Rheological behavior and stability of emulsions obtained from *Pereskia aculeata* Miller via different drying methods

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**ABSTRACT**

Hydrocolloid powders from *Pereskia aculeata* Miller were obtained by using drying in a vacuum oven and freeze-drying at different concentrations (0.5–3.0 g of hydrocolloids/100 g of aqueous phase) to produce oil-in-water emulsions. The rheological characteristics of the emulsions and their microstructures were determined. The freeze-dried hydrocolloids and higher hydrocolloid concentrations resulted in emulsions with higher viscosities. The drying method and hydrocolloid concentration influenced the thixotropic behavior, and the resulting emulsions exhibited shear-thinning behavior. Microstructural analysis showed that with increasing freeze-dried hydrocolloid concentration, the emulsions had droplets with lower average diameters and greater uniformity, suggesting greater system stability.

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**Introduction**

An emulsion is conventionally defined as a thermodynamically unstable system that consists of two immiscible liquids such as oil and water, which destabilize over time by various physicochemical mechanisms such as creaming, flocculation, coalescence, phase inversion, and Ostwald ripening. Therefore, the shelf life of useful emulsions is determined primarily by their physical stability. Emulsifiers and thickeners are usually added to improve the emulsion stability (ES), i.e., to make it kinetically stable.¹⁻⁴

The stability and physicochemical and sensory properties of emulsion-based food products are strongly influenced by the concentration and characteristics of the droplets they are made of and also by the effects of other food ingredients and the interactions between them. Emulsifiers and thickeners contribute to the structural and textural properties of foods through their aggregation and gelation behavior. Of these materials, proteins are known for their emulsifying capacity (EC) and foaming ability. Proteins reduce the interfacial tension between immiscible liquids due to their adsorption at the interface. Indeed, they can form films at the droplet surface, providing electrostatic repulsion between the droplets. Polysaccharides are used as stabilizers, thickeners, and gelling agents because of their water retention and thickening properties.⁵⁻⁶

The properties of protein–polysaccharide biopolymer complexes such as hydration (solubility and viscosity), structuration (aggregation and gelation), and interfacial phenomena (foaming and emulsifying) can be exploited in the formulation of new foods. Food colloids contain charged colloidal particles, i.e., oppositely charged polysaccharides and proteins, which interact and form particulate dispersions, foams, gels, and emulsions.⁶

*Pereskia aculeata* Miller is a climbing shrub commonly known as ora-pro-nobis (OPN) in Brazil. The species belongs to the family Cactaceae and genus *Pereskia*, and in Brazil, it grows in areas ranging from Bahia to the Rio Grande do Sul province.⁷ OPN has a tropical origin. It is consumed...
as a food and used as an emollient in folk medicine. This plant has been of interest to the food and pharmaceutical industry because of its high protein, lysine, and mucilage contents. Additionally, this plant can be easily bred and cultivated.\cite{8}

Almeida Filho and Cambraia\cite{9} were the first to study the composition of the OPN leaves, and they concluded that the leaves had a high nutritional value and a protein content of 25.5% dry mass. Sierakowski et al.\cite{10} extracted the mucilage polysaccharide OPN and showed the presence of arabinogalactan. Studies have also shown that this polysaccharide consists of a heteroglycan that is formed mainly from arabinose, galactose, rhamnose, and galacturonic acid.\cite{11} Due to this composition rich in biopolymers and protein, Lima Junior et al.\cite{12} have suggested that the species enables the extraction of hydrocolloids with significant emulsifying and stabilizing capacity.

Although some hydrocolloids with surface activity can act as emulsifiers in oil-in-water emulsions, the principal role of most of these biopolymers is that they act as structuring, thickening, or gelling agents in the aqueous phase. In other words, they modify the rheological behavior of the continuous phase and contribute to equalizing the densities of the oil and aqueous phases.\cite{2,4} Thus, the analyses of changes in the emulsion structure and rheology due to the addition of different hydrocolloids may be important for understanding their effects on stability.\cite{2,13}

In order to conserve the composition rich in biopolymers and protein, and their original constituents, special attention has been given to drying techniques that yield powdered OPN gum. Unlike conventional drying methods that use the evaporation of liquid water, freeze-drying promotes the sublimation of ice as the main drying mechanism. Freeze-drying is mainly applied to heat-sensitive foods and yields products with superior sensory characteristics.\cite{14} The aims of the present work were to determine the rheological behavior of the emulsions prepared with hydrocolloid powders obtained by vacuum oven (VO) drying and freeze-drying processes. Further, we sought to evaluate the effect of the powdered OPN hydrocolloid concentration on the EC, microstructure, and stability of the emulsions.

