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Rheological characterization and stability study of an emulsion made with a dairy by-product enriched with omega-3 fatty acids

Caracterização reológica e estudo da estabilidade de uma emulsão preparada com subproduto de indústria de laticínios enriquecido com ácidos graxos ômega-3

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Summary

This study involved a rheological characterization of a W/O emulsion manufactured on a pilot scale using omega-3 fatty acids as part of the oil phase and butter milk as the emulsifier. Polyunsaturated omega-3 fatty acids are essential to prevent cardiovascular diseases, improve pulmonary function and also form part of the neurological structure. Buttermilk is a by-product of the dairy industry and has a high organic load which possesses surfactant properties and constitutes a good substitute for conventional emulsifiers in the food industry. The microstructural nature of the emulsion was characterized from the viscoelastic parameters and mechanical spectra. The linear viscoelastic range was determined, from which the maximum stress that the emulsion could withstand from the processing conditions without altering its microstructure was established. In addition, the storage stability of the emulsion was studied to instrumentally predict the rheological behaviour before sensory destabilization of the emulsion was observed. At the frequencies used, a significant decrease in dynamic viscoelastic parameters was periodically observed (G 'and G''), showing a structural change during storage. Furthermore, a coalescence phenomenon was observed after 18 months. The formulation with added omega-3 fatty acids and buttermilk provided a basis for obtaining a functional food as well as adding value to an industrial by-product.

Key words: *Rheology; W/O emulsion (water/oil); Emulsifier; Butter milk; Omega- 3 fatty acids; Functional food.*

Resumo

Este estudo caracteriza reologicamente uma emulsão W/O preparada com ácidos graxos ômega-3 na fase oleosa e soro de manteiga, fabricada em escala piloto. Os ácidos graxos poli-insaturados ômega-3 são essenciais para prevenir doenças cardiovasculares, melhorar a função pulmonar e, além disso, formam parte da estrutura neurológica. O soro de manteiga é um subproduto da indústria de laticínios com alta carga orgânica, a qual possui propriedades surfactantes e constitui um bom substituto dos emulsificantes convencionais na indústria de alimentos. A natureza microestrutural da emulsão foi caracterizada através de parâmetros viscoelásticos e espectros mecânicos, caracterização que permitiu determinar a faixa viscoelástica linear e estabelecer a faixa de esforço que suporta a microestrutura, se mantendo íntegra às condições de processo. A estabilidade da emulsão foi estudada durante o armazenamento com a finalidade de predizer instrumentalmente o comportamento reológico antes da sua deterioração sensorial. Nas frequências utilizadas periodicamente foi observado um decréscimo significativo nos parâmetros viscoelásticos dinâmicos (G' e G''), evidenciando modificações na estrutura e coalescência após 18 meses de armazenamento. A formulação feita com adição de ácidos graxos ômega-3 e soro de manteiga funciona como base para obter um alimento com propriedades funcionais, além de agregar valor a um subproduto industrial.

Palavras-chave: *Reologia; Emulsão W/O; Emulsificante; Soro de manteiga; Ácidos graxos ômega-3; Alimento funcional.*

1 Introduction

Omega-3 fatty acids (ω3) are essential nutrients and are also considered functional ingredients because of their health benefits, including reductions in cardiovascular risk, mental illness and the symptoms of inflammatory disease. In addition, they may improve vision, cognitive development and child brain development and influence pregnancy and lactation, since they are constituents of the phospholipids in cell membranes and neurological structures (BLACK and RHODES, 2006; BOURRE, 2007; KRUTULYTE et al., 2008; RITTER-GOODER et al., 2008; SIRÓ et al., 2008; VON SCHACKY, 2011). The addition of these fatty acids to food formulations results in desirable functional characteristics for the consumer. In food processing these fatty acids are added by using dispersions (DALGLEISH, 2006). Within these dispersions the spherical oil droplets in the W/O emulsion must be stabilized in the aqueous phase by surfactants or hydrocolloid emulsifiers in order to increase the repulsive forces and prevent aggregation of the oil droplets, which would undesirably alter product quality. The active surface of these surfactants is adsorbed onto the oil-water interface and has a low surface tension (SUN and GUNASEKARAN, 2009). Gum Arabic, modified starches and certain proteins that are extracted from a wide variety of natural sources can be used as food emulsifiers due to their ability to facilitate the formation of water-oil emulsions, improve their stability and impart desirable physicochemical properties (SURH et al., 2006a, b).

