Determination of Nano-Collagen Quality from Sea Cucumber

Holothuria scabra

Desmelati¹, Sumarto¹*, Dewita¹, Dahlia¹, Syafrijal², and P A Sari²

¹Lecturer in Department of Fisheries Processing Technology, Faculty of Fisheries and Marine Science, Universitas Riau, Pekanbaru, 28293, Indonesia
²Student in Department of Fisheries Processing Technology, Faculty of Fisheries and Marine Science, Universitas Riau, Pekanbaru, 28293, Indonesia
*Corresponding author: sumarto1976@yahoo.co.id

Abstract. Sea cucumber contains high collagen protein and potential to be used in cosmetics and health. The aim of this study are to determine the quality of sea cucumber collagen using hydrochloric acid with different concentrations and the quality of nano-collagen using stirrer magnetic in different stirring times. This study was conducted by an experimental method using stages of the collagen extraction process and manufacture of nano-collagen. Process of extracting sea cucumber meat collagen using different concentrations of HCl (4%, 5%, and 6%), and process of making nano-collagen using 96% ethanol solution with different stirring times (one, two, three hours). Sea cucumber collagen with the 6% HCl extraction process obtained optimally results with collagen yield of 20.76%, white degree 73.97%, moisture level 10.99%, protein 98.3%, ash 0.86%, fat 0.59%, and collagen pH 3.92. The functional groups in collagen in accordance with the standard collagen compounds. The nano-collagen obtained was in the form of a creamy white powder (79.25% white degree), moisture 9.11%, ash 0.68%, fat 0.51%, protein 98.9%, pH 3.98, and particle size 285 nm.

1. Introduction
Sea cucumbers can be used as a functional food ingredient because it contains high protein. It can also serve as raw material for medicines and food supplements. In non-food application, sea cucumbers can be used as an ingredient for cosmetics and pharmaceutical industries.

As a commercial commodity, sea cucumber contains protein 79.55% (dry basis), fat 5.13%, ash 4.18%, and carbohydrates 11.13% [1].

Collagen is a protein in form fibrils or fibers as a primary structural component of the connective tissue that nearly 30% making up the body of vertebrate animals and invertebrates. Collagen found in many parts of the skin, tendons, blood vessels, bone, and cartilage. Collagen is widely used in the health scope as a medicine and as a cosmetic [2].

Research on the exploration of sea cucumber collagen is limited; some of them include the extraction and application of sea cucumbers S. hermanii collagen for skin moisturizer, extraction of collagen from sea cucumber S. variegatus [3], and activity inhibitor angiotensin-converting enzyme (ACE) and the antioxidant peptide S. variegatus collagen of sea cucumber [4]. Sea cucumber contains70% protein collagen. Sea cucumbers are the important components of marine ecosystems in oceans over the world [5].
Collagen is obtained by extraction using organic or inorganic acid. An acid solution serves to change structure of the collagen fibers becoming fragmented simpler, consequently simplifying the extraction process [3]. Type of acid solvent for extraction process uses a solution of hydrochloric acid (HCl). According to [6], the immersion process using HCl causes opening pores of the sea cucumbers to the maximum so that the formed spaces are easy to be achieved by the extractors (HCl). Increasing concentration of HCl causes more the amino acid bonds fragmented hence more protein dissolved during the extraction process to produce collagen. Hydrochloric acid for extract collagen in sea cucumber is rare, so it is necessary to use different concentrations of HCl to obtain the optimal extraction of collagen.

The main component of collagen consists of mollecules with diameter 1.5 μm, length 280 μm and weight 290,000 Dalton. Collagen level is three chains of polypeptide with more than 1,000 amino acids in each chain [7].

If the collagen particle size is smaller and homogeneous, collagen can be used effectively and more widely. Nanoparticle size on collagen is a very small granule or particle between 10-1000 nm[8]. Nanoparticle was developed in 1970 [9]. The main advantage of food or drug conductor in nanoparticle size is that the food (drug) can be delivered like the desired target in the body, a high absorption and broad capabilities to achieve the target in various areas of the body for a long time, biodegradable, and non-toxic [10].

