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Evaluación de aceites esenciales encapsulados con mucílago de chía por coacervación compleja utilizando ultrasonido y microfluidización para su aplicación como antimicrobianos en alimentos

Tesis presentada en cumplimiento parcial de los requisitos para obtener el Grado de Doctor en Ciencia de Alimentos

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Evaluation of essential oils encapsulated with chia mucilage by complex coacervation, using ultrasound and microfluidization, for application as antimicrobials in food products

In partial fulfillment of the requirements for the Degree of Doctor in Food Science

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La Dra. María Teresa Jiménez Munguía, profesora del Departamento de Ingeniería Química, Alimentos y Ambiental de la Universidad de las Américas Puebla, hace constar que:

la tesis titulada ***"Evaluación de aceites esenciales encapsulados con mucílago de chía por coacervación compleja utilizando ultrasonido y microfluidización para su aplicación como antimicrobianos en alimentos"***, presentada por Ruth Hernández Nava, para optar por el grado de Doctora en Ciencia de Alimentos por la Universidad de las Américas Puebla, ha sido realizada en dicha universidad, bajo su dirección, y que reúne las condiciones necesarias para ser defendida por su autora.

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DISSERTATION OUTLINE

The present doctoral thesis titled "Evaluation of essential oils encapsulated with chia mucilage by complex coacervation, using ultrasound and microfluidization, for application as antimicrobial in food products" is organized into four chapters. Chapter one is a review of complex coacervation as microencapsulation method. Chapter two present the study of the conditions to form complex coacervates between gelatin and chia mucilage. Chapter three presents the evaluation of the effect of oregano essential oil content in the formation of complex coacervates, as well as the effect of spray drying process conditions in the physicochemical properties of the powders obtained. Chapter four presents the study concerning the physicochemical properties of spray-dried coacervates, homogenized by ultrasound or microfluidization, containing essential oil of oregano or thyme. In addition, the antimicrobial activity of the reconstituted powders against *Escherichia coli* in green juice was also analyzed.

Since the chapters are published or being submitted to selected journals where styles vary, the format varies among chapters (sections and references). Full citations of the chapters included in this thesis are as follows:

Chapter 1

Hernández-Nava, R. & Jiménez-Munguía, M.T. (2017). Coacervación compleja: una altermativa como método de encapsulación. *Temas Selectos en Ingeniería de Alimentos*, *11*, 22-28 (Spanish in original version/ English translation in this document).

Chapter 2

Hernández-Nava, R., López-Malo, A., Palou, E., Ramírez-Corona, N., & Jiménez-Munguía, M. T. (2019). Complex Coacervation between Gelatin and Chia Mucilage as an

Alternative of Encapsulating Agents. *Journal of Food Science*, *84*(6), 1281-1287. DOI: 10.1111/1750-3841.14605.

Chapter 3

Hernández-Nava, R., López-Malo, A., Palou, E., Ramírez-Corona, N., & Jiménez-Munguía, M. T. (2020). Encapsulation of Oregano Essential Oil (*Origanum vulgare*) by Complex Coacervation between Gelatin and Chia Mucilage and its properties after spray drying. *Food Hydrocolloids*, accepted. DOI: 10.1016/j.foodhyd.2020.106077.

Chapter 4

Hernández-Nava, R., López-Malo, A., Palou, E., Ramírez-Corona, N., & Jiménez-Munguía, M. T. (2020). Essential oils encapsulated by complex coacervation using ultrasound or microfluidization homogenization and its evaluation as antimicrobials in green juice. Under review.

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ABSTRACT

Complex coacervation is an encapsulation technique that involves the electrostatic attraction between biopolymers of opposite charges, and that has been applied in several industries such as the pharmaceutical, pesticides, cosmetics, among others. Previous studies have proved that this encapsulation technique is useful protecting functional ingredients such as essential oils that are known for its antioxidant and antimicrobial properties. However, nowadays there is few information of the encapsulating agents that could be applied in complex coacervation, and how complex coacervates could be applied as antimicrobials. Hence, the formation conditions for a complex coacervate between gelatin and chia mucilage were studied. It was observed that mass rate and pH affect the formation of coacervates. Other factors studied that affect the formation of complex coacervates were the amount of essential oil used for core material, and the homogenization method. In general, particles with a small particle size could hinder the formation of complex coacervates. In addition, complex coacervates could be stabilized by spray drying; therefore, the effect of the drying operation conditions was studied to determine its effect in the final product. It was observed that inlet air temperature and feeding rate could affect the physicochemical properties of the powders obtained. Reconstituted powders of complex coacervates containing essential oil of oregano or thyme were tested against *Escherichia coli* in green juice. These powders resulted effective against this pathogenic bacteria. Therefore, complex coacervation is a promising encapsulation technology to be applied in food industry.

RESUMEN

La coacervación compleja es una técnica de encapsulación que involucra la atracción electrostática entre biopolímeros de cargas opuestas, y que se ha aplicado en varias industrias como la farmacéutica, pesticida, cosméticos, entre otras. Estudios anteriores han demostrado que esta técnica de encapsulación es útil para proteger ingredientes funcionales como los aceites esenciales que son conocidos por sus propiedades antioxidantes y antimicrobianas. Sin embargo, hoy en día existe poca información sobre los agentes encapsulantes que podrían usarse en la coacervación compleja y cómo los coacervados complejos podrían aplicarse como antimicrobianos. Por lo tanto, se estudiaron las condiciones de formación para un coacervado complejo entre gelatina y mucílago de chía. Se observó que la proporción de masa y el pH afectan la formación de coacervados. Otros factores estudiados que afectan la formación de coacervados complejos fueron la cantidad de aceite esencial utilizado como material del núcleo y el método de homogeneización. En general, las partículas con un tamaño pequeño podrían dificultar la formación de coacervados complejos. Por otra parte, los coacervados complejos pueden estabilizarse mediante el secado por pulverización; por lo tanto, se estudió el efecto de las condiciones de operación del secado para determinar su efecto en el producto final. Se observó que la temperatura del aire de entrada y la velocidad de alimentación podrían afectar las propiedades fisicoquímicas de los polvos obtenidos. Los polvos reconstituidos de coacervados complejos contiendo aceite esencial de orégano o tomillo se probaron contra *Escherichia coli* en jugo verde. Estos polvos resultaron ser efectivos contra esta bacteria patógena. Por lo tanto, la coacervación compleja es una tecnología de encapsulación prometedora para ser aplicada en la industria alimentaria.

INTRODUCTION

Essential oils obtained from different sources such as plants and seeds are complex mixtures of volatile compounds, which have a varied chemical composition and a strongly aromatic character. These give plants their characteristic odors and are a common source of bioactive compounds, responsible for their various functional properties (Misharina, 2003; Hyldgaard, Mygind and Meyer, 2012; Bakry et al., 2016).

By their nature, essential oils are chemically unstable, which makes them susceptible to oxidative rancidity and the loss of volatile compounds, especially when exposed to heat, oxygen or light. Such deterioration causes the loss of its antioxidant and antimicrobial properties, which are of interest in the preservation of food (Misharina, 2003; Hyldgaard, Mygind and Meyer, 2012; Bakry et al., 2016).

The microencapsulation can protect the essential oils from oxidation. It is a technique, which involves covering a solid, liquid or gaseous substance in a small capsule, giving stability to the encapsulated substance (Bakry et al., 2016).

One of the microencapsulation techniques applied to essential oils is complex coacervation, which involves electrostatic attraction between two biopolymers of opposite charges. This technique produces stable microcapsules with a thin surface and high oil content. These microcapsules also have excellent controlled release characteristics. In addition, this method is considered as simple, low cost, without solvent use, and reproducible for obtaining microencapsulated oils; therefore, it could be used industrially (Yan and Zhang, 2014, Bakry et al., 2016; Thies, 2016).

Among the biopolymers that have been used in complex coacervation are gelatin, arabic gum, carboxymethyl cellulose and chitosan. Chia mucilage could be explored as an encapsulating agent in complex coacervation, since it has shown to have properties similar

to guar gum, which is effective in the formation of complex coacervates (Xiao, Liu, Zhu, Zhou, and Niu, 2014; Bakry et al., 2016; Timilsena, Wang, Adhikari, and Adhikari, 2016).

Another area of study in complex coacervation is the use of drying techniques such as spray drying, to increase the stability and handling of complex coacervates (Dutra & Ferreira 2010; Anandharamakrishnan & Ishwarya, 2015; Kaushik, Dowling, McKnight, Barrow, and Adhikari, 2016). In this aspect, the use of spray drying and its effects on complex coacervates have been barely investigated. Likewise, the application and effects of homogenization methods such as ultrasound and microfluidization in coacervate formation have been not much explored; being microfluidization a new proposal never studied.

In addition, there is currently few information on food products that present microencapsulated essential oils by complex coacervation applied as antimicrobials.

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JUSTIFICATION

Nowadays, a tendency of consumers towards products with ingredients of natural sources and with functional properties exists. Complex coacervation is a technique that has proven effective in encapsulating essential oils, which have antioxidant and antimicrobial properties, and could have a potential application in food products.

Currently, several biopolymers are being studied in the application for complex coacervation. Among these, chia mucilage is a novel polysaccharide to be used in complex coacervation since it possesses encapsulation properties. Moreover, there are several fields of research for complex coacervation such as the homogenization method to form the complex coacervates or the effect of spray drying to stabilize the coacervates. In addition, there is still few information on the use of complex coacervates in food products and its application as antimicrobials, representing an area of opportunity for research.

GENERAL & SPECIFIC OBJETIVES

General objective

Evaluate the essential oils encapsulated by complex coacervation, using different sources of encapsulating agents (chia mucilage) and homogenization method; analyzing their application as antimicrobials in food products as well.

Specific objectives

1. Determine the physical and chemical conditions for the complex coacervates preparation between gelatin and chia mucilage.
2. Determine the effect of the oil concentration of oregano essential oil on the formation of complex coacervates.
3. Evaluate the effect of different operating conditions during spray drying on the physical properties of powders of oregano essential oil encapsulated by complex coacervation.
4. Determine the effect of different methods of homogenization (ultrasound and microfluidization) on the physical properties of powders of oregano and thyme essential oils encapsulated by complex coacervation.
5. Evaluate the antimicrobial activity in-vitro of powders of oregano and thyme essential oils encapsulated by complex coacervation against *Escherichia coli*, in green juice.

1. COMPLEX COACERVATION: AN ALTERNATIVE AS A MICROENCAPSULATION METHOD

Hernández-Nava, R. & Jiménez-Munguía, M.T. (2017). Coacervación compleja: una alternativa como método de encapsulación. *Temas Selectos en Ingeniería de Alimentos*, *11*, 22-28. (Spanish in original version/ English translation in this document)

Complex coacervation: an alternative as a microencapsulation method

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ABSTRACT

Complex coacervation is a technique involving the electrostatic attraction between two oppositely charged biopolymers that surround a compound of interest, which may be lipid in nature. It is considered as a simple and low-cost method, which produces microcapsules with a thin surface and high content of oil, with excellent characteristics of controlled release of the compound and resistance to the heat. This technique has been used to increase the shelf life of functional ingredients, such as flavors and polyunsaturated fatty acids, providing controlled release of these, and allowing alternative food processing. The objective of this review is to present how the process of complex coacervation is carried out; in addition to discussing critical factors that promote its stability.

Keywords: complex coacervation, oils, proteins, polysaccharides, encapsulation

Introduction

Microencapsulation is a method in which a material of interest is surrounded by a coating wall to form small capsules. This has been widely used for a variety of food applications such as odor masking, prolonging the organoleptic effects of taste or other sensory markers, and the protection of food ingredients that are chemically unstable under storage conditions (temperature, humidity, oxygen, etc.) (Yeo, Bellas, Firestone, Langer and Kohane, 2005; Bakry *et al*., 2016). One of the microencapsulation techniques applied to food is complex coacervation that involves the electrostatic attraction between two biopolymers of opposite charges. Gelatin, especially type A, as cation and arabic gum as anion are the most common encapsulants widely used in food by this technique (Tamjidi, Nasirpour and Shahedi, 2012). Complex coacervation has been used to encapsulate,

protect and supply functional ingredients, such as flavors and polyunsaturated fatty acids, in order to increase their shelf life under different storage conditions, permitting an alternative processing of food, to mask the taste or allowing the controlled release of the encapsulated ingredients (Yan and Zhang, 2014). However, the properties of the final capsules are very sensitive to parameters such as structure, molecular weight and density charge of proteins and polysaccharides used as encapsulating agents; also, ingredients contained in the capsule also play an important role in the stability of these. Therefore, studies continue in the application of this technique in food (Thies, 2016). The objective of this review is to present how the complex coacervation process is carried out; besides discussing the critical factors that promote its stability.

Review

1. Complex coacervation

In the year of 1929, Bungenberg de Jong and Kruyt introduced the term coacervation and are considered as the pioneers in investigating the phenomenon that occurred between a gelatin-arabic gum system (Bungenberg De Jong and Kruyt, 1929; Yan and Zhang, 2014). Subsequently, the complex coacervation has been studied and applied to various fields, being the first product the carbonless copy paper. In the food industry, various application possibilities have been studied, for example, in the protection of compounds (additives, nutrients, volatiles, vitamins, minerals, polyunsaturated fatty acids) to increase their shelf life, to mask the taste or to promote the controlled release of encapsulated components (Yan and Zhang, 2014). This technique has also been used to encapsulate essential oils, and studies have been done on the encapsulation of clove, spearmint, sweet orange, camphor, lavender, garlic, among others (Bakry *et al*., 2016).

1.1 Bases and advantages

Complex coacervation consists in the union of two biopolymers of opposite charges that interact to form a wall that encapsulates a material of interest. According to IUPAC (2014), coacervation is defined as the separation of a colloidal system into two liquid

parts; complex coacervation occurs with the interaction of two biopolymers (protein and polysaccharide) of opposite charges interacting in an aqueous medium. This results in two immiscible liquid phases, one poor in polymers and another rich phase in them, also known as coacervates (Yan and Zhang, 2014; Thies, 2016). Among the encapsulated materials by this technique are edible oils because they are chemically unstable and susceptible to deterioration especially when exposed to environmental conditions such as oxygen, humidity and heat; therefore, it has sought to maintain their biological and functional properties through microencapsulation. This technique produces stable microcapsules with a thin surface and high oil content. They also have excellent characteristics of controlled release and heat resistance. In addition, this method is considered as simple, low cost, without the use of solvents, and reproducible to obtain microencapsulated oils; consequently, it could be used at industrial level (Yan and Zhang, 2014, Bakry *et al*., 2016).

1.2. Encapsulation process by complex coacervation

Complex coacervation consists of three steps: emulsion formation, coacervate formation, wall formation or hardening (Figure 1).

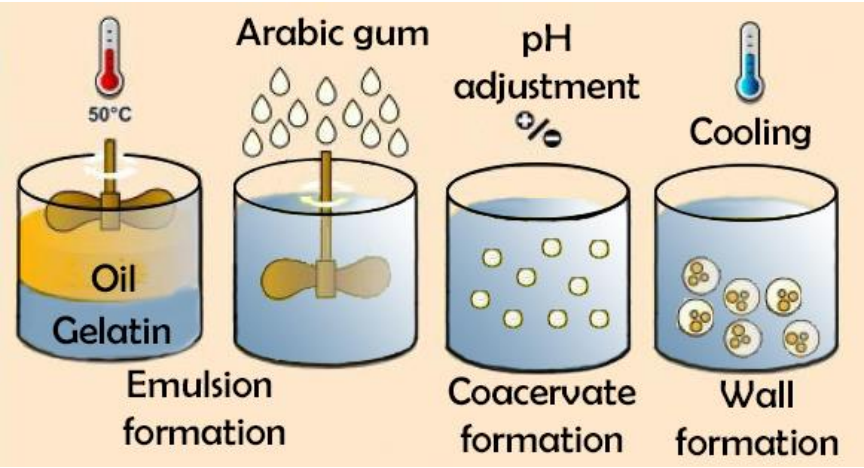

Figure 1. Stages of complex coacervation using gelatin and Arabic gum.

Emulsion formation. In this step, the edible oil or material to be encapsulated is added to a solution rich in biopolymers, at a temperature above the gelation point and a pH higher than the isoelectric point of the protein used as an encapsulating agent, keeping in constant agitation to obtain the desired drop size (Yan and Zhang, 2014; Bakry *et al*., 2016). For example, in the case of a coacervate formed by gelatin-arabic gum, initially the material to be encapsulated (edible oil) is mixed with a gelatin solution until homogenization is achieved, maintaining the temperature above the gelation point (50 ° C). To the previous mixture, the arabic gum is added and mixed until homogenization is achieved (Figure 1).

Coacervate formation. The pH is adjusted below the isoelectric point of the protein to initiate the electrostatic interactions between the polymers of opposite charges; in which the protein has a positive charge and the polysaccharide a negative charge. As a result, the droplets of the dispersed phase agglomerate and promote phase separation (Yan and Zhang, 2014; Bakry *et al*., 2016). For example, in a coacervate formed by gelatin-arabic gum, the pH is adjusted around 4.0 to propitiate the opposite charges between the protein and the polysaccharide.

Wall formation / hardening. The temperature of the system is slowly decreased below the gelation point of the protein, generally reaching refrigeration temperatures, resulting in the formation of the wall, due to the accumulation of the polymer-rich phase around the material of interest. Subsequently, hardening can be achieved through cross-linking that will be discussed later (Yan and Zhang, 2014; Bakry *et al*., 2016).

