Effect of chitosan as adsorben to bacto agar quality from *Gelidium sp.*

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Abstract. Gelidium sp. is a seaweed that produces agar with a high gel strength. Bacto agar must have a high purity, so it needs a right purification method. One of the ways by adsorption process using chitosan. The aim of this research was to determine the influence of chitosan as an adsorbent and the optimum condition of its adsorption process. Bacto agar was extracted with water at 90°C. An alkali and acid pretreatment were added in the extraction process in order to increase the physico-chemical properties of the agar gels. The agar extract was adsorbed by chitosan with various concentration of chitosan (0.5; 0.75; and 1%) and adsorption time (0, 30, and 60 minutes). The characteristic of bacto agar include moisture content, ash content, acid-insoluble ash content, sulphate content, gel strength, viscosity, syneresis, gelling and melting points, analysis of its molecular structure by FTIR and SEM. Bacto agar resulted in this research were tested as a bacterial culture medium using Total Plate Count (TPC) method. The best chitosan treatment process was obtained in bacto agar at 0.75% and 0 minute of adsorption which is had the physico-chemical properties that met the criteria of commercial bacto agar with 3.50% of bacto agar yield, 14.61% moisture content, 4.02% ash content, 0.93% acid-insoluble ash content, 1.34% sulphate content, 1054.96 g/cm² gel strength, 60 Cp viscosity, 4.62% syneresis, 65 °C and 20.75 °C of melting and gelling points respectively. The microbial analysis shows that bacto agar can be used as a bacterial culture medium.

1. Introduction

Seaweed is one of the superior marine products that has economic value that can drive the economic sector, starting from the level of farmers, producers, processors to users [1]. One type of seaweed that can be used is Rhodophyceae (red algae) which can produce agar. Agar has been widely used in the food industry either as a food product or as a food additive. Agar has also been used as a medium for bacterial growth to replace gelatin, which is unstable at hot temperatures [2]. The use of agar as a microbiological medium tends to increase, this is because only high quality pure agar can become a medium for bacterial growth.

Bacto agar is an agar that is used as a microbiological culture medium that has been purified by reducing as low as possible foreign substances, dyes, metal ions, impurity particles, coarse particles and mineral salts [3]. Bacto production so as not to meet domestic needs and to meet these needs still fully rely on imported products which are quite expensive [4]. One of the efforts to reduce imports of Bacto agar is to use local seaweed as raw material for domestic production of Bacto so that the domestic...
products are of the same quality as imported products so that it is expected to improve the welfare of the community, especially in coastal areas.

This research used Gelidium sp. for making bacto agar. According to Ahmad et al. (2011) [5], that agar extracted from the Gelidium type has the best quality, due to its high gel strength. However, due to its limited availability and high price (Ahmad et al., 2011) and its high impurity content [4], the use of Gelidium in agar production is less desirable than Gracilaria. The presence of impurities will affect the physico-chemical properties of the resulting agar and reduce its quality.

Increasing the quality of Bacto agar from Gelidium can be done by purifying agar by the adsorption method. Adsorbents derived from nature are potential materials for use in adsorption because they are more environmentally friendly [6]. One of the natural ingredients that can be used is chitosan. Chitosan is a modified biopolymer of chitin compounds found on the outer skin of crustacean animals [7]. Chitosan has properties as an adsorbent in handling heavy metal waste [8]–[10] and as an absorbent for textile dye waste because it is able to bind ions [11]–[13]. In addition, the nature of chitosan which is easy to absorb water is also used as a coating on food so it helps the process of storing and preserving food because it can reduce the moisture content of the material [14]. The use of chitosan as an adsorbent has increased, because of its good adsorption ability due to the presence of its amine and hydroxyl groups, which causes chitosan to be polyelectrolyte and has high chemical reactivity [8]. In addition, the raw materials for manufacture are abundant and environmentally friendly because they are biocompatible and biodegradable so they are not toxic and harmless [11], [15].

