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Increasing Antihypertensive (ACE Inhibitory) and Antioxidant Activity (DPPH, ABTS and FRAP) Potentials of *Spirulina platensis* Protein by Proteolytic Hydrolysis

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ABSTRACT

In recent years there is increasing interests on Microalgae as natural sources of bioactive compounds capable of addressing growing nutritional and health challenges. These bioactive constituents, often extracted through biotechnological approaches, have shown therapeutic potential against various diseases. In the present study, dried Spirulina platensissuspended in water was hydrolysed by using gastric (pepsin) and intestinal (trypsin) enzymes for two hours. Additionally, in model gastrointestinal digestion, the pepsin-treated hydrolysate underwent a second enzymatic treatment with trypsin under the same conditions. The obtained protein hydrolysates were evaluated for their angiotensin-I-converting enzyme (ACE) inhibitory activity and antioxidant capacity. The results showed that enzymatic hydrolysis significantly enhanced the bio-functional properties of Spirulina proteins. The degree of hydrolysis (DH) was measured as 2.65% for the untreated sample (PT0), 11.33% for the pepsin hydrolysate (P120), 11.13% for the trypsin hydrolysate (T120), and 13.18% for the sequential hydrolysate (PT240). ACE inhibition rates were determined as 92.40%, 89.96%, 94.81%, and 95.53% for PT0, P120, T120, and PT240, respectively. The IC50values, indicating the protein concentration required to inhibit 50% of ACE activity, were 2.92, 0.68, 0.79, and 0.51 mg protein/mL for PT0, P120, T120, and PT240, respectively. The lower IC_{50} value of PT240 indicated a stronger ACE inhibitory effect. Antioxidant analyses further supported the enhanced bioactivity of sequential hydrolysates. PT240 exhibited the highest values in all antioxidant assays: DPPH (0.91 mg Trolox equivalents/g DW), ABTS (3.47 mg TE/g DW), FRAP (3.92 mg FeSO4·7H2O/g DW), and total phenolic content (2.87 mg GAE/g DW). In conclusion, enzymatic hydrolysis improved the ACE inhibitory and antioxidant properties of S. platensis protein, with sequential hydrolysis by pepsin and trypsin yielding the most pronounced bioactive effects.

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Introduction

Microalgae have long been recognized for their nutritional and therapeutic value and are considered a rich source of naturally occurring bioactive compounds (Pangestuti & Kim, 2011). Numerous studies have demonstrated that bioactive substances derived from microalgae possess a wide range of health-promoting properties, including antioxidant, anti-inflammatory, antihypertensive, antimicrobial, and antiviral activities (Samarakoon and Jeon, 2012; Gil-Chávez et al., 2013; Raposo et al., 2013).

These microscopic organisms are particularly noted for their high protein content, typically comprising 40–70% of their dry weight. In addition, they contain 12–23% carbohydrates, 4–20% lipids, and 8–20% carotenoids. Microalgae also offer a substantial supply of essential vitamins, including B-complex vitamins, as well as vitamins E, K, and D (Becker, 2007). It has been reported that proteins that make up the major part of the microalgae composition have antioxidant, antihypertensive, anticoagulant, antitumor, and immunostimulatory properties. The bioactivity of microalgae proteins is not only directly linked to the intact protein but also related to the production of bioactive peptides (Gomes et al., 2020; Lordan, et al., 2011; Samarakoon & Jeon, 2012). Bioactive peptides obtained from microalgae proteins have been shown to have antioxidative and antihypertensive

properties in many studies and can be used to improve human health. These peptides can be obtained by three different hydrolysis processes; hydrolysis with digestive enzymes of animal origin, hydrolysis with proteolytic enzymes of microorganism or plant origin, and hydrolysis with proteolytic enzymes during fermentation (Korhonen & Pihlanto, 2006; Samarakoon & Jeon, 2012).

