IJC IJC

103337-Microwave-Assisted Extraction of Polysaccharides from Chlorella pyrenoidosa - Indonesian Jour

SDM Universitas Gadjah Mada

Document Details

Submission ID
trn:oid:::3618:77301957

Submission Date
Jan 6, 2025, 3:11 PM GMT+7

Download Date
Jan 6, 2025, 3:15 PM GMT+7

File Name
103337-Microwave-Assisted Extraction of Polysaccharides from Chlorella pyrenoidosa - Indonesia....pdf

File Size
845.0 KB

13 Pages

3,681 Words

21,739 Characters

17% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Match Groups

- **48** Not Cited or Quoted 16%
  Matches with neither in-text citation nor quotation marks
- **4** Missing Quotations 1%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

- 11% Internet sources
- 12% Publications
- 0% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

Match Groups

- **48** Not Cited or Quoted 16%
  Matches with neither in-text citation nor quotation marks
- **4** Missing Quotations 1%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

11% Internet sources
12% Publications
0% Submitted works (Student Papers)

Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	Internet	www.scitechnol.com 3%
2	Internet	www.ncbi.nlm.nih.gov 2%
3	Publication	Amanda de Sousa e Silva, Weuller Teixeira de Magalhães, Laís Marques Moreira, ... 1%
4	Internet	ijred.cbiore.id 1%
5	Publication	Jing Li, Jun Zhang, Minghui Tang, Junping Xin et al. "Hematopoietic Stem Cell Acti... 1%
6	Internet	link.springer.com 1%
7	Internet	www.mdpi.com 1%
8	Publication	Magdalena Kurzyna-Szklarek, Justyna Cybulska, Artur Zdunek. "Analysis of the ch... 0%
9	Publication	Yu-Ping Huang, Hsi-Mei Lai. "Bioactive compounds and antioxidative activity ofco... 0%
10	Internet	dokumen.tips 0%

11 Internet

www.neliti.com 0%

12 Publication

Meng, R.. "Investigation of ultrasound assisted regeneration of Ni-bentonite with... 0%

13 Publication

Nahid Najafian, Aazam Aarabi, Alireza Nezamzadeh-Ejhieh. "Evaluation of physico... 0%

14 Publication

Patrícia A. Caetano, Tatiele C. do Nascimento, Andrêssa S. Fernandes, Pricila P. Na... 0%

15 Internet

hdl.handle.net 0%

16 Publication

Chinglen Leishangthem, P. P. Sutar. " Innovative high power short time microwav... 0%

17 Internet

doi.nrct.go.th 0%

18 Internet

www.phcogj.com 0%

19 Publication

Jinlong Yang, Yu Feng. "An optimization high-resolution network for human pose ... 0%

20 Internet

bjbas.springeropen.com 0%

21 Publication

Bianca Zappulla Sabio, Raquel García Pacheco, Pau Vilardell Pàrraga, Itzel Alcarra... 0%

22 Internet

biotechnologyforbiofuels.biomedcentral.com 0%

23 Publication

Bing-Mei Su, Yi-Bing Shi, Wei Lin, Lian Xu, Xin-Qi Xu, Juan Lin. "A Chemoenzymatic... 0%

24 Publication

"Algae Biomass: Characteristics and Applications", Springer Science and Business ... 0%

25 Publication

Bing Yuan, Douglas A. Wicks. " Thermotropic color changing nanoparticles prepar... 0%

26 Publication

Harun, R.. "Bioprocess engineering of microalgae to produce a variety of consum... 0%

27 Publication

Kai Wu, Ke Yang, Siyu Wang, Jiajun Yu, Chenyang Chu, Bingbing Luo, Huiyan Zhan... 0%

28 Publication

Yu Fujii, Erina Yoshida, Toshiyuki Osakai. "A Strategy for in Silico Prediction of the... 0%

29 Internet

core.ac.uk 0%

30 Internet

pt.scribd.com 0%

31 Publication

Claudia L. Vargas-Serna, Claudia I. Ochoa-Martínez, Carlos Vélez-Pasos. "Microwa... 0%

32 Publication

Neha Sharma, Yugal Khajuria, Jitendra Sharma, Durgesh K. Tripathi et al. "Micros... 0%

33 Publication

Teresa Cecchi, Carla De Carolis. "Biobased Products from Food Sector Waste", Spri... 0%

Microwave-Assisted Extraction of Polysaccharides from *Chlorella pyrenoidosa* and Its Characterization

ABSTRACT

This study explored an efficient method for extracting polysaccharides from *Chlorella pyrenoidosa* using Microwave-Assisted Extraction (MAE) with water as the solvent, a technique consistent with green chemistry principles. The goal was to enhance the yield and quality of polysaccharides for their potential applications as multifunctional active ingredients in the pharmaceutical and functional food industries. Key extraction parameters, including extraction time (10, 20, and 30 minutes), temperature (80°C), and solid-to-liquid ratios (1:20, 1:30, 1:40 m/v), were systematically evaluated. The results indicated that a solid-to-liquid ratio of 1:40 m/v at 80°C for 10 minutes yielded the highest polysaccharide content (56.64%). FT-IR analysis confirmed the presence of pyranose rings in D-glucose and hydroxyl groups, while HPLC identified D-mannose (58.12%) as the predominant sugar, followed by D-glucose (34.46%), D-galactose (3.61%), and L-rhamnose (3.81%).

