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LAMINARIN CRUDE EXTRACT CHARACTERIZATION OF *Sargassum sp.* ORIGINATED FROM JEPARA-INDONESIA WITH THE LAMINARIN ACID EXTRACTION METHOD USING AN ACETIC ACID SOLVENT

Ratna Indria Sari*, Suryati, Indrian Rizka Amalia and Arief Taslihan

Research Center for Fishery, National Research and Innovation Agency, Bogor, Indonesia
Jl. Raya Bogor KM47, CIbonong Bogor, West Java, Indonesia

* Correspondence, email: ratn024@brin.go.id

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ABSTRACT

Laminarin is a bioactive compound found in *Sargassum* sp. whose existence is widespread in Indonesia, including in Jepara, Central Java, Indonesia. Laminarin can be used in food, pharmaceutical, cosmetic, and feed industries as it is beneficial as an anti-inflammatory, antioxidant and regulates gut microbiota. This study aimed to extract laminarin from Sargassum sp. that originated from Jepara-Indonesia by laminarin acid extraction procedure with an acetic acid solvent (CH_3COOH) which was safer, cheaper and easy to obtain. Characterization of laminarin crude extract use Fourier Transform Infrared (FT-IR); meanwhile, nutrition tested are moisture, ash, crude protein and crude fiber content. The results showed that laminarin extraction of *Sargassum* sp. from Jepara using acetic acid solvent (L-ACT) obtained the highest yield of 15.5% with a solvent ratio of 1:5 (w/v) but still lower than the yield extraction using hydrochloric acid (L-HCl) with a solvent ratio of 1:10 (w/v) that was 24.17%. FT-IR absorption band at 2359.96 cm-1 (-OH bending; transmission angle peak), 1538.54 cm-1 (C-C aromatic; carboxyl groups), 1409.35 cm-1 (C-H bending; carboxyl groups), 1230.89 cm-1 (C-O-C stretching; sugar region), 1022.58 cm-1 (R-O-R (acetal)), and at 944.94 cm-1 (O-R cyclic) of the L-ACT samples were similar to those of the L-HCl and standard laminarin references in other studies that used as a positive control, confirming the presence of laminarin. The L-ACT extract had lower ash, crude protein content and higher water content compared to L-HCl crude extract. Meanwhile, the crude fiber content in both L-ACT and L-HCL was not identified. The results showed that *Sargassum* sp. from Jepara was the potential source of laminarin for many industries (pharmacy, cosmetic, food), and acetic acid could be used as a suitable solvent to extract laminarin in the LAE method.

Keywords: *Laminarin, Sargassum* sp.*,* Acetic Acid, Edible Solvent, LAE

INTRODUCTION

Laminarin is a type of water-soluble polysaccharide, known as a β-glucan, that serves as a food reserve for the photosynthesis process within the cell wall [1]. The polysaccharide comprises of a linear structure of (1,3)-β-D-glucan with several β-(1,6)-intrachain linkages, consisting of 20 to 25 units of glucose moieties, which is a distinctive characteristic of polysaccharides found in brown algae [2,3]. Consequently, Laminarin exhibits a degree of polymerization (DP) that ranges between 20 to 25. From the reducing end, the polymer chains of Laminarin can be categorized into M-series

polymer chains terminated by D-mannitol residues and G-series polymer chains with D-glucose residues at the end of the chain [4,5]. Depending on the degree of polymerization, Laminarin has an average molecular weight of 5 kDa [3,6].

One of the brown algae which were detected to be having laminarin is *Sargassum* sp. [7]. Laminarin in *Sargassum* sp. is a high polysaccharide content derived from the cell wall [7]. *Sargassum* sp. is a type of Phaeophyceae or brown algae which is the largest genus in the family Sargassaceae that lives widespread in Indonesia, growing in protected waters or those with large waves in rocky habitats, in intertidal, subtidal, and coastal areas [1,8]. *Sargassum* sp. contains carbohydrates (47.73%), protein (11.20%), lipids (1.06%), polysaccharides (21.01%), fiber (4.83%), macro and microelements such as potassium, sodium, magnesium, and iron [7,9]. There is various bioactivity from laminarin *Sargassum* sp. that has been pharmacologically proven. *Sargassum crassifolium* laminarin from Talango Island, Madura, Indonesia shows anti-inflammatory activity that can be applied as edema-reducing agents [10]. Antioxidant activity is also shown by laminarin of *Sargassum* sp. from Malaysia with the presence of superoxide anion radical scavenging activities [11]. Moreover, the laminarin of *Sargassum henslowianum* is also reported to have the potential to regulate gut microbiota [12].

Laminarin, a low molecular weight water-soluble polysaccharide or β-glucan polysaccharide, has diverse potentials for use in the food, pharmaceutical, cosmetic, and feed industries [4]. The composition of laminarin has been found to stimulate, regenerate, energize human dermis fibroblasts and human epidermis keratinocytes, with optimal activity observed at a concentration of 10 µg/ml, making it useful in cosmetics and pharmaceuticals [13]. Furthermore, as a polysaccharide, laminarin is resistant to hydrolysis in the upper gastrointestinal tract. Therefore, it can serve as a dietary fiber and prebiotic for microflora and intestinal epithelium in humans and animals [14].

The content of laminarin varies depending on environmental factors, such as nutrient levels, frond age, water temperature, waves, depth of immersion, sea currents, and salinity, where brown algae grow [6,15]. In addition, the yield and quality of laminarin are also affected by the algae species, seasonal fluctuations, and extraction conditions [4]. Therefore, optimising the extraction method is crucial to achieving the highest yield of laminarin. There are two methods for extracting laminarin: heat extraction and cold extraction. Heat extraction uses high temperatures, even boiling water, while cold extraction employs chemical compounds [4]. Although water extraction produces relatively low yields, the material content is purer than other methods [4,16].