Materials and methods

Hydrocolloid preparation

The fresh OPN leaves were obtained in the city of Itutinga, MG, Brazil. The leaves were selected, washed, and frozen at −18°C in a freezer. The hydrocolloid extraction was conducted according to the optimized process provided by Lima Junior et al.\cite{12} The drying was performed using two different processes: (1) The extracts were dried in a VO (Nova Ética, Model: 440-2D, Vargem Grande Paulista, Brazil) at a temperature of 60°C ± 2°C for 24 h; (2) The extracts were homogenized, filtered, placed in glass plates, and frozen with static air (deep freezer; REVCO, Asheville, USA) at −60 ± 2°C. The frozen extracts were carried to a pilot freeze-dryer (Edwards High Vacuum, model L4KR, São Paulo, Brazil), which was maintained at −40°C with a vacuum pressure of 0.0998 kPa (0.998 mbar), where they remained for an average time of 24 h. During the secondary stage of freeze-drying, the product reached a final temperature of approximately 25°C.

Aqueous phase preparation

The gums were reconstituted with distilled water at 80°C using a magnetic stirrer for 30 min. The gums were maintained in a BOD oven (Electrolab, EL202, São Paulo, Brazil) at 4°C for 12 h for hydration (maturation). The solutions, at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g of hydrocolloids/100 g of aqueous phase, were prepared using the powdered hydrocolloids obtained via the two drying methods.
Emulsion preparation

The emulsions were prepared using 10 g of commercial soybean oil (Mazola, Cargill, São Paulo, Brazil) and 40 g of reconstituted gum. The sample was mixed in a mechanical stirrer (Ika Labortechnik, model RW 2.0, Germany) for 5 min and then homogenized in a grinder (Tecnal brand, model TE102, Piracicaba, Brazil) at 20,500 rpm for 5 min.

Rheological characterization

A HAAKE RheoStress Rheometer 6000 (Thermo Scientific) that was connected to a HAAKE UTM temperature controller (Thermo Scientific) was used to perform rheological measurements. The oscillatory shear tests were performed using a parallel plate geometry with a diameter of 34.997 mm and a 1 mm gap. All analyses were performed in duplicate at 20°C.

The shear stress was measured as a function of the shear rate to generate curves from 0 to 300 s\(^{-1}\) and from 300 to 0 s\(^{-1}\). The data were fitted to Newton’s law, power law, and Herschel–Bulkley rheological models. The thixotropic behavior of the emulsions was characterized by the hysteresis between the first and second curve which corresponded to increasing (0–300 s\(^{-1}\)) and decreasing shear rates (300–0 s\(^{-1}\)), respectively. Thixotropic behavior of the emulsions was further verified and quantified by the difference in the areas between the climbing curves (increased shear stress) and descent (reduction of shear stress). The apparent viscosity was analyzed at a constant shear rate of 100 s\(^{-1}\) because this is a typical strain rate for food processes, e.g., flow through tubes in the industry, and stirring and chewing processes.\(^{[11]}\) The linear viscoelastic region was identified by subjecting the samples to a shear stress sweep at a fixed frequency of 1 Hz at 20°C. Frequency sweeps were performed from 1 to 10 Hz, at a strain selected to be within linear viscoelastic range.

Emulsifying capacity and the stability of the formed emulsions

The EC of and the ES induced by the hydrocolloids from different drying methods were determined according to Pranee et al.\(^{[15]}\) and Lima Junior et al.\(^{[12]}\) using Eqs. (1) and (2), respectively.

\[
EC(\%) = \frac{\text{emulsion volume}}{\text{total fluid volume}} \times 100.
\]  
\[
ES(\%) = \frac{\text{final emulsion volume}}{\text{total fluid volume}} \times 100.
\]

Here, the emulsion volume is the volume after mechanical agitation and homogenization (mL). The total fluid volume is the volume after the soybean oil and gum were weighed together. The final emulsion volume is the volume of the emulsion after destabilization in the centrifuge.

Microstructure and droplets sizes

The microstructure and stability of the emulsions were analyzed using a light microscope (LM Meiji 5000, Meiji Techno America, Santa Clara, CA, USA) with a camera (Cole-Parmer 49901–35, Cole-Palmer, Vernon Hills, IL, USA) attached. Emulsion images were produced 10 min after preparation. The images were produced in triplicate, and droplets sizes were quantified using the SigmaScan 5.0 software (SPSS Science, Chicago, USA). The microstructural parameters measured as a function of the hydrocolloid concentrations were the number of droplets, the average diameter (Feret diameter),
the standard deviation, the area, droplet size distribution, and secondary parameters such as compactness and shape factors.