Research on the purification of bio-agar using chitosan adsorbent has been widely carried out. Abdullah and Suptijah [16] studied the effect of adding various concentrations of chitosan on making Bacto agar from Gracilaria seaweed and obtained the optimum conditions for the addition of 1.5% chitosan. Research by Abdullah et al. [17], added chitosan in making Bacto agar from Gracilaria seaweed and agar stems by modifying the chitosan concentration and absorption process time and produced the optimum treatment combination at a concentration of 1% with a heating time of 45 minutes for agar bacteria from Gracilaria sp. and 0.5% for the agar from agar stems without heating. Purification of bacto agar with chitosan is considered capable of reducing the ash and sulfate content in agar which will affect the purity and physical properties of the resulting gel.

This study aims to determine the effect of the addition of chitosan as an adsorbent on the purification of the agar made from Gelidium sp. Chitosan was added with a concentration difference of 0.5; 0.75 and 1% (“b”/”v”) and the adsorption time for 0, 30 and 60 minutes, respectively. The optimum condition for adding chitosan was determined to determine the efficiency of the treatment in making bacto so that it could reduce production costs without reducing its quality. Bacto agar produced is characterized by physical properties including gel strength, viscosity, melting point, and gelling point, its chemical properties include moisture content, ash content, acid insoluble ash content and sulfate content as well as analysis of surface structures. and the functional groups it contains. Bacto agar obtained was tested as a medium for bacterial growth using the Total Plate Number (ALT) method. The optimum treatment combination is determined based on the results of the characterization of the bacto agar which are closest to the standard of commercial agar in dipco.

2. Material and methods

2.1. Materials

The materials used in this research were commercial chitosan, seaweed type Gelidium sp. obtained from Pameungpeuk Beach, bacto agar commercial dipco, NaOH, CH3COOH, NaOCl, HCl, Butterfield's phosphate buffered (BFP) solution, mackerel obtained from Palmerah Market and aquades.

Seaweed Gelidium sp. obtained from Pameungpeuk Beach, Garut, dried in the sun and carried out an initial characteristic test of seaweed raw materials based on SNI 2690: 2015 [18] concerning dry seaweed which includes moisture content, impurities [19], and Clean Anhydrous Weed [20].
2.2. *Bacto* agar extraction

Pretreatment and extraction of agar (modified from [21]). A total of 1 kg of Gelidium sp. which has been dried weighed and cleaned under running water to remove impurities such as sand, gravel and other types of seaweed. Furthermore, the pretreatment was carried out by soaking seaweed in 5% NaOH with a ratio of 1:10 at 90 °C for 90 minutes. The seaweed samples were then washed under running water to remove the alkalis. Furthermore, seaweed was immersed in a 0.5% acetic acid (CH3COOH) solution for 1 hour at room temperature with a ratio of 1:10. The sample was then blanched by immersing in 4% NaOCl solution with a ratio of 1:10 for 30 minutes at room temperature, then washed with water to pH 6 and continued with the extraction process to obtain the agar extract. The extraction process is carried out by boiling seaweed using water with a ratio of 1:10 to seaweed. Sample extraction was carried out at 90 °C for 2 hours accompanied by stirring.

2.3. Adsorption process with chitosan

The filtrate obtained from the extraction process was purified by adding chitosan with a concentration of 0 (control); 0.5; 0.75; and 1% ("b" / "v"), which aims to bind the impurities present during the extraction process. The adsorption process of each filtrate was carried out for 0, 30, and 60 minutes at 90 °C with stirring to determine the effective adsorption time for the purification of *bacto* agar. The filtrate is filtered to separate the filtrate from chitosan while the mixture is still hot. The filtrate obtained is then made into *bacto* agar. The extraction process was carried out in two repetitions.

2.4. Analysis of *bacto* agar

Chemical characterization of *bacto* agar, water content [22], ash content [22], acid soluble ash content [22], sulfate content [23], physical characterization of *bacto* agar, gel strength [24], viscosity [25], syneresis [26], gelling point [26], melting point [26], analysis of *bacto* agar molecular structure: functional group analysis with FTIR [27], Microstructure analysis by SEM (modified from [21]).

2.5. Microbiological analysis of *bacto* agar as bacterial growth media [28]

Microbiological test for *bacto* agar was carried out to determine the ability of *bacto* agar as a medium for bacterial growth found in fresh fish, namely mackerel. The analysis was carried out using the Total Plate Number (ALT) test method based on the total microbial count, to determine the microbial growth in the bacterial medium so that the results of the study were then compared with the commercial agar.

