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Microfluidization and characterization of phycocyanin-based emulsions stabilised using a fumed silica

Patricia Tello [a], Rosa Sánchez [a], Luis A. Trujillo-Cayado [a], Jenifer Santos [b,*], Goran Vladisavljevic [c]

[a] Departamento de Ingeniería Química, Escuela Politécnica Superior, Universidad de Sevilla, c/ Virgen de África,7, E41011, Sevilla, Spain

[b] Departamento de Ciencias Biológicas y de la Salud, Facultad de Ciencias de la Salud, Universidad Loyola Andalucía, Avda. de las Universidades s/n, 41704, Dos Hermanas, Sevilla, Spain

[c] Chemical Engineering Department, Loughborough University, Loughborough, LE11 3TU, Leicestershire, UK

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ABSTRACT

Phycocyanin (PC), a protein pigment obtained from algae, is attracting attention due to the search for new plant-based alternatives to stabilise food products. Furthermore, PC presents surface activity and is able to reduce interfacial tension to create droplets in emulsions. However, PC is sensitive to degradation; one potential solution is to use it in combination with other materials. In this study, using PC in combination with Aerosil 200 to stabilise food-grade nanoemulsions was studied via rheology, laser diffraction and multiple light scattering. First, the microfluidization technique was used to reduce the droplet size of PC-based emulsions to a minimum of 243 nm after six passes. However, the resulting emulsion presented poor physical stability with an extensive creaming process. Incorporating Aerosil 200 reduced the creaming process at low concentrations and completely inhibited it above 5 g/100 g of Aerosil 200. This study shows that a combination of PC and Aerosil 200 was able to stabilise nanoemulsions, with potential applications for food products.

1. Introduction

Emulsion technology is commonly used to develop food products with required food qualities, i.e., sensorial characteristics and health-supporting properties. Low-energy and high-energy methods are used to prepare emulsions. Microfluidization is a high-energy food products development method that has recently attracted much attention (Kumar et al., 2021; Li, Deng, et al., 2021). This technology uses microchannels to reduce an emulsion's droplet size and a high-pressure pump to make the system pass through them. As a result, the nanometre scale for emulsions has been achieved (Jafari et al., 2007; Villalobos-Castillejos et al., 2018).

Phycocyanin (PC), a bioactive protein–pigment well-known for its blue colour, is obtained from the microalgae *Arthrospira platensis*. It is utilised in many fields, including healthcare, diagnosis, and nutraceuticals, as well as the biomedical and food industries (Ashaolu et al., 2021), due to its antioxidant properties. PC is used in food products to replace synthetic dyes, as it is a natural pigment. Furthermore, it has been proven that PC presents surface activity and can reduce interfacial tension at relatively lower bulk concentrations compared to common food proteins (Batista et al., 2006; Böcker et al., 2021; Chronakis et al., 2000). As a natural dye with interfacial properties, PC is an interesting emulsifier option for use in food products. However, PC is sensitive to degradation under light and high temperature, which might affect the stability of products formulated using PC (Ashaolu et al., 2021). One way to address this disadvantage is to incorporate other materials with PC to form a complex (Li, Zhang, & Abbaspourrad, 2021). In addition, different treatments can be applied to modify PC's structure, and hence, its physicochemical properties. This has been reported for other proteins (Cabra et al., 2007). Encouraged by the European Green Deal, the search for new sustainable and resource-efficient alternatives to animal-based products is attracting much attention (Chaudhary et al., 2018; Willett et al., 2019). As a result, the search for new plant-based compounds to stabilise food products is emerging.

Aerosil 200 is a silica compound well-known for its emulsification properties as well as its role as a stabiliser and thickener. It has been used in many products, such as rubbers, plastics, coatings, adhesives, cements, sealants and food products, to enhance physical stability

* Corresponding author.
E-mail address: jsgarcia@uloyola.es (J. Santos).

