ISBN 978-81-934174-2-3
6th International Conference on Healthcare, Environment, Food and Biological Sciences (HEFBS-17)
Istanbul (Turkey) Sept. 8-10, 2017

Performances of Colored Spirulina (*Spirulina platensis*) Soluble Protein Fractions as Surfactants at Liquid-Liquid Interface

Abakoura Barka[1], Christophe Blecker[1]

[1]University of Liège, Gembloux Agro-Bio Tech. Food Science and Formulation Laboratory. Passage des Déportés, 2. B-5030 Gembloux (Belgium).

***Abstract*:** *A study was carried out to evaluate the performances of colored spirulina (Spirulina platensis) soluble proteins fractions as surfactants at water/n-dodecane interface using a drop volume tensiometer. Also the mechanical behavior at this interface were studied via an automated drop tensiometer. Various concentrations (0.1%; 0.3% and 0.5% (w/w)) and pH levels (native, 3 and 5) were tested. Results showed that the interfacial tension decay increased with increased protein concentration. Also, at 0.3% (w/w) colored protein in the aqueous phase there were no significant difference in interfacial tension decay between the native pH and the pH3 levels while at pH5 the surface tension decay was greater. The viscoelastic moduli and the equilibrium interfacial tension of the fractions suspensions also decreased with concentration.*

***Key words**: surfactants, viscoelastic moduli, interface, proteins*

1. Introduction

Food preferences today are mostly directed towards the choice of natural ingredients (Rozin *et al.*, 2004). The value-added of a formulated food is tightly linked to its lower level of synthetic ingredients. Some synthetic ingredients are being banned or have their levels considerably reduced in formulated foods. Proteins in food systems, in addition to their nutritive value can achieve different roles in the sensory and organoleptic properties of processed foods. They can act as natural foaming, emulsifying, color, texture agents etc. Historically, proteins from animal sources such as those from milk, meat and eggs have being used for many applications including cheese, ice cream, dairy products, meat products etc. The use of proteins from other sources like plants, algae, microorganisms, insects, microalgae etc implies that investigations to understand their functions within food systems is obvious. This will help to know the foundation of their behavior at the molecular level.

Microalgae such as *Spirulina (Arthrospira) platensis* present good sources of protein (55.8- 77%) with a high production potential (Barka and Blecker, 2016). The soluble fractions of its proteins are of different colors including the green and blue fractions (Chronakis, 2001).

To tackle the major constraints limiting the use of any protein from new sources, its behavior within food systems must be investigated. Fundamental physicochemical properties governing protein functionalities must be evaluated to simulate their real behavior in food systems despite the complexity of the later. Studies in this area are seriously lacking, especially as far as Spirulina soluble proteins are concerned. Few studies including those of Nirmala *et al* (1992) on solubility; Chronakis (2000) on the interfacial behavior, Chronakis (2001) on some functional characteristics Spirulina water soluble proteins

were carried out. More recent studies include those of Benelhadj *et al* (2016); Bashir *et al* (2016) and Mahajan *et al* (2016) are so far available in the literature. To the best of our knowledge there is no study on the performances of spirulina colored soluble proteins at liquid-liquid interface.

The aim of the present study is to evaluate the behavior of spirulina colored soluble protein fractions at water/n-dodecane (non-polar liquid) interface using a drop volume tensiometer and an automated drop tensiometer.

2. Materials and Methods

2.1. Materials

Food grade Spirulina powder (2.5kg) was purchased here in Belgium (Laboratoires BIORES Liège Belgium) and kept under freezing temperature and only part of it is sampled and kept in fridge for the daily experiments. The liquid phase immiscible with water used under this study was n-dodecane 99+% from Alfa Aesar Industries (USA).

2.1.1 Extraction and fractionation procedure

Spirulina powder was suspended in an alkaline aqueous solution (pH10) and centrifuged to remove pellets. The latter was suspended again and centrifuged at 9050g for 30 minutes at 25°C. The supernatants from the two centrifugation steps were collected and their pH values were adjusted to 3.5 for the total fraction (TSSP) extraction or 4.5 for the Green fraction (GSSP) precipitation. The pH of the supernatant from the separation of the green fraction was again adjusted to 3.5 and the blue fraction (BSSP) was precipitated by centrifugation under the same conditions as above.

Fractions pellets obtained were suspended again, their pH adjusted to 7, and dialysed for 48 hours at 4°C. Dialysed fractions were freeze dried and defatted with n-hexane at 4°C. Dry Spirulina protein fractions were obtained and kept in fridge at 4°C awaiting analysis.

