Production and 3D printing of Pickering emulsions envisaging customized nutrition

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"*All our dreams can come true if we have the courage to pursue them"*

~ Walt Disney

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ABSTRACT

Personalized nutrition is gaining prominence as people seek tailor-made dietary solutions to meet their unique health and lifestyle needs. The use of Pickering emulsions in the production and 3D printing of personalized nutritional products has aroused significant interest in the food sector, especially emulsions incorporating bioactive compounds, such as vitamin D3, which influences the absorption of calcium through the digestive system and directly influences the maintenance of bones and teeth. Pickering emulsions, stabilized by solid particles instead of traditional surfactants, offer numerous advantages in customizing nutritional, namely by providing nutritional value, taste, and texture. The synthesis of particles based on biopolymers allows for the production of entirely natural formulations, as is the case of chitosan/gelatin type B (CH/GB) based systems, whose coacervation at specific pHs offers the opportunity to design Pickering stabilizers using simple and economical approaches. It is important to note that Pickering emulsions are highly resistant to coalescence and Ostwald ripening phenomena compared to other emulsions using conventional surfactants. In this context, the main objectives of this study were determining the printing parameters of CH/GB systems and the encapsulation of vitamin D3. After preliminary tests, two promising formulations were selected for printing, named E6 - 5.5% and E6 - 6%, where E means emulsion and the values represent the percentage of CH/GB in the solution. The work focused on finding the printing parameters for these two emulsions, evaluating the influence of the oil fraction, temperature on the printability. With the obtained results and the defined parameters, vitamin D3 was incorporated, and its influence in the emulsion was analyzed. Finally, 4 formulations named E6 - 5.5%, E6 - 5.5%D, E6 - 6% and E6 - 6%D (D addresses to the formulations with vitamin D3) were prepared and analyzed in terms of texture, encapsulation efficiency and visual inspection after printing analyzing their ability to serve as a customized food. The obtained results were promising and it was possible to verify that the vitamin caused a slightly increase in stability compared to the base emulsion, while the encapsulation efficiency was determined obtaining a value of 84.03% and 86.62% for E6 – 5.5D and E – 6D respectively. Through the texture analysis it was possible identify parameters such as hardness, springiness, cohesiveness, gumminess and resilience, which can help in the printing of different materials with different structures.

Key words: Pickering emulsions, Chitosan, Type B gelatine, Customized nutrition, 3D printing.

RESUMO

A nutrição personalizada está a ganhar destaque à medida que as pessoas procuram dietas sob medida para atender às suas necessidades de saúde e estilo de vida. O uso de emulsões Pickering na produção e impressão 3D de produtos nutricionais personalizados tem despertado um interesse significativo na área alimentar, principalmente no que respeita ao uso de emulsões que incorporam compostos bioativos, tais como a vitamina D3 que é reconhecida pela sua influência na absorção de cálcio no sistema digestivo, influenciando diretamente a manutenção dos ossos e dentes. As emulsões Pickering, estabilizadas por partículas sólidas em vez de surfactantes convencionais, oferecem inúmeras vantagens para a personalização do conteúdo nutricional, sabor e textura. A síntese de partículas à base de biopolímeros permite a produção de formulações totalmente naturais, como é o caso dos sistemas à base de quitosana/gelatina tipo B (CH/GB), cuja coacervação em pHs específicos oferece a oportunidade de desenhar estabilizadores Pickering por meio de abordagens simples e económicas. Assim, é importante destacar que as emulsões Pickering, possuem elevada resistência a fenômenos de coalescência e amadurecimento de Ostwald em comparação com as emulsões convencionais. Neste contexto, o presente trabalho teve como principais objetivos determinar parâmetros de impressão para emulsões preparadas com partículas CH/GB e realizar a encapsulação da vitamina D3. Com a realização dos testes preliminares foram selecionadas duas formulações promissoras para impressão, designadas por E6 – 5.5% e E6 – 6%, onde E significa emulsão, e a percentagem representa quantidade de partículas CH/GB. O trabalho focou-se no estabelecimento dos parâmetros de impressão que mais se adequavam à impressão destas duas emulsões, avaliando a influência da fração de óleo, temperatura e imprimibilidade. Com os resultados obtidos e os parâmetros definidos, foi realizada a incorporação da vitamina D3 e analisada a sua influência na emulsão analisada. Por fim, foram preparadas 4 formulações designadas por E6 – 5.5%, E6 – 5.5%D, E6 – 6% e E6 – 6%D, onde D indica a presença da vitamina, que foram analisadas quanto à textura, eficiência de encapsulação e inspeção visual após impressão com o objetivo de analisar a sua capacidade para atuar como um alimento customizado. Os resultados obtidos foram promissores e foi possível verificar que a vitamina causou um ligeiro aumento na estabilidade em comparação com a emulsão de base, enquanto a eficiência de encapsulamento foi determinada, obtendo um valor de 84,03% e 86,62 para E6 - 5,5D e E - 6D, respectivamente. Através da análise de textura foi possível notar que a emulsão apresentou inúmeros parâmetros como dureza, elasticidade, coesividade, gumosidade e resiliência que diferem cada uma delas, o que pode ajudar na impressão de diferentes materiais com diferentes estruturas.

Palavras chaves: Emulsões Pickering, Quitosano, Gelatina Tipo B, Nutrição customizada, Impressão 3D.

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LIST OF ABBREVIATIONS

3D	Three-dimensional
3DP	Printing
AcAc	Acetic acid
AM	Additve Manufacturing
CH	Chitosan
CH/GB	Chitosan/Gelatin type B
D	Vitamin D3
EE	Encapsulation efficiency
FTIR	Fourier transform infrared spectroscopy
GB	Gelatin type B
CI	Creaming index
HIPPEs	High internal phase Pickering emulsions
HLB	Hydrophilic-lipophilic balance
O/W	Oil in water
O/W/O	Oil in water in oil
PEs	Pickering emulsions
RP	Prototyping
UV-vis	Ultraviolet-visible
W/O	Water in oil
W/O/W	Water in oil water

Chapter 1

Introduction

1. Introduction

1.1.Motivation and objectives

Customized nutrition is gaining relevance in new social health and nutrition trends, and new products are being sought. As a consequence, progress has been made in the research of new processing approaches, highlighting 3D-printing as an innovative strategy. 3D-printing possibilities include the design of custom-made complex products, enabling the personalization of customized nutritional products at low cost.

This scenario also implies the need for developing novel bioinks, able to fulfil requirements related to the 3D-printing process and preserving the quality of the nutritional products. Among others, emulsions are positioned as promising materials with significant characteristics to comply with these aspects. More specifically, Pickering emulsions (Pes), which use solid particles as stabilizers instead of conventional synthetic surfactants, offer the opportunity to develop stable, green, and non-toxic products.

The opportunity to design the particles, namely the Pickering stabilizers, from biopolymers facilitates the development of totally natural products. Concurrently, synthesizing the particles via green chemistry routes (complexation or enzyme-induced reactions) enables formulations in mild conditions. This is the case of chitosan and gelatin (type B), whose complexation at specific pH ranges where both polymers' functional groups are protonated can result in the formation of nanoparticles. Their use in the preparation of Pickering emulsions is the following step; obtaining stable formulations, typically by adjusting the oil and water ratio and particles' concentration.

In this context, to achieve printable Pickering emulsions, specific rheological behaviors are needed, tunable in terms of internal phase content, the so-called high internal phase Pickering emulsions (HIPPEs). Moreover, it can be achieved by increasing particle content or adding other colloids to the external phase during the synthesis processes, acting as thickeners.

Emulsions, due to their compartmentalized structure, can act as versatile templates to protect and deliver active agents. Among the benefits of using Pickering stabilizers, the development of Pickering emulsions makes the combination of different active agents in the same product viable, which is not easily achieved with other delivery systems. This approach can preserve active agents of the same or opposite hydrophilic/hydrophobic nature in separate

compartments within the same formulation. This opens many opportunities, with potential for several applications, including customized nutrition.

In this case, the proof of concept will involve the incorporation of vitamin D3 (lipophilic). It is expected that the vitamin will bring stability to the emulsion, which would demonstrate promise for the incorporation of other compounds such as calcium (e.g., calcium gluconate, hydrophilic), which is known to have synergistic effects with the vitamin. This could contribute to solving recognized social and health problems related to calcium deficiency, such as osteoporosis.

General objective

The global objective of the work consists of conducting a study on the production of bioparticles to be used in the preparation of Pickering emulsions using natural polymers, able to be processed through 3D printing technology and use the most promising formulations to supplement with bioactive compounds to assess the potential of these systems in customized nutrition applications.

Specifics objectives

Considering the overall goal of developing functional Pickering emulsions for 3D printing focused on customized nutrition, this research project has the following specific objectives:

I. Produce nanoparticles based on chitosan and gelatin using physical interaction methodologies, namely coacervation and ionic gelation, and characterize the properties according to particle size, pH, morphology, and wettability.

II. Optimize stable Pickering emulsions using the prepared particles, diverse oil and water ratios, and particle concentrations. Characterize the systems regarding creaming index, morphology, droplet size, texture, and viscosity.

III. Obtain the 3D printing emulsions and adjust printing parameters to determine the most suitable printing conditions.

IV. Incorporate vitamin D3 in the most promising printable emulsions and measure encapsulation efficiency and emulsion properties, like stability and printability.

V. Analyze and compare the properties determined for the base and loaded emulsions and printed pieces to validate the proof of concept of designing nutritional customized products while maintaining product appearance/consistency and bioactive effectivity.

Dissertation Structure

This dissertation is organized into chapters that cover from an introductory approach to the topic, serving as a foundation for the development of the work, to the results and discussion of the analysed systems, including conclusions and proposals for future work. It is divided into the following chapters:

- Chapter 1 introduces the work developed.
- Chapter 2 refers to the literature review, discussing important definitions for the work developed and relevant analyses for addressing the topic.
- Chapter 3 addresses the description of the materials and methods used throughout the development of the work.
- Chapter 4 addresses the presentation and discussion of the results obtained.
- Chapter 5 pertains to the conclusions of the current study and indications for future work.

Chapter 2

Bibliographic Review

2. Bibliographic review

2.1. Emulsions

Traditional emulsions are composed of an oil and water phase, leading to oil in water (O/W) or water in oil (W/O) emulsions, aided by emulsifiers, responsible for compatibilized both phases over time. More complex systems can also be found, such as double emulsions, represented by oil in water in oil (O/W/O) or water in oil in water (W/O/W) systems. Figure 1 illustrates O/W and W/O emulsions and their use as primary emulsions for preparing W/O/W and O/W/O double emulsions, respectively. These double emulsions are characterized by having a more elaborate structure compared to conventional emulsions. However, due to this complexity, their production and stabilization become challenging (Friberg, 2007). Double emulsions are also known as multiple emulsions, and their formation mainly occurs when the first emulsion is dispersed again in an additional external phase (J. Zhang & Reineccius, 2016).

