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Effect of Ultrasonic-Assisted Enzymolysis on Protein, Phenolic Content, and Antioxidant Activity of *Chlorella* sp. Crude Extract

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Abstract

Chlorella sp. microalgae is a potential source of natural antioxidants and other bioactive compounds used in the food and pharmaceutical industries. Ultrasound pretreatments followed by enzymolysis are widely used to improve the functional properties of biological materials. This work aimed to investigate the effects of ultrasound pretreatments followed by bromelain hydrolysis on the proteins, phenolic content, and antioxidant activity of *Chlorella* sp. crude extract. The experimental design for the study involved two factors: ultrasound pretreatment time (0, 5, and 10 min) and bromelain hydrolysis time (0, 2, 4, and 6 hours). The protein was measured using Lowry assay while the total phenolic content was measured using a Folin–Ciocalteu assay. The highest protein content (7.09 ± 0.44 mg/mL) and phenolic concentrations (0.28 ± 0.01 ìg/mL) were obtained at 5 min ultrasound pretreatment and 6 hours enzymolysis. For antioxidant activity, 5 min ultrasound pretreatment followed by 4 hours of enzymolysis resulted in the highest DPPH (1,1-diphenyl-2-picrylhydrazyl radical) inhibitory activity (81.74%). Meanwhile, ultrasound pretreatment for 10 min followed by 6 hours of enzymolysis resulted in the highest ABTS (2,20 azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) inhibitory activity (94.10%). This study showed that variation in ultrasound pretreatments followed by bromelain hydrolysis could be used to improve the protein content, phenolic content, and antioxidant activity of *Chlorella* sp. crude extract.

Keywords: Antioxidant activity, bromelain, *Chlorella* sp., ultrasound

Introduction

Chlorella sp. is a species of green microalgae rich in essential amino acids with a protein content of around 40-58% dry basis (db) (Hildebrand et al., 2020). *Chlorella* sp. is also a good source of lipids (polyunsaturated fatty acids), carbohydrates (starch, cellulose, and glucan), pigments (chlorophyll and carotenoids), vitamins, and minerals (Lorenzo et al., 2023). The most significant bioactive ingredient in *Chlorella* sp. is the polysaccharide 5ØýÞ-1,3 glucan, which functions as an immunostimulator, reducing free radicals, and blood cholesterol. *Chlorella* sp. also contains sulfated polysaccharides that possess various biofunctional properties such as antiviral, antitumor, antioxidant, and anti-inflammatory effects (Andrade et al., 2018; Kiran & Venkata Mohan, 2021). *Chlorella* sp. is one of the primary sources of microalgae in marketed products (Widyaningrum & Prianto., 2021) and has been used as food or food ingredient because of its high nutritional value. The aqueous extract of *Chlorella* sp. exhibits various biological properties, including antioxidant, antimicrobial, and immunomodulatory activities (Yusuf et al., 2022; Andrade et al., 2021).

There are several methods of extracting microalgae, such as conventional methods and ultrasonic-assisted extraction (UAE). Conventional microalgae extraction methods, such as liquid-liquid or solid-liquid extraction, usually require long extraction times and massive amounts of solvents and can even cause the loss of some nutrients and bioactive compounds during extraction. Top of Form

Extraction using ultrasound (ultrasonic-assisted extraction or UAE) has been extensively studied to extract compounds with high added value from microalgae (Vernes et al., 2019). Ultrasound requires

less solvent consumption, short extraction times, and gives good extraction yields. Actually, ultrasound breaks down cellulose cell wall of microalgae hence bioactive compound can be extracted. This makes ultrasound an alternative method of sustainable extraction compared to conventional extraction methods (Pagano et al., 2021). Hildebrand et al. (2020) reported UAE technology as an effective tool for protein extraction from *Chlorella vulgaris*. Khawli et al. (2021) investigated the optimized extraction of proteins, carbohydrates, phenolic, and antioxidant compounds from the *Phaedoactylum tricornutum* under UAE pre-treatment.

