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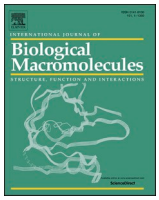

Characterization of emulgels formulated with phycocyanin and diutan gum as a novel approach for biocompatible delivery systems

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ABSTRACT

Phycocyanin (PC), a protein derived from algae, is non-toxic and biocompatible. Due to its environmental and sustainable properties, it has been studied as an alternative stabilizer for food emulsions. In this sense, the main objective of this work is to evaluate the effectiveness of PC and its use in combination with diutan gum (DG), a biological macromolecule, to prepare emulgels formulated with avocado oil. *Z*-potential measurements show that the optimum pH for working with PC is 2.5. Furthermore, the system exhibited a structured interface at this pH. The surface tension did not decrease further above 1.5 wt% PC. Interestingly, emulsions formulated with >1.5 wt % PC showed recoalescence immediately after preparation. Although 1.5 wt% had the smallest droplet size, this emulsion underwent creaming due to the low viscosity of the system. DG was used in combination with PC to increase viscosity and reduce creaming. As little as 0.1 wt% DG was sufficient to form an emulgel when incorporated into the previous emulsion, which exhibited pseudoplastic behaviour and viscoelastic properties with very low creaming rates. However, the use of PC in combination with DG resulted in a non-aggregated and stable emulgel with 1.5 wt% PC and 0.1 wt% DG.

1. Introduction

An emulsion is a colloidal dispersion system in which two immiscible liquids are stabilized by an emulsifier, resulting in tiny droplets of one liquid dispersed in the other. They can undergo various destabilization mechanisms such as coalescence, Ostwald ripening, creaming and phase inversion [1]. The chemistry and microstructure of the proteins are related to their functional properties. Protein functionality plays a crucial role in determining food products' texture, taste, and nutritional value. For instance, the ability of proteins to form gels, emulsions, or foams depends on their structural properties, affecting the sensory experience and shelf stability of food formulations. It is, therefore, necessary to understand the physical, chemical and functional properties of proteins in order to know how proteins can influence the interfaces formed in dispersed systems.

Phycocyanin (PC), a natural protein derived from a non-animal source (cyanobacteria and algae), has recently gained considerable attention as a stabilizer for dispersed systems [2–4]. PC is considered non-toxic and biocompatible, which addresses the growing demand for environmentally friendly and sustainable compounds. It also has antioxidant and anti-inflammatory properties that make it very interesting for biomedical applications [5]. Regarding its use as a stabilizer, PC can reduce the interfacial tension and prevent droplet growth. It appears that PC adsorbs at the interface and forms a protective layer that may prevent coalescence or Ostwald ripening, thereby improving physical stability [6]. In addition, PC has been used as a stabilizer for nanoparticles [7] and nanocarriers [8]. However, information on PC as a main stabilizer in emulsions or nanoemulsions is very limited.

Another destabilization mechanism that emulsions can suffer from is creaming, or the migration of oil droplets to the top of the product vessel. One way to solve this problem is to incorporate a thickener to increase the viscosity of the continuous phase. In this way, it can reduce the movement of the droplets and inhibit the creaming process. In addition, the formation of protein-polysaccharide complex contributes to the texture, stability, and nutritional profile of food products, serving as emulsifiers, thickeners, and encapsulation agents. These complexes

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have attracted much attention recently due to the application of Pickering emulsions with significant potential applications in drug delivery systems [9,10].

A biological macromolecule that acts as a thickener and has recently attracted a lot of attention is diutan gum. This biological macromolecule is obtained from the fermentation of *Xanthomonas campestris*. It has very useful properties, such as its resistance to extreme temperatures and pH values. In addition, it is considered an excellent thickener and stabilizer [11–14]. Due to these properties, diutan gum has been used as a stabilizer for nanoemulsions in various fields such as food, pharmaceuticals and agriculture [15–17].

Avocado oil is extracted from the flesh of avocados, the fruit of the *Persea Americana* tree. It is attracting a lot of attention due to its beneficial health properties. Avocado oil is rich in healthy fats that reduce cholesterol levels, vitamins (B, E and K) and antioxidants. [18]. This oil is used for culinary purposes, skin moisturizers, and beauty care [18]. This makes avocado oil a natural and nutritious alternative to more traditional oils.

The main objective of this work was to obtain emulgels using PC as the main stabilizer and diutan gum as a thickener since little work has been done to develop diutan gum-phycocyanin systems. An interfacial study and laser diffraction measurements were performed for PC-based emulsions. Then, a biological macromolecule (diutan gum) was used as a thickener to inhibit the creaming of these emulsions. This research proves that phycocyanin has emerged as a promising alternative to stabilize dispersed systems for the food industry.

2. Materials and methods

2.1. Materials

Phycocyanin (PC) was purchased from Naturegrail (Spain) and used as an emulsifier. Avocado oil used as dispersed phase was purchased from Bidah Chaumel (Spain) and used as received. Phycocyanin and avocado oil are natural products based on certificate of analysis. KELTROL® diutan gum was kindly provided by CP Kelco (Atlanta, GA, USA).

2.2. Phycocyanin composition

The different phycocyanin components have been determined by the methods laid down in A.O.A.C. International (2005) [19].

2.2.1. Proteins

Proteins were determined using a LECO CHNS-932 microanalyzer (Leco Corporation) to measure the nitrogen content and then multiply it by 5.95 to obtain the result [20].

2.2.2. Ashes

The ash content was determined by a standard gravimetric method carried out in triplicate. The crucibles were cleaned and dried in an oven at 100 °C for about 2 h and then cooled in a desiccator. After reaching room temperature, the crucibles were weighed and tared with the weight of the test sample. The flour samples were incinerated in a flask at 550 °C for 36–48 h and the amount of inorganic matter present was calculated from the difference in weight.