The particle size is an important factor to determine the effectiveness using collagen in biomedical scope and cosmetics. The particle size affects the cellular absorption; therefore, it needs a size reduction technology through nanotechnology to improve the effectiveness and the absorption. One of the methods to make collagen nanoparticles with simple and easy way to apply is mixing collagen with an ethanol solution through the system of continuous stirring using a magnetic stirrer, so that collagen nanoparticle size can be achieved [11].

Previous research on nano-collagen by [12] has managed to make the fish cork skin collagen nanoparticles with the smallest size 253.49 nm and stirring time for one hour, and the nanoparticle size of bamboo cone fish skin collagen 146.71 nm with stirring time for two hours [13]. Therefore, it needs to conduct research about determination the quality of sea cucumbers nano-collagen with various stirring time. This study aims to determine the quality of sea cucumber collagen using hydrochloric acid (HCl) with different concentrations and the quality of nano-collagen using magnetic stirrer with different stirring times.

2. Methodology

2.1. Materials dan Methods

The sea cucumber weighed between 350-400 grams is obtained from the waters of Pulau Terung, Batam, Riau Islands, Indonesia. The chemical substances used for extraction are NaOH solution, HCl solution, ethanol solution, sulfuric acid, Copper complexes, borax acid, methylene red and methylene blue indicator, hexane, and buffer solutions for pH.

This research uses an experimental method that consists of two stages of the research, namely: (a) Study extraction process of collagen using the difference solution of concentrated HCl (4%, 5% and 6%), and (b) Study process of making nano-collagen using 96% ethanol solution using a magnetic stirrer with different stirring variations (one, two, three hours) at 40°C.

2.2. Research Stages

The research stages in the process of extracting collagen and nano-collagen of sea cucumber meat include:

1) Weeding and preparing the sea cucumber sample. This stage is split and fillet the sea cucumber body using a knife. The needed part for this research is fresh sea cucumber meat. Sea cucumber meat is mashed using a blender until it is mixed well (mixing meat and aquades 1:2 w/v).

2) Extraction process of the sea cucumber collagen:
a) The mashed sea cucumber meat is added gradually with 35% NaOH solution using a drop pipette until pH 11 obtained.
b) Heating the sea cucumber meat using a hotplate at temperature 40°C while stirring using a magnetic stirrer for 30 minutes.
c) The mixture is centrifuged at 7500 rpm for 30 minutes, then a supernatant is obtained.
d) Supernatant (collagen solution) is added with HCl solution for adjustments close to pH 4 (isoelectric point of collagen) using HCl solutions with different concentrations (HCl 4%, 5% and 6%). HCl solution is added gradually using a dropper pipette while stirring using magnetic stirrer at temperature 40°C.
e) Centrifuging the process of separating the precipitates containing collagen with HCl solvent.
f) Evaporating the precipitates containing collagen through a rotary evaporator for 60 minutes.
g) Drying the collagen using oven at a temperature of 35-37°C for 60 minutes.

3) Process of making nano-collagen sea cucumber:
a) Adding sea cucumber meat collagen with 96% ethanol (ratio 1:2 w/v).
b) Heating collagen solution using a hotplate at temperature 40°C using variations stirring time (magnetic stirrer) at speed 1000 rpm for one, two and three hours.
c) Separating collagen solution using the solution at 7500 rpm for 30 minutes.
d) The centrifuge results obtained nano-collagen is evaporated using a rotary evaporator for 60 minutes, and dried using oven at temperature of 35-37°C to maximize the nano-collagen powder.

