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Effectiveness Inhibition of Fermentation Legen using Chitosan Nanoparticles

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Abstract

Legen is one of the traditional drink that has been widely known among the people of Indonesia. Beside from being a refreshing drink, legen also has many properties, such as the prevention of stomach ulcers, increase vitality, shed the pain of kidney stones, prevent hemorrhoids disease, and natural doping to maintain stamina. Legen could be alcoholic beverages if the storage period exceeds two days. So it takes a substance that can inhibit the fermentation process of the legen. One material that has potential as inhibitors of fermentation is chitosan. For the effectiveness of inhibitory fermentation process of the legen, then in this study used chitosan nanoparticles. Based on the results of the solubility test and characterization by FT-IR and PSA, it is known that chitosan nanoparticles have been synthesized. The addition of chitosan nanoparticles influence on the inhibition fermentation process of the legen. It can be seen from the testing alcohol levels and the quality of the samples which include aroma and flavor. After being left for 5 days, the alcohol content in the sample legen with treatment (addition of chitosan nanoparticles) was (1.414 ± 0.046)% v/v, while the alcohol content in the sample legen without treatment (control) was (4.243 ± 0.026)% v/v. Based on the quality of the sample test legen that have added a solution of chitosan nanoparticles, it can be seen that the addition of chitosan nanoparticles did not affect the aroma and flavor of legen.

Keywords: Legen; Alcoholic beverages; Chitosan; Nanoparticles; Fermentation

Introduction

Indonesia is a country rich in natural resources. Fisheries Indonesia is one sector that has a promising future. It was seen by the increase in export value of fishery products nationwide. As reported kkp.go.id, the value of total fishery exports to Indonesia by commodities from January to November 2013 reached US$ 3.77 billion, an increase of 6.985% of the US$ 3.53 billion in 2012. In that period, the shrimp turns out to be the main export commodity of Indonesian fishery with a value of US$ 1.280 million. Exports of shrimp increased by 25.46% from the previous year with the largest contribution value of frozen shrimp worth US$ 1.121 million. Along with the increase in shrimp production, the waste generated from processing the shrimp will also increase. The amount of waste produced is not processed immediately otherwise it will cause environmental pollution. During this time the shrimp waste treatment is only used as materials for crackers, shrimp paste, and supplements for forage. Whereas waste from the shrimp is a potential for shrimp shells contain chitin approximately 99.1% [1].

Chitin when further processing will produce chitosan that can be used as a preservative and stabilizer products. Chitosan can be used as a food or drinks preservative because it is able to inhibit the growth of microorganisms that makes rotten and simultaneously coating the product is preserved so that there is minimal interaction between the product and its environment [2].

Legen is one of the traditional drink that has been widely known among the Indonesia people's. Legen is a beverage derived from palm trees. Beside from being a refreshing drink turns legen has many properties, such as the prevention of stomach ulcers, increase vitality, shed the pain of kidney stones, prevent hemorrhoids disease, and natural doping to maintain stamina.

But legen would just be a refreshing and healthy drink when still aged 0 to 2 days. If it has been over two days, the legen will undergo fermentation turn into young wine has low alcohol content below 2%. The longer vulnerable time passed legen, the greater the alcohol content in it which means that the legend has become a heady wine. One of the materials that can be used to inhibit the fermentation process is chitosan. In this research are used in the form of nanoparticles of chitosan to inhibit the fermentation process at the legen. With the added chitosan nanoparticles into the legen expected inhibition of legen fermentation process can be run more effectively.

Method

Preparation shrimp shell powder

Shrimp shells washed clean of dirt, then dried in the sunlight. Once dried shrimp shells crushed into powder and sieved.

Isolation of chitin from shrimp shells

Removal of protein found in shrimp shell powder (deproteination): Shrimp shell powder put into a glass beaker and then added a solution of NaOH 7% with the comparison between shrimp shell powder with a solution of NaOH 1:10 (g powder/mL NaOH). This process is carried out with stirring for about 2 hours at a temperature of 65°C. Then the mixture is separated with filtered to take the precipitate. Furthermore, the precipitate was washed by using distilled water to neutral pH. Then the precipitate that is filtered and dried in an oven at 60°C [3].

