fixed capital investment (FCI)			
SFE+PLE−SAS unit extraction [a]	US$ 175,728.00	US$ 774,839.00	US$ 3,735,571.00
Depreciation rate [b]	10 %/year	10 %/year	10 %/year
Annual maintenance rate [b]	6 %/year	6 %/year	6 %/year
Operational labor (COL) [c]	US$ 5.95 h^{-1}	US$ 5.95 h^{-1}	US$ 5.95 h^{-1}
Number of workers	1	2	3
Cost of the raw material (CRM)			
Pre-Processing [d]	US$ 40 t^{-1}	US$ 40 t^{-1}	US$ 40 t^{-1}
Turmeric supplier 1 [e]	US$ 7.27 kg^{-1}	US$ 7.27 kg^{-1}	US$ 7.27 kg^{-1}
Turmeric supplier 2 [e]	US$ 1.59 kg^{-1}	US$ 1.59 kg^{-1}	US$ 1.59 kg^{-1}
Industrial CO_2 [e]	US$ 1.85 kg^{-1}	US$ 1.85 kg^{-1}	US$ 1.85 kg^{-1}
Ethanol [e]	US$ 0.85 kg^{-1}	US$ 0.85 kg^{-1}	US$ 0.85 kg^{-1}
Utilities (CUT)			
Electricity [e]	US$ 0.20 $(kWh)^{-1}$	US$ 0.20 $(kWh)^{-1}$	US$ 0.20 $(kWh)^{-1}$
Water steam (high pressure) [f]	US$ 20 t^{-1}	US$ 20 t^{-1}	US$ 20 t^{-1}
Water [f]	US$ 0.05 t^{-1}	US$ 0.05 t^{-1}	US$ 0.05 t^{-1}
$CaCl_2$ (refrigerant fluid) [f]	US$ 0.25 t^{-1}	US$ 0.25 t^{-1}	US$ 0.25 t^{-1}

[a] Estimated cost using the Eq. (1); [b] based on Peters et al., 2003 [28]; [c] Bureau of Labor Statistics, http://www.bls.gov/fls/country/brazil.htm, USA, last accessed on 06/02/2015; [d] base on Veggi et al., 2014 [30]; [e] direct quotation; [f] SuperPro designer 8.5® database

Figure 3 presents the Gantt chart (a bar chart to illustrate the star and finish of an operation) for SFE+PLE−SAS process, considering an extraction plant containing 1 extraction vessel for SFE+PLE section and 1 precipitation vessel for SAS section, each with capacity of 50 L. Processing times, mainly in extraction operations were established based in S/F ratios that guarantee the complete extraction of interest compounds from raw material. For SFE and PLE processes was used a S/F ratio of 2.4. This S/F ratio allows the extraction of almost the totality of TEO and curcuminoids using a smaller amount of solvent and in a process with short extraction time.

For a 10-fold increase in the production capacity, the processing times for pressurization, depressurization, discharging and particle recovery were increased of 50%.

This percent increase was based on the knowledge acquired by developing the experimental part of this paper and also based on the results of other studies conducted by our research group during 30 years.

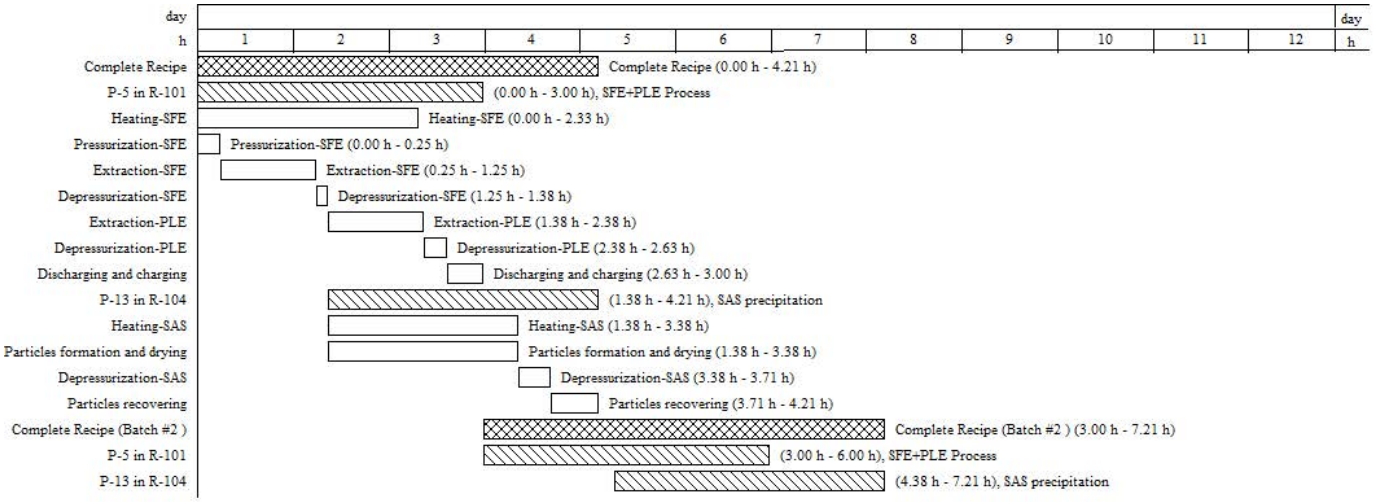

Figure 3. Operations Gantt chart obtained for a SFE+PLE−SAS process with capacity of 2×50 L.

3. Results and discussion

3.1. Economic evaluation of the SFE+PLE−SAS process

To determine the COMs of the TEO and PCE, the SFE+PLE−SAS process was simulated using the SuperPro Designer 8.5® software. For calculation of TEO and PCE COMs, to each product were charged the corresponding costs of each section where is produced. For TEO, the costs produced in SFE+PLE were considered and for PCE those produced in SAS section were considered. In other words, COM to each product was calculated as the relation between the annual operating cost of each section (CMR+FCI+COL+CUT) and the annual production rate of each product. Since both products come from the same raw material, the cost of ground turmeric was divided between the two products. In the same way, as operators are responsible for operation of the entire SFE+PLE−SAS process where TEO and PCE are produced, the COL was divided between the two products.

Integration of SFE, PLE and SAS processes allowed obtain products with a lower COM. The COM obtained for the production of TEO was 31.40% more economic than that obtained by Carvalho et al. [13] using a SFE process in a system with capacity of two extractors of 50 L. Although in the process proposed in this work, the objective was to use to the maximum the raw material, performing an exhaustive extraction of the essential oil and the curcuminoids, the result of the integration of the processes SFE, PLE and SAS was a more

economic process. For example, to guarantee the extraction of all essential oil, in this work, a S/F ratio of 2.4 was used, whereas in the work done by Carvalho et al. [13] was 1.31. Even though the cost of the raw material (turmeric) used in this work was slightly lower, the use of a higher quantity of solvent should have increased the COM, however, the process integration allowed the decrease of the COM due to the better use of raw material and equipment.

To the PCE, regarding to a previously work performed by Osorio-Tobón et al. [15], where the solvent was eliminated using rotaevaporation, the COM obtained in this work was superior. Although the extract obtained after rotaevaporation is an oleoresin, the COM calculated for that work was 46% and 38% more economic than that obtained for SFE+PLE-SAS process when was used systems with capacity of 2×50 L and 2×500 L, respectively. It is obvious that due to its higher duration, number of equipment involved and quantity of CO_2 used, the SAS process has a greater energetic expenditure in relation to the solvent elimination by rotaevaporation. However, the characteristics of the powdered extract are superior to the oleoresin, among them, higher curcuminoid content (2 times higher) and a much simpler storage. Similarly, the PCE COM has a higher value than the COM of the TEO produced in the same process. SAS process through which the solvent is eliminated and the powdered extract is obtained consumes more time, energy and solvent than the SFE+PLE process, representing a higher proportion of the annual operation cost (AOC). For example, using a turmeric purchasing cost of US$ 7.27 kg^{-1} and a system with capacity of 2×50 L, SAS process represents 61% of the AOC to entire system.

3.2. Influence of scale-up on COM

Initially, a turmeric purchasing cost of US$ 7.27 kg^{-1} and three different production scales (2×5 L, 2×50 L and 2×500 L) were considered as shown in Figure 4. It is possible to observe as COM decreases as production scale increases. For example, with this turmeric purchasing cost, the TEO COMs were estimated to be US$ 236.88 kg^{-1}, US$ 112.70 kg^{-1} y US$ 85.58 kg^{-1} for a SFE+PLE−SAS process with capacity of 2×5 L, 2×50 L and 2×500 L, respectively. Regarding to PCE production, when scale-up process was simulated, also was observed a COM decrease. COMs of 260.99 kg^{-1}, US$ 174.80 kg^{-1} and US$ 141.63 kg^{-1} were obtained for a SFE+PLE−SAS process with capacity of 2×5 L, 2×50 L and 2×500, respectively, considering a turmeric purchasing cost of US$ 7.27 kg^{-1}. Considering equipped units with an extractor and a precipitator of higher capacity, is possible increase the productivity and obtain lower COMs as show in Figure 4. However, it is necessary an

appropriate planning to ensure the supply of raw materials to guarantee the production of the final products.

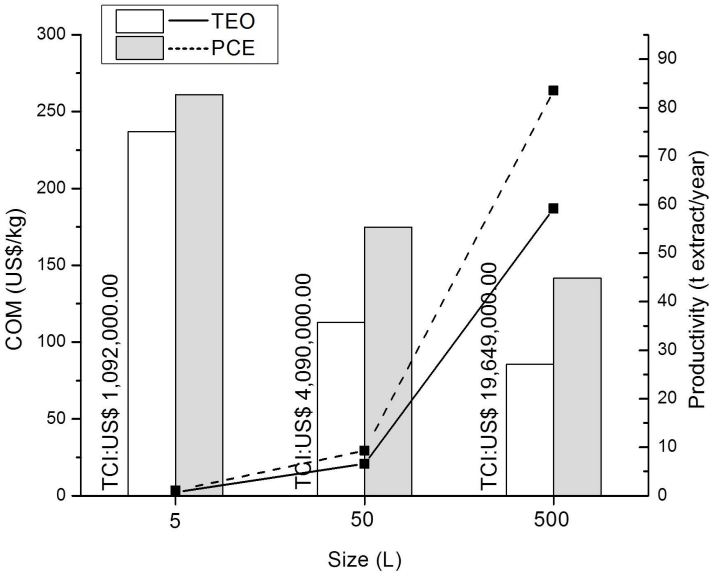

Figure 4. Influence of system capacity on the volatile oil and powdered curcuminoid extract COMs.

As shown in Figure 5, as the scale of the process increases, the CMR increases its impact on the COM for both process sections. The scale-up criteria used fixed the extraction time of the essential oil and the precipitation time of the curcuminoids. Only pressurization, depressurization, loading and unloading times were increased as scale was increased. Although with units with capacities of 2×50 L and 2×500 L the number of batches that can be performed annually decreased 11% and 20% regarding to integrated unit with capacity of 2×5 L, the quantity of raw material and solvent necessary significantly increases, and therefore their participation in the COM. Same as CMR, with scale-up, the CUT increased too. Obviously, as the quantity of raw materials and process times increased as the scale-up, the energetic expenditures and energy requirements were also increased, as can be observed in Figure 5. In important to highlight that in SAS section, the participation of the CUT in the COM is more representative than in SFE+PLE section. SAS process in compare with SFE and PLE processes has higher energy expenditure. The precipitation process involves the CO_2 pumping, maintaining of pressure and temperature during more time than SFE+PLE section, causing that SAS section have a higher participation in the COM for the entire process.

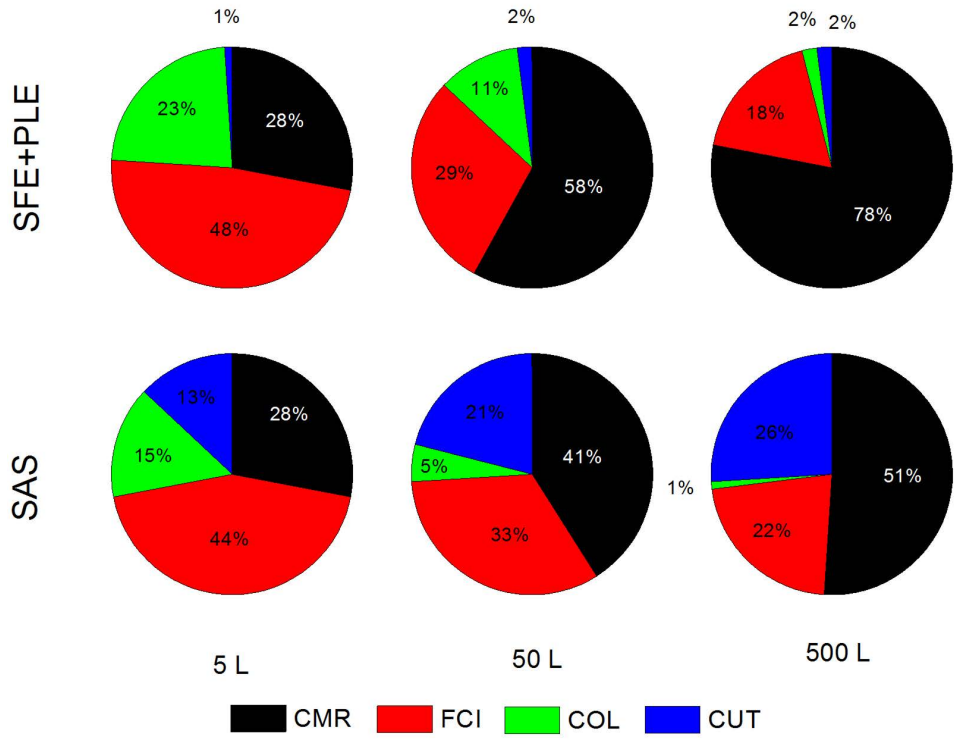

Figure 5. Influence of system capacity on the contribution of each component in COM in SFE+PLE and SAS process sections.

On the other hand, the other two components of the COM, the FCI and COL, decreased their participation in the COM as the process was scaled-up. This behavior is due to the high turmeric purchasing cost (US$ 7.27 kg^{-1}) and higher demand for raw materials at higher scales, the participation of FCI and COL in the COM is diluted. For example, the economic resources demanded by raw materials are 79 times higher for a 500 L system, than those required by a system of 5 L, whereas for FCI and COL, the economic resources required to 500 L system, are 21 and 2.9 times higher than 5 L system.

3.3. Influence of CRM on COM

Raw materials are generally the component that more contributes with the COM. The cost of the raw materials is often subjected to high variability and its behavior can be difficult to predict [33]. To evaluate the influence of turmeric purchasing cost on the COM, an alternative scenario using a purchasing cost of US$ 1.59 kg^{-1} was simulated. As shown in Figure 6, the COM for TEO and PCE strongly decreases as the CRM decreases. For a system with 2×50 L and when the cost of raw materials decreased from US$ 7.27 kg^{-1} to US$ 1.59 kg^{-1}, the COM for the TEO decreased from US$ 112.70 kg^{-1} to US$ 64.97 kg^{-1} and for PCE from US$ 174.80 kg^{-1} to US$ 140.96 kg^{-1}. This change in the CRM represented a diminished on the COM of 42% and 19%, for TEO and PCE, respectively. As presented in Figure 7, the CMR decreased its participation in the COM for both process sections. The other COM

components increased their participation and particularly, the FCI is the component that more increased its participation in the COM. The total investment cost does not increase and due to the significantly reduction in the CRM, the FCI participation in the COM is increased.

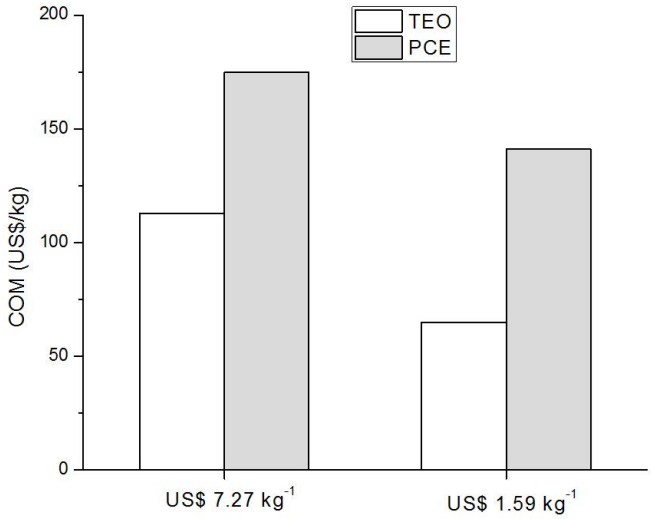

Figure 6. Influence of raw material purchasing cost on the volatile oil and powdered curcuminoid extract COMs.

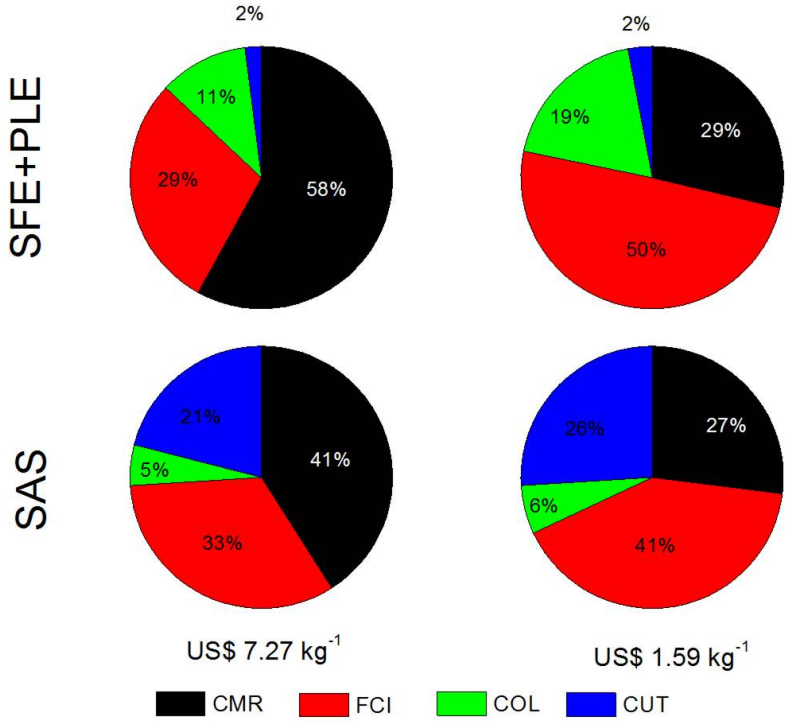

Figure 7. Influence of raw material purchasing cost on the contribution of each component in COM in SFE+PLE and SAS process sections.

3.4. Sensitivity study

Although is difficult establish a selling price to these type of products, two commercial products with similar characteristics to those produced in this work were used as reference. The market selling price for TEO produced by hydrodistillation is about US$ 265

kg^{-1} (Edens Garden, USA), whereas a PCE (95% purity) has an estimated selling price of US$ 324 kg^{-1} (Badmonkey Botanicals, USA). For this work, a selling price of US$ 265 kg^{-1} for the TEO was considered. On the other hand, due to that the purity of the PCE obtained through this process is 55%, a selling price of US$ 162 kg^{-1} was considered for the PCE. Two different sensitivity studies were performed varying the CRM in a SFE+PLE−SAS process with capacity of 2×50 L. In Table 4 is presented an executive summary of the project indices calculated after performed the simulation. Gross margin is an economic indicator that allow estimated the short-term possible benefit of a specific activity. Represents a company's total sales revenue minus its cost of goods sold, divided by the total sales revenue, expressed as a percentage [33]. In other words, this number represents the proportion of each dollar of revenue that the company retains as gross profit. When is used the most expensive raw material (US$ 7.27 kg^{-1}), the gross margin is 27.20%, which means that the company would retain US$ 0.27 from each dollar generated by selling of TEO and PCE. On the other hand, when the CRM decreased to US$ 1.59 kg^{-1}, the gross margin increased up to 46.54%. Therefore, a diminishing in the CRM allows significantly increase the proportion retained for the company for each dollar sold.

Table 4. Project indices of the SFE+PLE−SAS process model.

	Value	
Project indices	US$ 7.31 kg^{-1}	US$ 1.63 kg^{-1}
Gross margin (%)	27.20	46.54
Return on Investment (%)	21.18	30.69
Payback time (years)	4.72	3.26
Internal Rate of Return after taxes (%)	14.92	23.20
Net Present Value at 7.00% ($)	2,176,000.00	4,909,000.00

The ROI (Return of Investment) is a performance measure used to evaluate the efficiency of an investment. ROI is a very popular metric because of its versatility and simplicity. The ROI is defined as the annual profit generated by a unit of invested capital [34]. Clearly, the higher the ROI, the more desirable the project. In many cases, a minimum acceptable value of the ROI of 10% to 15% for the ROI is established to accept or denied a project [35]. For this study, regardless of the CRM used, the ROI for both scenarios was positive and superior to the minimum acceptable value commonly used, which allow consider the possible feasibility of the proposed integrated process. Additionally, the capital recovery period (payback time), which represents the length of time required to recover the cost of an investment can be calculated on the basis of the ROI. Although smaller values of the payback time are more attractive due to that the initial investment is more easily recovered, acceptable

payback times between 2 and 4 years are used. In this process, when was used a CRM of US$ 7.27 kg−1 a payback time of 4.42 years was obtained, whereas when the CRM decreased to US$ 1.59 kg−1, the payback time was reduced to 3.26 years, recovering the initial investment 31% faster. For both scenarios, the time at which the initial investment is recovered is quite acceptable.

The Net Present Value (NPV) represents the difference between the present value of cash inflows and the present value of cash outflow, in other words is the remaining surplus for the investor after to have regained the initial investment. If the NPV of a project is positive after assuming a discount interest of 7%, as shown in Table 4, the project should be considered as feasible [36]. In this case regardless of the CRM, the project is feasible, however the investment is more attractive when is used a less expensive raw material, which generates a NPV 56% higher. The internal rate of return (IRR) tries to measure the profitability of a project or asset. The IRR represents the average intrinsic profitability of the project and is defined as the rate that makes the NPV of all cash flows from a particular project equal to zero. In other words, the higher a project's IRR, the more desirable it is to undertake the project [37]. According to El-Halwagi [35] if the calculated value of IRR is higher or equal to the minimum value established for the ROI, the project is recommended because it has exceeded or met the minimum value established for the ROI. In this work, for both CRM, the IRR value is positive. When the more expensive CRM was used, the IRR value was 14.92%, which is slightly lower than the minimum value recommended ROI to the economic viability of the process. On the other hand, when a less expensive raw material was used, the IRR was 23.30%. This value exceeding the minimum recommended value, making this project is an attractive and economically viable project.

4. Conclusions

The integration of the SFE, PLE and SAS processes allowed the obtaining of more economic products due to better use of raw material and the equipment involved in the process. SFE+PLE− SAS process permits the obtaining of essential oil and the extraction of curcuminoids in one stage, operating at moderate temperatures and using solvents considered safe (GRAS). The scale-up process increased the processes productivity and caused a decreased in the COM. The raw material was the component that had more impact and influenced the COM. The lower estimated COM for TEO and PCE were US$ 37.85 kg^{-1} and US$ 107.79 kg^{-1}, respectively, when the CRM was US$ 1.59 kg^{-1} in a system with capacity of

2×500 L. The production process of TEO and PCE through the integrated SFE+PLE−SAS process is economically viable and it is shown as promissory, especially when is possible access to raw material with lower prices.

Conflict of interest

The authors confirm that there are no conflicts of interest regarding this paper.

Acknowledgements

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-CAPÍTULO 7-

Nanoencapsulation of flavors and aromas by emerging technologies

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Nanoencapsulation of flavors and aromas by emerging Technologies

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1. Introduction

Modern consumers are increasingly aware of the relationship between feeding and maintaining a healthy way of life. Consumers are also aware about environmental issues related with the processes through foods are produced. Therefore, these new consumers valorize products obtained from natural sources and if they have been produced using clean technologies.

In this context, the use of flavoring compounds such as essential oils (EOs) extracted from leaves, fruits and seeds, through emerging technologies of nanoencapsulation is an attractive investment that can bring major innovations in food industry. Nanoencapsulation involves a set of techniques that allow the formation of particles/emulsions with functional properties, consisting of an encapsulating matrix (carbohydrate, protein, lipid and others) and an active material (essential oil) distributed within these systems. Nanoencapsulation provides protection to compounds that composing the EOs (triglycerides, hydrocarbons, phenols, ether and others) against adverse conditions that can promote their volatilization and oxidation while is allowed the release of these compounds under controlled conditions of pH, temperature and desired ambient.

The use of supercritical technologies for nanoencapsulation of EOs has aroused great interest due to the fact that these techniques are suitable for the processing of heat sensitive compounds. The minimum process temperature of these methods is directly linked to the critical conditions (temperature and pressure) of the supercritical fluid used in the process. One of the supercritical fluids most used in the nanoencapsulation of several types of compounds is carbon dioxide. Supercritical carbon dioxide is recognized by its moderate critical properties (Tc = 304.2 K and Pc = 7.38 MPa) which are relatively easily achieved and allows to operate in conditions that do not contribute with the degradation of the bioactive compounds (Silva and Meireles, 2014).

The formation of nanoemulsions assisted by ultrasound is another emerging technology with great potential for EOs nanoencapsulation because it is an effective method in the reduction of the droplet size of the dispersed phase, and thus, leads to greater protection of the active compounds present in EOs.

In this context, this chapter aims the application of emerging technologies based on supercritical fluids and ultrasonication to form nanoparticles/nanoemulsions of EOs with application as flavor and aroma agents in food products, besides to add value to these

products and to promote innovation in food industry through the obtaining of flavorings considered safe obtained using clean technologies.

2. Issues relating to addition of flavors and aromas in foods

Pursuit of pleasure and well-being is a primitive instinct of the human being. In this context, foods need to be sensorially attractive to awaken the interest of the consumers. For example, flavor and aroma properties are parameters that determine the quality of food stuffs and, in many respects these characteristics are prioritized over the nutritional properties. On the other hand, the growing interest in replacing artificial additives by natural compounds has created the possibility to use compounds which besides having pleasant characteristics of flavor and aroma, provides other benefits such as the ability to prevent diseases or in food preservation through addition of compounds that besides improve the sensorial characteristics of the product, contributes with other functional properties. Particularly, flavoring and aroma agents obtained from flowers, herbs, seeds and spices have great potential for application in food industry and have acceptance by the market. The compounds responsible by flavor and aroma of these raw materials are contained in the EOs. Since ancient times, compounds of EOs extracted from citrus fruits, such as orange, lime and lemon have been used as flavoring agents.

As natural products, EOs have attractive functional properties, therefore, interest in the research and use of EOs in various areas such as food and pharmaceutical industries has gained an important place in recent years. EOs are used in a variety of processes and products. According to Baser and Buchbauer (2009) products such as perfumes, cosmetics, toiletries, detergents, household chemicals and related products have been perfumed with EOs. As a flavoring agent, the development of the soft drinks industry has been of great importance because it is a major consumer of EOs, especially those of citrus origin. Other food products such as ice creams, confectionery, bakery, chewing gum and a variety of fast foods also commonly use EOs in their formulations. Nowadays innovative antimicrobial packaging is being developed in order to extend the shelf-life of food through the antimicrobial activity of the EOs. EOs from of citrus, cinnamon, clove, ginger, anise, pepper, pimento, laurel, cardamom, ginger, basil, oregano, dill and fennel are used in the production of the products mentioned above.

As a result of their functional properties and the wide quantity of products where EOs are used, a great panorama about potential EOs applications in food industry is opened, therefore, this chapter will be limited to explore the nanoencapsulation of EOs.

2.1. Classification and properties

Flavor can be defined as a set of sensory sensations derived from the contact with sensory receptors in the nose and other structures from tactile and taste receptors in the mouth. These sensory receptors are capable of transmitting to the brain the combination of numerous biochemical reactions that occur at the moment of consuming a food. Additionally, flavor perception is influenced by numerous other factors such as color, temperature and texture, together with other psychological factors such as expectations and appetite. However, flavor is the result of the interaction of two main sensory properties, taste and flavor. Taste is the result of various biochemical reactions that occurs in the tongue, which are received and transmitted by receptors responsible for the taste perception. Five types of taste have been identified: salt, sweet, bitter, sour and umami. Aroma is the result of perception by nose receptors of volatile chemical compounds that are not related to the nutritional value of food. Sensory receptors in the nose are capable to identify a larger number of compounds that those located in the tongue, especially those that have low molecular weight (Cheetham, 2010).

A considerable amount of compounds have been identified as flavors and aromas. A practical way to differentiate between flavor and aroma compounds is through its volatility. For example, less volatile compounds contribute more to flavor than aroma. Nevertheless, chemical structures of these molecules show high diversity and even flavor compounds with highly similar chemical structures may have an entirely different sensorial effect (Cserháti and Forgács, 2003).

EOs are complex mixtures of organic compounds, often composed of more than 100 different terpenic compounds. In figure 1 are presented the chemical structures of the main compounds which compose the EOs. In this class of compounds, a considerable diversity of chemical structures have been found, among them, hydrocarbons, alcohols, aldehydes, ketones, esters, acids, phenolic compounds and heterocyclic compounds are part of the identified compounds. Although classification of these compounds according to their chemical structure is possible, but according to the scope of this chapter is irrelevant.

EOs due to its origin are classified as a natural flavor. According to United Estates code of federal regulation (CRF, 2013), the term natural flavor or natural flavoring means the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation

products thereof, whose significant function in food is flavoring rather than nutritional. On the other hand, the term artificial flavor or artificial flavoring means any substance, the function of which is to impart flavor, which is not derived from neither of the raw materials listed above. As previously stated, EOs are highlighted among natural flavors and aromas.

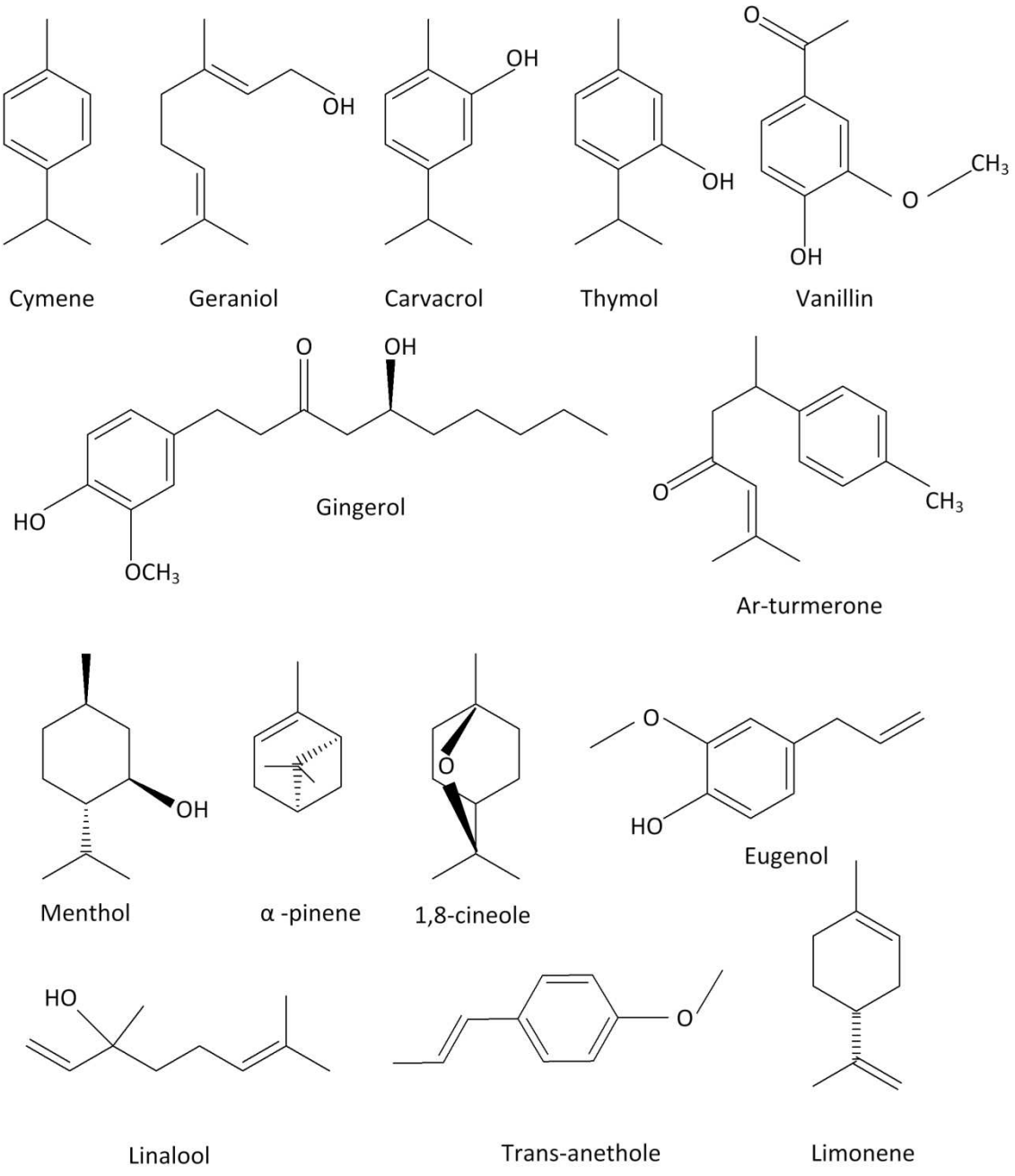

Figure 1. Major chemical constituents in EOs.

