FEDERAL UNIVERSITY OF RIO GRANDE DO SUL (UFRGS)
INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY (ICTA)
POSTGRADUATE PROGRAM IN FOOD SCIENCE AND TECHNOLOGY (PPGCTA)

ENCAPSULATION OF BIOACTIVE COMPOUNDS OF GARLIC AND GINGER USING DIFFERENT METHODS AND WALL MATERIALS

Loleny Tavares

Porto Alegre, 2020

Loleny Tavares

ENCAPSULATION OF BIOACTIVE COMPOUNDS OF GARLIC AND GINGER USING DIFFERENT METHODS AND WALL MATERIALS

Thesis presented to the Postgraduate Program in Food Science and Technology of the Federal University of Rio Grande do Sul, as a partial requirement for obtaining a Doctorate degree in Food Science and Technology.

Advisor: Prof. Dr. Caciano Pelayo Zapata Noreña

Porto Alegre, 2020

CIP - Catalogação na Publicação

Tavares, Loleny
Encapsulation of bioactive compounds of garlic and ginger using different methods and wall materials / Loleny Tavares. -- 2020.
314 f.
Orientador: Caciano Pelayo Zapata Noreña.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciência e Tecnologia de Alimentos, Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Porto Alegre, BR-RS, 2020.

1. Encapsulation. 2. Garlic. 3. Ginger. 4. Complex Coacervation. 5. Bioactive compounds. I. Noreña, Caciano Pelayo Zapata, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Approval of the thesis:

ENCAPSULATION OF BIOACTIVE COMPOUNDS OF GARLIC AND GINGER USING DIFFERENT METHODS AND WALL MATERIALS

Submitted by **Loleny Tavares** in partial fulfillment of the requirement for obtaining a Doctorate degree in **Food Science and Technology** at **Federal University of Rio Grande do Sul**, on November 27th 2020.

Examining Committee Members:

Prof. Caciano Pelayo Zapata Noreña, PhD (Advisor)

Federal University of Rio Grande do Sul (UFRGS)

Prof. Adriano Brandelli, PhD

Federal University of Rio Grande do Sul (UFRGS)

Prof. Nadya Pesce da Silveira, PhD

Federal University of Rio Grande do Sul (UFRGS)

Prof. Dr. Jose Maria Lagaron, PhD

Instituto de Agroquímica y Tecnología de Alimentos (IATA), Valencia - Spain.

DEDICATION

To Sara, Amacy and Christian

"O céu e o mar tornam-se pequenos
Ó minha mãe!
Frente à gratidão que tenho por ti
És a única capitã que segurou o leme
Do barco que carrega os sonhos dos teus filhos

Escolheste bem os tijolos
Para construir a vida dos teus filhos
Não na areia fofa de uma praia
Mas, com alicerces assentes em terreno sólido
És o pilar que tem suportado tempestades
Até as tempestades de saudades

A senhora não deixou faltar sorriso
Trabalhou para nos dar tudo
Tu és a minha maior inspiração
És sinônimo de superação

Quando me falta forças
É na tua fonte que busco energias
Para lutar e vencer cada desafio do dia-a-dia
Para continuar tendo a esperança
De escrever uma história
Que é minha, mas, também é sua

Gratidão por tudo que tens feito
Dinheiro algum pagará o teu esforço
Mas, saiba que dentro do meu peito
Há um coração que pula
Há sonhos que navegam em mares turbulentos
Mas, tu és sempre a minha bússola "
*Gift from my friend Lucindo Cardoso de Pina

ACKNOWLEDGMENT

First of all, I want to thank God Almighty for blessing me and giving me the opportunity to complete this journey.

Foremost, I would like to say a special thank you to my supervisor, Caciano Noreña, for believe in me, for their close support, excellent supervision, friendship and guidance during the running of this thesis.

I would like to thank my laboratory colleagues Andreia, Cassiano, Eduarda, Fernanda, Liliana and Mariane for their companionship, encouragement, knowledge exchange, friendship, partnership and insightful contributions in this thesis. Thank you for making my job more "easier".

Additionally, I would like to express my great gratitude to Lúcia Santos for all the support and the opportunity to develop part of this study (five months) at the Faculty of Engineering, University of Porto and all members' staff of the LEPABE (Laboratory for Process Engineering, Environment, Biotechnology and Energy) for all the support.

I would like to express my utmost gratitude to all members' staff and teachers of the Institute of Food Sciences and Technology of the Federal University of Rio Grande do Sul, for the support, guidance and insightful contributions in this thesis.

I want to thank my mother Sara, who I dedicate this thesis, for all the support during my entire life. I have a real queen in my life, who I dedicate this sentence: "*In life, having two pillars is incredible, but having one that is worth two is very rewarding and worthy of all the dedication"*.

Also, I expand my thank to my big brother Amacy, who presented me with a beautiful nephew Christian. Thank you brother for the partnership, friendship and support. Brother, thank you for everything!

I equally thank all of my parents, for the support, motivation, friendship, companionship and making me believe in a better future for us. You can always count on me for everything.

My sincere thanks to my friends Carlos Correia, Delmiro, Didi, José Pedro, Manuel, José Pedro, Didi, Manuel, compadre Delmiro, Alfredo and Patrick for the partnership, friendship and group conversations.

I would like to thank my African and Brazilian friends in Porto Alegre. All the friends are important, but I want to thank some who were more closer and had different contributions in this thesis: to my great friend Hélio (for his great contribution and partnership), Ana, António, Claudia, Deila, Emanuel, Ivanildo, Iraci, Jaell, Jocilene, Lavínea, Lucindo, Paul Michael, Shannon Lee, Stefon, Tania, Teresa, Vania and Zélia.

I would like to thank the research funding agency CAPES (Programa Estudantes-Convênio de Pós-Graduação (PEC-PG) for scholarship funding. I thank the CNPq and FAPERGS for financial support to our research laboratory, and the Primex (Siglufjordur, Iceland) and Arla Foods Ingredients by donations of chitosan and whey proteins isolates, respectively.

Last but definitely not the least, I would like to thank all my teachers, from childhood to this date, for the education, teachings and support.