**Fluorescence microscopy**

The microstructural analysis was performed on a Leica DM2000 vertical epi-fluorescence microscope using the LAS v.4 software with an Image Program AxioVision 4.6, Zeiss PEG camera. The filters used were a TX2 ET Leica – excitation BP 560/40, dichromatic mirror 595, suppression filter BP 645/75 (to red fluorescence works) and a L5 ET Leica – excitation BP 480/40, dichromatic mirror 505, suppression filter BP 527/30 (to green fluorescence works). The photomicrographs were obtained using the fluorochromes Rhodamine BP.A (Dinâmica CAS [81-88-9]) and Red Nile (Sigma-Aldrich N3013-100 mg). The Rhodamine was prepared per the method described by Schmitt et al.\(^\text{[16]}\) and the Red Nile according to technical information provided by the vendor.\(^\text{[17]}\)

**Statistical analysis**

Fitted curves, the rheological parameters of the fitted models, coefficients of determination \((R^2)\), and root mean square error (RMSE) were obtained using the SAS statistical software. A bi-factorial design (drying method and hydrocolloid concentration) was used for statistical analysis, which performed using the same software. All experiments were conducted at least in duplicate, and the values presented are averages from these experiments. Tukey tests were used to compare the mean values, and the results were only considered significant when \(p\)-values were less than 0.05. The rheological models were adjusted to a 1% significance level.

**Results and discussion**

**Rheological analysis**

Based on the fitting performed, the power law model yielded the best coefficients of determination \((R^2 > 0.94)\) and good results for the RMSE. All the model parameters obtained were significant in the treatments (Table 1). Figures 1a and 1b show the variation in shear rate as a function of shear stress in the emulsions prepared using oven-dried and freeze-dried (FD) hydrocolloids, respectively. The graphs show both the experimental data and the corresponding power law model fits. The behavior shown in the rheograms (Fig. 1) is typical of non-Newtonian fluids; i.e., the hydrocolloid emulsions

| Concentrations | Power law |
|----------------|-----------|
| G/100 g of aqueous phase | \(R^2\) | MSE | \(K\) | \(n\) | Apparent viscosity at 100 s\(^{-1}\) (Pa.s\(^{-1}\)) |
| Vacuum oven | 0.5 | 0.941 | 0.123 | 0.036 | 0.691 | 0.008 |
| 1.0 | 0.995 | 0.109 | 0.101 | 0.703 | 0.026 |
| 1.5 | 0.990 | 0.222 | 0.172 | 0.681 | 0.039 |
| 2.0 | 0.998 | 0.173 | 0.469 | 0.626 | 0.083 |
| 2.5 | 0.969 | 0.915 | 0.322 | 0.639 | 0.098 |
| 3.0 | 0.999 | 0.229 | 0.694 | 0.630 | 0.126 |
| Freeze-dryer | 0.5 | 0.965 | 0.128 | 0.054 | 0.679 | 0.011 |
| 1.0 | 0.991 | 0.175 | 0.196 | 0.635 | 0.035 |
| 1.5 | 0.988 | 0.450 | 0.367 | 0.657 | 0.073 |
| 2.0 | 0.999 | 0.151 | 0.669 | 0.628 | 0.119 |
| 2.5 | 0.999 | 0.248 | 0.970 | 0.598 | 0.152 |
| 3.0 | 0.998 | 0.401 | 2.279 | 0.507 | 0.236 |

\(K = \text{consistency index (Pa.s)}^n; n = \text{flow behavior index (dimensionless); } R^2 = \text{coefficient of determination; MSE = residual of mean square.} \)
obtained via the two drying methods at different concentrations did not show a linear relationship between shear stress and shear rate. From the rheograms, we observe that the relationship between shear stress and shear rate follows a convex curve suggesting pseudoplastic fluid behavior. The drying method and the concentration influenced the parameters of the power law model, resulting in significant differences ($p < 0.05$). The emulsions obtained from the FD hydrocolloids showed a higher consistency index ($k$) and lower flow indices ($n$), indicating that these emulsions are pseudoplastic. The values of $n$ of the emulsions were influenced by the concentration of the hydrocolloids. The smallest value (0.507) was observed when using a concentration of 3.0 g of hydrocolloids/100 g of aqueous phase for the FD samples. In the case of this emulsion, the reduction in $n$ is due to an increase in the concentration of proteins and polysaccharides in the continuous phase of the emulsion (water). In other words, when increasing the hydrocolloid concentration, emulsions show non-Newtonian fluid behavior, the value of the flow index becomes smaller ($n < 1$), and pseudoplastic behavior becomes more evident.

The increase in the hydrocolloid concentration influenced the consistency index values ($k$), and the highest value was observed at a concentration of 3.0 g of hydrocolloids/100 g of aqueous phase. The consistency index is a parameter that also indicates the viscosity of a fluid. Therefore, according to Medina-Torres$^{18}$ and İbanoğlu,$^{19}$ the $k$ values increase with increasing solid content and decrease with temperature. A higher solid content generally causes an increase in viscosity and results in an increased restriction of molecular motion due to entanglement between polymer

Figure 1. Shear stress as a function of shear rate and curves adjusted by power law for emulsions prepared with OPN hydrocolloids (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g OPN/100 g aqueous phase) that were (a) vacuum oven-dried and (b) freeze-dried.
chains. Similar results were found by İbanoğlu, who observed that increasing the Arabic gum concentration in stabilized emulsions of milk proteins resulted in an increase in the value of $k$. This behavior was also observed in the present study of emulsions that were stabilized by hydrocolloids extracted from OPN leaves.