Keywords: *Chlorella pyrenoidosa*, Green Chemistry, Mannose, Microwave-Assisted Extraction (MAE), Polysaccharide

INTRODUCTION

Currently, there is a large interest to develop nutritious functional foods as a result of rising public awareness towards healthy food. Functional foods are defined as food products enriched with additives that offer high nutritional and energy value, while also delivering physiological benefits to human health due to the biological activity of their active ingredients. Among these, polysaccharides are often considered as a key active component and thus caused higher demand in market. Polysaccharides composed of C5 and C6 sugars serve as essential precursors for

producing sugar-based platform chemical building blocks such as furfural, glutamic acid, xylonic acid, levulinic acid, xylitol, and sorbitol. These compounds have been recognized by the U.S. Department of Energy (DOE) as “Top Value Added Chemicals from Biomass” [1]. Consequently, investigating optimal methods for extracting polysaccharides is of great importance.

Polysaccharides can be sourced from various biomass types. Signoretto *et al*., (2019) classified biomass into three distinct generations. The use of polysaccharides derived from first-generation biomass has faced criticism, particularly in the chemical industry, as it competes directly with food supplies when sourced from food crops [2]. Second-generation biomass, which includes non-food materials like lignocellulosic biomass (e.g., wood, forest residues, and agricultural waste such as bagasse, plant residues, rice straw, banana waste, and rice husks) [3], presents its own challenges. While it is non-edible, cultivating these plants requires large land areas [4] and can negatively impact soil fertility [5]. These limitations of the first and second generations have driven the development of third-generation biomass, sourced from algae, including both macroalgae and microalgae. This third-generation biomass offers several advantages, including its lack of competition with food supplies, ease and cost- effectiveness of cultivation, and high concentration of active ingredients resulting from the production of complex compounds [6,7].

One type of microalgae with great potential as a raw material for polysaccharides is *Chlorella pyrenoidosa* [7–9]. According to Yuan *et al*. (2020), the biological activities of polysaccharides in *Chlorella pyrenoidosa* include antioxidant, immunomodulatory, hypolipidemic, antitumor, anticancer, anti-aging, anti-asthma, and antiviral properties. *Chlorella pyrenoidosa* is photoautotrophic organism, relying on log meaning it requires light as an energy source for cell growth and the synthesis of various essential substances. The characteristics of the light source, such as wavelength and intensity, are critical factors influencing the production of *Chlorella pyrenoidosa* and microalgae in general [11]. *Chlorella pyrenoidosa* was first described by Chick (1903), who distinguished it from *Chlorella vulgaris* based on the presence of pyrenoids in its chloroplasts. Pyrenoids are subcellular microcompartments found in the chloroplasts of algae, certain land plants, and hornworts. They are associated with the carbon concentration mechanism. The presence of pyrenoids in *Chlorella pyrenoidosa* contributes to its significant carbohydrate content, which includes polysaccharides.

Polysaccharide extraction is typically performed using conventional methods such as maceration, mechanical calcination, and thermal flux. Each of these techniques has its own set of advantages and disadvantages. Prolonged extraction times and high temperatures can lead to the decomposition of polysaccharides, which may reduce their effectiveness as active ingredients in the pharmaceutical industry [13].

Microwave-assisted extraction (MAE) is a cell disruption method for extracting polysaccharides from microalgae and is considered as a green technology. This technique offers several advantages, including higher yields and better quality of extracts, simple equipment,

shorter extraction times, and more efficient solvent use [14]. MAE is also ideal for extracting thermolabile organic materials [15]. The microwave extraction method has been applied to various other biomasses to extract biomolecules, including *Tremella* [14], *Auricularia auricular* [16], mung beans [17], and *Ascophyllum nodosum* [18].

The structure of polysaccharides can vary significantly based on cultivation conditions, harvest seasons, and different extraction methods [19]. Previous studies have shown that arabinogalactan can be extracted from hot water extracts of freeze-dried *Chlorella pyrenoidosa* cells [20]. When *Chlorella pyrenoidosa* was extracted using ultrasound-assisted extraction (UAE), the highest concentration of mannose was obtained [9], while extraction with UAE followed by ultrafiltration resulted in the highest galactose content [21]. These experiments were carried out using water as a solvent.

The use of water as a solvent offers several benefits, including lower costs and alignment with green chemistry principles [22]. Green chemistry principles include: 1) using renewable resources; 2) using alternative solvents (e.g., water or oil); 3) reducing energy consumption through innovative technologies; 4) producing by-products rather than waste; 5) minimizing unit operations; and 6) producing uncontaminated extracts that can biodegrade without contamination [23].

In this context, it is essential to assess the parameters of Microwave-Assisted Extraction (MAE) for obtaining polysaccharides from the microalgae *Chlorella pyrenoidosa* using water as a solvent. Determining the optimal extraction conditions and performing proper characterization are crucial to achieve the maximum yield of biomolecules in accordance with green chemistry principles. Factors such as solid-liquid ratio, extraction temperature, and extraction time play a significant role in influencing the efficiency of MAE and can substantially enhance the polysaccharide yield [24,25].

