Making Gelatin From Milkfish Bones (Chanos Chanos) and it Application in Soy Milk Pudding (Glycine Max)

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Abstract. Indonesia's dependence on imported gelatin is increasing. Based on the literature that as much 80% of imported gelatin uses non-halal raw materials in the form of pig skin, it is necessary to make innovations to produce gelatin from halal materials. The purpose of this study was to analyze the characteristics of gelatin from milkfish bones and produce soy milk pudding with characteristics that are acceptable to panelists. The research method consisted of making two types of gelatin using 3% acetic acid solution and 4% hydrochloric acid solution, then conducting physical analysis for gelatin and organoleptic, protein, and syneresis tests for soy milk pudding. The results showed that milkfish bone gelatin using 3% acetic acid solution had a gel strength of 181.5 g/bloom, a yield of 2.2%, and a pH of 7. Meanwhile, milkfish bone gelatin using 4% hydrochloric acid solution had a gel strength of 57.5 g/bloom, a yield of 13.97%, and a pH of 6. Then, the best results were obtained in soy milk pudding with the addition of milkfish bone gelatin in 3% acetic acid solution. In addition, milkfish bone gelatin using 3% acetic acid solution has almost the same structure as commercial gelatin based on FTIR results.

1 Introduction

Milkfish (*Chanos chanos*) is one type of fish that is very widely circulated in the market. This fish is known to have a high protein content and has an economical price so it is often consumed by the community. Currently, public knowledge of the development of milkfish marketing is increasing, for example by producing boneless milkfish, both fresh and processed. However, along with the high consumer demand for milkfish products, the waste of milkfish bones is increasing. Based on data from the Central Bureau of Statistics in 2018, it states that milkfish production as a whole has an amount of 701,319 tons. The composition of fish bone waste to all parts of the fish is 10-20% so that milkfish bone waste has an amount

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of around 88,000 tons [10]. This can cause environmental pollution both in waters and on land due to the wasteful disposal of milkfish bone waste. In fact, the milkfish bone waste still contains 32% protein and 24% collagen so that it can be utilized into a new product [7]. One alternative to utilize milkfish bone waste is to process it into gelatin.

Gelatin is a much-needed product in the food, pharmaceutical and cosmetic industries. At present, Indonesia's dependence on gelatin needs still relies on imported products from other countries because there is still a lack of gelatin manufacturing industry in Indonesia. This is evidenced by the gelatin import data from the Central Bureau of Statistics in 2019 which states that Indonesia received gelatin imports of 4,808 tons from various countries such as China, India, Thailand, Brazil, and Australia and will continue to increase for the following year. Based on research [8], 80% of gelatin imports use pig skin raw materials, 15% use cow skin raw materials, and 5% use cow, pig, and fish bone raw materials. Therefore, it is necessary to make new innovations to produce gelatin from halal materials so that it is safe for use in industries in Indonesia. In general, gelatin in circulation uses mammalian bones and skins, but in this study, bones from marine organisms were used because they have lower allergy levels, avoiding haram status and viruses from zoonotic mad cow disease from several gelatin import countries, and more stable viscosity levels. According to research [19], milkfish bones have the potential to be used as raw material for making gelatin because it has a high protein content of around 32% and the resulting gelatin can be used as a thickener. Meanwhile, other functions of gelatin are as a stabilizer, gelling agent and also as a preservative. Therefore, gelatin is often used in the food industry, especially in making pudding.

Pudding is a very popular dessert among the public. Pudding processing usually uses agar powder as a thickener in order to produce an optimal texture. However, currently there are several industries that produce pudding using gelatin as a thickener. In general, pudding production also often uses cow's milk so that it cannot be consumed by all people because it has a high lactose content. The solution to the problem is to make pudding using soy milk as raw material because soy milk does not contain lactose and is low in calories so it is safe for consumption by all people, from small children to the elderly. Therefore, the background of this research was made in order to produce good soy milk pudding characteristics with efforts to improve texture by adding gelatin from milkfish bones.

Gelatin is generally obtained by extraction from collagen that is heated into a simpler structure using the hot water extraction method. This extraction process will change the nature of collagen fibers from insoluble in water to soluble in water by cutting the crosslinking bonds contained in collagen. Therefore, gelatin has properties as a thickener and emulsifier so it is often used in several food and cosmetic industries. The novelty of this research is in the form of application to pudding products, because in previous studies only gelatin characterization and application to jelly candy and panna cotta products were carried out.

2 Research methodology

2.1 Tools and Materials

2.1.1 Tools

The tools used in this study consisted of tools for making gelatin, making pudding, and tools for analysis. The tools used for making gelatin are basin, Petri dish, funnel, beaker, grinder, scissors, oven, pan, ruler, thermometer, and waterbath. The tools used for making pudding are portable stoves, pans, refrigerators, spoons, thermometers, analytical scales, and containers. The tools used for analysis are porcelain cup, schott cup, erlenmeyer, beaker, measuring cup, kjhedal flask, magnetic stirrer, oven, refrigerator, centrifuge, centrifuge tube, texture analyzer, and analytical balance.