Butter milk is an abundant industrial by-product with remarkable emulsifying properties conferred by proteins that help to control the texture and also improve the nutritional quality (BOUTIN et al., 2007). The emulsifying properties result from the physical, mechanical and rheological properties of the whey proteins, as an interfacial film is formed. The mechanical strength and viscoelasticity have a marked influence not only on the emulsion properties but also on the effectiveness of its formation (especially its stability) (GU et al., 2005). The major proteins that act as emulsifiers in butter milk are β-casein and αs1-casein (GU et al., 2005). These proteins have a significant amphiphilic character and adsorb onto the oil-water interface to form stable emulsions (BOUTIN et al., 2007).

To maintain the stability of the emulsion, strict control of the homogenization conditions and the composition is required, and these factors significantly affect the protein concentration and performance of the water-oil interface (BOUTIN et al., 2007). By increasing the concentration, increased protein adsorption is facilitated, which enhances not only the emulsion stability but also the viscosity (GU et al., 2005).

The study of rheological emulsions using various tests and measurements can allow for an understanding and correlation of the different destabilization mechanisms that occur in an emulsion, providing complete knowledge regarding the structural state under study, predicting the stability and identifying the mechanism through which destabilization occurs (TADROS, 2004).

The aim of this study was to determine the rheological characterization and stability of a W/O emulsion formulated using ω3 fatty acids as part of the oil phase and butter milk as the emulsifier. This emulsion was manufactured on a pilot scale and stored under refrigeration.

2 Material and methods

2.1 Emulsion preparation

A water-in-oil emulsion was prepared using a commercial olive oil brand "The Spanish", that was enriched with ω3 fatty acids, as the oil phase. Butter milk, stabilized by spray drying (moisture 4.0 ±.0.7%, fat 8.5 ± 0.5%, protein 30.0 ± 0.5%), was used as the emulsifier, and these ingredients were added in proportions of 69% and 6.9%, respectively, of the total weight of the formula. The pilot scale components were incorporated using a Delmix brand-VK-7 colloidal mill (Aaron Equipment Company, Chicago, Il., USA).

The process started with the addition of the solid ingredients, followed by the aqueous phase and an initial homogenization. Oil was then added at a constant speed for 3 minutes for the final homogenization. The emulsification process was carried out at a speed of 2860 rpm and 20 ± 2 °C. The time for each of the stages was monitored.

After processing, the emulsion was allowed to stand for two hours for the internal temperature to decrease before the rheological characterization.

2.2 Rheological and microstructural characterization

The rheology of the emulsion was characterized using flow curves and mechanical spectra, and the microstructural level characterized by studying the droplet size distribution.

To determine the rheological properties of the emulsion, a ThermoHaake brand (USA) Haake Mars II controlled stress rheometer equipped with a thermostatic bath with a precision of ± 2 °C, and the software Haake RheoWin 4.0, Mars II, 2009, were used for data processing.

For flow testing, an oscillatory shear test (SAOS) with a 60 mm diameter (Gap = 1 mm) sensor system rugged plate-plate were applied to avoid slipping during the surface measurements of the food emulsions with high oil contents (RUIZ-MÁRQUEZ et al., 2010).

2.2.1 Flow curves

Flow curves were obtained using the CS-CR rotation method (Controlled Stress-Controlled Rate) at a shear stress range between 0.1 Pa and 80 Pa and 20 steps of 180 seconds each. The experimental conditions were adjusted in preliminary tests. The curves were obtained within an integration time of 30 seconds and with an equilibrium time of 5 minutes prior to sample testing at 20 ± 0.2 °C. The assays were repeated three times and the experimental data fitted to the Power Law model (Equation 1) to establish the type of non-Newtonian emulsion behavior. In order to adjust the data to the model, a nonlinear regression analysis was carried out using the software Origin Lab® V 8.1 SR1 2009.

$$\tau = K\gamma^n \quad (1)$$

Where τ is the shear stress, γ is the shear rate, K is the consistency index, and n is the performance index. From this model it can be inferred that if n>1, the fluid is showing dilatant behaviour; if n<1, it shows pseudoplastic behaviour; if n=1, it is exhibiting Newtonian behaviour (SCHRAMM, 2004).

