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JULIANA DOMINGUES DOS SANTOS CARVALHO

Use of mixtures of cellulosic polymers with chitosan for the preparation of structured systems: hydrogels and polymeric emulgels

Utilização de misturas de polímeros celulósicos com quitosana para a elaboração de sistemas estruturados: hidrogéis e emulgéis poliméricos

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Orientadora: Miriam Dupas Hubinger
Coorientadora: Renata Santos Rabelo

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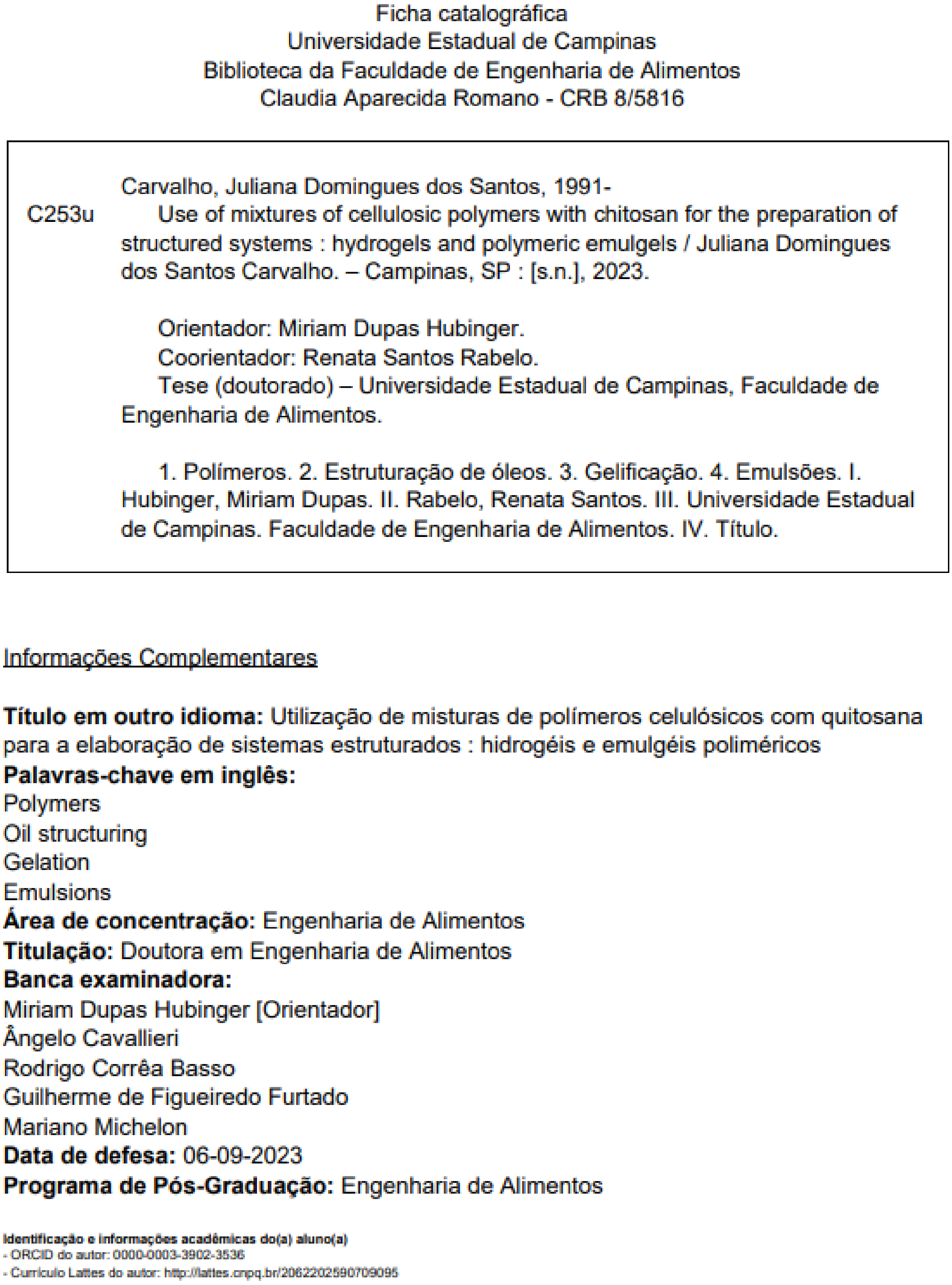

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DEDICATÓRIA

Não se entregue aos sonhos de ter o que você não tem,
mas calcule a principal das bênçãos que você possui, e então, felizmente,
lembre-se de como você ansiaria por elas se não fossem suas.

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Aos meus pais, Jurema e Robson,
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para me ajudar em meus sonhos.
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RESUMO

A inovação tecnológica de se produzir produtos *Lowsat* e *Lowfat*, através da estruturação de óleos insaturados, têm recebido grande interesse por parte da comunidade científica e pelas indústrias de alimentos. Por isso, novos métodos para substituição de gordura têm sido estudados, como os sistemas estruturados (hidrogéis e emulgéis). Nesse contexto, o objetivo deste trabalho é verificar o potencial de aplicação de hidroxipropilmetilcelulose (HPMC) e metilcelulose (MC) em conjunto com a quitosana (CHI) como sistemas estruturados para transporte de óleo (emulgéis), a fim de avaliar o desempenho desses sistemas como potenciais substitutos de gorduras em alimentos. Para obter esses resultados, foram produzidos e caracterizados hidrogéis de HPMC: CHI e MC: CHI nas seguintes proporções, 80: 20, 70:30 e 60:40. Observou-se que a adição da quitosana foi capaz de promover a redução e a expansão da temperatura de formação de gel desses sistemas, quando comparado aos polímeros celulósicos individuais (HPMC e MC puros). A maior redução ocorreu para os seguintes hidrogéis, 70HPMC: 30CHI (43,6 °C) e 70MC: 30 CHI (39,3 °C). Na etapa seguinte os hidrogéis foram utilizados como base para a obtenção de sistemas estruturados do tipo emulgéis. Os emulgéis obtidos foram do tipo óleo em água (O/W) e as análises reológicas revelaram emulgéis pseudoplásticos, dependentes da frequência e nenhum ponto de gel foi visualizado. A varredura de temperatura foi utilizada para investigar o efeito da adição de CHI aos emulgéis, já que nos sistemas hidrogéis foi visualizada a mudança no ponto de gel dos mesmos. Durante o aquecimento desses emulgéis foi observado que as amostras com MC eram mais estruturadas, formando géis mais fortes do que os sistemas com HPMC. Dentre as formulações estudadas, os emulgéis 80MC: 20CHI e 70MC:30 CHI formaram géis mais fortes (G'>>G"). Já no estudo de estabilidade durante 10 semanas, foi observado um aumento no tamanho de gota ($D_{[4,3]}$) nos sistemas armazenados a 40 °C. A microscopia revelou que as emulsões na 7ª e 10ª semana de armazenamento apresentaram características semelhantes à emulsão fresca. Portanto, esses resultados indicam que os emulgéis apresentam boa resistência térmica, predominância de comportamento elástico e podem reter altas concentrações de óleo em sua estrutura (96 a 99%). Devido a essas características, podem proporcionar estruturação a óleos vegetais e ser aplicados em matrizes alimentícias que requerem aquecimento (45 a 85 °C).

Palavras chave: polímeros celulósicos, estruturação de óleos, gelificação direta, gelificação indireta, emulsões.

ABSTRACT

The technological innovation of producing Lowsat and Lowfat products through structuring unsaturated oils has received significant interest from the scientific community and the food industry. Therefore, new methods for replacing fat have been studied, such as structured systems (hydrogels and emulgels). In this context, the objective of this work is to verify the potential application of hydroxypropylmethylcellulose (HPMC) and methylcellulose (MC) together with chitosan (CHI) as agents structured to produce emulgels. Subsequently, these systems are evaluated as possible substitutes for trans and saturated fat in food matrices. HPMC: CHI and MC: CHI hydrogels were produced and characterized in the following proportions, 80:20, 70:30, and 60:40. It was observed that the addition of chitosan was able to promote the reduction and expansion of the gel formation temperature of these systems when compared to individual cellulosic polymers (pure HPMC and MC). The most significant reduction occurred for the following hydrogels, 70HPMC: 30CHI (43.6 °C) and 70MC: 30 CHI (39.3 °C). In the next step, the hydrogels were used as a basis for obtaining structured emulsion-type systems. The emulsions obtained were of the oil-in-water (O/W) type, and the rheological analyzes revealed pseudoplastic emulsifiers, dependent on the frequency, and no gel point was visualized. The temperature scan was used to investigate the effect of adding CHI to the emulgels since the change in the gel point of the hydrogel systems was visualized. During the heating of these emulgels, it was observed that the samples with MC were more structured, forming stronger gels than the systems with HPMC. Among the studied formulations, the 80MC:20CHI and 70MC:30 CHI emulgels formed stronger gels (G'>>G"). In the stability study for ten weeks, an increase in droplet size (D[4,3]) was observed in the systems stored at 40 °C. Analyzing the microscopy, the emulsions at the end of storage (7th and 10th weeks) showed similarity to the newly produced emulsions. Thus, the results show that these systems have G' greater than G". That is, the emulsifiers are more elastic, have good thermal resistance, and manage to retain a high amount of oil in their elastic network (96 to 99%). Due to these characteristics, they can provide structure to vegetable oils and be applied in food matrices that require heating (45 to 85 °C).

Keywords: cellulosic polymers, oil structuring, direct gelation, indirect gelation, emulsions.

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SUMMARY

CHAPTER I

General introduction, objective, and thesis structure

1.1.GENERAL INTRODUCTION

Consumption of saturated and trans fats is associated with harmful health effects. Diseases such as obesity and cardiovascular problems are among the disorders associated with consuming foods that contain this type of fat. The harm caused by this consumption has led to the mobilization of health regulatory agencies worldwide (BHANDARI et al., 2020).

In Brazil, we have RDC nº 332 of 12/23/2019, which defines the requirements for using industrial trans fats in food in the country. This legislation defines that, as of July 1, 2021, the amounts of industrial trans fats in refined oils cannot exceed 2% (m/m) of total fat. For food intended for the final consumer and food services, the established limit is 2% (m/m) of total fat, and the established deadline is between July 1, 2021, and January 1, 2023 (BRASIL, 2019).

In Brazil, ANVISA (National Health Surveillance Agency) published RDC n° 514 of 05/28/2021, which changes some parameters of the previous resolution. Industrial fats manufactured until June 30, 2021, can be marketed until the end of their shelf life. The final products manufactured on June 30, 2021, with this type of fat are allowed to be commercialized during their expiration dates until December 31, 2022 (BRASIL, 2021). For the removal of trans fats from the Brazilian market, the production, import, use, and supply of partially hydrogenated oils and fats for use in food is prohibited from January 1, 2023 (BRASIL, 2019). As for saturated fats that are naturally present in the population's daily diet, health regulatory bodies guide the reduction of their consumption (PATEL & DEWETTINCK, 2016).

As a result, ANVISA published RDC No. 429 of 10/08/2020, which talks about the new labeling of packaged foods. The new front-of-package (FOP) magnifying glass model highlights the most relevant nutritional information. It highlights information about high fat, sugar, and sodium content to facilitate communication with the consumer. Furthermore, Brazilian food industries have until 10/09/2023 to adapt to this new legislation (ANVISA, 2020).

Based on this problem and the search for a healthier lifestyle by consumers, the food industry has sought to minimize the fat content and the use of health-promoting components in its products (PEHLIVANOĞLU et al., 2018; PUSCAS et al., 2020). With this, it is understood that the replacement of saturated and trans-fat by another lipid phase is of great importance.

Implementing structured vegetable oils in the food industry is a new approach to replacing saturated and trans-fat, possibility of creating new products with an improved nutritional profile (PINTO et al., 2021; PEHLIVANOĞLU et al., 2018). However, structuring vegetable oils is relatively recent and needs further studies. Depending on the choice of production process, it is possible to obtain systems with different characteristics and industrial applications (MATTICE & MARANGONI, 2018).

There are several strategies for structuring vegetable oils, but the production of concentrated emulsions (emulgels) has been gaining prominence due to its method of obtaining via direct emulsification. Polysaccharides with gel-forming properties are used as a structuring agent for their production. These polysaccharides stabilize emulsions mainly by steric hindrance. When structured emulsions have a predominance of elastic behavior over viscous behavior (G'>G"), they can be classified as emulgels or gel emulsions (SAID DOS SANTOS et al., 2021; PINTO et al., 2021). Because they have the mechanical properties of a solid, they can be used as a resource to replace saturated and trans-fat in food.

Some of the polysaccharides used in producing emulgels, hydroxypropylmethylcellulose (HPMC) and methylcellulose (MC), stand out. These polymers derived from cellulose have a hydrophobic character and exhibit amphiphilic behavior and a surfactant nature (PATEL & DEWETTINCK, 2016). Furthermore, HPMC and MC exhibit heating gelling properties, having an LCST (Lower Critical Solution Temperature) between 75 and 90°C and 40 and 50°C, respectively (DARGE et al., 2019; GOFF & GUO, 2019).

Another polysaccharide that can be used to obtain single gels or in combination with other hydrophilic polymers, such as HPMC and MC, is chitosan (CHI). CHI's ability to interact with vegetable oils and fats is related to its amphiphilicity. The molecular structure of chitosan is composed of amino groups (hydrophilic) and acetyl groups, which are hydrophobic; this molecular characteristic can influence its properties in solution and the solid state (HU et al., 2017).

However, the formation of these structured systems can be limited when using oils more sensitive to high temperatures due to lipid oxidation. Alternatively, strategies to reduce the gelation temperature of these systems are desired. So, as a technological alternative, cellulosic derivatives and chitosan can be used to obtain hydrogels (HPMC: CHi and MC: CHI). This combination can guarantee that these polymers will be in a gel state at a lower temperature and wide temperature range (DOS SANTOS CARVALHO et al., 2022). These hydrogels can be used later to obtain emulsifiers with a wide range of gelling temperatures. The applications of structured systems in processed foods must be studied so that they do not affect the quality properties of the final product. It is already known that the central role of fat in food formulations is to give structure to the product, a characteristic capable of affecting physical properties such as texture (hardness and spreadability) and sensory properties (melt-in-the-mouth effect, cooling effect, breaking emulsion effect, the release of flavors among others) (PATEL & DEWETTINCK, 2016; PEHLIVANOĞLU et al., 2018).

Given this problem, this work aims to produce methylcellulose and hydroxypropyl methylcellulose hydrogels with chitosan to verify the potential application of these mixtures as structured systems for oil entrapment, of the emulgel type. After studying emulgel systems, evaluate the behavior of these systems as a potential substitute for trans and saturated fats in foods.

1.2.GENERAL OBJECTIVE

To produce hydrogels using cellulosic polymers, hydroxypropylmethylcellulose, and methylcellulose in their composition, with chitosan. Moreover, evaluate the potential application of hydrogels to obtain structured systems of the emulgel type for use as a fat substitute in food.

1.2.1. SPECIFIC OBJECTIVES

- To evaluate, in the hydrogels, how the addition of chitosan can reduce the gelation temperature of pure cellulosic polymers.
- Apply hydrogels to obtain structured systems.
- Structure sunflower oil, by emulsification, from mixtures of hydroxypropyl methylcellulose and methylcellulose with chitosan.
- Evaluate the interactions between chitosan with hydroxypropyl methylcellulose and methylcellulose in different mixing proportions at 60% oil.
- Characterize the rheological, thermal, mechanical behavior, microstructure, and stability of emulgels.

1.3.THESIS STRUCTURE

The results obtained during the doctorate are presented in this thesis as chapters.

Chapter I – The present chapter brings a general introduction and the general and specific objectives.

Chapter II – Review of literature on using hydrophilic polymers to obtain structured systems.

Chapter III –Describes a study of the thermo-rheological properties of chitosan hydrogels with hydroxypropyl methylcellulose and methylcellulose.

Chapter IV – Explores the use of hydrogels to obtain oleogel systems by the indirect emulsion method

Chapters V and VI – In these chapters, this thesis's general discussion and conclusion were presented, respectively.

Chapters VII – General references.

REFERENCES

Bhandari, S. D., Delmonte, P., Honigfort, M., Yan, W., Dionisi, F., Fleith, M., … Bergeson, L. L. (2020). Regulatory Changes Affecting the Production and Use of Fats and Oils: Focus on Partially Hydrogenated Oils. JAOCS, Journal of the American Oil Chemists' Society, 97(8), 797–815.

Brasil. (2019). Resolução - RDC n^{o} 332 de 23 de Dezembro de 2019.pdf. ANVISA - Agência Nacional de Vigilância Sanitária - Diário Oficial da União.

Brasil. (2020). Resolução - RDC n^{o} 429 de 08 de outubro de 2020.pdf. ANVISA - Agência Nacional de Vigilância Sanitária - Diário Oficial da União.

Brasil. (2021). Resolução - RDC n^{o} 514 de 28 de Maio de 2021.pdf. ANVISA - Agência Nacional de Vigilância Sanitária - Diário Oficial da União.

Darge, H. F., Andrgie, A. T., Tsai, H. C., & Lai, J. Y. (2019). Polysaccharide and polypeptide based injectable thermo-sensitive hydrogels for local biomedical applications. International Journal of Biological Macromolecules, 133, 545–563.

dos Santos Carvalho, J. D., Rabelo, R. S., & Hubinger, M. D. (2022). Thermo-rheological properties of chitosan hydrogels with hydroxypropyl methylcellulose and methylcellulose. International Journal of Biological Macromolecules, 209(PA), 367–375.

Goff, D., & Guo, Q. (2019). Food Structure Development : The Interplay Between Processing Routes and Formulation Elements. Part A Food Structure Development: The Interplay Between Processing Routes and Formulation Elements, 1–28.

Huc-Mathis, D., Bousquières, J., Mamou, M., Bonazzi, C., & Michon, C. (2017). Multiscale evaluation from one bubble to the foam of surface active properties of cellulose derivatives used for a starchy model sponge cake. Food Hydrocolloids, 71, 129–140.

Mattice, K. D., & Marangoni, A. G. (2018). Oleogels in Food. In *Reference Module in Food Science*. Elsevier.

Patel, A.R., & Dewettinck, K. (2016). Edible oil structuring: An overview and recent updates. *Food and Function*, 7(1), 20–29.

Pinto, T. C., Martins, A. J., Pastrana, L., Pereira, M. C., & Cerqueira, M. A. (2021). Oleogel-Based Systems for the Delivery of Bioactive Compounds in Foods. *Gels*, 7(3), 86.

Pehlivanoğlu, H., Karasu, S., Konar, N., Toker, O.S., Demirci, M., & Sagdic, O. (2018). Oleogels, a promising structured oil for decreasing saturated fatty acid concentrations: Production and food-based applications. *Critical Reviews in Food Science and Nutrition*, *58*(8), 1–12.

Puscas, A., Muresan, V., Socaciu, C., & Muste, S. (2020) Oleogels in Food: A Review of Current and Potential Applications. Foods, v. 9, n. 1, p. 70.

Said Dos Santos, R., Bassi da Silva, J., Rosseto, H. C., Vecchi, C. F., Campanholi, K. da S. S., Caetano, W., & Bruschi, M. L. (2021). Emulgels containing propolis and curcumin: The effect of type of vegetable oil, poly(acrylic acid) and bioactive agent on physicochemical stability, mechanical and rheological properties. Gels, 7(3)

Singh, A.; Auzanneau, F.I.; Rogers, M.A (2017). Advances in edible oleogel technologies – A decade in review. *Food Research International*, 97 (4), p. 307–317.

CHAPTER II

Literature review

2.1.GELLED STRUCTURES

Gelled structure, gel, is defined as a semi-solid three-dimensional network that immobilizes a liquid phase with different degrees of polarity using hydrophilic or hydrophobic gelling agents (Rehman & Zulfakar, 2013). A gel is composed mainly of dispersed water particles, while the solid phase (dispersion medium) is the component of lower concentration. The solid phase can confer some mechanical properties, such as rigidity and elasticity, which are characteristic of solids to provide structuring properties (Rehman & Zulfakar, 2013; Ract, da Cruz & Pereira, 2019).

A gel's classification will depend on the characteristics of its liquid phase; in general, there are two significant groups: oleogels and hydrogels. Oleogels (also called organogel) immobilize organic solvents, typically vegetable oil, in a 3D network using a gelator or organogelator. Hydrogels, on the other hand, contain water (polar solvent) as a liquid phase, and their three-dimensional network is formed by a hydrophilic gelling agent (Rehman & Zulfakar, 2013; Ract, da Cruz & Pereira, 2019).

2.1.1. HYDROGELS

Hydrogels can be broadly defined as hydrophilic polymeric structures capable of forming a three-dimensional reticulated network that retains a large amount of water in its matrix. These gels can be obtained from natural and/or synthetic polymers crosslinked by chemical or physical bonds (ALI & AHMED, 2018; SHARMA & TIWARI, 2020).

These colloidal gels have two phases in their structure: a liquid, typically composed of water, and a solid phase consisting of a polymer or a polymeric mixture (BATISTA et al., 2019). The ability to retain water in their 3D structure depends on factors such as the amount and presence of hydrophilic groups, the type of bond involved, the polymer concentration, and the structure and functionality of the crosslinking agent (ALI & AHMED, 2018). These systems can be classified into different categories, but the most commonly used classification is based on the origin of their crosslinks: physically crosslinked hydrogels and chemically crosslinked hydrogels (DUQUETTE & DUMONT, 2019).

Physical hydrogels are stabilized by chain entanglement, ionic interactions and hydrogen and hydrophobic bonds responsible for their reversibility (DUQUETTE & DUMONT, 2019; SHARMA & TIWARI, 2020). Different conformational hydrogel structures can be formed depending on the medium polymer, concentration, and pH. These structures can have varied textures and gel strength, allowing different applicability in the food industry (SHARMA & TIWARI, 2020).

In chemically crosslinked hydrogels, also known as permanent gels, gelled structures form through covalent bonds between accessible, functional groups and crosslinking agents (RANJHA & KHAN, 2013). These hydrogels are stable, have greater mechanical strength than physical ones, and can be dissolved if the covalent bonds are cleaved (SHARMA & TIWARI, 2020; RANJHA & KHAN, 2013).

Hydrogels are directly related to applications in the pharmaceutical industry due to their hydrophilicity, flexibility, elasticity, softness, and high swelling capacity. However, its study is necessary for other practice areas, such as the food industry (PINTO et al., 2021).

In terms of the food industry, the application of hydrogel systems is still restricted, as the ingredients to be used must be GRAS (generally recognized as safe). That is, they must be food-grade components recognized by the FDA (Food and Drug Administration) and included in the EU list of permitted food additives established in EC Regulation 1333/2008 (HILAL, FLOROWSKA & WRONIAK, 2023).

The biopolymers considered food-grade used in producing hydrogels for food industry applications are proteins (whey, soy, and gelatin), polysaccharides (starch, pectin, alginate, cellulose, agar), or a mixture of these two (OKURO, 2021). These compounds have great potential to meet consumer health and environmental sustainability demands, as they are renewable, accessible, biocompatible, biodegradable, and edible, in addition to having a wide range of functionalities and structuring routes (gelation). Proteins and polysaccharides are widely used in elaborating colloidal systems in foods. These components can create and modify food structures such as textures, sensory properties, and shelf life (CAO & MEZZENGA, 2020; HILAL, FLOROWSKA & WRONIAK, 2023).

However, studies on food applications are still under development. With that, there is a need to integrate the knowledge of hydrogels from other segments to leverage food-grade hydrogels. Because of the in-depth knowledge of the polymeric composition and mechanism of formation of emulgels, it is possible to develop food matrices of better nutritional quality and increase the availability of bioactive compounds.

2.1.2. OLEOGELS

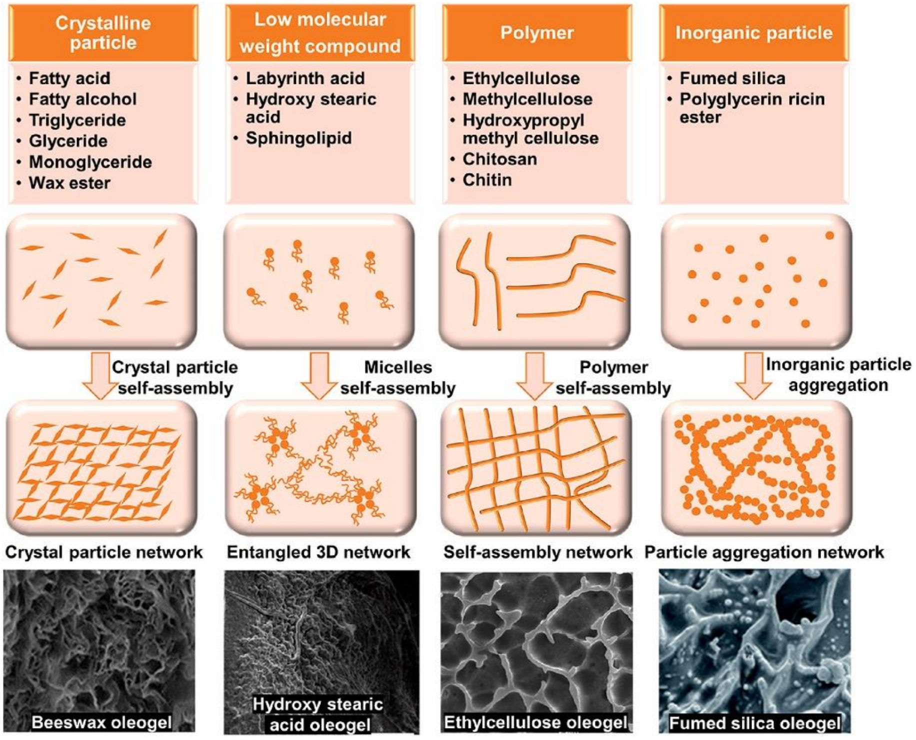
In a simplified way, converting an oil (liquid) into a solid/semi-solid structure (with gel characteristics) without changing its chemical properties characterizes an oleogel. An organic liquid (oil) is trapped in a three-dimensional thermoreversible gel network using a structuring agent of the lipid system at low concentration (<10% by weight) called oleogelator or just gelator (PEHLIVANOĞLU et al., 2018; SHORTZ et al., 2012). This system can behave like solid fat, depending on the method of obtaining it and the gelator used. It can have rheological properties and appearance similar to fats (SIRAJ et al., 2015).

Vegetable waxes, monoacylglycerols and diacylglycerols, alcohols or fatty acid esters, phospholipids, and phytosterols are the most known and used oleogelators in the literature (PEHLIVANOĞLU et al., 2018). According to Co & Marangoni (2012) and

Pehlivanoğlu et al. (2018), a gelator must present the following properties to be considered a structuring of the lipid system with food applications: the presence of lipophilic and interactive parts, surface activity, thermoreversible characteristics, natural origin, efficiency, versatility and be considered as GRAS (Generally Recognized as Safe), that is, safe.

Structuring agents (oleogelators or organogelators) can be categorized as (Figure 1): polymers and inorganic particles, crystalline particles, and low molecular weight components. The most commonly used polymeric structuring agents in foods are ethylcellulose, hydroxypropylmethylcellulose, and other ethers derived from cellulose. In contrast, the most used lipid-based oleogelators are waxes, fatty alcohols, fatty acids, and some emulsifiers (Guo, Cui & Meng, 2023).

Figure 1. Formation mechanism of different oleogels (GUO, CUI & MENG, 2023).



(i) **Crystalline particles**: In this method, structuring occurs by trapping the liquid oil phase in triacylglycerol particles (TAGs). The crystallization process of the structuring agent is the main reason for forming networks in oleogels elaborated by crystalline particles (PEHLIVANOĞLU et al., 2018; Xu et al., 2022). The liquid oil is trapped in the lattice structure as the crystalline particles develop to form agglomerates, which would create a compact crystalline lattice as they are cooled (XU et al., 2022). Such a mechanism can be found in waxes, fatty acids, fatty alcohols, monoacylglycerols, and diacylglycerols. (DAVIDOVICH-PINHAS, BARBUT & MARANGONI, 2016; PEHLIVANOĞLU et al., 2018).

(ii) **Self-assembly systems network**: In this type of oleogel, the gelation process occurs when the melting point of the solvent gelator (continuous phase) is below the melting point of the structuring agent, leading to the self-assembly of the gelator molecules in a nucleation process. 12-hydroxystearic acid and ceramides are examples of self-assembling gelators that control the flow of liquid oil by forming spiral or twisted crystalline fibers. Gelation (structuring) is stabilized through weak physical molecular interactions such as hydrogen bonds, van der Waals forces, electrostatic interactions, dipole forces, and hydrophobic forces (DAVIDOVICH-PINHAS, BARBUT & MARANGONI, 2016; XU et al., 2022).

(iii) **Self-assembled structures of polymers or polymeric filaments:** The fundamental condition in the generation of polymeric oleogels is the non-covalent crosslinking or self-assembly between polymeric structural components through chemical bonding (XU et al., 2022). This method is characterized by using self-assembled structures that use polymers or polymeric chains such as ethylcellulose. Ethylcellulose, proteins, and hydrophobic polysaccharides can also be used as structuring agents of the lipid system (PEHLIVANOĞLU et al., 2018).

(iv) **Miscellaneous:** The use of other methods for structuring oils, inorganic particles (fumed silica), and "High Internal Phase Emulsions" (HIPEs) emulsions are also reported (PATEL, 2015).

2.1.2.1.OBTAINING METHODS

According to Singh, Auzanneau & Rogers (2017), there are several classification procedures for oleogels. Oleogels can be categorized based on the chemical characteristics of the gelator (low molecular weight organic compounds, polymeric and inorganic compounds); chemical nature of the gelator (lipid and non-lipid), gelator association (single component and mixed system gels), type of building blocks and the structuring principles involved in gelation (formation of crystalline particles and network formation, crystallization induced by self-assembly or non-crystalline network and polymer chains) (PATEL & DEWETTINCK, 2016). The most studied methods are direct gelation method, indirect methods, and oil adsorption.

Direct gelation method is generally the most used method for obtaining oleogels. Gelators of lipid origin with direct structuring capacity (waxes, fatty acids, fatty alcohols, monoacylglycerols, and others) are directly dispersed in the oil phase at temperatures above their melting points, followed by cooling to lower temperatures under shear conditions (GUO, CUI & MENG, 2023). Some polymers of non-lipid origin can also be used for the direct gelation process, such as ethylcellulose and colloidal silicon dioxide (inorganic particles) (PATEL & DEWETTINCK, 2016). According to the study by Zhao et al. (2020), oleogels produced with mixtures of structuring agents can generate stronger networks than those capable of imitating solid fats, while systems with a single structuring agent hardly imitate the properties of traditional solid fats.

In indirect methods, the objective is to prepare emulsions that can be of the O/W or W/O type. For the preparation of these emulsions, polysaccharides, and proteins that have an active surface can be used from their dispersion in water in which their conformational structures are created. First, its adsorption is promoted at the oil-water interface, followed by water removal to obtain dry microstructures with the trapped oil phase. Emulsions can be formed from oil concentrations of 60% up to emulsions greater than 75% by volume. Emulsions with more than 75% oil are known as "High Internal Phase Emulsions" (HIPEs) (PATEL & DEWETTINCK, 2016). Molecular interactions (steric hindrance and electrostatic repulsion) are some of the mechanisms used to stabilize oil droplets at the oil-water interface of emulsions (GUO, CUI & MENG, 2023).

Structuring aided by oil absorption is developed using polymers in their constitution, such as aerogels, hydrogels, and cryogels. After the formation of the polymeric gel, it is dried until a porous network capable of absorbing oil is obtained. The oil absorbed by the network may or may not undergo strong agitation to obtain a softer texture with oil concentrations greater than 95% (PATEL & DEWETTINCK, 2016).

More research is still needed to understand the mechanisms of structuring and manipulation of macroscopic properties of oleogels. Therefore, the production and analysis of this new class of structured materials have attracted the academic community and industry.

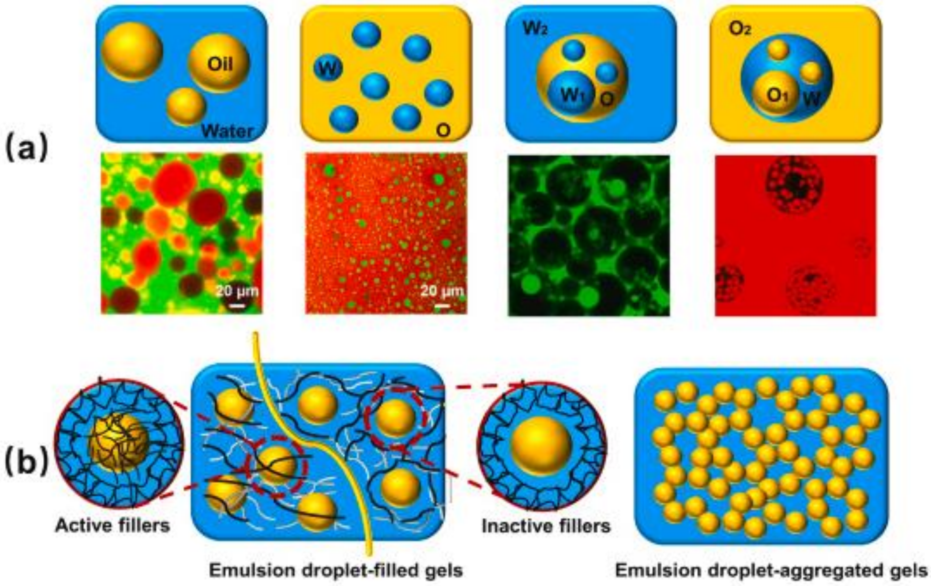
2.1.3. EMULGELS

Structured systems such as emulgel, emulsion gel, or emulsion gels are heterogeneous systems in which two phases coexist: gels and emulsions (Figure 2A). For its formation, the initial emulsification of the system is necessary, accompanied by a gelation process of the compounds present in the medium (PINTO et al., 2021).