2.2.3. Lipids

Lipid content was determined using the Soxhlet method by repeated extractions using petroleum ether as solvent. Approximately 5 g of sample is weighed and wrapped in filter paper. After that, it is transferred to the Soxhlet device. To remove the remaining petroleum ether from the sample, it is left in the odour extraction chamber until the solvent has completely evaporated. When dry, the sample is reweighed.

2.2.4. Carbohydrates

The percentage of carbohydrates was calculated as the difference between the percentages determined for the other components.

2.3. ζ-Potential

The isoelectric point of the protein in solution was determined by surface charge detection at different pH values (from 2.5 to 11.5) using a Zetasizer Nano ZS zeta potential analyzer (Malvern Instruments, Malvern, UK) and the Smoluchowski equation [21]. For this purpose, different samples were prepared by dispersing phycocyanin at a concentration of 1.0 wt% in water at different pH values. This measurement provides insight into the stability of a particle and indicates the potential required to penetrate the surrounding ionic layer of the particle to destabilize it. The point at which this occurs is called the isoelectric point, where this pH is the value at which the molecule dissociates evenly and solubility is virtually zero [22].

2.4. Surface tension

Surface tension was determined to evaluate the most appropriate pH and phycocyanin concentration for emulsion development. The surface tension was determined using the dynamic Wilhelmy platinum plate method [23], where the plate is inserted into the sample and slowly raised until the plate is completely separated from the liquid. Before this occurs, the interface on both sides of the plate is bent. The stress exerted by the interface at this point is measured and balanced against the lifting force of the plate.

Measurements were made using a Sigma 701 tensiometer (KSV, Castlemead, UK) with SGSErver software. Dispersions with different phycocyanin concentrations (0.005 %, 0.02 %, 0.05 %, 0.1 %, 0.5 %, 1 %, 2 % and 4 %) were prepared, all at pH 2.5, corresponding to the pH selected in the previous section. The solutions were stirred with magnetic stirrers for 20 min, using vessels suitable for carrying out the Wilhelmy plate method.

2.5. Interfacial rheology

The evaluation of rheological properties was performed to verify that the interfaces were structured to avoid destabilization effects such as coalescence [24]. The rheological properties were measured at the interface of the solution at different concentrations (between 1.0 and 2.5 wt%) at pH 2.5. Because the protein concentration was too small to obtain acceptable values, the measurement of the interfacial rheology of the solution below 1 % phycocyanin could not be carried out.

This rheological study was carried out by means of dynamic oscillatory tests using a DHR3 rheometer (TA Instruments, New Castle, DE, USA) and a ring-shaped accessory [25]. For this, the previously prepared solution was deposited after a rest time (about 30 min) and the ring was placed in the interface between. The results were obtained by means of frequency sweep tests at room temperature (20 ± 2 °C) in an interval between 0.08 and 8.0 rad/s in the linear viscoelastic range. For this, strain sweep tests were previously carried out at 0.1 Hz (0.628 rad/s) in order to determine the critical strain (the last strain in the linear viscoelastic range).

2.6. Emulgels preparation

The aqueous phase was prepared by using different concentrations of phycocyanin (0.5–2.5 wt%) in deionised water at a pH of 2.5. The oil phase consisted of avocado oil at 10 wt%. The selection of the optimum PC concentration was carried out in the first part of the study. These emulsions (200 g) were prepared by primary homogenisation using a Silverson L5M rotor-stator apparatus at 7000 rpm for 90 s. The emulsion with optimised values of PC concentration was used as the starting point for the addition of diutan gum to form emulgels. A primary gum solution

containing 2 wt% of the biological macromolecule was prepared using an IKa-Visc MR-D1 homogeniser for 8 h at 500 rpm at 20 °C. This solution was then kept at 5 °C for 48 h to allow complete hydration of the polysaccharide. The final emulgels were prepared by mixing the gum solution and the optimal emulsion using the Ikavisc MR-D1 at 700 rpm for 15 min. Final emulgels were obtained with different concentrations of diutan gum (0.1, 0.5 and 1 wt%).

2.7. Droplet size distributions

The droplet size distributions of the developed emulsions and emulgels were evaluated using a Malvern Mastersizer 2000 (Malvern). Each measurement was taken three times. In addition, the Sauter diameter ($d_{3,2}$) and volumetric mean diameter ($d_{4,3}$) were used to characterise the droplet mean sizes:

$$d_{3,2} = \frac{\sum_{i=1}^{N} n_i d_i^3}{\sum_{i=1}^{N} n_i d_i^2} \quad (1)$$

$$d_{4,3} = \frac{\sum_{i=1}^{N} n_i d_i^4}{\sum_{i=1}^{N} n_i d_i^3} \quad (2)$$

where d_i is the droplet diameter, n_i is the number of droplets of diameter d_i and N is the total number of droplets. To quantify the polydispersity of the droplet size distributions, the *v*alues of the span parameter were analysed.

$$Span = \frac{d(v,0.9) - d(v,0.1)}{d(v,0.5)} \quad (3)$$

where d(v,0.9), d(v,0.5) and d(v,0.10) are the diameters at 90 %, 50 % and 10 % cumulative volume.

2.8. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra in transmission mode were acquired over a wavenumber range from 600 to 4000 cm^{-1} at 4 cm^{-1} resolution using a FT/IR-4200 spectrometer (JASCO, Tokyo, Japan).

2.9. Physical stability

The destabilization of the emulsions has been monitored by multiple light scattering using an optical scanning instrument (Turbiscan Lab Expert, Formulaction). The emulsions and emulgels were filled into cylindrical glass tubes and stored at room temperature. The backscattering of light from the emulsions was then measured as a function of height at different times. The results can be presented either as backscattering (%BS) or in reference mode (delta backscattering, %ΔBS), i.e. by subtracting the first scan from all subsequent scans. Another data that can be provided from this technique is the Turbiscan Stability Index (TSI), which is obtained from the sum of the variations measured by the equipment:

$$TSI = \sum_j \left| scan_{ref}(h_j) - scan_i \left(h_j \right) \right| \quad (4)$$

where $scan_{ref}$ and $scan_i$ are the initial value of the backscattering and the value at a given time respectively, h_j is a given height of the measuring cell. The higher the TSI, the more variations there are and therefore the less stable the sample is.