Meanwhile, chemical extraction, especially acid, produces a high yield, but the crude material is still dissolved and remains inside the extract. The laminarin yield of species *Laminaria* sp. isolated with cold HCl and precipitated with ethanol can reach 36% of its dry weight according to the season [4,14]. Laminarin extraction with an acid

solution, namely sulfuric acid (Laminarin Acid Extraction = LAE) to *Sargassum duplicatum* originating from Talango Madura Island with a solvent ratio of 1:14, produces the highest yield of laminarin about 24.16 mg/g. [4,17] conducted laminarin extraction with hydrochloric acid solvents from *Eisenia bicyclis* from Korea that produced a 14.5% of yield.

However, using strong liquid acids, such as HCl and H_2SO_4, as solvents have drawbacks due to their costly reagents, corrosive nature, and harsh reaction. Furthermore, contact with these acids can cause harm to the skin, eyes, and upper respiratory tract, resulting in environmental and food safety concerns [18,19]. Therefore, the issue of environmental and food safety solvents is essential. Unfortunately, no research has yet reported the extraction of laminarin from *Sargassum sp*. originating from Jepara, Indonesia, using safe solvents. Hence, this study aims to extract laminarin from Sargassum sp. originating from Jepara-Indonesia using an acetic acid solvent (CH_3COOH) in the laminarin acid extraction procedure, which is safer, cheaper, and easier to obtain. Additionally, this research is expected to enhance the benefits of Sargassum sp. originating from Jepara-Indonesia by extracting its laminarin component.

METHOD

Laminarin extraction and nutrition (moisture, ash, crude protein, and crude fiber) tested at the Laboratory of Physics, Chemistry, Environment and Residue; Laboratory of Microbiology and Histopathology, in Main Center of Brackish Water Aquaculture Jepara, which was validated based on ISO 17025.2017 accreditation. In addition, analysis for FT-IR spectroscopic scanning was carried out at Lab—Chemical Engineering Research, Faculty of Industrial Engineering, at the Islamic University of Indonesia. Brown seaweed used in this research was *Sargassum* sp., collected from the Teluk Awur coastal, Jepara, Central Java, Indonesia. Acetic acid (CH3COOH) as a solvent in this study was a variable tested. The characterization of crude laminarin extract using acetic acid (L-ACT) was determined based on a comparison with extracts from HCl solvent (L-HCl). L-HCl is a standard solvent in laminarin extraction and used as a positive control in the research by Rajauria *et al*. [20]. The data obtained were analyzed quantitatively and qualitatively.

Preparation of *Sargassum* sp.

Sargassum sp. was cleaned with fresh water to eliminate sand, rocks, and other contaminants. *Sargassum* was air-dried without exposure to sunlight at a temperature of 28-30°C, then powdered with a grinder.

Extraction of Laminarin

Laminarin extraction was based on a modified method from [4] and Yudiati and Isnansetyo [21] that used an acid solvent. This method was *Laminarin Acid Extraction* (LAE). The principle of this extraction was to separate laminarin, which was polar, by using an acidic solvent that is also polar, as a material will easily dissolve in a solvent of the same polarity. Solvents were extracted dissolving the material with the solvent for a

certain time, followed by filtrate separation from the extracted residue [22]. The high yield was the advantage of using the acid solvent for laminarin extraction.

On the other hand, t acid caused lysis or degradation of the cell wall so that the laminarin could be dissolved maximally [4]. *Sargassum* powder weight of 100 g was extracted using 0.001 M HCl (pH 3) and 1 M CH_3COOH (pH 3) at 100°C for 60 minutes with stirring. Sargassum powder was extracted by comparing two solvent ratios (w/v) of 1:5 and 1:10 as variable tested. It aimed to determine the optimum solvent extraction capacity and the laminarin extraction's consistency and reproducibility.

The extract was filtered through a nylon mesh (74 µm pore size) and then cold-dried at 20°C for 3-4 days. The yield of laminarin crude extract was further determined by the dry weight ratio before and after extraction (%).

FT-IR Spectroscopic Scanning

Confirmation of laminarin presence and its chemical structure (functional groups) differences after dissolving with HCl solvent and acetic acid were determined by Fourier Transformed–Infra-Red (FT-IR) thermoscientific Nicolet iS5 spectroscopy scanning with reflector diamond named attenuated and total reflectance (ATR) iD7 at 4000 – 400 cm^{-1} area [20].

Water and Ash Contents

Water and ash contents were analyzed by a gravimetric method based on the Indonesian National Standard (SNI) 2354.2:2015 [23] for water contents and SNI 2345.1:2010 [24] for ash contents.

Crude Protein Contents

Crude protein content was analyzed using the Dumas method using Dumatherm protein analyzer (Gerhardt Dumatherm DT). Crude laminarin extract was weighed, wrapped using tin foil, and placed on a Dumatherm tray. Tweezers picked the sample and inserted it into the autosampler carousel with 980°C combustion furnace, 650°C reduction furnace, and 300°C degassing furnace.

Crude Fibre Contents

Crude fiber content was analyzed by a gravimetric method using the Fibretherm instrument (Gerhardt Fibretherm FT 12). The analysis started by separately weighing the empty fiber bag (C) and the crude laminarin extract (FO). Crude laminarin extract was put into a fiber bag and placed into the carousel, and then the program on Fibretherm Gerhardt could be operated. The next step was to put the sample into a porcelain crucible and dried using an oven at 105°C for 3 hours. Next, the sample was cooled in a desiccator and then weighed (A). After that, the sample was put into a furnace at a temperature of 550°C for 6 hours, cooled in a desiccator and weighed (B). The equation for determining crude fiber content was as follows:

$$\%\ crude\ fiber = \frac{A-B-C}{FO} \times 100\% \quad (1)$$

RESULTS AND DISCUSSION

Effect of Solvent Type and Ratio for Laminarin Extraction

The raw material used to produce laminarin in this study was brown seaweed, *Sargassum* sp., which grows wild in Teluk

Awur, Jepara, Indonesia. Crude laminarin from *Sargassum* sp. extracted by LAE method with different solvent types and ratios (w/v). Two solvent types that used in this research were hydrochloric acid (L-HCl), which is an inedible strong acid solvent, and acetic acid (L-ACT), which is a weak acid solvent. The solvent ratio used for this extraction was 1:5 and 1:10 (w/v). The yield of laminarin crude extract could be seen on Table 1.