2. Critical factors in complex coacervation

Coacervation is highly sensitive to many factors, such as the type of material to be encapsulated and its concentration, as occurs with edible oils; the nature of the proteins and the polysaccharides used as encapsulating agents, due to the ionic strength required for the attraction of opposite charges between these biopolymers to occur (Yan and Zhang, 2014; Thies, 2016).

2.1. Nature of lipid compound and its concentration

The adsorption in the oil phase can modify the efficiency of the coacervation, being important factors the nature of the lipid compound and the concentration of it. Prata and Grosso (2015) studied oils of different characteristics (vegetable, mineral and essential) finding different efficiencies of encapsulation, coacervation yields and morphological characteristics, being the essential oil the one that presented the best stability and the highest efficiency of encapsulation, due to the hydrophilic compounds present in it that can act as surfactants. On the other hand, at low concentrations of edible oil there are microcapsules containing less material in the core because there is an excess of encapsulating material. By increasing the concentration of edible oil, the amount of this in the core of the microcapsules increases. However, at high concentrations of edible oil and low proportions of encapsulating agents, the amount of encapsulated oil is reduced due to the decrease in coacervates formation. It has been shown that at concentrations of fish oil below 3% and greater than 10%, the amount of encapsulated oil is decreased (Tamjidi *et al*., 2012).

2.2. Encapsulating agents

Encapsulating agents are of great interest in the food industry, since they must comply with strict regulations and not affect the cost of the final product (Gouin, 2004; Kralovec, Zhang, Zhang and Barrow, 2012). Likewise, these affect the stability of the capsules, the efficiency of the process and the degree of protection of the encapsulated ingredients (Nesterenko, Alric, Silvestre and Durrieu, 2013). The encapsulating agents most used in the application of foods for complex coacervation are proteins and polysaccharides because they are natural and relatively cheap products. In addition, its combination is propitious for the formation of coacervates (Souza, Rojas, Melo, Gaspar and Lins, 2013; Bakry *et al*., 2016).

2.2.1. Proteins

Proteins are polymers whose structures depend on the sequence of amino acids present. It is essential to consider its electrical characteristics due to the electrostatic interactions

necessary to carry out the coacervation. Proteins have positive charge below their isometric point (IP), neutral charge in the IP, and negative charge above the IP (Yan and Zhang, 2014). Some examples of proteins used in complex coacervation are the following:

Gelatin. Those extracted from skin and bones of mammals have been the most used in the coacervation process, due to its excellent emulsifying capacity, gelling capacity and its high cross-linking activity through its primary amino group (Yan and Zhang, 2014; Thies, 2016). Gelatins formed by acid hydrolysis of collagen are classified as type A; while the gelatins formed by alkaline hydrolysis are classified as type B. For type A, their IP is generally between 8-9, meanwhile for type B, their IP is between 4-5. Although both types of gelatin produce suitable coacervates for the formation of microcapsules, type A gelatins are the most commonly used (Thies, 2007). However, gelatin has some limitations, for example, not being accepted for consumption by the vegetarian population, not being suitable for Kosher products, and not having an ideal sensory profile for all applications (Kralovec *et al*., 2012).

Proteins of vegetable origin. The use of vegetable proteins as encapsulating materials is a result of the current trend of consumers due to the increasing concern for the safety of animal products. In food applications, it is known that plant proteins are less allergenic compared to proteins derived from animals (Nesterenko *et al*., 2013). Among the vegetable proteins used as encapsulating materials in microencapsulation, are isolates of soy protein, pea protein, and cereal proteins; being flaxseed and chia protein isolates recently studied (Nesterenko *et al*., 2013; Kaushik, Dowling, McKnight, Barrow and Adhikari, 2016; Timilsena, Wang, Adhikari and Adhikari, 2016). Although proteins of vegetable origin have been shown to be effective for the complex coacervation process, they have some limitations, such as the cost of extraction to obtain high quality proteins or the low solubility of some of these proteins (Nesterenko *et al*., 2013).

2.2.2 Polysaccharides

Polysaccharides differ chemically from each other in number, sequence and type of units present in their chain. This gives them different functional properties, such as solubility, thickening, gelling, water retention capacity, emulsification, etc. Like proteins, for polysaccharides their electrical properties must be considered when selecting them to be used in complex coacervation. The electrical charge of the polysaccharides depends on the nature of the ionic groups present in the chain. Anionic polysaccharides have a neutral charge at pH values below their pKa value, becoming negative at values above their pKa. On the other hand, cationic polysaccharides have a neutral charge at pH values above their pKa value but a positive charge below it (Yan and Zhang, 2014). Polysaccharides are commonly used together with proteins to form coacervates, because they have several negatively charged carboxyl groups that can interact with cationic groups present in proteins. Chitosan is an exception, since it contains amino groups in its chain, allowing it to form coacervates using polysaccharides that have anionic groups (Thies, 2016).

Arabic gum. Arabic gum is a polysaccharide derived from tree exudates of the *Acacia senegal* and *Acacia seyal* species. It consists mostly of β-(1–3) galactopyranose which is highly branched with β-(1–6) galactopyranose with arabinose and glucoronic acid terminations (Xiao, Li, Zhu, Zhou and Niu, 2013; Yan and Zhang, 2014). Arabic gum is the most used polysaccharide at an industrial level due to its good solubility and low viscosity (Yan and Zhang, 2014). Together with gelatin are the most used biopolymers to carry out complex coacervation because, in aqueous conditions, arabic gum has a negative charge, while gelatin at a pH less than 4.75 has a positive charge (Xiao, Li, Zhu, Zhou and Niu, 2015). Also, the mechanism of arabic gum is the one that has been more studied compared to other polysaccharides. Although there are studies that characterize the structure and properties of other polysaccharides, the available database is not as extensive as that of arabic gum (Thies, 2016).

Chitosan. It is a cationic linear polysaccharide composed primarily of β(1-4) glucosamine bound to N-acetylglucosamine. Its pKa value is between 5.5 and 6.5, in which its amino groups are protonated giving the molecule a positive charge. Therefore, to carry out

complex coacervation it is necessary to lower the pH below 6.5 and associate it with a protein (gelatin type B, for example) to achieve coacervate formation (Xiao *et al*., 2013; Thies, 2016).

3. Stability and encapsulation efficiency of the coacervates

The stability of the coacervates is determined by factors such as the structure, size and distribution of the capsules, cross-linking and encapsulation efficiency.

3.1. Structure

The structure of the coacervates is influenced by the homogenization rate during the emulsification process. Depending on this, microcapsules with a single core or multiple core can be obtained. Normally, microcapsules with a single core are attained by applying low homogenization rates. By increasing the homogenization rate, smaller drops of the dispersed phase are obtained, increasing their surface area and promoting the formation of multiple cores contained within a single microcapsule. Coacervates with multiple cores have greater heat resistance and delayed release properties because these are more difficult to break completely, compared to single-core capsules (Yeo *et al*., 2005; Yan and Zhang, 2014; Prata and Grosso, 2015).

3.2. Capsule size and distribution

The size and distribution of the capsules affect the texture and sensory properties of food, being the bigger capsules undesirable in most cases (Yan and Zhang, 2014). The diameter of the coacervates can vary from nanometers to micrometers depending on the operating conditions for their preparation (Schmitt and Turgeon, 2011; Prata and Grosso, 2015). The concentration of encapsulating agents during emulsification is related to the size of the coacervates. It has been observed that the increase in protein concentration causes the increase in the size of the microcapsule, while the increase in the concentration of the polysaccharide causes the decrease in the size of the microcapsule (Tamjidi *et al*., 2012; Yan and Zhang, 2014).

3.3. Crosslinking

The walls of the microcapsules formed by the coacervates are usually unstable at high temperatures and of low mechanical resistance, due to the ionic nature of the interaction between the biopolymers; therefore, stabilization by crosslinking is recommended (Yan and Zhang, 2014). Stabilization by chemical crosslinking with glutaraldehyde has been the most used. In this process, glutaraldehyde reacts with the primary amino groups of the protein forming chemical bonds in the coacervate gel that increase the gel's resistance and prevent the gel from melting due to heat. However, there is a concern in the use of this compound due to its genotoxic and mutagenic effects (Speit, Neuss, Schutz, Frohler-Keller and Schmid, 2008; Thies, 2016). As a result, other alternatives have been sought to achieve crosslinking, being one of them the use of phenolic compounds like tannic acid. It quickly shrinks the coacervate walls reducing the water content present; although, it has the disadvantage of reacting rapidly with the protein, which makes the precise control of the formation process difficult (Thies, 2007). Also, this compound is prone to fading and has a characteristic flavor, which limits its use in foods (Yan and Zhang, 2014). Another alternative is the use of enzymes, being transglutaminase the most used. This enzyme catalyzes the formation of bonds between the ε-amino group of lysine and the γ-carboxamide group of glutamines, resulting in the formation of intra and intermolecular bonds of ε-(γ-glutamyl) lysine (Dutra and Ferreira, 2010; Yan and Zhang, 2014). It has been shown that this enzyme is effective in the formation of coacervates using gelatin or proteins of plant origin, giving them greater heat resistance (Lv, Yang, Li, Zhang and Abbas, 2014; Xiao *et al*., 2015; Timilsena *et al*., 2016).

3.4. Encapsulation efficiency

The encapsulation efficiency is an indicator to evaluate the quality of encapsulated products. This is defined as the relationship between the weight of the final compound of interest encapsulated, and the total amount of this compound used in the initial formulation. Regarding essential oils, the encapsulation efficiency is determined based on the percentage of non-encapsulated oil. This is a key parameter in the quality of the microencapsulates because the non-encapsulated oil, when it comes to an edible one, is

susceptible to develop rancidity, which limits the shelf life of the product. Microcapsules obtained by complex coacervation have a lower percentage of non-encapsulated oil and a higher content of encapsulated oil compared to spray drying. It also has an encapsulation efficiency above 99% (Kralovec *et al*., 2012; Yan and Zhang, 2014; Bakry *et al*., 2016).

4. Food applications

Food products manufactured by complex coacervation available in the market are very limited. The ONC (Ocean Nutrition Canada) has managed to microencapsulate fish oil through complex coacervation using gelatin-polysaccharide, and to produce it at industrial scale under the name of Powderloc™ (Yan and Zhang, 2014). Likewise, studies continue in the possible applications of complex coacervation in food. Dima, Cotârlet, Alexe and Dima (2014) found that encapsulation of essential oil of *Pimenta dioica* through the combination of chitosan/k-carrageenan is viable and possibly applicable for meat products. Qv, Zeng and Jiang (2011) encapsulated lutein through gelatin/arabic gum, giving this carotenoid greater resistance to light, temperature and humidity. They also observed an increase in the stability of the encapsulated product during storage for 30 days, where the percentage of lutein retention was 92.86% at 4°C and 90.16% at 25°C. Kralovec *et al*. (2012) point out that coacervates made with whey protein and arabic gum, show greater stability after being treated with ultrapasteurization; in addition to a better sensory quality in dairy products compared to coacervates made with gelatin. Wang, Adhikari and Barrow (2014) used gelatin/sodium hexametaphosphate to encapsulate tuna oil to delay the oxidation of omega-3 oils, obtaining an encapsulation efficiency of 88.03% and an oxidative and thermal stability (Rancimat) of 40.16 hours. Calderón, Pedroza, Escalona, Pedraza and Ponce (2017) managed to encapsulate a mixture of nisin and avocado's peel extract as an antimicrobial and antioxidant, respectively, using collagen/alginate and collagen/pectin.

Nowadays, most of the studies on complex coacervation applied in food have focused on obtaining coacervates and studying their characteristics, being their application in various food products still unexplored.

Conclusions

Complex coacervation is a possible alternative of microencapsulation in the food industry due to the advantages of the products obtained, for example, heat resistance and having controlled release of the encapsulated ingredients, from a simple and low-cost method. Currently, studies are still being done with possible encapsulating agents to be applied in this technique so that they meet the requirements demanded by consumers. Also, in the food sector, this technology continues to be explored because many of its potential applications are still unknown.

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2. COMPLEX COACERVATION BETWEEN GELATIN AND CHIA MUCILAGE AS AN ALTERNATIVE OF ENCAPSULATING AGENTS

Hernández-Nava, R., López-Malo, A., Palou, E., Ramírez-Corona, N., & Jiménez-Munguía, M. T. (2019). Complex Coacervation between Gelatin and Chia Mucilage as an Alternative of Encapsulating Agents. *Journal of Food Science*, *84*(6), 1281-1287. DOI: 10.1111/1750-3841.14605.

Complex Coacervation between Gelatin and Chia Mucilage as an Alternative of Encapsulating Agents

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Abstract: Complex coacervation between gelatin type B (GE) and chia mucilage (ChM) was studied. GE-ChM were mixed at mass ratios of 1:1, 2:1, 3:1, 4:1 and 1:2 in a pH range of 1.50-5.00, maintaining a total solid concentration of 0.2% (w/w), using turbidity and viscosity tests to obtain the highest yield of complex coacervates. To characterize the complex coacervates morphology and Fourier-transform infrared spectroscopy (FTIR) were determined. The optimum yield for complex coacervation was achieved with a GE-ChM mass ratio of 2:1 and pH value of 3.6. The critical pH values associated with the formation of soluble (pH_c) and insoluble ($pH_{\phi 1}$) complexes, and complete dissociation ($pH_{\phi 2}$) at the optimum GE-ChM ratio were found to be 4.50, 4.10 and 2.00, respectively. It was observed that increasing the mass ratio of GE or ChM, the yield of complex coacervates decreased; the higher yields were obtained with the proportions of 2:1 and 1:1 with values of 68.25 ± 0.05% and 61.04 ± 0.05%, respectively. Capsules formed at mass ratios of 1:1, 2:1, 3:1, had the characteristic grape agglomerate shape for complex coacervates. Further characterization with SEM showed a spherical shape for capsules. FTIR spectrum of complex coacervates at optimum conditions had a combination of bands corresponding to GE and ChM, suggesting an interaction between GE-ChM during the formation of complex coacervates. Therefore, complex coacervates between GE-ChM can be formed, and could be used as an alternative as encapsulating agents to be applied in the food industry.

Keywords: complex coacervation, gelatin, chia mucilage, coacervate yield

Practical Application: Complex coacervation is a technique that is being studied in several applications in the food industry. However, studies are still being made to explore different possibilities of natural sources to be used in complex coacervation. This study showed that the combination of gelatin and chia mucilage may be an alternative as encapsulating agents for complex coacervation to be applied in the food industry.

Introduction

Bungenberg de Jong and Kruyt, in the year 1929, introduced the term coacervation and are considered as the pioneers in investigating the phenomenon that occurred between gelatin and arabic gum systems. Complex coacervation is a technique that involves electrostatic attraction between two biopolymers of opposite charges that surround a compound of interest (McClements, Decker, & Weiss, 2007; Speranza et al., 2017). This results in two immiscible liquid phases, one poor in polymers and another phase rich in these, also known as coacervate. The coacervation process is widely used in the pharmaceutical, pesticide and cosmetics industries, being recently applied to foods (Yan & Zhang, 2014; Bakry et al., 2016; Shen et al., 2016; Thies, 2016).

The encapsulating agents most used in the application of foods for complex coacervation are proteins and polysaccharides, because they are natural and easily accepted as GRAS for food applications. Studies are still being made to explore different possibilities of natural sources to be used in complex coacervation.

In complex coacervation, proteins extracted from animal-derived products such as gelatin, whey, and casein; and proteins from vegetables such as soy, pea, lentil, canola and chia have been investigated. On the other hand, the most used polysaccharides in complex coacervation are arabic gum, pectin, chitosan, agar, alginate, and carrageenan (Xiao, Liu, Zhu, Zhou, & Niu, 2014; Timilsena, Wang, Adhikari, & Adhikari, 2016). There are plant proteins and polysaccharides that can be used as encapsulating agents in complex coacervation that are theoretically known to possess favorable characteristics, but they

have not been explored (Dickinson, 2003; Kaushik, Dowling, Barrow, & Adhikari, 2015; Jain, Thakur, Ghoshal, Katare, Singh & Shivhare, 2016).

The mucilage of chia seed is a complex anionic heteropolysaccharide obtained from chia (*Salvia hispanica L.*) which is an annual herbaceous plant from *Lamiaceae* family originally from southern Mexico. The mucilage is extracted when the seed is in contact with water, increasing the solution viscosity; even at low concentration, the mucilage contributes to the stabilization of food structure (Lin, Daniel, & Whistler, 1994; Capitani, Ixtaina, Nolasco, & Tomás, 2013). Mucilages can be used as dietary fiber sources, thickening, emulsifying, or stabilizer agents, as well as drug delivery excipients (Mirhosseini & Amid, 2012). A tentative structure of the basic unit of the polysaccharide was proposed by Lin et al. (1994) being a tetrasaccharide with 4-O-methyl-α-D-glucoronopyranosyl residues occurring as branches of β-D-xylopyranosyl on the main chain. This mucilage can be easily extracted and hydrated to achieve water retention and it has a great potential as a functional ingredient to be used in several food applications. Additionally, Timilsena, Wang, et al. (2016) proved the potential use of chia mucilage as encapsulating agent.

The structure and stability of complex coacervates are affected by different factors, including pH, protein-polysaccharide mass ratio, ionic strength, etc. (Duhoranimana et al., 2018). Consequently, is fundamental to understand these factors in the optimization of coacervation process. In this paper, complex coacervation between gelatin and chia mucilage were prepared to obtain the best conditions to produce coacervates by applying low frequency ultrasound obtaining a higher yield of coacervates, studying the effects of pH and mass ratio. Due to the actual demand of ingredients of natural sources accepted as GRAS, further investigation is needed. Hence, a system of gelatin and chia mucilage could be an alternative as encapsulating agents for complex coacervation to be applied in the food industry.