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(Markusík et al., 2023; Mohylyuk et al., 2020; Rempe et al., 2021; Winkler et al., 2016). Furthermore, it has been used in combination with polymeric emulsifiers (Santos et al., 2019; You et al., 2005) to improve the physical stability of dispersed systems, but never in combination with a protein. Studies have proven that using these particles to stabilise food products is efficient (Huang et al., 2018; Santos et al., 2019) and safe (Winkler et al., 2016). For example, it was demonstrated that fumed silica particles had no negative effects on rodents when added to their food (Winkler et al., 2016). Furthermore, Aerosil 300 (also a silica compound; with a different specific surface) has been used in food formulations as a solid self-emulsifying delivery system (Huang et al., 2018).

Essential oils, which are composed of terpenes, terpenoids and other organic components, possess antioxidant, antibacterial and antifungal properties, which make them interesting options for application in food products. However, their industrial applications are limited due to their hydrophobicity and high volatility. One way to address this is using essential oils in nanoemulsions as a dispersed phase. For example, lemon essential oil can be used as a natural preservative and flavouring agent (Jiang et al., 2020; Yazgan et al., 2019). Furthermore, this essential oil has been considered safe by the FDA since 2018, and its use in food products is attracting much attention.

This study's objective was to develop stable nanoemulsions based on a natural dye, phycocyanin, as a primary stabiliser and emulsifier to obtain an efficient alternative to non-plant-based products. The combination of phycocyanin with a thickener (Aerosil 200) to improve the systems' physical properties was studied. The influence of Aerosil 200 concentration on rheological properties (flow curves and oscillatory tests), droplet size distribution and the physical stability of phycocyanin-based nanoemulsions was studied. This research sets the stage for future studies of phycocyanin-based emulsions and the destabilization mechanisms these systems can suffer.

2. Materials and methods

2.1. Materials

Phycocyanin supplied by Naturegrail (UK) was used as an emulsifier. Pure lemon essential oil was purchased from Bidah Chaumel (Lorquí, Murcia, Spain) and used as received. Aerosil 200 nanoparticles (12 nm diameter), developed by Evonik Industries, were supplied by Quimidroga (Barcelona, Spain).

2.2. Methods

2.2.1. Development of nanoemulsions

For 250 g samples, 2 g/100 g of PC was added to deionized water at pH 2.5 to dissolve the total amount. Next, pure lemon essential oil (5 g/100g) was dispersed during the aqueous phase using the Silverson L5M (Silverson, Chesham, United Kingdom) equipped with a finer emulsification mesh at 6000 rpm for 90 s. Subsequently, the pre-emulsion was passed through the Microfluidizer M110P (Microfluidics, USA) at 20000 psi using a Y + Z configuration. The outflow sample tube was refrigerated using cold water (5 °C) to avoid recoalescence. Samples were passed through the M110P from 1 to 10 times.

Once the required number of passes was determined, the resulting nanoemulsion was selected to incorporate a fumed silica, Aerosil 200, in different concentrations (2, 3.5, 5 and 6.5 g/100g) to enhance its physical stability. This incorporation was carried out using a homogenizer IKA-Visc at 300 rpm for 5 min.

2.2.2. Droplet size distribution characterization

The Malvern Mastersizer 2000 (Malvern, Worcestershire, United Kingdom) was used to measure emulsions' droplets' size and nanoemulsions' droplets sizes greater than 200 nm based on laser light scattering technology (Eshel et al., 2004). The refraction index used for lemon essential oil was 1.473. Volumetric diameter ($D_{4,3}$) characterized these samples as follows:

$$D_{4,3} = \sum_{i=1}^{N} n_i d_i^4 \Big/ \sum_{i=1}^{N} n_i d_i^3 \qquad \text{Eq. (1)}$$

where d_i is the droplet diameter, N is the total number of droplets, and n_i is the number of droplets having a diameter d_i.

2.2.3. Rheological measurements

All measurements (flow curves as well as small amplitude oscillatory shear tests) were conducted using a Haake MARS II rheometer (Thermo Fisher Scientific, Waltham, USA). The geometry used was a 60 mm diameter serrated plate–plate. Flow curves were conducted using a multi-step protocol of 3 min/point. The protocol for frequency sweeps was from 20 to 0.05 rad/s at a stress in the linear viscoelastic range (LVR). The LVR was determined using stress sweeps at 0.1 and 1 Hz. All measurements were carried out in triplicate.

