

Identification Of β -Glucan Polysaccharides In Hanjeli (Coix Lacryma Jobi) Using The Colorimetric Method

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Abstract

β -Glucan is a polysaccharide that the human body uses as a food fiber. Beta β -Glucan plays an important role in the human digestive process, slowing the rate at which food is digested. Hanjeli's β -Glucan content was investigated in this study (*Coix lacryma jobi*). Soft shell Hanjeli (*Coix lacryma jobi* var. *ma-yuen*) and hard shell Hanjeli were discovered (*Coix lacryma jobi* var. *stenocarpa*). Using Fourier-transform infrared spectroscopy, crude glucan solids from each Hanjeli variety were analyzed qualitatively for their functional groups (FTIR). The study's findings revealed that there is a specific structure of the β -Glucan compound at wave number 898 cm^{-1} . The levels of β -Glucan in the hanjeli were analyzed using the Congo Red colorimetric method; the results of the analysis revealed that the β -Glucan levels in the hanjeli soft shell were 596,366 ppm (42% w/w), and in the hard shell hanjeli were 546,200 ppm (40.6% b/b). Hanjeli (*Coix lacryma jobi*) has good potential and prospects for development as a functional food because it contains an active compound β -Glucan, which functions as an immunomodulator, cholesterol lowering agent, and antioxidant.

Introduction

Hanjeli (*Coix lacryma jobi*) is a plant in the Gramineae family and the genus *Coix* that is white like a pearl. This plant has the potential to be developed as a functional food [1]. Hanjeli is divided into two varieties based on shell differences: hard shell (*Coix lacryma jobi* var. *stenocarpa*) and soft shell (*Coix lacryma jobi* var. *MA-yuen*) [2]. Hanjeli contains the active polysaccharide Coixan, β -Glucan [3]. The β -Glucan content of fungi and cereal has been shown to be a preventive agent (immunomodulator) [4], and heal cardiovascular disease by lowering cholesterol [5], acting as an antioxidant [6]. *Coix lacryma-Jobi* is a whole grain cereal used as food and medicine [7]. Colorimetric analysis of β -Glucan levels in cereals can be performed using the Megazyme Tools Kit and Congo Red. β -Glucan analysis with Congo Red has the advantage of being simple, requiring only the instruments needed by a standard spectrophotometer, relatively fast (around 3–4 hours), low cost (because Congo Red reagent is inexpensive and easy to obtain), and linear in sensitivity [8]. The soft shell hanjeli (*Coix lacryma jobi* var. *MA-yuen*) and the hard shell hanjeli (*Coix lacryma jobi* var. *stenocarpa*) were used to compare the β -Glucan levels produced by these two varieties.

The purpose of this study was to isolate the β -Glucan produced in the two varieties of hanjeli (*Coix lacryma jobi*), determine the levels of β -Glucan produced in the two varieties of hanjeli (*Coix lacryma jobi*) using the colorimetric method using Congo Red, and learn about yield functional group analysis. The β -Glucan extraction of both varieties of functional food hanjeli (*Coix lacryma jobi*) was measured using an FTIR Spectrophotometer.

Methods

Materials

The main ingredient for hanjeli seeds is obtained from the tourist village of Waluran, Jampang, Sukabumi Regency. The supporting materials used for the extraction of β -Glucan. Distilled water (Sigma Aldrich), ethanol (Sigma Aldrich), filter paper (Whatman 14), KBr (Merck), β -Glucan (Megazyme), Congo Red reagent (Merck), NaOH (Merck).

Equipment

The tools used are analytical balance, oven, blender, incubator, UV-Visible spectrophotometer, FTIR spectrophotometer, centrifugation, magnetic stirrer, water bath and other glassware.

Hanjeli β -Glucan extraction.

To extract β -Glucan from hanjeli, first the fresh hanjeli seeds are air-dried and dried in the sun until crisp dry, then baked for 2 hours at 45°C. Blending dried hanjeli until smooth and then filtering through a fine sieve yields flour. To 500 mL of distilled water, add 25 grams of hanjeli flour and heat to boiling for 2 hours. A filter is used to separate the filtrate and residue from the Hanjeli porridge. The filtrate was treated with 80% ethanol three times its original volume and incubated at 4°C for 24 hours. The filtrate was then centrifuged at 5000 rpm at 4°C for 10 minutes. The resulting precipitate is called water-soluble crude β -Glucan solids, dried with a freeze dryer and then weighed as the dry weight of the crude sample.