2.1.2 Materials

The materials used in this study consist of materials for making gelatin, making pudding, and materials for analysis. The materials used for making gelatin are distilled water, litmus paper, filter paper, CH_3COOH solution, HCl solution, and milkfish middle bone. The ingredients used for pudding making are cocoa powder, commercial bovine gelatin (Hakiki), sugar, and soy milk. The materials used for analysis were distilled water, Conway indicator, filter paper, label, H_2SO_4 solution, H_3BO_3 solution, 4 N NaOH solution, 40% NaOH solution, selenium, spoon, and panelist container.

2.2 Research procedure

2.2.1 Degremeasing

Degrameasing is the process of preparing raw materials and serves to break mineral components with high temperature treatment. The first step of the degrameasing process is to prepare the raw material of milkfish bones. Next, the raw materials are washed and cleaned from dirt, meat residue, and fat on the bones. After that, the milkfish bones are boiled at 80°C for 30 minutes, then the bones are cleaned and dried.

2.2.2 Demineralization

Demineralization is the process of removing calcium, fat components, non-collagen proteins, and mineral salts contained in the bone so that the resulting bone becomes soft. The demineralization process begins with soaking the bones in HCl solution with a concentration of 4% and another treatment using 3% CH3COOH solution for 48 hours. The ratio of bone and acid solution was 1:3. After that, the bones were washed with running water until the pH was neutral.

2.2.3 Extraction

Extraction in this study used the hot water extraction method, which is a process that uses hot water, where this process occurs denaturation, increased hydrolysis, and solubility of gelatin. Ossein with neutral pH was put into a beaker and water was added, the ratio of bone and water was 1:3 (w/b). After that, the ossein solution was extracted in a waterbath at 85°C for 6 hours. The extracted filtrate was then filtered using filter paper. After that, the filtrate was poured into a petri dish to be dried in an oven at 55°C for 24 hours to obtain gelatin sheets. Next, the gelatin sheets were pulverized using a grinder into powder form. Analytical methods of milkfish bone gelatin include gel strength, yield, FT-IR test, and pH test.

2.2.4 Making soy milk pudding

The process of making soy milk pudding starts with weighing gelatin as much as 10 grams and then dissolving gelatin in 40 ml of cold water and adding 20 ml of boiling water. Next, 50 grams of sugar, 20 grams of cocoa powder, 15 grams of chocolate bar, 1 gram of vanilla, and 250 ml of soy milk are weighed and then heated to boiling. After boiling, the pudding mixture is stirred for 5 minutes. Then, turn off the portable stove and add the gelatin solution and stir again for 5 minutes. Next, the pudding was cooled to 25°C and placed in a refrigerator for 24 hours. Soy milk pudding analysis methods include syneresis, protein content, and water content.

3 Results and discussions

3.1 Discussion of milkfish bone gelatin

3.1.1 Yield

The yield is the final result obtained from the collagen extraction process which shows the effectiveness of the method used. The higher the yield obtained, the more effective the method used. This yield value serves to determine production planning and capacity, raw material requirements, calculation of production costs, and the efficiency of the process carried out [17]. The yield can be influenced by the length of soaking time, raw materials, and the type and concentration of the solution used. Commercial gelatin has a yield level of around 22.12-30.27% because in general this gelatin is sourced from a mixture of cow skin and bones [12]. The research data ranged from 2.62% to 13.97%. The highest yield was obtained in milkfish bone gelatin using HCl solution while the lowest yield was obtained in milkfish bone gelatin using CH_3COOH solution. The yield results on milkfish bone gelatin can be seen in Figure 1.

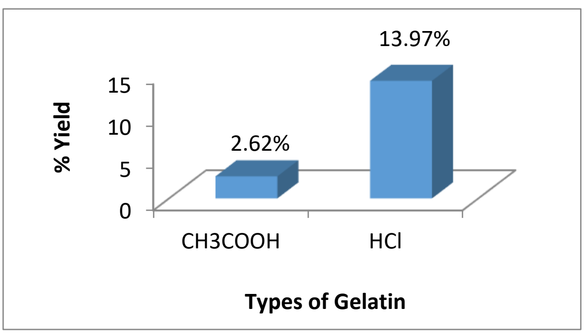

Fig. 1. Yield value of milkfish bone gelatin with 2 types of acid treatment.