Hypertension, often referred to as the "silent killer," is a major cardiovascular disorder and a leading risk factor for various health complications (Sheih et al., 2009). Its management typically involves the use of synthetic ACE inhibitors such as enalapril and captopril. While these synthetic drugs are effective in reducing blood pressure, they are also associated with a range of undesirable negative effects (Lahogue et al., 2010; Lu et al., 2010). In response to these limitations, there has been growing scientific interest in identifying natural alternatives to synthetic ACE inhibitors (Fitzgerald et al., 2011). Bioactive peptides with ACE-inhibitory properties have been successfully extracted from various food and food proteins, including dairy proteins, corn gluten, garlic, gelatin, mushrooms, porcine hemoglobin, potatoes, soybeans, and fish such as tuna (Lu et al., 2010). These food-derived bioactive peptides are increasingly recognized as potential natural agents for the dietary management of hypertension.

The aim of this study was to determine the ACE inhibitory potential and antioxidant properties of protein hydrolysates derived from *Spirulina platensis* powder, which possesses a high protein content, through individual and sequential enzymatic hydrolysis by using pepsin and trypsin.

Material and Methods

The powdered *Spirulina platensis* used in this study was sourced from CN Lab Nutrition Asian Group (Shaanxi, China). Enzymes including pepsin, trypsin, and angiotensin-I-converting enzyme (ACE, 0.25 units from rabbit lung), along with hippuric acid and N-hippuryl-His-Leu hydrate, were obtained from Sigma-Aldrich (Steinheim, Germany). All other chemicals utilized were of analytical grade.

Preparation of Enzymatic Hydrolysates

Protein hydrolysis was conducted based on protocol described by Wang and Zhang (2017). Ten percent (w/v) suspensions of *S. platensis* powder (10 g/100 mL) were prepared and then subjected to enzymatic hydrolysis under controlled conditions using pepsin and trypsin.

The suspensions were hydrolyzed with pepsin (pH 2) and trypsin (pH 7.5) enzymes at an enzyme-to-substrate ratio of 1:50 at 37°C for 120 minutes with stirring. The enzymatic reactions were terminated by heating the mixtures in a water bath at 85°C for 15 minutes. In addition, after adjusting the pH of the pepsin hydrolysate to 7.5 with 1N NaOH solution, it was hydrolyzed sequentially with trypsin under the same conditions. After enzymatic inactivation, all samples were cooled and centrifuged at 4000×g for 10 minutes. The resulting supernatants were collected and stored at –80°C until analyzed.

Determination of the Degree of Hydrolysis (DH)

The DH of pepsin and trypsin hydrolysates of *Spirulina platensis* was determined according to the method suggested by Ng et al., (2013). The principle of the method is based on the determination of the soluble protein content of the sample in 10% trichloroacetic acid (TCA). Equal volumes (10 mL each) of hydrolysate and 20% TCA were mixed and incubated for 30 minutes, followed by centrifugation at 4000×g for 20 minutes. The soluble protein content in the supernatants was measured using the Lowry method (Lowry et al., 1951), and a standard curve was prepared with bovine serum albumin (0–500 ppm). The total protein content of enzymatic hydrolysates was determined according to micro-Kjeldahl analysis method proposed in AACC-Method No.46.12 (AACC, 2000). The DH was calculated using the equation as follows:

$$\text{DH (\%)} = \left(\frac{\text{10\% TCA Soluble Protein of Hydrolysate}}{\text{Total Protein of Sample}}\right) \times 100$$

Determination of ACE Inhibitory Activity

In-vitro inhibitory activity of *S. platensis* protein hydrolysates on Angiotensin-I-Converting enzyme (ACE) was determined according to the method suggested by Wu and Ding (2002). This method is based on the measurement of the amount of hippuric acid (HA) released as a result of the hydrolysis of the synthetic substrate N-hippuryl-L-histidyl-L-leucine (HHL) by ACE activity, in the presence or absence of potential inhibitors. In the control sample without inhibitor, it is assumed that ACE completely hydrolyzes HHL to HA, and this is accepted as 100% enzyme activity. In contrast, when samples contain inhibitors, ACE activity is suppressed, thus decreasing HA released. The decrease in ACE activity was quantified using high-performance liquid chromatography (HPLC) in comparison with the control sample. The rate inhibition of ACE activity (INH %) was calculated according to the following equation:

$$\text{INH \%} = \left[1 - \left(\frac{HA_{Inhibitor}}{HA_{Control}}\right)\right] \times 100$$