2.2 Sample preparation

Colored proteins powder was dissolved in milli Q water and stirred for one hour with a magnetic stirrer (400tr/min) at ambient temperature. The pH values of the solutions were adjusted to 3, or 5 using HCl 0.1M and NaOH 0.1M solutions.

2.3 Interfacial adsorption kinetics (TVT1)

Measurements were carried out using a dynamic method at 25°C±0.1°C via a tensiometer TVT1 (Lauda, Königshofen Germany). A 2.5mL seringe and a capillary of 1.390 mm radius were used. Drop formation time was 0.07 to 2.5s/µL. Six drops were formed and values for surface tension and time of drop formation were the mean values of two measurements. Drops of the proteins fraction solutions were formed via the capillary immersed into the n-dodecane in a cuvette.

2.4 Viscoelastic properties of spirulina colored protein fractions (Tracker)

The viscoelastic interfacial properties of fractions solutions were determined using an automated drop tensiometer Tracker (L.T. Concept Instrument, Longessaignes, France). Measurements were carried out on oscillatory interfacial tension by periodical compression - expansion of the drop volume. The drop was formed via a syringe with its capillary immersed in the n-dodecane in a cuvette. Preliminary trials allowed us to fix the volume of the drop to 5µL and a 20% deformation of the drop and a frequency of 100 mHz. Measurements were carried out at 25°C±0.1°C.

3. Results and Discussions

3.1 Interfacial adsorption kinetics (TVT1)

3.1.1 Effect of concentration

N-dodecane was used as the non-polar liquid to simulate interfaces usually found in emulsions. It is preferred to vegetable oils because it is devoid of other surface active molecules such as mono or diglycerides that may affect the results of the protein performances. Interfacial tension measurements were carried by expelling a drop of the protein suspension via a capillary immersed in the n-dodecane filled in the cuvette of the TVT. The amphiphilic protein molecules migrate towards the interface once the drop is formed thereby causing the decay of the surface tension. The drop detaches from the capillary and falls to the bottom of the cuvette when the interfacial pressure is not strong enough to support the weight of the drop. The rate of proteins adsorption at interface is directly related to the rate of interfacial tension reduction and this allow for the evaluation of the overall interfacial adsorption kinetics.The interfacial tension reduction is expressed as a function of time ($\Upsilon = f(t)$). This is a measurable expression of the progressive diffusion and adsorption of protein to the interface orienting their hydrophobic tail towards the non-polar liquid and the hydrophilic head towards the aqueous drop. Some authors sectioned protein adsorption to interface into three steps including diffusion, adsorption and rearrangement (Dagorn, 1986; Damodaran, 1994). This stepwise fractionation of the adsorption process depends on the size and the molecular structure of the protein.

Figure 1 shows the adsorption kinetics of three spirulina protein fractions (TSSP, BSSP, GSSP) at different concentrations (0.5%; 0.3%; 0.1% (w/w)). This is concretely presented by the measure of the interfacial tension as a function of time ($\Upsilon = f(t)$). It clearly appear on the Figure 1 (A, B, and C) that the interfacial tension reduces with the age of the drop. As we stated early, for the protein molecules to adsorb to interface and reduce the interfacial tension it should undergo some steps that may take a laps of time depends on its distance to the interface, on the various forces due to its environment and on its molecular structure. This shows that the time taken by every individual protein molecule to arrive at interface and contribute to interfacial tension decay by adsorption is special. This explains the progressive decay of the surface tension with a relatively low slope. The pattern of the curves (A, B, and C) is the same and the decay is greater for the higher protein concentration (0.5%) followed by 0.3% and then 0.1% (w/w) concentrations. The slopes of the curves are greater at the beginning and reduce towards the end of the measurement. This can be explained by the fact that adsorption becomes more difficult when the interface is saturated and at this level the molecular rearrangement also contributes to the surface tension decay. This results corroborate with those reported on plant (Dagorn, 1986) and animal proteins (Blecker, 1988; Karamoko, 2014)

The adsorption kinetics comparison of the three fraction at 0.3% (w/w) concentration is presented in the Figure1D No significant difference is observed among the three tested Spirulina protein fractions. Despite their color difference, the extraction method used does not give pure fractions containing exclusively one type of protein. Also the differences in color of spirulina protein fractions are the nature of the pigment phycocyanobilin linked to the protein fragment. The pigment may not necessarily significantly influence the adsorption process of the pigment-protein complex (phycobiliprotein).