According to research [5], the yield of milkfish bone gelatin using citric acid solution is 9.74%. Based on the results of the study, the yield of milkfish bone gelatin using acetic acid (CH_3COOH) was 2.62% while the yield of milkfish bone gelatin using hydrochloric acid (HCl) was 26.7%. The yield results obtained show that the yield of gelatin from fish bones has a smaller value compared to the yield value of commercial gelatin because collagen in cattle bones and skin has a larger composition of around 24% and 89% while collagen from fish bones is only around 24% [17]. Meanwhile, soaking using hydrochloric acid produces higher yields compared to soaking using acetic acid. This is because the hydrochloric acid solution has a higher acid concentration level compared to acetic acid, which is a weak acid group indicated by the high concentration of H+ ions that will hydrolyze collagen from the triple helix chain to the mono helix chain so that it can produce higher gelatin yields. In addition, the higher acid concentration can also increase the yield obtained due to the process of binding calcium minerals in milkfish bones by hydrogen bonds and ionic bonds formed between polypeptide chains so that the molecular structure can open and can free collagen in larger quantities.

3.1.2 Gel strength

Gel strength is a physical property of gelatin that can determine the quality of gelatin produced. Gel strength testing serves to determine the ability of gelatin to change liquid form into solid form or change the form of sol into gel which has reversible properties. At low temperatures, gelatin molecules from the hydrolysis process will bind together and form a compact structure so that it can form a gel texture in the resulting product. The gelatin group is divided into three types based on the gel strength value, namely high bloom gelatin with a gel strength value of 220-300 g/bloom, medium bloom gelatin with a gel strength value of 150-220 g/bloom, and low bloom gelatin with a gel strength of <150 g/bloom [17]. The higher the gel strength value, the more chewy/solid the texture of the food product will be. Differences in gel strength values can be influenced by solvent concentration, heating time, and materials used. Commercial gelatin is classified as high bloom gelatin because it has a gel strength of around 220 g/bloom. The research data ranged from 57.5 g/bloom to 181.5 g/bloom. The highest gel strength value was obtained in commercial gelatin while the lowest gel strength value was obtained in milkfish bone gelatin using HCl solution. Commercial gelatin is gelatin sold commercially in the market which is generally made from a mixture of cow skin and bones. The results of the values of the different types of gelatin can be seen in Figure 2.

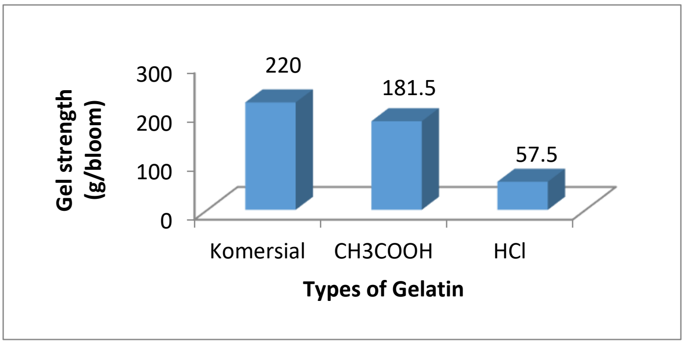

Fig. 2. Gel strength values of different types of gelatin.

According to research [16], the gel strength value of milkfish bone gelatin using acetic acid solution is 134.3 g/bloom. Based on the results of the study, the gel strength value of milkfish bone gelatin using hydrochloric acid (HCl) solution is 57.5 g/bloom, while the gel strength value of milkfish bone gelatin using acetic acid (CH_3COOH) solution is 181.5 g/bloom which indicates that soaking using acetic acid (CH_3COOH) solution produces a higher gel strength value. Based on the gelatin class described above, gelatin produced from soaking in acetic acid (CH_3COOH) solution is included in the medium bloom gelatin class, while gelatin produced from soaking in hydrochloric acid (HCl) solution is included in the low bloom gelatin class. This is because the HCl solution, which is a strong acid, has a lower pH level than the CH_3COOH solution, which is a weak acid. This pH value can affect the gel strength value because strong acids with low pH levels will cause further collagen hydrolysis and can produce amino acids with short chains that are easily degraded and damaged so that the gel strength value can decrease [11]. Meanwhile, a weak acid with a higher pH will protect the polypeptide chain bonds from damage so that it can produce a higher gel strength value. In addition, factors that can affect gel strength are also in the form of extraction temperature where this study used a temperature of 85°C and it is possible to increase the temperature even higher due to technical errors from the waterbath used so that it can interfere with the collagen hydrolysis process and can reduce the gel strength value.

3.1.3 pH

pH is an important parameter that can be used to determine gelatin quality standards. The pH test serves to determine the acidity of the gelatin produced. The pH value is important for gelatin because pH can affect the physical properties of gelatin such as gel strength and viscosity [15]. In addition, the pH value can also affect the application of gelatin in a product. The lowest pH result was obtained in milkfish bone gelatin using HCl solution while the highest result was obtained in milkfish bone gelatin using CH_3COOH solution and commercial gelatin. The pH results on various types of gelatin can be seen in Figure 3.

