Production, stability study, particles size distribution and rheological behavior of oil-in-water systems based on whey proteins

Produção, estudo de estabilidade, distribuição e tamanho de partículas e comportamento reológico de sistemas óleo/água adicionados de soro lácteo

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ABSTRACT
The present study evaluated the functionality of whey protein as an emulsifying and stabilizing agent in oil-in-water systems with the purpose of suggesting the use of whey in food, cosmetic or medicines emulsions. A basic emulsion with vegetable oil (20% w/w), whey protein (20% w/w) and xanthan gum (0.1% w/w) was prepared using a mixer equipament. Stability tests and storage conditions, flow test, freeze drying, particles size characterization, analyses of microscopic and rheological measurements were performed. Characterization analyses showed that the formed systems can be classified as emulsions with droplet sizes smaller than 100 µm, non-Newtonian fluids characterized as pseudoplastics and practically constant viscosity at high shear rates. In addition, characterization indicates that the use of Turrax results in more stable emulsions (more homogeneous droplets and reduced diameter) and lower apparent viscosity values. The results showed that emulsions formed by the use of whey include xanthan gum exhibit stability at room temperature, during freezing/thawing and the freeze drying process, suggesting the satisfactory application of these compounds information and stabilization of emulsions that undergo processes involving these unitary operations in pharmaceutical and food industries.
Keywords: Green Consumption, New Product Developments, Food Industries.

RESUMO
O presente estudo avaliou a funcionalidade do soro lácteo como agente emulsificante e estabilizante em sistemas óleo-em-água com o objetivo de sugerir a sua utilização em emulsões alimentícias, cosméticas ou medicamentosas. Uma emulsão básica com óleo vegetal (20% m/m), soro lácteo (20% m/m) e goma xantana (0,1% m/m) foi preparada. Foram realizados testes de estabilidade em condições de armazenamento, teste de escoamento, liofilização, caracterização do tamanho e distribuição de partículas, análises microscópicas e reológicas. A caracterização mostrou que os sistemas formados apresentaram tamanho de partículas < 100 µm. A reologia mostrou que esses sistemas são fluidos não newtonianos caracterizados como pseudoplásticos e viscosidade praticamente constante em altas taxas de cisalhamento. Além disso, a caracterização indicou que o uso do Turrax resultou em emulsões mais estáveis (gotículas de óleo mais homogêneas com diâmetro reduzido) e menores valores de viscosidade aparente. Os resultados mostraram que as emulsões formadas pelo uso do soro lácteo com adição de goma xantana apresentaram estabilidade à temperatura ambiente, durante o congelamento e descongelamento e no processo de liofilização, sugerindo a aplicação satisfatória dessas informações sobre sistemas emulsionados que passam por processos envolvendo essas operações unitárias na indústria farmacêutica e alimentícia.

Palavras-chave: Consumo Verde, Desenvolvimentos de Novos Produtos, Indústrias Alimentícia.

1 INTRODUCTION
Most of the products used in the food industries are produced as emulsions, consisting of two immiscible liquids, usually oil and water, which one of the liquids is dispersed as small spherical droplets in the other. They can be classified according to the distribution of the oil and aqueous phases. There is the oil in water system that consists of oil droplets dispersed in an aqueous phase which is used, for example, in the dairy products and mayonnaise. There is also the water in oil system that consists of water droplets surrounded by oil, for example, margarine and butter (MCCLEMENTS, 2015).

The quest for stability is one of the greatest challenges for the development of emulsions, because the more stable the emulsion, the more slowly its properties change. An emulsion may become unstable due to a variety of physicochemical mechanisms that results in an alteration in the spatial distribution or structural organization of the molecules and in an alteration in the kind of molecules present in it. Creaming, flocculation, coalescence, phase inversion and Ostwald ripening are examples of physical instability, whereas oxidation and hydrolysis are examples of chemical instability. Therefore, it is necessary to use raw materials that improve the stability of the product by increasing the shelf life (MCCLEMENTS, 2015; AULTON, 2005; MORAIS, 2006).
The stability study predicts the behavior of the emulsion under the different storage conditions, as well as confirming that there will be no changes in the three essential requirements of a product: quality, effectiveness and safety (SOUZA; FERREIRA, 2010). The current challenge is to introduce green consumption that consists in the replacement of the use of ingredients that were widely used for more sustainable alternatives, for example, the use of agro-industry waste, such as whey proteins (WEISS et al., 2009). Through the reuse of previously discarded materials one can create new products that change the production line by reducing environmental impacts and reducing costs, leading to constant innovation (BUSS; HENKES, 2015).

