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Physical Characteristic of Glucose Syrup from *Gracilaria sp.*

Intan Ramadhita Pramidanirwa[1] and Dedin Fitnasiyatull Rosida[1]*

[1]*Department of Food Technology, Faculty of Engineering and Science, Universitas Pembangunan Nasional Veteran Jawa Timur, Indonesia 60294*

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CORRESPONDING AUTHOR

*E-mail: dedin.tp@upnjatim.ac.id

A B S T R A C T

The growing gap between domestic sugar production and consumption in Indonesia underscores the need for alternative carbohydrate sources beyond sugarcane. Seaweed Gracilaria sp. represents a promising renewable biomass due to its high cellulose content; however, its rigid lignocellulosic matrix limits enzymatic conversion efficiency. This study examines the physical characteristics of glucose syrup produced from Gracilaria sp. via Microwave-Assisted Inorganic Salt Pretreatment, followed by cellulolytic hydrolysis with Viscozyme® L. The glucose syrup was assessed for yield, ash content, and total dissolved solids (TDS) based on standard analytical protocols. The results indicate that the pretreatment successfully enhanced enzymatic accessibility, yielding glucose syrup with physical properties comparable to conventional sweeteners used in food applications. These findings highlight the potential of Gracilaria sp. as a sustainable raw material for sweetener production and reinforce the importance of physical characterization as a determinant of product stability and compliance with food quality standards.

Contribution to Sustainable Development Goals (SDGs):
SDG 2: Zero Hunger
SDG 9: Industry, Innovation, and Infrastructure
SDG 12: Responsible Consumption and Production
SDG 14: Life Below Water

1. INTRODUCTION

1.1. Research Background

The global demand for sugar and carbohydrate-based sweeteners has continued to grow in line with population expansion and industrial development, particularly in the food, beverage, and pharmaceutical sectors. However, the increasing reliance on conventional sugarcane and starch-based feedstocks has raised significant concerns regarding land use competition, resource sustainability, and production costs [1]. As a result, attention has shifted toward renewable and non-terrestrial biomass sources such as marine macroalgae as potential substrates for sustainable sugar production [2]

Sugar consumption in Indonesia continues to increase in parallel with population growth; however, domestic production remains insufficient to satisfy national demand. The country's annual sugar output, estimated at approximately 2.5–3.0 million tons, results in a widening supply gap that is increasingly filled through imports [3]. The heavy reliance on sugarcane as the sole raw material has become a major challenge, particularly as cultivation areas have declined due to climatic limitations and suboptimal agricultural practices [4]. This situation underscores the urgency of developing alternative raw materials and implementing more efficient production methods to achieve national sugar self-sufficiency.

Gracilaria sp., a genus of red seaweed (*Rhodophyta*) widely cultivated in Indonesian coastal waters, encompasses several economically important species, including *Gracilaria verrucosa*, *Gracilaria edulis*, and *Gracilaria chilensis*. These species are well recognized for their high carbohydrate content, which can reach up to 70% of dry weight [5]. The principal components of *Gracilaria* sp. biomass include cellulose (approximately 14–18%), hemicellulose, lignin (~10%), and specific polysaccharides such as agar and galactan [6]. The abundance of carbohydrates positions *Gracilaria* sp. as a promising renewable biomass feedstock. Nevertheless, lignin may impede enzymatic hydrolysis by restricting enzyme access to cellulose fibres [7]. Hence, appropriate pretreatment is essential to enhance cellulose accessibility, improve hydrolysis efficiency, and optimize glucose conversion from this biomass.

The potential utilization of *Gracilaria sp.* as a feedstock for glucose syrup production has been explored using various hydrolysis strategies. For instance, acid-assisted pretreatment combined with enzymatic hydrolysis of *Gracilaria verrucosa* has been shown to enhance sugar recovery, albeit at the expense of the formation of inhibitory by-products such as hydroxymethylfurfural (HMF) and levulinic acid (LA) [8]. Studies on other lignocellulosic feedstocks, including sorghum leaves and coffee husks, have also demonstrated the potential of cellulase-mediated hydrolysis following chemical pretreatment, although process efficiency remains highly substrate- and condition-dependent [9,10]. However, limited research has investigated the application of environmentally friendly pretreatment methods particularly Microwave-Assisted Inorganic Salt Pretreatment, to improve enzymatic hydrolysis and glucose yield from marine biomass such as *Gracilaria sp.*

Therefore, this study aims to evaluate the effectiveness of MAISP in enhancing enzymatic hydrolysis efficiency and glucose syrup production from *Gracilaria sp.*, and to characterise the resulting syrup's physical properties to assess its potential as an alternative sweetener source.