3.1.2 Effect of pH

The effect of pH on Spirulina colored protein fractions adsorption kinetics at water/n-dodecane interface is presented in Figure 3. Suspensions (0.3% (w/w)) were prepared from the three Spirulina protein fractions and tested at three pH levels i.e: the native pH of the fractions suspensions, pH5 and pH3. The graphs are the presentation of the interfacial tension as a function of time. These shows that interfacial tension decreases with the drop age fairly quickly at the beginning and slowly towards the end of the experiment as

observed in previous experiments. At the same concentrations the interfacial tension decay for all the three fractions were closer at pH 3 and the native pH while at pH 5 the decay is significantly important ($p < 0.05$) and differs from the first two pH levels.

The surface tension measured at the milli-Q water/n-dodecane interface 44.53 ± 0.08 mN/m was considered as the starting point of the surface tension decay during the drop ageing of protein suspensions. Being that surface tension decay with the drop age is the manifestation of the adsorption kinetics at the water/n-dodecane interface the fast decay within the first ~10 seconds means protein adsorption is faster within this period of time. Ward and Tordai (1946) stated that within the first instants of adsorption processes, the surface tension decay reflects adsorption solutes diffuse from the aqueous phase to the interface. The surface tension is reduced from its initial value (45.53± 0.08 mN/m) by almost 60% within ~10 seconds. Once the drop is formed, the free energy of the system reaches its highest value and the protein diffusion towards the interface (the tendency to lower the free energy) is accelerated both by the gravitational force (for pendant drop) and the acceleration brought about by the change in free energy of the system represented by the newly formed drop. This fast adsorption at interface during the dynamic surface tension measurement is one of the peculiarities of the dynamic surface tension measurement method as compare to the static method. Measurements are carried out on a freshly formed interface and during its ageing. The method seems to be closer to real colloid systems during emulsions formation which are not equilibrium systems. The needed property in colloids as far as surface active agents are concerned is not only their ability to adsorb at interfaces but also the rate at which the adsorption proceeds i.e: the rate of surface tension reduction in addition to the strength of the film formed at interfaces. Graham and Phillips (1979a) presented a general adsorption pathway with an interpretation dividing the molecular adsorption into different phases - diffusion of the molecules from the aqueous phase to the interface - penetration into interfacial layer by new molecules and development of an energy barrier opposing this process - and the rearrangement of adsorbed molecules by conformational modifications. This shows that the more the drop age is increasing the more the process of adsorption of new molecules to the interface is becoming difficult because of the creation of barriers by already adsorbed molecules and the need for the molecule to rearrange and change their conformations at interfaces. This may explain the fall of the slope of the graphs representing the surface tension decay kinetic (adsorption kinetic). Adsorption process thus the surface tension decay proceeds at speeds higher at the beginning (freshly formed drops) and progressively slows down as the above mentioned barriers are becoming stronger.

Initial rate of adsorption at the water/n-dodecane interface of the fraction suspensions at different pH levels (pH3, pH5 and native pH) and concentrations are presented in the table 1 below. It is expressed as the change of interfacial tension from its initial value (45.53± 0.08 mN.m-1.s-1) to the first value measured (about 10 seconds after drop formation) during drop formation. It is therefore an estimation of the interfacial tension reduction per unit time and expressed as the differential of the interfacial tension over that of the first measurement time ($d\gamma/dt$). This gives an idea on how fast the interfacial tension drops when the drop is formed. Initial adsorption rate increases with increasing concentrations for all the fractions and are higher at pH5 for all the fractions. At 0.5% the blue fraction (BSSP) shows the highest initial speed (2.04±0.03 mN.m-1.s-1) compared to the other two fractions, while at pH5 the green fraction shows the highest initial speed of interfacial tension reduction. Despite their closeness in surface behavior, the spirulina colored protein fractions response to pH variation appear different. This may be due to the differences in the pigment response to pH change and that may bring about different electrostatic barriers and thus influence the adsorption process differently.

3.2 Effect of concentration on the viscoelastic properties of spirulina colored protein fractions

The viscoelastic properties of the spirulina colored soluble protein fractions were evaluated at the water/n-dodecane interface during oscillation of the pendant drop when its interfacial tension become stable ($\gamma = \gamma e$). The viscoelastic moduli and the equilibrium interfacial tension of the proteins aqueous solution at different concentrations are presented in table 2. For all the fractions the viscoelastic moduli and the equilibrium interfacial tension decrease when increasing concentration within the range of concentration tested.

