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Biopeptides with antioxidant activity extracted from the biomass of *Spirulina* sp. LEB 18

Cristiane Reinaldo Lisboa, Aline Massia Pereira and Jorge Alberto Vieira Costa*

Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande – FURG, Rio Grande, RS, Brazil.

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The microalga *Spirulina* has been marketed in many countries due to its application in foods, especially for its high protein content, which can be used to obtain protein hydrolysates. Protein hydrolysates are easily absorbed by the gastrointestinal tract and have significant physiological properties, because during the hydrolysis process, peptides with antioxidant activity can be obtained. The objective of this study was to obtain enzymatic hydrolysates from the biomass of *Spirulina* sp. LEB 18 with functional properties and antioxidant potential. This study was a 2^2 type central composite design, to determine the best conditions for obtaining hydrolysates. The hydrolysis reactions of *Spirulina* biomass were carried out using the enzyme Protemax 580 L. The enzymatic hydrolysis of the proteins resulted in increased digestibility, solubility, water-retention capacity and antioxidant activity of hydrolysates (which increased by 1.8, 2.8 and 8.5 using the reducing power methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2' azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), respectively). Therefore, biologically active peptides of *Spirulina* sp. LEB 18 biomass were produced.

Key words: Protein hydrolysate, microalga, active peptide.

INTRODUCTION

Microalgae are unicellular and multicellular from freshwater and marine systems that possess very large industrial potential (Pignolet et al., 2013). They are photosynthetic microorganisms capable of easily producing biomass from solar energy, CO_2 and nutrients. Microalgal biomass and its derivatives can be applied in various fields, such as supplements in animal feed, in human nutrition, medicines and biofuels (Mostafa, 2012). They have many benefits when used in foods, because they produce various substances such as vitamins, minerals, pigments, proteins and lipids.

The microalga *Spirulina* has a biomass that is rich in protein, with values above 50%. In addition, it has strong potential to be used in the extraction of valuable bio-compounds. This microalga is "generally recognized as safe" (GRAS) certified by the Food and Drug Administration (FDA), which guarantees that it can be used as a food and drug, when grown and processed within the required health and safety standards for foods (Fox, 1996). This microalga has been the subject of extensive research regarding: the extraction of phycocyanin and the benefits caused by the intake of this

*Corresponding author. E-mail: jorgealbertovc@terra.com.br

biocolorant (Kumar et al., 2011; Walter et al., 2011; Chaiklahan et al., 2012; Zeng et al., 2012), preparation of foods enriched with its biomass (Figueira et al., 2011; Lemes et al., 2012; Rabelo et al., 2013) the biological treatment of effluents (Mezzomo et al., 2010; Zeng et al., 2012); the development of nanofibers (Morais et al., 2014); beneficial health effects from ingestion of its biomass (Peiretti and Meineri, 2011; Nah et al., 2012; Zotte et al., 2013) and CO_2 biofixation (Morais et al., 2011; Ramanan et al., 2010; Zhao et al., 2011; Chen et al., 2012; Zeng et al., 2012). Some studies have reported that *Spirulina* is a source of natural antioxidants (Gad et al., 2011; Marco et al., 2014)

Antioxidant activity can be associated with bioactive peptides in certain protein sequences (Costa et al., 2007). Peptides are inactive in precursor protein and in order to have a physiological effect, they must be hydrolyzed; this can be achieved through the action of proteolytic enzymes. Bioactive peptides are composed of about 2 to 20 amino acid residues per molecule and their activity is based on the respective composition and placement of these residues (Korhonen, 2009).

Obtaining protein hydrolysates using microalgae as an alternative protein source is beneficial because protein hydrolysates from microalgae have a higher nutritional value than those from conventional protein sources. Because of this, the objective of this study was to obtain different enzymatic hydrolysates from the biomass of *Spirulina* sp. LEB 18, and to verify their antioxidant potential and functional properties.

MATERIALS AND METHODS

Microorganism

The microalga used for obtaining the protein hydrolysates was *Spirulina* sp. LEB 18, isolated from Mangueira Lagoon (Morais et al., 2008) and produced in the pilot plant at the Biochemical Engineering Laboratory, on the bank of Mangueira Lagoon (33° 30' 13''S and 53° 08' 59'' W) in the city of Santa Vitória do Palmar, Brazil (Morais et al., 2009). Cultivation was carried out using Zarrouk medium (Zarrouk, 1966) in raceway type open tanks, inside of a transparent greenhouse, exposed to environmental conditions. The dry biomass of *Spirulina* sp. LEB 18 drought, was ground in a ball mill (Model Q298, QUIMIS) and sieved (Tyler 60, opening 250 mm).

