Article

Physicochemical characterization of colored soluble protein fractions extracted from *Spirulina* (*Spirulina platensis*)

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Abstract

The aim of this study was to evaluate the physicochemical properties of *Spirulina* colored soluble protein fractions extracted from spray-dried *Spirulina* powder. Three fractions, including the blue soluble *Spirulina* protein, the green soluble *Spirulina* protein, and the total soluble *Spirulina* protein fractions were obtained. Investigations on their behavior at air/water interface were carried out using dynamic methods of drop volume and bubble pressure surface tension measurements. Evaluation of their monolayer films' mechanical behavior was done via compression isotherms using Langmuir film balance. The protein contents of the fractions were 82.76, 82.29, and 74.53% for the blue, the green, and the total fractions, respectively. Surface tension decay increased with increasing concentration for all the fractions. No significant difference in surface tension decay was observed between the samples at 0.3% (w/w). Surface tension decay was less important at pH 3 for all the fractions. The total fraction and the blue fraction appeared to form more elastic films than the green fraction. The blue soluble fraction also presented the highest collapse pressure and initial expansion area.

INTRODUCTION

New protein sources are being investigated in an attempt to supplement the existing limited global protein resources. Potential unexploited natural sources of protein such as insects and microalgae are today subjected to various scientific studies for their nutritional, functional, and safety properties for human consumption (Ademolu et al., 2010; Anand et al., 2008; Auerswald and Lopata, 2005; Gouveia et al., 2008).

Microalgae production was improved by investigations on industrial mass production but the expected level is yet to be reached (Richmond, 2004). Unlike some insects and microalgae species, which could be considered as novel foods and their safe consumption is a topic of controversial opinions, the use of *Spirulina platensis* in human food has an historical background (Hu, 2004). Its high nutrients content, high productivity, and its ability to survive and thrive under drastic climatic conditions make it a unique contender in supplementing the limited sources of conventional proteins (Barka and Blecker, 2016).

Food systems are usually colloidal mixtures made up of miscible and/or nonmiscible liquids and solid particles. The amphiphilic nature of proteins allows them

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to act as surfactants, thus leading to a significant effect on the textural outcome of processed foods. As surface active agents, proteins can contribute to foam and emulsion formation. These functionalities are linked to the ability of protein molecules to adsorb to interfaces and to form strong viscoelastic films that can contribute to the stabilization of foams and emulsions (Damodaran, 1994).

Proteins from microalgal biomass as well as those obtained from plant sources could substitute conventional animal proteins (e.g. egg, milk, and meat proteins). They have the advantage of being cheaper, abundant in nature, and friendly to the environment as compared to animal proteins (Barka and Blecker, 2016). Their use in formulated foods needs a proper understanding of their physicochemical and technofunctional behaviors. Colored protein from *S. platensis* could have a double impact on the organoleptic aspect of a food, when used as ingredients. They could more readily substitute synthetic colorants, as well as texture agents while improving the nutritional value of the food. As natural coloring agent, *Spirulina* proteins could be used to develop new foods, in response to the increasing consumer demand for more natural food products presenting health benefits (Batista et al., 2006). Phycocyanin, the blue *Spirulina* protein–pigments complex known for its nutraceutical effects (Liangqian et al., 2017; Romay et al., 2003) is also reported to act as a surface active agent by stabilizing food colloids such as emulsions (Batista et al., 2006).

Presently, there are very few investigations on surface and interfacial behaviors of colored *Spirulina* proteins. The few studies available so far on functional behaviors of *Spirulina* proteins were focused on the total soluble fraction. Chronakis (2001) evaluated visible absorption, differential scanning calorimetry, viscosimetry, and dynamic oscillatory rheological properties of the *Spirulina* protein aqueous solutions. Some functional properties of soluble *Spirulina* proteins including oil and water absorption capacities, foaming and emulsifying properties, surface hydrophobicity and nitrogen solubility were evaluated by Bashir et al. (2016). Benelhadj et al. (2016) also investigated nitrogen solubility, oil and water absorption capacities, and emulsifying properties of the soluble *Spirulina* proteins. To the best of our knowledge, the only study on interfacial behaviors of colored fractions is that of Chronakis et al. (2000), who investigated the colored fractions obtained by ultracentrifugation. However, the separation method of the colored fractions used in this study was an analytical method that could not be industrially feasible for large quantity food processing, due to the high cost and energy requirements of ultracentrifugation. Also, other testing methods (dynamic methods of measuring adsorption kinetics) closer to processes of

foam and emulsions preparation may be needed. This would allow a better appreciation of the behavior of *Spirulina*-colored proteins within colloidal systems during process.

In the present study, the soluble *Spirulina*-colored protein fractions were separated by a simple industrially feasible method inspired from that of Chronakis et al. (2000) and evaluated for their surface activity. Adsorption kinetics of these fractions at air/water interface were studied using dynamic methods. These included the dynamic methods of surface tension measurement with the drop volume (TVT1) and the bubble pressure (BP100) tensiometers under various concentrations and pH conditions. Moreover, the Langmuir film balance was used to investigate the behavior of fractions' monolayer films. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profiles and isoelectric points of the fractions were also determined.

MATERIALS AND METHODS

Materials

Food-grade spray-dried *Spirulina* powder (2.5 kg) was purchased in Belgium (BIORES Laboratories, Liège Belgium), kept under freezing temperature, and only part of it was sampled, and stored at 4 °C for the daily experiments.

Extraction procedure

Extraction is adapted from the method of Chronakis et al. (2000), using an alkaline aqueous solution of sodium hydroxide at pH 10. Details of the extraction procedure are presented in Figure 1.

Proximate composition of the fractions

The total proteins content of freeze-dried *Spirulina* protein fractions was evaluated using the DUMAS method, with a conversion factor of 6.25. The method was based on burning out about 200 mg of samples to evaluate its nitrogen content. The ash content of the samples was evaluated by incinerating 3 g of sample in a muffle furnace (Nabertherm, Bremen, Germany) at 500 °C overnight. Fat content was determined by Soxhlet extraction method. Dry matter content was determined by drying the samples in an oven at 105 ± 1 °C, until a constant mass was obtained. Experiments were repeated three times for every sample.