The objective of this study is to extract polysaccharides from *Chlorella pyrenoidosa* using microwave-assisted extraction (MAE) with water as the solvent. The research aims to evaluate the efficiency of MAE in maximizing the yield of polysaccharides, while maintaining their structural integrity and functional properties. Additionally, the study seeks to optimize the extraction parameters, including extraction time, temperature, and ratio solid-to water ratio establish a green, sustainable, and effective method for polysaccharide recovery.

EXPERIMENTAL SECTION

Materials and Chemicals

Dried *Chlorella pyrenoidosa* in powdered form, with a particle size of 100 µm, was obtained from PT Algaepark, Polanharjo, Klaten, Central Java, Indonesia. Additional materials, including ethanol, CTAB (cetyl trimethylammonium bromide), *d*-glucose (Glu), *l*-rhamnose (Rha), *d*-galactose (Gal), and *d*-mannose (Man), from Merck. All chemicals and reagents used were of analytical grade.

Microwave-Assisted Extraction

The schematic of the microwave extraction apparatus is presented in Figure 1. *Chlorella pyrenoidosa* powder was mixed with distilled water in the reactor, with variations in extraction time, solid-liquid ratio, and temperature. Each experiment was conducted in triplicate..

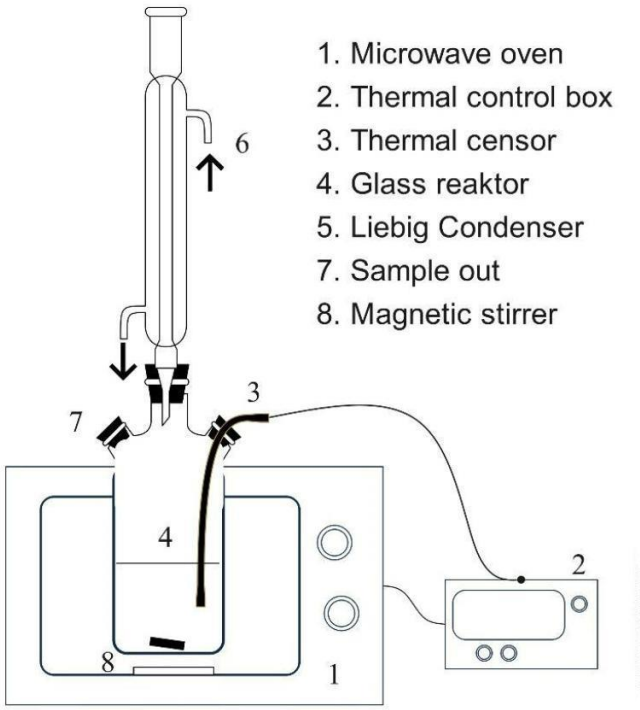


Fig 1. Schematic of Microwave-Assisted Extraction (MAE)

The equipment used in this study included a microwave with the following specifications: a maximum rated output power of 800 W, a voltage of 220 V, a rated input power of 1250 W, and a magnetron frequency of 2450 MHz (12.45 GHz). Pyrex three-neck flasks (1000 mL) served as the extraction reactors for the MAE method. These flasks were fitted with a condenser for cooling and two openings, one for a thermocouple and another for sampling. The commercial microwave employed in the extraction process was equipped with a thermocouple and a stirrer to ensure uniform mixing and temperature monitoring.

In this study, the variables were tested in stages. Extraction was carried out with varying times of 10, 20, and 30 minutes, keeping the polysaccharide-to-water ratio constant at 1:20 and the temperature at 80°C. Based on the yield results, the extraction time was used to determine the variation in solid-liquid ratios, which were adjusted to 1:20, 1:30, and 1:40 m/v at 80°C. Then, for the time and ratio that produced the best yield, further variations in extraction time were tested at 10, 20, and 30 minutes. All treatments were repeated three times.

After the polysaccharides were extracted using microwave-assisted extraction, the sample was centrifuged at 3500 rpm for 10 minutes, and the supernatant was collected. The extraction residue was then dried at 55°C for approximately 3 hours. After separation, the supernatant was deproteinized and precipitated using CTAB (Cetyltrimethylammonium bromide).

Characterization of *Chlorella pyrenoidosa* Cells

Proximate analysis was performed to quantitatively determine the chemical composition of the sample. The morphological characteristics of the microalgae particles before and after microwave extraction were examined in dry conditions using scanning electron microscopy (SEM) (Jeol, JSM-6510 (LA), Japan) [26]. The identification of functional groups within the carbohydrate components was carried out using FTIR. FTIR analysis was conducted with a Thermo Scientific Nicolet iS10 equipped with a Deuterated TriGlycine Sulfate (DTGS) detector. The testing was performed within the wave number range of 4000-400 cm^{-1} at room temperature (25°C) and 57% humidity, allowing for the absorbance of the sample to be measured [27].

Determination of carbohydrates from *Chlorella pyrenoidosa* extract

The concentration of polysaccharides was determined using the Phenol-Sulfuric Acid Method [28] employing a UV-Vis spectrophotometer at a wavelength of 490 nm, with distilled water as the blank solution and glucose as the standard solution. The polysaccharide composition was analyzed using High-Performance Liquid Chromatography (HPLC) with an Aminex YMC-Pack Polyamine II/S-5µm/12nm detector. The eluent consisted of a 75:25 acetonitrile/water mixture, with a flow rate of 1.00 mL/min at 40°C.