In this study, four samples were evaluated for their inhibitory effects: the untreated sample (PT0), the pepsin hydrolysate (P120), the trypsin hydrolysate (T120), and the sequential hydrolysate treated with both enzymes (PT240). Each reaction mixture consisted of 5 mM HA, 2 mM HHL, and 50 mU/mL ACE, all dissolved in a 0.1 M sodium borate buffer (pH 8.3) supplemented with 0.3 M NaCl. To initiate the reaction, 0.04 mL ACE, 0.04 mL of either inhibitor or buffer (for control), and 0.20 mL of HHL solution were mixed and incubated at 37°C for 60 minutes. The enzymatic reaction was then terminated by the addition of 0.10 mL of 1N HCl. In the control sample, all HHL was assumed to be converted to HA.

The amount of HA released was quantified using an HPLC system (Shimadzu-20AD, Japan) equipped with a UV/VIS detector. A 10 µL aliquot of each reaction mixture was injected onto a C18 reversed-phase column (Perkin Elmer, 4.0 × 150 mm, 5 µm, USA). Detection was performed at 228 nm.

The mobile phases used were (A) 0.05% trifluoroacetic acid (TFA) in water and (B) 0.05% TFA in acetonitrile. The chromatographic conditions included a gradient elution from 5% to 60% acetonitrile over 10 minutes, followed by a 2-minute hold at 60%, a return to 5% over 1 minute, and a final 4-minute isocratic phase at 5% acetonitrile. The flow rate was maintained at 0.7 mL/min.

Determination of Antioxidant Activity

The antioxidant activities of pepsin and trypsin hydrolysates of *Spirulina platensis* was determined by DPPH, ABTS, and FRAP methods.

DPPH-Radical Scavenging Activity

The radical scavenging activity against DPPH (1,1-diphenyl-2-picrylhydrazyl) was determined as described by Braca et al. (2001). A mixture of 100 µL hydrolysate and 2 mL of 0.04 mM methanolic DPPH was incubated in darkness at room temperature for 30 minutes. Absorbance of the mixtures was measured at 517 nm using a UV-Vis spectrophotometer (Cary 60, Agilent Technologies, Malaysia). DPPH radical scavenging activity was expressed as mg Trolox equivalents per gram of dry weight (mg TE/g DW).

Trolox Equivalent Antioxidant Capacity (TEAC) (ABTS-Radical Scavenging Activity)

The TEAC method was determined according to the assay of ABTS radical cation ($ABTS^+$) scavenging activity described by Re et al., (1999). $ABTS^+$ [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] was obtained by mixing 7 mM ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowing the solution to stand in the dark for 16–18 hours. The resulting solution was diluted with methanol to an absorbance of 0.70 at 734 nm. One milliliter of the diluted $ABTS^+$ was combined with 100 µL of hydrolysate, incubated for 6 minutes in darkness, and the absorbance of the mixtures was measured at 734 nm. The results were expressed as mg Trolox equivalents per gram of dry weight (mg TE/g DW).

Ferric Reducing-Antioxidant Power (FRAP)

The ferric-reducing power was determined according to the FRAP method proposed by Benzie and Strain (1996). FRAP solution was prepared by mixing 300mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM$FeCl_3 \cdot 6H_2O$ at a 10:1:1 ratio. Two hundred µl of hydrolysate was mixed with 1.8 ml of FRAP solution and incubated at 37°C for 10 minutes. Then the absorbance of the mixtures was determined at 593 nm in the spectrophotometer. The results were expressed as mg $FeSO_4 \cdot 7H_2O$equivalents per gram of dry weight ($FeSO_4 \cdot 7H_2O$/g DW).