Materials and Methods

Materials

Chia seeds (*Salvia hispanica* L.) were purchased from Verde Limón Trading Company (Mexico City, Mexico). Gelatin (type B) was purchased from Gelco S.A. (Bogota, Colombia). Other chemicals used in this study were analytical grade, and were purchased from Hycel (Jalisco, Mexico).

Extraction of chia mucilage

The modified method described by Timilsena, Wang, et al. (2016) was used. Chia seeds were soaked in distilled water in a ratio of 1:20 (w/v) and kept in constant agitation for 4 h at 35 ± 1.0 °C to let the mucilage absorb water and the seeds became swollen. Later it was freeze-dried (Triad™ Labconco, USA). The mucilage was mechanically separated from the seeds by sieving using a mesh #35 (500 μm), and stored at 25 ± 1.0 °C inside a sealed container until further use.

Preparation of coacervates

Gelatin (GE) and chia mucilage (ChM) were mixed at mass ratios of 1:1, 2:1, 3:1, 4:1, and 1:2, respectively. Total solid concentration (GE-ChM) was maintained at 0.2% (w/w) in aqueous solution; meanwhile the pH was varied from 1.50 to 5.00 by adding HCl 0.1 N. GE and ChM solutions were prepared individually by the reconstitution of the powders in distilled water with constant stirring (350 rpm) at 40 ± 1.0 °C until total dissolution. Both solutions were mixed and then homogenized by low frequency (20 kHz) ultrasound (CP-505, Cole-Parmer Instrumental Company, USA) for 10 min applying an intensity of 70%, which corresponds to an ultrasonic wave amplitude of 84 μm. The pH was adjusted as required by adding HCl 0.1 N dropwise maintaining the solution with constant stirring (250 rpm) for 5 min. Then a second ultrasound homogenization was applied for 5 min following the same methodology previously described. The system was cooled down to 25°C and stored at 4.0 ± 1.0 °C until further use.

Determining the best conditions to form complex coacervates

Measurement of turbidity. The pH controls the degree of ionization of the functional groups and the strength of the electrostatic interaction between them; therefore, optimum pH (pH_{opt}) corresponds to the pH value at which the highest turbidity was observed (Kaushik et al., 2015). Turbidity was measured using a colorimeter (DR/890, Hach, USA). Structure-forming transitions (pH_c, $pH_{\phi 1}$, and $pH_{\phi 2}$) were determined graphically from the curve according to Weinbreck, de Vries, Schrooyen, & de Kruif (2003).

Measurement of viscosity. The stronger interaction between opposite charges is registered at the lower viscosity, which occurs at the pH_{opt} (Shinde & Nagarsenker, 2009). This analysis was made as a confirmatory assay of the pH_{opt} obtained in the measurement of turbidity. Viscosity was measured with a Cannon-Fenske (350, Thomas Scientific, USA) viscometer where the time taken for the liquid to flow by capillarity through the viscometer is measured (Tadros, 2018). Dynamic viscosity was determined using the following equations:

$$Kinematic\ viscosity\ (cSt) = \ viscometer\ constant\ \times\ flow\ time$$

$$Dynamic\ viscosity\ (cP)\ = \ kinematic\ viscosity \times\ density$$

Measurement of the complex coacervate yield (CY). The coacervate was separated by vacuum filtration and then dried at 105 ± 1.0 °C until reaching constant weight as described by Huang, Sun, Xiao, & Yang (2012). The yield is calculated using the following equation:

$$CY(\%) = \frac{m_i}{m_0} \times 100$$

where, CY is the yield of the coacervate (%), m_i is the mass (g) of dried coacervates and m_0 is the initial total mass of both GE and ChM in the formulation.

Characterization of complex coacervates

FTIR spectra of GE, ChM and complex coacervate. An FTIR spectrometer (Cary 630, Agilent Technologies, USA), in the range of wave number from 1800 to 800 cm^{-1}, was used to determine the functional groups in GE, ChM and GE-ChM coacervate with the higher yield.

Morphology. An optical microscope (Axiovert 25, Zeiss, Germany), coupled to a digital camera controlled by Zen Lite software (v.2011, Zeiss, Germany) using the 100x/1.25 objective, was used for systems at pH_{opt} to establish a relation between coacervation yield and the shape of capsules. For further characterization of the system with the highest coacervation yield studied, a scanning electron microscope (MAIA3, Tescan, Czech Republic) was used. Coacervates were freeze dried, and powder samples were sprinkled on aluminum stubs using a double-sided adhesive carbon tape without metallic coating. The micrographs were taken at an accelerating voltage of 2.0 kV and a magnification of 3.50 kx.

Statistical analysis

Obtained data was statistical analyzed using Minitab (v.17, LEAD Technologies Inc., USA) performing analysis of variance (ANOVA) and Tukey's comparison tests, using a confidence level of 95%.

Results and discussion

When electrostatic attractive forces between the biopolymers GE and ChM become oppositely charged, complex coacervation is achieved. Critical factors that affect the attraction and subsequent complexation between these two biopolymers are pH and GE-ChM mass ratio. The pH and mass ratio play an important role in complex coacervation since the strength of the electrostatic interaction between the functional groups and the charge density of the biopolymers affect the physical-mechanical and thermal properties of the coacervates shell (Timilsena, Wang, et al. 2016; Shen et al., 2016). Therefore, these

two parameters were optimized to achieve the highest possible yield. As suggested in earlier studies (Shinde & Nagarsenker, 2009; Huang et al., 2012; Wang, Adhikari, & Barrow, 2014; Timilsena, Wang, et al., 2016), turbidity, viscosity and the yield of coacervates were measured and used in this study as the basis of optimization of the complex coacervation. These results showed that the optimum yield for complex coacervation maintaining total solid concentration at 0.2% (w/w) was achieved with a GE-ChM mass ratio of 2:1 and pH value of 3.6; at this condition the highest turbidity and the lowest viscosity were observed indicating the stronger interaction between opposite charges (Shinde & Nagarsenker, 2009; Kaushik et al., 2015), resulting in a CY% of 68.25 ± 0.05 (Table 1). Timilsena, Wang, et al. (2016) whom worked with chia seed protein isolate-chia seed gum; and Chang, Gupta, Timilsena and Adhikari (2016) whom worked with canola protein isolate-chitosan, reported a CY% higher than 70. The differences between the values reported can be attributed to the complex coacervation process applied and the encapsulating agents used.

Effect of pH and the identification of pH_c, $pH_{\phi 1}$, pH_{opt}, and $pH_{\phi 2}$

The highest value of turbidity was observed below the isoelectric point (Ip) of GE, in which positive charges are achieved. For gelatin type B, its pI is around pH 4 – 5 (Yan & Zhang, 2014). Mass ratio of 4:1 showed the highest value of pH_{opt} (Table 1) that could be due to the excess of gelatin in the system (there were more amino groups and a lack of carboxyl groups contributed by ChM). Therefore, generation of less positive charges were needed to neutralize the electrostatic charge of the system. As for ChM, carboxyl groups are contributed by uronic acid (pKa ~1.8). All values of pH_{opt} found were located above the pKa of uronic acid where negative charges are accomplished. At pH lower than 1.6 the turbidity presented the lowest values and remained almost constant in those systems. This could be explained since ChM become slightly positively charged at pH lower than 1.6, as reported by Timilsena, Wang, et al. (2016). At this point, GE and ChM had positive charges where no electrostatic interaction between these two biopolymers were achieved, giving as a result no change in turbidity ($P > 0.05$).

Table 1 – Physical properties and yield for different complex coacervates systems.

Mass ratio	pH_c	$pH_{\phi 1}$	pH_{opt}	$pH_{\phi 2}$	$pH_c - pH_{\phi 2}$	Maximum turbidity (Fau)	Minimum viscosity (cP)	CY (%)
01:01	4.30^a	3.80^a	3.40^a	2.50^a	1.8^a	647 ± 1.0^a	1.5105 ± 0.01^a	61.04 ± 0.05^a
02:01	4.50^b	4.10^b	3.60^b	2.00^b	2.5^b	742 ± 0.6^b	1.4151 ± 0.01^b	68.25 ± 0.05^b
03:01	4.40^c	4.20^c	3.80^c	2.60^c	1.8^a	475 ± 1.0^c	1.4703 ± 0.01^c	55.29 ± 0.10^c
04:01	4.90^d	4.70^d	4.50^d	3.10^d	1.8^a	336 ± 1.0^d	1.4445 ± 0.02^d	52.39 ± 0.05^d
01:02	4.10^e	3.60^e	3.30^e	2.50^a	1.6^c	527 ± 0.6^e	1.6423 ± 0.01^e	55.14 ± 0.10^c

Different letters in column show significant differences ($P < 0.05$) between samples.

When pH is lowered, proteins and polysaccharides form complexes. The critical point at which turbidity starts to increase sharply is known as pH_c, here the formation of complexes initiates. At lower pH when the interaction is stronger, phase separation takes place ($pH_{\varphi 1}$). Increase in turbidity continues until it reaches the highest value corresponding to the pH_{opt} where neutral complexes are formed as a result of two biopolymers oppositely charged reaching an electrical equivalence. Finally, the critical point at which the complexes completely dissociate is denoted as $pH_{\varphi 2}$ (Weinbreck et al., 2003; Timilsena, Adhikari, Kasapis & Adhikari, 2016; Eghbal & Choudhary, 2018). The stages of this process are shown on Figure 1.

In this study, it was observed that at $pH > pH_c$, the solution was clear, and the turbidity was almost constant. At this range, polymers are soluble in the aqueous solution in which no electrostatic interaction occurs. When pH was between pH_c and $pH_{\varphi 1}$, the solution of GE-ChM became slightly turbid, and turbidity started to increase sharply. In this zone GE and ChM have negative charges (pH of GE is above its Ip, and pH of ChM is above pKa of uronic acid). Due to the negative charges, no electrostatic interaction occurs, and the polymers remain soluble in the aqueous solution; however, Weinbreck et al. (2003) suggested the formation of soluble complexes. When pH reached the $pH_{\varphi 1}$, turbidity gradually increased, and the solution of GE-ChM became more turbid, presenting phase separation due to the formation of insoluble complexes. With further acidification pH_{opt} is observed (Figure 1); pH_{opt} for every system studied presented a significant difference ($P < 0.05$) (Table 1), this suggests that pH_{opt} is dependent of the mass ratio employed.

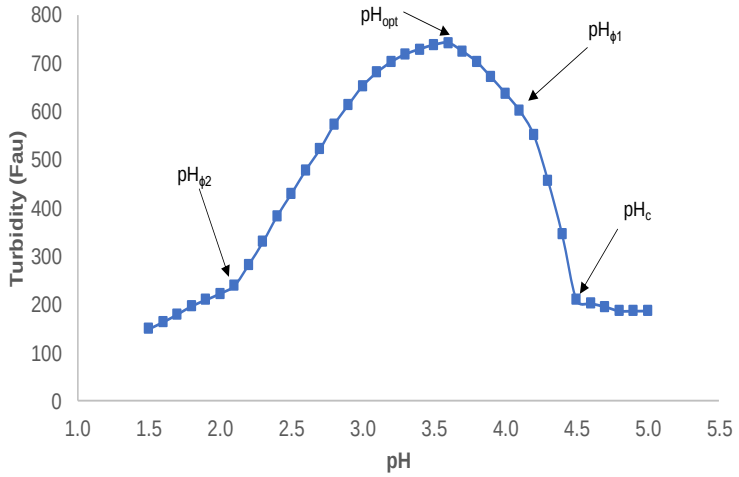

Figure 1 – Turbidity as function of pH and 2:1 mass ratio of GE-ChM, demonstrating the various structure-forming transitions (pH_c, $pH_{\phi 1}$, and $pH_{\phi 2}$) and the highest turbidity (pH_{opt}) (n=3)

Similar results were observed by other authors (Kaushik et al., 2015; Timilsena, Adhikari, et al., 2016; Liu et al., 2009) where by varying the mass ratio, the pH_{opt} was modified. As Timilsena, Adhikari, et al. (2016) demonstrated, when the pH is further lowered than the pH_{opt}, slow dissociation of complex coacervates began to occur due to the protonation of reactive sites in the anionic polysaccharide. Moreover, additional acidification of the dispersion of GE-ChM below the $pH_{\varphi 2}$ value, resulted in the loss of turbidity and the system became clear, similar as the first stage ($pH > pH_c$). To low the pH to the $pH_{\varphi 2}$ value, an important increase in acid addition was necessary. It could be due to a much stabilized system and to achieve complete dissociation of the complexes formed, more acid is needed to fully protonate the reactive sites in the anionic polysaccharide. Structure-forming transitions (pH_c, $pH_{\phi 1}$, and $pH_{\phi 2}$) were dependent of the mass ratio employed ($P < 0.05$), these results differ to the reported for other authors, whose studied systems were whey protein-arabic gum (Weinbreck et al., 2003), pea protein isolate-arabic gum (Liu et al., 2009), flaxseed protein isolate-flaxseed gum (Kaushik et al., 2015), and chia seed protein isolate-chia seed gum (Timilsena, Wang, et al., 2016). However, these differences can be attributed to the characteristics of proteins and polysaccharides used for each study.

Effect of mass ratios of GE-ChM

Another important factor affecting the process of complex coacervation is protein-to-polysaccharide mass ratio because different ratios affect the intensity of interaction and complexation, due to the charge balance between protein and polysaccharide (Liu et al., 2009; Kaushik et al., 2015). It was observed that increasing the mass ratio of GE shifted the turbidity curve to the right, while increasing the mass ratio of ChM shifted the curve to the left (Figure 2). This behavior was previously observed by other authors (Schmitt, Sanchez, Thomas, & Hardy, 1999; Liu, Low, & Nickerson, 2009) when proteins or polysaccharides were in excess, which could be attributed to the opposite charges in the system which modify the stability of it. Turbidity increased gradually when the GE-ChM mass ratio was increased until a ratio of 2:1. However, further increase in the GE or ChM mass ratios decreased the turbidity values significantly ($p < 0.05$). These results are aligned with the findings reported by Timilsena, Wang, et al. (2016) where the highest turbidity observed was at a mass ratio of 6:1, further increasing of mass ratio resulted in a lower turbidity, and a decrease of the coacervate yield. Moreover, with the increase of GE or ChM mass ratios, the curves became tight compared to mass ratio 2:1, which resulted in a narrow range of pH to form coacervates, and a decrease in the coacervate yield (Table 1). As suggested by Timilsena, Wang, et al. (2016) this decrease in the formation of complex coacervates is due to the inability of the excess protein to bind with the polysaccharide gum leaving increasingly higher amount of free protein in the dispersion.

Effect of viscosity

Formation of capsules was achieved at every GE-ChM mass ratio tested over pH range of 2.5 to 4.0 were a decrease of viscosity values were noted. Outside this range a rise in viscosity was observed indicating that there was sufficient interaction between the two biopolymers to cause rise in viscosity but not for coacervation to occur (Shinde & Nagarsenker, 2009). This could be attributed to the opposite charges in the system due to the dissociation of carboxylic groups and primary amino groups, present in chia mucilage

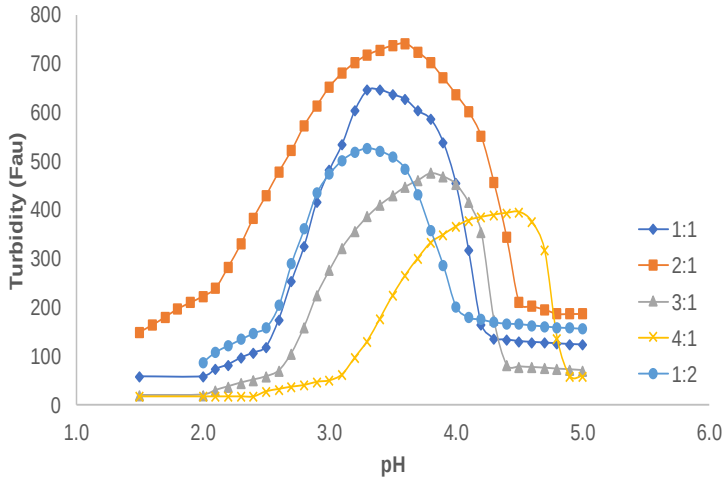

Figure 2 – Turbidity values as a function of pH and GE–ChM mass ratios (n = 3)

and gelatin, respectively. Viscosity behavior could be also explained with pH_c and $pH_{\phi 2}$ obtained in the viscosity curve (Figure 3). It was observed that at pH > pH_c, and pH < $pH_{\phi 2}$ the viscosity increased; this suggests that when there was a presence of soluble complexes the viscosity had higher values, meanwhile when insoluble complexes were formed, the viscosity tend to decrease until reaching the pH_{opt} where an electrical equivalence between the two biopolymers of opposite charges is achieved. Further acidification after the pH_{opt}, initially resulted in a slight increase in viscosity and then it remained almost constant; this could be due to the presence of soluble complexes and insoluble complexes in the system. After reaching a pH < $pH_{\phi 2}$, the viscosity tends to quickly increase since only soluble complexes are present. Lower viscosity values obtained are shown on Table 1, these values correspond to the same optimum pH observed at the maximum turbidity found. As shown on Table 1, viscosity decreased gradually when the GE-ChM mass ratio was increased until a mass ratio of 2:1; meanwhile further increase in the GE or ChM mass ratios increased the turbidity values significantly (P < 0.05). Similar results were observed by Devi & Maji (2011) where initially the viscosity decrease until reaching a minimum value; further increasing of the mass ratio of gelatin-sodium carboxymethyl cellulose resulted in the increase of viscosity due to the presence of unreacted sodium carboxymethyl cellulose in the system.