Ultrasonic technology has been widely applied in food processing. Ultrasonication can be combined with enzymolysis processes to increase the overall effectiveness of the enzymolysis process (ultrasonic-assisted enzymolysis). Enzymolysis is frequently used to extract, separate, and purify biological substances during food processing. Enzymolysis is based on the science that enzymes can selectively break down plant cell walls to dissolve the target compound, thereby increasing extraction quickly. One type of enzyme that can be used for the enzymolysis process is protease (Umego et al., 2021). Proteases can be used to hydrolyze proteins into peptides that have better biofunctional properties. This peptide has the potential as an antihypertensive compound, antioxidant, opioid, antibacterial, antithrombotic and immunomodulator (Zaky et al., 2022). Bromelain (EC 3.4.22.32), a pineapple-derived cysteine protease, enjoys widespread application in the food processing industry thanks to its Generally Recognized as Safe (GRAS) status. It has the capacity to achieve a significant degree of hydrolysis, making it a promising candidate for producing bioactive peptides (Mazorra-Manzano et al., 2018). Modification of microalgae extracts by enzymatic hydrolysis has been carried out to increase their bioactivity such as the hydrolysis of *C. sorokiana* protein using bromelain, pepsin, and thermolysin enzymes (Tejano et al., 2019).

Several studies have reported the application of ultrasonic-assisted enzymolysis to increase the bioactivity and nutritional components of food extracts. Koirala et al. (2021) reported that ultrasound pretreatment followed by protein hydrolysis of Caprine milk protein succeeded in increasing dissolved protein and Caprine milk protein's antioxidant properties. Likewise, according to Yu et al. (2012), peanut (*Arachin conarachin* L.) hydrolysate's antioxidant activity was increased using ultrasonic-assisted enzymolysis. However, only a few studies have reported using ultrasonication as a pretreatment approach followed by enzymatic hydrolysis on *Chlorella* sp's chemical characteristics and bioactivity. Therefore, this study aimed to determine the effect of the length time of ultrasound pretreatment followed by the enzymolysis process using bromelain on the dissolved protein content, phenolic content, and the antioxidant activity of intact *Chlorella* sp. crude extract.

Material and Methods

Materials

The *Chlorella* sp. powder was purchased from Asiaplant, Sukoharjo, Indonesia and they were cultured in freshwater. The powder also exhibits a rich, deep green color, and fine texture. The technical grade bromelain enzyme was purchased from Mainland, China with the activity of 10,000 U/g. The analytical chemicals used were 2,2-Diphenyl-1-picrylhydrazyl or DPPH (Sigma Aldrich, USA), methanol, gallic acid, sodium hydroxide (Merck, German), sodium carbonate (Merck, German), cupric sulfate pentahydrate (Merck, German), sodium tartrate dehydrate (Merck, German), Folin–Ciocalteu (Merck, German), and Bovine Serum Albumin (BSA) (Sigma Aldrich, USA), potassium persulfate (Merck, German), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) or ABTS (Sigma Aldrich, USA).

Methods

This study used a factorial completely randomized design (FCRD) with two factors (Table 1). The ultrasonication time factor consists of three levels (0, 5, and 10 minutes), while the hydrolysis time factor consists of four levels (0, 2, 4, and 6 hours). There were 12 treatments, three replications, and 36 experimental units in total.

Table 1. Factorial completely randomized design (FCRD) of this study

Samples	Ultrasonication (US) time (minutes)	Enzymolysis or Hydrolysis using Bromelain time (hours)
Crude extract of Chlorella sp.	0 minutes	0 hours
		2 hours
		4 hours
		6 hours
Crude extract of Chlorella sp.	5 minutes	0 hours
		2 hours
		4 hours
		6 hours
Crude extract of Chlorella sp.	10 minutes	0 hours
		2 hours
		4 hours
		6 hours

Ultrasound Pretreatment and Enzymatic Hydrolysis

Chlorella sp. powder was added to distilled water at a 1:10 (w/v) ratio. The mixtures were then subjected to ultrasound (sonicators, GT Sonic, VGT 1620QTD) pretreatment following Oliveira et al. (2022) with modifications, utilizing a 20 kHz frequency for a variable time following the experimental design. The hydrolysis process was then carried out according to Tejano et al. (2019) with a modified enzyme-to-substrate ratio and hydrolysis time. The samples were adjusted to pH 7.0 and 55 °C. Bromelain enzyme in the ratio of 1:4 (enzyme: substrate) was added to the ultrasonicated samples and was incubated at different times as per the design. After hydrolysis, the temperature was increased to 85 °C around 15 min to inactivate the enzyme. Then, the suspensions were centrifuged at 7,500 rpm (Eppendorf 5804R, USA, Rotor F-34-6-38) for 15 min. Finally, the supernatants were collected and subjected to the following analysis.