2.3 Droplet size distribution

A Malvern Instruments Ltd. MasterSizer X particle size analyser (UK) was used to obtain the droplet size distribution. A 1% sodium dodecyl sulphate (SDS) dispersing medium dissolved in 0.05 M Tris-HCl buffer (pH 8) was used with a 100 mm lens. The diluted solution was homogenized and then assessed using laser diffraction analysis. The conditions were set at 50% agitation and 80% pumping for 20 minutes. The experimental data were fitted to Equation 2. Six consecutive measurements were taken for each test.

$$y = 1 - e^{-(x/r)^n} \quad (2)$$

Where y is the percentage volume accumulated, x is the particle diameter, r is the diameter for which the function has a value of 0.632, and n is a parameter that is inversely proportional to the particle size dispersion in the medium. The Sauter diameter (D [3,2] (μm)), volumetric diameter (D [4,3] (μm)) and amplitude values (A) were obtained, the latter being the width of distribution based on the 10%, 50% and 90% quantiles (Equation 3).

$$A = \frac{D_{[v,0.9]} - D_{[v,0.1]}}{D_{[v,0.5]}} \quad (3)$$

2.4 Stability study

This study consisted of a macroscopic observation of the emulsion and the development of viscoelastic assays of low oscillatory shear amplitude. The study was dependent on the storage time, and thus the data obtained was able to indicate the physical destabilization of the emulsion.

To assess the emulsion stability, samples were placed in glass jars and stored under refrigeration (4±2 °C). During the first 3 months, the droplet size distributions were measured at one-monthly intervals. After the third month, frequency sweeps were applied every two months up to the 18th month of storage. The tests were carried out using the previously described conditions.

3 Results and discussion

3.1 Flow curve

The thixotropic behaviour was determined using ascending and descending scanning to constant speed. It was found that for the same shear rate value, two different values were obtained for shear stress, which indicated that the emulsion prepared presented thixotropic behaviour (data not shown).

Figure 1 shows the data for the fit to the power law model, which reflected pseudoplastic non-Newtonian behaviour in the shear rate range from 10^{-4} to 10^{3} s^{-1} and a stress range from 0.1 Pa at 80 Pa. The viscosity calculated at 1 s^{-1} and 100 s^{-1} was 53.72 and 91.63 ± 2.36 Pa s, respectively. The intercept or consistency index (K) was 1.730 ± 0.014, and the correlation coefficient (r^2) was 0.997. Therefore the flow behaviour index (n) was 0.115 and a n-value between 0 <n <1 indicates a pseudoplastic nature in the emulsion.

These results agree with the value obtained by Taherian et al. (2011), who used whey protein, gelatine and a mixture of both as emulsifiers for the water-in-oil emulsion. They also found that when only the protein was added, the emulsion showed pseudoplastic behaviour; however, when the mixture was used, Newtonian behaviour was observed. Martínez et al. (2007) worked

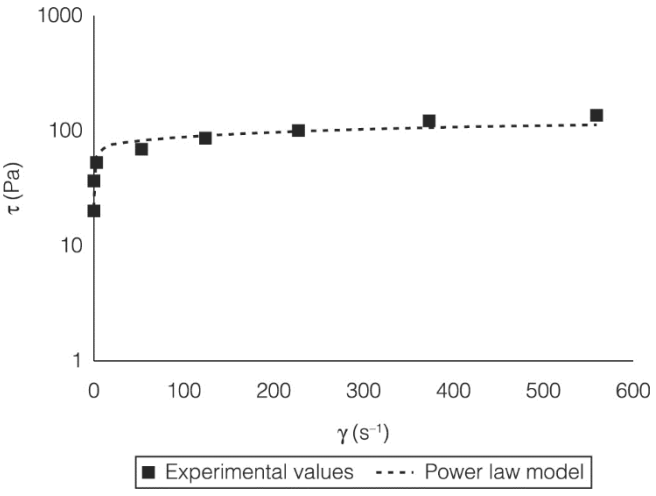

Figure 1. Flow behaviour curve and power law model fitting (T ° = 20 ± 2 °C).