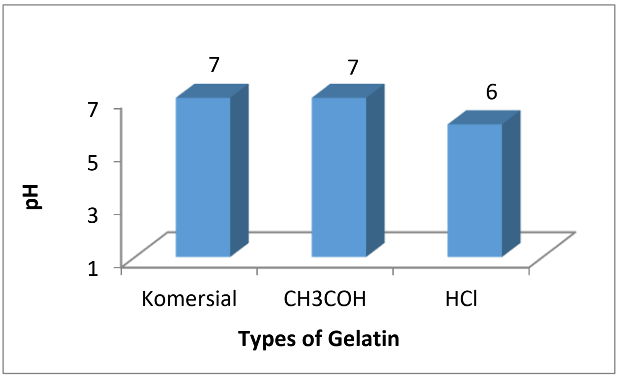

Fig. 3. pH value of milkfish bone gelatin with different types of treatment

The results showed that commercial gelatin and gelatin from milkfish bones using CH_3COOH solution had a pH value of 7 while milkfish bone gelatin using HCl solution had a pH value of 6. In the treatment of both types of gelatin using an acidic solution, the pH is neutral because when it has gone through the demineralization process, the ossein will be neutralized using running water so that the acid content in the ossein will decrease and can approach a neutral pH. Gelatin with a neutral pH tends to be preferred, based on this, the neutralization process is important to do in order to remove the remaining acid from the demineralization process [14]. Therefore, the gelatin produced is safe to use in pudding making because it does not contain acids that are harmful to the body.

3.1.4 FTIR

FT-IR testing is a test to determine the functional groups in a sample and prove that the tested sample contains compounds of pure gelatin. The results of the extraction of milkfish bone collagen into gelatin can be proven if the FT-IR test of gelatin has hydroxyl functional groups (-OH), amine groups (N-H), carbonyl groups (C=O), nitrile groups (C-N) and alkane groups (C-H) [9]. In the FT-IR measurement results, fish bone gelatin has 4 absorption regions in the form of amide A, amide I, amide II, and amide III. The results of FTIR testing on milkfish bone gelatin can be seen in Figure 4.

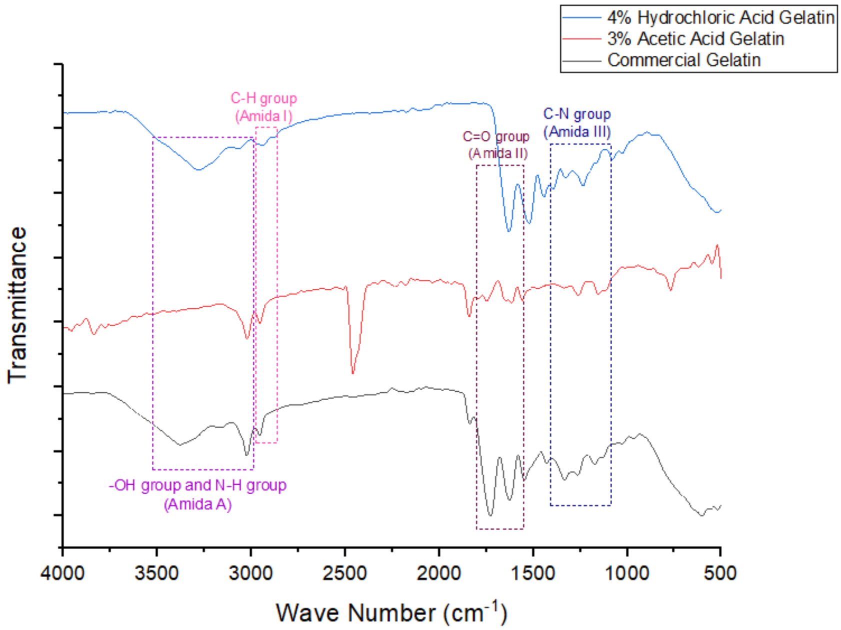

Fig.4. FTIR graph of milkfish bone gelatin.

The absorption area of amide A (-OH group and N-H group) can be seen from the absorption peak in the frequency range around 3600-3000 cm^{-1}. Commercial gelatin derived from cattle has an amide A absorption area of 3135.42 cm^{-1} [13]. Based on the results of FTIR testing, the absorption peak of amide A in milkfish bone gelatin using HCl solution is around 3280.57 cm^{-1} for the -OH group which gives a fairly deep peak and a small peak for the N-H group with a wave number of 3066.56 cm^{-1}, while the absorption peak of amide A in milkfish bone gelatin using CH3COOH solution is around 3280.57 cm^{-1} for the -OH group which gives a fairly deep peak and a small peak for the N-H group with a wave number of 3071.59 cm^{-1}. This shows that both types of gelatin have a fairly strong amide A absorption region. The -OH group shown in the FTIR spectrum indicates that the presence of the amino acid hydroxyproline, which is the main amino acid in gelatin, and the presence of OH compounds from the water used to extract gelatin [9].