In dairy industries, whey is produced after the milk coagulation process for cheese production, accounting for 80-90% of the total milk volume used for this production and containing about 55% of milk nutrients (ALVES et al., 2014). The great concern is when this material is released into the environment without proper reuse or a previous treatment, because it presents a high organic matter load that when discarded incorrectly can intensify the environmental problems. Furthermore, in the past the use of whey in animal feed and fertilizers was common. In recent years, the use of whey has grown and expanded to numerous food formulations, valuing a nutritionally rich co-product (COSTA et al., 2014).

Whey proteins are natural emulsifiers that have a globular structure containing disulfide bridges, which impart a certain degree of structural stability and have excellent nutritional properties. The major proteins are β-lactoglobulin (β-Lg) and α-lactalbumin (α-La) which constitute approximately 70% of the total serum proteins (COSTA et al., 2008; ALVES et al., 2014). Considering the emulsifier characteristics of these whey proteins, this study aimed to investigate the production of oil/water emulsions systems, evaluate the stability, rheological behavior and particle size distribution in order to reuse this residue avoiding the disposal in the environment, in addition to providing a sustainable alternative, the reduced use of synthetic materials and the development of new products and processes.

2 MATERIALS AND METHODS

2.1 PREPARATION OF EMULSIONS

The emulsion consists of deionized water (59% w/w), xanthan gum (1.0% w/w), vegetable oil (20% w/w) and whey powder (20% w/w). Whey powder and xanthan gum were diluted in the aqueous phase and then heated (65 °C/30min). The diluted material
was added to the Mixer (Fisatom®). Vegetable oil was added during the microfluidization 5 min/1000 rpm and then for another 5 min/1750 rpm. The emulsion was divided into 2 parts and one part sent to the Turrax apparatus (FSH - 2A) at 8 min/17000 rpm (WEISS et al, 2009; JUNQUEIRA et al., 2019).

2.2 RELATIONSHIP BETWEEN MASS AND TIME OF THAWING

The emulsions were frozen (-18 °C) for 24 hours. About 15.0 g of each emulsion was placed in a glass funnel (65 mm), the flow time was timed, and the weight of the beaker (in grams) was measured every 3 minutes until the material was completely transferred. This procedure was performed in a temperature controlled room.

2.3 FREEZE DRYING

All emulsions were freeze dried for 24 hours (LYOFLIZER LIJO, SCIENTIFIC JJ) at -44.4 °C and vacuum at 176 mmHg.

2.4 FREEZE/MELTING CYCLE STABILITY

Approximately 5.0 ml of the emulsions were used in transparent tubes and they were hermetically sealed. Samples were frozen (-5 °C) and melted (21 °C) in a daily cycle of 5 hours of melting and 24 hours of freezing for a total of 20 days to close the cycle. At each freezing and melting cycle, visual observation and photographic recording were performed.

2.5 STABILITY

In order to verify the stability, aliquots of 5 mL of the emulsions, with the respective pH values, and of the emulsions processed in the Mixer and Turrax were separated in hermetically sealed transparent tubes. They were conditioned under refrigerated conditions (5 °C) during 30 days in which every 2 days a sample was analyzed and the visual stability analysis was duly demonstrated by photos.

2.6 CHARACTERIZATION OF EMULSIONS

2.6.1 Particle size

To verify the size of the particles present in the emulsions, the Nanotrac apparatus was used. The LS Particle Size Analyzer - Beckman Coulter LS 13 320 was used to verify
the particle size and to analyze the reduction of the droplet diameter that make up the emulsions of both processes.

2.6.2 Microscopy

Cool SNAP-PRO Color devices was used, with Pro Plus imaging software and the Olympus DP72 microscope for identification of the droplet size formed in the emulsions. Fluorescence microscopy was also performed on the Olympus BX51 microscope apparatus and the FITC dye was used to label the proteins. In addition, the USB digital microscope was used to obtain the micrographs of the material after freeze drying.