Determination of Protein Content

Protein concentration was measured using a method according to Lowry et al. (1951). Lowry's reagent is composed of a total of five individual reagents. The first reagent was made by combining 2.8 g of sodium hydroxide and 14.3 g of sodium carbonate in 500 mL of distilled water. The second reagent, cupric sulfate pentahydrate weighing up to 1.4 g, was dissolved in 100 mL of purified water. The third reagent, 2.9 g of sodium tartrate, was dissolved in 100 mL of distilled water. Next, reagents 1:2:3 (100:1:3) were dissolved to create the Lowry reagent or the fourth reagent. The fifth reagent is the Folin–Ciocalteu reagent, which is diluted in distilled water (1:1). Firstly, 0.5 mL of sample solution was mixed with 0.7 mL of the Lowry reagent and allowed to stand for 20 minutes at room temperature. Then, 0.1 mL of the Folin–Ciocalteu reagent was added to the mixture. After 30 minutes, the absorbance was measured at 750 nm using a UV–visible spectrophotometer (Agilent Technologies Cary 60 UV-Vis, USA). Bovine serum albumin (BSA) was used as the protein standard.

Determination of Total Phenolic Content (TPC)

A method described by Singleton et al. (1999) was used to evaluate the total phenolic content. First, a 100 µL of sample solution was mixed with 250 µL of the Folin–Ciocalteu reagent (ratio of Folin–Ciocalteu reagent: water = 1:1, v/v) and incubated for 8 minutes. Then, a 750 µL of 10% sodium carbonate was added to the sample and incubated for 2 hours. After that, 3.9 mL of distilled water was added, and the absorbance was measured at 740 nm using a UV–visible spectrophotometer (Agilent Technologies Cary 60 UV-Vis, USA). Gallic acid was used as the standard.

Determination Antioxidant Activity

Determination of DPPH Radical Scavenging Activity

Antioxidant activity was estimated using a slightly modified version of the technique described by Yen et al. (2001). First, 0.1 mM of DPPH solution was prepared in methanol. Then, the sample solution (250 ìL) and 1750 ìL of methanol were added to 500 ìL of DPPH reagent. This was incubated for 30 minutes in the dark and the absorbance was measured at 517 nm using a UV–visible spectrophotometer (Agilent Technologies Cary 60 UV-Vis, USA). The DPPH inhibition percentage was calculated using the following equation:

$$\% \text{ DPPH inhibition} = \frac{A_{blank\text{-}}A_{sampel}}{A_{blank}} \times 100\% \quad ....(1)$$

A_{sample} is the sample's 517 nm absorbance, and A_{blank} is the blank's 517 nm absorbance.

Determination of ABTS Radical Scavenging Activity

Antioxidant activity based on ABTS free radical inhibition was carried out according to Delgado-Andrade et al. (2005). First, a 7 mM ABTS solution was prepared by weighing 72.1 g of ABTS and then dissolved in 20 mL distilled water. Next, 140 mM potassium persulfate solution was prepared by weighing 756.9 mg of potassium persulfate and was dissolved in 20 mL of distilled water. ABTS radicals were prepared by mixing 20 mL of 7 mM ABTS with 352 ìL of 140 mM potassium persulfate and stored in a dark room for 18 hours. A total of 1 mL of ABTS radicals was then diluted with 5 mL of distilled water until the absorbance of the solution reached 0.75 at 734 nm using a UV–visible spectrophotometer (Agilent Technologies Cary 60 UV-Vis, USA). Antioxidant assay of the sample was carried out by mixing 25 ìL of the sample solution and 1 mL of ABTS radical and was incubated for 6 minutes. After that, the absorbance of the sample was measured by a spectrophotometer (Agilent Technologies Cary 60 UV-Vis, USA) at a wavelength of 734 nm. The results are expressed in percent (%) of inhibition. The percentage of ABTS inhibition was calculated using the following equation:

$$\% \text{ DPPH inhibition} = \frac{A_{blank\text{-}}A_{sampel}}{A_{blank}} \times 100\% \quad ....(2)$$

A_{sample} is the sample's 734 nm absorbance, and A_{blank} is the blank's 734 nm absorbance.