2.3. Observation Analysis
Data were statistically analyzed using analysis of variance (ANOVA) with confidence level 99% (p<0.01) and SPSS software version 22.0. Parameters test are: yield[14], color and whiteness degree[15], proximate analysis (water level, protein, fat, ash)[14], pH[16], analysis of Particle Size Analyzer[17] (DelsaTM Nano, Cordoun), Viscometer (Brookfield LV), Fourier Transform Infrared Spectrophotometer (Bruker Tensor Type MBQ00)[18], Scanning Electron Microscopy (JEOL JSM-6360-LA)[19].

3. Results and Discussion
3.1. Proportion of Sea Cucumber
Results of weeding process and preparing sample sea cucumber H. scabra obtained 45% meat, 38.5% skin, 8.85% gonad innards and 7.65% other impurities.

The largest part of the sea cucumber body is meat with the percentage of 45%, which consists of moisture 78.75%, protein 79.77%, ash 6.68%, fat 4.88%, and carbohydrates 8.67%. Proportion of every sea cucumber body from the material yield different proportions, depends on the size of sea cucumbers H. scabra.

The larger size of the sea cucumber gives the proportion of meat slight decrease and the proportion of the skin increases. The results of the research of [1] using a sample of raw material sea size 525 g per tail obtained proportion of meat sea cucumber 43.53% and the proportion of skin reaches 40.1%.

The results of the research are known nutrients in the body of other parts for the dominant skin that contains a layer of lime and minerals reaching 61.44% and carbohydrate 25.84%, in the innards organ there are still proteins 56.95% and fat 23.28%. Based on the protein level that is high enough in the sea cucumber meat (79.77%), it is potential for extract the collagen proteins.

3.2. Characteristics of Collagen
3.2.1. Collagen Yield
The value of collagen yield is obtained from comparison dry weight collagen with material weight of sea cucumber meat. Collagen yield of sea cucumber with the use of different consentration HCL gives different amount of yield (Tabel 1).
The statistical analysis results show that the extraction process of collagen using HCl solution with different concentrations. It gives real effect ($p<0.01$) to the amount of collagen-meat sea cucumber sand. The use of a solution of HCl 4% gives real effect between HCl 5% and 6% solutions against each total yield (17.88%; 19.62% and 20.76%). The results appear that the use of higher concentrations of HCl provides a greater amount of yield. The number of low-yield tends to increase as the concentration of acidic acid increases [20].

The yield of sea cucumber meat collagen has better yield than the acid solubilized collagen (ASC) gamma sea cucumber collagen. The results of research by [4] obtained a collagen yield of 16.40%. The collagen yield of the sea cucumber is still higher than the collagen yield of the golden sea cucumber obtained only 0.66% [21].

The yield of sea cucumber meat collagen is still lower than the collagen yield of cured fish Nemipterus hexodon) by 24.9% [22], collagen scales of Chuan striatus fish scales by 1.94% [23], but higher when compared to the yield of dry skin collagen Onchorhynchus mykiss rainbow trout at 9.48% [24]. The different yield collagen in each material as a source of collagen is influenced by many factors. The factors that may affect the value of the yield that differences in methods of extraction include the length of the extraction, the comparison sample and solvent, the concentration of acid-base, and the type of raw materials used [25] and [26].

That high concentration of acid in the extraction process of collagen is able to increase the yield [20]. Higher acid concentrations cause hydrolysis process speeds are getting faster, so as to increase the amount of collagen molecules are converted into simple collagen products.

### 3.2.2. Color and Whiteness Degree

One of the characteristics of sea cucumber meat collagen is solvent extraction of sand with different concentrations of HCl produce collagen relatively in same color, i.e. creamy white.

Sea cucumber meat collagen is relatively same in color, that is, creamy white. Whiteness degree of each collagen with 4% HCl is 73.65%; HCl 5% has whiteness degree 73.82% and HCl 6% has whiteness degree 73.97%. This shows that difference concentration of HCl (4%, 5% and 6%) does not show any significant difference in color and whiteness degree of collagen from sea cucumber meat. This is due to the difference concentration HCl tend to affect more to the yield than the color.