A total of 3.5 g of *bacto* agar were dissolved in 200 mL of distilled water and heated until homogeneous. As a comparison, commercial agar is weighed as much as 3.5 g and dissolved in 200 mL of distilled water, then stirred and heated until homogeneous. The two solutions were sterilized in an autoclave at 121 °C for 15 minutes. A screw tube containing 9 mL of sterilized physiological saline was prepared and labeled with a dilution of 10x – 1000x.

The sample was prepared by weighing the sample aseptically as much as 10 grams and added with 90 mL of butterfield's phosphate buffered (BFP) solution and homogenized using vortex, this homogenate is a 10x dilution. 1 mL of the homogenate was taken and put into the 1st tube (100x) aseptically, then shaken. Followed by taking 1 mL of solution from the 1st tube into the 2nd tube (1000x) aseptically, then shaking it.

Each dilution was then put in 1 mL sterile empty petri dish, then the *bacto* medium was poured into the petri dish that already contained the sample and shaken until well blended. After the agar had solidified, the plates were placed in an incubator at 35-37 °C for 48 h. After 48 h, the number of colonies that grew was counted.
2.6. Data Analysis
The data obtained were analyzed using analysis of variance (ANOVA) with a confidence limit of 95% (α = 0.05) on IBM SPSS Statistics version 20.0 and continued with the Duncan test.

3. Results and discussion

3.1. Chemical characterization
The percentage yield, water content, ash content, acid insoluble ash and sulfate content of bacto can be seen in Figure 1.

Note:  
C1  =  Bacto agar + chitosan 0.5%  
C2  =  Bacto agar + chitosan 0.75%  
C3  =  Bacto agar + chitosan 1%  
T0  =  Adsorption time 0 min (10 sec)  
T30 = Adsorption time 30 min  
T60 = Adsorption time 60 min

Figure 1. Yield (a), water content (b), ash content (c), acid insoluble ash (d) and sulfate content (e) of bacto agar

The water content of bacto agar produced in this study has a value that tends to fluctuate. The adsorption time and the interaction between treatments (concentration of addition of chitosan and
adsorption time) had a significant effect (P<0.05) on the water content of bacto agar, while the concentration of addition of chitosan did not have a significant effect (P> 0.05) on the water content of bacto agar. The water content of bacto agar with chitosan treatment for 0 minutes was different from that of bacto agar which was adsorbed by chitosan for 30 minutes and bacto agar without adsorption, meanwhile, adsorption by chitosan for 60 minutes was not different from that of bacto agar which was adsorbed for 0 and 30 minutes. However, it has a different effect with the bacto content in the control order.

Based on the results, it was found that the moisture content of the bacto agar produced without the adsorption treatment showed a higher percentage of water content than that of the agar with the adsorption treatment. This can happen because chitosan as an adsorbent can reduce the moisture content in the material, by the presence of a hydrophilic group (OH) that is able to bind water [29]. The same results were obtained in the study of Abdullah et al. [17] using Gracilaria sp. as the raw material, which indicates that the reheating treatment with chitosan has a significant effect on the moisture content of agar, with the resulting moisture content of 14.34–23.79%. Chitosan has an amine group in its structure which causes chitosan to be hidrofilic, so it can bind water molecules [30]. The interaction between chitosan molecules and water molecules occurs through hydrogen bonds [31].

The adsorption time will affect the absorption process of the material, the more opportunities the adsorbent has to come into contact with impurity particles so that the number of impurity particles absorbed will increase [32]. However, the agar which is adsorbed for 0 minutes tends to have a lower water content. This is because in the adsorption process with chitosan for 0 minutes, the adsorption equilibrium has been reached so that the number of particles absorbed will be relatively constant [33] so that it does not cause a reduction in the number of water molecules again when the adsorption time is added.

Ash is an inorganic substance from the remains of the combustion of an organic material [34]. Ash content indicates the level of product purity [35][36]. The results showed that the ash content ranged from 4.02±0.14 to 6.93±0.14%. This result is not much different from the ash content in standard agar, which is 3.0–6.5%. The same results were obtained in the study by Abdullah et al. [17], on making Bacto agar from seaweed Gracilaria sp. with the addition of chitosan has an ash content of 3.45–7.1%. The ash content in agar should not exceed the standard value, this is because the excess ash content can inhibit the bacteria grown on the media [4].

