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## Effect of Soaking Time with Acetic acid on tuna skin gelatin (*thunnus albacores*)

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**Abstract** Gelatin is a protein-derived product obtained from the hydrolysis of animal collagen contained in bones and skin. Gelatin is useful for food, pharmaceutical, cosmetic, and photography industries. This study aims to determine the characteristics of gelatin with variations in soaking time and to determine the best soaking time variation produced. The design used in this study was a completely randomized design (CRD) with 4 treatments and 3 replications. Observation data were analyzed using ANOVA with F test and DNMRT further test at 1% real level. Variables observed: a. Yield, b. Moisture content, c. Ash content, d. pH and e. Viscosity. The results of this study showed that the variation of soaking time with 3% acetic acid had a very significant effect on the quality of tuna skin gelatin, namely: yield, water content, ash content, pH and viscosity. The best soaking time is at 24 hours soaking, namely: yield (11.78%), moisture content 7.4 ash content (0.37%), pH (5.95) and viscosity (1.57 cps).

**Keywords** tuna fish, gelatin, acetic acid, soaking method

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### Introduction

Tuna fish is a type of fish that contains very high protein. The nutritional composition of tuna fish varies depending on species, type, age, season, metabolic rate, movement activity, and level of gonadal maturity. Tuna is generally marketed as fresh product, loin, filet, steak and canned product. Tuna fish is a fish that has a high selling value and is among the most sought after types of fish from Indonesian seas, containing calcium, phosphorus, iron and sodium minerals, vitamin A and vitamin B, which are nutritious and healthy. In addition, this fish has a high selling value, due to its delicious taste. However, the edible part of tuna fish only ranges from 50% - 60% in the form of meat. This results in by-products such as head, scales, skin and bones. Fish bones and heads are by-products that dominate both industrial and household scales [1].

These tuna products produce by-products in the form of bones and skin. The by-products of tuna bones and skin in some industries are still not optimally utilized. The by-products of tuna skin are not utilized, just thrown away and become solid waste. Fish-processing waste can form an ideal raw material for gelatin preparation as fish skin and bone is a major by-product of the fish-processing industry [2]. Utilization of tuna skin can be processed into gelatin because it contains collagen, this can be seen from the structure of the skin which is very supple (elastic). Gelatin is a natural polymer derived from collagen processing that can be obtained from various animal sources including pig, cow and fish skin [3]. The traditional sources of gelatin include bovine and pig skins and demineralized bones and hooves. However, recent studies have shown that there are viable new sources of gelatin such as marine fish skins and bones [4].



The utilization of tuna fish skin waste as collagen is very important to develop because this fish skin waste in addition to being a source of collagen also encourages the utilization of natural materials so as to apply material effectiveness, reduce environmental pollution, apply clean processing and increase the selling value of fish skin from just waste to more valuable materials. Another advantage of making collagen with skin raw materials compared to raw materials from bones and scales is that it requires a faster process time with an acid soaking process. In addition, fish skin has a softer texture compared to bone so it requires a faster extraction process time [5].

The process of collagen hydrolysis by immersion in acidic solution takes shorter time than immersion in alkaline solution because acid is able to convert triple helix collagen fibers into single chain while alkaline immersion solution is only able to produce double chain. So that at the same time the collagen hydrolyzed by the acidic solution is more than the basic solution [6]. This series of collagen hydrolysis process contained in the skin collagen protein can be scientifically captured to be converted into gelatin [7].

Gelatin is a water-soluble conversion protein that can either be a gelling agent (gel-making ingredients) or a non-gelling agent [8]. Gelatin from fish bone including gelatin type A, so it is necessary to do the treatment process with an acid solvent that is hydrochloric acid to produce gelatin in accordance with standard and gelatin which halal.

The process of converting collagen into gelatin involves three changes, namely the breaking of a number of peptide bonds to shorten the chain, the breaking of a number of side bonds between chains, and a change in chain configuration. Gelatin derived from acid-treated precursors is known as type A and gelatin derived from alkaline-treated precursors is known as type B [9]. Type A, with isoionic point of seven to nine, is derived by using exclusively acid pretreatment. Type B, with isoionic point of four to five, is the result of an alkaline pretreatment.