When freeze-drying, the hydrocolloid temperature was kept low during the ice sublimation in the primary drying step until a temperature of 25°C was reached at the end of the secondary drying step. On the other hand, the entire hydrocolloid drying in the VO took place at a constant temperature of 60°C. The emulsions prepared using the hydrocolloids dried in the VO presented lower viscosities than those made using FD hydrocolloids. We believe the long high-temperature duration in the VO disintegrated the constituent polymers and denatured the proteins to form more single strands and resulted in emulsions with a greater ease of flow. For each drying method, the viscosity as a function of shear rate increases with an increase in the hydrocolloid concentration. OPN gum is rich in proteins, and per Goff & Jordan the increase in the amount of protein at the interface of the droplets reduces the interfacial tension and slightly increases the viscosity of the emulsion. Furthermore, a greater amount of hydrocolloid results in more interactions between the phases of the emulsion. An increase in the hydrocolloid concentration causes more water retention, resulting in a higher apparent viscosity.

The results of the thixotropic behavior study (Fig. 2 and Table 2) showed that the fluid properties of the emulsions were time dependent. When a constant shear rate is applied, the apparent viscosities of the emulsions decrease with application time. Thixotropic behavior is more evident when the hydrocolloid concentrations are increased in the emulsions, as evidenced by the increased difference in the area between the increasing and decreasing shear rate curves. The higher concentrations of hydrocolloids produced more intermolecular interactions and led to the formation of small aggregates. By increasing the applied shear stress, these interactions and aggregates can be broken, thereby decreasing the fluid flow resistance and decreasing the viscosity. This internal structure does not change when using a lower shear stress, thereby demonstrating the thixotropic behavior.

The statistical analysis showed that the drying method and concentration significantly influenced the thixotropy values ($p < 0.05$). The FD hydrocolloids (Fig. 2b) resulted in emulsions with the highest mean thixotropy value compared with emulsions that were obtained using the vacuum-dried ones (Fig. 2a). This difference suggests that in the emulsions that contain the FD hydrocolloids at higher concentrations, the continuous decrease in apparent viscosity with time, under a constant shear rate, occurs with greater intensity with the subsequent recovery of viscosity when the flow is discontinued. For the VO-dried hydrocolloid emulsions, the concentrations of 0.5, 1.0, and 1.5 g of hydrocolloids/100 g of aqueous phase showed no significant difference with respect to the areas under the curves. The emulsion with a concentration of 2.0 g of hydrocolloids/100 g of gum did not differ from the emulsions with concentrations of 0.5 and 1.0 g of hydrocolloids/100 g of gum. In the emulsions that contained the FD hydrocolloids, the concentrations of 0.5, 1.0, and 1.5 g of hydrocolloids/100 g of aqueous phase showed no significant difference between the thixotropy values; neither did the emulsions at concentrations of 2.0 and 2.5 g of hydrocolloids/100 g of gum.

From the values shown in Table 2, we note that the VO emulsions at concentrations of 2.0, 2.5, and 3 g of hydrocolloids/100 g of aqueous phase and the FD emulsions at 1.5, 2.0, 2.5, and 3.0 g of hydrocolloids/100 g of aqueous phase all exhibit clear thixotropic behavior, presenting a positive value for the area differences. Since the VO emulsions at concentrations of 0.5 g of hydrocolloids/100 g of gum, 1.0 and 1.5 g of hydrocolloids/100 g of aqueous phase, and the FD emulsions at 0.5 and 1.0 g of hydrocolloids/100 g of aqueous phase exhibited a negative difference in the areas, the decline curve (reduction of shear stress) was above the incline curve (increasing shear stress) (Figs. 2a and 2b). These emulsions therefore exhibit anti-thixotropic behavior.

According to Martin, anti-thixotropic behavior results from an increased frequency of collisions of the dispersed particles or polymer molecules in the suspension when the shear rate is increased. Over time, these collisions result in an increase in the binding between particles. With a few exceptions, anti-thixotropy is typically found in dispersions and polymer solutions near the edge...
Anti-thixotropy is contrary to thixotropy in the sense that shear induces aggregation of particles or molecules. This effect is well known and usually irreversible. In this work, while anti-thixotropy was observed in the emulsions with the lowest hydrocolloid concentrations, the values were very small and not significant.