ABSTRACT

The consumption of natural foods with bioactive compounds has been rapidly increasing over the word due to its biological effects to human organism. Garlic (*Allium sativum* L.) and ginger (*Zingiber officinale* R.) are two of these products rich in bioactive compounds and widely consumed around the world due to its multiple biological activities, prophylactic function and nutraceutical properties. However, after the extraction process, its bioactive compounds are exposed to adverse environmental conditions, such as high temperature, light and presence of oxygen, which may cause its degradation, decomposition, isomerization, volatilization, oxidation and polymerization. In this sense, this work presents different encapsulation methods and wall materials used in the protection of two derived garlic products (garlic extract (GE) and garlic essential oil (GEO)) and and ginger essential oil (GO) in order to protect its bioactive compounds and increase their stability. The encapsulation of GE was divided in three parts: in the first, the GE was encapsulated by complex coacervation method followed by spray draying using whey protein isolate (WPI) and chitosan (CH) with degrees of deacetylation (DD) of 83 (CH-83), 94 (CH-94) and 96% (CH-96) as wall materials; in the second, GE was encapsulated by complex coacervation followed by freeze-drying using complex of WPI/CH-96 and gum Arabic (GA)/CH-96 as wall materials; and in the third part, the GE was encapsulated in multilayer wall materials composed by complex of WPI/CH-96 and GA/CH-96 followed by addiction of polydextrose (POL) to act as bulking and stabilizer agent in order to reinforce the polymeric matrices of WPI/CH and GA/CH, followed by freeze-drying to obtain bioactive compounds-rich powders. In the first part, previously tests of coacervate yield and rheological measurements were realized before drying process, and the results of coacervate yield indicated that optimum complex coacervation occurred at the optimum CH:WPI mass ratios of 0.2:1 (w/w). The rheological measurements were analyzed in terms of viscosity and viscoelastic behavior determined by dynamic test (analyzing the elastic modulus G’ and viscous modulus G’’) and static test (creep and recovery). The results of viscosity indicated coacervates with shear-thinning behavior of pseudoplastic fluids, the viscoelastic behavior indicated formation of coacervate with an elastic gel structure (G’ higher than G’’), and the creep–recovery tests showed more compact and stronger internal structure for WPI/CH-96 coacervate. The dispersions of coacervate were spray-dried to obtain powders and the retention efficiency (*RE*) of phenolic compound ranged from 51 to 61%. The results showed that the high temperature used in the spray-drying process (160 ºC) caused degradation and decomposition of

some heat sensitive compounds present in GE. For this reason, in the second and third parts the studies were realized in order to reduce the use of high drying temperatures, applying the freeze-drying process to obtain powders. Thus, in the second study the *RE* of phenolic compounds were 84 and 78% for GA/CH and WPI/CH, respectively, whereas in the third study the *RE* were 84.30 and 92.64% for WPI/CH/POL and GA/CH/POL, respectively. Therefore, the use of freeze-drying instead spray drying revelead as a good alternative as dehydration method to obtain powders, since allowing to protect the heat sensitive compounds of GE and obtaining powders with higher concentration of phenolic compounds. In other study, GEO was encapsulated by molecular inclusion using β-cyclodextrin (βC) as wall material and complex coacervation method using soy proteins isolate (SPI) and chitosan (CH) at mass ratio of 1:0.125 (w/w) as wall materials. The rheological mensurements were performed before drying process, and the results indicated that the incorporation of GEO into wall systems resulted in dispersions with higher values of complex modulus (G*), complex viscosity (η*), G' and G'', indicating an increase in the intermolecular bonding and improvement in the biopolymers network structure. The *RE* of the freze-dryied powders were 82.03 and 71.74% using βC and complex of SPI/CH as wall materials, respectively. The βC/GEO and SPI/GEO/CH showed surface area of 4.74 and 4.41 $m^2 g^{-1}$ and an average pore diameter of 1.53 and 1.84 nm, respectively. Finally, it was studied the encapsulation of GO by complex coacervation followed by freeze-drying using complex of WPI/GA and GA/CH as wall materials. The best conditions for complex coacervation beween WPI/GA and GA/CH were obtained at mass ratios of 3:1 (w/w) and 5:1 (w/w), respectively. The determination of the rheological properties of the coacervate before freeze-drying process indicated that the Burgers model equation and exponential decay function were adequate to fit the experimental creep and recovery data, respectively. After freeze-drying of the coacervates, the *RE* of GEO present in the powders were 55.31 and 81.98% using complex of GA/CH and WPI/GA, respectively. In general, the results indicates that the bioactive compounds presents in GEO, GE and GO were incorporated into the dispersion matrices of the wall materials by physical interaction, according to Fourier-transform infrared spectroscopy (FTIR) analysis. The powders were thermally stable up to 220 °C, indicating that the wall materials contributed to the protection of heat-sensitive compounds present in GE, GEO and GO.

RESUMO

Nos últimos anos o consumo de alimentos naturais que contêm compostos bioativos aumentou em todo o mundo devido às suas propriedades biológicas benéficas para o organismo humano. Alho (*Allium sativum* L.) e gengibre (*Zingiber officinale* R.) são dois desses produtos ricos em compostos bioativos e que são consumidos em larga escala devido às suas múltiplas atividades biológicas, função profilática e propriedades nutricionais. No entanto, após o processo de extração e separação, esses compostos bioativos são expostos às condições ambientais adversas, tais como alta temperatura, presenças de luz e oxigênio, e podem sofrer degradação, decomposição, isomerização, volatilização, oxidação e polimerização. Nesse sentido, este trabalho apresenta diferentes métodos de encapsulamento e materiais de parede utilizados na proteção dos compostos bioativos presentes em dois produtos derivados do alho (extrato de alho (GE) e óleo essencial de alho (GEO)) e no óleo essencial de gengibre (GO). O encapsulamento do GE foi dividido em três partes: na primeira, o GE foi encapsulado pelo método de coacervação complexa seguido de atomização, através de utilização matérias de parede como proteína isolada do soro de leite (WPI) e quitosana (CH) com graus de desacetilação (DD) de 83 (CH- 83), 94 (CH-94) e 96% (CH-96); no segundo, o GE foi encapsulado por coacervação complexa seguido de liofilização usando complexos de WPI/CH-96 e goma arábica (GA)/CH-96 como materiais de parede; e na terceira parte, o GE foi encapsulado em materiais de parede formado por complexos de WPI/CH-96 e GA/CH-96, seguido de adição de polidextrose (POL) para atuar como agente estabilizador e de volume, e com isso reforçar as matrizes poliméricas dos dois complexos. Depois, realizou-se a liofilização de WPI/CH/POL e GA/CH/POL para a obtenção de pós ricos em compostos bioativos. Na primeira parte, testes preliminares de rendimento de coacervado e medições para caracterização das propriedades reológicas do coacervado foram realizados antes do processo de secagem, e os resultados do rendimento de coacervado indicaram que as condições ótimas de coacervação aconteceram nas proporções CH:WPI de 0,2:1 (p/p) para todos os coacervados. As propriedades reológicas dos coacervados foram analisadas em termos de viscosidade e comportamento viscoelástico, sendo a ultima determinada através de dois testes: dinâmico (analisando o módulo de elasticidade ou armazenamento (G') e o módulo de perdas ou viscoso (G")) e estático (fluência e recuperação). As análises de viscosidade indicaram que todos os coacervados têm comportamento de fluidos pseudoplásticos; as análises de propriedades viscoelásticas indicaram que os coacervados