Emulgels can be of the oil/water or water/oil type, and these systems form gels by adding gelling agents and thickeners in the aqueous phase. This means that in this process, the emulsion remains trapped in the matrix of a hydrogel (KHULLAR et al., 2012; LIGHT & KARBOUNE, 2021).

Emulgels can be further classified into emulsion gels filled with droplets and emulsion gels with aggregated droplets. Emulsion-filled gel systems (Figure 2B) can be defined as a gel matrix embedded in emulsion droplets. Its rheological properties will follow the characteristics of the continuous phase (FARJAMI & MADADLOU, 2019). The droplets formed during emulsification can be active. That is, they are connected to the three-dimensional gel network and contribute to the strength of the gel, and the inactive drops have limited or no interaction with the gel matrix. Emulgels of aggregated particles form a continuous three-dimensional network in gels aggregated by emulsion droplets, and the network is a crucial factor influencing the rheological properties (GUO, CUI & MENG, 2023).

Figure 2. Structure of emulgels (Adapted from GUO, CUI & MENG, 2023).



Emulgels are obtained by confining an organic solvent, such as vegetable oils, within a 3D network composed of hydrophilic molecules. With this, the hydrophobic components are trapped in the external phase that has the property of forming physical or chemical gels by entanglement or reticulation of the chains (KHULLAR et al., 2012).

This gelling ability offers some advantages, such as increased stability of the trapped emulsion, reduced interfacial tension, increased viscosity of the aqueous phase, additional protection for bioactive compounds, and control over emulsion release conditions. Due to the limited movement of oil droplets and oxygen diffusion, these systems limit lipid oxidation in immutable network structures (GUO, CUI & MEG, 2023). Therefore, these gels can trap hydrophobic and hydrophilic bioactive compounds in an aqueous environment, allowing their application in several areas, such as the food industry (LIGHT & KARBOUNE, 2021).

Emulgels are particularly important in the food area to promote the improvement of texture properties of reduced-fat matrices, calorie reduction, and as a fat substitute (GUTIÉRREZ-LUNA, ASTIASARÁN & ANSORENA, 2021).

The application of these systems as a substitute for fats still needs to be studied, since these systems focus mainly on the pharmaceutical area. However, in the scientific literature, it is possible to find some works involving emulgels as a fat substitute in meat products (PINTADO et al., 2018; PAGLARINI et al., 2018; PINTADO & COFRADES, 2020; YANG, LI, & TANG, 2020; CUI et al., 2022) and bakery products (CHEN et al., 2016; MARTÍNEZ-CERVERA, SALVADOR & SANZ 2015 YANG et al., 2022; BU et al., 2022). This fact justifies the study of these systems as potential substitutes for saturated and trans-fat in foods.

2.1.4. POLYSACCHARIDES IN THE PRODUCTION OF GELS

Obtaining a gel-like structure using polysaccharides is a promising strategy, as several economically viable polymers are approved for use in food. However, most polymers used in the food industry have the characteristic of hydrophilicity and, therefore, cannot be used directly in the gelation of oils (PINTO et al., 2021). Systems containing water as a continuous phase in emulsions can be used as molds to gel dispersed oil (SINGH, AUZANNEAU & ROGERS, 2017).

Some hydrophilic polymers, such as modified proteins and polysaccharides, have surface activity due to the presence of hydrophobic groups in their molecular structures. In short, to use hydrophilic polymers in the gelation of oils, it is necessary to pre-hydrate the polymers in the aqueous phase and then start with the dehydration of this system so that it can be used in the physical retention of the non-polar phase (PATEL, 2015; SINGH, AUZANNEAU & ROGERS, 2017).

For the production of hydrogels, some hydrophilic polymers and their derivatives can provide excellent structured systems. However, they lose their efficiency when used for structuring oils (hydrophobic components). Therefore, if any alternative is found for using hydrophilic polymers as structuring agents for vegetable oils, there will be an expansion in the application of these systems to other fields (Patel et al., 2014).

2.1.5. HYDROXYPROPYL METHYLCELLULOSE (HPMC), METHYLCELLULOSE (MC) AND CHITOSAN (CHI) AS STRUCTURING AGENTS

2.1.5.1.HPMC, MC AND CHI – STRUCTURE

Cellulose is the most abundant linear glucose homopolymer on the planet, insoluble in water, natural fiber (indigestible), biodegradable, non-toxic, low cost, and united by β (1,4) glycosidic linkages. Cellulose can be modified chemically by esterification or etherification of hydroxyl groups, producing cellulosic derivatives. In cold water, the cellulose-derived molecules preset lower molecular weights, mechanical resistance, and good solubility. Thus, these characteristics allow greater applicability in the food industry (SANNINO & MADAGHIELE, 2009; GOFF & GUO, 2020).

Cellulose-derived has an apparent density between 300 and 600 g /L, resistance to light, and a shelf life of 18 to 36 months. At high temperatures above 250 °C, they present signs of decomposition, and their degradability is influenced by the degree of substitution (DS) in their carbon chain (THIELKING & SCHMIDT, 2000).

Hydroxypropyl methylcellulose (HPMC) and methylcellulose (MC) are cellulosic polymers, synthetically prepared by substituting hydroxyl groups on the cellulose backbone with methoxy and hydroxyl propyl groups, as shown in Figure 3A (PATEL, 2015; PATEL & DEWETTINCK, 2016). This substitution gives the molecules a hydrophobic character, and thus, they show an amphiphilic behavior and active surface nature (PATEL, 2015; PATEL & DEWETTINCK, 2016).

In solution, cellulose esters can be swelled by modifying the viscosity and rheological properties of the solvent. Depending on DS (degree of substitution), cellulose derivatives may present different values of surface tension. The surface tension of the HPMC and MC is about 63 mN/m and 54 m /m, respectively (THIELKING & SCHMIDT, 2000).

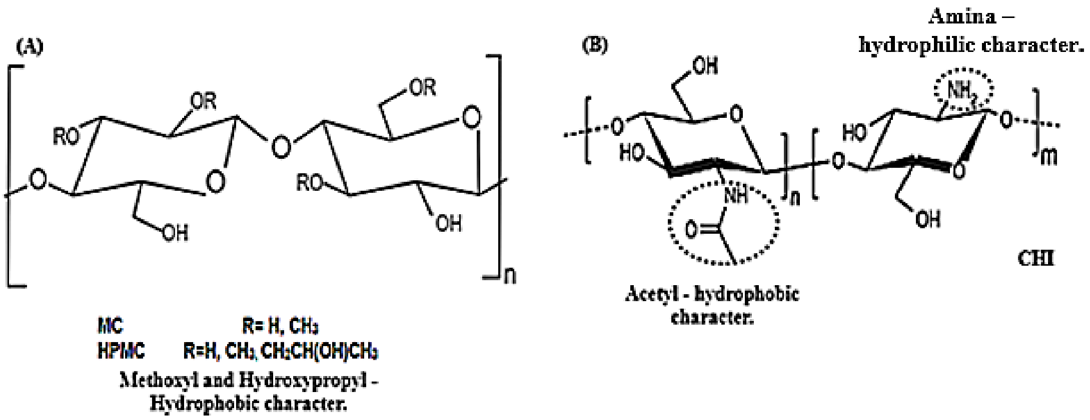
HPMC and MC possess thermal gelation properties (NISHINARI et al., 1977; SAKAR, 1979). This characteristic is found only in nonionic cellulose ethers (THIELKING & SCHMIDT, 2000). These abilities can confer foam and emulsion stabilizing properties for pharmaceutical, and food products, among others (PATEL, 2015; PATEL & DEWETTINCK, 2016).

Chitosan (CHI) is a polysaccharide composed of units of N-acetylglucosamine and D-glucosamine associated with β (1–4) bonds and can be obtained commercially from the deacetylation of chitin (Figure 3B) (HU et al., 2018; JAYAKUMAR; PRABAHARAN; MUZZARELLI, 2011). After this process, CHI can dissolve in an acidic environment and becomes the only polysaccharide with a high density of positive charges. This fact occurs due to the protonation of amino groups in its carbon chain. Of its cationic character, this polysaccharide can interact with anionic molecules (LUO & WANG, 2014). Due to this unique characteristic, CHI can react with other negatively charged molecules, like poly (acrylic acid) or opposing surfaces, such as fats, cholesterol, proteins, and macromolecules (ISLAM et al., 2020).

CHI's ability to interact with vegetable oils and fats is related to its amphiphilicity. The molecular structure of chitosan is composed of amino groups (hydrophilic) and acetyl groups that are hydrophobic, and this molecular characteristic can influence its properties in solution and solid state (ISLAM et al., 2020).

CHI has proven to have many other intrinsic properties, such as non-toxicity, biocompatibility, biodegradability, antibacterial and antifungal activity (LUO & WANG, 2014), and was considered GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration), making possible its application in the food industry (FDA, 2012).

Figure 3. Polymeric structure: (A) Hydroxypropyl methylcellulose (HPMC) and methylcellulose (MC), and (B) Chitosan (CHI). Adapted from Sannino, Demitri & Madaghiele, 2009; Islam et al. 2020.



2.1.5.2.HPMC, MC, and CHI - Gelling Properties

Hydrophilic cellulosic polymers are used to produce hydrogels and, consequently, can be used to obtain gelled structures, such as oleogels and emulgels. HPMC and MC have thermal gelling properties (NISHINARI et al., 1977; SAKAR, 1979), so they can be used to produce hydrogels. For this reason, hydrogels are obtained by physical or chemical stabilization from the preparation of aqueous solutions of these polymers (SANNINO, DEMITRI & MADAGHIELE, 2009; GOFF & GUO, 2020).

HPMC and MC in an aqueous solution produce gels when heated (SAKAR, 1979). These polymers form thermoreversible gels at low concentrations, between 1-10% by weight (SANNINO, DEMITRI & MADAGHIELE, 2009). According to Goff & Guo

(2020) and Sakar (1979), HPMC and MC need to be heated above gelation temperatures, which depend on factors such as the degree of substitution of methyl and hydroxypropyl groups, concentration, and the addition of salts. Gelation occurs after heating to a low critical solution temperature (LCST), at a temperature between 40 and 50 °C for MC that produces more consistent gels and 65-90 °C for HPMC (more hydrophilic) that produces softer gels (DARGE et al. al., 2019; GOFF & GUO, 2020).

The MC and HPMC gelling mechanisms occur through hydrophobic interactions between the methoxy groups in their molecular structure. At low temperatures, these molecules are hydrated, and low polymer-polymer interaction is formed. After heating this solution, water loss begins to happen between the molecules and leads to a loss of relative viscosity of the solution (SAKAR, 1977). The molecules begin to interact as the temperature increases (gelation temperature). At that moment, polymer-polymer interactions are promoted instead of polymer-solvent interactions (GOFF & GUO, 2020).

Consequently, the formation of an infinite network occurs, defining the gelation process. An increase in the relative viscosity of the solution characterizes this phenomenon. The strength of the gel increases with the temperature of the system. However, the gelation phenomenon can be reversed by cooling the gel that returns to the solution phase (Sanz, Falomir & Salvador, 2015).

The chitosan-based hydrogel can be prepared simply by mixing CHI with other non-anionic but water-soluble compounds (BHATTARAI, GUNN & ZHANG, 2010), such as cellulosic polymers, HPMC, and MC. CHI can form a gel on its own without the addition of any additives. The CHI amino groups need to be neutralized to avoid repulsion between polymer chains and hydrogen bonds, hydrophobic interactions, and chitosan crystals that form (RODRÍGUEZ-RODRÍGUEZ et al., 2018).

For CHI gels to produce hydrogen bonds between the hydrophilic groups of CHI and the water molecules of the solution at temperatures below the LCST is necessary (KONG & CHEN, 2018; BHATTARAI, GUNN & ZHANG, 2010). However, at elevated temperatures, CHI exhibits hydrophobic domains (acetyl groups) (Figure 3B). As a result, hydrophobic forces increase, and hydrogen bonds are reduced, resulting in the polymer chains forming the CHI-gel. Considering the opposite effect, the reduction in temperature causes a decrease in molecular mobility between CHI chains. Hydrophobic forces are weakened, hydrogen bonds are formed, and CHI chains are dissolved in the medium (DARGE et al., 2019; KONG & CHEN, 2018). Thus, CHI-based thermoreversible hydrogels can be produced by aggregating other polymers with hydroxyl, amine, amide, or carbonyl CHI functional groups (DARGE et al., 2019).

At temperatures above 50°C, CHI begins to form a gelled network. Rabelo et al. 2019 observed an increase in G' and G" at high temperatures in lipid nanoparticles (NLCs) coated with CHI, concluding that hydrophobic interactions are formed between CHI and lipids, being favored by the increase in temperature. Hydration water was removed from the CHI chairs, and the heat-induced the transfer of protons from protonated amino groups from the CHI to the lipid material (RABELO et al., 2019; ALIAGHAIE et al., 2012).

Thus, it is possible to conclude that the heating process induces hydrophobic interactions, which are essential for the confinement of the oil within the NLCs (RABELO et al., 2019). Analogously to this result, we can infer that CHI can be used as an alternative in structuring vegetable oils, together with HPMC and MC, for forming gelled systems, such as emulgels.

The combination of CHI, HPMC, and MC aims to improve the gel properties, increase the "gel window" of these combined systems and adjust the LCST temperature (CHENITE et al., 2000; WANG et al., 2016). Consequently, it is expected to prolong the gelling temperature of these blended polymers and improve stability and mechanical properties. In other words, it is expected to obtain structured systems that can retain the vegetable oil inside, characterizing an emulgel.

2.1.5.3. Regulation of HPMC, MC, and CHI in Brazil

Regarding Brazil, we have IN No. 211 of 03/01/2023, which establishes that HPMC and MC are food additives with the following technological functions: gelling or suspending agent, emulsifier, stabilizer, and thickener. These polymers have a limit of use. The necessary amount can be added to obtain the desired technological effect.

CHI is also considered a food additive when used as a fining or microbiological control agent in wines. For each technological function, the CHI presents a usage limit. According to RDC IN No. 28 of 7/26/2018, CHI is also considered a dietary supplement (dietary fiber), and its use limit is 3g/day.

REFERENCES

Ali, A., & Ahmed, S. (2018). Recent Advances in Edible Polymer Based Hydrogels as a Sustainable Alternative to Conventional Polymers. Journal of Agricultural and Food Chemistry, 66(27), 6940–6967.

Aliaghaei, M., Mirzadeh, H., Dashtimoghadam, E., & Taranejoo, S. (2012). Investigation of gelation mechanism of an injectable hydrogel based on chitosan by rheological measurements for a drug delivery application. Soft Matter, 8(27), 7128–7137.

Batista, R. A., Espitia, P. J. P., Quintans, J. de S. S., Freitas, M. M., Cerqueira, M. Â., Teixeira, J. A., & Cardoso, J. C. (2019). Hydrogel as an alternative structure for food packaging systems. Carbohydrate Polymers, 205(October 2018), 106–116.

Bhattarai, N., Gunn, J., & Zhang, M. (2010). Chitosan-based hydrogels for controlled, localized drug delivery. Advanced Drug Delivery Reviews, 62(1), 83–99.

Brasil. (2018). Instrução Normativa – IN nº 28 de 26 de julho de 2018.pdf. ANVISA - Agência Nacional de Vigilância Sanitária - Diário Oficial da União.

Brasil. (2023). Instrução Normativa – IN nº 211 de 01 de março 2023.pdf. ANVISA - Agência Nacional de Vigilância Sanitária - Diário Oficial da União.

Bu, N., Huang, L., Cao, G., Pang, J., & Mu, R. (2022). Stable O/W emulsions and oleogels with amphiphilic konjac glucomannan network: preparation, characterization, and application. Journal of the Science of Food and Agriculture, 102(14), 6555–6565.

Cao, Y., & Mezzenga, R. (2020). Design principles of food gels. *Nature Food*, *1*(2), 106–118.

Chen, X. W., Fu, S. Y., Hou, J. J., Guo, J., Wang, J. M., & Yang, X. Q. (2016). Zein based oil-in-glycerol emulgels enriched with β-carotene as margarine alternatives. Food Chemistry, 211, 836–844.

Chenite, A., Chaput, C., Wang, D., Combes, C., Buschmann, M. D., Hoemann, C. D., … Selmani, A. (2000). Novel injectable neutral solutions of chitosan form biodegradable gels in situ. Biomaterials, 21(21), 2155–2161. https://doi.org/10.1016/S0142-9612(00)00116-2

Co, E.D., & Marangoni, A.G. (2012). Organogels: An alternative edible oil-structuring method. *JAOCS, Journal of the American Oil Chemists' Society, 89*(5), 749–780.

Cui, B., Mao, Y., Liang, H., Li, Y., Li, J., Ye, S., … Li, B. (2022). Properties of soybean protein isolate/curdlan based emulsion gel for fat analogue: Comparison with pork backfat. *International Journal of Biological Macromolecules*, *206*(March), 481–488.

Darge, H. F., Andrgie, A. T., Tsai, H. C., & Lai, J. Y. (2019). Polysaccharide and polypeptide based injectable thermo-sensitive hydrogels for local biomedical applications. International Journal of Biological Macromolecules, 133, 545–563.

Davidovich-Pinhas, M., Barbut, S., & Marangoni, A.G. (2016). Development, Characterization, and Utilization of Food-Grade Polymer Oleogels. *Annual Review of Food Science and Technology*, *7*(1), 65–91.

Duquette, D., & Dumont, M. J. (2019). Comparative studies of chemical crosslinking reactions and applications of bio-based hydrogels. Polymer Bulletin, 76(5), 2683–2710.

Farjami, T., & Madadlou, A. (2019). An overview on preparation of emulsion-filled gels and emulsion particulate gels. *Trends in Food Science and Technology*, *86*(July 2018), 85–94.

FDA (Food and Drug Administration), Gras notices (GRN No. 443), (Food Drug Adm. GRN No. 443), Available online: https://wayback.archiveit.org/7993/20171031005742/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm347791.htm

Goff, D., & Guo, Q. (2019). Food Structure Development: The Interplay Between Processing Routes and Formulation Elements. Part A Food Structure Development: The Interplay Between Processing Routes and Formulation Elements, 1–28.

Guo, J., Cui, L., & Meng, Z. (2023). Oleogels/emulsion gels as novel saturated fat replacers in meat products: A review. *Food Hydrocolloids*, *137*(October 2022).

Gutiérrez-Luna, K., Astiasarán, I., & Ansorena, D. (2020). Gels as fat replacers in bakery products: a review. Critical Reviews in Food Science and Nutrition, 0(0), 1–14.

Hilal, A., Florowska, A., & Wroniak, M. (2023). Binary Hydrogels: Induction Methods and Recent Application Progress as Food Matrices for Bioactive Compounds Delivery—A Bibliometric Review. *Gels*, *9*(1).

Hu, Y., Zhang, S., Han, D., Ding, Z., Zeng, S., & Xiao, X. (2018). Construction and evaluation of the hydroxypropyl methyl cellulose-sodium alginate composite hydrogel system for sustained drug release. Journal of Polymer Research, 25(7), 1-12.

Islam, M. M.; Shahruzzaman, M.; Biswas, S.; Nurus Sakib, M.; Rashid, T. U (2020). Chitosan Based Bioactive Materials in Tissue Engineering Applications-A Review. Bioact. Mater, 5 (1), 164–183.

Jayakumar, R.; Prabaharan, M.; Muzzarelli, R.A.A. (2011). Chitosan for Biomaterials I. Berlin, Heidelberg: Springer Berlin Heidelberg.

Khullar, R., Kumar, D., Seth, N., & Saini, S. (2012). Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharmaceutical Journal, 20(1), 63–67.

Kong, M., & Chen, X. (2018). The Novel Medical Thermoresponsive Hydrogel Derived from Chitosan. Current Organic Chemistry, 22(7), 620–627.

Light, K., & Karboune, S. (2021). Emulsion, hydrogel and emulgel systems and novel applications in cannabinoid delivery: a review. Critical Reviews in Food Science and Nutrition, 0(0), 1–31.

Luo, Y., & Wang, Q. (2014). Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. International Journal of Biological Macromolecules, 64, 353–367.

Martínez-Cervera, S., Salvador, A., & Sanz, T. (2015). Cellulose ether emulsions as fat replacers in muffins: Rheological, thermal and textural properties. LWT - Food Science and Technology, 63(2), 1083–1090.

Nishinari, K., Hofmann, K. E., Moritaka, H., Kohyama, K., & Nishinari, N. (1997). Gel-sol transition of methylcellulose. Macromolecular Chemistry and Physics, 198(4), 1217–1226.

Okuro, P. K., Santos, T. P., & Cunha, R. L. (2021). Compositional and structural aspects of hydro- and oleogels: Similarities and specificities from the perspective of digestibility. *Trends in Food Science and Technology*, *111*(February), 55–67.

Paglarini, C. de S., Furtado, G. de F., Biachi, J. P., Vidal, V. A. S., Martini, S., Forte, M. B. S., Pollonio, M. A. R. (2018). Functional emulsion gels with potential application in meat products. Journal of Food Engineering, 222, 29–37.

Patel, A.R., Cludts, N., Bin Sintang, M.D., Lewille, B., Lesaffer, A., &Dewettinck, K. (2014). Polysaccharide-based oleogels prepared with an emulsion-templated approach. ChemPhysChem, 15(16), 3435–3439.

Patel, A.R. (2015). Alternative Routes to Oil Structuring. In *Springer*.

Patel, A.R., & Dewettinck, K. (2016). Edible oil structuring: An overview and recent updates. *Food and Function*, *7*(1), 20–29.

Pehlivanoğlu, H., Karasu, S., Konar, N., Toker, O.S., Demirci, M., & Sagdic, O. (2018). Oleogels, a promising structured oil for decreasing saturated fatty acid concentrations: Production and food-based applications. *Critical Reviews in Food Science and Nutrition*, *58*(8), 1–12.

Pintado, T., Herrero, A. M., Jiménez-Colmenero, F., Pasqualin Cavalheiro, C., & Ruiz-Capillas, C. (2018). Chia and oat emulsion gels as new animal fat replacers and healthy bioactive sources in fresh sausage formulation. Meat Science, 135(August 2017), 6–13.

Pintado, T., & Cofrades, S. (2020). Quality characteristics of healthy dry fermented sausages formulated with a mixture of olive and chia oil structured in oleogel or emulsion gel as animal fat replacer. Foods, 9(6).

Pinto, T. C., Martins, A. J., Pastrana, L., Pereira, M. C., & Cerqueira, M. A. (2021). Oleogel-Based Systems for the Delivery of Bioactive Compounds in Foods. Gels, 7(3), 86.

Rabelo, R. S., Oliveira, I. F., da Silva, V. M., Prata, A. S., & Hubinger, M. D. (2018). Chitosan coated nanostructured lipid carriers (NLCs) for loading Vitamin D: A physical stability study. International Journal of Biological Macromolecules, 119, 902–912.

Ract, J. N. R., Cruz, R. G. d., & Pereira, C. G. (2018). Phase equilibrium of organogels. In Thermodynamics of Phase Equilibria in Food Engineering.

Ranjha, N. M., & Khan, S. (2013). Review Article Chitosan / Poly (vinyl alcohol) Based Hydrogels for Biomedical Applications: A Review. Journal of Pharmacy and Alternative Medicine, 2(1), 30–42.

Rehman, K., Cairul, M., Mohd, I., & Zulfakar, M. H. (2014). Development and Physical Characterization of Polymer-Fish Oil Bigel (Hydrogel / Oleogel) System as a Transdermal Drug Delivery Vehicle. Journal of Oleo Science, 970(10), 961–970.

Rodríguez-Rodríguez, R., García-Carvajal, Z. Y., Jiménez-Palomar, I., Jiménez-Avalos, J. A., & Espinosa-Andrews, H. (2019). Development of gelatin/chitosan/PVA hydrogels: Thermal stability, water state, viscoelasticity, and cytotoxicity assays. Journal of Applied Polymer Science, 136(10), 1–9.

Sannino, A., Demitri, C., & Madaghiele, M. (2009). Biodegradable cellulose-based hydrogels: Design and applications. Materials, 2(2), 353–373.

Sanz, T., Falomir, M., & Salvador, A. (2015). Reversible thermal behaviour of vegetable oil cellulose ether emulsions as fat replacers. Influence of glycerol. Food Hydrocolloids, 46, 19–27.

Sarkar, N. (1979). Thermal gelation properties of methyl and hydroxypropyl methylcellulose. Journal of Applied Polymer Science, 24(4), 1073–1087.

Sharma, S., & Tiwari, S. (2020). A review on biomacromolecular hydrogel classification and its applications. International Journal of Biological Macromolecules, 162, 737–747.

Singh, A.; Auzanneau, F.I.; Rogers, M.A (2017). Advances in edible oleogel technologies – A decade in review. Food Research International, 97 (4), p. 307–317.

Siraj, N., Shabbir, M.A., Ahmad, T., Sajjad, A., Khan, M.R., Khan, M.I., & Butt, M.S. (2015). Organogelators as a Saturated Fat Replacer for Structuring Edible Oils. *International Journal of Food Properties*, *18*(9), 1973–1989.

Stortz, T.A., Zetzl, A.K., Barbut, S., Cattaruzza, A., & Marangoni, A.G. (2012). Edible oleogels in food products to help maximize health benefits and improve nutritional profiles. *Lipid Technology*, *24*(7), 151–154.

Thielking, H., & Schmidt, M. (2012). Ullmann's Encycl. Ind. Chem. - Heterogenous Catalysis and Solid Catalysts 1. Fundamentals. Ullmann's Encyclopedia of Industrial Chemistry, 565–582.

Xu, H. J., Li, T., Zhang, H. X., Shi, C. H., Cao, J. Q., & Zhang, X. R. (2022). The application of oleogels in food products: Classification, preparation, and characterisation. Acta Alimentaria, 51(4), 462–478.

Wang, T., Chen, L., Shen, T., & Wu, D. (2016). Preparation and properties of a novel thermo-sensitive hydrogel based on chitosan/hydroxypropyl methylcellulose/glycerol. International Journal of Biological Macromolecules, 93, 775–782.

Yang, T., Li, X. T., & Tang, C. H. (2020). Novel edible pickering high-internal-phase-emulsion gels efficiently stabilized by unique polysaccharide-protein hybrid nanoparticles from Okara. *Food Hydrocolloids*, *98*(August 2019), 105285.

Yang, Y., Zhang, M., Li, J., Su, Y., Gu, L., Yang, Y., & Chang, C. (2022). Construction of egg white protein particle and rhamnolipid based emulsion gels with β-sitosterol as gelation factor: The application in cookie. *Food Hydrocolloids*, *127*(August 2021).

Zhao, M., Lan, Y., Cui, L., Monono, E., Rao, J., & Chen, B. (2020). Physical properties and cookie-making performance of oleogels prepared with crude and refined soybean oil: A comparative study. Food and Function, 11(3), 2498–2508.

CHAPTER III

Thermo-rheological properties of chitosan hydrogels with hydroxypropyl methylcellulose and methylcellulose

THERMO-RHEOLOGICAL PROPERTIES OF CHITOSAN HYDROGELS WITH HYDROXYPROPYL METHYLCELLULOSE AND METHYLCELLULOSE

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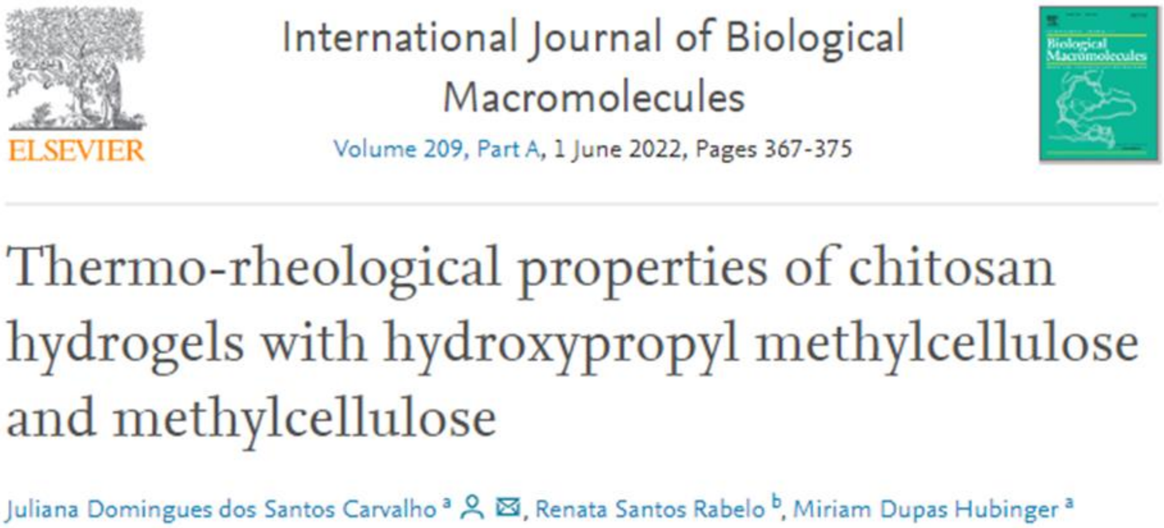

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ABSTRACT

Thermal and rheological properties of methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) hydrogels with chitosan (CHI) were investigated to verify the potential application of these blends as structured systems for oil transport (emulgel, oleogels, and bigels). FTIR confirmed hydrophobic interactions of cellulosic polymers with chitosan. In the temperature sweep, the thermosensitive hydrogels showed their reduced gel point compared to the original polymers. The gelation temperature was reduced from 66.9 °C for pure HPMC to 43.6 °C and 43.6 °C (MC pure polymer) to 39.3 °C when 30% CHI was added for both cases. The addition of 20 and 30% chitosan is enough to modify the extension of the gelation temperature of these polymers. These results indicate that the addition of chitosan enables MC and HPMC to form gels at lower temperatures, which could allow milder thermal conditions to be applied in processing oil carrier systems.

Keywords: LCST; polysaccharides; gel window.

1. Introduction

Methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) are examples of polymers that form thermo-reversible and physically crosslinked hydrogels at a LCST (lower critical solution temperature) within 40 and 50 °C, and 75 and 90 °C, respectively [1-4]. Thus, these polymers are used to obtain gelled structures, such as oleogels, emulgels, and bigels, at temperatures above their LCST [5].

However, the temperature at which these polymeric gels are formed can limit the application of more sensitive oils to high temperatures in structured systems to replace fat in food matrices. As an alternative, it is possible to use other components together with HPMC and MC to promote modifications in their LCST. This addition can ensure that these polymers will be in a gel state at a lower temperature and over a wide temperature range.

Chitosan (CHI) may be a candidate to form thermoreversible gels with these cellulosic polymers and reduce their LCST [2, 6, 7]. This polysaccharide is obtained from chitin deacetylation and presents a high concentration of positive charges. This polymer has other relevant characteristics, such as the presence of amino groups (hydrophilic) and acetyl groups (hydrophobic), which ensure their interaction with vegetable oils and fats [7-9].

The use of CHI to prepare aqueous solutions with disodium ß-glycerophosphate (ß-GP) as gelled systems for the encapsulation of living chondrocytes was reported by Chenite et al. [10]. Upon heating (5 to 70°C), the elastic modulus (G') increased sharply, and at 37 °C, the liquid solution turned into a gel. During cooling, the opposite trend was observed (gel-solution), confirming the thermoreversibility of the gel. The authors emphasized that this system can gel and undergo the solution/gel transition due to the hydrophobic interactions favored with increasing temperature. CHI combined with pure HPMC and HPMC with glycerol to prepare thermoreversible hydrogels was investigated by Wang et al. [11]. In the rheological characterization with temperature variation (20 to 80 °C), the hydrogels of HPMC (2 w/w%) and CHI (1 w/w%) without additives showed a LCST of 56 °C. Thus, the addition of CHI promoted a reduction in the HPMC gelation temperature (LCST temperature) compared to the pure polymer whose LCST is above 60°C.

Apart from forming hot gels, HPMC and MC have hydrophobic groups in their structure [2, 6, 10]. These hydrophobic groups (methyl and hydroxypropyl) are exposed during heating these materials to favor polymer-polymer interactions. Therefore, these materials can interact with non-polar components such as vegetable oils sensitive to temperature [2, 3]. Thus, as a technological alternative, hydrogels composed of mixtures of HPMC: CHI and MC: CHI are potential systems to be used in obtaining gelled structures capable of retaining liquid oil.