**Table 2.** Thixotropy mean values for the emulsions that were obtained from the OPN hydrocolloids that were vacuum oven-dried and freeze-dried at different concentrations.

| Concentration | Vacuum oven (Pa.s⁻¹) | Freeze-drier (Pa.s⁻¹) |
|---------------|----------------------|---------------------|
| 0.5           | 12 abA*              | 15 aA               |
| 1.0           | 34 aA                | 33 aA               |
| 1.5           | 18 abA               | 99 aB               |
| 2.0           | 119 cA               | 764 bB              |
| 2.5           | 42 bA                | 899 bB              |
| 3.0           | 356 dA               | 1289 cB             |

*Means followed by the same lower letter in the column do not differ by Tukey test (p < 0.05).
Means followed by the same capital letter in the row do not differ by Tukey test (p < 0.05).

**Figure 2.** Thixotropic behavior of emulsions prepared with different OPN hydrocolloid concentrations (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g OPN/100 g aqueous phase) and drying methods (a) vacuum oven (VO)-dried and (b) freeze-dried (FD).
In summary, we observed that the emulsions began to display thixotropic behavior at a concentration of 1.5 g of hydrocolloids/100 g of aqueous phase when prepared with the FD hydrocolloids. This behavior began at 2.0 g of hydrocolloids/100 g of aqueous phase when using the hydrocolloids that were dried in a VO. For both drying methods, the more concentrated emulsions with 3.0 g of hydrocolloids/100 g of aqueous phase showed higher thixotropy and had higher structural degree.\cite{25}

Oscillatory tests were performed to study the viscoelastic properties of the samples. All emulsions exhibited a behavior similar to that shown in Fig. 3, where $G'$ and $G''$ increased with an increasing frequency. For the two drying processes that were evaluated, the values of the elastic modulus ($G'$) were greater than the storage modulus ($G''$) over the entire frequency range. The results showed that the behaviors were primarily elastic for all the emulsions prepared using OPN gum. According to Steffe,\cite{26} a more stable emulsion exhibits a higher internal rheological resistance, resulting in a storage modulus, $G'$, that is greater than its loss modulus, $G''$. This behavior is characteristic of rigid gels.

The tangent of the phase angle ($\tan \delta$) is a common parameter used to characterize the viscoelastic behavior of a material. The value of $\tan \delta$ is calculated by the ratio of the loss modulus $G''$ to the storage modulus $G'$. $\tan \delta < 1$ indicates a predominantly elastic behavior, whereas $\tan \delta > 1$ indicates a predominantly viscous behavior.\cite{27} Figure 3 shows the variation in $\tan \delta$ as a function of frequency for the VO (Fig. 3a) and FD (Fig. 3b) hydrocolloid concentrations of 1.0, 2.0, and 3.0 g of OPN/100 g of aqueous solution. Values of $\tan \delta > 1$ were observed for VO emulsions at lower hydrocolloid concentrations, which would indicate that at low concentrations these emulsions

![Figure 3](image1.png)

Figure 3. (1) Elastic modulus ($G'$), viscous modulus ($G''$), and (2) $\tan \delta$ as function of frequency for emulsions prepared with different concentrations of OPN hydrocolloids (1.0, 2.0, and 3.0 g OPN/100 g aqueous phase) obtained from different drying methods: (a) Vacuum oven and (b) freeze-drying.
display a weakly elastic gel-like behavior.\textsuperscript{[27]} For all FD emulsions, the values of tan $\delta$ were less than 1, which indicates predominantly elastic behavior.

**Emulsifying capacity and the stability of the emulsions**

There was no significant difference ($p > 0.05$) in the EC between the differently dried hydrocolloids, the various hydrocolloid concentrations, or due to a combination of these factors. The average ability to form an emulsion using OPN gum reached 99.11 $\pm$ 0.40%. The ES analysis, from measuring changes in the emulsions over a time period at different concentrations, showed that the drying method was not a significant ($p > 0.05$) factor and that it did not influence the stability of the formed emulsions. The concentration was found to have a significant influence ($p < 0.05$) on the stability of the formed emulsions (data not shown). For the interaction between the drying method and the concentrations, the analysis of variance showed no significant difference ($p > 0.05$).

The stability of the OPN gum emulsions at a concentration of 0.5 g of hydrocolloids/100 g of gum was the lowest. An increasing hydrocolloid concentration increased the stability of the formed emulsion, which reached a maximum stability at a concentration of 3.0 g of hydrocolloids/100 g of gum. At a concentration 2.5 g of hydrocolloids/100 g of gum, the emulsion remained stable (data not shown). Thus, at a certain concentration, the adsorption of the macromolecule at the droplet interface can be difficult, and the aggregates that form do not interfere with the stability of the emulsion.