The amide I absorption area (C-H group) can be seen from the absorption peak in the frequency range of around 3000-2850 cm^{-1} in commercial gelatin derived from cattle has an amide I absorption area of 2876.95 cm^{-1} [13]. Based on the results of FTIR testing, the amide I absorption peak in milkfish bone gelatin using HCl solution is around 2924.36 cm^{-1} with a deep peak, while the amide I absorption peak in milkfish bone gelatin using CH3COOH solution is around 2930.11 cm^{-1} with a rather deep peak which indicates that milkfish bone gelatin using HCl solution and CH3COOH solution has the α-helix chain content of the gelatin structure with moderate intensity [9].

The absorption area of amide II (C=O group) can be seen from the absorption peak in the frequency range around 1800-1600 cm^{-1}. Commercial gelatin derived from cattle has an amide II absorption area of 1638.6 cm^{-1} [13]. Based on the results of FTIR testing, the amide II absorption peak in milkfish bone gelatin using HCl solution is around 1628.08 cm^{-1} with a very deep peak, while the amide II absorption peak in milkfish bone gelatin using CH3COOH solution is around 1643.16 cm^{-1}. Based on this, it can be concluded that milkfish bone gelatin produced using HCl solution and CH3COOH solution has a very strong amide II absorption region and can form -NH- bond deformations to produce α-helix chains [9].

The absorption area of amide III (C-N group) can be seen from the absorption peak in the frequency range around 1400-1100 cm^{-1}. Commercial gelatin derived from cattle has an amide A absorption area of 1325.15 cm^{-1} [13]. Based on the results of FTIR testing, the absorption peak of amide III in milkfish bone gelatin using HCl solution is around 1164.86 to 1329.32 cm^{-1} with a fairly stable wave peak, while the absorption peak of amide III in milkfish bone gelatin using CH3COOH solution is around 1239.55 cm^{-1} with a small peak. Based on this, it can be concluded that milkfish bone gelatin produced using HCl solution and CH3COOH solution has a fairly strong amide III absorption region [9].

In the frequency range of about 2400 cm^{-1}, the O=C=O stretching functional group was obtained in acetic acid gelatin which indicates the presence of residues extracted from acetic acid, but this does not affect the characteristics of the gelatin produced because gelatin with acetic acid solution has good characteristics and functional properties of gelatin. Based on the results obtained from the FTIR test, it can be concluded that milkfish bone gelatin using HCl solution and CH3COOH solution shows functional groups that are in accordance with commercial gelatin because it has met the standard range of each amide region and has a wave number value that is almost the same as commercial gelatin.

3.2 Discussion of soy milk pudding

3.2.1 Organoleptic

3.2.1 Texture

Texture is a parameter of sensory analysis produced by a product. Texture is one of the physical properties that can determine the taste in food that can be detected using the sense of touch in the food product being tested. Poor texture of food products can give a bad impression so that it can affect the acceptability and level of liking of panelists and consumers. The research data ranged from 2.81-3.75. The lowest texture organoleptic results were obtained in pudding using milkfish bone gelatin HCl solution, while the highest results were obtained in pudding using commercial gelatin. The organoleptic results of texture in soy milk pudding using various types of gelatin can be seen in Figure 5.

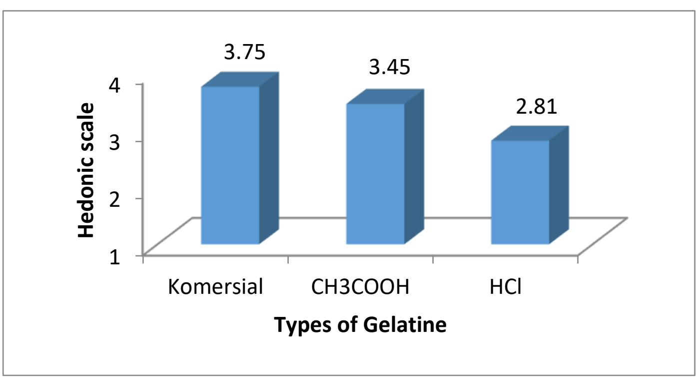

Fig. 5. Effect of gelatin type on the texture of soy milk pudding.

Based on the statistical results show that the type of gelatin in making pudding has a significant effect (sig. 0.000) at the 5% level ($p < 0.05$) on the texture of the resulting pudding. Then, based on the hedonic scale that has been converted, it is known that soy milk pudding using commercial gelatin and gelatin acetic acid solution (CH_3COOH) has the same texture, but is different from pudding using hydrochloric acid solution (HCl). The results obtained show that commercial pudding has the highest score of 3.75 (like) which has a soft and dense texture, while pudding using HCl solution gelatin has the lowest score of 2.81 (rather like) which has a rather liquid texture. This is due to the different gel strength values of the three types of gelatin used. Gelatin using HCl solution has a low pH which at a low pH level will cause a further collagen hydrolysis process so that it can undergo a degradation process which causes the gel strength value to decrease [17]. Therefore, if the lower the gel strength value, the texture of the resulting pudding will be more liquid and not preferred by consumers.