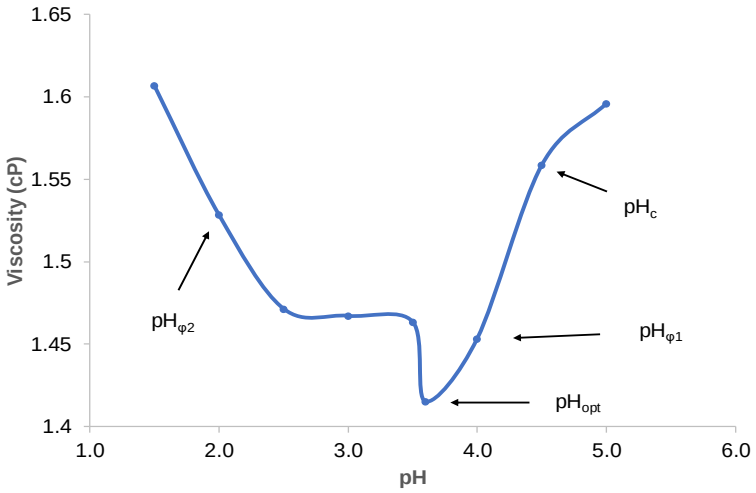

Figure 3 – Viscosity curve as function of pH at 2:1 mass ratio of GE-ChM, demonstrating the various structure-forming transitions (pH_c, $pH_{\phi 1}$, and $pH_{\phi 2}$) and the lowest viscosity (pH_{opt}) (n=3)

FTIR spectra of GE, ChM and GE-ChM complex coacervate

FTIR data were obtained in the range of wave number from 1800 to 800 cm^{-1} (Figure 4). Spectra of GE had the major absorption band in the amide region, which is known as a characteristic pattern of gelatin (Figure 4A). The band 1626.9 cm^{-1} represents the CN stretching and in-plane N-H bending of the amide I. The spectra also show that the CN stretching with contribution of N-H bending formed the amide II band, which was located at the wavenumber 1525.1 cm^{-1}. The amide III bands, mainly referring to the CN stretching, were in the region 1232.7 cm^{-1} (Pongjanyakul & Puttipipatkhachorn, 2007). For ChM spectra, peaks 1597.0 cm^{-1} and 1418.4 cm^{-1} represent the symmetric stretching of carboxyl group of uronic acid, which are characteristic of polysaccharides from seed gums (Figure 4B). Peak at 1150.3 cm^{-1} represent the bending vibration of C-O-C present in the pyranose ring (Timilsena, Adhikari, et al., 2016). The band at 1037.2 cm^{-1} is assigned to C-O-C stretching of 1→4 glycosidic bond and C-O-H bending, considered as the characteristic of polysaccharide compounds (Toğrul & Arslan, 2003; Fonseca et al.,

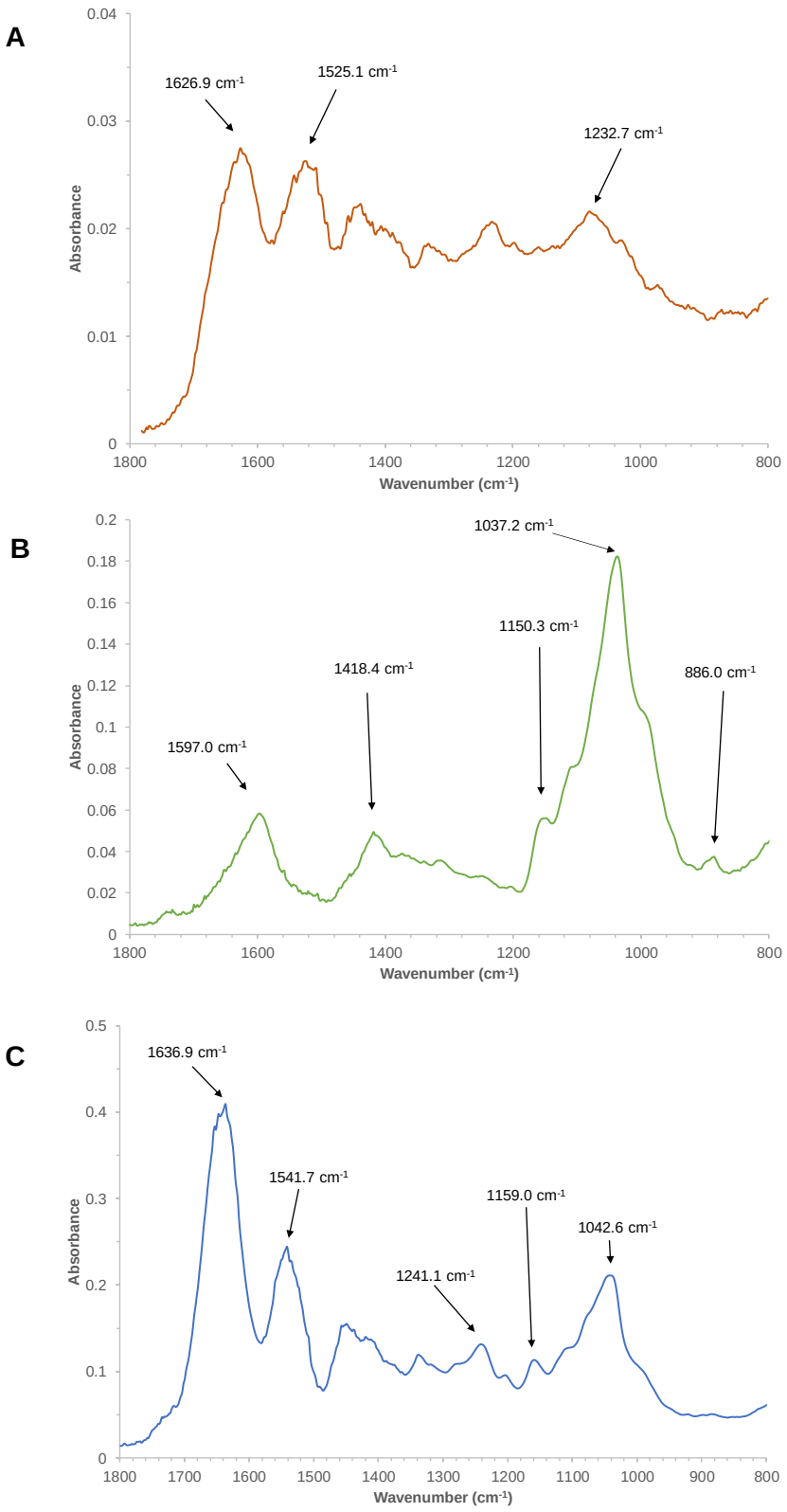

Figure 4 – FTIR spectra of GE (A), ChM (B), and GE-ChM complex coacervate at mass ratio 2:1 (C)

2011). The band at 886 cm^{-1} represents the β-anomeric C-H deformation and glycosidic linkages attributable to glucoryranose and xylopyranose units (Cerqueira et al., 2011; Timilsena, Adhikari, et al., 2016). FTIR spectra of complex coacervate at optimum conditions (mass ratio GE-ChM 2:1, pH 3.6) had a combination of bands corresponding to GE and ChM with a domination by GE functional groups due to the high GE-ChM mass ratio (Figure 4C). In addition, it presented a slight shift towards left and increased intensity of bands regarding the individual polymers. Since no new peaks were found, chemical interaction of GE-ChM could be discharged, suggesting an electrostatic interaction between GE-ChM during the formation of complex coacervates.

Morphology of complex coacervates at pH_{opt}

Capsules formed at mass ratios of 1:1, 2:1, and 3:1 had the characteristic grape agglomerate shape for complex coacervates (Figure 5). However, mass ratios 1:1 and 3:1 presented individual spherical shapes, as well. Different to mass ratio 1:1 that only exhibited grape agglomerate shapes. Mass ratios of 1:2 and 4:1 showed individual spherical and oval shapes, respectively. Since no characteristic grape agglomerate shapes were observed, these ratios were discarded in the formation of complex coacervates, which is related to the lower coacervation yield.

In the SEM micrograph of complex coacervate at optimum conditions (mass ratio GE-ChM 2:1, pH 3.6), capsules shown a spherical shape (Figure 6). This result agrees with observations reported by other authors (Jain, Thakur, Goshal, Katare & Shivhare, 2015; Timilsena, Wang, et al., 2016; Xiao, Li, Zhu, Zhou & Niu, 2016) where spherical shapes for complex coacervates were observed. The appearance of freeze-dried ChM as overlapping sheets was reported by Capitani et al. (2013) in the characterization of chia mucilage. In the complex coacervate at optimum conditions, these overlapping sheets were observed along with the capsules formed by coacervates, this could be explained since not all ChM interacts in the coacervation, resulting in a CY% of 68.25 ± 0.05.

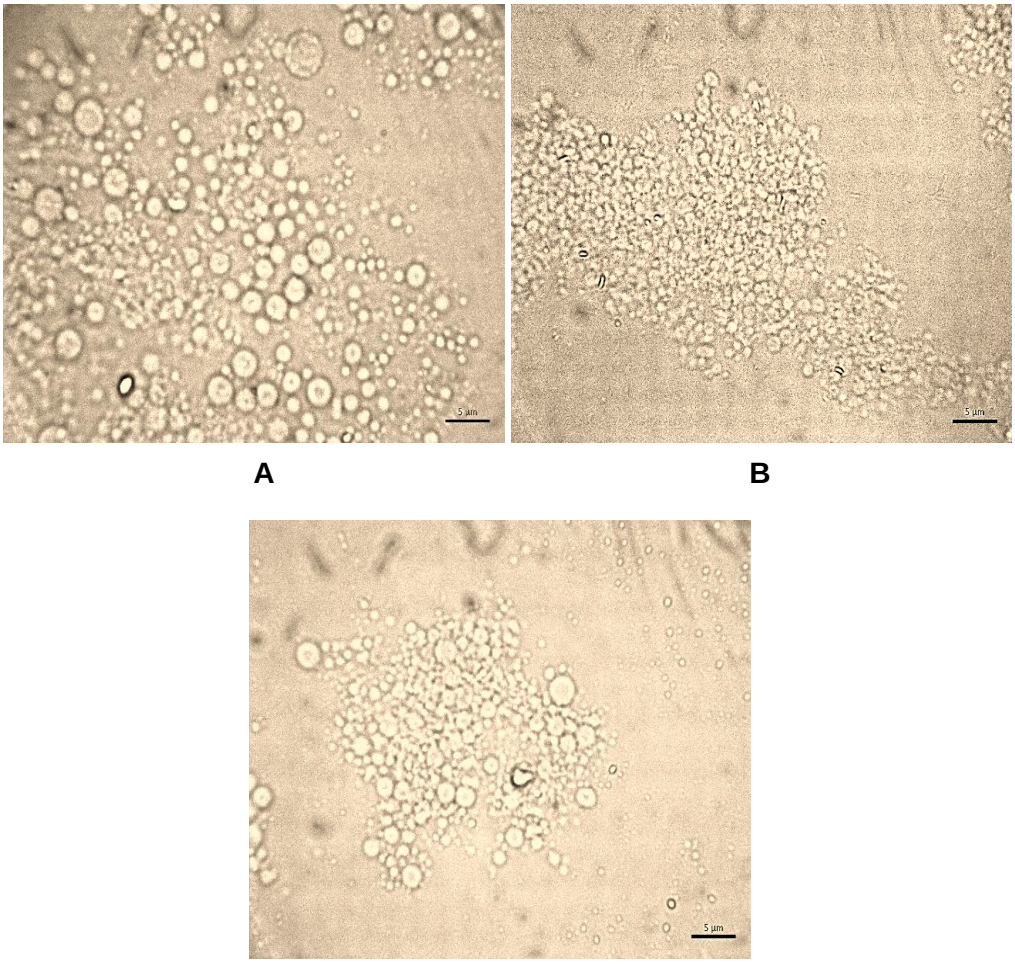

Figure 5 – Micrographs of GE-ChM complex coacervates at mass ratios 1:1 (A), 2:1 (B), and 3:1 (C)

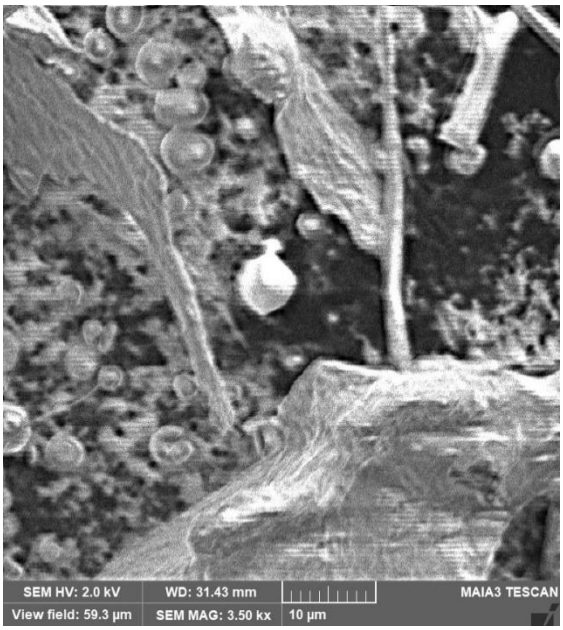

Figure 6 – SEM micrographs of GE-ChM complex coacervate at mass ratio 2:1 and pH 3.6

Conclusions

The variation of mass ratios affected the structure-forming transitions (pH_c, $pH_{\phi 1}$, and $pH_{\phi 2}$) and optimum pH to form complexes influencing the CY%. The optimum conditions studied to form complex coacervates of GE-ChM were with a mass ratio of 2:1 and a pH value of 3.6. FTIR analysis showed that electrostatic interaction was responsible for the formation of complex coacervates between GE and ChM rather than chemical reaction. Morphology of the coacervate had a characteristic grape agglomerate shape; furthermore, SEM showed a spherical shape of the capsules formed. This study demonstrated that gelatin (GE) and chia mucilage (ChM) can be successfully used to form complex coacervates. Besides, complex coacervates formed by GE-ChM may be an alternative as encapsulating agents to be applied in food industry, such as delivery vehicles for active and unstable food ingredients. However, further research is required to study the practical applications of this complexed system, and the ability to microencapsulate and protect sensitive ingredients.

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Author Contributions

R. Hernández-Nava contributed to the study design, conducted the experiments and wrote the manuscript draft. A. López-Malo contribute to the interpretation of the results and revised the manuscript. E. Palou and N. Ramírez-Corona revised the draft manuscript. M. T. Jiménez-Munguía designed the study and revised the data information, as well as the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

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3. ENCAPSULATION OF OREGANO ESSENTIAL OIL (*ORIGANUM VULGARE*) BY COMPLEX COACERVATION BETWEEN GELATIN AND CHIA MUCILAGE AND ITS PROPERTIES AFTER SPRAY DRYING

Hernández-Nava, R., López-Malo, A., Palou, E., Ramírez-Corona, N., & Jiménez-Munguía, M. T. (2020). Encapsulation of Oregano Essential Oil (*Origanum vulgare*) by Complex Coacervation between Gelatin and Chia Mucilage and its properties after spray drying. *Food Hydrocolloids*, accepted. DOI: 10.1016/j.foodhyd.2020.106077.

Encapsulation of oregano essential oil (*Origanum vulgare*) by complex coacervation between gelatin and chia mucilage and its properties after spray drying

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Abstract

Oregano essential oil (OEO) was encapsulated by complex coacervation between gelatin and chia mucilage (mass ratio 2:1, solids concentration 0.2% w/w). It was compared with a system of gelatin-arabic gum (GE-GA) as encapsulating agents. Two essential oil concentrations (5 and 7.5% w/w) were studied to determine its effect in the formation of complex coacervates by its encapsulation efficiency (EE). To characterize the complex coacervates Fourier-transform infrared spectroscopy (FTIR), particle size and morphology were determined. FTIR spectrum of complex coacervates showed characteristics peaks of carvacrol, one of the majority components present in OEO. OEO concentration and emulsifier added influenced the particle size and morphology of the coacervates, being the sample with gelatin-chia mucilage (GE-ChM) and 7.5% of OEO, the complex with the highest EE obtained (91.79 ± 0.05%). Complex coacervates with the highest EE were spray dried, testing two inlet temperatures (180 and 160°C) and two feeding rates (5 and 7.5 g/min). Dried coacervates were characterized in moisture content, solid yield, EE, particle size, bulk density, tapped density, compressibility index, and Hausner ratio. Inlet temperature and feeding rate affected the physical properties studied, being the sample with GE-ChM (160°C and 5 g/min) the one with the highest encapsulation efficiency observed (95.6 ± 0.39%). Moreover, GE-ChM powders had the best flow properties (compressibility index and Hausner ratio). Therefore, GE-ChM resulted in a better system than GE-GA to encapsulate OEO, being an alternative as encapsulating agents.

