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The Characteristic of Physicochemical, Chemical and Organoleptic Gelatin: Stingray (*DasyatisSp*) and Unicorn Leatherjacket (*Aluterusmonoceros*) Skin.

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ABSTRACT

Gelatin is a type of protein extracted from collagen tissue in animal skin and bones. *Dasyatissp* and *Aluterusmonoceros* skin waste can be utilized as gelatin. Gelatin quality is determined based on the characteristics of physicochemical, chemical and organoleptic. The research method uses factorial Completely Randomized Design (CRD) with three replications. Statistical data processing results of the study determine the best combination used the effectiveness index method with the De Garmo procedure. The results showed the best combination of treatments based on the characteristics of physicochemical, chemical and organoleptic obtained on gelatin made from *A. Monoceros* skin with a soaking time of 36 hours and extraction temperature of 60°C, namely: gel strength 18,21 mm/g.s;gelling point 11,67°C; viscosity 5,83 cps; yield 6,11%; pH value 3,9; protein content 92,65%; fat content 1,08%; water content 3,89%; ash content 3,47%, color 6,43 and the odor of 5,83.

Keywords: Gelatin,*Dasyatissp, Aluterus Monoceros*, Physicochemical, Chemical, Organoleptic

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INTRODUCTION

Gelatin is a type of protein extracted from collagen tissue in animal skin and bones [9], [19]. Gelatin is widely used in various fields, namely for food products, cosmetics, photography and pharmaceuticals [10]. The process of making gelatin itself begins with swelling, which is the soaking process using chemical solutions aimed at opening collagen tissue in order to remove thewater in fish skin. Therefore, collagen is more easily extracted. This demineralization process can be carried out by soaking in 4-7% hydrochloric acid concentrations [12], [20], [25]. In the soakingstage, hydrolysis of the main chain occurs and the breakdown of the material crosslinking so that during heating the gelatin will dissolve easily. The soaking solutions commonly used are hydrochloric acid (HCl) and sodium hydroxide [6], [2].

Another stage which is determining in making gelatin is the extraction step. The extraction step aims to convert collagen to gelatin. The physicochemical quality of gelatin (viscosity, gelling point, melting point and gel strength) is strongly influenced by the type of fish and the method used during the extraction process. During extraction, there will be several factors which influence the gelatin result, namely: treatment concentration, treatment time and temperature [7], [26], [23]. The extraction step to convert collagen to gelatin was carried out by using the waterbath at 40 – 80^{0}C [15].

The availability of waste from the fishing industry has the potential to replace gelatin from mammals. Waste of fish skin can be utilized as gelatin so it is expected to be able in increasing its economic value. Moreover, it can be a safe and halal alternative gelatin also to reduce industrial dependence on gelatin derived from mammals. The improvement of gelatin quality and the importance of the utilization of fish skin waste is the basis for research on the effect of soaking time and extraction temperature on the quality of gelatin from fish skin waste, especially stingray (*Dasyatissp*) and unicorn leatherjacket (*Aluterusmonoceros*) skin waste. The quality of gelatin is usually based on the characteristic ofphysicochemical, chemical (proximate) and organoleptic of the gelatin itself.

MATERIALS AND METHODS

Material

The fish skin was used in this study is *A. monoceros* obtained from PT. VariaNiaga Nusantara Beji - Pasuruan, East Java, Indonesia and *Dasyatissp* obtained from Kenjeran Beach in Surabaya, East Java, Indonesia. The raw material was used for fish skin is stored in the 0-5^{0}C cold chain and then used in the process of making gelatin.

Manufacture of Gelatin

The raw material in the form of fish skin was washed first and then cut into small pieces with a size about 1-2 cm. The skin of the fish that had been cut into pieces wassoaked in HCl solution of 4% concentration with a different soaking time for about 24 hours and 36 hours. Fish skin was washed thoroughly with running water and continued with the extraction stage. Extraction was carried out at different temperatures about 60^{0}C and 80^{0}C, each for ± 5 hours [19], [12], [23]. Moreover, the extraction results were filtered and dried using an oven at 60^{0}C until a gelatin sheet is formed. This gelatin sheet was mashed to be used as gelatin powder.

Yield (%)

The percentage calculation of gelatin yield wasderived from the dry weight ratio of gelatin to the wet weight of the raw material which wasused for fish skin and stated as a percentage.

The Characteristic of Physicochemical, Chemical and Organoleptic.