Tabel 1. Crude laminarin extraction of *Sargassum* sp. from different solvent types and ratio

Sample Code	Dry Crude Laminarin Extract (g)	Crude Laminarin Yield (%)
L-HCL (1:5)	10,87	10,87
L-HCL (1:10)	24,17	24,17
L-ACT (1:5)	15,5	15,5
L-ACT (1:10)	9,92	9,92

The result showed that the use of different solvent types and ratios gave different yield percentages. Extraction of L-HCl (1:10) was able to produce the highest yield value at 24.17% compared to extraction of L-HCl (1:5), L-ACT (1:5), and L-ACT (1:10) at 10.87%, 15.5%, and 9.92%, respectively. The laminarin crude extracted by L-HCl was having brown color, the texture was easily crushed to form powder, and easier to dry completely. Meanwhile laminarin crude extracted by L-ACT was blackish brown, the texture was slightly sticky to form lumps and difficult to dry completely even though it has evaporated through a drying process that takes 1 day longer than the L-HCl extract. The laminarin crude L-HCl and L-ACT could be seen in Figure 1.

(a) (b)

Figure 1. *Sargassum* sp. crude laminarin extraction with (a) HCl solvent (L-HCl) and (b) acetic acid solvent (L-ACT).

Laminarin contained in *Sargassum* sp. can be extracted optimally using hydrochloric acid compared to acetic acid because hydrochloric acid is a strong acid that can cause lysis or degradation of the cell wall [4,25], thus the laminarin becomes more soluble in the extraction solution. Hydrochloric acid has an acid dissociation constant (pKa) of 7, while acetic acid is a weak acid with a pKa of 4.74. The stronger the acid, the greater cell degradation efficiency [26], andthe greater the pKa value, the higher amount of an acid that dissociates. The high dissociation causes an increase in the number of hydrogen ions, so the ability of acid to attract divalent ions and replace them with hydrogen ions becomes stronger. Hydrogen ions functioned to hydrolyze the cell wall, thus the yield of laminarin can be produced [27,28].

Apart from the influence of solvent types, the larger solvent ratio gave a significant difference in the extraction results. Solvent ratio of 1:10 (w/v) with hydrochloric acid solvent had the highest yield of *Sargassum* sp. crude laminarin compared to other treatments. Due to the character of dry *Sargassum* powder that had high water absorption capacity, less dry *Sargassum* powder used in the extraction made the less solvent absorbed into it.

Therefore, the filtrate extraction yield was huge. The number of solvents used facilitates the stirring process during the extraction. Stirring functioned to increase the interaction of hydrogen ions in the solvent with *Sargassum* sp. extract. The greater interaction occurs, the greater the laminarin that dissolve in extraction solvents thus optimum yield can be obtained.

In a previous study [29], the laminarin extraction of *L. gurjanovae* using cold extraction method with HCl solvent obtained a laminarin yield of 17.6%, while hot extraction with water obtained 8.7%. Meanwhile, crude laminarin from *Eisenia bicyclis* which is extracted with 0.09 N HCl at room temperature produced a 14,5% yield of the dry weight of seaweed [17]. Another study performed laminarin extraction using the Ultrasound assisted extraction (UAE) method with hydrochloric acid which produced laminarin concentrations at 5.82% and 6.24% respectively of *A. nodosum* and *L. hyperborea* dry weight of [2]. If compared to three studies above, the research laminarin extraction results from Sargassum sp. originated from Jepara, Indonesia in this research was much higher, 0.87 - 24.17% through extraction used HCL and 9.92 - 15.5% through extraction used acetic acid. One of the reasons was the different sources of laminarin used. Variations in the seaweed laminarin content can be influenced by the type of seaweed, harvest season, living habitat, and extraction method [2].

Characterization of Crude Laminarin

Characterization of crude laminarin could be analyzed by the pattern of functional groups determined from infrared absorption of an FT-IR spectrometer with a frequency between 4000 – 400 cm^{-1}. This FT-IR method is sensitive to anomeric structure (position and configuration) of glycosidic bonds in glucan. It is possible to analyze glucan in high molecular fraction crude isolated from various raw materials [30]. The analysis of functional group absorption band of L-HCl and L-ACT samples has been compared to absorption bands from standard laminarin (sigma) from other studies [20]. The FT-IR absorption band produced (Figure 2) showed that L-HCl and L-ACT had infrared absorption bands that were similar to other laminarin standard absorption bands in previous studies [20].

Based on the absorption band, L-HCl and L-ACT samples showed wide absorption peaks at wave numbers of 3278.36 cm^{-1} and 3311.74 cm^{-1} respectively, which vibrated from the stretching of O-H functional group from carbohydrate [7]. The wide absorption peak near 3400 cm^{-1} represents O-H of hydroxyl stretching for all polysaccharide compounds [7,30]. The absorption band was also identified in standard laminarin [20]. The bending vibration of the -OH group was detected in the L-HCl sample at a wave number of 2360.62 cm-1, while in the L-ACT sample, there was an absorption peak in the wave number region at 2359.96 cm^{-1}.Vibration of aromatic C-C functional group in both samples of laminarin (L-HCl at 1618.00 cm^{-1} and L-ACT at 1538.54 cm^{-1}) showed anisomeric stretching with absorption peak around 1620 cm^{-1} which was also found in standard laminarin [20,31]. Peak absorbance of L-HCl and L-ACT at 1406.74 cm-1 and 1409.35 cm-1 respectively indicated the presence of carboxyl group (C-H bending vibration) as well as standard laminarin with peaks approaching 1420 cm^{-1} [20].