apresentaram estruturas semelhantes a um gel rígido e elástico (G' maior que G''); e os testes de fluência e recuperação mostraram que o coacervado WPI/CH-96 tem uma estrutura interna mais compacta e forte em relação aos demais coacervados (WPI/CH-83 e WPI/CH-94). As dispersões de coacervados foram secas por atomização para a obtenção de pós e a eficiência de retenção (*RE*) dos compostos fenólicos presentes nos pós variou de 51 a 61%. Os resultados mostraram que a alta temperatura utilizada no processo de atomização (160 ºC) causou degradação e decomposição de alguns compostos termossensíveis presentes no GE. Por este motivo, na segunda e terceira partes deste trabalho foram realizados estudos no sentido de eliminar o uso de alta temperatura de secagem, sendo aplicado o processo de secagem por liofilização para a obtenção dos pós. Assim, no segundo estudo os resultados de *RE* dos compostos fenólicos foram de 84 e 78% para GA/CH e WPI/CH, respetivamente, enquanto que no terceiro estudo os resultados de *RE* foram de 84,30 e 92,64% para WPI/CH/POL e GA/CH/POL, respetivamente. O processo de secagem por liofilização permitiu obter pós com maiores concentrações de compostos fenólicos e revelou-se eficaz na proteção dos compostos termossensíveis presentes no GE. Em outro estudo, GEO foi encapsulado por dois métodos: inclusão molecular usando β-ciclodextrina (βC) como material de parede e coacervação complexa através do emprego de proteína isolada de soja (SPI) e quitosana (CH) na proporção de massa de 1:0,125 (p/p) como materiais de parede. A caracterização das propriedades reológicas dos coacervados foram realizadas antes do processo de secagem, e os resultados indicaram que a incorporação de compostos de GEO nas matrizes poliméricas dos biopolímeros resultou em coacervados com magnitudes superiores em termos de módulo complexo (G*), viscosidade complexa (η*), G' e G'', o que indica que houve um incremento nas ligações intermoleculares e consequente formação de coacervados com estruturas de rede mais rígida e compacta. Os resultados da eficiência de retenção de compostos de GO presentes nos pós foram de 82,03 e 71,74% usando βC e complexo de SPI/CH, respetivamente. Os pós de βC/GEO e SPI/GEO/CH apresentaram área superficial de 4,74 e 4,41 m^2g^{-1} e diâmetro médio de poros de 1,53 e 1,84 nm, respetivamente. Por fim, foi estudado o encapsulamento de GO por coacervação complexa seguido de liofilização através da utilização dos complexos de WPI/GA e GA/CH como materiais de parede. As melhores condições para coacervação complexa entre WPI/GA e GA/CH foram obtidas nas proporções de massa de 3:1 (p/p) e 5:1 (p/p), respetivamente. A determinação das propriedades reológicas dos coacervados antes do processo de liofilização indicou que o modelo de Burger e a função de decaimento exponencial foram adequados nos ajustes dos dados

experimentais de fluência e recuperação, respetivamente. Após o processo de liofilização dos coacervados, os resultados da eficiência de retenção dos compostos de GEO presentes nos pós foram de 55,31 e 81,98% usando o complexo de GA/CH e WPI/GA, respetivamente. No geral, os resultados de espectroscopia de infravermelho com transformada de Fourier (FTIR) indicaram que os compostos bioativos presentes no GEO, GE e GO foram incorporados nas matrizes dos biopolímeros através de interações físicas. Todos os pós apresentaram estabilidade térmica até 220 °C, o que indica que os materiais de parede contribuíram para a proteção dos compostos bioativos termossensíveis presentes no GE, GEO e GO.

FIGURE CAPTIONS

TABLE CAPTIONS

CONTENTS

INTRODUCTION

Actually, the consumption of natural food among the society has been rapidly increasing over the world due to an increase in public awareness of environmental issues and interest in healthy foods with biological effects. Garlic (*Allium sativum* L.) and ginger (*Zingiber officinale* R.) are two of these products widely consumed due to its multiple biological activities, prophylactic function and nutraceutical properties (QUESADA et al., 2020; SRINIVASAN, 2017). Garlic contains diverse bioactive compounds such as organosulfur compounds (the main constituents include alliin, allicin, ajoene, allylpropyl disulfide, diallyl polysulfides, vinyldithiins, diallyl sulfide, diallyl disulfide, diallyl trisulfide, allylmethyl trisulfide, diallyl tetrasulfide), saponins (proto-eruboside B and eruboside B) and phenolic compounds such as caffeic, ferulic, vanillic, *p*-hydroxybenzoic, *p*-coumaric and sinapic acids) (BEATO et al., 2011; CASELLA et al., 2013; KUETE, 2017; MATSUURA, 2001).

Ginger is a rich source of bioactive compounds, such as phenolic compounds (mainly composed by gingerol, shogaol, paradol, quercetin, zingerone, gingerenone-A, 6-dehydrogingerdione) and terpene compounds (composed by β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene), which are esponsable for their biological activities (ALI et al., 2008; GUNATHILAKE & RUPASINGHE, 2015; PRASAD & TYAGI, 2015; STONER, 2013). These compounds are responsible for the biological activities of garlic and ginger, including antioxidant, cardiovascular protective, anticancer, anti-inflammatory, immunomodulatory, anti-diabetic, anti-obesity, and antibacterial properties (GUNATHILAKE & RUPASINGHE, 2015; MAO et al., 2019; MARTINS et al., 2016; TOULOUPAKIS & GHANOTAKIS, 2010).

The bioactive compounds of garlic and ginger are unstable and susceptible to losing its biological activities when exposed to adverse environmental conditions, such as high temperature and presence of oxygen and light (FERNANDES et al., 2016; ILIĆ et al., 2017; TAVARES et al., 2019; TAVARES & NOREÑA, 2019, 2020). The encapsulation technology have been used in a wide range of applications to protect the unstable active compounds within a protective matrix (ZUIDAM & HEINRICH, 2010). Encapsulation is defined as a process that involves the coating or entrapment of one or mixture compounds (core material) within another carrier material (wall material) with capacity to protect the functional properties of the encapsulated compounds (SAIFULLAH et al., 2019).