Keywords: complex coacervation, oregano essential oil, encapsulation, spray drying

1. Introduction

Essential oils are a mixture of aromatic and volatile compounds obtained from plants, including flowers, roots, leaves, seeds, among others. These are a common source of bioactive ingredients with several functional properties such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antihypertension, among others. In the food industry, these components gain particular attention due to their safe status and wide acceptance by consumers. Moreover, due to their antioxidant and antimicrobial properties, essential oils are an alternative as substitutes for chemical additives in the food industry. However, essential oils are easily volatile during processing and storage, and have low water solubility. Therefore, encapsulation is a way to provide an effective and viable method to enhance their solubility in water, increasing physical and chemical stabilities, by protecting them from air, light, heat and humidity that can lead to volatilization or undesirable reactions such as oxidation (Liolios, Gortzi, Lalas, Tsaknis, & Chinou, 2009; Bakry et al., 2016).

Complex coacervation is an encapsulation technique in which an electrostatic attraction between two biopolymers of opposite charges, normally a protein and a polysaccharide, is involved; as a result, a coacervate is formed. It consists of two immiscible liquid phases, one rich in polymers and another phase poor in these (Yan & Zhang, 2014; Bakry et al., 2016; Thies, 2016). Gelatin, especially type A, as cation and arabic gum as anion are the most common encapsulants widely used in food within the coacervation technique (Tamjidi, Nasirpour, & Shahedi, 2012). As a result of the current trend of consumers for the increasing concern for the safety of animal products, the use of vegetable proteins as encapsulating materials has arisen, since it is known that plant proteins are less allergenic compared to proteins derived from animals in food applications (Nesterenko, Alric, Silvestre, & Durrieu, 2013). Among the vegetable proteins used as encapsulating materials in microencapsulation are proteins of vegetable origin such as isolates from soy, pea, and cereals. Being flaxseed and chia protein isolates two recently studied since these possess good foaming, excellent water and oil holding capacities, and the amino acid profile of these proteins are nutritionally desirable (Nesterenko et al., 2013;

Kaushik, Dowling, McKnight, Barrow, & Adhikari, 2016; Timilsena, Wang, Adhikari, & Adhikari, 2016). For polysaccharides, carrageenan, chitosan, carboxymethylcellulose, pectins are commonly used for complex coacervation; being mucilages a new proposal since these have unique properties of forming a gelatinous mass when soaked in water and excellent water-holding properties, functioning as an emulsifier and stabilizer of emulsions. As polysaccharide gums, mucilages are negative charged in a wide pH range and have the potential to interact with the positive charge of another biopolymer (such as proteins) to form complex coacervates (Nesterenko et al., 2013; Xiao, Liu, Zhu, Zhou, & Niu, 2014; Kaushik et al., 2016; Timilsena et al., 2016; González-Martínez et al., 2017; Maestrello et al., 2018; Hernández-Nava, López-Malo, Palou, Ramírez-Corona, & Jiménez-Munguía, 2019).

Complex coacervation has been used to encapsulate, protect and supply functional ingredients, such as flavors and polyunsaturated fatty acids, in order to increase their shelf life under different storage conditions, leading an alternative processing of food, to mask the taste or allowing the controlled release of the encapsulated ingredients (Tamjidi et al., 2012). In complex coacervation, the formation of capsules is affected by various factors such as the emulsion composition including the wall materials concentration, core oil and the quantity of emulsifiers added (Ties, 2016). In addition, drying the coacervates can improve their shelf-life, solubility and release properties (Dutra & Ferreira 2010; Anandharamakrishnan & Ishwarya, 2015; Kaushik et al., 2016).

Compared with microcapsules, nanocapsules represent more advantages since they are more effective at preventing oil materials to deteriorate due to undesired environmental conditions; these enhance the physical and chemical stability of the active substances; also, their bioactivity may increase due to the subcellular size achieved; having potential applications in many fields including the food industry (Donsì, Annunziata, Sessa, & Ferrari, 2011; Xiao, Li, Zhu, Zhou, & Niu, 2016). The purpose of the present study was to determine the effect of essential oil concentration in complex coacervation, using different encapsulating agents (gelatin-gum, arabic and gelatin-chia mucilage), through the formation of nanocapsules and their encapsulation efficiency, and

the effect of different conditions of operation during spray drying in the encapsulation efficiency, and the physical properties of the powders obtained.

2. Materials and methods

2.1. Materials

Chia seeds (*Salvia hispanica* L.) were purchased from Verde Limón Trading Company (Mexico City, Mexico). Gelatin (type B) was purchased from Gelco S.A. (Bogota, Colombia). Gum arabic (GA) was purchased from Caragum International (Marseille, France). Oregano essential oil (OEO) was purchased from Laboratorios Hersol (Mexico City, Mexico). Tween 80 used in this study was purchased from Sigma-Aldrich (USA). Other chemicals used were analytical grade, and were purchased from Hycel (Jalisco, Mexico).

2.2. Extraction of chia mucilage

The method described by Hernández-Nava et al. (2019) was used. Chia seeds were hydrated in distilled water in a ratio of 1:20 (w/v), and freeze-dried (Triad™ Labconco, USA). Using a mesh #35 (500 μm) the mucilage was mechanically separated from the seeds and stored at 25 ± 1.0°C inside a sealed container until further use.

2.3. Preparation of coacervates

Complex coacervation process was made following the method described by Hernández-Nava et al. (2019) with minor modifications. Encapsulating agents, gelatin (GE) and chia mucilage (ChM) were mixed at mass ratio of 2:1, with solid concentration of 0.2% (w/w) in aqueous solution. A solution of GE (0.13% w/w) and another of ChM (0.07% w/w) + Tween 80 (2.5% w/w) were prepared individually dissolving the components in distilled water with constant stirring (350 rpm) at 40 ± 1.0°C until dissolved. Both solutions were mixed with oregano essential oil (5 or 7.5% w/w), and then homogenized by low frequency (20 kHz) ultrasound (CP-505, Cole-Parmer Instrumental Company, USA) for

10 min applying an intensity of 84 μm of wave amplitude (70% of intensity). The pH was adjusted to 3.6 by adding HCl 0.1 N dropwise maintaining the solution with constant stirring (250 rpm) for 5 min. Following the same methodology previously described, a second ultrasound homogenization was applied for 5 min; then, the system was cooled down to 25°C and stored at 4.0 ± 1.0°C until further use. Maltodextrin (20% w/w) was added to the coacervate (with excess of water) before drying to help the spray drying of the coacervate (Adhikari, Howes, Bhandari, & Troung, 2004; Kaushik et al., 2016). The coacervate was spray-dried using a mini spray drier (B-290, BUCHI Labortechnik, Switzerland). Two inlet temperatures (180 and 160°C) and two feeding rates (5 and 7.5 g/min) were tested. The spray-dried coacervates were collected and stored inside an amber flask at 25 ± 1.0°C until further characterization. To prepare coacervates of gelatin-gum Arabic (GE-GA) a mass ratio of 1:1, and total solid concentration of 2% was used (Xiao, Li, & Zhu, 2015). A solution of GE (1% w/w) and another of GA (1% w/w) + Tween 80 (2.5% w/w) were prepared individually dissolving the components in distilled water with constant stirring (350 rpm) at 40 ± 1.0°C until total dissolve. Once both solutions were obtained, these were mixed together with oregano essential oil (5 or 7.5% w/w). Homogenization and spray drying processes were the same as the GE-ChM system described above. System of gelatin-gum Arabic was studied for comparative purposes.

2.4. Characterization of liquid coacervates

2.4.1. FTIR spectra of complex coacervates

An FTIR spectrometer (Cary 630, Agilent Technologies, USA), in the range of wave number from 3800 to 800 cm^{-1}, was used to determine the functional groups in OEO, tween 80 and complex coacervates (with 5 or 7.5% w/w of OEO). A diamond ATR accessory (Agilent Technologies, USA) was used. 10 μL of sample was added to the diamond crystal and the determination was carried out using the MicroLab PC Software (v.5.3, Agilent Technologies, USA). Analysis was carried out by triplicate.

2.4.2. Particle size distribution

Size distribution of the liquid coacervates containing OEO was determined by a dynamic light scattering particle analyzer (Nanotrac Wave II, Microtrac Inc., USA). Analysis was carried out by triplicate.

2.4.3. Encapsulation efficiency

The encapsulation efficiency (EE) was determined according to the method described by Xiao et al. (2016) with some modifications. The maximum absorbance for OEO was observed at 271 nm using an UV/VIS (Cary 100, Varian Inc., USA). A standard curve using different concentrations (0.5 – 5 μL) of OEO dissolved in n-hexane was made. The encapsulation efficiency was calculated by determining the free essential oil in the coacervate by dissolving 1 mL of coacervate in 10 mL of n-hexane with constant stirring (250 rpm) for 30 s. An aliquot of 1 mL was taken from the surface and was dissolved in 9 mL mL of n-hexane. This solution was scanned at the maximum absorbance observed. Determinations were made by triplicate. EE was calculated using the following equation:

$$EE\ (\%) = \frac{TEO - FEO}{TEO} \times 100 \quad (1)$$

Where TEO is the theoretical essential oil content in the coacervate formulation, and FEO is the free essential oil measured in the coacervate.

2.4.4. Morphology

An optical microscope (Axiovert 25, Zeiss, Germany), coupled to a digital camera controlled by Zen Lite software (v.2011, Zeiss, Germany) using the 100X/1.25 objective, was used for systems to establish a relation between encapsulation efficiency and the shape of capsules.

2.5. Characterization of dried coacervates

2.5.1. Moisture content

To determine the moisture content, A.O.A.C. (2000) method 926.12 was used. Analysis was carried out by triplicate.

2.5.2. Solid yield

To determine the losses during the spray drying process the solid yield was calculated as the ratio of the powder mass collected after drying process to the initial content of solids in the liquid coacervate before drying.

2.5.3. Encapsulation efficiency (EE)

It was calculated by measuring the surface oil (SO) and total oil (TO) of the dried coacervates. SO was determined by the modified method described by Liu, Low, & Nickerson (2010), as follows. A sample of 0.5 ± 0.001 g of dried coacervates was dispersed in 20 mL of n-hexane with constant stirring (80 rpm) for 15 s. The oil phase with n-hexane was filtered (Whatman #41); then, centrifuged at 2,500×g at 25 ± 1.0 °C for 10 min. The oil phase was recovered, and the excess of n-hexane was evaporated using a vacuum oven (G0553-10, Cole-Parmer, USA) at 70°C ± 1.0°C. SO content was determined gravimetrically. TO content in the dried coacervates was determined by acid digestion using the modified method described by Eratte, Wang, Dowling, & Adhikari (2015). A sample of 0.5 ± 0.001 g of dried coacervates was dissolved in 3 mL of HCl 4 N with constant stirring (350 rpm) for 5 min. After total dilution, 20 mL of n-hexane was added and maintained with constant stirring (250 rpm) for 1 h. HCl was separated with a separation funnel, and the oil phase with n-hexane was recovered. Then, the sample was centrifuged at 2,500 ×g at 25 ± 1.0 °C for 10 min, and the oil phase was recovered in a flask, previously set at constant weight. Excess of n-hexane was evaporated with a vacuum oven (G0553-10, Cole-Parmer, USA) at 70°C ± 1.0 °C. TO content was determined gravimetrically. Determination of TO and SO was carried out by triplicate. The percent of EE was calculated using the following equation:

$$EE\ (\%) = \frac{TO - SO}{TO} \times 100 \quad (2)$$

2.5.4. Particle size distribution

Particle size distribution of the dried coacervates (containing OEO) was determined using a laser diffraction particle analyzer (Bluewave, Microtrac Inc., USA). Analysis was carried out by triplicate.

2.5.5. Bulk density and tapped density

The bulk density (ρ_{bulk}) was determined by measuring the volume occupied by a known quantity of powder in a 10 mL test tube, measuring its weight before and after adding the powder. Similarly, the tapped density (ρ_{tap}) was determined, with the difference of the volume of powder measured once it does not change after mechanically tapping (WHO, 2018). Determinations were made by triplicate.

2.5.6. Compressibility index and Hausner ratio

According to the WHO (2018), the compressibility index (CI) and Hausner ratio (HR) are measures of the tendency of a powder to be compressed. Determinations were made by triplicate. To calculate the CI and HR, ρ_{bulk} and ρ_{tap} the following the equations were used:

$$CI = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}} \times 100 \quad (3)$$

$$HR = \frac{\rho_{tap}}{\rho_{bulk}} \quad (4)$$

2.6. Statistical analysis

Analysis of variance (ANOVA) and Tukey's comparison tests, using a confidence level of 95%, were performed to statistical analyze the obtained data using Minitab (v.17, LEAD Technologies Inc., USA).

3. Results and discussion

3.1. Characterization of liquid coacervates

3.1.1. FTIR spectra of OEO, tween 80 and OEO complex coacervates

Spectra of tween 80 (Fig. 1A) shown characteristic peaks. The bands 2920.3 cm^{-1} and 2856.2 cm^{-1} represent the C–H stretching of the methylene group. Peaks 1734.5 cm^{-1}, 1457.7 cm^{-1} and 1093.2 cm^{-1} are assigned to C=O stretching, C–H bending of methylene group, and C–O–C stretching, respectively (Choudhury, Mandal, Chakravorty, Gopal, & Goswami, 2013). For OEO (Fig. 1B), the spectra exhibited characteristic peaks for carvacrol (810.4, 993.7, 1115.5, 1173.8 and 1251.5 cm^{-1}) which is expected to be an abundant component present in this essential oil. Peaks 2959.1 cm^{-1}, 2925.8 cm^{-1} and 2869.3 cm^{-1} are assigned to C-H stretching that are common in essential oils (Solano & Rojas 2017). FTIR spectra of complex coacervates of GE-ChM and GE-GA with 5% of OEO (Fig. 1C and Fig. 1D) and 7.5% of OEO (Fig. 1E and Fig. 1F) are dominated by bands corresponding to carvacrol. Peaks presented a slight shift towards right that can be attributed to the interaction between OEO and tween 80. Since no new peaks in the complex coacervates were found, the denaturation of the OEO is discharged. Moreover, the peaks of complex coacervates of OEO showed a slight decrease in absorbance compared to the free oil, suggesting the encapsulation of the essential oil.

3.1.2. Effect of the concentration of essential oil and emulsifier added on the particle size distribution

Complex coacervates with 5 and 7.5% of OEO, prepared with GE-ChM and GE-GA, showed unimodal distributions with shift toward the left (to the smaller size) for their particle size distributions. D_{10}, D_{50}, and D_{90} values showed a significant difference ($p < 0.05$) between samples, being GE-GA with 5% of OEO the one with the smallest particle size (Table 1). The difference of particle sizes between samples of GE-ChM and GE-GA could be because of interaction between the amount of tween 80 and the essential oil used.

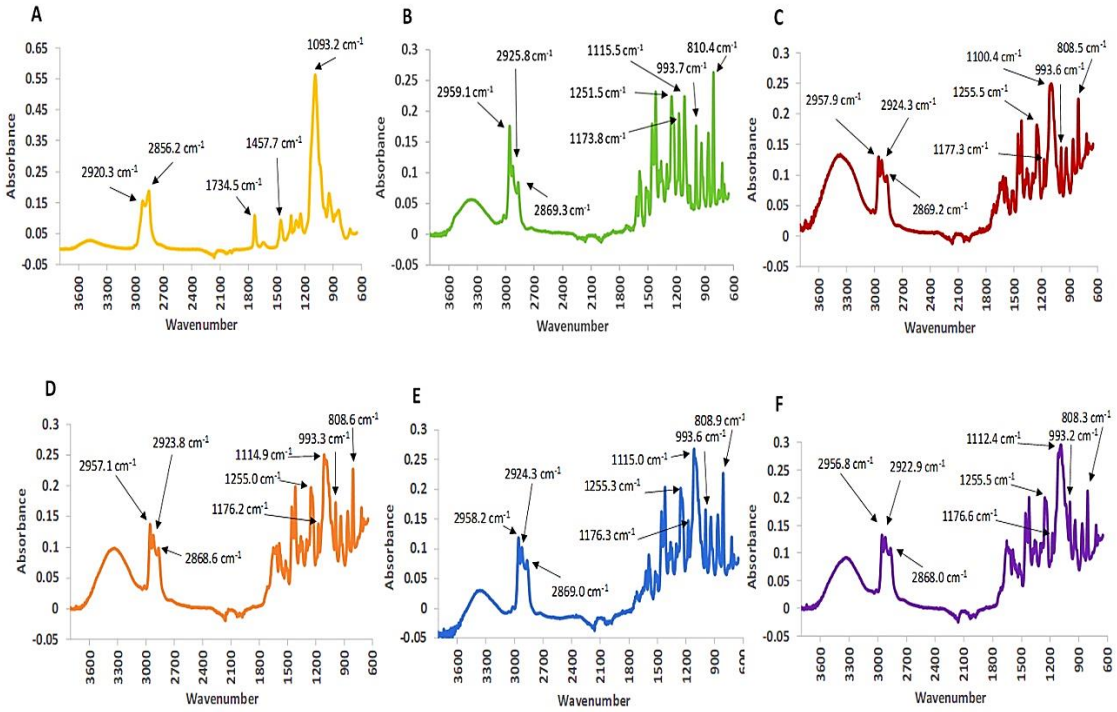

Fig. 1. FTIR spectra of tween 80 (A), OEO (B), complex coacervate GE-ChM 5% OEO (C), complex coacervate GE-GA 5% OEO (D), complex coacervate GE-ChM 7.5% OEO (E), complex coacervate GE-GA 7.5% OEO (F).

Table 1
Particle size and encapsulation efficiency of coacervates of GE-ChM and GE-GA at different oil concentrations.