3.2.2 Taste

Taste is one of the parameters of sensory analysis that can be detected using the human sense of taste by tasting the food product being tested. Each food ingredient has a different flavor depending on the composition of the food ingredients and additives used. The research data ranged from 3.13-3.83. The lowest organoleptic taste results were obtained in pudding using milkfish bone gelatin HCl solution, while the highest results were obtained in pudding using commercial gelatin. The results of organoleptic taste in soy milk pudding using various types of gelatin can be seen in Figure 6.

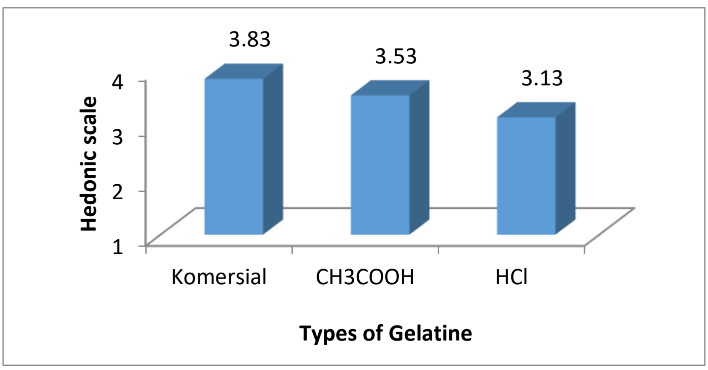

Fig. 6. Effect of gelatin type on the taste of soy milk pudding.

Based on the statistical results show that the type of gelatin in making pudding has a significant effect (sig. 0.000) at the 5% level ($p < 0.05$) on the taste of the resulting pudding. Then, based on the hedonic scale that has been converted, it is known that soy milk pudding using commercial gelatin and gelatin acetic acid solution (CH_3COOH) has the same taste, but is different from pudding using hydrochloric acid solution (HCl). The results obtained show that the commercial pudding has the highest score of 3.83 (like) which has a sweet taste, while the pudding using HCl solution gelatin has the lowest score of 3.13 (somewhat like) which has a moderately sweet taste. The use of gelatin did not affect the taste of the pudding produced because commercial gelatin and gelatin extracted from fish bones have a neutral taste [2]. However, the difference in taste of the pudding can be caused by the texture where the pudding using commercial gelatin and milkfish bone gelatin using CH_3COOH solution has a dense texture so that it has a taste that is preferred by consumers. Meanwhile, the milkfish bone gelatin pudding using HCl solution has a rather liquid texture so that the sweetness of the pudding is reduced and can affect the panelists assessment.

3.2.3 Aroma

Aroma is one of the parameters of sensory analysis that can be detected using the sense of smell by smelling the aroma of the food product tested. The aroma of a product can be caused by volatile compounds that evaporate. The research data ranged from 3.55-3.76. The lowest organoleptic flavor results were obtained in pudding using milkfish bone gelatin HCl solution, while the highest results were obtained in pudding using commercial gelatin. The organoleptic results of aroma in soy milk pudding using various types of gelatin can be seen in Figure 7.

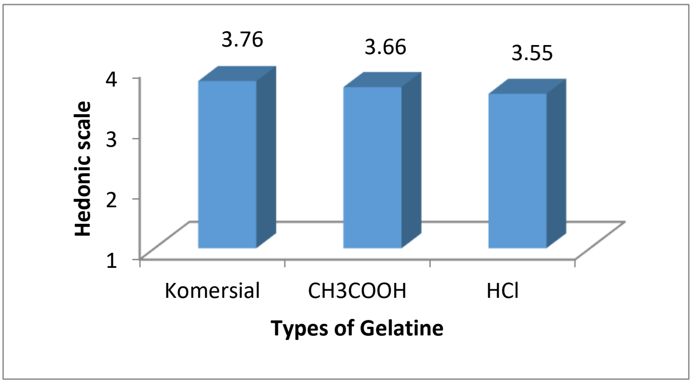

Fig. 7. Effect of gelatin type on the aroma of soy milk pudding.

Based on the statistical results, it shows that the type of gelatin in pudding making does not have a significant effect (sig. 0.000) at the 5% level ($p < 0.05$) on the aroma of the pudding produced. The results obtained show that commercial pudding has the highest score of 3.76 (like) which has a chocolate aroma, while pudding using HCl solution gelatin has the lowest score of 3.55 (like) which has a chocolate aroma. This is due to the use of the same chocolate powder and chocolate bar in all soy milk pudding treatments, resulting in the same distinctive chocolate aroma. In the process of heating the pudding, the volatile compounds from the additives used will evaporate, resulting in a distinctive chocolate aroma [2]. Therefore, panelists could not distinguish the pudding samples presented which could be influenced by panelists habits in consuming pudding which is generally marketed in the form of chocolate pudding.