Zeta potential of the fraction solutions

The zeta potential (ζ potential) of the fractions solutions was measured using the Delsa Nano C Beckman Coulter (Miami, USA) coupled with an automatic
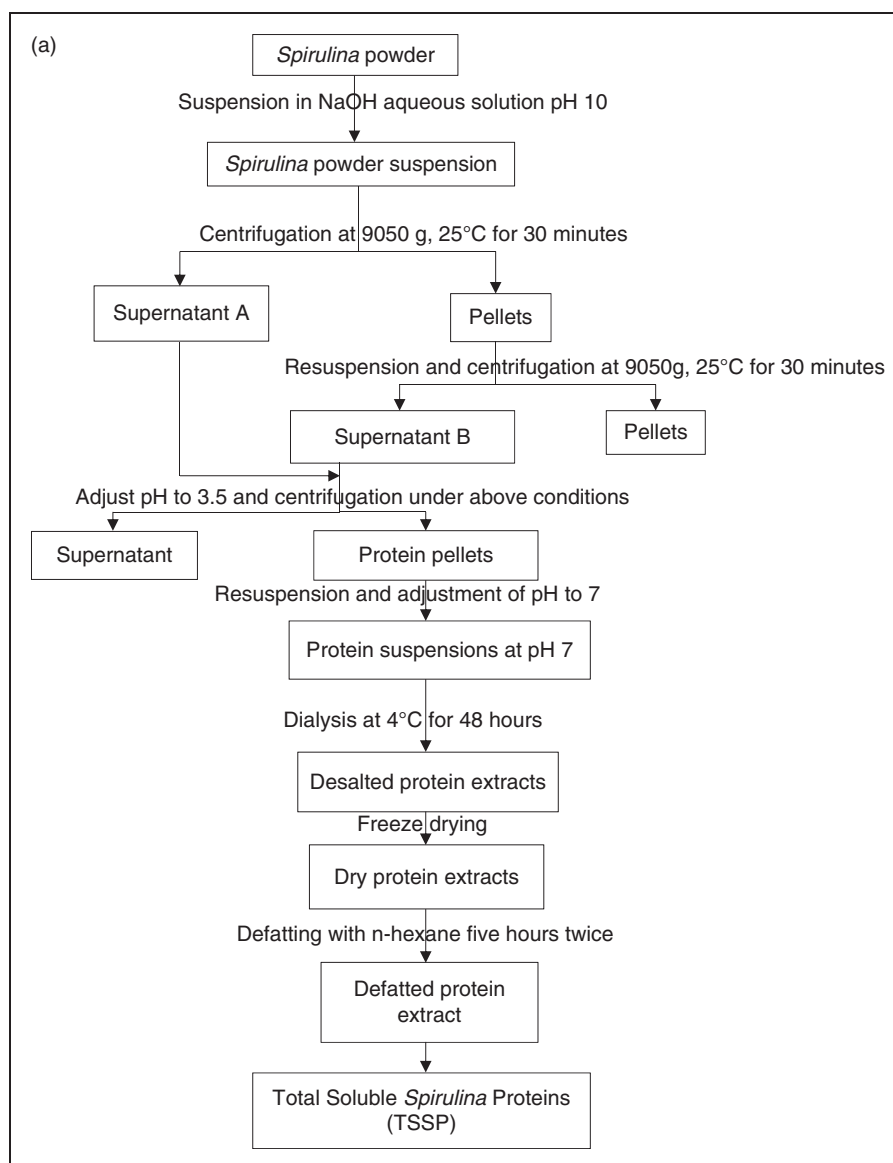
Figure 1. (a) Synoptic extraction diagram of total soluble *Spirulina* proteins (TSSPs) from *Spirulina* powder (adapted from Chronakis et al. (2000)) and (b) synoptic extraction diagram of green soluble *Spirulina* proteins (GSSPs) and blue soluble *Spirulina* proteins (BSSPs) from *Spirulina* powder.

titrator Delsa Nano AT (Miami, USA). Fraction solutions (0.05% (w/w) were prepared in Milli-Q water. Tested pH ranged from 2 to 10 and data were plotted in a graph ζ potential $= f$ (pH). The isoelectric pH (pHi) of the fractions was identified as the point at which the ζ potential equals zero.

SDS-PAGE

SDS-PAGE protein analysis of *Spirulina*-colored protein fractions was performed according to the method of Laemmli (1970) using 12% acrylamide gel, under reducing conditions in a Mini-Protean II electrophoresis cell (BioRad, Hercules, CA). Gels were run at 160 V for 45 min and stained with Coomassie brilliant blue. Densitometry analysis of bands obtained in SDS-PAGE profiles was performed using Image J software.