2.2.4. Physical stability

The samples' physical stability was determined using the multiple light scattering technique (Mengual et al., 1999) (Turbiscan Lab Expert, Formulaction, Toulouse, France). The samples' backscattering (BS) at every measuring cell height was measured for a maximum of two weeks and a minimum of 5 h, considering the instability of the systems. BS differences in the low zone of the measuring cell were directly related to the creaming process and height. BS peaks and aging times were analysed using Turbiscan Soft software to determine the creaming height with aging time.

2.2.5. Statistical analysis

Results obtained by laser diffraction measurements and rheological tests were analysed using one-way analysis of variance (ANOVA) using Microsoft Excel. Every sample was measured in triplicate for the laser diffraction technique, and in duplicate for rheological tests. All statistical calculations were carried out at a significance level of $p < 0.05$.

3. Results and discussion

This section first discusses results regarding the influence of the number of passes through the microfluidization process for phycocyanin-based emulsions. Next, it discusses the impact of Aerosil 200 incorporation as a stabiliser on the physical stability and rheological measurements of PC-based emulsions.

3.1. Microfluidization of phycocyanin-based emulsions

Fig. 1 shows the droplet size distributions for emulsions formulated using phycocyanin as emulsifier as a function of the number of passes through the Microfluidizer M100P. The 0 pass emulsion (pre-emulsion) was processed using only the Silverson L5M (the first step in the homogenization procedure). Bimodal droplet size distributions were observed for all systems, regardless of the number of passes. Although the main peak was centred at approximately 10 μm for the pre-emulsion, it was centred below 1 μm for the systems processed using the Microfluidizer, thus proving the effectiveness of using a microchannel device. However, a monomodal distribution could not be obtained at this pressure. This might have been a condition of the formulation; i.e., a monomodal distribution could not be obtained using this formulation via microfluidization. Furthermore, as shown in Fig. 1, the droplet size distributions for emulsions passed through the Microfluidizer six, seven and eight times were similar. Fig. 2 provides deeper insight into droplet results, illustrating volumetric diameters with the number of passes. Volumetric diameter decreased with the number of passes until the sixth pass, when an increase in volumetric diameter was observed. Taking these results into account, the sixth pass showed the significantly lowest

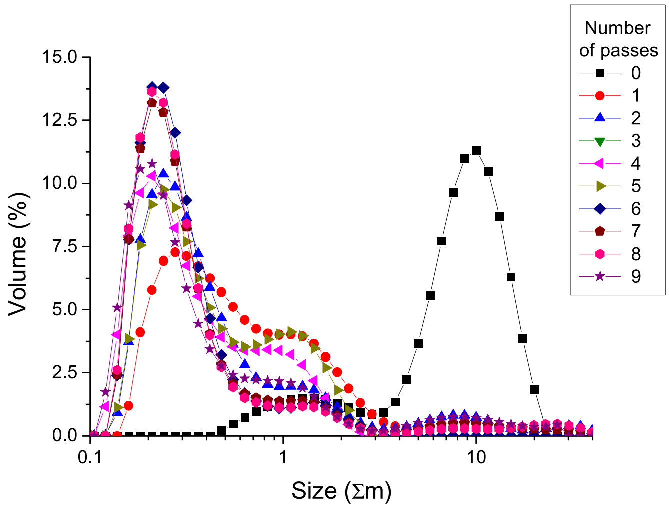

Fig. 1. Droplet size distributions of phycocyanin-based emulsions formulated without Aerosil 200 (aged 1 day) as a function of the number microfluidization passes.

droplet size; this finding was supported by ANOVA testing. Hence, six passes optimally reduced the droplet size for this system. Additionally, recoalescence due to over-processing was taking place at the same time. This happens in many systems (Karsli et al., 2022; van der Schaaf et al., 2020) and is determined by the formulation. When over-processing occurs, there is insufficient surfactant to recover the interface to stabilise the droplets formed; as a result, some droplets merge. Many studies have shown a relationship between smaller droplet size and improved physical stability (such as reducing or inhibiting different destabilization mechanisms) and with improved rheological properties (Liu et al., 2019; Shahavi et al., 2019). Hence, the starting point for the following step, to facilitate applying this system to a commercial product, was six passes via microfluidization.