Enzyme

The enzyme Protemax 580 L, an endopeptidase serine of *Bacillus licheniformis* bacterial origin (Prozyn, São Paulo - SP) was used. Its activity was defined as the quantity of enzyme that releases 1 µg of tyrosine per minute, under the conditions used in the study, according to the method described by Ma et al. (2007).

Proximal composition

The analyses carried out to assess the proximal composition of the microalgae were total protein, ash, moisture and lipids, determined according to the methods described by the Association of Official Analytical Chemists (AOAC) (2005). For the quantification of protein, the method of determination of micro-Kjeldahl total nitrogen was used with a conversion factor of 6.25. Ash was determined by the gravimetric method in an oven (550 to 600°C) and the moisture content by gravimetric method in an oven (105°C). Lipids were extracted with 2:1 (v/v) chloroform/methanol, purified with 0.9% (w/v) KCl and 2:1 (v/v) methanol/water (Folch et al., 1957) and transferred to a rotary evaporator. The solvent was removed at approximately 37°C. The lipid content was determined gravimetrically. Carbohydrates were determined by difference.

Enzymatic hydrolysis

The reactional system consisted of the enzyme Protemax 580 L and biomass of *Spirulina* sp. LEB 18, in a sodium carbonate bicarbonate buffer, pH 9.5. The reactors were arranged in a "shaker" (Certomat BS-1) with agitation of 180 rpm and a constant temperature of 60°C (optimum temperature of the enzyme activity), for all experiments. Upon completion of the reaction, the enzyme was thermically inactivated at 85°C for 10 min. The protein hydrolysates of *Spirulina* sp. LEB 18 that were obtained were frozen and freeze-dried. The amount of biomass added and the reaction time were established using the central composite design (CCD). The quantity of enzyme added was 5 $U.mL^{-1}$, a quantity established in preliminary experiments (data not shown). The 2^2 type CCD was carried out with three repetitions at the central point, totaling 7 experiments. The enzyme concentration used was 5 $U.mL^{-1}$ for all assays. The initial content of the substrate and the reaction time were the variables studied using CCD, according to the coded and actual values presented in Table 2. The degree of hydrolysis of biomass proteins was considered as a dependent variable. Analyses were carried out in duplicate and the results of the statistical analysis, applied to the experimental data of degree of hydrolysis, were determined by overall error.

Determination of degree of hydrolysis

After the elapsed time according to the CCD for each assay, 1 ml aliquots of hydrolyzate were inactivated by the addition of 9 ml of trichloroacetic acid (TCA) solution 6.25% w/v, and left to stand for 10 min. Subsequently, the aliquots were centrifuged for 5 min at 5000 rpm to remove the insoluble material precipitated by TCA. The content of soluble proteins in the filtrate was determined using the method of Lowry et al. (1951) expressed as mg of albumin. The degree of hydrolysis (DH) was estimated according to the method described by Hoyle and Merritt (1994) according to Equation 1:

$$\mathrm{DH}(\%) = \frac{(\mathrm{PS}_{\mathrm{timet}} - \mathrm{PS}_{\mathrm{time0}})}{\mathrm{P}_{\mathrm{total}}} \times 100 \quad (1)$$

Where: the white, $PS_{time\ 0,}$ corresponds to the amount of soluble protein in TCA 6.25% w/v before the addition of enzyme; $PS_{time\ t}$ is the amount of soluble protein at a certain time after the addition of the enzyme and P_{total} is the amount of total protein in the sample determined by micro-Kjeldahl.

Digestibility of proteins

The *in vitro* digestibility of the proteins of the biomass of *Spirulina* sp. LEB 18, and of their hydrolysates from the assays that obtained the highest and lowest DH, was evaluated according to Akeson and Stahmann (1964). Quantification of the soluble protein was carried

out using the method of Lowry et al. (1951), taking 0.5 ml of the solution hydrolysed by pepsin and 0.5 ml of the solution hydrolyzed by pancreatin. Tyrosine was used as a reference.