Adsorption kinetics measurement

Drop volume method. A drop volume tensiometer TVT1 (Lauda, Königshofen, Germany) was used. Measurements were carried out using a dynamic method at $25 \pm 0.1\,^\circ$C. Drops were formed through a 2.5 ml syringe (Lauda, Königshofen, Germany)
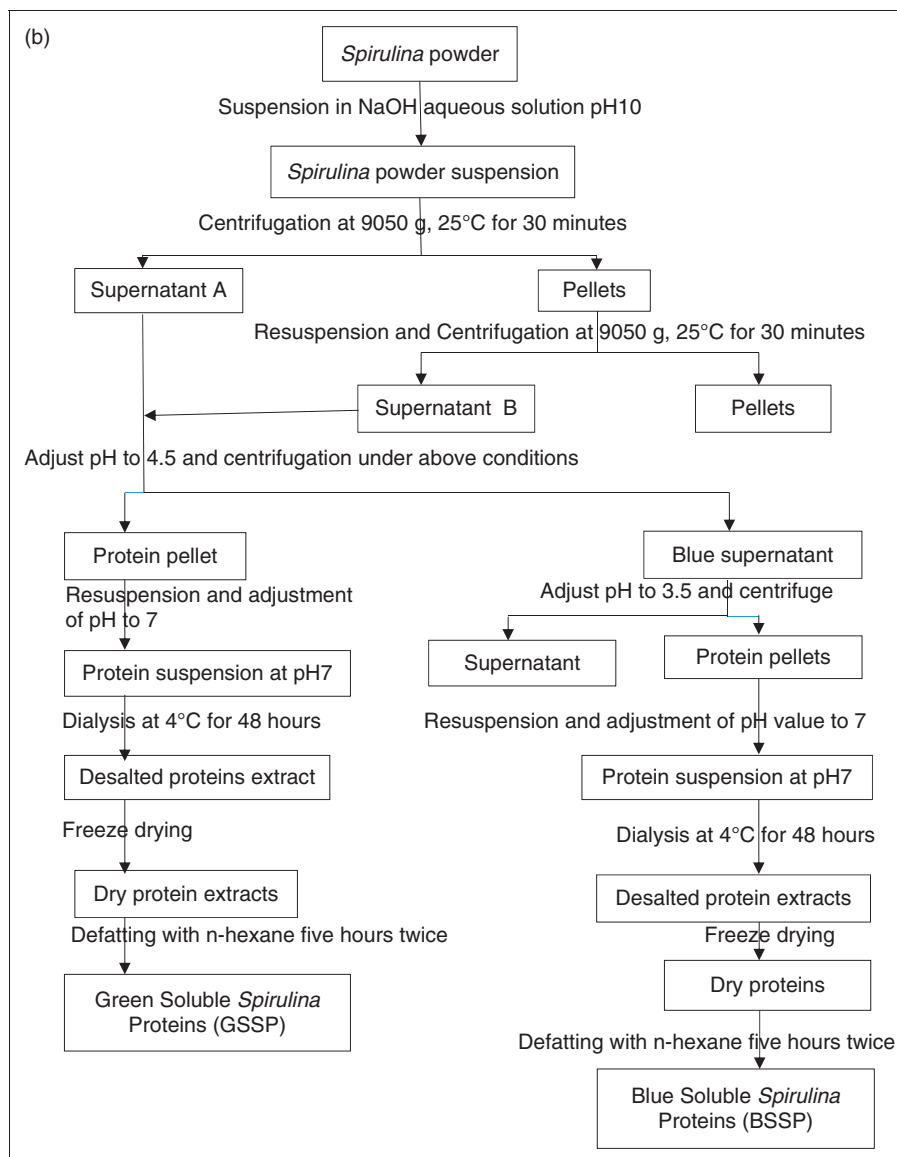
Figure 1. Continued.

equipped with a 1.055 mm radius capillary. Six drops were formed to evaluate the surface tension and drop formation time.

Colored protein powders were dissolved in Milli-Q water and stirred for 1 h with a magnetic stirrer at 25 °C. The pH values of the solutions were adjusted to 3, 5, or 7, using HCl 0.1 M and/or NaOH 0.1 M solutions. Also, different concentrations were tested including 0.05, 0.1, 0.3, and 0.5% (w/w).

Bubble pressure. Measurements of dynamic surface tension were carried out using a pressure tensiometer BP100 (KRÜSS, Germany) within milliseconds time scale to closely evaluate the surface tension decay kinetics once the bubble was formed. Bubble formation was done by injecting air in the protein solution through an immersed capillary. Pressure in the capillary increased from its initial value to the maximum and then fell rapidly to its initial value. This enabled the calculation of the surface tension of the protein solution. Twelve milliliters protein solution (0.3%, w/w) at two pH levels (pH 3 and 5) was used for the measurement at 25 ± 0.5 °C.

Compression isotherm

A Langmuir film *waage LFW2* $3''5$ (Lauda, Königshofen Germany) was used for the determination of compression isotherms of the three *Spirulina*-colored protein fractions.

Aqueous solutions 0.3% (w/w) (at their native pH ≈ 6.5) of each of the three fractions were prepared

using Milli-Q water as stated earlier. Eighty microliters of the protein solution was deposited drop by drop on the Milli-Q water surface used as subphase. The dropped samples were allowed to spread for 30 min before a 5 min compression was initiated.

All measurements were carried out at a temperature of 25 °C and were repeated three times with a maximum standard deviation of ±0.01 °C. A plot of the surface pressure as a function of spreading area per protein quantity was presented for the three fractions.

Data analysis

Data conversion and determination of some characteristic parameters such as initial film expansion area (for compression isotherms) and equilibrium surface tensions (for surface tension decay kinetics) were done using Gabriel Data Analysis software (Olive, 2016). All experiments were performed in triplicate.

Statistical analysis was assessed using SPSS. 21. One-way analysis of variance was used and Duncan's tests were used for comparison of means. The results were considered significant if the associated P-value was below 0.05.

RESULTS AND DISCUSSIONS

Proximate composition of the extracts

Out of every 100 g of crude *Spirulina* powder (62.55% proteins) used, 25.50 g of total soluble proteins fraction, 14.75 g blue fraction, and 10.10 g of green fraction were obtained using the extraction method outlined in Figure 1(a) and (b). These represented the major extracted soluble protein fractions extractible under alkaline conditions (in aqueous solution pH 10) where solubility was found to be maximum (Chronakis et al., 2000). Colored soluble *Spirulina* proteins carry covalently linked linear tetrapyrrole pigments related structurally to biliverdin (Glazer, 1994). The term soluble was used here in the denomination of the fractions to highlight the difference with proteins not soluble under the extractions conditions used

in the present study. It clearly appeared that more than half of the proteins contained in the original powder remained in the pellets obtained after extraction, together with other nutrients that could be used as food or feed ingredients.

Fractions subjected to this study were in fact mixtures of various proteins and other molecules such as carbohydrates and fat (Table 1) that could influence adsorption kinetic at interfaces. Our investigations were limited to the whole protein contributions in surface tension reduction, which is an important aspect, as far as the technological application in foam and emulsion is concerned (Damodaran, 1994). The fundamental mechanisms of molecular adsorption at interfaces could not be fully discussed in this study.

The total soluble fraction presented a higher carbohydrate content (11.06%) than the other fractions while the green fraction was rather higher in fat content. The protein contents of the green and the blue fractions are close (82.29 and 82.76%, respectively) and higher than that of the total fraction (74.53%) ($P < 0.05$). In fact, the total fraction was obtained by precipitation of the whole soluble components extracted from the suspended powder. This process minimized the loss in soluble materials such as carbohydrates, thus giving out a total fraction with a higher carbohydrates content.