3.2.4 Color

Color is one of the parameters of sensory analysis that can be detected using the sense of sight by directly seeing the color or appearance of the food product being tested. Each food product has a certain color as its characteristic which serves as a parameter of quality or quality by consumers. The research data ranged from 4.01-4.08. The lowest color organoleptic results were obtained in pudding using milkfish bone gelatin HCl solution, while the highest results were obtained in pudding using commercial gelatin. Color organoleptic results on soy milk pudding using various types of gelatin can be seen in Figure 8.

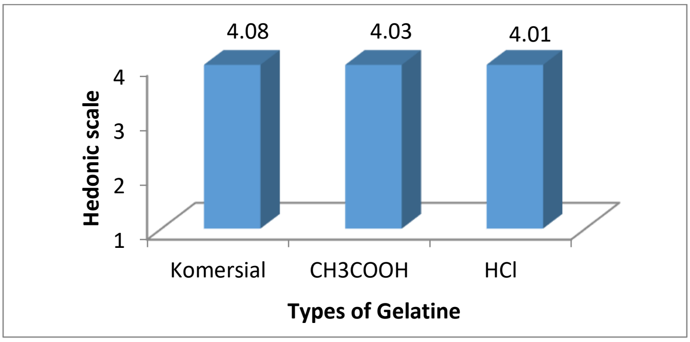

Fig. 8. Effect of gelatin type on the color of soy milk pudding.

Based on the statistical results showed that the type of gelatin in making pudding did not have a significant effect (sig. 0.000) at the 5% level ($p < 0.05$) on the color of the pudding produced. The results obtained show that commercial pudding has the highest value of 4.08 (like) which has a brown color, while pudding using HCl solution gelatin has the lowest value of 4.01 (like) which has a brown color. This is due to the addition of the same chocolate powder in soy milk pudding treatments resulting in the same color. Therefore, panelists could not distinguish the pudding samples presented which could be influenced by panelists habits in consuming pudding which is generally marketed in the form of chocolate pudding [17].

3.2.2 Syneresis

Syneresis is a parameter that shows the slow shrinkage of the gel which is influenced by time and the release of liquid in the gel. The smaller the syneresis value, the better the product will be because it is more difficult to release water [3]. Syneresis testing serves to see the amount of water that can be released by a gel product. The research data ranged from 4.099%-8.042%. The lowest syneresis result was obtained in pudding using commercial gelatin, while the highest result was obtained in pudding using milkfish bone gelatin HCl solution. The results of the syneresis value in soy milk pudding using various types of gelatin can be seen in Figure 9.

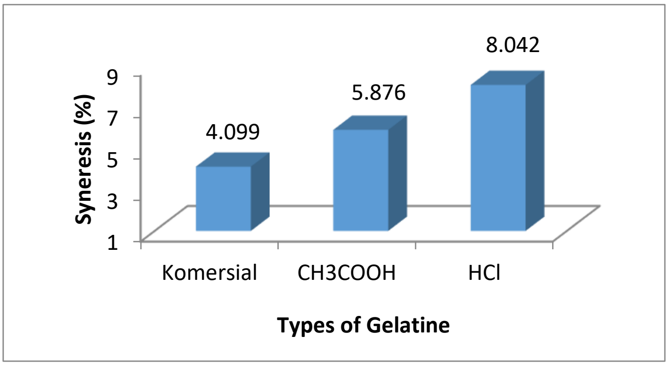

Fig 9. Syneresis value of soy milk pudding.

Based on the statistical results showed that the type of gelatin in pudding making had a very significant effect (sig. 0.000) at the 5% level ($p < 0.05$) on the percentage of pudding syneresis produced. Then, based on the results of Duncan's further test at the 5% level, it was found that pudding using commercial gelatin was significantly different from pudding using

HCl solution gelatin. The results obtained show that there is an increase in the percentage of syneresis which is highest in pudding using HCl gelatin solution. This is because the pudding using HCl solution has gelatin properties with lower gel strength so that it is easier to release water compared to commercial gelatin and gelatin using CH_3COOH solution which has higher gel strength. The syneresis value is inversely proportional to the gel strength value where the higher the syneresis value, the lower the gel strength value [6]. The low gel strength indicates that the binding power of gelatin water to the pudding will decrease so that the syneresis content will increase.

3.2.3 Moisture content

Water is one of the components often found in food ingredients. Moisture content is very important in determining the quality of pudding products. The lower the moisture content, the better the quality of a product because it is more difficult for microorganisms to grow [4]. The principle of testing water content is by heating the sample at high temperature until a constant weight is obtained. The research data ranged from 39.1%-54%. The lowest water content results were obtained in pudding using commercial gelatin, while the highest results were obtained in pudding using milkfish bone gelatin HCl solution. The results of moisture content in soy milk pudding using various types of gelatin can be seen in Figure 10.