Protein solubility

The protein solubility was determined using the method of Morr et al. (1985) with varying pH in the range of 3 to 11. A 500 mg sample was homogenized with 2 ml of 0.1 mol L^{-1} NaCl solution. Then, 40 ml of phosphate buffer was added at pH 3, 5, 7, 9 and 11, the suspension was maintained for 45 min in a magnetic stirrer and the volume was measured at 50 ml. Afterwards, the suspension was centrifuged at 5000 rpm for 30 min and the concentration of soluble protein in the supernatant was measured using the method of Lowry et al. (1951). The percentage of soluble protein was calculated according to Equation 2.

$$P.S(\%) = \frac{[A(mg/mL) \times 50]}{[W(mg)S/100]} \times 100 \quad (2)$$

Where: P.S = content of soluble proteins present in the sample [%]; A = the protein concentration of the supernatant = (mg/ml); W = sample weight [mg]; S = concentration of protein in the sample (%).

Water retention capacity

The water retention capacity (WRC) was determined according to the method of Regenstein et al. (1984). Protein dispersions at 1% were homogenized with 2 ml of 0.1 mol L^{-1} NaCl solution and 38 ml of phosphate buffer at pH 3, 5, 7, 9 and 11. The protein dispersion was agitated for 15 min and centrifuged at 5000 rpm for 25 min. The WRC was expressed as volume of water retained per amount of total protein present in the sample (ml of water/g of protein).

Electrophoresis (SDS-PAGE)

Electrophoresis (SDS-PAGE) was based on the method of Laemmli (1970). Electrophoresis in sodium dodecyl sulfate polyacrylamide gel (SDS) was carried out in a continuous buffer system: 1.5 mol L^{-1} Tris buffer and 10% SDS (w/v). The gel was prepared with 12% separation and 4% concentration. The electrophoresis analysis was carried out in a vertical electrophoresis unit (GSR/300STS) with a current of 40 mA for 3 h. Afterwards, the gel was removed from the apparatus and placed in staining solution for 2 h under constant agitation. A mixture of proteins (Bench Mark Ladder protein, California, USA) ranging from 10 to 220 kDa was used as a reference.

Antioxidant activity

The antioxidant activity was determined using the methods of capacity to sequester free radicals DPPH, capture of free radicals ABTS and reducing power. These analyses and the calculation of the antioxidant potential were carried out in triplicate. The results were evaluated by analysis of variance and comparison of means using the Tukey test at a 5% significance level.

Reducing power

The antioxidant activity was determined by the reducing power method according to Oyaizu (1986). A sample of 2 ml of hydrolysate solution 5 mg/ml was mixed with 2 ml of 0.2 mol L^{-1} sodium phosphate buffer (pH 6.6) and 2 ml of 1% potassium ferrocyanide w/v. This mixture was incubated at 50°C in a water bath for 20 min. Afterwards, 2 ml of TCA 10% w/v was added. Aliquots of 2 ml of the incubated samples were mixed with 2 ml of distilled water and 0.4 ml of ferric chloride of 0.1% w/v. After 10 min, the absorbance of the resulting solution was read at 700 nm in a spectrophotometer (Quimis, Q-108 DRM model, Brazil). The increase of the absorbance of the mixture from the reaction indicates an increase of reducing power.

Capacity to sequester the free radical DPPH

The sequestering effect of free radical 2,2-diphenyl-1-picryl-hidrazol (DPPH) was measured as described by Rufino et al. (2007b). Samples of 0.1 ml (2.5 mg ml^{-1}) were added to 3.9 ml of 0.06 mmol L^{-1} DPPH in methanol, in the dark. The mixture was homogenized in a vortex and maintained at room temperature for 60 min. The resulting absorbance of the solution was measured at 517 nm in a spectrophotometer (Quimis, Q-108 DRM model, Brazil). The capacity to sequester DPPH was calculated according to Equation 3.

$$\text{Inhibition (\%)} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \quad (3)$$

Capture of the free radical ABTS

The capacity to capture free radical ABTS was determined according to Rufino et al. (2007a). A stock solution of ABTS, 2,2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) 7 mmol L^{-1} was prepared. The cation radical ($ABTS^{+}$) was prepared from the reaction of 5 ml of ABTS stock solution with 88 µl of 140 mM of potassium persulfate solution, and the mixture was left to stand in the dark at room temperature for 16 h. Afterwards, 1 ml of this mixture was diluted with ethanol until absorbance of 0.70 ± 0.05 nm to 734 nm. Under dark conditions, a 30 µl aliquot of sample (2.5 mg ml^{-1}) was transferred to test tubes with 3.0 ml of $ABTS^{+}$ radical, and agitated in a vortex. A spectrophotometer (Quimis, Q-108 DRM model, Brazil) was used to take a reading after 6 min. For the standard solution, the synthetic antioxidant Trolox was used and the results were expressed as mM Trolox/g of sample.