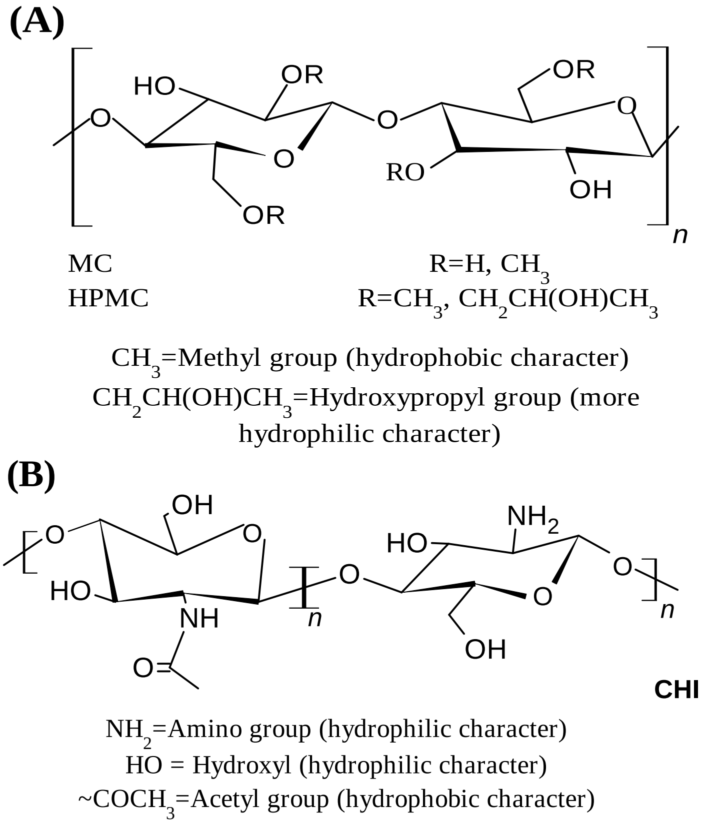
This work aimed to obtain thermoreversible hydrogels based on HPMC, MC, and CHI that can be applied in food processing systems. In order to understand the thermogelation characteristics of these hydrogels based on cellulosic polymers and chitosan, rheological assays, Fourier transform infrared spectroscopy (FTIR), dynamic interfacial tension, and thermal behavior by DSC were studied.

2. Material and Methods

2.1.Material

The raw materials used as structuring agents of the hydrogels were chitosan (CHI, CAS 9012-76-4, Deacetylation degree = 85%), acquired from Sigma-Aldrich (São Paulo - Brazil), hydroxypropyl methylcellulose (HPMC K4M, CAS 9004-65-3), and methylcellulose (MC A4M, CAS 9004-67-5) donated by Ashland Chemical Specialty Trade in Brazil LTDA (São Paulo - Brazil). The letters A and K refer to the chemical identity of the substituent groups, methyl, and methyl hydroxypropyl, respectively, and 4 M means typical viscosity of 4000 mPAs^{-1} in a 2% aqueous solution. Phosphoric acid (PA, 7664-38-2) was purchased from Synth (São Paulo – Brazil). The selected sunflower oil (SO) was purchased at a local market. All the experiments used Milli-Q water (resistivity 18.2 MΩ.cm). Figure 1 shows HPMC, MC and CHI polymeric structure.

Figure 1. Polymeric structure: (A) Hydroxypropyl methylcellulose (HPMC) and methylcellulose (MC), and (B) Chitosan (CHI). Adapted from Sannino, Demitri & Madaghiele [1] & Islam et al. [8].



MC R=H, CH_3
HPMC R=CH_3, $CH_2CH(OH)CH_3$

CH_3=Methyl group (hydrophobic character)
$CH_2CH(OH)CH_3$=Hydroxypropyl group (more hydrophilic character)

NH_2=Amino group (hydrophilic character)
HO = Hydroxyl (hydrophilic character)
~$COCH_3$=Acetyl group (hydrophobic character)

2.2.Hydrogels preparation

The polymer concentration was fixed at 2% (w/w) [12], in solutions and final hydrogels composition. The CHI solution was dispersed in phosphoric acid solution (1% v/v). The polysaccharides HPMC and MC were prepared in Milli-Q water, and the mix was heated up to 80–85 °C with constant stirring. These solutions were mechanically stirred for 12 h (300 rpm) at room temperature to ensure complete hydration of the polymers. After that, hydrogels were prepared using blends of HPMC: CHI and MC: CHI, according to Table 1. The process was conducted in duplicate.

Table 1. Formulation of the hydroxypropyl methylcellulose-chitosan (HPMC: CHI) and methylcellulose-chitosan hydrogels (MC: CHI).

Hydrogels HPMC: CHI	**Hydrogels MC: CHI**
80:20	80:20
70:30	70:30
60:40	60:40

2.3.Hydrogels characterization

2.3.1. Rheological assays

A Physica MCR301 controlled tension rheometer (Anton Paar, Graz, Austria) with a Peltier system and a water bath (Julabo, Seelbach, Germany) was used for rheological tests. A rough 50 mm diameter stainless steel flat plate geometry (PP50/S) was selected from preliminary tests. The GAP used was 0.3 mm. All tests were performed in duplicate for each process, and a new sample was used for each trial.

Flow curves of the hydrogels were acquired along three shear rate ramps using the following order: increasing-decreasing-increasing in a range of 0 and 300 s^{-1}. The third curve was used to assess rheological behavior at a steady state. The results obtained from the third curve were adjusted according to the power-law model (Equation 1).

$$\sigma = k\dot{\gamma}^{n} \quad (1)$$

Where σ (Pa) is the shear stress, k (Pa.s^n) is the consistency index, $\dot{\gamma}$(s^{-1}) is the shear rate, *n* is the flow behavior index (dimensionless).

The linear viscoelastic (0.1%) region was determined in a strain scan of 0.01–100% at 1 Hz for the oscillatory assays. Frequency sweeps (0.1 Hz and 10 Hz) were made to obtain the mechanical spectra at room temperature. A temperature sweep was made to determine structural changes due to thermal effect at a rate of 5 °C/min. The equipment was preheated up to 85 °C with equilibrium for 1 min. After that, the samples were cooled from 85 to 5 °C and then left isothermically for 14 min, and heated up again to 85 °C. Low viscosity silicone oil was used to seal round the sample to avoid evaporation. The elastic component (G') and the viscous component (G") were determined.

2.3.2. Sample lyophilization

The lyophilization technique can influence the polymer-solvent interactions of hydrogen bonds [38]. For polymer-polymer interactions (hydrophobic interactions) there are no reports in the scientific literature [13,15, 39, 40, 41]. Therefore, lyophilization was used as a DSC and FTIR analysis sample preparation method. Since the equipment requires dry samples, this technique is widely used to analyze hydrogel systems [15, 30,40].

After preparing the gels (or pure polymeric solutions), the samples were freeze-dried in a lyophilizer LS 3000 (Terroni Equipamentos Científicos, São Carlos, Brazil) at −40 °C and 100 µm.Hg for 48 h. Then, the samples were kept in a desiccator for subsequent characterization by FTIR and DSC.

2.3.3. Fourier Transform Infrared Spectroscopy (FTIR)

The chemical structure of dried gel and pure polymers were analyzed qualitatively using FTIR IRPrestige-21version 1.60 spectrometer (Kyoto, Japan) with KBr pastille. For analysis, 2 mg of sample was macerated with 200 mg KBr using grail and agate pistil and transferred to a 13 mm diameter stainless steel mold. The sample was pressed at 80 kN for 10 minutes using a Shimadzu hydraulic press (model SSP-10A) operated under vacuum to form the pellet. The spectrum was recorded in the wave range between 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1} and 40 scans.

2.3.4. Dynamic Interfacial Tension

The interfacial tension was evaluated between the aqueous phase HPMC, MC, CHI and the oil phase, composed of sunflower oil (SFO). The concentration of 0.2% (m/m) was chosen to promote the contrast of the equipment samples. The drop volumes were 30 mm^3 for HPMC, 17 mm^3 for MC, and 23 mm^3 for CHI. 17, 18, and 17 mm^3 were the drop volumes found for the proportion of HPMC: CHI 80:20, 70:30, and 60:40, respectively. For the MC: CHI samples, 22, 20, and 17 mm^3 were found for the proportions of 80:20, 70:30, and 60:40, respectively. All measurements were performed at 25 °C for 3,600 s. The interfacial tension experiments were conducted using a Tracker-S tensiometer (Teclis, France) using the pendent droplet method for HPMC, MC, and their mixtures with CHI. Due to the high opacity of pure CHI, the rising method was used. All measurements were performed in duplicate.

2.3.5. Thermal behavior

Differential scanning calorimetry (DSC) characterized thermal events and glass transition temperature of the dried pure polymers and gels. DSC measurements were carried in a liquid nitrogen (N_2) atmosphere using the DSC instrument (Mettler Toledo, Schwerzenbach, Switzerland). The calibration was made with indium as standard. Samples were weighed and sealed in aluminum pans with a perforated lid. Operational conditions were: heated from 25 °C to 200 °C and maintained for 2 min, cooled from 200 °C to 25 °C (10 °C/min), in an inert atmosphere of N_2 at 50 ml/min.

2.3.6. Statistical analysis

The results were subjected to analysis of variance (ANOVA) and Tukey's test with a significance level of 5%, using Minitab16.1.0 (Minitab Inc., StateCollege, PA, USA).

3. Results and Discussion

3.1.Rheological characterization

3.1.1. Steady-shear behavior

The fit parameters of the power-law model to the flow curves at room temperature are shown in Table 2. All the systems showed no thixotropic behavior because the up, down, and up-flow curves were similar.

Table 2. Consistency index (k), flow behavior index (n), determination coefficient (R^2) and viscosity (μ) of hydrogels of 2% HPMC, MC, and CHI and their different mixtures.

Samples	***n***	***k* (Pa.s^{n})**	**R^2**	**μ_{300} s^{-1} (Pa. s)**
Polymer solution (2%)				
HPMC	0.552±0.006^{b}	11.522±0.746^{d}	0.999±0.000^{a}	0.866±0.047^{c}
MC	0.483±0.008^{c}	11.715±0.557^{d}	0.999±0.001^{a}	0.472±0.002^{d}
CHI	0.807±0.006^{a}	0.562±0.005^{g}	0.999±0.000^{a}	0.181±0.003^{e}
Hydrogels – HPMC e CHI				
80HPMC:20CHI	0.326±0.025^{f}	60.206±1.223^{a}	0.996±0.002^{b}	1.470±0.06^{a}
70HPMC:30CHI	0.368±0.011^{e}	48.199±1.586^{b}	0.999±0.000^{a}	1.258±0.029^{a,b}
60HPMC:40CHI	0.408±0.024^{d}	40.211±0.775^{c}	0.999±0.000^{a}	1.080±0.066^{b,c}
Hydrogels – MC e CHI				
80MC:20CHI	0,550±0.001^{b}	6.812±0.284^{f}	0.999±0.001^{a}	0.505±0.013^{d}
70MC:30CHI	0.519±0.012^{b}	7.974±0.605^{e,f}	0.999±0.000^{a}	0.531±0.041^{d}
60MC:40CHI	0.533±0.018^{b}	8.907±0.429^{e}	0.999±0.000^{a}	0.559±0.052^{d}

Different letters indicate a significant difference ($p < 0.05$) in the same column. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; CHI: chitosan.

Hydrogels, HPMC, and MC dispersions presented pseudoplastic behavior ($n < 1$), with n between 0.326 and 0.550. Low "n" values suggest more structured gels. The 80HPMC: 20CHI and 70HPMC:30CHI samples have a higher elastic character due to their higher concentration of cellulosic polymer. Regardless CHI concentration, hydrogels prepared with MC exhibited similar "n" values (no statistical difference). For the chitosan dispersion (2%), the "n" value was 0.80, suggesting a pseudoplastic fluid with weak structure.

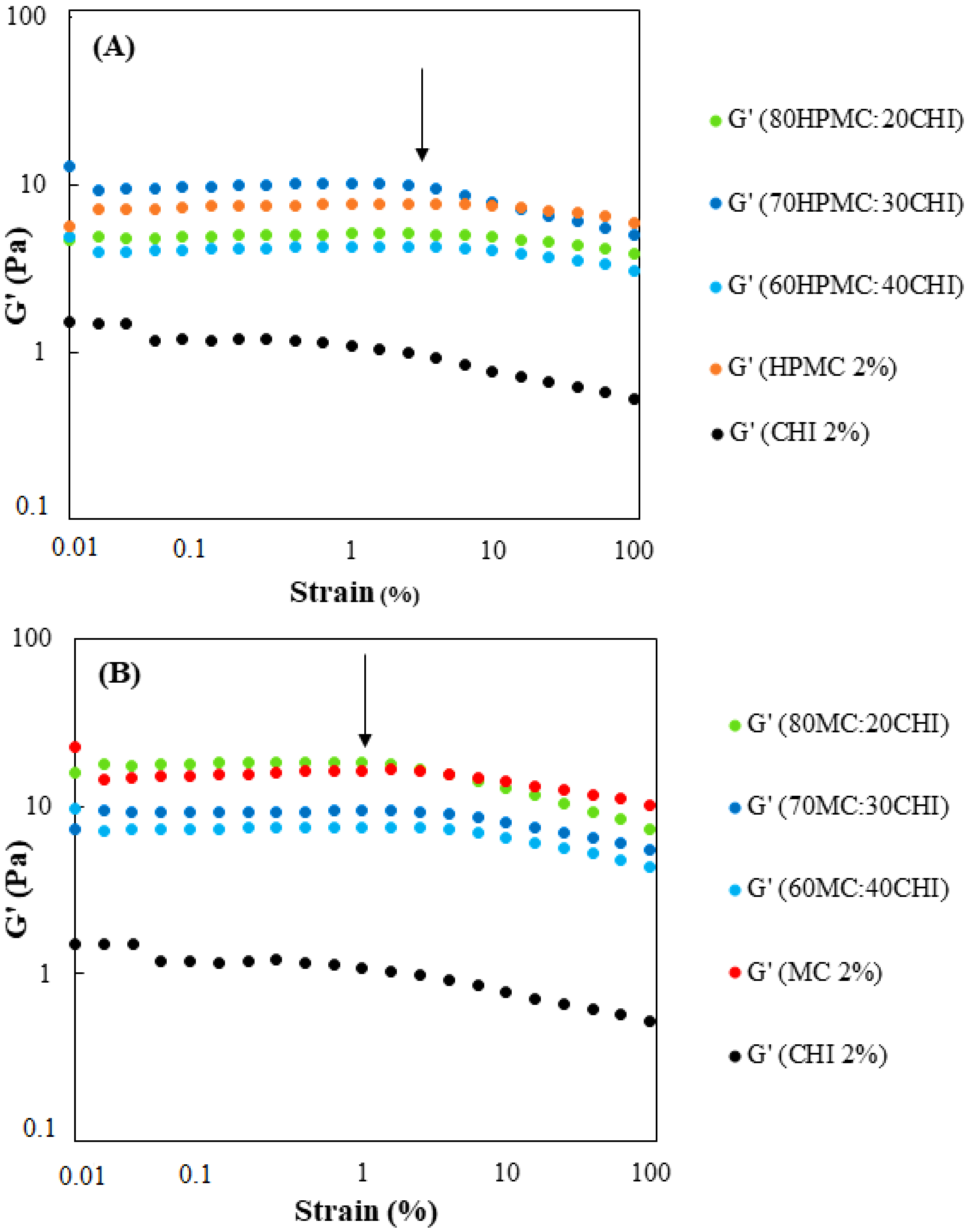
The "k" parameter has a relationship with the apparent viscosity of the materials. Analyzing HPMC and MC dispersions, we can see higher "k" values, 11.522 and 11.715 (Pa.sn), respectively, than the CHI value, which is 0.562 (Pa.sn). Furthermore, there was an increase in the "k" value for the HPMC hydrogels at the highest concentrations of cellulosic polymer. Hydrogels with MC had low values of "k" when compared to systems with HPMC, meaning that HPMC hydrogels are more structured than MC hydrogels.

For shear rate, the range from 0 to 300 s^{-1} was chosen to guarantee a laminar regime, and this value covers most processes in the food industry. The values of apparent viscosity are in Table 2. Analyzing the viscosity of the individual polymers, it is remarkable that HMPC has the highest value, followed by MC and CHI. This fact resulted in HPMC: CHI hydrogels with higher apparent viscosity and more structured systems than those produced with MC: CHI.

3.1.2. Oscillatory shear behavior

Figure 2 shows the strain scan to determine the linear viscoelastic region of the hydrogels with HPMC: CHI and MC: CHI; it also exhibits results for HPMC, MC, CHI dispersions and their G' (storage modulus) and G" (viscous modulus). Tests obtained within this region ensured that the structure of the polymeric material was intact and within the linear strain range.

Figure 2. Stress sweeps of (A) HPMC: CHI, (B) MC: CHI hydrogels and polymer dispersions. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose and CHI: chitosan.



G' is constant (Figure 2A e B) at a given strain for all the samples; meanwhile, G' decreased when the output region of linear viscoelasticity occurs. When the force applied to the material is increased, the structure of the polymeric material tends to break. This fact is more related to the rupture of the material's elastic network (G') than to the viscous properties expressed by G". The hydrogels 70HPMC: 30CHI (Fig. 2A) and 80MC:20CHI system and 2% MC dispersion (Fig. 2B) system showed the highest G' values. Thus, these materials are closer to solid-like behavior. Consequently, these samples were more elastic and stable. CHI has little influence on the elastic property of the material, as the hydrogel samples have similar breaking points (Fig. 2). Nevertheless, CHI is the first to lose its structure. That is, it is more susceptible to the mechanical forces as the strain rate increases.

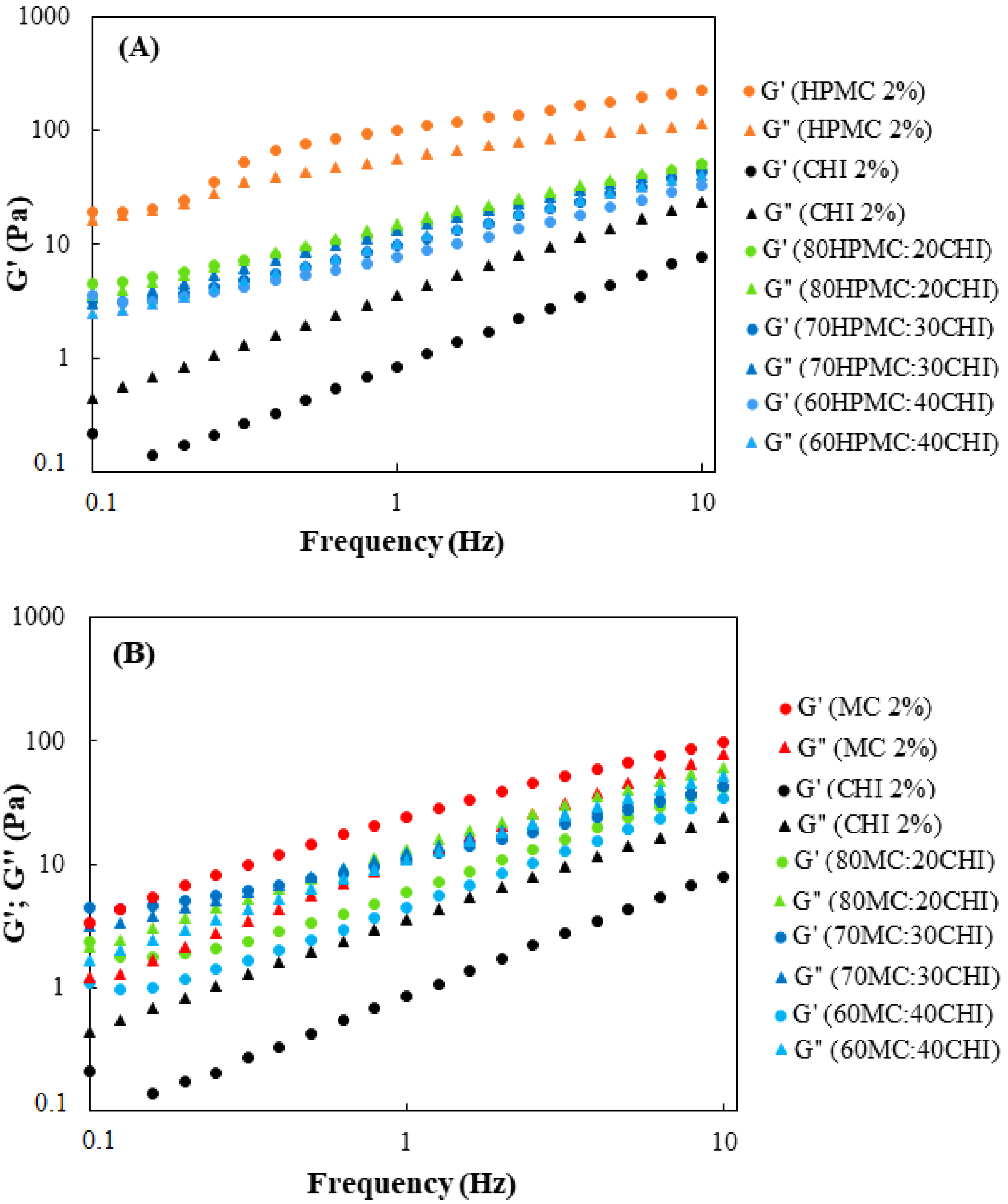
Figure 3 represents the frequency scans at a 0.1% strain (linear viscoelastic region) where all samples showed frequency dependence. This behavior is typical of an interlaced polysaccharide solution [13].

However, each gel, pure polymers, or hydrogels (MC: CHI and HPMC: CHI) showed different behavior. HPMC: CHI hydrogels and 2% HPMC dispersion (Fig. 3A) exhibited, at low frequencies, at long observation times, close values for G' and G" across the entire frequency ramp (G'/G"<10). These samples exhibit stable but weak gel behavior. The 2% HPMC dispersion was the only one that showed G'>G" with increasing frequency, characterizing it as a more structured material with gel behavior.

Analyzing the samples of hydrogels produced with MC, all samples presented G" values greater than G', except 70MC: 30 CHI sample (G'>G"). Pure MC (2%) presented an elastic behavior (G') predominant over the viscous one (G"). For CHI (2%), the opposite behavior was observed.

Lindblad et al. [14] prepared hydrogels from modified cellulosic polymers (hemicellulose). During the frequency sweep tests (0.1 to 16 Hz), the authors observed that G' was greater than G" in all its extensions. These data led to the conclusion that the hydrogel systems showed predominantly solid behavior. This conclusion is similar to the present work, which observed G'>G" in HPMC: CHI and MC: CH hydrogels.

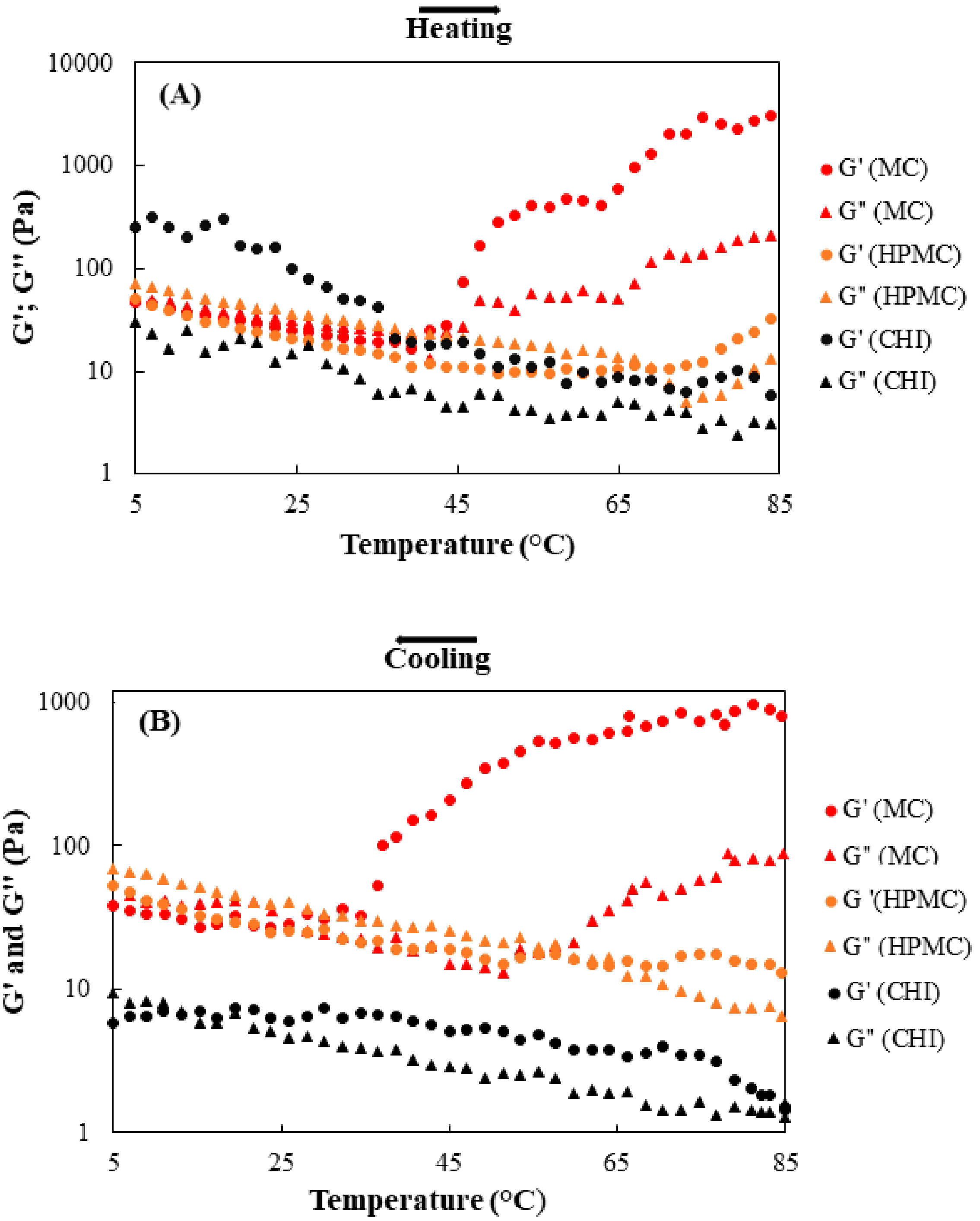
Figure 3. Storage (G') and loss (G") modulus of (A) HPMC: CHI and (B) MC: CHI hydrogels by frequency sweeps. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose and CHI: chitosan.



3.1.3. Analysis of thermal properties

The oscillatory behavior during heating and cooling ramps (thermo-reversibility) is shown in Figure 4 for the individual polymers and Figure 5 for the MC: CHI and HPMC: CHI hydrogels.

Figure 4. Storage (G') and loss (G") modulus for pure polymers at (A) heating and (B) cooling sweeps. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose and CHI: chitosan.



For temperatures from 5 to 40 °C (Fig. 4A), the changes in the viscoelastic behavior of pure cellulosic polymers were less pronounced. The MC showed approximate G' and G" values until 40 °C, and in this interval, G' and G" decreased. However, at temperatures above 45 °C, an increase in G' and G" values is observed. This increase in the viscoelastic behavior correlated with the interconnected polymer chains to create a continuous network [15]. At 43.6 °C, G' becomes equal to G" (G'=G"), this crossing can be defined as gel point. With the progressive increase in temperature, there is a predominance of elastic behavior over the viscous one. The correlation between G' and G" was performed (G'/G"). If this parameter is lower than 10, we have a weak gel; values above 10 characterize a strong gel [16]. At 85 °C, we got a G'/G" ratio >10 for MC, proving that this polymer forms a solid and well-structured gel.

Figure 4A also shows the results for HPMC (2%) solution. Compared to the MC solution, there are significant differences in the curves during the sol-gel transition from the temperature of 45 °C. For HPMC, at temperature values from 5 to 60 °C, the G" is greater than G', indicating a viscoelastic system dominated by the liquid component (viscous). The same behavior was observed by Hussain, Keary & Craig [13] in their study on the thermo-rheological behavior of three grades of HPMC material (METHOCEL E4M, F4M, K4M) at 2% concentration. However, G' and G" progressively decreased with increasing temperature. At 66.9 °C, HPMC showed a gel point (G'=G") and formed weaker gels due to the low G' values. Then G' and G" continue to increase to 85°c, which is the maximum study temperature.

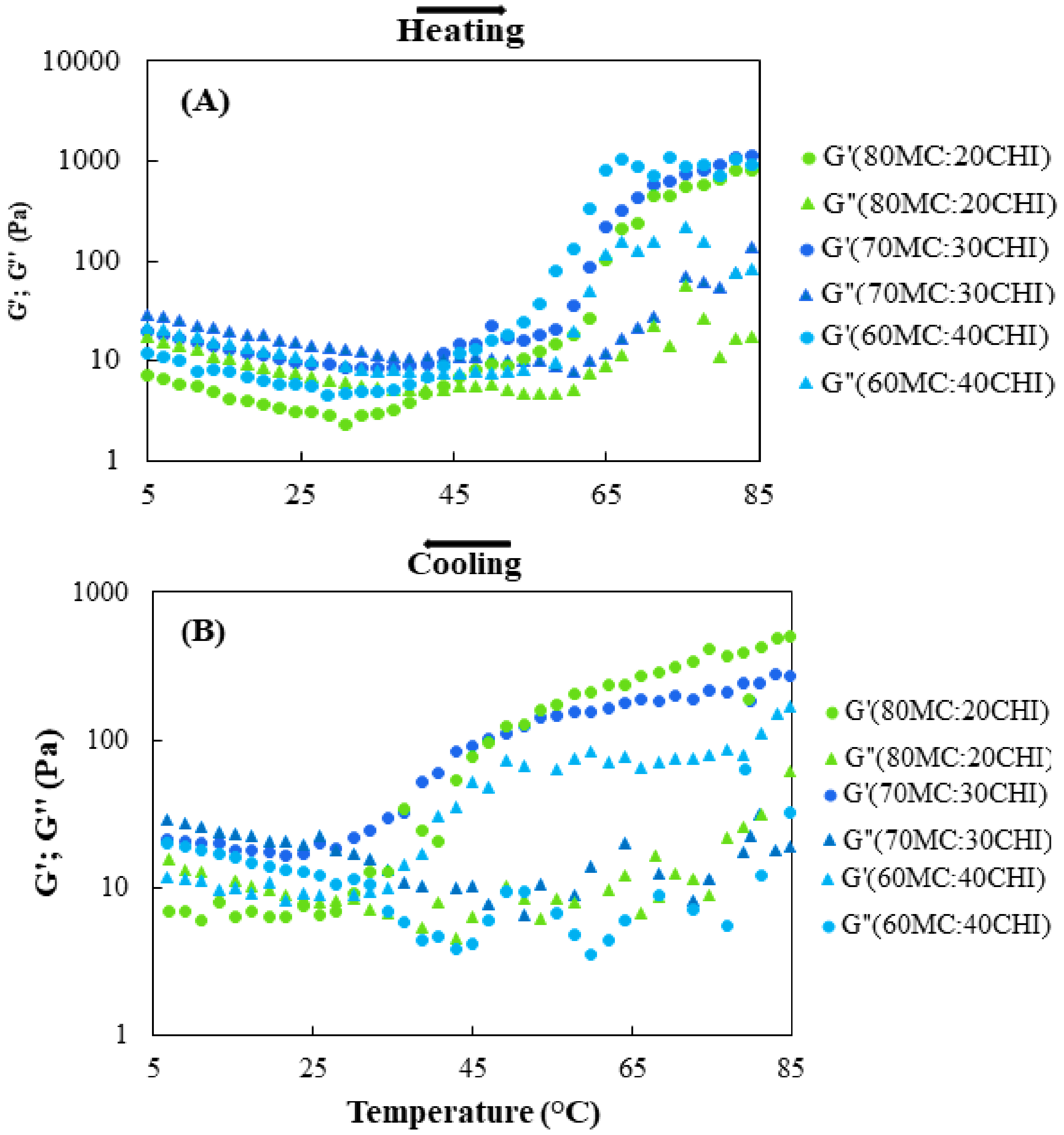
Methyl and hydroxypropyl methylcellulose in an aqueous solution are known to form gel upon heating [17]. These polymers can form thermoreversible gels in a low concentration, 1-10% by weight [1]. According to Goff & Guo [3], HPMC and MC need to be heated up above their gelation temperature. Gelation occurs upon heating to a LCST, at temperatures between 40 and 50 °C for MC that produces more structured gels, and 65-90 °C for HPMC (more hydrophilic) that produces soft gels [2, 3].

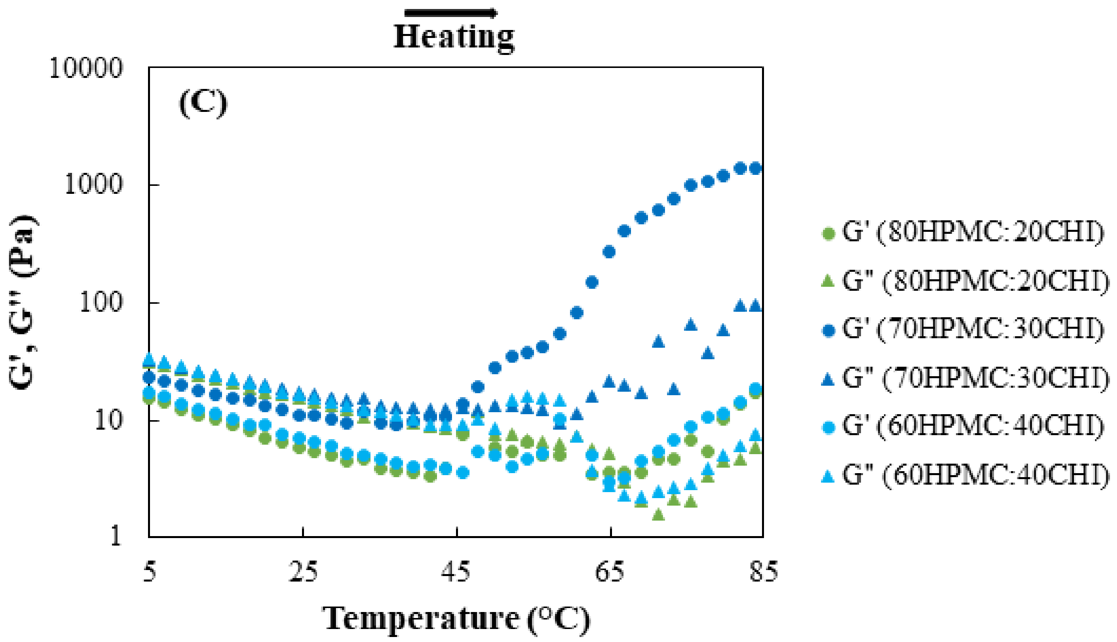
On MC cooling (Fig 4B), the G' and G" had a sharp drop with the decrease in temperature as the pre-formed polymeric network began to dilute in water. At 25.9 °C, the destabilization point was reached; and with that, the transition from the gel state to liquid occurred. This temperature (25.9 °C) is defined as dissolution temperature. Below 35 °C, both modules recover their original values, showing that they are thermo-reversible gels (passage from the gel stage to the liquid). For HPMC, G' showed a slight reduction after cooling, and at 55.6 °C, the dissolution temperature was observed (thermo-reversible). A gel point at 43.6 and 66.9 °C was observed on heating for MC and HPMC, respectively. While cooling, the following dissolution temperatures were obtained: 25.9 °C for MC and 55.6 °C for HPMC. Wang et al. [18] related this change to hysteresis and/or chain condensation at a lower temperature.