with binary mixtures of an emulsifier consisting of egg yolk and different amphiphilic molecules (Tween 20, sucrose laurate, and pea protein), and reported pseudoplastic behaviour which adjusted to the Carreau model. Sosa-Herrera et al. (2012) described Newtonian behaviour when mixing sodium caseinate (1% and 2%) and sodium alginate (0.1%), whereas the addition of $CaCl_2$ (5 mM) resulted in pseudoplastic behaviour with viscoelastic characteristics. The power law model was used to describe the flow properties of W/O mayonnaise type emulsions, and found pseudoplastic behaviour (GUILMINEAU and KULOZIK, 2007; IZIDORO et al., 2008), results agreeing with those obtained in the present work.

3.2 Determination of a linear viscoelastic zone

During the sweeping stress analysis, it was established that the emulsion showed elastic behaviour that overrode the viscous component at an effort greater than 10 Pa, and the structural destruction of the emulsion was evident.

During the time sweep, a stress of 0.2 Pa was used to ensure that the stress was within the linear viscoelastic zone during the frequency sweep. Since the storage modulus and loss modulus were held constant, this test ensured a linear zone and required 300 seconds of equilibration time. Results for the frequency sweep were not obtained at frequencies above 8 rad s^{-1} (1,27Hz) due to inertia problems. A linear fit was obtained for the double logarithmic representation of G' versus frequency; with a slope value of 0.075 ± 0.002 and a correlation coefficient of 0.997. The G' value at a frequency of 6.28 rad s^{-1} corresponded to 238 Pa (results not shown).This was possibly due to the sensitivity of these emulsions and to coalescence, and may require the use of an energy adjustment per unit volume according to the processing conditions (SAIKI et al., 2007).

All the mechanical spectra showed a weak gel-like behaviour with an elastic component (G') but were frequency dependent, and the viscous component (G'') was lower and showed a minimum at lower frequency. Table 1 summarizes the optimum conditions obtained during the rheological characterization of the emulsion.

The characteristics described in Table 1 are important during the processing of the emulsion in order to determine the processing conditions. During homogenization the collisions between droplets are particularly rapid due to the intense mechanical energy to which the emulsion is subjected, resulting in deformation of the oil droplets, a phenomenon that can be controlled by the viscosity of the emulsion, droplet size, the type of emulsifier and the oil phase used (SAIKI et al., 2007). If the drops are not sufficiently protected by a membrane produced by the interfacial emulsifier, they may coalesce more easily with neighbouring drops. The membrane interfacial resistance against coalescence depends largely on the concentration of the emulsifier molecules, their chemical properties and structural dimensions, the electrical charge of the emulsifier and the intermolecular interactions (SAIKI et al., 2007; THANASUKARN et al., 2004). In addition, Ruiz-Márquez et al. (2010), prepared oil-in- water emulsions made with tuna proteins and found that the dynamic viscoelastic functions showed behaviour similar to that of the emulsion of the present study.

Table 1. Values obtained in the characterization of the linear viscoelastic zone.

Parameter	Optimal value
Maximum shear stress	0.5 Pa
Equilibration time	300 s
Maximum frequency	8 rad s^{-1} (1.27 Hz)
Behaviour	Weak gel

According to Taherian et al. (2011), the addition of whey protein improved the emulsion stability by increasing electrostatic repulsion (pH 6.8) and is useful for delivering ω3 fish oil into the milk beverages. Similarly, serum proteins combined with fish gelatine to coat the oil phase and provide stability to the emulsion, allow for the addition of omega-3 fish oil to fruit drinks.