Data Analysis

The analysis of variance (ANOVA) was used to statistically examine the collected data. Duncan's new multiple range test and the SPSS 22.0 program were used to calculate the significant differences. The $P<0.05$ was defined as significant differences between the samples.

Results and Discussion

Protein Content of The *Chlorella* sp. Crude Extract

The protein content values from different experimental conditions are shown in Figure 1. Ultrasound pretreatment and enzymolysis time had a significant effect ($p<0.05$) on the protein concentration of *Chlorella* sp. crude extract. It can be observed from Figure 1 that at six hours of enzymolysis, all the protein content in the extract had the highest significant ($p<0.05$) value compared to 0, 2, and 4 hours of enzymolysis. The highest protein concentrations of 7.09 ± 0.44 mg/mL were obtained at 5 min ultrasonication and six hours enzymolysis. The protein enzymolysis aims to break down the peptide bonds and to increase the free amino acid and carboxyl groups. The result is more soluble and digestible (Contreras-López et al., 2020). The rise in protein content over the enzymolysis time aligns with the findings of Koirala et al. (2021), which used pepsin to hydrolyze Caprine milk protein.

Bromelain (EC 3.4.22.32) is frequently used in food processing and can create a significant degree of hydrolysis to produce bioactive peptides (Abadía-García et al., 2021). Studies have shown that the protein isolate of *C. sorokiniana* was successfully hydrolyzed using bromelain for 4 hours with a degree of hydrolysis of 15.93% (Tejano et al., 2019). Bromelain typically hydrolyzes proteins, amides, peptides, and esters with preferential cleavage sites at the carbonyl ends of lysine, alanine, tyrosine, and glycine (Yu & Mikiashvili, 2020).

Umego et al. (2021) proposed that in ultrasonic-assisted enzymolysis, the bubbles from the acoustic cavitation phenomenon implode due to mechanical action generated by the interaction of micro-jets and shock waves, providing a highly significant mechanical shearing force. As a result, the hydrophilic groups in the protein are released during denaturation, boosting solubility, and making it more straightforward for enzymes to attach to substrates, thus enhancing the efficiency of the enzymolysis process. The shear produced by the ultrasonic waves improves the mass transfer of extractable components during enzymolysis by producing forces proportionate to the target protein or enzyme in the media. Compared to ultrasonication for 0 and 5 minutes, it is observed that there is a decrease in protein content after ultrasonication for 10 minutes and during hydrolysis time of 2, 4, and 6 hours (Figure 1). Liang et al. (2021) explained that excessive ultrasound intensity and treatment period may promote denaturation and aggregation of the unfolded protein. Changes in protein structure affect enzyme activity. The structure of a protein is critical to its function because it determines whether the protein can interact with other molecules.

TPC Analysis of *Chlorella* sp. Crude Extract

The effect of ultrasonication and hydrolysis on the TPC of *Chlorella* sp. crude extract is shown in Figure