Qualitative Analysis Of Crude Glucan

Crude solid β -Glucan from hanjeli as much as ± 20 mg is mixed with KBr powder, crushed in an agate mortar until well blended and taken a small amount to put into a disc and pressed with a press holder. After the thin film was formed, the KBr disc was inserted into the KBr disc holder and the sample spectrum was recorded at a wavelength of 400–4000 cm^{-1} with an FTIR spectrophotometer.

Measurement Of β -glucan Concentration

0.02 g of solid β -Glucan solid was weighed and put into a vial (polypropylene bottle) dissolved with 1.4 mL of NaOH and 0.6 mL of distilled water (1% β -Glucan solution) then stirred until dissolved. Take 1 mL of 1% β -Glucan solution with a pipette, then put it into 1.5 mL of eppendorf pipette. The solution was centrifuged, and the filtrate was separated. The filtrate was added 0.5 mL of 1M NaOH, then added 0.4 mL of 1% Congo Red. The absorbance of the solution can be measured at a wavelength of 510 nm using a UV-Visible spectrophotometer. The level of β -Glucan can be calculated based on the calibration curve obtained from a standard solution of β -Glucan from barley by entering the absorbance obtained from the measurement results into the linear regression.

$$y = ax + b$$

The concentration results obtained in ppm units are then converted to % b / b units.

Results

Preparation and Characterization of β -Glucan

Based on the solubility properties of the β -Glucan compound to be extracted, extraction was carried out using ethanol solvent. β -Glucan is a polysaccharide that dissolves in hot water and NaOH. This solubility occurs because heating causes a branched chain-1,3;1.6 glucan to open, allowing water to enter the structure and interact with the hydrogen bond with the -OH group. This occurs during gelatinization, as evidenced by the extracted thickened supernatant [9].

The addition of ethanol to the extraction of β -Glucan from hanjeli separates the β -Glucan compound dissolved in distilled water. With the addition of ethanol, the interaction between the hydrogen β -Glucan and distilled water is replaced by the interaction between ethanol and distilled water, causing the β -Glucan to precipitate. Because of its low solubility, β -Glucan is unable to interact with ethanol. The lower the ethanol temperature for precipitation, the more polysaccharides that can be precipitated. Optimally 80% ethanol is kept in an incubator (4°C) for 1 night to precipitate more crude β -Glucan.

The liquid-liquid phase separation process is driven by centrifugal force (rotary force). Apart from its role in separation, centrifugation is also involved in material drying. During the separation process, particles of various sizes and densities of the solution mixture are separated from the liquid by centrifugal force. For 10 minutes, the centrifuge was set at 5000 rpm and 4°C. The Hectic Universal 320R centrifugation has a maximum speed of 5000 rpm; the higher the centrifugation speed and the lower the temperature, the higher the crude yield obtained. The freeze drying method was chosen for drying wet hanjeli crude so that the structure of the hanjeli dry crude was preserved. The wet crude of soft shell hanjeli was 5.52 grams with a dry weight yield of 2.29 grams (0.09% w/w from 25 grams of wet weight of soft shell hanjeli) and the wet crude of hard shell hanjeli was 25.6 grams with a dry weight yield of 2.85 grams (0.1% w/w from 25 grams of hard shell hanjeli weight).

Ftir Spectra

The β -Glucan solid samples obtained from the extraction results were characterized by comparing their spectra to β -Glucan standards. The KBr disc method was chosen because it is suitable for sample preparation in the form of solid powder.

The pattern of the FTIR spectrum from crude in (See Fig. 1 shows that glucan compounds have several main functional groups. According to glucan standard, (Fig. 1. a) is an alcohol and OH (Hydroxy) bond at wavelength 3750 – 3000 cm^{-1} , CH alkane bonds at wavelength 3000 – 2700 cm^{-1} , COC bonds at wavelength 1260 – 1050 cm^{-1} , and β -Glucan bonds at wavelength 895 – 865 cm^{-1} . Depicts the glucan compound's structure, with the specific β -Glucan compound located at wave number 898 cm^{-1} in both soft shell samples (Fig. 1. b) and hard shell samples (Fig. 1.c). This wavelength is the focus of this research because it is the compound that you want to isolate. There are also several other functional

groups, including OH at wave number 3427 cm⁻¹, CH alkanes at wave numbers 2958 cm⁻¹ and 1458 cm⁻¹, and COC at wave number 1165 cm⁻¹. The FTIR characterization of the two samples of soft shell jelly and hard shell jelly revealed the presence of β -Glucan compounds in both.