Table 1
Elemental composition of the phycocyanin, on a dry basis, and concentration of each component.

Component	Concentration (wt%)
Carbohydrates	54.3
Proteins	42.0
Lipids	0.8
Ashes	2.1

2.10. Rheological characterization

Rheological measurements were carried out using an AR2000 controlled stress rheometer (TA Instruments). Flow curves for emulsions (without diutan gum) were performed using a sandblasted Z-20 coaxial cylinder. Emulgels tested with different concentrations of diutan gum were measured using a serrated plate sensor (60 mm diameter, 1 mm gap). Rheological characterization for the emulgels included stress and frequency sweeps in small amplitude oscillatory shear (SAOS) experiments and flow curves. The equilibration time prior to the rheological tests was 5 min. All measurements were performed at 25 °C.

3. Results and discussion

3.1. Phycocyanin composition

Table 1 shows the composition of phycocyanin (PC) on a dry basis, obtaining that the major component of the PC is carbohydrates, 54.3 wt % of the total, closely followed by proteins, 42.8 wt%. The lipid content of the PC is practically insignificant at 0.8 wt%. The ash corresponds to inorganic compounds and represents 2.1 wt% of the total composition. The moisture of the PC, although not shown, was necessary to obtain the dry weight.

Of all the elementary components, proteins are the ones that really act as emulsifier in emulsion formation. Therefore, the effect of phycocyanin as an emulsifier will be limited as it contains less than half of its total protein weight. In emulsions, therefore, higher concentrations of the protein will have to be used than if it had a higher protein content. The presence of ash indicates inorganic compounds. Inorganic components could significantly influence the emulsifying properties and overall stability of emulsions. Some inorganic components, such as certain salts or metals, can act as emulsifiers, stabilizers, or modifiers of the interfacial properties, thereby affecting the stability and properties of emulsions [26]. Nevertheless, surface tensions were determined to evaluate and determine the concentrations used to make the emulsions.

3.2. Phycocyanin characterization

Fig. 1 shows the *Z*-potential of phycocyanin (1 wt%) in water as a function of pH. The z-potential value follows a decreasing trend with increasing pH, starting from the highest value (pH 2.5; lowest pH value evaluated) and reaching its minimum at pH 11.5. The most significant drop in zeta potential is between pH 2.5 and pH 4. This suggests that the isoelectric point of the protein will be in this range (between 3 and 3.5). This value coincides with that found by other authors in the literature, who found the isoelectric point to be around pH 3 [27].

Concerning the acid pH, pH 2.5 shows the highest value of z-potential in absolute value. This pH is suitable for food products since some products need it for various purposes, including preservation, flavor, enhancement, texture modification, and safety assurance. This effect leads to an electrostatic repulsion between the molecules, which could prevent their aggregation [28]. Therefore, this fact indicates that a pH of 2.5 is adequate for preparing food emulsions using phycocyanin.

Fig. 2 shows the surface tension values of phycocyanin solutions as a function of concentration. This information allows the selection of an appropriate phycocyanin concentration for the preparation of

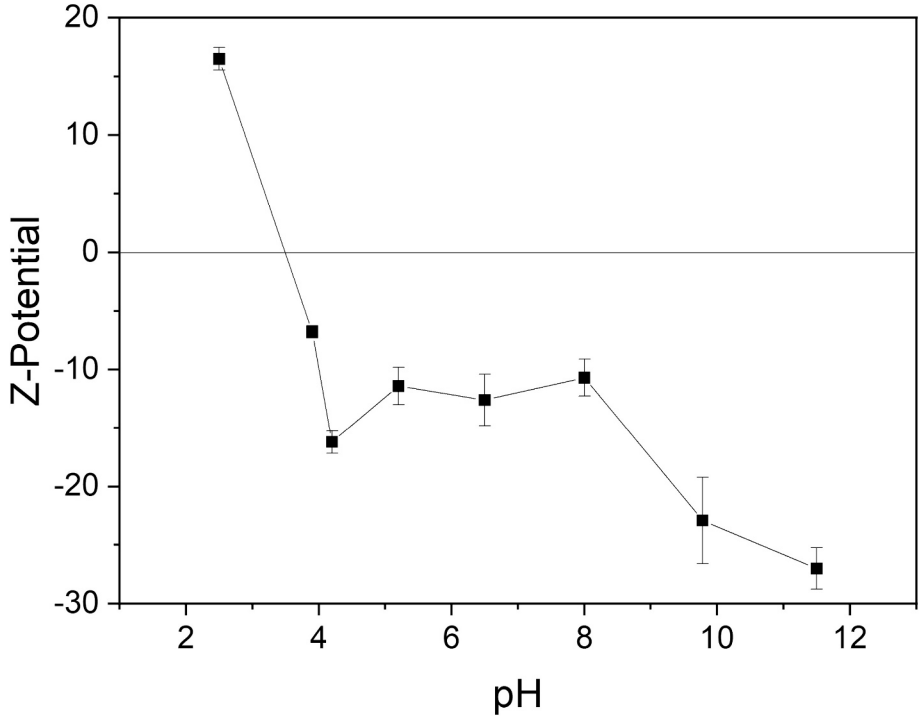

Fig. 1. Zeta potential as a function of pH values for PC in water dispersion.