Electrophoresis of the different Spirulina-colored protein fractions

The electrophoretic profile of the three *Spirulina* protein extracts revealed bands of molecular weight ranging from 10 to ~55 kDa. These results corroborate with those obtained by Benelhadj et al. (2016) who reported the presence of subunits of molecular weight within this range. Bands revealed at ~45, ~38, and ~55 kDa are light and common to all the three fractions. Subunits weighing ~ 10 kDa are revealed only in the total (Figure 2, Lane 2) and the blue (Figure 2, Lane 3) fractions, but absent in the green fraction (Figure 2, Lane 4). This may be due to differences in the fraction

Table 1. Proximate composition of colored soluble protein fractions from *Spirulina* powder.

Constituent	*Spirulina* powder	Blue fraction (%)	Green fraction (%)	Total fraction (%)
Protein	62.55 ± 0.74a	82.76 ± 0.20b	82.29 ± 0.93b	74.53 ± 0.58c
Fat	3.75 ± 0.17a	0.269 ± 0.06b	1.02 ± 0.01c	0.46 ± 0.12d
water	10.01 ± 0.10a	10.8 ± 0.09b	10.29 ± 0.22c	10.46 ± 0.05c
Ash	6.12 ± 0.05a	3.90 ± 0.00b	3.34 ± 0.03c	3.49 ± 0.07d
Carbohydrates by difference	17.57	2.27	3.06	11.06

Values given are means of three repetitions (x ± SD) (SD: standard deviation) except that of carbohydrates obtained by difference. Values with same letters within the same line are not significantly different ($P > 0.05$).
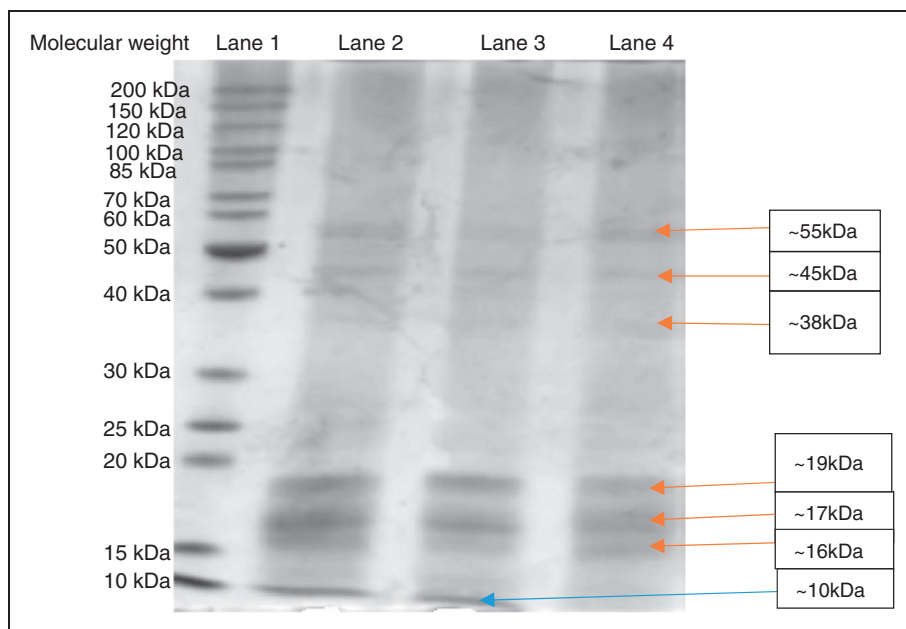
Figure 2. Electrophoretic profile of *Spirulina* protein fractions: 10–200 kDa molecular weight marker (Bio Basic Inc.) (Lane 1), TSSP fraction (Lane 2); BSSP fraction (Lane 3), and GSSP fraction (Lane 4).

separation method based on pH adjustment and centrifugation.

The proteins subunits of ∼ 16, ∼17, and ∼19 kDa are the main bands of all the three fractions. The ∼19 kDa band may correspond to α subunits of C-phycocyanins (Moreno et al., 1997).

Definitely, the composition of the two fractions (TSSP and BSSP) only differed in the concentrations of the protein subunits. In fact, based on densitometry analysis of the protein bands in each fraction, it was found that the ∼17 kDa represented 25.91% of the TSSP fraction and 16.66% of the BSSP fraction; the subunits weighing ∼19 kDa made of the 30.60% TSSP (Figure 2, Lane 2) and 40.43% of the BSSP fraction (Figure 2, Lane 3) and 17.08% of the GSSP (Lane 4). The GSSP is devoid of the smaller subunits weighing (∼10 kDa). These light subunits probably remained soluble at the first precipitation pH (pH 4.5 in Figure 1(b)) and were separated from the green pellets.

Zeta potential of Spirulina soluble fractions

All the fractions presented a maximum negative charge around pH 7 and a maximum positive potential around pH 2.5 (Figure 3). Their pHi were revealed at 3.5 for the TSSP and BSSP and 3.3 for the GSSP fraction. These values were close to that reported by Chronakis et al. (2000). At these pH values, the net charge of the protein molecules is zero. Shaw et al. (2001) reported that proteins were expected to be least soluble near their
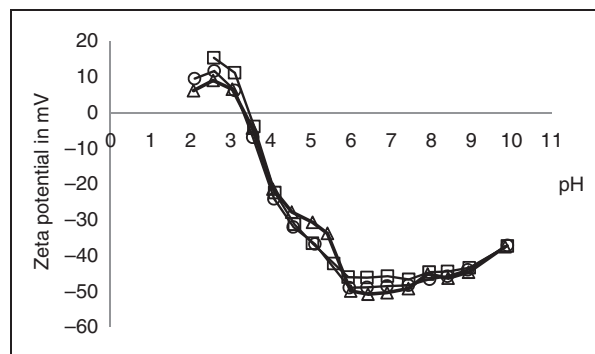

Figure 3. Zeta potential of *Spirulina* soluble protein fractions (using 0.05% w/w protein aqueous solutions) proteins: ○ TSSP, △ BSSP, ◇ GSSP.

isoelectric points, and the solubility should increase as the pH was raised or lowered with increasing net charge. Knowing the zeta potential of the fractions is therefore important as far as interfacial properties of proteins are concerned (Zhu et al., 2006). Protein–protein and protein–solvent interactions are likely to be affected, as well as proteins' ability to adsorb to interface. At electrokinetic potentials close to pHi protein–protein interaction was favored and protein molecules tend to precipitate. Under these conditions, where solubility is low, protein could be separated from the solvent by centrifugation. In the present study, the protein powder was solubilized in an alkaline (NaOH) solution whose pH was initially at 10, from which a suspension

with a pH around 8 was obtained. It could be observed that this pH value of 8 corresponded to a zeta potential of about −45 mV which was close to the maximum negative potential of the total fraction, i.e. the maximum net negative charge of the *Spirulina* protein molecules. These results were the basis of the extraction method used in this study.