Analyzing the CHI on heating, G' is higher than G" across the full range of temperature sweep, but these values are close in order of magnitude, leading to the formation of a weak gel. However, the structure of CHI on cooling shows a dissolution point at 17 °C. During heating, the CHI reorganized itself, bringing the molecules together due to water loss through hydrophobic forces between the polymeric chains [19, 20].

Figure 5A presents the heating ramp data for the MC: CHI hydrogels. The hydrogels constituted by MC and CHI showed similar behavior in every temperature scan. G" was slightly higher than G' in the range of 5 to 40 °C for all samples. That is, with increasing temperature, there is a reduction in the viscoelastic parameters of all samples. However, after 40 °C, there was a predominance of elastic behavior over the viscous one (G'>G"), that is, structured gels with different strengths were formed. At the maximum temperature of analysis (85 °C), the calculation for the gel strength of these systems was performed, taking into account the ratio between G' and G". For 80MC: 20CHI, the G'/G" ratio was greater than 10, precisely 45, showing that at this temperature this system forms well-structured gels with solid behavior. For the samples 60MC: 40CHI, G'/G"> 10 and for 70MC: 30 CHI G'/G," it was less than 10. As a gel point, all samples (80MC: 20CHI, 70MC: 30CHI, and 60MC: 40CHI) showed lower values than pure MC hydrogel (no addition of CHI).

Figure 5. Storage (G') and loss (G") modulus for MC: CHI hydrogels at (A) heating and (B) cooling and for HPMC: CHI hydrogels at (C) heating and (D) cooling. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; and CHI: chitosan.




Heating
(C)
10000
1000
100
10
1
G', G" (Pa)
5
25
45
65
85
Temperature (°C)
G' (80HPMC:20CHI)
G" (80HPMC:20CHI)
G' (70HPMC:30CHI)
G" (70HPMC:30CHI)
G' (60HPMC:40CHI)
G" (60HPMC:40CHI)

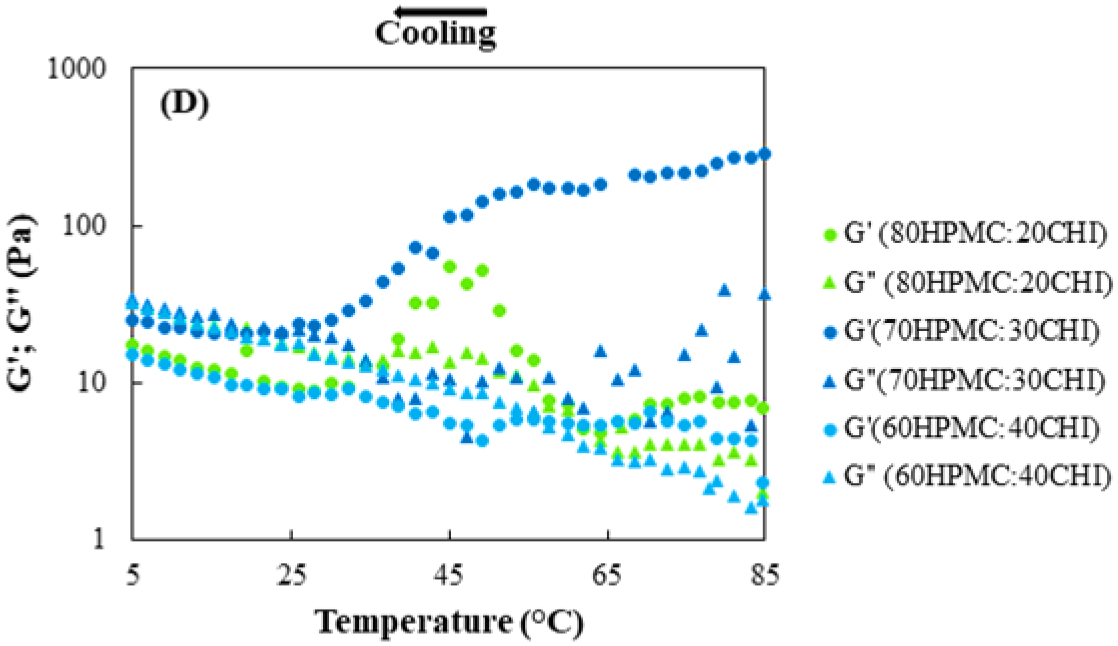

Cooling
(D)
1000
100
10
1
G'; G" (Pa)
5
25
45
65
85
Temperature (°C)
G' (80HPMC:20CHI)
G" (80HPMC:20CHI)
G'(70HPMC:30CHI)
G"(70HPMC:30CHI)
G'(60HPMC:40CHI)
G" (60HPMC:40CHI)

Figure 5C shows the rheological behavior of hydrogels prepared with HPMC: CHI. 80HPMC: 20CHI hydrogels show a drop in G' and G" values, but with G">G' from 5 to 45 °C. After reaching the gel point at 45.7 °C, there was a reduction in G" values and an increase in G'. Such behavior was similar to the 60HPMC: 40CHI sample. Both showed G'/G" gel strength <10, forming weak gels. However, 70HPMC: 30CHI hydrogel had approximately G' and G" up to its gel point (43.6 °C). After that, G' and G" increased progressively. Furthermore, these systems form strong and well-structured gels because the G'/G" ratio is >10. The gel point of these samples was also lower than pure HPMC.

In the cooling curve for the hydrogels prepared with MC and CHI (Fig. 5B), the samples 80MC: 20CHI and 70MC: 30CHI had a marked slope in the values of G' and G" and presented dissolution temperatures at 25.90 and 23.75 °C, respectively. Both samples, below 25 °C, returned to have similar values obtained in the heating step for G' and G", indicating the thermo-resistance of the gel. 80HPMC: 20CHI (Fig. 5D) showed a decrease in G' and G" up to 60 °C and two dissolution points, at 62 °C and another at 36.5 °C. 70HPMC: 30CHI and 60HPMC: 40CHI also exhibited decreased viscoelastic parameters, exhibiting dissolution temperature at 21.6 and 55.6 °C, respectively. At low-temperature values (in the cooling ramp), the hydrogels exhibited behavior analogous to CHI, and at high temperatures, they approached pure MC or HPMC.

Based on Figure 5 (A, B, C, and D) and in the previous discussion, it is possible to conclude that CHI addition causes a significant change in the G' and G" behavior of HPMC and MC. For systems with MC, the gel point occurs at the following temperatures: 41.4, 39.3, and 41.5 °C for 80MC: 20CHI, 70MC:30CHI, and 60MC: 40CHI, respectively. For samples with HPMC, 80HPMC: 20CHI, 70HPMC: 30CHI, and 60HPMC: 40CHI, the gel point obtained was at 45.7, 43.6, and 64.8 °C, respectively. In both cases, gel formation is observed at a lower temperature after the addition of CHI. There is in these systems a decrease in the LCST temperature of the polymers.

Thus, it is possible to conclude that heating induces hydrophobic interactions between CHI and cellulosic polymers (carbohydrate-carbohydrate system), favored by increasing the temperature, promoting the extension of the gelation temperature of these polymers. The cooling curves show the reduction in gel temperature for all samples and the thermo-reversibility characteristic of the hydrogels.

3.2.Fourier transform infrared spectroscopy (FTIR)

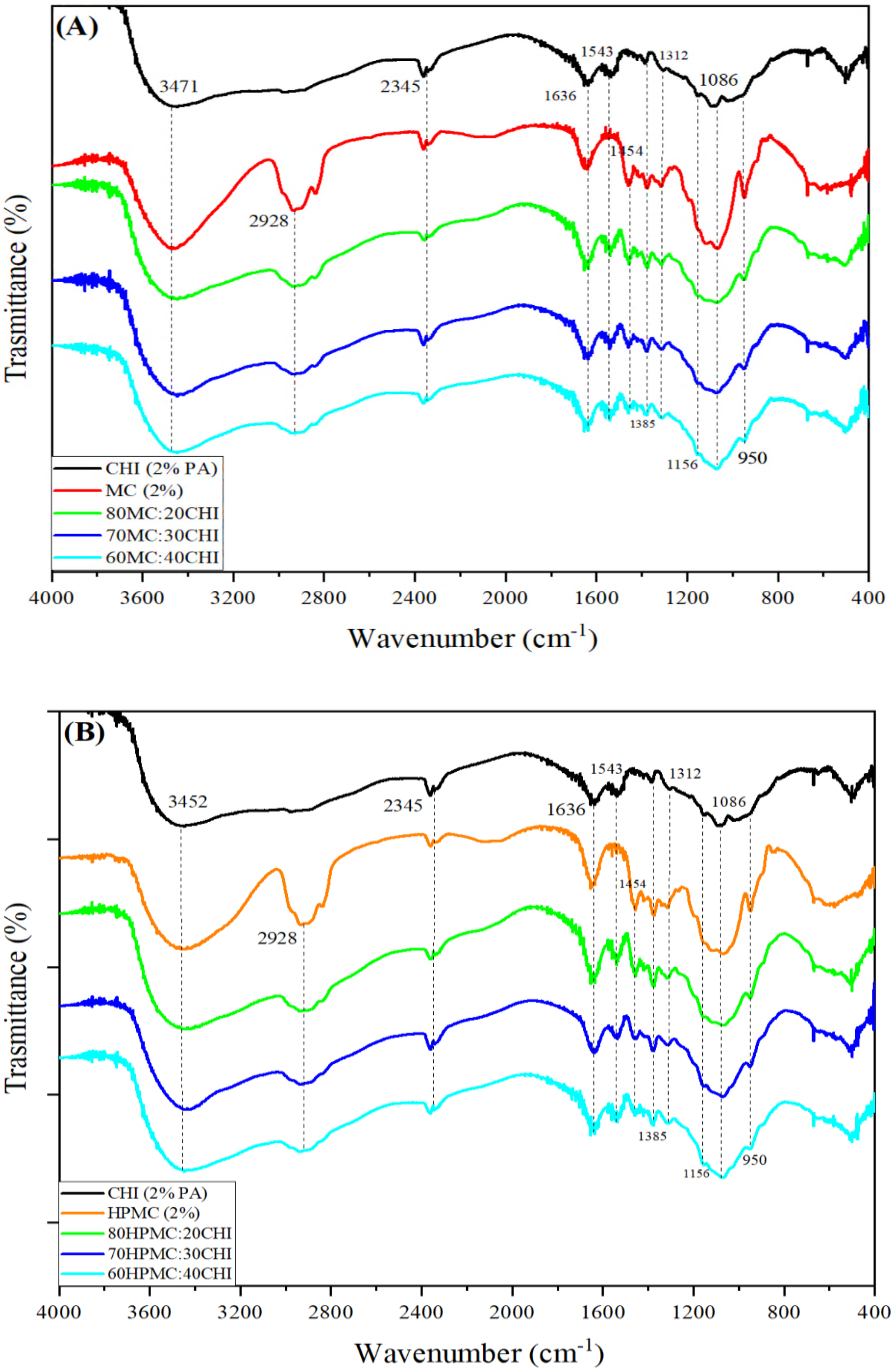
Each functional grouping absorbs energy at a specific wavelength exhibiting a unique spectrum like a fingerprint of a molecule in the infrared region [21, 22]. Figure 6 (A and B) shows the FTIR spectroscopy analysis to characterize specific chemical groups in the individual polysaccharides and hydrogels, after the gelling process and drying in a freeze dryer.

For the structural analysis of carbohydrates, five spectral regions are specified in the 4000-400 cm^{-1} regions: (a) region of OH and CH stretching vibrations at 3600-2800 cm^{-1}; (b) region of local symmetry at 1500-1200 cm^{-1}; (c) carbohydrate region or C–0 stretching region at 1200-950 cm^{-1}; (d) anomeric region or fingerprint at 950-700 cm^{-1}; and (e) skeletal below 700 cm^{-1} [22-24]. Figure 6 shows peaks around 1156-890 cm^{-1} corresponding to the saccharide structure of CHI, HPMC, MC, and their combinations.

CHI spectrum (Fig 6A and B) exhibited characteristic peaks at 1636 cm^{-1} (C=O stretching) and 1543 cm^{-1} (N–H bending), referring to amide I and amide II, respectively. The peak at 1312 cm^{-1}, typical of CHI, indicates the amide III groups. Other peaks at 950, 1086, and 1156 cm^{-1} reveal the C–O stretching vibration in chitosan [25, 26]. A peek at

3432 cm^{-1} regarding OH–stretching (data not shown) was identified, overlapping NH–stretching in the same region [27].

Figure 6. Infrared spectra of (a) MC: CHI and (b) HPMC: CHI hydrogels. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose and CHI: chitosan.



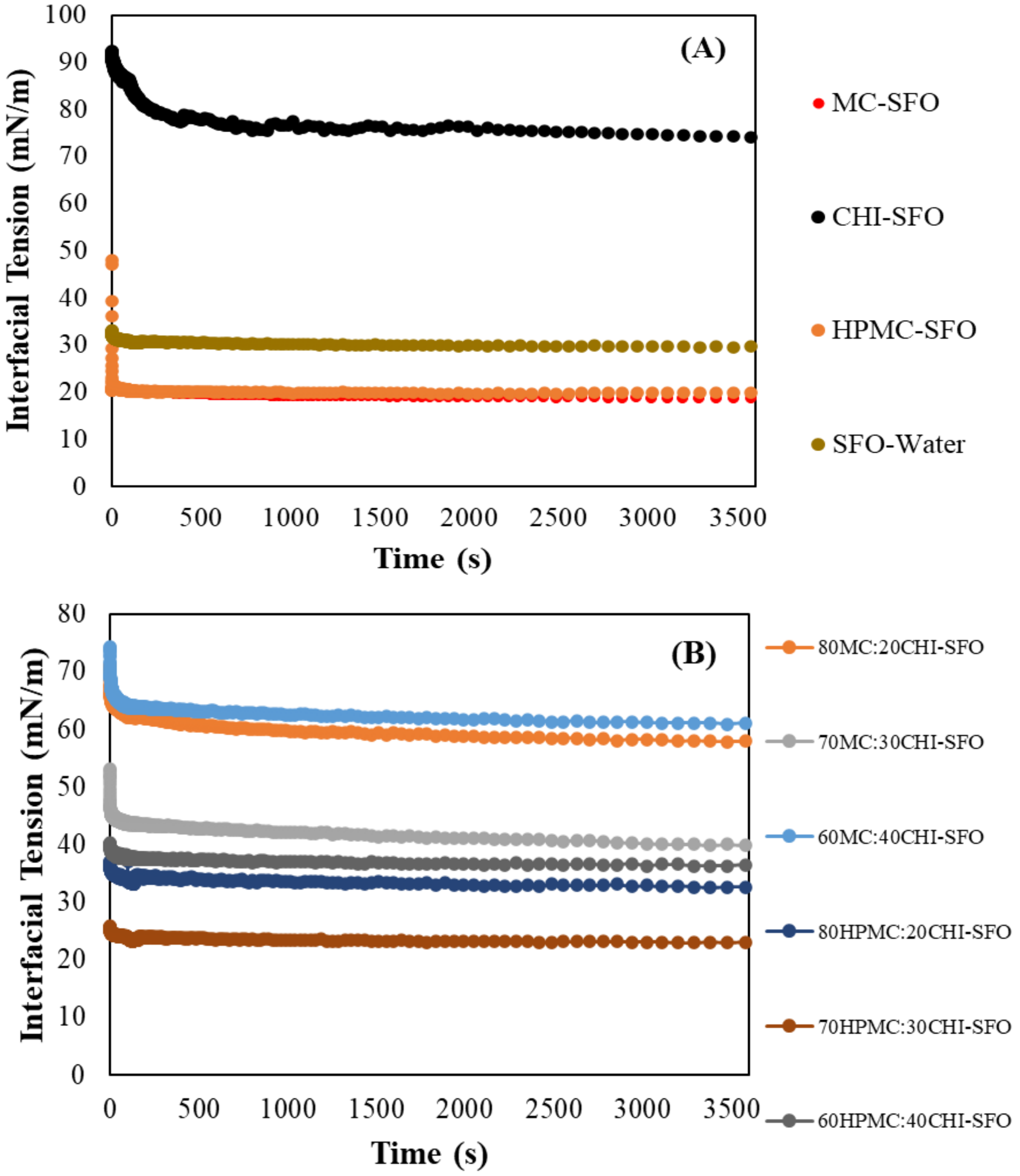
The spectra obtained were similar for cellulosic polymers, MC (Fig. 6A), and HPMC (Fig. 6B). The spectrum of pure MC and HPMC shows the presence of the bands at 2928, 2345, 1636, 1454, 1385,1 312, 1156, 1086, and 950 cm^{-1}. Both had O–H stretching at 3439 cm^{-1} (data not shown), and 2928 cm^{-1} corresponds to C–H stretching. A peak at 1636 cm^{-1} was related to water in the amorphous region [27]. The region between 1400 and 900 cm^{-1} represents the fingerprint of these polymers. Thereby, the peak 1454 cm^{-1} indicates methyl groups (C-H bending), 1385 and 1312 cm^{-1} indicate –OH vibrations [28-29]. A weak peak at 1156 cm^{-1} was assigned to the C–O–C bridge (anti-symmetric stretching) [27]. The most pronounced peak in the HPMC and MC spectra was at 1056 cm^{-1}. According to Akinosho, Hawkins & Wicker [28], this peak represents the out-of-phase vibrations of an alkyl-substituted cyclic ring containing ester bonds. Furthermore, the peak 950 cm^{-1} matches the in-phase vibrations from ether bonds.

After the mixture of MC: CHI and HPMC: CHI in different proportions, all the characteristic bands of pure cellulosic polymer are in FTIR spectrums. The peak at 1543 cm^{-1} (amino-group band) is typical of pure chitosan. It is absent in the spectra of pure HPMC and MC. After mixing CHI to HPMC or MC, it was possible to identify the presence of this peak (1543 cm^{-1}) in these hydrogels in all the mixtures, 80:20, 70:30, and 60:40. This peak in HPMC: CHI and MC: CHI hydrogels shows some interactions between CHI and the cellulosic polymers under study. Thus, it can be inferred that this interaction between CHI and HPMC and MC can stabilize hydrogel systems due to the exposure of their functional groups during heating (Item 3.1.3) since there was some miscibility [27] between these components. Yin et al. [27] studied the miscibility of mixtures of CHI with MC and HPMC to prepare films on poly(tetrafluoroethylene) surfaces. They used the following ratios between CHI: MC or MC, 100:0, 80:20, 50:50, 20:80, and 0:100. The authors identified the same peak at 1543 cm^{-1} and attributed it to the interaction between CHI and cellulosic polymers.

3.3.Interfacial tension

Figure 7 shows the interfacial tension of the polysaccharides dispersions, CHI: HPMC, and CHI: MC mixtures. The interfacial tension between oil (SFO) and water phases with the addition of CHI, MC, HPMC, and their mixtures in different proportions was evaluated to investigate if these polymers show surface activity.

Figure 7. Interfacial tension of (A) pure polymers, (B) MC: CHI, and HPMC: CHI hydrogels at room temperature. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose and CHI: chitosan.



The interfacial tension of the SFO-pure water system has an initial point of 35.26±0.25 mN/m and an equilibrium point of 30.29±0.26 mN/m (Fig. 7A). As shown in (Fig. 7A), the cellulosic polymers HPMC and MC showed the lowest initial interfacial tension values of 47.94 mN/m and 24.99 mN/m. The equilibrium point was 20.34 mN/m for HPMC and 18.80 mN/m for MC.

CHI presented an interfacial tension of 91.46 mN/M, with a slow dynamic, and this property changes its value over time until reaching equilibrium at 75.56 mN/m. CHI, a cationic polymer, is not generally considered a polysaccharide with surface properties [30, 31], as electrolytes with insufficient hydrophobic character have a low capacity to position at the interface (oil and water) and reduce interfacial tension [30]. However, Payet & Terentjev [32] studied the interfacial tension of different solutions of CHI (0.1, 0.5, 1.0 and, 2.0 w/w%) and paraffin. The authors concluded that CHI has no surface activity at concentrations below 0.1% by weight (w/w). The interface tension decreases slowly at higher concentrations, and the overall decrease is small when compared to traditional amphiphilic molecules. Such conclusions are in agreement with the present work, which used a 0.2% CHI solution.

In Figure 7B, the 70HPMC:30CHI system reduced the interfacial tension by 21.84% (25.73 mN/m) and reached equilibrium before 1000s. The other samples showed high surface tension values, and the surface activity in increasing order is as follows: 60MC: 40CHI, 80MC: 20CHI, 70MC: 30CHI, 60HPMC: 40CHI, and 80HPMC: 20CHI. Therefore, systems prepared with HPMC: CHI are more active than systems with MC: CHI regarding the surface activity. This behavior is associated with the presence of hydroxypropyl groups that confer a hydrophobic character and increase surface activity compared to samples with MC [12, 33].

3.4. Thermal events and glass transition temperature (T_g) by DSC

Table 3 shows the melting temperatures obtained from the heating thermogram (Figure 8 A and B), the enthalpies corresponding to these endothermic peaks, and the glass transition temperature for MC, HPMC, pure CHI, MC: CHI, and HPMC: CHI hydrogels. This analysis was performed to determine how chitosan influences the thermal events of these cellulosic polymers.

Table 3. Thermal analysis for MC: CHI, HPMC: CHI thermoreversible hydrogels and pure polymers.

Samples	Endothermic peak I		Endothermic peak II		Glass Transition (T_g) (°C)
	T_{peak} (°C)	ΔH (J/g)	T_{peak} (°C)	ΔH (J/g)	
Polymer (2%)					
MC	97.61	[a]	-	-	-
HPMC	98.04	[a]	-	-	117.64
CHI	107.76	[a]	173.55	-11.94	-
Hydrogels – MC and CHI					
80MC:20CHI	102.44	[a]	182.01	-5.59	117.50
70MC:30CHI	104.96	[a]	178.67	-7.23	118.07
60MC:40CHI	107.90	[a]	176.04	-5.69	-
Hydrogels – HPMC and CHI					
80HPMC:20CHI	104.30	[a]	185.25	-2.94	118.01
70HPMC:30CHI	102.80	[a]	180.55	-9.78	117.94
60HPMC:40CHI	103.87	[a]	178.20	-7.33	117.72

[a] This parameter was impossible to be determined. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; CHI: chitosan; T_{peak}: peak melting temperature; ΔH: melting enthalpy.

For pure cellulosic polymers, thermograms were similar, and only one endothermic event was identified with T_{peak} at 98.04 °C for HPMC (Figure 8A) 97.61 °C for MC (Figure 8B). The presence of this peak is related to the evaporation of water still present [34]. This peak is seen in the thermogram of pure CHI at 107.76 °C. The second endothermic peak of CHI, T_{peak} at 173.55 °C, is related to polymer decomposition. For pure MC and HPMC, the second endothermic peak was not identified due to the amorphous state of these polymers [35].

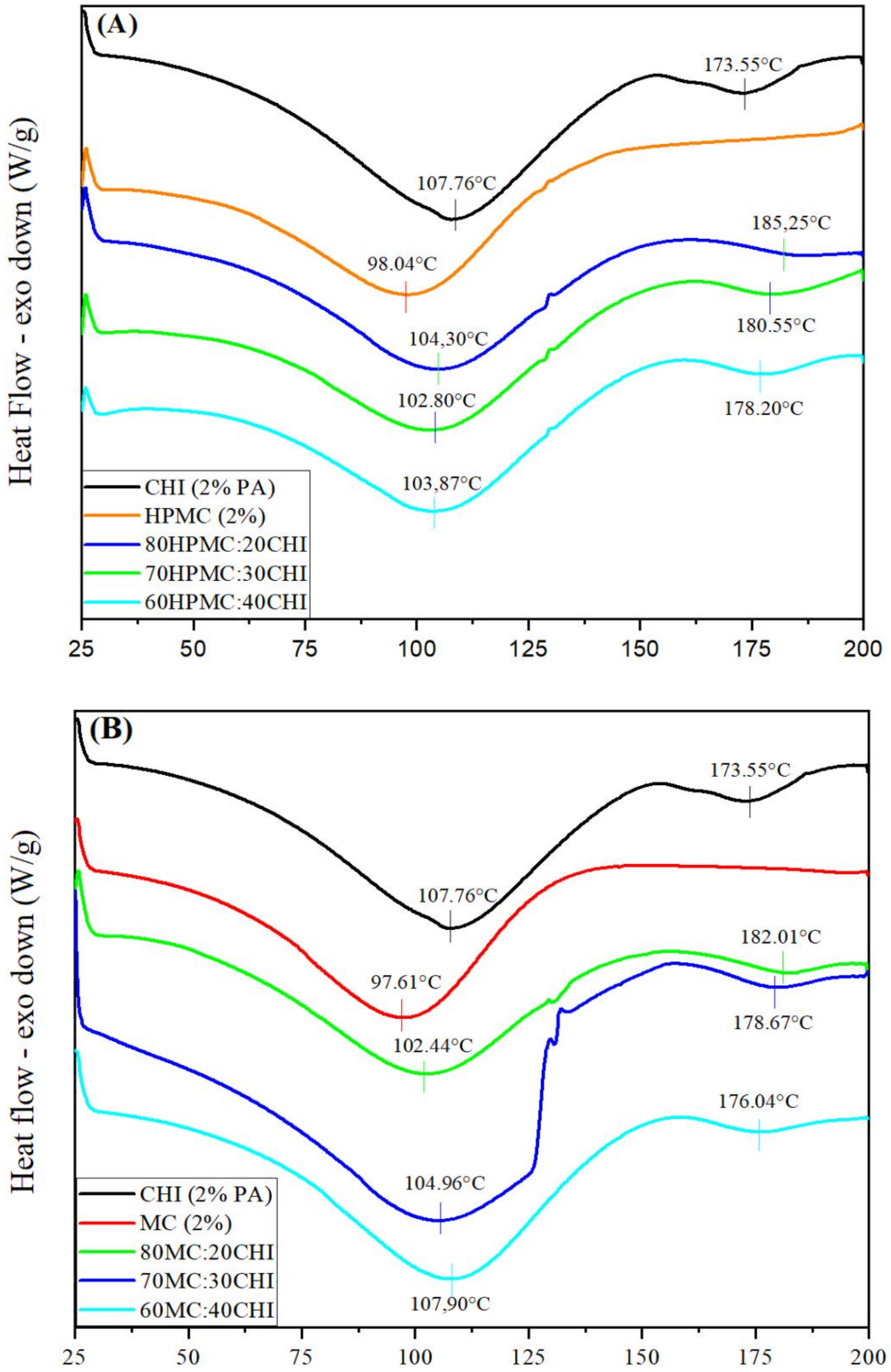
The mixed samples of MC: CHI and HPMC: CHI exhibited a broad endothermic peak ranging from 102.44 to 107.90 °C and the second thermal event of these mixtures with T_{peak} between 176.04 to 185.25 °C, referring to the evaporation of phosphoric acid and water still present during the preparation of hydrogels and sample degradation. However, samples with higher proportions of CHI had a reduction in T_{peak} in the second thermal event.

As samples were lyophilized before analysis, knowing that both polymers present hygroscopicity, it is plausible to say that they adsorbed water during storage until their analysis in DSC. The observations made above support the idea that water is present in different states. The water in a hydrogel is present in free form, freezing bound water, and bound water. Free water behaves like pure water and does not make a hydrogen bond with the polysaccharide. Freezing water forms weak bonds with the polymer. While bound water is strongly bound to the substrate, it is difficult to be disposed of and not frozen, so it is not accounted for in DSC [35]. Therefore, the water adsorbed by the MC: CHI and HPMC: CHI samples affects their thermal properties and their glass transition temperature (T_g).

The T_g determination is a necessary study parameter for polymeric materials. At values below T_g, the molecular mobility is low, and the material is in its glassy state. However, at a temperature above T_g, we increase the mobility of molecules (rubbery state), and with that, we increase the water mass transfer rates [36].

Among the pure polymers, only HPMC presented T_g at 117.64°C in the evaluated temperature range. Dharmalingam & Anandalakshmi [37] find a TG of ~146°C for pure HPMC (K15M). Siepmann & Peppas [36] report that T_g can vary according to the viscosity of the polymer. HPMC and MC blends, a slight shift in the T_g value was found with an increase in the concentration of CHI at 118.01, 117.94, and 117.72°C for 80HPMC: 20CHI, 70HPMC: 30 CHI and 60HPMC:40 CHI, respectively.

Figure 8. DSC melting curves for (A) HPMC: CHI and (B) MC: CHI hydrogels. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose and CHI: chitosan.



For

MC: CHI, the following T_g was obtained 117.50 and 118.07 °C for 80MC: 20CHI and 70MC: 30CHI, respectively. The 60MC: 40CHI sample in the temperature scan studied did not show T_g. However, the T_g peaks for both MC, pure CHI, and 60 MC: 40CHI can be masked by peaks of water evaporation and material degradation and therefore have not been identified [35].

However, based on these data and the small displacement of the T_g peak, it is possible to say that the samples showed stability in the studied temperature range.

4. Conclusions

The individual polymers, HPMC and MC, showed a LCST of 66.9 and 43.6 °C, respectively. The addition of CHI resulted in a reduction of the LCST temperatures of all the studied formulations. The minimum temperature reached was 43.6 °C for 70HPMC: 30CHI and 39.3 °C for 70MC: 30CHI. Thus, it is possible to conclude that the combination of CHI with HPMC and MC can improve the gelling properties of these cellulosic polymers and reduce the gel point of these combined systems. These hydrogels form hydrophobic interactions between carbohydrate-carbohydrate and reduce the environment's interfacial tension, showing low LCST values. This property gives this system the ability to trap heat-sensitive nonpolar components (vegetable oils) in its polymeric structure.

5. Acknowledgments

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6. References

[1] A. Sannino, C. Demitri, M. Madaghiele, Biodegradable cellulose-based hydrogels: Design and applications, Materials (Basel). 2 (2009) 353–373. https://doi.org/10.3390/ma2020353.

[2] H.F. Darge, A.T. Andrgie, H.C. Tsai, J.Y. Lai, Polysaccharide and polypeptide based injectable thermo-sensitive hydrogels for local biomedical applications, Int. J. Biol. Macromol. 133 (2019) 545–563. https://doi.org/10.1016/j.ijbiomac.2019.04.131.

[3] D. Goff, Q. Guo, Food Structure Development: The Interplay Between Processing Routes and Formulation Elements, Part A Food Struct. Dev. Interplay Between Process. Routes Formul. Elem. (2019) 1–28.

[4] H.N. Lee, T.P. Lodge, Lower critical solution temperature (LCST) phase behavior of poly(ethylene oxide) in ionic liquids, J. Phys. Chem. Lett. 1 (2010) 1962–1966. https://doi.org/10.1021/jz100617t.

[5] K. Rehman, M.H. Zulfakar, Recent advances in gel technologies for topical and transdermal drug delivery, Drug Dev. Ind. Pharm. 40 (2014) 433–440. https://doi.org/10.3109/03639045.2013.828219.

[6] M. Kong, X. Chen, The Novel Medical Thermoresponsive Hydrogel Derived from Chitosan, Curr. Org. Chem. 22 (2018) 620–627. https://doi.org/10.2174/1385272821666170811111651.

[7] Y. Luo, Q. Wang, Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery, Int. J. Biol. Macromol. 64 (2014) 353–367. https://doi.org/10.1016/j.ijbiomac.2013.12.017.

[8] M.M. Islam, M. Shahruzzaman, S. Biswas, M. Nurus Sakib, T.U. Rashid, Chitosan based bioactive materials in tissue engineering applications-A review, Bioact. Mater. 5 (2020) 164–183. https://doi.org/10.1016/j.bioactmat.2020.01.012.

[9] R.S. Rabelo, I.F. Oliveira, V.M. da Silva, A.S. Prata, M.D. Hubinger, Chitosan coated nanostructured lipid carriers (NLCs) for loading Vitamin D: A physical stability study, Int. J. Biol. Macromol. 119 (2018) 902–912. https://doi.org/10.1016/j.ijbiomac.2018.07.174.

