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Bioactive Peptides Isolated from Microalgae *Spirulina platensis* and their Biofunctional Activities

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ABSTRACT

Spirulina platensis is symbiotic, multicellular, and filamentous blue-green microalgae. It is a rich source of some high-value bioactive molecules containing essential amino acids, pigments like β -carotene and phycobiliproteins and polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). *Spirulina platensis* and bioactive peptides derived from these microalgae are gaining more attention because of their nutritional and various medicinal properties like antioxidant, antihypertensive, antimicrobial, anti-diabetes and anti-obesity activities. This study provides an overview about bioactive peptides derived from microalgae *Spirulina platensis* and their some biological activities with health benefits.

Keywords: *Spirulina platensis*, Microalgae, Bioactive peptides, Antioxidant activity, Antihypertensive activity

Mikroalg *Spirulina platensis*'ten Elde Edilen Biyoaktif Peptitler ve Biyofonksiyonel Aktiviteleri

ÖZ

Spirulina platensis simbiyotik, çok hücreli ve iplikli yapıda mavi-yeşil mikroalgdır. Esansiyel aminoasitler, β -karoten ve fikobiliproteinler gibi pigmentler ve eikosapentaenoik (EPA) ve dokosaheksaenoik asit (DHA) gibi çoklu doymamış yağ asitleri gibi bazı değerli biyoaktif bileşiklerin zengin bir kaynağıdır. *Spirulina platensis* ve ondan elde edilen biyoaktif peptitler besinsel ve antioksidan, antihipertansif, antimikrobiyal, antidiyabetik ve antiobezite etkiler gibi bazı medikal faydaları nedeniyle sağlıklı gıda bileşeni olarak gittikçe önem kazanmaktadır. Bu çalışma, *Spirulina platensis*'ten elde edilen biyoaktif peptitler ve onların bazı sağlık faydaları hakkında bilgi vermeyi amaçlamaktadır.

Anahtar Kelimeler: *Spirulina platensis*, Mikroalg, Biyoaktif peptitler, Antioksidan aktivite, Antihipertansif aktivite

INTRODUCTION

Spirulina platensis is a blue-green microalgae owing to chlorophyll (green) and phycocyanin (blue) pigments in its chemical composition [1]. It is one of multicellular cyanobacterium microalgae with ability of photosynthesis that can grow well in either fresh water and seawater [2]. Because of its rich pigments like chlorophyll, it is placed in the plant kingdom. On the

other hand, it is also classified as a member of the bacteria kingdom owing to its biochemical properties [3].

Spirulina platensis, which is one of the more important microalgae, has been used as a food because of its high nutritional value at Asian and Pacific Asian countries for long time and it is gaining importance in European countries, which is new trend [4]. It contains numerous high-value bioactive components like proteins, lipids,

carbohydrate, pigments, phenolics, vitamins and other phytonutrients [3, 5, 6].

Spirulina platensis has both a nutritional value and also it used in cosmetic applications due to its antioxidant, anti-inflammatory, neuroprotective, antiviral, antitumor and hepatoprotective activities [4]. This study provides an overview about bioactive peptides extracted from microalgae *Spirulina platensis* and its biofunctional properties with health benefits.

CHEMICAL COMPOSITION OF *SPIRULINA PLATENSIS*

Spirulina platensis is an excellent source of protein, carbohydrates, polyunsaturated fatty acids (PUFAs), and essential minerals and vitamins and a whole spectrum of natural mixed carotene and xanthophylls phytopigments [5, 7, 8]. Table 1 shows the basic compounds of *Spirulina platensis*.

Table 1. The basic compounds of *Spirulina platensis* [1, 3].