The characteristic of *A. monoceros*and *Dasyatisspc*ollagen skin are important to know about the characteristic of physicochemical, chemical and organoleptic. The characteristic of physicochemical observed included gel strength [8], [18], the gelling point test [31],[19] and the viscosity test [26]. The characteristic of chemical observed included tests of pH (power of Hydrogen), fat content [29], protein content tests [3] and

ash content tests [28]. The characteristic of organoleptic gelatin from *A. monoceros* and *Dasyatissp* skin were carried out on the color and odor by using a preference test with the scoring test method.

Statistical Analysis.

The research onthe characteristic of physicochemical, chemical and organoleptic through*Dasyatissp* and *A. Monoceros* gelatin skin used a factorial Completely Randomized Design(CRD) with three replications. Statistical data processing results of the study determine the best combination used the effectiveness index method with the De Garmo procedure.

RESULTS AND DISCUSSION

The characteristic of physicochemical, chemical and organoleptic of *A. monoceros*and *Dasyatissp*gelatin skin with different soaking times and extraction temperatures (Table 1).

Table 1: The characteristic of physicochemical, chemical and organoleptic gelatin

Parameter	Average Treatment								Gelatin Quality Standards
	*Dasyatis*sp (A1)				*A. monoceros* (A2)				
	24 hours (B1)		36 hours (B2)		24 hours (B1)		36 hours (B2)		
	60^0C (C1)	80^0C (C2)	60^0C (C1)	80^0C (C2)	60^0C (C1)	80^0C (C2)	60^0C (C1)	80^0C (C2)	
Gel strength (mm/g.s)	28,10	18,70	40,90	41,80	17,48	13,66	18,21	21,38	50-300 bloom [27]
Gelling point (^{0}C)	11	11,17	12,50	12,83	8,50	8	11,67	11	15 [18]
Viscosity(cps)	5,43	4,63	4,68	4,90	9,60	6,17	5,83	5,17	1,5-7,5 [27]
pH Value	2,73	2,97	2,73	2,60	3,03	3,03	3,90	3,73	3,8-5,5 [27]
Yield(%)	10,54	9,99	9,55	11,74	5,29	7,81	6,11	7,63	-
Protein content (%)	83,86	84,48	90,43	88,22	90,89	91,43	92,65	92,64	88 [23]
Water content (%)	7,44	7,43	9,67	9,83	5,74	5,48	3,89	5,71	5,10 [18]
Fat content (%)	0,99	0,63	1,43	0,21	0,92	0,54	1,08	0,89	0,18-0,29 [21]
Ash content (%)	3,74	4,09	3,46	4,70	5,35	5,34	3,47	4,03	0,3-2 [27]
Color	5,47	4,77	4,70	5,30	6,13	6,60	6,43	6,20	Colorless to yellowish [27]
Odor	3,43	3,87	3,40	3,53	5,23	5,90	5,83	5,10	-

The Characteristic of Physicochemical.

In analyzing the characteristic of physicochemical by using the parameters of gel strength test, gelling points and viscosity of raw materials for *Dasyatissp*and *A. Monoceros* skin. The range of analysis onthe characteristic physicochemical of *Dasyatissp*gelatin skin, namely: strength of gelatin gel 18,7 – 41,8 mm/g.s, gelling point 11 – 12,83 °C and viscosity 4,63 – 5,43 cps. The range of analysis on the characteristic physicochemical of *A. Monoceros* gelatin skin, namely: strength of gelatin gel 18,7 -41,8 mm/g.s, gelling point 8 – 11,67°C and viscosity 5,17 – 9,6 cps (Figure 1).