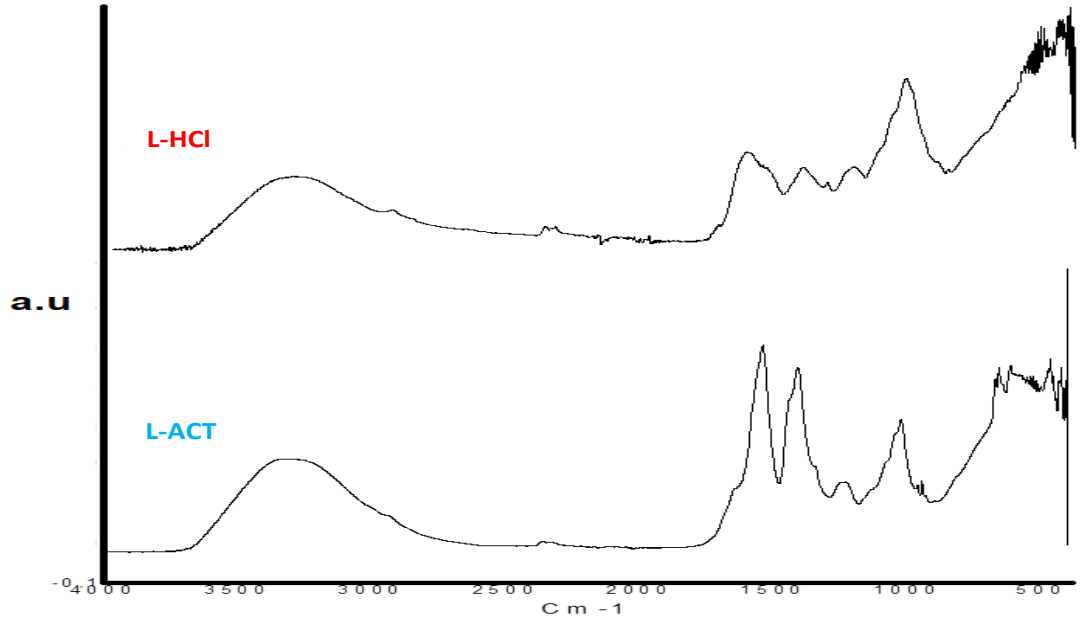

Figure 2. FT-IR spectrum of L-HCl and L-ACT.

Tabel 2. interpretation of FT-IR spectra of laminarin crude extract

Wave Number (cm^{-1})			possible functional groups
L-ACT	L-HCl	Reference [20]	
3311.74	3278.36	3400	-OH stretching; -OH Groups
2359.96	2360.62	2410	-OH bending; Transmitting angle peak
1538.54	1618.00	1620	C-C aromatic; anisomeric stretching
1409.35	1406.74	1420	C-H bending; carboxyl groups
1230.89	1220.94	1200	C-O-C Stretching; sugar region
1022.58	1030.27	1030	R-O-R (Acetal);
944.94	910.24	950	O-R cyclic

There are two important absorption area to characterize structural polysaccharides, namely the sugar area with 1200 – 950 cm^{-1} of wavelength and the anomeric area with 950 – 750 cm^{-1} of wavelength [30]. The stretching vibration of C-O-C and R-O-R (Acetal) on glycosidic bond and pyranoid ring produced absorption bands for two samples, with L-HCl number at 1220.94 cm^{-1} and 1030.27 cm^{-1}, while L-ACT at 1230.89 cm^{-1} and 1022.58 cm^{-1}. Meanwhile, the glycosidic bond and pyranoid ring of standard laminarin dominated at peaks of 1200 cm^{-1} and 1030 cm^{-1} [20,32]. The occurrence of vibrations in the sugar area indicates the presence of alduronic acid (sugar acid) such as guluronic acid or mannuronic acid in polysaccharides. Uronic acid is a constituent component of alginate polymers in the form of alginic acid [20,33]. Alginic acid is observable on standard laminarin [20]. Furthermore, a weak absorption band at 910.24 cm-1 from L-HCl and a strong absorption band at 944.94 cm-1 from L-ACT indicated vibration of cyclic O-R in glucose anomeric structure [30]. Meanwhile, on the standard laminar, the presence of a glucose anomeric structure is indicated by a weak band at 880 cm^{-1} [30]. Band shifting towards a higher wave number

is a marker of an amorphous state [30]. Thus, extracting Sargassum from the sea waters of Jepara using either a strong or weak acid extraction method could produce laminarin.

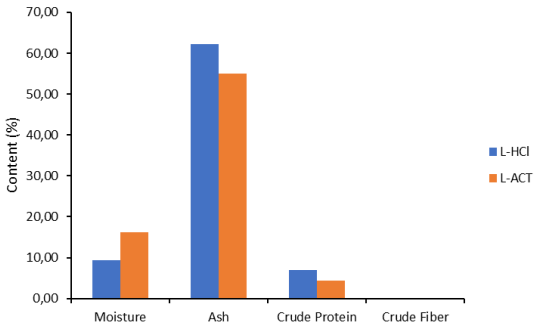

Figure 3. Nutrition content from crude laminarin extract.

Laminarin has been known to have various bio-functional activities such as antitumor, anti-apoptotic, anti-inflammatory, anticoagulant, and antioxidant activity [2]. Research on feeding laminarin-rich extract to animals has shown its suitability as a functional food and feed [2]. Besides its yield and chemical functional groups, the characteristics and quality of laminarin crude extract from Sargassum sp. could be observed based on its nutritional components, such as moisture, ash, crude protein, and crude fiber content. Therefore, the nutrition composition could determine the potential of such material as a nutraceutical ingredient. Factors that can affect the nutritional composition of a seaweed extract are the species and the extraction process; in this study, the extraction process was conducted in three steps which were: the heating process with acid solvents (acetic acid and hydrochloric acid), the filtering to separate filtrate from residue, then the cold drying to obtain laminarin crude extract. The chemical composition of laminarin crude extract as a nutritional value consisting of water and ash content, crude protein content and crude fiber content of laminarin crude extract was shown in Figure 3.