The blue fraction appears to be more sensitive to concentration changes followed by the total fraction and the green fraction as far their viscoelastic moduli are concerned. This results is somehow contradictory to what we expected during our investigations. The viscoelastic modulus which is the expression of the viscosity combined with the elasticity of the liquid is rather expected to increase with increase solution concentration but surprisingly it decreases within the range of the tested concentrations. This results those not corroborate with that reported by Karamoko (2014) on proteose-peptone who reported an increase in viscoelastic modulus with increasing concentration.

On the other hand the total fraction equilibrium interfacial tension response to concentration changes is more important than that of the other two fractions. The equilibrium interfacial tension of all the three fractions also decreases with increasing concentration in protein of the tested suspensions. The higher the protein concentration, the more proteins adsorb to the interface and the less susceptibility they are to desorb back from the interface to the aqueous subphase due to the reduced concentration gradient between the interface film and the aqueous phase. At equilibrium, protein concentration at interface is therefore increases whith the increase of its concentration in the aqueous subphase.

4. Conclusion

Adsorption kinetics and interfacial viscoelastic properties of soluble proteins are the fundamental basis of surface active molecules at interfaces. Spirulina proteins considerably reduce interfacial tension, a condition necessary for the stabilization of emulsions in colloid systems which are thermodynamically unstable. The reduction in interfacial tension brought about by spirulina colored proteins will ease emulsion formation and enhance the stability of an emulsion. The viscoelastic properties of the monolayer films are also important parameters as far as the stability of an emulsion is concerned. They are one of the fundamental properties affecting the ability of the film to resist emulsion destabilization phenomena such as coalescence, flocculation and creaming. The stronger the film surrounding fat globules in an emulsion the more stable it is. Spirulina colored protein fractions appear to be less viscoelastic at higher concentration within the tested concentration range. Tests on a larger concentration range are needed for a better appreciation of the effect of spirulina protein concentration on the viscoelastic modulus of Spirulina protein in aqueous systems.

5. References

[1] Barka Abakoura and Blecker Christophe. Microalgae as a potential source of single-cell proteins. A review. *Biotechnology, Agronomy, Society and Environment* 20 (3), 427-436 (2016).

[2] Bashir, S. , Sharif, M.K., Butt, M.S., Shahid, M. Functional properties and amino acid profile of spirulina platensis protein isolates. Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences Volume 59, Issue 1, 12-19 (2016).

[3] Benelhadj Sonda, Adem Gharsallaou, Pascal Degraeve, Hamadi Attia, Dora Ghorbel. Effect of pH on the functional properties of Arthrospira (Spirulina) platensis protein isolate. *Food Chemistry* (2016) 194, 1056-1063. https://doi.org/10.1016/j.foodchem.2015.08.133

[4] Blecker Christophe. Etude de la modification des propriétés fonctionnelles du lactoserum par hydrolyse enzymatique de la matière grasse résiduelle. Thèse de Doctorat soutenue à la faculté Universitaire de Gembloux (1998).

[5] Chronakis Ioannis S. Gelation of Edible Blue-Green Algae Protein Isolate (*Spirulina platensis* Strain 387 Pacifica): Thermal Transitions, Rheological Properties, and Molecular Forces Involved *J. Agric. Food* 388 *Chem. 49*, 888-898(2001).
https://doi.org/10.1016/S0927-7757(00)00548-3

[6] Chronakis I.S., Galatanu A.N., Nylander T. & Lindman B., 2000. The behaviour of protein preparations from blue-green algae (Spirulina platensis strain Pacifica) at the air/water interface. Colloids Surf., A, 173, 181-192.
https://doi.org/10.1016/S0927-7757(00)00548-3

[7] Chronakis I.S., Galatanu A.N., Nylander T. & Lindman B. The behaviour of protein preparations from blue-green algae (Spirulina platensis strain Pacifica) at the air/water interface. Colloids Surf., A, 173, 181-192 (2000).
https://doi.org/10.1016/S0927-7757(00)00548-3

[8] Dagorn-scavinier C. Etudes des propriétés tensioactives des gliadines du pois. Application aux isolats protéiques utilizes comme agent d'émulsification ou de moussage. *Thèse de Doctorat, Université de Nantes (*1986).

[9] Damodaran S. Structure-function relationship of food proteins. In: Protein functionality in food systems. Marcel Decker Inc. New York, eds, N.S. Hettiarachchy, G.R. Ziegler, P1-38 (1994).