EOs usually exist in liquid form at room temperature and usually they are separated from the aqueous phase by a physical method that does not lead to significant change in its chemical composition. Due to their hydrophobic nature and their density often

lower than that of water, EOs are generally lipophilic, soluble in organic solvents and immiscible in aqueous medium (Asbahani et al., 2015). Besides providing flavor and aroma, they have several biological properties that can be explored in the development of applications to food and pharmaceutical industries. EOs are recognized by its anticancer, antimicrobial, anti-inflammatory, antioxidant, antiviral and antinociceptive activities (Baser and Buchbauer, 2009). One of the most important properties of the EOs is its antimicrobial activity; this property can be explored in food packaging industry in order to develop novel antimicrobial packaging. This type of packaging can maintain the nutritional and sensory quality of food while the shelf-life is extended. All plants have the ability to produce volatile compounds, however, particularly herbs and spices have been commonly used as raw materials for obtaining EOs. In Table 1 are presented some examples of herbs and spices most commonly used as a source of EOs and its major flavoring compounds.

Due to its chemical characteristics, EOs are unstable and fragile volatile compounds. Consequently, its stability, solubility and interactions with other food components are related with the intensity which they are perceived by senses and defined the conditions under their degradation are triggered. In next paragraphs are presented some important topics regarding the behavior of EOs and how its perception could be changed or how their compounds could be easily degraded if they are not protected from external factors.

2.1.1. Stability

The stability of EOs is determined by physical and chemical factors. Physical factors such as evaporation, separation of the phases in emulsions and it adsorption in complex matrices leads to loss their characteristics. Temperature is the most important parameter that could affect the stability of these compounds. Heat causes the loss of the volatile compounds and leads to chemical changes, decreasing the intensity which they are perceived by senses (Taylor and Linforth, 2009). On the other hand, in contact with water and air, oxidation and hydrolysis reactions are triggered, causing the degradation of the compounds. Additionally, these compounds can also react with other compounds or simply rearranging at molecular level, losing their characteristics (Rowe, 2005).

Table 1. Principal herbs and spices sources of EOs.

Herbs and Spices	Part of Plant Used	Major flavoring compounds	Reference
Cinnamon (*Cinnanamon zeylanicum*)	Leaves	Trans-cinnamaldehyde, 3-methoxy-1,2-propanediol, o-methoxy-cinnamaldehyde, coumarin and benzeneethanol	(Wang et al., 2009)
Vanilla (*Vanilla fragans*)	Beans of the orchid	Vanillin and *p*-hydroxibenzaldehyde	(Longares-Patrón e Cañizares-Macías, 2006)
Ginger (*Zingiber officinale* Roscoe)	Rhizomes	Gingerol, zingerone and shogaol	(Mesomo et al., 2013)
Turmeric (*Curcuma Longa*)	Rhizomes	Ar-turmerone and turmerone	(Ravindran et al., 2007)
Oregano (Origanum vulgare)	Flowers and leaves	Carvacrol, thymol, limonene, pinene, ocimene, and caryophyllene	(Almeida et al., 2013)
Mints (*Mentha spicata* Huds)	Leaves	Menthone, menthofuran and menthol	(Costa et al., 2014)
Rosmary (*Rosmarinus officinalis*)	Leaves	Alpha-pinene, 1,8-cineole, camphor, borneol, trans-caryophyllene, carnosic and rosmarinic acids, carnosol and rosmanol	(Taylor e Linforth, 2009)
Cardamom (*Elettaria cardamomum*)	Seeds	1,8-cineole, α-pinene, β-pinene, sabinene, myrcene and α-phellandrene	(Parthasarathy et al., 2008)
Clove (Syzygium aromaticum (L.))	Dried unopened flower buds	Eugenol, eugenyl acetate and b-caryophyllene	
Coriander (Coriandrum sativum)	Seeds	Linalool, limonene, camphor and geraniol	(Pavlić et al., 2015)
Pepper black and white	Seeds	α-pinene, β-pinene, 1-α-phellandrene, dllimonene, piperonal and dihydrocarveol	(Ferreira et al., 1999)
Tea (*Camellia sinensis*)	Leaves	Linalool, genariol, α-damascone, linalool oxide, cis-jasmone, maltol, anethole, α-terpineol, nerolidol	(Pripdeevech e Wongpornchai, 2013)
Aniseed (*Pimpinella anisum* L.)	Seeds	Trans-anethole, γ-himachalene, cis-isoeugenol and linalool	(Samojlik et al., 2012)

2.1.2. Solubility

EOs are slight soluble in water, therefore, is necessary provide suitable conditions for their incorporation in food, ensuring the quality of the products. The chemical characteristics of the compounds and the system temperature govern the solubility of the compounds of the EOs in water. The solubility of EOs in aqueous solutions is influenced by their chemical structure, which could affect the thermodynamic equilibrium of the compounds in the mixture. the distribution of the compounds that compose the EOs between the different phases of foodstuff governs flavor release from food and therefore, the intensity in their perception (Covarrubias-Cervantes et al., 2005). Encapsulation of this type of compounds contributes with the solubility increase of the EOs in food systems.

2.1.3. Interactions with other food components

Natural flavors such as EOs contains various chemical compounds with different polarities, therefore, when are added to food, they may react with other components, causing their degradation and the loss of bioactivity. For example, one of the most important parameters is the fat content. Intensity of flavor and aroma is much greater in fat than in water, therefore, is important avoid the dispersion of the flavor and aroma compounds in the aqueous phase (Taylor and Linforth, 2009). On the other hand, proteins and lesser extent carbohydrates can form chemical bonds with other composites such as reversible weak interactions or strong irreversible covalent interactions. These interactions lead to a dramatic reduction in the intensity of flavor and aroma, as a result, the perception of the compounds by consumers is affected (Wang and Arntfield, 2015).

2.2. EOs extraction methods

Among the most widely used methods for obtaining EOs are hydrodistillation, entrainment by water steam, organic solvent extraction and cold pressing (Asbahani et al., 2015). Although the main advantage of hydrodistillation is that as EOs are immiscible in water and thus, after condensation, EOs could be easily separated, hydrodistillation is recognized as a tedious and expensive operation. Entrainment by water steam is a method based on hydrodistillation but without direct contact between plant and water, in a process with lower extraction time. In organic solvent extraction, the EOs are extracted using an organic solvent; then, the extract is concentrated by removing the solvent under reduced pressure. Cold pressing is the traditional method to extract EOs from citrus fruits. During

extraction, oil sacs break and release EOs. This oil is removed mechanically by cold pressing yielding a watery emulsion. However, these techniques cause EOs alterations due to the use of high temperatures. Other drawbacks such as the use of organic solvents or the limitation to being applied only with a particular type of raw material require the developing on new extraction techniques. As it will be show in section 4.1, a relatively new extraction technique to obtain EOs as supercritical fluid extraction (SFE) has been successfully applied. This technique allows reducing extraction times and using solvents considered as safe (GRAS).

3. Nanoencapsulation of EOs

As was previously stated, EOs are susceptible to various types of degradation under action of oxygen, light and temperature. In addition to that, similarly with almost all bioactive compounds, the EOs are not soluble in water, which limits their application in food. Therefore, through nanoencapsulation technique is possible increase the solubility of the EOs, decreasing the necessity of use surfactants and enhancing the possible use of EOs as food additives. However, in some applications (e.g. food packaging), the strong flavor of the EOs would change or alter the original taste of food. To overcome this limitation, is possible to entrap the EO compound into a capsule in order to mask their undesirable flavor. For example, a cinnamon EO nanofilm developed by Wen et al. (2016) contributed to increase the shelf-life of strawberries without affect significantly the flavor of the product. Thus, the use encapsulation techniques allow taking advantage of the bioactive properties of the EOs without affecting the sensorial properties during the product storage.

Additionally, in certain applications is necessary the controlled release of the compounds in specific conditions. In that sense, is a duty of food industry overcome these limitations and provides products with longer shelf-life maintaining its palatability during this period. A feasible alternative is the stabilization of these compounds using encapsulation techniques. Besides protects the compounds against adverse environmental conditions, the purpose of encapsulation is to potentiate the action of the compounds, controlling their release rate and/or transforming liquids products in solids materials such as particles (Nedovic et al., 2011).

Nanoencapsulation is an important field of nanotechnology, and it can be defined as the isolation process of compounds inside carrier materials with nanoscale dimension. Among the nanoencapsulation systems, nanoemulsions and lipid nanoparticles particularly appears suitable for food applications (Spigno et al., 2013). According to Cushen et al. (2012) a nanoparticle is defined as a discrete entity that has three dimensions in the range of 100 nm

or less. On the other hand, a nanoemulsion can be considered to be a conventional emulsion that contains very small particles. They are characterized as a thermodynamically unstable colloidal dispersion consisting of two immiscible liquids, with one of the liquids being dispersed as small spherical droplets (r < 100 nm) in the other liquid (McClements, 2012).

Besides guarantee excellent protection of EOs against degradation or evaporation, due to the subcellular size, nanoencapsulation techniques may increase the passive cellular absorption mechanisms, thus reducing resistance to mass transfer and increase antimicrobial activity (Donsì et al., 2011). On the other hand, flavor nanoencapsulation also can reduce fat absorption, allowing the delivery of flavor and aroma without an increased in the caloric value of the food, in addition to other undesired effects (Coles and Frewer, 2013).

3.1. Encapsulation materials

There is innumerable quantity of materials that have potential to be used as a carrier material in nanoencapsulation of many compounds. Encapsulation materials besides protect and release appropriately the EOs, should be water soluble, biodegradable, forming suspensions of low viscosity, not being reactive and have a low cost. However, only a limited number of encapsulation materials can be recognized as GRAS materials, and therefore applied in food processing. Is important to highlight this fact because limits the development of new products by food industry when compared to the pharmaceutical industry. The development of new pharmaceutical products is less restrictive and is possible the application of a large diversity of materials in drug encapsulation (Wandrey et al., 2009).

The majority of materials used for encapsulation in the food sector are biomolecules. These materials have to provide maximal protection of EOs against environmental conditions, to maintain the bioactivity of the compounds during processing or storage under various conditions. Regarding nanoencapsulation of EOs, among the materials most commonly used in the encapsulation of EOs, there are several biopolymers such as modified starches, β-cyclodextrins, maltodextrins and various gums (Martín et al., 2010).

3.1.1. Carbohydrates

Among all materials, the most widely used for encapsulation in food applications are polysaccharides. In food industry their consumption exceeds the production of synthetic polysaccharides; therefore these polymeric carbohydrate molecules composed of long chains of monosaccharides became materials of great economic importance. Starch and their

derivates such as amylose, amylopectin, dextrins, maltodextrins, polydextrose, syrups and cellulose and their derivatives are commonly used in encapsulation of all type of compounds (Nedovic et al., 2011).

Compared with lipid carriers, for instance, systems that used as encapsulating material carbohydrates can interact with a wide range of bioactive compounds via their functional groups, which makes them versatile materials to encapsulate hydrophilic and hydrophobic compounds such as EOs or even others food ingredients with bioactive compounds. Additionally, they are considered as a suitable encapsulation material to processes that uses high temperature due to their temperature stability in comparison to lipids or proteins which might be melted or denatured (Fathi et al., 2014). For example, cyclodextrins are carbohydrates recognized by its ability to entrap hydrophobic molecules such as EOs. Several EOs components such as limonene, eucalyptol, linalool and α-pinene, among others, were encapsulated by Kfoury et al. (2015). After the encapsulation process, the radical scavenging ability of the EOs was enhanced and the volatility of the EOs was significantly reduced. These characteristics would allow reducing the EOs losses during storage or processing and developing bioactive packaging as well as enhance the aroma power of the EOs. β-cyclodextrin was used by Wen et al. (2016) to develop a cinnamon EO antimicrobial film with diameter of 240 nm. To building the film, a highly biocompatible polymer known as polyvinyl alcohol was used to prepare the antimicrobial material. The film showed antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* and contributed to enhance the thermal stability of the EO.

Other encapsulation material considered safe for food application is n-octenyl succinic anhydride (OSA)-modified starch. OSA has superficial activity, being capable of reduce the superficial tension between water and oil, therefore, it can be used as an efficient emulsifier and consequently as a suitable encapsulation material. OSA has been used in the nanoencapsulation of several compounds with application in food such as carotenoids (Santos et al., 2012) and curcuminoids (Abbas et al., 2015). OSA also has been used in essential oil nanoencapsulation of lavandin, improving performance of the bioactive compounds (Varona et al., 2010, Varona et al., 2011, Varona et al., 2013).

Polysaccharides of microbial or animal origin such as chitosan and dextran, and various gums have also been used successfully in nanoencapsulation. Among them, chitosan a derivative of chitin gives singular chemical and biological characteristics, such as: biocompatibility, antibacterial properties and hydrophobicity. Recently, chitosan has attracted a great attention in the encapsulation of bioactive compounds because of its general

recognition as GRAS and other features such as abundance, low toxicity, biodegradability and biocompatibility. For example, in order to enhance antifungal activity and stability of the *Zataria multiflora* Boiss EOs against the causal agent of gray mould disease, the EOs was nanoencapsulated using chitosan as encapsulation material (Mohammadi et al., 2015). Woranuch and Yoksan (2013) confirmed the improved thermal stability of encapsulated eugenol compared with pure eugenol. These results suggest that eugenol-loaded chitosan nanoparticles could possibly be used as antioxidants for various thermal processing applications, including bioactive plastics for food packaging. Turmeric and lemongrass EOs were encapsulated in chitosan-alginate nanocapsules with size below 300 nm (Natrajan et al., 2015). The nanocapsules showed good stability with encapsulation efficiency between 71% and 86.5%. In this case, these capsules have a potential use for pharmaceutical applications, due to the antiproliferative activity of the EOs and the controlled release that allows the nanoencapsulation technique.

Nanogels are another important application where polysaccharides are used. Nanogels have several applications, particularly in pharmaceutical industry, mainly by the fact that they can trapping bioactive substances such as EOs in their nanometric net, improving its efficiency at low concentration, stability and release. In another research, the *Cuminum cyminum* EO, an herbaceous plant used commercially as a flavoring agent was encapsulated by Zhaveh et al. (2015) in a chitosan – caffeic acid nanogel. After encapsulation, the antimicrobial activity against *Aspergillus flavus* was improved as well its stability. The results showed that the chitosan – caffeic acid nanogel is a feasible material to encapsulate the EO because besides showing a slow-release, the process had an encapsulation efficiency of 85%, producing capsules with size below 100 nm. Although the use of nanogels has been focused mainly in pharmaceutical industry, nowadays some interesting applications are being developing to apply nanogels in vegetables and fruits in order of preserving their quality. Specifically, is possible enhancing the antifungal effects of the EOs through the controlled release of the EOs from nanogels and extend the storage period. For example, thyme EO nanoencapsulated in a chitosan – benzoic acid nanogel was able of inhibiting the growth of *Aspergillus flavus* in tomatoes after 1 month of storage under sealed conditions (Khalili et al., 2015). However, due to the high volatility of the EOs, under non-sealed conditions its antifungal activity in nanogels was affected. In tomato under non-sealed conditions, the thyme EO was not able to totally inhibit the growth of the fungi. Therefore, to truly enhance the biological activity of the EOs, the contact with air and other environmental conditions should be avoided or controlled.

Cashew gum is a heteropolysaccharide extracted from the exudate of the Brazilian tree Anacardium occidentale, whose structure resembles gum Arabic. Nowadays cashew gum has great interest due to may be a suitable substitute for gum Arabic, which is more expensive. Among their properties, cashew gum is recognized by its ability to interact with water and thus act as stabilizer, emulsifier and adhesive. Herculano et al. (2015) obtained nanocapsules of Eucalyptus EOs using cashew gum as encapsulation material. The nanoparticles obtained in this study have potential for use as a natural food preservative due to they showed a high antimicrobial activity and storage stability over 365 days. In another approach, Abreu et al. (2012) developed nanogel nanoparticles of *Lippia sidoides* EOs using cashew gum and chitosan as encapsulation materials with sizes ranged from 335 nm to 558 nm.

Inulin is other carbohydrate with potential use as carrier material in nanoencapsulation of EOs. Inulin is a natural ingredient commonly used in food industry as a prebiotic compound. It also can be used as excipient and stabilizer, but it has great potential as carrier material for encapsulation and controlled release of EOs. For example, oregano EO have been encapsulated using inulin as carrier material by Beirão-da-Costa et al. (2013). The particles had a particle size ranging between 3 and 4.5 μm after spray drying using temperatures from 393 to 463 K. The temperature had a significant effect in the structure and the morphology of the capsules and due to these changes in the structure, different profiles of release were observed. Thus, further efforts are necessary in order to obtain feasible formulations using inulin as carrier material and establish the best processing conditions for production of encapsulates suitable for use in food industry.

3.1.2. Proteins

Proteins can be used in their natural state, or they can be chemically, physically, or enzymatically modified to modulate their functional attributes. Therefore, is possible according to the specific application, to improve the functional performance of proteins. In the same sense, as proteins are easily digested by the human body, it is possible to take advantage of the bioactive properties of the EOs during an eventual release of EOs after ingestion. However, protein particles are often highly sensitive to alterations in pH, ionic strength, and/or temperature because these trigger changes in their surface charge and hydrophobicity (Joye and McClements, 2014).

Zein is a corn protein that has ability to form films. Besides is also recognized by being biodegradable and biocompatible. For this reasons, zein has been successfully applied

in several researches as well in food and pharmaceutical industries. Thymol and carvacrol, two predominant compounds in oregano and thyme EOs were encapsulated in nanoparticles of zein using the liquid-liquid dispersion method by Wu et al. (2012). This research allowed the dispersion of both EOs in water, enhancing their potential use in food preservation and control of human pathogenic bacteria. For example, solubility of oregano EOs increased up to 14 fold without damaging their ability to scavenge free radicals or to control *E. coli* growth.

In another approach, due to its abundance, nutritional value and acceptance by consumers, milk proteins have been widely applied in the encapsulation of several compounds due to its versatility and excellent functional properties (Tavares et al., 2014). Particularly, whey protein isolates (WPI) and whey protein concentrates (WPC) have demonstrated improve heat stability of encapsulated compounds. WPI was used in the obtaining of nano dispersions of thymol (Shah et al., 2012). In this research, upon hydration of the spray-dried powder, transparent and heat stable nano dispersions were formed at thymol concentrations well above its solubility limit. Therefore, this technology is applicable to disperse various lipophilic compounds such as EOs in transparent beverages like clear fruit juices. In another studies, although the nanometric size was not reached, WPI and WPC has been successfully used in the EOs encapsulation. Hundre et al. (2015) used WPI to encapsulate vanillin (3-methoxy-4-hydroxy-benzaldehyde) extracted from the pods of *Vanilla planifolia*. This research indicated that there was no interaction between the encapsulation material (WPI) and core (vanillin) materials. Moreover, microencapsulated vanillin + WPI by spray–freeze drying technique yielded better thermal stability than spray dried and freeze-dried samples. On the other hand, the mixture of WPC and other proteins or polysaccharides could enhance the characteristics of the emulsions and thus, the encapsulation process. Chia (*Salvia hispanica* L.) EO was encapsulated using a mixture of WPC, mesquite gum or gum Arabic (Rodea-González et al., 2012). The use of a binary mixture provides better stability to droplet coalescence due to that the interaction among the proteins contributes to produce more stable emulsions. After spray-drying capsules with encapsulation efficiency higher than 70% were obtained from emulsions made with an EO to carrier material of 1:3.

3.1.3. Lipids

Nanoencapsulation using lipids as encapsulation material is among the more developed nanotechnology fields related to application in food systems. Nanoencapsulation using lipid-based systems has several advantages, for example, hydrophobic and highly unstable compounds as EOs are difficult to incorporate in aqueous systems, however through

using lipids as encapsulation materials is possible to trap materials that have different solubility, using natural ingredients at industrial scale (Zuidam and Nedovic, 2009). According to Martín et al. (2010), phospholipids are a class of amphiphilic lipids formed by a hydrophilic head (a phosphate group, a diglyceride and a simple organic molecule) and a hydrophobic tail (long fatty acid) are less toxic than other encapsulation materials and thus an excellent option for EOs encapsulation. These compounds may be used in the formation of liposomes, by encapsulating hydrophobic materials such as EOs. Liposomes are closed spherical vesicles arranged in one or more concentric bilayers of phospholipids with an internal aqueous phase. Particularly due to their easy biodegradation and their similarity with biomebranes, the use of liposomes constitutes a suitable system for encapsulation of volatile unstable EOs constituents and it may allow enhance the functional properties of EOs. Donsì et al. (2011) encapsulated a terpenes mixture and D-limonene into nanoemulsions based on food-grade ingredients such as sunflower oil, palm oil and soy lecithin. According to the results, the addition of low concentrations of the nanoencapsulated terpenes was able to delay the microbial growth or completely inactivate the microorganisms while minimally altering the organoleptic properties of the fruit juices. In another approach, Sebaaly et al. (2015) developed suitable formulations of natural soybean phospholipid vesicles to improve the stability of clove EOs and its main component, eugenol. It was found that liposomes exhibited nanometric spherical shaped vesicles and protected eugenol from degradation induced by UV exposure; maintaining their stability after stored for 2 months at 277 K.

4. Emerging technologies

4.1. Supercritical fluids (SCFs)

A pure compound is considered a supercritical fluid (SCF) when its temperature and pressure are above than critical values, Tc and Pc, respectively. The critical temperature is defined as the higher temperature in which a gas can be transformed in a liquid, due a pressure increase. The critical pressure consists in the higher pressure in which a liquid can be converted in a gas, due a temperature increase. These properties characterize the critical point (CP) (Figure 2). In the supercritical region, there is no equilibrium between liquid and gas phases; however, the physical-chemical properties of the SCFs are intermediaries between gas and liquid. Additionally, around CP, little pressure changes causes huge changes in density, viscosity, solvation power and diffusivity, allowing a selective precipitation of solute, and thus enabling the solvent recycle (Brunner, 2005).

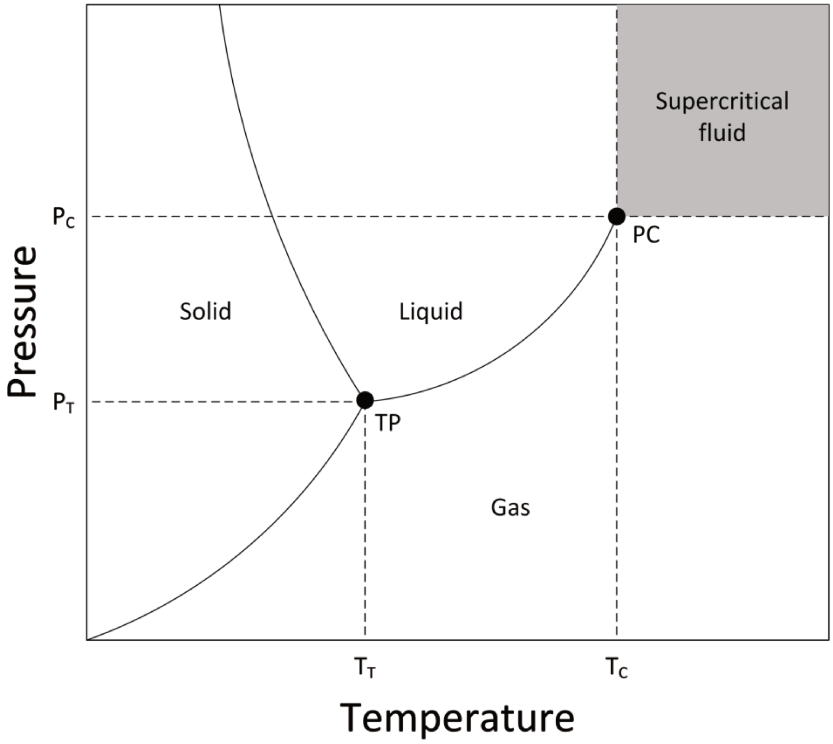

Figure 2. Pressure-temperature phase diagram for pure substances.

The SCF more used is carbon dioxide (CO_2). CO_2 is considered inert, nonflammable, nontoxic and is available in large quantities, with low cost and high purity, having a relatively low CP, 304.2 K and 3.38 MPa. Carbon dioxide is the most suited solvent for SFE of thermolabile compounds because of its favorable properties (including nontoxic and nonflammable character, high availability at low cost, and high purity) and to its ability to produce isolates with optimal physicochemical, biological, and therapeutic properties. Additionally, due its moderate critical temperature, it allows for operation near to room temperature with a slight critical pressure (Meireles, 2008). In general, supercritical CO_2 ($scCO_2$) behaves like a lipophilic solvent, but compared with liquid solvents, its main advantage is that its selectivity and solvation power are adjustable and can be controlled through temperature and pressure changes (Reverchon, 1997). For example, because the high solubility of the EOs in SCFs, these compound have been isolated successfully using SFE. EOs from raw materials such as rosemary (Zabot et al., 2014), hops (Van Opstaele et al., 2012), eucalyptus (Zhao and Zhang, 2014), coriander (Pavlić et al., 2015), ginger (Mesomo et al., 2013), peppermint (Gañán et al., 2015) and turmeric (Carvalho et al., 2014) have been successful obtained using SFE. Process conditions that use pressures between 8 and 15 MPa and temperatures between 293 and 333 K are feasible for its use as extraction solvent in the extraction of volatile and thermolabile compounds such as EOs, prevents their thermal

degradation. On the other hand, as SFE process is performed at the absence of light and oxygen, oxidation reactions, a problem of major importance in antioxidant extraction can be avoided using this technology. Therefore SCFs display unique characteristics that enable them to be used as solvent to extraction of bioactive compounds as well as feasible antisolvent to precipitate compounds and encapsulate EOs as will show in next section.

4.1.1. SCFs in EOs encapsulation

Several techniques have been studied and used to form capsules include spray-drying, spray chilling, jet milling, fluidized bed coating, liposome entrainment, coacervation, thermal and ionic gelation, etc. However, some disadvantages such as the difficulty in producing capsules with a narrow particle size distribution as well as use of high operating temperatures, generate the necessity of develop novel techniques to overcome these drawbacks. The use of SCFs as an alternative technique for EOs encapsulation would improve the results obtained with other techniques, overcoming the drawbacks and even create novel formulations and products. Particular features of the $scCO_2$, such as the possibility of adjust its solvation power, low viscosity, moderate or high diffusion coefficient and no product contamination; have become the use of SCFs in a feasible alternative for the encapsulation of EOs.

In the encapsulation processes using a SCF, a solution that contains the solute and the carrier material is dissolved in a SCF. Due to the particular behavior of the solution in the supercritical phase, the solution reaches a higher supersaturation, the solubility of the compounds is reduced drastically, making the carrier material precipitates and the entrapment of the compounds of interest is caused (Vinjamur et al., 2013).

However, owing to the high sensibility to the changes in the process conditions on the phase equilibrium for this type of systems, a further knowledge of the phase equilibrium of the EOs + carrier material + CO_2 is a requirement for the development of processes where SCF are used for encapsulation. According to Reverchon et al. (2003) the formation of a single supercritical phase is the key step for the successful production of nanoparticles. In complex systems such as EOs + carrier material + CO_2, the phase diagram of the system could change due to presence of other soluble solids different than EOs or carrier materials in the solution or emulsion or due to some changes in the proportions of the mixture components. For example, depending on the system temperature and pressure and due to the large differences in size, shape, and polarity among the EOs molecules and the carriers materials, three or more phases could be produced causing no capsules formation. In the same

context, depending of the process conditions a cosolvent effect also could be observed. In that case, the dissolution of some compounds in the mixture could be increased and therefore a higher fraction could be dragged out the system by the $scCO_2$. A similar behavior was observed by Lévai et al. (2015) in a quercetin encapsulation process using SCF. In the performed process some low molecular weight antioxidant compounds of lecithin were dragged out by the $scCO_2$. In consequence although the antioxidant activity of the capsules obtained by SFEE was higher than pure quercetin, the antioxidant activity of the SFEE capsules had lower antioxidant activity than the mixture of quercetin and lecithin from the oil in water emulsion.

Consequently, one of the more important aspects of use SCFs in encapsulation processes is that the process must be initially based in the knowledge of phase equilibrium behavior and the solubility of the substrate and the polymer matrix in the SFE. This feature is one of the main limiting factors of supercritical techniques described below.

4.1.2. SCFs encapsulation techniques

Various techniques using SCFs have been proposed to precipitate and to encapsulate several compounds. According to Silva and Meireles (2014) these techniques can be classified in accordance with the function of the supercritical fluid in the process: solvent (Rapid Expansion of a Supercritical Solution, RESS); antisolvent (Gas Antisolvent, GAS; Supercritical Antisolvent SAS); cosolvent or solute (Particles from Gas-Saturated Solutions, PGSS); nebulization compound (Carbon Dioxide Assisted Nebulization with a Bubble Dryer, CAN-BD) and extractor and antisolvent techniques (Supercritical Fluid Extraction of Emulsions, SFEE). Many of these processes were originally developed to produce solid compounds, however, with some modifications is possible to obtain solid-liquid compounds, for example, liposomal nanocapsules of EOs.

4.1.2.1. Rapid expansion of supercritical solution (RESS)

RESS process consists in the saturation of a SCF with a solid substrate in a saturation vessel; afterwards, the saturated solution is pressurized through an expansion heated nozzle into a low pressure chamber or ambient pressure vessel (Figure 3). The pressure change causes a faster nucleation of the substrate in very small particles, which are collected in the gas current. The fast injection of the substrate into gas phase should guarantee the production of very small particles. This process is attractive mainly due to the no use of

organic solvents, the use of low temperatures and the narrow particle size distribution (Reverchon and Adami, 2006).

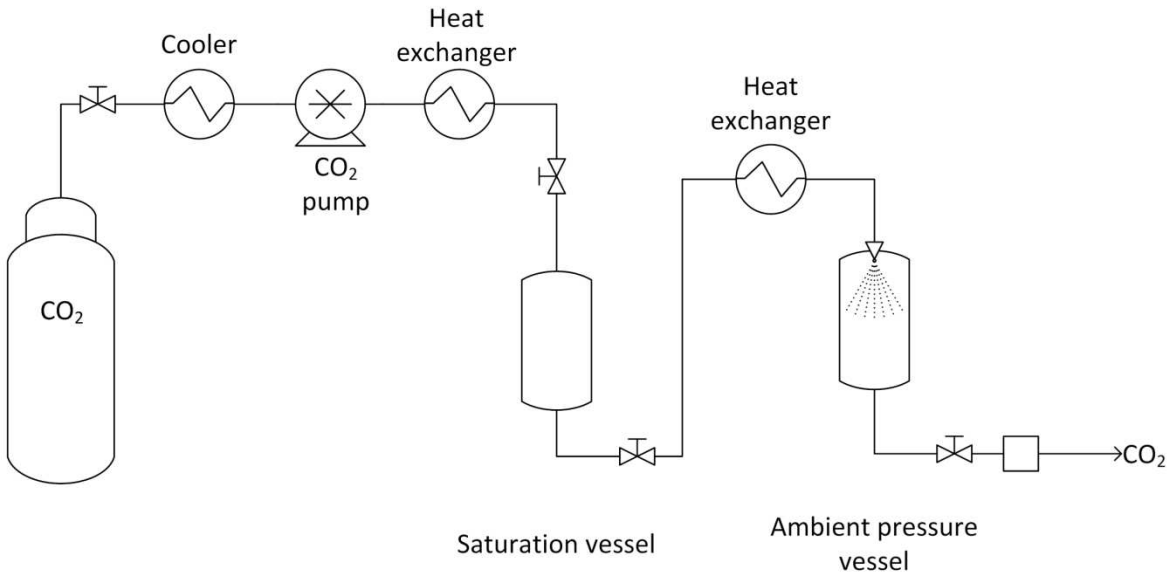

Figure 3. Schematic flowsheet of the Rapid Expansion of Supercritical Solution (RESS) process.

Some disadvantages of the RESS process such as: difficulties for the scale-up and nozzle design, as well as the energy cost associated with the recompression of CO_2 for its recirculation into the process, could limit its utilization (Rodríguez-Rojo et al., 2013), however, several compounds have been micronized using the RESS process. Most of these micronized compounds are pharmaceutical substances or high economic value materials. The application of the RESS process in the food industry and related products is limited because substances such fat-soluble vitamins (vitamin E, for instance) are moderately soluble in CO_2 (Weidner, 2009). Additionally, high molecular weight compounds as polymers and phospholipids, which are essential in the encapsulation process of EOs are slightly soluble in CO_2, restricting the use of the RESS process in the encapsulation of EOs. For example, phospholipids are not easily dissolved in pure $scCO_2$. These compounds are used in the formation of liposomes in aqueous medium; therefore, the conventional RESS process is not applicable in the encapsulation of EOs using liposomes.

