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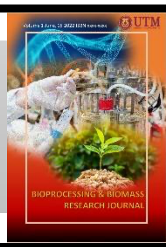

Research Article

# Carbohydrate Extraction from Brown Algae Using Green Technology Approach - Ultrasonic Assisted Extraction (UAE)

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**ABSTRACT**

Over the past decade, conventional methods have been widely employed for extracting carbohydrate from brown algae, involving significant quantities of acid and alkaline chemicals, lengthy processing times, and high energy requirements. However, in line with the current global emphasis on environmental friendliness, the focus has shifted towards green technologies in the extraction process. Green technologies usually refer to non-conventional methods, with Ultrasonic-Assisted Extraction (UAE) being one of the most commonly utilized techniques. This research paper focuses on the extraction of carbohydrates from brown algae using a green technology approach, specifically UAE. Central composite design (CCD) was used in this study with different combinations of parameters such as temperature (30-60 °C), time (15-45 min) and material to solvent ratio (1:15-1:35). The best operating parameters that obtained the highest carbohydrate yield are the material-to-solvent ratio of 1:35 g/mL, extraction time of 30 min, and extraction temperature of 45 °C. The highest yield of carbohydrate obtained was 7.73 mg/g. These findings will contribute to the development of sustainable and efficient methods for carbohydrate extraction from brown algae using green technology approaches.



## INTRODUCTION

As the awareness of functional ingredients derived from food continues to rise, seaweeds are garnering attention as a promising source of bioactive compounds. The consumption of seaweed as both a standalone food and as an ingredient was initially documented in Asian regions. Carbohydrates from brown seaweeds ranked among the crucial and extensively utilized compounds in the food industry and nutraceutical companies.

These compounds are employed as natural additives to improve food quality and as ingredients to augment nutritional value, particularly due to their dietary fiber content. Studies found that there are valuable components from carbohydrates that able to be extracted from brown seaweed, specifically fucoidan, alginate and laminarin (Li et al., 2021).

The varied biochemical composition of carbohydrates has contributed to a wide range of scientific investigations exploring its potential bioactivities and applications. Fucoidan, alginates, and laminarin have demonstrated promising biological and pharmacological properties, including heparin-like antioxidant, anticoagulant, antitumor, anti-angiogenic, anti-inflammatory, anti-hyperglycaemic, antiviral, and immunomodulatory bioactivities. Furthermore, products containing fucoidan, alginates, or laminarin are widely consumed for their nutraceutical and health-promoting benefits, attributed to unique molecular mechanisms.

The effectiveness of the components extracted such as fucoidan depends on various factors, such as the source of seaweed, its composition and structure, the concentration and location of sulphate substitutions, and the purity of the

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extracted product (Zhang & Row, 2015). Therefore, it is crucial to utilize an appropriate extraction process to preserve the structural integrity and relevant features necessary for specific biological activity. A standardized extraction methodology is also required to understand the relationship between polysaccharide structures and biological functions. The absence of such a standardized extraction process hinders a detailed comparison and, more importantly, impedes the comprehension of the correlation between polysaccharides structure and biological activity. Overall, studies currently highlighted the need to figure out the extraction method for carbohydrates in effective ways with greener and cheaper technologies to use the carbohydrates in various industries, especially the food and pharmaceutical industries.

Ultrasonic extraction also known as ultrasound-assisted extraction (UAE), is currently a common conventional technique used to extract solid samples, including medicines derived from plants. This method is based on the application of sound waves that travel through the solvent molecules and undergo a number of cycles of compression and rarefaction (Garcia-Vaquero et al., 2017). This method has several advantages such as being cost-effective, easy to use, reproducible, suitable for thermolabile compounds, and reducing extraction time, energy, chemicals, and solvents. Additionally, it can increase the yield of extracted materials.