	Coacervate GE – ChM		Coacervate GE – GA	
Particle Size (nm)				
	5% OEO	7.5% OEO	5% OEO	7.5% OEO
	D_{10} 38.9^{a}	D_{10} 76.5^{b}	D_{10} 17.7^{c}	D_{10} 34.8^{d}
	D_{50} 57.1^{a}	D_{50} 94.6^{b}	D_{50} 21.4^{c}	D_{50} 48.1^{d}
	D_{90} 87.5^{a}	D_{90} 119.8^{b}	D_{90} 26.9^{c}	D_{90} 85.2^{d}
Encapsulation efficiency (%)				
	5% OEO	7.5% OEO	5% OEO	7.5% OEO
	76.39 ± 0.02^{a}	91.79 ± 0.05^{b}	89.93 ± 0.06^{c}	91.23 ± 0.04^{d}

Different letters in row show significant differences ($p < 0.05$) between samples.

When the proportion of tween 80 was closer to the proportion of OEO added in the formulation, it was observed a decrease in the particle size. Similar results were reported by Hu et al. (2011) for nanocapsules of polybutylcyanoacrylate (PBCA) using sorbitan monolaurate as emulsifier (tween 20). This is explained since the formation of smaller droplets of OEO are promoted by the increase in the concentration of Tween 80 which enhances the interfacial tension reduction and droplet breaking (Malmsten, 2002; Celis, Contreras, Forgiarini, Rosenzweig, & Garcia-Rubio, 2016).

Complex coacervates with 5 and 7.5% of OEO, prepared with GE-ChM and GE-GA, showed unimodal distributions with shift toward the left (to the smaller size) for their particle size distributions. D_{10}, D_{50}, and D_{90} values showed a significant difference ($p <$

In addition, Lv, Yang, Li, Zhang, & Abbas (2014) obtained values of D_{50} from 74.58 ± 0.40 to 384.14 ± 8.28 nm in the nanoencapsulation of jasmine essential oil by complex coacervation without the use of crosslinking. Results obtained in this study are in the range of D_{50} reported by the previous authors applying complex coacervation.

3.1.3. Encapsulation efficiency

Complex coacervates with 5 and 7.5% of OEO with GE-ChM and GE-GA showed a significant difference ($p < 0.05$) for encapsulation efficiency. It was observed that the particle size had an effect in the EE, as particle size decreased EE showed lower values (Table 1). Xiao et al. (2016) and Hu et al. (2011) observed similar results by adding emulsifiers in the nanoencapsulation of styrallyl acetate and rose fragrance, respectively. With a smaller particle size exists a greater space between nanocapsules that hinders their agglomeration and the formation of coacervates, decreasing EE of this technique. It was observed that samples with GE-GA had lower particle size than those of GE-ChM; for a concentration of 5% of OEO with GE-GA a better EE than those of GE-ChM, was obtained. For this study, EE of 91.79 ± 0.05% using GE-ChM and 91.23 ± 0.04% with GE-GA were achieved, which are higher compared with the values reported of 63.68% (Xiao et al., 2016) and 67.65% (Hu et al., 2011) for nanoencapsulation of components of essential oils.

Other important factor in encapsulation efficiency is the nature and the concentration of the oil used. Prata & Grosso (2015) reported that essential oils (EO) presented more stability and encapsulation efficiency compared to vegetable and mineral oils, since EO contain hydrophilic compounds that can act as surfactants. On the other hand, at low concentrations of oil there are microcapsules containing less material in the core because there is an excess of encapsulating material. By increasing the concentration of oil, the amount of this in the core of the microcapsules increases. However, at high concentrations of oil and low proportions of encapsulating agents, the amount of encapsulated oil is reduced due to the decrease in coacervates formation. Tamjidi et al. (2012) reported that at concentrations of fish oil below 3% and greater than 10%, the amount of encapsulated oil in complex coacervation decreased. Our preliminary research found similar results, in which concentrations below 3% and greater than 10%, the encapsulation efficiency decreased in the encapsulation of essential oils by complex coacervation.

3.1.4. Morphology of complex coacervates

For both systems, GE-ChM and GE-GA, it was observed that complex coacervates with 5% of OEO had the characteristic grape agglomerate shape for complex coacervates along with individual capsules (Fig. 2A and Fig. 2C); while the complex coacervates with 7.5% of OEO only shown grape agglomerate shapes (Fig. 2B and Fig. 2D). Xiao et al. (2016) reported the formation of individual capsules in the nanoencapsulation of styrallyl acetate by complex coacervation, obtaining a lower EE compared to the results achieved in this study. For both studies, the lower encapsulation efficiency in the complex coacervates can be attributed to the individual capsules formed because of the decrease in the particle size and the electrostatic interactions between biopolymers.

3.2. Characterization of dried coacervates

For GE-ChM and GE-GA, 7.5 % of OEO had the highest value of EE observed, and presented the characteristic morphology of complex coacervates. Only this oil content was used for further analysis with spray drying. There are several important properties for food

powders, among them moisture content, solid yield, encapsulation efficiency, particle size, and flow properties are commonly used for the characterization of powders.

3.2.1. Effect of inlet temperature and feeding rate in moisture content and solid yield

Moisture content is an important variable to the shelf life of powders since it may prevent changes in the physicochemical characteristics during storage (Alvarenga-Botrel et al., 2012). Solid yield represents the powder recovered in the spray dryer after the encapsulation process (Jafari, He, & Bhandari, 2007; Roccia, Martínez, Llabot, & Ribotta, 2014; Asensio et al., 2017).

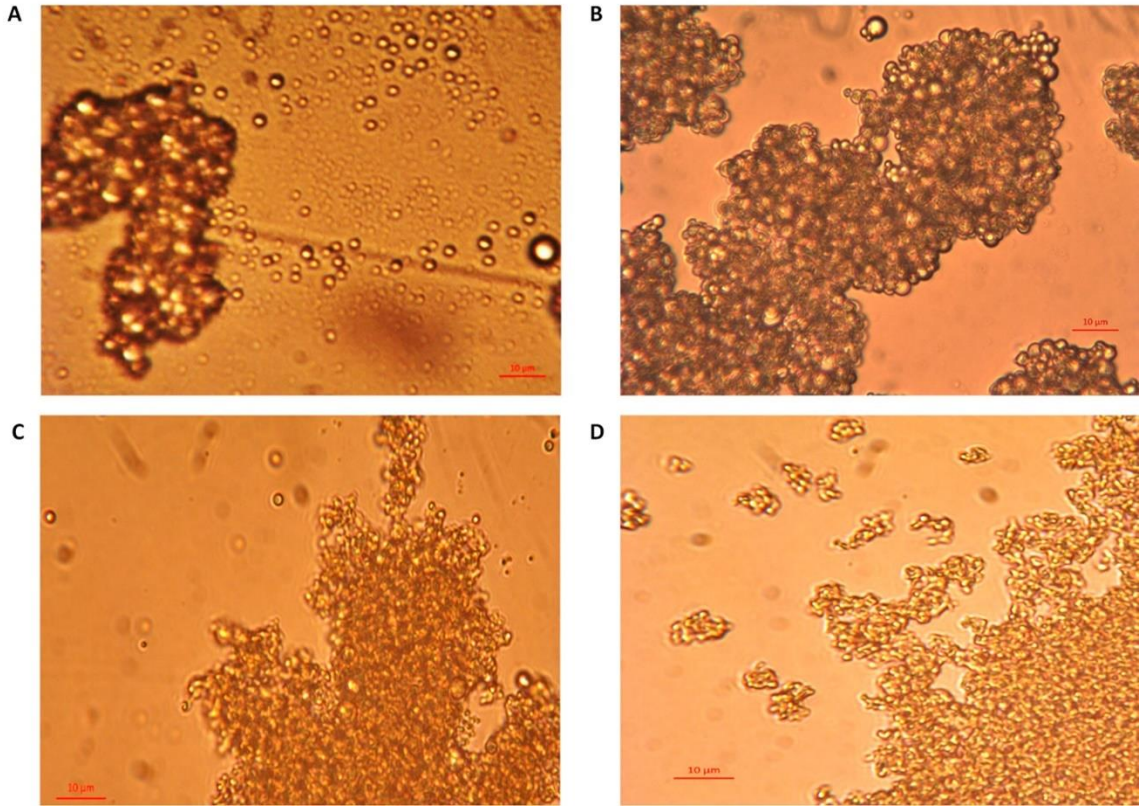

Fig. 2. Micrographs of complex coacervates with 5% OEO – GE-ChM (A), 7.5% OEO – GE-ChM (B), 5% OEO – GE-GA (C), and 7.5% OEO – GE-GA (D).

Results of moisture content are shown on Table 2. For the samples with GE-ChM moisture content varied in a range from 3.49 to 4.58% (w.b.), while GE-GA ranged from 3.86 to 4.55% (w.b.), showing a significant difference between samples ($p < 0.05$); these values are within the range of 1-5% of moisture content generally accepted for food powders (Opaliński, Chutkowski, & Hassanpour, 2016). For both mixes of encapsulating agents, GE-ChM and GE-GA, the increasing of inlet temperature decreased the moisture content; high temperatures increase the mass transfer and moisture is removed from the system. However, when the feeding rate increase, the heat transfer decrease resulting in the increase of moisture content. Similar results were observed by Alvarenga-Botrel et al. (2012) in the microencapsulation of oregano essential oil evaluating different spray drying conditions. For solid yield (Table 2), using GE-ChM the maximum value obtained in this study was 88.5%, and for GE-GA was 88.0%. It was observed that decreasing the feeding rate increased the solid yield for both mixes of encapsulating agents. Alvarenga-Botrel et al. (2012) reported moisture contents of 1.3 to 3.65% and a solid yield of 59.7%; meanwhile Asensio et al. (2017) reported values of 3.1 to 5.1% for moisture content and 31.1 to 51.8% for solid yield. These authors worked in the microencapsulation of oregano essential oil by emulsification and spray drying. Kaushik et al. (2016) reported values from 3.20 to 3.70% of moisture content and ranges from 35.46 to 52.60% for solid yield in the spray drying of microencapsules of flaxseed oil by complex coacervation. Differences between the moisture contents and solid yields observed in this study and the reported by other authors could be due to the nature of the encapsulated oil, the encapsulation method, and the operation conditions during spray drying.

3.2.2. Encapsulation efficiency

Encapsulation efficiency is the amount of essential oil encapsulated inside the powder particles; it is of interest for essential oils since they are volatile compounds highly susceptible to be lost during the drying process (Jafari et al., 2007; Roccia, Martínez, Llabot, & Ribotta, 2014; Asensio et al., 2017).

Table 2
Characterization of powders of oregano essential oil encapsulated by complex coacervation.

Gelatin – Chia mucilage				
	180°C – 7.5 g/min	**180°C – 5.0 g/min**	**160°C – 7.5 g/min**	**160°C – 5.0 g/min**
% Moisture (w.b.)	4.23 ± 0.02[a]	3.49 ± 0.01[b]	4.58 ± 0.01[c]	3.68 ± 0.04[d]
Bulk density (g/cm^3)	0.244 ± 0.002[a]	0.234 ± 0.001[b]	0.282 ± 0.001[c]	0.265 ± 0.002[d]
Tapped density (g/cm^3)	0.348 ± 0.003[a]	0.335 ± 0.002[b]	0.403 ± 0.002[c]	0.379 ± 0.003[d]
Compressibility index	30 ± 0.01[a]	30 ± 0.01[a]	30 ± 0.01[a]	30 ± 0.01[a]
Hausner ratio	1.43 ± 0.01[a]	1.43 ± 0.01[a]	1.43 ± 0.01[a]	1.43 ± 0.01[a]
Particle size (μm)				
D_{10}	1.92 ± 0.02[a]	1.65 ± 0.02[b]	2.30 ± 0.03[c]	1.55 ± 0.02[d]
D_{50}	3.61 ± 0.02[a]	2.93 ± 0.02[b]	4.20 ± 0.03[c]	2.74 ± 0.02[d]
D_{90}	8.20 ± 0.02[a]	7.34 ± 0.02[b]	8.83 ± 0.03[c]	6.73 ± 0.02[d]
Solid yield (%)	81.0*	88.5*	80.5*	86.0*
EE (%)	82.2 ± 0.61[a]	85.2 ± 0.71[b]	88.2 ± 0.29[c]	95.6 ± 0.39[d]
Gelatin – Gum Arabic				
	180°C – 7.5 g/min	**180°C – 5.0 g/min**	**160°C – 7.5 g/min**	**160°C – 5.0 g/min**
% Moisture (w.b.)	4.44 ± 0.04[a]	3.86 ± 0.05[b]	4.55 ± 0.05[c]	4.19 ± 0.02[d]
Bulk density (g/cm^3)	0.178 ± 0.001[a]	0.157 ± 0.001[b]	0.202 ± 0.001[c]	0.158 ± 0.001[b]
Tapped density (g/cm^3)	0.357 ± 0.001[a]	0.314 ± 0.001[b]	0.404 ± 0.001[c]	0.316 ± 0.001[b]
Compressibility index	50 ± 0.01[a]	50 ± 0.01[a]	50 ± 0.01[a]	50 ± 0.01[a]
Hausner ratio	2.00 ± 0.01[a]	2.00 ± 0.01[a]	2.00 ± 0.01[a]	2.00 ± 0.01[a]
Particle size (μm)				
D_{10}	6.73 ± 0.07[a]	7.14 ± 0.06[b]	8.11 ± 0.08[c]	6.08 ± 0.08[d]
D_{50}	14.68 ± 0.07[a]	14.45 ± 0.06[b]	16.34 ± 0.08[c]	14.30 ± 0.08[b]
D_{90}	26.83 ± 0.07[a]	25.12 ± 0.06[b]	29.93 ± 0.08[c]	30.31 ± 0.08[d]
Solid yield (%)	72.0*	88.0*	74.0*	87.0*
EE (%)	94.5 ± 0.44[a]	87.5 ± 0.47[b]	90.3 ± 0.32[c]	88.6 ± 0.48[b]

Different letters in row show significant differences ($p < 0.05$) between samples.
*All properties were calculated by triplicate except for solid yield

Samples with GE-ChM had an encapsulation efficiency in the range of 82.15 to 95.6%; meanwhile samples with GE-GA had values from 88.6 to 94.5% (Table 2). Inlet temperature and feeding rate had significant difference ($p < 0.05$) for EE in GE-ChM samples, while GE-GA samples with feeding rate of 5 g/min did not have significant difference ($p > 0.05$) in EE. For GE-ChM, the decrease of inlet temperature and feeding rate resulted in the highest EE, while for GE-GA was the opposite. This difference could be due to the interaction between the encapsulation agents, in which the microcapsules of GE-ChM were more susceptible to high temperatures than GE-GA. In the thermogravimetric analysis (TGA) of GE, Qi, Quian, Zhao & Wei (2015) reported that the degradation starts at 275.8 °C; as for GA and ChM, it starts at the temperatures of 309°C and 270°C, respectively (Timilsena et al., 2016; Barra et al., 2019). Therefore, the degradation of the encapsulating agents used in this study is discharged. However, the use

of elevated temperatures during spray drying gives as a result capsules with fissures or cracks which could decrease the encapsulation efficiency (Jafari et al., 2007). The higher EE for GE-ChM was achieved at 160°C and 5 g/min; meanwhile for GE-GA it was obtained at 180°C and 7.5 g/min (Table 2). This could be because a lower mass transfer rate is needed in the GE-ChM system to allow the water bound in the mucilage gel to evaporate and form capsules with a smooth surface. Contrary to the GE-GA systems which needs an increase in the mass transfer rate allowing a quick drying of the wall, but it is combined with a feeding rate that permits enough moisture in the capsule to avoid the formation of cracks on the surface.

Rojas-Moreno, Osorio-Revilla, Gallardo-Velázquez, Cárdenas-Bailón, & Meza-Márquez (2018) reported values of EE of 56.02 ± 0.81% for the microencapsulation by complex coacervation between whey protein isolate-chitosan using glutaraldehyde as crosslinking agent of orange essential oil after being spray dried. A value of EE of 87.6 ± 3.06% was observed by Kaushik et al. (2016) in the spray drying of microcapsules of flaxseed oil with flaxseed protein-flaxseed gum complex coacervates using glutaraldehyde as crosslinking agent. Alvarenga-Botrel et al. (2012) reported values ranged from 5.1 to 33.9% for EE in the microencapsulation of oregano essential oil by emulsification and spray drying. These values reported by other authors are lower than those obtained in this study using mixtures of encapsulating agents of GE-ChM and GE-GA without using a crosslinking agent. These differences with the values reported in the bibliography could be due to the nature of the essential oil used, the encapsulation technique applied, the operation conditions during spray drying, and the nano size of the capsules before being spray dried which resulted in a stable system during the drying process.

3.2.3. Particle size distribution

Another important property is particle size since it affects the stability of the powder and the flow properties of it. The particle size distribution is shown on Table 2. GE-ChM and GE-GA samples shown a unimodal distribution with shift toward the left (to the smaller

size). GE-ChM samples shown significant difference ($p < 0.05$) for D_{10}, D_{50} and D_{90} values, while GE-GA samples with feeding rate of 5 g/min did not have significant difference ($p > 0.05$) for D_{50}. It was observed that increasing the feed rate increased the particle size, possibly due to the amount of moisture present in the drying chamber that allows an accumulation of material on the surface of the particle as a result of the increase of cohesivity. Moreover, when the inlet temperature is increased, the particle size increases because there is more swelling caused by high temperatures (Kurozawa, Morassi, Vanzo, Park, & Hubinger, 2009). Qi et al. (2015) reported that in the TGA of GE, the stage of mass loss due to the evaporation of water occurs in a temperature ≈70°C. In the case of GA, Barra et al. (2019) reported that this phenomena occurs in the range of temperature of 72-103°C; meanwhile, Timilsena et al. (2016) reported that this stage of mass loss due to the evaporation of water in ChM occurs in the range of 60-120°C. In this study was observed that the particle size of ChM systems was smaller compared to the GA ones. This could be explained since ChM starts to lose water at a lower temperature (60°C) than GA (72°C), giving as a result a less swelling of the particles due to the effect of water available and the drying temperature in the ChM systems.