The impurities in the spectrum of crude hanjeli hard shell, in addition to identifying the functional group of the β -Glucan compound, showed the C = O group at a wavelength of 1597 cm⁻¹ and the N-H group at a wavelength of 1726 cm⁻¹. These two peaks are most likely amide compounds bound in glucans (proteoglycans). The presence of CO-NH vibrations of proteins or proteoglycans at around a wavelength of 1650 cm⁻¹ – 1540 cm⁻¹ indicates the presence of amide vibrations of proteins in the determination of β -Glucan [10].

The quantitative test for crude samples using the colorimetric method with Congo Red aims to determine the levels of β -Glucan compounds in the sample. Samples dissolved in NaOH and then treated with Congo Red reagent produced a red color, indicating a reaction to form a polysaccharide-Congo Red complex [11]. The absorbance of this solution was measured using a UV-Visible spectrophotometer at 510 nm. A standard β -Glucan was used to determine the levels of β -Glucan in soft shell jelly and hard shell jelly samples (Fig. 2).

A good linearity relationship is achieved if the method parameter value verification criteria is 0.9 [12]. Because the standard β -Glucan curve has a linear equation, $y = 6.10 \cdot 10^{-6}x + 0.0338$, with a regression coefficient (R²) of 0.9517, slope (a) $6.10 \cdot 10^{-6}$, and intercept (b) 0.0338, it can be concluded that the standard β -Glucan curve This β -Glucan meets the parameter values of the linearity test criteria and can be accepted by a valid analytical method.

Table 1
Sample absorbance measurement data

Sample	A1	A2	A3	A Average	Concentration	
					ppm	% b/b
Blanko	0	0	0	0		
HC soft	3.612	3.612	3.612	3.612	596.366	42
HCK hard	3.311	3.311	3.311	3.311	546.200	40,6

The concentration of β -Glucan (Table 1) in soft shell jelly and hard shell jelly samples differed. The difference in concentrations produced by the two varieties can be calculated using the percentage yield obtained. Although the yield of hard shell hanjeli was higher, it was related to the level of β -Glucan contained, which was lower than that of soft shell hanjeli. This is due to the presence of impurities in the hard shell during the extraction process, which is visible on the FTIR spectrum.

Based on the content of β -Glucan in two types of hanjeli, soft shell hanjeli and hard shell hanjeli, hanjeli can now be consumed as a functional food because β -Glucan has been shown to be a preventive agent

(immunomodulator) [4], lowers cholesterol levels [5] and as an antioxidant [6]. Hanjeli has good potential and prospects for development into functional food because the crude β -Glucan content present in both varieties of hanjeli (42% w/w and 40.6% w/w) is higher than barley (3.40% w/w) [13], and even though it is lower than wheat content (92.39% w/w) [14], hanjeli is a good prospect for Indonesian plants that can be alternative functional food.

Conclusion

According to the findings of research on both soft shell jelly (*Coix lacryma jobi* var. MA-yuen) and hard shell jelly (*Coix lacryma jobi* var. stenocarpa):

1. Extraction of β -Glucan yielded a dry weight of 2.29 grams (0.09% w/w from 25 grams of soft shell jelly) and 2.85 grams (0.1% w/w from 25 grams of hard shell jelly weight).
2. The results of the FTIR characterization test revealed that the bond position of 1,3 -D β -Glucan, the main feature of the β -Glucan compound, is located at wave number 898 cm^{-1} in both the soft and hard shell shells.
3. Testing for crude β -Glucan content with Congo Red yielded a concentration of 596,366 ppm.
4. Hanjeli has good potential and prospects for development into functional food because the crude β -Glucan content present in the two varieties of hanjeli (42% w/w and 40.6% w/w) is higher than barley (3.40% w/w)[13], and even though it is lower than wheat content (92.39% w/w)[14], hanjeli is a good prospect for a local plant from Indonesia that can be alternative functional food.

Declarations

Ethics approval and consent to participate.

Ethics approval and consent to participate were approved by the ethics committee at the Faculty of Science and Technology Muhammadiyah University of Sukabumi.

Consent for publication Not applicable

Availability of data and material

Supporting research methods carried out by researchers in their research. As well as the data generated from the results of the research.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Reni Mulyani and Lela Mukmilah analyzed and collected the experiment data. Reni Mulyani identified the structure of Beta glucan using FTIR, and measured the concentration of Beta glucan using UV Visible. Lela Mukmilah performs sample preparation, extraction, and manufacture of KBR pellets for identification using FTIR. All the authors critically reviewed and approved the final manuscript

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Conflict of interest The authors report no expressions of interest. We declare that the authors have no competing interests as defined by Springer, or other interests that might be considered to influence the results and/or discussions in this document.