RESULTS AND DISCUSSION

Bioactive peptides with antioxidant activity are usually extracted from animal proteins (milk and fish) and higher plants (soy), which need to be processed and concentrated to obtain high protein content. The biomass of *Spirulina* sp. LEB 18 has a high concentration of protein and the conditions required to grow it can be altered to stimulate a higher concentration of this bioproduct. Protein concentration (51.66%) (Table 1) was used to calculate the amount of biomass needed to obtain the suspensions of each assay.

Table 2 shows the CCD matrix with real and coded variables and DH response obtained in each experiment. The study of the influence of the independent variables enabled variation of 38.0 to 62.8% in the DH of the proteins of the *Spirulina* sp. LEB-18 biomass. Assay 3 had the greatest DH 3 (62.8%) and its reaction medium consisted of the lowest concentration (4%) of substrate

Table 1. Proximal composition of the biomass of *Spirulina* sp. LEB 18.

Determinations	*Spirulina* sp. LEB 18 (% w/v)*
Humidity	11.99±0.07
Protein	51.66±1.87
Ash	7.84±0.02
Lipids	7.06±0.11
Carbohydrates	21.45±2.07

*means ($\overline{X}$) ± standard deviation (SD).

Table 2. Matrix of the 2^2 central composite design for the degree of hydrolysis response.

Assay	Coded and real values		DH (%)
	CS (%)	t (min)	
1	-1 (4)	-1 (100)	55.1
2	+1 (8)	-1 (100)	38.0
3	-1 (4)	+1 (200)	62.8
4	+1 (8)	+1 (200)	42.5
5	0 (6)	0 (150)	47.3
6	0 (6)	0 (150)	47.6
7	0 (6)	0 (150)	47.0

*CS = concentration of substrate; t = reaction time.

and 200 min reaction time. The minimum DH was found in Assay 2, where the substrate concentration corresponded to 8%.

Analysis of variance showed that the F calculated was 9.44 times greater than F tabulated, thus the model is significant ($p < 0.05$), with the correlation coefficient equal to 0.97. Therefore, it was possible to obtain the linear coded model presented in Equation (4). This represents the model's regression coefficients that describe the response as a function of the independent variables, making it possible to obtain the response surface (Figure 1).

Figure 1 shows that the DH values close to 60% are achieved when the reaction system consists of the lowest substrate concentration (4%) and longest reaction time. This may be possible due to the non-competitive inhibition of the substrate by the enzyme. When the substrate concentration is high, the enzyme can bind to a substrate unit, in an affinity region other than the active center, preventing it from properly forming the enzyme-substrate complex with another substrate unit, thereby preventing the formation of the product.

$$DH\ (\%\ p/p) = 48.61 - 9.35CS + 3.05T \quad (4)$$

Within the working range selected, ranging from 4 to 8%

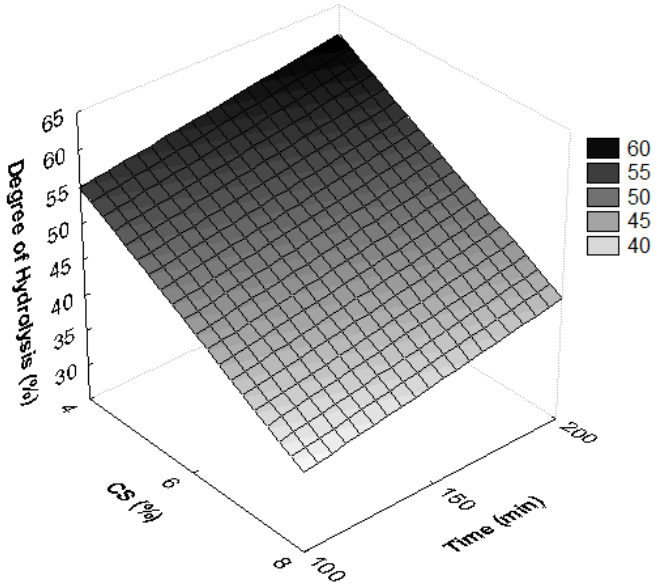

Figure 1. Response surface as a function of concentration of substrate (CS) and reaction time for the degree of hydrolysis.