Moreover, to overcome the limitation that the RESS process only is suitable to compounds with low polarity or moderate solubility in $scCO_2$, changes as the addition of cosolvents such as ethanol or acetone in a process named Rapid Expansion of a Supercritical Solution with a Nonsolvent (RESS-N) has been proposed. The RESS-N process was used by Wen et al. (2010) to form liposomes containing EOs from rhizomes of *Atractylodes*

macrocephala Koidz, a Chinese plant commonly used as supplement in food and folk medicine. In this modified method, the EOs solution and the materials of the liposomes are dissolved in the $scCO_2$-ethanol mixture and are pulverized into a phosphate buffer solution through a coaxial nozzle to incorporate the essential oil into the liposomes. The entrapment efficiency, EOs loading and average particle size of liposomes were found to be 82.18%, 5.18% and 173nm, respectively, under the optimum conditions of at a pressure of 30 MPa, a temperature of 338 K and an cosolvent mole fraction in $scCO_2$ of 15%.

4.1.2.2.Supercritical solvent impregnation (SSI)

This technique is based in the fact that a polymer can be impregnated with another compound. In the beginning, the compound of interest is dissolved into a SCF in a saturation vessel and, immediately, the compound + SFC mixture are put in contact with the polymers particles to be impregnated in an impregnation vessel (Figure 4). The two main items of the setup are a column, in which $scCO_2$ is saturated with the compound of interest, and the impregnation column, in which compound + SFC mixture is brought into contact with the polymer. In the same way that the RESS process, in the SSI process is also possible to use cosolvents to enhance the solubility or to improve the dispersion of the compound or carrier material in the polymer (Cocero et al., 2009).

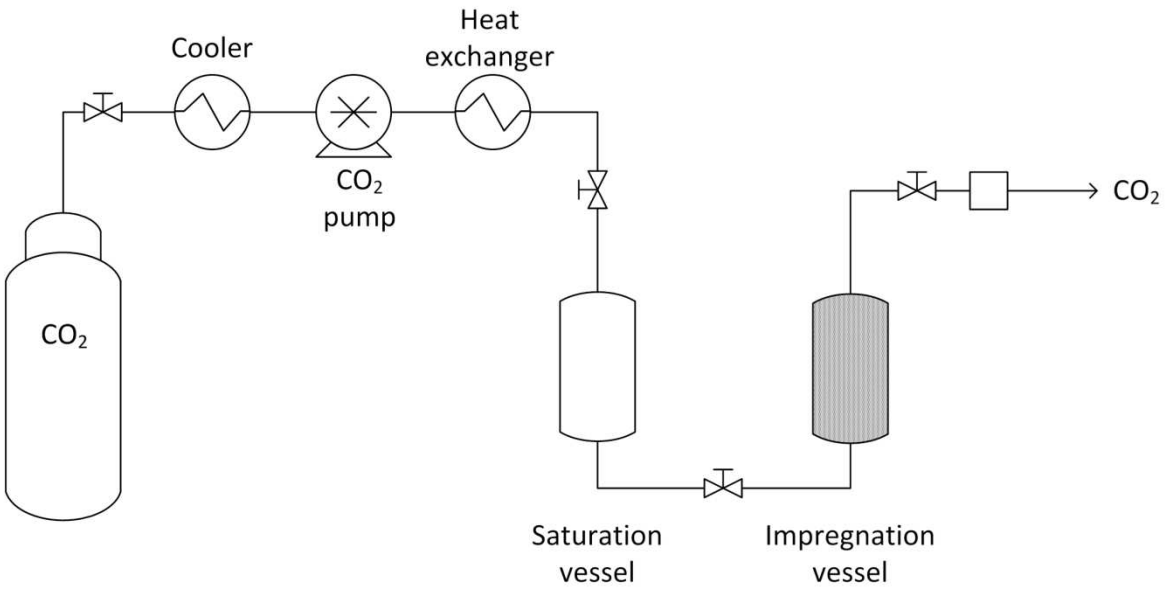

Figure 4. Schematic flowsheet of the Supercritical Solvent Impregnation (SSI) process.

Particularly, this technique could benefit from the high solubility of EOs into $scCO_2$, and the high capacity of diffusion of the SCF through the carrier material, which

generally is a powdered polymer with a pre-formed morphology. In fact, this process is used to produce micro and nano compounds, through the introduction of an active compound into pre-formed particles using diverse carrier materials. Due to this characteristic, the SSI process cannot be considered strictly an encapsulation technique (Rodríguez-Rojo et al., 2013). Nevertheless, it can be particularly advantageous in relation to the conventional encapsulation processes. For example, the EOs encapsulation using spray drying from an oil-water emulsion (o/w) and some carrier material, can trigger the degradation of the EOs due the high process temperature (above 353 K) and to oxidation of the compounds due to the presence of oxygen in the compressed air using during the process. Particularly, oregano and the compounds of its EOs have been presented as special interest to this technique. Almeida et al. (2013) demonstrated that SSI process is an attractive technique to impregnation of natural matrices with EOs. In this work, oregano EOs was impregnated in microspheres of different types of starch (sorghum and rice) using the SSI process. Mild operating conditions (10 MPa and 313 K) avoid EOs degradation and the high diffusivity of CO_2 in the solid matrix (starch) ensures a deep impregnation of the essential oil. The product was characterized for maintain a high antioxidant activity even during storage. Besides, the scale up process is considered simple and therefore, SSI process has potential for the production of ingredients for the food industry. In this type of process, depending of the EOs quantity impregnated is possible to change the polymer morphology impregnated due the formation of new chemical bonds that can change the solid structure of the polymer. Milovanovic et al. (2015) impregnated oregano EOs, containing mainly thymol in cellulose acetate using the SSI process. Although higher impregnation yield was obtained (72% at 308 K and 20 MPa), the morphology of the samples changed significantly with the high impregnation yields, impregnated near the surface of the bead to induce the change of polymer solid structure via participating in the intermolecular implying the impact of thymol on the cellulose acetate. Thymol impregnated near the surface induces the change of polymer solid structure, changing the intermolecular hydrogen bonds, and thus, the morphology of this part of the bead is modified. Lavandin (*Lavandula hybrida*) EOs was impregnated in OSA (Varona et al., 2011). After testing pressures between 10 and 12 MPa and temperatures between 313 and 323 K, it was stated that the distribution coefficient of EOs between the starch and the supercritical phase as well as the EOs load depended on the density of CO_2. The quantity of EOs impregnated increased when temperature was increased and pressure was decreased.

4.1.2.3.Supercritical Antisolvent (SAS)

In SAS process, $scCO_2$ is used as an antisolvent to reduce the solute solubility dissolved in a solvent. In the SAS process a liquid solution that contains the compound to be encapsulated and the carrier material is injected into a SCF. In this process, $scCO_2$ is pumped into a precipitation vessel using specific process parameters such as temperature, pressure, solution flow rate and CO_2 flow rate. Afterwards, the solution that contains the interest compound, the carrier material and the organic solvent is pulverized through an expansion nozzle into the precipitation vessel. The solvent diffuses quickly from the drops of solution to supercritical phase. This leads to supersaturation of the solute, which is compensated by nucleation and the compound of interest is precipitated within the carrier material. The formed particles are collected using a filter fixed at the bottom of the precipitation vessel whereas the residual organic solvent and the CO_2 are removed from the system (Figure 5). SAS process is characterized by operating in a semi-continuous mode, where the solution and the $scCO_2$ are continuously injected into the precipitation vessel. To the SAS process to be successful, the solute must be soluble in the organic solvent at the process temperature and must be insoluble in the SCF. Another important aspect is that the solvent must be completely miscible with the SCF, because if the solute is just partially soluble into the SCF, depending of the process conditions, two or more fluid phases could be formed and the solute may remain dissolved or partly dissolved.

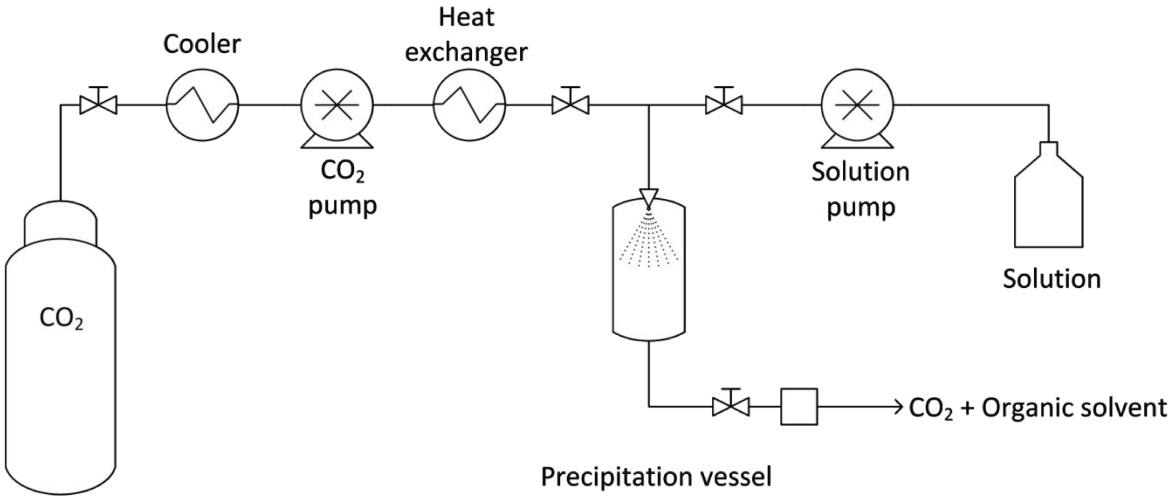

Figure 5. Schematic flowsheet of the Supercritical Antisolvent (SAS) process.

A process variation is the operation in batch mode, which is known as Gas antisolvent (GAS) process. In GAS process, the precipitation vessel is loaded with a given quantity of the liquid solution and, then, the supercritical antisolvent is added until the final pressure is obtained (Reverchon and Adami, 2006).

In SAS process is possible to obtain nanoparticles due to the fast supersaturation and nucleation caused by the high mass transference rates of the SCF. On the other hand, narrowed size distributions can be obtained by controlling process parameters. For example, using this technique, is possible the formation of dry lipid particles, which is more advantageous than forming liposomes in suspension because of the improved stability upon storage and transport (Beh et al., 2012). According to Kalani and Yunus (2011) the major disadvantage of this technique is the long washing period prior to the agglomeration and aggregation of particles. However, this problem can be minimized by intensively mixing the supercritical antisolvent and the solution, which increases the mass transfer and thus produces smaller particle size. Despite this disadvantage, several compounds such as carotenoids (Mezzomo et al., 2012, Mattea et al., 2009b, Martin et al., 2007, Santos and Meireles, 2013) and various antioxidant compounds (Visentin et al., 2012, Zu et al., 2012, Zhao et al., 2011, Sosa et al., 2011) have been successfully precipitated and encapsulated using the SAS process.

Specifically, SAS process has not been applied in the production of dry particle of EOs. Despite this, the use of the SAS process appears to be an efficient and environmentally-friendly process to produce liposomes. Lesoin et al. (2011) developed a process to produce liposomes of lecithin using the SAS process. In this work, the process was performed using a precipitation temperature of 308 K, range of precipitation pressure from 9 to 13 MPa and CO_2/solvent (ethyl alcohol) molar ratio range from 50 to 100. The liposome size distribution was included in the range of 0.1–100 μm and encapsulation efficiency was about 20%. Moreover, when SAS process is compared with conventional process (Bangham method), SAS process is carried out under mild temperature conditions unlike the Bangham method (308 K for the SAS process and 323 K for the Bangham process) and SAS liposomes were more stable than those obtained by Bangham method. According to these results, SAS process could be a feasible method with potential application in the production of EOs liposomes.

4.1.2.4.Particles from Gas-Saturated Solutions (PGSS)

PGSS is a process that allows from a saturated supercritical fluid solution to obtain nanoparticles. In the PGSS process the solute is initially saturated with $scCO_2$ in a high pressure vessel denominated as static mixer (Figure 6). Afterwards, the saturated solution is expanded at moderate pressure through an expansor nozzle into a spray tower causing the formation of solid particles or liquid particles. The rapid expansion of the saturated solution cause an intense cooling effect caused by Joule-Thomson effect (Cocero et al., 2009). For example, in an encapsulation process of β-carotene with poly-(ε-caprolactone) by PGSS process, de Paz et al. (2012) established that when a higher CO_2 content is used, the Joule–Thomson cooling effect, which is the driving force for particle formation by PGSS process, becomes more intense, promoting the formation of smaller particles. Therefore, the knowledge and control of this phenomenon is a key factor to success of PGSS process.

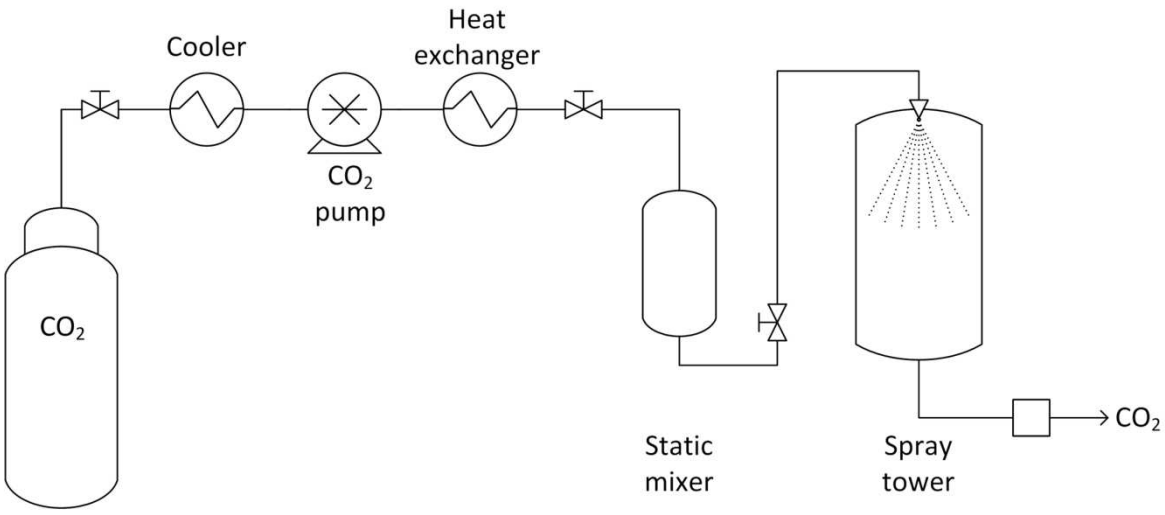

Figure 6. Schematic flowsheet of the Particles from Gas-Saturated Solutions (PGSS) process.

The advantages of PGSS process are related with low SFC consumption and low to medium pressure process. Because of simplicity of this process, the processing cost is very low compare to other processes. It can be used with suspensions or emulsion of active ingredients in polymers or other carrier substances leading to composite particles. Among the disadvantages of PGSS process are included the difficult of producing submicron-sized particles and the control of particle size (Fahim et al., 2014). However, PGSS process has a great potential in the production of particles and capsules of many molten fats, lipids or polymers due to the high solubility in $scCO_2$ of these compounds at moderate pressures

(Martín et al., 2010). For example, a saturated mixture of $scCO_2$ and an emulsion of EOs + carrier material can be formed. Afterwards, this saturated solution can be expanded, precipitating the carrier material entrapping the EOs in capsules.

PGSS process was successfully applied in the lavandin EOs encapsulation using as carrier materials polyethylene glycol (PEG) and OSA modified starches (Varona et al., 2010). In this process was evaluated a PGSS process modification named PGSS-drying. The main difference between the two processes is that in PGSS-drying an oil-in-water emulsion is intensively mixed with $scCO_2$ using a static mixer, encapsulating the EOs in starch by removing the water from the emulsion. The EOs were effectively encapsulated through the PGSS-drying process using OSA modified starches as carrier material. Between the two carries materials, PEG had a better performance reaching higher encapsulation efficiencies than OSA (14–66% of initial EOs encapsulated). Besides, spherical particles with narrow particle size distribution were obtained, which is a key factor in order to control the release of the lavandin oil for further applications. In other work about lavandin EOs encapsulation, demonstrated that the antibacterial activity of lavandin EOs against three pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*) could be enhanced by encapsulation, due to the protection and control release of the EOs (Varona et al., 2013). In another approach, menthol was encapsulated using beeswax as the wall material (Zhu et al., 2010). The experiments were performed at 333 K with pressure in the range from 6 to 20 MPa, mass fraction of menthol in the menthol/beeswax mixture from 10 to 40 % and flow rate of solution from 0.21 to 0.81 cm^3/min. Results indicated that in the range of studied conditions, increase of the pressure, decrease of the gas-saturated solution flow rate, and decrease of the menthol mass fraction can decrease the particle size and narrow particle size distribution of the produced menthol/bees wax microparticles. Although in this work were not obtained nanoparticles (2–50 μm), the microparticles produced have an obvious protection against menthol volatilization.

Choi et al. (2010) obtained PEG microparticles containing coriander EOs using the PGSS process. In this work, temperatures in the range from 310 to 333 K and pressure from 10 to 25 MPa were evaluated. At these process conditions, the stability of the coriander EOs was improved and microparticles with size between 0.1 and 10 μm were produced. On the other hand, they observed a positive influence on the formation of spherical microparticles and highest entrapment efficiency with increasing temperature and decreasing pressure. Gitin et al. (2011) encapsulated garlic EOs using PEG as wall material. In this study, temperatures between 324 and 335 K and pressures between 15.7 and 20.3 MPa were used. Particularly,

although the encapsulation efficiency of the process had good performance (26.10 – 48.93%), it was observed that when the quantity of the EOs was increased, some fraction of oil that was not encapsulated produced particle agglomeration. Therefore, it was impossible to reach nanometric scale and particle sizes ranging from 71.124 µm to 205.64 µm were obtained.

In summary, PGSS process is one of the emerging methods most used to encapsulate. Particularly, although the encapsulation efficiency of the process had good performance, however, further research in order to achieve nanometric scale and avoid particle agglomeration is necessary. EOs protection and the subsequent controlled release of the bioactive compounds are two characteristics of the PGSS process that could be explore in the encapsulation of EOs and other flavor and aroma compounds in order to develop potential applications to food industry.

4.1.2.5.Supercritical Fluid Extraction of Emulsions (SFEE)

SFEE process is a combination of the conventional emulsion precipitation process with the SAS process, where $scCO_2$ is used to eliminate the organic solvent from the emulsion droplets (Figure 7). Emulsion techniques usually involve large quantities of organic solvents, and the removal of them involves additional separation techniques and the use of high temperatures. On the other hand, the particles obtained using SFC sometimes present agglomeration problems generating large particles or it cannot reach nanometric size. However, the application of SCF in the particle technology with the particle formation process from emulsions allows overcome the main problems of each separated technologies (Cocero et al., 2009).

SFEE can benefit from the excellent transport properties of the SFC and cause the precipitation of the particles inside the emulsion droplets. Therefore, the growth of the particles is limited by the size of the emulsion droplets and agglomeration is reduced thanks to the surfactants forming the emulsion. One of the main drawbacks of the SFEE process is that instead of obtaining dry particles as SAS process, the final product usually consists of a suspension of the desired compound in water (Mattea et al., 2009a). Santos et al. (2012) demonstrated that sub-micrometer (344 - 366 nm) particles of carotenoids (β-carotene and lycopene) with high stability and solubility in aqueous media can successfully produce by SFEE process. These results indicate that lycopene used presents a higher solubility in CO_2 and stability than β-carotene under the same operating conditions (323 K and pressures between 7 and 13 MPa). Quercetin is other compound with low water solubility that have been successfully encapsulated the SFEE process (Lévai et al., 2015). After encapsulation,

particles with mean size around 100 nm and encapsulation efficiency around 70% were obtained.

In the same way that SAS process, SFEE process is in principle suitable to obtain micelles loaded with EOs. For example, the solubility of many EOs compounds in CO_2 at moderate pressures is much lower than that of organic solvents; therefore, in any case the fraction of EOs compound extracted during micelle formation can be easily recovered and recycled by depressurization of the gas effluent (Martín et al., 2010). Although at this time there is not specific works about EOs encapsulation, SFEE process could explore the properties of EOs and its behavior in supercritical mixtures to obtain micelles loaded with EOs to food application.

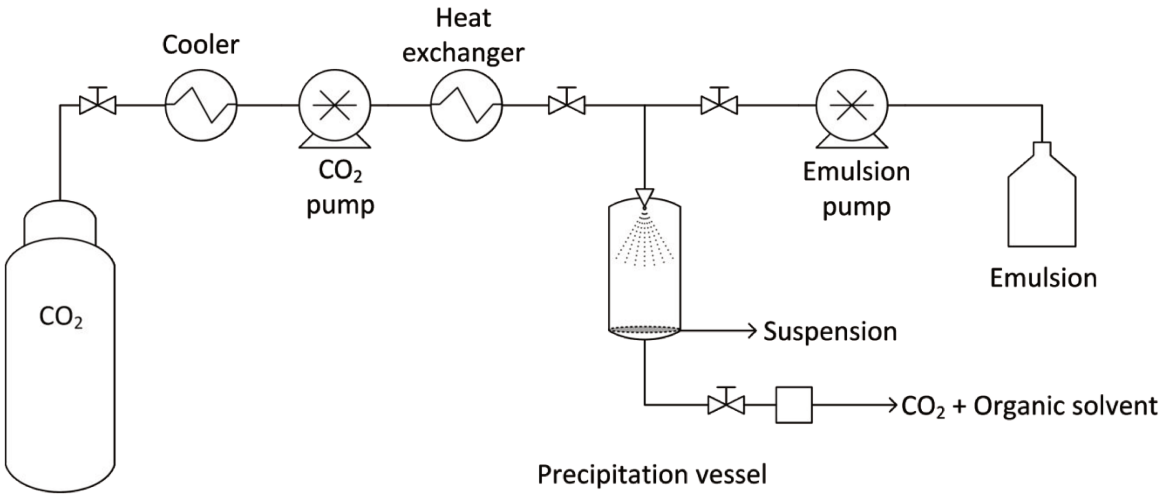

Figure 7. Schematic flowsheet of the Supercritical Fluid Extraction of Emulsions (SFEE) process.

4.2 Ultrasonication

Ultrasonication technology have been evaluated to diverse applications such as extraction of bioactive compounds (Santos et al., 2015), particle formation (Jordens et al., 2015), dehydration of waste oil (Xie et al., 2015), biodiesel production (Sarve et al., 2015), Sulfur removal from bauxite water slurry (Ge et al., 2015), among others.

Depending of the type of process, cavitation is produced due to the formation of high frequency waves in several expansion and contraction cycles, which could causes various effects in the vegetal matrix or liquid medium when ultrasonication is being applied. In most cases these effects are related with enhancing the mass transfer. For example, in the extraction of bioactive compounds, the cell walls of the vegetal matrix are disrupted, the extraction solvent penetration and the mass transfer are favored, and thus the overall yield and

the extraction rates are increased (Toma et al., 2001). On the other hand, in precipitation processes, ultrasonication enhances the nucleation rate which results in a larger amount of fine particles and breakage of the already formed particles into smaller particles by the large shock-waves and micro jets (Horst et al., 2007). However, the use ultrasonication for the formation of nanoemulsions with EOs is a technology almost unexplored. Through research in this area could lead to significant advances in supply of new systems for food flavoring agents.

Emulsions by definition are colloidal systems thermodynamically unstable due to interfacial tension (enthalpy) observed between the phases of different chemical nature. The use of emulsifiers and surfactants allows stabilization of these systems by various mechanisms such as adsorption of the molecules in water-oil interface with a reduction in the interfacial tension; electrostatic interaction of the molecules that act as a barrier to coalescence of the droplets in dispersed phase; combination of the two mechanisms mentioned above; viscosity increase of the continuous phase providing a physical barrier to coalescence. However, the emulsification method used is directly related with the reach of the kinetic stability because a reduction in droplet size of the dispersed phase decreases the velocity of system phase separation (McClements, 2012). This effect is related with Stokes law applied to colloidal systems, that indicates that the velocity of the droplet of the dispersed phase is proportional to the square of its radius (Desrumaux and Marcand, 2002).

Emulsification using high-intensity ultrasound with a frequency range of 16 - 100 kHz and a power of 10 - 1000 W/cm^2 has the ability to produce fine emulsions with size distribution of highly uniform drops (Chandrapala et al., 2012, Silva et al., 2015). Figure 8 shows the schematic flowsheet and the type of energy involved in each piece of an ultrasound equipment. Emulsification via ultrasound has distinct effects on the breakdown of the oil droplets in an emulsion due to the mechanisms involved in this technique. Homogenization occurs through two main mechanisms associated with the generation of an acoustic field and the application of low-intensity ultrasound frequency. The acoustic field is responsible for generating unstable interfacial waves that promotes the mixture of oil with water (continuous phase) leading to the formation of droplets in the system. The application of low frequencies of ultrasound generates an acoustic cavitation phenomenon, which is primarily responsible for the reduction in the oil droplets size. The rapid formation and collapse of microbubbles are promoted by the intense shear rates associated with cavitation. The collapse causes extreme levels of turbulence highly localized and this acts as an effective method to the breakdown the droplets of the dispersed phase in droplets with sizes that can be lower than the sub-micron

range or even reaching the nanoscale (Li and Fogler, 1978a, Li and Fogler, 1978b). Therefore, the superiority of ultrasound as an emulsification method can be associated with microshear resulting of cavitation. The intense shear involved in this technique along with other effects of cavitation, such as heating, may cause the rupture of chemical bonds and physical changes in the polymers used as emulsifiers (Arzeni et al., 2012, Gülseren et al., 2007).

Therefore, when ultrasound is applied as emulsification method using polymers with biological activity associated, such as whey proteins, soy and egg proteins, for example, an evaluation about the maintaining of the activity of these macromolecules after ultrasound processing must be done. This can be considered the only disadvantage associated with use of this technology (Arzeni et al., 2012).

Nanoemulsions obtained can be considered as final process products and are characterized by having diverse possibilities of application in food products. In addition, since most systems are directed to form emulsions type oil-in-water, drying the continuous phase of the EOs nanoemulsions by different techniques (spray-drying and freeze-drying, among others) allows the particle formation with high encapsulation efficiencies of the bioactive compound of interest (Soottitantawat et al., 2003, Soottitantawat et al., 2005).

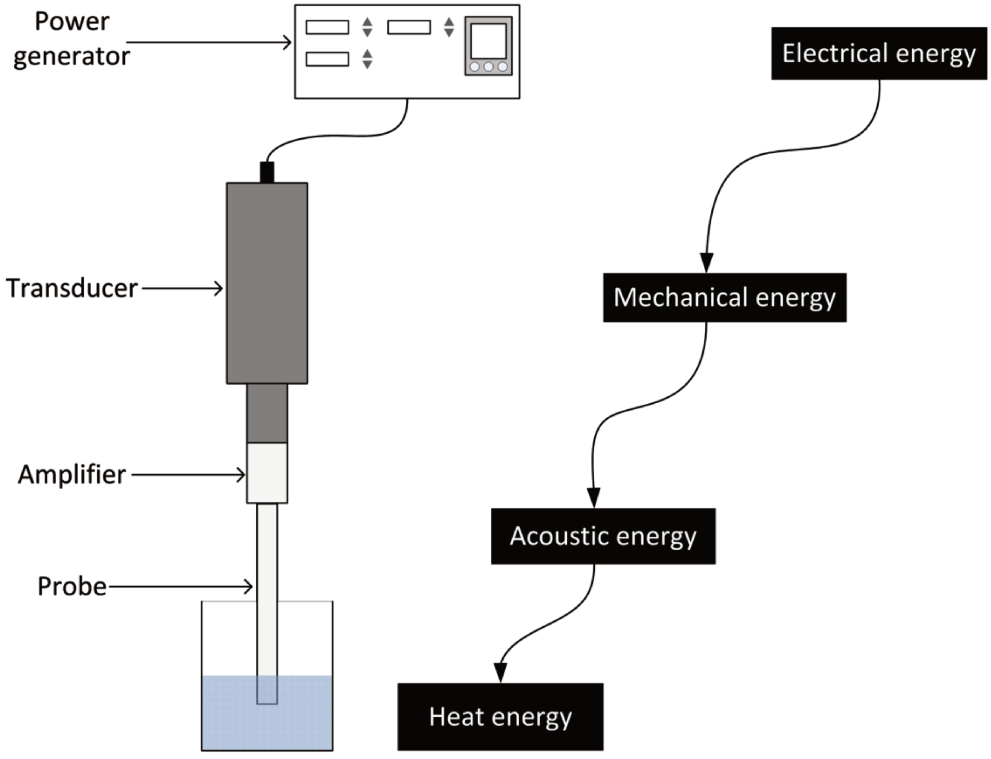

Figure 8. Schematic flowsheet and the type of energy involved in ultrasonication.

4.2.1 Effects of the application of ultrasound in oils

Besides the modifications in the molecular structure of the emulsifiers (gums, starches, proteins, and others) previously mentioned, the use of ultrasound may also cause changes in oily compounds such as EOs. Physicochemical effects such as appearance of off-flavors, metallic taste, production of free radicals, breakdown of compounds (structure modification) and some changes in physical parameters are associated with the use of ultrasound in food processing. These changes may be related with process conditions (temperature and pressure) and microshear mechanisms produced for the acoustic cavitation phenomenon (Pingret et al., 2012, Pingret et al., 2013). For example, after pasteurization of pineapple, grape and cranberry juices using thermo-sonication, Bermúdez-Aguirre and Barbosa-Cánovas (2012) observed changes in color and pH of the samples. Thus, ultrasound may promote the formation of chemical products that affect the pH or cause the destruction of pigments and generate non-enzymatic browning. In other research, Anese et al. (2013) observed after processing of tomato pulp with ultrasound an increase in the viscosity due to the formation of a stronger fibre network, however this network entraps the lycopene and the lycopene bioaccessibility decreased. Cravotto et al. (2011) studied the extraction of kiwi seed oil using Soxhlet and other four different non-conventional techniques, including ultrasound-assisted extraction. The authors observed formation of off-flavors and presence of oxidation compounds in oil extracted with ultrasound due to the partial degradation of the kiwi seed oil. On the other hand, Metherel et al. (2009) showed that the cavitation phenomenon produced by the ultrasound device led to the generation of free radicals in flaxseed oil, and although peroxide levels were increased, the fatty acid composition almost was not affected by ultrasound. Therefore, although the application of ultrasound can promote the nanoencapsulation of EOs, the formation of off-flavors, induction of oxidation in lipid chains, and formation of free radicals are key factors that have to be considered in order to avoid some negative effect on the EOs.

4.2.2 Applications of ultrasonication in obtaining of EOs nanoemulsions

Hashtjin and Abbasi (2015) studied the formation of natural orange peel EOs (OPEO) nanoemulsion assisted by ultrasound. OPEO is among the most common important EOs used in food industry and is recognized by being limonene-rich. To perform the nanoemulsions were used Tween 80 and native gums such as Persian gum (PG) and gum tragacanth (GT) as emulsifiers. Nanoemulsions were prepared using OPEO (1% w/w), as the

oil phase, and mixture of Tween 80 (2% w/w), combined soluble fractions of PG and GT (0.25%w/w) and deionized water (96.75% w/w), as the aqueous phase. The effects of sonication amplitude (70 – 100%) and different process times (90 – 150 s) were evaluated. OPEO nanoemulsions with size of 13 nm were obtained using sonication amplitude of 94% and process time of 138 s at 310 K. In this work, the authors established that sonication amplitude and process time, as well as their interaction had a significant effect over emulsion droplet size. OPEO nanoemulsions showed Newtonian behavior and were physically stable at 278 K and 298 K over three months of storage. The authors concluded that the obtained results strengthen the potential of ultrasonic technique for application in food and pharmaceutical products. Through ultrasonic technique is possible to produce nanoscale emulsions of EOs with long term kinetic stability.

Salvia-Trujillo et al. (2014) evaluated the antimicrobial activity of lemongrass EOs nanoemulsions against *Escherichia coli*. Nanoemulsions were prepared using sodium alginate (1% w/v), Tween 80 (1% v/v) and lemongrass EOs (1% v/v). When the sonication was performed at 400 W and 180 s, nanoemulsions with droplet size of 4.3 ± 0.2 nm were obtained. However, the lowest droplet size of emulsion diminished the antimicrobial potential of lemongrass essential oil nanoemulsions against *E. coli* when compared with nanoemulsions with droplet size above 34.9 ± 8.5 nm obtained at 120 W and 30 s of sonication.