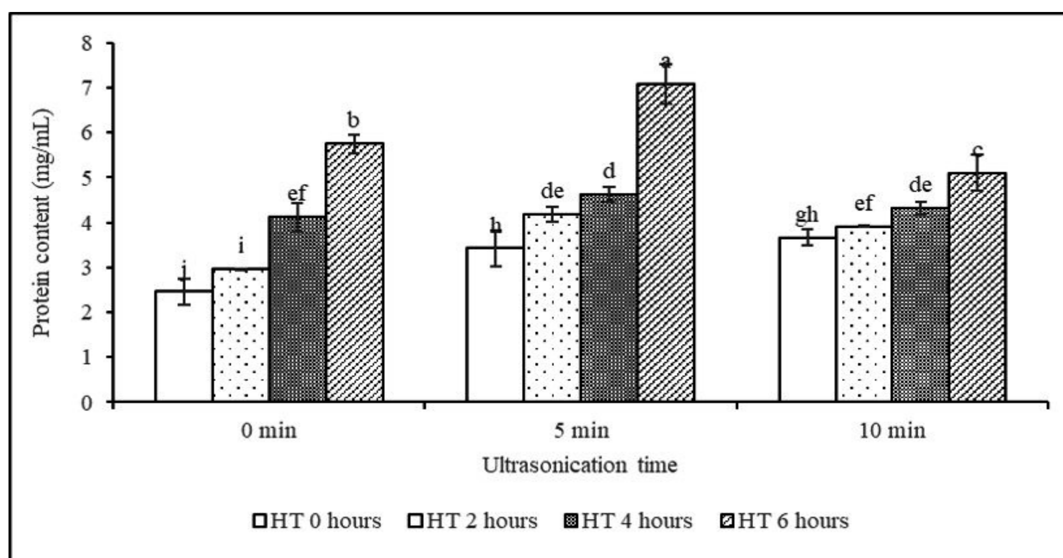

Figure 1. Total protein content of *Chlorella* sp. crude extract. HT: hydrolysis time. Different letters indicate statistically significant differences ($p < 0.05$) among the samples.

2. The TPC is influenced by the combined treatment ($p<0.05$). At each ultrasonication time, hydrolysis for 6 hours gave the best phenolic content. Meanwhile, 5 minutes of ultrasonication at each hydrolysis time provided the best phenolic content. The highest TPC of 0.28 ± 0.01 ìg/mL was obtained at 5 min of ultrasonication and 6 hours of enzymolysis. Nag and Sit (2018) also studied the polyphenols extraction on pomegranate peels using ultrasound-assisted enzymatic methods. The result showed that ultrasonication followed by enzymatic treatment can be considered a promising method to extract polyphenols. The result is also consistent with studies from Shahram et al. (2019) and Gao et al. (2022) on orange processing waste and *Empetum nigrum* phenolic compounds, respectively. Hu et al. (2020) observed that the longer the hydrolysis time with protease, the higher the polyphenol content of the hydrolysate. In their investigation, corn gluten meal was hydrolyzed using three different protease enzymes (bromelain, papain, and ficin), with bromelain providing the most excellent TPC in the fourth hour of hydrolysis. Ee et al. (2019) hydrolyzed roasted butterfly pea seeds for 2 hours with bromelain. Bromelain-treated hydrolysates had a two-fold increase in TPC compared to the non-hydrolyzed sample. Ultrasonication helps to break down the cell walls, release polyphenols, and make the substrate more accessible to the enzyme (Singla et al., 2023). The combined action of enzyme hydrolysis and ultrasonication yielded extracts with higher total phenol levels to liberate bound and unbound phenolics (Pradhan et al., 2022).

Enzymatic hydrolysis with protease, i.e., neutrase, alcalase, flavorzyme, papain, pepsin, and pancreatin, has been shown to increase the release of phenolic chemicals from flaxseed meal (Ribeiro et al., 2013). Enzymes hydrolyze and break the connection between cell components, conjugated compounds, and phenolics, releasing more phenolic compounds. The increase in non-proteinogenic parts after proteolytic treatment of the plant matrix suggests that the bound and soluble conjugated phytochemicals are released (Ee et al., 2019). Wang et al. (2010) also extracted antioxidants from the red algae *Palmaria palmata*. In comparison to cold-water extraction (control) and carbohydrases, the evaluated proteases (umamizyme, alcalase, protamex, kojizyme, neutrase, flavourzyme, and viscozyme) significantly improved the extraction of polyphenols and other active components. Protein-polyphenol complexes may be formed during extraction. When the algal cell wall is ruptured, intracellular components such as proteins are liberated, which generally combine with polyphenols, generating aggregation, and ultimate deposition. The efficiency of phenolic compound extraction using protease enzymes can be due to the conversion of proteins into small peptides and free amino acids (Wang et al., 2010). Therefore, the interaction of protein polyphenols and algae can be reduced. In this study, the longer the hydrolysis time, it is suspected the shorter peptides and amino acids may form, which could reduce the interaction of proteins with polyphenols and ultimately enhance the effectiveness of polyphenol extraction. According to Al-Dhabi et al. (2017), extending the ultrasound pretreatment period resulted in a greater breakdown of the cell components and aids in the diffusion of the polyphenols into the solvent. Nevertheless, in our study ultrasonication for 10 minutes was seen to slightly reduce TPC at hydrolysis times of 2, 4, and 6 hours.

