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Comparison of Extraction Techniques for Determining Bioactive Compounds and Antioxidant Activity of *Spirulina platensis*

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ABSTRACT

Spirulina platensis (S. platensis) is a high-nutrient blue-green algae that has been used as a food supplement for a long time. It contains carbohydrates, lipids, proteins, vitamins, minerals, and bioactive compounds essential for basic human nutrition. It is known to have anti-cancer, antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, and hypocholesterolemic properties due to the bioactive compounds it contains. In this study, the effects of freeze-thawing, a rapid freezing (-20°C) and thawing (4°C) process, and ultrasonically assisted extraction techniques on the color, antioxidant capacity, total phenolic content, and phenolic composition of *Spirulina platensis* extracts were investigated. The antioxidant capacity of the extracts obtained was determined by two different methods, DPPH (2,2-diphenyl-1-picryl hydrazyl) and ABTS (2,2-azinobis (3-ethylbenzothiazollin-6-sulfonic acid)). The sugar profile was determined by HPLC-RID and phenolic composition was determined by HPLC-ESI-DAD-MS/MS. The antioxidant activity and total phenolic content of samples prepared by the freeze-thawing were higher than those prepared by ultrasonic-assisted conventional extraction technique. In addition to ferulic acid 4-O-glucuronide and brevifolin carboxylate, an isocoumarin derivative, as the dominant phenolic compound in *S. platensis* extracts, a total of 10 phenolic compounds including catechin isomer, resveratrol C-hexoside, myricetin, ferulic acid, gallic acid, phloroglucinol, and lutein were detected. Glucose was the predominant sugar in both samples. The total sugar content was higher in the freeze-thawed samples (217.92 mg/100 g DW) than in the ultrasonic-assisted conventional extraction technique (182.91 mg/100 g DW). *S. platensis* has a significant amount of antioxidants, valuable secondary metabolites, and potential commercial applications and medicinal properties, but releasing these compounds is difficult due to the cell wall. This study was carried out to determine how different extraction techniques alter the release of bioactive compounds.

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Introduction

Microalgae are seen as one of the sources with the highest biological activity in nature and contain many bioactive compounds in their structure (Alajil Alslibi, 2019). *Spirulina platensis* is a single-celled, filamentous, prokaryotic microalgae that thrives natively in alkaline waters of warm lakes and consists of about 15 species (Maddina et al., 2016). *S. platensis*, also known as *Arthrospira platensis*, is a protein-rich algae that the Aztecs historically collected from Lake Texcoco in Mexico (Saranraj & Sivasakthi 2014). This particular plant-based protein is unique in terms of its protein content. It contains all essential amino acids, including leucine, valine, isoleucine, tryptophan, methionine, phenylalanine, theanine, and lysine, as well as non-essential amino acids such as glycine, proline, arginine, cysteine, tyrosine, glutamine, alanine, serine, glutamate, and aspartate. It has a protein content of approximately 55-70% and is often referred to as a "superfood" since of provides abundant nutritional content (Maddina et al., 2016; Saranraj & Sivasakthi 2014).

S. platensis contains bioactive compounds, especially carotenoids, responsible for antioxidant activities, phycocyanin, an active protein, and phenolic acids (Maddiboyina et al., 2023). Phycocyanin acts as antioxidants that scavenge free radicals and prevent oxidative stress. These antioxidants can transform into pro-oxidants and protect the body against oxidative stress (Maddiboyina et al., 2023). Phenolic compounds are

naturally occurring antioxidants that are important sources of polyphenols synthesized by microalgae. These compounds contain one or more hydroxyl groups directly attached to the aromatic ring. According to studies by Ferreres et al. (2012) and Heffernan et al. (2015), polyphenols are widely recognized as crucially important chemical compounds.

S. platensis is easy for humans to digest as its cell wall consists of 86% digestible polysaccharides without hard cellulose. However, breaking down the cell wall with conventional extraction techniques is very difficult. Therefore, it is necessary to hydrolyze the covalent bonds in the structure to release phenolics or to disrupt the cell wall matrix (Arranz et al., 2010; Shahidi & Yeo, 2016). It is very important to use green extraction processes to increase efficiency, reduce processing time, and save energy. Due to its ability to lower process temperature, duration, and solvent consumption, ultrasound-assisted extraction (UAE) is gaining popularity as a substitute method (Ahmed et al., 2022). This study used freeze-thawing, which has been widely used in recent studies, in addition to ultrasonic-assisted conventional extraction (UACE). In UACE, ultrasonic waves disrupt the cell wall and accelerate mass transfer, enabling the desired bioactive components to be obtained in a shorter time and yield higher than classical techniques (Purdi et al., 2023). When a sound wave is intense, it can create empty spaces in a liquid by overcoming the gravitational forces that cause the liquid to relax. These empty spaces are known as 'cavitation bubbles' and grow larger with each sound wave cycle until they reach a critical size. Bubbles reaching the critical size collapse violently, and as a result of this collapse, the increase in temperature and pressure in the environment leads to the formation of microjets. Microjets cause surface peeling, erosion, cell wall destruction, and leakage of cell contents, thus enabling the extraction of natural compounds from various sources (Purdi et al., 2023). The freeze-thawing technique consists of rapid freezing at -20 °C and thawing at 4°C. This lysis method causes the cells to swell and eventually break by forming intracellular ice crystals during freezing and shrinking during thawing. Multiple cycles are required for effective lysis, and the process can be pretty long (Tan et al., 2020). This study investigated the effect of ultrasonically assisted conventional extraction and freeze/thaw technique on antioxidant activity, total phenolic content, sugar, and phenolic composition of *S. platensis* extracts. The antioxidant activity of the extracts was determined by 2 different methods, DPPH and ABTS, sugar profile by HPLC/RID, and phenolic composition by HPLC-MS/MS.