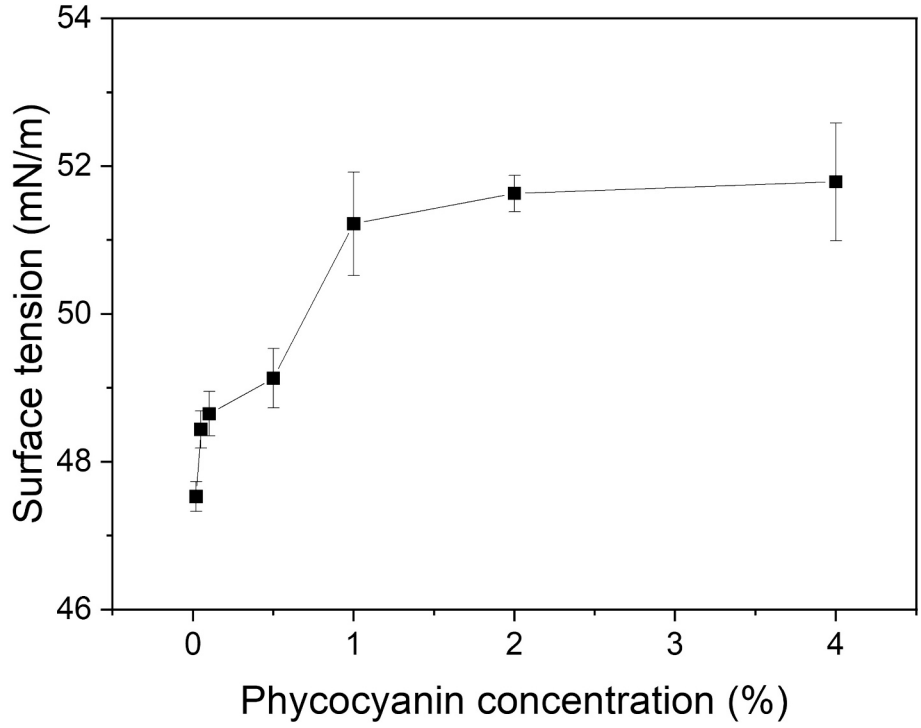

Fig. 2. Surface tension values of PC dispersions as a function of concentration at pH 2.5.

emulsions. It was observed that the surface tension decreased rapidly at the beginning, proving its superficial activity. Then, the superficial tension increases slightly with phycocyanin concentration and finally stabilises above 2 wt%. This subsequent increase might relate to repulsions and the formation of aggregates of phycocyanin [27]. This fact could destabilize the future formation of emulsions using PC as emulsifier. The aggregation of the emulsifier may not effectively protect the interface and hence, a possible fusion of droplets could take place. Comparing with other plant protein concentrates and isolates with typical values around 30–45 mN/m [29] or proteins of animal origin e.g. β-lactoglobulin or lysozyme (~42 mN and ~ 46 mN/m) [30], phycocyanin shows a low superficial activity [5]. Because of that, it may need a cosurfactant.

Fig. 3 shows the values of the interfacial elastic and viscous moduli (G'_i and G''_i, respectively) as a function of frequency for the different phycocyanin dispersions (1.0, 1.5, 2.0 and 2.5 wt% phycocyanin

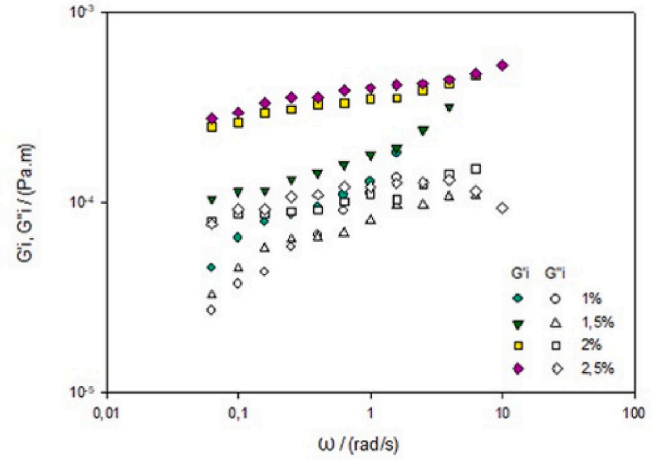

Fig. 3. Elastic (G'_i) and viscous (G''_i) moduli versus frequency for PC dispersions versus concentrations at pH 2.5.

concentrations). It can be observed the elastic modulus in the surface (G'_i) is higher than the viscous modulus (G''_i) in all the frequency range studied, suggesting a gel-like character of the surface water/phycocyanin-air, which is a typical behaviour in protein-based systems [31]. In addition, there is an increase in the superficial elastic modulus and a less dependency frequency with phycocyanin concentration with a tendency to level-off above 1.5 wt%. This is a positive aspect since as protein acts as an emulsifier, the interface is structured and the emulsions become more stable due to the formation of stronger intermolecular interactions [32]. Similar results were obtained by Dai et al. [33]. However, the values are very low in comparison with the ones obtained for example vegetable proteins [34] or animals proteins [35]. This lower modulus could impact the mechanical stability of an emulsion formulated with PC as a unique emulsifier. Low values of elastic and viscous moduli in the interfacial rheology can weaken the strength of the interfacial film and increase the tendency of emulsion droplets to coalesce and break, ultimately leading to an unstable emulsion. The lower decrease in surface tension and worse interfacial rheological properties compared to animal and vegetable proteins lead to seek strategies such as the inclusion of stabilizers to improve the properties of the resulting emulsion.

3.3. Development of emulsions containing avocado oil as dispersed phase and phycocyanin as stabilizer

Fig. 4 shows the droplet size distributions for emulsions containing avocado oil as the dispersed phase as a function of phycocyanin concentration. Emulsions formulated with 0.5 w.t% to 1.5 wt% showed monomodal distributions centered around 10 μm. Above 1.5 wt% there is a shift to higher droplet sizes and the appearance of a second peak. This fact suggests that above 1.5 wt% the concentration is too high to stabilize this system and it provokes the coalescence of the protein aggregates and the droplets. The possible aggregation of phycocyanin was previously suggested in the protein characterization part since the superficial tension did not decrease above 1.5 wt% PC. In addition, the impact of droplet size on the sensory properties of emulsions can be significant, affecting texture, mouthfeel and appearance. Smaller droplet sizes can result in a smoother and creamier texture, whereas larger droplet sizes may lead to a more coarse or gritty texture.