5. Conclusions and future perspectives

Flavor and aroma compounds have a fundamental place in relation with food quality and its acceptability by consumers. The change in perception, way of buying and how food are produced have motivated the consumers to looking for food produced using natural additives obtained through processes environmentally friendly that use nontoxic solvents. Besides, herbs and spices have been used since ancestral times as flavoring and aromatics agents in food-making. Due to the characteristics of their EOs, nowadays they have become in a valuable source of bioactive compounds and the industrial use of EOs is a very promising area. However, because its volatility and sensibility to temperature, EOs require greater care for preservation. Nanoencapsulation allows increasing their solubility and offers protection against factors that triggers their degradation, enhancing its performance as flavoring agents and taking advantage of their pharmacological properties. The developing of novel formulations that enhance its flavoring and aroma characteristics as well as the use of its bioactive properties for increasing shelf-life or even prevent diseases through controlled release allows to think in a promisor future of the field of EO nanoencapsulation. Although

currently is relatively limited the application of emerging techniques in the nanoencapsulation of EOs; the obtaining of nanoparticles and nanoemulsions using supercritical fluids as well as nanoemulsion formation assisted by ultrasound open an attractive panorama to develop new food products with enhanced properties. According to the discussion in this chapter, these two technologies have great potential for application in nanoencapsulation of EOs with application in food industry. The possibility of using encapsulation materials and solvents recognized as safe (GRAS) in processes performed at operating conditions that guarantee the integrity of the compounds is a valuable opportunity to continue the development of a promising area such as nanoencapsulation of natural flavors and aromas for food industry.

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-CAPÍTULO 8-

Discussão Geral

DISCUSSÃO GERAL

De acordo com a pesquisa bibliográfica apresentada no **Capítulo 2**, a extração via PLE é uma técnica adequada para a extração de compostos bioativos tais como os curcuminóides. Os curcuminóides são um grupo de compostos fenólicos responsáveis pela coloração amarela dos rizomas da cúrcuma. As temperaturas elevadas utilizadas pelo processo PLE aumentam a transferência de massa enquanto a alta pressão mantem o solvente no seu estado liquido. Quando comparada com outros métodos convencionais de extração, o processo PLE apresenta varias vantagens tais como a utilização de menores quantidades de solvente em um processo que não tem contato nem com a luz e nem com o ar. Esta técnica tem potencial particular para a extração de curcuminóides como foi demostrado no **Capítulo 4**.

Utilizando uma coluna de núcleo fundido é possível desenvolver um método rápido e confiável na identificação e quantificação dos curcuminóides, como foi apresentado no **Capítulo 3**. Inicialmente foi testado um gradiente linear com uma fase móvel de agua com 0,1% de acido acético (solvente A) e metanol ou solução de acetonitrila com 0,1% de acido acético (Solvente B). Quando a acetonitrila foi utilizada, o sistema apresentou uma menor pressão em relação a solução de metanol. Por outro lado, uma melhor resolução foi atingida utilizando acetonitrila com gradiente entre 40 e 60%. Foram testadas temperaturas entre 308 e 328 K, onde o melhor desempenho foi apresentado na maior temperatura (328 K) resultando em uma diminuição do tempo de retenção dos compostos e na pressão do sistema. Da mesma maneira, foram testadas vazões de fase móvel entre 1.0 e 2.5 $mL \cdot min^{-1}$, onde o melhor desempenho foi com a vazão de 2.5 $mL \cdot min^{-1}$. Este comportamento só é possível nas colunas de núcleo fundido, as quais permitem a utilização de vazões altas sem afetar a resolução. Após tentativa e erro, o método foi otimizado utilizando um gradiente linear (0 min, 45% de solvente B; Etapa 1: 1.5 min, 65% solvente B; Etapa 2: 2.5 min, 90% solvente B; Etapa 3: 4.0 min, 90% solvente B; Etapa 4: 5.0 min, 45% solvente B) com uma temperatura de 328 K, utilizando uma vazão de 2.5 $mL \cdot min^{-1}$ e 2 minutos de tempo de reequilíbrio.

A otimização da extração dos curcuminóides via PLE foi apresentada no **Capítulo 4**. Os rizomas de cúrcuma foram inicialmente desaromatizados utilizando SFE e posteriormente no mesmo equipamento, o processo PLE foi realizado e os curcuminóides foram extraídos utilizando etanol como solvente. O efeito de parâmetros operacionais

temperatura (333–353 K) e pressão (10–35 MPa) foram avaliados sobre o rendimento de extração e o rendimento de curcuminóides. De acordo aos resultados, só a temperatura teve um efeito significativo no rendimento de extração (p_{valor} = 0,000), enquanto para o rendimento de curcuminóides, a interação entre a temperatura e pressão teve um efeito significativo (p_{valor} = 0,000). Como era de se esperar ocorreu um aumento da temperatura incremento o rendimento de extração, no entanto, o processo promoveu a extração de outros compostos diferentes aos curcuminóides, de tal maneira, que a pureza do extrato diminui com o aumento da temperatura. Por outro lado, a pressão teve um efeito negativo no processo de extração de curcuminóides. O aumento da pressão compacta o leito, diminuindo a superfície especifica, criando canais preferenciais e, portanto evitando o contato adequado entre o solvente e a matriz. As condições menos severas de temperatura (333 K) e a pressão de (10 MPa) foram selecionadas como condições ótimas de operação. Após uma análise econômica utilizando as condições otimizadas, foi possível confirmar que o ajuste cinético utilizando um spline linear que o t_{fer} é um parâmetro adequado para fazer uma primeira estimativa do COM. Quando comparado com processos convencionais de extração tais como LPSE e Soxhlet, o processo PLE foi o mais vantajoso economicamente. Devido ao tempo de extração do processo PLE ser mais curto, após 30 minutos de extração e com um custo da matéria-prima de US$ 7,91 kg^{-1} resultou em um COM de US$ 94,92 kg^{-1}, enquanto para os processo LPSE e Soxhlet foram de US$ 245,38 kg^{-1} e US$ 193,55 kg^{-1}, respectivamente.

A próxima etapa foi eliminar o solvente e precipitar os curcuminóides do extrato etanólico obtido no **Capítulo 4**. O processo de precipitação dos curcuminóides foi estudado utilizando o processo SAS como foi apresentado no **Capítulo 5**. Para determinar o efeito dos parâmetros operacionais sobre o processo de precipitação foi utilizado um planejamento estatístico Split-plot. Foram testados dois tipos de injetor (T-mixer e coaxial), duas temperaturas (313 e 333 K), duas pressões (10 e 12 MPa) e duas vazões de CO_2 (500 e 800 g/h). A vazão do extrato etanólico foi fixada em 0,5 $mL \cdot min^{-1}$, devido ao fato de que não foram formadas partículas quando foram utilizadas vazões diferentes. Quando foram utilizadas vazões maiores, o extrato etanólico foi arrastrado para fora do sistema, enquanto quando foram utilizadas vazões menores, os injetores apresentaram problemas de entupimento. No entanto, quando o processo foi realizado utilizando o injetor T-mixer a 313 K, 10 MPa e vazão de CO_2 de 500 g/h foram obtidas partículas com o menor tamanho (111 ± 1 μm) e a distribuição de partículas mais uniforme. Nestas condições de processo, aproximadamente 93,5% dos curcuminóides presentes no extrato etanólico injetado são

precipitados obtendo um extrato em pó com alta pureza (535.5 $mg_{curcuminóides}/g_{extrato}$). Adicionalmente, quando os experimentos foram realizados utilizando estes parâmetros operacionais, o sistema apresentou uma melhor estabilidade em relação à manutenção da pressão e da vazão de CO_2. Por outro lado, considerando o processo integrado, trabalhar com as condições menos severas representa vantagens tais como o requerimento de menos energia e a não desestabilização dos compostos.

As condições otimizadas nos **Capítulos 4 e 5** foram utilizadas para realizar a análise econômica do processo integrado SFE-PLE+SAS, a qual é apresentada no **Capítulo 6**. Este processo integrado permite a obtenção de óleo volátil de cúrcuma e extrato em pó rico em curcuminóides utilizando tecnologias emergentes sem afetar a integridade dos compostos. A integração de processos permitiu produtos com menor COM, por exemplo, em relação com o óleo volátil, o COM obtido após a análise econômica foi de 31,40%, mais econômico que aquele obtido em um trabalho anterior do nosso grupo que considerava só o processo SFE. O extrato em pó rico em curcuminóides, quando comparado com o processo de eliminação do solvente por Soxhlet, o COM do processo integrado é maior, no entanto, quando comparado o extrato em pó com a oleoresina obtida no processo Soxhlet, o extrato em pó apresenta varias vantagens. Por exemplo, o extrato em pó tem um conteúdo de curcuminóides duas vezes maior e sua aplicação e estocagem é mais simples. O COM diminui quando a escala do processo aumentou. Quando é utilizado um custo de compra da matéria-prima de US$ 7,27 kg^{-1} e a escala do aumenta de 2×5 L até 2×500 L, o COM do óleo volátil diminui de US$ 236,88 kg^{-1} para US$ 85,58 kg^{-1}, enquanto para o extrato em pó diminuiu de 260,99 kg^{-1} para US$ 141,63 kg^{-1}. O custo da matéria-prima tem uma influência muito forte no COM. Quando o custo da matéria prima diminui de US$ 7,27 kg^{-1} para US$ 1,59 kg^{-1} em uma escala de 2×50 L, o COM do óleo volátil diminui de US$ 112,70 kg^{-1} para US$ 64,97 kg^{-1}, enquanto para o extrato em pó diminuiu de US$ 174,80 kg^{-1} para US$ 140,96 kg^{-1}. A análise de sensibilidade feita para uma escala de 2×50 L mostrou que o projeto é economicamente viável, principalmente quando é utilizado uma matéria-prima de menor custo.

De acordo com os resultados obtidos neste trabalho, foi possível estabelecer uma metodologia para análise dos curcuminóides por CLAE, usar o processo PLE para a extração destes compostos e por fim a utilização da tecnologia supercrítica através do processo SAS para a formação de partículas de curcuminóides. No entanto, como a maioria dos compostos bioativos, os curcuminóides apresentam uma baixa solubilidade na agua e, portanto, seu uso na indústria esta acompanhado da adição de emulsificantes. Com o objetivo de estudar este

fator limitante, o **Capítulo 7** apresentou uma revisão de literatura sobre processos de nanoencapsulação utilizando tecnologias emergentes. Foi apresentada uma revisão crítica sobre tecnologias emergentes com potencial de aplicação na industrialização de alimentos, como a encapsulação assistida por ultrassom a partir da formação de emulsões do tipo óleo em água e a encapsulação empregando-se tecnologias supercríticas. Foi apresentada uma visão global dos diferentes tipos de materiais de parede (carboidratos, lipídios e proteínas), sendo discutidas suas propriedades físico-químicas, desempenho, vantagens e desvantagens. Finalmente, foram discutidas as características das técnicas emergentes propostas neste estudo e foram analisados detalhadamente os estudos recentes de nanoencapsulação.

-CAPÍTULO 9-

Conclusões gerais

CONCLUSÕES GERAIS

No **Capítulo 1** foi exposto brevemente o estado da arte da extração de curcuminóides via PLE e o processo posterior de precipitação dos extratos utilizando a tecnologia de fluidos supercríticos. O processo PLE é uma técnica adequada para a extração de compostos bioativos, por empregar tempos menores de extração e menores quantidades de solvente. Por outro lado, a utilização de fluidos supercríticos para a eliminação do solvente e a precipitação de compostos bioativos é uma excelente alternativa às técnicas tradicionais.

O estado da arte sobre o processo de extração de curcuminóides via PLE foi apresentado no **Capítulo 2**. De acordo com a pesquisa bibliográfica, o processo PLE possui aplicabilidade no quesito analítico e de processo, apesar da pouca informação existente sobre extração de curcuminóides. Para a otimização do processo PLE é fundamental a compreensão dos parâmetros operacionais tipo de solvente, temperatura, tempo de extração pressão e tamanho de partícula da matéria-prima.

De acordo aos resultados do **Capítulo 3,** foi possível desenvolver um método rápido e confiável na identificação e quantificação dos curcuminóides para os experimentos feitos nos **Capítulos 4** e **5**. A separação dos três curcuminóides principais (Curcumina, demetoxicurcumina e bisdemetoxicurcumina) foi atingida em curto período (1,3 minutos). Apesar da robustez do método não ter sido satisfatória em relação ao volume de injeção, o método desenvolvido apresentou eficiência na resolução, seletividade e simetria dos picos. O tempo total de análise por amostra foi otimizado em 7 minutos. Quando comparado com as técnicas convencionais de quantificação, o método desenvolvido no Capítulo 3 incrementa significativamente a eficiência e a confiabilidade dos resultados.

Extratos etanólicos ricos em curcuminóides foram obtidos com sucesso a partir do uso do processo PLE, conforme apresentado no **Capítulo 4.** Rendimentos entre 4,4 e 2,2 % (b.s.) foram obtidos empregando cúrcuma desaromatizada como matéria-prima, etanol como solvente de extração e tempo estático de 20 min. A temperatura de 333 K e a pressão de 10 MPa foram as condições ótimas de operação, por serem condições de extração menos severas e por resultarem em menos gasto energético em relação às outras condições avaliadas. De acordo com os resultados, o aumento da temperatura aumenta o rendimento de extração, enquanto que a eficiência do processo é afetada negativamente por pressões elevadas. O tempo de extração do processo foi 3 e 6 vezes menor que as técnicas de extração Soxhlet e

LPSE, respectivamente. O custo da matéria-prima teve um grande impacto sobre o COM: quando utilizado o custo da matéria-prima de 0,85 US$ / kg (em um sistema com capacidade de 2 x 0,05 m^3 e 40 minutos de tempo de extração), o COM foi de 15,55 US$ / kg de extrato, ou seja, aproximadamente 4 e 7 vezes mais econômico em relação ao custo da matéria-prima de 4,4 e 7,91 US$ / kg, respectivamente.

Maior concentração de ativos, estabilidade e simplicidade de estocagem são algumas das vantagens dos extratos secos em relação aos extratos líquidos obtidos no **Capítulo 4.** Portanto, o processo SAS é uma excelente alternativa para a eliminação de solvente e precipitação dos curcuminóides, conforme apresentado no **Capítulo 5**. Em função das condições operacionais foi possível precipitar entre 54% e 97% dos curcuminóides presentes no extrato etanólico injetado por meio do processo SAS. Além de permitir a precipitação dos curcuminóides, o processo SAS permitiu o fracionamento da solução. Porém, é necessário o conhecimento prévio do equilíbrio de fases do sistema CO_2 + etanol + extrato etanólico. Mediante submissão do processo a pressão, temperatura e vazão de CO_2 elevados, foi obtido maior rendimento global de sólidos, porém em menor quantidade de curcuminóides nas partículas, devido ao efeito cossolvente gerado pelas condições de operação.

Os parâmetros injetor T-mixer, temperatura de 333 K, pressão de 10 MPa e vazão de 500 g/h de CO_2 foram eleitos como condições ótimas de operação. Nestas condições, o equipamento apresenta maior estabilidade, são produzidas as partículas de tamanho mínimo (100 μm) e distribuição uniforme, e a eficiência de precipitação é por volta de 90%.

A integração e a intensificação de processos, que resultam em gasto energético e custos reduzidos, favorecem a obtenção de produtos de alta qualidade. De acordo aos resultados do **Capítulo 6**, a integração dos processos SFE, PLE e SAS permite a obtenção de produtos economicamente viáveis devido ao aproveitamento intensificado da matéria-prima e dos equipamentos envolvidos. No processo simulado, denominado SFE-PLE+SAS, diante da ampliação de escala, o COM diminuiu; da mesma maneira foi possível observar maior influência do custo da matéria-prima sobre o COM. O menor COM foi obtido na utilização da matéria prima com custo de US$ 1.59 kg^{-1} e no sistema com capacidade de 2×500 L. Nestas condições, o COM para o óleo volátil e o extrato pulverizado rico em curcuminóides foi de US$ 37.85 kg^{-1} e US$ 107.79 kg^{-1}, respectivamente. Após análise de sensibilidade, foi demonstrado que o processo integrado proposto é economicamente viável e se apresenta

como promissor, particularmente em casos nos quais há disponibilidade de matérias-primas com custos relativamente baixos.

O crescente interesse pela substituição de aditivos sintéticos por aditivos naturais converte o produto final obtido nesta tese em um forte candidato para inserção no mercado de produtos naturais. Adicionalmente, de acordo aos resultados do **Capítulo 6**, o extrato em pó rico em curcuminóides, tem potencial de aplicação industrial. No entanto, os curcuminóides, semelhantemente a maioria dos compostos nutracêuticos, possui grande limitação na indústria alimentícia ocasionada pela redução da sua solubilidade em meios aquosos. Portanto, o **Capítulo 7** apresenta uma revisão de diversos métodos de nanocapsulação empregando tecnologias emergentes. Estas tecnologias, baseadas no uso de fluidos supercríticos e na formação de emulsões assistidas com ultrassom, mantém a integridade dos compostos bioativos e aumentam sua solubilidade em meios aquosos. Adicionalmente, a liberação controlada desses compostos permitiria o maior aproveitamento de suas propriedades funcionais para formulação de alimentos ou fármacos.

MEMÓRIA DO PERÍODO DE DOUTORADO

O doutorando Juan Felipe Osorio Tobon, realizou as atividades de pesquisa apresentadas neste projeto de pesquisa no laboratório LASEFI, com auxílio financeiro da CAPES, com vigência de março de 2012 a fevereiro de 2016.

Foram obtidos 21 créditos. As disciplinas cursadas durante o período do doutorado foram: TP320 (Termodinâmica), TP322 (Fenômenos de Transporte I), TP323 (Fenômenos de Transporte II), TP199 (Seminários), IQ323 (Equilíbrio de Fases) e TP121 (Tópicos em Engenharia de Alimentos com ênfase em Estatística).

No primeiro período letivo de 2013 e no segundo período de 2014, participou do Programa de Estágio Docente, grupo C (PED C) com atividades de apoio parcial à docência na disciplina TA 331-A (Termodinâmica), atuando como voluntário (em 2013) e bolsista (em 2014) com carga horária de 08 horas semanais. Além disso, foi aluno ouvinte do curso Metodologia Seis Sigma (Formação *Green Belt*), com 100 h de carga horária, ministrada pelo Prof. Dr. Ademir J. Petenate, no IMECC/UNICAMP.

Em 2013, participou no evento SFE'13 (*Workshop on Supercritical Fluids and Energy*), realizado em Campinas (Brasil). Em 2015, participou no evento *12th International Congress on Engineering and Food* (ICEF12), sediado na cidade do Quebec (Canadá), cujo trabalho foi destaque dentre os mais de 500 trabalhos aceitos e 1040 trabalhos submetidos, e agraciado com o prêmio de melhor apresentação de pôster, nos quesitos conteúdo e design de pôster, e habilidades do apresentador.

As pesquisas referentes, tanto ao projeto de Doutorado, quanto a parceria com o projeto de doutorado de Pedro Ivo Nunes de Carvalho, do mesmo grupo de pesquisa (LASEFI), resultaram, até o presente momento, em 1 artigo de revisão publicado no periódico *Food and Public Health*, 2 artigos experimentais publicados no *Journal of Supercritical Fluids*, 1 artigo experimental submetido ao *Journal Food Chemistry*, 1 artigo experimental aceito para publicação no *Journal of Supercritical Fluids*, 1 artigo experimental que será submetido a um periódico Food and Bioproducts Processing, 1 Capítulo de livro aceito para ser publicado no livro *NanoScience and Food Industry* (*Multi-Volume* SET, Elsevier) e 4 trabalhos publicados em anais de eventos, sendo 1 trabalho completo e 3 resumos, com participação no evento *Workshop on Supercritical Fluids and Energy*, (Campinas, Brasil) em 2013 e no *12th International Congress on Engineering and Food* (Quebec, Canadá) em 2015.

Artigos completos publicados em periódicos

OSORIO-TOBON, J. F., MEIRELES, M. A. 2013. Recent applications of pressurized fluid extraction: curcuminoids extraction with pressurized liquids. Food and Public Health, 3, 303. 289-

OSORIO-TOBON, J. F., CARVALHO, P. I. N., ROSTAGNO, M. A., PETENATE, A. J. & MEIRELES, M. A. A. 2014. Extraction of curcuminoids from deflavored turmeric (*Curcuma longa* L.) using pressurized liquids: Process integration and economic evaluation. The Journal of Supercritical Fluids, 95, 167-174.

CARVALHO, P. I. N., **OSORIO-TOBON, J. F.**, ROSTAGNO, M. A., PETENATE, A. J. & MEIRELES, M. A. A. 2015. Techno-economic evaluation of the extraction of turmeric (*Curcuma longa* L.) oil and ar-turmerone using supercritical carbon dioxide. The Journal of Supercritical Fluids, http://dx.doi.org/10.1016/j.supflu.2015.03.020.

Trabalhos completos publicados em anais de congressos

CARVALHO, P. I. N., **OSORIO-TOBON, J. F.**, ROSTAGNO, M. A., PETENATE, A. J., MEIRELES, M. A. A. Optimization of the ar-turmerone extraction from turmeric (*Curcuma longa* L.) using supercritical carbon dioxide. In: 14th European Meeting on Supercritical Fluids, Anais do 14th European Meeting on Supercritical Fluids, 2014, Marseilles (França).

Resumos publicados em anais de congressos

OSORIO-TOBON, J. F., ROSTAGNO, M. A., MEIRELES, M. A. A.. Extraction, micronization and encapsulation of curcuminoids from turmeric (*Curcuma longa* L.) by pressurized liquids and supercritical fluids. In: Workshop on Supercritical Fluids and Energy, 2013, Campinas (Brasil).

ROSTAGNO, M. A.; **OSORIO-TOBON, J. F.**, CARVALHO, P. I. N.; MEIRELES, M. A. A. . Ultrasound-Assisted Extraction of Curcuminoids from *Curcuma Longa*. In: Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (PITTCON), 2014, Chicago (EUA).

OSORIO-TOBON, J. F., CARVALHO, P. I. N., ROSTAGNO, M. A., PETENATE, A. J. & MEIRELES, M. A. A. Production of a powdered extract of curcuminoids by supercritical

antisolvent process. In: 12th International Congress on Engineering and Food (ICEF12), 2015, Québec (Canadá).

Prêmios e reconhecimentos

Best poster award for the presentation entitled: Production of a powdered extract of curcuminoids by supercritical antisolvent process. Session: Advances in Food Engineering. 12th International Congress on Engineering and Food (ICEF12). Student poster competition. 2015, Québec City, Canada.

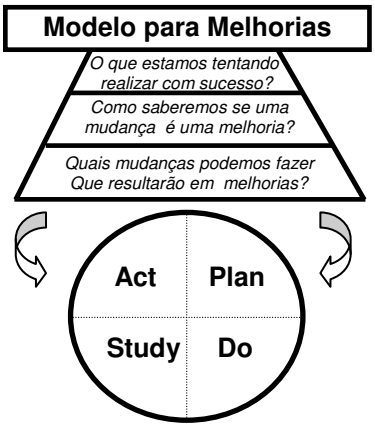
Apêndice

APÊNDICE A

UTILIZAÇÃO DA FERRAMENTA PDSA (PLAN, DO, STUDY AND ACT) PARA ARTIGO DE REVISÃO PLE

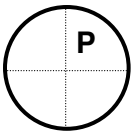
UNICAMP
Programa Black Belt

CICLO DE MELHORIA
PDSA



Data: 10-09-12
Equipe: Juan Felipe Osorio Tobón
Ciclo nº: 01: <u>Pesquisa Bibliográfica</u>

Associates in Process Improvement



PLANEJAR

OBJETIVO DO CICLO

Realizar uma pesquisa bibliografia sobre o conhecimento atual na área de extração de curcuminoides dos rizomas da *Curcuma longa* L. por PLE [Pressurized Liquid Extraction] e SFE [Supercritical Fluid Extraction] com CO_2 e etanol como co-solvente, além da posterior encapsulação utilizando as técnicas SAS [Supercritical Antisolvent Process] e SFEE [Supercritical Extraction From An Emulsion], estabelecendo uma base de artigos sobre os quais se fará a revisão de literatura.

- *Que conhecimento adicional é necessário para levar a ação?* Uso do sistema. Conhecimento das bases de dados disponíveis.

QUESTÕES A SEREM RESPONDIDAS A PARTIR DOS DADOS OBTIDOS NESTE CICLO

1. Existem informações experimentais ou teóricas publicadas sobre o assunto?
2. As publicações estão em fontes referenciáveis?
3. Os dados disponíveis são provenientes de laboratórios cuja credibilidade já está estabelecida?
4. Quem são os autores mais importantes da área? Em que países estes autores realizaram o trabalho?
5. Existem patentes sobre o tema? De que locais são elas?
6. As publicações aconteceram num período determinado? Qual é a situação atual?
7. Qual é o cenário futuro?
8. Os curcuminoides encapsulados terão um possível impacto no futuro?
9. Quais tipos de substancias têm sido encapsuladas utilizando fluidos supercríticos? As técnicas SAS [Supercritical Antisolvent Process] e SFEE [Supercritical Extraction From An Emulsion] têm sido utilizadas para extrair o que tipos de sustâncias.
10. Os curcuminoides encapsulados terão um possível impacto no futuro?

PREDIÇÕES

1. Serão encontrados muitos artigos relacionados com a extração supercrítica de compostos de interesse que podem ser utilizados em diversas indústrias como a alimentar e farmacêutica e que terão potencial aplicação na escala industrial.
2. Devido à aparição de química verde, os processos de extração supercrítica tem chamado a atenção dos pesquisadores e o conhecimento se tem expandido através das bases de dados.
3. A busca sobre a extração supercrítica indicará periódicos relacionados com fluidos supercríticos, engenheira de alimentos e indústrias farmacêuticas.
4. Serão muitas as publicações relacionadas com a extração supercrítica de compostos de todo tipo a partir de fontes

naturais.

5. É provável que as informações obtidas indiquem os produtos obtividos ainda não têm aplicação direita na indústria de alimentos e que sua produção não é escada industrial.
6. É presumível não encontrar muitos artigos sobre processos de encapsulação de curcuminoides utilizando fluidos supercríticos.
7. É possível não achar artigos comparando os custos de diferentes técnicas de extração de curcuminoides
8. As publicações encontradas estarão escritas em inglês.

- *Há dados históricos disponíveis para responder às questões acima?*
- *A equipe concorda com estas predições?*

DESENVOLVA UM PLANO PARA RESPONDER ÀS QUESTÕES (Quem, O que, Onde, Quando, Como)

1. Procurar as teses, relatórios e trabalhos em feitos no LASEFI - DEA / FEA – UNICAMP na Base Alimentarium a fim de obter trabalhos relacionados feitos pelo grupo de pesquisa, serão utilizadas as seguintes palavras chave *Curcuma, Extração supercrítica, Encapsulação, Extração com liquido Pressurizado.*
2. Buscar nas bases de dados ISI Web of Knowledge e Scopus, caso o número de itens obtido seja aumentado, refinar a pesquisa pela opção *Food Science Technology*;
3. Utilizar as palavras chave *Curcuma* e *Curcuminoids* a fim de se obter artigos relacionados a todas as propriedades e características da matéria prima e os compostos alvos de extração;
4. Utilizar a palavra chave *Supercritical Fluid Extraction (SFE)* a fim de se obter artigos relacionados a todas as aplicações dessa tecnologia;
5. Utilizar as palavras-chave *Supercritical Fluid Extraction (SFE)* e *Curcuminoids* a fim de obter artigos relacionados com a utilização dos fluidos supercríticos na extração de curcuminoides;
6. Utilizar a palavra chave *Pressurized Liquid Extraction (PLE)* a fim de se obter artigos relacionados a todas as aplicações dessa tecnologia;
7. Utilizar as palavras-chave *Pressurized Liquid Extraction (PLE)* e *Curcuminoids* a fim de obter artigos relacionados com a utilização de fluidos líquidos pressurizados na extração de curcuminoides
8. Utilizar as palavras-chave *Encapsulation, co-precipitation* e *Supercritical Fluid Extraction (SFE)* a fim de obter relacionados com a utilização de fluidos supercríticos na obtenção de partículas de tamanho nanométrico, os materiais parede e os compostos obtidos;
9. Utilizar a palavra chave *Supercritical Antisolvent Process (SAS)* a fim de se obter artigos relacionados a todas as aplicações dessa tecnologia;
10. Utilizar as palavras-chave *Supercritical Antisolvent Process (SAS)* e *Curcuminoids* a fim de obter artigos relacionados com a utilização de fluidos líquidos pressurizados na extração de curcuminoides
11. Utilizar a palavra chave *Supercritical Extraction From An Emulsion (SFEE)* a fim de se obter artigos relacionados a todas as aplicações dessa tecnologia;
12. Utilizar as palavras-chave *Supercritical Extraction From An Emulsion (SFEE)* e *Curcuminoids* a fim de obter artigos relacionados com a utilização de fluidos líquidos pressurizados na extração de curcuminoides;
13. Em todos os itens anteriores selecionar as patentes relacionadas com o tema de pesquisa;
14. Ter cuidado de selecionar os artigos que estão relacionados diretamente com o tema de pesquisa;
15. Limitar a busca para artigos publicados a partir de 2005. Caso o número de itens obtido seja reduzido, aumentar o período da busca;
16. Será utilizado o programa EndNote para organizar os artigos e serão divididos nas seguintes pastas:
    16.1 Curcuma
    16.2 SFE
    16.3 PLE
    16.4 Encapsulação co-precipitação
        16.4.1 SAS

16.4.2 SFEE

16.5 Patentes

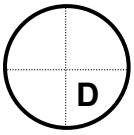
16.6 Livros

17. Selecionar os itens mais interessantes através da leitura dos resumos e da quantidade de citações que tenha o artigo;
18. Prestar atenção nos nomes de autores que aparecem com maior frequência e países em que se localizam os laboratórios;
19. Redigir a lista de referências bibliográficas;
20. Este processo de pesquisa possibilitará a construção da revisão de literatura.

- *O seu plano considerou os seguintes métodos:*

- Formulários Coleta de Dados: ( ) Sim ( ) Não	- Experimentação Planejada: ( ) Sim ( ) Não	- Diagramas de Dispersão: ( )Sim ( )Não
- Diagramas de Pareto: ( ) Sim ( ) Não	- Métodos de Pesquisa: ( ) Sim ( ) Não	- Gráficos de Tendências: (x)Sim ( )Não
- Gráficos de Controle: ( ) Sim ( ) Não	- Simulação/Modelagem: ( ) Sim ( ) Não	- Análise de Engenharia: ( )Sim ( ) Não
- Histogramas: (x) Sim ( ) Não		

- *Você definiu responsabilidades para a coleta e análise dos dados?*
- *É necessário treinamento?*
- *O plano é consistente com o contrato?*
- *O plano pode ser conduzido em pequena escala?*
- *Você considerou as pessoas de fora da equipe que serão afetadas por este plano?*



FAZER

OBSERVAÇÕES AO CONDUZIR O PLANO

1. O LASEFI - DEA / FEA – UNICAMP tem desenvolvido as seguintes teses relacionadas com o tema:

   Braga, Mara Elga Medeiros. Extração supercrítica de curcuminoides de Curcuma longa L. usando como solvente mistura de CO2 + etanol e/ou isopropanol: um estudo da cinética e das propriedades funcionais do extrato/ Mara Elga Medeiros Braga. Dissertação (mestrado) - Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos. Campinas, SP: [s.n.], 2002.

   Braga, Mara Elga Medeiros. Obtenção de compostos bioativos de Curcuma longa L. e Lippia alba M. por tecnologia supercrítica : rendimento global, cinética de extração, composição química e aproveitamento do resíduo amilaceo. Dissertação (doutorado) - Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos. Campinas, SP: [s.n.], 2005.

   Santos, Diego Tresinari dos. Extração, micronização e estabilização de pigmentos funcionais = construção de uma unidade multipropósito para desenvolvimento de processos com fluídos pressurizados. Dissertação (doutorado) - Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos. Campinas, SP: [s.n.], 2011.