**Microstructural characterization**

**Sizes and distribution of droplets**: According to Chanamai and McClements,\textsuperscript{[28]} the droplet size in an emulsion contributes to macroscopic properties such as its color, opacity, rheology, ES, and coalescence. Since the drying methods did not influence the stability of the emulsions, the analysis of droplet sizes was performed in defined areas of the FD OPN emulsion micrographs only. The droplets sizes were measured for each concentration of FD emulsion using the corresponding micrographs (Fig. 4). The results for the number of droplets (ND), the area, the Feret diameter ($D_{AV}$), the standard deviation (SD), and secondary parameters such as compactness and shape factor are presented in Fig. 5a. Figure 5a shows an exponential increase in the number of droplets and an exponential reduction in the values of area, Feret diameter, and standard deviation as a function of the OPN concentrations in the emulsions. The exponential behavior of these parameters can be represented by Eq. (3) of Table 3. The increase in the number of droplets (Fig. 5a) is caused by the increase in concentration and is justified by the OPN adsorption at the oil–water interfaces that decreases the interfacial energy, facilitating the shearing of the droplets.\textsuperscript{[29]}

Figure 5a also shows the decrease in the Feret diameter of droplets as function of OPN concentrations. A notable reduction in the Feret diameter can be observed between the concentrations of 0.5–1.5 g hydrocolloids/100 g aqueous phase in which the average diameter decreased from 29.3 to 10.89 $\mu$m, a reduction of approximately 63%. The analysis of the standard deviation of the Feret diameter of the droplets allows to evaluate the homogeneity in the average diameter of the emulsion droplets. Figure 5a shows that increasing the hydrocolloid concentration in the emulsions decreases the standard deviation of the average diameter of the droplets, indicating that the emulsions with low hydrocolloid concentrations had more heterogeneously distributed diameters. The fall becomes less notable going from the concentration of 1.5 (standard deviation 7.38 $\mu$m) to 3.0 g OPN/100 g aqueous phase (standard deviation 2.60 $\mu$m), indicating a considerable increase in homogeneity of the droplet diameters. It appears from Fig. 5a that the increase in the hydrocolloid concentration in the preparation of the emulsion resulted in the decrease of the perimeter (data not shown) and area of the droplets. The analyses of these parameters assist in the geometric and morphological understanding of the droplets, represented as compactness and shape factor or circularity.

Figure 5b presents the compactness data that directly relates the size parameters with droplet shape, the anisotropy (primarily), and intrinsic viscosity. With regard to compactness, the closer the
value to 12, the more compacted are the droplets. In Fig. 5b, the value corresponding to the concentration of 2.5 g OPN/100 g of aqueous phase is the one with the most compact and least anisotropic droplets. The shape factor indicates the uniformity of the droplets. Droplets with shape factor approaching 1 are more uniform. In Fig. 5b, it is observed that the emulsion with 2.5 g OPN/100 g aqueous phase has the highest shape factor value (0.95). This emulsion also has the highest compression and, therefore, the most stable morphological structure among the other emulsions evaluated.

**Distribution of droplet diameters:** The droplet size distribution is shown as a histogram plot of relative frequency versus binned sizes (Fig. 6). In Fig. 6a, a broad diameter distribution of the droplets for the concentration of 0.5 g OPN/100 g aqueous phase is observed. The size is found to vary by approximately 80 µm between the smallest diameter (5.54 µm) to the largest diameter (87.80 µm). The diameter class with the highest frequency (30.45%) is found to be between 1 and 11 µm. Other smaller peaks can also be observed, such as in the diameter class ranging from 41 to 51 µm with a relative frequency of 13.05%. Figure 6b shows that the droplet diameter distribution
range for the emulsion prepared with a hydrocolloid concentration of 1 g OPN/100 g aqueous phase decreased considerably compared to 0.5 g OPN/100 g aqueous phase. The most frequent diameter class was between 11 and 21 µm, with a relative frequency of 61.45%. Additionally, in these emulsions, the droplet sizes were heterogeneous. The effect of increased concentration on the droplet diameters is verified by the increase in the relative frequency in the 1–11 µm class, resulting in more homogeneous emulsions (Figs. 6c–6f and 4).

Table 3. Parameters of exponential model adjusts applied in the droplet size analyses.

|       | $y_0$   | $A$    | $B$   | $R^2$ |
|-------|---------|--------|-------|-------|
| ND/area* | -334.5  | 304.4  | 0.598 | 0.993 |
| Area ($\mu m^2$) | 86.0    | 5597.7 | -2.53 | 0.999 |
| $D_{AV}$ (µm) | 0.839   | 3.567  | 0.164 | 0.992 |
| SD (µm)   | 3.637   | 58.475 | -2.541| 0.953 |