RESULTS AND DISCUSSION

Effect of Various Temperatures

Polysaccharide yield was determined using the Dubois phenol-sulfuric acid method with a UV-VIS spectrophotometer and D-glucose as the standard. A standard curve was generated based on various glucose concentrations (Figure 2).

Figure 2 shows that the regression equation obtained was 퓋 = ㄷㅅ ㄷㅈ ㄹㄱㄱ ㄸㄸ. ㄷㅅ퓋 − ㄷㅂ ㄹㅎ ㄷㅈ. ㄷㅅ 퐑ㄷㅅ = with an ㄸㄸ. . These results were used to calculate the polysaccharide concentration in the MAE ㄹㄹㄱ ㄷㅈ ㄷㄷ ㄷㅈ extract. The R^2 value being close to 1 indicates that the curve can be used as a standard curve for determining yield in this experiment. In this study, the effect of temperature (with a reaction time of 10 minutes and a solid-liquid ratio of 1:30) on the yield of polysaccharides was observed using the Dubois method [28]. Figure 3 shows the impact of temperature on polysaccharide yield at different reaction times.

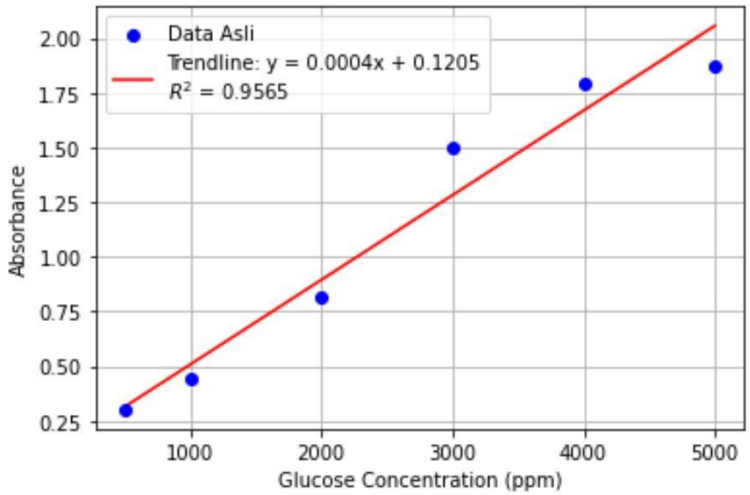


Fig 2. Mean absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 490 nm for the standard D-glucose solution, with three repetitions.

Figure 3 shows the effect of the extraction temperature on polysaccharide yield. As seen in Figure 3, the highest yield reach at extraction temperature 70°C.

Fig 4. Polysaccharide yield (%) as a function of solid-liquid ratio (m/v). Data shown are the averages from triplicate experiments.

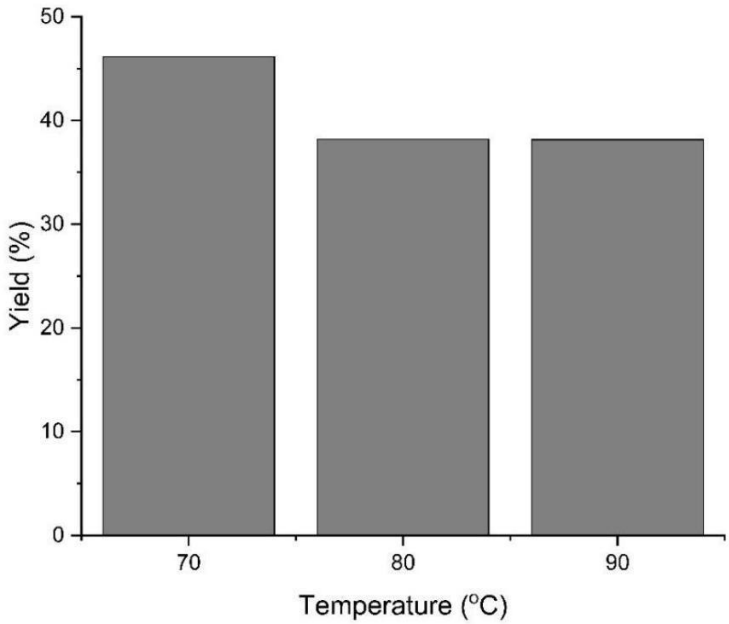


Fig 3. Polysaccharide yield (%) as a function extraction temperature (°C) Data shown are the averages from triplicate experiments.