[10] Graham D.E., Phillips M.C. Proteins at liquid interfaces. 1 Kinetics of adsorption and surface denaturation. *J. Colloid Interface Sci*., 70, (3), 403-414 (1979a).
https://doi.org/10.1016/j.appet.2004.03.005

[11] Karamoko Gaoussou. Etude des propriétés de surface et techofonctionnelles des fractions proteose-peptones. Thèse de Doctorat soutenue à la Faculté agronomique de Gembloux, Université de Liège (2014).

[12] Mahajan, A., Neetu, Ahluwalia A.S. Effect of processing on functional properties of Spirulina protein preparations. *African Journal of microbiology Rsearch* Vol.4(1), pp. 055-060 , (2010)

[13] Nirmala, C., Prakash, V., Venkataraman, L.V. Physico-chemical and functional properties of proteins from spray dried algae (Spirulina platensis). *Molecular Nutrition Food Research*, Volume 36, Issue 6 (1992).

[14] Rozin, P., Spranca, M., Krieger, Z., Neuhaus, R., Surillo, D., Swerdlin, A., *et al*.. Preference for natural: instrumental and ideational/moral motivations, and the contrast between foods and medicines. Appetite, 43, 147-154 (2004).
https://doi.org/10.1016/j.appet.2004.03.005

[15] Ward A.F.H, Tordai L. Time-dependence of boundary tensions of solutions. The role of diffusion in time effects. *J. Chem. Phys*. 14, (7), 453-461 (1946).
https://doi.org/10.1063/1.1724167

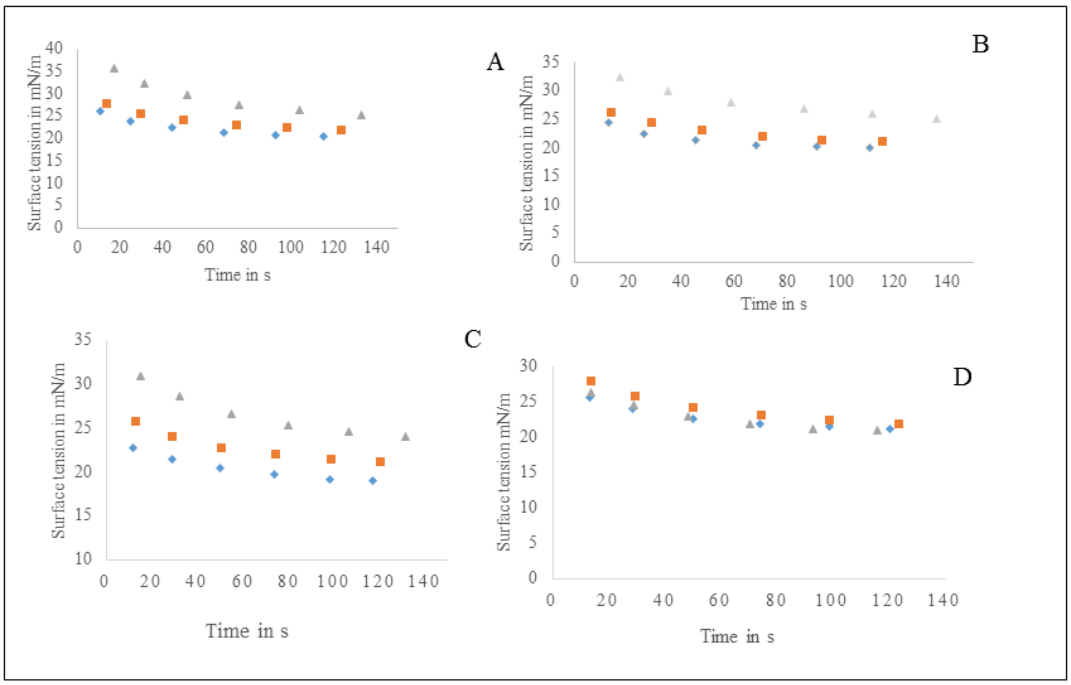

Figure 1: Effect of concentration on adsorption kinetics of different Spirulina colored soluble protein fractions at water/n-dodecane interface measured with drop volume tensiometer (TVT1): A TSSP; B GSSP; CBSSP(◆ 0.5%, ■ 0.3% , and ▲ 0.1% (w/w); D : comparison of the three fractions at 0.3% (w/w): ■ TSSP;◆BSSP; ▲GSSP