2.6.3 Rheological behaviour

The rheological measurements were carried out using a rotational viscometer with concentric cylinders Brookfield DVIII Ultra (Brookfield Engineering Laboratories, Stoughton, USA), using the adaptor for small samples 13R/RP (19.05 mm diameter and 64.77 mm depth; Brookfield Engineering Laboratories, Stoughton, USA) and the shear coaxial sensor (9.39 mm diameter and 24.23 mm length; Brookfield Engineering Laboratories, Stoughton, USA). The sample temperature (5°C) was controlled using thermostatic bath (Nova Etica, Vargem Grande Paulista, Brazil) that was coupled to the viscometer. Data generated in the flow curve were fitted to the rheological models presented in Eq. (1) (Newton), Eq. (2) (power law) and Eq. (3) (Herschel-Bulkley), the given informations were fluid classification and the parameters of adjustment.

\[ \tau = \mu \dot{\gamma} \]  
\[ \tau = k (\dot{\gamma})^n \]  
\[ \tau = \tau_0 + k (\dot{\gamma})^n \]

Where \( \tau \) is shear stress (Pa), \( \dot{\gamma} \) is shear rate (s\(^{-1}\)), \( \mu \) is viscosity (Pa.s), \( k \) is the consistency index (Pa.s), \( n \) is the flow behavior index (dimension less) and \( \tau_0 \) is the yield stress (Pa).

2.7 STATISTICS

Samples and analyzes were carried out in triplicate, experimental results were subjected to analysis of variance (ANOVA) and the mean differences were compared by
the Tukey test at 5% significance level using the software R (R Development Core Team, Vienna, Austria).

3 RESULTS AND DISCUSSION

3.1 RELATIONSHIP BETWEEN MASS AND TIME OF MELTING (MASS/TIME)

This procedure was performed to check the flow time of the first drop after melting, the results are arranged in the Table 1. After draining the first drop of each sample, the amount of mass of the drained sample was weighed in grams every 3 minutes. According to Table 1, the emulsion obtained after being processed in Turrax has a first droplet flow time greater than the emulsion obtained by the Mixer, as well as the total flow time.

Table 1. Average flow test results of emulsions processed in Mixer and Turrax equipments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1° drop mass (g)</th>
<th>Total mass drained (g)</th>
<th>Loss Mass (g)</th>
<th>Drained Time 1° drop (min)</th>
<th>Total drained time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixer</td>
<td>15.98±1.93a</td>
<td>0.11±0.15a</td>
<td>13.56±0.59a</td>
<td>8’06”a</td>
<td>23’13”a</td>
</tr>
<tr>
<td>Turrax</td>
<td>15.57±3.14a</td>
<td>0.09±0.01a</td>
<td>14.08±4.12a</td>
<td>10’11”b</td>
<td>27’14”b</td>
</tr>
</tbody>
</table>

In addition, the mass of the first drop of the Mixer emulsion is heavier than the Turrax emulsion, but the total drained mass is smaller because it is more adhered to the funnel. This can be explained by the relationship between the increase in energy used to form the emulsions and the size of the droplets. According to McClements (2015), the size of the droplets in an emulsion can be reduced by increasing the intensity during homogenization.

3.2 FREEZING/THAWING AND STABILITY STUDY

The emulsion samples were subjected to the freeze/thaw cycle, in which the objective was to verify the stability of the emulsions under drastic temperature variations in a short period of time, since the whey proteins can undergo denaturation affected by changes in temperature and, consequently, they end up losing its emulsifying function, causing emulsion breakage (CHEN et al., 2018). Thus, the samples were frozen and thawed the day after at room temperature. According to Figure 1, it is possible to verify that the Mixer and Turrax samples remained unchanged after the complete cycle. In this way, it was verified that both emulsions remained homogeneous, indicating a uniform
droplet size distribution and a more stable emulsion, which reduces the probability of droplet coalescence and phase separation (DICKINSON, 2003; GOMES et al., 2018).