Up to now, the information on the best operating conditions for the highest carbohydrate that could be obtained from brown algae using UAE is limited. Brown algae such as *Sargassum mcclurei* and *Nizamuddinia Zarnardinii* have been used for the extraction of polysaccharides using the UAE technology as stated in the papers of Thao My et al. (2020) and Alboofetileh et al. (2019). However, the operating conditions for the highest polysaccharide extraction vary between brown algae species. Operating parameters such as temperature, the solvent used, extraction time, and the material-solvent ratio varies in different papers. Besides, the polysaccharide's composition extracted using various operating conditions also varies. A longer extraction time or a very high temperature might cause these polysaccharides to degrade, which then reduces the number of polysaccharides that could be obtained. An optimum extraction time and temperature are needed to prevent the degradation or loss of desired products Owing to this, proper control of UAE operating conditions is required to enhance the process and obtain the desired product of interest, which is carbohydrate. The objective of this research is to determine the optimum UAE operating condition in the extraction of carbohydrates from brown algae *Padina sp*.

## MATERIALS AND METHOD

### Materials and Apparatus

The materials used in this study included *Padina sp.* (brown algae), distilled water (solvent), 5% phenol (Sigma-Aldrich), 96% sulfuric acid (Sigma-Aldrich), and glucose (Sigma-Aldrich) stock solution. The apparatus used included a conical flask, pipette, spatula, thermometer, test tube, volumetric flask, volumetric flask stopper, measuring cylinder, beakers, filter funnel, electronic balance, ultrasonic generator, centrifuge, High-Performance Liquid Chromatography (HPLC), and UV-VIS spectrophotometer.

### *Padina sp.* Sample Preparation

The brown algae *Padina sp*. in this study was collected at the Blue Lagoon, Port Dickson. The fresh *Padina sp*. was washed with clean water to remove sand and debris. Then, the clean *Padina sp.* was sun-dried for several days and milled into desired particle sizes by a blender.

### Ultrasonic-Assisted Extraction of Carbohydrate

The experiment was performed based on the experimental design generated by Design Expert Version 13.0 (Stat Ease Inc., USA). The central composite design (CCD) was used to design a total of 20 experimental runs with different combinations of parameters as shown in Table 1. After extraction, each sample undergoes centrifugation at 5000 rpm for 30 min at 4 °C to separate the extract into supernatant and pellets. The supernatant was collected as a sample to continue for carbohydrate content.

### Analysis of Carbohydrate Content

The carbohydrate content was analysed using phenol-sulfuric method with D-glucose as the standard (Nielsen. 2010). A 2 mL aliquot of carbohydrate solution was mixed with 1 mL 5% aqueous solution of phenol in a test tube. Subsequently, 5 mL of concentrated sulfuric acid is added rapidly to the mixture. Then, the test tube was allowed to stand for 10 min and water bath for 20 min at room temperature for colour development. The absorbance value was determined using a spectrophotometer at 490 nm.

## RESULTS AND DISCUSSION

**Figure 1** shows the calibration curve for the standard glucose stock solution. The linear equation of the calibration curve with an equation of y = 0.0159x + 0.0551 with a $R^2$ value of 0.9843. This implies that the standard calibration curve can represent the carbohydrate content concentration with high reliability.

**Table 1** illustrates the yield of the carbohydrate for the actual and predicted values under 20 various conditions. In Central Composite Design (CCD), the predicted value is determined using the model equation generated from the regression analysis of the experimental data. The model equation generated in this study is shown in **Equation 1**. From **Table 1**, run 2 obtained the highest carbohydrate content (7.73 mg/g), while run 5 obtained the lowest carbohydrate content which was 3.10 mg/g. The run with the highest carbohydrate content was extracted with the operating condition of material to solvent ratio of 1:35, extraction time of 30 min, and extraction temperature of 45 °C. Thao My et al. (2020), obtained 7.1203 mg/g of total polysaccharides using UAE method with ethanol as solvent to extract polysaccharides from *Sargassum mcclurei*. The optimal conditions are material to solvent ratio of 1:24 g/mL, extraction time of 49 min, and extraction temperature of 54 °C (Thao et al., 2020).