This synergistic protein can be used as an effective emulsifier to formulate food emulsions under acidic conditions, and thus the results of this study may have practical applications in the design of industrial dispersions to incorporate functional ingredients into emulsified beverages (TAHERIAN et al., 2011). It is noteworthy that whey proteins act as a butter emulsifier, mainly due to electrostatic and steric interactions, which confer resistance to the emulsion against destabilization phenomena, such as flocculation and coalescence, through the accumulation of particles at the oil-water interface as a dense layer (MATSUMIYA et al., 2010). This layer of coarse particles prevents flocculation and coalescence of the droplets through a steric mechanism. The extent of the steric barrier depends on how difficult it is to remove the particles from the oil-water interface, which would form monolayers, different structures or arrangements oriented to the continuous phase and prevent the displacement of particles outside or inside the layer (FISCHER and WINDHAB, 2011). In turn, the interface may also form a rigid disordered network or particles may become absorbed in the oil-water interface due to attractive forces between particles (FISCHER and WINDHAB, 2011).

3.2.1 Stress sweep at different frequencies

Figure 2 shows that of the frequencies tested, the linear zone is narrower for that corresponding to 0.1 Hz, thus ensuring that the sample structure has not been

altered. In this situation, the elastic (G') and viscous (G'') components remain practically constant up to a certain deformation value (called the critical strain, γ_c) at which point G' decreases and G'' increases. If one knows the critical strain and stress values, the extreme conditions during emulsion processing that could cause structural damage to the product (TCHOLOKOVA et al., 2002) can be determined.

Table 2 shows the values corresponding to the critical strain (γ_c) and critical stress (σ_c) evaluated at different frequencies, and the frequencies (ω) of 0.1 Hz (0.63 rad.s^{-1}) and 0.5 Hz (3.14 rad.s^{-1}) were those showing smaller critical values. However, for the purposes of this study, the frequency of 0.1 Hz was selected as the most suitable for providing a rapid drop in the G' values in contrast to the other frequencies tested, thus narrowing the linear region with a corresponding increase in the G'' values.

Previous studies on emulsions containing whey proteins (TAHERIAN et al., 2011), gluten, soy and crab concentrate (BENGOECHEA et al., 2008, 2010) showed higher values for G' as compared to G'', and are consistent with those obtained in the present work, thus demonstrating that the emulsion has a greater elastic component effect on the viscous component (G''), an effect which is predominant in the solid state. The values for G' explained a semisolid structure and showed the interaction between protein, oil, polysaccharides and the other components that form the emulsion (FERNANDEZ et al., 2012).

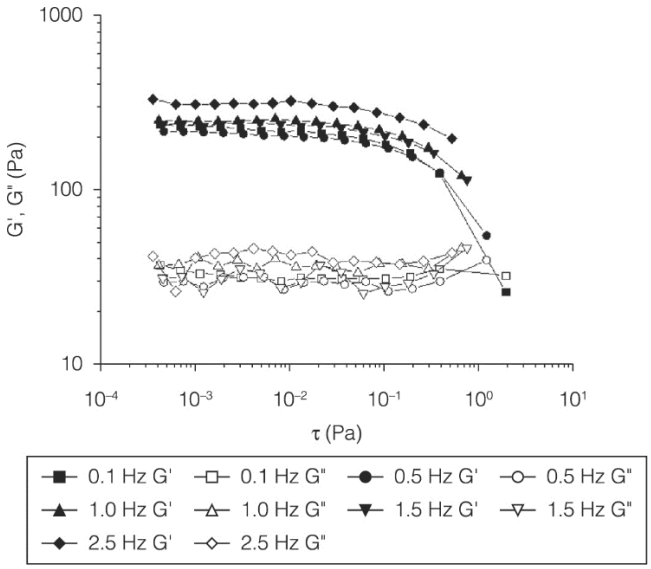

Figure 2. Stress sweep at different frequencies.