The water content of *Sargassum* sp. extracted with HCl solvent (L-HCl) was lower at 9,41% compared to extraction with acetic acid solvent (L-ACT) at 16,19%. The high water content of L-ACT showed that the drying method was less effective to be applied to *Sargassum* sp. extracted with acetic acid solvent. The high or low value of water content in a product can be influenced by drying conditions (drying temperature, drying time, and a combination of both) so that treatment with the right drying conditions will produce the desired moisture content [34–36]. However, with the same drying method, L-HCl had low water content. It may be due to differences in hydrophilicity and volatility between the acetic acid and hydrochloric acid contained in the filtrate that affected water content in the extract [37,38]. The hydrophilicity and volatility of acetic acid were lower than hydrochloric acid. Thus, it was more difficult to separate the laminarin extract from acetic acid solvent by cold drying. This water content is an important characteristic in determining the quality of laminarin extract as it can affect the shelf life of the laminarin crude extract [34]. The water contained in the laminarin crude extract can trigger microbiological activity, affectinghe storage period and the quality of laminarin [39]. Products with a low water content can have a longer shelf life than those with high water content [40]. Maximum moisture content for dry products is 10%, and products

with less than 14% of moisture can prevent mold growth [41].

Besides water content, ash content also affected the quality of seaweed laminarin, which can determine the quality of product purity from undesired components [40]. Ash content can also be related to the number of inorganic compounds and salts in the aquatic environment where algae grow [42]. The ash content of L-HCl extraction in this study was 62.16%, higher than L-ACT which was 55.05%. Meanwhile, other studies reported that hot water extract from *Sargassum polycystum* originating from the coast of Bo Mao Bay, Thailand has a very low ash content value of 0.19 ± 0.04% [43]. The high ash content in L-HCl was probably due to extraction using hydrochloric acid which was strong acid. L-HCL solvent dissolved the minerals contained in seaweed better compared to extraction using acetic acid solvent which is a weak acid. High mineral content affects high ash content vice versa. If the mineral content is small, the ash content of the material is also small [40,44]. The high or low ash content is affected by the presence of other mineral salts attached to seaweed such as sodium and calcium [34,45]. High ash content in seaweed extract flour mostly comes from salts and other minerals that bound to seaweed polymers (polysaccharides), such as K, Mg, Ca, Na, and ammonium galactose and their sulfate content [34,35]. Furthermore, the ash content in the final product of processed algae may be affected by the washing procedure after algae harvesting. In addition, the residue from the use of inorganic acid solvents such as HCl can increase the ash content of the crude laminarin.

The brown seaweed has a lower protein content, ranging from 3-15% of dry weight, compared to green and red seaweed, which have 10-47% dry weight protein content [42,46]. According to MacArtain et.al. [47], the protein content of seaweed ranges from 4.6% of dry weight and varies depending on the season and species. For Sargassaceae seaweed such as Sargassum binderi, Sargassum cinereum, Sargassum crassifolium, and Sargassum sp., the protein content varies between 5.19 – 16.72% [46]. The highest protein levels are obtained during the winter and spring, while the lowest protein levels are obtained during summer [46]. Environmental conditions, such as light intensity and nitrogen storage, influence the protein content of seaweed during different seasons. In winter and spring, the presence of nutrients reaches a maximum, which results in maximum protein formation. Conversely, during summer, the high intensity of sunlight causes protein degradation, and thus protein storage in the thallus is not maximized [46]. In tropical regions, such as Indonesia, the protein content of seaweed is higher during the rainy season compared to the dry season.

The protein content of a product can determine its quality [41]. Therefore, a higher protein content is associated with better product quality [41]. In this study, the crude protein content of L-ACT was 4.3%, lower than the protein content of L-HCl (7.00%). However, the protein level of L-ACT was not significantly different from the lowest protein range of seaweed from the Sargassaceae

family, which is around 5.19% [46]. Chamidah et al. [4], which produced a protein content of 4.33% in laminarin extracted with sulfuric acid solvent, the use of a weak acid solvent in the form of acetic acid in this study was able to extract laminarin without decreasing the protein content. During the extraction process, the solubility of a compound in a solvent can be influenced by polar and nonpolar bonds [48]. Protein is a polar compound, and thus it can easily bond with polar solvents. HCl is more polar than acetic acid so it can bind more protein molecules. The high amount of protein dissolved in the HCl solvent is due to the many hydrogen bonds formed between HCl and protein molecules. In acetic acid, the number of hydrogen bonds formed is lower due to the presence of hydrocarbon bonds that cannot form hydrogen bonds [48].

The results of crude fiber analysis both in L-HCl and L-ACT were not identified (0%). This indicated that HCl and acetic acid at pH 3 did not leave crude fiber inside the extract. The crude fiber content in a material can change due to the processing on the original material [49]. Crude fiber is part of food that cannot be hydrolyzed by chemicals or strong acids and strong bases [49]. Crude fiber consists of lignin which is not soluble in alkali, which is related to nitrogen and cellulose [50]. The crude fiber fraction contains cellulose, hemicellulose and lignin, depending on the species and growth phase of the plant material [51]. Crude fiber can play a role in blocking the absorption of other nutrients such as fat, carbohydrates and protein thus all nutrients can be absorbed [49]. Crude fiber is important in assessing the quality of food ingredients because this number is an index to determine food's nutritional value [49]. The high crude fiber content in a product affects the slow rate of nutrition digestion and absorption [52]. The absence of identified crude fiber in the laminarin indicates that the product had a high level of digestibility.