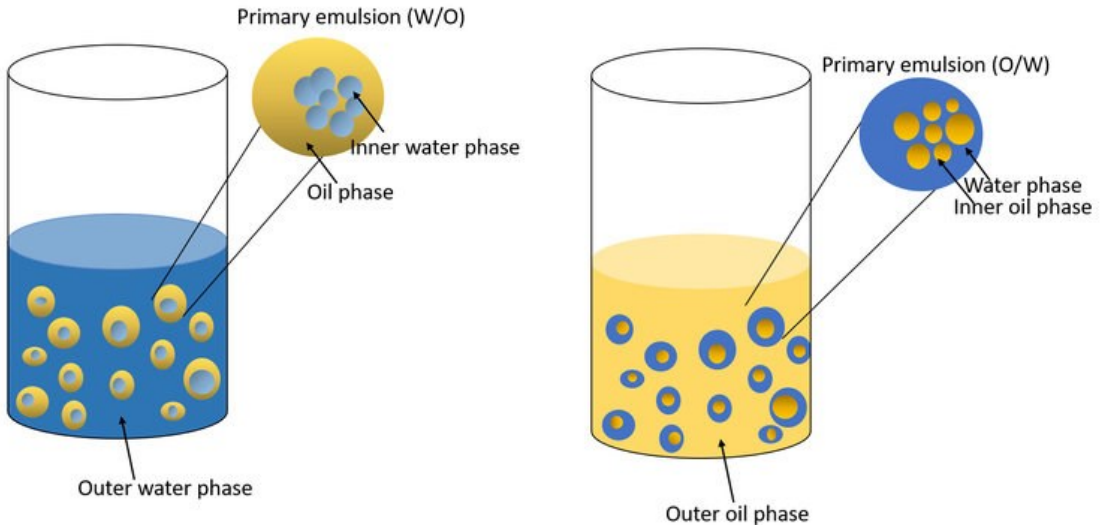


Figure 1 – Types of emulsions. Adapted from Mudrić et al., 2019.

The formation of emulsions is achieved through a homogenization process by mixing two immiscible liquids (mainly oil and water), for which high-pressure homogenizers are typically used. Phase separation tends to occur, with the denser liquid (water) settling below the less dense one (oil). This happens because the mixing of the phases is thermodynamically unfavorable (McClements, 2015). To slow down the rate at which the emulsion destabilizes, it is common to use emulsifiers or thickeners (Benetti, 2018).

Emulsifiers are molecules with surfactant activity that act at the interface of the droplets formed during homogenization. Their primary function is to reduce the interfacial tension, helping to create a protective barrier around the droplet and preventing destabilization during molecular collisions (Benetti, 2018). Emulsifiers have polar and non-polar regions, making them amphiphilic molecules. In recent years, there has been significant industrial interest in using Pickering particles, namely solid particles acting as emulsions' stabilizers, as part of the pursuit of innovation.

The phase in which the emulsifier tends to be more soluble is indeed the continuous or external phase, as described by Bancroft's Rule. In this phase, the emulsifier plays a crucial role in stabilizing the whole system (Schramm, 2005). The hydrophilic-lipophilic balance (HLB) scale is indeed responsible for representing the amphiphilic nature of emulsifiers. HLB values below 6 indicate that the surfactant is classified as lipophilic and tends to form oil-in-water emulsions. On the other hand, HLB values above 10 are interpreted as hydrophilic surfactants that tend to form water-in-oil emulsions. This HLB scale is a valuable tool in formulating emulsions and helps to determine the most suitable emulsifier for a specific application (Shi et al., 2019).

2.1.1. Emulsions destabilization

Emulsions can become destabilized through various physical-chemical mechanisms that alter their properties. Among these processes, notable ones include creaming, flocculation, coalescence, sedimentation, phase inversion, and Ostwald ripening (McClements, 2015). In Figure 2, the most encountered destabilization types are represented.

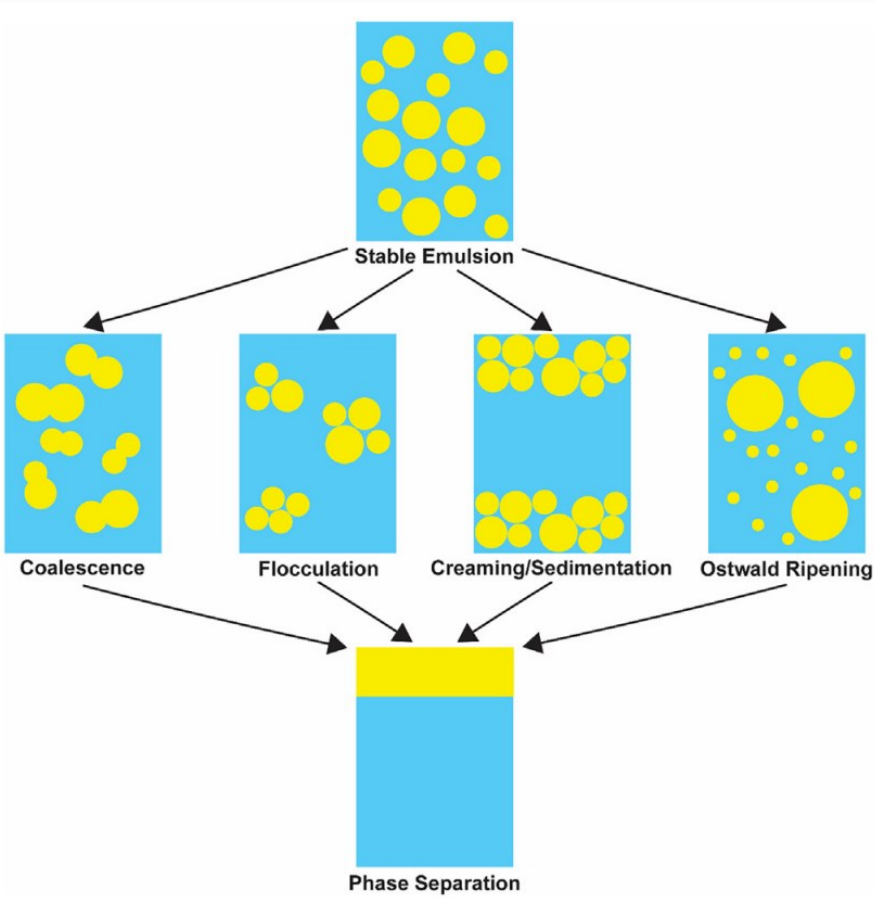


Figure 2 – Type of emulsion destabilization. Adapted from Wilson et al., 2022.

External forces, such as centrifugation and gravity, can destabilize the particles, moving them to the top of the container (creaming) if their density is lower than that of the emulsion or to the bottom (sedimentation) if their density is greater. This occurs due to the chemical and physical characteristics of the materials involved, including their density and viscosity (Damodaran et al., 2010).

Coalescence occurs when there is a rupture of the films surrounding the droplets, leading to the merging or fusion of these droplets into larger ones (Langevin, 2019). Flocculation is the process where droplets accumulate when there isn't enough repulsion to keep them individualized (Tadros, 2009). Ostwald ripening occurs through the merging of smaller and larger droplets, decreasing the number of dispersed droplets (Han et al., 2018). Phase separation occurs when the two phases become entirely or partially differentiated (McClements, 2015).

2.1.2. Pickering emulsions

Pickering emulsions are systems formed by homogenizing two immiscible liquids stabilized by colloidal solid particles. In classical emulsions, the role of the emulsifier is to reduce the interfacial tension, thereby lowering the Gibbs free energy and providing stability to the emulsion. In the case of Pickering emulsions, solid particles are irreversibly adsorbed at the interface between the two liquids, forming a rigid barrier that surrounds the droplets. Like classical emulsions, they reduce the Gibbs free energy, ensuring greater stability for the system (Hu et al., 2016; Lv et al., 2020).

For Pickering emulsions, the particles don't need to be completely soluble in either of its phases. These particles should exhibit an appropriate partial wettability and small droplet size and need to be added at adequate concentrations to ensure the stability of the systems (Chevalier & Bolzinger, 2013). The wettability of the particles can be quantified through Young's equation, defined by the three-phase contact angle (θ) formed between the particle/oil/water using Equation 1, where γρo, γρw, and γow correspond to the interfacial tensions between particle-oil, particle-water, and oil-water, respectively (Berton-Carabin & Schroën, 2015).

$$\cos\theta = \frac{\gamma\rho o - \gamma\rho w}{\gamma ow} \qquad \textbf{(Equation 1)}$$

For values of θ less than 90°, the particles are considered hydrophilic and will form O/W emulsions. Particles with θ > 90° values, W/O emulsions will form considering their more hydrophobic character. Theoretically, to be regarded as a stable Pickering emulsion, the particles should generate angles as close to 90° as possible, where the adhesion at the interface between the liquids is stronger, promoting a more durable stability (Berton-Carabin & Schroën, 2015).

To achieve irreversible adsorption at the interface, particles should have a contact angle (θ) between 30° and 150°, where the energy required for desorption is greater than the thermal energy associated with Brownian motion. Equation 2 is used to calculate the energy (ΔE) needed to remove particles with a known radius (R) from the interface, where γow represents the interfacial tension and θ is the three-phase contact angle (Ning et al., 2020).

$$\Delta E = \pi R^2 \gamma ow(1 \pm \cos\theta)^2 \qquad \textbf{(Equation 2)}$$

The particle concentration is an important factor that directly influences the characteristics of the formed emulsion. When low particle concentration is used, only a tiny interfacial area is stabilized, resulting in instability and the formation of very large particles. At intermediate concentrations, the interfacial region is slightly larger than the area particles can cover, leading to the coalescence effect until the droplets are entirely coated. When enough particles can cover a large interface area at higher particle concentrations, finer emulsions are formed (Chevalier & Bolzinger, 2013b). In this case, it is pursued not to remain "free" particles suspended in the continuous phase, although they can provide a thickening effect, favoring their stability over time.

The drop size is inversely proportional to the number of particles, and this phenomenon is called "limited coalescence," which helps control the droplet size. To demonstrate that the homogeneous particle size distribution is proportional to the particle mass and the droplet coverage, Equation 3 can be applied (Albert et al., 2019; French et al., 2015).

$$\frac{1}{D} = \frac{mp}{6\ x\ C\ x\ pp\ x\ Vd} \frac{ap}{\vartheta p} \qquad \textbf{(Equation 3)}$$

Where C is the surface coverage, pp is the particles' density, Vd is the dispersed phase volume, ϑp is the particles' volume, D is the final droplet diameter, ap is the particles' area and mp is the particles' mass.

2.1.3. Pickering stabilizers

For the preparation of Pickering emulsions, polysaccharides, proteins, food fats, flavonoids, and composite particles are often used (Meng et al., 2023). Among them is to be highlighted chitosan (CH), a linear cationic polysaccharide derived from the deacetylation of chitin, which is found in the exoskeleton of crustaceans, the epidermis of insects, and the cell walls of some fungi. This polymer is described as a potent bioactive macromolecule with antibacterial, antifungal, and gel-forming properties (Sharkawy et al., 2021). Chemically, chitosan is a hydrophilic polymer composed of β-(1→4) D-glucosamine and N-acetyl-D-glucosamine, as shown in Figure 3.