The addition of chitosan adsorbent with different concentration and adsorption time had a significant effect (P <0.05) on the ash content, while the interaction between the two treatments had no significant effect (P> 0.05). The ash content in the control (without adsorption) has a different effect from the agar which is adsorbed by chitosan with a chitosan 0.75 and 1%, but has the same effect as the bacto agar content adsorbed by chitosan 0.5%. The ash content of the bacto agar for the control (without adsorption) had a different effect from the ash content of the agar which was adsorbed by chitosan for 60 and 30 minutes, but had the same effect as the agar which was adsorbed for 0 minutes. According to Suptijah [37], chitosan is able to absorb salt components up to 50% during extraction, so it is possible to use it in the refining process. This causes the diffusion mechanism to be low so that additional reactions are needed by activating chitosan using acid to expand the pore surface so that its adsorption power is increased [37].

Acid insoluble ash is acid insoluble salts, some of which are heavy metals and silica [38]. Determination of acid insoluble ash content is a further test of total ash content, which is carried out to determine the specific types of minerals present in materials such as sand or other impurities which are determined by dissolving materials that have become ash, with acid (HCl) [39]. In addition, it also aims to determine the presence of acid-insoluble heavy metal contamination, which refers to the quality of raw materials and the level of cleanliness in the processing of these products [40]. The ash content which is adsorbed by chitosan tends to fluctuate. The acid insoluble ash content of the study was higher than the standard value with a maximum value of 0.5%. Research conducted by Murdinah et al. [4] using Gelidium rigidum as raw material, the agar contains 0.18–0.38% acid insoluble ash. Whereas in the research Kumala et al. [41] using Gracilaria verrucosa as raw material, the resulting agar contains 0.38–
0.76% acid insoluble ash. When compared with other studies, the acid insoluble ash content of the research results is still higher, which can be caused by different processing processes, such as the washing of raw materials and drying which is done manually using the sun's heat so that the product is contaminated with other minerals from the environment such as dust particles in the air.

The presence of sand in the agar extract will reduce the purity of the agar. Sand is one type of impurity found in Gelidium sp. because the seaweed habitat generally attaches to coral rocks in the sea [42]. Meanwhile, the addition of chitosan can reduce mineral contamination such as heavy metals in bio-agar. This is due to the functional groups that chitosan has, namely the hydroxyl group (-OH) and the amine group (-NH₂), which can bind metal ions by ion exchange [43]. According to Laksono [44], the amine group has free electrons that can bind protons or metal ions and form a complex. The N atoms in NH₂ tend to easily donate their electron pairs, which then interact with metal ions as electron acceptors [44]. The adsorption treatment by chitosan with different concentrations, adsorption time, and the interaction between the two treatments had a significant effect (P <0.05) on the acid insoluble ash content in the agar. The insoluble ash content of bacto agar in control (without adsorption) had the same effect as that of bacto agar which was adsorbed by 0.5% chitosan, but both had different effects from bacto agar which was adsorbed by 0.75 and 1% chitosan. Meanwhile, the insoluble ash content of bacto agar which was adsorbed by chitosan 0.75% had the same effect as adsorption by 1% chitosan. Meanwhile, the adsorption time shows that the bacto agar control has a different effect from the insoluble ash content which is adsorbed by chitosan for 0, 30, and 60 minutes. Meanwhile, the insoluble ash content of bacto agar which was adsorbed for 30 minutes had the same effect as adsorption for 60 minutes. However, the bacto agar which was adsorbed by chitosan had a higher acid insoluble ash content than the control agar. This shows that chitosan is less able to absorb metal maximally, which may be due to the particle size which is less fine and not uniform. According to Handayani and Yusnimar [45], the absorption capacity of chitosan can be increased by reducing the size of the chitosan particles so that the surface area is larger and the absorption capacity is maximum.