CONCLUSION

Sargassum sp. originated from the Jepara, Central Java could produce a crude laminarin extract around 9.92 – 24.17% of dry weight through the *Laminarin Acid Extraction* (LAE) method, so it was potential to be used as a source of raw material for producing laminarin. The use of acetic acid (pH 3) in the LAE method as an alternative of edible solvent to produce crude laminarin extract from *Sargassum* sp. up to 15.5 % of dry weight with a solvent ratio of 1:5 (w/v). The crude laminarin extract produced from extraction using acetic acid solvent (L-ACT) had a lower ash content at 55.05% compared to extraction using hydrochloric acid (L-HCl) at 62.16%. The protein content in L-ACT about 4.3%, was not different from most of the algae protein studied which was 4.3% of the dry weight, although still lower than the protein content contained in L-HCl (7.00%). Meanwhile, crude fiber levels in both L-ACT and L-HCl were not identified. On the other hand, the water content in L-ACT was still high (16.19%), even though it had gone through a longer drying time than L-HCl. from this study, it could be seen that acetic acid had a potency to replace hydrochloric acid as a solvent in the LAE method in many previous studies to produce laminarin from *Sargassum* sp. with several advantages such as the cost of extraction becomes lower, safer, and

allows it to be applied on an industrial scale. Nonetheless, further studies could focus on optimizing the extraction process with acetic acid as a solvent to reduce the water content in the laminarin crude extract to below 10%. In this study, the origin of the raw materials and the differences in the extraction process may give different results.

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REFERENCES

[1] C. S. Pakidi and H. S. Suwoyo, "Potensi dan Pemanfaatan Bahan Aktif Alga Cokelat *Sargassum sp*," *Octopus : Jurnal Ilmu Perikanan*, vol. 6, no. 1, pp. 551-562, Jun. 2017, doi: 10.26618/OCTOPUS.V6I1.708.

[2] S. U. Kadam, B. K. Tiwari, and C. P. O'Donnell, "Extraction, structure and biofunctional activities of laminarin from brown algae," *Int. J. Food Sci. Technol.*, vol. 50, no. 1, pp. 24–31, Jan. 2015, doi: 10.1111/IJFS.12692.

[3] L. A. Hussein, "Novel prebiotics and next-generation probiotics: opportunities and challenges," *Functional Foods and Nutraceuticals in Metabolic and Non-Communicable Diseases*, pp. 431–457, Jan. 2022, doi:10.1016/B978-0-12-819815-5.00055-0.

[4] A. Chamidah, Y. Marsono, and E. Harmayani, "Pengaruh Metode Ekstraksi terhadap Karakteristik Crude Laminaran dari Sargassum duplicatum," *AGRITECH*, vol. 33, no. 3, 2013, doi: 10.22146/agritech.9545.

[5] Z. Li, R. Anbuchezhian, and V. Karuppiah, "Prospect of Marine Algae for Production of Industrially Important Chemicals," *Algal Biorefinery: An Integrated Approach*, pp. 195–217, Jan. 2016, doi: 10.1007/978-3-319-22813-6_9.

[6] L.-E. Rioux, S. L. Turgeon, and M. Beaulieu, "Characterization of polysaccharides extracted from brown seaweeds," *Carbohydr. Polym.*, vol. 69, no. 3, pp. 530–537, Jun. 2007, doi: 10.1016/j.carbpol.2007.01.009.

[7] R. S. Panjaitan and L. Natalia, "Ekstraksi Polisakarida Sulfat dari *Sargassum polycystum* dengan Metode Microwave Assisted Extraction dan Uji Toksisitasnya," *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan*, vol. 16, no. 1, Jun. 2021, doi: 10.15578/jpbkp.v16i1.692.

[8] A. Kadi, "Beberapa Catatan Kehadiran Marga Sargassum di Perairan Indonesia," *Oseana*, vol. XXX, no. 4, pp. 19–29, 2005.

[9] Y. Peng, "Nutritional and Chemical Composition and Antiviral Activity of Cultivated Seaweed Sargassum naozhouense Tseng et Lu," *Mar. Drugs*, vol. 11, no. 1, pp. 20–32, 2013, doi: 10.3390/MD11010020.

[10] A. Chamidah, A. Awaludin Prihanto, H. Hardoko, and A. A. Prihanto, "Edema-reducing activity of Sargassum crassifolium B-glucan (Laminaran) on edema-induced Rattus norvegicus," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 10, 2017, doi: 10.22159/ajpcr.2017.v10i7.18523.

[11] N. A. Mohd Fauziee, L. S. Chang, W. A. Wan Mustapha, A. R. Md Nor, and S. J. Lim, "Functional polysaccharides of fucoidan, laminaran and alginate from Malaysian brown seaweeds (*Sargassum polycystum, Turbinaria ornata* and *Padina boryana*)," *Int. J. Biol. Macromol.*, vol. 167, pp. 1135–1145, Jan. 2021, doi: 10.1016/j.ijbiomac.2020.11.067.

[12] Y. Cui, "Structure of a laminarin-type β-(1→3)-glucan from brown algae Sargassum henslowianum and its potential on regulating gut microbiota," *Carbohydr. Polym.*, vol. 255, p. 117389, Mar. 2021, doi: 10.1016/j.carbpol.2020.117389.

[13] J.-C. Yvin, F. LeVasseur, and F. Hud'Homme, "Use of laminarin and oligosaccharides derived therefrom in cosmetics and for preparing a skin treatment drug," May 11, 1999

[14] C. Devillé, J. Damas, P. Forget, G. Dandrifosse, and O. Peulen, "Laminarin in the dietary fibre concept," *J. Sci. Food Agric.*, vol. 84, no. 9, pp. 1030–1038, Jul. 2004, doi: 10.1002/jsfa.1754.

[15] A. O. Chizhov *et al.*, "Structural analysis of laminarans by MALDI and FAB mass spectrometry," *Carbohydr. Res.*, vol. 3, no. 310, pp. 203–210, Aug. 1998, doi: 10.1016/S0008-6215(98)00177-3.

[16] Mohsen, M. S. Asker, Sahar, F. M. ali, and O. H. El-Sayed, "Chemical structure and antiviral activity of water-soluble sulfated polysaccharides from *Sargassum latifolium* Chemical Structure and Antiviral Activity of Water-soluble Sulfated Polysaccharides from Surgassium latifolium," *J. Appl. Sci. Res.*, vol. 3, no. 10, pp. 1178–1185, 2007.