The gelatin production process can be divided into an acid process (type A) and an alkaline process (type B). The acid process will convert collagen into gelatin in a short time, because the acid will change the triple helix collagen fiber into a single chain, while the alkaline process will maximize the converted collagen but the resulting gel strength will be low and the time required is relatively long [10].

The use of acetic acid for hydrolysis has several advantages. First, the price of weak acid solvents, which are cheaper than strong acids, can reduce the cost of gelatin production. Secondly, the gelatin produced has no pungent odor and no dark color. Third, acetic acid is categorized as green chemistry so it does not damage the environment [11]. This study was conducted to determine the effect of soaking time in acetic acid solution on gelatin from tuna fish bones.

## Materials and Methods

The design used in this study was a completely randomized design (CRD) with 4 treatments and 3 replicates. The data obtained were analyzed using ANOVA (F test) and Duncan's New Multiple Range Test (DNMRT) at 1% real level. Preparation of tuna skin gelatin with acid process using 3% acetic acid [12], the treatment is the length of soaking of tuna skin with acetic acid, as follows: 6, 12, 18, and 24 hours.

## Results and Discussion

### *Rendemen, moisture content, and ash content of tuna skin*

Experiments on the length of soaking with acetic acid on the yield, moisture content and ash content of tuna skin are presented in Table 1.

**Table 1:** Average yield, moisture content, and water content of tuna skin gelatin treated with soaking time with acetic acid

Soaking Time with Acetic Acid (hours)	Rendemen (%)	Moisture Content (%)	Ash Content (%)
6	7.06a	10.18a	2.05a
12	7.60b	9.51b	1.73b
18	8.58c	8.40c	0.92c
24	11.78d	7.45d	0.37d

Column numbers followed by the same letter are not different according to DNMRT test at 1% level.



Table 1 shows that the yield of gelatin produced ranged from 7.06-11.78%. The longer soaking time with acetic acid led to an increase in gelatin yield, as shown in Figure 1(a).