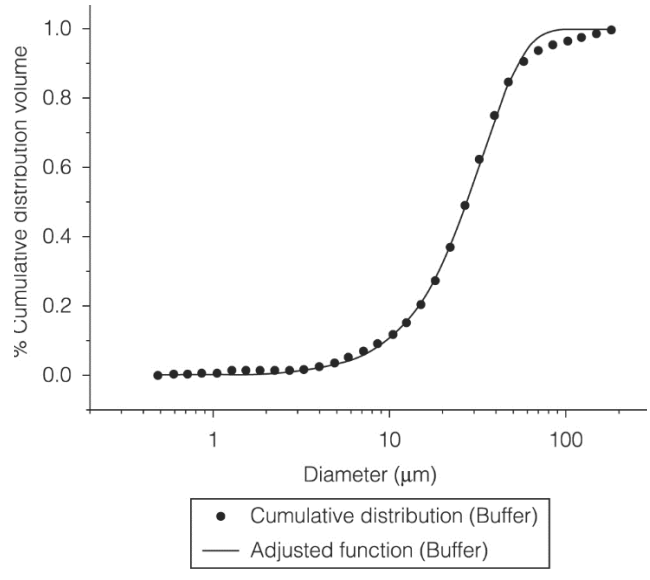

Figure 3. Cumulative droplet size distribution.

3.3 Droplet size distribution

Figure 3 shows the cumulative droplet size distribution and the data fitted to Equation 2 with R^2=0.9987.

Table 3 shows the parameters of the droplet size distribution obtained from the experimental data, with the values for the Sauter diameter (D [3.2] (μm)), volumetric diameter (D [4.3] (μm)) and amplitude (A) for a freshly prepared emulsion.

Thus the values reported were within the range for oil droplet diameters and agree with the data obtained by Fernandez et al. (2012), who measured the Sauter diameter for two commercial mayonnaises. The first sample had 77% oil and added starch and a value for D [3.2] = 3.24 μm, and the second sample contained 30% oil and a value for D [3.2] = 6.22 μm. It is important to note that the droplet size influences the emulsion properties, such as the rate of degradation, the textural characteristics, the stability after lengthy storage and resistance to the creaming phenomenon (FERNANDEZ et al., 2004). Guggisberg et al. (2012) found that emulsions made with buttermilk and sunflower oil showed destabilization when D [3.2] was

Table 2. Stress and critical strain values at different frequencies.

ω (rad.s^{-1})	σ_c (Pa)	γ_c
0.63	4.6	0.021
3.14	4.6	0.023
6.28	12.3	0.052
9.42	12.9	0.060
15.70	14.2	0.047

Table 3. Droplet size distribution parameters.

Parameter	SDS – Buffer
r (μm)	25.5
N	1.3
R^2	0.998
D[3.2] (μm)	4.5 ± 1.7
D[4.3] (μm)	19.6 ± 3.7
A	1.407 ± 0.075

over 6.2 µm and D [4.3] over 33 µm. The results obtained in this study are in the range where the product is stable, indicating that the droplet size obtained in this formulation was comparable to commercial mayonnaises.

3.4 Stability study

Table 4 presents the values for the Sauter diameter (D [3.2] (µm)), the volumetric diameter (D [4.3] (µm)) and the amplitude (A) obtained from the microstructural analysis. There was a slight increase in droplet size, indicating a gradual growth of droplet diameters up to the fourth month, which does not reflect emulsion destabilization. Due to their high molecular weight, proteins develop much thicker interfacial films, which provide cohesiveness to the different system phases. Thus, prolonged stability against the coalescence phenomena that are typical in emulsions with high oil contents (FERNANDEZ et al., 2004) was achieved, and according to Guggisberg et al. (2012) the emulsion had good storage stability.

3.4.1 Frequency sweeps

Figure 4 shows the frequency sweeps between months 4 and 18. The figure shows that during the first 12 months, there was no significant difference between the values obtained for the storage modulus G', while from months 14 to 18, there was variability, with a clear decreasing tendency for both the storage (G') and loss (G'') moduli. This could occur due to an increased coalescence rate, which is associated with a net charge reduction and consequent reduction in repulsion amongst coated oil droplets, as a result of the pH being near the isoelectric point (PI) of the whey proteins (THANASUKARN et al., 2004).