Nutrient	Percent Composition (wet basis)	Details
Proteins	60-65	Essential amino acids: lysine, methionine, threonine, phenylalanine, isoleucine, leucine, tryptophane and valine and 10 nonessential amino acids: arginine, cystine, aspartic acid, glycine, glutamic acid, proline, serine, histidine, alanine and tyrosine.
Carbohydrates	13.5-15	Glucose, mannose, rhamnose, galactose, xylose and two unusual sugars (2-O-methyl-L-rhamnose and 3-O-methyl-L-rhamnose)
Lipids	5-6	PUFAs: gamma-linolenic acid, linoleic acid, stearidonic acid, EPA, DHA and arachidonic acid
Vitamins	<1	Vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B9, vitamin B12, vitamin C, vitamin D and vitamin E
Minerals	~7	Essential minerals: calcium, potassium, copper, chromium, magnesium, iron, phosphorus, manganese, sodium, selenium and zinc
Pigments	<1	Alpha and beta-carotenes, xanthophylls, echinenone, cryptoxanthin, zeaxanthin and lutein, porphyrin, chlorophyll, phycocyanin, phycoerythrin, phytionadione and tetrapyrrole
Moisture	6-13.5	-

NUTRITIONAL QUALITY OF ALGAL PROTEIN FROM *SPIRULINA PLATENSIS*

Spirulina platensis has a high protein content, with about 60-70% of its dry weight. It is considered as a complete protein, containing various essential amino acids, including leucine, isoleucine, and valine, although with reduced amounts of methionine, cysteine, and lysine when compared with standard proteins such as those from soybean, eggs, etc. [3, 9]. Table 2 presents amino acid composition of *Spirulina platensis* as compared with vegetable and animal protein sources.

Phycobiliproteins found in *Spirulina platensis* are divided into three main classes according to their structure: phycoerythrins, phycocyanin and allophycocyanins. *Spirulina platensis* is an excellent source of phycocyanin, a blue photosynthetic pigment. The phycocyanin composes up to 20% of its dry weight [5, 10, 11, 12]. The phycocyanin is used mainly as a natural blue colorant. Moreover, phycocyanin has proven to possess some therapeutic properties like antioxidant, anti-inflammatory and anti-cancer activities [10].

Table 2. Amino acid profile of *Spirulina platensis* as compared with conventional protein sources an Food and Agriculture Organization of the United Nations (FAO) (g per 100 protein) [13, 14]

Source	Ile	Leu	Val	Lys	Phe	Tyr	Met	Cys	Try	Thr	Ala	Arg	Asp	Glu	Gly	His	Pro	Ser
FAO	4.0	7.0	5.0	5.5	6.0		3.5		1.0	4.0								
Egg	6.6	8.8	7.2	5.3	5.8	4.2	3.2	2.3	1.7	5.0		6.2	11.0	12.6	4.2	2.4	4.2	6.9
Soybean	5.3	7.7	5.3	6.4	5.0	3.7	1.3	1.9	1.4	4.0	5.0	7.4	1.3	19.0	4.5	2.6	5.3	5.8
<i>S. platensis</i>	6.7	9.8	7.1	4.8	5.3	5.3	2.5	0.9	0.3	6.2	9.5	7.3	11.8	10.3	5.7	2.2	4.2	5.1

ISOLATION AND PURIFICATION OF BIOACTIVE PEPTIDES FROM *SPIRULINA PLATENSIS* PROTEIN

Bioactive peptides can be generated by enzymatic hydrolysis reactions using protease enzymes obtained from microbes, plants and animals [16-18]. Enzymatic

hydrolysis of proteins allows developing bioactive peptides using certain enzymes and physico-chemical conditions such as optimum temperatures and respective pHs [4, 16]. Table 3 presents optimum conditions of various enzymes for enzymatic hydrolysis in protein solution of *Spirulina platensis*.

Table 3. Optimum conditions of various enzymes for enzymatic hydrolysis in protein solution of *Spirulina platensis*

Enzyme	Optimum Conditions		References
	pH	Temperature (°C)	
Pepsin	2	37	19
	2	45	20
Alcalase	8.5	50	19
Papain	6.5	60	21
	6.5	55	19
Protamex	6	40	22
Trypsin	8	42	19
Alkaline protease	7	55	21
Protease K	8.5	55	22

Bioactive peptides usually include approximately 3–40 amino acids residues per molecule, their amino acid composition and sequence are important factors for their physiological activities [15]. For example, in a study of Suetsuna and Chen [19], amino acid sequence of peptic fractions isolated from the peptic digests of *Spirulina platensis* were determined as Ile-Ala-Glu, Phe-Ala-Leu, Ala-Glu-Leu, Ile-Ala-Pro-Gly, and Val-Ala-Phe. These peptides exhibited angiotensin I-converting enzyme (ACE) inhibitory activity in hypertensive rats.