2. Utilizando a ferramenta “citation report” da base de dados ISI “Web of Knowledge” obtiveram-se os seguintes gráficos:

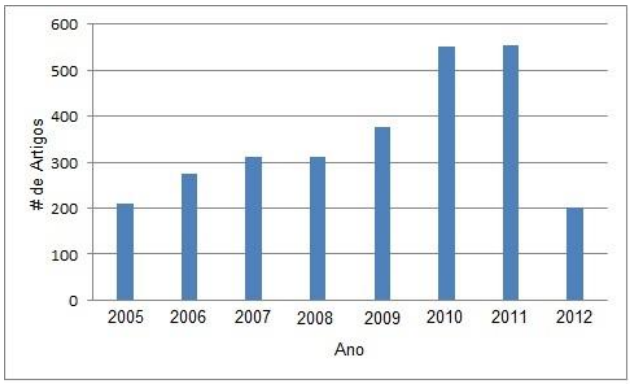


Figura 1. Histograma do número de artigos publicados sobre curcuma

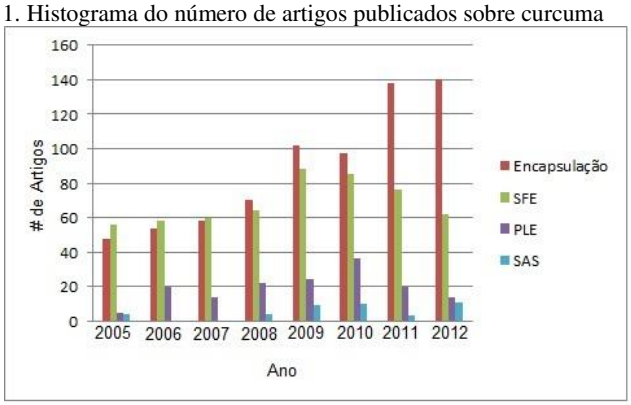


Figura 2. Histograma do número de artigos publicados sobre encapsulação e as técnicas de extração supercrítica

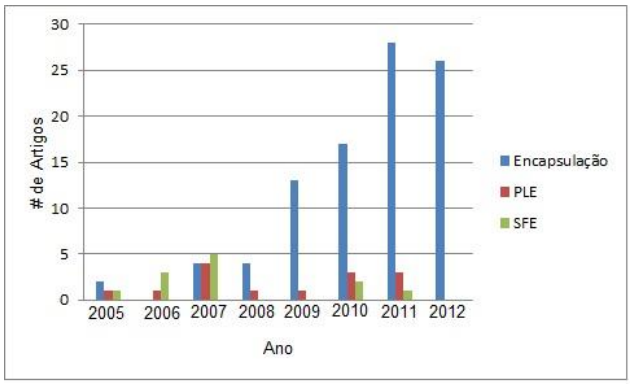


Figura 3. Histograma do número de artigos publicados sobre encapsulação e as técnicas de extração supercrítica de curcuminoides

A encapsulação de curcuminoides é uma matéria que tem focado a atenção de pesquisadores, baseados nas propriedades funcionais dos curcuminoides e a alta seletividade dos fluidos supercríticos, o numero de pesquisas tem aumentando ao longo dos anos, apresentado a encapsulação de curcuminoides uma porcentagem representativa (13%) entre os artigos publicados nas áreas de farmácia, agricultura e ciência e tecnologia de alimentos, embora no momento de procurar com as palavras chave Encapsulation* curcuma* supercritical fluids não tenha gerado nenhum resultado.

3. Os autores mais representativos da área são:
   Chattopadhyay, P., B., Cocero, M. J., Brunner, G., Meireles, M. A. A., Perrut, M., Reverchon, E., Santos, D. T., Wang, Y. L.,
4. Os laboratórios de pesquisa em que esses autores operam são:

   i. Ferro Corporation, Pharmaceutical Technology, 7500 East Pleasant Valley Road, Independence, OH 44131, USA.

ii. Departamento de Ingeniería Química y Tecnología del Medio Ambiente, Facultad de Ciencias, Universidad de Valladolid, 47011 Valladolid, Spain.
iii. Thermal Separation Processes, Technische Universita¨ t Hamburg-Harburg, Eissendorfer Str. 38, D 21073 Hamburg, Germany.
iv. LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas). Cidade Universitária "Zeferino Vaz", R. Monteiro Lobato, 80, 13083 862 Campinas, SP, Brazil.
v. Separex 5, rue Jacques Monod F-54250, Champigneulles, France.
vi. Dipartimento di Ingegneria Chimica e Alimentare, Universit`a di Salerno, Via Ponte Don Melillo 1, 84084 Fisciano (SA), Italy.
vii. New Jersey Center for Engineered Particulates, New Jersey Institute of Technology, Newark, NJ 07102.

5. Os periódicos mais importantes da área são:

a. Journal of Supercritical Fluids
b. Journal of Agricultural and Food Chemistry
c. Journal of Food Engineering
d. Trends in Food Science & Technology

6. A investigação de textos científicos, selecionados pela importância, resultou na seguinte descrição:

Pasta	Itens encontrados	Itens eleitos
Curcuma	24 artigos/5 livros	6 artigos/5 livros
SFE	38 artigos/3 livros	11 artigos/3 livros
PLE	17 artigos	9 artigos
Encapsulação co-precipitação	54 artigos/4 livros	12 artigos/4 livros
SAS	18 artigos	5 artigos
SFEE	4 artigos	2 artigos
Livros (capítulos)	19	19
Patentes	12	*

7. Foram eleitos os seguintes artigos relacionados com a curcuma e curcuminoides:

Al-Reza, S. M., A. Rahman, et al. (2010). "Essential oil composition and antioxidant activities of Curcuma aromatica Salisb." Food and Chemical Toxicology 48(6): 1757-1760.

Araujo, C. A. C. and L. L. Leon (2001). "Biological activities of Curcuma longa L." Memorias Do Instituto Oswaldo Cruz 96(5): 723-728.

Chang, L. H., T. T. Jong, et al. (2006). "Supercritical carbon dioxide extraction of turmeric oil from Curcuma longa Linn and purification of turmerones." Separation and Purification Technology 47(3): 119-125.

Chattopadhyay, I., K. Biswas, et al. (2004). "Turmeric and curcumin: Biological actions and medicinal applications." Current Science 87(1): 44-53.

Jayaprakasha, G. K., L. Jagan, et al. (2005). "Chemistry and biological activities of C-longa." Trends in Food Science & Technology 16(12): 533-548.

Mulik, R., K. Mahadik, et al. (2009). "Development of curcuminoids loaded poly(butyl) cyanoacrylate

nanoparticles: Physicochemical characterization and stability study." European Journal of Pharmaceutical Sciences 37(3-4): 395-404.

8. Foram eleitos os seguintes artigos relacionados com o processo SFE:

Began, G., M. Goto, et al. (2000). "Response surfaces of total oil yield of turmeric (Curcuma longa) in supercritical carbon dioxide." Food Research International 33(5): 341-345.

Braga, M. E. M., M. Angela, et al. (2007). "Accelerated solvent extraction and fractioned extraction to obtain the Curcuma longa volatile oil and oleoresin." Journal of Food Process Engineering 30(4): 501-521.

Braga, M. E. M., P. F. Leal, et al. (2003). "Comparison of yield, composition, and antioxidant activity of turmeric (Curcuma longa L.) extracts obtained using various techniques." Journal of Agricultural and Food Chemistry 51(22): 6604-6611.

Brunner, G. (2005). "Supercritical fluids: technology and application to food processing." Journal of Food Engineering 67(1-2): 21-33.

Hauthal, W. H. (2001). "Advances with supercritical fluids review." Chemosphere 43(1): 123-135.

Herrero, M., J. A. Mendiola, et al. (2010). "Supercritical fluid extraction: Recent advances and applications." Journal of Chromatography A 1217(16): 2495-2511.

Kao, L., C.-R. Chen, et al. (2007). "Supercritical CO2 extraction of turmerones from turmeric and high-pressure phase equilibrium of CO2+turmerones." Journal of Supercritical Fluids 43(2): 276-282.

Palmer, M. V. and S. S. T. Ting (1995). "APPLICATIONS FOR SUPERCRITICAL-FLUID TECHNOLOGY IN FOOD-PROCESSING." Food Chemistry 52(4): 345-352.

Perrut, M. (2000). "Supercritical fluid applications: Industrial developments and economic issues." Industrial & Engineering Chemistry Research 39(12): 4531-4535.

Reverchon, E. and I. De Marco (2006). "Supercritical fluid extraction and fractionation of natural matter." Journal of Supercritical Fluids 38(2): 146-166.

Rosa, P. T. V. and M. A. A. Meireles (2005). "Rapid estimation of the manufacturing cost of extracts obtained by supercritical fluid extraction." Journal of Food Engineering 67(1-2): 235-240.

9. Foram eleitos os seguintes artigos relacionados com o processo PLE:

Barbero, G. F., M. Palma, et al. (2006). "Pressurized liquid extraction of capsaicinoids from peppers." Journal of Agricultural and Food Chemistry 54(9): 3231-3236.

Carabias-Martinez, R., E. Rodriguez-Gonzalo, et al. (2005). "Pressurized liquid extraction in the analysis of food and biological samples." Journal of Chromatography A 1089(1-2): 1-17.

Cheah, E. L. C., P. W. S. Heng, et al. (2010). "Optimization of supercritical fluid extraction and pressurized liquid extraction of active principles from Magnolia officinalis using the Taguchi design." Separation and Purification

Technology 71(3): 293-301.

Choi, M. P. K., K. K. C. Chan, et al. (2003). "Pressurized liquid extraction of active ingredients (ginsenosides) from medicinal plants using non-ionic surfactant solutions." Journal of Chromatography A 983(1-2): 153-162.

Hu, J., Z. Guo, et al. (2011). "Pressurized liquid extraction of ginger (Zingiber officinale Roscoe) with bioethanol: An efficient and sustainable approach." Journal of Chromatography A 1218(34): 5765-5773.

Jaime, L., I. Rodriguez-Meizoso, et al. (2010). "Pressurized liquids as an alternative process to antioxidant carotenoids' extraction from Haematococcus pluvialis microalgae." Lwt-Food Science and Technology 43(1): 105-112.

Mustafa, A. and C. Turner (2011). "Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review." Analytica Chimica Acta 703(1): 8-18.

Rodriguez-Meizoso, I., L. Jaime, et al. (2008). "Pressurized fluid extraction of bioactive compounds from Phormidium species." Journal of Agricultural and Food Chemistry 56(10): 3517-3523.

Santos, D. T., P. C. Veggi, et al. (2012). "Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba skins." Journal of Food Engineering 108(3): 444-452.

10. Foram eleitos os seguintes artigos relacionados com encapsulação:

Lertsutthiwong, P., K. Noomun, et al. (2008). "Preparation of alginate nanocapsules containing turmeric oil." Carbohydrate Polymers 74(2): 209-214.

Lertsutthiwong, P., P. Rojsitthisak, et al. (2009). "Preparation of turmeric oil-loaded chitosan-alginate biopolymeric nanocapsules." Materials Science & Engineering C-Biomimetic and Supramolecular Systems 29(3): 856-860.

Martin, A. and M. J. Cocero (2008). "Micronization processes with supercritical fluids: Fundamentals and mechanisms." Advanced Drug Delivery Reviews 60(3): 339-350.

Mattea, F., A. Martin, et al. (2009). "Supercritical antisolvent precipitation from an emulsion: beta-Carotene nanoparticle formation." Journal of Supercritical Fluids 51(2): 238-247.

Nayak, A. P., W. Tiyaboonchai, et al. (2010). "Curcuminoids-loaded lipid nanoparticles: Novel approach towards malaria treatment." Colloids and Surfaces B-Biointerfaces 81(1): 263-273.

Onwulata, C. I. (2012). Encapsulation of New Active Ingredients. Annual Review of Food Science and Technology, Vol 3. M. P. Doyle and T. R. Klaenhammer. 3: 183-202.

Reverchon, E. and R. Adami (2006). "Nanomaterials and supercritical fluids." Journal of Supercritical Fluids 37(1): 1-22.

Sanguansri, P. and M. A. Augustin (2006). "Nanoscale materials development - a food industry perspective." Trends in Food Science & Technology 17(10): 547-556.

Vitaglione, P., R. B. Lumaga, et al. (2012). "Curcumin Bioavailability from Enriched Bread: The Effect of

Microencapsulated Ingredients." Journal of Agricultural and Food Chemistry 60(13): 3357-3366.

Wang, Y., Z. Lu, et al. (2009). "Study on microencapsulation of curcumin pigments by spray drying." European Food Research and Technology 229(3): 391-396.

Weidner, E. (2009). "High pressure micronization for food applications." Journal of Supercritical Fluids 47(3): 556-565.

Yeo, S. D. and E. Kiran (2005). "Formation of polymer particles with supercritical fluids: A review." Journal of Supercritical Fluids 34(3): 287-308.

11. Foram eleitos os seguintes artigos relacionados com o processo SAS:

Guha, R., M. Vinjamur, et al. (2011). "Demonstration of Mechanisms for Coprecipitation and Encapsulation by Supercritical Antisolvent Process." Industrial & Engineering Chemistry Research 50(2): 1079-1088.

Martin, A., F. Mattea, et al. (2007). "Co-precipitation of carotenoids and bio-polymers with the supercritical anti-solvent process." Journal of Supercritical Fluids 41(1): 138-147.

Reverchon, E. (1999). "Supercritical antisolvent precipitation of micro- and nano-particles." Journal of Supercritical Fluids 15(1): 1-21.

Reverchon, E., R. Adami, et al. (2008). "Spherical microparticles production by supercritical antisolvent precipitation: Interpretation of results." Journal of Supercritical Fluids 47(1): 70-84.

Reverchon, E., I. De Marco, et al. (2007). "Nanoparticles production by supercritical antisolvent precipitation: A general interpretation." Journal of Supercritical Fluids 43(1): 126-138.

12. Foram eleitos os seguintes artigos relacionados com o processo SFEE:

Chattopadhyay, P., R. Huff, et al. (2006). "Drug encapsulation using supercritical fluid extraction of emulsions." Journal of Pharmaceutical Sciences 95(3): 667-679.

Della Porta, G., N. Falco, et al. (2011). "Continuous Supercritical Emulsions Extraction: A New Technology for Biopolymer Microparticles Production." Biotechnology and Bioengineering 108(3): 676-686.

13. Foram encontradas as seguintes patentes.

Extraction of curcumin from Curcuma Longa|Includes putting powder of Curcuma Longa L in extract container, heating to 60-70 deg. C, contacting carbon di:oxide in super-critical state, etc, Okinawa Ken Kinousei Shokuhin.

Cheng, Y., J. Dou, et al. Active substance of Curcuma for treating tumor, obtained by extracting Curcuma using ethyl acetate and ethanol, extracting condensate by ethanol, subjecting extract to column chromatography using water and acetonitrile as elute, Tianjin Tianshili Pharm Co Ltd; Tianjin Tasly Pharm Co Ltd.

Choi, G. I. and D. H. Mun Extract of curcuma longa l. Containing ingredients having anti-inflammatory effects for manufacturing safe anti-inflammatory cosmetics and an efficient extraction method thereof, Choi G I; Pio Korea Co Ltd; Ind Education Coop Org Daegu Health C.

Huang, C., F. Chen, et al. Curcuma volatile oil extraction and curcumin-compounds purification by using polar solvent to extract curcuma extract, using solid adsorbent, e.g. silica gel, and using non-polar solvent, e.g. ether to remove volatile oil, Univ Gaungxi Normal.

Hyeon, B. K. Method for preparing salted Mackerel using Curcuma longa rhizoma, involves removing intestine of Mackerel, pulverizing Curcuma longa rhizoma, drying pulverized Curcuma longa rhizoma powder, and preparing Curcuma longa extract, Hyeon B K.

Janiscki Da Lozzo, E. Curcuma longa hydrogel preparation used for human and animals for treating diseases, and also used as cosmetic and pharmaceutical product, is obtained by soaking dry powder of rhizome of Curcuma longa in hydroalcoholic solution, Janiscki Da Lozzo E.

Kuang, C., X. Li, et al. Extraction and separation of curcuma oil for cosmetics, involves extracting curcuma oil from curcuma raw material using supercritical carbon dioxide in distillation column operated at preset pressure and temperature, Univ Cent South Forestry & Technology; Univ Cent South Forestry Technology.

Lian, Y., J. Liu, et al. Extraction of fine curcumin and curcuma oil, involves washing and extracting curcuma, condensing filtered extraction liquid, separating upper liquid to form curcuma oil, and absorbing and crystallizing lower liquid to form curcumin, Chenguang Natural Pigment Group Co Ltd.

Oei, B. L. Potentiating analgesic effect of curcuminoid|by removing bis-des:methoxy curcumin and adding extracts of Curcuma domestica and/or Curcuma xanthorrhiza, Pt Daria-Varia Lab; Pt Darya Varia Lab; Pt Daria Varia Lab; Pt Darya-Varia Lab.

Park, K. J. Method for fermenting Curcuma domestica while removing bitter taste and unfavorable odor, comprises using Aspergillus oryzae, Park K J.

Perrut, M. Microparticle coating comprising supercritical fluids, Separex Sa; Perrut M.

Quintanilla Almagro, E. and J. Diaz Alperi Potentiating analgesic effect of curcuminoid|by extraction of dried and milled rhizomes, pref. under supercritical conditions using aqueous or organic solvents, Asac Pharm Int Aie; Asac Pharm Int Aie Sagitario.

Sheng, Y., N. Pan, et al. Method for extracting volatile oil from micron-level curcuma zedoary powder using CO2 supercritical extracting technology, Univ Sichuan.

Wei, F., X. Chen, et al. Method of extracting element in curcuma zedoary by supercritical carbon dioxide fluid extraction-rectification, Univ Hebei Sci & Technology.

14. Embora a busca tenha sido limitada a artigos acima do ano 2005, vários artigos com data de publicação mais antiga tem sido citados de maneira constante e poderiam importantes também devido a sua relevância:

Braga, M. E. M., P. F. Leal, et al. (2003). "Comparison of yield, composition, and antioxidant activity of turmeric (Curcuma longa L.) extracts obtained using various techniques." Journal of Agricultural and Food Chemistry 51(22): 6604-6611.

Camel, V. (2001). "Recent extraction techniques for solid matrices-supercritical fluid extraction, pressurized fluid

extraction and microwave-assisted extraction: their potential and pitfalls." Analyst 126(7): 1182-1193.

Chattopadhyay, I., K. Biswas, et al. (2004). "Turmeric and curcumin: Biological actions and medicinal applications." Current Science 87(1): 44-53.

Perrut, M. (2000). "Supercritical fluid applications: Industrial developments and economic issues." Industrial & Engineering Chemistry Research 39(12): 4531-4535.

Reverchon, E. (1999). "Supercritical antisolvent precipitation of micro- and nano-particles." Journal of Supercritical Fluids 15(1): 1-21.

Reverchon, E. and G. Della Porta (1999). "Production of antibiotic micro- and nano-particles by supercritical antisolvent precipitation." Powder Technology 106(1-2): 23-29.

Rydberg, J., M. Cox, et al. (2004). Solvent Extraction Principles and Practice, Taylor & Francis Group.

15. Os seguintes livros (capítulos) foram encontrados e servirão de apoio durante a elaboração e desenvolvimento do projeto:

Aguilar, M. and J. L. Cortina (2008). Solvent Extraction and Liquid Membranes: Fundamentals and Applications in New Materials, Taylor & Francis.

Bagchi, D. and H. G. Preuss (2004). Phytopharmaceuticals in Cancer Chemoprevention, Taylor & Francis.

Cazes, J. (2001). Encyclopedia of Chromatography, Marcel Dekker.

Deibler, K. D. and J. Delwiche (2003). Handbook of Flavor Characterization: Sensory Analysis, Chemistry, and Physiology, Marcel Dekker.

Khoury, F. M. (2004). Multistage Separation Processes, Third Edition, Taylor & Francis.

Lebovka, N., E. Vorobiev, et al. (2011). Enhancing Extraction Processes in the Food Industry, Taylor & Francis.

Luthria, D. L. (2004). Oil Extraction and Analysis: Critical Issues and Comparative Studies, AOCS Press.

Martinez, J. L. (2007). Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounds, Taylor & Francis.

Meireles, A. A. (2008). Extracting Bioactive Compounds for Food Products: Theory and Applications, Taylor & Francis.

Mukhopadhyay, M. (2000). Natural Extracts Using Supercritical Carbon Dioxide, Taylor & Francis.

Packer, L., M. G. Traber, et al. (1996). Proceedings of the International Symposium on Natural Antioxidants: molecular mechanisms and health effects, AOCS Press.

Pathak, Y. (2011). Handbook of Nutraceuticals: Scale-Up, Processing and Automation, Taylor & Francis.

Ravindran, P. N., K. N. Babu, et al. (2007). Turmeric: The genus Curcuma, Taylor & Francis.

Rydberg, J., M. Cox, et al. (2004). Solvent Extraction Principles and Practice, Taylor & Francis Group.

Shi, J. (2006). Functional Food Ingredients and Nutraceuticals: Processing Technologies, Taylor & Francis.

Shi, J., G. Mazza, et al. (2002). Functional Foods: Biochemical and Processing Aspects, Taylor & Francis.

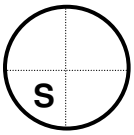
Socaciu, C. (2007). Food Colorants: Chemical and Functional Properties, Taylor & Francis.

Tzia, C. and G. Liadakis (2003). Extraction Optimization in Food Engineering, Taylor & Francis.

Zhang, S. M., R. R. Cao, et al. (2007). Fabrication and characterization of biodegradable nanospheres containing curcumin. Nanoscience and Technology, Pts 1 and 2. C. Bai, S. Xie and X. Zhu. 121-123: 767-770.

16. A busca com os termos *supercritical extraction* e *supercritical extraction fluid* resultou em um grande número de artigos, com estudos bastante atuais, fornecendo um retrato muito satisfatório da utilização desta tecnologia em diversas frentes de pesquisa.
17. O termo Supercritical Fluid Extraction of an Emulsion (SFEE) não forneceu um grande número de artigos, e só foram encontrados não mais de 4 artigos relacionados.
18. Apesar de terem sido encontradas várias publicações relacionadas a diversos aspectos do processo supercrítico, ainda muitos compostos não são produzidos a escada industrial e sua aplicação em alimentos comerciais é pequena, mas e uma grande oportunidade de pesquisar sobre este tema.
19. A procura com as palavras chave *Supercritical Antisolvent Process (SAS,) Supercritical Fluid Extraction of an Emulsion (SFEE)* e *Curcuminoids, curcuma, curcumin, turmeric* não obteve resultados.

- *Identifique as coisas observadas que não fazem parte do plano.*
- *Documente o que saiu errado durante a coleta de dados.*
- *Comece a análise inicial dos dados assim que forem coletados.*
- *Avalie os dados quanto a mudanças ao longo do tempo (gráfico de controle ou gráfico de tendência).*



ESTUDAR

O QUE FOI APRENDIDO COM OS DADOS?

1. O tema selecionado representa uma oportunidade para o avanço do conhecimento?

   A extração supercrítica tem-se tornado uma área de interesse com a aparição da química verde sendo uma importante matéria prima a curcuma, a qual tem muitas propriedades funcionais com um amplo potencial de aplicação na indústria alimentar e farmacêutica, se encontrando um grande numero de artigos relacionados com a encapsulação de muitos compostos e alguns derivados da curcuma, mas a maioria empregando técnicas tradicionais ou não relacionadas com o uso de fluidos supercríticos; a técnica SAS tem ganhado espaço nas pesquisas e junto com a técnica SFEE apresenta uma oportunidade de obter partículas de diferentes tamanhos e morfologias além de manter a propriedades funcionais e garantir seu efeito.

2. Você considera que as ferramentas necessárias para a realização deste trabalho estão disponíveis ou poderiam ser

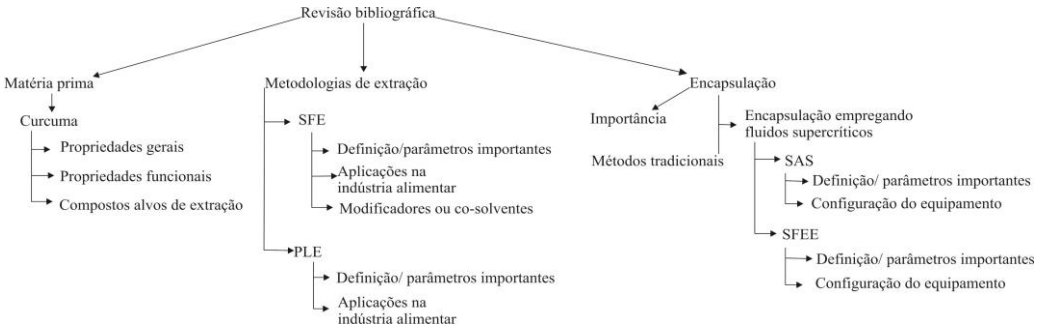
disponibilizadas para a execução da pesquisa proposta?

Sem, o LASEFI tem trabalhado com processos de encapsulação de outras sustâncias e possui os equipamentos necessários para o desenvolvimento da pesquisa.

3. Existem muitas patentes sobre o assunto?
   A curcuma é um condimento muito usado na cozinha há muito tempo e é conhecida por ter muitas propriedades funcionais e participar na prevenção e tratamento de doenças, portanto comercialmente os extratos de curcuma são importantes e as empresas farmacêuticas têm desenvolvido processos de extração de curcuminoides e outros compostos dela para sua aplicação na elaboração de maquiagem, corantes e medicinas.

4. Quais itens devem ser inseridos na próxima pesquisa para uma busca mais detalhada?

   Procurar a variedade especifica de cúrcuma que será utilizada para a obtenção dos extratos e os materiais de parede mais utilizados. Incluir as palavras chave: *Curcuma longa* L. e *Coating materials*.
5. O fluxograma proposto para a redação da revisão de literatura é o seguinte:



- *Os resultados deste ciclo estão de acordo com as predições feitas na fase de planejamento?*
- *Sob quais condições as conclusões deste ciclo poderiam ser diferentes?*
- *Quais são as implicações das observações e problemas não planejados durante a coleta de dados?*
- *Os dados ajudam a responder as questões colocadas no plano?*

RESUMA O NOVO CONHECIMENTO OBTIDO NESTE CICLO

Faça uma lista das oportunidades para estudo e uma lista dos assuntos que devem ser evitados. Justifique!

Faça uma lista das oportunidades para estudo e uma lista dos assuntos que devem ser evitados. Justifique

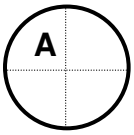
- As palavras chave usadas geraram uma grande quantidade de artigos, mas a usar duas palavras chave como, por exemplo, supercritical fluid extration* curcuma, o numero de artigos foi reduzido; além de que foi de muita ajuda restringir a procura na área de ciência e tecnologia de alimentos.

- Ao classificar os artigos no site ISI pelas citações que tinha cada artigo por ano, ficaram no primeiro lugar geralmente os artigos dos pesquisadores mais importantes ou aqueles artigos de revisão ou pesquisas novas, mas nem todos os casos o texto completo em pdf estava disponível.

- Uma pesquisa inicial sem utilizar o programa Endnote, seria improdutiva porque o programa evita os erros no momento da citação e permite organizar os artigos em pastas segundo as áreas de interesse, porem artigos lidos antes da elaboração do PDSA foram um obstáculo ao ter que procura-los de novos na base de dados e exporta-los no Endnote.

- Continuar coletando informação sobre a extração supercrítica de curcuminoides, sua encapsulação utilizando tecnologia supercrítica, a medição das propriedades dos extratos e partículas obtidas e suas possíveis aplicações na industria alimentar e farmacêutica;

- *Revise o conhecimento atual para refletir este aprendizado (atualize fluxogramas e diagramas de causa e efeito).*
- *Este novo conhecimento se aplicará em todos os lugares?*



AGIR

1. **Quais áreas devem ser estudadas?**
   No próximo ciclo devem ser estudados a avaliação de custos num processo de extração supercrítica e o uso de materiais de parede.
2. **Que outras áreas poderiam ser estudadas?**
   Seria muito interessante para o projeto estudar a modificação de amidos na área de encapsulação com o objetivo de utilizar o amido dos rizomas da curcuma como material de parede no processo SFEE, caso que o extrato obtido não seja solido.
3. **Quais áreas não devem ser estudadas pois o conhecimento sobre o assunto é satisfatório?**
   Não há a necessidade de construir o equipamento para os processos SAS e SFEE, uma vez que o equipamento já foi construído anteriormente pelo grupo de pesquisa do LASEFI

- *O sistema de causas é suficientemente compreendido?* ☐Sim ☐Não
- *As mudanças foram testadas em pequena escala?* ☐Sim ☐Não
- *As responsabilidades para implementar e avaliar as mudanças foram comunicadas?* ☐Sim ☐Não
- *Uma mudança ou ação apropriada foi desenvolvida ou selecionada?* ☐Sim ☐Não
- *Há forças na organização que ajudarão ou dificultarão as mudanças?* ☐Sim ☐Não
- *As mudanças ou ações melhorarão o desempenho no futuro?* ☐Sim ☐Não

OBJETIVOS DO PRÓXIMO CICLO

Seleção do Periódico.

APÊNDICE B

MATERIAL SUPLEMENTAR DO ARTIGO FAST ANALYSIS OF CURCUMINOIDS FROM TURMERIC (CÚRCUMA LONGA L.) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING A FUSED-CORE COLUMN

1 CURVAS PADRÃO UTILIZADAS NAS ANÁLISES CLAE

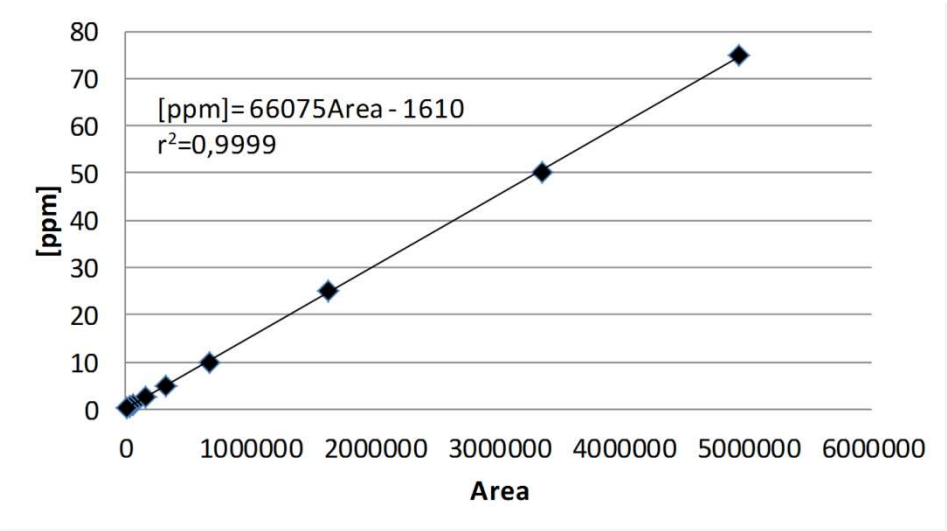


Figura A1. Curva padrão de curcumina para método de determinação do conteúdo de curcuminóides.

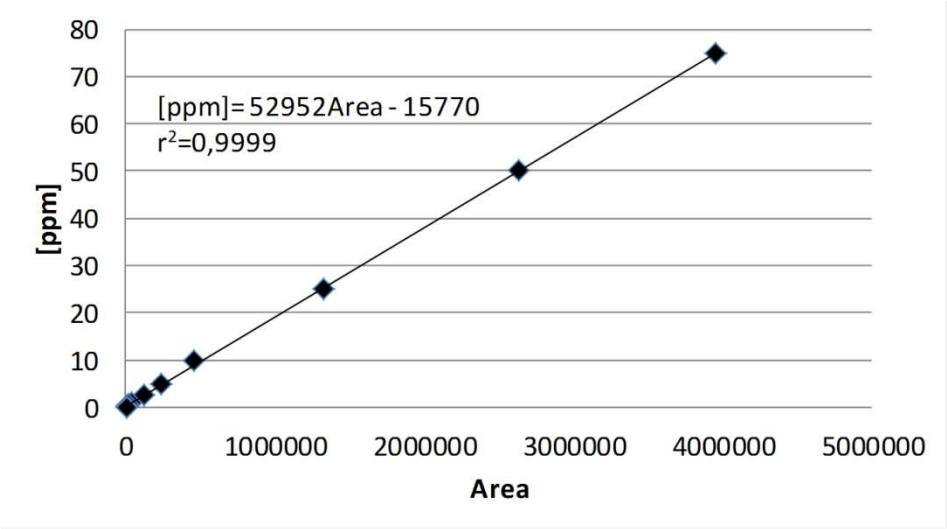


Figura A2. Curva padrão de demetoxicurcumina para método de determinação do conteúdo de curcuminóides.

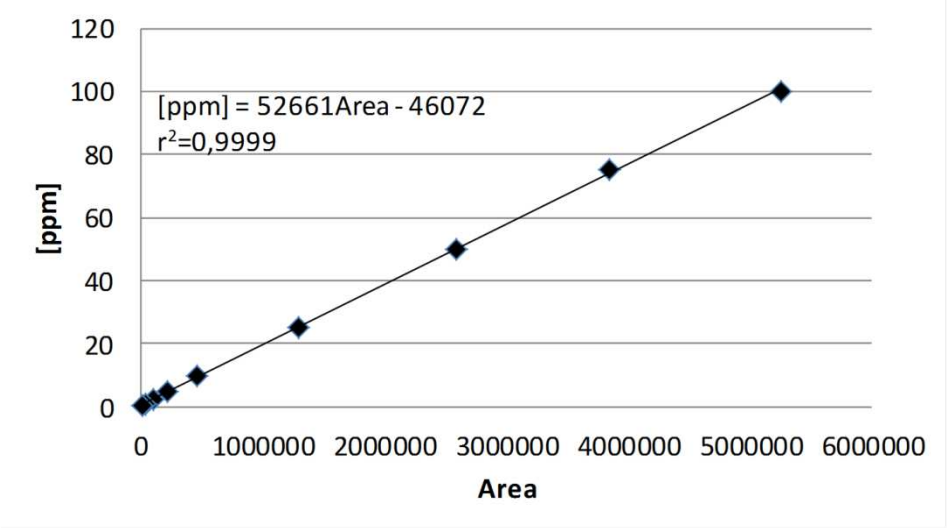


Figura A3. Curva padrão de bisdemetoxicurcumina para método de determinação do conteúdo de curcuminóides.