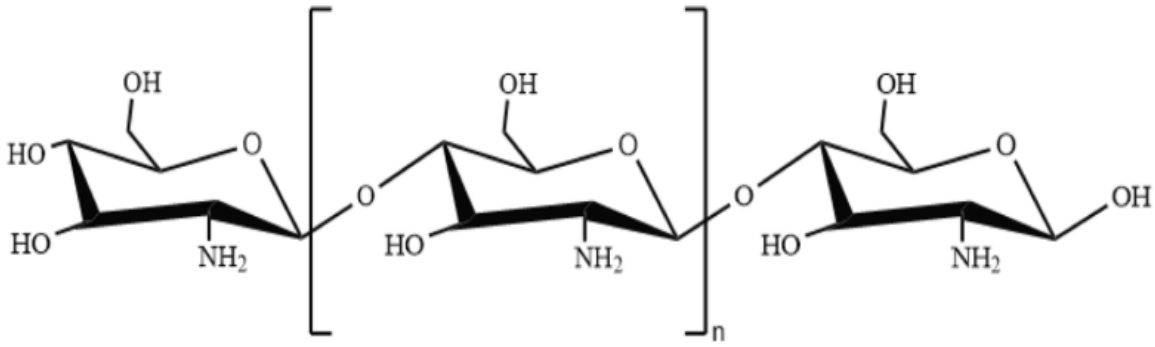


Figure 3 - Structure of chitosan. Adapted from Meng et al., 2023.

The presence of hydrophilic amino groups in the deacetylation process of chitosan imparts water solubility characteristics in an acidic medium, distinguishing it from chitin, which is highly insoluble in water (Elieh-Ali-Komi & Hamblin, 2016). Due to chitosan's hydrophilicity, several methods can be employed to address the preparation of particles focused on oil/water emulsions. The main approaches include self-aggregation, ionic gelation, polyelectrolyte complexation, and hydrophobic modification of chitosan, commonly used to achieve chitosan-based particles with the potential to provide stable emulsions (Tabatabaei et al., 2022).

Chitosan undergoes a deprotonation process at alkaline pH, which impedes the formation of colloidal particles (Meng et al., 2023). By contrast, when protonated in an acidic medium, ionic gelation, a process that occurs due to the ionic attraction between the protonated amino groups and anionic groups (derived from another compound), is employed (Shah et al., 2016). Among the biopolymers able to provide the anionic functional groups to the formulation, gelatin type B (GB) can be highlighted. GB has a high surface activity but tends to dissolve quickly in an aqueous medium(H. Liu et al., 2014).

Gelatin type B is produced from the partial hydrolysis of collagen at an alkaline media, with numerous benefits, including biodegradability, non-toxicity, and low cost (Ahmed et al., 2020; D. Liu et al., 2015). Gelatin is rich source of proline, hydroxyproline, and glycine in its polymeric chain structure Figure 4. Type B gelatin exhibits high surface activity, emulsifying oil holding viscoelastic properties while showing promising affinity in aqueous medium (Roy et al., 2017). As an example, it has been reported the microencapsulation capacity of chitosan/gelatin-based formulations allows the entrapment of 50% of the core substance with an encapsulation efficiency of 66% when using a 1:1 ratio (Hussain & Maji, 2008). In addition, the utilization of gelatin has been expanded to cover advanced techniques like additive

manufacturing (AM) during the last decade. Three-dimensional (3D) printing (3DP) is among the most recognized AM methods, facilitating the bio-fabrication of tissues and organs, as well as the printing of food additives and food packaging materials (Ahmed et al., 2020).

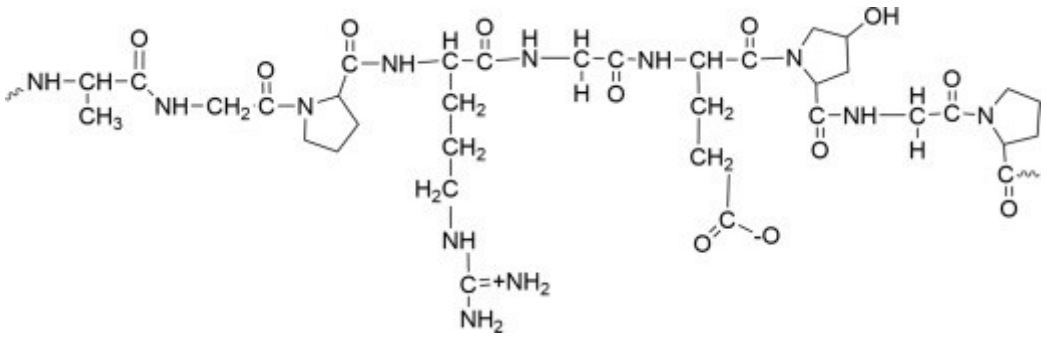


Figure 4 - Structure of gelatin. Adapted from (Verma et al., 2021).

2.2. 3D Printing in food applications

The three-dimensional printing technology, also known as additive manufacturing and rapid prototyping (RP), has been garnering increasing attention and interest from researchers, industries, and the public due to its diverse applications in areas such as medicine, gastronomy, engineering, manufacturing, art, and education (Murphy & Atala, 2014). Notably, this technology allows the creation of objects layer by layer from a 3D design program and is opening new perspectives in the customization of food products (Dankar et al., 2018).

In gastronomy, 3D food printing is a rising field that challenges the boundaries of creativity (Kietzmann et al., 2015). Foods are complex in physicochemical properties, but researchers have been working on expanding the applications of 3D printing to diversify food products. This includes the printing of chocolate, cookie dough, cereals, processed cheese, meat gels, and even fruits and vegetables (Hamilton et al., 2018; Hao et al., 2010; Holland et al., 2018; Lipton et al., 2010; Tudorache & Bala, 2007; Yang et al., 2018).

Furthermore, 3D food printing technology has the potential for "Innovation Democratization," enabling independent designers and a new economy of customized products to thrive (Periard et al., 2007). However, challenges still need to be overcome, such as optimizing the printing process, especially for food, and understanding the limitations aiming to adopt strategies to be surpassed (Gurrala & Regalla, 2014).

While reviews on 3D food printing have been conducted, a real knowledge gap exists in the relationship between the critical process variables and the materials' structure (Severini,

Derossi, et al., 2018). Thus, optimizing the food printing process and specific substrate control are areas that have received attention to expand the possibilities of this technology, which is also applied to emulsions-based products.

2.3.Customized nutrition

Customized nutrition, driven by technological advancements like 3D food printing, is emerging as an innovative way to enhance the quality of our diet and promote well-being. A revolutionary concept called "Edible growth" involves printing layers of food using a customized 3D printer (Baiano, 2022).

Additional studies have explored 3D printing to enhance products such as lemon juice gel, vegetable puree, cheese, ground meat, and dairy (Le Tohic et al., 2018; Z. Liu et al., 2018; Ross et al., 2019; Severini et al., 2018; L. Wang et al., 2018). Innovation continues with the 3D-printing of nutritious snacks, using composite millet flour, green gram, fried gram, ajwain seeds, and cookies with altered chewing times to influence satiety perception (Keerthana et al., 2020). For example, Japan has developed a 3D food printing system called "FoodFab," which allows controlling the food intake by adjusting the internal density of foods to affect chewing time and, consequently, the perception of satiety (Lin et al., 2020).

Furthermore, personalized meal customization with digital technology is gaining interest, even involving companies like Singapore's Anrich 3D and Universities such as Nanyang Technological University, offering 3D food printing solutions based on mathematical models to cater to individual nutritional and aesthetic needs (Eswaran et al., 2023).

In this context, customized nutrition is gaining relevance as a new and current field by incorporating bioactives with a specific role in humans' health, particularly in the area of emulsions. Few works are found in literature relating both concepts (i.e., 3 articles are recognized in the SCOPUS Database when "customized nutrition" AND "emulsions" terms are searched, 13/10/2023). This fact demonstrates the importance of this work in developing knowledge and innovation in a field with great potential that encompasses both nutrition and technology.

Customized nutrition involves the possibility of delivering specific dosages of bioactive compounds to certain people through strategies. As an example of these compounds, vitamin D3 is responsible for favoring osteogenesis, calcium homeostasis, and immune responses of the body, among others (Harrison et al., 2020). Concurrently, it is known that

vitamin D3 combined with other compounds can provide synergistic activities to promote their effectiveness and, as a consequence, the well-functioning of the organism. For instance, some works demonstrated that combining vitamin D3 with calcium source compounds, namely calcium gluconate, can promote calcium absorption, an essential stage in bone metabolism (Liao et al., 2017).

Concurrently, the combination of compounds involves the need for compartmentalized structures that are able to maintain both compounds of different hydrophobic/hydrophilic natures, as is the case of vitamin D3/calcium sources, in the same product. To this purpose, Pickering emulsions offer a high versatility for customized nutrition systems, having the possibility to be entirely produced from natural compounds, opening a new framework to design novel 3D printable formulations to be applied in customized nutrition products.

Chapter 3

Materials and Methods

3. Materials and Methods

For the preparation of the emulsions, chitosan 90/200/A1 produced by Biotechnologie GmbH, with a viscosity of 91.9% (1% at 20°C and 1% AcAc solution), and type B gelatine (SIGMA-ALDRICH, 100%) were used. Acetic acid purchased from Honeywell Fluka™ (Germany) was used to prepare the acid solutions to dissolve the chitosan. Miglyol 812N oil (density 0.96g/mL, Acofarma) was used to prepare the Pickering emulsions. The bioactive compound incorporated was vitamin D3 99% (Alfa Aesar). The water used to dissolve the gelatine was treated in the Milli-Q water purification system.

3.1.Nanoparticles preparation

The nanoparticles were prepared following the methodology described in the work of Tan et al, 2016 with some modifications. The solutions of chitosan and type B gelatine were prepared individually. The particles were prepared at concentrations of 5.5% and 6% (w/v). For that, the chitosan was dissolved in 0.3 M acetic acid using a magnetic stirrer at 650 rpm for 2 hours at room temperature until it was completely dissolved. Type B gelatine was dissolved in deionized water at room temperature using a magnetic stirrer at 400 rpm for 30 minutes.

The CH:GB solution was prepared by mixing the two solutions using a peristaltic pump (Ismatec) with a flow rate of 9 mL/min. The mixture was kept under stirring for a further 1 hour at 600 rpm to allow the chitosan and gelatine to complex. The pH of the mixture was adjusted to 5.5 using NaOH or HCl in concentrations ranging from 0.1 to 1 M.

3.2.Characterization of particles

3.2.1. pH measurement

The pH of the particle's dispersion was be determined using an Inolab pH 720, WTW pH-meter. The measurements were done in triplicate at room temperature.

3.2.2. Formation of coacervation complex

Fourier transform infrared spectroscopy (FTIR) was used to analyze the formation of the CH/GB coacervates. The spectra were collected on an ABB Inc. FTIR, model MB3000 (Quebec, Canada), operating in ATR mode using an ATR cell equipped with a diamond crystal. The spectra acquisition was done by co-adding 32 scans, with a resolution of 16 cm^{-1}, between 4000 – 550 cm^{-1}. To process the data, Horizon MB FTIR software was used.

3.2.3. Wettability

The contact angle of the particles was assessed using the optical contact angle measuring device (OCA15 plus, Dataphysics, Germany) (Figure 5), which aims to evaluate the hydrophilic/hydrophobic nature of the samples. For this evaluation, the produced particles were deposited on a glass slide and dried at room temperature. The dried film was placed on the platform of the device and then 5 µm of deionized water was deposited using a high-precision injector. The images were taken using a digital camera attached to the equipment and the measurements were taken automatically by the equipment's software.