In Figure 3, it can be seen that the highest yield was obtained at an extraction temperature of 70°C per unit time, which was 46.17%. When the temperature was increased to 80°C and 90°C, the polysaccharide yield was lower. The use of MAE allows for precise temperature control, minimizing the risk of polysaccharide degradation and ensuring optimal yield. A stable temperature also helps in producing consistent extract products [29]. A temperature of 70°C facilitates the rapid breakdown of cell walls and membranes, thus accelerating the polysaccharide extraction process

[30]. This rapid heating improves reaction kinetics, allowing the active ingredient to be extracted more efficiently. It also helps maintain the molecular structure of the polysaccharide, preserving its quality and biological activity. Polysaccharides extracted at this temperature have better potential for use in pharmaceutical and nutraceutical applications [7,31]. Temperatures lower than 70°C can reduce the efficiency of cell wall and membrane breakdown, thus lowering the yield of extracted polysaccharides. Additionally, lower temperatures may require a longer extraction time, which can increase the risk of polysaccharide degradation. When the extraction temperature exceeds 70°C, the yield decreases due to the risk of polysaccharide degradation at higher temperatures. Elevated temperatures can damage the molecular structure of polysaccharides, diminishing the quality of the resulting extract. Furthermore, excessively high temperatures can cause burning or unwanted chemical changes in the polysaccharides [32].

Effect of Biomass-to-Water Ratios on Polysaccharide Yield

Figure 4 shows the effect of the solid-liquid ratio of microalgae to water on polysaccharide yield. As seen in Figure 4, the yield increases with the solid-liquid ratio [33].

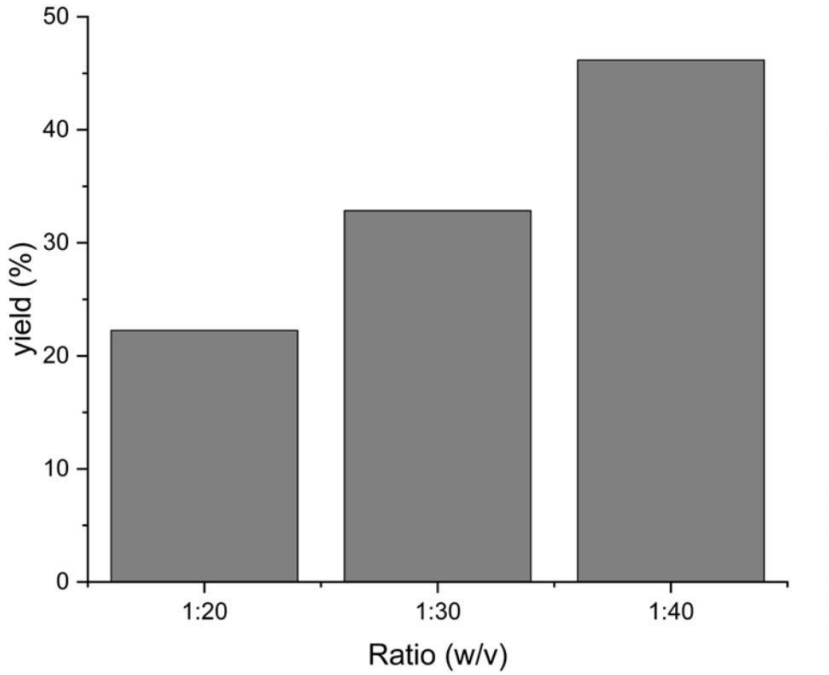


Fig 4. Polysaccharide yield (%) as a function of solid-liquid ratio (m/v). Data shown are the averages from triplicate experiments.

The data presented in Figure 4 shows the yields from the extraction of polysaccharides from *Chlorella pyrenoidosa* at a constant temperature of 80°C and a 10-minute extraction time, using various solid-liquid ratios. The variation in the solid-liquid ratio affected the yield. At a ratio of 1:20, the yield was relatively low, averaging around 22%. As the ratio increased to 1:30, the yield significantly improved, reaching values around 32-35%. The highest yield was observed at a ratio of 1:40, with values ranging from 36% to 56%.

Higher solvent ratios likely improve the solubility of the polysaccharides, leading to increased yields. The 1:40 ratio appeared to be optimal in this experiment, indicating the point at which solvent volume maximizes the yield. As the solid-liquid ratio increases, the diffusion of

polysaccharides from the cell wall into the solvent becomes more efficient, suggesting that solvent volume plays an important role in increasing yields [34]. Extraction with a higher water content results in more efficient diffusion of polysaccharides from the surface of solid particles. Increasing the water-to-raw material ratio enhances solvent penetration into the cells and improves the desorption of polysaccharides from the particles [35].

Effect of Times on Polysaccharide Yield

Figure 5 shows the effect of times on polysaccharide yield. Reaction time is a critical factor in determining the efficiency and outcome of chemical processes. In many catalytic reactions, the duration of the process significantly influences the yield and product quality. Reaction kinetics, intermediate formation, and side reactions are all time-dependent factors that must be optimized to enhance process efficiency. For industrial applications, determining the optimal reaction time is essential to balancing yield and cost-effectiveness. This study investigates the effect of reaction time on yield percentage at three intervals: 10, 20, and 30 minutes. The findings aim to provide insights into the optimal time for achieving maximum yield under specified conditions.