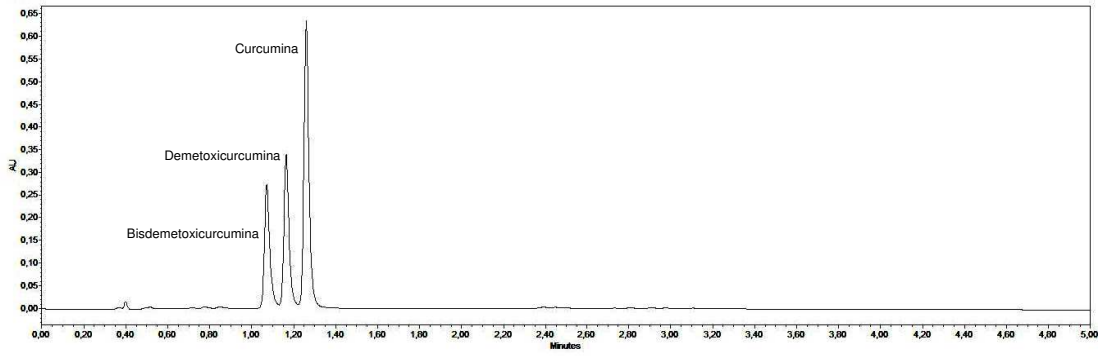


Figura A4. Cromatograma característico.

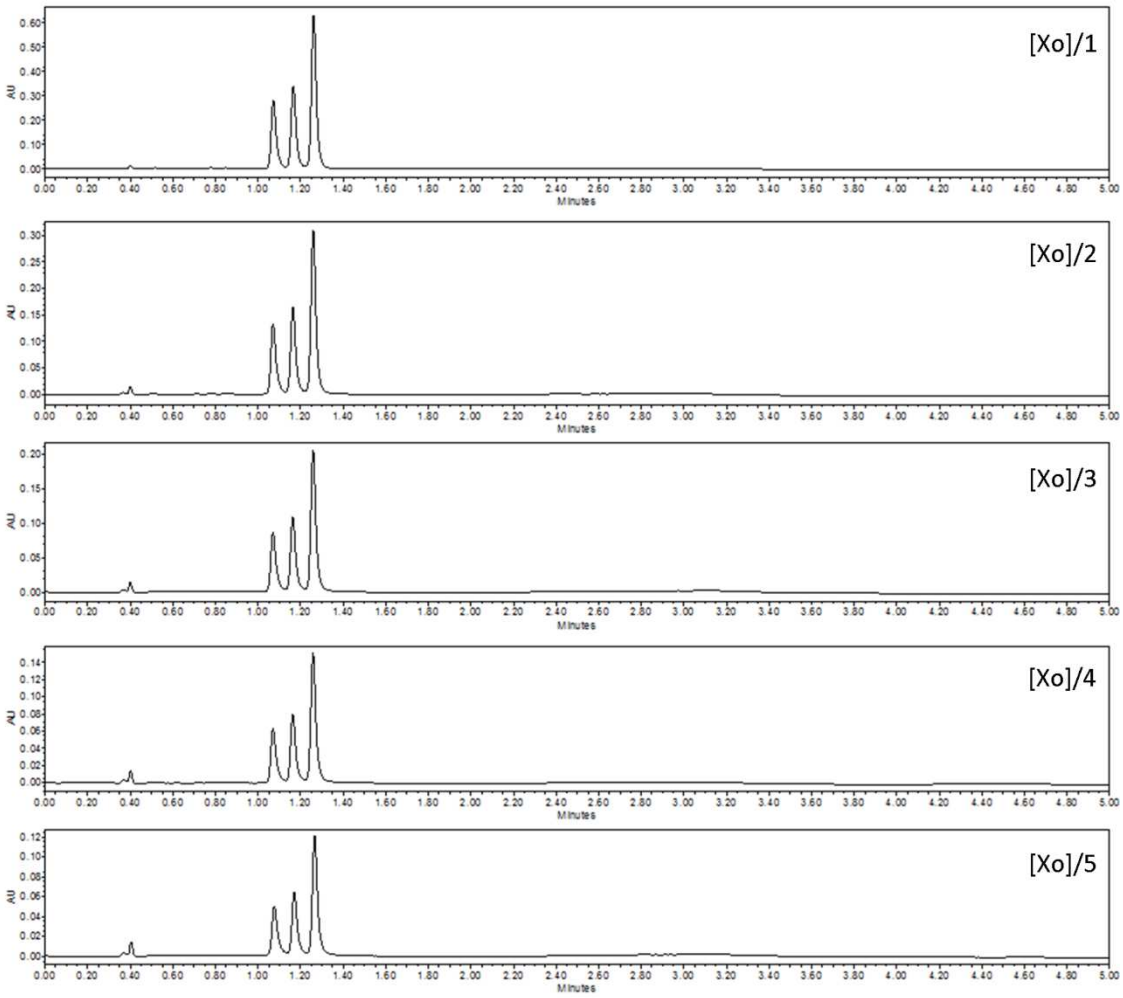


Figura A5. Cromatogramas da Tabela 3 do artigo fast analysis of curcuminoids from turmeric (cúrcuma longa l.) by high-performance liquid chromatography using a fused-core column.

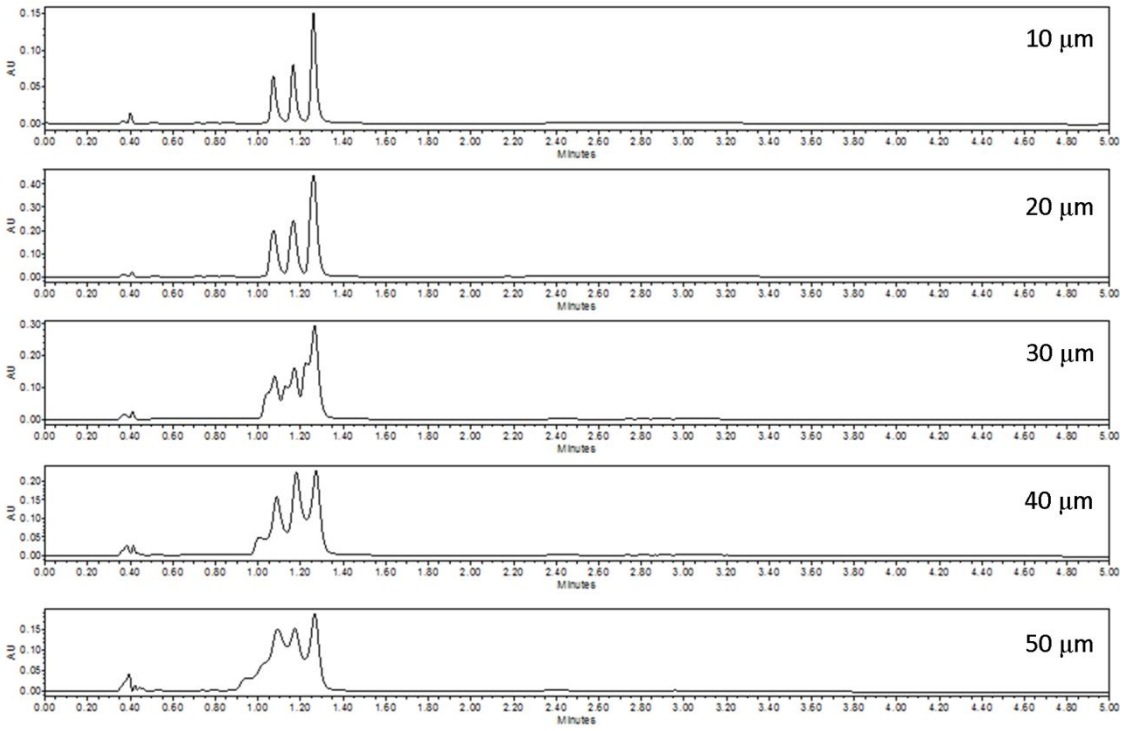


Figura A6. Cromatogramas da Tabela 4 do artigo fast analysis of curcuminoids from turmeric (cúrcuma longa l.) by high-performance liquid chromatography using a fused-core column.

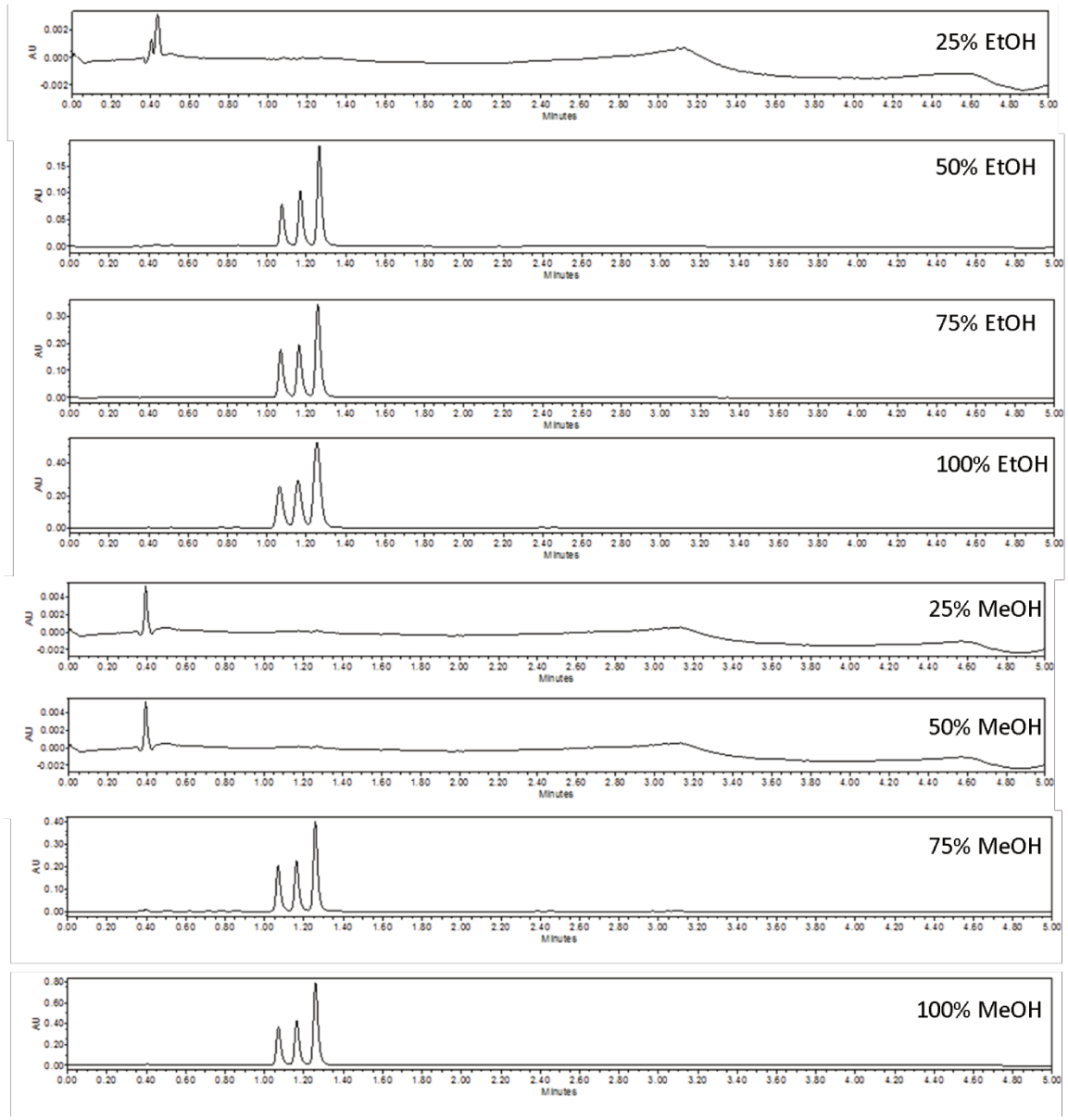


Figura A7. Cromatogramas da Tabela 5 do artigo fast analysis of curcuminoids from turmeric (cúrcuma longa l.) by high-performance liquid chromatography using a fused-core column.

Tabela A1. Dados suplementares que foram utilizados na validação do método de análise por CLAE.

Compound	Retention Time	Area	% Area	Height	Start Time	End Time	Baseline Start	Baseline End	K Prime	Selectivity
CC-Met-1	Cúrcuma 1ml 40 10 min 50A									
Bisdemethoxycurcumin	2,521	1792734	21,33	525707	2,348	2,673	2,348	3,413	5,30E+00	
Demethoxycurcumin	2,759	2402227	28,58	653734	2,673	2,937	2,348	3,413	5,90E+00	1,11E+00
Curcumin	3,028	4209225	50,08	1076274	2,937	3,413	2,348	3,413	6,57E+00	1,11E+00
CC-Met-2	Cúrcuma 2ml 50 6 min 50 A									
Bisdemethoxycurcumin	1,154	876284	21,28	446708	1,093	1,228	1,093	1,615	1,89E+00	
Demethoxycurcumin	1,275	1170357	28,42	550853	1,228	1,358	1,093	1,615	2,19E+00	1,16E+00
Curcumin	1,413	2071282	50,3	907631	1,358	1,615	1,093	1,615	2,53E+00	1,16E+00
CC-Met-3	Cúrcuma 23 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,211	744846	21,21	469289	1,158	1,27	1,158	1,582	2,03E+00	
Demethoxycurcumin	1,306	996398	28,37	626380	1,27	1,365	1,158	1,582	2,27E+00	1,12E+00
Curcumin	1,404	1771091	50,42	1118827	1,365	1,582	1,158	1,582	2,51E+00	1,11E+00
CC-Met-4	Cúrcuma 23 ml 55 3 min 55 A									
Bisdemethoxycurcumin	1,119	742975	21,21	561881	1,077	1,16	1,077	1,367	1,80E+00	
Demethoxycurcumin	1,187	993968	28,38	759500	1,16	1,225	1,077	1,367	1,97E+00	1,09E+00
Curcumin	1,254	1765912	50,41	1362663	1,225	1,367	1,077	1,367	2,13E+00	1,09E+00
CC-Met-5	Cúrcuma 23 ml 55 5 min 50A									
Bisdemethoxycurcumin	0,975	749593	21,25	422821	0,932	1,038	0,932	1,375	1,44E+00	
Demethoxycurcumin	1,079	1001093	28,38	520225	1,038	1,15	0,932	1,375	1,70E+00	1,18E+00
Curcumin	1,2	1776405	50,36	841141	1,15	1,375	0,932	1,375	2,00E+00	1,18E+00
CC-Met-6	Cúrcuma 23 ml 55 4 min 50 A									
Bisdemethoxycurcumin	1,125	734845	22,42	290878	1,04	1,245	1,04	1,625	1,81E+00	
Demethoxycurcumin	1,352	958334	29,24	244132	1,245	1,625	1,04	1,625	2,38E+00	1,31E+00
Curcumin	1,886	1584738	48,35	117302	1,638	2,313	1,638	2,313	3,71E+00	1,56E+00

Tabela A1. Dados suplementares que foram utilizados na validação do método de análise por CLAE.

Compound	Retention Time	Area	% Area	Height	Start Time	End Time	Baseline Start	Baseline End	K Prime	Selectivity
CC-Met-7	Cúrcuma 23 ml 55 5 min 55A v2									
Bisdemethoxycurcumin	1,155	742819	21,3	536449	1,118	1,2	1,118	1,392	1,89E+00	
Demethoxycurcumin	1,23	990153	28,39	722323	1,2	1,273	1,118	1,392	2,08E+00	1,10E+00
Curcumin	1,306	1754619	50,31	1296556	1,273	1,392	1,118	1,392	2,26E+00	1,09E+00
CC-Met-8	Cúrcuma 23 ml 55 5 min 50A v3									
Bisdemethoxycurcumin	0,954	750455	21,28	474179	0,912	1,007	0,912	1,265	1,39E+00	
Demethoxycurcumin	1,038	999633	28,34	613906	1,007	1,092	0,912	1,265	1,60E+00	1,15E+00
Curcumin	1,128	1776778	50,38	1070951	1,092	1,265	0,912	1,265	1,82E+00	1,14E+00
CC-Met-9	Cúrcuma 23 ml 55 5 min 55A v2									
Bisdemethoxycurcumin	1,102	742274	21,23	485372	1,063	1,152	1,063	1,388	1,76E+00	
Demethoxycurcumin	1,183	991671	28,36	658620	1,152	1,23	1,063	1,388	1,96E+00	1,11E+00
Curcumin	1,262	1762348	50,41	1194857	1,23	1,388	1,063	1,388	2,16E+00	1,10E+00
CC-Met-10	Cúrcuma 25 ml 55 5 min 45A									
Bisdemethoxycurcumin	0,706	680445	20,92	503500	0,675	0,738	0,675	0,92	7,64E-01	
Demethoxycurcumin	0,761	918860	28,25	647556	0,738	0,795	0,675	0,92	9,03E-01	1,18E+00
Curcumin	0,822	1652849	50,82	1121210	0,795	0,92	0,675	0,92	1,05E+00	1,17E+00
CC-Met-11	Cúrcuma 25 ml 55 5 min 50A v2									
Bisdemethoxycurcumin	0,873	689156	21,3	475721	0,84	0,917	0,84	1,1	1,18E+00	
Demethoxycurcumin	0,945	918382	28,38	623916	0,917	0,988	0,84	1,1	1,36E+00	1,15E+00
Curcumin	1,02	1628116	50,32	1103526	0,988	1,1	0,84	1,1	1,55E+00	1,14E+00
CC-Met-12	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,127	683800	21,31	453610	1,088	1,177	1,088	1,388	1,82E+00	
Demethoxycurcumin	1,216	911884	28,42	602801	1,177	1,267	1,088	1,388	2,04E+00	1,12E+00
Curcumin	1,307	1612463	50,26	1074600	1,267	1,388	1,088	1,388	2,27E+00	1,11E+00

Tabela A1. Dados suplementares que foram utilizados na validação do método de análise por CLAE.

Compound	Retention Time	Area	% Area	Height	Start Time	End Time	Baseline Start	Baseline End	K Prime	Selectivity
CC-Met-13	Cúrcuma 25 ml 55 4 min 50A									
Bisdemethoxycurcumin	0,852	689373	21,3	564583	0,82	0,89	0,82	1,038	1,13E+00	
Demethoxycurcumin	0,913	919438	28,4	756068	0,89	0,951	0,82	1,038	1,28E+00	1,14E+00
Curcumin	0,975	1628358	50,3	1361110	0,951	1,038	0,82	1,038	1,44E+00	1,12E+00
Cúrcuma etanol 25% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,08	183	33,67	112	1,057	1,113	1,057	1,113	1,70E+00	
Demethoxycurcumin	1,179	187	34,51	99	1,152	1,213	1,152	1,213	1,95E+00	1,14E+00
Curcumin	1,269	173	31,83	96	1,243	1,303	1,243	1,303	2,17E+00	1,12E+00
Cúrcuma etanol 50% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,076	128081	20,86	76830	1,032	1,137	1,032	1,432	1,69E+00	
Demethoxycurcumin	1,17	173278	28,23	103179	1,137	1,23	1,032	1,432	1,93E+00	1,14E+00
Curcumin	1,266	312505	50,91	187339	1,23	1,432	1,032	1,432	2,16E+00	1,12E+00
Cúrcuma etanol 50% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,073	128129	20,92	76442	1,028	1,132	1,028	1,402	1,68E+00	
Demethoxycurcumin	1,167	173665	28,35	102680	1,132	1,227	1,028	1,402	1,92E+00	1,14E+00
Curcumin	1,262	310787	50,73	186216	1,227	1,402	1,028	1,402	2,16E+00	1,12E+00
Cúrcuma etanol 75% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,07	318386	24,33	174432	1,01	1,128	1,01	1,415	1,67E+00	
Demethoxycurcumin	1,163	358511	27,4	192437	1,128	1,222	1,01	1,415	1,91E+00	1,14E+00
Curcumin	1,258	631645	48,27	339398	1,222	1,415	1,01	1,415	2,15E+00	1,12E+00
Cúrcuma etanol 75% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,065	318147	24,44	174408	1,018	1,125	1,018	1,385	1,66E+00	
Demethoxycurcumin	1,158	356376	27,38	192067	1,125	1,217	1,018	1,385	1,90E+00	1,14E+00
Curcumin	1,254	626971	48,17	338486	1,217	1,385	1,018	1,385	2,13E+00	1,13E+00

Tabela A1. Dados suplementares que foram utilizados na validação do método de análise por CLAE.

Compound	Retention Time	Area	% Area	Height	Start Time	End Time	Baseline Start	Baseline End	K Prime	Selectivity
Cúrcuma etanol 100% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,065	593759	22,58	252444	1,003	1,12	1,003	1,413	1,66E+00	
Demethoxycurcumin	1,159	711453	27,05	290708	1,12	1,212	1,003	1,413	1,90E+00	1,14E+00
Curcumin	1,255	1324495	50,37	524985	1,212	1,413	1,003	1,413	2,14E+00	1,13E+00
Cúrcuma etanol 100% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,063	592358	22,58	251634	0,997	1,117	0,997	1,403	1,66E+00	
Demethoxycurcumin	1,156	711984	27,14	288603	1,117	1,21	0,997	1,403	1,89E+00	1,14E+00
Curcumin	1,252	1318568	50,27	520926	1,21	1,403	0,997	1,403	2,13E+00	1,13E+00
Cúrcuma metanol 25% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,078	189	23,34	120	1,052	1,11	1,052	1,11	1,70E+00	
Demethoxycurcumin	1,176	260	32,03	144	1,142	1,217	1,142	1,217	1,94E+00	1,14E+00
Curcumin	1,27	362	44,63	194	1,247	1,313	1,247	1,313	2,18E+00	1,12E+00
Cúrcuma metanol 25% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,083	257	28,46	146	1,06	1,122	1,06	1,122	1,71E+00	
Demethoxycurcumin	1,176	210	23,22	131	1,153	1,215	1,153	1,215	1,94E+00	1,14E+00
Curcumin	1,274	437	48,33	214	1,242	1,323	1,242	1,323	2,19E+00	1,13E+00
Cúrcuma metanol 50% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,075	144	15,77	71	1,058	1,117	1,058	1,117	1,69E+00	
Demethoxycurcumin	1,175	226	24,81	157	1,157	1,213	1,157	1,213	1,94E+00	1,15E+00
Curcumin	1,272	541	59,41	308	1,248	1,322	1,248	1,322	2,18E+00	1,12E+00
Cúrcuma metanol 50% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,081	166	15,34	85	1,055	1,122	1,055	1,122	1,70E+00	
Demethoxycurcumin	1,17	271	25,01	163	1,147	1,212	1,147	1,212	1,93E+00	1,13E+00
Curcumin	1,267	646	59,65	333	1,245	1,333	1,245	1,333	2,17E+00	1,13E+00

Tabela A1. Dados suplementares que foram utilizados na validação do método de análise por CLAE.

Compound	Retention Time	Area	% Area	Height	Start Time	End Time	Baseline Start	Baseline End	K Prime	Selectivity
Cúrcuma metanol 75% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,069	333810	24,71	206172	1,01	1,128	1,01	1,417	1,67E+00	
Demethoxycurcumin	1,162	368993	27,32	225684	1,128	1,218	1,01	1,417	1,91E+00	1,14E+00
Curcumin	1,258	647994	47,97	399693	1,218	1,417	1,01	1,417	2,15E+00	1,13E+00
Cúrcuma metanol 75% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,077	333403	24,78	204682	1,023	1,137	1,023	1,393	1,69E+00	
Demethoxycurcumin	1,172	368736	27,41	224635	1,137	1,23	1,023	1,393	1,93E+00	1,14E+00
Curcumin	1,268	643071	47,8	398115	1,23	1,393	1,023	1,393	2,17E+00	1,12E+00
Cúrcuma metanol 100% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,07	626994	23,25	366111	1,018	1,128	1,018	1,37	1,68E+00	
Demethoxycurcumin	1,164	733048	27,19	426767	1,128	1,222	1,018	1,37	1,91E+00	1,14E+00
Curcumin	1,259	1336447	49,56	791952	1,222	1,37	1,018	1,37	2,15E+00	1,12E+00
Compound	Retention Time	Area	% Area	Height	Start Time	End Time	Baseline Start	Baseline End	K Prime	Selectivity
Cúrcuma metanol 100% 40X	Cúrcuma 25 ml 55 5 min 55A									
Bisdemethoxycurcumin	1,068	628658	23,24	366669	1,017	1,127	1,017	1,378	1,67E+00	
Demethoxycurcumin	1,162	734107	27,14	427076	1,127	1,218	1,017	1,378	1,90E+00	1,14E+00
Curcumin	1,258	1342516	49,63	790515	1,218	1,378	1,017	1,378	2,14E+00	1,13E+00

APÊNDICE C

MATERIAL SUPLEMENTAR DO ARTIGO EXTRACTION OF CURCUMINOIDS FROM DEFLAVORED TURMERIC (CURCUMA LONGA L.) USING PRESSURIZED LIQUIDS: PROCESS INTEGRATION AND ECONOMIC EVALUATION

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PROCEDIMENTO OPERACIONAL PADRÃO (POP) – UNIDADE COSSOLVENTE

O processo de extração de curcuminóides via PLE foi realizado na unidade de extração com cossolvente (Figura A8), equipamento concebido e montado no LASEFI representado esquematicamente na Figura A9. A unidade de extração consiste em duas bombas de alta pressão para solvente (Thermo Separation Products, modelo 2000, Flórida, EUA), uma para solventes líquidos e outra refrigerada para CO_2, dois banhos termostáticos programáveis (PolyScience, modelo 9510, Niles, EUA e Marconi, modelo MA-184, Piracicaba, SP) responsáveis pela manutenção da temperatura do extrator de aço inox e dos cabeçotes da bomba de CO_2, respectivamente, um totalizador de vazão (LAO, modelo G0,6 ± 0,001 m3, São Paulo, SP), termopares, válvulas e manômetros (Record, 50 MPa ± 0,5, São Paulo, SP).

Figura A8. Unidade de extração Cossolvente.

1.1 Procedimento operacional

No caso do processo PLE a unidade foi operada somente com a parte destacada na Figura A2, correspondente à parte responsável pela injeção do cossolvente em processos SFE, uma vez que para processos PLE, foram utilizados somente solventes no estado líquido.

Os rizomas desaromatizados foram submetidos à extração por PLE usando etanol como solvente. O procedimento padrão foi o seguinte:

1) Conectar toda a linha;

2) Ajustar a vazão de solvente na bomba de alta pressão (BC) ;

3) Abrir a válvula V-5 para liberar a entrada de solvente no extrator;

4) Ligar a bomba e programá-la para operar à pressão requerida do processo. O sistema é pressurizado a partir da entrada de solvente no extrator;

5) Assim que o sistema atingir a pressão de operação (M3) desligar a bomba e fechar a válvula de bloqueio V-5;

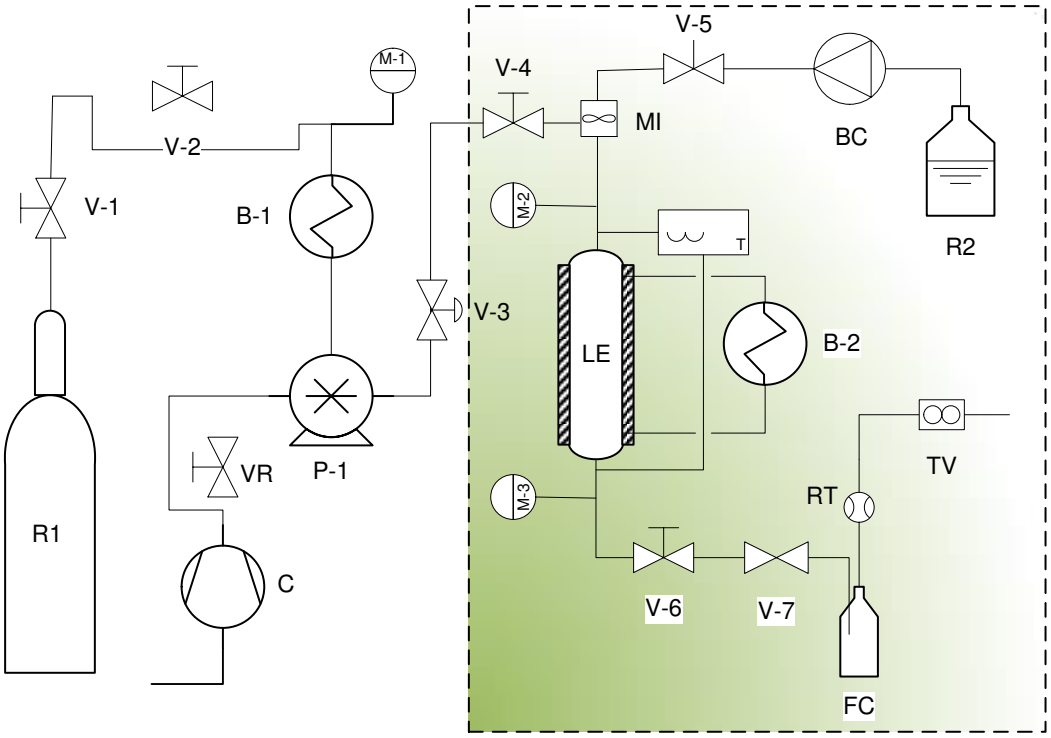
6) Cronometrar o tempo estipulado para o período estático;

7) Após o período estático, abrir a válvula V-5, ligar a bomba de alta pressão, abrir cuidadosamente a válvula V-6 e controlar o fluxo de solvente com válvula micrométrica V-7 monitorando a pressão do sistema (M3);

8) Ao final do processo (após o tempo pré-estabelecido de extração), retirar o frasco coletor, desligar a bomba de alta pressão e fechar a válvula V-5;

9) Abrir as válvulas V-6 e V-7 para despressurizar o sistema, desligar o banho de aquecimento do extrator e o aquecimento da válvula micrométrica.

10) Coletar, pesar e guardar os extratos em freezer doméstico à temperatura igual ou menor que zero para posterior análise.



R1 – Reservatório de solvente (CO_2)
R2 – Reservatório de cossolvente
P-1 – Booster
C – Compressor
BC – Bomba de alta pressão
B-1 – Banho de resfriamento (CO_2)
B-2 – Banho de aquecimento do extrator
LE – Leito de extração
T - Termopares
MI – Misturador
FC – Frasco coletor
M - Manômetros
RT - Rotâmetro
TV – Totalímetro
V-1 – Válvula de bloqueio (cilindro de CO_2)
V-2 - Válvula de retenção
V-3 – Válvula back pressure
V-4 - Válvula de bloqueio (entrada de CO_2)
V-5 - Válvula de bloqueio (entrada de cossolvente)
V-6 - Válvula de bloqueio (saída de extrato)
V-7 – Válvula micrométrica aquecida

Figura A9. Diagrama esquemático da unidade Cossolvente, adaptado de Veggi (2013).

1.2 Preparo do leito de extração

O preparo do leito seguiu um procedimento padrão para todos os ensaios de extração com líquidos pressurizados para a determinação do rendimento global. Na extremidade inferior da célula de nylon, que possui mesmo diâmetro do extrator, a qual estava fechada, foi depositada uma camada de lã de vidro e em seguida aproximadamente 47 g de matéria-prima foram cuidadosamente empacotadas com o auxílio de um bastão de aço inoxidável, também com mesmo diâmetro do extrator. Outra camada de lã de vidro foi depositada em cima da matéria-prima empacotada e por fim o volume da célula de nylon foi completado com esferas de vidro. Essa célula foi então fechada e então inserida na parte

inferior do extrator. Também foi inserida na parte superior do extrator, porém na parte superior, uma coluna de teflon que representa aproximadamente 70% do volume do mesmo. A densidade aparente do leito foi mantida constante e a lã de vidro foi utilizada como um filtro para evitar que partículas pequenas obstruíssem a tubulação. Um esquema do leito de extração pode ser visualizado na Figura A10.