Recently, bioactive peptides obtained from *Spirulina platensis* have received much attention because of their health benefits and biological activities. Especially, antihypertensive, antioxidative, antitumor, antiproliferation and antimicrobial effect of these peptides has been proved by numerous studies carried out *in vitro* and *in vivo* [8, 19-27]. Amino acid sequence and bioactivities of peptides obtained from *Spirulina platensis* are shown in Table 4.

Table 4. Amino acid sequence and bioactivities of peptides obtained from *Spirulina platensis*.

Enzyme	Bioactivity	Amino acid composition and sequence	References
Pepsin	ACE-I inhibitory activity	Ile-Ala-Glu, Phe-Ala-Leu, Ala-Glu-Leu, Ile-Ala-Pro-Gly and Val-Ala-Phe	19
	Antitumor effect	Not available	20
Alcalase	Antitumor effect	Not available	20
Papain	Antibacterial activity	Lys-leu-Val-Asp-Ala-Ser-His-Arg-Leu-Ala-Thr-Gly-Asp-Val-Ala-Val-Arg-Ala	21
	Antitumor effect	Not available	19
Protamex	ACE-I inhibitory activity	Not available	22
Trypsin	Antitumor effect	Not available	20
Alkaline protease	Antibacterial activity	Lys-Leu-Val-Asp-Ala-Ser-His-Arg-Leu-Ala-Thr-Gly-Asp-Val-Ala-Val-Arg-Ala	21
ProteaseK	Antioxidant activity	Pro-Asn-Asn	23
Papain	Antiproliferation activity on cancer cells	Tyr-Gly-Phe-Val-Met-Pro-Arg-Ser-Gly-Leu-Trp-Phe-Arg	24
Alcalase	ACE-I inhibitory activity	Ile-Gln-Pro	25
Alcalase and Flavourzyme	Iron-chelating activity	Thr-Asp-Pro-Ile(Leu)-Ala-Ala-Cys-Ile(Leu)	26
Papain	ACE-I inhibitory activity	Val-Glu-Pro	27

The molecular weight and size of the bioactive peptides are other important factors for obtaining desired bio-functionality peptides. Hence, ultrafiltration membrane system is an appropriate method for preparation of peptides with desired molecular size-weight and biological activity [4, 15, 16]. Moreover, various chromatographic methods such as ion exchange, gel chromatography and reverse-phase high performance liquid chromatography (RP-HPLC) can be utilized for further purification of obtained peptides. In addition, the spectrophotometric techniques like liquid chromatography-mass spectrophotometry (LC-MS) and mass-mass spectrophotometry (MS-MS) are usually used for identification bioactive peptides at the molecular level [4].

BIOLOGICAL ACTIVITIES OF BIOACTIVE PEPTIDES DERIVED FROM *SPIRULINA PLATENSIS*

Bioactivities of peptides are defined as mimic hormones and they can alter the physiological functions or increase a positive effect with binding to certain receptors and interact on target cells or inhibition by enzyme actions. Bioactive peptides isolated from *Spirulina platensis* have various biological activities such as antioxidant activity, antihypertensive activity, antimicrobial activity, anti-diabetes activity and anti-obesity activity [4, 18].