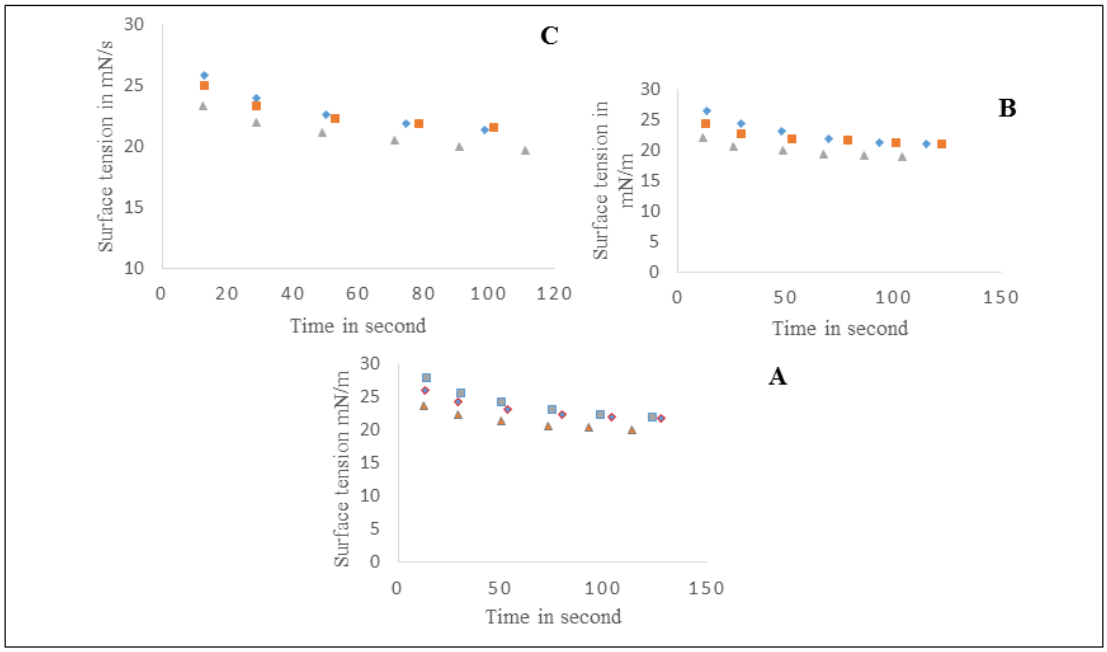

Figure 2: Effect of pH on adsorption kinetics of different Spirulina colored soluble protein fractions at water/n-dodecane interface measured the drop volume tensiometer (TVT1): A TSSP; B GSSP; C BSSP: ■ pH 3,◆ native pH, and ▲pH 5

Protein suspension at different % (w/w) and pH values	Initial adsorption rate dγ/dt
TSSP 0.1%	0.59±0.02
TSSP 0.3%	1.29±0.02
TSSP 0.5%	1.87±0.32
TSSP 0.3% pH 3	1.51±0.11
TSSP 0.3% pH5	1.70±0.04
GSSP 0.1%	0.77±0.06
GSSP 0.3%	1.39±0.17
GSSP 0.5%	1.67±0.09
GSSP 0.3% pH 3	1.62±0.03
GSSP 0.3% pH5	1.94±0.03
BSSP 0.1%	0.94±0.06
BSSP 0.3%	1.50±0.00
BSSP 0.5%	2.04±0.03
BSSP 0.3% pH 3	1.58±0.04
BSSP 0.3% pH5	1.78±0.06

TABLE I: Initial rate of adsorption (in $mN.m^{-1}.s^{-1}$) of different Spirulina colored proteins suspended in milli Q water calculated from data obtained by a drop volume tensiometer TVT

Note: Where the pH value is not mentioned means it is the native pH of the fraction suspension in milli-Q water

TABLE II: Effect of concentration on the equilibrium interfacial tension and viscoelatic modulus of Spirulina colored soluble protein fractions at water/ndodecane interface

Sample	Protein concentration (% w/w)	Equilibrium intefafial tension mN/m	Viscoelastic modulus mN/m
TSSP	0.1	14.26±0.11	24.37±0.30
	0.3	13.51±0.13	23.405±0.67
	0.5	11.05±0.05	19.825±0.03
BSSP	0.1	15.66±0,01	27.475±0.02
	0.3	14.28±0,01	24.955±0.28
	0.5	13.01±0,00	20.13±0.02
GSSP	0.1	14.41±0.00	21.45±0.06
	0.3	13.02±0.00	20.14±0.00
	0.5	12.23±0.00	20.00±0.00