[17] Y.-M. Kim, Y.-S. Choi, and J.-H. Park, "Purification and Chemical Characterisation of Laminaran from Eisenia bicyclis in Korea," *Journal of the Korean Society of Food Science and Nutrition*, vol. 35, no. 1, pp. 78–86, Jan. 2006, doi: 10.3746/JKFN.2006.35.1.078.

[18] M. Himaja, D. Poppy, and K. Asif, "Green Technique-Solvent Free Synthesis and Its Advantages," *Int. J. Res. Ayurveda Pharm.*, vol. 2, no. 4, pp. 1079–1086, 2011.

[19] S. Utomo, "Bahan Berbahaya dan Beracun (B-3) dan Keberadaannya di dalam Limbah," *KONVERSI*, vol. 1, no. 1, pp. 37–46, 2012, doi: 10.24853/konversi.1.1.%25p.

[20] G. Rajauria, R. Ravindran, M. Garcia-Vaquero, D. K. Rai, T. Sweeney, and J. O'Doherty, "Molecular characteristics and antioxidant activity of laminarin extracted from the seaweed species Laminaria hyperborea, using hydrothermal-assisted extraction and a multi-step purification procedure," *Food Hydrocoll.*, vol. 112, p. 106332, Mar. 2021, doi:10.1016/J.FOODHYD.2020.106332.

[21] E. Yudiati, A. Isnansetyo, Murwantoko, Ayuningtyas, Triyanto, and C. R. Handayani, "Innate immune-stimulating and immune genes up-regulating activities of three types of alginate from Sargassum siliquosum in Pacific white shrimp, Litopenaeus vannamei," *Fish Shellfish Immunol.*, vol. 54, pp. 46–53, Jul. 2016, doi: 10.1016/j.fsi.2016.03.022.

[22] A. T. Septiana and A. Asnani, "Kajian Sifat Fisikomimia Ekstrak Rumput Laut Coklat *Sargassum duplicatum* Menggunakan Berbagai Pelarut dan Metode Ekstraksi," *AGROINTEK*, vol. 6, no. 1, pp. 22–28, 2012, doi: 10.21107/agrointek.v6i1.1950.

[23] Badan Standardisasi Nasional (BSN), "Cara uji kimia-Bagian 2: Pengujian kadar air pada produk perikanan. SNI 2345.2:2015," Jakarta, 2015, SNI: 2354.2:2015

[24] Badan Standardisasi Nasional (BSN), "Cara uji kimia- Bagian 1: Penentuan kadar abu dan abu tak larut dalam asam pada produk perikanan. SNI 2345.1:2010," Jakarta, 2010, SNI: 2345.1:2010

[25] Basito, "Efektivitas Penambahan Etanol 95% dengan Variasi Asam dalam Proses Ekstraksi Pigmen Antosianin Kulit Manggis (*Garcinia mangostana L.*)," *Jurnal Teknologi Hasil Pertanian*, vol. IV, no. 2, pp. 84–93, Aug. 2011, doi: 10.20961/jthp.v0i0.13570.

[26] M. Michelon "Extraction of Carotenoids from *Phaffia rhodozyma*: A Comparison between Different Techniques of Cell Disruption," *Food Sci. Biotechnol.*, vol. 21, no. 1, pp. 1–8, 2012, doi: 10.1007/s10068-012-0001-9.

[27] R. A. Wulaningrum, W. Sunarto, and M. Alauhdin, "Pengaruh Asam Organik dalam Ekstraksi Zat Warna Kulit Buah Manggis (Garcinia mangostana)," *Indo. J. Chem. Sci.*, vol. 2, no. 2, 2013, doi: 10.15294/ijcs.v3i1.2866.

[28] K. Y. Kesuma, N. W. R. Widarta, and I. D. G. M. Permana, "Pengaruh Jenis Asam dan pH Pelarut terhadap Karakteristik Pektin dari Kulit Lemon (*Citrus limon*)," *Jurnal Ilmu dan Teknologi Pangan*, vol. 7, no. 4, pp. 192–203, Dec. 2018, doi: 10.24843/itepa.2018.v07.i04.p06.

[29] N. M. Shevchenko, S. D. Anastyuk, N. I. Gerasimenko, P. S. Dmitrenok, V. V Isakov, and T. N. Zvyagintseva, "Polysaccharide and Lipid Composition of the Brown Seaweed *Laminaria gurjanovae*," *Russ. J. Bioorg. Chem.*, vol. 33, no. 1, pp. 96–107, 2007, doi: 10.1134/S1068162007010116.

[30] A. Synytsya and M. Novak, "Structural analysis of glucans," *Annals. of Translational Medicine*, vol. 2, no. 2. AME Publishing Company, Feb. 01, 2014. doi:10.3978/j.issn.2305-5839.2014.02.07.

[31] R. B. Xu, "Chemical Composition and Antioxidant Activities of Three Polysaccharide Fractions from Pine Cones," *International Journal of Molecular Sciences,* vol. 13, no. 11, pp. 14262–14277, Nov. 2012, doi: 10.3390/IJMS131114262.

[32] X. Zhang, Y. Liu, X. Q. Chen, J. J. Aweya, and K. L. Cheong, "Catabolism of *Saccharina japonica* polysaccharides and oligosaccharides by human fecal microbiota," *LWT*, vol. 130, p. 109635, Aug. 2020, doi: 10.1016/J.LWT.2020.109635.

[33] M. Sterner and U. Edlund, "Multicomponent fractionation of *Saccharina latissima* brown algae using chelating salt solutions," *J. Appl. Phycol.*, vol. 28, no. 4, pp. 2561–2574, Jan. 2016, doi:10.1007/S10811-01507850/TABLES/10.

[34] K. P. Hermanto, "Pengaruh Perbedaan Konsentrasi Larutan Alkali $Ca(OH)_2$ terhadap Mutu Tepung Karagenan yang Dihasilkan *Euchema cottonii*," *Jurnal Akuatek*, vol. 2, no. 1, pp. 51–57, 2021, doi: 10.24198/akuatek.v2i1.33750.