1.2. Literature Review

Glucose syrup is a carbohydrate-based liquid sweetener widely used in the food, beverage, and pharmaceutical industries due to its functional properties, including sweetness, viscosity, and stability [1]. Conventionally, glucose syrup is produced from starch-rich substrates such as corn and cassava, involving gelatinization, liquefaction, and enzymatic saccharification steps [11]. In contrast, seaweed biomass, such as Gracilaria sp., contains relatively low levels of starch but high levels of cellulose and complex polysaccharides, which can be hydrolysed directly without gelatinisation [2].

Nevertheless, the recalcitrant structure of seaweed biomass, particularly the presence of lignin, often limits the efficiency of enzymatic hydrolysis [7]. Pretreatment is therefore required to enhance cellulose accessibility and improve hydrolysis performance. One emerging and promising approach is Microwave-Assisted Inorganic Salt Pretreatment, which integrates microwave irradiation with inorganic salts such as NaCl, KCl, or $CaCl_2$ to effectively disrupt lignin networks, increase cellulose recovery, and improve subsequent enzymatic saccharification [12, 10]

The working principle of MAISP relies on two synergistic phenomena: dielectric heating generated by microwave energy and ionic conductivity enhancement from dissolved inorganic salts. When exposed to microwave radiation, polar molecules, such as water and hydroxyl groups within lignocellulosic structures, oscillate rapidly, producing localized heating (hot spots) that soften and weaken the plant cell wall matrix [12].

Cations such as Na^+, K^+, and Ca^{2+} play a pivotal chemical role in facilitating lignin disruption and cellulose exposure. Monovalent ions (Na^+, K^+) mainly enhance ionic conductivity, while divalent ions (Ca^{2+}) act as Lewis acids capable of coordinating with oxygen atoms in lignin and hemicellulose, promoting partial depolymerization and cleavage of ether (C–O–C) bonds [13]. Simultaneously, chloride anions (Cl^-) may participate in mild nucleophilic substitution reactions that break hemicellulosic glycosidic linkages, thereby increasing surface porosity and enzymatic accessibility [17]. As a result, MAISP facilitates partial delignification and structural loosening of cellulose fibrils, leading to significantly improved hydrolysis efficiency

1.3. Research Objective

This study aims to characterise the physical properties of glucose syrup produced from Gracilaria sp. via Microwave-Assisted Inorganic Salt Pretreatment followed by enzymatic hydrolysis with cellulase (Viscozyme® L).

2. MATERIALS AND METHODS

This research was conducted from March to July 2025 at the Laboratory of Food Technology, Faculty of Engineering and Science, UPN "Veteran" Jawa Timur, Indonesia. The main raw material used was red seaweed (*Gracilaria sp.*) obtained from local aquaculture farmers in East Java, Inorganic salts (NaCl, KCl, and $CaCl_2$,) were used as pretreatment agents, Cellulase enzyme (Viscozyme® L, Sigma-Aldrich, USA) was applied for the enzymatic hydrolysis process.

The process of glucose syrup production it self consisted of several stages as follows:

1. Pretreatment
   10 g dried Gracilaria sp. powder was mixed with 100 mL of a 3% inorganic salt solution (NaCl, KCl, or $CaCl_2$) at a 1:10 (w/v) ratio. The mixture was subjected to microwave irradiation at 600 W for 5 minutes.
2. Enzymatic hydrolysis
   a. *Liquefaction*
      50 g of *Gracilaria sp.* powder was suspended in 1 L of distilled water and heated for 5 minutes. The pH was adjusted to 4.8–5.0 using citrate buffer, and 50 mL of cellulase enzyme was added. The mixture was hydrolyzed in a water bath shaker at 60 °C and 180 rpm for 1 hour.
   b. *Saccharification*
      The liquefied slurry was further hydrolysed by adding cellulase at concentrations of 3%, 5%, and 7% (v/v) of the total slurry volume, and incubated for 72 hours.
3. Decolorization and Purification
   The slurry was heated at 80–90 °C for 10 minutes to inactivate the enzyme. Then, 5% activated carbon was added and the mixture was stirred at the same temperature for 30 minutes using a magnetic stirrer. The slurry was allowed to stand for 3-5 hours to optimize adsorption. It was then centrifuged at 4000 rpm for 10 minutes to separate the solid residue from the sugar hydrolysate.