Based on the overview described in the preview paragraph, the main objectives of this study were to encapsulate two derived garlic products (garlic essential oil (GEO) and garlic extract (GE)) and ginger essential oil (GO) in order to protect its bioactive compounds using three encapsulation methods (complex coacervation, spray drying and molecular inclusion) and six wall materials (chitosan, whey protein isolate, polydextrose, gum Arabic, soybean protein isolate and β-cyclodextrin). These wall materials are products with functional characteristics and desired physicochemical properties to be used as a protective agent in the encapsulation of the bioactive compounds obtained from garlic and ginger. Chitosan (CH) is a cationic polysaccharide obtained by alkaline N–deacetylation of chitin, and extensively applied in the food and pharmaceutical industries due to its physicochemical property of dietary fiber, biocompatibility, biodegradability, non-toxicity, antioxidant and antimicrobials activities (ABDELMALEK et al., 2017; MUJTABA et al., 2019; TAN et al., 2020). Whey protein isolate (WPI) is a coproduct of the dairy industry, obtained during the industrial production of cheese or casein; It consists in a mix of globular proteins with a minimum of 90% of proteins, mainly composed by β-lactoglobulins and α-lactalbumin as major proteins which are an important source of bioactive peptides responsible for its biological activities (BRANDELLI et al., 2015; DE CASTRO et al., 2017; NORWOOD et al., 2016). WPI is widely used in the food industry due to its high nutritional properties and its capacity for gelatinization, film formation, emulsification, solubility in water and other functional activities (BRANDELLI et al., 2015; DE CASTRO et al., 2017; ROCHA et al., 2014). Gum Arabic (GA) is a natural polysaccharide obtained from trunks and branches of two species of acacia trees: *Acacia senegal* and *Acacia seyal* (SHI et al., 2017). GA have been used as wall material due to its low cost, non-toxicity, biocompatibility, biodegradability, good capacity to act as a stabilizer, dietary fiber, emulsifier and protection against oxidation (ALI et al., 2009; FERNANDES et al., 2016; WU et al., 2018). Soybean protein isolate (SPI) has been widely used in the food industry due to its nutritional benefits, and its functional properties such as gelation, water solubility, emulsifiability and foamability (NISHINARI et al., 2014). Polydextrose (POL) is a bulking and stabilizer with high solubility in water, prebiotic effect, and functional properties such as dietary fiber and low glycemic index (MITCHELL, 1996). β-cyclodextrin (β-CD) is a dietary fiber (not absorbed in the upper gastrointestinal tract, and are completely metabolized by the colon microflora) and represent the most common type of cyclodextrin used as wall material due to its non-toxicity, capacity to reduce foaming, stabilize emulsion, decrease enzymatic browning, and enhancing solubility,

controlled release and stability of the encapsulated compounds (SAFFARIONPOUR, 2019; SZENTE & SZEJTLI, 2004).

In this context, it is important to carry out studies that aim to produce powders with encapsulated bioactive compounds of garlic and ginger to be used as active ingredients for application in food products in order to pretect its biological activities during the processing, handling and storage conditions.

MAIN OBJECTIVE

The main objective of this study was to encapsulate the bioactive compounds obtained from two by-product of garlic (garlic extract (GE) and garlic essential oil (GEO)) and ginger essential oil (GO) using different encapsulation methods and wall materials.

SPECIFIC OBJECTIVES

- Encapsulate the garlic extract (GE) using protective wall materials (chitosan (CH), whey protein isolate (WPI), gum Arabic (GA) and polydextrose (POL)) and method of complex coacervation followed by either spray drying or freeze-drying.
- Study the effect of the degree of deacetylation (DD) of CH according with its positive amino groups (NH_3^+) to interact electrostatically with negative carboxyl groups ($–COO^–$) of whey protein isolate in order to produce high coacervate yield to be used as wall materials in the protection of GE.
- Encapsulate garlic essential oil (GEO) by the methods of molecular inclusion and complex coacervation using β-cyclodextrin (βC) and complex of soybean protein isolate (SPI)/CH, respectively, as wall materials;
- Encapsulate ginger essential oil (GO) by the method of complex coacervation followed by freeze-drying using the complex of WPI/GA and GA/CH as wall materials.
- Study the rheological properties of the dispersions before drying process and the physcochemical, structural, thermal, storage stability and functional properties of the produced bioactive-riched powders.
- Study the influence of GEO incorporations on the network structure of the WPI/CH by applying different rheological measurements to determine the viscosity and viscoelastic properties of the coacervate with and without GEO.

THESIS STRUCTURE AND CONTENT

The present thesis is organized in four parts that include a total of ten Chapters and seven scientific Articles (Fig. 1).

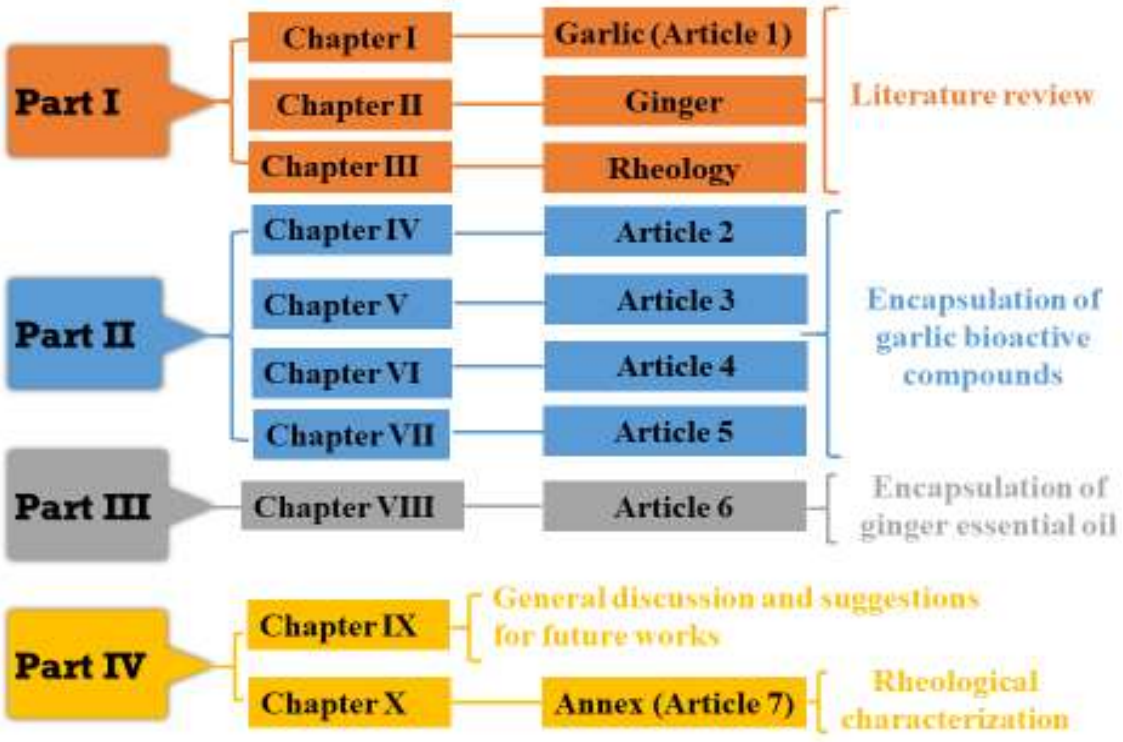

Fig. 1. Scheme representing the organization of the thesis.