Adsorption kinetics

Effect of concentration. Adsorption kinetics of freshly formed drops of *Spirulina*-colored protein fractions were measured at different concentrations (0.05, 0.1, 0.3, and 0.5% w/w) using a dynamic method of drop volume tensiometer (Figure 4).

For all the three tested protein fractions (Figures 4(a) to (c)), the surface tension decay significantly increased with increasing protein concentration (P < 0.05). The rate of decay was low at lower protein concentration (0.05% (w/w)). At this protein concentration, the curves were almost linear for the three fractions. The initial rate of the decay can be considered to be the slope of the plotted curve, i.e. the greater the slope of the curve, the higher the rate of surface tension decay.

A 20.7% surface tension decay from that of Milli-Q water (72 mN/m) was observed within 6 s, when using 0.5% aqueous solution of total *Spirulina* protein fraction (Figure 4(a)). The surface tension decay for the other *Spirulina*-colored protein fractions underwent the same trend (about 20% within 6 s) at 0.5% (w/w) (Figure 4(b) and (c)). The same decay pattern was followed at 0.3 and 0.1% concentrations except for the 0.05% solution. For the later concentration, the decay was slower showing a lag phase at the beginning, before a uniformly fairly low decay rate was observed.

The diffusion speed of the subphase molecules toward the surface could be one of the factors influencing adsorption kinetics. Dagorn-Scaviner et al. (1986) presented protein adsorption to the interface as a two-step process. The first step is characterized by the adsorption of the molecules to the surface and their concentration to form a monolayer film on the surface, thereby inducing a rapid surface tension decay. The second step corresponded to penetration and conformational rearrangement of the adsorbed proteins. At the second phase, the slope of the surface tension decay curve dropped and the surface tension tended to stabilize to an equilibrium value. This was more or less
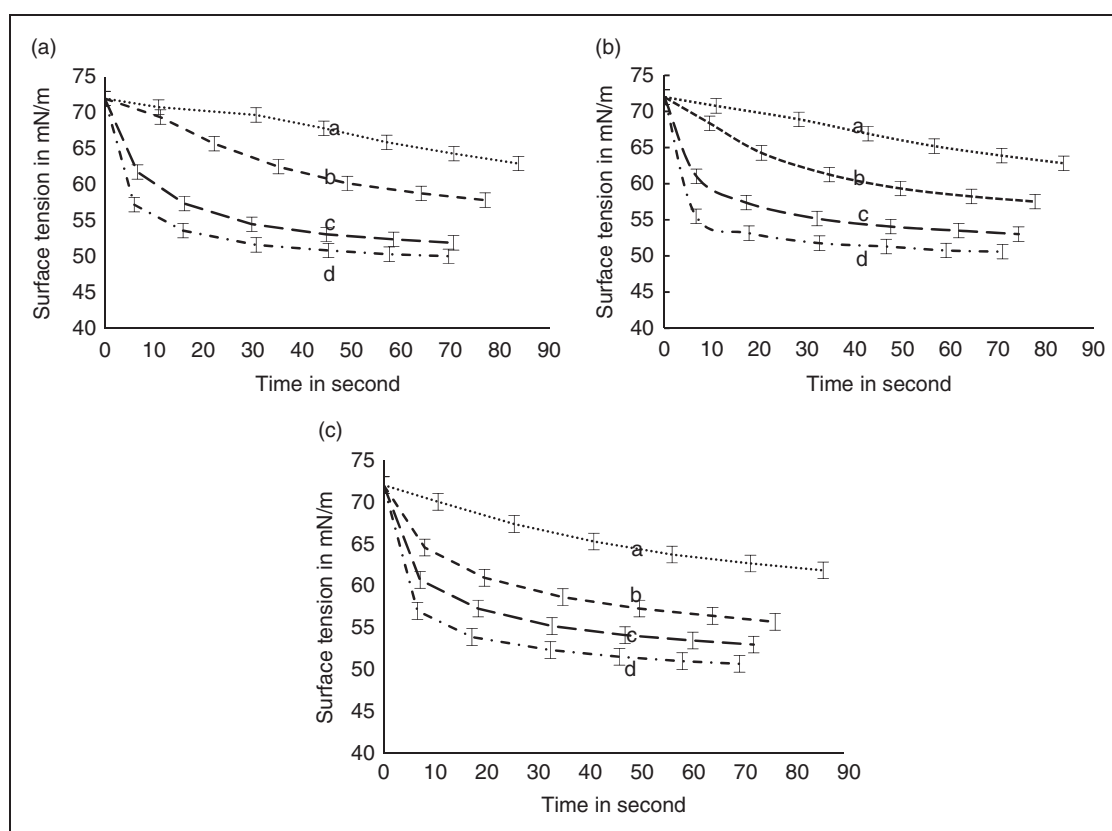

Figure 4. Surface tension decay kinetics of *Spirulina*-colored soluble protein fractions at different concentrations (a: 0.05%, b: 0.1%, c: 0.3%, and d: 0.5% (w/w)) using drop volume method: (a) TSSP, (b) BSSP, and (c) GSSP.

verified by the behavior of all tested *Spirulina*-colored protein fractions, for which the surface tension decay curves tended to be asymptotic toward the end of the measurement. Vogler (2013) reported that the decrease in protein adsorption (i.e. decrease in surface tension reduction) was due to the "energetic cost" of the surface adsorption–dehydration process. As interface water molecules are being replaced by proteins, the energy needed to remove the remaining water molecules became higher and the adsorption rate decreased.

Another observation was that despite the color differences of the fractions, their behavior at the interface remained similar. Their color difference was just a matter of distribution of the various *Spirulina* protein molecules among the fractions with predomination of a given color agent in any of the fractions. This could be probably because fractions are not purified protein molecules, but rather they are still mixtures with different color agent predominations.

The three fractions being all mixtures of *Spirulina* soluble proteins, they behave the same way on surface tension decay under these experimental conditions at the air/water interface.