### 3.2.3. Chemical Characteristics of Collagen

Change of chemical characteristics to proximate analysis of sea cucumber meat collagen can be seen in Table 2.

| Chemical Characteristics | Concentration, HCl | Reference** | Collagen Standard)* |
|--------------------------|-------------------|-------------|---------------------|
|                          | 4%               | 5%          | 6%                  |                     |
| Moisture (%wb)           | 11.26±0.22        | 11.22±0.26  | 10.99±0.17          | 13.64               |
| Protein (%db)            | 97.4±0.24         | 97.8±0.20   | 98.3±0.28           | 67.88               |
| Fat (%db)                | 0.67±0.08         | 0.61±0.05   | 0.59±0.07           | ns                  |
| Ash (%db)                | 1.28±0.09         | 0.93±0.11   | 0.86±0.07           | 4.15                |
| pH                       | 5.73±0.05         | 5.39±0.07   | 4.92±0.05           | 7.37                |

* [28]; ** [3]; ns= not specified; wb=wet base; db= dry base

Table 2 indicates that different concentrations of HCl solution did not significant effect to water level and fat collagen of sea cucumber meat, while using different concentrations HCl solution had significant effect to protein, ash, and pH of sea cucumber meat collagen ($p<0.01$). Using higher
concentration HCl solution may increase collagen protein levels of sea cucumber meat and decrease trend of moisture fat, ash and pH collagen.

Based on the results obtained, the characteristics of sea cucumber meat collagen which were in accordance with ISO standards [27] were water level, protein, fat, and ash, while pH of collagen products is not in accordance with the standards [22].

Sea cucumber collagen produced moisture level relatively in the same range, between 10.99-11.26%. This indicates that using different HCl concentration did not give variety of water level collagen and moisture level was affected by the drying system. Using different HCl concentrations (p<0.01) produced relatively the same range of fat collagen, between 0.59-0.67%. Fat collagen sea cucumbers meat in this research was lower than fat collagen in results study on catfish skin which had fat level 8.85% [28], and the fat of sea cucumber collagen was higher than skate fish skin collagen (Raja kenojei) [29].

Protein collagen of sea cucumber meat tended to increase along the use of HCl concentration. This was due to the increase of HCl concentration causing the amino acids more fragmented so that the protein was more dissolved in extraction process. The high dissolved protein caused the increase of protein level. In sea cucumber meat, the protein level was higher than collagen level in the sea cucumber which was 68.54% gamma [3].

In this study, the ash level of sea cucumber meat collagen was 6.70%, and it was lower than gamma sea cucumber [3]. High and low of ash level depended on several factors, such as the difference between habitat and environment, as well as the solvent extraction [30]. According to [31], ash is inorganic substances of burning an organic materials. Determination of ash level is closely related to the minerals contained in the ingredients. If the mineral in food is high, the ash level is high too [32].

pH value of the sea cucumber meat collagen is influenced by the acid concentration, i.e. increase in acid value (decrease pH). Range of pH value of the sea cucumber collagen is between 4.92-5.73% and it is lower than sea cucumber collagen gamma pH 6.08 [33]; pH of S. variegatus sea cucumber collagen is 7.37[3]; pH of golden sea cucumber collagen is 6.91 [21].

pH value of H. scabra collagen is lower than pH value in other studies, because the collagen extraction process in this study used HCl solution, while other research used acetic acid (gamma and golden sea cucumber). However, using the same solvent will have different result depending on several factors: concentration and extraction temperature that affects the pH product.

Some commercial collagen for cosmetics have the pH between 3.8 to 4.7 [34], and pH value is related to the minerals as a buffer collagen solution. The different pH values is due to the different acid or base type and concentration. The pH value is related to the level of collagen solubility [24].