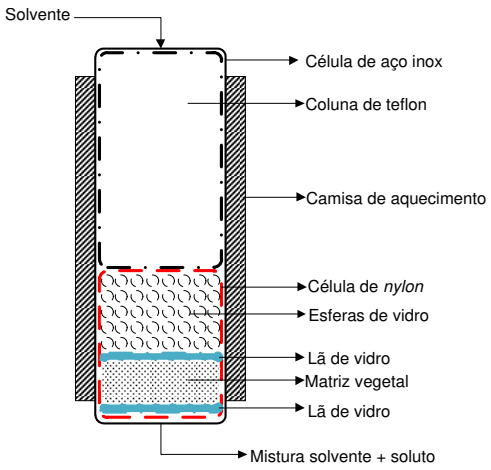


Figura A10. Esquema do leito de extração[1].

[1] Comunicação pessoal de Isabel C. N. Debien

1.3 Resultados Experimentais: PLE

A seguir estão apresentados os resultados experimentais utilizando cúrcuma desaromatizada e que resultaram na publicação do artigo que está apresentado no **Capítulo 4**.

Tabela A2. Dados dos ensaios do planejamento fatorial completamente aleatorizado correspondentes ao rendimento global (X0) e rendimento de curcuminóides (%CC).

Ensaio	T (°C)	P (bar)	Massa amostra (g)	Massa extrato (g)	Massa curcuminóides (g)	X_0 (%, b.s.)	%CC (%, b.s.)
1	70	350	47,0714	4,2304	1,0320	10,33	2,52
2	80	350	47,0512	4,7279	1,2160	11,55	2,97
3	70	200	47,0402	4,5918	1,6616	11,22	4,06
4	70	250	47,0515	4,6625	1,3140	11,39	3,21
5	60	200	47,0787	3,5347	1,2324	8,63	3,01
6	60	350	47,0006	3,0177	0,9115	7,38	2,23
7	60	300	47,0792	2,7238	0,8642	6,65	2,11
8	70	300	47,0477	5,3538	1,0642	13,08	2,60
9	60	100	47,0459	4,9157	1,8387	12,01	4,49
10	60	250	47,0350	3,8302	1,0152	9,36	2,48
11	60	300	47,0698	2,5512	0,9501	6,23	2,32
12	70	350	47,0380	4,9722	1,1868	12,15	2,90
13	60	200	47,0668	5,2946	1,3043	12,93	3,19
14	60	100	47,0492	4,3184	1,7167	10,55	4,19
15	60	250	47,0473	2,7874	1,1911	6,81	2,91
16	80	100	47,0570	6,7387	1,5475	16,46	3,78
17	70	100	47,0162	5,8902	1,8770	14,4	4,59
18	80	300	47,0759	6,0410	1,6423	14,75	4,01
19	70	150	47,0494	4,7400	1,7669	11,58	4,32
20	70	150	47,0651	5,3476	1,7434	13,06	4,26
21	80	150	47,0560	6,2759	0,8270	15,33	2,02
22	80	150	47,0432	3,3765	0,9700	8,25	2,37
23	60	150	47,0353	3,6706	1,6205	8,97	3,96
24	70	300	47,0350	5,3933	1,2440	13,18	3,04
25	80	300	47,0240	5,2366	1,6760	12,8	4,10
26	70	250	47,0781	4,7880	1,4540	11,69	3,55
27	80	250	47,0186	4,0661	1,3223	9,94	3,23
28	80	200	47,0368	6,8217	1,5346	16,67	3,75
29	60	350	47,0433	2,5048	0,8976	6,12	2,19
30	70	100	47,0620	4,5652	1,7035	11,15	4,16
31	60	150	46,9994	3,9458	1,4189	9,65	3,47
32	80	250	47,0492	7,2819	1,4011	17,79	3,42
33	80	200	47,0573	4,5853	1,4493	11,2	3,54
34	80	100	47,0657	5,3313	1,3513	13,02	3,30
35	70	200	47,0825	6,4392	1,4787	15,72	3,61
36	80	350	47,0612	5,6993	1,4084	13,92	3,44

Tabela A3. Dados de massa e rendimento do extrato de curcuminóides obtidos utilizando as técnicas de extração Soxhlet e leito agitado.

	Soxhlet			Leito Agitado		
Experimento	1	2	3	1	2	3
Massa de amostra (g)	17,0852	17,0616	17,0064	4,7027	4,7077	4,7001
Frasco vazio (g)	87,2272	89,6105	88,8416	93,1364	92,8686	91,9826
Frasco + extrato (g)	87,7568	89,8601	89,352	93,3916	93,3661	92,2241
Extrato (g)	0,5296	0,2496	0,5104	0,2552	0,4975	0,2415
Rendimento b.s. (%)	12,83	12,11	12,42	12,48	12,15	11,81
Tempo (h)		6			3	
Média		12,5			12,1	
Desvio		0,4			0,3	

Tabela A4. Dados de massa e teor de curcuminóides obtidos utilizando as técnicas de extração Soxhlet e leito agitado.

	Soxhlet			Leito Agitado		
Experimento	1	2	3	1	2	3
Massa de amostra (g)	17,0852	17,0616	17,0064	4,7027	4,7077	4,7001
Curcuminóides (g)	0,64	0,62	0,63	0,15	0,15	0,15
Rendimento b.s. (%)	4,32	4,2	4,24	3,63	3,68	3,71
Média		4,2			3,7	
Desvio		0,06			0,04	

Tabela A5. Dados de saída do analises do planejamento fatorial do rendimento global (X_0) do software Minitab 16.

General Linear Model: Xo (%) versus Temperatura. Pressão

```
Factor       Type   Levels  Values
Temperatura  fixed       3  60. 70. 80
Pressão      fixed       6  100. 150. 200. 250. 300. 350

Analysis of Variance for Xo (%), using Adjusted SS for Tests
Source               DF   Seq SS   Adj SS  Adj MS      F      P
%SFE                  1   23,078   43,552  43,552  10,46  0,005
Temperatura           2  145,417  145,459  72,730  17,47  0,000
Pressão               5   39,997   44,389   8,878   2,13  0,111
Temperatura*Pressão  10   40,761   40,761   4,076   0,98  0,495
Error                17   70,792   70,792   4,164
Total                35  320,044

S = 2,04064   R-Sq = 77,88%   R-Sq(adj) = 54,46%
Term         Coef  SE Coef      T      P
Constant   15,398    1,236  12,45  0,000
%SFE      -0,7558   0,2337  -3,23  0,005
```

Tabela A6. Dados de saída do analises do planejamento fatorial do teor de curcuminóides (%CC) do software Minitab 16.

General Linear Model: %CC versus Temperatura. Pressão

```
Factor       Type   Levels  Values
Temperatura  fixed       3  60. 70. 80
Pressão      fixed       6  100. 150. 200. 250. 300. 350


Analysis of Variance for %CC, using Adjusted SS for Tests

Source               DF   Seq SS  Adj SS  Adj MS      F      P
%SFE                  1   2,8586  0,0192  0,0192   0,32  0,581
Temperatura           2   1,4107  1,6043  0,8021  13,23  0,000
Pressão               5   5,8612  6,4763  1,2953  21,36  0,000
Temperatura*Pressão  10   7,9005  7,9005  0,7901  13,03  0,000
Error                17   1,0307  1,0307  0,0606
Total                35  19,0617


S = 0,246225   R-Sq = 94,59%   R-Sq(adj) = 88,87%


Term         Coef  SE Coef      T      P
Constant   3,2331   0,1492  21,67  0,000
%SFE      0,01589  0,02820   0,56  0,581

%SFE: Quantidade de óleo tirado na etapa de desaromatização da matéria prima.
```

Tabela A7. Dados de saída do analises do teste de Tukey para o rendimento do processo do software Minitab 16.

General Linear Model: Rend.Processo versus Temperatura. Pressão

```
Factor       Type   Levels  Values
Temperatura  fixed       3  60. 70. 80
Pressão      fixed       6  100. 150. 200. 250. 300. 350


Analysis of Variance for Rend.Processo, using Adjusted SS for Tests

Source               DF   Seq SS   Adj SS  Adj MS      F      P
%SFE                  1  1001,82    34,56   34,56   1,14  0,301
Temperatura           2   753,46   875,59  437,79  14,41  0,000
Pressão               5  3154,36  3554,51  710,90  23,39  0,000
Temperatura*Pressão  10  4528,74  4528,74  452,87  14,90  0,000
Error                17   516,62   516,62   30,39
Total                35  9955,00


S = 5,51268   R-Sq = 94,81%   R-Sq(adj) = 89,32%


Term         Coef  SE Coef      T      P
Constant   79,177    3,340  23,70  0,000
%SFE      -0,6733   0,6313  -1,07  0,301
```

Tabela A7. Dados de saída do analises do teste de Tukey para o rendimento do processo do software Minitab 16.

```
Grouping Information Using Tukey Method and 95,0% Confidence

Temperatura  Pressão  N    Mean  Grouping
70           100      2  100,28  A
60           100      2  100,15  A
70           150      2   99,06  A
80           300      2   93,21  A B
70           200      2   87,85  A B C
60           150      2   85,39  A B C D
80           200      2   84,22  A B C D E
80           100      2   81,13  A B C D E F
70           250      2   77,95  A B C D E F
80           250      2   75,13    B C D E F
80           350      2   73,07    B C D E F
60           200      2   69,66      C D E F G
70           300      2   62,67        D E F G
70           350      2   62,55          E F G
60           250      2   61,49            F G
60           350      2   50,52              G
60           300      2   50,45              G
80           150      2   48,76              G

Means that do not share a letter are significantly different.
```

Tabela A8. Dados do ensaio cinético 1 obtido via extração com fluido supercrítico com etanol como solvente a 333 K e 10 MPa.

Tempo (min)	Massa de extrato acumulada (g)	Rendimento de extração (%, b.s.)	Massa de solvente acumulada (g)	S/F
0	0	0	0	0
5	0,7702	1,16	36,5	0,5
10	1,2832	1,93	73	1,0
15	1,7356	2,61	109,5	1,4
20	2,0685	3,11	146	1,9
25	2,2966	3,46	182,5	2,4
30	2,4548	3,69	219	2,9
40	2,6281	3,95	292	3,8
50	2,6907	4,05	365	4,8
60	2,7138	4,08	438	5,7
80	2,7409	4,12	584	7,6
100	2,7588	4,15	730	9,5
120	2,7707	4,17	876	11,5
150	2,7822	4,19	1095	14,3
180	2,7905	4,20	1314	17,2

Tabela A9. Dados do ensaio cinético 2 obtido via extração com fluido supercrítico com etanol como solvente a 333 K e 10 MPa.

Tempo (min)	Massa de extrato acumulada (g)	Rendimento de extração (%, b.s.)	Massa de solvente acumulada (g)	S/F
0	0	0	0	0
5	1,1650	1,8	36,5	0,5
10	1,9262	2,9	73	1,0
15	2,3183	3,5	109,5	1,4
20	2,5338	3,8	146	1,9
25	2,6614	4,0	182,5	2,4
30	2,7255	4,1	219	2,9
40	2,7615	4,2	292	3,8
50	2,7823	4,2	365	4,8
60	2,7976	4,2	438	5,7
80	2,8192	4,2	584	7,6
100	2,8322	4,3	730	9,5
120	2,8421	4,3	876	11,5
150	2,8522	4,3	1095	14,3
180	2,8585	4,3	1314	17,2

Tabela A10. Rotina de programação usada no SAS 9,2 (SAS Institute, Inc.) para o ajuste dos dados experimentais da OEC (333 K/10 MPa) a um spline de 3 linhas retas.

```
/* ---------------------------------------------------------------------
/* Ajuste das curvas experimentais no SAS
/* Juan Felipe Osorio Tobón  - LASEFI                          */
   Options NoDate NoNumber PS=100 LS=100 FormDLim='-';
   Title'Ensaio Cinético PLE Cúrcuma: 100bar/60oC EtOH';
   FootNote;
DATA DADOSNLIN;
INPUT TEMPO RendPLE;
CARDS;
0     0
5     0.97
10    1.60
15    2.03
20    2.30
25    2.48
30    2.59
40    2.69
50    2.74
60    2.76
80    2.78
100   2.80
120   2.81
150   2.82
180   2.82
;
PROC PRINT DATA = DADOSNLIN;
RUN;
```

Tabela A10. Rotina de programação usada no SAS 9,2 (SAS Institute, Inc.) para o ajuste dos dados experimentais da OEC (333 K/10 MPa) a um spline de 3 linhas retas.

```
Comment Reta E2_100 ------------------------------------------------------
-----------------------------------------;

PROC NLIN DATA = DADOSNLIN;
TITLE 'RendPLE NLIN';
PARMS                    b0 = 0.2477
                                 b1 = 0.1132              /*----termo de
primeira ordem do período tcer---*/
                         b2 = 0.0099              /*----termo de primeira
ordem do período tfer---*/
                         b3 = 0.0005              /*----termo de primeria
ordem do período difusional---*/
                         C1 = 20                  /*----tcer---*/
                         C2 = 50;                /*----tfer---*/
                                  INT = MIN(Tempo,C1);
                                  AL1 = MAX(Tempo-C1,0);
                                  AL2 = MAX(Tempo-C2,0);
                         AL3 = MAX(Tempo-C2,0);

MODEL RendPLE = b0 + b1*INT + b2*(AL1-AL2) + b3*AL3;
        Output out = a p=RendPLE_hat r= Mres;
        Axis order = (0 to 100 by 10);
        run;
```

Tabela A11. Dados de saída do procedimento PROC NLIN do software SAS 9,2 (SAS Institute, Inc.).

Ensaio Cinético PLE Cúrcuma: 100bar/60oC EtOH

Obs	TEMPO	Rend PLE
1	0	0.00
2	5	0.97
3	10	1.60
4	15	2.03
5	20	2.30
6	25	2.48
7	30	2.59
8	40	2.69
9	50	2.74
10	60	2.76
11	80	2.78
12	100	2.80
13	120	2.81
14	150	2.82
15	180	2.82

Tabela A11. Dados de saída do procedimento PROC NLIN do software SAS 9,2 (SAS Institute, Inc.).

RendPLE NLIN

The NLIN Procedure

Dependent Variable RendPLE

Method: Gauss-Newton

Iterative Phase

Iter	b0	b1	b2	b3	C1	C2	Sum of Sq
0	0.2477	0.1132	0.00990	0.000500	20.0000	50.0000	0.2098
1	0.2526	0.1123	0.0115	0.000604	19.6213	44.9471	0.1757
2	0.1973	0.1233	0.0151	0.000604	17.4674	42.0340	0.1101
3	0.1482	0.1332	0.0182	0.000604	15.9507	41.3616	0.0848
4	0.1421	0.1344	0.0186	0.000604	15.8721	41.4273	0.0825
5	0.1420	0.1344	0.0186	0.000604	15.8712	41.4271	0.0825
6	0.1420	0.1344	0.0186	0.000604	15.8712	41.4271	0.0825

NOTE: Convergence criterion met.

Estimation Summary

Method	Gauss-Newton
Iterations	6
Subiterations	1
Average Subiterations	0.166667
R	1.043E-8
PPC	2.31E-9
RPC(b0)	3.076E-6
Object	1.93E-10
Objective	0.082514
Observations Read	15
Observations Used	15
Observations Missing	0

Source	DF	Sum of Squares	Mean Square	F Value	Approx Pr > F
Model	5	9.4880	1.8976	206.98	<.0001
Error	9	0.0825	0.00917		
Corrected Total	14	9.5705			

Parameter	Estimate	Approx Std Error	Approximate 95% Confidence Limits	
b0	0.1420	0.0801	-0.0392	0.3232
b1	0.1344	0.00856	0.1150	0.1538
b2	0.0186	0.00647	0.00398	0.0333
b3	0.000604	0.000822	-0.00126	0.00246
C1	15.8712	1.1154	13.3479	18.3945
C2	41.4271	6.3572	27.0461	55.8081

Approximate Correlation Matrix

	b0	b1	b2	b3	C1	C2
b0	1.0000000	-0.8017837	-0.0000000	-0.0000000	0.2235729	0.0000000
b1	-0.8017837	1.0000000	0.0000000	0.0000000	-0.5551791	-0.0000000
b2	-0.0000000	0.0000000	1.0000000	0.0000000	-0.6456601	-0.7162544
b3	-0.0000000	0.0000000	0.0000000	1.0000000	-0.0000000	-0.4611385
C1	0.2235729	-0.5551791	-0.6456601	-0.0000000	1.0000000	0.3075532
C2	0.0000000	-0.0000000	-0.7162544	-0.4611385	0.3075532	1.0000000

APÊNDICE D

MATERIAL SUPLEMENTAR DO ARTIGO PRECIPITATION OF CURCUMINOIDS FROM AN ETHANOLIC TURMERIC EXTRACT USING A SUPERCRITICAL ANTISOLVENT PROCESS

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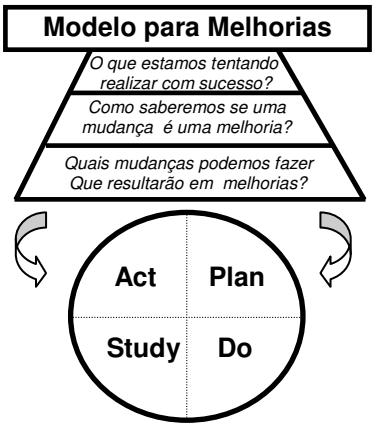

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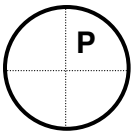


PLANEJAR

OBJETIVO DO CICLO

Realizar uma pesquisa bibliografia sobre o as principais variáveis que interferem no tamanho das partículas obtidas via SAS [Supercritical Antisolvent Process].

- *Que conhecimento adicional é necessário para levar à ação?* Uso do sistema. Conhecimento das bases de dados disponíveis.

QUESTÕES A SEREM RESPONDIDAS A PARTIR DOS DADOS OBTIDOS NESTE CICLO

1. Existem informações experimentais ou teóricas publicadas sobre o assunto?
2. As publicações estão em fontes referenciáveis?
3. Os dados disponíveis são provenientes de laboratórios cuja credibilidade já está estabelecida?
4. Quem são os autores mais importantes da área? Em que países estes autores realizaram o trabalho?
5. Quais variáveis interferem no tamanho de partícula? De que maneira?
6. Existem diferenças entre as substancias empregadas nos artigos publicados e os curcuminóides a serem precipitados?
7. Quais condições poderiam ser testadas para diminuir o tamanho das partículas e aumentar o rendimento de precipitação?
8. Existem artigos relacionados com a produção de partículas a partir de extratos naturais?

PREDIÇÕES

1. Serão encontrados muitos artigos relacionados com o uso da técnica SAS na formação de partículas e o emprego de materiais de parede para obter compostos encapsulados.
2. Serão encontrados muitos artigos relacionados com a aplicação da técnica em possíveis usos na indústria farmacêutica e de alimentos.
3. A busca sobre o uso de fluidos supercríticos em processo de formação de partículas indicará periódicos relacionados com fluidos supercríticos, engenheira de alimentos e indústrias farmacêuticas.
4. É provável que as informações obtidas indiquem que os produtos estejam voltados à aplicação na indústria farmacêutica.
5. Serão encontrados muitos arquivos relacionados com o encapsulamento de curcuminóides, no entanto nenhum ou muito poucos sobre a produção de partículas de curcuminóides usando fluidos supercríticos.
6. As publicações encontradas estarão escritas em inglês.

- *Há dados históricos disponíveis para responder às questões acima?*

- *A equipe concorda com estas predições?*

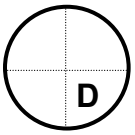
DESENVOLVA UM PLANO PARA RESPONDER ÀS QUESTÕES (Quem, O que, Onde, Quando, Como)

1. Primeiro buscar nas bases de dados Web of Science e ScienceDirect;
2. Utilizar a palavra chave Supercritical Antisolvent Process *(SAS)* a fim de se obter artigos relacionados a todas as aplicações dessa tecnologia;
3. Utilizar a palavra-chave Supercritical Antisolvent Process*process parameters a fim de obter artigos relacionados com o efeito dos principais parâmetros que interferem no processo de formação de partículas;
4. Utilizar a palavra-chave Supercritical Antisolvent Process*antioxidant compounds recovery a fim de obter artigos relacionados com a formação de partículas de compostos bioativos;
5. Utilizar a palavra-chave Supercritical Antisolvent Process*curcuminoids a fim de obter artigos relacionados com a formação de partículas de curcuminóides.
6. Limitar a busca para artigos publicados a partir de 2010. Caso o número de itens obtido seja reduzido, aumentar o período da busca. Selecionar os itens mais interessantes através da leitura dos resumos. Prestar atenção nos nomes de autores que aparecem com maior frequência e países em que se localizam;
7. Fazer a leitura completa dos artigos selecionados;
8. Redigir brevemente a lista de parâmetros e descrever de que maneira influenciam o tamanho de partículas e rendimento;
9. Este processo de pesquisa possibilitará definir os parâmetros a serem avaliados no processo de obtenção das partículas dos curcuminóides via SAS.

- *O seu plano considerou os seguintes métodos:*

- Formulários Coleta de Dados: ☐Sim ☐ Não
- Experimentação Planejada: ☐ Sim ☐ Não
- Diagramas de Dispersão: ☐Sim ☐ Não
- Diagramas de Pareto: ☐Sim ☐ Não
- Métodos de Pesquisa: ☐Sim ☐ Não
- Gráficos de Tendências: ☐Sim ☐ Não
- Gráficos de Controle: ☐ Sim ☐ Não
- Simulação/Modelagem: ☐Sim ☐ Não
- Análise de Engenharia: ☐Sim ☐ Não
- Histogramas: ☐Sim ☐ Não

- *Você definiu responsabilidades para a coleta e análise dos dados?*
- *É necessário treinamento?*
- *O plano é consistente com o contrato?*
- *O plano pode ser conduzido em pequena escala?*
- *Você considerou as pessoas de fora da equipe que serão afetadas por este plano?*



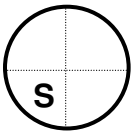
FAZER

OBSERVAÇÕES AO CONDUZIR O PLANO

1. A busca com o termo *Supercritical Antisolvent Process* resultou em um grande número de artigos, com estudos bastante atuais, fornecendo um retrato muito satisfatório da utilização desta tecnologia em diversas frentes de pesquisa.
2. A busca com os termos *Supercritical Antisolvent Process*process parameters* resultou em um limitado numero de artigos, a maioria do autor E. Reverchon, nos quais descrevia o processo com base em uma perspectiva termodinâmica. Outro autor com um numero grande de artigos publicados na área é María José Cocero.
3. Os termos Process*antioxidant compounds recovery forneceram poucos artigos em que o solvente de um extrato previamente obtido por um processo de extração era eliminado via SAS.

4. O termo Supercritical Antisolvent Process*curcuminoids não forneceu nenhum artigo,
5. A maioria das publicações está focada no processo de micronização de compostos com fins farmacêuticos.
6. Apesar de terem sido encontradas muitas publicações relacionadas a diversos aspectos do processo SAS em si, atualmente poucos processos são levados para a escala industrial e sua aplicação na obtenção de precipitados a partir de extratos ricos em compostos bioativos. Por isso, esse tema torna-se uma grande oportunidade de pesquisa.

- *Identifique as coisas observadas que não fazem parte do plano.*
- *Documente o que saiu errado durante a coleta de dados.*
- *Comece a análise inicial dos dados assim que forem coletados.*
- *Avalie os dados quanto a mudanças ao longo do tempo (gráfico de controle ou gráfico de tendência).*



ESTUDAR

O QUE FOI APRENDIDO COM OS DADOS?

1. Como no processo de precipitação com líquido antissolvente, o sucesso do processo de micronização via SAS depende da solubilidade do solvente líquido no antissolvente supercrítico e da insolubilidade do soluto no antissolvente (Kim et al., 2012). Em outras palavras, os compostos a precipitar devem ser solúveis no solvente porem muito pouco solúveis no CO2.
2. Embora a geração de partículas empregando o processo SAS tem sido amplamente estudada, os mecanismos que controlam a formação de micro/nanopartículas e sua morfologia ainda são pouco conhecidos.
3. De acordo a Reverchon et al. (2010) o processo SAS é complexo, uma vez que envolve: A) o conhecimento do equilíbrio a altas pressões do sistema binário (solvente/CO2) e o sistema ternário (solvente/CO2/soluto), B) a mistura dos jatos, quando a solução de líquido é injetada no vaso de precipitação e, C) a transferência de massa entre as fases de líquido injetado e o fluido supercrítico, o que provoca a sobressaturação e a precipitação do soluto;
4. O tamanho e morfologia das partículas são afetados principalmente pelos parâmetros de extração: pressão, temperatura, concentração na solução e as vazões de solvente e CO2.
5. Além dos parâmetros de extração, o tamanho da partícula é dominado principalmente por dois possíveis mecanismos: evaporação do solvente dentro da fase do antissolvente e a difusão do antissolvente dentro das gotas da solução (Zhao et al., 2012).
6. Embora dados da mistura CO2-etanol-curcuminóides não esteja disponível na literatura, dados de equilíbrio da mistura CO2-etanol poderiam ser utilizados inicialmente para tratar a viabilidade do processo.
7. Micropartículas são produzidas quando a pressão de operação do SAS é próxima ao ponto crítico da mistura; no entanto, quando a pressão aumenta para além da pressão crítica da mistura são formadas nanopartículas. No entanto com aumentos da pressão, a eficiência de precipitação e encapsulação podem mudar. Mezzomo et al. (2012) observou que aumentando a pressão de 8 ate 10 MPa, a eficiência da encapsulação aumentou. Por outro lado, com a mudança na pressão de 10 para 12 MPa a eficiência de encapsulação diminuiu.
8. A maneira de resumo, na tabela 1 são apresentados os efeitos dos parâmetros de operação sob o tamanho e a morfologia das partículas produzidas via SAS.

Tabela 1. Efeitos dos parâmetros de operação do processo SAS sob o tamanho e morfologia

Parâmetro operacional	Efeito
Pressão	Altas pressões e partículas menores (Zu et al., 2012, Zhao et al., 2010): A alta pressão incrementa o rompimento das gotas da solução em partículas menores.

	Pressões menores e partículas menores (Wang et al., 2013): Em uma situação acima do ponto crítico, uma redução da pressão diminui a solubilidade o que resulta em uma sobressaturação mais elevada do soluto; por conseguinte, partículas menores são produzidas.
Temperatura	Partículas com menor tamanho e de forma esférica são obtidas geralmente quando são usadas altas temperaturas (Reverchon e De Marco, 2011). O aumento da temperatura reduz a solubilidade do soluto na solução e, portanto, aumenta a sobressaturação máxima, de modo que são obtidas partículas menores. Além disto, ao aumentar a temperatura o tempo de evaporação do solvente diminui. É importante se forem utilizados polímeros como material de parede, utilizar temperaturas menores do que a temperatura de transição vítrea (Tg) do polímero (Kalani e Yunus, 2011). Por outro lado, se forem utilizados solventes com alta volatilidade, partículas com menor tamanho podem ser obtidas empregando baixas temperaturas (Kalani e Yunus, 2011).
Concentração	Em concentrações mais elevadas, menores tamanhos de partícula podem ser obtidos devido a que o aumento da concentração inicial aumenta a sobressaturação máxima e, por conseguinte, as partículas menores vão ser formadas (Miguel et al., 2008). A concentração do soluto na solução influencia significativamente no tamanho das partículas. Geralmente quanto menor a concentração de soluto na solução menor é o tamanho da partícula (Zhao et al., 2010). Embora a concentração da solução inicial parecesse desempenhar um papel importante no rendimento global de precipitação, nem sempre o teor de compostos bioativos no precipitado está relacionado com o rendimento global. Por exemplo, Visentin et al. (2012) observaram um aumento do rendimento de 17,9% a 90% quando a concentração de sólidos na solução aumentou de 2,7 a 7,4 wt.%, porem o teor de compostos bioativos no precipitado só foi de 0,039 g/g. Por outro lado, quando o processo é realizado a baixas concentrações, a supersaturação do soluto acontece muito lentamente. Por tanto, a precipitação é adiada e o fenômeno de nucleação domina o crescimento das partículas (Wang et al., 2013).
Geometria do bico injetor	Diâmetro menor do bico injetor reduz o tamanho da partícula e produz partículas mais esféricas . Quanto menor o diâmetro do injetor maior é a velocidade de saída e menor o tamanho da gota, gerando um menor tamanho de partícula (Guha et al., 2011).
Vazões de solvente e CO2	Incrementar a vazão do CO2 por encima da vazão do reduz o tamanho de partícula (Reverchon et al., 2007). Menores tamanhos de partículas são obtidos quando a vazão de CO2 aumenta, no entanto um menor rendimento pode ser obtido devido ao pouco tempo de residência da solução dentro do vaso de precipitação. Algumas vezes não tem efeito nenhum sobre a morfologia nem o tamanho (Boonnoun et al., 2013).

1. Rendimentos próximos a 100%, concentrações de compostos alvos no precipitado de 100% e morfologias mais uniformes são obtidos geralmente quando no processo de precipitação via SAS foram utilizadas soluções obtidas mediante a diluição de compostos puros em algum solvente (Tabela 2). Por exemplo: pó de A. paniculata em etanol (Imsanguan et al., 2010), antibióticos em diclorometano e etanol, luteína em etil acetato (Miguel et al., 2008), licopeno em diclorometano (Miguel et al., 2006), β-caroteno em diclorometano (Mattea et al., 2009), oxeglitazar em agua (Majerik et al., 2007) e nafmefene em etanol (Adami et al., 2008). Por outro lado, a maioria dos trabalhos publicados não mencionam o rendimento de precipitação.

Tabela 2. Resumo de trabalhos SAS onde foi utilizado um composto puro na solução injetada.

Composto/Solvente	Concentração solução	Parâmetros de operação	Rendimento de precipitação	Morfologia e tamanho	Ref
Taxifolina/ EtOH	5-20 g/L	35-65°C,	Não disponível	Forma de agulha quando T e P	(Zu et al.,

		10-25 MPa, 3-6 cm^3/min (solução), 8,5 kg/h (CO2)		aumentaram (40-200 µm) Tamanho diminuiu quando concentração diminuiu.	2012)
Camptotecina/ DMC	1,25-5 g/L	35-68°C, 10-25 MPa, 3,3-13,2 cm^3/min (solução), 8,5 kg/h (CO2)	Não disponível	Esferas com tamanho entre 0,38 e 0,93 µm. Tamanho aumentou quando concentração diminuiu, a T aumento e P diminuiu.	(Zhao et al., 2010)
Taxol/ EtOH	2,5-10 g/L	35-68°C, 10-25 MPa, 3,3-13,2 cm^3/min (solução), 8,5 kg/h (CO2)	Não disponível	Esferas e partículas amorfas com tamanho de 150 µm. O tamanho aumentou e diminuiu conforme o aumento do valor dos parametros	(Zhao et al., 2012)
Ginkgo biloba/ EtOH Extracto de Ginkgo biloba: 6%terpenoides e24% glicosídeos flavonóides	1,25-5 g/L	35-80°C, 10-40 MPa, 3-12 cm^3/min (solução), 2,1-4,3 kg/h (CO2)	98% de terpenoides e glicosídeos flavonoides	Partículas esféricas com um diâmetro entre 20 e 160 nm Conforme T, P e concentração e vazão de solução aumentaram o tamanho diminuiu gradualmente.	(Zhao et al., 2011)
Cafeina/ EtOH e DMC	2,7-8 g/L	30-60°C, 8-12 MPa, 4 cm^3/min (solução), 4 kg/h (CO2)	99%	Cristais simples com tamanhos entre 1 e 40 µm. Com o aumento ate 2,4 g/L da concentração inicial o tamanho diminui, após este valor aumento. Altas T e P diminuíram o tamanho	(Weber Brun et al., 2012)
Hidroxamptotecina/ EtOH e DMC	1,2-3 g/L	35-40°C, 7,5-14 MPa, 0,3-1,7 cm^3/min (solução), 1 g/L (CO2)	32%	Esferas com diâmetro de 50 µm (15°C,8 MPa) Quando T ou P é maior do que um determinado valor, as partículas tendem a agregar.	(Wang et al., 2013)
Etilcelulose/ DMC	10-40 g/L	35°C, 8 MPa, 2	Não disponível	O tamanho das partículas aumentou com o aumento da	(Montes et al., 2012b)

		cm^3/min (solução), 11 g/min (CO2)		concentração de solução Partículas amorfas com tamanho entre 3.8 e 5.0 μm	
Camptotecina/ DMF, NMP, CHF, AA, e EtOH	1 g/L	40°C, 14 MPa, 0,8 mL/h (solução), 20 g/min (CO2)	Não disponível	Escamas com tamanho entre 0,39 e 2,14 μm Escamas relativamente aglomeradas com AA e NMP Partículas menores com DMF e EtOH	(Liu et al., 2013)
Acetaminofeno/ EtOH e acetona	1-5 wt.%	40°C, 10-30 MPa	Não disponível	O polimorfismo dos cristais pode ser ajustado entre monoclínico e ortorrômbico, variando o teor de etanol na solução.	(Rossmann et al., 2013)
Amoxicilina e Etilcelulose/ DMC	Amoxicilina 1,33 g/L Etilcelulose 10 g/L	35-55°C, 10-25 MPa, 2 cm^3/min (solução), 11 g/min (CO2)	Rendimento de precipitação entre 5,5 e 84%	Independente da pressão foram obtidas partículas amorfas menores a baixas temperaturas (1-3 μm).	(Montes et al., 2012a)
Paclitaxel/DMC e EtOH Material de parede: PLLA	5-12 g/L	30-45°C, 8-14 MPa, 0,2-1,5 cm^3/min (solução), 2 g/L (CO2)	Eficiência de encapsulamento entre 39,9%	Menor tamanho com DMC puro do que com misturas. Partículas agregadas de maior tamanho em T acima de 40°C. P não apresentou um efeito significativo sobre a morfologia o u tamanho.	(Li et al., 2012)
Atorvastatina de cálcio/THS, acetona, AA, DMF, NMP, DMSO	100-300 g/L	40°C, 12 MPa, 0,5 g/min (solução), 45 g/L (CO2)	>90%	Esferas com THS e acetona Aglomerados com NMP e DMSO O tamanho aumento com o aumento da concentração da solução 91-1493 nm	(Kim et al., 2012)
Gadolínio acetato/DMSO	20-300 g/L	35-60°C, 9-20 MPa,	Não disponível	Partículas esféricas O tamanho diminuiu com o aumento da P 12-18 MPa Micropartículas (0,28-0,52 μm) 20 MPa nanopartículas (90-210 nm) O tamanho aumento com o aumento da concentração e da T	(De Marco e Reverchon, 2011)

Ibuprofeno/EtOH	20-40 g/L	40-50°C, 10-12 MPa, 0,5-1 cm^3/min (solução), 0,5-0,8 kg/h (CO2)	14-73%	Partículas com forma de agulha, escamas e laminas de 1 µm.	(M. S. Gomes et al., 2014)

NMP n-metil-2-pirrolidona, DMF dimetilformamida, CHF clorofórmio, AA acido acético, EtOH etanol, DMC diclorometano, PLLA acido poli láctico, THF tetrahidrofurano, DMSO dimetil sulfóxido

2. No entanto quando no processo SAS são utilizados extratos previamente obtidos empregando outras técnicas de extração (por exemplo, Soxhlet) são obtidos rendimentos menores, extratos com uma menor pureza e partículas com morfologias desuniformes ou amorfas, como é apresentado na Tabela 3.