Part I contains three **Chapters** (**I**, **II** and **III**) corresponding to the literature review. The **Chapter I** presents the **Article 1** that contains the literature review of encapsulation process of bioactive compounds present in garlic extract (GE) and garlic essential essential oil (GEO). The **Chapters II** and **III** presents a literature review about ginger and rheology, respectively. These three **Chapters** (**I**, **II** and **III**) contains an overview of the main concerns of this work and the theoretical concepts applied in the elaboration of six research papers. Therefore, the **Article 1** presents a literature review relating to the bioactive compounds of garlic, their biological activities, and four main encapsulation methods (molecular inclusion, spray drying, complex coacervation and liposome entrapment) used in the encapsulation of two derived garlic products (garlic extract (GE)

and garlic essential oil (GEO)). The review paper presents the fundamental principles, advantages, disadvantages/limitations of each encapsulation methods used in the encapsulation of GE and GEO in order to protect its bioactive compounds and mask its pungent smell. Each of these methods is discussed, and its influences on the structural, antimicrobial and physicochemical properties of produced nano/microparticles were highlighted, with special focus on the characterization techniques of zeta potential, rheological analyses, Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The review contains the potential applications of GE and GEO nano/microparticles as active ingredients in food products. The **Chapter II** presents literature review about ginger and an overview of encapsulation technologies used in the protection of its bioactive compounds. The **Chapter III** presents literature review about rheology, experimental devices and rheological measurements.

The **Part II** presents the Chapter **IV** to **VII** corresponding to the four research papers published or submitted to an international *peer-reviewed* journals about to the encapsulation of garlic extract (GE) and garlic essential oil (GEO). Each of these Chapters provide a specific sections of "abstract", "introduction", "material an methods", "results and discussion", and a specific list of references. The **Chapter IV** contains the **Article 2** elaborated based on the encapsulation of GE by complex coacervation followed by spray drying using the complex of whey protein isolated (WPI) with chitosan (CH) as wall materials. In this article, preliminary studies were carried out to determine the optimal conditions of pH and biopolymers mass ratio (between WPI and CH with degree of deacetylation (DD) of 83, 94 and 96%, representing by WPI/CH-83, WPI/CH-94 and WPI/CH-96, respectively). The effect of chitosan DD to interact electrostatically with whey protein isolate (WPI) was investigate in order to produce high coacervate yield. Subsequently, the zeta potential of the dispersions containing GE, WPI, CH was determined to assess the existence of electrostatic interactions between each CH (with positively charged amino groups ($–NH_3^+$) and WPI (with negatively charged carboxyl groups ($-COO^–$). Measurements of rheological properties were employed in order to evaluate the viscosity and viscoelastic properties of the coacervates. The viscoelastic properties were determined by two tests: dynamic (analyzing the elastic module G' and the viscous module G") and static (by analyzing creep and recovery phases). The Burgers model and the exponential decay function were used to adjust experimental creep and recovery data, respectively. Then, the three types of dispersions containing GE (WPI/CH-83, WPI/CH-94 and

WPI/CH-96) were spray-dried to obatain powders. The obtained powders were characterized using different characterization techniques, such as FTIR, TGA, SEM, differential scanning calorimeter (DSC), antioxidant activity by the ABTS method, content of total phenolic compounds, solubility, moisture content, hygroscopicity and water activity.

The **Chapter V** contains the **Article 3** based on the encapsulation of garlic extract (GE) by complex coacervation followed by freeze-drying using complexes of WPI/CH and gum Arabic (GA)/CH as wall materials. This study was developed following the **Articles 2** with the main purpose to substitute the dehydration process of spray drying by the freeze-drying. In this study, the complex of WPI/CH-96 was also used as wall material since from the **Article 2** was possible to conclude that the WPI/CH-96 powders have better physicochemical properties than WPI/CH-83 and WPI/CH-94 powders. In addition to the characterization techniques used in the **Article 2,** from the **Article 3** were used others characterizations techniques to evaluate the properties of the produced freeze-dried microparticles powders, such as: a) the nitrogen adsorption/desorption isotherm for determining the area and pore size of the microparticles; b) the water sorption isotherm to assess the storage stability of the powders when exposed to higher levels of relative humidity; c) particle size distribution to determine the size of the produced powders; and d) diffraction of X-rays to study the physical structure of the powders. The GAB (Guggenheim, Anderson and Boer), Halsey, Henderson, Smith and Oswin models were used to adjust the experimental water adsorption isotherm data.

The **Chapter VI** presents the **Article 4**, where the GE was encapsulated into the matrices of two types of multilayer walls composed by WPI/CH/polydextrose (POL) and GA/CH/POL. This study was developed following the **Articles 2** and **3**, with the main purpose to study the storage stability of the produced powders when exposed to higher levels of relative humidity and tempetarure of 30, 40 and 50 ºC. The structures of the polymeric matrices of WPI/CH and GA/CH were reinforced with polydextrose (POL) to act as bulking volume and stabilizer agent, followed by freeze-drying to obtain bioactive compounds-rich powders denominated by WPI/CH/POL and GA/CH/POL. The storage stability of the produced powders was evalued according to the analyses of water adsorption isotherms and integral thermodynamic properties determined at 30, 40 and 50 °C. The surface area and pore sizes of the powders were determined using the water and nitrogen experimental adsorption data.

The **Chapter VII** contains the **Article 5** based on the encapsulation of garlic essential oil (GEO) using two encapsulation methods: molecular inclusion using β-cyclodextrin (βC) as wall material and complex coacervation using soy proteins isolate (SPI) and chitosan (CH) as wall materials. This study was developed following the **Articles 2**, **3** and **4**, with the main propuse to use GEO instead GE. The GE is a crude mixture of several polar and nonpolar compounds with different molecular size and properties. In this sense, the proposal of the **Article 5** was to use a derived garlic product rich in organosulfur compounds, namely GEO. From the **Article 5**, dynamic rheological analysis was performed for the dispersions with GEO (βC/GEO and SPI/GEO/CH) and without GEO (βC and SPI/CH) before freeze-drying process to obtain microparticle powders. The produced powders were characterized by entrapment yield, particle size distribution, moisture content, water activity, hygroscopicity, solubility, surface area, average pore diameter, FTIR, TGA and SEM.

The **Part III** presents the **Chapter VIII** that contains the **Article 6** based on the encapsulation of ginger essential oil (GO) by complex coacervation using WPI/GA and GA/CH-96 as wall materials. This article was designed as a way to evaluate the capacity of WPI, GA and CH-96, used as wall materials in the **Article 2** and **3**, in the protection of the bioactive compounds present in GEO. From **Article 6**, premilinary studies of rheological mensurements were carried out to determine the viscosity and viscoelastic properties of coacervates with and without GO. The Burgers model and the exponential decay function were used to adjust experimental fluency and recovery data, respectively. The microparticles powders were obtained by freeze-dryng and then characterized through analysis of their physical, chemical and structural properties through tests of FTIR, TGA, SEM, solubility, humidity and hygroscopicity.

Part IV contains the **Chapter IX** and **Chapter X**. The **Chapter IX** provides a general dissussion and conclusion, suggestions for future works and a specific list of references used in the introduction of this thesis. The **Chapter X** presents the Annex with the **Article 7** elaborated in order to understand the influence of GEO incorporation on the network structure of the complex of WPI/CH by applying different rheological measurements. The viscoelastic properties were determined by the dynamic oscillatory test (stress, frequency, temperature and time sweeps) and static test (creep and recovery). Arrhenius model was used to estimate the relationship of viscosity and temperature (to determine the energy of activation), the Carreau-Yasuda model was used to fit the experimental complex viscosity data, and Burgers model and the exponential decay function were used to adjust experimental creep and recovery data, respectively.