Particle size distribution of the dried coacervates was wider than liquid coacervates; this was expected since the addition of solids generates a larger particle size because of its effect in the increase of viscosity (Koç, Sakin-Yilmazer, Kaymak-Ertekin, & Balkir, 2014), causing a wider distribution.

3.2.4. Effect of particle size in ρ_{bulk}, ρ_{tap}, CI and HR

It was observed that at an inlet temperature of 160°C and a feed rate of 7.5 g/min, there was more moisture content in the drying chamber which resulted in a larger particle size, with that increase, the bulk and tapped densities also increased (Table 2). In this study, similar results in the behavior of bulk and tapped densities were observed, to those reported by Mitra et al. (2017). These authors observed that tapped density was higher than bulk density since smaller particles occupy the voids between larger particles and

attain a dense packing condition as a result of the tapping. According to Carr (1965) and Hausner (1967), the flowability of the GE-ChM powders is classified as bad and its cohesiveness as intermediate, while the flowability of GE-GA powders are classified as very bad and its cohesiveness is high. Even if particle size had no effect in CI and HR, which had no significant difference ($p > 0.05$) between samples (Table 2), as Seerangurayar, Manickavasagan, Al-Ismaili, & Al-Mulla (2017) reported, a small and uniform particle size, as seen in the GE-ChM powders, resulted in high bulk density and low value of compressibility index and Hausner ratio compared to the GE-GA powders. The above is explained since small and uniform particle sizes offer a larger contact surface with the surroundings as a result of the decrease in inter-particle voids (Seerangurayar et al., 2017).

4. Conclusions

Complex coacervation between gelatin and chia mucilage could be used as an alternative of the most used system in complex coacervation (gelatin and arabic gum), as the encapsulation efficiency (>90%) of oregano essential oil suggested in the liquid and spray-dried coacervates. The amount of emulsifier (tween 80) and essential oil added influenced the formation of coacervates. Physicochemical properties of the powders were affected by the inlet temperature, feeding rate and the mix of encapsulating agents used. Further research is needed to study the practical applications of this complexed system in food products, as delivery vehicles for active and unstable ingredients.

Conflict of interest

The authors declare no conflict of interest.

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4. ESSENTIAL OILS ENCAPSULATED BY COMPLEX COACERVATION USING ULTRASOUND OR MICROFLUIDIZATION HOMOGENIZATION AND ITS EVALUATION AS ANTIMICROBIALS IN GREEN JUICE

(Under review)

Essential oils encapsulated by complex coacervation using ultrasound or microfluidization homogenization and its evaluation as antimicrobials in green juice

Abstract

Complex coacervation between gelatin and chia mucilage was used to encapsulate 7.5% (w/w) of oregano essential oil (OEO) or thyme essential oil (TEO). Complex coacervates were homogenized by ultrasound (wave amplitude of 84 μm) or microfluidization (20,000 psi and two passes), and then spray-dried at 160°C (air inlet temperature) with a feeding rate of 5 g/min. Powders were characterized by its physicochemical properties, moisture content, water activity, solid yield, encapsulation efficiency and particle size. Antimicrobial activity of the reconstituted powders was tested against *Escherichia coli* ATCC 25922, in a commercial green juice determining its minimum inhibitory concentration (MIC). Powders with OEO, homogenized by ultrasound or microfluidization, showed a significant difference ($p < 0.05$) for moisture content, water activity and particle size; meanwhile, powders with TEO, homogenized by ultrasound or microfluidization, showed a significant difference ($p < 0.05$) for water activity and particle size. Reconstituted powders of OEO or TEO, homogenized by ultrasound, had MIC values of 274 ppm and 186 ppm, respectively. These values were lower than the ones homogenized by microfluidization, 367 ppm for OEO and 280 ppm for TEO. Based on the results, ultrasound and microfluidization could be an alternative as homogenization techniques applied in complex coacervation to be used to encapsulate essential oils to be utilized in various foods to avoid microbial contaminations.

1. Introduction

Food safety is a permanent concern for consumers and the food industry due to the increasing prevalence of foodborne diseases, which lead to economic and social problems (Burt, 2004; Lamine, Rahali, Hammami and Mliki, 2019). Being juices products that have a short shelf life due to their susceptibility to microbial and enzymatic deterioration (Winniczuk and Parish, 1997). Thermal processing is the most used method for microbial control in juices; however, it can cause loss of nutrients and change the sensory properties of the product (Raybaudi-Massilia, Mosqueda-Melgar and Martín-Belloso, 2006). Since synthetic chemical preservatives can be a threat to the human health and harmful to the ecological environment,

a growing interest to replace synthetic chemicals by natural preservatives such as essential oils extracted from plants which possess bioactive properties have prompted (Willcox, Ash and Catignani, 2004; Gaglio et al., 2019). Essential oils are volatile substances from plants with antimicrobial activity against food-borne pathogens and food spoilage bacteria (Burt, 2004; Wu, Luo, and Wang, 2012; Gedikoğlu, Sökmen and Çivit, 2019). Two essential oils that have proven antimicrobial effects are oregano and thyme. The main volatile compounds in these essential oils are carvacrol, thymol and γ-terpinene for oregano essential oil, and thymol for thyme essential oil (Božik, Nový and Klouček, 2017; Gedikoğlu et al., 2019; İşcan, Demirci and Köse, 2020).

However, essential oils can loss their antimicrobial properties since these are chemically unstable when exposed to certain environmental conditions such as elevated temperatures, light, moisture, and oxygen (Misharina, Polshkov, Ruchkina, and Medvedeva, 2003). To limit the composition loss during processing and storage, and alternative is the use of encapsulation techniques (Bakry et al., 2016).

Complex coacervation is an encapsulation technique that involves electrostatic attraction between two biopolymers of opposite charges (Bakry et al., 2016; Thies, 2016). In addition, these coacervates could be dried to improve their shelf-life, solubility and release properties (Dutra & Ferreira 2010; Anandharamakrishnan and Ishwarya, 2015; Kaushik, Dowling, McKnight, Barrow, and Adhikari, 2016; Hernández-Nava, Ruiz-González and Jiménez-Munguía, 2020). However, the formation of complex coacervates is affected by different factors, being one of them the type of homogenization (Ach et al., 2015; Prata & Grosso, 2015). In the homogenization of complex coacervates, various equipments have been used, including rotor systems, high-speed mixers, ultrasound homogenizers, among others (Brzozowska, 2019); being microfluidization an alternative not yet studied.

Microfluidization is a homogenization technique that uses high pressure. A fluid is forced through an interaction chamber that creates high-speed microcurrents, generating turbulence, large shear forces and cavitation resulting in a reduced particle size (McCrae, 1994; Zhang, Peppard, and Reineccius, 2015; Villalobos-Castillejos et al., 2018).

Nowadays, few information of the application of complex coacervation in the encapsulation of essential oils to be applied as antimicrobials in food products has been reported (Dias-Gonçalves et al., 2017). Moreover, there are no studies reporting the application of microfluidization in the formation of complex coacervates.

Therefore, the aim of this study was to characterize spray-dried complex coacervate powders of oregano essential oil or thyme essential oil, homogenized by ultrasound or microfluidization, and to compare the antimicrobial activities of the reconstituted powders in green juice against *Escherichia coli*.

2. Materials and methods

2.1. Materials

Gelatin (type B) was purchased from Gelco S.A. (Bogota, Colombia) and chia seeds (*Salvia hispanica* L.) were purchased from Verde Limón Trading Company (Mexico City, Mexico). Oregano essential oil (OEO) and thyme essential oil (TEO) were purchased from Laboratorios Hersol (Mexico City, Mexico).

2.2 Chemical analysis of essential oils

Essential oils were analyzed using a gas chromatographer (6850N, Agilent Technologies, USA), coupled with a mass-spectrometer detector (5975 C, Agilent Technologies, USA) with a split-splitless injector (10:1 split ratio). A fused silica capillary column (HP-5MS, Agilent Technologies, USA) with the characteristics of 30 m (length), 250 mm (width) and 0.25 μm (film thickness) was used. Carrier gas (helium) had a flow rate of 1.1 mL/min. Injector was set at 300°C. The column oven temperature was set at 60°C for 2 min to 250°C at a rate of 10°C/min. Compounds of essential oils were identified using the National Institute of Standards and Technology Mass Spectral Database (Ruiz-Gonzalez, Lopez-Malo, Palou, Ramirez-Corona, Jimenez-Munguia, 2019).

2.3. Extraction of chia mucilage

Chia mucilage was obtained following the method described by Hernández-Nava, López-Malo, Palou, Ramírez-Corona and Jiménez-Munguía (2019). In a ratio of 1:20 (w/v), chia seeds were hydrated in distilled water and freeze-dried (Triad™ Labconco, USA). The mucilage was stored inside a sealed container at 25 ± 1.0°C until further use.

2.4. Preparation of coacervates

A solution of gelatin (0.133% w/w) and another of chia mucilage (0.063% w/w) were prepared. Once both solutions were obtained, these were mixed together at a speed of 250 rpm until complete homogenization, maintaining the temperature at 40 ± 1.0°C. Then, oregano essential oil or thyme essential oil (7.5% w/w) was added to the solution containing gelatin and chia mucilage. The sample was homogenized using two techniques, low frequency ultrasound and microfluidization. For ultrasound homogenization, the method described by Hernández-Nava et al. (2020) was used. An ultrasound (CP-505, Cole-Parmer Instrumental Co., USA) applying an intensity of 70% (wave amplitude of 84 μm) was utilized, and the pH was adjusted to 3.6 by adding HCl 0.1 N maintaining a temperature of 40 ± 1°C. For the microfluidization process, after the preparation of the solutions of gelatin and chia mucilage, these were mixed with the essential oil and kept under constant stirring (1100 rpm) for 15 min at 40 ± 1°C. Then, a pressure of 20,000 psi and two passes, as suggested by Zhang et al. (2015), were applied to the system using a microfluidizer (M-110P, Microfluidics, USA). The pH was adjusted to 3.3 using a 0.1 N HCl solution, keeping the solution under constant stirring (250 rpm) for 10 min at 40 ± 1°C. Samples homogenized by ultrasound or microfluidization were cooled to 25°C and stored at 4.0 ± 1.0°C until further use.

2.5 Drying of complex coacervates

Before drying, maltodextrin (20% w/w) was added to the coacervate (with excess of water) to help the spray drying of the coacervate (Adhikari, Howes, Bhandari, and Troung, 2004; Kaushik et al., 2016). The coacervate was spray-dried using a mini spray drier (B-290, BUCHI Labortechnik, Switzerland) using an air inlet temperature of 160°C and a feeding

rate of 5 g/min. Powders of coacervates were stored inside an amber flask at 25 ± 1.0°C until further characterization.

2.6. Characterization of dried coacervates

2.6.1. Moisture content and water activity

A.O.A.C. (2000) method 926.12 was used to determine the moisture content. Water activity was determined using the method 978.18 from A.O.A.C. (1995).

2.6.2. Solid yield

The solid yield was calculated as the ratio of the powder mass collected after drying process to the initial content of solids in the liquid coacervate before drying to determine the losses during the spray drying process.

2.6.3. Encapsulation efficiency (EE)

It was calculated by measuring the surface oil (SO) and total oil (TO) of the dried coacervates following the method described by Hernández-Nava et al. (2020). SO and TO content were determined gravimetrically. The percent of EE was calculated using the following equation:

$$EE\ (\%) = \frac{TO - SO}{TO} \times 100 \quad (1)$$

2.6.4. Particle size distribution

A laser diffraction particle analyzer (Bluewave, Microtrac Inc., USA) was used to determine the particle size distribution of the dried coacervates. To express the polydispersity of the powder, span was calculated using the following equation:

$$Span = \frac{D_{90} - D_{10}}{D_{50}} \times 100 \quad (2)$$

2.7. Evaluation of the antimicrobial activity of reconstituted powders of essential oils

2.7.1. Microbial culture preparation

Escherichia coli ATCC 25922 strain culture was provided by the Food Microbiology Laboratory of Universidad de las Americas Puebla, Mexico. On tryptone soy agar (BD

BIOXON, Mexico), bacterial strains were cultivated and incubated at 37 ± 1°C for 24 h and stored at 4°C for a maximum of three weeks. Two loops of the bacterial cultures were taken to inoculate tubes containing 10 mL of tryptone soy broth (BD BIOXON, Mexico); the tubes were incubated at 37 ± 1°C for 24 h.

2.7.2. Calculation of minimum inhibitory concentration (MIC) of reconstituted powders in green juice

MIC of reconstituted powders of OEO or TEO was evaluated as follows. 10 g of powder was reconstituted in 40 mL of sterilized distilled water, keeping it under constant stirring (350 rpm) for 30 min. This stock solution was added to tubes containing green juice (JUMEX®, Mexico) to obtain concentrations of 0.1-1 µL/mL of essential oil. Then, 200 µL of the bacterial suspensions at 10^8 CFU ml^{-1} concentrations were added to each tube to achieve a final concentration of 10^6 CFU ml^{-1}, obtaining a final volume of 20 mL per tube. Tubes were homogenized at 3,000 rpm for 15 seconds and incubated at 25 ± 1°C for 24 h by duplicate. After the incubation period, microbial growth was determined with the plate count agar method using tryptone soy agar (BD BIOXON, Mexico). MIC value corresponds to the lowest essential oil concentration that has negative growth after incubation of the plates at 37 ± 1°C for 24 h. This analysis was made by duplicate.

2.8 Statistical analysis

Obtained data was statistical analyzed performing an analysis of variance (ANOVA) and Tukey's comparison tests with a confidence level of 95%, using Minitab (v.17, LEAD Technologies Inc., USA).

3. Results and discussion

3.1. Characterization of essential oils

More than 30 components were identified for oregano essential oil (OEO) and thyme essential oil (TEO). Table 1 shows the results of the major components identified in the essential oils (EO). OEO major component was carvacrol (46.13%), meanwhile for TEO, it

was thymol (30.73%). These components are the characteristics ones present in these essential oils (Božik et al., 2017; Gedikoğlu et al., 2019; İşcan et al., 2020). It is important to identify the major components of EO used as antimicrobials since the chemical composition, varies by the geographical origin, growth stage, ecological conditions, and extraction method (Gaglio et al., 2017).

3.2 *Characterization of dried coacervates*

3.2.1. Moisture content and water activity

Moisture content and the water activity are important properties since the remaining amount of water in the product can affect its shelf life (Basu, Shivhare, and Mujumdar, 2006; Alvarenga-Botrel et al., 2012). Results of moisture content and water activity are shown on Table 2. Moisture content for samples with OEO varied from 1.72 to 2.48% (w.b.) while samples with TEO ranged from 1.41 to 1.52% (w.b.). Only OEO samples showed significant difference ($p < 0.05$) between homogenization methods for moisture content. On the other hand, values within the range of 1-5% of moisture content are generally accepted for food powders (Opaliński, Chutkowski, and Hassanpour, 2016); values of moisture content obtained in this study are inside that range.

Table 1
Chemical characterization of essential oils

Essential oil	**Major component**	**Area (%)**
OEO	Carvacrol	46.13
	Syn linalol	26.19
	Syn m-cymene	13.10
	Syn δ-terpinen	7.48
	β-pinene	1.71
	Caryophyllene oxide	1.37
TEO	Thymol	30.73
	m-cymene	21.75
	1r-α-pinene	9.30
	Syn-linalol	6.15
	Syn δ-terpinen	6.10
	δ-terpinen	4.60
	Caryophyllene oxide	2.20
	Caryophyllene	2.05

OEO: oregano essential oil; TEO: thyme essential oil

Table 2

Characterization of powders of oregano essential oil or thyme essential oil encapsulated by complex coacervation homogenized by ultrasound or microfluidization

Gelatin – Chia mucilage	OEOU	OEOM	TEOU	TEOM
% Moisture (w.b.)	2.48 ± 0.14^{a}	1.72 ± 0.10^{b}	1.52 ± 0.10^{a}	1.41 ± 0.09^{a}
Water activity	0.238 ± 0.012^{a}	0.178 ± 0.001^{b}	0.145 ± 0.002^{a}	0.134 ± 0.001^{b}
Solid yield (%)	76.81 ± 0.93^{a}	73.09 ± 2.18^{a}	77.24 ± 1.57^{a}	73.40 ± 0.06^{b}
EE (%)	91.36 ± 0.61^{a}	91.84 ± 0.59^{a}	93.02 ± 0.63^{a}	93.26 ± 0.23^{a}
Particle size (μm)				
D_{10}	4.40 ± 0.32^{a}	3.42 ± 0.14^{b}	3.39 ± 0.14^{a}	3.03 ± 0.10^{b}
D_{50}	12.22 ± 0.32^{a}	10.17 ± 0.14^{b}	8.99 ± 0.14^{a}	8.22 ± 0.10^{b}
D_{90}	130.5 ± 0.32^{a}	61.97 ± 0.14^{b}	67.31 ± 0.14^{a}	78.36 ± 0.10^{b}

OEOU: oregano essential oil homogenized by ultrasound; OEOM: oregano essential oil homogenized by microfluidization;
TEOU: thyme essential oil homogenized by ultrasound; TEOM: thyme essential oil homogenized by microfluidization
Different letters in row show significant differences ($p < 0.05$) between samples of the same essential oil
All properties were calculated by triplicate

For water activity, it varied from 0.178 to 0.238 for samples with OEO and ranged from 0.134 to 0.145 for samples with TEO, showing a significant difference ($p < 0.05$) between homogenization methods for both EO. These values of water activity indicate a good stability of the product, because food products with a water activity less than 0.6 are considered microbiologically stable (Quek, Chok, and Sweldund, 2007).