[35] D. Yuarni, K. Kadirman, and J. P. J. P, "Laju Perubahan Kadar Air, Kadar Protein dan Uji Organoleptik Ikan Lele Asin Menggunakan Alat Pengering Kabinet (Cabinet Dryer) Dengan Suhu Terkontrol," *Jurnal Pendidikan Teknologi Pertanian*, vol. 1, no. 1, pp. 12–21, Aug. 2015, doi: 10.26858/JPTP.V1I1.5139.

[36] M. Lisa, M. Lutfi, and B. Susilo, "Pengaruh Suhu dan Lama Pengeringan terhadap Mutu Tepung Jamur Tiram Putih (Plaerotus ostreatus)," *Jurnal Keteknikan Pertanian Tropis dan Biosistem*, vol. 3, no. 3, pp. 270–279, 2015, doi: 10.21776/jkptb.v3i3.293.

[37] F. J. Polnaya, H. Hilda, and C. G. C. Lopulalan, "Konsentrasi Asam Asetat Memengaruhi Karakteristik Fisikokimia Pati Sagu Ihur Terasetilasi," *Jurnal Teknologi dan Industri Pangan*, vol. 31, no. 2, pp. 180–187, Dec. 2020, doi: 10.6066/jtip.2020.31.2.180.

[38] I. M. T. W. Aristya, B. Admadi, and I. W. Arnata, "Karakteristik Mutu dan Rendemen Alginat dari Ekstrak Rumput Laut *Sargassum sp.* dengan Menggunakan Larutan Asam Asetat," *Rekayasa dan Manajemen Agroindustri*, vol. 5, no. 1, pp. 81–92, 2017.

[39] S. A. Saputra, M. Yulian, and K. Nisahi, "Karakteristik dan Kualitas Mutu Karaginan Rumput Laut di Indonesia," *Lantanida Journal*, vol. 9, no. 1, pp. 25–37, 2021, doi: 10.22373/lj.v9i1.9189.

[40] R. Masrikhiyah and A. D. Wahyani, "Karakteristik Kimia dan Fisik Bubuk Rumput Laut *Gracilaria sp.* dengan Agen Pemucat NaOCl," *Jurnal Teknologi Perikanan dan Kelautan*, vol. 11, no. 1, pp. 93–98, 2020, doi: 10.24319/jtpk.11.93-98.

[41] F. G. Winarno, *Kimia Pangan dan Gizi*. Jakarta: Gramedia Pustaka Utama, 1997.

[42] S. H. Manteu, N. Nurjanah, and T. Nurhayati, "Characteristics of Brown Seaweeds *Sargassum policystum* and Padina minor from Pohuwato Water, Gorontalo," *J. Pengolahan Hasil Perikanan Indonesia*, vol. 21, no. 3, p. 296, Dec. 2018, doi: 10.17844/JPHPI.V21I3.24709.

[43] M. Yangthong, J. Ruensirikul, and G. Kaneko, "The Hot-Water Extract of Sargassum sp. as a Feed Ingredient for Spotted Scat (Scatophagus argus Linnaeus, 1766) Reared in Songkhla Lake: Effects on Growth, Feed Efficiency, Hematological Data and Body Composition," *Fishes*, vol. 7, no. 4, p. 170, Jul. 2022, doi: 10.3390/fishes7040170.

[44] Warkoyo, "Studi Ekstraksi Karaginan dari Rumput Laut *Eucheuma cottonii* (Kajian Jenis Larutan Perendaman dan Lama Perendaman)," *Jurnal Protein*, vol. 14, no. 1, pp. 49–56, 2007.

[45] A. P. Romenda, R. Pramesti, and A. Susanto, "Pengaruh Perbedaan Jenis dan Konsentrasi Larutan Alkali Terhadap Kekuatan Gel dan Viskositas Karaginan Kappaphycus alvarezii, Doty," *JMR*, vol. 2, no. 1, pp. 127–133, 2013, doi: 10.14710/JMR.V2I1.2065.

[46] T. Handayani, "Kandungan Nutrisi Pada Rumput Laut," *Oseana*, vol. XXXVI, no. 2, pp. 1–10, 2011.

[47] P. MacArtain, C. I. R. Gill, M. Brooks, R. Campbell, and I. R. Rowland, "Nutritional Value of Edible Seaweeds," *Nutr. Rev.*, vol. 65, no. 12, pp. 535–543, Dec. 2007, doi10.1111/J.1753-4887.2007.TB00278.X.

[48] M. D. Masyitoh, I. D. A. R. Dewanti, and D. Setyorini, "Analisis Profil Protein Ekstrak Aquades dan Etanol Daun Mimba (Azadirachta Indica A. Juss) dengan Metode SDS-PAGE," *Jurnal Pustaka Kesehatan*, vol. 4, no. 3, pp. 533–539, 2016.

[49] Hardiyanti and K. Nisah, “Analisis Kadar Serat Pada Bakso Bekatul Dengan Metode Gravimetri,” *AMINA*, vol. 1, no. 3, pp. 103–107, 2019.

[50] D. J. R. Cherney, “Characterization of forages by chemical analysis.,” *Forage evaluation in ruminant nutrition.*, pp. 281–300, Jan. 2000, doi: 10.1079/9780851993447.0281.

[51] Y. Pasaribu and I. I. Pratiwi, “Kandungan Serat Kasar Centrosema pubescens dan Capologonium mucunoides di Kampung Wasur,” *Agricola*, vol. 4, no. 1, pp. 33–40, 2014.

[52] G. G. Maradon, R. Sutrisna, and dan Erwanto, “Pengaruh Ransum Dengan Kadar Serat Kasar Berbeda Terhadap Organ Dalam Ayam Jantan Tipe Medium Umur 8 Minggu,” *Jurnal Ilmiah Peternakan Terpadu*, vol. 3, no. 2, pp. 6–11, 2015, doi: 10.23960/jipt.v3i2.p%25p.