Material and Method

Chemicals

Folin-Ciocalteu reagent was obtained from Merck (Darmstadt, Germany), Trolox and 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazollin-6-sulfonic acid) (ABTS), phenolic compounds and sugar standards (sucrose, glucose, and fructose) were obtained from Sigma-Aldrich Chemical Co (St. Louis, USA). All solvents and chemicals used in this study were of chromatographic and analytical purity and were prepared daily.

Preparation of S. platensis Extracts

Commercial *Spirulina* samples were obtained as powder from a local market in Adana. Four grams of samples were weighed into a 100 ml Erlenmeyer flask, 100 ml of water was added and mixed for 15 minutes in a magnetic stirrer. The samples were kept in an ultrasonic water bath with an ultrasonic power of 60 W and a frequency of 40 kHz for 1 hour at 25 °Cice was added to prevent temperature rise, and then one of the samples was stirred overnight at room temperature in a magnetic stirrer, and the other was subjected to a rapid freezing (-20°C) and thawing (4°C) process repeated 4 times. At the end of the period, both samples were centrifuged at 7000 rpm for 15 minutes at 4°C. The resulting extracts were filtered through a 0.45 µm membrane filter and stored in a refrigerator at 4°C until analysis.

Antioxidant Capacity and Total Phenolic Content

DPPH method: DPPH (2,2, diphenyl 1-picry hydrazyl), which can measure the ability to inhibit free radicals, was used and the absorbance values were recorded in a UV-Vis spectrophotometer (BMG LABTECH, SPECTROstar Nano, Ortenberg, Germany) according to the results of measuring the change of the reaction in methanol against time (Brand-Williams et al., 1995; Kelebek et al., 2013). Briefly, 100 µl of each extract was mixed with 3.9 ml of DPPH solution and incubated in the dark at room temperature for 1 hour. The absorbance was then measured at 515 nm with a UV-visible spectrophotometer and the results are given as µmol Trolox/100g DW. *ABTS method:* It was performed according to the method of Saafi et al. (2009). To perform this method, mix 7 mM of ABTS (2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) with 2.45 mM of potassium sulfate and keep it in the dark for around 12 to 16 hours. Then, dilute this solution with sodium acetate buffer (pH 4.5) to an absorbance of 0.700±0.01 at a wavelength of 734 nm in a spectrophotometer. After that, add 2.98 mL of the prepared buffer to 20 µL of the sample extract, wait for 10 minutes, and measure the absorbance in a UV-Vis spectrophotometer (BMG LABTECH, SPECTROstar Nano, Ortenberg, Germany) at a wavelength of 734 nm. The absorbance values obtained should be calculated by using a trolox (10-100 µmol/L) standard slope chart, and the results were expressed in µmol Trolox/ 100g.

Determination of total phenolic content: The basic principle of the analysis is based on the oxidation of phenolic compounds by reduction of the Folin-Ciocalteu reagent in a basic medium. To perform a spectrophotometric analysis, 200 µl of either extract or standard solution was added into a cuvette. Then, 1.5 ml of Folin-Ciocalteu reagent (diluted at a 1:10 ratio) was added to the cuvette and left for five minutes. Following that, 1.5 ml of 6% sodium carbonate solution was added to the cuvette. The mixture was then kept at room temperature and in the dark for 90 minutes. With increasing phenolic content, the blue-colored solution becomes darker. Absorbance differences were measured at 765 nm on a UV-Vis Spectrophotometer (BMG LABTECH, SPECTROstar Nano, Ortenberg, Germany). A 500 ppm solution of gallic acid was prepared for the calibration curve. Phenolic content was calculated based on the slope obtained from the calibration curve (Shahidi, 2015) and the results were expressed in mg GAE/ 100g.