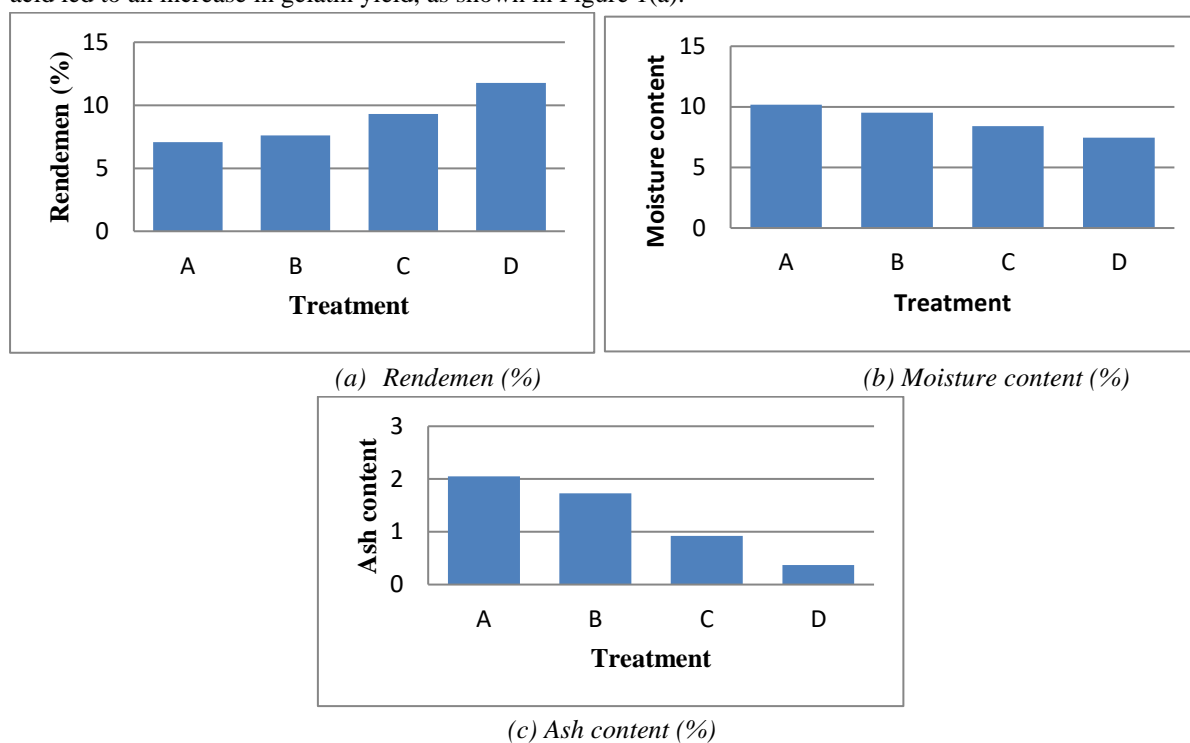


Figure 1: Average yield, moisture content, and ash content of tuna skin gelatin in various treatments of soaking time in acetic acid.

From Figure 1(a), it can be seen that the longer the soaking time with 3% acetic acid, the higher the yield. This is because the yield produced is influenced by the length of soaking time with 3% acetic acid and extraction time, so that the gelatin produced increases. During soaking,  $H^+$  ions from the acid solution will interact with collagen, where some of the hydrogen bonds in the tropocollagen will be hydrolyzed resulting in tropocollagen chains that begin to lose their triple helix structure, as well as the cross-links that connect the tropocollagen with one another, tissues containing collagen that are treated with acid and given heating will cause the collagen fibril structure to be broken down continuously, so as to increase the yield and protein value [13]. The higher the acid concentration and the longer the soaking time, the more hydrogen bonds and hydrophobic bonds that are stabilizing bonds in the triple helix will be broken down into  $\alpha$ ,  $\beta$ ,  $\gamma$  components, making it easier and more converted into gelatin [14].

Table 1(b) also explains the moisture content of tuna skin gelatin produced ranged from 7.45-10.18%. The longer the soaking, the lower the water content. This is because the longer the soaking time causes the reaction to run quickly so that the free water available is also more which results in the water content decreasing during drying. According to [15] moisture content is influenced by water loss during the drying process and water absorption during soaking. The decrease in gelatin moisture content is due to the open and weak collagen structure that produces gelatin with a weak structure, as a result the water binding power of gelatin is less strong [16]. The water content of gelatin will affect the shelf life, because it is closely related to the metabolic activity that occurs while the gelatin is stored [17].

Table 1(c) also explains the ash content of tuna skin gelatin produced ranged from 0.37-2.05%. It can be seen that the ash content is decreasing. This is because the ash content is determined by the demineralization process, the more minerals that are dissolved, the lower the ash content. The high or low ash content comes from the minerals in the test sample and also the demineralization process during gelatin processing [18]. During soaking in acid solution, there is a reaction between acid and calcium phosphate. The reaction between the two produces soluble calcium salts so that the ash content of the gelatin obtained is lower. According to [19], the ash content



of gelatin and other proximate parameters are influenced by the content of the raw materials, the filtration method, and the extraction performed.

### *pH and Viscosity of Tuna Skin Gelatin*

Experiments on the duration of soaking with acetic acid on the pH and viscosity of tuna skin are presented in Table 2.

Table 2: Average pH and viscosity of tuna skin gelatin treated with soaking time with acetic acid.

Soaking Time with Acetic Acid (hours)	pH	Viscosity (cps)
6	5.28a	3.08a
12	5.35b	2.84b
18	5.51c	2.25c
24	5.95d	1.57d

Column numbers followed by the same letter are not different according to DNMRT test at 1% level.