[10] A. Chenite, C. Chaput, D. Wang, C. Combes, M.D. Buschmann, C.D. Hoemann, J.C. Leroux, B.L. Atkinson, F. Binette, A. Selmani, Novel injectable neutral solutions of chitosan form biodegradable gels in situ, Biomaterials. 21 (2000) 2155–2161. https://doi.org/10.1016/S0142-9612(00)00116-2.

[11] T. Wang, L. Chen, T. Shen, D. Wu, Preparation and properties of a novel thermo-sensitive hydrogel based on chitosan/hydroxypropyl methylcellulose/glycerol, Int. J. Biol. Macromol. 93 (2016) 775–782. https://doi.org/10.1016/j.ijbiomac.2016.09.038.

[12] A.R. Patel, K. Dewettinck, Comparative evaluation of structured oil systems: Shellac oleogel, HPMC oleogel, and HIPE gel, Eur. J. Lipid Sci. Technol. 117 (2015) 1772–1781. https://doi.org/10.1002/ejlt.201400553.

[13] S. Hussain, C. Keary, D.Q.M. Craig, A thermorheological investigation into the gelation and phase separation of hydroxypropyl methylcellulose aqueous systems, Polymer (Guildf). 43 (2002) 5623–5628. https://doi.org/10.1016/S0032-3861(02)00430-5.

[14] M.S. Lindblad, A.C. Albertsson, E. Ranucci, M. Laus, E. Giani, Biodegradable polymers from renewable sources: Rheological characterization of hemicellulose-based hydrogels, Biomacromolecules. 6 (2005) 684–690. https://doi.org/10.1021/bm049515z.

[15] J.P.A. Fairclough, H. Yu, O. Kelly, A.J. Ryan, R.L. Sammler, M. Radler, Interplay between Gelation and Phase Separation in Aqueous Solutions of Methylcellulose and Hydroxypropylmethylcellulose, Langmuir. 28 (2012) 10551–10557. https://doi.org/10.1021/la300971r.

[16] I. Tavernier, C.D. Doan, D. Van De Walle, S. Danthine, T. Rimaux, K. Dewettinck, Sequential crystallization of high and low melting waxes to improve oil structuring in wax-based oleogels, RSC Adv. 7 (2017) 12113–12125. https://doi.org/10.1039/c6ra27650d.

[17] N. Sarkar, Thermal gelation properties of methyl and hydroxypropyl methylcellulose, J. Appl. Polym. Sci. 24 (1979) 1073–1087. https://doi.org/10.1002/app.1979.070240420.

[18] Y. Wang, L. Yu, F. Xie, S. Li, Q. Sun, H. Liu, L. Chen, On the investigation of thermal/cooling-gel biphasic systems based on hydroxypropyl methylcellulose and hydroxypropyl starch, Ind. Crops Prod. 124 (2018) 418–428. https://doi.org/10.1016/j.indcrop.2018.08.010.

[19] N. Bhattarai, J. Gunn, M. Zhang, Chitosan-based hydrogels for controlled, localized drug delivery, Adv. Drug Deliv. Rev. 62 (2010) 83–99. https://doi.org/10.1016/j.addr.2009.07.019.

[20] T. Irimia, C.E. Dinu-Pîrvu, M.V. Ghica, D. Lupuleasa, D.L. Muntean, D.I. Udeanu, L. Popa, Chitosan-based in situ gels for ocular delivery of therapeutics: A state-of-the-art review, Mar. Drugs. 16 (2018). https://doi.org/10.3390/md16100373.

[21] B.M. Prado, S. Kim, B.F. Özen, L.J. Mauer, Differentiation of carbohydrate gums and mixtures using fourier transform infrared spectroscopy and chemometrics, J. Agric. Food Chem. 53 (2005) 2823–2829. https://doi.org/10.1021/jf0485537.

[22] M. Kačuráková, R.H. Wilson, Developments in mid-infrared FT-IR spectroscopy of selected carbohydrates, Carbohydr. Polym. 44 (2001) 291–303. https://doi.org/10.1016/S0144-8617(00)00245-9.

[23] M. Mathlouthi, J.L. Koenig, Vibrational spectra of carbohydrates, Adv. Carbohydr. Chem. Biochem. 44 (1987) 7–89. https://doi.org/10.1016/S0065-2318(08)60077-3.

[24] E. Gómez-Ordóñez, P. Rupérez, FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds, Food Hydrocoll. 25 (2011) 1514–1520. https://doi.org/10.1016/j.foodhyd.2011.02.009.

[25] H.S. Mansur, E. de S. Costa Jr., A.A.P. Mansur, E.F. Barbosa-Stancioli, Cytocompatibility evaluation in cell-culture systems of chemically crosslinked chitosan/PVA hydrogels, Mater. Sci. Eng. C. 29 (2009) 1574–1583. https://doi.org/10.1016/j.msec.2008.12.012.

[26] R.S. Rabelo, G.M. Tavares, A.S. Prata, M.D. Hubinger, Complexation of chitosan with gum Arabic, sodium alginate and κ-carrageenan: Effects of pH, polymer ratio and salt concentration, Carbohydr. Polym. 223 (2019) 115120. https://doi.org/10.1016/j.carbpol.2019.115120.

[27] J. Yin, K. Luo, X. Chen, V. V. Khutoryanskiy, Miscibility studies of the blends of chitosan with some cellulose ethers, Carbohydr. Polym. 63 (2006) 238–244. https://doi.org/10.1016/j.carbpol.2005.08.041.

[28] H. Akinosho, S. Hawkins, L. Wicker, Hydroxypropyl methylcellulose substituent analysis and rheological properties, Carbohydr. Polym. 98 (2013) 276–281. https://doi.org/10.1016/j.carbpol.2013.05.081.

[29] M. Akhlaq, F. Maryam, A. Elaissari, H. Ullah, M. Adeel, A. Hussain, M. Ramzan, O. Ullah, M.Z. Danish, S. Iftikhar, N. Aziz, Pharmacokinetic evaluation of quetiapine fumarate controlled release hybrid hydrogel: A healthier treatment of schizophrenia, Drug Deliv. 25 (2018) 916–927. https://doi.org/10.1080/10717544.2018.1458922.

[30] N. Calero, J. Muñoz, P. Ramírez, A. Guerrero, Flow behaviour, linear viscoelasticity and surface properties of chitosan aqueous solutions, Food Hydrocoll. 24 (2010) 659–666. https://doi.org/10.1016/j.foodhyd.2010.03.009.

[31] J. Nilsen-Nygaard, S.P. Strand, K.M. Vårum, K.I. Draget, C.T. Nordgård, Chitosan: Gels and interfacial properties, Polymers (Basel). 7 (2015) 552–579. https://doi.org/10.3390/polym7030552.

[32] L. Payet, E.M. Terentjev, Emulsification and stabilization mechanisms of O/W emulsions in the presence of chitosan, Langmuir. 24 (2008) 12247–12252. https://doi.org/10.1021/la8019217.

[33] D. Huc-Mathis, J. Bousquières, M. Mamou, C. Bonazzi, C. Michon, Multiscale evaluation from one bubble to the foam of surface active properties of cellulose derivatives used for a starchy model sponge cake, Food Hydrocoll. 71 (2017) 129–140. https://doi.org/10.1016/j.foodhyd.2017.03.038.

[34] E. Pinho, S. Machado, G. Soares, Smart Hydrogel for the pH-Selective Drug Delivery of Antimicrobial Compounds, Macromol. Symp. 385 (2019) 1–7. https://doi.org/10.1002/masy.201800182.

[35] S.C. Barros, A.A. da Silva, D.B. Costa, C.M. Costa, S. Lanceros-Méndez, M.N.T. Maciavello, J.L.G. Ribelles, F. Sentanin, A. Pawlicka, M.M. Silva, Thermal–mechanical behaviour of chitosan–cellulose derivative thermoreversible hydrogel films, Cellulose. 22 (2015) 1911–1929. https://doi.org/10.1007/s10570-015-0603-5.

[36] J. Siepmann, N.A. Peppas, Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC), Adv. Drug Deliv. Rev. 64 (2012) 163–174. https://doi.org/10.1016/j.addr.2012.09.028.

[37] K. Dharmalingam, R. Anandalakshmi, Fabrication, characterization and drug loading efficiency of citric acid crosslinked NaCMC-HPMC hydrogel films for wound healing drug delivery applications, Int. J. Biol. Macromol. 134 (2019) 815–829. https://doi.org/10.1016/j.ijbiomac.2019.05.027.

[38] A. Merivaara, J. Zini, E. Koivunotko, S. Valkonen, O. Korhonen, F.M. Fernandes, M. Yliperttula, Preservation of biomaterials and cells by freeze-drying: Change of paradigm, J. Control. Release. 336 (2021) 480–498. https://doi.org/10.1016/j.jconrel.2021.06.042.

[39] S.C. Barros, M.M. Silva, Seeking the lowest phase transition temperature in a cellulosic system for textile applications, Cellulose. 25 (2018) 3163–3178. https://doi.org/10.1007/s10570-018-1763-x.

[40] S.M.C. Silva, F. V. Pinto, F.E. Antunes, M.G. Miguel, J.J.S. Sousa, A.A.C.C. Pais, Aggregation and gelation in hydroxypropylmethyl cellulose aqueous solutions, J. Colloid Interface Sci. 327 (2008) 333–340. https://doi.org/10.1016/j.jcis.2008.08.056.

[41] M. Hirrien, C. Chevillard, J. Desbrières, M. A. V. Axelos, M. Rinaudo, Thermogelation of methylcelluloses: new evidence for understanding the gelation mechanism, Polymer (Guildf). 39 (1998) 6251–625.

CHAPTER IV

Oil-in-water emulsion gels stabilized with cellulosic polymers and chitosan: themorheological and physical-chemical evaluation

OIL-IN-WATER EMULSION GELS STABILIZED WITH CELLULOSIC POLYMERS AND CHITOSAN: THEMORHEOLOGICAL AND PHYSICAL-CHEMICAL EVALUATION

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Oil-in-water emulsion gels stabilized with cellulosic polymers and chitosan: Themorheological and physical-chemical evaluation

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ABSTRACT

This research evaluated the use of different polymer ratios, hydroxypropyl methylcellulose (HPMC) and methylcellulose (MC) with chitosan (CHI), in the production of emulgel by emulsification. The concentration was fixed at 2% (w/v) for all polymers. 60/40 sunflower oil-in-water emulgels were made with a cellulosic polymer: chitosan ratio of (80:20), (70:30), and (60:40), respectively. The objective was to study how different proportions of a cellulosic polymer combined with chitosan can affect the stability, microstructure, and rheology of the emulgels to be potential oil carrier systems. Droplet size and microscopy results show oil-in-water (O/W) emulgels, and their interface was stabilized by mixing polymeric pairs, HPMC: CHI or MC: CHI. In the thermal analysis, it was identified in the entire temperature range studied (5 to 85ºC) that both emulgels, HPMC: CHI and MC: CHI, were presented as gels (G'>G"). Thus, the addition of CHI to the systems modified their gelling behavior. Microscopy revealed that the emulsions at the 7th and 10th week of storage showed similar characteristics to the fresh emulsion. Therefore, these results indicate that the emulgels present good thermal resistance, the predominance of elastic behavior, and can retain high concentrations of oil in their structure (96 to 99%).

Keywords: Emulgels, cellulosic polymers, sol-gel transition, rheology, mechanical properties.

1. Introduction

Hydroxypropylmethylcellulose (HPMC) and methylcellulose (MC) are cellulosic polymers, synthetically prepared by replacing hydroxyl groups in the cellulose structure with methoxy and hydroxypropyl groups. This substitution gives the molecules a hydrophobic character, which presents an amphiphilic behavior and surface-active nature [1], [2]. Due to these characteristics, HPMC and MC can provide foam and emulsion stabilizing properties to pharmaceutical and food products, among others [1], [2]. Furthermore, HPMC and MC have thermal gelling properties [3], [4]; which is an intrinsic characteristic of non-ionic cellulose ethers [5].

Studies that report the use of hydrophilic polymers to obtain structured systems are still limited and need further research. Therefore, using HPMC and MC as structuring agents is a promising approach to obtaining structured systems [6], [7].

Among the strategies used for structuring vegetable oils, emulgels have stood out since they can be obtained by indirect emulsification. These emulsions are produced by gel-forming polysaccharides that stabilize these systems by steric hindrance. When these gelled emulsions have the mechanical properties of a solid, they are defined as emulgels, emulsion gels, or gelled emulsions [8], [9] and can be applied to replace saturated and trans-fat in foods.

Another polysaccharide that can be used to obtain unique gels or in combination with other hydrophilic polymers, such as HPMC and MC, is chitosan (CHI). Chitosan is a polysaccharide commercially obtained from the deacetylation of chitin [10], [11]. After this process, CHI can dissolve in an acidic environment and becomes the only polysaccharide with a high density of positive charges [12]. CHI's ability to interact with vegetable oils and fats is related to its amphiphilicity. The molecular structure of chitosan is composed of amino groups (hydrophilic) and acetyl groups, which are hydrophobic; this molecular characteristic can influence its properties in solution and solid state [13].

The use of HPMC and MC as structuring agents has already been studied to obtain oleogels using the emulsion and foam model [14], [15], [16], [17], [18], [19], [20]. However, so far, no work has been found in the scientific literature that uses structured systems, emulgels stabilized by mixtures of HPMC or MC with the cationic polymer CHI. The combination of these polysaccharides can be used to obtain structured oils (solid character) without changing the fatty acid composition of the oil to be modified in substitution for saturated fat in food matrices. These physical transformations lead to gelled systems that can be applied over a wide temperature range, enabling the maintenance of the oxidative quality of unsaturated fats.

In this context, the objective of this work was to evaluate different proportions of HPMC: CHI and MC: CHI and their effects as structuring agents in the formation of gelled systems and the mechanical strength of sunflower oil-based emulgels. The micro and macrostructure, mean droplet diameter and size distribution, and rheological behavior were studied to apply these systems as potential substitutes for saturated and trans-fat in foods or other applications.

2. Material and Methods

2.1.Material

The raw materials used as a structuring agent (gelators) of the lipid system were chitosan (CHI, CAS 9012-76-4), acquired from Sigma-Aldrich (São Paulo - Brazil), hydroxypropyl methylcellulose (HPMC, CAS 9004-65-3), and methylcellulose (MC, CAS 9004-67-5) donated by Ashland Chemical Specialty Trade in Brazil LTDA (São

Paulo - Brazil). The selected sunflower oil (SO) was purchased at a local market. All the experiments were done with Milli-Q water (resistivity 18.2 mΩ.cm at 25°C).

2.2.Emulgel preparation

The HPMC and MC dispersions were prepared in Milli-Q water, except CHI, dispersed in phosphoric acid solution (1% v/v). All polymeric solutions concentration was fixed at 2% (w/v) for all formulations. After preparation, HPMC, MC, and CHI dispersions were mechanically stirred for 12h (300 rpm) at room temperature to ensure complete hydration of the polymers. Emulgels were obtained from 150g of oil-in-water emulsions (60/40) stabilized with mixtures of cellulosic polymers (HPMC or MC) and CHI in proportions 80:20, 70:30 and 60:40. An Ultraturrax® (IKA®-Werke GmbH & Co. KG, Germany) at 13.000 rpm for 8 min was used to mix and homogenize the system. The process was carried out in duplicate.

2.3.Emulgels characterization

2.3.1. Microstructure

The microstructures of the emulgels were performed by optical and fluorescence microscopy using a Carl Zeiss optical microscope (Axio Scope A1, Lower Saxony, Germany). For fluorescence analysis, the emulgels were stained with a drop of Nile red (0.1 g/L in polyethylene glycol) and a drop of FITC (0.2 g/mL in ethanol) (Sigma Aldrich, Wicklow, Ireland). Sunflower oil was dyed with Nile red (red), and the polymers were dyed with FITC (green). The micrographs were observed in 10x (optical microscope) and 40x (fluorescence microscope) amplitude and were evaluated with the software AxioVision Rel. 4.8 (Carl Zeiss, Germany).

2.3.2. Size of emulgel drops

The size distribution was performed by laser diffraction using the Mastersizer S equipment (Malvern Instruments, Malvern, UK). The average diameter was considered based on the average diameter of a sphere of the same area ($D_{[3,2]}$) and the surface area/volume ratio, using $D_{[4,3]}$ (Eq. (1) and (2), respectively). The Span value (polydispersity index), which gives the size distribution's width, was calculated from Eq. (3). The larger peak's Mode was determined based on the size distribution.

$$D_{32} = \frac{\sum_{i=1}^{n} n.d_i^3}{\sum_{i=1}^{n} n.d_i^2} \qquad (1)$$

$$D_{43} = \frac{\sum_{i=1}^{n} n.d_i^4}{\sum_{i=1}^{n} n.d_i^3} \qquad (2)$$

$$Span = \frac{d(0{,}9)-d(0{,}1)}{d(0{,}5)} \qquad (3)$$

Where d_i is the diameter of the droplet and, n is the number of droplets. The size distribution will be given through the parameters: d (0.1), d (0.5), and d (0.9), which represent the diameter of the accumulated distribution of 10%, 50%, and 90% of the accumulated volume. Each sample was analyzed in triplicate.

2.3.3. Rheological measurements

Rheological measurements for emulgels were performed with the Physica MCR301 controlled tension rheometer (Anton Paar, Graz, Austria) equipped with a Peltier system and a water bath (Julabo, Seelbach, Germany) for temperature control. The geometry used was that of a rough 50 mm diameter stainless steel flat plate geometry (PP50/S), and the GAP used was 0.3 mm, adopted based on preliminary results. The samples of the emulgels were introduced into the rheometer with the aid of a spatula. These samples were left to stand for 5 minutes before measurements. All tests were performed duplicated for each process, and a new sample was used for each test.

2.3.3.1.Steady shear

Steady-shear flow curves of the emulgels were obtained along three shear rate ramps performed in the following order: increasing-decreasing-increasing in a range of 0 and 300 s^{-1}. The first and second curves were made to eliminate possible thixotropic effects. The third curve was used to assess rheological behavior at a steady state. The results obtained from the third curve were adjusted according to the power-law model (Eq. (4)).

$$\sigma = k\dot{\gamma}^n \quad (4)$$

Where σ (Pa) is the shear stress, k (Pa.s^n) is the consistency index, $\dot{\gamma}$(s^{-1}) is the shear rate, *n* is the flow behavior index (dimensionless).

2.3.4. Oscillatory assays

The oscillatory measurements were performed using a strain value within the linear viscoelastic region (0.1%). This value was previously determined by a strain scan performed from 0.01 to 100% at a frequency of 1.0 Hz. Then, frequency sweeps were performed between 0.1 Hz and 10 Hz at constant temperature (25 °C) to obtain the mechanical spectra. The temperature sweep was obtained at a rate of 5° C/min. Firstly, the equipment was preheated to 90°C with equilibrium for 1 min. Secondly, the samples were cooled from 90 to 5 °C, and then left isothermally for 14 min, and heated again to 90 °C. The elastic component (G') and the viscous component (G") were determined.

2.3.5. Oil binding capacity

After its production, about 1g of emulgels was weighed in Eppendorfs and subjected to oil loss determination. This analysis was made by mass difference (Eq. (5))

due to the samples' centrifugation at 10,000 rpm for 30 minutes (Adapted from [19]). Each analysis was conducted in triplicate.

$$LO = \frac{m_1 - m_2}{m_1 - m} \, x \, 100 \quad (5)$$

Where m_1 is the mass of initial sample and the Eppendorf, m_2 is the mass of Eppendorf with sample after centrifugation and m is the mass of the Eppendorf.

2.3.6. Stability study of emulgels

After its production, about 1g of emulgels were placed in Eppendorf tubes for monitoring its stability for ten weeks. These samples were stored in BOD incubators (Tecnal brand, model TE-391, Piracicaba, São Paulo Brazil) at 25 ºC and 40 °C. The emulgels were subjected to microstructure, drop size, and oil loss analyzes, performed after 0, 2, 4, 7, and 10 weeks of storage. The analyzes were performed in triplicate.

2.3.7. Statistical analysis

The results were subjected to analysis of variance (ANOVA) and Tukey's test with a significance level of 5%, using Minitab16.1.0 (Minitab Inc., State College, PA, USA).

3. Results and Discussion

3.1.Macro and Micro Structure

Fig. 1 shows the macroscopic characteristics (color and general appearance) of the emulgels obtained after the emulsification process. All emulgels showed a structure of semi-solid consistency, homogeneous, firm, shiny appearance, and similar to cream/spread materials. No phase separation or oil exudation is seen in all studied formulations.

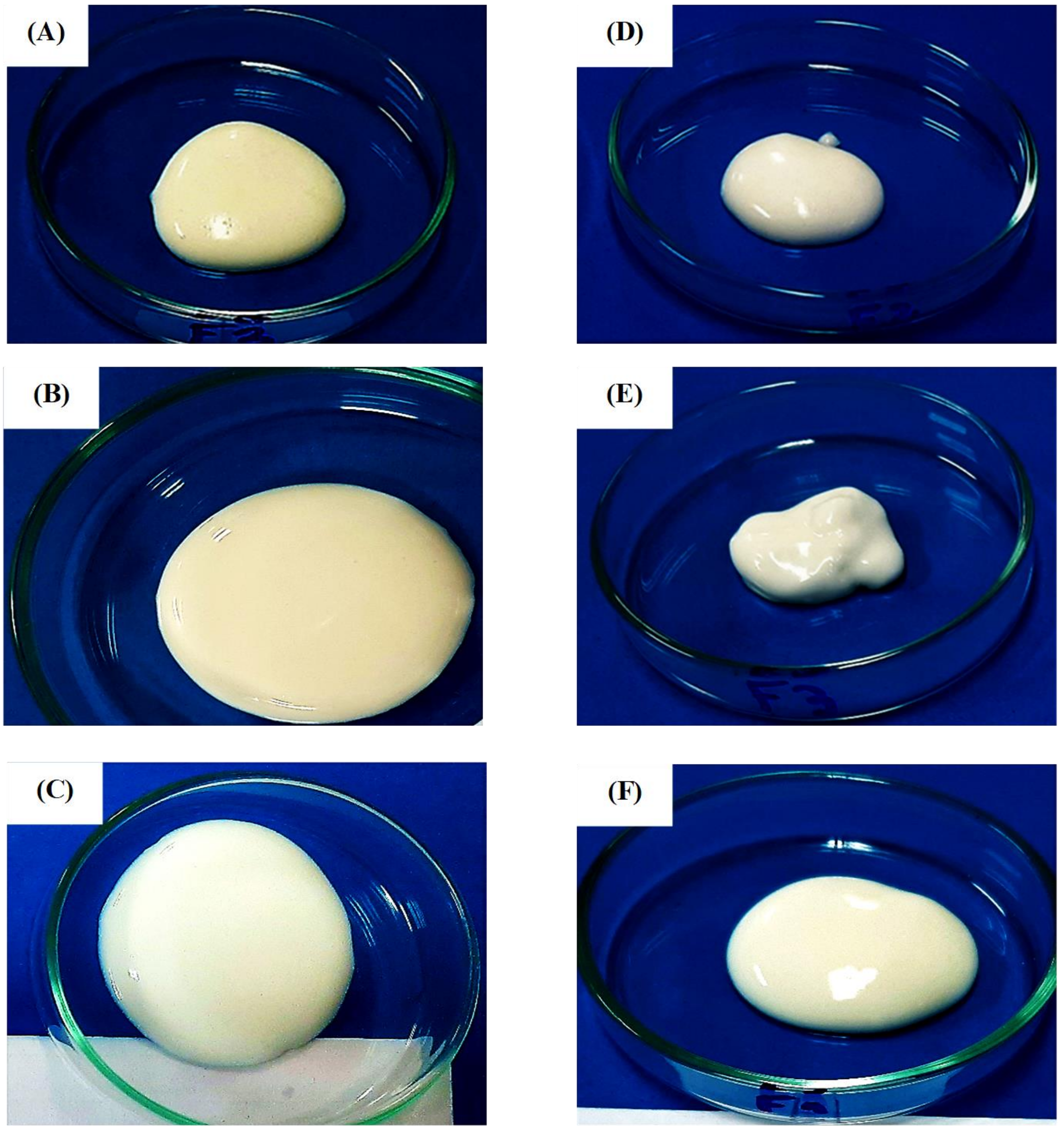


Fig. 1. Macroscopic appearance of the emulgels (A) 80HPMC: 20CHI, (B) 70HPMC: 30CHI, (C) 60HPMC: 40CHI, (D) 80MC: 20CHI, (E) 70MC: 30CHI and (F) 60MC: 40CHI. Where, HPMC: hydroxypropyl methylcellulose, MC: methylcellulose and CHI: chitosan.

Posteriorly, the morphological characteristics of the emulgels stabilized with different polymeric pairs, HPMC: CHI and MC: CHI, were investigated by light microscopy (Fig. 2) and by fluorescence (Fig. 3).

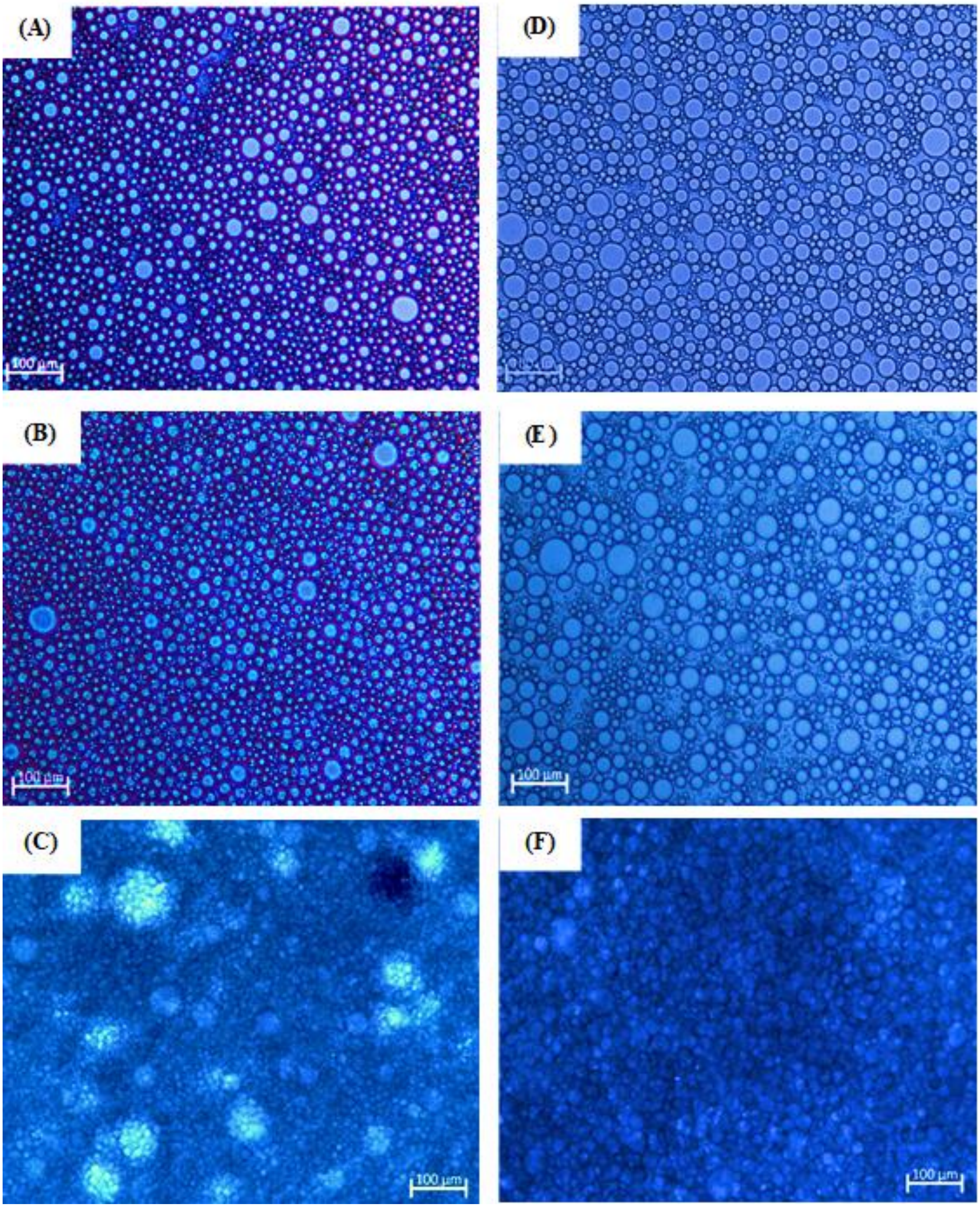


Fig. 2. Emulgels micrographs (amplitude 10x) in 100 µm scale: (A) 80HPMC: 20CHI, (B) 70HPMC: 30CHI, (C) 60HPMC: 40CHI, (D) 80MC: 20CHI, (E) 70MC: 30CHI and (F) 60MC: 40CHI. Where, HPMC: hydroxypropyl methylcellulose, MC: methylcellulose and CHI: chitosan.

Fig. 2 reveals the presence of spherical droplets dispersed with different sizes and distributed in the aqueous gel phase. In general, the morphological structures of the emulgels are similar to each other. That is, the oil drops are packed together and without any presence of coalescence.

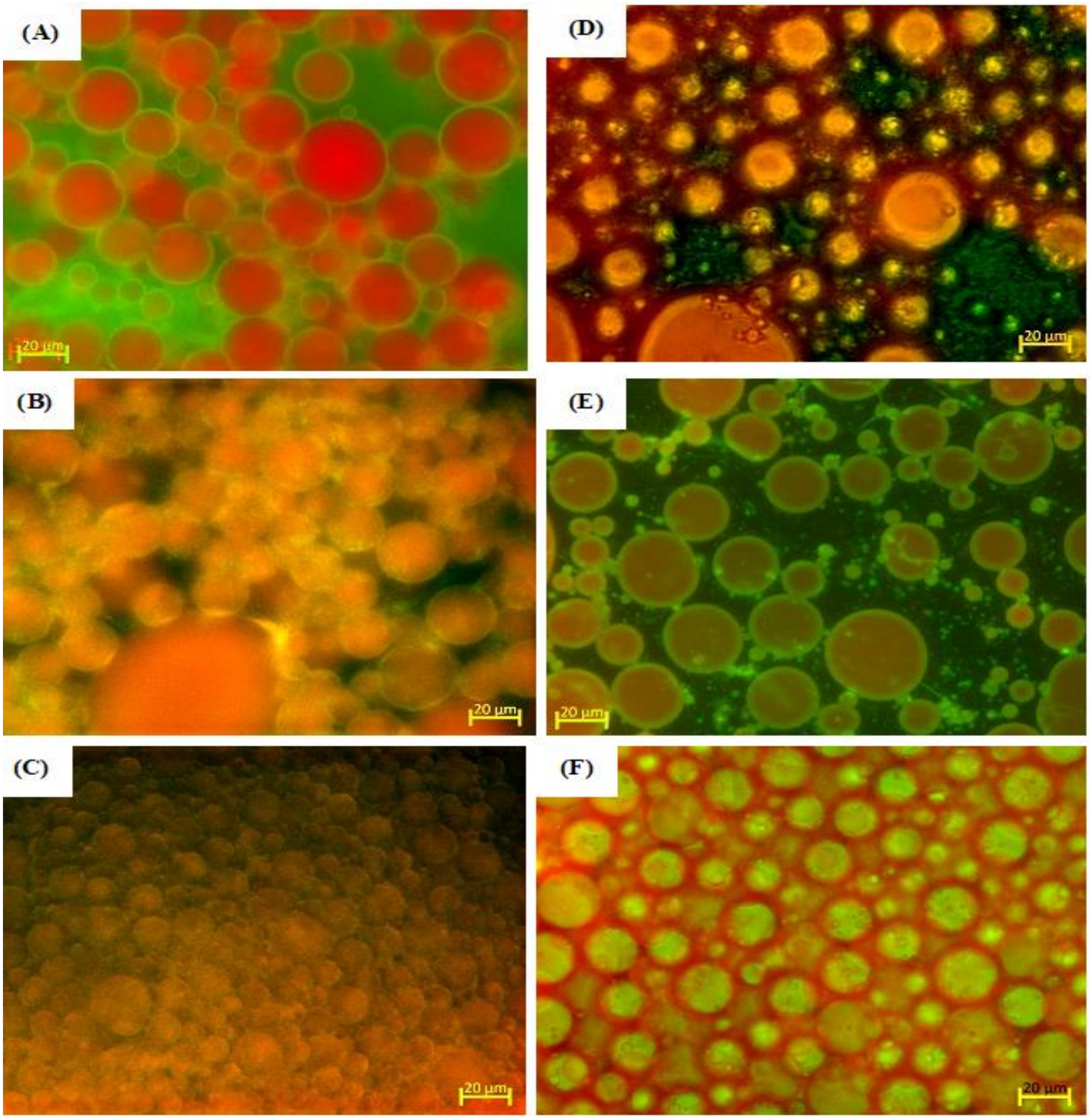


Fig 3. Fluorescence Micrographs of O/W Emulgels (40x amplitude) in 20 µm scale: (A) 80HPMC: 20CHI, (B) 70HPMC: 30CHI, (C) 60HPMC: 40CHI, (D) 80MC: 20CHI, (E) 70MC: 30CHI and (F) 60MC: 40CHI. Where, HPMC: hydroxypropyl methylcellulose, MC methylcellulose and CHI: chitosan; O/W: oil-in-water emulsion-type.

Fig. 3, on the other hand, shows that all systems, regardless of the combination of polymeric pairs, presented an oil-in-water emulsion-type (O/W) structure since the oil droplets (red) are dispersed in the continuous phase of water and polymer (green). Thus, it is possible to say that the formed emulgels were coated with a combination of polymers, HPMC: CHI and MC: CHI, and the system remained stable due to the steric effects provided by the polysaccharides.