To further understand the droplet size results, Fig. 4B is presented. Fig. 4B shows the Sauter diameter, volumetric diameter and span values for the emulsions developed as a function of phycocyanin concentration. A decrease in both droplet diameters is observed with phycocyanin concentration up to 1.5 wt%. Then, an increase in the three parameters studied is observed. Analyzing these results, the Sauter diameter and the volumetric diameter show a minimum at 1.5 wt% phycocyanin concentration. At this concentration, a medium value of span is observed. In order to achieve a stable emulsion, the main factor to consider is the mean droplet sizes [36]. Hence, emulsion containing 1.5 wt% of phycocyanin was considered as the starting point to obtain stable systems.

Variations in Backscattering (BS) are shown in Fig. 5 to detect the main destabilization process in the emulsion formulated with 1.5 wt% of phycocyanin. A marked drop in BS at the low part of the measuring cell is observed from the very beginning of aging time. The decrease of BS in the low zone of the vial is related to a clarification process. Thus, a creaming process where the droplets are moving to the top is taking place. This fact is related to the low interfacial elastic modulus shown in Fig. 3. The microstructure formed with PC is not enough to reduce the movement of the droplets. The most efficient way to reduce and prevent creaming is to incorporate a gelling agent to increase the viscosity of the continuous phase and reduce the movement of the droplets. In this sense, diutan gum was incorporated in the following part of this study.

3.4. Development of emulgels containing diutan gum as thickener

To improve the physical stability of these emulsions, a biological macromolecule (diutan gum) was added to the formulation. The influence of the diutan gum concentration (0.1–1 wt%) was studied for the emulsion that showed the best physical droplet sizes (1.5 wt%). Fig. 6 shows the FTIR profiles for combinations of the different elements present in the emulgel in order to analyze chemical bonds, functional groups, and structural changes induced during formulation. All the possible combinations of elements studied presented a similar profile with two intense and broad peaks at 3351 cm^{-1} and 1645 cm^{-1}. The first peak is attributed to the O—H vibrations, whereas the second one refers to the H-O-H bending vibrations of water molecules [37]. On the other hand, the emulsion system exhibited four additional peaks. Two of them at 2923 and 2858 cm^{-1} are related to the C—H symmetrical stretching from CH_3 groups and the other two at 1723 cm^{-1} and 1130 cm^{-1} for C═O stretching and C—O stretching, respectively [37,38]. Nevertheless, the characteristic peak of avocado oil at 3468 cm^{-1} attributed to the -OH stretching vibration of β-cytosterol [38] is masked by the broad peak of O—H vibration of water molecules previously described.

Fig. 7 shows the flow curves of all samples as a function of diutan gum concentration. The difference between the behaviour of the systems studied is quite clear. First, an almost constant values of viscosity versus shear rate was observed in a shear rate range between 20 and 200 s^{-1} for the emulsion without the biological macromolecule, showing a Newtonian behaviour and a viscosity value of 0.0019 Pa·s. This constant viscosity value is called Newtonian viscosity and is related to the stability of the emulsions against creaming. For the emulsions with Diutan gum, a pseudoplastic behaviour is observed for all the concentrations studied, where the viscosity decreases with increasing shear rate. It is observed that the curves tend to a constant value of viscosity at low shear rates (at rest), which would be analogous to the Newtonian viscosity, in this case called the Newtonian limit viscosity. This change in viscosity tendency means that a new microstructure between PC and diutan gum is formed, i.e., a 3D network. This fact also occurs in other systems formulated with diutan gum and other protein [39]. Interestingly, this network could reduce the movement and the migration of the droplets and may be determining the physical stability of these systems.

An increase in the stabilizer concentration in the emulsions led to an increase of the Newtonian limit viscosity, which is attributed to the formation of a much stronger biopolymer network. The estimated values in the Newtonian limiting viscosity are also shown in Table 2. The values obtained for the emulgel of 0.1 wt% diutan gum concentration are similar to others found in the literature [40]. The parameter m, which is related to the flow rate, implies that the greater the pseudoplastic character the higher the value, although in this case very similar values are observed regardless of the concentration.