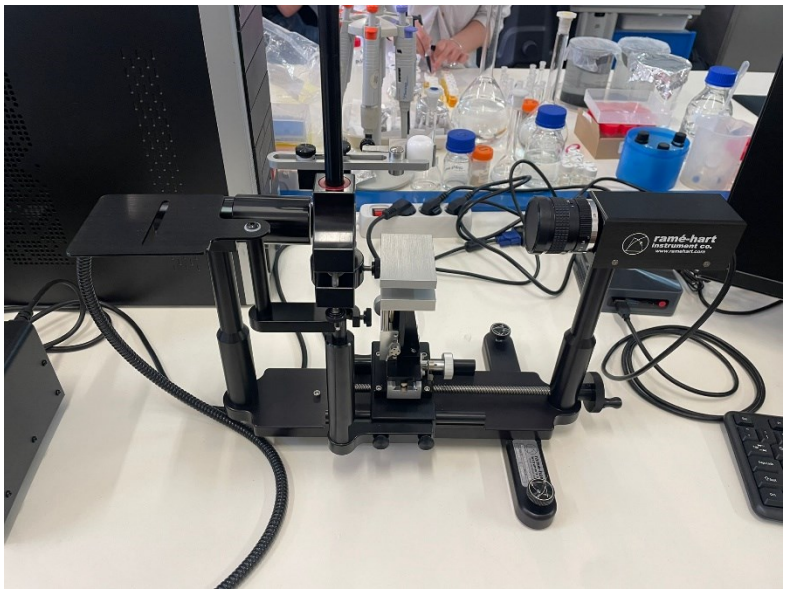


Figure 5 – Goniometer used to determine the contact angle of the particles.

3.3. Preparation of Pickering emulsions

Pickering emulsions contain the basic properties of conventional emulsions stabilized by surfactants (emulsifiers). It is essential that it remains stable over time against destabilization phenomena (coagulation, coalescence, flocculation) and that the emulsification process is possible (Chevalier & Bolzinger, 2013a).

A stator rotor was used to reduce particle size and achieve homogenization. The stator rotor consists of a rotor with blades and an open stator (Albert et al., 2019b). The rotation speed and homogenization time are of great importance to obtain a Pickering emulsion with relevant characteristics and properties. The rotation parameters used to produce emulsions varied between 5000 and 30000 rpm (Maa & Hsu, 1996).

The use of the rotor-stator has numerous advantages. The most important of which is the low operating cost and the disadvantages are the high shear rate generating an increase in temperature, which can destabilize the emulsion (Bröckel et al., 2013; Thompson et al., 2011). In Pickering emulsions preparation, the CH:GB mixture (at concentrations from 2% to 6%) was homogenised, mixed with the oil using a peristaltic pump at a 40/60, 50/50 and oil/water ratio, and then homogenized at a rotation speed of 13500 rpm for 6 minutes.

The incorporation of vitamin D3 was carried out in the oil phase at a concentration of 1% w/v. The preparation procedure was the same as that described for the base emulsion without the presence of the vitamin.

3.4.Characterization of Pickering emulsions

3.4.1. Optical microscopy

Optical microscopy analysis was carried out using a Nikon Eclipse 50i optical microscope (Kawasaki, Japan) equipped with a Nikon Digital Sight camera. The images were obtained at magnifications of 200x and 400x, being used to evaluate and monitor the morphology and size of the particles over time.

3.4.2. Emulsion type

The method used to determine the type of emulsion was that cited by (Feng & Lee, 2016; Lv et al., 2020b). Two containers were used, one containing distilled water and the other oil. A drop of the emulsion was added to each container to assess its solubility. By visual analysis, if the emulsion dissolves in the oil, it will be classified as a W/O emulsion; otherwise, it will be considered an O/W emulsion.

3.4.3. Creaming index

The creaming index is used to indicate the stability of the emulsions and was determined as described by Nikolovski et al. (2016) with modifications. The method involves assessing the stability of the emulsion by measuring phase separation, which includes determining the serum (aqueous) layer and the emulsion layer. After preparing the emulsions, they were transferred to 25 mL graduated cylinders, sealed to prevent evaporation and kept at room temperature for 30 days. The creaming index (CI) was calculated as the ratio between the height of the aqueous layer (Hs) and the total height (Ht), expressed in percentage, as described in Equation 4.

$$CI(\%) = \frac{Hs}{Ht} \times 100 \qquad \textbf{(Equation 4)}$$

3.4.4. Droplet size

The DLS Mastersizer 3000 equipped with a Hydro MV unit, Malvern Instruments (Worcestershire, UK), was used to quantify the size and distribution of the emulsion droplets by laser diffraction. The samples were diluted in deionized water and introduced in the equipment using a Pasteur pipette. Analyses were carried out on the four most promising formulations with an average of 5 measurements at room temperature, obtaining the particle size in volume and number for 10, 50 and 90% of the sample distribution, D10, D50 and D90, respectively.

3.4.5. Encapsulation efficiency of bioactive compounds (vitamin D3)

The encapsulation efficiency was measured according to Zhang et al., (2022) with some modifications. A calibration curve was carried out by dissolving vitamin D3 in hexane (Carlos Erba, 99%) which was stirred at 300 rpm for 2 hours. Linear regression calculations were carried out using Excel software to determine the linear equation.

To determine the encapsulation efficiency of vitamin D3, 5 mL of the freshly prepared vitamin D3 emulsion was added to a Falcon tube, dissolved in 3 mL of ethanol, vortexed for 2 minutes, after which 4 mL of hexane were added, and the sample vortexed again for 2 minutes. After stirring, the sample was centrifuged (Eppendorf, centrifuge 5810R) at 14,000 rpm for 15 minutes. After destabilization, the supernatant hexane was extracted and analyzed by ultraviolet-visible (UV-vis) spectrophotometry (UV-Visible Spectrophotometer V-730 Jasco Inc) at 265 nm. This process was carried out in triplicate. The base solution was the supernatant of the emulsion contain vitamin D3 in its composition.

The encapsulation efficiency (EE) of vitamin D3 was determined according to Equation 5, considering the quantity of vitamin D3 incorporated into the emulsion and the quantified one as determined by UV-Vis.

$$EE\ (\%) = \frac{Total\ vitamin\ D3 - quantified\ vitamin\ D3}{Total\ vitamin\ D3} \cdot 100$$

(Equation 5)

Where the total vitamin D3 is the concentration of vitamin incorporated into the emulsion, and the quantified of vitamin D3 is the value found after destabilization.

3.4.6. 3D Printing

The prepared emulsions were printed using a REGEMAT 3D BIO V1 3D printer (Figure 6). Two printing shapes (cube and cylinder) were selected for printing the optimized emulsions, which were developed and supplied by the printer manufacturer. The parameters followed in printing models were extrusion speed, height, width, length and others. For the 9-layer cube print, the parameters used were 2 mm height, 18 mm width, 18 mm length, 3x3 mm pores, 0.35 mm layer height and 3 mm/s flow speed. For printing a cylinder with 21 layers, the parameters used were a height of 7 mm, a diameter of 10 mm, pores of 1x1 mm, a layer height

of 0.25 mm and a flow speed of 3 mm/s. The prints were made on a 0.41 mm nozzle and the ambient temperature was controlled at 21 °C.

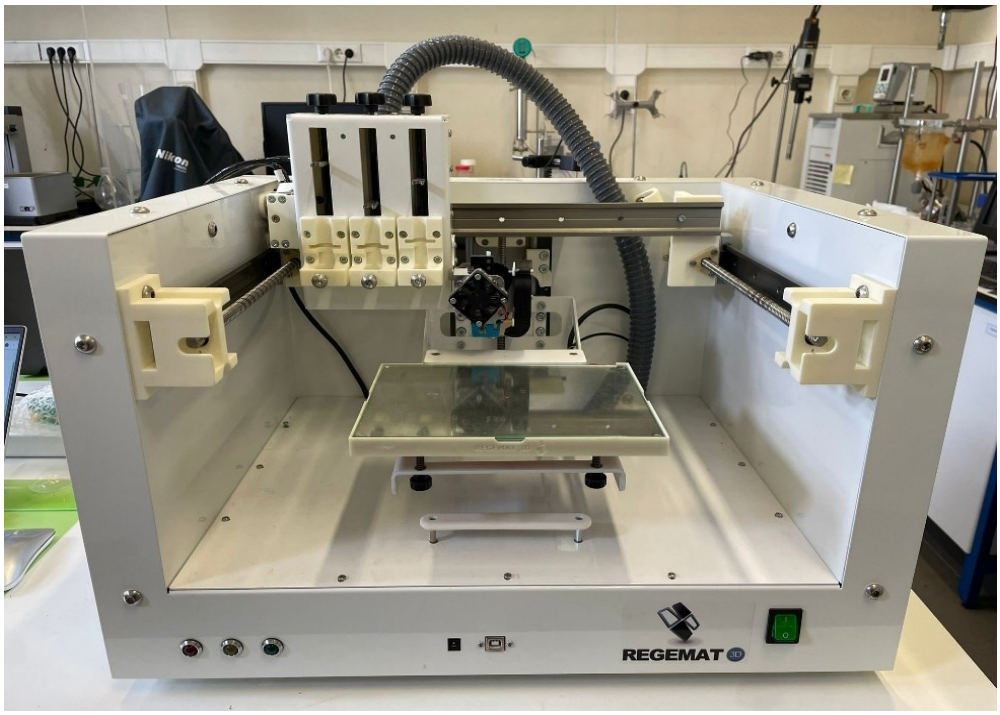


Figure 6 - 3D printer used for analysis.

3.4.7. Texture analysis

The texture analysis of the optimized emulsions was carried out using the TA - XT plus texture analyzer from Stable Micro Systems (Vienna Court, Godalming UK) (Figure 7), equipped with a 5 kg load ballot, according to the method of Roriz et al. (2020) with some modifications. The test was carried out on the compression model, measuring the following parameters: hardness, elasticity, cohesion, gumminess, and resilience. A 45 mm P/45 aluminum probe and a rear extrusion cell with a 35 mm diameter compression disc were used. The samples were printed with a height of 1 cm and a diameter of 2 cm and kept in the freezer for 24 hours before the test. The distance set was 5 mm and the trigger mode was set at 5 g. The samples were compressed twice with an interval of 3 seconds between them, and the test speed was 5 mm/s. The results were obtained using Exponent software version 6.1.11.0, proprietary to Stable Micro Systems.

Figure 7 - Texture analyzer.

Chapter 4

Results and Discussion

4. Results

Preliminary tests were carried out with chitosan dissolved in 0.3 M acetic acid and subsequently mixed with type B gelatin. The Pickering emulsions were prepared with Mygliol 812N oil. Based on these tests, the most promising formulations were defined and optimized for printing and subsequent incorporation of the bioactive compound.

4.1. Emulsions formulation

Numerous tests were carried out varying the concentration of CH/GB particles, pH, acetic acid concentration and O/W ratio in order to determine a formulation with potential to be printed (Table 1). The emulsions were coded as E (emulsion) - percentage of CH/GB particles.