3.2.Size Distribution and Average Diameter

The average droplet size and the size distribution of the emulgels prepared with HPMC: CHI and MC: CHI are shown in Table 1 and Fig. 4.

Table 1. Average drop diameter (D [3,2] and D [4,3]), mode (Mo) and Span of the emulgels.

Samples	D[43] (µm)	D[32] (µm)	Mo (µm)	Span
Emulgels – HPMC e CHI				
80HPMC:20CHI	12.473±0.129^{d}	5.239±0.043^{e}	14.331±1.066^{d}	1.623±0.008^{a}
70HPMC:30CHI	19.011±0.765^{c}	8.518±0.129^{c}	23.970±2.070^{c}	1.419±0.049^{c}
60HPMC:40CHI	19.565±0.103^{c}	10.039±0.317^{d}	26.892±1.839^{b,c}	1.546±0.015^{b}
Emulgels – MC e CHI				
80MC:20CHI	22.051±0.483^{b}	9.261±0.160^{b}	29.770±1.427^{a,b}	1.430±0.022^{c}
70MC:30CHI	22.448±0.738^{b}	9,240±0.178^{a}	26.890±3.990^{b,c}	1.489±0.051^{b,c}
60MC:40CHI	27.398±0.506^{a}	10.835±0.136^{a}	32.620±2.750^{a}	1.481±0.013^{b,c}

Different letters indicate a significant difference ($p < 0.05$) in the same column. HPMC: hydroxypropylmethylcellulose; MC: methylcellulose; CHI: chitosan, Mo:moda.

Based on Table 1 and Fig. 4 it can be seen that the size distribution of HPMC emulgels: CHI and MC: CHI is bimodal with two distinct peaks (in accordance with Figure 3), indicating two predominant sizes, peak 1 with volume < 2% and between 9 and 12 % volume for peak 2. This behavior is also proven in the micrographs shown in Fig. 2. Overall, emulgels had similar droplet size distribution and narrow polydispersion (low span values).

Table 1 shows that D[4,3], the mean volume diameter value, is the parameter that most representatively describes the emulgel droplet size, as its value is similar to the values found for the mode (Mo). The droplet size of the emulgels ranged from 12.473 to 27.398 µm in the following order: 80HPMC: 20CHI, 70HPMC: 30CHI, 60HPMC: 40CHI, 80MC: 20CHI, 70MC: 30CHI and 60MC: 40CHI. Thus, it is possible to see that the systems composed of HPMC and CHI had the smallest values of D[4,3]diameter .

Droplet size and distribution are determining factors for the stability of emulsified systems. McClements [21] reported that droplets with low diameter values would have a slow precipitation velocity, resulting in more stable emulsions.

Meng et al., [19] prepared emulsions with 60% of soybean oil stabilized with HPMC (0.2; 0.4; 0.6; 0.8 and 1%) and xanthan gum with constant concentration (0.3 % by weight). The authors observed that as the concentration of HPMC increased, the average droplet size decreased. This reduction was attributed to the lipophilic (methoxyl) and hydrophilic (hydroxypropyl) segments present in the HPMC molecule. These two groups were able to stabilize these systems composed of water and oil. The results found by these authors are similar to the D [4,3] diameter data found in this work for HPMC emulgels, since this polymer has hydroxypropyl and methoxyl groups in its structure. MC is mainly composed of lipophilic groups (methoxyl).

On the other hand, the addition of chitosan could also change the droplet size of the structured systems since emulgels with higher concentrations of CHI had higher values of D [4,3]. However, the effect of adding CHI was more pronounced in MC and CHI emulgels.

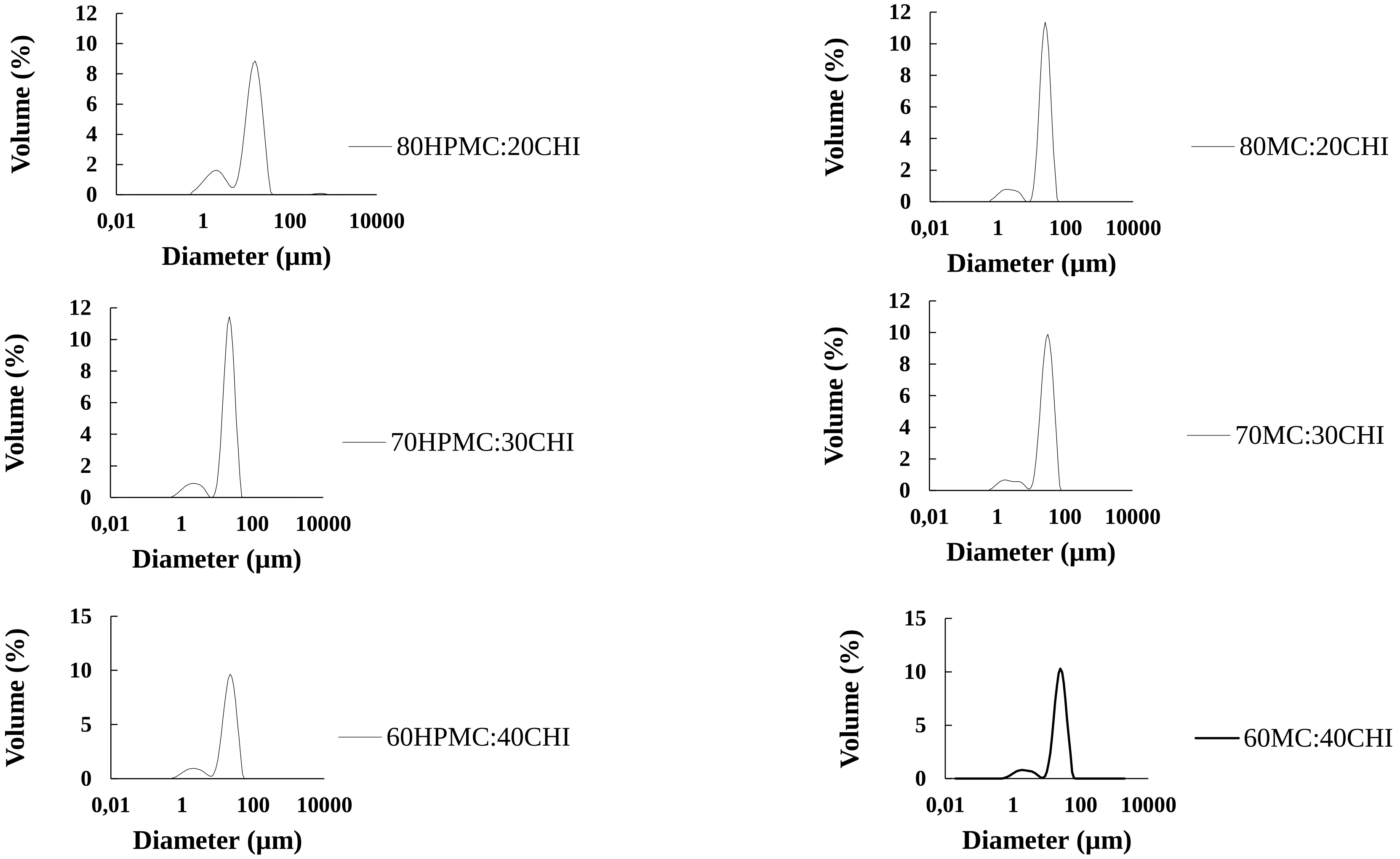


Fig. 4. Size distribution of emulgels composed of HPMC: CHI and MC: CHI. Where HPMC: hydroxypropyl methylcellulose, MC methylcellulose, and CHI: chitosan.

3.3.Rheological Characterization

3.3.1. Steady-shear behavior

The analysis of the emulgels structures stabilized by HPMC: CHI and MC: CHI flow curves were performed. The fit parameters of the Power Law model to the flow curves are present in Table 2. All samples did not show thixotropy as the ascending-descending-ascending curves were similar.

Table 2. Consistency index (k). flow behavior index (n). and determination coefficient (R^2) of HPMC: CHI and MC: CHI emulgels in different proportions.

Samples	***n***	***k* (Pa.s^n)**	**R^2**	**μ_{300} s^{-1} (Pa.s)**
Emulgels – HPMC e CHI				
80HPMC:20CHI	0.323±0.026^{c,d}	59.706±1.287^{c}	0.996±0.002^{a}	1.463±0.067^{b}
70HPMC:30CHI	0.368±0.011^{b}	47.949±1.896^{d}	0.999±0.000^{a}	1.258±0.029^{c}
60HPMC:40CHI	0.408±0.024^{a}	27.210±2.156^{e}	0.999±0.000^{a}	1.080±0.066^{d}
Emulgels – MC e CHI				
80MC:20CHI	0.311±0.004^{d}	85.437±4.910^{a}	0.999±0.000^{a}	1.665±0.006^{a}
70MC:30CHI	0.320±0.001^{c,d}	76.320±1.474^{b}	0.999±0.019^{a}	1.563±0.025^{a,b}
60MC:40CHI	0.349±0.006^{b,c}	60.505 ±2.620^{c}	0.997±0.004^{a}	1.455±0.031^{b}

Different letters in the same column indicate a significant difference ($p < 0.05$). HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; CHI: chitosan.

The Power Law model was used to adjust the rheological parameters of the emulsion flow curve, and this model presented high values of R^2. Parameter n represents the behavior of fluids, and these can be Newtonian ($n=1$), pseudoplastic ($n<1$), or dilating ($n>1$). All emulgels showed pseudoplastic behavior ($n<1$), with n ranging from 0.311 to 0.408. Low values of “n” are related to the formation of more elastic gelled structures. The 80HPMC: 20CHI and 80MC:20CHI emulgels were the samples with the lowest n values among the systems studied. Therefore, they are the most structured samples. This behavior is confirmed by their highest apparent viscosity values, 1.463 and 1.665 Pa.s, respectively, and these samples had the smallest droplet size (Table 1).

The k parameter reveals structural information about the materials. High k values show a more structured material. The emulgels samples with 80% HPMC or MC and 20% CHI had the highest k, 59.706, and 85.437 $Pa.s^n$, so they are the most consistent samples.

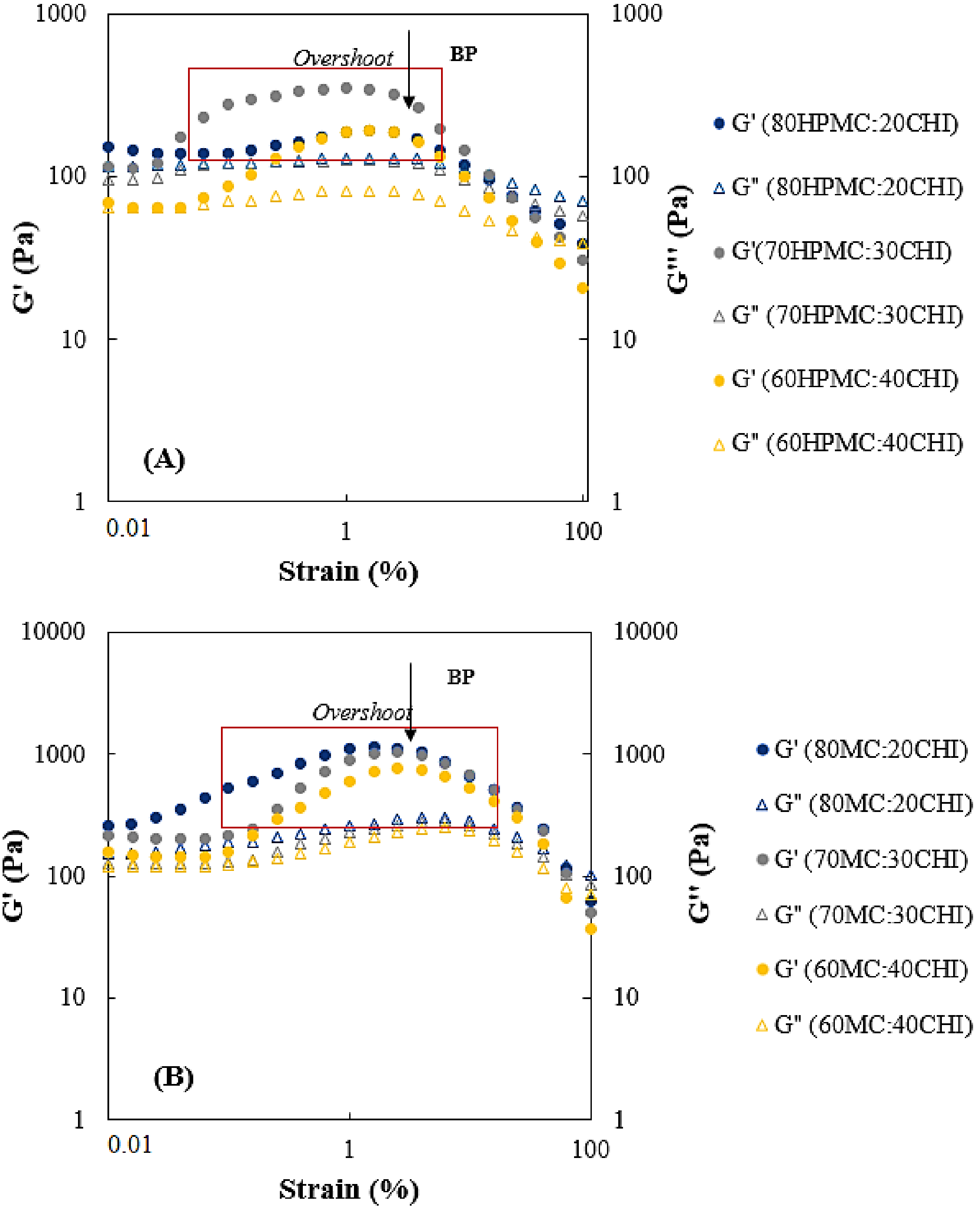
Gomes et al. [22] reported the correlation between emulsion droplet size and system viscosity. In systems with large droplet sizes, there is less exposure of the surface area to droplet interaction. Therefore, there is a reduction in the pseudoplastic behavior and the viscosity of the systems. This fact was observed for the emulgels with the highest proportions of CHI, 60MC:40 CHI, and 60HPMC: 40CHI, which had the largest droplet sizes, 27.398 and 19.565 µm respectively (Table 1), and the lowest apparent viscosity values (Table 2). Conversely, the emulgel with the highest concentration of MC (80MC: 20 CHI) had the highest apparent viscosity among the studied formulations. Therefore, it is the most stable among the studied emulgels.

3.3.2. Oscillatory behavior

Fig. 5 shows the strain scan performed for all emulgels to determine the linear viscoelasticity region.

The G' parameter (elastic modulus) portrays the ability of materials to store energy, that is, the solid structure. The G" (viscous modulus) describes the energy dissipation of materials, viscous behavior (liquid). For all emulgels samples, the G' remained more significant than the G" throughout the strain range studied. High G' values indicate that emulgels show a predominant elastic (solid) over viscous (liquid) behavior. Emulgels also exhibited overshoots (Fig. 5). Subsequently, the systems showed a sharp drop in the values of G' and G", characterizing the breaking point (BP) of these materials and exit from the linear viscoelastic region.

Fig. 5. Stress sweeps of (A) HPMC: CHI and (B) MC: CHI emulgels. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose, CHI: chitosan and, BP: breaking point.



However, they disrupt their structure in the same region for all emulgels prepared with HPMC (80HPMC: 20CHI, 70HPMC: 30CHI, and 60HPMC: 40CHI). Based on Figure 5B, we observe that the MC: CHI emulgels behaved similarly to the systems prepared with HPMC and, at their breakpoint, all the emulgels (80MC: 20CHI, 70 MC: 30CHI, and 60 MC: 40CHI) had a loss of the elastic structure in the same region.

Emulgels made with MC and CHI had the highest values of viscosity (Table 2) and G' compared to emulgels made with HPMC: CHI. Thereby, these emulgels are more structured (solid) and stable.

Fig. 6 shows the frequency scan data at 0.1% strain (linear viscoelasticity region) for the HPMC: CHI and MC: CHI emulgels.

All emulgels showed frequency-dependent behavior and no crossing point between G' and G" (gel point). In regions with low-frequency values (region I) and high observation time, the 70HPMC:30CHI and 70MC:30CHI emulgels behaved as elastic materials (G' > G"). The others (80HPMC: 20CHI, 60HPMC: 40CHI, 80MC: 20CHI and 60MC: 40CHI) had close values for G' and G", showing that their elastic network is weaker. With the increase in frequency and shorter observation times (region II), the elastic modulus predominates over the viscous one, describing the gel behavior. In region III, this behavior is maintained until the samples raise values close to G' and G" between them. HPMC: CHI and MC: CHI emulgels are stable at high-frequency values.

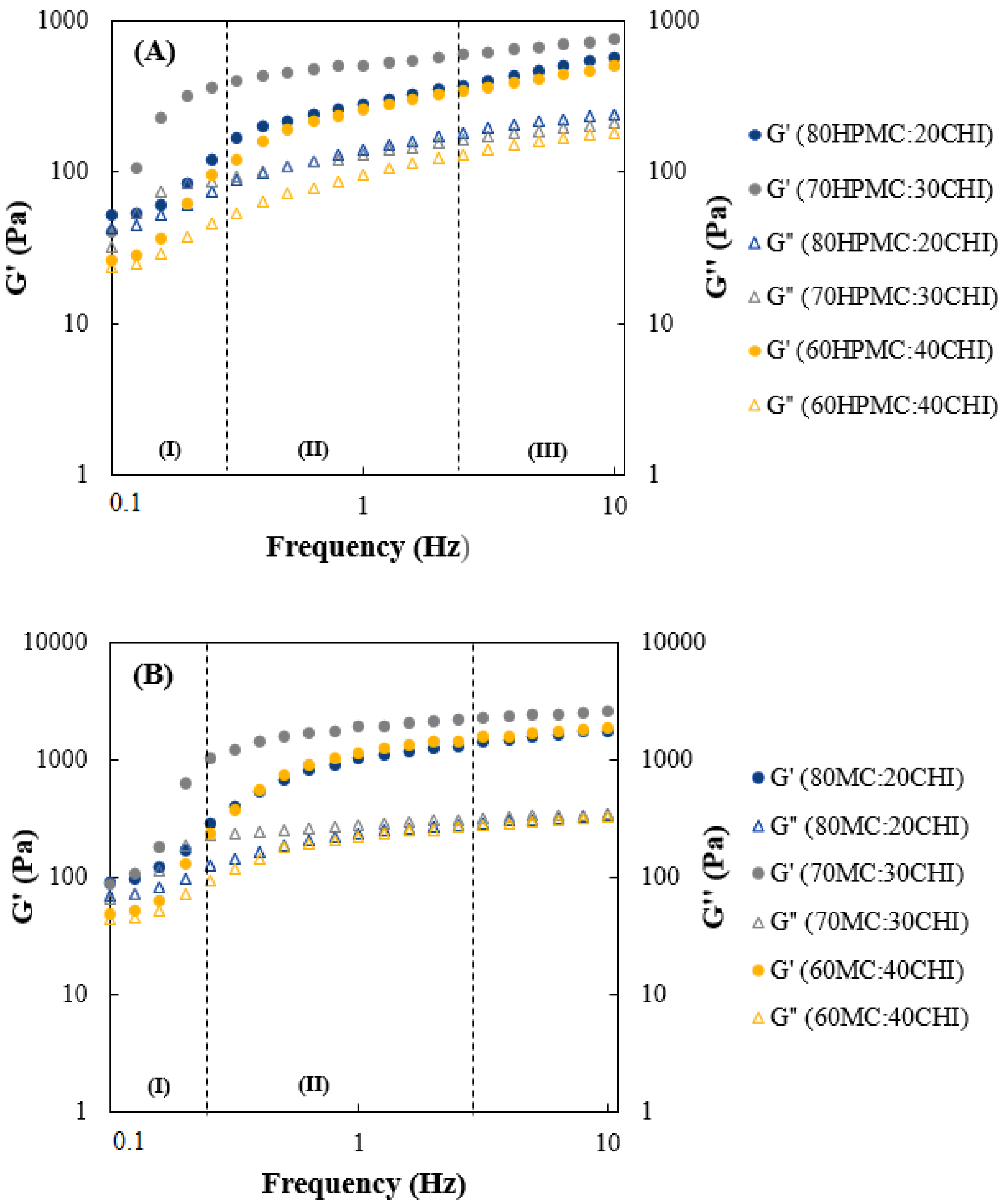


Fig. 6. Storage (G') and loss (G") modulus of (A) HPMC: CHI and (B) MC: CHI emulgels by frequency sweeps. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose, and CHI: chitosan.

All emulgels exhibit the typical behavior of a weak gel, as the ratio between G' and G" (G'/G") is lower than 10; values above 10 indicate a strong gel [23]. The MC: CHI emulgels were more structured than HPMC: CHI. The gel strength (G'/G") obtained was 5.28, 7.39 and 5.76 for 80MC: 20 CHI, 70MC: 30 CHI and 60MC: 40CHI, respectively.

Therefore, the elastic rule (represented by G') of emulgels prepared with MC is stronger than that of HPMC.

Tan δ values were included (Fig. 7) and this parameter correlates G" and G'. For Tan values δ < 1, it means that G' is greater than G". All emulgels samples had values less than 1 for this factor, showing the behavior of a viscoelastic solid. The Tan δ (Fig. 7) agrees with the experimental data obtained in Fig. 6, in which the 70HPMC: 30CHI and 70MC: 30CHI systems were the most elastic.

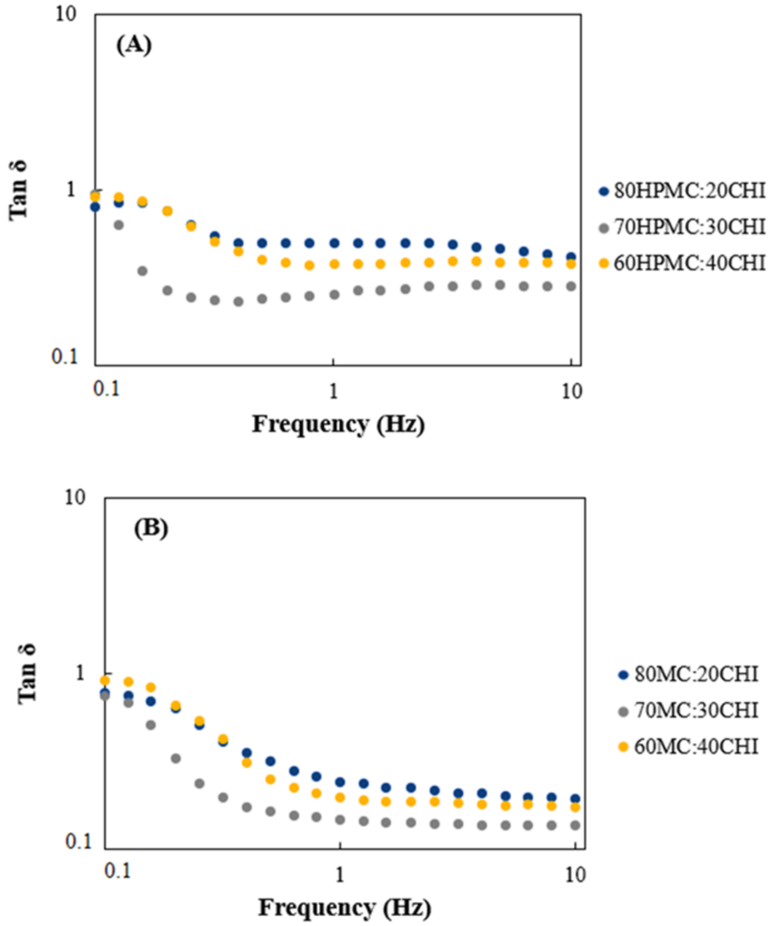


Fig. 7. Tan δ (G"/G') of (A) HPMC: CHI and (B) MC: CHI emulgels. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; CHI: chitosan; G': elastic modulus, and G": viscous modulus.

Patel and Dewetrick [16] prepared sunflower oil oleogels using a polymeric solution of HPMC (2% m/m) of different viscosities (0.015, 0.05, 0.1, and 4 Pa.s). The authors reported that all systems showed “gel-like” consistency as the Tan δ values were less than 1. Espert, Salvador & Sanz [20] obtained oleogels by the emulsion model using HPMC (F 4M) and MC (A 4M) at different concentrations (0.5; 1 and 2%) and sunflower oil as non-polar phase (47 and 67%). For all systems, G' values greater than G" were identified. Regardless of the oil concentration and type of cellulose, the determining factor was the increase in the concentration of the cellulosic polymer, which increased the modulus of elasticity and brought Tan δ to values close to zero.

Polymer concentration is a factor that strongly influences on the viscoelastic properties of structured systems. This fact has already been highlighted by other authors whom claim that in order to obtain structured systems, with greater mechanical strength and capacity to trap the oil phase it is necessary to increase the polymer concentration [19], [20], [24].

3.3.2.1.Temperature sweep

Cellulose derivatives, HPMC and MC, are known to form gels during the heating of their solutions. The formation of these thermo-reversible gels occurs after heating to a temperature above the lowest critical solution temperature (LCST) [25], [26]. Figs. 8 and 9 show the thermal behavior in heating and cooling. Table 3 shows the gel strength of the emulgels at different temperatures.

In general, all the HPMC and CHI emulgels (Fig. 8 A) showed similar behavior, in which G' and G" increased with the heating of the samples. The G' was larger and with similar curves for all emulgels and G", and the gel spot was not identified (G'=G"). However, the 70HPMC: 30CHI emulgel sample showed the greatest difference in G' and G" at 85 °C (Table 3), 8.53, showing that this system forms a stronger gel than the other ones. HPMC is a polymer with more hydrophilic groups and has an LCST temperature between 65 to 90 °C. However, adding a hydrophobic component (vegetable oil) intensifies the hydrophobic associative interactions at high temperatures. Therefore, this polymer forms less structured (soft) gels than MC gels [25], [26].

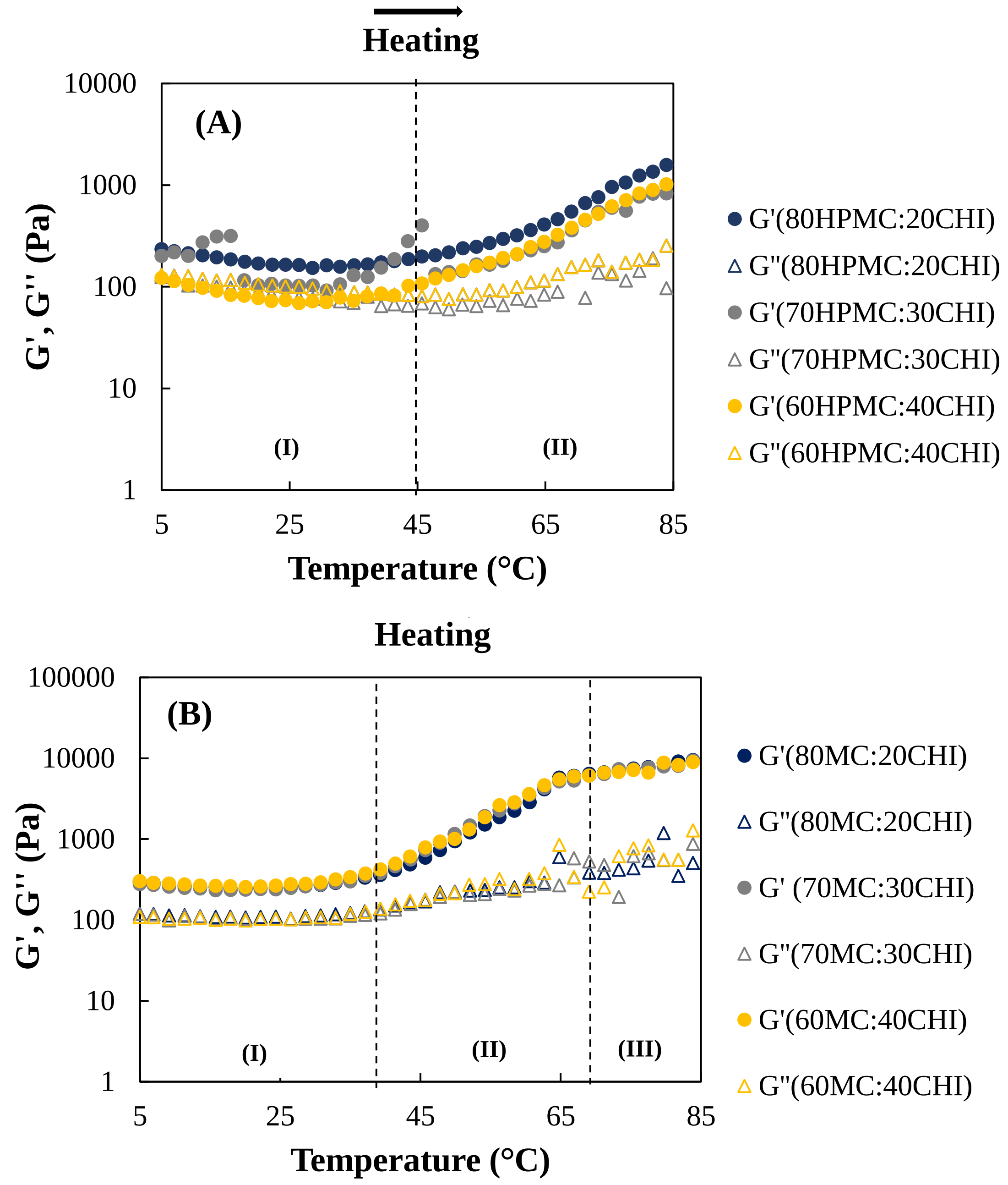


Fig. 8. Storage (G') and loss (G'') modulus for (A) HPMC: CHI and (B) MC: CHI emulgels at heating. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; and CHI: chitosan.

Emulgels composed of MC and CHI (Fig. 8 B) exhibited similar behavior concerning viscoelastic parameters. However, with higher G' values than G'', the solid part predominated in the systems during the temperature scan. The ratio between G' and G" differences was greater than 10 for the emulgels: 80MC: 20CHI and 70MC: 30CHI, at temperatures 85, 75, and 65 °C, as shown in Table 3. Thus, these emulgels are strong (true gels) and do not break easily.

The sample with the highest CHI content (60MC: 40CHI) had a gel strength of 7.50; therefore, it forms weaker emulsions with greater ease of taking out of the structure. The formation of the structured network for MC occurs at an LCST between 40 and 50 °C [25], [26], and this polymer, as it has more hydrophobic groups exposed to heating, interacts with the oil and takes to a strong development of the elastic component (G'). With that, more consistent gels are formed.

Table 3. Gel strength (G'/G") emulgels of HPMC: CHI and MC: CHI in different proportions. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; CHI: chitosan.

Samples	**Gel strength (G'/G'')**					
	85 °C	**75°C**	**65°C**	**55 °C**	**45 °C**	**35 °C**
Emulgel – HPMC e CHI						
80HPMC:20CHI	6.20	6.82	3.55	2.91	2.44	1.84
70HPMC:30CHI	8.53	4.49	3.07	2.58	5.86	1.87
60HPMC:40CHI	5.82	5.16	3.36	2.99	2.16	1.40
Emulgel – MC e CHI						
80MC:20CHI	18.30	17.01	17.92	7.37	3.47	2.51
70MC:30CHI	10.74	11.85	19.28	9.27	6.20	2.64
60MC:40CHI	10.63	9.31	6.44	8.20	4.47	2.88

In area I, the HPMC emulgels: CHI and MC: CHI showed invariability in the values of G' and G" at 5 to 45 °C (Fig. 8 A) and 5 to 35 °C (Fig. 8 B), respectively. At this interval, the gel-like structure (G'>G") was not altered with temperature changes, suggesting that all samples are thermostable up to this interval.