Tabela 3. Resumo de trabalhos SAS onde foi utilizado um extrato rico no composto alvo.

Matéria prima (Compostos bioativos)/Solvente	Concentração solução	Parâmetros de operação	Rendimento	Morfologia e tamanho	Método de obtenção do extrato	Ref
Resíduos de uva (Antocianinas)/Metanol	Antocianinas 15,6 g/kg	40°C, 11 MPa, 0,7 cm^3/min (solução), 25 ml/min (CO2)	Global 99%	Não disponível	As antocianinas foram separadas empregando uma coluna C18 e metanol foi utilizado para sua dessorção	(Floris et al., 2010)
Alecrim (polifenóis)/Etanol	Conteúdo de sólidos do extrato etanólico 2,7 wt% 110 g/kg de polifenóis 25 ml injetados	25-50°C, 8-12 MPa, 1 cm^3/min (solução), 0,7 kg/h (CO2)	Extrato precipitado: 13,3% polifenóis (35°C e 12 MPa) Global 17,9% (50°C e 10 MPa)	Aglomerados com tamanho superior a 200 µm (25°C,10 MPa) até 50 µm (50°C,10 MPa)	Alecrim desengordurado com micro-ondas. Extração com etanol a baixa pressão. Filtrado 0,45 µm.	(Visentin et al., 2012)
Sementes de uva (polifenóis)/Etanol	Conteúdo de sólidos do extrato etanólico 3 wt% 110 g/kg de polifenóis	35-60°C, 8-15 MPa, 1 cm^3/min (solução), 2,38 kg/h (CO2)	Global 6,8-62% Extrato precipitado: 63% de polifenóis (15 MPa e 40 °C)	Microesferas com diâmetro médio de 100 nm	Desengorduradas com hexano. Extração com etanol utilizando Soxhlet.	(Marqués et al., 2013)
P. indica (ryanodol, inseticida)/Etanol	Conteúdo de sólidos do extrato etanólico 3 wt%	35-60°C, 8-15 MPa, 5,7 cm^3/min (solução), 2,38 kg/h	Global 56,1% Extrato precipitado: 37,7 % de ryanodol (15 MPa e 35°C)	Não disponível	Extração com etanol a baixa pressão	(Martín et al., 2011)

		(CO2)				
Resíduo de camarão (carotenoides)/Acetona	2-12 g/L de extrato Pluronic F127 (material de parede)	35-45°C, 8-120 MPa, 1-3 cm^3/min (solução), 1 kg/h (CO2)	Eficiência de encapsulamento de74% a 35°C e 10 MPa	Partículas amorfas de tamanho micrométrico maior a 100 µm	Extração com maceração com acetona	(Mezzomo et al., 2012)

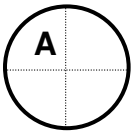
3. É possível observar que embora os extratos utilizados no processo SAS sejam ricos nos compostos alvos, o extrato precipitado não tem um rendimento muito alto nem uma concentração muito elevada quando comparados com aqueles trabalhos onde foram empregadas soluções contendo o composto puro. Pode ser que devido à maior complexidade da solução, existam outros componentes que sejam precipitados junto com os compostos de interesse, prejudicando a pureza do precipitado. No teste prévio de precipitação do extrato de curcuminóides via SAS, foi utilizada uma solução com uma concentração de 7,44 g/L de curcuminóides em um processo utilizando 40°C, 12 MPa, 0,5 cm^3/min de solução e 0,6 kg/h de CO2. O extrato precipitado teve um conteúdo de curcuminóides por massa de sólidos de 0,5 g/g e um rendimento global de precipitação de curcuminóides de 54%. Resultado que é consistente com os resultados apresentados na Tabela 3 e que ainda é comparável com os rendimentos de precipitação obtidos em trabalhos onde foram empregadas soluções feitas diluindo o composto puro. Por exemplo, Wang et al. (2013), Montes et al. (2012a) e M. S. Gomes et al. (2014) obtiveram rendimentos de precipitação de 32%, 5,5-84% e 14-73%, respectivamente.

- *Os resultados deste ciclo estão de acordo com as predições feitas na fase de planejamento?*
- *Sob quais condições as conclusões deste ciclo poderiam ser diferentes?*
- *Quais são as implicações das observações e problemas não planejados durante a coleta de dados?*
- *Os dados ajudam a responder as questões colocadas no plano?*

RESUMA O NOVO CONHECIMENTO OBTIDO NESTE CICLO

1. Temperatura, pressão, concentração da solução, vazão de CO2, são parâmetros importantes que interferem no processo SAS;
2. O processo SAS é geralmente realizado sob temperaturas e pressões que variam entre 35-60°C e 8-15 MPa
3. A composição da solução a ser injetada tem um papel fundamental no rendimento do processo e na morfologia das partículas;
4. Rendimentos globais de precipitação menores são obtidos quando é precipitada uma solução cuja composição tem outros compostos além dos compostos alvos. Devido à presença de outros compostos na solução a ser precitada, são obtidas purezas menores no extrato precipitado.
5. No caso de soluções provenientes de processos de extração como, PLE, LPSE e Soxhlet parece ser necessário um processo de purificação adicional com a finalidade obter uma solução só com os compostos a serem precipitados.

- *Revise o conhecimento atual para refletir este aprendizado (atualize fluxogramas e diagramas de causa e efeito).*
- *Este novo conhecimento se aplicará em todos os lugares?*



AGIR

QUAIS ÁREAS DEVEM SER ESTUDADAS?

1. Melhorar a pureza da solução de curcuminóides a ser precipitada. Embora a unidade cossolvente tenha um filtro na saída, baseado no trabalho de Visentin et al. (2012), o extrato rico em curcuminóides foi submetido a uma filtragem empregando um filtro de 0,45 µm para diminuir a quantidade de sólidos presentes na solução, com o objetivo de obter um precipitado com maior teor de curcuminóides ao eliminar material não considerado composto alvo. Como resultado, atualmente tem-se a disposição 2,7 L de extrato etanólico filtrado de curcuminóides com um conteúdo de sólidos de 5,5 wt. % e 207 g/kg de curcuminóides (7,8 g/L).
2. Os parâmetros de processo a serem avaliados devem estar em concordância com as condições citadas anteriormente, principalmente a temperatura (35-60°C) e a pressão (8-15 MPa), além dos parâmetros utilizados no trabalho de M. S. Gomes et al. (2014): 0,5-0,8 kg/h de CO2, 0,5-1 cm^3/min de solução e injetor coaxial e T.
3. Estudar a cinética do processo de precipitação.

QUE OUTRAS ÁREAS PODERIAM SER ESTUDADAS?

1. Estudar a solubilidade dos curcuminóides na fase etanol-CO2.
2. Estudar a cinética do processo de precipitação.

- *O sistema de causas é suficientemente compreendido?* ☐Sim ☐Não
- *As mudanças foram testadas em pequena escala?* ☐Sim ☐Não
- *As responsabilidades para implementar e avaliar as mudanças foram comunicadas?* ☐Sim ☐Não
- *Uma mudança ou ação apropriada foi desenvolvida ou selecionada?* ☐Sim ☐Não
- *Há forças na organização que ajudarão ou dificultarão as mudanças?* ☐Sim ☐Não
- *As mudanças ou ações melhorarão o desempenho no futuro?* ☐Sim ☐Não

OBJETIVOS DO PRÓXIMO CICLO

Seleção dos parâmetros de operação.

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PROCEDIMENTO OPERACIONAL PADRÃO (POP) – UNIDADE SAS/SFEE

2 UNIDADE SAS/SFEE

O processo de precipitação de curcuminóides via SAS foi realizado na unidade SAS/SFEE (Figura A4), equipamento concebido e montado no LASEFI representado esquematicamente na Figura A5. A unidade de extração consiste em duas bombas de alta pressão, uma para solvente uma bomba HPLC (Jasco, PU-2080, Japan) e outra refrigerada para CO2 (Maximator, M111 CO2, Germany), dois banhos termostáticos programáveis (PolyScience, modelo 9510, Niles, EUA e Marconi, modelo MA-184, Piracicaba, SP) responsáveis pela manutenção da temperatura do extrator de aço inox e dos cabeçotes da bomba de CO2, respectivamente, um totalizador de vazão (LAO, modelo G0,6 ± 0,001 m3, São Paulo, SP), termopares, válvulas e manômetros (Record, 50 MPa ± 0,5, São Paulo, SP).

Figura A11. Unidade de precipitação SAS/SFEE.

2.1 Procedimento operacional

Na Figura A5 é apresentado o diagrama da unidade SAS/SFEE. Os curcuminóides foram precipitados utilizando o seguinte procedimento operacional:

1) Ligar o banho de resfriamento (5) e programá-lo para operar a – 3.5 ºC;

2) Ligar o banho de aquecimento (7) e programá-lo para operar na temperatura desejada;

3) Conectar a tubulação T no orifício central da câmara de precipitação (VP-2) e suas ramificações na válvula V-5 e na bomba BL;

4) Elaborar e inserir no fundo do vaso de precipitação um cartucho de papel de filtro (10 μm) para evitar uma perda por arraste das partículas formadas;

5) Conectar a tubulação de saída da válvula micrométrica (14) um frasco coletor lacrado (50 ou 100 mL) de massa conhecida, imerso em um banho de gelo;

6) Verificar se as válvulas de bloqueio (3) e micrométrica (14) estão fechadas;

7) Realizar a pressurização do CO2.

8) Ligar o controlador do sistema de aquecimento da válvula micrométrica (14) e programá-los para operar a uma temperatura que evite congelamento da linha de saída (120 ºC);

9) Quando a pressão e temperatura do sistema estiverem estabilizadas, abrir as válvulas localizadas após do vaso de precipitação cuidadosamente até que a vazão desejada seja alcançada (Medição através do rotâmetro);

10) Estabilizada a vazão desejada, ligar a bomba HPLC (9) e programá-la na vazão desejada;

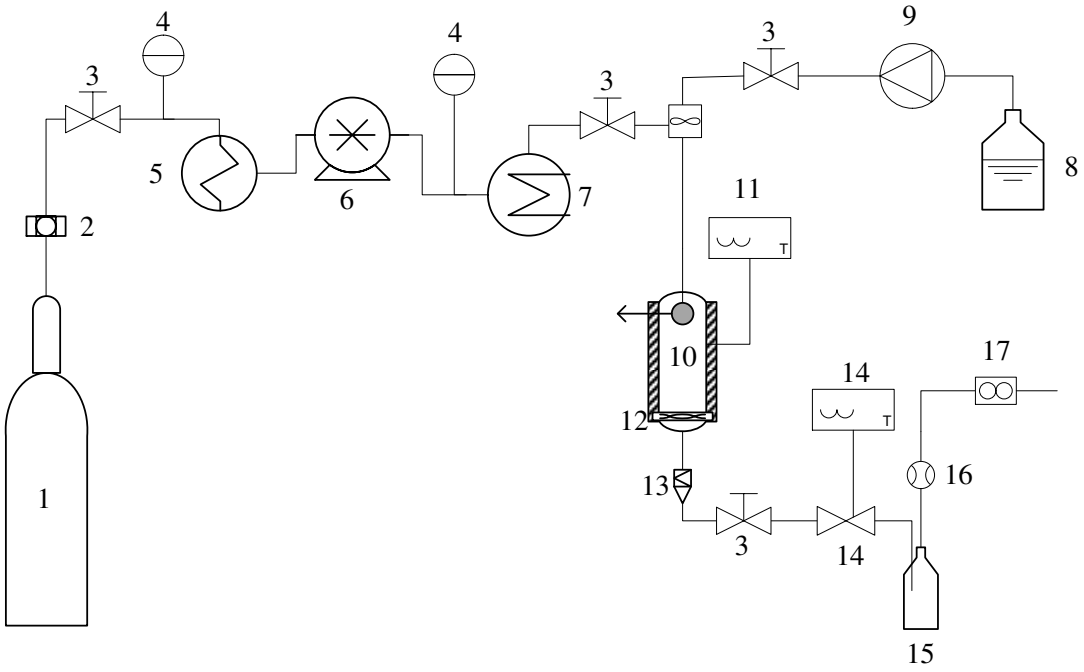
11) Após o tempo pré-estabelecido de formação de partículas, desligar a bomba HPLC (9) para interromper a alimentação da solução;

12) Por um determinado tempo (1,25 h), continuar alimentando a câmara de expansão somente com CO2 supercrítico a mesma vazão para eliminar o solvente residual nas partículas formadas;

13) Decorrido o tempo de eliminação do solvente orgânico residual, desligar o banho (5), o banho (7) e o controlador de aquecimento (14);

14) Despressurizar o vaso de precipitação (10) empregando a mesma vazão utilizada no processo de formação de partículas e eliminação do solvente residual, para não provocar uma perda, por arraste, das partículas formadas na câmara de precipitação;

20) Deslacrar o vaso de precipitação (10), coletar, pesar e guardar as partículas formadas em frascos de vidro lacrados e protegidos da luz.



1 – Reservatório de solvente (CO_2)
2 – Reservatório de cossolvente
3 – Válvulas de bloqueio
4 – Manômetros
5 –Banho de resfriamento (CO_2)
6 – Bomba de alta pressão
7 – Banho de aquecimento
8 – Reservatório da solução
9 –Bomba HPLC
10 – Vaso de precipitação
11 –Sistema de aquecimento do vaso de precipitação
12 – Filtro de aço inox
13 – Filtro de saida
14 – Válvula micrométrica
15 – Frasco coletor do solvente
16 – Rotâmetro
17 – Totalímetro

Figura A12. Diagrama esquemático da unidade de precipitação SAS/SFEE.

2.2 Resultados Experimentais: SAS

A seguir estão apresentados os resultados experimentais da precipitação de curcuminóides via SAS e que resultaram no artigo submetido que está apresentado no **Capítulo 5**.

Tabela A12. Dados da caracterização do extrato etanólico rico em curcuminóides utilizado.

Frasco	Peso Frasco (g)	Frasco + 5 mL (g)	Frasco + extrato	Massa extrato	Massa solução	wt. %	Teor de curcuminóides (g/L)
1	34,5776	38,5443	34,7657	0,1881	3,9667	5,4	13,7
2	34,1207	37,9801	34,309	0,1883	3,8594	5,6	14,4

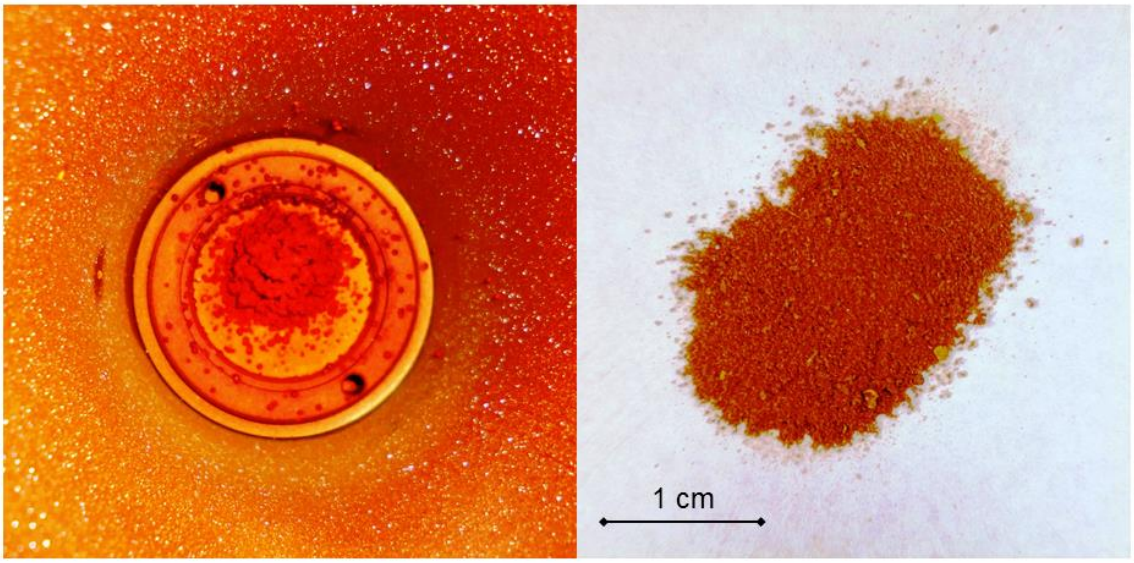


Figura A13. Fotografias das partículas a) no vaso de precipitação e b) após coletar com pincel.

Tabela A13. Dados dos ensaios do planejamento split plot correspondentes ao rendimento global de sólidos (GYSOL), teor de curcuminóides (CCC) e eficiência de precipitação (PE).

Exp	Injetor	Temperatura (K)	Pressão (MPa)	Vazão de CO2 (g/h)	Frasco (g)	Frasco + partículas (g)	GY_{SOL} (%)	C_{CC} (mg/g)
1	T-mixer	313	12	500	24,8893	25,6125	48,2	415
2	T-mixer	313	10	500	25,9806	26,7778	53,1	524
3	T-mixer	333	12	800	25,9852	26,6969	47,4	397
4	T-mixer	333	12	500	25,7092	26,3566	43,2	446
5	T-mixer	313	10	800	25,5388	26,5821	69,6	485
6	T-mixer	333	10	500	26,1703	-	-	-
7	T-mixer	313	12	800	26,1702	27,1725	66,8	344
8	T-mixer	333	10	800	25,3891	-	-	-
9	Coaxial	333	10	800	25,6037	26,4429	55,9	558
10	Coaxial	333	12	800	25,8127	-	-	-
11	Coaxial	333	12	500	24,2852	24,8005	34,4	316
12	Coaxial	333	10	500	25,7578	26,2695	34,1	474
13	Coaxial	313	10	800	25,8135	26,4177	40,3	392
14	Coaxial	313	10	500	25,8708	26,4961	41,7	445
15	Coaxial	313	12	800	24,5859	25,2926	47,1	443
16	Coaxial	313	12	500	21,2125	21,7907	38,5	528
17	Coaxial	333	12	800	24,8837	-	-	-
18	Coaxial	313	12	500	25,7378	26,3084	38,0	487
19	Coaxial	313	10	500	24,4501	25,0636	40,9	426
20	Coaxial	333	12	500	24,5489	25,0765	35,2	303
21	Coaxial	313	12	800	25,9615	26,6951	48,9	469
22	Coaxial	333	10	800	25,7453	26,5495	53,6	551
23	Coaxial	333	10	500	25,3779	25,9187	36,1	470
24	Coaxial	313	10	800	23,6022	24,2765	45,0	420
25	T-mixer	333	10	800	26,0055	-	-	-
26	T-mixer	333	10	500	25,6521	-	-	-
27	T-mixer	313	10	800	26,0062	27,0264	68,0	490
28	T-mixer	333	12	500	24,4316	25,0552	41,6	486
29	T-mixer	313	12	800	25,9208	26,9245	66,9	337
30	T-mixer	313	10	500	24,8952	25,7044	53,9	547
31	T-mixer	333	12	800	26,1518	26,9508	53,3	395
32	T-mixer	313	12	500	21,1672	21,8941	48,5	427

Tabela A14. Dados da analise do tamanho de partícula feitos no equipamento Master sizer.

Exp	span	D43	Uniformity	Specific surface area	D32	d (0.1)	d (0.5)	d (0.9)
1	2.9	206	2,855	205,932	0,949	0,0534	112,334	59,101
2	2.2	111	0,738	0,162	36,924	31,511	83,148	210,411
3	3.2	134	0,927	0,21	28,607	22,65	91,84	318,005
4	2.2	236	0,683	0,0599	100,208	61,351	189,737	483,239
5	11.1	287	2,33	0,0982	61,085	35,912	98,517	1131,504
6	-	-	-	-	-	-	-	-
7	1.4	850	0,519	0,0618	97,108	41,477	1121,034	1600,089
8	-	-	-	-	-	-	-	-
9	2.4	331	0,765	0,0717	83,637	52,425	267,548	707,457
10	-	-	-	-	-	-	-	-
11	3.4	245	1,05	0,141	42,55	25,778	164,35	588,744
12	2.6	238	0,791	0,103	58,516	32,565	190,349	518,339
13	5.7	432	1,71	0,0876	68,46	35,77	204,467	1201,245
14	3.4	522	1,08	0,0885	67,828	45,586	359,662	1266,967
15	4.9	212	1,66	0,161	37,311	28,302	96,234	498,771
16	4.3	356	1,26	0,0662	90,608	43,067	206,712	940,415
17	-	-	-	-	-	-	-	-
18	3.9	244	1,22	0,126	47,682	29,887	145,598	593,239
19	2.5	512	0,782	0,0372	161,136	75,243	415,052	1111,998
20	4.5	142	1,25	0,202	29,658	20,588	79,699	377,307
21	5.3	229	1,71	0,148	40,585	28,87	101,883	571,424
22	2.6	315	0,833	0,097	61,861	39,075	249,692	695,451
23	2.6	322	0,805	0,0674	89,078	48,019	254,596	701,846
24	3.5	297	1,05	0,0659	91,081	47,166	191,177	711,689
25	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-
27	1.4	300	0,563	0,0655	91,558	44,401	1061,16	1579,678
28	3.7	364	1,11	0,0599	100,202	48,712	230,12	897,749
29	1.4	829	0,517	0,0559	107,393	40,817	1069,406	1549,539
30	2.6	111	0,84	0,15	39,872	28,066	77,331	226,261
31	2.9	222	1	0,0778	77,125	40,052	145,376	465,009
32	2.3	126	0,778	0,0889	67,526	36,755	90,592	247,671

Tabela A15. Dados de saída do analises do planejamento Split-Plot do rendimento global de sólidos (GYSOL) do software SAS 9,2 (SAS Institute, Inc.).

Dependent Variable: GY_{SOL}

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	2816.652060	216.665543	62.53	<.0001
Error	12	41.581786	3.465149		
Corrected Total	25	2858.233846			

R-Square	Coeff Var	Root MSE	Y Mean
0.985452	3.871282	1.861491	48.08462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Tipo	1	1078.641108	1078.641108	311.28	<.0001
rep(Tipo)	2	3.588214	1.794107	0.52	0.6086
Temperatura	1	238.095238	238.095238	68.71	<.0001
Pressao	1	153.015000	153.015000	44.16	<.0001
VAZ_CO2	1	858.217204	858.217204	247.67	<.0001
Tipo*Temperatura	1	215.275896	215.275896	62.13	<.0001
Tipo*Pressao	1	8.867626	8.867626	2.56	0.1356
Tipo*VAZ_CO2	1	13.003233	13.003233	3.75	0.0766
Temperatura*Pressao	1	28.366875	28.366875	8.19	0.0143
Temperatura*VAZ_CO2	1	8.840833	8.840833	2.55	0.1362
Pressao*VAZ_CO2	1	2.660208	2.660208	0.77	0.3981
Tipo*Tempera*Pressao	0	0.000000	.	.	.
Tipo*Tempera*VAZ_CO2	1	208.080625	208.080625	60.05	<.0001
Temper*Pressa*VAZ_CO	0	0.000000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Tipo	1	891.5653099	891.5653099	257.29	<.0001
rep(Tipo)	2	3.5882143	1.7941071	0.52	0.6086
Temperatura	1	76.3831406	76.3831406	22.04	0.0005
Pressao	1	2.3884298	2.3884298	0.69	0.4226
VAZ_CO2	1	835.0008333	835.0008333	240.97	<.0001
Tipo*Temperatura	1	0.8228571	0.8228571	0.24	0.6348
Tipo*Pressao	1	22.0900000	22.0900000	6.37	0.0267
Tipo*VAZ_CO2	1	128.8225000	128.8225000	37.18	<.0001
Temperatura*Pressao	1	1.2014286	1.2014286	0.35	0.5669
Temperatura*VAZ_CO2	1	8.8408333	8.8408333	2.55	0.1362
Pressao*VAZ_CO2	1	33.6400000	33.6400000	9.71	0.0089
Tipo*Tempera*Pressao	0	0.0000000	.	.	.
Tipo*Tempera*VAZ_CO2	0	0.0000000	.	.	.
Temper*Pressa*VAZ_CO	0	0.0000000	.	.	.

Tabela A15. Dados de saída do analises do planejamento Split-Plot do rendimento global de sólidos (GYSOL) do software SAS 9,2 (SAS Institute, Inc.).

Dependent Variable: GY_{SOL}

Tests of Hypotheses Using the Type I MS for rep(Tipo) as an Error Term

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Tipo	1	1078.641108	1078.641108	601.21	0.0017

Tests of Hypotheses Using the Type III MS for rep(Tipo) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Tipo	1	891.5653099	891.5653099	496.94	0.0020

Tabela A16. Dados de saída do analises do planejamento Split-Plot do conteúdo de curcuminóides das partículas (CCC) do software SAS 9,2 (SAS Institute, Inc.).

Dependent Variable: C_{CC}

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	122494.4546	9422.6504	43.89	<.0001
Error	12	2576.1305	214.6775		
Corrected Total	25	125070.5851			

R-Square	Coeff Var	Root MSE	CC Mean
0.979403	3.291007	14.65188	445.2096

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Tipo	1	384.36134	384.36134	1.79	0.2057
rep(Tipo)	2	471.58431	235.79216	1.10	0.3647
Temperatura	1	643.99795	643.99795	3.00	0.1089
Pressao	1	29283.56551	29283.56551	136.41	<.0001
VAZ_CO2	1	1544.40158	1544.40158	7.19	0.0200
Tipo*Temperatura	1	2867.06103	2867.06103	13.36	0.0033
Tipo*Pressao	1	8592.33247	8592.33247	40.02	<.0001
Tipo*VAZ_CO2	1	15460.39600	15460.39600	72.02	<.0001
Temperatura*Pressao	1	52464.89763	52464.89763	244.39	<.0001
Temperatura*VAZ_CO2	1	4504.68750	4504.68750	20.98	0.0006
Pressao*VAZ_CO2	1	3736.50521	3736.50521	17.41	0.0013
Tipo*Tempera*Pressao	0	0.00000	.	.	.
Tipo*Tempera*VAZ_CO2	1	2540.66403	2540.66403	11.83	0.0049
Temper*Pressa*VAZ_CO	0	0.00000	.	.	.

Tabela A16. Dados de saída do analises do planejamento Split-Plot do conteúdo de curcuminóides das partículas (CCC) do software SAS 9,2 (SAS Institute, Inc.).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Tipo	1	18938.76397	18938.76397	88.22	<.0001
rep(Tipo)	2	471.58431	235.79216	1.10	0.3647
Temperatura	1	6548.48100	6548.48100	30.50	0.0001
Pressao	1	40892.19306	40892.19306	190.48	<.0001
VAZ_CO2	1	2869.99470	2869.99470	13.37	0.0033
Tipo*Temperatura	1	34514.53441	34514.53441	160.77	<.0001
Tipo*Pressao	1	36900.48902	36900.48902	171.89	<.0001
Tipo*VAZ_CO2	1	576.72022	576.72022	2.69	0.1271
Temperatura*Pressao	1	32247.07316	32247.07316	150.21	<.0001
Temperatura*VAZ_CO2	1	4504.68750	4504.68750	20.98	0.0006
Pressao*VAZ_CO2	1	769.23022	769.23022	3.58	0.0827
Tipo*Tempera*Pressao	0	0.00000	.	.	.
Tipo*Tempera*VAZ_CO2	0	0.00000	.	.	.
Temper*Pressa*VAZ_CO	0	0.00000	.	.	.

Dependent Variable: C_{CC}

Tests of Hypotheses Using the Type I MS for rep(Tipo) as an Error Term

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Tipo	1	384.3613366	384.3613366	1.63	0.3299

Tests of Hypotheses Using the Type III MS for rep(Tipo) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Tipo	1	18938.76397	18938.76397	80.32	0.0122

Tabela A17. Dados de saída do analises do planejamento Split-Plot do tamanho de partícula (d[4,3]) do software SAS 9,2 (SAS Institute, Inc.).

Dependent Variable: $d_{[4,3]}$

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	816743.5037	62826.4234	9.79	0.0002
Error	12	77022.3810	6418.5317		
Corrected Total	25	893765.8846			

R-Square	Coeff Var	Root MSE	Tamanho Mean
0.913823	25.48647	80.11574	314.3462

Tabela A17. Dados de saída do analises do planejamento Split-Plot do tamanho de partícula (d[4,3]) do software SAS 9,2 (SAS Institute, Inc.).

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Tipo	1	2.2894	2.2894	0.00	0.9852
rep(Tipo)	2	6767.1190	3383.5595	0.53	0.6033
Temperatura	1	57905.7202	57905.7202	9.02	0.0110
Pressao	1	759.3750	759.3750	0.12	0.7368
VAZ_CO2	1	59816.8816	59816.8816	9.32	0.0100
Tipo*Temperatura	1	5672.6784	5672.6784	0.88	0.3657
Tipo*Pressao	1	235021.9561	235021.9561	36.62	<.0001
Tipo*VAZ_CO2	1	151492.0672	151492.0672	23.60	0.0004
Temperatura*Pressao	1	1260.7500	1260.7500	0.20	0.6655
Temperatura*VAZ_CO2	1	50960.3333	50960.3333	7.94	0.0155
Pressao*VAZ_CO2	1	1564.0833	1564.0833	0.24	0.6305
Tipo*Tempera*Pressao	0	0.0000	.	.	.
Tipo*Tempera*VAZ_CO2	1	245520.2500	245520.2500	38.25	<.0001
Temper*Pressa*VAZ_CO	0	0.0000	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Tipo	1	8363.9339	8363.9339	1.30	0.2759
rep(Tipo)	2	6767.1190	3383.5595	0.53	0.6033
Temperatura	1	185232.1000	185232.1000	28.86	0.0002
Pressao	1	23159.3058	23159.3058	3.61	0.0818
VAZ_CO2	1	18096.3333	18096.3333	2.82	0.1190
Tipo*Temperatura	1	10569.1429	10569.1429	1.65	0.2236
Tipo*Pressao	1	231361.0000	231361.0000	36.05	<.0001
Tipo*VAZ_CO2	1	295936.0000	295936.0000	46.11	<.0001
Temperatura*Pressao	1	31557.1429	31557.1429	4.92	0.0467
Temperatura*VAZ_CO2	1	50960.3333	50960.3333	7.94	0.0155
Pressao*VAZ_CO2	1	79524.0000	79524.0000	12.39	0.0042
Tipo*Tempera*Pressao	0	0.0000	.	.	.
Tipo*Tempera*VAZ_CO2	0	0.0000	.	.	.
Temper*Pressa*VAZ_CO	0	0.0000	.	.	.

Dependent Variable: d[4,3]

Tests of Hypotheses Using the Type I MS for rep(Tipo) as an Error Term

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Tipo	1	2.28937729	2.28937729	0.00	0.9816

Tests of Hypotheses Using the Type III MS for rep(Tipo) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Tipo	1	8363.933884	8363.933884	2.47	0.2565

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