ND/area = number of droplets/area; $D_{AV}$ = Feret diameter; SD = standard deviation.
Fluorescence microscopy: Fluorescence microscopy provided a qualitative evaluation of the emulsion microstructures by showing the presence of compounds using fluorochromes. Fluorochromes are substances that bind to specific compounds, and when activated and viewed using the appropriate wavelengths, they fluoresce at the binding site, highlighting the compound of interest. The microscopic examination of the OPN emulsions using the fluorochrome Red Nile (Fig. 7a) showed the fluorescence of lipids covering all the droplet surfaces. Rhodamine B (Fig. 7b) showed fluorescence around the emulsion droplets, demonstrating that the protein molecules were adsorbed around the droplets. The adsorption of these molecules around the droplets shows that the OPN hydrocolloids in the emulsions acted as emulsifiers and stabilizers. Figure 7 shows that OPN gum proteins are responsible for stabilizing oil/water-type emulsions. At the interface, the non-polar groups interact with the oil droplets, while the charged terminal groups that protrude are presented to the aqueous phase. The involvement of the contrary ions forms a double layer, which prevents the aggregation of the oil droplets as observed in Fig. 7a. Protein molecules can be oriented in such a way that their hydrophobic and hydrophilic extremes are placed on the oil/water interface (Fig. 7b).

Conclusion

The emulsions prepared using higher concentrations of FD OPN hydrocolloids exhibited more evident thixotropic behavior. The formed emulsions were determined to be pseudoplastic fluids.
based on the power law model and exhibited predominantly elastic behavior. The EC did not change based on the drying method used in the extraction process or the OPN concentration. However, the emulsions exhibited a high stability at higher concentrations of hydrocolloids.

The micrographs of the emulsions with FD hydrocolloids showed that with an increase in the hydrocolloid concentration, there was an increased number of emulsion droplets and a greater uniformity in droplet size. The emulsion with 2.5 g OPN/100 g aqueous phase had the highest compression and, therefore, the most stable morphological structure among the other emulsions evaluated.

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**Figure 7.** Fluorescence photomicrograph of emulsions constituted of 2.0 g of OPN gum/100 g of aqueous phase stained with (a) Red Nile (fluorescence of lipids) and (b) Rhodamine B (fluorescence of proteins) dyes.
References