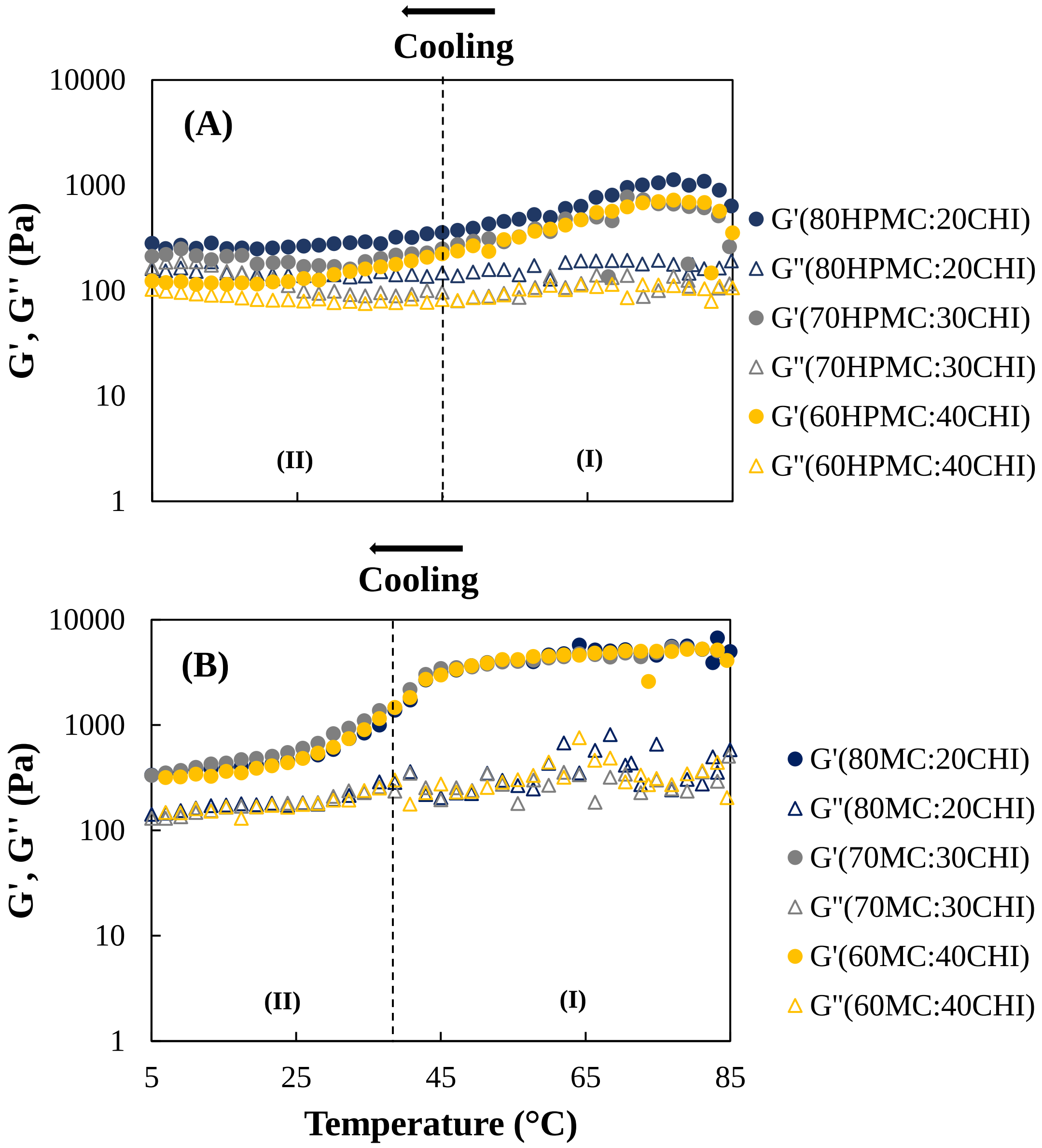
Fig. 8 (area II) shows that G' constantly grows at higher temperatures, 85 °C for HPMC and 65 °C for MC. In Fig. 8B, the MC: CHI emulgels show constant viscoelastic parameter values, represented by area III.

The addition of CHI to the systems possibly modified their gelling behavior. It was identified that in all the studied temperature ranges (5 to 85ºC), both emulgels with HPMC: CHI and MC: CHI were presented as gels. These data are in agreement with dos Santos Carvalho et al. [27]. The authors studied different ratios of HPMC and MC hydrogels with CHI (80:20, 70:30, and 60:40). Analyzing the thermal properties by the temperature scan of these systems, the authors concluded that the addition of CHI causes a significant transformation in the behavior of the viscoelastic parameters (G′ and G″) of HPMC and MC. For both systems, there was a reduction in the LCST (lower critical solution temperature) temperature. For the systems with HPMC: CHI, the gel point ranged from 43.6 to 64.8 °C, and for the MC: CHI hydrogels from 39.3 to 41.5 °C.

The extent of gelation in these systems makes the application of these emulgels favorable in several food matrices that involve heating. CHI has amino (hydrophilic) and acetyl (hydrophobic) groups that can interact with vegetable oils and cellulosic polymers [27]. HPMC emulgels had a considerable increase in G' from 45 °C and 35 °C for MC. These values are lower than the LCST values found in the literature for the solution of pure polymers, which are 65-90°C for HPMC and 40-50°C for MC [26], [27].

On cooling (Fig. 9), all emulgels reduced viscoelastic parameters, which was expected as HPMC and MC are known to form gels and increase their elastic property on heating. In area I (Fig. 9A), all HPMC emulgels showed approximate G' and G" values up to 45 °C. With the decrease in temperature, G' and G" decreased and reached a new area with constant G' and G" (area II). Similarly, MC emulgels (Fig. 9B) showed continuous G' and G" up to 36 °C. Later, these parameters were also reduced until they reached a temperature of 5°C. Both samples of emulgels produced with HPMC: CHI and MC: CHI again had similar values of G' and G" obtained in the heating step at 45 and 36 ºC, respectively, indicating the thermal resistance of these systems.

Fig. 9. Storage (G') and loss (G") modulus for (A) HPMC: CHI and (B) MC: CHI emulgels at cooling. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; and CHI: chitosan.



Although hydrogels of HPMC, MC and their combinations with CHI are thermo-reversible [25], [28], [29], [30], emulgels of HPMC: CHI and MC: CHI did not show the same behavior in the temperature range from 5 to 85°C. In summary, the absence of a G' and G' crossing point (gel point) indicates that the emulgels did not exhibit the characteristic term of reversibility. Thus, there was no solution-gel transformation on heating (Fig. 8) and gel-solution on cooling (Fig. 9). Likewise, Bascus et al. [7] prepared emulsions and oils based on 2% HPMC (4 Pa.s) and xanthan gum (0.6%) using 60g of sunflower or linseed oil. For all samples, it was identified that the G' remained constant throughout the temperature scan (up to 120 °C), and the gel spot (G' = G") was not visualized, indicating the absence of thermal reversibility of these systems when compared to its hydrogels.

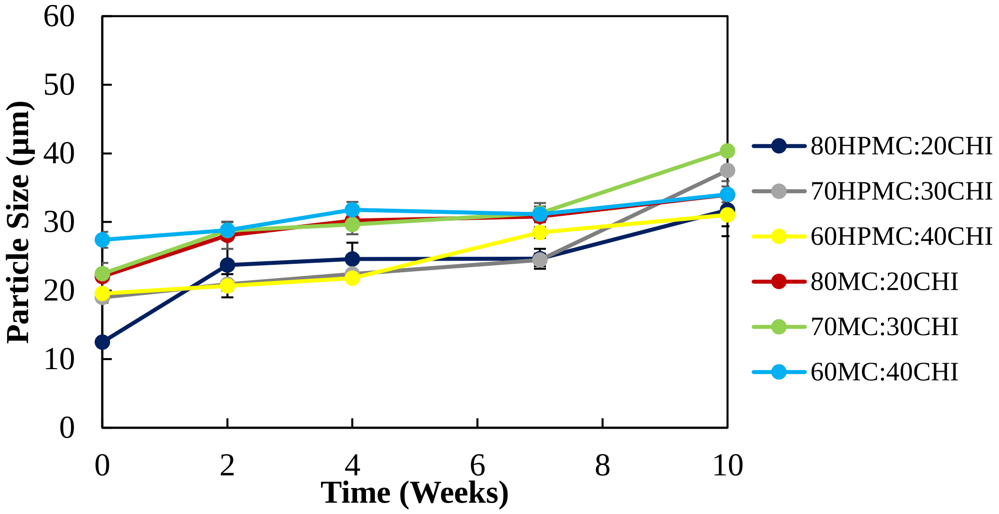
3.4.Emulgel stability study

All emulgels were stored at two different temperatures, 25°C, and 40°C, for ten weeks. The 0, 2, 4, 7, and 10 weeks were evaluated for all systems.

3.4.1. Droplet size and morphology

The mean volume diameter values (D [43]) were chosen to evaluate the emulgels droplet sizes during the storage (as can be seen in Fig. 10) due to a better representation of the size features, as discussed in section 3.2. Fig. 10A shows that on storage at 25°C smaller droplets than at 40°C were obtained (Fig. 10B) for all the emulgels. The systems prepared with 70% cellulosic polymer and 30% chitosan had the highest values of D [43], regardless of the storage condition. In the tenth week, the following results were obtained 37.50µm and 40.38µm for 70HPMC: 30CHI and 70MC:30CHI at 25°C, respectively. While at 40°C, the following values of D [43] 58.09µm for 70HPMC:30CHI and 39.67µm for 70MC:30CHI were obtained.

A) 25°C



B) 40°C

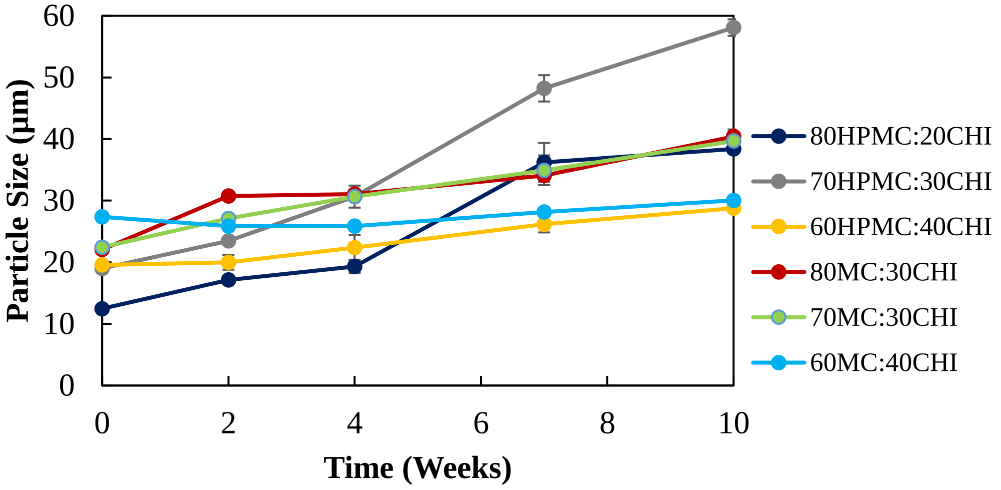


Fig. 10. Average drop diameter (D [43]) of (A) HPMC: CHI and MC: CHI emulgels at 25°C and (B) HPMC: CHI and MC: CHI emulgels at 40°C stored for ten weeks. HPMC: hydroxypropyl methylcellulose, MC: methylcellulose and CHI: chitosan.

Analyzing Fig. 10, it is also possible to observe that, regardless of temperature, all emulgels showed an increase in droplet size D [43] over the weeks. However, 70HPMC: 30CHI emulgels increased in D [4,3] at week 7 and 10 at 40°C. The increase in droplet size during emulsion storage at high temperatures is expected, since droplets become more mobile due to the reduction in stiffness and an increase in fluidity in the emulsifier layers, expanding the coalescence rate [31]. The coalescence is an emulsion instability mechanism where droplets of different sizes come together to form a larger one [32]. Due to the increase in droplet size, it begins to undergo gravitational actions, and in oil-in-water emulsions cases, oil droplets tend to migrate to the surface, producing cremeation. These instability mechanisms, called momentum, lead to phase separation, a thermodynamically stable mechanism for components (water and oil) with different polarities.

Regarding morphological images, it can be observed that the aged emulsions (7th and 10th weeks; Fig. 11) showed the same features as the fresh emulsion (0 weeks; Figure 2), where spherical droplets of several sizes were distributed in the aqueous gel phase. However, there is an increase in oil droplets' size in almost all formulations except for 80HPMC:20CHI. The high proportion of HPMC raises the viscosity of the continuous phase and, consequently, can increase the effect of steric impediment [33]. Additionally, it can be noted that the high values of D [4,3] could be explained by the droplet aggregation caused by the gelation of HPMC. According to Silva et al. [34], the HPMC solution exposed to high temperatures increases its hydrophobic character, which is responsible for the polymeric aggregation, forming a three-dimensional network structure.

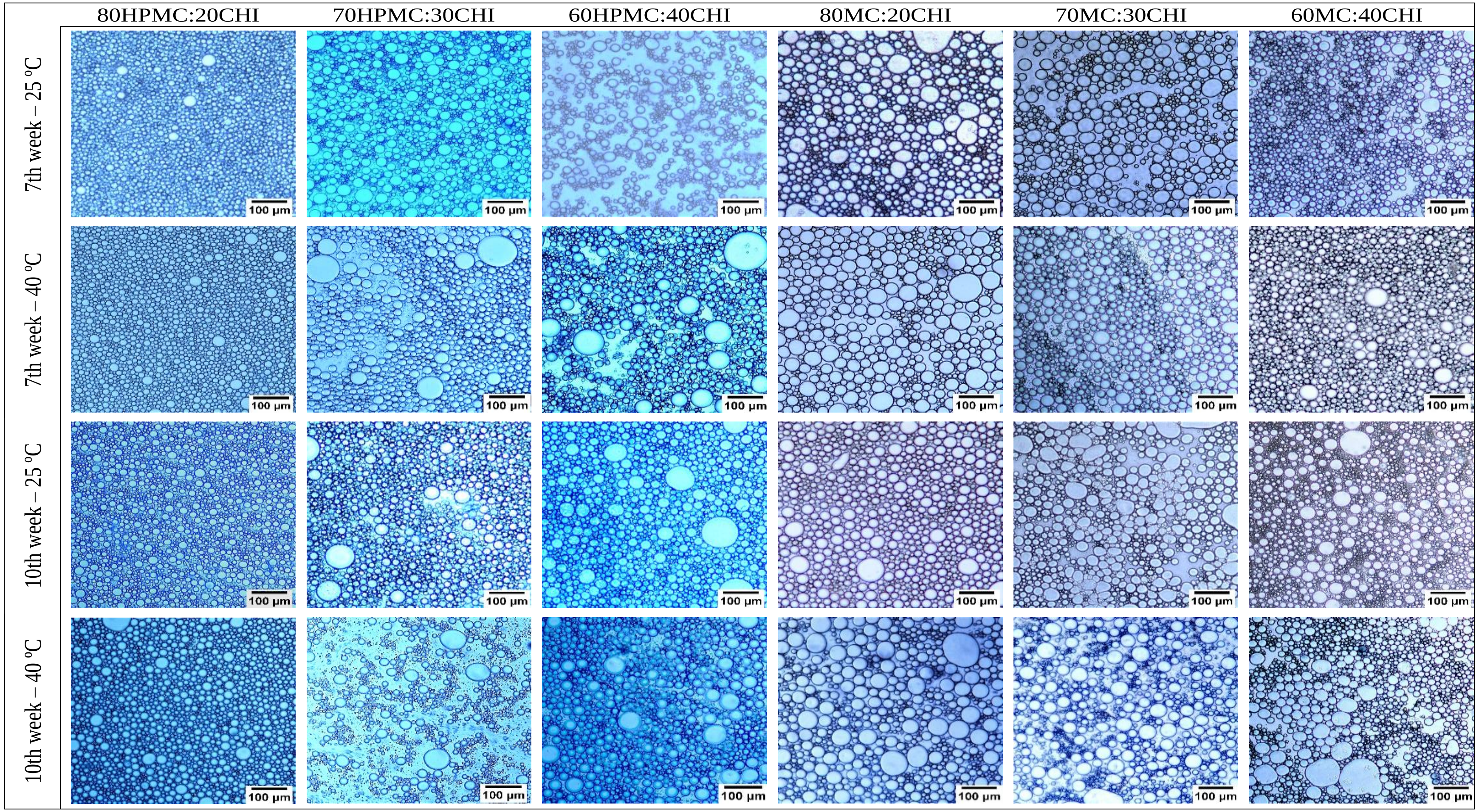


Fig. 11. Emulgel micrographs during stability at 10x magnification.

3.4.2. Oil Loss

Fig. 12 shows the oil loss for emulgels storage for systems prepared with HMPC: CHI and MC: CHI at 25°C and 40°C.

In time zero, both systems lost less than 1% of oil. However, evaluating systems with MC: CHI, these emulgels lost less oil than HPMC: MC. Systems with 80MC: 20CHI, 70MC: 30CHI and 60MC: 40CHI released 0.27%, 0.45% and 0.28%, respectively, while systems with 80HPMC: 20CHI, 70HPMC: 30CHI and 60HPMC: 40CHI lost 1.04%, 0.13% and 0.54%, respectively.

At 25°C storage, all emulgels maintained a stable oil release behavior. At the end of 10 weeks of storage, emulsions prepared with HPMC: CHI (Fig. 12A) systems had oil retention of around 97%, and systems with MC: CHI at the storage end had 99% oil retention. At 25 ºC, systems with MC: CHI (Fig. 12B), regardless of polymer concentration, had higher mechanical strength and, consequently, better bonding with the oil.

An increase in oil loss was found for emulgels stored at 40°C (Fig. 12 C-D). The 70HPMC:30 CHI emulgel showed high oil loss from weeks 2 to 4 (1.04 and 2.88%, respectively), and at the end of storage, it lost 4.56% of oil. However, at the end of the tenth week, the oil retention was approximately 96% for all emulgels.

Systems prepared with 80MC: 20 CHI and 70MC: 20CHI had similar curves, with high oil loss over the ten weeks of storage, compared to 60MC:40 CHI emulgel. Such systems at week 10 showed 4.18, 4.27, and 3.87 oil losses for 80MC:20 CHI, 70MC: 30CHI, and 60MC: 40 CHI. All MC: CHI emulgels at week ten had retention of around 96%, similar to systems with HPMC: CHI.

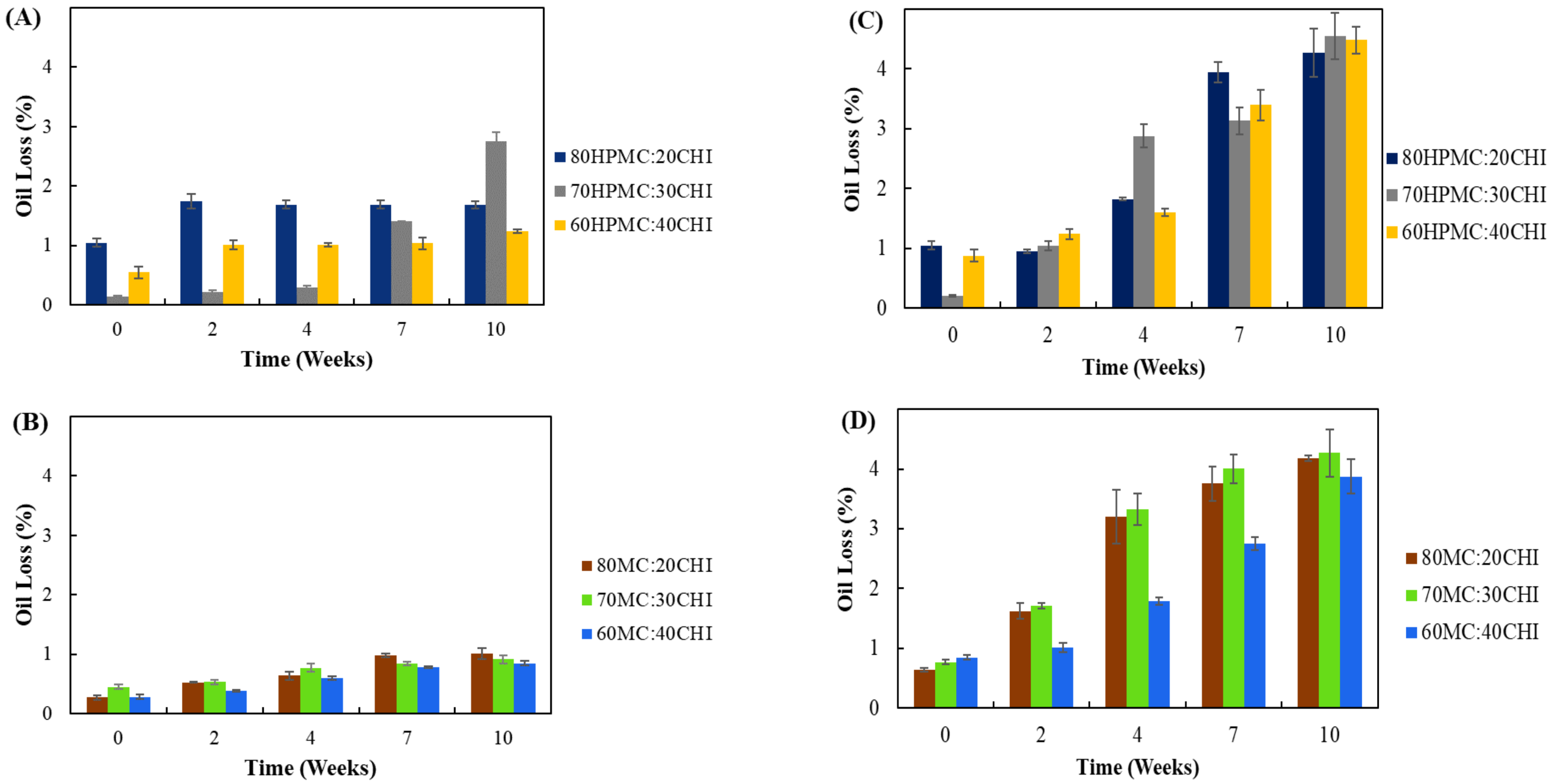


Fig. 12. Oil loss of (A) HPMC: CHI and (B) MC: CHI emulgels stored for ten weeks at 25 °C; (A) HPMC: CHI and (B) MC: CHI emulgels stored for ten weeks at 40 °C. HPMC: hydroxypropyl methylcellulose, MC: methylcellulose and CHI: chitosan.

From Fig. 10, we show that the largest droplet sizes were obtained for the 70HPMC or MC and 30CHI systems, and for these systems, regardless of temperature, there was less oil retention at the end of storage (tenth week). As a result, drops with high D[4,3] values resulting from the coalescence phenomenon release more oil.

Overall, retention at 25°C was 99% for systems with MC: CHI and 97% for HPMC: CHI. At 40°C, retention was around 96% for all systems. These results confirm that these emulgels can retain a large amount of oil in their structure, presenting high physical stability.

This high oil retention of emulgels can be explained by the presence of hydrophobic groups (methyl and hydroxypropyl) present in the molecular structure of HPMC and MC. These functional groups can interact with nonpolar components (such as vegetable oils) through hydrophobic interactions, leading to the entrapment of oil in its structure [27].

4. Conclusion

The emulgels presented spherical drops with two predominant sizes, and the emulsion type found was oil in water (O/W). The addition of CHI was able to modify the droplet size and the rheological properties of the emulgels, especially in their thermal properties, which showed that in the temperature range studied, the systems presented themselves as gels (G'>G"). The 80MC: 20CHI and 70MC:30 CHI systems formed the strongest (true) emulgels among all those studied. This change in the gelling behavior of MC is attributed to CHI presence, which has amino (hydrophilic) and acetyl (hydrophobic) groups that can interact with vegetable oils and cellulosic polymers. Therefore, emulgels have good thermal stability and oil retention (>96%) during storage for ten weeks and can be an alternative to provide a solid structure to vegetable oils in industrial processes that require heating.

5. Acknowledgments

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6. References

[1] A.R. Patel, Alternative Routes to Oil Structuring, 2015.

[2] A.R. Patel, K. Dewettinck, Edible oil structuring: An overview and recent updates, Food Funct. 7 (2016) 20–29.

[3] K. Nishinari, K.E. Hofmann, H. Moritaka, K. Kohyama, N. Nishinari, Gel-sol transition of methylcellulose, Macromol. Chem. Phys. 198 (1997) 1217–1226.

[4] N. Sarkar, Thermal gelation properties of methyl and hydroxypropyl methylcellulose, J. Appl. Polym. Sci. 24 (1979) 1073–1087.

[5] H. Thielking, M. Schmidt, Ullmann’s Encycl. Ind. Chem. - Heterogenous Catalysis and Solid Catalysts 1. Fundamentals, Ullmann’s Encycl. Ind. Chem. (2012) 565–582.

[6] M. Davidovich-Pinhas, Oil structuring using polysaccharides, Curr. Opin. Food Sci. 27 (2019) 29–35.

[7] S. Bascuas, I. Hernando, G. Moraga, A. Quiles, Structure and stability of edible oleogels prepared with different unsaturated oils and hydrocolloids, Int. J. Food Sci. Technol. 55 (2020) 1458–1467.

[8] R. Said Dos Santos, J. Bassi da Silva, H.C. Rosseto, C.F. Vecchi, K. da S.S. Campanholi, W. Caetano, M.L. Bruschi, Emulgels containing propolis and curcumin: The effect of type of

vegetable oil, poly (acrylic acid) and bioactive agent on physicochemical stability, mechanical and rheological properties, Gels. 7 (2021).

[9] T.C. Pinto, A.J. Martins, L. Pastrana, M.C. Pereira, M.A. Cerqueira, Oleogel-Based Systems for the Delivery of Bioactive Compounds in Foods, Gels. 7 (2021) 86.

[10] Y. Hu, S. Zhang, D. Han, Z. Ding, S. Zeng, X. Xiao, Construction and evaluation of the hydroxypropyl methyl cellulose-sodium alginate composite hydrogel system for sustained drug release, J. Polym. Res. 25 (2018).

[11] R. Jayakumar, M. Prabaharan, R.A. Muzzarelli, Chitosan for Biomaterials I | R. Jayakumar | Springer, 2011.

[12] Y. Luo, Q. Wang, Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery, Int. J. Biol. Macromol. 64 (2014) 353–36 [13] M.M. Islam, M. Shahruzzaman, S. Biswas, M. Nurus Sakib, T.U. Rashid, Chitosan based bioactive materials in tissue engineering applications-A review, Bioact. Mater. 5 (2020) 164–183.

[14] A.R. Patel, N. Cludts, M.D. Bin Sintang, B. Lewille, A. Lesaffer, K. Dewettinck, Polysaccharide-based oleogels prepared with an emulsion-templated approach, ChemPhysChem. 15 (2014a) 3435–3439.

[15] A.R. Patel, N. Cludts, M.D. Bin Sintang, A. Lesaffer, K. Dewettinck, Edible oleogels based on water soluble food polymers: Preparation, characterization and potential application, Food Funct. 5 (2014b) 2833–2841.

[16] A.R. Patel, K. Dewettinck, Edible oil structuring: An overview and recent updates, Food Funct. 7 (2016) 20–29.

[17] Z. Meng, K. Qi, Y. Guo, Y. Wang, Y. Liu, Physical Properties, Microstructure, Intermolecular Forces, and Oxidation Stability of Soybean Oil Oleogels Structured by Different Cellulose Ethers, Eur. J. Lipid Sci. Technol. 120 (2018a).

[18] Z. Meng, K. Qi, Y. Guo, Y. Wang, Y. Liu, Effects of thickening agents on the formation and properties of edible oleogels based on hydroxypropyl methyl cellulose, Food Chem. 246 (2018b) 137–149.

[19] Z. Meng, K. Qi, Y. Guo, Y. Wang, Y. Liu, Macro-micro structure characterization and molecular properties of emulsion-templated polysaccharide oleogels, Food Hydrocoll. 77 (2018c) 17–29.

[20] M. Espert, A. Salvador, T. Sanz, Cellulose ether oleogels obtained by emulsion-templated approach without additional thickeners, Food Hydrocoll. 109 (2020) 106085.

[21] D.J. McClements, Emulsion Formation, 2015.

[22] A. Gomes, A.L.R. Costa, F. De Assis Perrechil, R.L. Da Cunha, Role of the phases composition on the incorporation of gallic acid in O/W and W/O emulsions, J. Food Eng. 168 (2016) 205–214.

[23] I. Tavernier, C.D. Doan, D. Van De Walle, S. Danthine, T. Rimaux, K. Dewettinck, Sequential crystallization of high and low melting waxes to improve oil structuring in wax-based oleogels, RSC Adv. 7 (2017) 12113–12125.

[24] H. Huang, R. Hallinan, F. Maleky, Comparison of different oleogels in processed cheese products formulation, Int. J. Food Sci. Technol. 53 (2018) 2525–2534.

[25] H.F. Darge, A.T. Andrgie, H.C. Tsai, J.Y. Lai, Polysaccharide and polypeptide based injectable thermo-sensitive hydrogels for local biomedical applications, Int. J. Biol. Macromol. 133 (2019) 545–563.

[26] D. Goff, Q. Guo, Food Structure Development: The Interplay Between Processing Routes and Formulation Elements, Part A Food Struct. Dev. Interplay Between Process. Routes Formul. Elem. (2019) 1–28.

[27] J.D. dos Santos Carvalho, R.S. Rabelo, M.D. Hubinger, Thermo-rheological properties of chitosan hydrogels with hydroxypropyl methylcellulose and methylcellulose, Int. J. Biol. Macromol. 209 (2022) 367–375.

[28] M. Kong, X. Chen, The Novel Medical Thermoresponsive Hydrogel Derived from Chitosan, Curr. Org. Chem. 22 (2018) 620–627.

[29] T. Wang, L. Chen, T. Shen, D. Wu, Preparation and properties of a novel thermo-sensitive hydrogel based on chitosan/hydroxypropyl methylcellulose/glycerol, Int. J. Biol. Macromol. 93 (2016) 775–782.

[30] M. Davidovich-Pinhas, S. Barbut, A.G. Marangoni, Development, Characterization, and Utilization of Food-Grade Polymer Oleogels, Annu. Rev. Food Sci. Technol. 7 (2016) annurev-food-041715-033225.

[31] P.K. Okuro, A. Gomes, R.L. Cunha, Hybrid oil-in-water emulsions applying wax(lecithin)-based structured oils: Tailoring interface properties, Food Res. Int. 138 (2020) 109798.

[32] T.M. Ho, A. Razzaghi, A. Ramachandran, K.S. Mikkonen, Emulsion characterization via microfluidic devices: A review on interfacial tension and stability to coalescence, Adv. Colloid Interface Sci. 299 (2022) 102541.

[33] T. Futamura, M. Kawaguchi, Characterization of paraffin oil emulsions stabilized by hydroxypropyl methylcellulose, J. Colloid Interface Sci. 367 (2012) 55–60.

[34] S.M.C. Silva, F. V. Pinto, F.E. Antunes, M.G. Miguel, J.J.S. Sousa, A.A.C.C. Pais, Aggregation and gelation in hydroxypropylmethyl cellulose aqueous solutions, J. Colloid Interface Sci. 327 (2008) 333–340.

CHAPTER V

General discussion

GENERAL DISCUSSION

The use of saturated and trans fats in processed foods is necessary to give these products structure in terms of texture, stability, palatability, solidity, and plasticity (Stortz et al., 2012; Siraj et al., 2015). However, excessive consumption of this type of fat harms human health, causing diseases such as cardiovascular problems, obesity, and cancer, among others (Otto et al., 2012; Siraj et al., 2015). In this context, there has been a growing demand for the development of healthier products with reduced saturated and trans fats that have similar functionalities to fats in food production (Pehlivanoğlu et al., 2018).

As a result, techniques have been developed for obtaining structured vegetable oils without producing trans fatty acids. These techniques offer a new approach to replacing this type of fat in foods (Puscas et al., 2020) and creating new products with an improved nutritional profile.

Various techniques for structuring vegetable oils are presented in the scientific literature, which only changes the physical state of the oil without altering its chemical composition. The direct method (most commonly explored) and indirect methods use proteins and polysaccharides to stabilize these systems. Therefore, in this thesis, a system of structured oils, the emulgels, was chosen to be studied. Emulgels are emulsions with high oil content in their structure and do not require the drying step, which is common in obtaining structured oils by the indirect method, using cellulosic polymers (HPMC and MC) in combination with CHI.

These polymeric pairs were chosen because no works have studied this combination. Furthermore, HPMC and MC polymers derived from cellulose are prepared by replacing hydroxyl groups in the cellulose structure with methoxy and hydroxypropyl groups. This substitution gives the molecules a hydrophobic character, which presents an amphiphilic behavior and surface-active nature. In comparison, CHI’s ability to interact with vegetable oils and fats is related to its amphiphilicity. The molecular structure of chitosan is composed of amino groups (hydrophilic) and acetyl groups, which are hydrophobic. So, the combination of these polymeric pairs: HPMC: CHI and MC: CHI, are engaging in the elaboration of hydrogel systems, and consequently, due to their hydrophobic properties, they have potential application in structured oil systems (emulgel).

Chapter I introduces the objectives of the doctoral thesis. **Chapter II** reviewed the scientific literature on structuring vegetable oils, emphasizing using polymers with hydrophilic characteristics to obtain structured systems. However, since this topic is relatively new, there still needs to be a greater understanding of the methodologies for obtaining structured systems and a better differentiation of each one concerning the definition, processes, and characteristics.

Chapter III presents the project's first stage, "*Thermo-rheological properties of chitosan hydrogels with hydroxypropyl methylcellulose and methylcellulose,*" which examines the formation of hydrogels between HPMC and MC polymers with chitosan. The hypothesis was that adding chitosan could reduce the gelation temperature of cellulosic polymers and increase the LCST temperature (low critical solution temperature) to apply these hydrogels to obtain structured systems (emulgels).

The polymeric pairs, HPMC: CHI and MC: CHI, were chosen to study the thermal gelling properties and the hydrophobic interactions in their hydrogels in three different ratios, 80:20, 70:30, and 60:40, respectively. Rheology, FTIR, interfacial tension, and DSC tests were performed for this. Initially, in the analysis of the flow curves, the pure polymers (HPMC and MC) and the hydrogels of their mixtures with CHI exhibited shear thinning behavior ($n < 1$), that is, their viscosity decreases with the increase in processor speed. Pure CHI showed behavior similar to a Newtonian fluid (n close to 1). Hydrogels with higher proportions of cellulosic polymer had the highest apparent viscosity values.