Determination of Total Phenolic Content (TPC)

Total phenolic content was measured spectrophotometrically using the colorimetric method of Folin-Ciocalteu reagent as described by Velioglu et al., (1998). Briefly, 200 µL of µl of hydrolysate and 1.5 mL of Folin-Ciocalteu reagent (diluted 10-fold with deionized water) were mixed. After a 5-minute incubation, 1.5 mL of 6% (w/v) sodium carbonate solution was added, followed by thorough mixing. The mixture was left at room temperature for 90 minutes, after which absorbance was determined at 725 nm in spectrophotometer. A calibration curve was prepared using gallic acid (0–100 ppm), and results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW).

Statistical Analysis

All statistical analyses were conducted using JMP® Statistical Software (version 13.0). Analysis of variance was employed to examine the differences between applications at $p<0.05$ level. Student's t multiple-comparison tests were used for comparing the differences among the means.

Results and Discussion

ACE Inhibitory Activity and Hydrolysis Degree of S. Platensis Protein Hydrolysates

Some food-derived bioactive peptides are involved in blood pressure regulation by inhibiting ACE in the renin-angiotensin-aldosterone system (RAAS) (Ejike et al., 2017). ACE catalyses angiotensin-I to form the vasoconstrictor peptide angiotensin-II, and this resulting peptide causes an increase in blood pressure. Also, ACE breaks down the vasodilator peptide bradykinin (Kwon et al., 2006; Hartmann & Meisel, 2007; Mora, Aristoy, & Toldrá, 2018). Some ACE-inhibitory peptide containing drug therapy is used for hypertension by reducing blood pressure (Udenigwe & Aluko, 2012). These peptides have been reported to be absorbed in the gastrointestinal tract and to be highly biofunctional due to some other bioactive properties (Jamdar et al., 2010).

Enzymatic hydrolysis processes have been used for production of many kinds of bioactive peptides from different food proteins and it has been reported that their biological activity is strongly dependent on the hydrolysis process (Barkia et al., 2019). Therefore, hydrolysis parameters such as the type of proteolytic enzymes and reaction time are important for the peptides to exhibit higher bioactive properties (e.g., antioxidant and antihypertensive effects). In this study, ACE inhibitory activity, degree of hydrolysis, antioxidant activity, and total phenolic content of hydrolysates obtained by pepsin and trypsin hydrolysis of *Spirulina platensis* were determined. The degree of hydrolysis and ACE inhibitory activity values of Spirulina protein hydrolysates are shown in Figure 1.

The DH of Spirulina protein hydrolysates was determined by measuring the soluble protein concentration in 10% trichloroacetic acid solution. The highest DH was found in the PT240 hydrolysate, while the lowest was found in the PT0 sample ($p<0.05$). There was no a significant difference between P120 and T120 hydrolysates in terms of the DH ($p>0.05$). The DH of P120, T120 and PT240 hydrolysates were 11.33%, 11.13% and 13.18%, respectively. It was observed that the PT0 sample had 2.65% of the DH even though it didn't hydrolyze (Fig. 1). It is thought that this value observed before hydrolysis is due to the dissolved protein formed as a result of acidic hydrolysis.

ACE inhibition values of PT0, P120, T120, and PT240 samples were found as 92.40%, 89.96%, 94.81%, and 95.53%, respectively (Fig. 1).