Determination of Phenolic Compounds by HPLC-ESI-MS/MS

An Agilent Technologies HPLC system (model 1100) controlled by ChemStation software, was used to analyze phenolic compounds. The HPLC setup consisted of a degasser, a binary pump, and a diode array detector. A Phenomenex Luna C18 column (4.6 mm × 250 mm, 5 µm) was used. Two solvents made up the mobile phase: Solvent B was a mixture of solvent A and acetonitrile (60:40, v/v) and Solvent A was a mixture of water and formic acid (99:1, v/v). The phenolic compounds were extracted using the following conditions: a flow rate of 0.5 mL/min at 25°C, isocratic conditions with 0% B from 0 to 5 minutes, and gradient conditions for the following steps: from 0% to 5% B in 20 minutes, from 5% to 15% B in 18 minutes, from 15% to 25% B in 14 minutes, from 25% to 50% B in 31 minutes, from 50% to 100% B in 3 minutes. After the extraction process, the column was washed and reconditioned. The temperature was kept at 25°C and the flow rate was set to 0.5 ml/min. UV-visible spectra were recorded for all peaks between 200 nm to 800 nm (Tanrıseven et al., 2020). By comparing each compounds UV spectra and retention durations to real standards and was identified. Additionally, an Agilent 6430 LCMS/MS spectrometer equipped with an electrospray ionization source was used to confirm the chemicals. The detection process for electrospray ionization mass spectrometry (ESI-MS) was optimized and executed in negative ion mode. Using authentic standards and the external standard approach, the chemicals were quantified and following Sonmezdag et al. (2019) approach, the phenolic content was calculated. To measure each phenolic compound, we used the calibration curves of the standard phenolic compounds. However, as it was not feasible to provide a standard substance for all compounds, we made use of calibration curves prepared with structurally comparable chemicals to quantify these compounds. The performance of the method developed was determined by using standard solutions, spiked and non-spiked samples (Barwick, 2016). The method was fully validated in terms of linearity, accuracy (recovery), inter-day and intra-day precision (repeatability), limits of detection and quantification (LOD/LOQ), and relative standard uncertainty (95% confidence level). The current chromatographic settings' detection (LOD) and quantification (LOQ) limits were calculated with signal-to-noise ratios (S/N) of about 3 and 10, respectively. Using commercial standards at concentrations often seen in microalgae samples (ranging from 1 to 100 mg/L) and with R^2 values over 0.995, standard curves were produced. As per the findings of Sonmezdag et al. (2019) and Tanrıseven et al. (2020), the measurements were conducted three times.

Determination of Sugar Profile by HPLC-RID

Extracts were injected into HPLC (Shimadzu Prominence-i LC-2030C) with a RID detector to detect and quantify sugar profiles. The analysis was conducted using a BIORAD Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad; Hercules, CA, USA) with a flow rate of 0.5 mL/min and H2SO4 (5 mM) as the carrier phase. To find the amounts of the compounds, solutions were made at five different concentrations for each reference substance, and the amounts of the compounds were computed using calibration curves (Lee & Coates, 2000).

Statistical Analysis

Statistical analysis was performed by One-Way ANOVA using SPSS 22.0 (version 22, SPSS Inc., Chicago, IL, USA). Duncan's test measured differences in the content levels of the results and means with *p*-values less than 0.05 were indicated to be statistically significant.

Results and Discussion

Antioxidant Activity and Total Phenolic Content

S. platensis is recognized as a rich source of nutritious phenolic and flavonoid compounds due to its higher production capacity than traditional plant-based sources. *S. platensis* contains phycocyanin and phenolic compounds which are known to have antioxidant properties. Two methods, DPPH and ABTS, were used to determine the antioxidant activity of the samples. The antioxidant capacity and total phenolic content of the samples are shown in Table 1. According to DPPH and ABTS methods, *S. platensis* extracts (2195.83 µmol Trolox /100g DW, and 2150.69 µmol Trolox /100g DW) applied to freeze-thawing technique was determined approximately 1.9 times higher antioxidant activity than ultrasonic-assisted conventional extraction technique (1143.75 µmol Trolox /100g DW, and 1099.30 µmol Trolox /100g DW) ($p<0.05$).

In Table 1 shows the total phenolic content (TPC) of samples extracted by freeze-thawing and ultrasonic-assisted conventional techniques. Significant differences existed in the total phenolic matter amounts between the samples ($p<0.05$). The freeze-thawing sample of *S. platensis* was showed the highest TPC, measuring 726 mg GAE/100g DW. When comparing the total phenolic matter results, it was found that the freeze-thawing process resulted in 1.9 times higher phenolic content than the sample subjected to ultrasonic-assisted classical extraction, which measured at 326.22 mg GAE/100 g DW. In the freeze-thawing technique, ice crystals are formed during the freezing process, causing the cells to swell and eventually break down, followed by shrinkage of the cells during thawing. Thus, it is thought that the release of bioactive compounds in the structure is facilitated and the amount of antioxidant capacity and phenolic compounds increases.