3.2. *Incorporation of Aerosil 200 to stabilise microfluidized phycocyanin-based emulsions*

The flow behaviours of emulsions formulated using phycocyanin are shown in Fig. 3 as a function of Aerosil 200 concentration. The emulsion that did not contain fumed silica (Aerosil 200) exhibited Newtonian properties, with a 13.3 mPa s viscosity. All emulsions formulated using fumed silica illustrated shear-thinning behaviours with a trend to plateau at low shear rates (<0.001 s-1), which indicates that these emulsions were non-Newtonian fluids (Ren et al., 2022). This viscosity is called zero-shear viscosity; it increased with Aerosil concentration, as expected, as Aerosil provoked the creation of 3D-network structures (Aliabadian et al., 2018; Galindo-Rosales et al., 2009; Santos et al., 2019). Some authors claim that Aerosil particles form a gel structure in the continuous phase that can prevent the movement of the drops or bubbles (network stabilization) (Nushtaeva, 2015). In addition, data regarding viscosity–shear rate plots of some orders of magnitude are lacking. This is because measurements are stress-controlled; hence, a small increase in stress provokes a significant increase in shear rate. This is typical of fluids with a yield point (Calero et al., 2017). The occurrence of a yield point (as also shown in Fig. 4); is usually related to a longer physical stability; i.e., systems do not flow until the yield stress. This flow behaviour involves a structural collapse of the microstructure, and is well-known in other systems such as detergents (Calero et al., 2017), polymers (Léopoldès et al., 2015), dispersions of nanoparticles (Taheri et al., 2016) and emulsions containing clays (Han et al., 2015). Yield stress increased with Aerosil concentration, suggesting an enhanced physical stability using fumed silica concentration.

Fig. 5 illustrates the mechanical spectra of emulsions studied as a function of Aerosil concentration. Notably, results for 0, 2 and 3.5 g/100g emulsions are not shown because their linear viscoelastic ranges were so short the rheometer could not measure them, indicating that these systems' (0, 2 and 3.5 g/100g emulsions) microstructures were very weak. In contrast, for emulsions containing 5 and 6.5 g/100g of Aerosil, a frequency sweep in which the elastic modulus (G′) was higher than the viscous modulus (G″) was observed in all frequency range