The removal of minerals from shrimp shell powder (demineralization): The precipitate results deproteination put into a glass beaker and then added a solution of HCl 1N with a comparison

between the sample with a solution of HCl 1:15 (g sample/mL HCl). This process is carried out with stirring for 30 minutes at room temperature. Then the mixture is separated with filtered to take the precipitate. Furthermore, the precipitate was washed by using distilled water to neutral pH. Then the precipitate that is filtered and dried in an oven at 60°C [3].

The removal of dyes from shrimp shell powder (bleaching): The demineralized precipitate extracted with acetone for 1 hour at room temperature with a ratio between solid and solvent 1:10 (g solid/ml solvent). Then the mixture is separated with filtered to take the precipitate. Furthermore, the precipitate was washed by using distilled water to neutral pH. Then the precipitate that is filtered and dried in an oven at 60°C. Results of drying is called as chitin [3].

Deacetylation chitin into chitosan

Chitin which has been produced in the previous process put into a glass beaker then added solution of NaOH 60% with a ratio between chitin and NaOH 1:10 (g chitin/mL NaOH). This process is carried out with stirring for about 2 hours at a temperature of 95°C. Then do the filtering to be taken the precipitate. The precipitate was then washed by using distilled water to neutral pH. After this the precipitate dried in oven at 60°C. Results of drying is called as chitosan [3].

Synthesis chitosan nanoparticles

A total of 200 mg of chitosan put into a glass beaker and then dissolved in 1 L acetic acid 1%. Process is done with an agitator for 30 minutes. After obtaining a homogeneous chitosan solution, then the solution is dripped with 10 mL concentrated ammonia. Thus forming a white solution called Chitosan Nanoparticles solution. The solution is then put into erlenmeyer then placed in the ultrasonic bath which aims to eliminate the remaining ammonia. Then solutions is filtered and dried for 1 week.

Characterization chitin, chitosan and chitosan nanoparticles

To determine whether or not to form chitin and chitosan nanoparticles, then tested the solubility and IR spectroscopy test. Solubility test conducted by dissolving the powder obtained from the process of deacetylation into a dilute acetic acid solution. If it does not dissolve then the powder is chitin and contrary if the powder is dissolved then the powder is chitosan [4]. While IR spectroscopy is used to determine the functional groups and the degree of deacetylation of chitin and chitosan. The determination of the degree of deacetylation is determined by baseline method [5]. As for knowing whether or not chitosan nanoparticles formed, then samples of chitosan nanoparticles characterization with PSA (Particle Size Analyzer).

Inhibition of fermentation legen with chitosan nanoparticles

For sample preparation, 0.1 grams of chitosan nanoparticles which have been dissolved in 1 L acetic acid 1% put in 100 ml legen. Then these samples were left for 5 days and then the filtrate taken to test alcohol contents using GC. To further determine the effect of chitosan nanoparticles in the inhibition of fermentation legen, then eventually the alcohol content in legen with treatment (addition of chitosan nanoparticles) will be compared with the alcohol content in legen without treatment (control).

Beside tested the alcohol content, the effect of adding chitosan nanoparticles in the inhibition of fermentation legen can be seen from the quality of the samples which include aroma and flavor. The tests are carried out by comparing the aroma and flavor of legen with treatment (addition of chitosan nanoparticles) with legen without treatment (control).

Results and Discussion

Preparation shrimp shell powder

The initial steps in chitin isolation process is to prepare the shrimp shell. Shrimp shells are then cleaned by washing to remove dirt that is still attached. After the shrimp shells are cleaned, the shrimp shells dried in the sunlight. Furthermore, shrimp shells that have been dried finely ground with the purpose of expanding the field of shrimp shells surface so that the process of isolation can be performed optimally.

Isolation of chitin from shrimp shell powder

In the isolation of chitin from shrimp shell, shrimp shell sample is processed in several stages, namely the removal of proteins, removal of minerals and removal of the dye. At this stage of the removal of the protein, the protein in chitin cannot be completely eliminated because these proteins are bound by chitin by covalent bonds and form a stable complex. The residue obtained was washed with distilled water until neutral pH. Results of rough chitin (crude chitin) obtained after extraction with NaOH has a brown color.