A study was conducted to analyze the impact of various drying methods on physical properties, the DPPH capacity of *S. platensis* was determined as 69.82 mg/100g DW (Kuatrakul et al., 2017). Uzlasir et al. (2023) determined the antioxidant capacity of *S. platensis* grown using different salt concentrations as 137-173 mM Trolox/g DW by DPPH radical scavenging capacity method and 373-655 mM Trolox/g DW by ABTS capacity method. It was determined that antioxidant capacity decreased with increasing salt concentration. In the study conducted by Martins et al. (2023), it was found that the TPC of extracts obtained from *S. platensis* using ultrasonic-assisted extraction was 36.50 mg GAE/g DW. Additionally, the antioxidant activity of the extracts was found to be 37.98 mg Trolox/ g DW.

Phenolic Compounds

Phenolic compounds are divided into different subgroups, such as phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids according to their chemical structures (De la Rosa et al., 2019).

Table 1. Antioxidant activity and total phenolic content of *S. platensis* extracted by different extraction techniques

Analyses	F&T	UACE	Sig.
DPPH (µmol Trolox /100g DW)	2195.83±69.90	1143.75±75.06	*
ABTS µmol Trolox /100g DW)	2150.69±9.50	1099.30±4.21	*
TPC (mg/100 g DW)	726.00±7.09	368.22±4.02	*

(*) The symbol in the row indicates statistical differences ($p<0.05$*); TPC: Total phenolic content; F&T: Freeze&Thawing; UACE: Ultrasonic-assisted conventional extraction

Table 2. Phenolic profile and amounts of *S. platensis* extracts by HPLC-ESI-MS/MS (mg/100g DW)

No	R_T	Compounds	$[M-H]^-$ /$[M-H]^{+*}$	MS^2	F&T	UACE	Sig.
1	4,1	Catechin (isomer1)	289[a]	267/245/172.9/154.9	0.15±0.03	0.39±0.05	*
2	4,89	Resveratrol C-hexoside	389[a]	293/210/147/96	1.41±0.03	0.15±0.00	*
3	5,93	Catechin (isomer 2)	289[a]	267/245/172.9/154.9	1.74±0.05	0.49±0.14	*
4	6,25	Mirisetin	317[a]	179/151/137/107	1.28±0.03	0.61±0.02	*
5	11,51	Ferulic acid	195[b]	177/145	5.84±0.73	3.76±0.49	*
6	12,32	Ferulic acid 4-O-glucuronide	369[a]	193/178	10.73±0.25	8.41±0.52	*
7	14,09	Gallic acid	169[a]	125	0.38±0.01	0.59±0.02	*
8	18,62	Phloroglucinol	127[b]	108	2.01±0.02	2.72±0.02	*
9	29,45	Brevifolin carboxylate	291[a]	219/174	9.13±0.03	5.12±0.22	*
10	44,16	Lutein	569[b]	551/533/578/495/119/145/121	0.38±0.00	0.21±0.01	*
				Total	34.06±3.79	22.45±2.78	*

(*) The symbol in the row indicates statistical differences ($p<0.05$*); R_T: Retention time; a: Negative ion mode, b: Positive ion mode; F&T: Freeze-Thawing; UACE: Ultrasonic-assisted conventional extraction

Table 3. Sugar profile of *S. platensis* extracts by HPLC-RID (mg/100g DW)

Peak no	R_T	Compounds	F&T	UACE	Sig.
1	9,30	Sucrose	93.71±4.09	46.81±3.68	*
2	10,88	Glucose	131.74±2.30	115.77±5.90	*
3	11,65	Fructose	8.45±0.10	4.36±0.50	*
		Total	233.89±1.70	166.94±6.49	*

(*) The symbol in the row indicates statistical differences ($p<0.05$*), R_T: Retention time; F&T: Freeze-Thawing; UACE: Ultrasonic-assisted conventional extraction