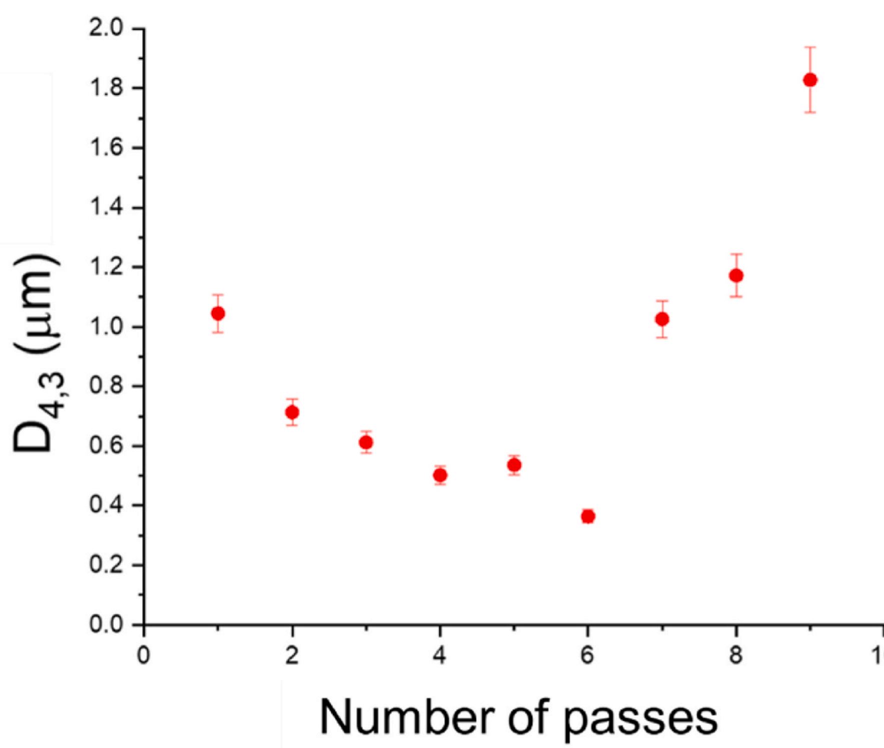

Fig. 2. Volumetric diameters of phycocyanin-based emulsions formulated without Aerosil 200 (aged 1 day) as a function of the number of microfluidization passes.

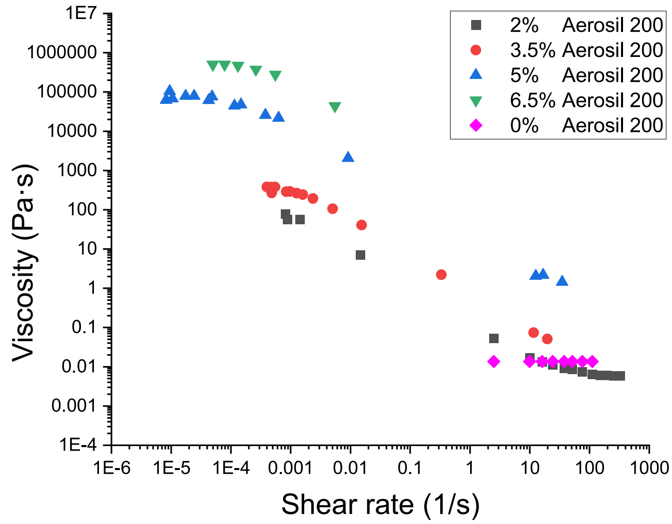

Fig. 3. Flow curves (viscosity vs. shear rate plot) for phycocyanin-based emulsions (aged 1 day) as a function of Aerosil 200 concentration. The emulsion formulated without Aerosil presented a 13.3 mPa s Newtonian viscosity.

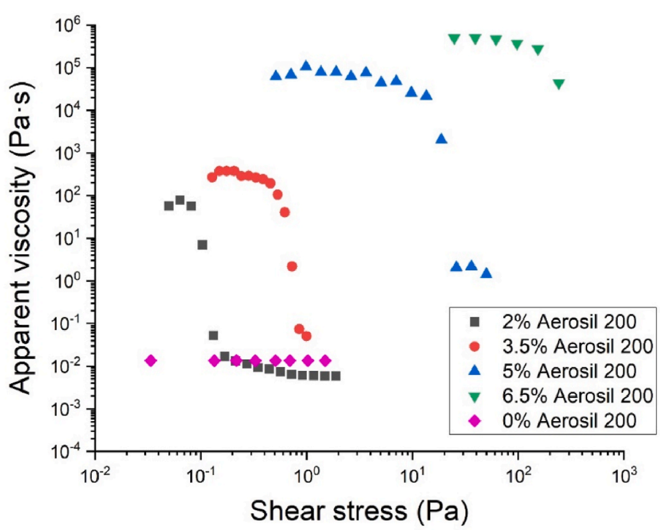

Fig. 4. Flow curves (viscosity vs. stress plot) for phycocyanin-based emulsions (aged 1 day) as a function of Aerosil 200 concentration. The emulsion formulated without Aerosil presented a 13.3 mPa s Newtonian viscosity.

studied. This suggests a gel-like network structure with mainly elastic behaviour, typical of food systems (Batista et al., 2006; Ren et al., 2021; Santos et al., 2019). Furthermore, the slope of G' was almost zero, suggesting strong gel-type characteristics for both systems. Viscoelastic properties increased as Aerosil concentration increased, as expected. This was related to a structuration grade increase in the systems. Recently, a structure formed using Aerosil was observed in emulsions with droplets embedded in a 3D network of Aerosils (Santos et al., 2020).