Sulfate is bound to one of the constituent components of agar, namely in the agaropeptin structure. According to Praiboon et al. [46] stated that the sulfate content in agar can cause interference with the gel formation process because sulfate will cause stiffness in the helix structure. The sulfate content in seaweed varies, due to differences in the ratio of the amount of agarose and agaropeptin in the agar molecule [47], which depends on the origin and type of seaweed [21]. Sulfate content values ranged from 1.16±0.18-2.06±0.38%. The lowest value was obtained for bacto agar which was adsorbed by 1% chitosan for 60 minutes and the highest was 0.75% chitosan for 30 minutes. The sulfate content of bacto agar tended to fluctuate, but this value was not much different from the standard commercial agar sulfate level of 1.778%. Similar results were also obtained in the study conducted by Abdullah et al. [17], who used Gracilaria sp. to make bacto agar accompanied by adsorption by chitosan, which resulted in sulfate levels in the agar of 0.58-1.98%. Meanwhile, the results of research by Murdinah et al. [4] which used Gelidium rigidum as raw material for making bacto so that without adsorption by chitosan, the sulfate content contained was 3.6-3.98%. This shows that the addition of chitosan is able to reduce the sulfate content in Bacto agar.

In an acidic solvent, the -NH₂ group of chitosan protonates to become polycationic –NH₃, which can react with negatively charged species such as citrate, succinate, and tripolyphosphate via electrostatic bonds to produce cross-linking networks [48]. Soaking with NaOH before extraction can also remove sulfate esters [51]. NaOH will hydrolyze the sulfate ester to a certain level during the pretreatment process and change the chemical structure of the agar [21]. During this process, the bonds between agarose and agaropeptin are broken. The unstable sulfate ester in the C-6 chain of the L-galactose-6-sulfate agaropeptin molecule will disappear due to the hydrolysis of NaOH which is then converted into 3,6-anhydrogalactose when the C-6 chain is deficient in ions due to sulfate removal [52].

The addition of different concentrations had a significant effect (P <0.05) on the sulfate levels in the agar. Meanwhile, the treatment of adsorption time and the interaction between the two treatments did not have a significant effect (P> 0.05) on the sulfuric acid levels of agar. The sulfate level of bacto agar which was adsorbed by chitosan with a concentration of 1% had a different effect from the adsorption
by 0.75% (w/v) of chitosan, but the two concentration treatments did not show any difference in effect with other treatments. Bacto agar which is adsorbed by 1% chitosan has a lower sulphate content value than Bacto agar which is adsorbed by chitosan with concentrations of 0.5% and 0.75% which indicates that the more concentration of chitosan is added, the number of chitosan particles increases so that the number More and more sulfate salts will be absorbed [53].

3.2. Physical characteristics of bacto agar

The results of the physical characteristics of bacto agar are shown in Figure 2.

Gel strength is the maximum load required to break the polymer matrix in the area under load, and can be expressed as a "breaking force" [17]. The strength of the bacto agar gel produced ranged from 1025±288 to 1445±68 g/cm². The strength was in accordance with the standard criteria for commercial
agar with a gel strength value of 600-800 g/cm². Similar results were obtained from the research conducted by Murdinah et al. [4] who used Gelidium rigidum, which produced a gel strength of 115.8 - 670.72 g/cm². The high value of gel strength indicates good agar quality [5].

Adsorption treatment with chitosan on both treatment parameters did not have a significant effect (P>0.05) on the gel strength. The gel strength was only affected by the sulfate content of the agar.[21] stated that Viscosity is a measure that states the thickness of a liquid or fluid, if the viscosity value is high, the liquid is getting thicker [54]. The viscosity values ranged from 20.5 ± 2.08 to 80.0 ± 9.63 Cp. The highest viscosity value was obtained which was adsorbed by 0.5% chitosan for 30 minutes and the lowest was obtained with 1% adsorption by chitosan for 0 minutes. In contrast to research conducted by Suptijah et al. [55] who used Gracilaria seaweed as raw material for making bacto agar, which only produced a viscosity value of 8.9-12.5%.

The addition of chitosan had a significant effect on the viscosity of bacto agar. Meanwhile, the treatment of adsorption time and the interaction between the two treatments did not have a significant effect on the viscosity of the bacterium agar. The viscosity value of bacto agar which is adsorbed by chitosan 0.5% has a higher value compared to that of control agar (without adsorption), which is 40.4%, whereas for bacto agar which is adsorbed by chitosan 0.75% and 1% has a value lower viscosity compared to bacto agar control. Viscosity tends to decrease and fluctuate with increasing concentration of chitosan addition. The decrease in viscosity value along with the increase in the concentration of chitosan as an adsorbent is probably due to the low level of bacto agar purity due to the remaining impurities that are not absorbed by chitosan because the absorption of chitosan has reached the maximum. The level of purity also affects the viscosity value, the higher the purity of a fluid, the greater the viscosity value [56], [57].