Phenolic compounds such as flavonoids and phenolic acids have antioxidant potential and their amounts vary according to the microalgae species and growing conditions. Table 2 shows the phenolic profile and amounts of *S. platensis* extracts. In addition to ferulic acid 4-O-glucuronide and brevifolin carboxylate, an isocoumarin derivative, as the dominant phenolic compound in *S. platensis* extracts, a total of 10 phenolic compounds including catechin isomer, resveratrol C-hexoside, myricetin (LOD-LOQ: 0.30-0.11 g/mL, R^2: 0.995), ferulic acid (LOD-LOQ: 0.18–0.60 g/mL, R^2: 0.995), gallic acid (LOD-LOQ: 1.89–6.30 g/mL, R^2: 0.995), phloroglucinol (LOD-LOQ: 0.32–0.44 g/mL, R^2:0.995) and lutein (LOD-LOQ: 0.08–0.28 g/mL, R^2: 0.995) were identified and quantified. Total phenolic compounds were 1.5 times higher in the freeze/thawing process than in the ultrasonic-assisted classic extraction technique ($p<0.05$). Catechins are important secondary metabolites found in plants and belong to the group of flavan-3-ols (or simply flavanols), which are part of the chemical family of flavonoids. Catechin is a molecule that has four diastereoisomers. Two of the isomers are in the *trans* configuration and are called catechin. The other two are in the *cis* configuration and are called epicatechin (Bernatoniene & Kopustinskiene, 2018; Tsuchiya, 2001). Catechin and epicatechin are known as the building blocks of proanthocyanidins, which are a type of condensed tannins. As flavonoids, catechins can act as antioxidants, but their antioxidant potential is relatively low when compared to other flavonoids, especially at lower concentrations. The ability to quench singlet oxygen is related to the catechin's chemical structure, a catechol moiety in the B ring, and a hydroxyl group in the C ring that activates the double bond (Pietta, 2000). With a colorless or slightly yellow crystalline form, gallic acid is one of the most prevalent phenolic acids in the kingdom of plants and finds widespread application in the food and pharmaceutical industries. It has been reported to have neuropsychological, metabolic, therapeutic activities, and cardiovascular disorders, including anti-inflammatory, antineoplastic, and antioxidant properties (Choubey et al., 2015). Lutein is a yellow-colored organic compound known as a vitamin carotenoid found in plants, which is covalently bonded with fatty acids. It is found in many organisms, including plants, yeasts, bacteria, and algae (Ochoa Becerra et al., 2020). Phloroglucinol is a naturally occurring secondary metabolite in certain plant species (Wong & Morita, 2019). The LC-ESI-MS/MS chromatograms of the phenolics identified in *S. platensis* samples are shown in Figure 1.

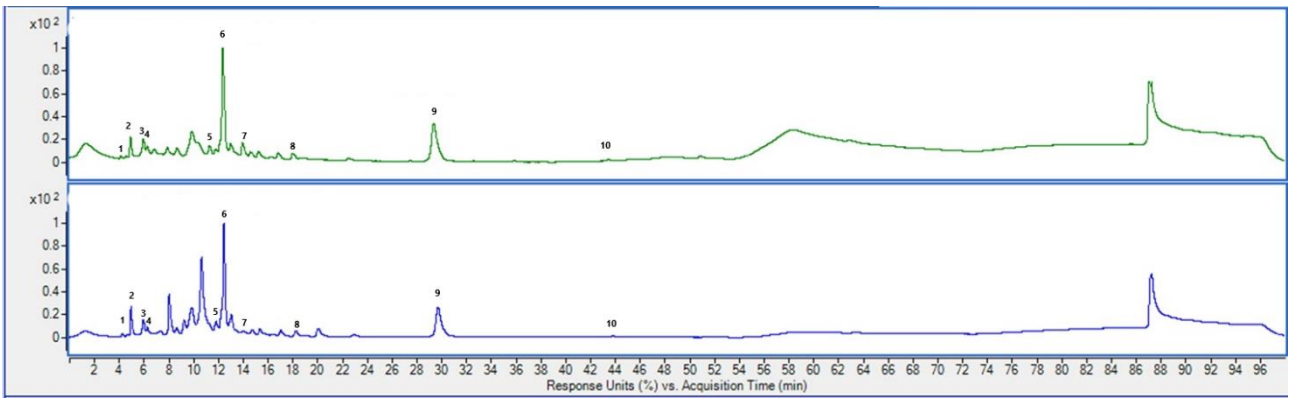

Figure 1. LC-ESI-MS/MS chromatograms of phenolic compounds of *S. platensis*
(1: Catechin (isomer1), 2: Resveratrol C-hexoside, 3: Catechin (isomer 2), 4: Mirisetin, 5: Ferulic acid, 6: Ferulic acid 4-O-glucuronide, 7: Gallic acid, 8: Fluoroglusinol, 9: Brevifolin carboxylate, 10: Lutein) (x: mAU, y: Aquisition time (min))