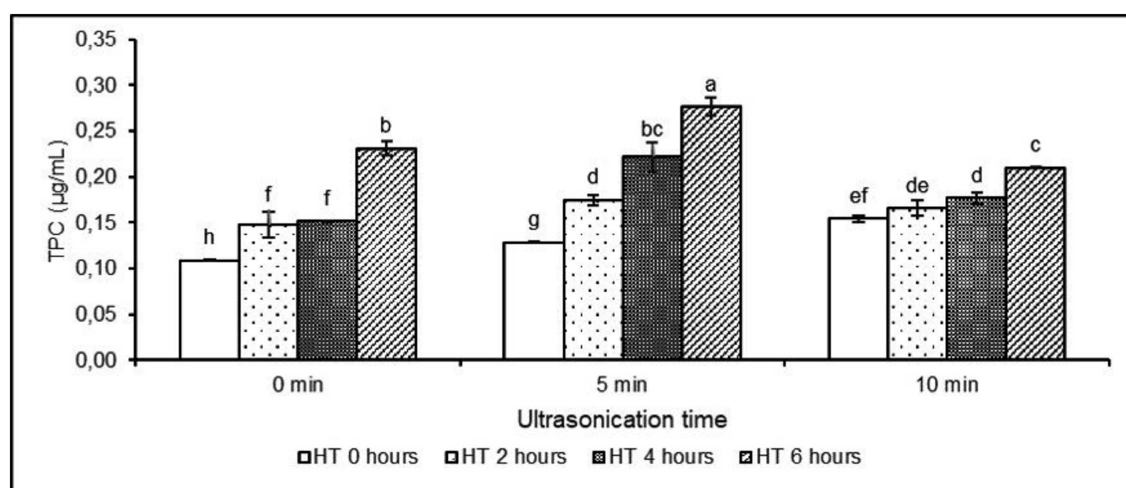

Figure 2. Total phenolic content of *Chlorella* sp. crude extract. HT: hydrolysis time. Different letters indicate statistically significant differences ($p < 0.05$) among the samples.

Determination of Antioxidant Activity of *Chlorella* sp. Crude Extract

The effect of ultrasonication and hydrolysis on the DPPH and ABTS inhibition assay of *Chlorella* sp. crude extract is shown in Figure 3. The dual effects of ultrasonication and enzymolysis affect the antioxidant properties of the *Chlorella* sp. crude extract. As shown in Figure 3, at the same ultrasound time, the percent of DPPH inhibiton of *Chlorella* sp. hydrolysates increases as the hydrolysis time ascended from 0 to 4 hours,after that the antioxidant activity of *Chlorella* sp. hydrolysates started to decline after 6 hours of hydrolysis. In the same hydrolysis time, ultrasound increases the DPPH inhibition value for up to 5 minutes of ultrasonication, and then the DPPH inhibition value will decrease, except on the 0-minute ultrasound. The highest DPPH inhibition value of *Chlorella* sp. hydrolysate (81.52 ± 1.50%) was obtained at 5 min of ultrasonication and 4 hours of enzymolysis time.

The ABTS assay (Figure 4) yields a different pattern from the DPPH assay, in which the ABTS inhibition value rises in direct proportion to the extension of the hydrolysis time at the same ultrasonication time. In this study, ABTS also yields antioxidant outcomes that exhibit a statistically more substantial disparity compared to DPPH, suggesting that the combined treatments exert a more significant influence on ABTS antioxidants. It is reported that the ABTS method offers additional flexibility as it can be employed at various pH levels, unlike DPPH, which is sensitive to acidic pH conditions. ABTS is also soluble in both aqueous and organic solvents, making it valuable for evaluating antioxidant activity across different environments, while the DPPH assay is typically carried out in a methanol/ water mixture (Shalaby & Shanab, 2013). At 4 and 6 hours of hydrolysis, Figure 4 shows that the ABTS inhibition value rises with ultrasonication time. However, for hydrolysis durations of 0 and 2 hours, the ABTS inhibition value increases up to 5 minutes of ultrasonication and then declines. The highest ABTS inhibition value of *Chlorella* sp. hydrolysate (94.10 ± 0.62%) was obtained at 10 minutes of ultrasonication and 6 hours of hydrolysis. Fadimu et al. (2021) reported that ultrasound pretreatment accompanied by flavourzyme hydrolysis increased lupin protein hydrolysates antioxidants activity with a higher ABTS inhibition value than DPPH inhibition.