3.2.4. Collagen Functional Group
The determination of functional groups sea cucumbers meat collagen used Fourier-Transform Infrared analysis (FTIR). The results show that collagen had absorption peaks distribution at several wave numbers that show certain functional groups.

In this research, the collagen showed absorption peaks including amide A, amide B, amide I, amide II and amide III. FTIR absorption region of sea cucumber meat collagen (Table 3).

| Functional Groups | Wave Number (cm⁻¹) | Information               |
|-------------------|---------------------|---------------------------|
|                   | HCl 4%              | HCl 5%                    | HCl 6%                    |
| Amide A           | 3418.97             | 3418.97                   | 3418.97                   | N-H stretch |
| Amide B           | 2930.66             | 2928.11                   | 2928.07                   | Asimetrikal stretching CH₂ |
| Amide I           | 1652.10             | 1634.74                   | 1652.10                   | C-O stretch  |
| Amide II          | 1558.55             | 1558.55                   | 1544.08                   | C-N stretching dan N-H bending |
| Amide III         | 1227.74             | 1227.74                   | 1236.42                   | N-H bending dan C-H stretching |
The FTIR spectra show the amide groups, namely: amide A, amide B, amide I, amide II and amide III of all treatments in sea cucumber meat collagen. Amide A absorption peaks was detected at wave number 3418.97 cm\(^{-1}\), showing the N-H stretching vibration. This result is not much different from the research by [3] stating that sea cucumber collagen gamma had amide A with wave number 3417.27 cm\(^{-1}\). According to [35], amide A was in the range of 3400 cm\(^{-1}\) to 3440 that occurred at N-H stretching vibration. Amide B of sea cucumber meat collagen was indicated from the absorption at wave numbers ranging from 2928.07 to 2930.66 cm\(^{-1}\). The range functional group amide B was between wave number 2935 cm\(^{-1}\) to 2915 cm\(^{-1}\) which was the asymmetric stretching of CH\(_2\) groups [36].

The FTIR also show the absorption peaks at wave number 1652.10 cm\(^{-1}\) and 1634.74 cm\(^{-1}\) that indicate the amide I. Range of amide I peak is between 1600 cm\(^{-1}\) to 1700 cm\(^{-1}\) and show the vibration C = O stretching along the polypeptide chain and it is a marker of the peptide secondary structure [35].

Spectrum Amide II from sea cucumber meat collagen was between the wave numbers 1544.08 cm\(^{-1}\) and 1558.55 cm\(^{-1}\). This was in accordance with amide II absorption contained in range of 1480 cm\(^{-1}\) to 1575 cm\(^{-1}\) and showed the presence of C-N stretching and N-H bending [37]. Amide group III was in the range of 1200 cm\(^{-1}\) to 1400 cm\(^{-1}\) that showed the presence of N-H bending and C-H stretching. Amide III of sea cucumber meat collagen was detected at wave number 1227.74 cm\(^{-1}\) and 1236.42 cm\(^{-1}\).

The functional group analysis with FTIR was used to confirm triple helix structure, which was detected from amide III absorption ratio values (1227.74 and 1236.42). The intensity ratio showed collagen triple helix structure. Ratio value approach to 1.0 indicates that collagen had a triple helix structure [38]. The collagen was not degraded into gelatin as it can be seen from its triple helix structure.

### 3.3. Characteristics of Nano-Collagen

#### 3.3.1. Color and Whiteness Degree

Characteristics of sea cucumber meat nano collagen with stirring system for one, two, three hours using a magnetic stirrer at 40°C showed relatively different color and whiteness degree.

The sea cucumber meat nano-collagen showed relatively different in color. The color was creamy white to white with whiteness degree of each nano-collagen using magnetic stirrer and stirring time one, two, three hours having whiteness degree was 78.47%; 78.81% and 79.25%, respectively. The results show that the different stirring time had significant effect on the color and whiteness degree of nano-collagen. This is due to difference of stirring time that affected the nano-collagen appearance.