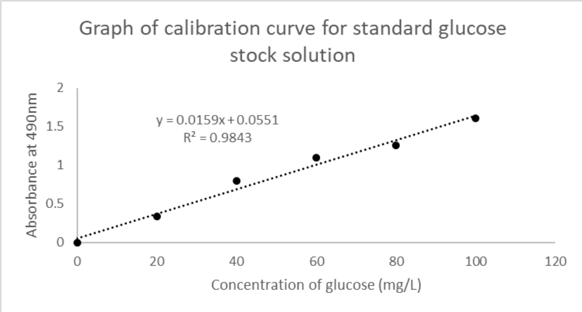


**Figure 1** Calibration curve for standard glucose stock solution

**Table 1** Experimental design parameter with response of actual and predicted carbohydrate content.

| Run | Material to solvent ratio (g/ml) | Time (min) | Temperature (°C) | Carbohydrate content (mg/g) | |
|---|---|---|---|---|---|
| | | | | Actual | Predicted |
| 1 | 1:25 | 30 | 60 | 5.65 | 5.31 |
| 2 | 1:35 | 30 | 45 | 7.73 | 7.33 |
| 3 | 1:25 | 30 | 30 | 5.24 | 5.21 |
| 4 | 1:25 | 30 | 45 | 5.14 | 5.22 |
| 5 | 1:15 | 45 | 60 | 3.10 | 3.16 |
| 6 | 1:15 | 30 | 45 | 3.25 | 3.28 |
| 7 | 1:35 | 45 | 60 | 7.16 | 7.29 |
| 8 | 1:25 | 15 | 45 | 5.17 | 5.04 |
| 9 | 1:25 | 45 | 45 | 5.25 | 5.02 |
| 10 | 1:15 | 45 | 30 | 3.12 | 3.07 |
| 11 | 1:25 | 30 | 45 | 5.08 | 5.22 |
| 12 | 1:25 | 30 | 45 | 5.13 | 5.22 |
| 13 | 1:35 | 45 | 30 | 7.02 | 7.11 |
| 14 | 1:25 | 30 | 45 | 5.10 | 5.22 |
| 15 | 1:35 | 15 | 60 | 7.09 | 7.24 |
| 16 | 1:25 | 30 | 45 | 5.04 | 5.22 |
| 17 | 1:15 | 15 | 30 | 3.21 | 3.17 |
| 18 | 1:15 | 15 | 60 | 3.19 | 3.19 |
| 19 | 1:35 | 15 | 30 | 7.09 | 7.12 |
| 20 | 1:25 | 30 | 45 | 5.08 | 5.22 |

**Table 2** shows the result of the ANOVA, goodness-of-fit, and adequacy of the model for the response surface model. A high F-value (F=92.83) and a very low *p*-value ($p$<0.0001) stated that the proposed model was significant. The goodness-of-fit of the model evaluated by the value of $R^2$ which is 0.9882 and the adjusted $R^2$ was 0.9775, indicating that 98.82% of the variance of the dependent variable being studied is able to be explained by the variance of the independent variable. The difference in value between adjusted $R^2$ (0.9775) and predicted $R^2$ (0.9340) is less than 0.2, indicating that the regression model was adequate to derive predictions within the range of the experimental variables. From **Table 2**, it can be seen that the independent variable of material to solvent had a remarkable effect on the carbohydrate content with $p$<0.0001. The material-to-solvent ratio has a remarkable effect on the extraction process because it influences the efficiency of the solubilization, diffusion, and recovery of the target compounds (e.g., carbohydrates) from the material into the solvent. If the solvent volume is too low relative to the material, the solvent can become saturated with the extracted compounds. This saturation reduces the driving force for further diffusion, limiting the yield of the extraction. An adequate solvent volume ensures that the solubilization process continues effectively, allowing for higher recovery of the target compounds. Moreover, the driving force for mass transfer is the concentration gradient between the material and the solvent. A lower material-to-solvent ratio (more solvent) maintains a higher concentration gradient, enhancing diffusion of the compounds into the solvent. A high material-to-solvent ratio (less solvent) can result in overcrowding, restricting solvent access to the internal structure of the material. Meanwhile, the other independent variables (extraction time, extraction temperature, and quadratic terms ($X_1X_2, X_1X_3, X_2X_3, X_1^2, X_2^2, X_3^2$) did not have *p*-value smaller than 0.05, which are not significant. Hence, in this study, the only significant parameter of the carbohydrate extraction using UAE was the material-to-solvent ratio.