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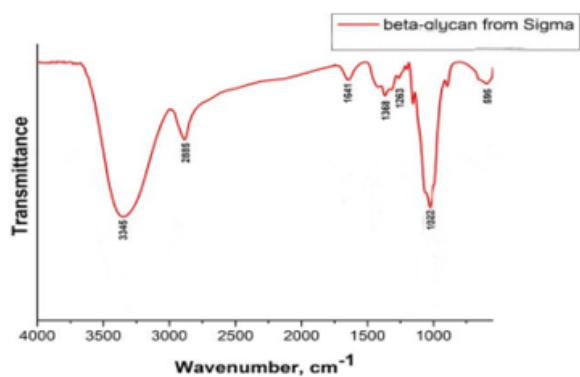
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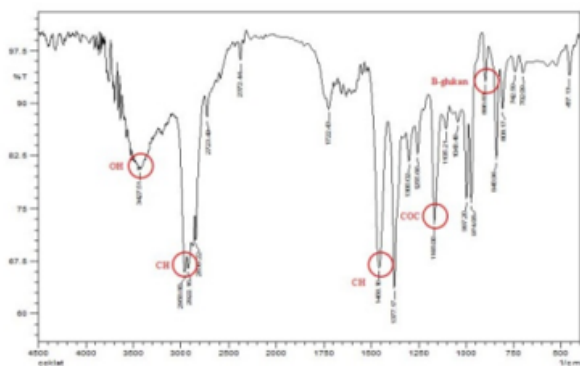
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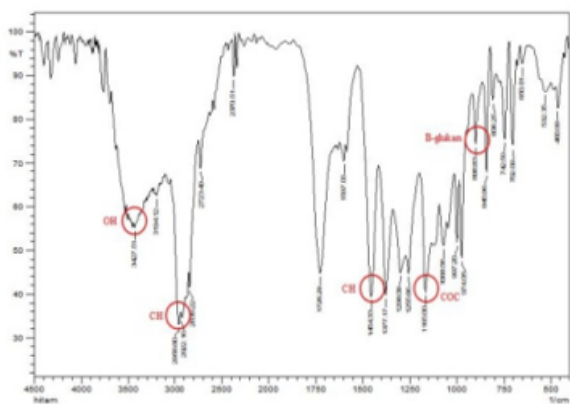
Figures



(a)



(b)



(c)

Figure 1

FTIR spectrum a) standard Beta-glucan b) Soft shell hanjeli, c) hard shell hanjeli

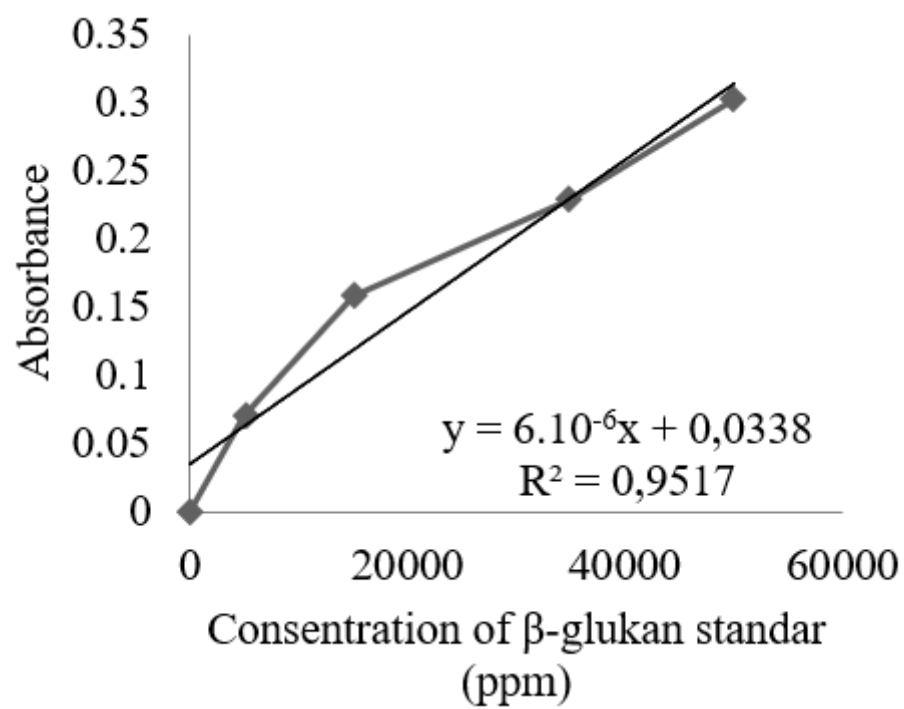


Figure 2

Calibration Curve