Syneresis is an event of water discharge from the gel which will affect the stability of the material throughout the storage and use period, to ensure the identity, strength, quality, and purity of the product [58]. The syneresis value of bacto agar is shown in Figure 2c. All of the bacto agar undergoes syneresis during storage which causes the texture to shrink. The highest percentage of syneresis (4.62±0.43%) occurred at 0.75% chitosan for 0 minutes and the lowest (1.99 ± 0.17%) occurred at 0.75% chitosan for 60 minutes. The high syneresis number indicates the amount of water that comes out of the agar gel so that physically the agar is less stable [58].

The addition of chitosan had no significant effect (P>0.05) on the bacto agar syneresis. Meanwhile, the treatment of adsorption time by chitosan and the interaction between the two treatments did not have a significant effect (P<0.05) on the bacto agar syneresis. The bacto agar syneresis adsorbed by chitosan for 60 minutes had a different effect from the bacto agar which was adsorbed for 0 minutes, but both had the same effect on the bacto agar syneresis with other treatments. The syneresis in agar gel is a symptom caused by water molecules that are not bound to agar leaving the gel matrix structure [59]. This can be caused by prolonged storage in the open air. The content of heavy metals or other impurities in the gel can also reduce the ability of the water-binding capacity in the agar structure, caused by the impurities present in the agar, it will interfere with the gel in order to bind water, so that it can reduce the physical stability of the agar gel [58]. The longer the adsorption time, the lower the percentage of bacto syneresis. This shows that the adsorption process with chitosan in a long time will increase the absorption of impurities by chitosan. The longer the adsorption time, the longer the contact intensity between the adsorbate (chitosan) and the molecules so that more impurities are absorbed by the chitosan molecules [32], so that the impurity content in the agar decreases and increases the water-binding power of the gel.

The gelling point is the temperature which a solution at a certain concentration begins to form a gel [38]. The low temperature of gel formation is useful for its application as a medium for bacterial growth and biotechnology [60], because as a medium for bacterial growth, a material with high stability is needed which will remain liquid at 42 °C and remain solid when used at a temperature 37 °C [61]. The results of the analysis of the gelling point is shown in Figure 2d. The result showed that the gelling point ranged from 15±1.19–23±3.13 °C. The point value of the bacto agar is lower than the standard point for commercial agar (35 °C). Meanwhile, different results were obtained for bacto agar made from other
seaweed, namely *Gelidium rigidum* ranging from 25-34 °C [4], while in *Gracilaria chilensis* it was 33-34 °C [62]. The adsorption treatment by chitosan with different concentrations had a significant effect (P <0.05) on the gelling point. However, the adsorption time and the interaction between the two treatments did not effect (P>0.05) on the gelling point.

Yampakdee et al. [21] reported that the agar gel formation temperature was influenced by the presence of a methoxy group (R-O-CH3) which is one of the residues from the formation of the bond bridge between C-3 and C-6 carbon when the agaropeptin structure changes by alkaline treatment. The methoxy group is considered as a group that affect gelling point, if high levels cause high gel formation temperature [46], [63], [64]. The results showed that the gelling point increased with the increasing concentration of chitosan addition. The increase in concentration is related to the amount of chitosan adsorbent added to the process of refining the agar, which indicates that the greater the concentration or the amount of chitosan added causes more residues of agar formation such as methoxy groups, which are absorbed in the chitosan molecule so that some important residues affect the formation agar gel decreases and reduces gelling point.

Melting point is the temperature when a solution melts at a certain concentration [38]. The melting point value of agar is shown in Figure 2e. The melting point ranged from 60.30±4.05–65.85±2.02 °C. This temperature is lower than the commercial agar standard with a melting point of 88 °C. Similar results were obtained in the study using *Gelidium rigidum* which produced an agar melting point of 67-77 °C [4], while in the other research [62], which used *Gracilaria chilensis*, the melting point was higher (83-85 °C). The melting point is influenced by the molecular weight and hydrogen bonds contained in the material [62]. The high molecular weight will cause the melting temperature to be higher. Hydrogen bonds formed from a polysaccharide polymer chain with other polymer chains cause the formation of complex polymer networks so that high temperatures are required to break down these networks.