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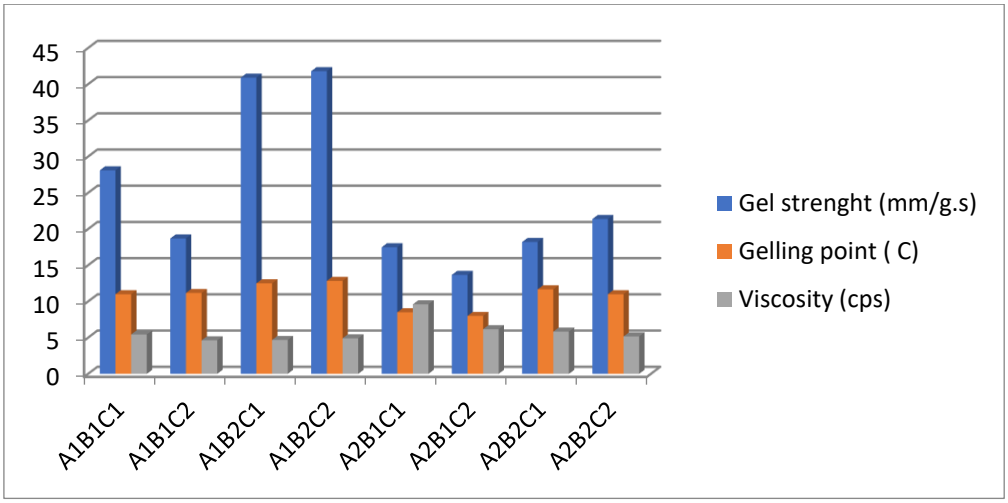
Figure 1. Graph of Test Results on the Characteristic of Physicochemical Gelatin



The results of the analysis on raw materials showed a significantly different effect on the value of gel strength and soaking time ($p < 0,05$). Therefore, further tests were carried out with Honestly Significant Difference (HSD). HSD further tests on the gel strength showed significantly different results, this is due to differences in the length of the amino acid chain contained in fish skins, where *Dasyatissp* and *A. monoceros* have different habitats that affect the number of amino acid chains. Gelatin which is produced from low temperature fish collagen has a low number of hydrogen bonds. The characteristic ofgelatin rheological (gel strength, gelling pointpoint and viscosity) are related to the hydroxyproline and proline collagen content of different species [13]. These amino acids show higher amounts in fish that live in warm waters. HSD further test results on soaking showed the strength value of gelatin gel with different soaking time gave significantly different results, this was due to the longer soaking the more hydrogen bonds between separate peptides. Therefore, collagen would be more easily extracted. Water entering the collagen fibers is caused by electrostatic forces between the polar groups of collagen fibers and H^+ from acids [14].

Table 2: Further test results of the interaction between the raw material with the soaking time and extraction temperature on the gel strength

Treatment		Average	Notation
A. Monoceros (A_2)	24 hours (B_1)	15,57	A
	36 hours (B_2)	19,795	B
*Dasyatis*sp (A_1)	24 hours (B_1)	23,4	B
	36 hours (B_2)	41,35	C
24 hours (B_1)	80°C (C_2)	16,18	A
	60°C (C_1)	22,79	B
36 hours (B_2)	60°C (C_1)	29,55	C
	80°C (C_2)	31,59	C

Note: notations shown with the same letters indicate no significant difference

The extraction temperature did not provide a significant difference in the gel strength values ($p > 0,05$). The higher extraction temperature tends to increase the strength value of the gelatin gel. This is caused when during heating/extraction, hydrogen bonds that exist between peptides decompose. Therefore, the higher extraction temperature, the more peptides decompose. Damage to hydrogen and covalent bonds due to collagen heating causes the stability of the triple helical structure to be disrupted and turned into rolls and eventually collagen is degraded into water-soluble gelatin [11]. Further test results on interaction of raw materials of *Dasyatissp* and *A. Monoceros* skin with different soaking times and extraction temperatures to gel strength showed differences in each combination (Table 2). The difference in raw material, soaking time and extraction temperature affect the length of the amino acid chain and the decomposition of hydrogen bonds between peptides.

The gelling point and length of soaking each showed a significantly different effect on the value of the gelling point (p <0,05). Therefore, further tests were done by HSD. The test results obtained that the gelling point value of *Dasyatissp* and *A. Monoceros*gelatin skin gave significantly different results, this is due to differences in the content of amino acids (proline and hydroxyproline) in the collagen of two species with different living habitats. Gelatin produced from low-temperature collagen has a number of low hydrogen bonds in water solutions and lower melting points compared to gelatin which is made from fish species from high-temperature environments [5], [34], [21].

The soaking time value and the different gelling points were significantly caused by the triple helical structure of amino acids that more binding to water. Therefore, the longer soaking in acid solutions,the more amino acids that bind to the water released during soaking also the faster it will form a gel. The longer ofsoaking tends to increase the value of gelatin gelling point. Different extraction temperatures do not provide a significant difference in the value of the gelling point (p> 0,05). The higher of extraction temperature tends to decrease the value of the gelatin gelling point.