The chemical strutures of wall materials used in this thesis, such as polydextrose, β-cyclodextrin, gum Arabic and chitosan are represented in Fig. 2. For soy protein isolate and whey protein isolate, the general structure of the amino acids that make up the proteins are represented in Fig. 3.

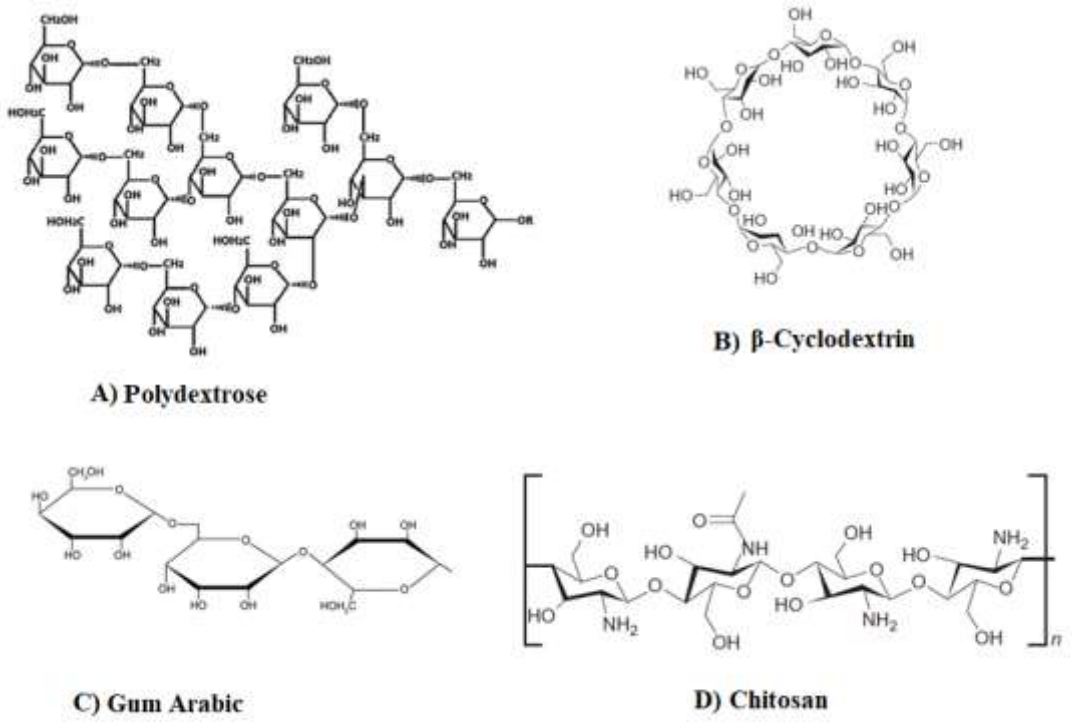

Fig. 2. The structures of A) Polydextrose adapted from DEMARCHI et al.(2014) ; B) β-Cyclodextrin adapted from HU (2007); C) Gum Arabic adapted from (2018); D) Chitosan adapted from LALANI and MISRA (2011).

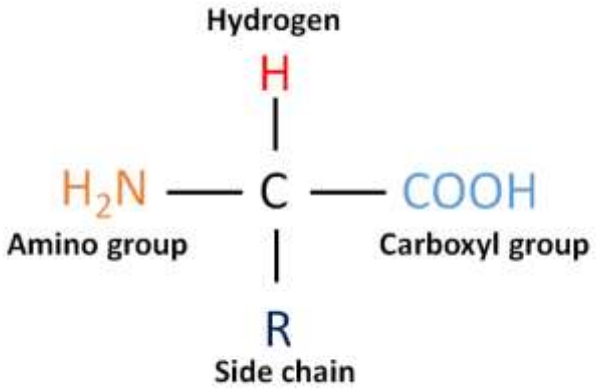

Fig. 3. General structure of the amino acids that make up the soy protein isolate and whey protein isolate. Adapted from MUTHARASAPPAN et al. (2020).

Part I

*This section provides three chapters: The **Chapter I** presents the **Article 1** that contains a literature review of garlic, different encapsulation methods and wall materials used in the encapsulation of two derived garlic products (garlic extract (GE) and garlic essential oil (GEO)) in order to protect its bioactive compounds and mask its pungent smell. **Chapter II** presents the literature review of ginger and its bioactive compounds. **Chapter III** provides a literature review of rheology and the theoretical concepts to be used in the next sections.*

Chapter I

The **Chapter I** was based on the following **Article 1** submitted to an international *peer-reviewed* journal:

Tavares, L., Santos, L., & Noreña, C. P. Z. (2020). Bioactive compounds of garlic: a comprehensive review of encapsulation technologies and characterization of the encapsulated garlic compounds.

Chapter II

The **Chaper II** contains a literature review of the following food product:

Ginger

Chapter III

The **Chaper III** contains a literature review of the following science that describes the deformation and flow behavior:

Rheology

Part II

*This section presents the **Chapter IV** to **VII** corresponding to the four scientific papers published or submitted to in international peer-reviewed journals about to the encapsulation of garlic extract and garlic essential oil (GEO). Each of these Chapters provides a specific sections of "abstract", "introduction", "material and methods", "results and discussion", and a specific list of references.*

Chapter IV

The **Chapter IV** was based on the following published **Article 2**:

Tavares, L., & Noreña, C. P. Z. (2019). Encapsulation of garlic extract using complex coacervation with whey protein isolate and chitosan as wall materials followed by spray drying. ***Food Hydrocolloids***, *89*, 360-369. doi: 10.1016/j.foodhyd.2018.10.052

Chapter V

The **Chapter V** was based on the following published **Article 3:**

Tavares, L., Barros, H. L. B., Vaghetti, J. C. P., & Noreña, C. P. Z. (2019). Microencapsulation of garlic extract by complex coacervation using whey protein isolate/chitosan and gum Arabic/chitosan as wall Materials: Influence of Anionic Biopolymers on the Physicochemical and Structural Properties of Microparticles. ***Food and Bioprocess Technology,*** *12*(12), 2093-2106. doi: 10.1007/s11947-019-02375-y.

Chapter VI

The **Chapter VI** was based on the following **Article 4** published to an international *peer-reviewed* journal:

Tavares, L., & Noreña, C. P. Z. (2020). Characterization of the physicochemical, structural and thermodynamic properties of encapsulated garlic extract in multilayer wall materials. ***Powder technology***, *378*, 388-399. Doi: 10.1016/j.powtec.2020.10.009.

Chapter VII

The **Chapter VII** was based on the following the **Article 5** submitted to international *peer-reviewed* journal:

Tavares, L., Santos, L., & Noreña, C. P. Z. (2020). Microencapsulation of organosulfur compounds from garlic essential oil using β-cyclodextrin and complex of soy protein isolate and chitosan as wall materials: a comparative study.