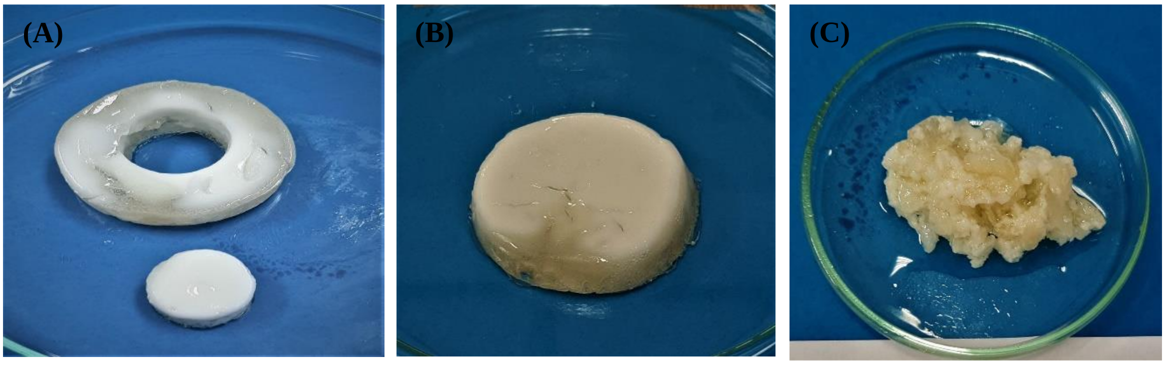
In the oscillatory tests, a deformation sweep was performed for all hydrogels and pure polymers to determine the region of linear viscoelasticity (0.1%). A predominance of G' was observed for samples with higher concentrations of cellulose ethers, which approached their pure polymers. All hydrogels were also frequency dependent. The hydrogels were thermoreversible in the thermal behavior analysis carried out by temperature scanning during heating and cooling. That is, they exhibited the behavior of a solution gel. Comparing HPMC: CHI and MC: CHI hydrogels with their constituent polymers (pure HPMC and MC), a reduction in gelation temperature was observed for all systems. The pure HPMC and MC gelation temperature was 66.9 and 43.6 °C, respectively. With the addition of CHI, the most significant reduction occurred for the following hydrogels 70HPMC: 30CHI (43.6 °C) and 70MC: 30 CHI (39.3 °C). These results indicate that adding CHI modifies and expands the gel formation temperature of thermoreversible hydrogels, allowing their application in food systems that use different process temperatures.

FTIR analysis revealed hydrophobic interactions between cellulosic polymers and CHI, characterized by a peak at 1543 cm^{-1} (amino group). All hydrogels also showed the ability to reduce the interfacial tension of sunflower oil, showing their potential for application in obtaining structured systems.

Chapter IV, entitled *"Oil-in-water emulsion gels stabilized with cellulosic polymers and chitosan: themorheological and physical-chemical evaluation"*, discusses the use of hydrogels obtained in the previous step (**Chapter III**) to produce structured systems.

The objective of this chapter was to obtain oleogels by the indirect emulsion method (Emulsion-templated approach), proceeding with oven drying of conventional emulsions (65°C for 48 h) to obtain solid structured, oleogel, with a low proportion of water and high concentration of oil. After the oven drying process (Figures 1A and 1B), obtaining a visually solid system was possible. However, for the actual characterization of an oleogel, it is necessary to test the oil retention capacity of that structure. After shearing the samples in a stator rotor, the studied systems became unstructured and lost their solid conformation, resulting in oil exudation (Figure 1 C). After this preliminary test, we concluded that obtaining oleogels by the indirect method using these polymer pairs is unfeasible. That is why we chose to work with emulgels, which do not need to go through the drying step.

Figure 1. (A) and (B) oleogels after the drying step and (C) oleogels after shearing.



The use of hydrogels as a basis for obtaining emulgels was successful. Emulgels were structured and had a final cream appearance. Microscopy revealed spherical droplets and O/W-type emulsions. Emulgels with higher concentrations of CHI had the highest $D_{[43]}$ values, and the 60MC: 40 CHI sample (27.398 µm) had the largest diameter among the studied formulations. The Power Law model was adjusted for all emulgels, which were pseudoplastic and their hydrogels. Emulgels with lower CHI concentrations, 80HPMC: 20CHI and 80MC: 20 CHI, were the most structured samples with the highest viscosity values.

The strain scan confirmed that emulgels prepared with MC and CHI (higher viscosity values) had the highest G' values compared to emulgels produced with HPMC: CHI, making them more structured (solid) and stable. The frequency analysis showed that all emulgels displayed frequency-dependent behavior and no crossover point between G' and G" (gel point).

The temperature scan was used to investigate the effect of adding CHI to the emulsifiers since, in the hydrogel systems (**Chapter III**), the gel point of these systems was visualized. In the thermal analysis, it was identified in the entire temperature range studied (5 to 85°C) that both emulgels, HPMC: CHI and MC: CHI, were presented as gels (G'>G"). Emulgels produced with MC were more structured than systems with HPMC. This behavior is due to the greater amount of hydrophilic groups in the molecular structure of HPMC. Emulgels 80MC: 20CHI and 70MC:30 CHI formed stronger gels (G'>>G") as they had a gel strength of more than 10. At the following temperatures, 85, 75, and 65 °C, high gel strengths were shown to these emulgels. Thus, the addition of CHI to the systems modified their gelling behavior.

After characterizing the emulgel systems, a stability study was conducted at two different temperatures, 25 and 40 °C. During ten weeks, the emulgels' droplet size, morphology, and oil loss were monitored.

Generally, systems stored at 25°C had droplet sizes ($D_{[43]}$) smaller than at 40°C, as shown in **Chapter IV** (Figure 10). Furthermore, systems with 70 HPMC or MC: 30CHI had the highest diameter value, both at 25° C and 40°C at the storage end. At the end of storage (week 10), the following droplet sizes were obtained at 25°C ($D_{[43]}$): 37.50µm and 40.38µm for 70HPMC: 30CHI and 70MC:30CHI, respectively. At the end of storage at 40°C, the following results were obtained: 58.09µm for 70HPMC:30CHI and 39.67µm for 70MC:30CHI.

However, it is possible to say that regardless of the analyzed temperature, the systems showed an increase in the size of their drops over time, but the increase was more considerable at high temperatures (40°C). This increase at high temperatures is expected since the droplets become more mobile due to reduced stiffness and increased fluidity in the emulsifier layers, increasing the coalescence rate (Okuro, Gomes & Cunha, 2020).

The morphology reveals that the emulsions in the 7th and 10th weeks condition (Chapter IV, Fig.11) presented similar characteristics to the emulsions produced in week zero. Spherical oil droplets with various size distributions dispersed in the aqueous gel were obtained.

The oil loss of emulgels prepared with HPMC: CHI and MC: CHI were investigated. Analyzing Fig. 12 present in Chapter IV, both systems lost approximately 1% of oil. At 25°C, all emulsifiers maintained a stable oil release behavior. In the 10th week, emulsions prepared with HPMC had an oil retention of about 97%, and systems with MC: CHI had 99% oil retention. At 40°C, an increase in oil loss from these systems was observed. Both emulgels have finished storage experiments with around 96% of the oil.

The systems generally lost little oil during storage, resulting in high retention values. This high oil retention of emulgels can be explained by the presence of hydrophobic groups (methyl and hydroxypropyl) present in the molecular structure of HPMC and MC. These functional groups can interact with nonpolar components (such as vegetable oils) through hydrophobic interactions, leading to the entrapment of oil in its structure (dos Santos Carvalho, Rabelo & Hubinger, 2022).

CHAPTER VI

General conclusion

GENERAL CONCLUSION

This doctoral work presents some information on the formation of hydrogels composed of HPMC and MC in combination with CHI in an attempt to use these polymeric pairs in different proportions, 80:20, 70:30, and 60:40, in the stabilization of structured systems, emulgels.

Chapter III confirmed that adding CHI can reduce the LCST (lower critical solution temperature) of cellulosic polymers and extend the period that methylcellulose: chitosan and hydroxypropyl methylcellulose: chitosan is in the gel conformation. Moreover, the methylcellulose hydrogels obtained were more structured than the systems with hydroxypropyl methylcellulose.

These hydrogel systems were applied to structure sunflower oil by emulsification to obtain emulsifiers. HPMC and MC can form gels while heating their polymeric solutions. Therefore, a temperature scan was performed for these emulgels. As a result, it was identified that these systems were in the gel conformation (G'>G") throughout the studied temperature range. The 80MC: 20CHI and 70MC:30 CHI samples formed the strongest (true) emulgels among all those studied, as they presented a G'/G" ratio > 10 at the following temperatures 85, 75, and 65 °C.

In the stability study, we concluded that there was an increase in the size of the drops during storage, mainly at 40°C. Microscopy revealed that the drops remained spherical, and the emulsions at the 7th and 10th week of storage showed similar characteristics to the fresh emulsion. In the oil loss analysis, it was seen that these systems are capable of retaining a high amount of oil inside (96 to 99%). With that, we can conclude that these systems present good thermal resistance.

Therefore, MC emulgels may be good candidates to partially or even wholly replace saturated and trans fats from partially hydrogenated vegetable oils. This replacement can occur in food processes that require heating at high temperatures, as these emulsifiers would be in their gelled form and have excellent thermal resistance.

CHAPTER VII

General references

REFERENCES

Ali, A., & Ahmed, S. (2018). Recent Advances in Edible Polymer Based Hydrogels as a Sustainable Alternative to Conventional Polymers. Journal of Agricultural and Food Chemistry, 66(27), 6940–6967.

Aliaghaei, M., Mirzadeh, H., Dashtimoghadam, E., & Taranejoo, S. (2012). Investigation of gelation mechanism of an injectable hydrogel based on chitosan by rheological measurements for a drug delivery application. Soft Matter, 8(27), 7128–7137.

Batista, R. A., Espitia, P. J. P., Quintans, J. de S. S., Freitas, M. M., Cerqueira, M. Â., Teixeira, J. A., & Cardoso, J. C. (2019). Hydrogel as an alternative structure for food packaging systems. Carbohydrate Polymers, 205(October 2018), 106–116.

Bhandari, S. D., Delmonte, P., Honigfort, M., Yan, W., Dionisi, F., Fleith, M., … Bergeson, L. L. (2020). Regulatory Changes Affecting the Production and Use of Fats and Oils: Focus on Partially Hydrogenated Oils. JAOCS, Journal of the American Oil Chemists' Society, 97(8), 797–815.

Bhattarai, N., Gunn, J., & Zhang, M. (2010). Chitosan-based hydrogels for controlled, localized drug delivery. Advanced Drug Delivery Reviews, 62(1), 83–99.

Brasil. (2019). Resolução - RDC nº 332 de 23 de Dezembro de 2019.pdf. ANVISA - Agência Nacional de Vigilância Sanitária - Diário Oficial da União.

Brasil. (2021). Resolução - RDC nº 514 de 28 de Maio de 2021.pdf. ANVISA - Agência Nacional de Vigilância Sanitária - Diário Oficial da União.

Bu, N., Huang, L., Cao, G., Pang, J., & Mu, R. (2022). Stable O/W emulsions and oleogels with amphiphilic konjac glucomannan network: preparation, characterization, and application. Journal of the Science of Food and Agriculture, 102(14), 6555–6565.

Cao, Y., & Mezzenga, R. (2020). Design principles of food gels. *Nature Food*, *1*(2), 106–118.

Chen, X. W., Fu, S. Y., Hou, J. J., Guo, J., Wang, J. M., & Yang, X. Q. (2016). Zein based oil-in-glycerol emulgels enriched with β-carotene as margarine alternatives. Food Chemistry, 211, 836–844.

Chenite, A., Chaput, C., Wang, D., Combes, C., Buschmann, M. D., Hoemann, C. D., … Selmani, A. (2000). Novel injectable neutral solutions of chitosan form biodegradable gels in situ. Biomaterials, 21(21), 2155–2161. https://doi.org/10.1016/S0142-9612(00)00116-2

Co, E.D., & Marangoni, A.G. (2012). Organogels: An alternative edible oil-structuring method. *JAOCS, Journal of the American Oil Chemists' Society*, *89*(5), 749–780.

Cui, B., Mao, Y., Liang, H., Li, Y., Li, J., Ye, S., … Li, B. (2022). Properties of soybean protein isolate/curdlan based emulsion gel for fat analogue: Comparison with pork backfat. *International Journal of Biological Macromolecules*, *206*(March), 481–488.

Darge, H. F., Andrgie, A. T., Tsai, H. C., & Lai, J. Y. (2019). Polysaccharide and polypeptide based injectable thermo-sensitive hydrogels for local biomedical applications. International Journal of Biological Macromolecules, 133, 545–563.

Davidovich-Pinhas, M., Barbut, S., & Marangoni, A.G. (2016). Development, Characterization, and Utilization of Food-Grade Polymer Oleogels. *Annual Review of Food Science and Technology*, *7*(1), 65–91.

dos Santos Carvalho, J. D., Rabelo, R. S., & Hubinger, M. D. (2022). Thermo-rheological properties of chitosan hydrogels with hydroxypropyl methylcellulose and methylcellulose. *International Journal of Biological Macromolecules*, *209*(PA), 367–375.

Duquette, D., & Dumont, M. J. (2019). Comparative studies of chemical crosslinking reactions and applications of bio-based hydrogels. Polymer Bulletin, 76(5), 2683–2710.

Farjami, T., & Madadlou, A. (2019). An overview on preparation of emulsion-filled gels and emulsion particulate gels. *Trends in Food Science and Technology*, *86*(July 2018), 85–94.

FDA (Food and Drug Administration), Gras notices (GRN No. 443), (Food Drug Adm. GRN No. 443), Available online: https://wayback.archiveit.org/7993/20171031005742/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm347791.htm

Goff, D., & Guo, Q. (2019). Food Structure Development: The Interplay Between Processing Routes and Formulation Elements. Part A Food Structure Development: The Interplay Between Processing Routes and Formulation Elements, 1–28.

Guo, J., Cui, L., & Meng, Z. (2023). Oleogels/emulsion gels as novel saturated fat replacers in meat products: A review. *Food Hydrocolloids*, *137*(October 2022).

Gutiérrez-Luna, K., Astiasarán, I., & Ansorena, D. (2020). Gels as fat replacers in bakery products: a review. Critical Reviews in Food Science and Nutrition, 0(0), 1–14.

Hilal, A., Florowska, A., & Wroniak, M. (2023). Binary Hydrogels: Induction Methods and Recent Application Progress as Food Matrices for Bioactive Compounds Delivery—A Bibliometric Review. *Gels*, *9*(1).

Hu, Y., Zhang, S., Han, D., Ding, Z., Zeng, S., & Xiao, X. (2018). Construction and evaluation of the hydroxypropyl methyl cellulose-sodium alginate composite hydrogel system for sustained drug release. Journal of Polymer Research, 25(7), 1-12.

Huc-Mathis, D., Bousquières, J., Mamou, M., Bonazzi, C., & Michon, C. (2017). Multiscale evaluation from one bubble to the foam of surface active properties of cellulose derivatives used for a starchy model sponge cake. Food Hydrocolloids, 71, 129–140.

Islam, M. M.; Shahruzzaman, M.; Biswas, S.; Nurus Sakib, M.; Rashid, T. U (2020). Chitosan Based Bioactive Materials in Tissue Engineering Applications-A Review. Bioact. Mater, 5 (1), 164–183.

Jayakumar, R.; Prabaharan, M.; Muzzarelli, R.A.A. (2011). Chitosan for Biomaterials I. Berlin, Heidelberg: Springer Berlin Heidelberg.

Khullar, R., Kumar, D., Seth, N., & Saini, S. (2012). Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharmaceutical Journal, 20(1), 63–67.

Kong, M., & Chen, X. (2018). The Novel Medical Thermoresponsive Hydrogel Derived from Chitosan. Current Organic Chemistry, 22(7), 620–627.

Light, K., & Karboune, S. (2021). Emulsion, hydrogel and emulgel systems and novel applications in cannabinoid delivery: a review. Critical Reviews in Food Science and Nutrition, 0(0), 1–31.

Luo, Y., & Wang, Q. (2014). Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. International Journal of Biological Macromolecules, 64, 353–367.

Martínez-Cervera, S., Salvador, A., & Sanz, T. (2015). Cellulose ether emulsions as fat replacers in muffins: Rheological, thermal and textural properties. LWT - Food Science and Technology, 63(2), 1083–1090.

Mattice, K. D., & Marangoni, A. G. (2018). Oleogels in Food. In *Reference Module in Food Science*. Elsevier.

Nishinari, K., Hofmann, K. E., Moritaka, H., Kohyama, K., & Nishinari, N. (1997). Gel-sol transition of methylcellulose. Macromolecular Chemistry and Physics, 198(4), 1217–1226.

Okuro, P. K., Gomes, A., & Cunha, R. L. (2020). Hybrid oil-in-water emulsions applying wax(lecithin)-based structured oils: Tailoring interface properties. *Food Research International*, *138*(PB).

Okuro, P. K., Santos, T. P., & Cunha, R. L. (2021). Compositional and structural aspects of hydro- and oleogels: Similarities and specificities from the perspective of digestibility. *Trends in Food Science and Technology*, *111*(February), 55–67.

Otto, M. C. O. *et al*. (2020). Dietary intake of saturated fat by food source and incident cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. The American journal of clinical nutrition, v. 96, n. 2, p. 397-404.

Paglarini, C. de S., Furtado, G. de F., Biachi, J. P., Vidal, V. A. S., Martini, S., Forte, M. B. S., Pollonio, M. A. R. (2018). Functional emulsion gels with potential application in meat products. Journal of Food Engineering, 222, 29–37.

Patel, A.R. (2015). Alternative Routes to Oil Structuring. In *Springer*.

Patel, A.R., & Dewettinck, K. (2016). Edible oil structuring: An overview and recent updates. *Food and Function*, 7(1), 20–29.

Patel, A.R., Cludts, N., Bin Sintang, M.D., Lewille, B., Lesaffer, A., &Dewettinck, K. (2014). Polysaccharide-based oleogels prepared with an emulsion-templated approach. ChemPhysChem, 15(16), 3435–3439.

Pehlivanoğlu, H., Karasu, S., Konar, N., Toker, O.S., Demirci, M., & Sagdic, O. (2018). Oleogels, a promising structured oil for decreasing saturated fatty acid concentrations: Production and food-based applications. *Critical Reviews in Food Science and Nutrition*, *58*(8), 1–12.

Pintado, T., Herrero, A. M., Jiménez-Colmenero, F., Pasqualin Cavalheiro, C., & Ruiz-Capillas, C. (2018). Chia and oat emulsion gels as new animal fat replacers and healthy bioactive sources in fresh sausage formulation. Meat Science, 135(August 2017), 6–13.

Pinto, T. C., Martins, A. J., Pastrana, L., Pereira, M. C., & Cerqueira, M. A. (2021). Oleogel-Based Systems for the Delivery of Bioactive Compounds in Foods. *Gels*, 7(3), 86.

Puscas, A., Muresan, V., Socaciu, C., & Muste, S. (2020) Oleogels in Food: A Review of Current and Potential Applications. Foods, v. 9, n. 1, p. 70.

Rabelo, R. S., Oliveira, I. F., da Silva, V. M., Prata, A. S., & Hubinger, M. D. (2018). Chitosan coated nanostructured lipid carriers (NLCs) for loading Vitamin D: A physical stability study. International Journal of Biological Macromolecules, 119, 902–912.

Ract, J. N. R., Cruz, R. G. d., & Pereira, C. G. (2018). Phase equilibrium of organogels. In Thermodynamics of Phase Equilibria in Food Engineering.

Ranjha, N. M., & Khan, S. (2013). Review Article Chitosan / Poly (vinyl alcohol) Based Hydrogels for Biomedical Applications: A Review. Journal of Pharmacy and Alternative Medicine, 2(1), 30–42.

Rehman, K., Cairul, M., Mohd, I., & Zulfakar, M. H. (2014). Development and Physical Characterization of Polymer-Fish Oil Bigel (Hydrogel / Oleogel) System as a Transdermal Drug Delivery Vehicle. Journal of Oleo Science, 970(10), 961–970.

Rodríguez-Rodríguez, R., García-Carvajal, Z. Y., Jiménez-Palomar, I., Jiménez-Avalos, J. A., & Espinosa-Andrews, H. (2019). Development of gelatin/chitosan/PVA hydrogels: Thermal stability, water state, viscoelasticity, and cytotoxicity assays. Journal of Applied Polymer Science, 136(10), 1–9.

Said Dos Santos, R., Bassi da Silva, J., Rosseto, H. C., Vecchi, C. F., Campanholi, K. da S. S., Caetano, W., & Bruschi, M. L. (2021). Emulgels containing propolis and curcumin: The effect of type of vegetable oil, poly(acrylic acid) and bioactive agent on physicochemical stability, mechanical and rheological properties. Gels, 7(3)

Sannino, A., Demitri, C., & Madaghiele, M. (2009). Biodegradable cellulose-based hydrogels: Design and applications. Materials, 2(2), 353–373.

Sanz, T., Falomir, M., & Salvador, A. (2015). Reversible thermal behaviour of vegetable oil cellulose ether emulsions as fat replacers. Influence of glycerol. Food Hydrocolloids, 46, 19–27.

Sarkar, N. (1979). Thermal gelation properties of methyl and hydroxypropyl methylcellulose. Journal of Applied Polymer Science, 24(4), 1073–1087.

Sharma, S., & Tiwari, S. (2020). A review on biomacromolecular hydrogel classification and its applications. International Journal of Biological Macromolecules, 162, 737–747.

Singh, A.; Auzanneau, F.I.; Rogers, M.A (2017). Advances in edible oleogel technologies – A decade in review. *Food Research International*, 97 (4), p. 307–317.

Siraj, N., Shabbir, M.A., Ahmad, T., Sajjad, A., Khan, M.R., Khan, M.I., & Butt, M.S. (2015). Organogelators as a Saturated Fat Replacer for Structuring Edible Oils. *International Journal of Food Properties*, *18*(9), 1973–1989.

Stortz, T.A., Zetzl, A.K., Barbut, S., Cattaruzza, A., & Marangoni, A.G. (2012). Edible oleogels in food products to help maximize health benefits and improve nutritional profiles. *Lipid Technology*, *24*(7), 151–154.

Thielking, H., & Schmidt, M. (2012). Ullmann's Encycl. Ind. Chem. - Heterogenous Catalysis and Solid Catalysts 1. Fundamentals. Ullmann's Encyclopedia of Industrial Chemistry, 565–582.

Wang, T., Chen, L., Shen, T., & Wu, D. (2016). Preparation and properties of a novel thermo-sensitive hydrogel based on chitosan/hydroxypropyl methylcellulose/glycerol. International Journal of Biological Macromolecules, 93, 775–782.

Xu, H. J., Li, T., Zhang, H. X., Shi, C. H., Cao, J. Q., & Zhang, X. R. (2022). The application of oleogels in food products: Classification, preparation, and characterisation. Acta Alimentaria, 51(4), 462–478.

Yang, T., Li, X. T., & Tang, C. H. (2020). Novel edible Pickering high-internal-phase-emulsion gels efficiently stabilized by unique polysaccharide-protein hybrid nanoparticles from Okara. *Food Hydrocolloids*, *98*(August 2019), 105285.

Yang, Y., Zhang, M., Li, J., Su, Y., Gu, L., Yang, Y., & Chang, C. (2022). Construction of egg white protein particle and rhamnolipid based emulsion gels with β-sitosterol as gelation factor: The application in cookie. *Food Hydrocolloids*, *127*(August 2021).

Zhao, M., Lan, Y., Cui, L., Monono, E., Rao, J., & Chen, B. (2020). Physical properties and cookie-making performance of oleogels prepared with crude and refined soybean oil: A comparative study. Food and Function, 11(3), 2498–2508.

APPENDIX

Figure A represents the flow curves, and Figure B shows the relationship between apparent viscosity and the shear rate of hydrogels prepared with HPMC: CHI and MC and CHI.

Figure A. Flow curves of shear thinning hydrogels of HPMC: CHI and MC: CHI. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; and CHI: chitosan.

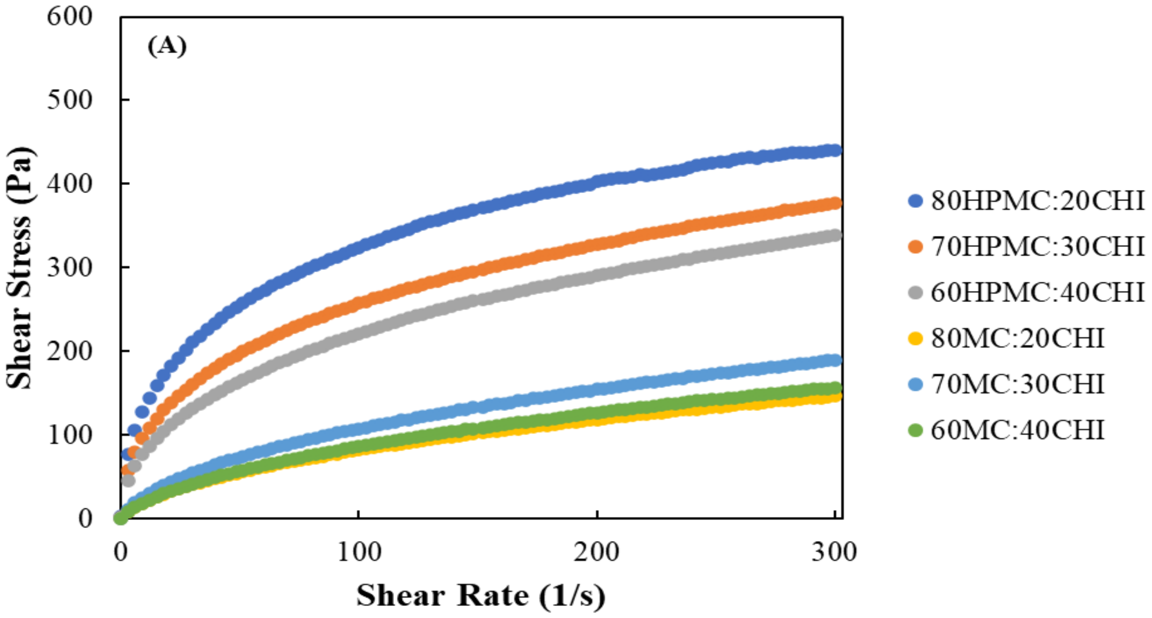


Figure B. Reduction in viscosity as the processing speed **(**0 a 300 s^{-1}). increases for hydrogels. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; and CHI: chitosan

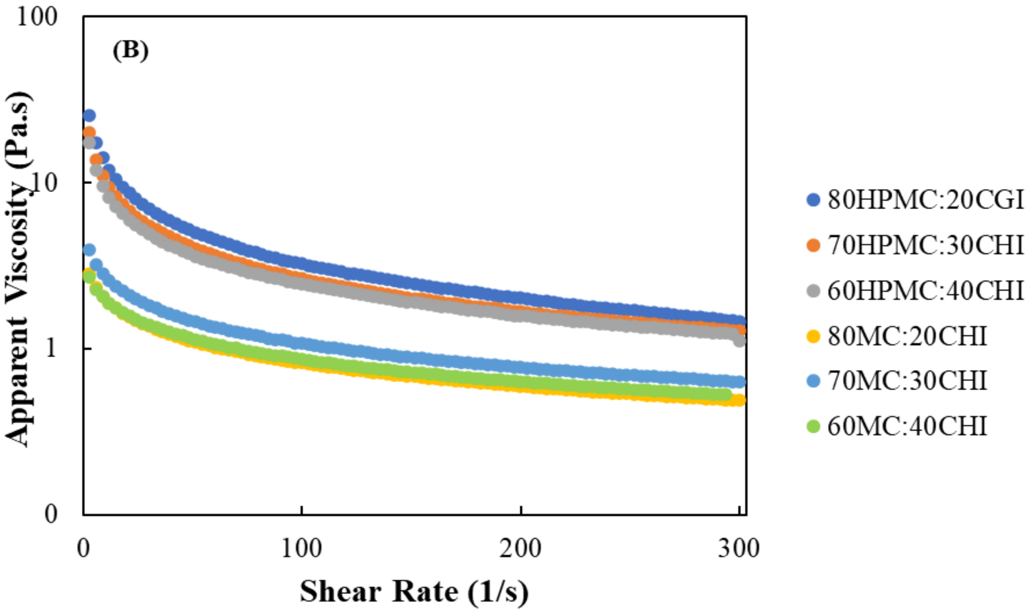


Figure C represents the flow curves, and Figure D shows the relationship between apparent viscosity and the shear rate of emulgels prepared with HPMC: CHI and MC and CHI.

Figure C. Flow curves of shear thinning emulgels of HPMC: CHI and MC: CHI. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; and CHI: chitosan.

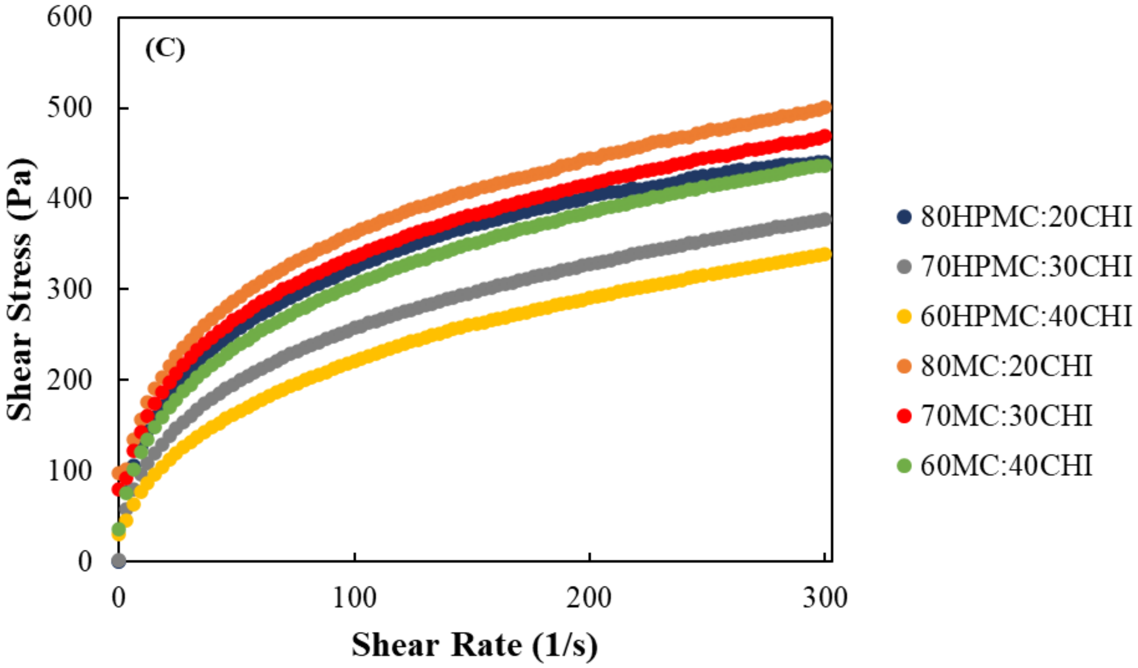
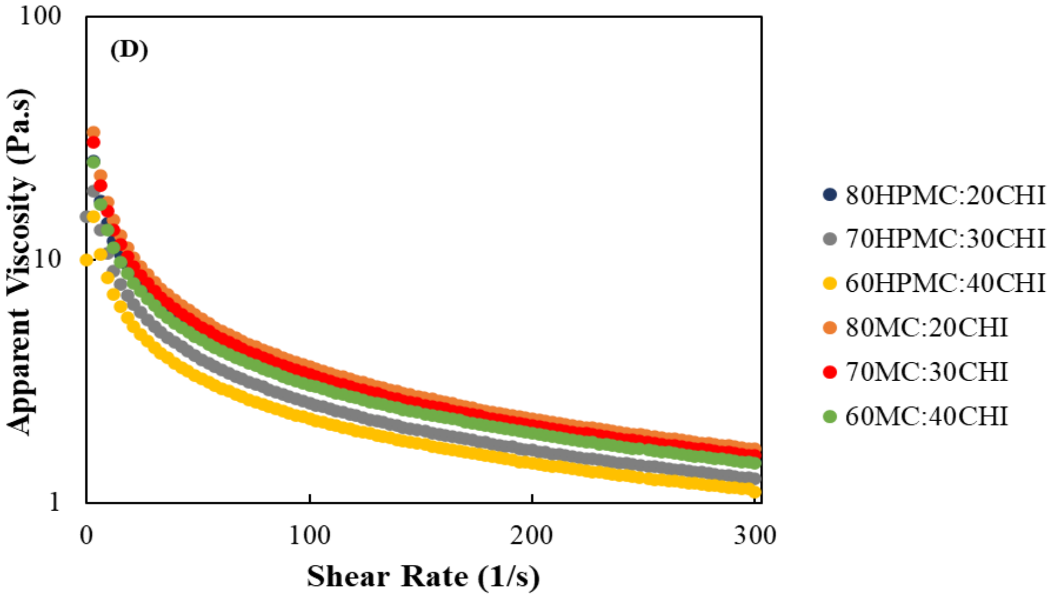


Figure D. Reduction in viscosity as the processing speed (0 a 300 s^{-1}). increases for emulgels. HPMC: hydroxypropyl methylcellulose; MC: methylcellulose; and CHI: chitosan.



From the data on the flow curves (Figures A and C), it was possible to determine that all systems (hydrogels and emulgels) made up of HPMC: CHI and MC: CHI are shear thinning, that is, they exhibit the behavior of non-Newtorian fluids independent of the time. These hydrogels and emulgels do not present residual stress for flow.

Shear thinning fluids are characterized by a reduction in apparent viscosity with an increase in the deformation rate, as shown in Figures B and D.

Such statements are confirmed based on the model of the flow curves of these systems, which were adjusted according to the Power Law model (chapters III and IV). These polymeric hydrogels and emulgels had parameter values “n” behavior index, lower than 1, indicating the pseudo-plasticity of these systems.