The multiple light scattering technique was used to study the developed systems' physical stability. Fig. 6 shows the variation in backscattering (BS) using the measuring cell's height as a function of aging time for emulsions formulated without Aerosil 200. This system presented a BS decrease in the measuring cell's low zone, related to the creaming process (droplets were moving to the measuring cell's upper zone). This is a typical destabilization process in systems with low viscosity and low viscoelastic properties. Analysis of these raw data determined the height of the cream layer as a function of Aerosil 200

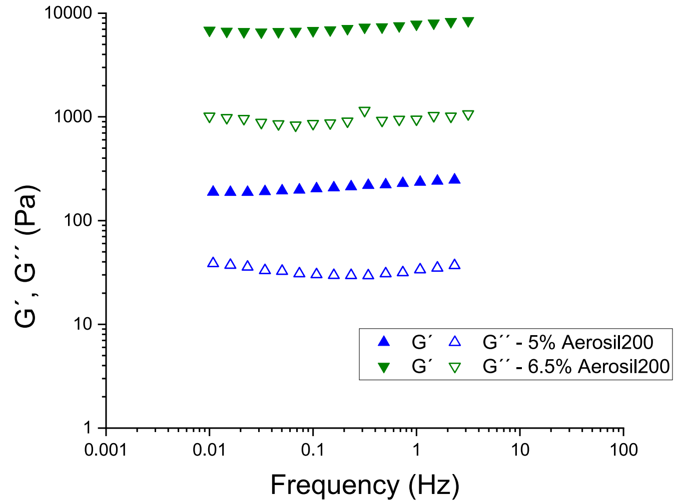

Fig. 5. Frequency sweeps of phycocyanin-based emulsions (aged 1 day) as a function of Aerosil 200 concentration. Emulsions formulated using 0, 2 and 3 g/100 g of Aerosil 200 did not present measurable viscoelastic properties.

concentration, as illustrated in Fig. 7. Both the pre-emulsion and the emulsion without Aerosil 200 had fast creaming processes (marked increases are apparent in Fig. 7), which supports the idea that these systems needed a thickener to enhance their physical stability. On the other hand, the incorporation of Aerosil 200 reduced the creaming process, which was completely inhibited when 5 and 6.5 g/100g of Aerosil 200 were used. This confirms that 5 g/100g of Aerosil 200 is enough to obtain stable phycocyanin-based emulsions.

4. Conclusions

Microfluidized nanoemulsions with a medium droplet size of 250 nm were prepared using phycocyanin as an emulsifier. This is a promising result for using PC as an emulsifier in the food industry. Six passes in the Microfluidizer were optimal, which presented the smallest droplet size. Next, different concentrations of the stabiliser Aerosil 200 were added to the resulting emulsions to prevent break-down processes that could occur in nanoemulsions.

Nanoemulsions formulated using only PC as an emulsifier presented Newtonian behaviour and weak microstructures. In contrast, nanoemulsions formulated using Aerosil showed non-Newtonian pseudoplastic behaviour. Moreover, the larger the Aerosil 200 concentration, the larger the viscosity was, increasing the systems' structures' stability. Frequency sweeps for 5 and 6.5 g/100 g of Aerosil 200 emulsions showed that their solid character was greater than their liquid character, therefore, these emulsions could be classified as gels.

This study of emulsions' physical stability showed that nanoemulsions without Aerosil underwent a creaming process as they aged. However, the creaming processes of emulsions with Aerosil 200 were reduced. Two kinetically stable nanoemulsions, with concentrations of 5 and 6.5 g/100 g of Aerosil 200, were prepared. These systems possessed properties, such as nanometric scales, weak gel properties and long physical stabilities, that make them interesting options for use in the food and cosmetic industries.