This is thoughtof the bonds contained in collagen have damage due to the heating process that affects the formation of gel on gelatin. It can be seen that the gelling point of *Dasyatissp* and *A. Monoceros* gelatin skin is lower than the commercial gelatin of 15°C [19]. The difference in the value of the gelling point is due to the characteristics of the raw material and the extraction method used. With this lower level gelling point, fish gelatin can be widely applied, especially in the fields of medicine and food coating.

Viscosity showed that raw material of *Dasyatissp* and *A. Monoceros* had a significantly different effect on the value of viscosity (p <0,05). This was caused by the molecular weight of amino acid in each species of fish differing so that it influenced in the formation of solution. Viscosity value of *Dasyatissp* gelatin skin is lower than *A. Monoceros* gelatin skin. The difference in the value of gelatin viscosity is due to differences in molecular weight of each type of fish. The results of the analysis by Kruskal Wallis showed that the different soaking times did not give a real difference to the value of viscosity (p-value> 0,05). The longer of soaking tends to decrease the value of gelatin viscosity. The decrease in viscosity value is related to the raw material of *Dasyatissp* and *A. Monoceros* skin which has weak cross-linking so that the longer of soaking process, the weak cross-linking will be easily damaged. Weak cross-linking makes collagen easily hydrolyzed, this hydrolysis can reduce the molecular weight of gelatin which will reduce the viscosity of gelatin solution [4].

The analysis showed that the different of extraction temperatures did not provide a significant difference in the viscosity value (p> 0,05). The higher of extraction temperature tends to decrease the value of the gelatin viscosity. It was caused by increasing the temperature made the bonds between peptides and collagen will be more broken.Therefore, the easier ofthe peptide binds to water so that it affects viscosity. Commercial gelatin has a viscosity value of 7,5 cps [19]. Gelatin extracted from the skin of fresh stingray (*Dasyatissp*) has a viscosity below 8 cps [22]. The research results obtained viscosity values between 5.17 - 9.6 cps, low viscosity values can be influenced by extraction solutions used such as acids. The value of viscosity is influenced by molecular weight and amino acid chain length. The addition of acid solution in the process of making gelatin can break the peptide bonds of amino acids into molecules with short chains [33].

The Characteristic of Chemical.

Analysis of chemical properties used the test parameters of pH values, yield, protein content, water content, fat content and ash content of the raw materials of *Dasyatissp* and *A. Monoceros* skin. The range of analysis of the chemical properties of *Dasyatis sp.*Gelatin skinwere: pH value 2,6 – 2,97, yield 9,55 – 11,74%, protein content 83,86 – 90,43%, water content 7,43 - 9,83%, fat content of 0,21 – 1,43% and ash content of 3,46 – 4,70. The range of analysis values of the chemical properties of *A. Monoceros*gelatin skin, namely: pH values 3,03 – 3,9, yield 5,29 – 7,81%, protein content 90,89 – 92,65%, water content 3,89 - 5,74%, fat content 0,54 – 1,08% and ash content 3,47 – 5,35 (Figure 2 and Figure 3).

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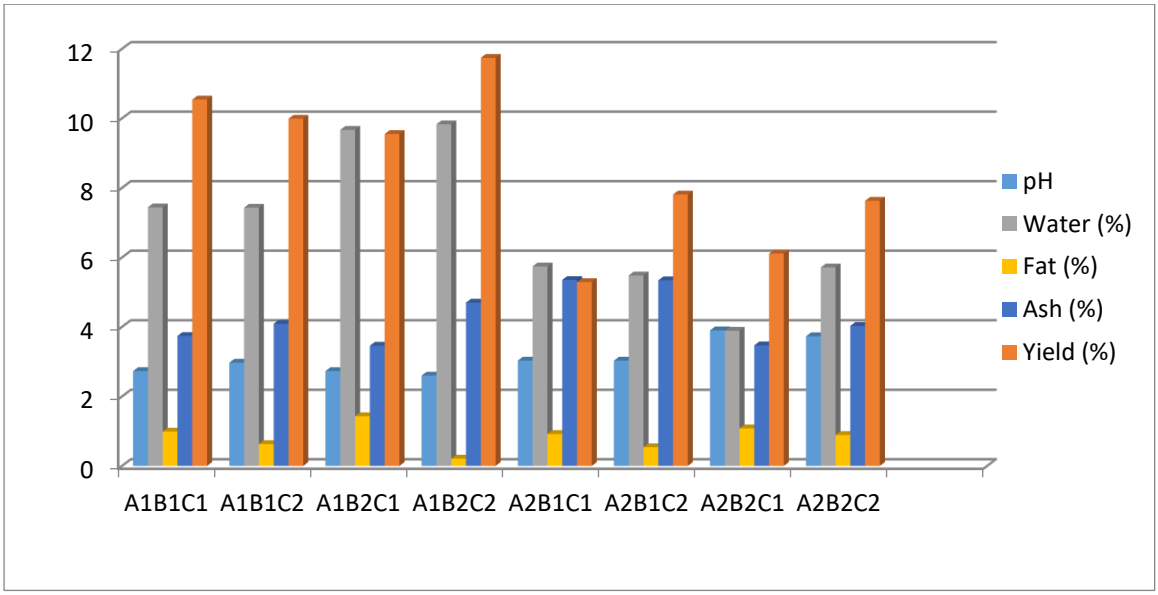
Figure 2. Graph of Test Results on the Characteristic of Gelatin Chemical