for the substrate concentration and 100 to 200 min for the reaction time, and taking into consideration that the best conditions for the process are those that enable the greatest DH, the optimal conditions for obtaining proteins with high DH are: substrate concentration of 4% and a reaction time of 200 min. Protein hydrolysates have an important physiological property, because they are more readily absorbed by the gastrointestinal tract, when compared with intact proteins, or even with the intake of free amino acids. They are widely used due to their functional (high digestibility and the presence of bioactive peptides) and technological (high solubility) properties.

The digestibility of the proteins of *Spirulina* sp. LEB 18 biomass was 74.1%. Both of its hydrolysates had higher digestibility than the digestibility of the original biomass: approximately 100% for Assay 3 test (highest DH), and 91.5% for Assay 2 (lowest DH). In addition, the assay with the highest DH also presented the highest digestibility, thereby proving that the hydrolysis of the protein made them more digestible. These results are in line with those found by Negrão et al. (2005), who evaluated the digestibility of meat protein mechanically separated from chicken and its hydrolysates; they also found that the hydrolyzed proteins were more digestible than the non-hydrolyzed ones.

According to Furlan and Oetterer (2002), Candido and Sgarbieri (2003) and Roman and Sgarbieri (2005), the main determining factor of the functional properties of hydrolysates is the size of hydrolyzed protein. Depending on the extent of hydrolysis, endo-peptidic enzymes, as used in this study, may produce a mixture of low molecular mass peptides, thus favoring solubility,

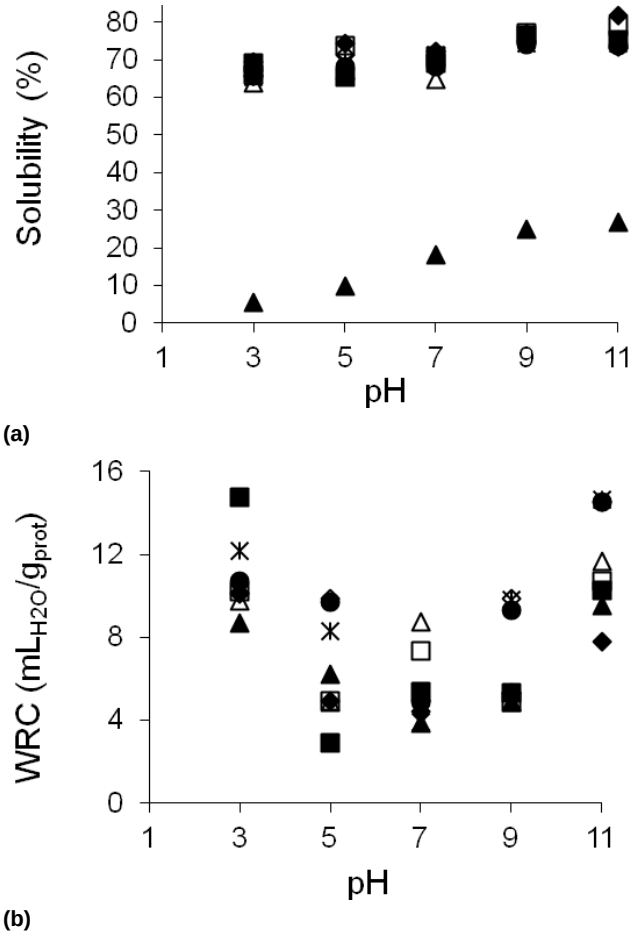

Figure 2. Solubility curve (a) and water retention capacity (b) of the hydrolysates of *Spirulina* Assay 1 (□) Assay 2 (△), Assay 3 (♦), Assay 4 (■), Assay 5 □∗□ Assay 6 (✹), Assay 7 (◇) And unhydrolyzed *Spirulina* (▲).