#### 3.3.2. Nano-Collagen Chemical Content

The chemical contents of sea cucumbers H. scabra meat nano-collagen include moisture, protein, fat, ash and pH as presented in Table 4.

| Chemical Characteristics | Stirring Time (hours) |
|--------------------------|-----------------------|
|                          | 1                     | 2                     | 3                     |
| Moisture (%wb)           | 9.85±0.25\(^a\)       | 9.62±0.19\(^b\)       | 9.11±0.21\(^a\)       |
| Protein (%db)            | 98.4±0.17\(^a\)       | 98.7±0.12\(^a\)       | 98.9±0.15\(^a\)       |
| Fat (%db)                | 0.58±0.07\(^a\)       | 0.54±0.09\(^a\)       | 0.51±0.09\(^a\)       |
| Ash (%db)                | 0.82±0.09\(^a\)       | 0.76±0.11\(^b\)       | 0.68±0.08\(^a\)       |
| pH                       | 3.91±0.05\(^a\)       | 3.96±0.07\(^a\)       | 3.98±0.05\(^a\)       |

The results show that different stirring time (magnetic stirrer) for one, two, and three hours had very significant effect in water level and ash level of sea cucumber meat nano-collagen, whereas there was no significant effect in protein level, fat, and pH value of sea cucumber meat nano-collagen (p<0.01). The longer stirring time affected the decrease of moisture, fat and ash sea cucumber nano-collagen and increased the protein level and pH value of sea cucumbers meat nano-collagen. This
indicates that longer stirring was more optimal to break down the compound particles so the surface area of the compound became more widespread and reduced moisture level, fat, and ash.

The range of water level value in nano-collagen of sea cucumber was between 9.11-9.85%. The different stirring time (one, two and three hours) produced significant difference of water level amount in each nanoparticles collagen or nano-collagen. The longer the stirring time the less water content produced. This can be seen in the decrease average water level value between 9.85% to 9.11%. The nano-collagen or nanoparticle collagen still meets the collagen water level quality standards that has been set, i.e. ≤12% [27].

The stirring time process produced smaller nanoparticles collagen so the water absorption rate was higher. This was related to the smaller size of nanoparticle collagen thereby increasing the surface area to absorb water. The smaller nanoparticle collagen, the greater surface area produced so the water ability to evaporate during drying process was greater [39]. The high water level was due to the way collagen storage that allowed the absorption of water [39].

The range of protein level value of sea cucumber nanoparticle collagen was higher from 98.4%-98.9% than the levels of protein nanoparticle collagen of stingray skin, i.e. 86.18% [39]. Based on these results, protein levels of nanoparticles collagen was high, so it met the quality standards established, i.e. collagen 80-88% [27]. Fat level in the process of making nano-collagen was relatively similar at stirring time of one to 3 hours. This indicates that stirring time did not have effect to reduce fat left on sea cucumber meat collagen. It is due to strong bond conjugates occurs between fat and protein, so it is difficult to escape from the compound.

The time of stirring caused reduction the collagen size into nanoparticles that could increase protein sea cucumbers H. scabra meat nanoparticles collagen. The smaller particle size, the more protein extracted. It is due to small particle size have large extensive contact area volume so the mass transfer between solute from solids to the solvent is bigger [40]. In addition, the high levels of the protein in sea cucumber meat nanoparticle collagen was because the collagen protein in sea cucumber meat was well hydrolyzed by the hydrochloric acid solution, so the protein collagen was drawn perfectly [41].

The ash level of sea cucumber meat H. scabra nanoparticle collagen with different stirring time had low ash level meeting the quality standards that has been set, i.e. ≤1% [27].

The low value of ash level in nanoparticle collagen from sea cucumber meat shows that the longer stirring time was effective to reduce minerals in sea cucumber meat. The constant stirring would cause the hydrochloric acid solvent bind minerals perfectly. If the stirring is not constant, the reaction of mineral binder will be imperfect [42].