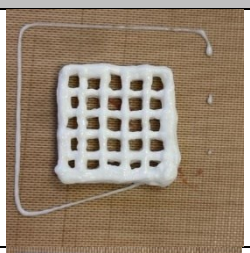
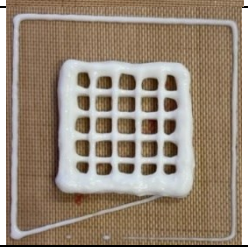
Table 1 - Tests carried out to define formulations to be optimized.

Formulations	Acetic acid (mol)	pH	O/W ratio
E1 - 2%	0.1	5	60/40
E2 - 2%	0.1	5	50/50
E3 - 2%	0.1	5.5	60/40
E4 - 2%	0.1	5.5	50/50
E1 – 3.5%	0.3	5	60/40
E2 – 3.5%	0.3	5	50/50
E3 – 3.5%	0.3	5.5	60/40
E4 – 3.5%	0.3	5.5	50/50
E1 - 4%	0.3	5	60/40
E2 - 4%	0.3	5	50/50
E3 - 4%	0.3	5.5	60/40
E4 - 4%	0.3	5.5	50/50
E2 – 4.5%	0.3	5	50/50
E5 - 4.5%	0.3	5	40/60
E4 – 4.5%	0.3	5.5	50/50
E6 – 4.5%	0.3	5.5	40/60
E6 - 5%	0.3	5.5	40/60
E5 - 5%	0.3	5	40/60
E6 – 5.5%	0.3	5.5	40/60
E5 – 5.5%	0.3	5	40/60
E6 - 6%	0.3	5.5	40/60
E5 - 6%	0.3	5	40/60

Initial tests showed that the emulsions prepared at pH 5 were unable to maintain their structure after printing; these samples had a more liquid appearance compared to the samples with a pH of 5.5. When analysing the influence of the O/W ratio on the final printing result, it was possible to observe that the samples with an oil ratio of 60/40 exuded oil when printed, effect not observed in the emulsions at 50/50 and 40/60. Increasing the concentration of particles directly influenced the firmness of the emulsion, so the higher the concentration, the harder the emulsion.

As a result, the samples with a pH of 5.5 and a ratio of 40/60 showed satisfactory results, being firmer, more defined and also able to maintain their structure after printing. According to the objective of the work to produce Pickering emulsions with potential for nutrition applications, it was decided to use lower oil concentrations to balance the nutritional values. The formulations with the best results are shown in Table 2.

Table 2 - Result of the Pickering emulsions produced.

Formulations	Solids content (Ch/GB 1:1 m/m)	pH	O/W	Printed emulsion
E6 – 5.5%	5.5%	5.5	40/60	
E6 – 6%	6%	5.5	40/60	

4.2. Particle formation and characterization

4.2.1. Analysis of the coacervates formation

The formation of the coacervation complexes was analysed using FTIR. Figure 8 shows the spectrum of CH/GB coacervates. The CH peak present at 1649 cm^{-1} and 1571 cm^{-1} is caused by C = O stretching (Amide I) and -NH bending (Amide II), respectively. Analysing

the GB spectrum shows bands in the 1635 – 1402 cm^{-1} range, representing the secondary characteristic of amide groups. The evidence of CH:GB complexation is represented by the bands present at 1652 cm^{-1} and around 1409 cm^{-1}, caused by $-NH_3^+$, which represents an angular deformation of chitosan and a stretching of the COO^- of gelatin, respectively.

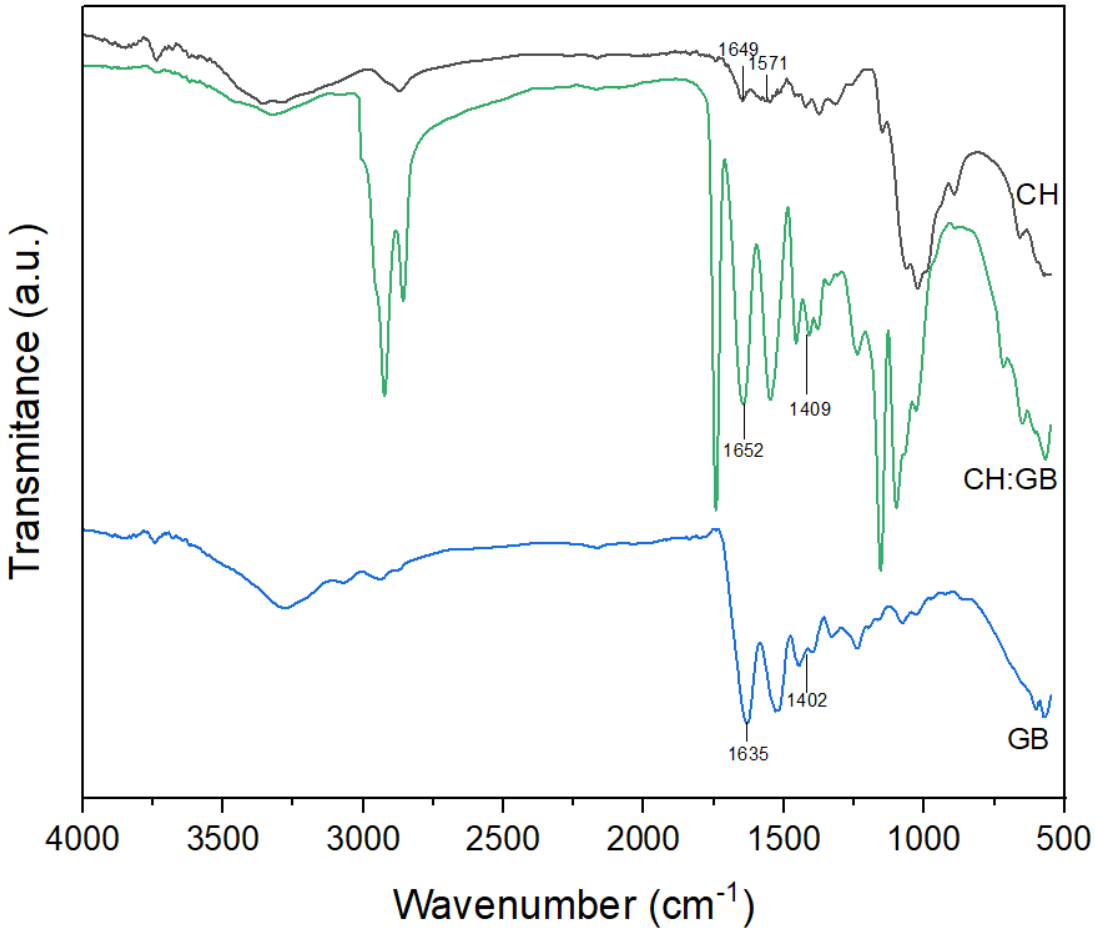


Figure 8 - FTIR Ch/GB formation of coacervation complexes.

4.2.2. Contact angle

The results of the particle contact angle test are shown in Figure 9. The contact angle values can be used to estimate the type of Pickering emulsion that can be formed. Typically, for hydrophilic particles the contact angle is lower (< 90°), and they tend to stabilize O/W emulsions. Particles with a larger contact angle (> 90°) are hydrophobic particles with greater potential for W/O emulsions. (Dickinson, 2017; Sharkawy et al., 2019).

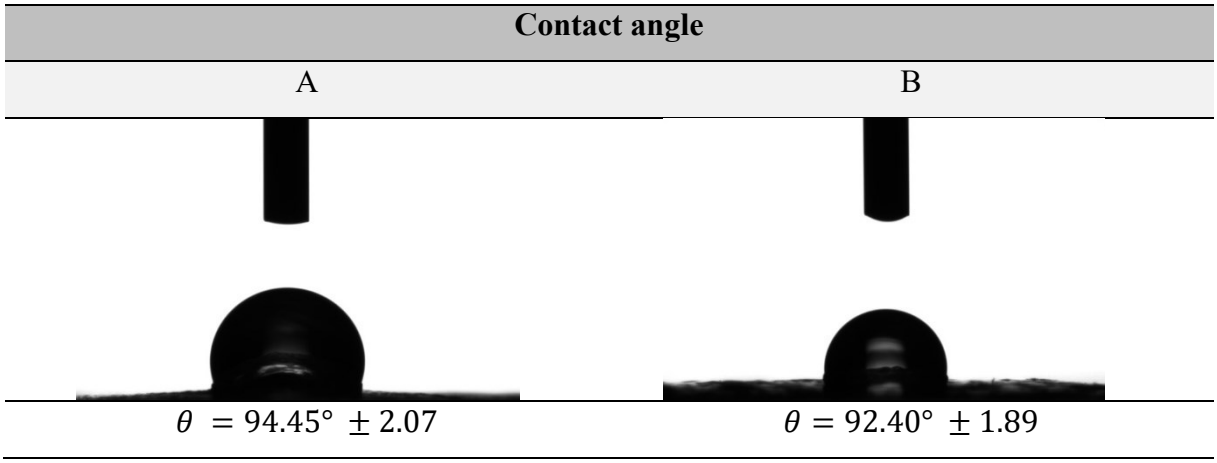


Figure 9 - Contact angles of the produced particles, A) Particle 5.5% and B) Particle 6%.

According to Binks (2002), particles with contact angle closer to 90° are able to generate more stable emulsions. As the values found are between 30° and 150°, this presupposes that the particles tend to be irreversible adsorbed at the O/W interface, meaning that the desorption energy is many times greater than the thermal energy of Brownian movement (Xiao et al., 2016). With the obtained results, it can be understood that the produced particles are hydrophobic.