**Table 2** ANOVA for the quadratic model of carbohydrates extracted using UAE

| Source | Sum of square | df | Mean Square | F-value | P-value |
|---|---|---|---|---|---|
| Model | 41.06 | 9 | 4.56 | 92.83 | <0.0001 |
| $X_1$-Material to solvent ratio | 40.92 | 1 | 40.92 | 832.77 | <0.0001 |
| $X_2$-Time | 0.0010 | 1 | 0.0010 | 0.0198 | 0.8908 |
| $X_3$-Temperature | 0.0259 | 1 | 0.0259 | 0.5268 | 0.4846 |
| $X_1X_2$ | 0.0036 | 1 | 0.0036 | 0.0725 | 0.7932 |
| $X_1X_3$ | 0.0043 | 1 | 0.0043 | 0.0867 | 0.7745 |
| $X_2X_3$ | 0.0023 | 1 | 0.0023 | 0.0467 | 0.8332 |
| $X_1^2$ | 0.0222 | 1 | 0.0222 | 0.4522 | 0.5165 |
| $X_2^2$ | 0.0930 | 1 | 0.0930 | 1.89 | 0.1997 |
| $X_3^2$ | 0.0061 | 1 | 0.0061 | 0.1251 | 0.7309 |
| Residual | 0.4914 | 10 | 0.0491 | | |
| Lack of fit | 0.4851 | 5 | 0.0970 | 76.07 | 0.0001 |
| Pure Error | 0.0064 | 5 | 0.0013 | | |
| Cor Total | 41.55 | 19 | | | |
| R-squared | 0.9882 | | | | |
| Adj. R-squared | 0.9775 | | | Std Dev | 0.2217 |
| Pred R-squared | 0.9340 | | | Mean | 5.19 |
| Adeq. Precision | 27.1654 | | | C.V% | 4.27 |

The correlation between the response and independent variables was done using multiple regression analysis. Based on the sequential model fitting, the correlation of carbohydrate extracted with material-to-solvent ratio, time, and temperature was expressed using a second-order polynomial equation. The second-order polynomial equation or empirical equation generated by RSM can be used to predict the value of response variables by substituting the value of independent variables. The uncoded or empirical equation that describes the carbohydrate extracted from *Padina sp.* using UAE is shown in Equation **(1).**

$$Y = 5.22 + 2.02X_1 - 0.0099X_2 + 0.0509X_3 + 0.0211X_1X_2 + 0.0231X_1X_3 + 0.0169X_2X_3 + 0.0899X_1^2 - 0.1838X_2^2 + 0.0473X_3^2 \quad \textbf{(1)}$$

where Y (mg/g) is carbohydrate content concentration, $X_1$ is material to solvent ratio (g/ml), $X_2$ is time (min), $X_3$ is temperature (°C).

**Analysis of Response Surface Method**

Three-dimensional (3-D) graphical representations of the response surfaces generated by the model for carbohydrates extracted using UAE are shown in **Figure 2, 3,** and **4**. **Figure 2** shows the relationship between the material-to-solvent ratio and the extraction time. The trend shows the yield of carbohydrate content increases as the material-to-solvent ratio increases and no significant changes can be observed as time increases from 15 to 45 min. The results indicate that the volume of water used greatly affected the extraction of the carbohydrates. The contour lines indicate that the material-to-solvent ratio has a stronger influence on carbohydrate than time. This is evident from the steep slope along the material-to-solvent ratio axis and the gentler slope along the time axis. Increasing the material-to-solvent ratio extracts more carbohydrates, likely because higher ratios enhance the solubility or availability of carbohydrates during the process. Time has a smaller influence, suggesting that the extraction process might reach a plateau. For optimal carbohydrate content, the material-to-solvent ratio is the primary parameter to adjust. In general, a lower material-to-solvent ratio generally reduces reaction time by increasing the extraction rate, while a higher material-to-solvent ratio slows down the process, requiring more time to achieve the same yield. However, in this study time is insignificant parameter and does not have an effect on the extraction of carbohydrate.