3. RESULT AND DISCUSSION

3.1. Yield

The determination of yield reflects the overall efficiency of biomass conversion and the effectiveness of the extraction and hydrolysis processes in producing glucose syrup from *Gracilaria sp*. As presented in **Table 1**, the observed differences in yield were primarily governed by the ionic characteristics of the inorganic salts used during the microwave-assisted pretreatment.

Table 1. Yield of glucose syrup

Type of Inorganic Salt	Cellulase Enzyme Addition (%)	Ash Content (%)
NaCl	3%	0.59 ± 0.01
	5%	0.61 ± 0.00
	7%	0.69 ± 0.02
KCL	3%	0.83 ± 0.11
	5%	0.84 ± 0.14
	7%	0.89 ± 0.09
$CaCl_2$	3%	1.09 ± 0.01
	5%	1.10 ± 0.01
	7%	1.11 ± 0.01

As shown on **Tabel 1,** pretreatment using $CaCl_2$ produced the highest yield due to the dual functionality of Ca^{2+} ions. On one hand, these divalent ions facilitated partial disruption of lignin and phenolic linkages within the cell wall, promoting the release of soluble organic compounds. On the other hand, Ca^{2+} ions formed crosslinking interactions with carboxyl groups in polysaccharides particularly alginate resulting in the formation of semi-rigid, gel-like matrices. This structural modification restricted complete enzymatic penetration, leading to partial hydrolysis and accumulation of soluble oligosaccharides and partially degraded polysaccharides. These intermediate products, possessing relatively high molecular mass, contributed to an apparent increase in the total syrup mass, thereby enhancing the yield even in the absence of complete saccharification [14, 15].

Samples pretreated with KCl exhibited the second-highest yield. The monovalent K^+ ions interact more weakly with the carboxyl groups of alginate than Ca^{2+}, forming less stable crosslinks that improve matrix permeability and enzyme accessibility. This increased solubilization facilitates more efficient hydrolysis while minimizing polysaccharide loss during washing. Moreover, potassium ions have been reported to enhance cellulase activity and structural stability by maintaining ionic balance in the reaction medium [16, 17], which likely contributed to the higher yield compared with NaCl treatment.

Conversely, pretreatment with NaCl resulted in the lowest yield. The smaller, highly hydrated Na^+ ion tends to form strong hydration shells, limiting its interaction with the carboxyl and hydroxyl groups of polysaccharides. Consequently, the pretreatment was less effective in disrupting the lignocellulosic and phenolic matrix, leading to reduced enzyme accessibility and lower product recovery [18]. In addition, increasing enzyme concentration generally led to higher yields, as greater enzyme levels increased substrate–enzyme interactions, thereby enhancing hydrolytic efficiency. However, beyond a certain point, the effect became limited by substrate saturation, consistent with the Michaelis–Menten enzymatic kinetics.

3.2. Ash Content

The determination of ash content is an essential step in food composition analysis, as it reflects the level of inorganic minerals remaining after organic components are removed through combustion. A high ash content not only indicates mineral richness but may also suggest the presence of inorganic contaminants or processing inefficiencies, both of which directly affect product quality.

Table 2. Ash content of glucose syrup

Type of Inorganic Salt	Cellulase Enzyme Addition (%)	Yield (%)
NaCl	3%	56.16 ± 0.60
	5%	56.75 ± 0.65
	7%	62.34 ± 0.81
KCL	3%	66.18 ± 0.14
	5%	67.86 ± 0.78
	7%	70.85 ± 0.63
$CaCl_2$	3%	67.03 ± 0.74
	5%	68.73 ± 0.42
	7%	71.37 ± 0.23

As shown in **Table 2**, $CaCl_2$ treatment resulted in the highest ash levels among the treatments. This can be attributed to the divalent and hygroscopic nature of Ca^{2+} ions, which are more difficult to remove during washing and tend to react with organic acids, phosphates, and sulfates, forming poorly soluble salts. Such reactions lead to a greater accumulation of inorganic residues in the final product [19, 20, 21]. In contrast, NaCl and KCl, which only contribute monovalent cations, produced lower mineral residues and thus lower ash levels. These findings align with the literature, which has highlighted that ionic complexity plays a critical role in mineral accumulation in food products [22, 16].

Additionally, the raw material's inherent mineral composition played a role. Seaweed contains high levels of essential minerals such as calcium, magnesium, potassium, and sodium, all of which contribute directly to ash values [23]. Another factor is the use of buffers for pH adjustment. Buffers often contain stable inorganic compounds, such as phosphates or citrates, that are resistant to thermal degradation and remain as ash residues after combustion [24].