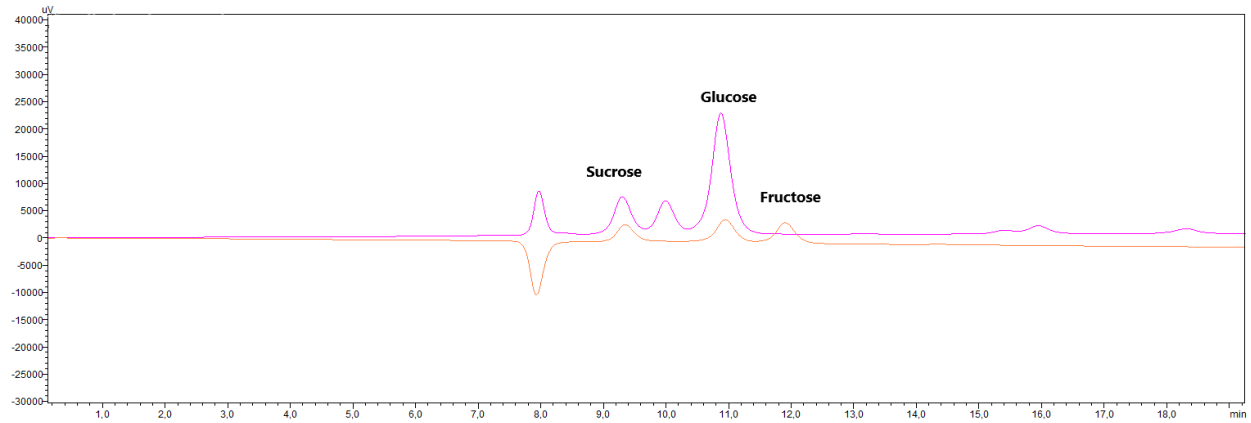

Figure 2. HPLC-RID chromatograms of the sugar profile of *S. platensis*

These natural phenolic compounds likely have a diverse range of chemical and biological properties, such as antioxidant and free radical-scavenging activities. Therefore, the total phenolic content was measured and correlated with the antioxidant capacity results of the samples. All the differences between the samples were found to be statistically significant. In our previous study in which we examined the effect of salt stress on the growth of *S. platensis*, a total of 24 phenolic compounds (catechin isomer, phloroglucinol, lutein, etc.) were identified, and their concentrations varied between 73 and 124 mg/100 g DW. A positive and strong correlation was found between antioxidant capacity and the amounts of total phenolic compounds (Uzlasir et al., 2023). Seghiri et al. (2019), discovered the presence of phenolic compounds like resveratrol, gallic acid, catechin, etc. in samples of *S. platensis*. They found that these compounds are usually found in extracts with higher polarity and are linked to antioxidant activity or a synergistic effect through their redox properties.

Sugar Profile

In general, microalgae contain approximately 10% carbohydrates on a dry weight basis. Microalgae species contain varying amounts and types of carbohydrates, with rhamnose, xylose, glucose, and mannose being the most abundant monomers (Villarruel-López et al., 2017). Sucrose, glucose, and fructose were determined in *S. platensis* using different extraction techniques. Statistically significant differences between the sugar contents ($p<0.05$) and the total sugar content were determined as 233.89 mg/100g DW in the freeze-thawing technique and 166.94 mg/100g DW in the ultrasonic-assisted conventional extraction technique (Table 3). The predominant sugar was glucose for both samples, and its amount was determined to be 115-131 mg/100g DW. The sugar chromatogram was given in Figure 2. Microalgae such as *Chlorella*, *Dunaliella*, *Nannochloropsis*, and *Spirulina* contain oligo and polysaccharides that make them potential prebiotics (Caporgno & Mathys, 2018; Gupta et al., 2017). In a study with commercial *Spirulina* by Al-Dhabi & Valan Arasu (2016), it was reported that the total sugar content of thirty-seven different *Spirulina* samples ranged between 309 and 1221 mg/100 g DW, and galactose, rhamnose, glucose, xylose, ribose, and fructose were detected as sugars. In another study, it was reported that rhamnose constitutes 53% of the total sugar in *Spirulina*, in addition to ribose, xylose, maltose, mannose, galactose, and glucose (Chaiklahan et al. 2013).

Conclusions

Spirulina platensis is a potential source of bioactive compounds, total phenolic content, and antioxidants with documented nutritional, physiological, and pharmacological benefits. Different extraction processes should be investigated to utilize these compounds more effectively. Green extraction processes are crucial to enhance efficiency, reduce processing time, and save energy. The antioxidant activity and total phenolic content

of the extracts prepared by the freeze-thawing technique were 1.9 times higher than those prepared by the ultrasonic-assisted conventional extraction technique. The most dominant sugar in both samples was glucose and the total sugar content was higher in the samples prepared by the freeze-thawing technique (233.89 mg/100 g DW) than by ultrasonic-assisted conventional extraction technique (166.94 mg/100 g DW). Ferulic acid 4-O-glucuronide and brevifolin carboxylate, an isocoumarin derivative, were determined as the dominant phenolic compounds in *S. platensis* extracts and the amount of total phenolic compounds was 1.5 times higher in the freeze/thaw method than in the ultrasonically assisted conventional extraction technique. *S. platensis* is known to have significant amounts of antioxidants, valuable secondary metabolites, and potential commercial applications and medicinal properties, but releasing these compounds is difficult due to the cell wall. This study determined that freeze-thawing can be a promising alternative to release bioactive compounds.