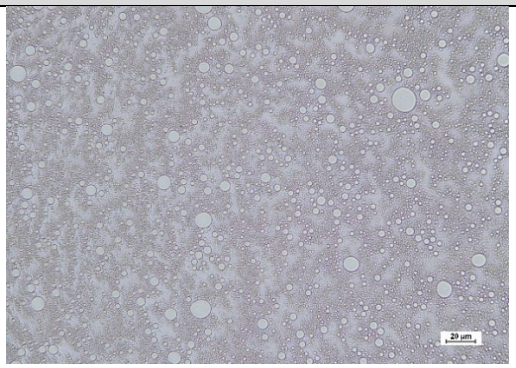
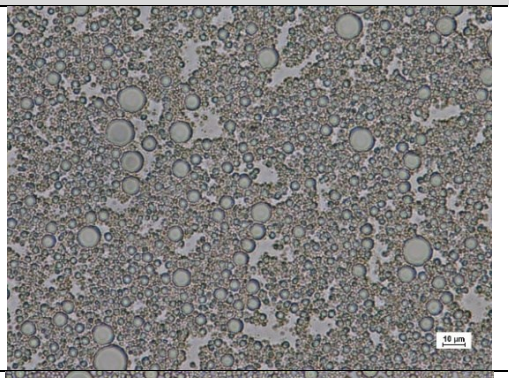
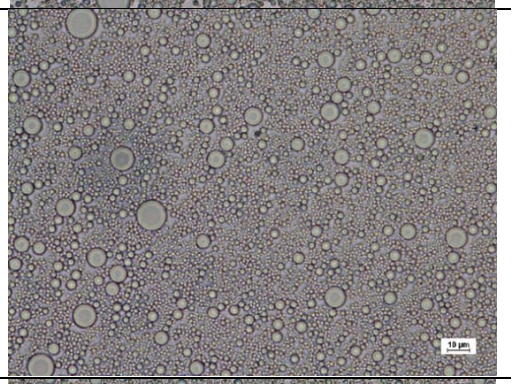
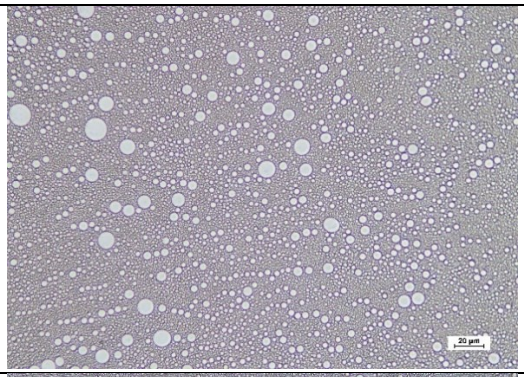
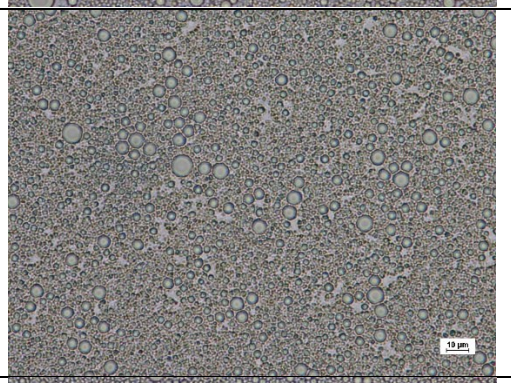
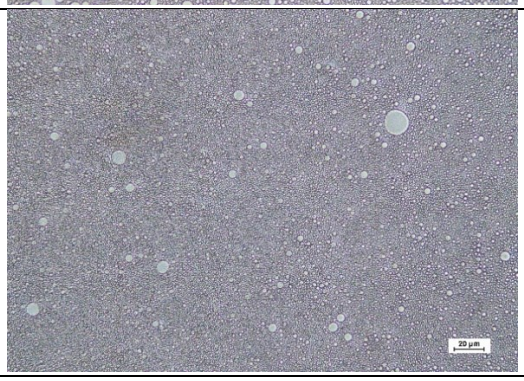
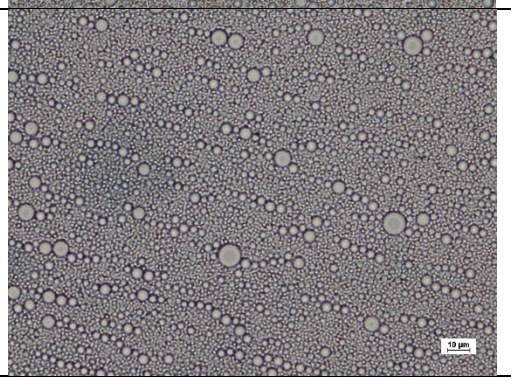
4.3. Stability of emulsions

The stability of Pickering emulsions was evaluated over a period of 30 days based on optical microscopy analysis, aiming to assess changes in the emulsion morphology and also by determining the creaming index (CI%).

4.3.1. Optical microscopy analysis

The results obtained from the microscopic analysis are shown in Table 3.

Table 3 - Morphology of the produced Pickering emulsions.

Formulation	200x	400x
E6 – 5.5%		
E6 – 6%		
E6 – 5.5%D		
E6 – 6%D		

Through microscopic analysis, it was possible to observe the formation of a Pickering emulsion with a non-uniform spherical standard size. It is known that higher concentrations of particles result in smaller droplet sizes, which is confirmed by comparing the emulsions with

concentrations of 5.5% and 6% (Chen et al., 2020). The incorporation of the vitamin brought greater uniformity in droplet size compared to the emulsions without the vitamin.

4.3.2. Drop test

Right after the emulsion production, a drop test was conducted to identify whether the emulsion formed was O/W or W/O. Visual analysis showed a rapid dispersion of the emulsion with concentration 5.5% and 6% in water, which is characteristic of O/W emulsions, as shown in Figure 10.

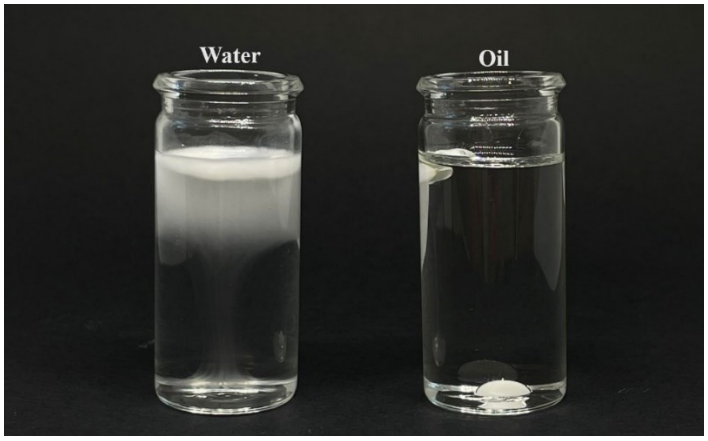


Figure 10 - Drop test.

4.3.3. Creaming index

Emulsions consist of an oil and an aqueous phase, presenting different densities. It is known that phase separation can occur, with oil droplets tending to rise to the top of the container while the aqueous phase becomes accumulated at the bottom (Krebs et al., 2013). Creaming index analysis was conducted over a period of 30 days. All four samples under study were analysed. The results show that no phase separation was observed at the two analysed time points, as depicted in Table 4. The results demonstrate great potential for these Pickering emulsions, which showed a CI of 0% during the evaluation period, indicative of high stability.

Table 4 - Results of creaming index.

Time (days)	E6 – 5.5%	E6 – 5.5%D	E6 – 6%	E6 – 6%D
0				
30				

4.3.4. Particle size

The particle sizes were analysed at T0, T7, and T30, corresponding to days 0, 7, and 30, respectively. The analyses were conducted in number and volume by DLS, determining the at D10, D50, and D90, corresponding to 10, 50, and 90% of particle volume or number, respectively.

Pickering particles have a significant influence on emulsion properties. When particles of large size are present, this implies forces that distinctly affect the formation of a stable emulsion (Wu & Ma, 2016). Conversely, emulsions produced with smaller particles exhibit better stability, mainly due to greater surface contact area (Li et al., 2013).

The volume sizes D10, D50, and D90 presented in Table 5 for the emulsions proved to be promising particles, with a variation between 1.85 to 1.98 µm, thus indicating a great

potential for Pickering emulsion production. These values represent the values analysed over the 30-day period.

Table 5 – Droplets size distribution of the emulsions in volume.

	Day 0			Day 7			Day 30		
Formulation	**D10 (µm)**	**D50 (µm)**	**D90 (µm)**	**D10 (µm)**	**D50 (µm)**	**D90 (µm)**	**D10 (µm)**	**D50 (µm)**	**D90 (µm)**
E6 – 5.5%	1.08 ± 0.01	1.89 ± 0.3	3.50 ± 0.02	1.03 ± 0.00	1.90 ± 0.00	10.6 ± 0.38	1.05 ± 0.00	1.93 ± 0.00	8.50 ± 0.35
E6 – 5.5%D	1.07 ± 0.00	1.85 ± 0.00	3.35 ± 0.01	1.05 ± 0.00	1.85 ± 0.00	5.56 ± 0.24	1.04 ± 0.00	1.90 ± 0.00	8.19 ± 0.65
E6 – 6%	1.07 ± 0.00	1.88 ± 0.00	3.65 ± 0.03	1.00 ± 0.00	1.92 ± 0.00	28.8 ± 1.83	1.01 ± 0.00	1.95 ± 0.00	33.9 ± 2.02
E6 – 6%D	1.08 ± 0.00	1.88 ± 0.00	3.43 ± 0.01	1.02 ± 0.00	1.94 ± 0.00	18.8 ± 0.73	1.02 ± 0.00	1.98 ± 0.00	35.5 ± 2.05

The volume distribution profile of the samples with a particle concentration of 5.5% is shown in Figures 11 and 12. It can be seen that the E6 - 5.5% samples remained stable in size over time, and Table 5 shows that when analysing the D50 there was a variation from 1.89 to 1.93. The emulsions with the presence of vitamin (E6 - 5.5%D) also obtained values similar to those presented by the formulation without the incorporation of the compound, however they obtained lower values ranging from 1.85 to 1.90.

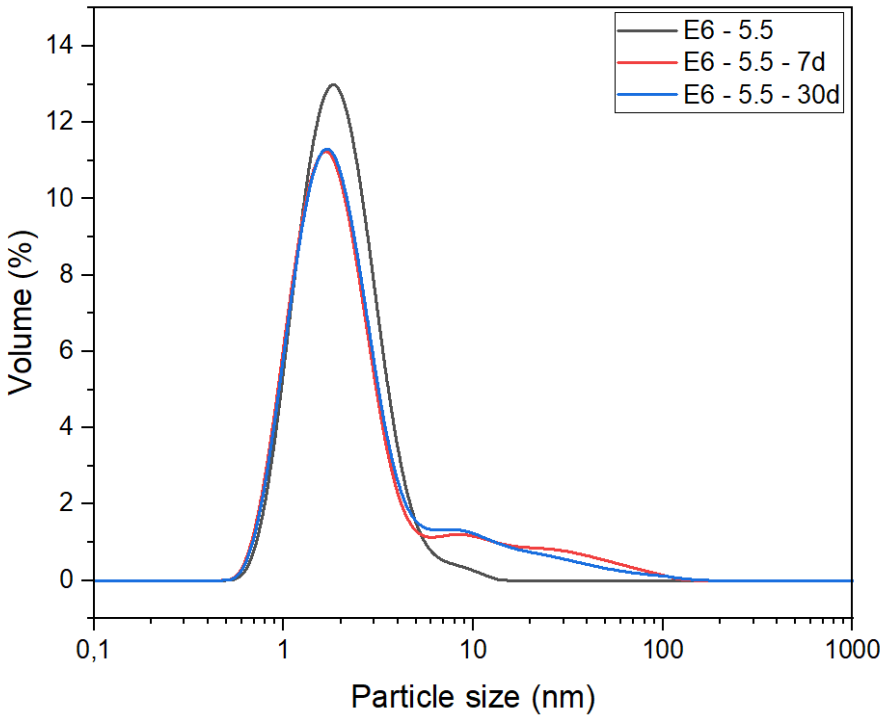


Figure 11 - Particle size distribution in volume for emulsions with 5.5% of particles at pH of 5.5.