The main mineral content contained in the shrimp shell is CaCO3 by 40-50% (wt). Other minerals contained in the shrimp shell is Ca3(PO4)2. In the isolation of chitin from shrimp shell, HCl solution will react with these minerals, thus forming salts are soluble in the solvent and easily removed or formed CO2 gas can out of the mix in the form of air bubbles. Mineral reaction with HCl is as follows:

$$CaCO_{3(s)} + 2\ HCl_{(aq)} \rightarrow CaCl_{2(aq)} + H2O_{(l)} + CO_{2\ (g)}$$

$$Ca_3(PO_{4)2(s)} + 4\ HCl_{(aq)} \rightarrow 2\ CaCl_{2(aq)} + Ca\ (H_2PO_{4)2(l)} + CO_{2(g)}$$

The removal of soluble salts carried out by filtration, while the gas will come out when the dissolution. This can be done because chitin is insoluble in HCl. The removal of dye (bleaching) is done by soaking the rough chitin (crude chitin) in acetone showed significant results. It can be seen from the change in color, where rough chitin (crude chitin) that before the bleaching process has brown color and then after the bleaching process becomes brownish yellow color.

Deacetylation chitin into chitosan

Deacetylation of chitin is the removal of the acetyl group binds to the amine group using a concentrated strong base. The process of deacetylation is done by using a solution of NaOH 60% for 2 hours at a temperature of 95°C. The use of a strong base solutions which NaOH 60% of the deacetylation process is due weak base cannot break acetamide C-N bond group at atom C-2 on acetamide of chitin, whereas a strong base will break bonds between atoms N acetyl group, thus forming the amine group ($-NH_2$) on chitosan. The amount of acetyl group is missing indicates the percentage of deacetylation of chitosan. Transformation chitin into chitosan is a hydrolysis reaction.

Chitosan is produced from the deacetylation process has more white color when compared with chitin. From the results of the solubility test, it can be seen that chitosan obtained dissolved in acetic

acid 1%. Whereas chitin does not dissolve in acetic acid 1%. To further determine formed or absence of chitin and chitosan then be characterized with IR spectroscopy to determine functional groups and degree of deacetylation.

Characterization of chitin, chitosan, and chitosan nanoparticles

Chitin and chitosan characterized by FT-IR, with spectra that can be seen in Figure 1. From these spectra can be seen the peaks owned by chitin functional group in Table 1 and peaks owned by chitosan in Table 2.

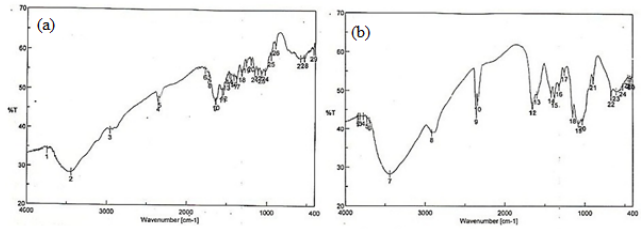

Figure 1: FT-IR spectra of (a) chitin and (b) chitosan.

The wave numbers (cm^{-1})	Type Vibration	Vibrating group
3500-3000	Stretching	OH and NH
2929.87	Stretching	CH, CH3
1658.78	Stretching	C=O amide
1417.68	Bending	C-H
1200-1075	Stretching	C-O-C

Table 1: The vibration of functional groups contained in the chitin.

The wave numbers (cm^{-1})	Type Vibration	Vibrating group
3500-3000	Stretching	OH and NH
2881.65	Stretching	CH, CH_3
1656.85	Stretching	C=O amide
1421.54	Bending	C-H
1089.78	Stretching	C-O-C

Table 2: The vibration of functional groups present in chitosan.

From the analysis of PSA (Particle Size Analyzer) obtained particle diameter data of chitosan nanoparticles is 50 nm. Based on the size of the particle diameter, it can be said that the chitosan nanoparticles have been synthesized. Because chitosan nanoparticles is actually chitosan which has a particle diameter size between 10-100 nm. PSA (Particle Size Analyzer) spectra of chitosan nanoparticles can be seen in Figure 2.

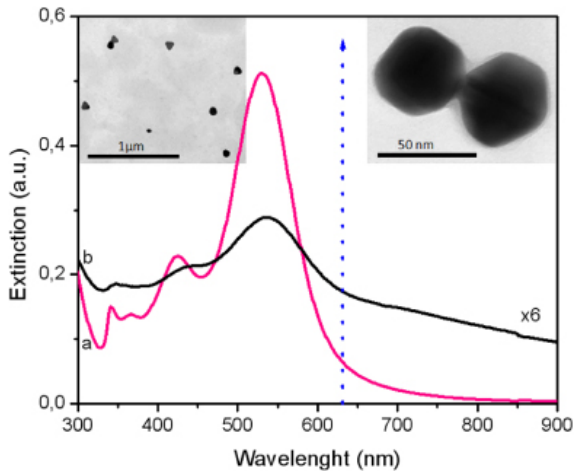

Figure 2: PSA (Particle Size Analyzer) spectra of chitosan nanoparticles.