All the fractions were made up of the same protein molecules and only the individual protein proportions varied from one fraction to another, for example level of subunits weighing ~19 kDa revealed in the SDS-PAGE and identified as phycocyanins subunits by Moreno et al. (1997) was higher in the BSSP fraction than in the other fractions. Another hypothesis could be that the various *Spirulina* proteins behave the same way as surface active agent. These results were quite different from those of Chronakis et al. (2000), who showed differences among the fractions. This was probably due to the fractions separation methods used. According to their study, one of the two blue fractions presented a higher surface tension reduction than the two green fractions. This means that the fractions performance as surface active agents is not an effect of a single type of molecule, but rather a contribution of the various types of surface active molecules present in the mixture, i.e. the more the fractions are purified the clearer will be their difference in reducing surface tension.

To further analyze the adsorption kinetics of the three *Spirulina*-colored soluble protein fractions, we estimated the equilibrium surface tension and the initial rate of surface tension decay (Table 2).The equilibrium surface tension (γ_e) was obtained by extrapolating surface tension to infinity time. It is in other words the least surface tension that can be brought about during the decay if sufficient time is given to the molecules to diffuse through the subphase and adsorb to the surface at a given concentration. The initial rate of surface tension decay is defined here as the decrease in drop surface tension per second. It is calculated as the ratio of the change in surface tension (mN/m) from its initial value to the change in time(s). The easier the protein molecules diffuse, adsorb, and rearrange at the surface the faster the surface tension decay. While the equilibrium surface tension is the lowest possible surface tension obtainable under specific operating conditions (concentration, pH, temperature, etc.), the rate reveals the quickness in attaining equilibrium under these conditions. Both are important parameters as far as foam and emulsion formation and stability are concerned. It has been shown that the rate of surface tension decay is correlated to foaming capacity of either low molecular weight surfactants (Kitabatake and Doi, 1982; Lech et al., 2015) or proteins (Dagorn-Scaviner et al., 1986).

Although comparison of the overall surface tension decay did not show significant difference ($p > 0.05$) among the samples at 0.3% (w/w), the total fraction showed the lowest equilibrium tension and lower initial rate of decay surface tension. This may be attributed to a synergic effect of a carbohydrates contribution whose proportion in this fraction was revealed higher (Zajic et al., 1977).

Table 2. Equilibrium surface tension and initial rate of surface tension decay of 0.3 and 0.5% (w/w) *Spirulina* protein fractions solutions calculated from data obtained by drop volume method.

Sample	Percent protein	Equilibrium surface tension γ_e (mN/m)	Initial rate of surface tension decay (mN/m/s)
Blue soluble *Spirulina* protein	0.3	49.39 ± 0.01a	1.63 ± 0.04a
	0.5	48.17 ± 0.19b	2.35 ± 0.01b
Green soluble *Spirulina* protein	0.3	48.46 ± 0.23b	1.59 ± 0.03c
	0.5	47.53 ± 0.03c	2.28 ± 0.05d
Total soluble *Spirulina* protein	0.3	47.16 ± 0.41cd	1.63 ± 0.02a
	0.5	46.84 ± 0.13d	2.49 ± 0.05e

All values given are means of three repetitions ($\bar{x} \pm$ SD) (SD: standard deviation). Values with same letters within the same column are not significantly different ($P > 0.05$).
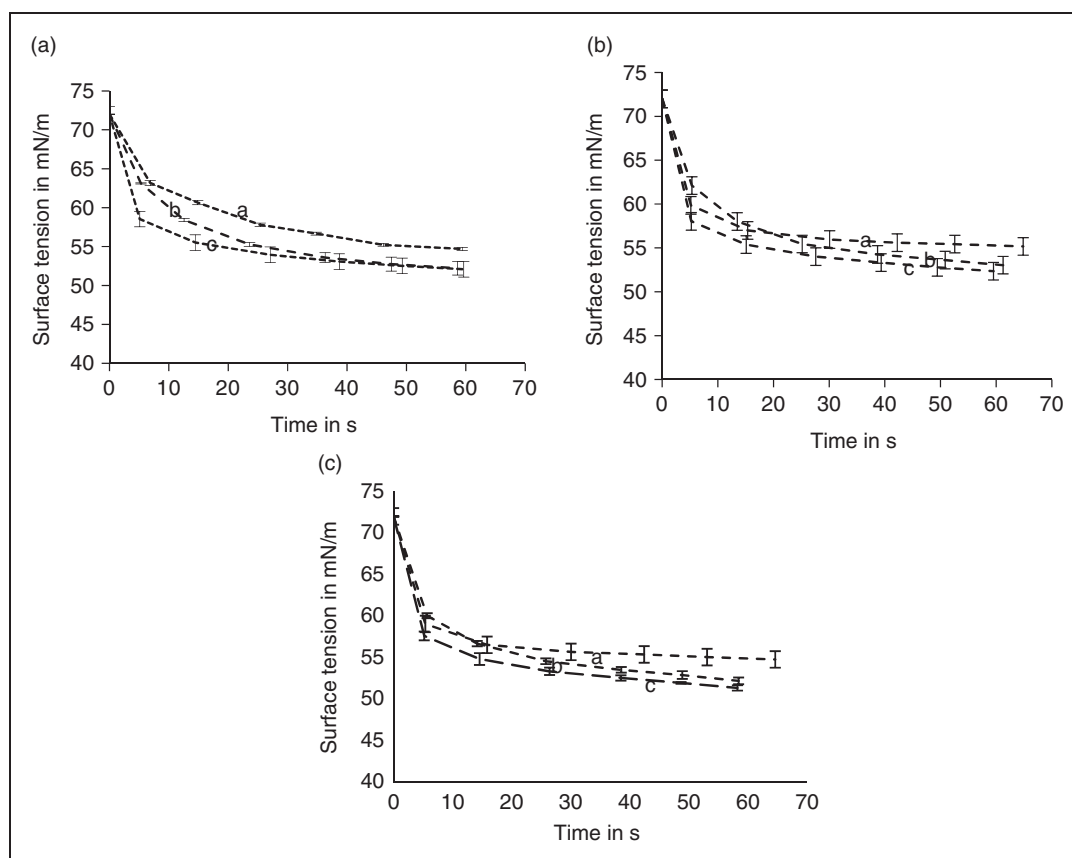
Figure 5. Effect of pH (a: pH 3; b: pH 7; c: pH 5; on surface tension decay of *Spirulina*-colored soluble protein fractions aqueous 0.3% (w/w) solutions using drop volume method: (a) TSSP, (b) BSSP, and (c) GSSP.