Antioxidant Activity

Antioxidants are compounds that inhibit the oxidation of other molecules and compounds and, they have an important impact for the prevention of different diseases like cardiovascular disease [4]. Numerous studies have shown that *Spirulina* or its protein extracts have antioxidant activity [28-31]. Gad and colleagues [29] evaluated the antioxidant activity of aqueous extract, containing the protein phycocyanin of *Spirulina platensis* *in vitro* and its hepatoprotective effects *in vivo*. In this study, it was shown that the *in vitro* antioxidant capacity of the aqueous extract of *Spirulina platensis* showed a strong inhibition of ferrozine- Fe^{2+} complex formation and *in vivo* study showed that *Spirulina platensis* succeeded in preventing liver damage. Estrada and coworkers [28] observed that an increase in the amount of phycocyanin caused an increase in the antioxidant activity, and therefore phycocyanin is the compound mainly responsible for the antioxidant activity of the protein extract of *Spirulina platensis*. Similarly, Bermejo et al. [31] proved antioxidant activity of a *Spirulina platensis* protein extract.

Antihypertensive Activity

High blood pressure is one of the important reasons of cardiovascular diseases [16]. The angiotensin I-converting enzyme (ACE) provides regulating blood pressure in the renin-angiotensin system [20]. In some studies mentioned below, the peptides with the ACE-inhibitory activity were obtained by enzymatic hydrolysis of *Spirulina platensis* protein extracts. According to Suetsuna and Chen [20] the peptides derived from *Spirulina platensis* showed antihypertensive activity by oral administration in spontaneously hypertensive rats. Oral administration of the peptidic fraction (200 mg/kg) reduced blood pressure in spontaneously hypertensive rats. He et al. [22] determined the IC_{50} values for ACE inhibitory activity of various hydrolysates including *Spirulina platensis* protein hydrolysates. They found that ACE inhibitory peptides obtained from *Spirulina platensis* showed the lowest IC_{50} value.

Antidiabetes and Antiobesity Activities

Nowadays, obesity is one of the significant health threats for human health, with more than 1 billion overweight adults and at least 300 million of them clinically obese. One of the most important methods to struggle with obesity is production of natural inhibitors of digestive enzymes such as alpha-amylase, alpha-glucosidase and lipase involved in the digestion of carbohydrates and lipids. Briefly, carbohydrate and lipid digestive enzyme inhibitors play an effective and active role for preventing obesity and obesity-related diseases. Recently, *Spirulina platensis* has also evidenced to possess anti-diabetes and anti-obesity activities. The water-soluble fraction of *Spirulina platensis* was found to be effective in lowering the serum glucose level at fasting while the water insoluble fraction suppressed the glucose level at glucose loading [3, 32]. Layam and Reddy [33] evaluated *in vivo* the antidiabetic property of *Spirulina platensis*. According to the results of this study,

the activity of hexokinase in liver decreased significantly, whereas the activity of glucose-6-phosphatase increased markedly in diabetic control. El-Desouki et al. [8] conducted an *in vivo* study to assess the effect of erythropoietin (EPO) and/or *Spirulina* to treat alloxanized-diabetic rats. According to the results of this study, diabetic rats treated with EPO or *Spirulina* showed a slight improvement of pancreatic islets and acinar cells.

Antimicrobial Activity

In vitro and animal studies suggest that *Spirulina* possesses antimicrobial effect. Pagnussatt et al. [34] investigated the inhibition of *Fusarium graminearum* growth and mycotoxin production by phenolic extract from *Spirulina* sp. They observed greatly reduced radial growth of fungal colonies and average reductions of 40% and 62% in the glucosamine levels and the amylase activity. Sun et al. [21] obtained an antibacterial peptide from *Spirulina platensis* with enzymatic hydrolysis using alkaline protease and papain enzymes. They showed that the minimum inhibitory concentration of the antibacterial peptide from *Spirulina platensis* was 8 mg/mL for *Escherichia coli* and 16 mg/mL for *Staphylococcus aureus*. Based on all these features, the *Spirulina* peptides can be considered to be potential promising antimicrobial agents.

CONCLUSION

Spirulina platensis is a microalgae with the wide range of physiological and biochemical characteristics. Bioactive peptides derived from *Spirulina platensis* have induced biological activities such as antioxidant activity, antihypertensive activity, antimicrobial activity, anti-diabetes activity and anti-obesity activity. It is one of the more important microalgae owing to its high nutritional value and remarkable health benefits.

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