In the stability studies, after eight months Lorenzo et al. (2008) were able to visually observe low fat emulsion destabilization in a blend of guar gum and xanthan gum (2% maximum),

Some studies have demonstrated a dependence on the storage modulus and loss modulus related to the frequency, as well as a close relationship with the nature of the emulsifier used, and the method used to manufacture the emulsion (RUIZ-MÁRQUEZ et al., 2010). As in the case of Taherian et al. (2011), the addition of whey protein resulted in a decrease in G' and G'' as the storage time progressed, which is indicative of a destabilizing type of emulsion, consistent with the results of the present study.

The emulsion pH evaluated in this study was between 3.3 and 3.7, and the PI of the butter milk protein was in the range from 4.40 to 4.76 and from 4.83 to 5.07 for αs_1-casein and β-casein, respectively (GU et al., 2005). Whatever the nature of the emulsion, once the emulsion is formed, the main factor determining its stability to coalescence is the repulsive nature or other interactions between droplet surfaces, without neglecting the thickness of the stabilizing layer (DICKINSON, 2010). If the droplet surface is covered by a dense layer of particles (or is barely covered) it may be influenced significantly by adjusting the pH and/or ionic strength (DICKINSON, 2010).

Table 4. Droplet diameter during the first four months of storage.

Month	D[3.2] (µm)	D[4.3] (µm)	A
1	4.5	19.3	1.576
2	4.7	20.6	1.668
3	4.9	23.6	1.823
4	5.1	25.5	1.927

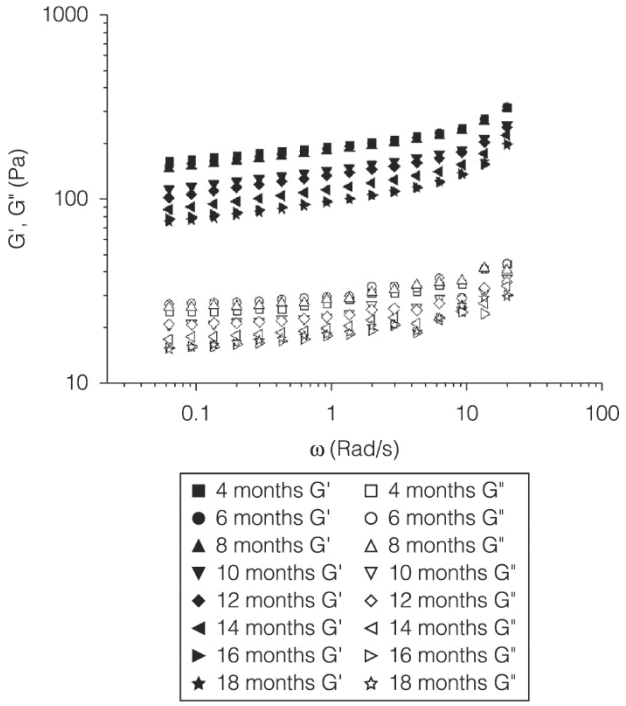

Figure 4. Frequency sweep to study sample stability over time (G '▪ and G''▫).

4 Conclusions

This work contributed to the study of the rheological properties of a W/O emulsion formulated with fatty acids, using ω3 as the oil phase and butter milk as the emulsifier, which stabilized the emulsion. This occurred through electrostatic and steric interactions that conferred resistance to the product against destabilization phenomena such as flocculation and coalescence, which are predominant destabilizing mechanisms associated with a net load reduction with time. In the periodical frequency sweeps, a significant decrease was observed in the dynamic viscoelastic parameters (G' and G'') as shown by the structural changes during storage and the coalescence phenomenon observed after 18 months.

Using the proposed formulation, it is feasible to obtain a functional food using buttermilk as the emulsifier, showing favourable characteristics for stability over long periods of time during processing and storage. Buttermilk is a by-product of the dairy industry. This work contributes to the design of the processing operations and to an understanding of the textural properties resulting from the interactions of emulsion components, and provides useful information related to the sensory quality and characteristics of the final product.

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