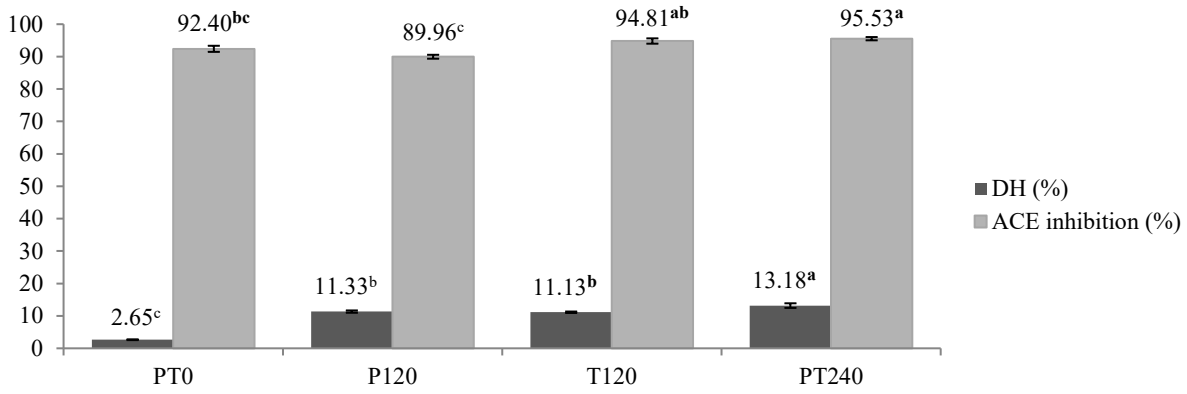

Figure 1. Percent of the degree of hydrolysis (DH) and ACE inhibition values of *S. platensis* protein hydrolysates.PT0 (untreated sample); P120 (pepsin hydrolysate); T120 (trypsin hydrolysate); PT240 (sequential hydrolysate). Different small letters in each column indicate statistical discrepancy (p<0.05).

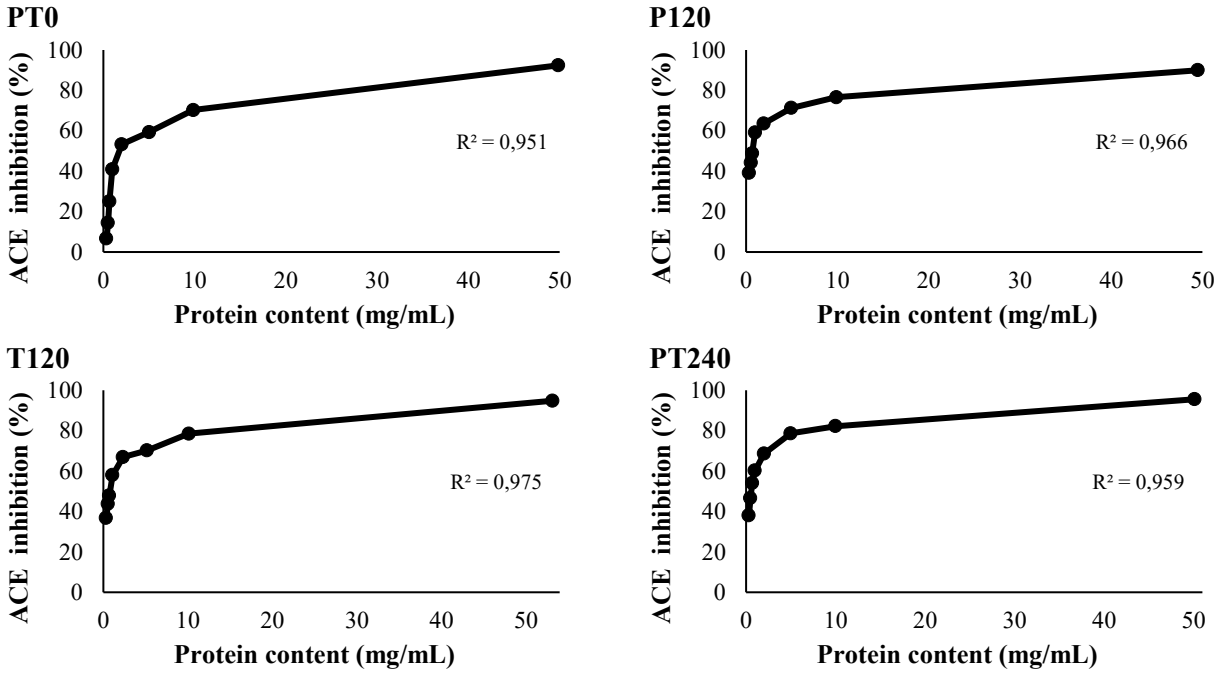

Figure 2. Curves showing the relationship between protein content and ACE inhibition (%) of *S. platensis* protein hydrolysates. PT0 (untreated sample); P120 (pepsin hydrolysate); T120 (trypsin hydrolysate); PT240 (sequential hydrolysate).