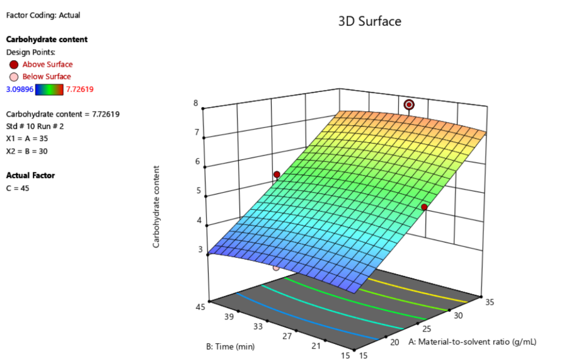


**Figure 2** 3D surface plot of material-to-solvent ratio and the extraction time

**Figure 3** shows the 3D surface plot for the relationship of the material to solvent ratio and the extraction temperature. As shown in the 3D surface graph, the carbohydrate content increases significantly as the material-to-solvent ratio increases. This suggests that increasing the concentration of the material in the solvent facilitates a greater extraction of carbohydrates. The carbohydrate content appears to increase moderately as the temperature rises. The extraction temperature does not have a significant effect on the carbohydrate yield. However, in general, if the temperature is too high, it may lead to the degradation of carbohydrates (Thao et al., 2020). The material-to-solvent ratio has the strongest influence on carbohydrate, suggesting that optimizing this parameter will yield significant improvements in extraction efficiency.

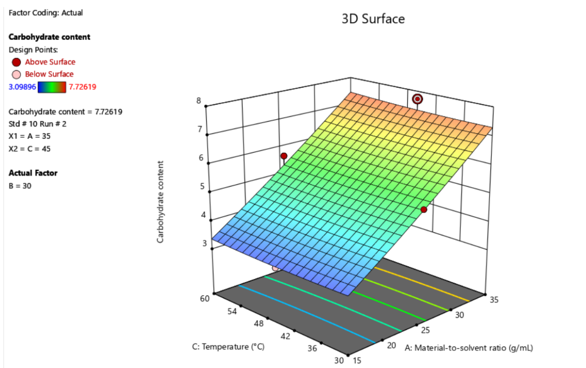


**Figure 3** 3D surface plot of material to solvent ratio and the extraction temperature

**Figure 4** illustrate 3D surface plot of time and temperature. There was no significant peak on the 3D plot for the relationship between extraction time and extraction temperature. This correlates with the fit statistic where extraction time and extraction temperature were not significant in this study. The surface plot is flat, suggesting no significant interaction between temperature and time. Contour lines in the 2D view show minimal variation, indicating that the carbohydrate content is not highly sensitive to changes in temperature or time within the displayed range. The process or reaction might not be dependent on these parameters in the experimental conditions tested.

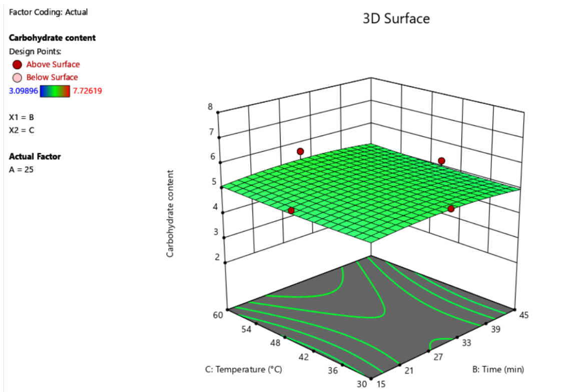


**Figure 4** 3D surface plot of time and temperature

## CONCLUSION

The best operating condition in this study for the carbohydrate extraction from brown algae using UAE was determined at material-to-solvent ratio of 1:35 g/mL, extraction time of 30 min, and extraction temperature of 45 °C. Water was the solvent used as green solvent and the highest yield of carbohydrate content obtained was 7.73 mg/g. The parameters in this study need to be adjusted more to get the optimum condition.

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