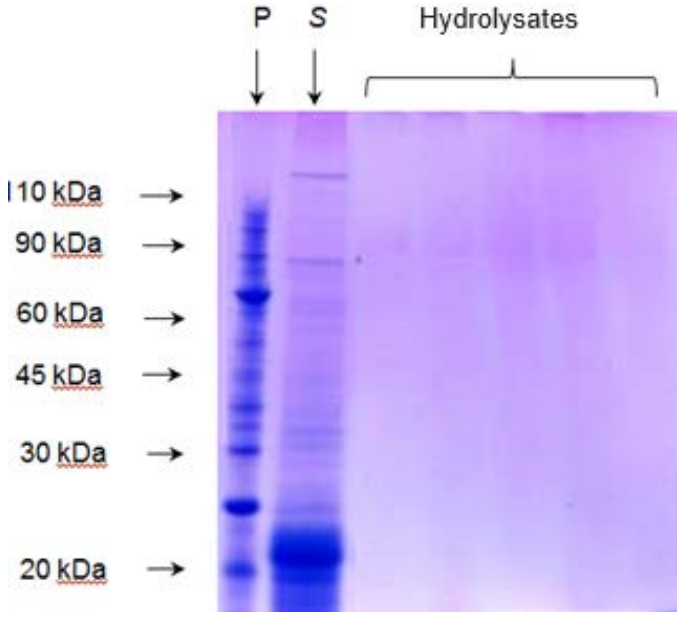

Figure 3. Electrophoretic profile of the proteins present in the standard (P), in the *Spirulina* biomass (S) and the proteins of the hydrolysates of *Spirulina* of assays 1, 2, 3 and 5.

foaming capacity and digestibility of these hydrolysates. Figure 2 shows the solubility curve of the protein hydrolysates obtained with the Protemax 580 L and non-hydrolyzed *Spirulina* biomass, at pH 3, 5, 7, 9 and 11. The lowest solubility values were at pH 3 and 5, and they increased as the pH reached the alkalinity range. This occurs with most proteins, because at an alkaline pH, there is an increase in interactions between protein and water molecules, increasing solubility.

Higher solubilities, for all assays, were obtained at pH 11. There was also a correlation between DH and solubility of proteins, because the greater the DH, the higher the solubility. Assay 3, which had the highest solubility (81%), also had the highest degree of hydrolysis (62.8%). The breakdown of the *Spirulina* sp. LEB 18 biomass proteins by the action of the enzyme Protemax 580 L increased their solubility. According to Martins et al. (2009), this enzymatic breakdown causes a structural change, and the protein is gradually cleaved into smaller peptide units, with increased solubility. Protein solubility is recognized as one of the best methods of evaluating the processing of a food rich in protein and indicates the percentage available for absorption. According to Mendes et al. (2004), the optimal range of variation of solubility for animal food is 73 to 85%. Thus, enzymatic hydrolysis of *Spirulina* biomass proteins is an important process to enhance the solubility and consequently the absorption of proteins.

All the assays presented the minimum WRC at pH 5 and pH 7. This may be a consequence of the protein's reduced ability to bond to water due to intermolecular interactions and also for being close to the region of the isoelectric point of these proteins. The high amount of low molecular mass peptides, formed during hydrolysis, may also influence the low WRC, because they end up reducing the absorption of water. Enzymatic hydrolysis of biomass increased the WRC in virtually all of the assays at the different pHs. The best results were found at pH 11. There was no relationship between solubility and WRC, or with the DH.

After enzymatic hydrolysis, the SDS-PAGE electrophoresis of both the hydrolysate and the unhydrolyzed biomass was carried out. Analysis of the electrophoretic profile (Figure 3) showed hydrolysis of the microalga's proteins. Figure 3 shows that the SDS-PAGE method enabled the tracking of changes of *Spirulina* biomass subjected to enzymatic hydrolysis reactions. For the non-hydrolyzed *Spirulina,* various molecular mass fragments ranging from 20 to 110 kDa were found. In all of the hydrolysates, it was not possible to detect

Table 3. Antioxidant activity of hydrolysates of the microalgae determined by the reducing power method, DPPH and $ABTS^{\cdot+}$.