Part III

*This section provides the **Chaper VIII**, corresponding to the **Article 6** based on the encapsulation of ginger essential oil (GO) by complex coacervation in order to protect its bioactive compounds against adverse environmental conditions, such as light, pH, oxygen, light, heat and high shear mixer.*

CHAPTER IX

This **Chapter VI** was based on the following published **Article 6**:

Tavares, L., & Noreña, C. P. Z. (2020). Encapsulation of ginger essential oil using complex coacervation method: Coacervate formation, rheological property, and physicochemical characterization. ***Food and Bioprocess Technology***, 1-16. doi: 10.1007/s11947-020-02480-3.

Part IV

*This section provides two Chapters: the **Chaper IX** with a general discussion, conclusion suggestions for future work and general references used in the section of introduction, and t*he **Chapter X** that contains the Anenex of this thesis with the **Article 7** (in progress):

Chaper IX

GENERAL DISCUSSION AND CONCLUSION

The work outlined in this thesis is the result of a research proposal designed to study the encapsulation of two derived garlic products (the garlic extract (GE) and garlic essential oil (GEO)) and ginger essential oil (GO) with bioactive compounds in order to protect them against adverse environmental conditions, such as high temperature, oxygen and light, and mask their pungent smell. The encapsulation of GE were employed using three different methods: a) complex coacervation followed by spray draying using whey protein isolate (WPI) and chitosan (CH) with degrees of deacetylation (DD) of 83, 94 and 96%, represented by CH-83, CH-94 and CH-96, as wall materials (Chapter IV; Article 2); b) complex coacervation followed by freeze-drying using complex of WPI/CH-96 and gum Arabic (GA)/CH as wall materials (Chapter V; Article 3); and c) complex coacervation using complex of WPI/CH-96 and GA/CH-96 followed by addition of Polydextrose (POL) to act as bulking and stabilizer agent in order to reinforce the polymeric matrices of WPI/CH and GA/CH, followed by freeze-drying to obtain bioactive compounds-rich powders of WPI/CH/POL and GA/CH/POL (Chapter VI; Article 4). The encapsulation of GEO was made through molecular inclusion and complex coacervation methods using β-cyclodextrin (βC) and complex of soy proteins isolate (SPI) and chitosan (CH), respectively, as wall materials (Chapter VII; Article 5). The encapsulation of ginger essential oil (GO) was performed by complex coacervation using WPI/GA and GA/CH-96 as wall materials (Chapter VIII; Article 6).

The first approach involved the study of optimum conditions for complex coacervation between CH with amino sites positively charged ($–NH_3^+$) and WPI with negatively charged carboxyl groups (–COO−) in order to produce high coacervate yield (Chapter IV, Article 2, Fig. 13). CH with a higher DD (CH–96) has a greater number of free amino groups in the polymer backbone and the results of coacervate yield of CH/WPI complex increased significantly ($p < 0.05$), attaining the highest value at a CH/WPI mass ratio of 0.2:1 (w/w), followed by a decrease in yield until there was no coacervate formation at a CH:WPI mass ratio of 1:1 (w/w) (Article 2, Fig. 13). The optimum condition for the formation of CH/WPI complex was verified at mass ratio of 0.2:1 (w/w) for all coacervates, revealing that this mass ratio is crucial for maximum interactions between CH and WPI (Fig. 13). The zeta potential values of all the dispersions reached zero, confirming the

formation of agglomerations as a result of electrostatic interactions between CH with positively charged amino groups ($–NH3^+$) and WPI with negatively charged carboxyl groups ($–COO^–$). This agglomeration confirms the existence of complex coacervate in the dispersion containing GE compounds. The coacervates showed the typical shear-thinning behavior of a pseudoplastic fluid (Fig. 14), and the results of dynamic viscoelasticity measurement showed dominant elastic behavior with G′ values higher than G″ values, implying the formation of an elastic gel structure promoted by electrostatic interactions (Fig. 15). The creep properties of the coacervates were better for complex coacervate produced with CH/96, which is consistent with the dynamic viscoelasticity measurement results (Fig. 15). These characteristics are desirable during the microparticles productions, where microparticles with strength network structure and high shear resistance are less affected by structural disintegration of the network chain. The CH-96/WPI powder revealed a superior capacity for phenolic compound retention and better antioxidant properties, indicating that CH with DD of 96% is the most indicate to interact with WPI (Table 6). The produced microparticles showed spherical morphology, no evidence of cracking and craters, and non-porous surfaces (Fig. 17), and revealing good protection of bioactive garlic compounds. The microparticles are thermally stable up to 220 °C, indicating that the wall materials contributed to the protection of heat-sensitive compounds present in GE (Fig. 18). TGA profile indicated there stages of weight loss, where the weight loss in the first stage depends strongly on the encapsulation efficiency, moisture content of the powders, and the capacity of the wall materials in the protection of the volatiles compounds (Fig. 16). The weight loss in the second and third stages result as degradation and decomposition of the wall materials components. These results can be used as one of the indicators to choose the most suitable food products for microparticles application, since the results provide important information about the thermal stability of the powders, allowing to improve the processing and storage conditions. The garlic compounds interacted physically with wall systems, and the absence of new chemical bonds was confirmed by FTIR analysis (Fig. 20).

The microparticles showed high water solubility, enabling their application in different formulations in food industries (Table 7). However, the high temperature used in the spray-drying process caused degradation and decomposition of some heat sensitive compounds present in GE, obtaining retention efficiency (*RE*) of phenolic compound ranged from 51% to 61% (Table 6).

Attending that the main goal of this work is protect the bioactive compounds of GE, in the Chapter V and Article 3, the GE compounds were encapsulated by complex coacervation followed by

freeze-drying using complex of WPI/CH-96 and gum Arabic (GA)/CH as wall materials. The use of freeze-drying as dehydration method revealed a good alternative for spray drying, since this method allowed to obtain WPI/CH and GA/CH microparticles with high concentration of phenolic compounds with *RE* values of 84 and 78%, respectively (Chapter IV, Article 3, Table 8). The GA was used in this study due to its low cost, non-toxicity, biocompatibility, biodegradability, good capacity to act as a stabilizer, dietary fiber, emulsifier and protection against oxidation. The use of the negative carboxyl groups ($–COO^–$) of GA was better than WPI for coacervation with positive amino groups (NH_3^+) of CH, allowing yo produce powders with less hygroscopicity, smaller particle size and higher retention of garlic phenolic compounds (Table 8). The microparticles revealed a non-spherical morphology and resembles blocky with irregular shape and different sizes, characteristic of powders obtained by the freeze-drying process (Fig. 22). The WPI/CH and GA/CH microparticles showed with amorphous structure, with surface area of 2.23 and 2.40 $m^2 g^{-1}$ and average pore diameter of 5.20 and 5.37 nm, respectively. The sorption characteristics of microparticles followed the type II isotherm and Guggenheim-Anderson-de Boer (GAB) model was the best model to fit the experimental data (Fig. 24; Table 10). It was verified that microparticles showed higher capacity to adsorb water at high water activities, and the equilibrium moisture content increased with the increase of water activity (Fig. 24; Table 10). These results are mainly due to the high hygroscopic capacity of the microparticles, resulting in powders with a great capacity to adsorb larger quantities of water at high relative humidity conditions (Table 8). The microparticles showed low water solubility (Table 8). However, the WPI/CH and GA/CH microparticles can be used as ingredients and applied into solid, semisolids and liquid food products, such as soup and bakery products due to its high content of phenolic compounds and antioxidant activity.