The addition of chitosan with various concentrations had a significant effect (P <0.05) on the melting point of bacto agar. However, the treatment of the length of adsorption time and the interaction between the two treatments did not have a significant effect (P> 0.05) on the melting point. Bacto agar for control has a different effect from that of bacto agar which is added with adsorption treatment by chitosan. Meanwhile, bacto agar with adsorption treatment by chitosan with all concentration have the same effect. According to Gaol et al. [65], one that affects the melting temperature of a substance is the purity of the substance. The more impurities or foreign substances contained in the material, the melting point will decrease [66]. The melting point of bacto agar tends to have an adjacent value at each addition of chitosan concentration, but this value increases with increasing concentration.

3.3. *Analysis of bacto agar functional groups*

The spectral pattern of the bacto agar which was adsorbed with chitosan at various concentrations and different adsorption times showed the same results as the spectrum for the control. The bacto agar spectrum band for control shows an absorption peak in the 3434 cm\(^{-1}\) region which indicates a stretching vibration of O-H due to hydrogen bonds which causes the peak to widen and a shift towards shorter wave numbers. The absorption peak that occurs in the 2917 cm\(^{-1}\) area so that the control is the absorption band for the CH2 functional group, in the 1646 cm\(^{-1}\) area indicates that there is absorption caused by stretching vibrations of the double bond on the carbonyl group (C=O), which is characterized as a primary amide, which indicates the presence of protein in the agar [67]. The absorption of the carbonyl group generally occurs in the 1700 cm\(^{-1}\) area, the shift in the absorption area is caused by structural changes that occur in the agar bacto.
Note:  
C1 = Bacto agar + chitosan 0.5%  
C2 = Bacto agar + chitosan 0.75%  
C3 = Bacto agar + chitosan 1%  
T0 = Adsorption time 0 min (10 sec)  
T30 = Adsorption time 30 min  
T60 = Adsorption time 60 min  

Figure 3. FTIR spectrum for bacto agar

The absorption peak that occurs in the area of 1376 cm\(^{-1}\) indicates the absorption of sulfate esters. According to Suptijah [37], the sulfate ester uptake area should occur in an area of 1200 cm\(^{-1}\), the shift in wave numbers indicates that the sulfate ester present in the agar has been reduced. This can be seen from the low levels of sulfate obtained in Bacto agar from the results of the study (Figure 13). The decrease in the amount of sulfate in bacto agar can be caused by the pretreatment process using NaOH which functions to bind sulfates to agaropectin molecules so that they change the structure of the agar. In addition, it is also caused by the use of chitosan in the process of refining agar, which functions to bind sulfate ions to agar, thereby reducing its sulfate content.

Meanwhile, the absorption that occurred at 930 cm\(^{-1}\) showed that the C-O-C group was absorbed in the 3,6-anhydrogalactose structure. The absorption peak in the 890 cm\(^{-1}\) area shows the presence of C-H bending absorption which is bound as a methoxy group in the D-galactose structure [68].

3.4. Microstructure characteristics of bacto agar

The microstructural characteristics of the resulting bacto agar were carried out using SEM (Figure 3). This analysis aims to determine the effect of chitosan addition so that the extraction results of Gelidium sp. seen from the microstructure of the agar.

There are fibers indicating carbon particles in the agar polymer structure consisting of gabactose monomers that are bound to each other by α-1,4 and β-1,3 glycosidic bonds. The figure shows the presence of small cavities as well as a rough and asymmetrical surface structure. The small cavities are probably related to the moisture content contained in the bacteria agar control. The rough surface of the bacto agar for control is caused by the softer gel texture due to the amount of water contained in the bacterium for more control so that it interferes with the binding of carbon atoms to the agar molecule itself.