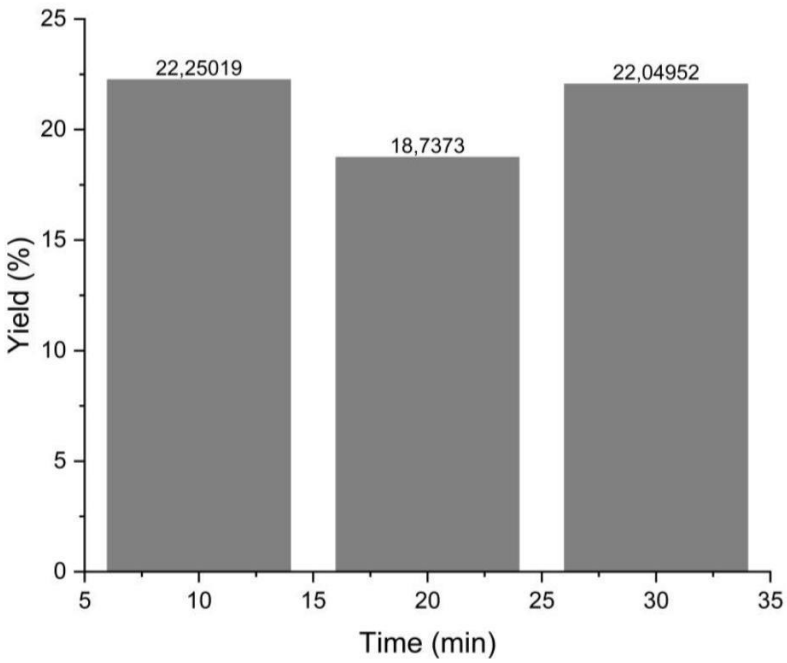


Fig 5. Polysaccharide yield (%) as a function of time (min). Data shown are the averages from triplicate experiments.

Fig 5 illustrates the influence of reaction time on yield percentage, evaluated at three distinct intervals: 10, 20, and 30 minutes. The highest yields were observed at 10 minutes (22.25%) and 30 minutes (22.05%), while the lowest yield (18.74%) occurred at 20 minutes. The results suggest that the yield demonstrates a non-linear trend with respect to time, peaking at the initial and final measured intervals. This behavior might be indicative of reaction kinetics where equilibrium is reached at certain time points, leading to either higher product formation or stabilization of intermediate species. The drop in yield at 20 minutes could be attributed to potential side reactions, incomplete reaction progress, or an intermediate stage before reaching equilibrium. The marginal

difference between the yields at 10 and 30 minutes could signify that extending the reaction beyond 10 minutes offers minimal improvement in yield. This insight is crucial for optimizing reaction time in industrial processes, as prolonged reactions might unnecessarily increase operational costs without significant gain in yield. Future studies could benefit from exploring additional time intervals and analyzing other factors, such as temperature, catalyst concentration, and reactant ratios, to better understand the reaction dynamics and optimize conditions for maximum yield.

Proximate Characterization

The selection of *Chlorella pyrenoidosa* as the raw material was based on its carbohydrate content, as summarized in Table 1. Among the microalgae species, *Botryococcus braunii* has the lowest carbohydrate content at 2%, while *Spirogyra* sp. has the highest, ranging from 33% to 64%. *Chlorella pyrenoidosa* contains approximately 26% carbohydrates, placing it in the medium range compared to other microalgae. Its carbohydrate content is notably higher than that of *Chlorella vulgaris*, by 12-17%. This makes *Chlorella pyrenoidosa* a suitable choice, offering a clear distinction from other *Chlorella* species based on carbohydrate content.

Table 1. *Chlorella pyrenoidosa* Composition [36,37]

Strain	Protein	Carbohydrate	Lipid
Anabaena cylindrica	43–56	25–30	4–7
Botryococcus braunii	40	2	33
Chlamydomonas rheinhardtii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	41–58	12–17	10–22
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Dunaliella tertiolecta	29	14	11
Euglena gracilis	39–61	14–18	14–20
Porphyridium cruentum	28–39	40–57	9–14
Prymnesium parvum	28–45	25–33	22–39
Scenedesmus dimorphus	8–18	21–52	16–40
Scenedesmus obliquus	50–56	10–17	12–14
Scenedesmus quadricauda	47	–	1.9
Spirogyra sp.	6–20	33–64	11–21
Spirulina maxima	60–71	13–16	6–7
Spirulina platensis	42–63	8–14	4–11
Synechoccus sp.	63	15	11
Tetraselmis maculata	52	15	3

The results of the proximate analysis of the Chlorella pyrenoidosa contents used in this study are shown in Table 2. The proximate analysis was conducted to determine the potential of Chlorella pyrenoidosa based on the composition of its carbohydrate and polysaccharide contents.

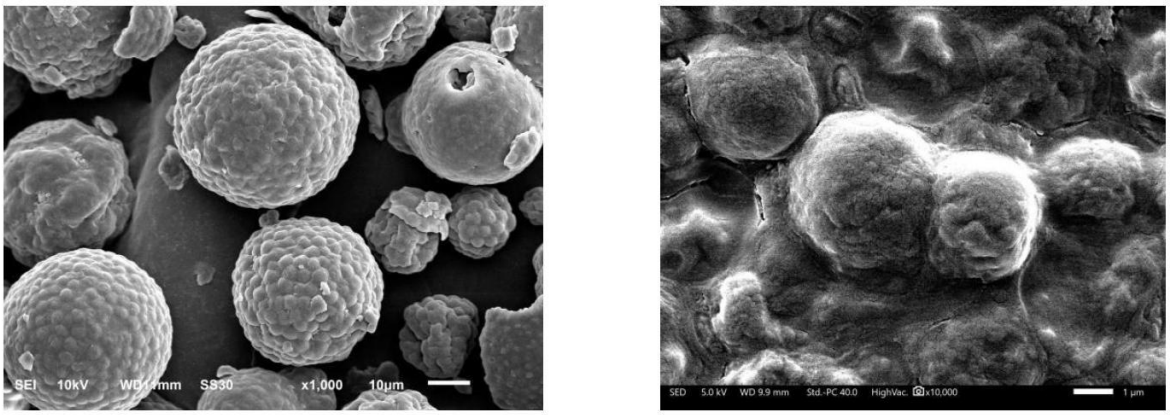
Table 2. *Chlorella pyrenoidosa* Characteristics