**Figure 1.** Stability study: A1 (Mixer = 1° day); A2 (Mixer = 30° day); B1 (Turrax = 1° day); B2 (Turrax = 30° day).

In relation to the stability study, the samples were placed under refrigeration (5 °C) and maintained for 30 days, they were observed and apparently did not show visible changes, as shown in Figure 2. The stabilization mechanism of the protein emulsion is mainly through electrostatic repulsion due to the presence of charged groups in the protein structure, which are adsorbed between the oil/water phases, as well as via steric repulsions (MCCLEMENTS, 2005; GOMES et al., 2018).
The results of both freezing/thawing and stability studies can also be explained by the presence of the added raw materials, such as xanthan gum, the polysaccharide hydrocolloid most used in the industrial environment (PAXIMADA et al., 2016). Xanthan gum increases the viscosity of the medium, preventing large droplet movement and destabilization, also increasing the stability of the emulsions in the face of temperature oscillation. This is due to the formation of an interfacial tension that generates a delay in the formation of droplets (KRSTONOŠIĆ et al., 2016).

Many factors can influence viscosity, the dispersed phase volume fraction, the rheology of the component phases, the drop size, the colloidal interactions and the drop load can be highlighted (NEJADMANSOURI et al., 2016). It is also worth mentioning that milk proteins have a great surface activity (GRIGORIEV et al., 2007), which together with the emulsifier helps to stabilize the emulsion, consequently reducing the interfacial tension, by reducing the surface energy between the two immiscible phases, preventing the coalescence of the particles and increasing the stability of the droplets (GAJO et al., 2016; PENA et al., 2017; MARTINS et al., 2021).

3.3 PARTICLE SIZE

The particle size was influenced according to the interfacial tension of the emulsion. Thus, it was known that whey protein will move to the interface and will
influence the interfacial tension on an oil/water surface, separately or synergistically and this is possible because of the amphiphilic characteristic present in whey protein (ZYCHOWSKI et al., 2018). According to McClementes (2002), “Food emulsion droplets usually have an average diameter in the range of 0.1 to 100 μm”.

The values found for an emulsion that passed through Turrax are in this range, showing that in this process the emulsion was combined with efficiency. However, the Mixer treatment has a larger droplet size until the initial treatment and a portion of its droplets is larger than 100 μm. This fact can be visually confirmed by microscopy (Figure 3), where larger droplets and a larger variation in droplet size can be seen. And it can result in less stability of these systems.

To evaluate the influence of particle size (Table 2), one can use the generated average particle diameter and the dispersion quality of the particles. With the addition of the emulsifier in the production process, a higher stability in interfacial tension, increased emulsion viscosity and consequent reduction in the chances of coalescence (particle agglutination phenomena and subsequent phase separation) were obtained, avoiding the formation of droplets with larger diameters (MCCLEMENTS, 2015; GUERRA-ROSAS et al., 2016).

| Table 2. Comparison between the size and the distribution of the particles processed by Mixer and Turrax. |
|---------------------------------------------------|--------|--------|--------|--------|--------|
| Average particle size distribution (μm)          | D_{10%} | D_{25%} | D_{50%} | D_{75%} | D_{90%} |
| Mixer                                            | 13.05±0.08<sup>a</sup> | 21.68±0.08<sup>a</sup> | 35.98±0.19<sup>a</sup> | 60.64±1.03<sup>a</sup> | 128.55±28.45<sup>a</sup> |
| Turrax                                           | 2.48±0.03<sup>b</sup> | 3.18±0.03<sup>b</sup> | 4.21±0.01<sup>b</sup> | 5.50±0.02<sup>b</sup> | 6.80±0.09<sup>b</sup> |

However, it was found that not only the material used as a emulsifying and stabilizing agent results in emulsion stability, but also the process by which mechanical energy is supplied to the emulsion forming system.

3.4 MICROSCOPY

A microscopic analysis was performed in order to obtain data concerning the size of the emulsion particles and if there was a formation of a droplet that forms protein halo. Thus, it was verified the formation of micro-droplets in both emulsions, however in the emulsion processed by Turrax it was possible to verify that there was a reduction in the size of these droplets according to Figure 3, confirming the particle size results. The size of the droplet can be observed through the home microscopy of the fat present in the
emulsion that forms a crystal lattice in optical response to polarization due to light birefringence and is widely used in food matrices (ZYCHOWSKI et al., 2018).