Effect of pH. A plot of surface tension (mN/m) against drop age (in seconds) is presented (Figure 5) for the three protein fractions at different pH levels (7, 5, and 3). The kinetic curves showed similar patterns for the three fractions at the tested pH values. All fractions appeared to be less effective in reducing surface tension at pH 3. At pH 7, the surface tension reduction was lower compared to pH 5 during the first 30 s and tended toward that of pH 5 at longer drop ages. Whereas for pH 3, at these drop ages, surface tension reduction was clearly revealed to be less effective than the other tested pH levels.

To further investigate the effect of pH on the surface tension decay, the two pH values (pH 3 and 5) that showed important differences in affecting surface tension decay kinetics were tested. This evaluation was done via another dynamic measurement with the bubble pressure tensiometer within the first seconds of which differences were not clearly revealed with the first method (TVT1). Results for this analysis are presented in Figure 6.

The kinetics of the surface tension decay for the three fractions followed the same pattern; the proteins were less effective as surface active agents and also for

all the fractions solutions at pH 3. This confirms previous results obtained with the drop volume tensiometer for longer drop ages. The closeness of this pH value to the pHi of the protein fractions (3.5 for the GSSP and 3.3 for TSSP and BSSP) could justify this observation.

These results corroborate with observations reported on pure milk proteins such as proteose–peptones, for which the pH level significantly affects surface tension decay (Karamoko et al., 2013). For the *Spirulina* proteins, the significant difference of the effect on surface tension decay was revealed only at the pHi. The decline of the electrostatic barrier due to the pHi would rather contribute to the protein precipitation and did not favor adsorption to the surface. At this pH level, the proteins were less soluble and the diffusion of individual molecules to the surface was limited, compared to the almost completely solubilized molecules at pH 5 (far from the pHi). Also, at pHi surface protein molecules may aggregate and desorb back to the subphase. Despite the presence of a relatively important electrostatic barrier at pH 5, molecules are more susceptible to remain adsorbed to the surface and induce a more pronounced surface tension decay. This was clearly
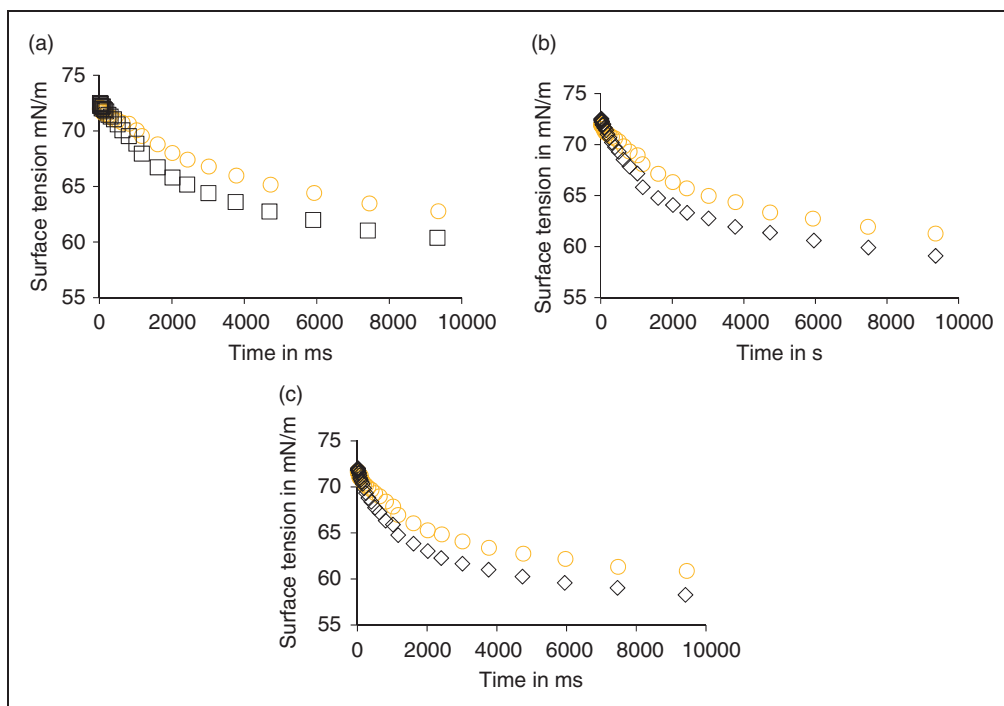
Figure 6. Effect of pH on surface tension decay of aqueous solutions (0.3%, w/w) of *Spirulina*-colored soluble proteins measured with a bubble pressure tensiometer (BP100). (a) TSSP, (b) BSSP, (c) GSSP. ○; pH 3; ◇; pH 5.

observed within the few milliseconds of measurement by the bubble pressure tensiometer (Figure 6(a) to (c)). Other adsorptions factors such as the steric barrier during longer adsorption periods of time may contribute to minimize the effect of pH change on surface tension decay. This was observed on the drop volume tensiometer (TVT1) measurements for which the differences due to pH 5 and 7 variations were not revealed at longer drop ages.

Mechanical behavior of Spirulina soluble proteins

Compression isotherms of monolayer films formed by spreading of surface active molecules to fluid interfaces are important factors as far as foam and emulsion stability is concerned (Kinsella and Phillips, 1989). Mechanical behavior of monolayer films at air–water interface can be evaluated via a spreading and compression method using Langmuir film waage (Boyd et al., 1973). The objective here was to evaluate the ability of the colored protein fraction to form cohesive films when spread on the water surface. Compression isotherms for the three samples at 0.3% (3 mg/ml) were illustrated in Figure 7. A monolayer film was formed by spreading aqueous solutions of the fractions on a Milli-Q water surface. When molecules of the film were compressed, the surface area per milligram of protein decreased, and the surface pressure (π) increased from an initial value A_0.
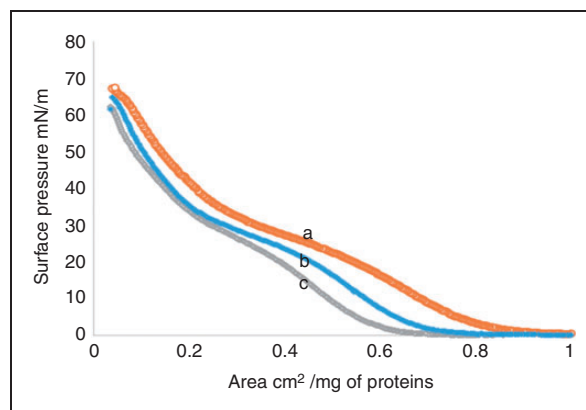

Figure 7. Compression isotherms of different *Spirulina*-colored soluble protein fractions dissolved in Milli-Q water (3 mg/ml) at $25 \pm 1\,^\circ$C on a Milli-Q water subphase via Langmuir film balance: a: BSSP, b: TSSP, and c: GSSP.