The results of the analysis on different raw materials gave significantly different effects on each pH value and the soaking time (p <0,05) so that further HSD tests were carried out. HSD further test results obtained pH values of gelatin with different raw materials gave significantly different results. The pH value of gelatin with *Dasyatissp* skin raw material is lower than *A. Monoceros* skin. Raw materials for *Dasyatissp* skin came from the drying process waste which was dried and it was suspected that the drying process can reduce the pH value on the skin. HSD further test results obtained that the pH value of gelatin with different soaking time gave significantly different results. The longer ofsoaking tends to increase the pH value of gelatin. It is because of the longer soaking so that the water contained in the skin tissue comes out and causes the concentration of acid solution decrease.

Different extraction temperatures did not provide a significant difference in the pH value (p> 0,05). Gelatin pH value with extraction temperature 60°C tends not to be different from gelatin pH value with extraction temperature 80°C. The pH value based on temperature did not differ due to the washing process after soaking. Therefore, the pH in collagen that had undergone swelling will be constant so that the pH value at the time of extraction did not differ much even with different temperature ranges. Further test results on interaction of raw materials and soaking time resulted in different pH values (Table 3). The difference in pH value was due to the influence of the type of acid solvent and the soaking time in the solution and the condition of the raw material.

Table 3: Further testing to the interaction of raw materials and soaking time on the pH value

Treatment		Average	Notation
*Dasyatis*sp(A_1)	36 hours (B_2)	2,665	A
	24 hours (B_1)	2,85	A
A. Monoceros (A_2)	24 hours (B_1)	3,03	B
	36 hours (B_2)	3,815	B

Note: notations shown with the same letters indicate no significant difference

The results of the analysis on different raw materials gave significantly different effects on yield (p <0,05). Therefore, further HSD tests were obtained that the yield of gelatin with different raw materials gave significantly different results. The difference in the value of this yield depend on the type of fish or raw material used. On *Dasyatissp* skin was thicker than *A. Monoceros* skin. The thickness of this skin affected the amount of collagen in the skin, where collagen if hydrolyzed produces gelatin. Therefore, the higher ofcollagen amount make the yield of gelatin is also higher.

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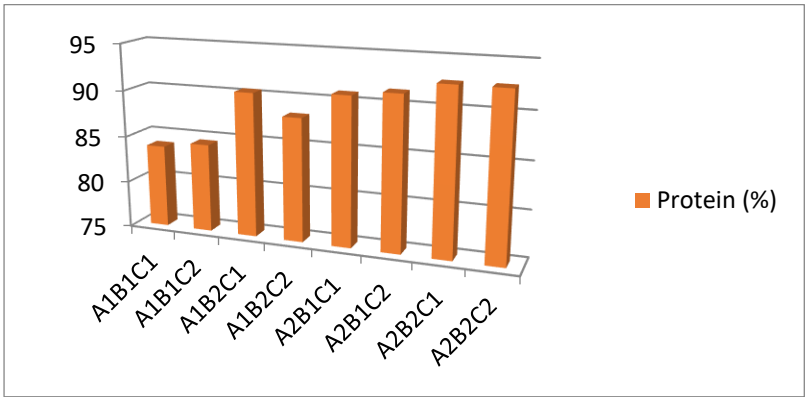
Each soaking time and extraction temperature did not give a significant difference to the yield (p> 0,05). The longer ofthe soaking and the higher of extraction temperature tends to increase the value of gelatin yield. However, long soaking caused more collagen to swellso that the resulting gelatin wasalso large. Whereas, the higher of temperature which wasused, the gelatin extraction process would be more perfect and producedmore gelatin.