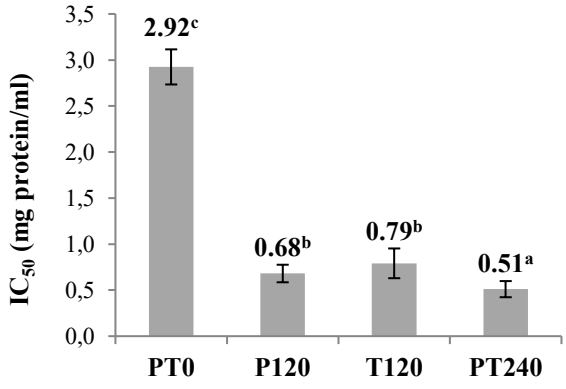

Figure 3. IC_{50} values of *S. platensis* protein hydrolysates. Different small letters in each column indicate statistical discrepancy (p<0.05).

It is seen that the ACE inhibition values are above 90% on average in undiluted samples both before and after hydrolysis. For a better understanding of the ACE inhibition effect of the samples, it is necessary to calculate the protein concentration that can inhibit ACE activity by 50% (IC_{50}). For this purpose, S. platensis protein hydrolysates were diluted at rates varying from 1-fold to 150-fold. IC_{50} value was calculated after the ACE inhibition value of the diluted samples was found. The relationship between the protein content of diluted P0, P120, T120, and PT120 hydrolysates and ACE inhibition is given in Figure 2.

According to the results, the IC_{50}value of the untreated PT0 sample was found to be 2.92 mg protein/mL (p<0.05). While there was no significant difference between the IC_{50} values of P120 and T120 hydrolysates (p>0.05), the lowest value was found in PT240 hydrolysate (p< 0.05).

Table1.Antioxidant activity and total phenolic content values of *S. platensis* protein hydrolysates.

Sample	DPPH (mg TE/g DW)	ABTS (mg TE/g DW)	FRAP (mg $FeSO_4 \cdot 7H_2O$/g DW)	TPC (mgGAE/g DW)
PT0	0.10 ± 0.01^{c}	0.29 ± 0.01^{c}	1.08 ± 0.05^{c}	0.77 ± 0.02^{d}
P120	0.51 ± 0.08^{b}	1.46 ± 0.03^{b}	2.53 ± 0.07^{b}	1.92 ± 0.04^{c}
T120	0.81 ± 0.01^{a}	$3.44 \pm 0,01^{a}$	3.77 ± 0.11^{a}	2.55 ± 0.06^{b}
PT240	$0.91 \pm 0,01^{a}$	3.47 ± 0.01^{a}	3.92 ± 0.07^{a}	2.88 ± 0.03^{a}

DPPH (DPPH-radical scavenging activity); ABTS (ABTS-radical scavenging activity); FRAP (ferric reducing-antioxidant power); TPC (total phenolic content); Different superscript letters indicate statistical discrepancy ($p<0.05$) in within each column. Data represent the means of three replicates ±the standard deviation (± SD).

Table2.Pearson's correlationcoefficients (r^2) between the analyzed *S. platensis* protein hydrolysates.

	DH (%)	IC_{50}	DPPH	ABTS	FRAP
IC_{50}	-0.980^{**}				
DPPH	0.882^{**}	-0.886^{**}			
ABTS	0.774^{*}	-0.799^{*}	0.976^{**}		
FRAP	0.861^{**}	-0.884^{**}	0.994^{**}	0.986^{**}	
TPC	0.900^{**}	-0.913^{**}	0.991^{**}	0.965^{**}	0.990^{**}

*Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level.

The IC_{50}values of P120, T120, and PT240 hydrolysates decreased compared to PT0 and were found to be 0.68, 0.79, and 0.51mg protein/ml, respectively (Fig. 3). A decrease in the IC_{50} value means that the inhibition effect is increased. Thus, it can be said that P120, T120, and PT240 samples have 3.30, 2.70, and 4.73 times more inhibition effects than PT0, respectively. When the correlation between IC_{50} values and the DH was examined, it was found that there was a significant negative correlation (r^2 = -0.980; Table 2).