Table 2 explains the pH of the tuna skin gelatin produced ranged from 5.28-5.95. The longer the immersion, the pH value increased, as shown in Figure 2(a).

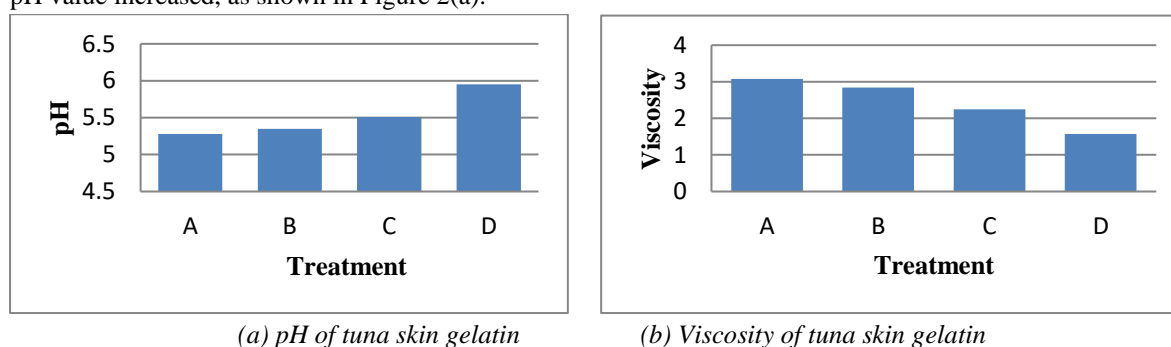


Figure 2: Average pH and viscosity of tuna skin gelatin at various immersion lengths in acetic acid

In Figure 2(a), it can be seen that the longer the immersion with 3% acetic acid, the more the pH value increases. The difference in pH value of tuna skin gelatin is because during the demineralization process, acetic acid is still carried during the extraction process, thus affecting the acidity of the resulting gelatin. Measurement of the pH value of gelatin is important because pH can affect the application of gelatin in products. The pH of the medium strongly affects many enzymic processes and transport of various components across the cell membrane [20].

The pH value of collagen obtained (5.28 - 5.95) is lower when compared to [21], who reported that the pH value of parang-parang fish skin collagen isolated by hydro-extraction method was 5,47. This is because the hydro-extraction method uses a lower concentration of acid, then neutralization and extraction using water so that the pH value of collagen tends to be neutral [22].

Table 2 also explains the viscosity of tuna skin gelatin produced ranged from 1.57-3.08 cps. It can be seen that the longer the soaking time with acetic acid, the lower the viscosity of tuna skin gelatin. This can be caused by the longer the soaking, the longer the hydrolysis time so that it is possible that the gelatin that has been formed continues to hydrolyze so that the polymer is split, the molecular weight becomes smaller so that the viscosity decreases. The higher the acid concentration, the more open the amino acid chain structure is which causes more amino acid chain cuts resulting in shorter chains and the molecular weight of collagen will be reduced which results in a low viscosity value [23].

Content the viscosity of gelatin is affected by the molecular weight of gelatin, and the molecular weight of gelatin is affected by the length of its amino bonds. The acid solvent used during demineralization breaks the amino bonds of collagen so that when collagen is hydrolyzed into gelatin, it will produce a low molecular weight so that the viscosity will decrease. The length of demineralization affects the number of collagen amino bonds that are broken due to the solvent used [24] and [15]. High viscosity values are required for various industries, such as food stabilizers, pharmaceuticals and photographic emulsions. Low viscosity is required for the sugar industry [16].



## Conclusions

Soaking with 3% acetic acid is best for producing tuna skin gelatin for 24 hours, namely, yield (11.78%), moisture content (7.45%), ash content (0.37%), pH (5.95) and viscosity (1.57 cps). The characteristics of tuna skin gelatin produced by soaking time of 6-24 hours with 3% acetic acid are: Yield (7.06-11.78), moisture content (7.45-10.18), ash content (0.37-2.05), pH (5.28-5.95) and viscosity (1.57-3.08 cps).

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