Figure 3. Graph of Test Results on Gelatin Protein Levels



Protein content (Figure 3) showed that different raw materials have significantly different effects on protein content (p<0,05). This iscaused by the protein content of each type of fish is different, so that it affects the protein content of the gelatin. Gelatin obtained from *A. Monoceros* skin has 88% protein content, while *Dasyatissp* skin has a protein content of 83,86% [24], [22].

Different soaking time gave a significant difference to the value of protein content (p<0,05).Meanwhile, the different extraction temperatures did not provide a significant difference to the protein content (p>0,05). Long soaking in acid, the water content in the material will be more come out, causing higher levels of protein in the material. Long soaking tends to increase levels of gelatin protein. The temperature of gelatin protein content with extraction temperature of 60°C tends not to be different from the level of gelatin protein with extraction temperature of 80°C.

The analysis of water content showed that different raw materials have significantly different effects on water content (p <0,05). Therefore, further testing has done by HSD. The difference in water content was caused by each type of fish certainly has a different water content as well, so that it affects the water content of gelatin produced. *Dasyatissp* skin water content of 8,48%, this value is higher than the water content of *A. Monoceros*[22]. Different soaking and extraction temperatures did not provide a significant difference in water content (p> 0,05).

The long soaking process tends to increase in gelatin water content. Soaking caused more water to be absorbed and this water be morebinds with hydrogen and amino acids that are decomposed from hydrolysis of collagen. The higher extraction temperature tends to increase gelatin water content. This happens because of the higher extraction temperature allowed the amount of water absorbed during the extraction process.

Gelatin water content in this study still meets the gelatin quality standards (see Table 1) where the water content of gelatin originating from the skin or bone of fish is in the maximum range of 9-10%. Whereas in the commercial gelatin "Hann" derived from cow bones has a water content of 5,1%.

The results of the analysis on fat content showed that different raw materials did not have a significantly different effect on fat content (p> 0,05). Gelatin fat content with *A. Monoceros* skin is higher than *Dasyatis sp*. The value of high fat content in *A. Monoceros* due to the type of *teleostei*whichhas a greater fat content than the types of fish *elasmobranchii* (cartilage) such as *Dasyatis sp*.

Different soaking duration did not have a significantly different effect on fat content (p> 0,05). The long soaking process causes more water to come out, so that the fat content in the soaked material gets

higher. Besides, the effect of the type of soaking solution that causes the hydrolysis of fat. Moreover, the different of extraction temperatures provide significant differences in fat content (p <0,05) so that the higher of extraction temperature tends to decrease gelatin fat levels. Therefore, the longer soaking tends to increase levels of gelatin fat.

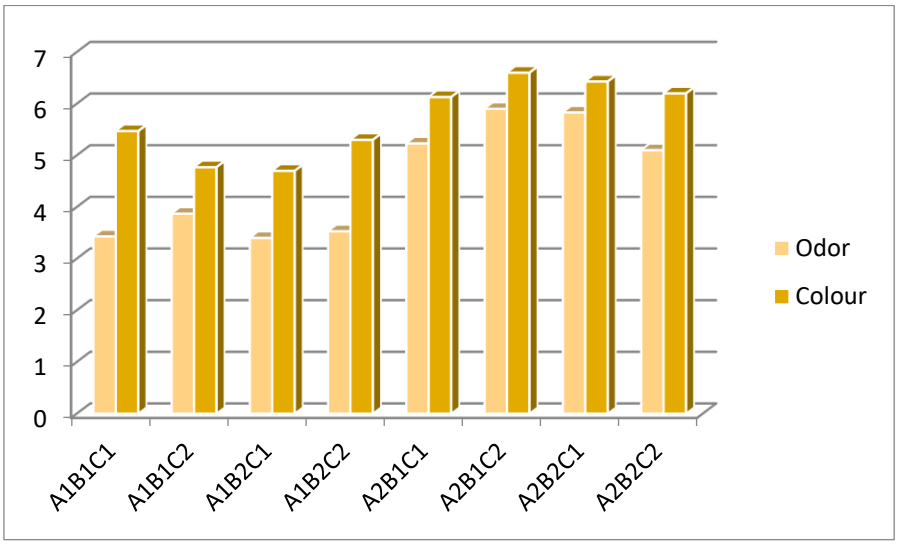
The range of fat content is quite good, because it does not exceed 5% which is the maximum value limit for gelatin quality requirements [19]. The higher levels of fat in gelatin, the quality of gelatin will be reduced because fat will cause the product to oxidize easily. Gelatin from leatherjacket (*A. Monoceros*) has a fat content ranging from 0,18 to 0,29% [24], while the fat content from *Dasyatissp* gelatin skin is 0,95% [22].