Various studies have investigated the angiotensin-I-converting enzyme (ACE) inhibitory potential of *S. platensis* and its derived products. In a study, the IC_{50} values of ACE inhibition activity of *S. platensis* and isolated phycocyanin were 2.93 mg protein/mL and 3.96 mg protein/mL, respectively (Arslan et al., 2021). More potent inhibitory effects have been observed in enzymatic hydrolysates of S. platensis, depending on the different proteases used. For instance, hydrolysates obtained through digestion with Protamex®, SM98011(He et al., 2007), and alcalase (Lu et al., 2010) demonstrated IC_{50} values of 0.17, 0.22, and 0.47 mg/mL, respectively. Moreover, extracts derived via supercritical CO_2 extraction exhibited an IC_{50} value of 0.274 mg/mL (Mallikarjun Gouda et al., 2014). These changes in activity may be attributed to the specific enzymes used and the conditions applied during hydrolysis. Proteolytic enzymes differ in their cleavage specificity, which leads to the release of distinct peptide profiles with variable bioactive properties (Mazorra-Manzano et al., 2018). Moreover, hydrolysis parameters (such as enzyme-to-substrate ratio, pH, reaction duration, and temperature) play a crucial role in forming peptides with ACE-inhibitory properties (Boschin et al., 2014). Pepsin, for example, tends to generate peptides having hydrophobic and aromatic amino acids, including proline, tryptophan, phenylalanine, and tyrosine residues known to interact effectively with the active site of ACE, thereby contributing to stronger inhibition. In contrast, trypsin, a highly specific serine endopeptidase, preferentially cleaves peptide bonds at the carboxyl side of lysine and arginine, often producing different peptide fragments (Boschin et al., 2014). Thus, it can be said that hydrolysed peptides obtained with pepsin cause higher ACE inhibition activity than the hydrolysed peptides obtained with trypsin (Chalamaiah et al., 2015).

In this study, untreated *S. platensis* proteins (PT0) showed a certain ACE inhibitory activity, whereas hydrolysed peptides (P120, T120 and PT240) released by digestion of proteins using different enzymes inhibited ACE were at the higher rate. Although there was no statistically significant difference between them ($p>0.05$), the ACE inhibitory activity of P120 hydrolysate obtained with pepsin enzyme was found to be higher than T120 hydrolysate obtained with trypsin enzyme.PT240 hydrolysate obtained by sequential hydrolysis showed the highest activity. The higher ACE inhibitory activity of PT240 can be explained by using different enzymes as well as by doubling the hydrolysis time which caused an increase in the DH.

Antioxidant Activities of S. Platensis Protein Hydrolysates

Bioactive peptides with ACE inhibitory activity have generally been suggested to display synergistic antioxidant activity (Barkia et al., 2019). In this study, the antioxidant effects of Spirulina protein hydrolysates were evaluated using various assays. Results showed that the untreated PT0 sample consistently exhibited the lowest antioxidant activity ($p< 0.05$), while enzymatic hydrolysis markedly improved the values across all tested methods, including DPPH, ABTS, FRAP, and total phenolic content (Table 1).

While the DPPH-radical scavenging activity value of the untreated PT0 sample was 0.10mg TE/g DW, it increased to 0.51mg TE/g DW after pepsin hydrolysis, 0.81 mg TE/g DW after trypsin hydrolysis, and 0.91mg TE/g DW after sequential hydrolysis. The ABTS assay revealed similar trends: values rose from 0.29 mg TE/g DW in PT0 to 1.46, 3.44, and 3.47 mg TE/g DW in P120, T120, and PT240 samples, respectively. FRAP values also increased progressively with hydrolysis, ranging from 1.08 mg $FeSO_4 \cdot 7H_2O$/g DW (PT0) to 3.92 mg $FeSO_4 \cdot 7H_2O$/g DW (PT240). According to statistical analysis results, PT0 sample showed lower antioxidant activity, followed by P120 sample ($p<0.05$), while differences between the T120 and PT240 samples were not statistically significant ($p>$ 0.05).