Component	*C. pyrenoidosa* (%)
Water	5.57
Ash	4.79
Lipid	1.052
Protein	57.91
Hemicellulose	30.68

The high hemicellulose content, which is part of the polysaccharides to be extracted, makes Chlorella pyrenoidosa used in this study noteworthy. As a type of freshwater green algae found in tropical regions, it is likely to have a higher hemicellulose content compared to microalgae cultivated in other areas [38][39].

SEM Characterization

Figure 6 illustrates the microalgae *Chlorella pyrenoidosa* before and after MAE. The rapid heating during MAE generates high internal pressure, weakening the cell wall of *Chlorella*



(a) (b)

Figures 6. (a) *C. pyrenoidosa* powder as the raw material; (b) *C. pyrenoidosa* crude polysaccharide obtained after MAE

pyrenoidosa and making it more prone to breakage. This disruption enhances the efficiency of extracting active compounds such as polysaccharides and lipids**.**

Figure 6(a) shows *Chlorella pyrenoidosa* powder used as the raw material, analyzed by SEM at 10,000x magnification, while Figure 6(b) depicts the crude polysaccharide obtained after MAE, also at 10,000x magnification. The images reveal that the particle size after extraction becomes smaller, more fragile, and agglomerated. The application of MAE leads to a reduction in the particle size of *C. pyrenoidosa* cells, increasing the surface area available for interaction with the solvent and thereby enhancing the extraction yield. Furthermore, microwaves improve cell membrane permeability, facilitating the release of intracellular components. This enhanced permeability reduces the diffusion barrier between the cell's active ingredients and the solvent, expediting the extraction process [29,40].

FTIR Characterization

Figure 7 presents the infrared spectra of the MAE purification results for the *Chlorella pyrenoidosa* polysaccharide fraction, which has undergone deproteinization and precipitation using CTAB. The spectra were recorded in absorbance mode within the mid-infrared region (4000–400 cm^{-1}), with triplicate recordings performed for each sample.

Infrared spectroscopy of mono- and oligosaccharides plays a significant role in elucidating small molecule structures and analyzing polymers representing structural units. In carbohydrate analysis, the anomeric region (950–750 cm^{-1}) is particularly important, as it reveals characteristic bands of *α* and *β* conformers, as well as vibrations of pyranoid and furanoid rings in mono- and polysaccharides. For instance, the *α* and *β* conformers of glucose, galactose, and mannose can