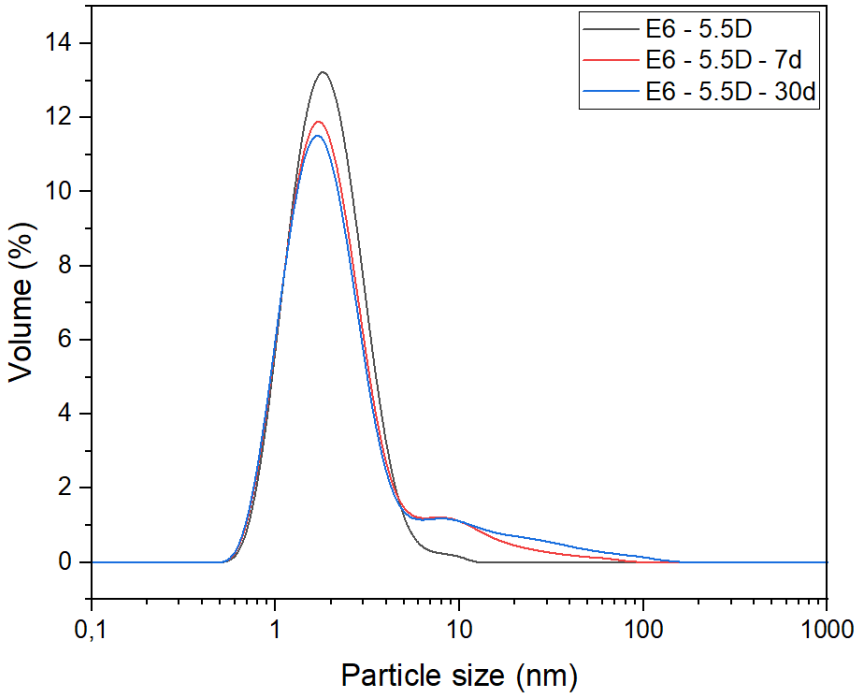


Figure 12 - Particle size distribution in volume for emulsions with 5.5% of particles at pH of 5.5. loaded with vitamin D3.

Regarding the formulations with a 6% concentration (Figures 13 and 14), analysing Table 5, we can observe that the E6 – 6% formulation showed a small variation over the analysis period, ranging from 1.88 to 1.95 µm, while E6 – 6%D had similar values, ranging from 1.88 to 1.98 µm. From the results, it is possible to observe that the emulsions with a 6% concentration achieved slightly higher values compared to the 5.5% concentration.

Analysing the incorporation of the vitamin, E6 – 5.5%D had slightly lower values compared to E6 – 5.5%, with the largest variation being 1.90 and 1.85 µm, respectively. On the other hand, E6 – 6%D had slightly higher values compared to E6 – 6%, with the largest variation being 1.98 and 1.95 µm.

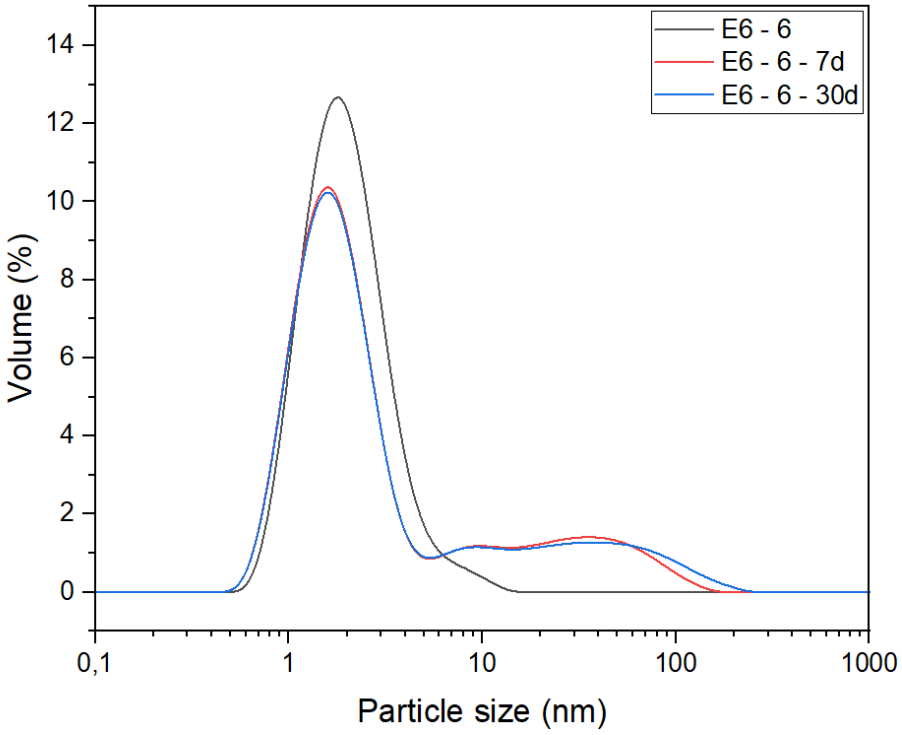


Figure 13 - Particle size distribution in volume for emulsions with 6% of particles at pH of 5.5.

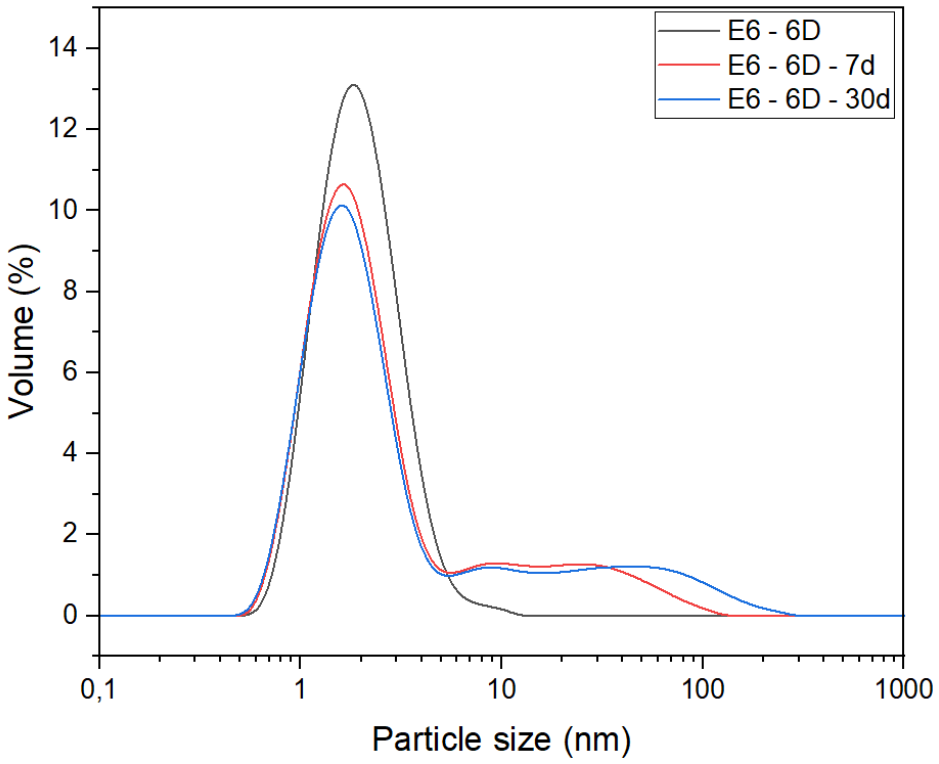


Figure 14 - Particle size distribution in volume for emulsions with 6% of particles at pH of 5.5 loaded with vitamin D3

Analysing the particle size in number (Table 6), it is possible to verify that there was no difference between the samples with a 5.5% concentration of particles, which had a distribution value of 1.09 µm. However, the emulsions with a 6% concentration of particles resulted in a small variation, with E6 – 6% having a value of 1.04 µm and E – 6%D having 1.06 µm, this difference is not a great variation.

Table 6 – Droplet size distribution of the prepared emulsions.

Formulation	D10 (µm)	D50 (µm)	D90 (µm)
E6 - 5.5%	0.75 ± 0.03	1.09 ± 0.05	1.54 ± 0.45
E6 - 5.5%D	0.75 ± 0.02	1.09 ± 0.04	1.82 ± 0.06
E6 – 6%	0.71 ± 0.06	1.04 ± 0.08	1.73 ± 0.14
E6 – 6%D	0.73 ± 0.05	1.06 ± 0.08	1.77 ± 0.14

4.3.5. Encapsulation efficiency

The encapsulation of vitamin D3 through Pickering emulsion is important for solving issues such as the compound's poor solubility in water and the protection provided by the Pickering emulsion, shielding the vitamin from heat, light, and oxygen which can cause rapid degradation (Abbasi et al., 2014). To analyse the encapsulation efficiency of vitamin D3, a calibration curve of known concentrations of vitamin D3 in UV-Vis was performed, correlating the obtained results with the known concentrations.

The calibration curve was constructed in the concentration ranging from 0.1 to 1 (g/L) using hexane as the solvent (Figure 15), resulting the in mathematical equation $y = 7.0982 * x - 0.2404$ and a coefficient of determination R^2 of 0.9912.

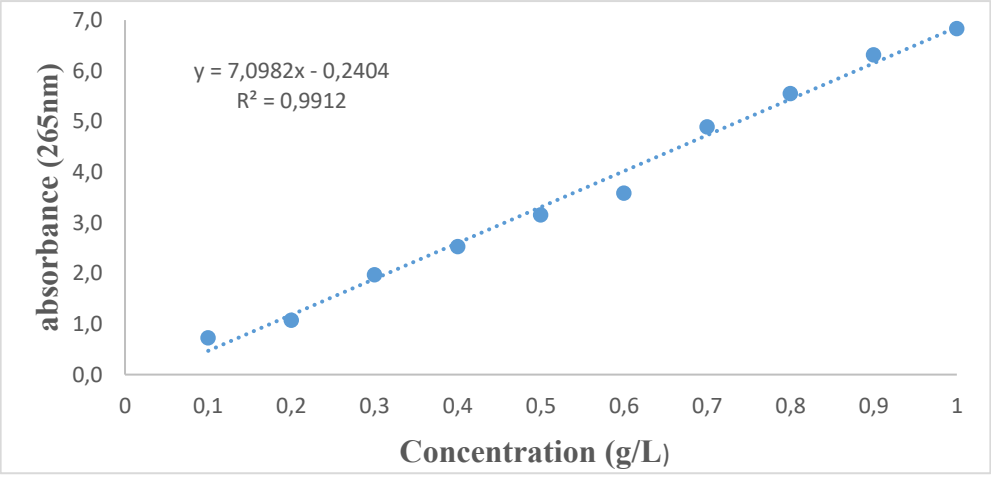


Figure 15 - Standard calibration curve of vitamin D3

The results found in Table 7 were performed in triplicate and demonstrated a significant encapsulation efficiency compared to other studies, such as the work conducted by S. Hu et al., 2022 on the incorporation of vitamins into emulsions using chitosan, which achieved an encapsulation efficiency between 63.4% and 80.4%. This demonstrates the possibility to disperse the hydrophobic vitamin D3 in the continuous external water phase, through its efficient encapsulation in the oil phase.