Different raw materials do not have significantly different effect on ash content, soaking time on ash content and extraction temperature on ash content (p-value> 0.05). The level of gelatin ash with raw materials of *Dasyatissp* skin is lower than *A. monoceros* skin. *Aluterusmonoceros* is includinga reef fish, so it has higher mineral content. The long soaking process tends to decrease gelatin ash levels. It is thoughtbecause of the longer soaking make the skinminerals will be more released and wasted during washing. High extraction temperatures tend to increase levels of gelatin ash. High ash content of raw material gelatin is due to the mineral component bound to collagen which has not been released during the washing process. Therefore, it is extracted and carried away during the ash process. The levels of gelatin ash produced from *leatherjacket* skin ranged from 0,60 to 0,71% [24].

The Characteristic of Organoleptic.

Organoleptic properties analysis used color and odor test parameters for *Dasyatissp* and *A. Monoceros* gelatin skin. The organoleptic range of gelatin from the skin of *Dasyatissp*, namely: colors 4,7 – 5,47 and odors 3,4 – 3,87. The range of organoleptic analysis values of *A. Monoceros* gelatin skin, namely: color 6,13 – 6,6 and odor 5,1-5,9 (Figure 4).

Figure 4. Graph of Organoleptic Gelatin Test Results



The results of the color analysis (Figure 4) with the Kruskal Wallis method showed that different raw materials give significant differences in the color change in gelatin (p <0,05). Gelatin discoloration was caused by raw materials of *Dasyatissp* skin that are not fresh. This is because of previously the drying and fuming processes have been carried out. This test color is to facilitate the application of gelatin to food types. On the other hand, different soaking length and extraction temperature did not show significant differences in the color of gelatin (p> 0,05). There was no difference in organoleptic (color) values indicating that the panelists could not distinguish the difference in color due to the different soakingtime and extraction temperature.

Figure 5. *Dasyatissp* skin gelatin (left) and *A. Monoceros* skin gelatin (right)

The results of this gelatin were yellow to slightly brownish. This was very different from the gelatin quality standards that gelatin is colorless. The difference in color was influenced by the quality of the raw material used for the skin, where the use of gelatin raw material is recommended to use fresh raw materials that have not been through any treatment. Therefore, it will obtain gelatin with a brighter color. The organoleptic test results of the panelists stated neutral and they like it. The application of gelatin fish skin is recommended depending on the type of food products that do not feature commercial color.

The odor analysis was performed by using the Kruskal Wallis method. Different raw materials gave significant differences in changes in the odor of gelatin ($p < 0,05$). This difference was due to *Dasyatissp* containing ammonia in the body and skin so that the process of handling raw materials that were less than perfect still leaves a residual odor on the gelatin. Organoleptic test results showed that the panelists did not distinguish the difference in odor due to the soaking time and the different extraction temperatures ($p > 0,05$).

Gelatin from *Dasyatissp* skin was not favored by panelists, but gelatin from the skin of leatherjacket was neutral and somewhat preferred by panelists. According to the standard quality of gelatin which was stated that the odor of gelatin is normal. Therefore, the appropriate gelatin is that comes from the skin of *A. Monoceros*.

CONCLUSION

The treatment of different raw materials gives a real influence on the gel strength, the gelling point, viscosity, pH value, yield, protein content, water content, color and odor. The different soaking treatment has a significant effect on the gel strength, the gelling point, the pH value and protein content. Different extraction temperature treatments have a significant effect on fat content. The best combination of treatments in the study was gelatin made from *A. Monoceros* skin with a soaking time of 36 hours and extraction temperature of 60°C, namely: gel strength 18,21 mm/gs, gelling point 11,670°C, viscosity 5,83 cps, yield 6,11%, pH value 3,9, protein content 92,65%, fat content 1,08%, water content 3,89%, ash content 3,47%, color 6,43 and odor 5,83.

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