ABTS and DPPH inhibition techniques were used to evaluate the antioxidant activity of *Chlorella* sp. crude extract. The chemical reaction involved in ABTS and DPPH radical-scavenging activity is a single electron transfer reaction-based test. The DPPH test employs a radical dissolved in an organic medium, thus compatible with hydrophobic systems. On the other hand, ABTS are soluble in aqueous and organic environments and can be used to evaluate the overall antioxidant activity of hydrophilic and lipophilic systems (Makuch et al., 2020; Khongdetch et al., 2022).

The increasing antioxidant activities of extracts by the combined treatments were attributed to the existence of the small protein and phenolic compounds from *Chlorella* sp. hydrolysate. Hu et al. (2020) hydrolyzed corn gluten meal using bromelain, and the highest antioxidant activity (DPPH) also occurs at the fourth hour of hydrolysis. The low-frequency energy of ultrasonication (20 kHz–1 MHz) leads to more excellent mass transfer rates due to the creation of localized pressure that causes plant tissue to be broken down and the release of bioactive cellular components (Ajila et al., 2011). Protein ultrasonication before enzymatic hydrolysis can enhance the release of bioactive peptides due to the unfolding protein and

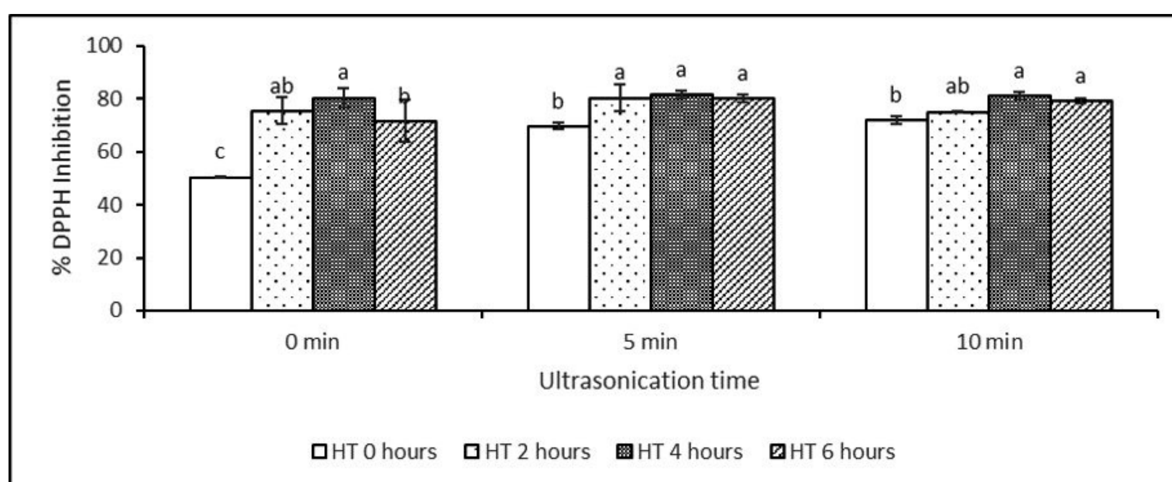

Figure 3. DPPH inhibitory activity of *Chlorella* sp. crude extract. HT: hydrolysis time. Different letters indicate statistically significant differences ($p < 0.05$) among the samples.