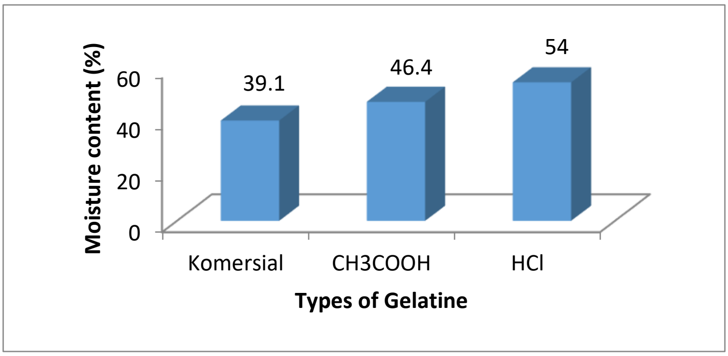

Fig. 10. Moisture content value of soy milk pudding.

Based on the statistical results showed that the type of gelatin in making pudding had a very significant effect (sig. 0.000) at the 5% level ($p < 0.05$) on the percentage of water content of the pudding produced. Then, based on the results of Duncan's further test at the 5% level, it was found that pudding using commercial gelatin was significantly different from pudding using HCl solution gelatin. The results obtained show that there is an increase in the percentage of water content that is highest in gelatin using HCl solution. This is because the value of syneresis is directly proportional to the value of water content, where the lower the strength of the gelatin gel to bind water, the higher the water content produced in a product [18]. In addition, the oven process on the gelatin produced can also affect the application of gelatin where the results on gelatin using HCl solution are more moist than gelatin using CH_3COOH solution.

3.2.4 Protein content

Protein is a component of which consists of amino acids and is connected by peptide bonds and serves to compose all parts of the human body. The protein test used is the Kjeldahl method which serves to determine the amount of protein contained in soy milk pudding with the addition of various types of gelatin. The research data ranged from 5.63%-6.52%. The lowest protein content results were obtained in pudding using commercial gelatin, while the highest results were obtained in pudding using milkfish bone gelatin HCl solution. The results of protein content in soy milk pudding using various types of gelatin can be seen in Figure 11.

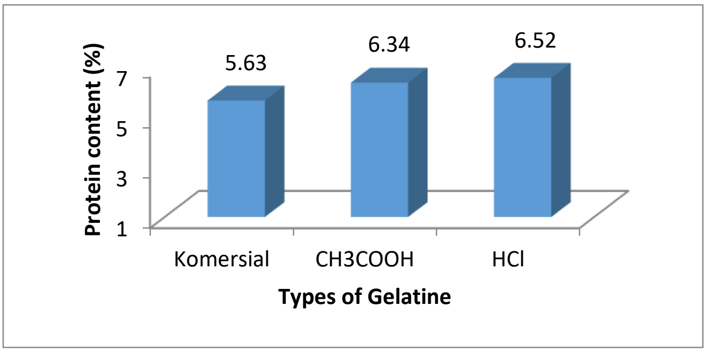

Fig. 11. Protein content value of soy milk pudding.

Based on the statistical results showed that the type of gelatin in making pudding had a very significant effect (sig. 0.000) at the 5% level ($p < 0.05$) on the percentage of pudding protein content produced. Then, based on the results of Duncan's further test at the 5% level, it was found that pudding using commercial gelatin was significantly different from pudding using HCl solution gelatin. The results obtained show that there is an increase in the percentage of protein content that is highest in gelatin using HCl solution. The difference in protein content can be influenced by the raw material of the gelatin used. Commercial gelatin made from cow bone has a protein content of about 24%, while fish bone has a high protein content of about 32% [1]. In addition, the gelatin extraction process using HCl solution has a high acid concentration so that it will break more amino acid bonds. This causes the soluble protein content in the extraction process to increase so that the protein content of gelatin will increase [7]. Therefore, the pudding produced from gelatin using HCl solution has the highest protein content.

4 Conclusions and suggestions

4.1 Conclusions

The conclusions obtained from this research are:

1. The characteristics of milkfish bone gelatin with acetic acid treatment had a gel strength of 181.5 g/bloom, a yield of 2.2%, and a pH of 7. Meanwhile, the characteristics of milkfish bone gelatin with hydrochloric acid treatment had a gel strength of 57.5 g/bloom, a yield of 13.97%, and a pH of 6. Both types of gelatin have almost the same structure based on FTIR testing.
2. The best result obtained is pudding with the addition of acetic acid milkfish bone gelatin because it has almost the same organoleptic as pudding made from commercial gelatin and can be accepted by panelists.

4.2 Suggestions

Suggestions for further research are to make gelatin using other types of fish bones and conduct more specific gelatin characterization in order to produce new innovations for industry in Indonesia. In addition, the composition of the use of gelatin powder is analyzed so that it can be applied in the food industry.

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