In the subsequent part of work (Chapter VI, Article 4), the GE compounds were encapsulated using multilayer matrices composed by WPI/CH-96/POL and GA/CH-96/POL followed by freeze-drying to obtain powders. The values of *RE* were 84.30 and 92.64% for WPI/CH/POL and GA/CH/POL, respectively (Table 13). The higher values of *RE* obtained in this study when compared with the above study (Chapter IV, Article 3, Table 8), indicates losses of some phenolic compounds in the supernatant phase separated from the biopolymer rich phase during the complex coacervation process ocourred in the Article 3. The water adsorption isotherms and thermodynamic properties of WPI/CH/POL and GA/CH/POL powders were studied in order to evaluate its surface

structure, prediction of shelf life and better conditions of drying, processing and storage. The compounds of GE were incorporated into the matrices of the wall materials by physical interactions, confirmed by FTIR analysis (Fig. 26). The WPI/CH/POL and GA/CH/POL powders exhibited a type II sigmoid shape, which are common to most foods products (Fig. 29). Different models were used to adjust the experimental water sorption data and the GAB model gave the best fit for all the adsorption isotherm, giving the lowest values of average relative deviation (*E*) and coefficient determination (R^2) higher than 0.99, whereas the Halsey, Smith, Oswin and Peleg models failed to describe some of the three sorption isotherms (Table 12). Thermodynamic properties from adsorption data provide important information about water binding behaviors and the energy requirements to remove the moisture content from the WPI/CH/POL and GA/CH/POL powders. The water adsorption processes of both powders are enthalpy-controlled (values of isokinetic temperature higher than harmonic mean temperature) and non-spontaneous process (positive values of Gibbs free energy positive) (Fig. 31). The results of surface areas and pore sizes of the powders were higher when determined using the water adsorption data instead nitrogen adsorption data, and these results indicated that the water molecules were able to penetrate higher narrow pores than nitrogen molecules (Table 15).

Taking the results of GE microparticles in consideration, the Article 5 (Chapter VII) aimed to encapsulate GEO. The approach of this study aimed to work with GEO instead of GE, since the last is a crude mixture of several polar and nonpolar compounds with different size and properties. GEO was successfully encapsulated through molecular inclusion and complex coacervation methods using β-cyclodextrin (βC) and complex of soy protein isolate (SPI) and chitosan (CH), respectively, as wall materials (Table 16). The results showed significant differences in the structural and physicochemical properties of the microparticles obtained by these two encapsulation methods. The molecular inclusion using βC as wall material resulted in microparticles with better physical-chemical characteristics, in terms of higher solubility, and lower moisture content, hygroscopicity and water activity than complex of SPI/CH (Table 16). The rheological behavior of the dispersions showed that the incorporation of GEO into βC and SPI/CH resulted in higher values of storage modulus (G') than loss modulus (G''), revealing viscoelastic solid-like behavior (Fig. 35). Incorporation of GEO into βC and SPI/CH had substantial effects on their structure, and the SPI/GEO/CH dispersion exhibited the stronger gel strength and formation of strand-type network structure. The electrostatic interaction between carboxyl groups (COO^-) of

SPI and protonated amine groups (NH_3^+) resulted in dispersion with network structure with stronger gel strength. These characteristics are desirable during the microparticles productions, where microparticles with strength network structure and high shear resistance are less affected by structural disintegration of the network chain. FTIR study of microparticles revealed no chemical cross-linking reaction between garlic compounds and wall materials complexes, indicating that the garlic compounds were incorporated into complex wall materials by physical interaction (Fig. 39). The Article 6 (Chapter VIII) was designed as a way to understand the properties of the wall materials used in the Articles 2 and 3 in other to protecte ginger essential oil (GO) instead GE or GEO. Therefore, the study aimed to encapsulate GO by complex coacervation using WPI/GA and GA/CH-96 as wall materials. The rheological characterization of coacervate, before drying process, using the oscillatory frequency sweep tests revealed that G'' predominated over G' for the both complex coacervate at low frequency values, and a crossover between the G' and G'' occurred at about 5 Hz for GA/CH and at 60 Hz for WPI/GA (Fig. 45). The magnitudes of both G' and G'' were higher for GA/CH than obtained for WPI/GA. The creep–recovery tests showed that the coacervates with GO resulted in higher compliance values and weaker internal network structures (Fig. 46). The Burgers model equation and exponential decay function adequated well the experimental data and describe the coacervates creep and recovery behavior, respectively (Table 17 and 18). TGA showed that wall materials contributed to a significant increase in the GO thermal stability and also evidenced some non-encapsulated GO present on the surface of WPI/GO/GA powders (Fig. 47). FTIR analyses revealed that only physical interactions occurred between the functional groups of GO and of WPI/GA and GA/CH complexes (Fig. 49). The entrapment efficiency was 55.31 and 81.98% using complex of GA/CH and WPI/GA, respectively, revealing GA/CH as a more efficient complex for the GO protection ($p < 0.05$) (Table 19). The Article 5 the results demonstrated that incorporation of GEO into the matrices of wall systems resulted in dispersions with higher values of complex modulus (G*), complex viscosity (η*), G' and G'', indicating increasing in the molecular entanglements and formation of more compact strutures. However, for the Article 6, the creep–recovery tests showed that the coacervates with GO resulted in higher compliance values and weaker internal network structures. In this sense, it is possible to conclude that the network structure of coacervate is affected by the the nature, composition, charge, molecular size and density of the essential oil, and also by the type of wall material, ratio and molecular interactions between the wall material and essential oil. Cryo-scanning electron

microscopy (cryo-SEM) technique is presented in the next section as a way to resolve this issue, since this technique allows to analyze the morphology of the network structure of the coacervate with and without essential oil. In this sense, the Article 7 (in progress and localized at the annex of this thesis) is being developed as a way to understand the main phenomena associated to the network structure of the coacervate with GEO.

Overall, the reported results demonstrated that GE, GEO, GO powders could be used as active ingredients to improve the functional properties of food products and this study can be used for optimizing the processing and storage conditions of the microparticles and to improve the quality and increase the shelf life of the bioactive compounds-rich powders.

Chapter X

The **Chapter X** contains the Anenex of this thesis with the **Article 7** (in progress):

Annex

Article 7

Characterization of the rheological properties of the complex coacervates composed by whey protein isolate, garlic essential oil and chitosan