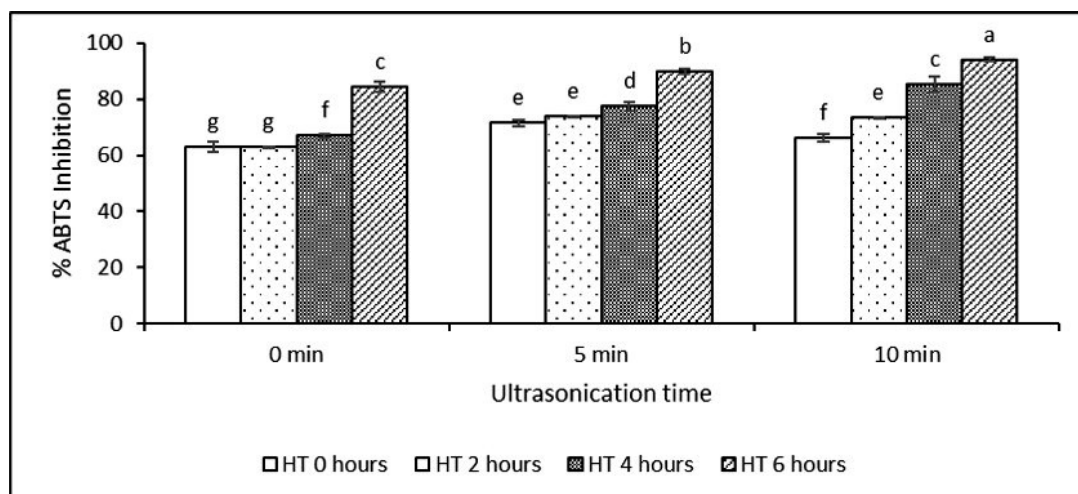

Figure 4. ABTS inhibitory activity of *Chlorella* sp. crude extract. HT: hydrolysis time. Different letters indicate statistically significant differences ($p < 0.05$) among the samples.

enhanced accessibility of the enzymes (Tawalbeh et al., 2022). Ultrasonication can cause acoustic cavitation, opening hydrophobic protein groups and facilitating enzyme peptide cleavage. Ultrasonication has been observed to impact the structural properties of protein hydrolysates, including changes in the á-helix, â-sheet, and unfolded compositions, as well as protein amide band I and II regions (Fadimu et al., 2021). In Figures 1 and 2, the total protein and phenolic contents follow a consistent pattern reflecting the ABTS antioxidant trend (Figure 4), with all three components showing an increase in content as hydrolysis or enzymolysis time extends while keeping the ultrasonication duration constant.

The structure of peptides and polyphenols affects their antioxidant properties. Different sizes and lengths of individual amino acids and peptides can be produced during the breakdown of proteins by enzymes, and these molecules are assumed to be directly related to biological activities (Kong et al., 2008). The features of the released peptides, such as their molecular weights, amino acid content, and amino acid sequence, are tightly related to the efficiency of the hydrolysates as antioxidants (Yang et al., 2021). The increase of antioxidants in *Chlorella* sp. extract could be due to the more exposed active amino acid R groups (aliphatic, aromatic, and uncharged groups, including imidazole and thiol groups) during hydrolysis (Sarmadi & Ismail, 2010). Budiari et al. (2019) also explained that the quantity of hydroxyl groups does not solely determine antioxidant activity in flavonoids and phenolics. Instead, it hinges on the configuration of phenolic hydroxyl groups and other functional groups within the molecule. In this work, *Chlorella* sp. hydrolysate with ultrasound pretreatment and bromelain hydrolysis yielded 50-90% antioxidant levels. This study demonstrates the efficacy of the combined treatments in increasing the antioxidant activity of *Chlorella* sp. crude extract. This result is also aligned with the study of Ee et al. (2019).

Conclusion

The combined treatments of ultrasonication and bromelain hydrolysis affected the protein content, phenolic content, and antioxidant activity of the *Chlorella* sp. crude extract. Ultrasonication for 5 minutes and bromelain hydrolysis for 6 hours gave the highest protein and phenolic content. In addition, the maximum DPPH inhibitory activity (81.74%) was obtained after 5 minutes of ultrasound pretreatment followed by 4 hours of hydrolysis, whereas the highest ABTS inhibitory activity (94.10%) was obtained after 10 minutes of ultrasound pretreatment followed by 6 hours of hydrolysis. ABTS yields antioxidant outcomes that exhibit a statistically more substantial disparity compared to DPPH, suggesting that ultrasonication and hydrolysis treatments exert a more significant influence on ABTS antioxidants. We conclude that the best treatment is ultrasonication for 10 minutes and bromelain hydrolysis for 6 hours because this treatment provides the best ABTS radical inhibition value with considerable total phenolic and protein content.

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Supplementary Materials

Supplementary materials is not available for this article.

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