Inhibition of fermentation legen with chitosan nanoparticles

Inhibition of fermentation legen with chitosan nanoparticles can be determined by comparing the alcohol content in legen with treatment (addition of chitosan nanoparticles) with the alcohol content in legen without treatment (control). The alcohol content of the sample that had been left for 5 days was tested by using GC. Results of analysis using GC instruments can be seen in Table 3.

From the results of alcohol content test in the sample that had been left for 5 days, it is known that the addition of chitosan nanoparticles can inhibit the fermentation process at the legen. It can be seen from the alcohol content in the sample legen with treatment can be said to be much lower when compared to the alcohol content in the sample without treatment (control). The alcohol content of legen sample with treatment (addition of chitosan nanoparticles) are (1.414 ± 0.046)% v/v, while the alcohol content in legen without treatment (control) was (4.243 ± 0, 026)% v/v. The use of chitosan in the form of nanoparticles in this study has the goal of keeping inhibition in legen fermentation process can take place more effectively. Inhibitory activity of the fermentation process due to chitosan nanoparticles have properties as an antimicrobial material. The ability to suppress the growth of bacteria due to the chitosan nanoparticles have poly-cations with a positive charged that is able to inhibit the growth of bacteria and fungi. One mechanism that may occur in the inhibition of the fermentation process is a molecule of chitosan nanoparticles that have the ability to interact with the compounds on the surface of bacterial cells and then adsorbed forming a layer (layer) which inhibits cells transport channels so that cells deficient substance to growing and cause cells death.

In addition to the chitosan nanoparticles have antimicrobial properties, the selection of chitosan nanoparticles as a material that can inhibit the fermentation process at the legen due to chitosan nanoparticles also has non-immunogenic and non-carcinogenic properties so suitable for use in food technology.

Sample code	Peak Area			Standard Area Ratio	Alcohol concentration (% v/v)	Average (% v/v)	SD (% v/v)	RSD (% v/v)
	Ethanol	Toluene (ISTD)	Ratios					
Treatment 1	19230438	456729684	0.0421		1.466			
Treatment 2	19195547	477211304	0.0402		1.400	1.414	0.046	1.10
Treatment 3	18796689	475833189	0.0395		1.375			
Control 1	55840932	459670488	0.1215		4.230			
Control 2	56467300	460097791	0.1227		4.273	4.243	0.026	0.62
Control 3	57021492	469809455	0.1214		4.226			
C2H5OH 5% (1)	66001796	458787333	0.1439					
C2H5OH 5% (2)	66078022	461154973	0.1433	0.1436				
C2H5OH 5% (3)	65029823	452605227	0.1437					

Table 3: Results of the analysis of the alcohol content by GC

It is also supported by the research conducted by Wardaniati and Setianingsih [6], which states that the addition of chitosan in the preservation of meatballs in addition to already meet microbiological standards, in terms of the chemical properties is also safe because in the process chitosan only need dissolved with dilute acetic acid (1%) to form a homogeneous solution of chitosan which has properties that are relatively safe. So that based on the results of this study can be said that the addition of chitosan nanoparticles in inhibiting the fermentation process in legen also have met the microbiological standard and safe in terms of its chemical properties because in the process chitosan nanoparticles only need dissolved with dilute acetic acid (1%) to form a homogeneous solution of chitosan nanoparticles. While based on the quality test of the sample legen that have added with a solution of chitosan nanoparticles which include aroma and flavor, it can be seen that the addition of chitosan nanoparticles did not affect the aroma and flavor of legen.

Conclusion

Chitosan nanoparticles can be synthesized by reacting chitosan with concentrated ammonia. Based on the analysis of PSA (Particle Size Analyzer), the particle diameter of chitosan nanoparticles is 50 nm. Chitosan nanoparticles from shrimp shells have the potential to inhibit the fermentation process in legen. The fermentation process in legen can be hampered due to chitosan nanoparticles have properties as an anti-microbial. The addition of chitosan nanoparticles to inhibit the fermentation process in legen does not affect the quality of legen which include aroma and flavor.

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