CRediT authorship contribution statement

Patricia Tello: Methodology, Software, Investigation. **Rosa Sanchez:** Data curation, Software, Investigation. **Luis A. Trujillo-Cayado:** Conceptualization, Supervision, Visualization, Investigation, Validation, Resources, Project administration, Funding acquisition. **Jenifer Santos:** Writing – original draft, Supervision, Writing – review & editing, Formal analysis. **Goran Vladisavljevic:** Supervision.

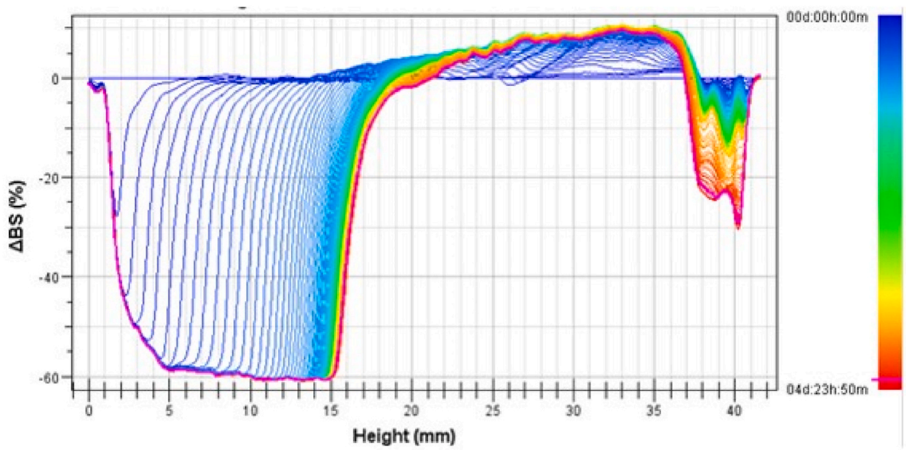

Fig. 6. Variations in backscattering (BS) with measuring cell heights of phycocyanin-based emulsions formulated without Aerosil 200 and developed only via primary homogenization (not microfluidization) as a function of ageing time.

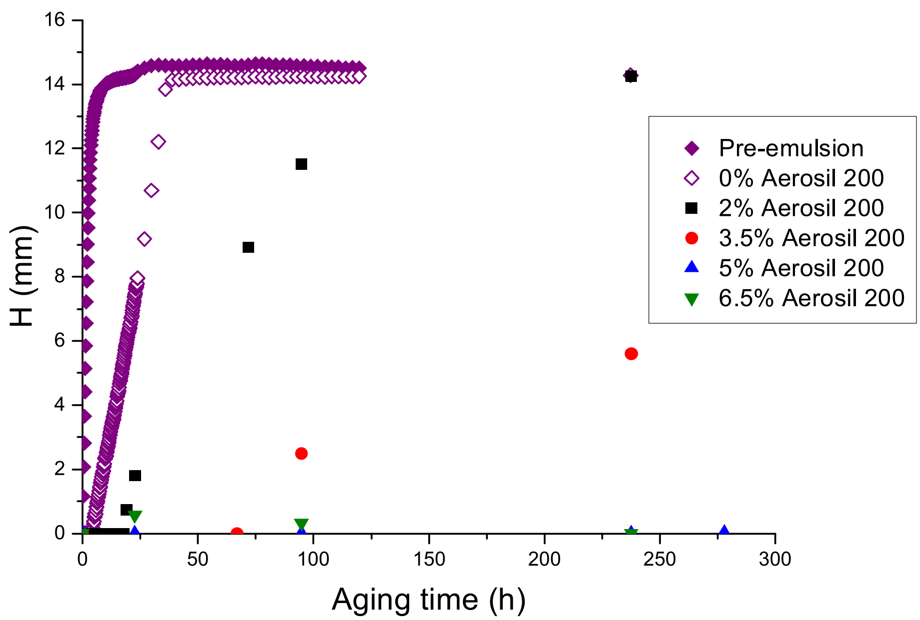

Fig. 7. Heights of cream layers (H) with aging times of phycocyanin-based emulsions as a function of Aerosil 200 concentration. The pre-emulsion system was not microfluidized.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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