1. McClements, D. J. Food Emulsions. Principles, Practices, and Techniques, 2nd ed.; CRC Press: Boca Raton, 2005.
2. Choi, M. J.; Briançon, S.; Bazile, D.; Royere, A.; Min, S. G.; Fessi, H. Effect of Cryoprotectant and Freeze-Drying Process on the Stability of W/O/W Emulsions. Drying Technology 2007, 25(5), 809–819. DOI: 10.1080/0737393071370183.
3. Martínez-Cervera, S.; Salvador, A.; Sanz, T. Cellulose Ether Emulsions as Fat Replacers in Muffins: Rheological, Thermal and Textural Properties. LWT - Food Science and Technology 2015, 63, 1083–1090. DOI: 10.1016/j.lwt.2015.04.067.
4. Dickinson, E. Hydrocolloids at Interfaces and the Influence on the Properties of Dispersed Systems. Food Hydrocolloids 2003, 17, 25–39. DOI: 10.1016/S0260-8774(01)00120-5.
5. Saha, D.; Bhattacharya, S. Hydrocolloids as Thickening and Gelling Agents in Food: A Critical Review. Journal of Food Science and Technology 2010, 47(6), 587–597. DOI: 10.1007/s13197-010-0162-6.
6. Wu, J.; Gan, N.; Hu, L.; Chen, S.; Pan, H.; Liu, H. Effect of Lyophilization Process on the Physical Properties and Stability of Tanshinone: A Micro-Emulsions. Drying Technology 2016. DOI: 10.1080/07373937.2016.1238387.
7. Duarte, M. R.; Hayashi, S. S. Estudo Anatômico de Folha E Caule de Pereska Aculeata Mill. (Cactaceae). Revista Brasileira de Farmacognosia 2005, 15(2), 103–109. DOI: 10.1590/S0102-695X2005000200006.
8. de Souza, M. R. M.; Correa, E. J. A.; Guimarães, G.; Gomes, P. R. O Potencial Do Ora-Pro-Nobis Na Diversificação Da Produção Agrícola Familiar. Revista Brasileira de Agroecologia 2009, 4(2), 3550–3554.
9. Almeida Filho, J.; Cambraia, J. Estudo Do Valor Nutritivo Do “Ora-Pro-Nobis” (Perakia Aculeata Mill.). Revista Ceres 1994, 21(114), 105–111.
10. Sierakowski, M. R.; Gorin, P. A. J.; Reicher, F.; Corrêa, J. B. Some Structural Features of a Heteropolysaccharide from the Leaves of the Cactus Pereska Aculeata. Phytochemistry 1987, 26, 1709–1713. DOI: 10.1016/S0031-9422(00)82273-6.
11. Sierakowski, M. R.; Gorin, P. A. J.; Reicher, F.; Corrêa, J. B. C. Location of O Acetyl Groups in the Heteropolysaccharide of the Cactus Pereska Aculeata. Carbohydrate Research 1990, 201, 277–284. DOI: 10.1016/0008-6215(90)84243-N.
12. Lima Junior, F. A.; Conceição, M. C.; Vilela de Resende, J.; Junqueira, L. A.; Pereira, C. G.; Torres Prado, M. E. Response Surface Methodology for Optimization of the Muclilage Extraction Process from Pereskia aculeata Miller. Food Hydrocolloids 2013, 33(1), 38–47. DOI: 10.1016/j.foodhyd.2013.02.012.
13. Singh, N.; Kaur, M.; Sandhu, K. S. Physicochemical and Functional Properties of Freeze-Dried and Oven Dried Corn Gluten Meals. Drying Technology 2005, 23(4), 975–988. DOI: 10.1081/DRT-200045253.
14. Oikonomopoulou, V. P.; Krokida, M. K. Structural Properties of Dried Potatoes, Mushrooms, and Strawberries as a Function of Freeze-Drying Pressure. Drying Technology 2012, 30(4), 351–361. DOI: 10.1080/07373937.2011.639475.
15. Thanatcha, R.; Franee, A. Extraction and Characterization of Mucilage In. International Food Research Journal 2011, 18, 201–212.
16. Schmitt, C.; Sanchez, C.; Lamprecht, A.; Renard, D.; Lehr, C. M.; De Kruijf, C. G.; Hardy, J. Study of B-Lactoglobulin/ACacia Gum Complex Coacervation by Diffusing-Wave Spectroscopy and Confocal Scanning Laser Microscopy. Colloids and Surfaces B: Biointerfaces 2001, 20(3), 267–280. DOI: 10.1016/S0927-7755(00)00200-9.
17. ATTI Bioquest. Nile Red * Ultra Pure Grade * Assay Protocol with Nile Red, Sunnyvale, CA, 2012.
18. Medina-Torres, L.; Brito-De La Fuente, E.; Torrestiana-Sanchez, B.; Katthain, R. Rheological Properties of the Mucilage Gum (Opuntia ficus Indica). Food Hydrocolloids 2000, 14, 417–424. DOI: 10.1016/S0268-005X(00)00151-5.
19. İbanoğlu, E. Rheological Behaviour of Whey Protein Stabilized Emulsions in the Presence of Gum Arabic. Journal of Food Science and Technology 2002, 52(3), 273–277. DOI: 10.1007/S12074-000-00115-7.
20. Bhattacharya, S.; Bhat, K. K.; Raghuvan, K. G. Rheology of Bengal Gram Cicerarietinum Flour Suspensions. Journal of Food Engineering 1982, 17, 83–96. DOI: 10.1016/0260-8774(92)90054-A.
21. Maskan, M.; Gogus, F. Effect of Sugar on the Rheological Properties of Sunflower Oil-Water Emulsions. Journal of Food Engineering 2000, 43, 173–177. DOI: 10.1016/S0260-8774(99)00147-8.
22. Goff, H. D.; Jordan, W. K. Action of Emulsifiers in Promoting Fat Destabilization during the Manufacture of Ice Cream. Journal of Dairy Science 1989, 72(1), 18–29. DOI: 10.3168/jds.S0022-0302(89)79075-5.
23. Mewis, J. Thixotropy - A General Review. Journal of Non-Newtonian Fluid Mechanics 1979, 6, 1–20. DOI: 10.1016/0377-0257(79)87001-9.
24. Martin, A. N. *Physical Pharmacy*, 4th ed.; Lea & Febiger: London, 1993; p 473.
25. Bellalta, P.; Troncoso, E.; Zúñiga, R. N.; Aguilera, J. M. Rheological and Microstructural Characterization of WPI-stabilized O/W Emulsions Exhibiting Time-Dependent Flow Behavior. *LWT - Food Science and Technology* **2012**, *46*(2), 375–381. DOI: 10.1016/j.lwt.2011.12.017.
26. Steffe, J. F. *Rheological Methods in Food Process Engineering*, 2nd ed.; Freeman Press: Michigan, 1996.
27. Capitani, M.; Corzo-Rios, L. J.; Chel-Guerrero, L.; Betancur-Ancona, D.; Nolasco, S. M.; Tomás, M. Rheological Properties of Aqueous Dispersions of Chia (*Salvia hispanica* L.) Mucilage. *Journal of Food Engineering* **2015**, *149*, 70–77. DOI: 10.1016/j.jfoodeng.2014.09.043.
28. Chanamai, R.; McClements, D. J. Dependence of Creaming and Rheology of Monodisperse Oil-In-Water Emulsions on Droplet Size and Concentration. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2000**, *172*(1–3), 79–86. DOI: 10.1016/S0927-7757(00)00551-3.
29. Mirhossein, H.; Tan, C. P.; Hamid, N. S. A.; Yusof, S. Effect of Arabic Gum, Xanthan Gum and Orange Oil on Flavor Release from Diluted Orange Beverage Emulsion. *Food Chemistry* **2008**, *107*, 1161–1172.