In general, moisture content in samples homogenized by ultrasound had a slight increase compared to the ones homogenized by microfluidization. Similar results were observed by Jafari, He, and Bhandari (2007) in spray-dried emulsions of d-Limonene homogenized by ultrasound or microfluidization. As expected, the increase of moisture content affected water activity, in which the same trend, as with moisture content, was observed between homogenization techniques.

3.2.2. Solid yield

After the encapsulation process, the powder recovered in the spray dryer is represented by the solid yield which helps to determine the losses during the spray drying (Jafari et al., 2007; Asensio et al., 2017). Obtained values of solid yield are shown on Table 2. These values

varied from 73.09-76.81 and 73.40-77.24 for samples of OEO and TEO, respectively. No significant difference ($p > 0.05$) between homogenization methods was observed for solid yield. Similar to moisture content and water activity, in solid yield, samples homogenized by ultrasound showed a slight increase compared to the ones homogenized by microfluidization. Observed values of solid yield in this study are higher compared to the ones reported by other authors (Alvarenga-Botrel et al., 2012; Asensio et al., 2017; Rojas-Moreno, Osorio-Revilla, Gallardo-Velázquez, Cárdenas-Bailón, and Meza-Márquez, 2018) whose values ranged between 51.8 to 62.04% in the encapsulation of essential oils by spray drying. Therefore, both ultrasound and microfluidization process conditions, along with the drying conditions used in this study resulted viable for practical application in the industry due to its results in solid yield.

3.2.3. Encapsulation efficiency (EE)

Encapsulation efficiency is of interest for essential oils since these are volatile compounds highly susceptible to be lost during the drying process (Jafari et al., 2007; Asensio et al., 2017). Samples with OEO had an encapsulation efficiency in the range of 91.36 to 91.84%; meanwhile samples with TEO had values from 93.02 to 93.26% (Table 2). No significant difference ($p > 0.05$) between homogenization methods was observed. As stated by Jafari et al. (2007), the similar encapsulation efficiencies between homogenization methods are justified by having the same composition in the formulation, the same particle size in the coacervate before drying and similar spray-drying conditions. All these conditions were fulfilled in the present study since ultrasound and microfluidization process conditions were selected to obtain a similar particle size ($D_{50} \approx 57$ nm) in the coacervate before drying.

3.2.4. Particle size distribution

Powders with OEO or TEO had a bimodal distribution (Figure 1) with a wide range of droplet sizes (span >5). It was observed that the particle size was smaller for the systems homogenized by microfluidization compared to the ones homogenized by ultrasound (Table 2). Significant difference ($p < 0.05$) between homogenization methods was observed for

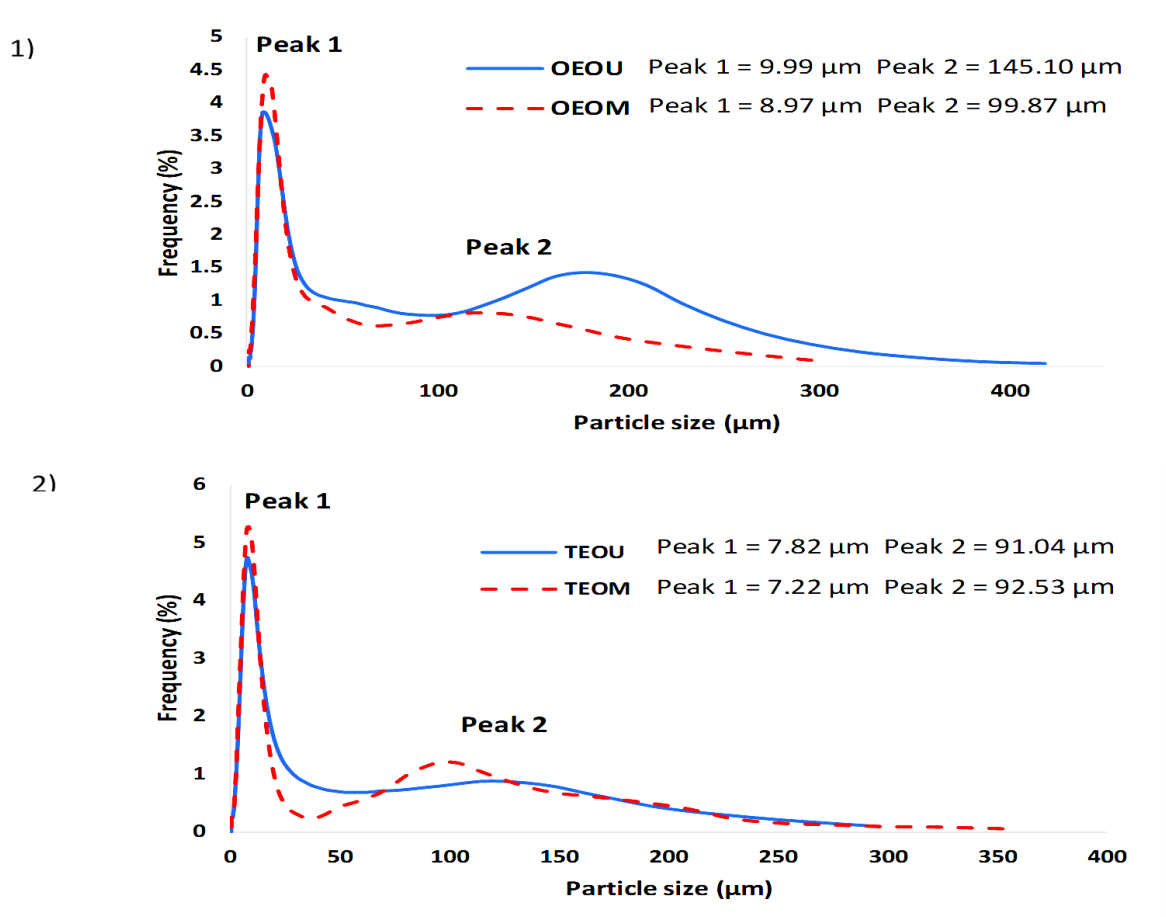

Figure 1. Particle size distribution of spray dried essential oils encapsulated by complex coacervation homogenized by ultrasound or microfluidization. OEOU: oregano essential oil homogenized by ultrasound; OEOM: oregano essential oil homogenized by microfluidization; TEOU: thyme essential oil homogenized by ultrasound; TEOM: thyme essential oil homogenized by microfluidization

particle size. These difference could be explained since even if two complex coacervate systems with the same composition and same particle size before drying is achieved, these could produce two different encapsulated powders due to the stability of the coacervates during the spray drying process (Jafari et al., 2007).

3.3. Antimicrobial activity of reconstituted powders in green juice

Minimum inhibitory concentration (MIC) for powders of coacervates of OEO homogenized by ultrasound and microfluidization were 274 ppm and 367 ppm, respectively; while for powders of coacervates of TEO homogenized by ultrasound and microfluidization were 186

ppm and 280 ppm, correspondingly. These could be explained as a result of the homogenization techniques used, in which the one with ultrasound is not uniform across the entire system and there is more energy surrounding the probe; meanwhile in microfluidization, inside the interaction chamber only a small volume of the sample passes through the emulsification zone allowing uniform process, giving as a result less free oil in the final product compared to the homogenized by ultrasound (McCrae, 1994; Jafari et al. (2007; Zhang, Peppard, and Reineccius, 2015; Villalobos-Castillejos et al., 2018). Since in this study coacervates homogenized by ultrasound presented a bigger particle size compared to the ones homogenized by microfluidization, it can be suggested that oil not encapsulated by the coacervate, was encapsulated by the maltodextrin used in the formulation. Once the powders of coacervates homogenized by ultrasound were reconstituted, the oil encapsulated by maltodextrin got free and acted against *Escherichia coli* and reduced the MIC compared to the systems homogenized by microfluidization. These results are similar to the ones reported by Ruiz-Gonzalez et al. (2019) in which oregano essential oil encapsulated by emulsification had a better MIC against *E. coli* compared to other essential oils due to the combination of different factors such as particle size, encapsulation efficiency and essential oil composition.

Conclusions

Complex coacervation between gelatin and chia mucilage homogenized by ultrasound or microfluidization was effective to encapsulate oregano essential oil (OEO) or thyme essential oil (TEO), as the encapsulation efficiency (>90%) suggested in the spray-dried coacervates. Reconstituted powders of OEO and TEO were effective against *Escherichia coli* ATCC 25922. In general, reconstituted powders obtained from coacervates homogenized by ultrasound were more effective in the inhibition of *E. coli*, compared to the ones homogenized by microfluidization. Further research is needed to determinate the release properties of the reconstituted powders of essential oils encapsulated by complex coacervation and homogenized by different techniques to stablish its relationship with antimicrobial effects.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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CONCLUSIONS

This research has endeavored in the use of complex coacervation between gelatin and chia mucilage to encapsulate essential oils to be applied as antimicrobials in food products. General conclusions are the following:

- The formation of complex coacervates is affected by the encapsulating agents, the amount of the core material, and the homogenization method.
- Complex coacervation between gelatin and chia mucilage can be achieved using a mass ratio of 2:1 and a pH value of 3.6.
- The combination of gelatin-chia mucilage as encapsulating agents can be an alternative to the most common ones used (gelatin-gum Arabic) in complex coacervation, as the solid yield was demonstrated to be > 80%, being viable for practical application.
- The operating conditions evaluated of microfluidization to prepare gelatin-chia mucilage complex coacervates to encapsulate OEO and TEO, produced the higher yields (91- 93%) when applying 20,000 psi and 2 passes and stabilized by spray drying.
- In the spray drying of complex coacervates, the inlet air temperature and the feeding rate affect the physicochemical properties of the final product. Suggested conditions to stabilize coacervates of gelatin-chia mucilage by spray drying are 160°C and 5 g/min.
- Reconstituted powders of oregano and thyme essential oils encapsulated by complex coacervation between gelatin and chia mucilage proved to be effective against *Escherichia coli* ATCC 25922 (10^6 CFU/mL) in green juice.
- Complex coacervation could be an alternative encapsulation method for antimicrobials, to be applied in the food industry.

RECOMMENDATIONS FOR FUTURE WORK

Complex coacervation has a promising future as encapsulating method to be applied in food industry. However, there are many research areas to be covered regarding complex coacervation. The recommendations based on the results of the present work are the following:

- Continue studying different alternatives for encapsulating agents in complex coacervation such as different natural sources of mucilages of seeds or fruits, due to their chemical composition.
- Use the complex coacervation between gelatin and chia mucilage in the encapsulation of different core materials such as vitamins, pigments, among others.
- Do further research on the effect of different homogenizing and drying methods.
- Do further research on the effect of microfluidization in the formation of complex coacervates.
- Continue characterizing the spray-dried coacervates used in the present study, such as its release properties, stability during storage and sensory acceptance by consumers.
- Apply the spray-dried coacervates in different food products as flavorings, antimicrobials, or antioxidants.

PUBLICATIONS AND PARTICIPATION IN CONFERENCES

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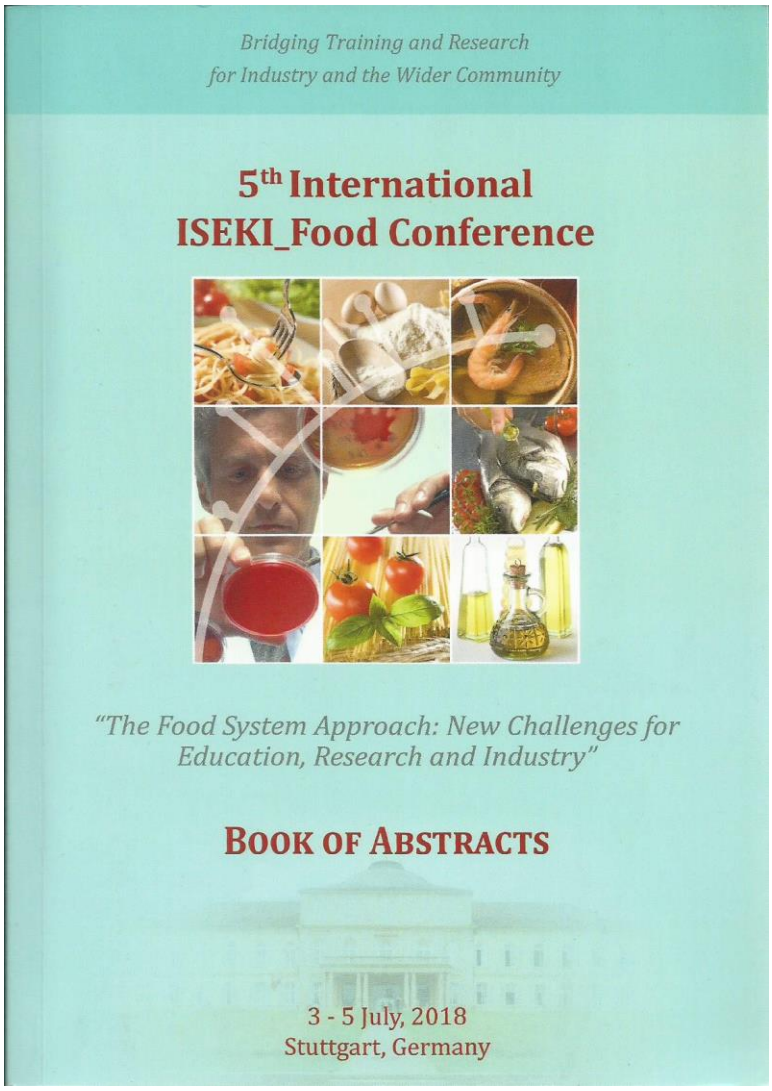

#132: Complex coacervation between gelatine and chia mucilage

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Complex coacervation is a technique that has been studied in the food industry for applications such as protection of compounds and to promote the controlled release of encapsulated components. However, studies are still being made to explore different possibilities of natural sources to be used in complex coacervation for food applications. Hence, complex coacervation between gelatin type B (GE) and chia mucilage (ChM) was studied. GE-ChM were mixed at mass ratios of 1:1, 2:1, 3:1, 4:1 and 1:2 in a pH range of 1.50-5.00, maintaining a total solid concentration of 0.2% (w/w), using turbidity and viscosity tests to obtain the highest yield of complex coacervates. To characterize the complex coacervates, micrographs and Fourier-transform infrared spectroscopy (FTIR) were determined. The optimum yield for complex coacervation was achieved with a GE-ChM mass ratio of 2:1 and pH value of 3.6. It was observed that increasing the mass ratio of GE or ChM, the yield of complex coacervates decreased; the higher yields were obtained with the proportions of 2:1 and 1:1 with values of 68.25 ± 0.05% and 61.04 ± 0.05%, respectively. Capsules formed at mass ratios of 1:1, 2:1, 3:1 had the characteristic grape agglomerate shape for complex coacervates; meanwhile mass ratios of 1:2 and 4:1 showed individual spherical and oval shapes, respectively. FTIR spectrum of complex coacervates at optimum conditions had a combination of bands corresponding to GE and ChM with predominant GE functional groups due to the high GE-ChM mass ratio, suggesting an interaction between GE-ChM during the formation of complex coacervates. Therefore, complex coacervates between GE-ChM present an alternative as encapsulating compounds to be applied in the food industry to protect sensible ingredients.

Keywords
complex coacervation, gelatin, chia mucilage, coacervate yield

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Chapter

Effect of Oil Content in the Physicochemical Characteristics of Spray-Dried Powders of Anise (*Pimpinella anisum* L.) Essential Oil Encapsulated by Complex Coacervation

Ruth Hernández-Nava, Nancy Ruíz-González and María Teresa Jiménez-Munguía

Abstract

Complex coacervation is a technique that involves the electrostatic attraction between two biopolymers of opposite charges that surround a compound of interest and can be stabilized by spray drying. This technique has been used to increase the shelf life of functional ingredients, such as essential oils, providing controlled release and allowing an alternative food processing. The aim of this work was to evaluate the effect of essential oil content present in the coacervate, and its effect in the physicochemical characteristics of spray-dried powders of anise essential oil. Complex coacervates between gelatin and chia mucilage were used to encapsulate 5.0 and 7.5% (w/w) of anise essential oil. These coacervates were spray-dried with an inlet air temperature of 160°C and a feeding rate of 5 g/min. Powders were characterized by particle size, moisture content, solid yield, flow properties, and encapsulation efficiency. The powder with 7.5% of anise essential oil had the highest encapsulation efficiency (96.6 ± 0.02%). All physicochemical characteristics of the powders were influenced by the essential oil content in the complex coacervates. Complex coacervation between gelatin and chia mucilage resulted in an effective method to encapsulate anise essential oil stabilized by spray drying with high encapsulation efficiencies.

Keywords: anise essential oil, complex coacervation, spray drying

1. Introduction

Essential oils are secondary metabolites of aromatic plants, obtained from different plant materials [1]. Their chemical composition can be influenced by the climate and the soil where the plants are grown, as well as the extraction processes used [2]. These have achieved an increasing interest in the food industry because of