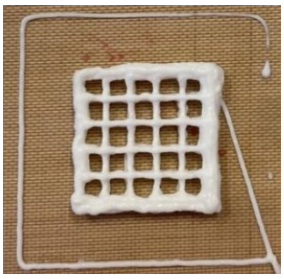
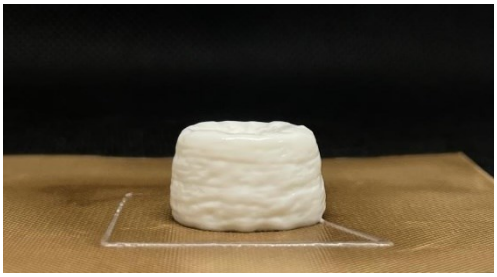
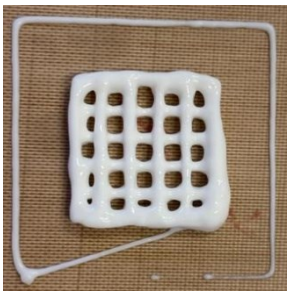
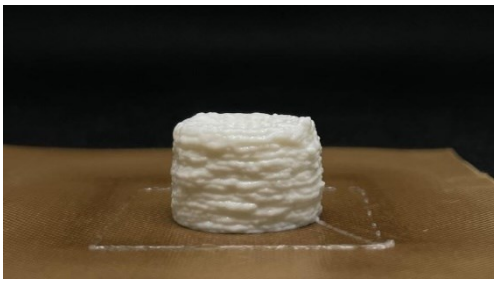
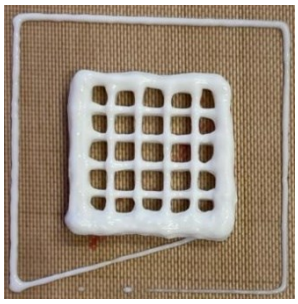
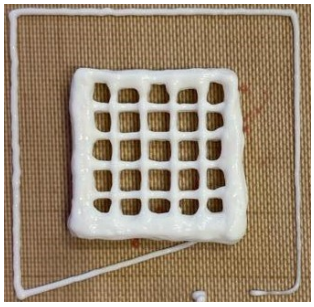
Table 7 – Results of vitamin D3 encapsulation efficiency

Formulation	Absorbance	Encapsulation efficiency
E6 – 5.5%D	4.5313	84.03%
E6 – 6%D	4.6784	86.62%

4.3.6. 3D printing

One of the main objectives of this work was to produce printable emulsions with a high degree of precision, ensuring good layer deposition and the ability to maintain their structure and shape after printing. To evaluate these parameters and determine which emulsion exhibited most promising properties, two types of structures were printed (Table 8).

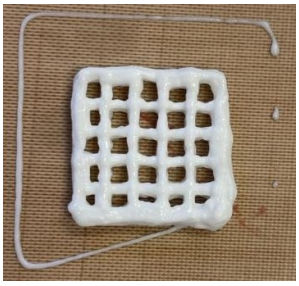
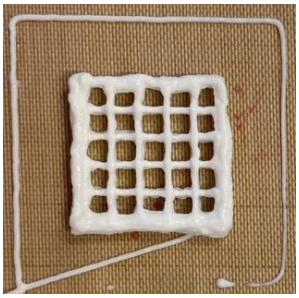
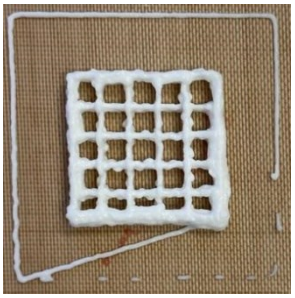
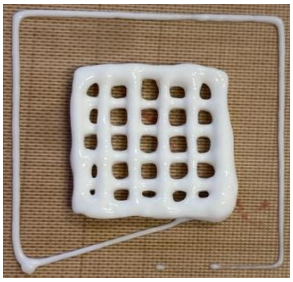
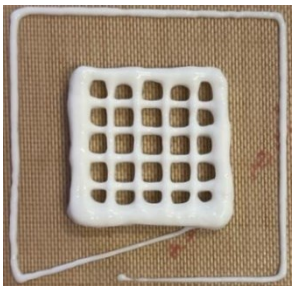
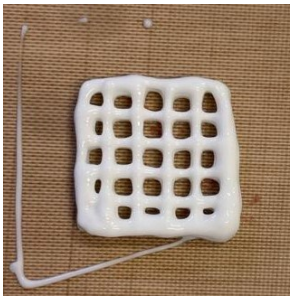
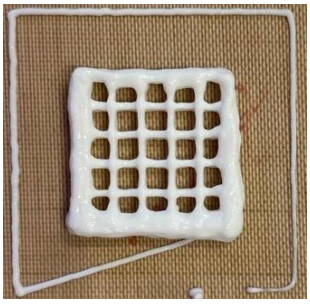
Table 8 – Printing results of the Pickering emulsions

Formulation	Cylinder	Cube
E6 – 5.5%		
E6 – 5.5%D		
E6 – 6%		
E6 – 6%D		

The visual analysis showed that the emulsions at 5.5% of particles, , spread out slightly at the bottom, when printed in a cylindrical format, while at 6% the emulsions maintained their structure even at the bottom. In addition, it was observed that the layers at 6% were better defined, which visually appeared to merge.

When examining the cube print, greater support was observed in the 6%D (E6 - 6%D) and 5.5% (E6 - 5.5%) emulsions. Both emulsions maintained their structure, however, in sample E6 - 5.5%, the emulsion appeared more granular compared to the others. This is mainly due to its high degree of hardness, hampering its extrusion during printing. The printed emulsions were able to maintain their structure without deforming for an analysed period of 30 days. The printed samples were stored in a freezer, refrigerator and at room temperature. It is important to note that the room temperature did surpass the 20°C. Tests were carried out to assess the influence of time on the printing of the samples. For this purpose, three prints were made on day 1 after the emulsion preparation, day 3 and day 7. The prints were made in two formats, cylindrical with a diameter of 1 cm and a deposit of 22 layers and cube-shaped with a diameter of 2 cm x 2 cm and a deposit of 7 layers (Table 9).

Table 9 - Test results on the influence of time on the printing of the emulsions

Formulation			
Time	Day 1	Day 3	Day 7
E6 - 5.5%			
E6 – 5.5%D			
E6 – 6%			
E6 – 6%D			

Through visual analyses, it was observed that at day 3, the samples showed better print quality compared to the others. It's important to note that on day 7, the prints also achieved

satisfactory results; however, the emulsions become too rigid, particularly the E6 - 5.5%, which made printing difficult.

4.3.7. Texture analysis

Texture analysis is an important indicator for assessing the properties and structural characteristics of emulsions (Zheng et al., 2022). The studied formulations were molded into cylindrical shapes with a height of 1 cm and a diameter of 2 cm (Figure 16), with the main difference between them being the morphology and the absence or presence of vitamin D3 in their composition, considering that the procedure was the same for all. To evaluate the emulsions produced at different concentrations and the influence of vitamin D incorporation, texture analysis tests were conducted, and the results are presented in Table 10. The hardness parameter is the maximum force that occurs during the first compression. Elasticity is the ability of a product to physically return to its original form after being deformed during its first compression. Cohesiveness is the ability of the product to withstand a second deformation relative to its resistance under the first deformation. Resilience is the parameter that analyzes how well the product recovers its original height, and Gumminess quantifies how viscous the material appears.

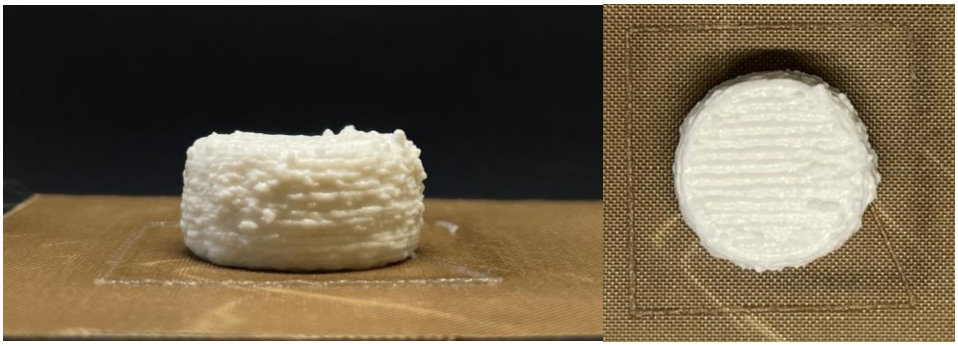


Figure 16 - Structure of the printed emulsion for the texture analysis test

Table 10 - Results of texture analysis

Formulation	Hardness (g)	Springiness (g)	Cohesiveness (g)	Gumminess (g)	Resilience (g)
E6 - 5.5%	1284.97 ± 251.30	0.91 ± 0.11	0.60 ± 0.05	768.71 ± 133.7	0.46 ± 0.07
E6 - 5.5%D	1040.23 ± 218.74	0.86 ± 0.06	0.49 ± 0.01	517.79 ± 119.9	0.24 ± 0.00
E6 – 6%	1012.29 ± 35.55	0.92 ± 0.05	0.67 ± 0.00	679.03 ± 21.01	0.54 ± 0.06
E6 – 6%D	910.08 ± 34.11	0.94 ± 0.03	0.68 ± 0.01	624.14 ± 15.25	0.59 ± 0.09

Analysing the textural properties of the samples, particularly the hardness, it was observed that the absence of vitamin D in the formulation led to higher hardness, with sample E6 - 5.5% being the toughest, presenting a value of 1284.97 g. The most flexible resulted the sample E6 – 6%D with a hardness of 910.08 g. However, sample E6 – 6%D showed promising values in terms of springiness, cohesiveness, and resilience, with the latter property being of paramount importance as it demonstrates that this sample can maintain its structure more easily compared to the other formulations.

Chapter 5

Conclusions and future work

5. Conclusions and future work

5.1. Conclusions

This work aimed to develop a printable food product as an alternative for customized nutrition through Pickering emulsion systems stabilized using chitosan and type B gelatin nanoparticles. Based on the literature review conducted and the knowledge acquired during the study, this strategy has never been used for the purpose of the present work. Pickering emulsion-related products are extensively studied in the pharmaceutical field to develop films, gels, and creams, which are usually employed to incorporate compounds. However, this system is less used in the food sector. In this study chitosan/type B gelatine nanoparticles were used as Pickering stabilizers to prepare Pickering emulsions, which would help make these systems to be more biodegradable and potentially useful for food applications.

The work was developed in the following phases: (i) Production of nanoparticles using chitosan/type B gelatin; (ii) Characterization of the nanoparticles; (iii) Development and optimization of stable Pickering emulsions; (iv) Characterization of the optimized Pickering emulsions; (v) Printing of the prepared emulsions; (vi) Incorporation of vitamin D3 and analysis of its influence on the characteristics of the obtained emulsions.

The results indicated the viability of using chitosan and type B gelatin as Pickering stabilizers, which yielded promising results as an emulsion. Some stable samples were developed, with two concentrations showing promise for printing: E6 – 5.5% and E6 – 6%. Based on these emulsions, printing parameters were determined to match their rheology and structure, ensuring the material was deposited in layers and maintained its structure over time. Encapsulation with vitamin D3 was achieved, yielding promising results in texture analysis and encapsulation efficiency tests, demonstrating potential for food applications and protection of hydrophobic compounds.

Overall, it was possible to develop a stable Pickering emulsion that can be printed using chitosan/type B gelatine as the solid particles. The system proved to be promising and innovative, with the ability to print foods in any shape and structure being particularly innovative for the food industry. Incorporating vitamin D3 adds value to the product by enhancing its functionality.

5.2. Future work

The current work explored innovative solutions for the development of new products aimed at the food industry, seeking to meet current needs. Therefore, the following points can be considered for future work:

- Incorporation of calcium: Calcium is an important bioactive that aids the absorption of vitamin D in the digestive system, allowing for rapid absorption of the vitamin by the body.
- Digestion tests: Conduct tests to define how the studied material behaves in the digestive system and how vitamin D is released.
- Microbiological analyses: Define the necessary precautions to ensure food safety.
- Complete nutritional analysis: Identify the compounds present in the emulsion for a comprehensive nutritional profile.

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