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References

Ahmed, T., Rana, M. R., Maisha, M. R., Sayem, A.S.M., Rahman, M., Ara, R. (2022) Optimization of ultrasound-assisted extraction of phenolic content & antioxidant activity of hog plum (*Spondias pinnata* L. f. kurz) pulp by response surface methodology. *Heliyon*, 8, e11109. https://doi.org/10.1016/j.heliyon.2022.e11109

Alajil Alslibi, Z. (2019). Influence of *Spirulina* and whey protein hydrolysate on growth rate and activity of some probiotic bacteria in ayran. Master of Science Thesis, Gaziantep University, Graduate School of Natural Sciences, Department of Biochemistry Science and Technology, Turkey.

Al-Dhabi, N. A., & Valan Arasu, M. (2016). Quantification of phytochemicals from commercial Spirulina products and their antioxidant activities. *Evidence-Based Complementary and Alternative Medicine*, 1-13. https://doi.org/10.1155/2016/7631864

Arranz, S., Silván, J. M., & Saura-Calixto, F. (2010). Nonextractable polyphenols, usually ignored, are the major part of dietary polyphenols: a study on the Spanish diet. *Molecular Nutrition & Food Research*, 54(11), 1646-1658. https://doi.org/10.1002/mnfr.20090058

Barwick, V. (2016). Eurachem/CITAC Guide: Guide to quality in analytical chemistry: An aid to accreditation (3rd edition). Available from www.eurachem.org.

Bernatoniene, J., Kopustinskiene, D. M. (2018). The role of catechins in cellular responses to oxidative stress. *Molecules*, 20;23(4):965. https://doi.org/10.3390/molecules23040965

Brand-Williams, W., Cuvelier, M.E., Berset, C.L.W.T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Caporgno, M.P., Mathys, A. (2018). Trends in microalgae incorporation into innovative food products with potential health benefits. *Frontiers in Nutrition*, 5, 58. https://doi.org/10.3389/fnut.2018.00058

Chaiklahan, R., Chirasuwan, N., Triratana, P., Loha, V., Tia, S., & Bunnag, B. (2013). Polysaccharide extraction from Spirulina sp. and its antioxidant capacity. *International Journal of Biological Macromolecules*, 58, 73–78. https://doi.org/10.1016/j.ijbiomac.2013.03.046

Choubey S, Varughese LR, Kumar V, Beniwal V. (2015). Medicinal importance of gallic acid and its ester derivatives: a patent review. *Pharmaceutical patent analyst*, 4(4):305-15. https://doi.org/10.4155/ppa.15.14

De la Rosa, L. A., Moreno-Escamilla, J. O., Rodrig o-García, J., & Alvarez-Parrilla, E. (2019). Phenolic compounds. *Postharvest Physiology and Biochemistry of Fruits and Vegetables*, 253–271. https://doi.org/10.1016/B978-0-12-813278-4.00012-9

Ferreres, F., Lopes, G., Gil-Izquierdo, A., Andrade, P. B., Sousa, C., Mouga, T., & Valentão, P. (2012). Phlorotannin extracts from fucales characterized by HPLC-DAD-ESI-MSn: approaches to hyaluronidase inhibitory capacity and antioxidant properties. *Marine Drugs*, 10, 2766-2781. https://doi.org/10.3390/md10122766

Gupta, S., Gupta, C., Garg, A.P., Prakash, D. (2017). Probiotic efficiency of blue-green algae on probiotics microorganisms. *Journal of Microbiology and Experimentation*, 4(4), 00120. https://doi.org/10.15406/jmen.2017.04.00120

Heffernan, N., Brunton, N. P., FitzGerald, R. J., & Smyth, T. J. (2015). Profiling of the molecular weight and structural isomer abundance of macroalgae-derived phlorotannins. *Marine Drugs*, 13, 509-528. https://doi.org/10.3390/md13010509

Kelebek, H., Jourdes, M., Selli, S., Teissedre, P.L. (2013). Comparative evaluation of the phenolic content and antioxidant capacity of sun-dried raisins. Journal of the Science of Food and Agriculture, 93(12), 2963–2972. https://doi.org/10.1002/jsfa.6125

Kuatrakul, I., Kuarthongsri, P., Yabuuchi, C., Somsai, K., Utama-ang N. (2017). Sensory descriptive analysis and physicochemical properties of *Spirulina platensis* from different drying processes: hot air drying and microwave vacuum drying, *Current Applied Science and Technology*, 17 (2).