Total phenolic content (TPC) also increased as a function of hydrolysis. The lowest TPC was found in PT0 (0.77 mg GAE/g DW), whereas PT240 exhibited the highest value (2.88 mg GAE/g DW). P120 and T120 hydrolysates had intermediate values of 1.92 and 2.55 mg GAE/g DW, respectively (Table 1). These differences were statistically significant ($p< 0.05$).

The antioxidant activity of protein-derived peptides is influenced by several factors, including amino acid composition, molecular weight, hydrophobicity, and the type of reactive oxygen species (Ejike et al., 2017). Hajimahmoodi et al., (2010), reported that FRAP and total phenolic content values of the extracts obtained from 12 microalgae species were between 1.33 and 74.34 µmol Trolox/g and 0.43-19.82 mg GAE/g, respectively. Lisboa et al., (2016) found that hydrolysed proteins from *Spirulina* spp. exhibited ABTS scavenging activity 8.5 times greater than that of untreated samples. In a study, it was reported that the total phenolic content of *Spirulina platensis* tryptic protein hydrolysates ranged from 23.97CAE/g DW to 41.11CAE/g DW, and FRAP values from 2.58 mg TE/g DW to 4.91mg TE/g DW. In addition, it was reported in the study that the total phenolic contents of pepsin hydrolysates were in the range of 27.34-40.28CAE/g DW, and FRAP values were in the range of 1.55-5.20mg TE/g DW (Yücetepe et al., 2018).

As detailed in Table 2, strong positive correlations ($r^2 > 0.9$) were observed between antioxidant assays and TPC values, with the highest correlation found between FRAP and DPPH ($r^2 = 0.994$). Correlation coefficients between DH and antioxidant assays ranged from 0.774 to 0.900, indicating that enzymatic hydrolysis not only increases DH but also enhances antioxidant activity. Furthermore, the IC_{50} values of S. platensis hydrolysates were negatively correlated with antioxidant parameters ($r^2 = –0.799$ to $–0.980$), suggesting that lower IC_{50}values are associated with stronger antioxidant potential.

Phenolic compounds are known to act as antioxidants. Thus, several studies have proposed a direct relationship between phenolic content and antioxidant activity in microalgal extracts (Goiris et al., 2012; Hajimahmoodi et al., 2010; Kepekçi and Saygideger, 2012; Wang et al., 2009). Nevertheless, some researchers have reported weak or no correlation, attributing the antioxidant effect to other bioactive components (Norzagaray-Valenzuela et al., 2017). For example, Alzahrani et al., (2018) found a significant positive correlation between TPC and ABTS activity in Alcalase-treated hydrolysates of Nitzschia, Spirulina, and Chlorella, with correlation coefficients of 0.9451, 0.9558, and 0.9769, respectively. Their study also reported a TPC of 2.31 mg GAE/100 g for Spirulina hydrolysates after 90 minutes of enzymatic treatment.

Conclusion

The findings of this study demonstrate that *Spirulina platensis* protein is a valuable source of bioactive compounds with antihypertensive, antioxidant, and phenolic properties, suggesting its potential application in the development of functional foods and nutraceutical products. Enzymatic hydrolysis of *S. platensis* protein significantly enhanced its ACE inhibitory capacity, antioxidant efficiency, and total phenolic content. Among the tested methods, the sequential enzymatic treatment with pepsin followed by trypsin yielded the most pronounced bioactive effects. Additionally, diverse peptide fractions with potential nutraceutical or functional uses may be produced through further processing techniques, such as ultrafiltration, applied after hydrolysis using various proteolytic enzymes. Future studies involving chromatographic identification of Spirulina-derived peptides may enable further exploration of their pharmacological potential and assessment in clinical settings.

Declarations

Author Contribution Statement

Rıdvan Arslan: Conceptualization; Data collection; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Software; Validation; Writing-original draft; Writing-review & editing.

Salih Aksay: Conceptualization; Funding acquisition; Data collection; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing-review & editing.

Evren Caglar Eroglu: Conceptualization; Data collection; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Software; Validation; Writing-original draft; Writing-review & editing.

Conflict of Interest

The authors have declared no conflicts of interest for this article.

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