Protein hydrolysate	Reducing Power (Abs 700 nm)	DPPH (% Inhibition)	$ABTS^{\cdot}$ + (mMol of Trolox/g of the sample)
Assay 1	0.504^{c}±0.004	55.61^{a}±0.65	0.199^{a}±0.004
Assay 2	0.575^{d}±0.011	68.02^{d}±2.01	0.179^{b}±0.001
Assay 3	0.528^{a}±0.008	60.14^{b}±0.75	0.248^{e}±0.002
Assay 4	0.539^{a}±0.004	73.25^{e}±0.34	0.155^{b}±0.003
Assay 5	0.547^{a}±0.011	$58.25^{a.b}$±1.01	0.193^{a}±0.004
Assay 6	0.543^{a}±0.006	$57.87^{a.b}$±1.72	0.192^{a}±0.001
Assay 7	0.544^{a}±0.006	55.18^{a} ±1.54	0.169^{d}±0.005
Non-hydrolyzed *Spirulina*	0.308^{b}±0.004	25.35^{c}±0.76	0.029^{c}±0.001

*For all the assays, the same letter in the same column indicates that there is no significant difference and different letters indicate a significant difference at 95% confidence interval.

fragments with molecular mass below 20 kDa. Thus, it was found that hydrolysis created peptides with a molecular size lower than 20 kDa. Antioxidant activity may be associated with the bioactive peptides in certain protein sequences, released after enzymatic hydrolysis (Costa et al., 2007). The protein hydrolysates produced using *Spirulina* sp. LEB 18 biomass as a protein source may present antioxidant activity due to the molecular mass of their peptides.

According to Table 3, in all the methods of antioxidant activity studied (reducing power, DPPH and ABTS), there was an increase of the antioxidant activity of the hydrolysates, compared to the non-hydrolyzed biomass, and this difference was significant at 95% confidence interval. These results show that the enzymatic hydrolysis of *Spirulina* proteins created peptides with antioxidant activity. Protein hydrolysates with higher DH had better antioxidant activity for the methods of DPPH and ABTS. It is known that hydrolysates with high DH have a greater amount of low molecular mass peptides, and thus greater potential for oxidation inhibition, compared with low DH hydrolysates.

The different hydrolysates with varying DH probably have peptides with different sizes and an amino acid sequence that might determine their antioxidant capacity. According to Je et al. (2009), the highest reducing power can be attributed to the high content of electron or hydrogen donor peptides. Therefore, the larger the absorbance, the higher the antioxidant activity of the sample. DPPH is a stable radical that has been widely used to test the ability to capture free radicals from different samples (Cao et al., 2012; Panzella et al., 2012; Bukman et al., 2013). The results were expressed as percentage of inhibition. The sequestering effect of the DPPH radical for the experiments with the hydrolyzed microalga (48.5 to 73.2%) increased greatly when compared with the intact biomass (25.3%). Table 3 shows that as in the antioxidant text for the method of capture of DPPH radicals, the protein hydrolysates reached peak values of TEAC, 0.248 mMol of Trolox/g of sample, and this result was 8.5 times greater when compared with unhydrolyzed biomass.

The enzymatic hydrolysis of proteins results in a mixture of free amino acids, di-, tri- and oligopeptides. This increases the number of polar groups and the solubility of the hydrolysate and, thus, modifies the functional characteristics of proteins, which usually improves the functional quality (Schmidt and Salas-Mellado, 2009). The microalga *Spirulina* sp. LEB 18 has been widely studied due to its high protein content. It can be used as a source of protein, and includes all the essential amino acids for human growth and health. The enzymatic hydrolysis of proteins is important for obtaining protein products that can be used in dietary supplements, increasing the functional and nutritional characteristics of foods, especially due to the formation of bioactive peptides during the hydrolysis process. These protein hydrolysates with functional activity, such as those obtained in this study, are more easily digested and absorbed by the human body compared to intact proteins.

The ingestion of hydrolysates rich in small peptides may be important, in order to provide a better utilization of proteins, especially in individuals with allergies to certain proteins or food intolerance, in the case of enzyme deficiency.

Conclusion

This study enabled us to increase the antioxidant activity and functional properties of the hydrolysates. DH of proteins was directly proportional to the digestibility and solubility, thereby demonstrating that the hydrolysis of proteins makes them more digestible. The increase in antioxidant activity of the protein hydrolysates was

caused by the presence of bioactive peptides formed during enzyme hydrolysis. Protein hydrolysates containing bioactive peptides can be used as ingredients in foods for individuals with allergies to certain proteins or food intolerances. Therefore, hydrolysates with a high DH obtained from the biomass of *Spirulina* sp. LEB-18 can be used for this application.

Conflict of interest

The authors have not declared any conflict of interest

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