The value of A_0 (Table 3) obtained from the compression isotherm was an indication of the degree of expansion of the monolayer film at the surface during compression (MacRitchie and Ter-Minassian-Saraga, 1983). A greater expansion of the monolayer film was observed for the blue fraction ($A_0 = 0.861\,\text{cm}^2$/mg protein). All values of A_0 obtained for the various *Spirulina* soluble proteins were within the range reported for whey protein concentrates by Blecker et al. (1997).

Table 3. Characteristic compression isotherm parameters of three *Spirulina* soluble protein fractions.

Spirulina protein fractions	A_0 (cm²/mg protein)	A_t (cm²/mg protein)	π_t (mN/m)	π_c (mN/m)	ε (mN/m)
Green soluble	$0.62 \pm 0.01a$	$0.36 \pm 0.03a$	$22.68 \pm 0.30a$	$61.70 \pm 0.07a$	$12.96 \pm 0.94a$
Blue soluble	$0.86 \pm 0.00b$	$0.45 \pm 0.02b$	$25.34 \pm 0.55b$	$67.03 \pm 0.59b$	$17.08 \pm 0.23b$
Total soluble	$0.67 \pm 0.05a$	$0.39 \pm 0.01a$	$23.87 \pm 0.71c$	$64.28 \pm 0.71c$	$17.59 \pm 0.30c$

A_0: expansion area; A_t: transition area; π_c: collapse pressure; π_t: transition pressure; ε: film elasticity.
All values given are means of three repetitions ($\bar{x} \pm SD$) (SD: standard deviation). Values with same letters within the same column are not significantly different ($P > 0.05$).

The film elasticity is the expression of its individual molecule flexibilities under compression or expansion constraints. The films elasticity (ε) was estimated at the linear part of the curve following the transition point and ending at the collapse point (Blecker et al., 1997). The green fraction presented the least elasticity (12.96 mN/m), while elasticities of 17.08 and 17.59 mN/m were observed for the total soluble and the blue soluble fractions, respectively (Table 3). Highly flexible molecules exhibit poor elasticity (Damodaran, 1994). Less flexible film molecules are less susceptible to conformational change from loops to trains and vice versa during compression and expansion of the film, thus preventing the film from local stresses (Philips, 1981). This will delay liquid drainage and reduce film thinning. Consequently, high elasticity (low flexibility) could positively contribute to foam stability.

All the three isotherms obtained followed the same pattern with the BSSP fraction showing higher surface pressure for equivalent surface areas. The general shape of the isotherms was comparable to those reported for other proteins, such as those from milk, e.g. proteose–peptones (Karamoko et al., 2013) and whey proteins (Blecker et al., 1997). The surface pressure almost linearly increased and then changed at the transition point (A_t, π_t) to a greater slope until it reached its collapse value (A_c, π_c). This later point is lower for the green and the total fractions, compared to that of the blue fraction.

The transition point (A_t, π_t) is a point usually observed on protein compression isotherms (MacRitchie and Ter-Minassian-Saraga, 1983). As compression proceeds, available surface area for individual molecules at the surface becomes narrower. Molecules at the surface undergo spatial rearrangement and conformational change. Further compression brings about an increase in the steric barrier and desorption of the monolayer film molecules toward the subphase. At the end of the compression, the monolayer film is not able to support compression constraints anymore and it collapses thereby making the pressure to brutally fall. The transition point (A_t, π_t) could therefore be the point from which the increase in pressure during compression is determined by the steric barrier. No further spatial rearrangement and conformational changes are possible and compressibility of the film was limited. Many authors reported this point as that of minimum compressibility of the monolayer film (Blecker et al., 1995; MacRitchie and Ter-Minassian-Saraga, 1983; Nitsch and Maksymiw, 1990).

The blue soluble fraction showed the highest collapse pressure ($\pi_c = 67.03$ mN/m) followed by the total soluble ($\pi_c = 64.28$ mN/m) and the green fraction ($\pi_c = 61.60$ mN/m) (Table 2). This showed that the blue fraction formed a stronger film that better resisted the mechanical constraints due to compression. The film formed by the blue fraction is expected to be more cohesive under these conditions. Indeed, phycocyanins expected to be present in the blue fraction at a higher proportion was reported to improve emulsion stability—a property linked to viscoelastic film formation at interfaces (Batista et al., 2006). This property greatly contributes to foam and emulsion stability (Blecker et al., 1997). A more cohesive film could readily hold gas bubbles in foams and oil droplets in emulsion matrices, usually encountered in foams and emulsions.

CONCLUSION

Three colored soluble protein fractions were extracted from *Spirulina* powder, and their physicochemical properties were evaluated. All fractions exhibited surface tension reduction. The total fraction showed an equilibrium surface tension lower than the two fractions. The blue soluble fraction showed a greater initial adsorption rate and better cohesive films, with higher elasticity compared to the other fractions. Their use in foam and emulsion may be boosted by their ability to form strong organic films which could be needed in coating sensitive molecules. On the other hand, at all tested pH values, fractions were able to bring about significant surface tension decay with less effectiveness at pH 3. The different colored fractions separation did not cause loss in surface activity. It rather sometimes improved surface activities and produced different colored protein products that could be attractive for some specific industrial applications.

For a better appreciation of the fractions as surface active agents, and for their proper implementation in

foams and emulsions, knowledge of their functional properties sustained by their interfacial behavior should be evaluated. These include, solubility, foaming, and emulsion properties. Their comparison to conventional protein under the same experimental conditions will be highly instructive.

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