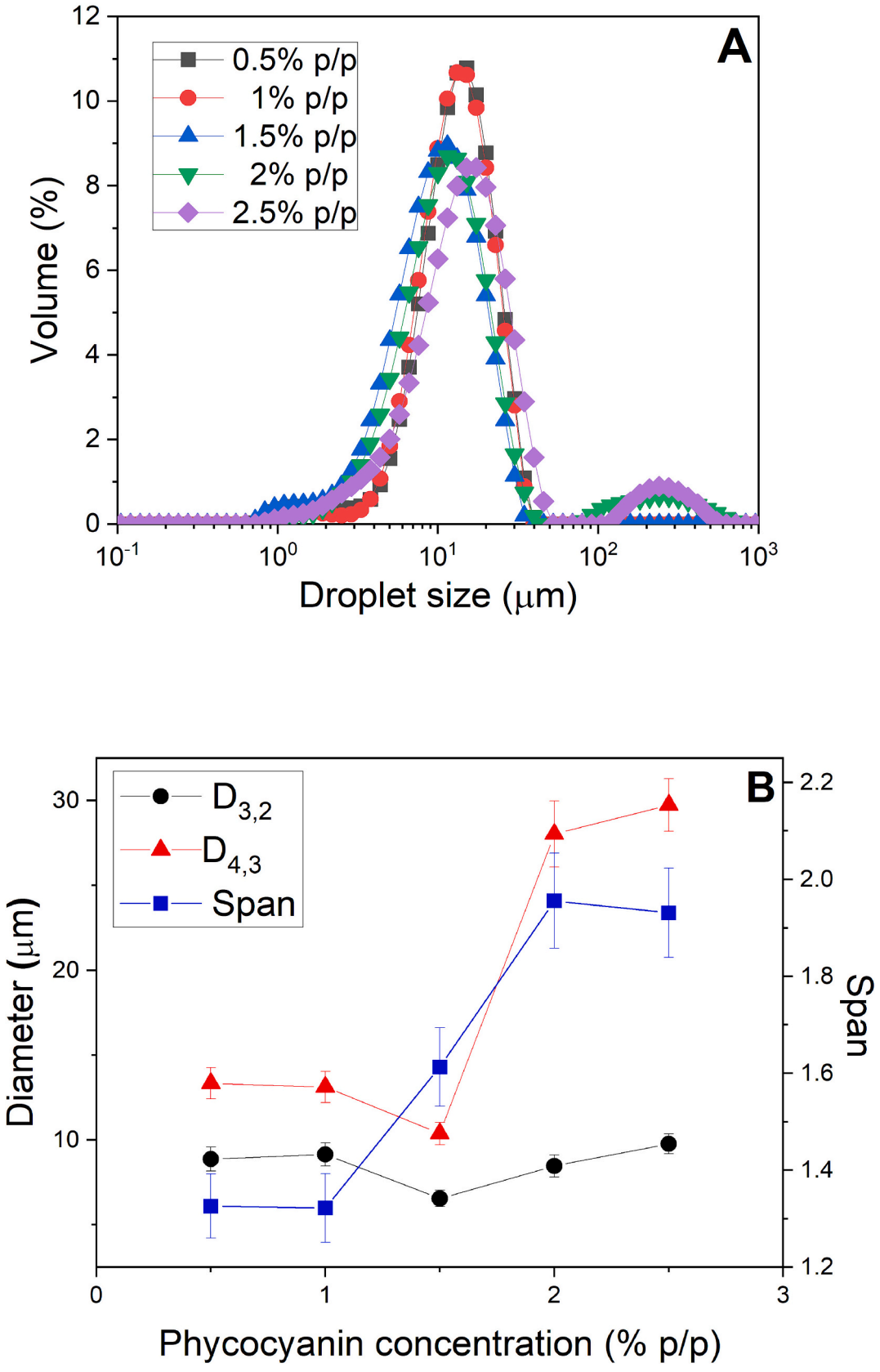

Fig. 4. (A) Droplet size distributions for emulsions containing 10 wt% avocado oil as a function of phycocyanin concentration immediately after preparation. (B) Sauter diameters, volumetric diameters and span values for emulsions containing 10 wt% avocado oil as a function of phycocyanin concentration immediately after preparation.

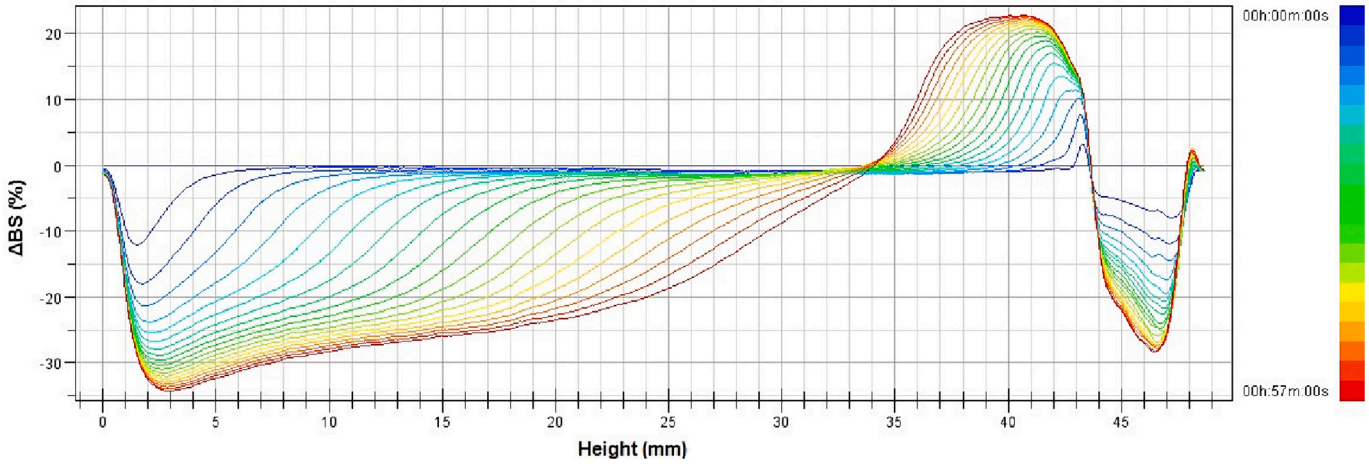

Fig. 5. Variation of backscattering as a function of measuring cell height with aging time for emulsion formulated with 1.5 wt% PC.

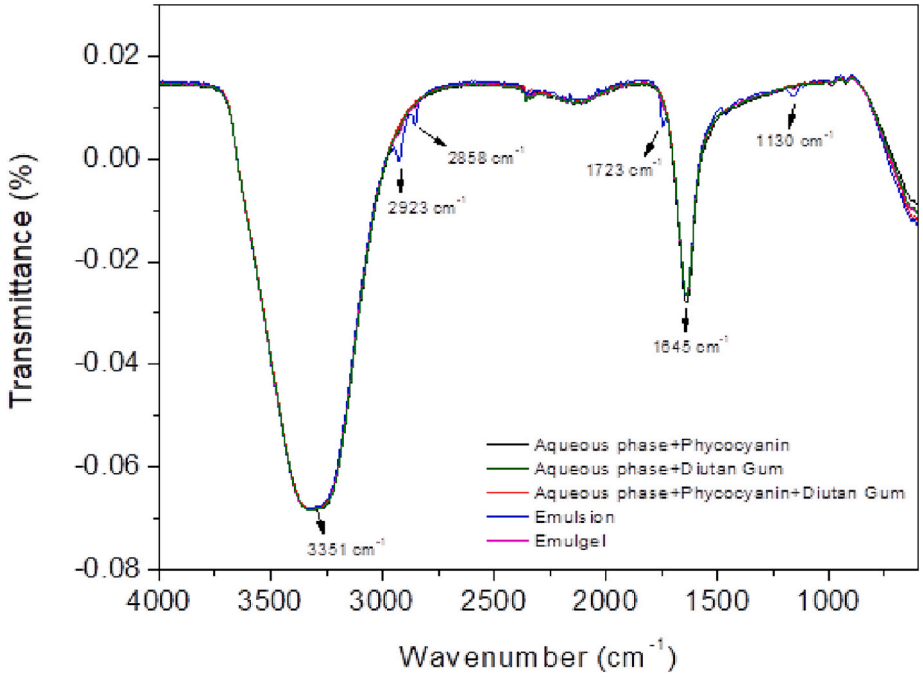

Fig. 6. FTIR profiles of combinations of the different elements present in the emulgel.