The addition of chitosan can reduce the water content contained in agar, by forming hydrogen bonds with water molecules [30], making it easier for the galactose monomers in agar to bind so that the gel chain is more numerous and tighter. This can be seen in the arrangement of the polymer matrix in order to show more uniform fibers. These results indicate that the concentration of chitosan and the adsorption time have an effect on the efficiency of the absorption of impurities in the agar by chitosan molecules. The more the number of adsorbents, the more adsorbate the adsorbate [11], and the longer the adsorption time, the longer the contact time between the adsorbent and the adsorbate so that the absorption becomes maximum [32].
3.5. Application of bacto agar as a media for bacterial growth

Microbiological analysis was carried out to determine the ability of bacteria to grow bacteria [4]. The bacto agar product produced in this study was applied as a medium for the growth of bacteria found in mackerel. Tests were carried out using the Total Plate Number (ALT) calculation method, to determine the number of bacterial colonies present in the mackerel fish sample.

| Treatment  | Total Colonies (cfu/mL) |
|------------|-------------------------|
| Kontrol    | 1.14 x 10^5             |
| C1T0       | 1.04 x 10^5             |
| C1T30      | 1.51 x 10^5             |
| C1T60      | 1.34 x 10^5             |
| C2T0       | 0.39 x 10^5             |
| C2T30      | 0.53 x 10^5             |
| C2T60      | 0.64 x 10^5             |
| C3T0       | 0.07 x 10^5             |
| C3T30      | 0.44 x 10^5             |
| C3T60      |                         |

Note:  
C1 = Bacto agar + chitosan 0.5%  
C2 = Bacto agar + chitosan 0.75%  
C3 = Bacto agar + chitosan 1%  
T0 = Adsorption time 0 min (10 sec)  
T30 = Adsorption time 30 min  
T60 = Adsorption time 60 min

**Figure 4.** The microstructure of the agar bacto

**Figure 5.** Total plate number on bacto agar
All of the bacto agar samples had the ability to grow bacteria. The number of bacterial colonies growing on agar ranges from 0.07x10^5-1.5x10^5 cfu/mL. Based on the results of the analysis, the number of bacterial colonies that grew on Bacto agar which was adsorbed by chitosan 0.5% for 0 and 60 minutes and chitosan 0.75% for 0 minutes, had a number that was not too different from control (1.14 x 10^5 cfu/mL). However, the number of colonies that grew on bacto agar so that the results of the study was still smaller than the number of colonies that grew on the bacterial agar for commercial difco based on the experiment was 1.81x10^5 cfu/mL. This is probably due to the level of bacto agar purity so that the research results are lower than the commercial agar in difco.

The growth of bacteria in agar showed a decrease in the number of colonies along with the increasing concentration of chitosan addition. The decrease in the number of colonies in the bacto agar media along with the addition of the chitosan adsorbent concentration can be caused by the ash content in the agar. The ash content obtained in this study increased along with the increasing concentration of chitosan addition. The decrease in the number of colonies in the bacterial agar for commercial difco based on the analysis of 1.34x10^5 cfu/ml. Based on the results of the analysis, the number of bacterial colonies that grew on the agar which was adsorbed by chitosan 0.5% for 0 and 60 minutes, had a number that was not too different from control (1.14 x 10^5 cfu/mL). However, the number of colonies that grew on bacto agar so that the results of the study was still smaller than the number of colonies that grew on the bacterial agar for commercial difco based on the experiment was 1.81x10^5 cfu/mL. This is probably due to the level of bacto agar purity so that the research results are lower than the commercial agar in difco.

4. Conclusion
Chitosan adsorbent can be used to increase the of the bacto-agar purity produced from Gelidium sp. Different concentrations and adsorption times have an effect on the physical and chemical properties of the bacto-agar produced and are able to improve its quality. The optimum treatment for bacto agar from Gelidium sp. resulted in adsorption by chitosan with a concentration of 0.75% and an adsorption time of 0 minutes (10 seconds). Bacto agar adsorbed by 0.75% chitosan for 0 minutes has a characteristic that is close to the commercial agar standard with a yield of 3.50%, water content of 14.61%, ash content of 4.02%, non-ash content. soluble acid 0.93%, sulfuric content 1.34%, gel strength 1054.96 g/cm², viscosity 60 Cp, syneresis 4.62%, melting point 65 °C and general point 20.75 °C and can be used as bacterial growth media with an average colony count of 1.34x10^5 cfu/mL.

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