Overall, the ash content of glucose syrup was primarily determined by the type of inorganic salt used during pretreatment, the raw material's intrinsic mineral content, and the use of pH buffers, whereas variations in enzyme dosage made no significant contribution. Therefore, the choice of inorganic salt is a key factor in controlling mineral levels and ensuring compliance with ash content quality standards for glucose syrup.

3.3. Total Dissolved Solids (TDS)

The Total Dissolved Solids (TDS) test is an important parameter that reflects the overall amount of dissolved substances in a solution, expressed in °Brix. This value represents the contribution of simple sugars, oligosaccharides, and non-carbohydrate compounds, such as minerals and soluble proteins. In this study, TDS was used to evaluate the quality of glucose syrup derived from *Gracilaria sp.* via a combination of microwave-assisted inorganic salt pretreatment and enzymatic hydrolysis, providing insight into both the conversion process efficiency and the physical characteristics of the final product.

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Table 3. Total Dissolved Solids (TDS) of Glucose Syrup

Type of Inorganic Salt	Cellulase Enzyme Addition (%)	TDS
NaCl	3%	10.00 ± 0.00
	5%	11.50 ± 0.71
	7%	14.50 ± 0.71
KCL	3%	12.50 ± 0.71
	5%	15.50 ± 0.71
	7%	19.00 ± 0.00
$CaCl_2$	3%	21.00 ± 0.00
	5%	21.50 ± 0.71
	7%	25.00 ± 0.00

As shown in Table 3, among all treatments, $CaCl_2$ consistently produced the highest TDS values, while NaCl produced the lowest. The elevated TDS under $CaCl_2$ treatment can be explained by the unique properties of Ca^{2+} ions, which are able to form cross-linking with carboxyl groups in pectin or other polysaccharides, resulting in a gel-like "egg-box" structure [25]. This structure facilitates the release of more non-monosaccharide fractions, such as oligosaccharides, soluble pectin, and other structural components, into the hydrolysate, thereby increasing TDS. However, the formation of such cross-linking networks simultaneously restricts cellulase accessibility to cellulose, leading to incomplete hydrolysis.

Overall, TDS values increased with higher enzyme dosages. The greater the enzyme concentration, the more glycosidic bonds can be hydrolyzed, thereby increasing the release of both simple sugars and soluble oligosaccharides [26]. Nevertheless, the effectiveness of this process remained strongly influenced by the type of inorganic salt used.

In addition to carbohydrate fractions, non-carbohydrate components also contributed to TDS values. Pretreatment with $CaCl_2$, for example, likely dissolved more calcium ions into the medium, thereby increasing the amount of soluble solids. This is consistent with ash analysis, which showed the highest ash content in $CaCl_2$ treatments. $CaCl_2$ is highly soluble and hygroscopic, making Ca^{2+} ions more strongly bound to the product matrix and difficult to completely remove during washing [19]. Furthermore, Ca^{2+} ions can react with organic acids, phosphates, or sulfates to form less soluble salts, increasing the accumulation of inorganic residues [20, 21].

This phenomenon aligns with the literature [27], which reported that the mineral content in biomass can increase soluble solids by dissolving during pretreatment and becoming part of the TDS. In addition, the presence of minerals may lower hydrolysis efficiency through buffering or neutralization effects while also increasing water retention. TDS does not solely represent sugars produced during hydrolysis but also includes minerals such as calcium, phosphorus, magnesium, and iron that remain suspended in the hydrolysate [28]. Therefore, the high TDS observed in $CaCl_2$ treatments is more representative of a combination of soluble non-monosaccharide fractions and dissolved minerals rather than solely the conversion of polysaccharides into simple sugars.

4. CONCLUSION

This study demonstrated that both the type of inorganic salt and cellulase enzyme concentration significantly influenced the physical characteristics of glucose syrup derived from *Gracilaria sp.* Pretreatment using $CaCl_2$ consistently produced the highest yield, ash content, and total dissolved solids, followed by KCl and NaCl. The superior performance of $CaCl_2$ was attributed to the dual role of Ca^{2+} ions, which facilitated partial lignin disruption while forming crosslinks with polysaccharide carboxyl groups, leading to the accumulation of soluble intermediates that increased product mass. Meanwhile, variations in enzyme concentration enhanced yield up to a saturation level but had a limited effect on mineral accumulation. Overall, the combination of microwave-assisted inorganic Salt Pretreatment and enzymatic hydrolysis with cellulase proved effective in producing glucose syrup with favourable physical properties.

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