The mechanical spectrum for the studied systems that exhibited measurable viscoelasticity is presented in Fig. 8. To determine the viscoelasticity, stress sweeps were first carried out to determine their linear viscoelastic range. Only emulsions formulated with diutan gum exhibited this range measurably.

As expected, G' (elastic modulus) was higher than G" (viscous modulus) in the whole frequency range studied, thus showing a gel behaviour already found for this biological macromolecule in aqueous dispersions and emulsions [39,41]. In addition, the appearance of a very low G' slope also demonstrates the gel character of these systems. The viscoelastic properties increased with diutan gum concentration, which supports the results of the flow curves. The marked jump in both viscoelastic functions from 0.1 wt% to 0.5 wt% biopolymer is related to the increase in Newtonian viscosity shown in Fig. 7. This jump suggests the existence of a much stronger three-dimensional network, similar to those of strong gels. The formation of a weak gel-like three-dimensional network disfavors the mobility of the droplets and, thus, the migration of the droplets toward the top of the sample or the collisions between the droplets. The formation of this network could be due to an electrostatic interaction since PC is positively charged at pH 2.5 and diutan gum is an anionic biopolymer. The network structure within emulgels provides mechanical strength, prevents phase separation, and contributes to overall stability due to the reduction of the movement of the droplets. This produces a very stable emulgel. Also, the presence of a well-developed network can contribute to a creamy and smooth texture in emulgels, enhancing their sensory appeal. The network helps to distribute the oil phase evenly throughout the gel matrix, resulting in a homogeneous texture. It should be noted that emulgels with a diutan gum content higher or equal to 0.1 wt% showed physical stability for 44 h much higher than that presented by emulsions without stabilizer in 1 h (see Figs. 5 and 9). In fact, the decrease of backscattering in the whole sample vial is practically negligible within the first 44 h of testing. The use of diutan gum and the associated increase in viscosity not only provides greater stability against gravitational separation due to the reduction of the droplets movement, but also modifies its rheological properties, broadening its spectrum of possible practical applications

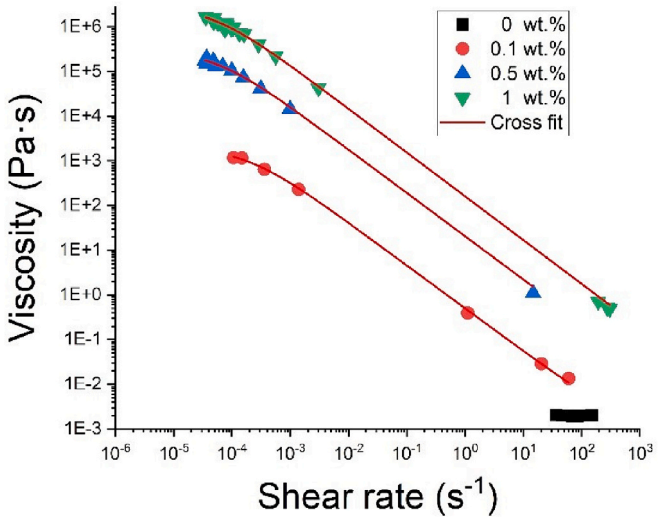

Fig. 7. Flow curves for the optimum emulsion and emulgels formulated with phycocyanin as a function of diutan gum concentration.

Table 2
Fitting parameter values for the Cross model. The values were obtained by a non-linear regression analysis.

Diutan gum concentration (wt%)	η_0 (Pa·s)	η_∞ (Pa·s)	k (s)	m
0.1	1991	<0.001	5614	0.96
0.5	301,540	<0.001	19,724	0.97
1	2,905,004	<0.001	22,007	0.98

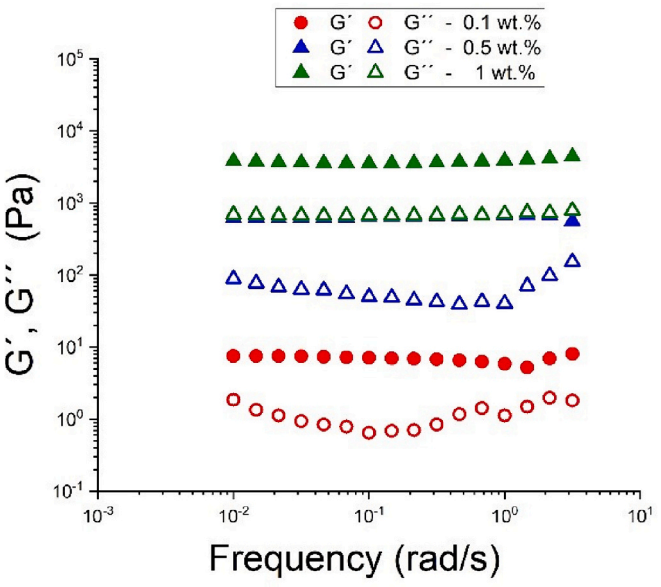

Fig. 8. Mechanical spectra for emulgels formulated with phycocyanin as a function of diutan gum concentration.

such as creams, sauces or other high-viscosity products.