This pH value is lower than gamma sea cucumber collagen from the study by [3] showing pH of 7.61 and pH value 6.85 [43]. The different pH value was caused by the acid concentration for hydrolysis process. The high acid concentrations made the acidic collagen. The low pH value was caused by imperfect neutralization process [44].

In this study, the lower pH value of nanoparticle collagen was in accordance with the ISO standards [27], the range between 6.5-8. That pH values in some collagen brands range from 3.8 to 4.7. The low pH value of the sea cucumber meat nanoparticle collagen can be caused by the type of solvent (acid or base) in hydrolyze process the protein collagen [34]. Acid process tends to produce low pH value, otherwise alkaline process will tend to produce high pH value. Combination of acids and bases tends to produce neutral pH [20].

### 3.3.3. Nano-Collagen of SEM and PSA

Several characteristics of collagen nanoparticles from nano-chitosan sea cucumber meat can be seen in Table 5.

Collagen nanoparticles can be visually distinguished by using SEM. Working principle of SEM is the wave characteristic of electrons in the form of diffraction at very small angles [19].
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Table 5. Particle Size of Nano-Collagen from H.scabra

| Stirring Time (Magnetic Stirrer) | Particle Size (nm) |
|---------------------------------|-------------------|
| 1                               | 1240±89^a         |
| 2                               | 950±41^b          |
| 3                               | 285±35^c          |

The test results of SEM (Figure 1) was the sample using nano-collagen with different stirring time (one, two, three hours) with magnification up to 20,000 times. The nano-collagen had a particle shape in the form of spheres resembling grains, solid colloids, smooth and whole round. Nano-collagen had particle size distribution that was relatively homogeneous.

The sea cucumber nanoparticle collagen was made by adding 96% ethanol as a desolvation agent. This desolvation agent was intended to reduce the presence of water in collagen particles and keep hydrated the nanoparticle collagen solution [45]. Meanwhile, ethanol can affect protein structure through the changes of dielectric constant, solvation power, influence the hydrophobic interactions, hydrogen bonding, dipole moment, and the salt bridge [46]. Adding 96% ethanol in manufacturing process of nanoparticle collagen is required as desolvation agent with ratio of 1:2 (w/v).

Generally, particle calculation uses image analysis or some type of particle counting. The size of nano-collagen was tested using Particle Size Analyzer (PSA) tool. The test shows that the average size of nano-collagen in one hour stirring was 1240 nm, two hours stirring was 950 nm and three hours stirring was 285 nm. Those nanoparticles were solid particles with size range of 10-1000 nm [8].

Preparation method is very influential in technology of making nanoparticles. Reducing size with a magnetic stirrer can produce more stable particles also in size, under 1000 nm [47]. The effect of reduction particle size with magnetic stirrer in high speed is generalizing the received energy by all parts of the solution, so the particle size is more homogeneous [48].

Longer stirring time using magnetic stirrers in the ionic glation process is due to provide wider opportunity to reduce particle size. The benefits of magnetic stirrer are homogenization process between chitosan solution with gelation ionic material, evenly controlled at high speed to produce homogeneous particles, stable and unagglomerated. Therefore, drying process is only the stable particles forming the nano particles, instead of the unagglomerated particles [49].

4. Conclusion

Extraction process using HCl with different concentrations has significant effect on the characteristics of sea cucumber meat collagen. The 6% HCl extraction obtained optimal results with collagen yield 20.76%, white degree 73.97%, moisture 10.99%, protein 98.3%, ash 0.86%, fat 0.59%, and collagen pH 3.92. The functional group in accordance with the standard collagen compound have amide A, amide B, amide I, amide II, and amide III. The long stirring time has significant effect to the characteristics of nano-collagen powder, with creamy white color (79.25% whiteness degree), moisture 9.11%, ash 0.68%, fat 0.51%, protein 98.9%, pH 3.98, and particle size 285 nm.
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