The lightness of an emulsion tends to be higher with increasing droplet concentration, and has a maximum value at a given droplet size, as it can be seen in micrographs in which the droplet concentration is higher in Turrrax emulsion, where the droplets are smaller, while Mixer emulsion visually presents much larger droplets, as shown in Figure 3.

**Figure 3.** Microstructure of drop oil dispersion of the emulsion. A = Mixer; B = Turrrax. Barr = 1mm.

Thus, completing the microscopy analysis, another one was also performed with the stirred emulsions in the Mixer and Turrrax to verify the difference of the micrographs of the samples. With this, it was possible to see that the analysis of the emulsion in the Mixer remained very similar to the emulsion B, with formation of well delimited droplets, formed by a halo of protein and emulsifier; however Turrrax emulsion showed extremely small droplets, almost imperceptible, well dispersed and homogeneous (RESENDE; COELHO; COSTA, 2021).

After the freeze drying process, it was found that both treatments resulted in a homogeneous and oily product with visually very similar pores (Figure 4). The presence of holes in the freeze drying structure may possibly arise from the destabilization of some droplets of the emulsion during freezing, since upon freezing it may have formed ice crystals in the aqueous phase which has penetrated the oily droplets, thus breaking the protein halo and the interfacial membrane (FIORAMONTI et al., 2017).
3.5 RHEOLOGICAL BEHAVIOR

Figure 5 shows a non-linear trend between a shear stress and shear rate, as explained by the Law of Power (Table 3) and a rheological model that normally explains the flow properties of oil-in-water food emulsions (JUNQUEIRA et al., 2019; CAMPELO et al., 2017; CAPITANI; NOLASCO; TOMÁS, 2016; FERNANDES et al., 2016; MASKAN, 2000; NIU et al., 2016; SILVA et al., 2015), which characterizes non-Newtonian fluids with pseudo plastic behavior.

Figure 5. Apparent viscosity as function of the shear rate of the emulsions. A: Mixer; B: Turrax.
In these systems, as the shear rate increases sufficiently to overcome the Brownian motion, the emulsion droplets become more orderly along the flow providing lower flow resistance and hence lower viscosity. After a sharp reduction, the viscosity change is flattened at high shear rates, common behavior in food emulsions, as evidenced in Figure 6 (HAYATI, 2007; MCCLEMENTS, 2005; SUN; GUNASEKARAN; RICHARDS, 2007).

Table 3. Rheological parameters (mean values) of the emulsions.

<table>
<thead>
<tr>
<th>Test</th>
<th>Power Law</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>k (Pa.s)</td>
<td>n (less dimension)</td>
</tr>
<tr>
<td>Mixer</td>
<td>0.9859</td>
<td>3.20+0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.29+0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Turrax</td>
<td>0.9899</td>
<td>1.06+0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94+0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Food emulsions are usually characterized by pseudoplastic behavior, for example, JUNQUEIRA et al., 2019; CAMPELO et al., 2017; HAYATI et al., 2007; LIANG et al., 2013; NIU et al., 2016; SUN; GUNASEKARAN; RICHARDS, 2007. This behavior was evidenced by values of consistency index greater than zero (k > 0) and values of flow behavior index between zero and one (0 < n < 1) (Table 3). The consistency index decreased when the Turrax was used and the flow behavior index increased, and became close to equation 1, indicating a tendency to Newtonian behavior. This and the lower values of apparent viscosity are the results of the values of lower than average droplet diameter obtained when the mechanical energy required to form the emulsion comes from Turrax, as evidenced by droplet size analysis and microscopy.

4 CONCLUSION

The study with the whey protein confirmed that there was formation of the stable microemulsion. They were found to remain stable during the shelf test as expected without change in homogeneity and consistency. In the freeze/melt and flow test they also presented a good viscosity without impairing the flow ability of the product. In addition, particle size and microscopy tests were performed to prove the reduction of microemulsion droplet size. Thus, the work achieved the main objective, since the emulsions were stable with size particles reduced, which will allow further studies and applications.
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