Lee, H. S., & Coates, G. A. (2000). Quantitative study of free sugars and myo-inositol in citrus juices by HPLC and a literature compilation. *Journal of Liquid Chromatography & Related Technologies*, 23(14), 2123–2141. https://doi.org/10.1081/JLC-100100476

Maddiboyina, B., Vanamamalai, H.K., Roy, H., Ramaiah Gandhi, S., Kavisri, M. (2023). Food and drug industry applications of microalgae *Spirulina platensis*: a review. *Journal of Basic Microbiology*,63: 573–583. https://doi.org/10.1002/jobm.202200704

Maddina, B.Y., Asthana, G.S., Asthana, A. (2016). A review on current scenario of *Spirulina* drug delivery systems. *World Journal of Pharmaceutical Sciences*, 4: 86–9.

Martins, R., Mouro, C., Pontes, R., Nunes, J., Gouveia, I. (2023). Ultrasound-assisted extraction of bioactive pigments from *Spirulina platensis* in natural deep eutectic solvents. Bioresources and Bioprocessing 10. https://doi.org/10.1186/s40643-023-00692-x

Ochoa Becerra, M., Mojica Contreras, L., Hsieh Lo, M., Mateos Díaz, J., & Castillo Herrera, G. (2020). Lutein as a functional food ingredient: Stability and bioavailability. *Journal of Functional Foods*, 66, 103771. https://doi.org/10.1016/j.jff.2019.10377

Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products*. 63 (7): 1035–42. https://doi.org/10.1021/np9904509

Purdi, T.S., Setiowati, A.D., Ningrum, A., 2023. Ultrasound-assisted extraction of Spirulina platensis protein: physicochemical characteristic and techno-functional properties. *Journal of Food Measurement and Characterization* 17, 5474–5486.. https://doi.org/10.1007/s11694-023-02051-y

Saafi, E.B., El Arem, A., Issaoui, M., Hammami, M., Achour, L. (2009). Phenolic content and antioxidant activity of four date palm (*Phoenix dactylifera* L.) fruit varieties grown in Tunisia. *International Journal of Food Science & Technology*, 44, 2314–2319. https://doi.org/10.1111/j.1365-2621.2009.02075.x

Saranraj, P., & Sivasakthi, S. (2014). *Spirulina platensis* – food for future: a review. *Asian Journal of Pharmaceutical Science & Technology*, 4(1), 26-33.

Seghiri, R., Kharbach, M., Essamri, A. (2019). Functional composition, nutritional properties, and biological activities of MoroccanSpirulinaMicroalga. *Journal of Food Quality*, 1–11. https://doi.org/10.1155/2019/3707219

Shahidi, F. (2015). Antioxidants. In Handbook of Antioxidants for Food Preservation, 1st ed.; Shahidi, F., Ed.; Woodhead Publishing Series in Food Science; Technology and Nutrition: Cambridge, UK, 1–14.

Shahidi, F., & Yeo, J. (2016). Insoluble-bound phenolics in food. *Molecules*, 21(9), 1216. https://doi.org/10.3390/molecules21091216

Tan, H.T., Khong, N.M.H., Khaw, Y.S., Ahmad, S.A., Yusoff, F.M., 2020. Optimization of the freezing-thawing method for extracting phycobiliproteins from *Arthrospira* sp. *Molecules* 25, 3894. https://doi.org/10.3390/molecules25173894

Tsuchiya H. (2001). Stereospecificity in membrane effects of catechins. *Chemico-Biological Interactions*, 14;134(1):41-54. https://doi.org/10.1016/s0009-2797(00)00308-2

Uzlasir, T., Selli, S., Kelebek, H. (2023). Effect of salt stress on the phenolic compounds, antioxidant capacity, microbial load, and ın vitro bioaccessibility of two microalgae species (*Phaeodactylum tricornutum* and *Spirulina platensis*). *Foods*, 12, 3185. https://doi.org/10.3390/foods12173185

Villarruel-López, A., Ascencio, F., Nuño, K. (2017). Microalgae, a potential natural functional food source – a review. *Polish Journal of Food and Nutrition Sciences*, 67(4), 251-263. https://doi.org/10.1515/pjfns-2017-0017

Wong, C. P., & Morita, H. (2019). Bacterial type III polyketide synthases. Reference Module in Chemistry, *Molecular Sciences and Chemical Engineering*. https://doi.org/10.1016/B978-0-12-409547-2.14640-2

Tanrıseven, D., Kadiroglu, P., Selli, S., Kelebek, H. (2020). LC-DAD-ESI MS/MS-assisted elucidation of the phenolic compounds in shalgams: Comparison of traditional and direct methods. *Food Chemistry*. 305,1555, doi.org/10.1016/j.foodchem.2019.125505.

Sonmezdag, A.S., Kelebek, H., Selli, S. (2019). Effect of hulling methods and roasting treatment on phenolic compounds and physicochemical properties of cultivars ‘Ohadi’ and ‘Uzun’ pistachios (Pistacia vera L.). Food Chemistry, 272, 418 426. https://doi.org/10.1016/j.foodchem.2018.08.065.