Table 3 shows the Turbiscan Stability Index (TSI) values for 1 h and 24 h of aging of the samples as a function of the diutan gum concentration. Again, the addition of a small amount of the biological macromolecule significantly improves the physical stability of the emulsions. Furthermore, the physical stability is further improved as the concentration of diutan gum increases. The same tendency follows the viscosity of the samples, as mentioned above. Thanks to the presence of diutan gum, the only destabilization mechanism present, creaming, is inhibited to a greater extent. The incorporation of diutan gum creates a 3D network that reduces the movement of the droplets. This provokes the inhibition of creaming at these concentrations. There is no need to increase the concentration further as the goal is achieved.

4. Conclusions

The analysis of the physicochemical properties of phycocyanin showed that this protein has an isoelectric point around pH 3, which would allow its relatively easy use in the food industry. Furthermore, according to the surface tension results, it can be stated that although the saturation of the interface in the solutions is reached at low concentrations of phycocyanin (1.5 wt%), the interfacial tensions do not substantially decrease between solutions compared to other animal or vegetable proteins. However, higher phycocyanin concentrations are required to find more structured interfaces. Furthermore, the interfacial results indicate that there is an aggregation of PC particles above 1.5 wt %.

The study of the properties of the emulsions showed that the use of 1.5 wt% PC concentration obtained the emulsion with the smallest droplet size since it seems that above this concentration, aggregation of PC takes place. However, the physical stability of this emulsion can be improved since it showed creaming with aging time. Since coalescence is not present in the emulsions, PC proved its role to protect the interface. The method to solve the drawback of creaming in this work was to form a complex between PC and diutan gum. The presence of this thickener was able to form emulgels with pseudoplastic behaviour. The gel-type structure of the systems was able to reduce creaming even at 0.1%wt of diutan gum. In addition, more diutan gum concentration, more stable emulgels.

This research has been limited to the preparation, stabilization and characterization of avocado oil emulgels with an algae extract (phycocyanin) as emulsifier and a macromolecule of biological origin (diutan gum) as rheology modifier. Thus, further studies could use these systems as matrices for controlled release systems of bioactive principles. Therefore, this work contributes to continued development efforts to uncover further insights into the versatility and efficacy of phycocyanin as a stabilizer, paving the way for its widespread use in different dispersed systems.

CRediT authorship contribution statement

Patricia Tello: Writing – original draft, Investigation, Formal analysis, Data curation. **Jenifer Santos:** Writing – original draft, Supervision, Formal analysis, Conceptualization. **Víctor M. Perez-Puyana:** Writing – original draft, Investigation, Formal analysis, Data curation. **Alberto Romero:** Writing – original draft, Validation, Supervision, Methodology, Formal analysis. **Luis A. Trujillo-Cayado:** Writing – original draft, Supervision, Software, Resources, Project administration, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Luis Alfonso Trujillo-Cayado reports article publishing charges and equipment, drugs, or supplies were provided by Universidad de Sevilla. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

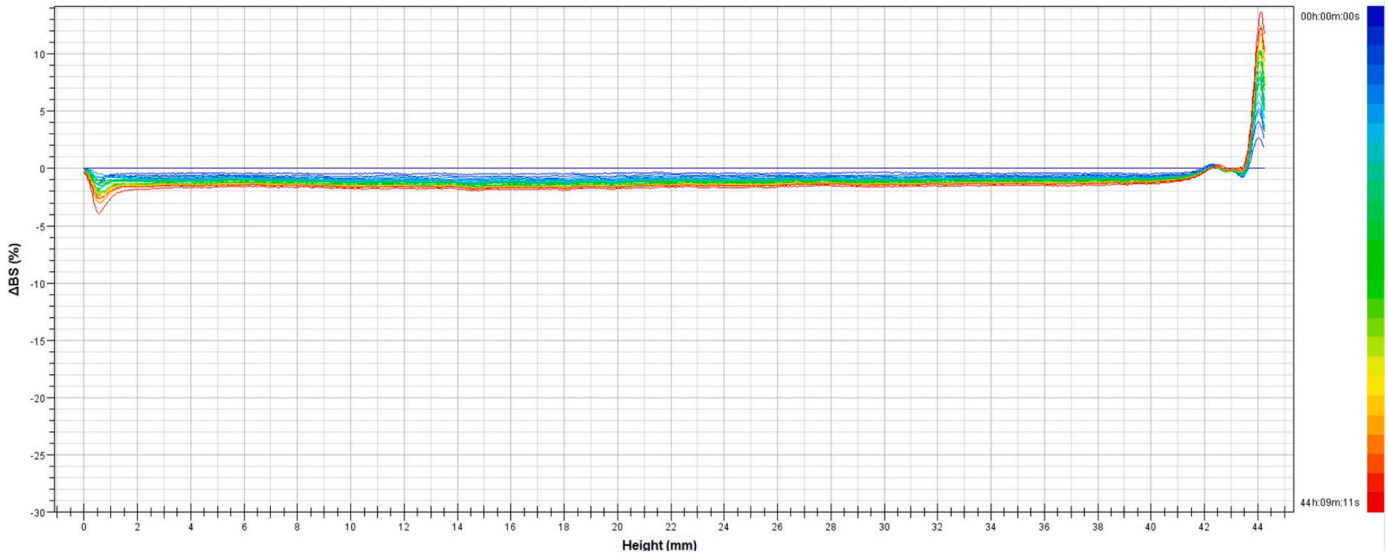

Fig. 9. Variation of backscattering as a function of measurement cell height with aging time for emulgel formulated with 1.5 wt% PC and 0.1 wt% diutan gum.

Table 3
Turbiscan Stability Index at 1 h and 24 h for all samples as a function of diutan gum concentration.

Diutan gum concentration (wt%)	TSI_{1h}	TSI_{24h}
0	28.4	58.5
0.1	1.2	1.6
0.5	0.9	1.4
1	0.3	0.8

Data availability

Data will be made available on request.

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