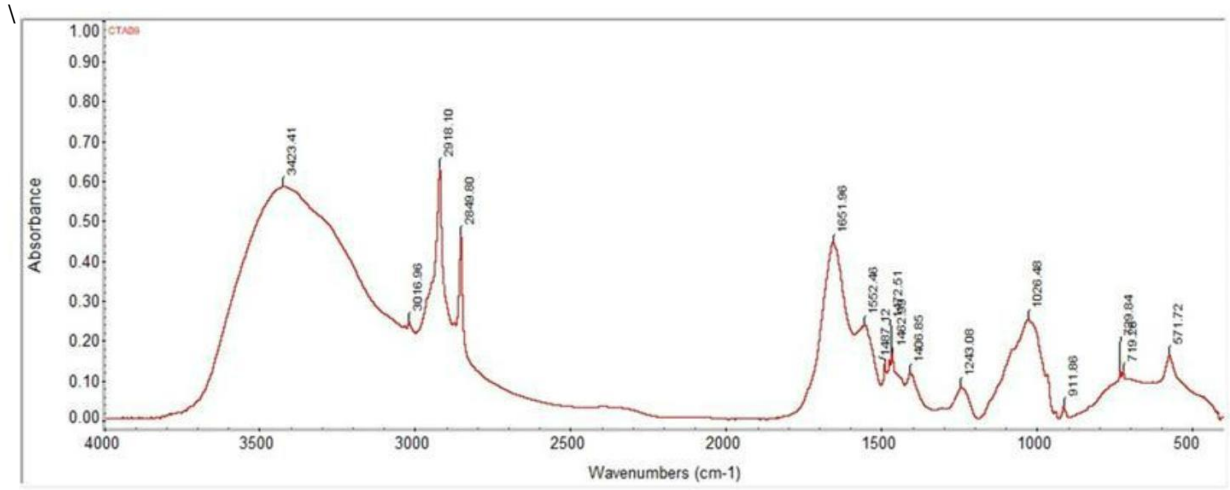


Fig 7. The IR spectra of polysaccharide of *Chlorella pyrenoidosa*

be distinguished by bands 2a and 2b at 870–840 cm^{-1} and 890 cm^{-1}, respectively [41]. The FT-IR spectrum of the sample shows a peak at 911 cm^{-1}, attributed to the stretching vibration of the pyranose ring, identifiable as D-glucose [41]. Distinguishing oligosaccharides from their hydrolysis products is facilitated by prominent spectra in the regions of 1160–1150 cm^{-1} and 1000–960 cm^{-1}, which can also differentiate non-reducing sugars from reducing sugars [42]. Additionally, a peak at 1651 cm^{-1} corresponds to the stretching vibration of the carbonyl bond in the amide group of the N–H bond, indicating the presence of protein. The band at 3423 cm^{-1} arises from the stretching vibration of the hydroxyl group in polysaccharides, while the band at 2918 cm^{-1} is due to the stretching vibration of C–H bonds [43].

HPLC Characterization

Based on the experimental results, the optimal yield was obtained at a temperature of 80°C, an extraction time of 10 minutes, and a solid-liquid ratio of 1:40 m/v (sp-15). Subsequently, characterization was carried out to determine the composition of monosaccharides and disaccharides using HPLC (Figure **8**).

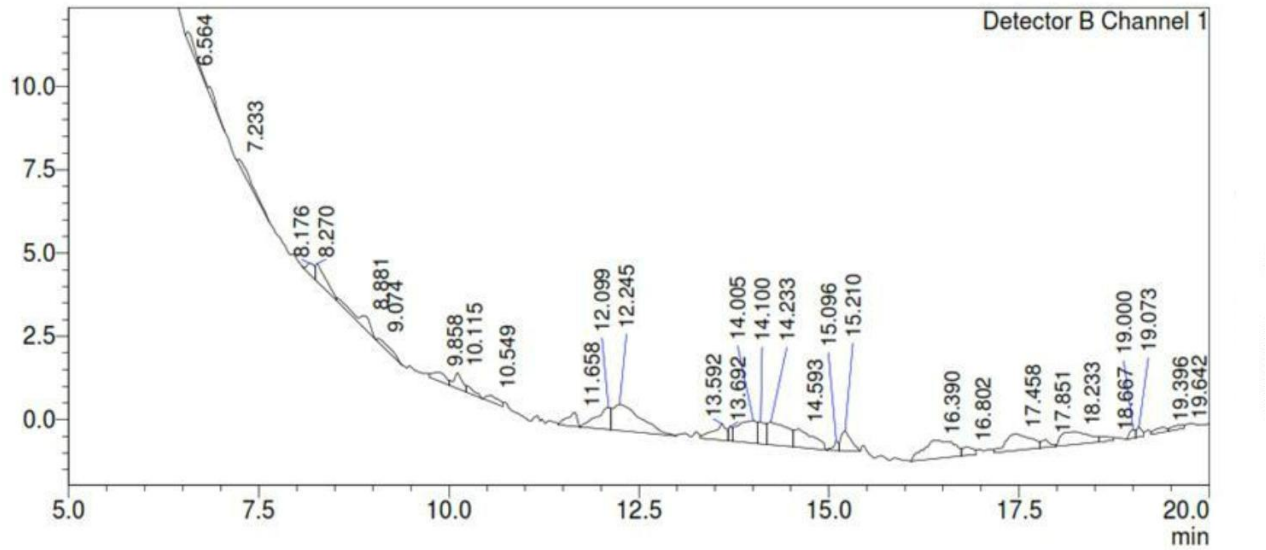


Fig 8. HPLC analysis of polysaccharides from *Chlorella pyrenoidosa* extracted using MAE for 10 minutes, with a solid-liquid ratio of 1:40 (m/v) at 80°C (sp-15).

HPLC calibration was performed using standard saccharide solutions of D-glucose, D-galactose, D-mannose, and L-rhamnose in triplicate to generate a standard curve and determine the retention times for each standard. When sp-15 was injected into the HPLC, the resulting peaks identified the retention times of each component, allowing the calculation of peak areas, which correspond to the concentrations in the sample. The retention time analysis for sp-15 revealed that the polysaccharide composition consisted of D-glucose, D-galactose, D-mannose, and L-rhamnose.

The experimental results from the HPLC analysis are summarized in Table 3. It is evident that the dominant monosaccharide in the sample is D-mannose, accounting for 58.12%, followed by D-glucose at 34.46%, L-rhamnose at 3.81%, and D-galactose at 3.61%. The polysaccharide yield was determined to be 56.64%.

Table 3. Yields and sugar composition of *C.pyrenoidosa* extraction using MAE

Sample	Yield (%)	Sugar Component (%)			
		D-Glucose	D-Galactose	D-Mannose	L-Rhamnose
Sp-15	56.64	34.46	3.61	58.12	3.81

CONCLUSION

The MAE method proved effective in extracting polysaccharides from *Chlorella pyrenoidosa*, achieving a yield of 56.64% under optimal conditions (80°C, 10 minutes, solid-liquid ratio 1:40 m/v). D-mannose was identified as the dominant monosaccharide component in the extracted

polysaccharides, highlighting its significant potential for pharmaceutical and nutraceutical applications.